



Seed production in the northern light

Proceedings of the Sixth International Herbage Seed Conference, Gjønnestad, Norway
18 - 20 June 2007

Trygve S. Aamlid, Lars T. Havstad & Birte Boelt (eds)

Seed production in the northern light

Proceedings of the Sixth International Herbage
Seed Conference, Gjenestad, Norway
18 - 20 June 2007

Trygve S. Aamlid, Lars T. Havstad & Birte Boelt (eds)



The publisher of Bioforsk Fokus is:

Bioforsk, Fredrik A Dahls vei 20, 1432 Ås, Norway
post.bioforsk@no
Editor in charge: Research Director Nils Vagstad

Editors of this issue:

Trygve S. Aamlid, Lars T. Havstad & Birte Boelt

ISBN: 978-82-17-00234-5

ISSN: 0809-8654

Photos on front cover: Lars T. Havstad

Print works: Birkeland Trykkeri

This issue can be ordered from:

Bioforsk Øst Landvik
Reddalsveien 215
N-4886 Grimstad
Norway

Price: 300 Nkr

Our sponsors:

Felleskjøpet Agri
Felleskjøpet Rogaland Agder
Strand Brænderi AS
DLF-Trifolium

PREFACE

Since the formation of the International Herbage Seed Group (IHSG, formerly IHSPRG) in 1978, International Herbage Seed Conferences have been organized, mostly at four year intervals. The Sixth Conference was held at Gjennestad Horticultural College in Vestfold, Norway, from 18 to 20 June 2007. As for other IHSG activities, the objective of the conference was 'to encourage co-operation and communication between workers actively engaged in herbage seed production research'.

About 80 delegates from 20 countries attended the conference. Four invited and almost sixty voluntary papers were presented, either orally or as posters. The topics were split into the following sessions:

1. Opening session with overview over herbage seed production and seed trade, world wide.
2. Herbage seed for the future: Biodiversity, GMOs and the role of seed yield capacity in herbage breeding programs.
3. Seed production of tropical species and species for stressful environments.
4. Physiological restraints to seed set and seed filling.
5. Establishing the potential for high and pure seed yields.
6. Fertility, plant growth regulators, and plant protection.
7. Statistical methods, seed harvest, and post-harvest issues.

The title of this volume, 'Seed production in the northern light', eludes to the fact that the conference venue is located at 59°N. The light conditions at northern latitudes have consequences, not only for seed crops, but also for conference delegates experiencing the long mid-summer days in Norway.

These proceedings include, mostly as full-text, all papers presented at the conference. The manuscripts have been reviewed and edited, mostly for style, but in some cases also for clarification of the scientific content, by a committee consisting of IHSG-president Birte Boelt, and Lars T. Havstad and Trygve S. Aamlid of Bioforsk, the conference's Norwegian host institute.

Besides the editors of the proceedings, the conference organizing committee has consisted of IHSG treasurer Christian Haldrup, Denmark, Agnar Kvalbein and Geir Fossnes from Gjennestad Horticultural College, Per Bjerkø from Vestfold Seed Growers' Union, and John Ingar Øverland from the Vestfold Farmers Extension Service Group. I would like to thank all of you for helping organize the various aspects of the conference, including pre- and post-conference tours, program for accompanying persons, excursions and social events.

I would also like to acknowledge the Norwegian seed companies Felleskjøpet Agri, Felleskjøpet Rogaland Agder and Strand Brænderi AS and the Danish seed company DLF-Trifolium for their financial support of the conference.

Finally, I thank all IHSG members and delegates for their active involvement and valuable contributions, both ahead of and during the conference.

Trygve S. Aamlid

Chairman of the Organizing Committee
Sixth International Herbage Seed Conference

Table of contents:

Opening session

Seed production and seed trade in a globalised world Invited speaker: Ole Bech Bondesen	9
---	----------

Session 2: Herbage seed for the future

GMOs and the role of seed yield capacity in herbage breeding programs Invited speaker: Odd Arne Rognli	13
--	-----------

Strategies to combine improved reproductive and agronomic traits in forage plant breeding programmes Athole H. Marshall, Michael T. Abberton, Daniel Thorogood, Ian P. Armstead & Terence P.T. Michaelson-Yeates	22
--	-----------

Relationship among seed yield and seed components using path analysis in <i>Poa angustifolia</i> collected from Zanjan, Iran Parviz Moradi, A.A.Jafari & H.M. Nadoushan,	28
--	-----------

Seed and herbage yield in <i>Bromus tomentellus</i> Boiss grown under optimal and drought stress conditions A. Jafari, A. Bafandeh Rouzbahani & E. Rahmani	33
--	-----------

Evaluation of seed yield and seed components in tall fescue (<i>Festuca arundinacea</i> Schreb.) through correlation, regression and path analysis S. Afkar, A. Jafari & G. Karimzadeh	39
--	-----------

Evaluation for seed yield and seed components among Iranian accessions of <i>Bromus persicus</i> Boiss. ex Steud. A. Jafari, A. Bafandeh, E. Rahmani & H. Panahpour	44
---	-----------

Effect of self incompatibility on seed yield in a perennial ryegrass F₂ population Bruno Studer, Louise Bach Jensen & Thomas Lübberstedt	49
---	-----------

Seed yield and seed yield components in winter cultivars of common vetch (<i>Vicia sativa</i> L.) Vojislav Mihailović, Đura Karagić, Aleksandar Mikić, Slobodan Katić, Dragan Milić & Vukašin Draganović	53
--	-----------

Development of Kentucky bluegrass for non-burn seed production William J. Johnston, Richard C. Johnson, Charles T. Golob, Mathew C. Nelson, Karine Paré & Kathleen L. Dodson	57
--	-----------

Improved seed production in a new Italian cultivar of lucerne (<i>Medicago sativa</i> L.) Renzo Torricelli, Nicola Colesanti & Mario Falcinelli	61
---	-----------

Experience of perennial ryegrass seed production in Finland of cultivars from central Europe Markku Niskanen & Oiva Niemeläinen	67
---	-----------

Forage and seed yield of sulla (<i>Hedysarum coronarium</i> L.) varieties and landraces in a semi-arid Mediterranean environment Gaetano Amato, Dario Giambalvo, Dario Graziano & Paolo Ruisi	68
---	-----------

Modeling pollen dispersal between white clover fields relevant to co-existence with GM-white clover	73
Christina L. Løjtnant, Christian F. Damgaard & Rikke B. Jørgensen	
Session 3: Seed production of tropical species and species for stressful environments	
Plant breeding and seed production of apomictic tropical forage grasses	74
Invited speaker: John W. Miles & Michael D. Hare	
Sources of new tropical forage cultivars: Past, present and future	82
Donald S. Loch	
Seed and seedling response to salinity effects in two species of <i>Atriplex halimus</i> and <i>Atriplex semibaccata</i>	88
F. Arab, A. Jafari, M. Jafari, M.H. Assareh & A.Tavili	
Development of new <i>Chloris gayana</i> cultivars with improved salt tolerance from ‘Finecut’ and ‘Topcut’	92
Margaret Zorin & Donald S. Loch	
Development of a Texas bluegrass hybrid for turf usage	97
Jay B. Burr	
Session 4: Physiological restraints to seed set and seed filling.	
Achieving forage ryegrass seed yields of 3000 kg ha⁻¹ and limitations to higher yields	100
Phil Rolston, Jason Trethewey, Bede McCloy & Richard Chynoweth	
Carbohydrates and seed yield limits in forage ryegrass	107
Jason A. K. Trethewey & Phil Rolston	
Translocation of nitrogen (N) to the seeds of perennial ryegrass in relation to the availability of N	113
René Gislum & Birte Boelt	
Session 5: Establishing the potential for high and pure seed yields	
Seed production in the northern light: Implications of temperature and daylength for flower induction and seed yield	117
Invited speaker: Ola M. Heide	
Seed yield contribution in smooth brome grass (<i>Bromus inermis</i> Leyss.) and meadow fescue (<i>Festuca pratensis</i> Huds.) established on different dates and with different plant densities	125
Trygve S. Aamlid & Lars T. Havstad	
The effect of barley ratios (<i>Hordeum vulgare</i> L.) as a companion crop on seed yield and yield components of common vetch (<i>Vicia sativa</i> L.) sown at different rates	131
Huseyin Ozpinar, Hikmet Soya & Zeki Acar	
Winter wheat: its effect on ryegrass seed yield and control options	136
Richard Chynoweth, Nick Pyke & Phil Rolston	

Vacuum seed planter to plant ryegrass seed in space plant nursery Lloyd R. Nelson	140
Effect of cutting schedule on seed yield in alfalfa Đura Karagić, Slobodan Katić, Sanja Vasiljević, Dragan Milić	142
Weed regulation and establishment of organic seed crops of <i>Trifolium pratense</i> L., <i>Phleum pratense</i> L. and <i>Festuca pratensis</i> Huds. Ann-Charlotte Wallenhammar & Per Ståhl	147
Seed production of various timothy (<i>Phleum pratense</i> L.) cultivars as affected by seeding rates Ann-Charlotte Wallenhammar & Lars Eric Anderson	152
Weed regulation by cutting in organic seed crops of <i>Trifolium pratense</i> L. and <i>Trifolium repens</i> L. Ann-Charlotte Wallenhammar, Per Ståhl, Bo Christiansson & Lars Andersson	156
Cover crops and weed control in establishment of organic seed crops of meadow fescue (<i>Festuca pratensis</i> Huds.) Kari Bysveen, Trygve S. Aamlid & Svein Ø.Solberg	160
Subterranean clover (<i>Trifolium subterraneum</i>) as a companion crop during establishment of organic seed crops of timothy Svein Ø. Solberg, Kari Bysveen & Trygve S. Aamlid	165
Alternating forage and seed production as a method to improve feasibility of tall fescue seed production at northern latitudes Oiva Niemeläinen & Markku Niskanen	168
Prediction of herbicide selectivity in grass seed crops from pot experiments Solvejg K. Mathiassen, Mette Rabølle & Per Kudsk	169
Selectivity of some herbicides in eight grass species grown for seed in the conditions of Central Europe - preliminary results Radek Macháč & Bohumír Cagaš	172
Control of grass weeds in seed production of <i>Phleum pratense</i> , <i>Poa pratensis</i> and <i>Festuca rubra</i> Kirsten S. Tørresen, John Ingar Øverland & Trygve S. Aamlid	178
Seed production of <i>Koeleria gracilis</i> Pers. Ants Bender & Rene Aavola	183
Importance of Ecological Conditions for Herbage Seed Production in Turkey Ilknur Ayan, Zeki Mut & Zeki Acar	188
 Session 6: Fertility, plant growth regulators and plant protection	
Economic damage thresholds for the clover seed weevil (<i>Apion fulvipes</i> Geoff.) and the lesser clover leaf weevil (<i>Hypera nigrirostris</i> Fab.) attacking white clover (<i>Trifolium repens</i> L.) seed crops Lars Monrad Hansen & Birte Boelt	193

Factors affecting the activity of Moddus M in red fescue	197
Solvejg K. Mathiassen, Mette Rabølle, Birte Boelt & Per Kudsk	
Response of creeping red fescue (<i>Festuca rubra</i> L.) and perennial ryegrass (<i>Lolium perenne</i> L.) to spring nitrogen fertility and plant growth regulator applications in Oregon	201
William C. Young III, Tom B. Silberstein, Tom G. Chastain & Carol J. Garbacik	
Economical optimal nitrogen (ECO-N) application rate is all that matters for the growers	206
René Gislum, Phil Rolston, John M. Hart, Richard Chynoweth, Bede McCloy & William C. Young III	
Growth regulation, fungicides and nitrogen in interaction in seed crop production	211
Christian Haldrup	
White clover - growing it in competition	214
Nick Pyke, Tabitha Armour, Richard Chynoweth & Bede McCloy	
Fungicides in Czech grass seed production	220
Bohumír Cagaš & Radek Macháč	
Plant growth regulators and insect control in seed production of red clover (<i>Trifolium pratense</i>)	226
John Ingar Øverland & Trygve S. Aamlid	
Effects of trinexapac-ethyl (Moddus) in seed crops of Italian ryegrass and timothy	231
Georges Rijckaert	
Use of plant growth regulators on annual ryegrass: The Oregon experience	236
Mark E. Mellbye, Gale A. Gingrich & Tom B. Silberstein	
Comparison of soil N tests for prediction of spring N rate in perennial ryegrass seed production	239
John M. Hart, Phil Rolston, Mark E. Mellbye, Tom B. Silberstein, William C. Young III, Bede L. McCloy, Gale A. Gingrich, Neil W. Christensen & René Gislum	
Nitrogen fertilization of Italian ryegrass (<i>Lolium multiflorum</i>) for seed production: Requirement evaluation for N supply calculation	244
Marie-Laure Casals, Francois Deneufbourg, Georges Rijckaert & Geroges Sicard	
Correlation between seed yield and biomass-nitrogen - a case study using multiple field experiments	248
René Gislum & Birte Boelt	
Sulphur fertilization in seed crops of <i>Lolium perenne</i>	252
Jan Rinze van der Schoot, Gerard Borm & Wim van Dijk	
 Session 7: Fertility, plant growth regulators and plant protection	
Implications of correlation between yield from the same plot from year to year	256
Kristian Kristensen & Lise C. Deleuran	
Straw residue management in seed production of meadow fescue (<i>Festuca pratense</i> Huds.) and timothy (<i>Phleum pratense</i> L.)	261
Lars T. Havstad	

Post-harvest treatments in smooth-stalked meadow grass (<i>Poa pratensis</i> L.) - effect on carbohydrates and tiller development Birte Boelt	266
Using seed moisture to determine optimum swathing time in annual ryegrass (<i>Lolium multiflorum</i> Lam.) seed production Tom B. Silberstein, Mark E. Mellbye, William C. Young III & Tom G. Chastain	270
Optimum harvest time of tall wheatgrass seed Leandro M. Cardoso, Sara I. Alonso, Andrea M. Clausen & Jorge Castaño	274
Effects of harvest date on seed yield and quality of <i>Scorpiurus muricatus</i> L. Giuseppe Di Giorgio, Paolo Ruisi, Dario Graziano, Dario Giambalvo & Gaetano Amato	278
Harvest time, harvest method and germination losses during storage of fodder galega (<i>Galega orientalis</i> Lam.) seed Jonas Slepetyš	282
Influence of harvest timing and storage location on the longevity of timothy (<i>Phleum pratense</i> L.) seed Jon Atle Repstad, Trygve S. Aamlid & Lars T. Havstad	287
Hardseededness and patterns of seed softening in burr medic (<i>Medicago polymorpha</i> L.) Dario Graziano, Paolo Ruisi & Gaetano Amato	292
Patterns of hardseed breakdown within <i>Scorpiurus muricatus</i> L. in a semi-arid Mediterranean environment Giuseppe Di Giorgio, Paolo Ruisi & Dario Giambalvo	297
Longevity of seeds of <i>Poa pratensis</i> and <i>Vulpia bromoides</i> under field conditions as related to burial depth and straw cover Peter Kryger Jensen	302
Appendix : World and EU herbage seed acreage and production	306

Seed production and seed trade in a globalised world

Ole Bech Bondesen, Denmark

seedcouncil@seedcouncil.dk

ABSTRACT

The localization of seed production is affected by climatic conditions, economic returns in the seed crop as well as in competitor crops, and agricultural and environmental policies. In the globalised market the price of the seed and of the competitor is likely to be the same, hence the driving force for optimal localization is the yield of the seed crop in comparison with the competitor crops (usually cereals). The world market for herbage seed has increased 50-60% in volume since the 1990'es - in large due to an increased demand and hence production of turf seed. This increase in production is mainly localised in USA and Europe. There is a tendency to an increasing production of proprietary varieties.

Key words: agricultural economics, localization of seed production, seed markets

SEED - SPEARHEAD FOR GLOBALISATION IN AGRICULTURE

Globalisation in seed production and seed trade is not a novelty. At world level the big consumption centres for forage and turf seed differ from the optimum locations for production and this of course involves the need for trade. Noticeable is that almost the whole Oregon production is consumed outside Oregon, 95 % of the Danish production is exported, and most of the Canadian and New Zealand production is exported as well.

Ever since the production of quality seed started about a century ago the transfer of seed over borders worldwide has been an increasing business. A number of prerequisites and reasons have been crucial for the process towards globalisation in the seed sector such as:

- Establishment of seed certification based on ISTA principles
- Harmonization of seed laws at international level as manifested in the OECD seed schemes
- World trade agreements (GATT or WTO) having abolished duty payments on seed and fixed limits for direct/coupled subsidies to agriculture including seed
- The reformation of the Common agricultural policy to a decoupled support system
- "The fall of the Wall" paving the road for socialist countries to join

agreements regulating international seed trade

No doubt, the seed sector has been a front runner in the liberalization process of agricultural trade. Today, almost all international trade barriers have been lifted for seed while for other agricultural commodities substantial support mechanisms are still in place.

LOCALIZATION OF FORAGE AND TURF SEED PRODUCTION WORLD WIDE

Based on the principles of the globalised market, seed production of cool season grasses and white clover has, over the years, found its best agro-climatic production sites in a belt between the 35th and 60th parallel in the Northern and Southern Hemisphere, respectively. Probably due to the existence of the Gulf Stream, the European production sites are in general situated at higher parallels, 48 to 58 °N, than those elsewhere in the world. The big production centres of the US northwest are situated at 44 to 48 °N, and the New Zealand production, the biggest in the Southern Hemisphere, is carried out between 43 and 45 °S. Differences are seen among species, timothy for instance being produced mainly at high parallels in Canada, Norway, Sweden and Finland.

A number of factors are affecting the localization of seed production worldwide:

- Climatic conditions
- Economic returns in proportion to returns from other productions
 - cereals
 - milk, cattle and sheep
 - set-aside
- Agricultural and environmental policies affecting prices and costs

Seed growing farmers anywhere in the world are producing seed for business. Farmers make their decisions on what to grow/what to produce based on gross margin calculations, i.e. the economic return per ha. This means that a farmer will not opt for seed production unless he can expect to obtain a remuneration per ha, which can match the return from the main agricultural crops or other productions. In most seed production areas of the world cereal is the main competitor crop to seed production. Certainly, this is the case for the European production and to a large extent also for the production in Oregon and Canada. Reports from New Zealand suggest that the main competitor to the grass and clover seed production areas at Canterbury Plains, South Island, nowadays comes from milk production on the rise in this region.

Assuming that in the globalised market the price for a certain quality of seed (final product) is the same worldwide and the same being the case for the competitor crop, cereals, it becomes evident that the seed yield compared to that of cereals is a significant factor for the localization of seed production. No doubt Denmark's position as the main European seed producer of grasses and clovers is due to the fact that the seed yields of these species are greater in Denmark compared to the seed yields of cereals than in other European countries.

As production cost is a determining factor for the seed growers economic result at "the bottom line" it becomes evident that the whole range of variable cost factors related to seed production can influence the localization of the production too. This also includes environmental based policies already experienced such as bans on straw burning and the use of certain chemicals in seed production.

SIZE AND DEVELOPMENT OF THE GLOBAL SEED MARKET

The Danish Seed Council has for a number of years made an effort to keep track of the seed production in the major production areas of the world. The results of the work are to be found in Appendix of these proceedings. Comparable figures are shown for the period since 1993.

It is to be noticed that the US figures in the tables in excess of registered production in Oregon include Kentucky bluegrass in the states of Washington and Idaho; that the Canadian figures include areas for certification only and an estimate for production of certified red fescue; that European figures are complete (certified production in Europe only) and that the New Zealand figures cover certified production. Certified area figures for the small production in Australia are incorporated for the last 3 years. In excess to the figures shown in the tables some production is carried out in Argentina (approximately 35,000 ha) and a small production in Chile. Certainly, some production is accomplished in Russia and Ukraine but the size is unknown. However, their production does not appear in the world market.

The total registered area and production at world level in 2005 sums up to about 623,000 ha and 649,000 tons. However, figures for the quite large production of uncertified seed in Canada, mainly red fescue and timothy, are not available. Likewise, figures for Argentina and some Australian figures are not included. If these shortages are taken into account the total 2005 world area and production of forage and turf seed can be estimated to 750,000 ha and about the same in tons. Preliminary figures for 2006 indicate a small increase of 3 % in the world area from 2005.

The world market for grass and clover seed has been on a steady increase as long as production figures can be traced backwards. In this context be aware that production figures are equal to those of consumption as seeds of grasses and clovers have no other outlet than seeds for sowing. Since the beginning of 1990'es the world market has increased 50-60 % in volume. No doubt, this increase is related to the turf seed segment of the industry while consumption of forage seed presumably has been quite stable

following the stagnation in the dairy industry, at least in Europe.

The shares of the seed production areas 2005 among countries and species are shown in the table below:

Percentage of world total			
USA	37	Peren. ryegrass	26.5
EU	36	Italian ryegrass	14.6
- DK	12	Red fescue	12.9
- DE	5	Tall fescue	11.7
- NL	4	Kentucky bluegrass	9.6
- FR	4	Timothy	8.3
- CZ	3	Red clover	5.2
- SE	2	White clover	3.7
- PL	2	Orchard grass	3.3
- UK	1	Meadow fescue	1.5
- FI	1	Hybrid ryegrass	1.3
- HU	1	Bent grass	0.6
CDN	17	Sheep's fescue	0.5
AR	5	Festulolium	0.4
NZ	4	Rough bluegrass	0.2
AUS	2		

The increasing trend of production in the 2 main production centres, USA and the European Union, has been very similar. Inside Europe the increase in production has taken place almost exclusively in Denmark. The production areas have been quite static in Canada and New Zealand. However, in the case of Canada, quite an increase for perennial ryegrass has appeared during the last years while New Zealand has lost almost half of the white clover seed area since the beginning of the 1990'es. It is to be noted that cyclic fluctuations appear in all production areas.

With regard to species the most remarkable increase in production since beginning of the 1990'es has appeared for tall fescue, more than 100 %. Substantial increases are also attributed to Kentucky bluegrass and perennial ryegrass, more than 50 %. More modest increases have occurred for red fescue, meadow fescue and Italian ryegrass, while the market has stagnated for timothy, cocksfoot, red and white clover. It is to be noted that Festulolium has been on a steep upwards trend since its introduction to the market in the mid 90'es.

SEED COMPANIES AND GLOBALISATION

Seed companies have a catalyst function in the global seed market as they are in charge of the international seed trade.

The international market includes marketing options for:

- Certified production of proprietary varieties
- Certified production of public varieties
- Common production, i.e. on certified seed

Seed production of proprietary varieties being in the hands of seed companies/variety owners exclusively is conducted according to contracts. As the main objective of the companies is to maximize the economic outcome of their varieties they search for those places in the world where multiplication can be carried out at the lowest cost for the needed quality.

Traditionally, multiplication of foreign varieties has been conducted via local seed companies or agents. However, nowadays multinational companies like DLF-TRIFOLIUM and Barenbrug possess production facilities in all major multiplication centres of the world enabling them to contract seed production directly with local farmers.

Seed production in Europe almost entirely takes place on proprietary varieties. Furthermore, according to EU seed legislation only certified seed of grasses and clovers can be marketed at the EU territory.

Seed production on public varieties plays an important, although shrinking, role in the other major production areas, New Zealand, Canada and USA. In this case seed growers have access to basic seed without company restrictions. However, marketing to the final consumer normally takes place through a seed company.

Production of common or uncertified seed has a big impact at the North American market due to the huge common seed production in Canada of especially red fescue and timothy. Considered by local experts about 90 % of the total production of these species is common seed. Also in this case the seed sale to the

final consumer normally takes place through a seed company but the seed is in the seed grower's possession, often several years on stock, until this final stage.

To conclude, the European production market is controlled by companies while in other parts of the world seed growers are to a great extent operating on their own. Furthermore, in Europe the operators in the production

segment are few. Thus in Denmark, the largest producer country in Europe, we have only 3 seed multiplying contractors, namely DLF-TRIFOLIUM, Hunsballe and Barenbrug, compared to more than 300 in Oregon.

However, all parties need the seed scientists to come up with the best solutions wherever the seed production is located around the world.

GMOs and the role of seed yield capacity in herbage breeding programs

Odd Arne Rognli

Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, PO Box 5003,
N-1432 Ås, Norway

odd-arne.rognli@umb.no

ABSTRACT

The potential tradeoffs between vegetative and reproductive growth is a constant challenge for the forage plant breeders. Breeding for seed production has inevitably played a secondary role compared to improvements of the vegetative production. In this paper the current status regarding genetic variation, genotype x environment interactions, heritability estimates and mapping of quantitative trait loci (QTL) for seed yield and seed yield components in grasses and legumes are reviewed, with special focus on important forage grasses. Investigations of seed yield components have shown that components contributing to an increased utilization of the reproductive potential, like seed set and seed retention, seems efficient in increasing seed yield without adverse effects on the vegetative production. The generation of transgenic plants (GMO) have been reported for many forage species, and genetic engineering will increasingly be used to manipulate traits like nutritive value, resistance to fungal and viral diseases, and the reproductive system like male and female sterility and apomixis. Turf and forage grasses, and forage legumes are outcrossing species with prolific pollen production and pollination facilitated by wind or insects. They are potentially some of the most problematic crops when it comes to gene flow by pollen especially during the generations of seed multiplication. GM plants with engineered reproductive systems will pose new challenges for the seed producers. Co-existence of GM grasses and legumes with conventional and organic seed production will be very difficult to establish, and commercialization of GM cultivars will therefore certainly require gene containment technologies that prevent or reduce transgene escape. Mapping of QTLs, identification of markers and candidate genes associated with seed yield components, and the utilization of comparative genomics with cereal species have revealed several key components which may facilitate development of markers for marker-assisted breeding for the improvement of seed yield.

Key words: co-existence, comparative genomics, gene flow, genetic variation, genotype x environment interaction, indirect selection, quantitative trait loci (QTL), seed yield components

INTRODUCTION

In contrast to breeders of grain crops, the fodder plant breeder is mostly concerned with the vegetative aspects of the plant. Improvement of leafiness, tillering capacity and persistency may lower the ability to produce seeds. Thus the most important characteristics of forage plants from a farmer's perspective can be a problem for the seed producer. However, it has for a long time been well established that outstanding cultivars will hardly be commercialized if the

seed production is not satisfactory. This constraint and the potential tradeoffs between vegetative and reproductive growth is a constant challenge for the forage plant breeders, and breeding for seed production has inevitably played a secondary role. The lack of deliberate breeding for seed productivity may also be due to the opinion that seed and forage production are negatively correlated (Bugge 1987). However, it has been quoted that this negative correlation probably can be changed by selection. Andersen (1981) reported a modest

negative correlation between seed yield and dry matter yield or persistency, based on a survey of a number of commercial cultivars, mainly perennial ryegrasses (*Lolium perenne* L.). He concluded that it should be possible to combine high seed yield with high dry matter production in grasses. Griffiths (1965) pointed out that overall seed yield was not correlated with forage production in perennial ryegrass. Elgersma's investigations in perennial ryegrass documented that high seed-yielding capacity and high dry-matter yield were not mutually exclusive (Elgersma 1990a). Recent studies of selection for seed yield in perennial ryegrass support these conclusions (Marshall & Wilkins 2003).

Although progress in developing transformation technologies for forage grasses and legumes has been slower than for major crops, generation of transgenic plants have been reported for many species, e.g. tall fescue, perennial ryegrass, Italian ryegrass, red fescue, creeping bentgrass and white clover (for references, see Wang *et al.* 2004). However, few of these have been tested in field trials and none have, to my knowledge, been commercialised so far. Herbicide resistant (glyphosate) creeping bentgrass has been close to deregulation in the US, but the discovery of long-distance gene flow, hybridization with closely related species, and the potential of creeping bentgrass as a glyphosate resistant weed in other crops have halted deregulation. Since many of the grasses and legumes are native species and can exist as weeds in other crops, herbicide resistance should probably not be a major target for developing GM cultivars. Because gene flow by pollen is the major route of transgene escape, the generations of seed multiplication constitute a major challenge. Despite these challenges, genetic engineering will increasingly be used to manipulate traits like nutritive value (lignin, cellulose and sugar composition for feed and bioenergy purposes), resistance to fungal and viral diseases, and the reproductive system (male and female sterility and apomixis). Manipulation of the reproductive system will make it possible to utilise heterosis better than in the current synthetic cultivars, and will also pose new challenges for the seed producers.

SEED YIELD AND SEED YIELD COMPONENTS

Seed yield is a highly complex trait which is influenced both by numerous interacting genetical, physiological and environmental factors. The seed production capacity varies with species and with type of cultivar, e.g. turf and forage cultivars. In grasses seed yield can be divided into components like the numbers of fertile tillers, spikelets per panicle and florets per spikelet, which determine the seed yield potential, and fertility and 1000-seed weight, which determine the utilization of the seed yield potential (Bean 1972). Utilization of the yield potential is thus heavily dependent on successful pollination, fertilization and seed growth, which is influenced by physiological and genetical factors like assimilate allocation, source-sink competition, self-incompatibility and pollen production. In addition, seed yield is influenced directly or indirectly by a number of agronomic traits such as plant height, leaf area, dry-matter yield, heading date, lodging resistance and proneness to seed shattering (Griffiths 1965).

GENETIC VARIATION AND SELECTION FOR SEED YIELD AND SEED YIELD COMPONENTS

Many studies of seed yield and its component traits have demonstrated large genetic variation and high heritability for these traits, and these estimates are often larger than for vegetative production traits. The larger genetic variation for seed production traits is usually attributed to the lower selection pressure than for vegetative production during breeding. In a study of high-latitude populations of timothy, Rognli (1987) estimated broad sense heritabilities of seed yield, date of ear emergence and plant height to 0.64, 0.86 and 0.80, respectively with corresponding genotypic coefficients of variation (GCV%) as 24.8, 13.2 and 5.8. Elgersma (1990b) found that narrow-sense heritabilities, estimated from parent-offspring regressions, were highest for earliness, flag leaf width, ear length and the number of spikelets per ear in perennial ryegrass.

Fang *et al.* (2004) studied phenotypic and genotypic variation for seed yield and associated traits (heading date, plant height,

number of fertile tillers, 1000-seed weight, panicle length, seed weight per panicle, fertility, flag-leaf length and flag-leaf width) in a full-sib family of meadow fescue (*Festuca pratensis* Huds.) grown at two locations in Norway. Their estimates of broad sense heritabilities (H_B^2) for the traits were high,

and highest (0.80) for seed yield per plant (Table 1.). Seed yield per plant and reproductive components like seed weight per panicle and fertility exhibited the largest genotypic coefficients of variation (GCV %), being around 34%.

Table 1. Estimates of genotypic and genotype x location variance components, broad sense heritabilities (H_B^2) and genotypic coefficient of variation (GCV %) of seed production and related traits in a full-sib family of meadow fescue (Fang *et al.* 2004).

Traits	Genotypic variance	G x L variance	H_B^2	GCV (%)
Plant height (cm)	28.41	-1.76 ^{ns}	0.80	4.0
Heading date (days)	1.15	0.52 ^{**}	0.71	3.2
Seed yield/plant (g)	45.52	6.78 [*]	0.80	35.4
# Fertile tillers	948.96	232.22 ^{**}	0.69	12.8
1000-seed weight (g)	0.0230	0.002 ^{ns}	0.78	8.8
Panicle length (cm)	2.86		0.75	7.3
Seed weight/panicle (mg)	2634.5		0.74	34.1
Panicle fertility (mg cm ⁻¹)	5.10		0.76	34.7
Flag leaf length (mm)	152.09		0.50	7.2
Flag leaf width (mm)	0.28		0.70	7.6
HD_01 (days)	1.22		0.76	2.4

All variance components were significant at $P=0.001$, except estimates of genotype x location variance components (*, ** significant at $P=0.05$ and $P=0.01$, respectively; ns, not significant).

Table 2. Path coefficients showing direct and indirect effects of number of fertile tillers/plant (NFT), panicle fertility (PF), plant height (PH), and flag-leaf width (FLW) on seed yield/plant (PSW) in meadow fescue (Fang *et al.* 2004)

Trait	Direct effect ¹	Indirect effect via				Total correlation with PSW
		NFT	PF	PH	FLW	
NFT	0.27	-	0.03	0.00	0.06	0.36
PF	0.60	0.01	-	0.07	0.07	0.75
PH	0.24	0.00	0.16	-	0.02	0.42
FLW	0.23	0.08	0.19	0.02	-	0.52

¹ Residual effect = 0.23

Fang *et al.* (2004) also conducted a path coefficient analysis in meadow fescue (Table 2). This analysis showed that fertility (measured as seed weight/panicle divided by panicle length) was the most important component trait contributing to seed yield with a direct effect of 0.60, while number of fertile tillers, plant height and flag-leaf width were also important with direct effects around 0.25. Variation in 1000-seed weight had little influence on seed yield per plant. The indirect path coefficients were in general small; however both flag-leaf width and plant height had sizeable indirect effects via

panicle fertility on seed yield per plant. Since panicle fertility is highly correlated with seed weight per panicle, this component trait could be used instead since it is easier to handle in selection programmes for seed production.

Our path analysis in meadow fescue demonstrated that flag-leaf width (FLW) has an important effect directly on seed yield and indirectly through panicle fertility (PF). This indicates that large flag leaves contribute to a good seed-set (panicle fertility) through assimilate reallocation via the stems to the

inflorescence in the period of anthesis, and that this contributes to higher seed yields. The importance of the flag-leaf for grain yield in cereals is well-known, recently demonstrated by Quarrie *et al.* (2006) in wheat, and it is not surprising that this is the case also in grasses. In perennial grasses the competition for assimilates is probably stronger than in annual cereal crops since the seeds have to compete with other sinks, i.e. actively growing organs like roots and new vegetative tillers, for assimilates. Indeed, studies in herbage grasses have shown that the number of florets that are produced is very large, but that a high percentage is aborted probably due to lack of fertilization and/or competition for nutrients and assimilates (Elgersma 1990a). The path analysis also showed that plant height (PH) had an indirect positive effect on seed yield via panicle fertility, and this effect was nearly as large as the direct effect. This might be explained by the fact that taller plants will have a better chance of capturing pollen, which will increase the proportion of florets being successfully fertilized.

The major importance of fertility and seed set has been confirmed in several studies. Elgersma (1990a) found that variation in seed yield was more related to variation in seed number than to variation in seed weight in a study of nine diploid late-flowering perennial ryegrass cultivars. Elgersma *et al.* (1994) found that the number of spikelets per ear was negatively correlated with seed yield. Marshall & Wilkins (2003) conducted two generations of recurrent phenotypic selection for seed yield per plant under controlled pollination in the perennial ryegrass cultivar AberDart. Selected and unselected varieties (AberDart and AberElan, respectively) and control varieties were grown for seed in pots in a glasshouse experiment and in two field plot experiments over 5 harvest years. Selection gave significant improvements in seed yield both in green house and in field plots and the increased seed yield of AberDart was attributed to a higher seed set, greater seed number per tiller and more reproductive tillers per plant.

It can be concluded that seed component studies have demonstrated that after the establishment of a sufficient number of fertile tillers, panicle fertility is the most important determinant of seed yield. It should therefore be possible to breed for an increased efficiency of the reproductive

system rather than an increased size of the reproductive system, without negative effects on the forage production.

GENOTYPE X ENVIRONMENT INTERACTIONS

Early selection of promising genotypes is usually performed on single plants in herbage breeding programmes. Early selection for high seed yield on spaced plants would be valuable for breeders, but only if this potential is also expressed in derived progenies sown later in drilled plots. Similar as for dry matter yield, a major problem in improving seed yield by breeding is environmental interactions, i.e. inconsistent correlations between estimates obtained in spaced plantings and in dense stands.

Selection in spaced plants, either by a component of seed yield or seed yield directly, constitutes a form of indirect selection. A prerequisite for successful indirect selection is a high genetic correlation between yield on spaced plants and yield in plots, i.e. absence of genotype x environment interactions. Elgersma (1990c) studied seed yield and seed yield components using spaced plants and drilled plots of nine perennial ryegrass cultivars, and found that spaced-plant traits in general showed poor correlation to corresponding traits in drilled plots. Cultivars with contrasting seed yield in plots could not be distinguished on the basis of their spaced-plant traits and therefore no criteria for indirect selection for seed production in drilled plots could be identified in spaced plants. Spaced-plant traits in two perennial ryegrass cultivars were assessed using clones and their open-pollinated progenies in four environments by Elgersma *et al.* (1994). They found significant differences among half-sib families for seed yield, 1000-seed weight, spring performance and earliness. However, multiple regression analyses revealed that no combinations of plant traits consistently explained a major proportion of the variation for seed yield of the progenies. The results indicated that spaced-plant data was of limited value in predicting seed production. They concluded therefore that direct selection for seed yield in drills of progenies in later stages of the breeding programme was the best method for obtaining varieties with sufficient seed production. I think this reflects the current

opinion and practice among forage grass breeders.

On the other hand, in lucerne, Annicchiarico (2006) reported that indirect selection for seed yield based on spaced plants was only 19% less efficient than direct selection in dense stands and the heritability was higher on spaced plants. However, indirect selection for seed yield components was not efficient. As pointed out by Marshall & Wilkins (2003), the poor correspondence between seed set of individual spaced plants and genetically related plants grown as drilled plots observed in perennial ryegrass by Bugge (1987) and Elgersma (1990c), could well be attributed to the lack of pollination control in field spaced plants. These plants are more likely to receive pollen from genetically unrelated plants than plants grown closely together in drilled plots. Perennial ryegrass (and many other grass species) is highly self-incompatible, and the male parent is as important as the female parent in determining whether a particular floret sets a seed. Marshall & Wilkins (2003) argue that phenotypic selection for improved seed set can be effective provided that pollination is closely controlled. In addition, lodging and insufficient pollination leading to abortion and low seed set is much more of a problem in dense stands, and this lowers the heritability and makes correlations between spaced plants and dense stands unreliable. The effect of lodging seems to differ among species. Griffith (2000) found that lodging depressed seed yield much more in perennial ryegrass than in tall fescue.

GMOs

Many transgenic plants of grasses and legumes have been developed but very few have been tested rigorously in field tests let alone reached the market. Recent examples of genetic engineering in forage and turf species are improved digestibility by downregulation of lignin biosynthesis in tall fescue (Chen *et al.* 2004), marker genes (Wang *et al.* 2004), repression of flowering in perennial ryegrass (Andersen *et al.* 2004), and herbicide tolerance and male sterility in creeping bentgrass (Luo *et al.* 2004). Field studies to obtain data for risk assessment have been reported for tall fescue using a marker gene (Wang *et al.* 2004), and for resistance against alfalfa mosaic virus (AMV) in white clover (Emmerling *et al.* 2004). Field studies to

obtain data for modelling gene flow using traditional marker genes, e.g. isozymes, have been conducted in perennial ryegrass (Giddings *et al.* 1997) and meadow fescue (Nurminiemi *et al.* 1998, Rognli *et al.* 2000).

In general, the major problems of GM cultivars are: i) gene flow between GM and traditional cultivars of the same crop; ii) gene flow between GM cultivars and crop wild relatives and weedy forms of the target crop species; and iii) contamination by spread of GM seeds through commercial and local traditional seed systems. Turf and forage grasses, and forage legumes are outcrossing species with prolific pollen production and pollination facilitated by wind or insects. Most of these species will also have natural populations with which they can hybridize. As such they are potentially some of the most problematic crops when it comes to gene flow by pollen. Long distance gene flow by pollen has been documented for herbicide resistant creeping bentgrass in Oregon. Watrud *et al.* (2004) found that most of the gene flow occurred within 2 km in the direction of the prevailing wind. However, maximal gene flow distances were 21 km on sentinel plants and 14 km on resident plants that were located in natural habitats. Also hybridization with *Agrostis gigantea* was observed with a frequency of 4 hybrids per 10 000 seedlings. *Agrostis gigantea* is also classified as a subspecies under *A. stolonifera* which may explain the relatively high frequency of hybridization. It is clear that gene flow will constitute a major problem for seed producers since this specialized production often is concentrated in certain regions, e.g. Oregon in the US. It follows that gene flow might be a serious issue only during seed production and not when grasses and legumes are used in leys, pastures and lawns and are harvested or cut before they reach the reproductive stage. Despite this fact, it will be difficult to secure that all plants are kept completely vegetative through a production cycle, and it is virtually impossible to contain genes under field conditions. Commercialization of GM cultivars will therefore certainly require gene containment technologies that prevent or reduce transgene escape, e.g. non-flowering plants (prevent gene flow completely by repressing both male and female reproduction), or male sterility or transformation of chloroplasts (prevent gene flow by pollen, not by seed). Great progress has been made in the development of such gene containment or mitigation

techniques, e.g. chloroplast engineering, mitigation genes that reduce fitness, and genetic use restriction technologies (GURT) (Lee & Natesan 2006). GURT or terminator technologies are designed to make seeds sterile but have not been used in practice due to strong political and environmental protests. GURT technologies are most useful in grain crops and can not be used to contain genes during seed production of grasses and legumes since fertile seeds are needed to establish production fields. However, it is possible to engineer inducible repressor systems that can be induced in the production fields to prevent reproduction. Currently chloroplast engineering of transgenes is the most developed technique, and has been successfully used to target transgenes to the chloroplast genome of several crops, e.g. cotton, carrots and rice. Gene containment strategies have their weaknesses, and in no case have these methodologies been field-tested and/or been shown to be 100% effective. Maternal transmission of chloroplasts is not complete, and paternal chloroplast transmission occurs in plants at low but variable frequency depending on species (Ruf *et al.* 2007). The chloroplast genome of creeping bentgrass has been sequenced recently and will be used to develop of a chloroplast transformation system for this species (Saski *et al.* 2007).

The regulation of GM crops varies but most countries have developed biosafety regulations alongside deliberate release of GM crops. In Europe governments are currently developing regulations for co-existence of GM-crops with conventional and organic productions. The term co-existence refers to the ability of farmers to choose between conventional, organic or GM-based crop productions, in compliance with the relevant EU legislation on labelling and/or purity standards. EU regulations have introduced a 0.9% labelling threshold for the adventitious presence of GM material in non-GM products. Suitable technical and organisational measures during cultivation, harvest, transport and storage will be necessary to ensure co-existence, and these measures will be different for different crops determined mainly by their reproductive biology. Adventitious presence of transgenes from GM forage grasses will primarily come from gene flow through pollen during seed production. Seeds of legumes keep viable in the soil much longer than grass seeds, and GM forage legumes might therefore also pose a

significant problem with adventitious presence of GM from volunteers in the soil originating from seed banks.

Most studies of pollen dispersal in wind-pollinated species have found that the majority of pollen is deposited within relatively short distances (75-100 m) from the pollen source although occasional long distance movements occur as a result of strong winds and turbulence. Studies in meadow fescue using isozyme markers have shown that plant density has a large effect on effective pollination (gene flow) (Rognli *et al.* 2000). At a distance of 75 m the frequency of pollen captured by single plants (trap plants) was 14.9 % while it was only 1.6 % on plants that had a neighbour plant that produced pollen. At a distance of 155 m the same figures were 5.9 % and 0.7 %. Predicting gene flow by pollen is difficult since there are so many factors involved, some of them are stochastic in nature. It seems to be even more difficult in insect pollinated species. However, population size, degree of overlapping anthesis and wind are major factors to consider. Estimates of pollen dispersals have been used to model gene flow in grasses (Giddings *et al.* 1997, Nurminiemi *et al.* 1998), and can also be used to design sampling strategies for monitoring gene flow when GM cultivars are being commercialized (Rognli *et al.* 1999, Watrud *et al.* 2004, Godfree *et al.* 2006). Even if it is clear that pollen dispersal and gene flow will be large from GM forage grasses and legumes, the effects will be different depending on whether gene flow between occurs to other cultivars, or to natural or weedy populations. Gene flow between cultivars constitutes a management issues, the effect of gene flow to weedy and natural populations is a question of fitness. The fitness effects of a gene in the wild are a far more important consideration than the overall rate of gene flow, and will determine whether a transgene will establish in a population and if so, how the gene will spread in generations to follow. Obtaining fitness estimates of transgenes in natural plant communities constitutes a formidable challenge, especially for future GM traits that will have a larger ecological significance than herbicide resistance. Unfortunately, I don't think we will obtain proper estimates without doing large-scale GM cultivation experiments and it will be very difficult to get regulatory permission for such experiments in these species.

QUANTITATIVE TRAIT LOCI (QTL) MAPPING AND COMPARATIVE STUDIES WITH CEREALS

QTLs for seed yield and seed yield components have been mapped both in forage grasses and legumes. Fang (2003) found a total number of 34 chromosomal regions contained QTLs for plant height, heading date, seed weight/plant, number of fertile tillers, 1000-seed weight, panicle length, seed weight/panicle, panicle fertility, flag leaf length and flag leaf width in meadow fescue. Most of the QTLs for seed production and related traits clustered in a few chromosomal segments, most evident on linkage groups 1F, 4F and 5F. This indicates that there must be one or a few major gene(s) in these regions that affect development of the reproductive apparatus and that have pleiotropic effects on many traits. QTLs for panicle fertility and seed yield that have the same positions were detected on chromosomes 1F, 2F, 4F and 6F, and these should be interesting for the future development of molecular markers for improved seed yield. Comparisons of the QTL positions with positions of QTLs of identical or similar traits in other grass (cereal) species, using common anchor markers, identified a number of putatively orthologous QTLs.

Mapping of an orthologue of the wheat vernalization gene *Vrn1* in perennial ryegrass (Jensen *et al.* 2005) and in meadow fescue (Ergon *et al.* 2006) and their association with vernalization and seed yield related traits demonstrate conservation across grass species and the value of comparative genomics approaches. In red clover Herrmann *et al.* (2006) identified 38 QTLs for eight seed yield

components. QTLs for several traits were often detected in the same genome region. Two genome regions contained several QTLs for different seed yield components and represent candidate regions for further characterisation of QTLs. These studies have revealed several key components which may facilitate development of markers for marker-assisted breeding for the improvement of seed yield.

Recent studies in rice have revealed molecular mechanisms involved in determining seed yield components that in the future can be utilized by comparative genomic approaches to improve seed yield components in grasses. Ogara *et al.* (2004) characterized a QTL associated with the protein content of cytosolic glutamine synthetase (GS1) in senescing leaves, panicle number, and panicle weight in rice. The structural gene for GS1 mapped in the QTL region for single-spikelet weight, suggesting that the gene function in senescing leaves is tightly related to grain filling, probably via its capacity for nitrogen export. Song *et al.* (2007) showed that a QTL for rice grain width and weight (GW2) encodes a previously unknown RING-type E3 ubiquitin ligase that regulates spikelet hull size and indirectly influences regulating grain size and grain yield. Konishi *et al.* (2006) revealed that the *qSH1* gene, a major QTL of seed shattering in rice, encodes a BEL1-type homeobox gene and demonstrated that a single-nucleotide polymorphism (SNP) in the 5' regulatory region of the gene caused loss of seed shattering owing to the absence of abscission layer formation. The SNP was highly associated with shattering among japonica subspecies of rice, implying that it was a target of artificial selection during rice domestication.

LITERATURE

- Andersen, C.H., Jensen, C.S., Didion, T., Petersen, K., Storgaard, M., Folling, M., Gao, C., Martin, J., Lenk, I. & Nielsen, K.K. 2004. Genetic control of flowering in perennial ryegrass. Plant & Animal Genomes XII Conference, San Diego, Abstract W73 (http://www.intl-pag.org/pag/12/abstracts/W21_PAG12_73.html).
- Annicchiarico, P. 2006. Prediction of indirect selection for seed and forage yield of lucerne based on evaluation under spaced planting. *Plant Breeding*: 641-643.
- Bean, E.W. 1972. Clonal evaluation for increased seed production in two species of forage grasses, *Festuca arundinacea* Schreb. and *Phleum pratense* L. *Euphytica* 2: 377-383.
- Bugge, G. 1987. Selection for seed yield in *Lolium perenne* L. *Plant Breeding* 98: 149-155.
- Chen, L., Auh, C-H., Dowling, P., Bell, J. & Wang, Z-Y. 2004. Improving forage quality of tall fescue (*Festuca arundinacea*) by genetic manipulation of lignin biosynthesis. *In*: Hopkins, A., Wang, Z.Y.,

- Mian, R. Sledge, M. & Barker, R.E. (eds.). Molecular Breeding of Forage and Turf. Developments in Plant Breeding 11: 181-188.
- Elgersma, A. 1990a. Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). Plant Breeding 105: 117-125.
- Elgersma, A. 1990b. Heritability estimates of spaced-plant traits in 3 perennial ryegrass (*Lolium perenne* L.) cultivars. Euphytica 51: 163-171.
- Elgersma, A. 1990c. Spaced-plant traits related to seed yield in plots of perennial ryegrass (*Lolium perenne* L.). Euphytica 51: 151-161.
- Elgersma, A., Winkelhorst, G.D. & van den Nijs, A.P.M. 1994. The relationship between progeny seed yield in drilled plots and maternal spaced-plant traits in perennial ryegrass (*Lolium perenne* L.). Plant Breeding 112: 209-214.
- Emmerling, M., Chu, P., Smith, K., Kalla, R. & Spangenberg, G. 2004. Field evaluation of transgenic white clover with AMV immunity and development of elite transgenic germplasm. In: Hopkins, A., Wang, Z.Y., Mian, R. Sledge, M. & Barker, R.E. (eds.). Molecular Breeding of Forage and Turf. Developments in Plant Breeding 11: 359-366.
- Ergon, Å., Fang, C., Jørgensen, Ø., Aamlid, T.S. & Rognli, O.A. 2006. Quantitative trait loci controlling vernalisation requirement, heading time and number of panicles in meadow fescue (*Festuca pratensis* Huds.). Theoretical and Applied Genetics 112: 232-242.
- Fang, C. 2003. Comparative genome analyses, QTL mapping and genetic analyses of seed yield and related traits in meadow fescue (*Festuca pratensis* Huds.). Agricultural University of Norway. Doctor Scientiarum Thesis 2003:10.
- Fang, C., Aamlid, T.S., Jørgensen, Ø. & Rognli, O.A. 2004. Phenotypic and genotypic variation in seed production traits within a full-sib family of meadow fescue (*Festuca pratensis* Huds.). Plant Breeding 123: 241-246.
- Giddings, G.D., Sackville Hamilton, N.R. & Hayward, M.D. 1997. The release of genetically modified grasses. Part 1. Pollen dispersal to traps in *Lolium perenne*. Theoretical and Applied Genetics 94: 1000-1006.
- Godfree, R. C., Vivian, M. & Lepschi, B.J. 2006. Risk assessment of transgenic virus-resistant white clover: Non-target plant community characterisation and implications for field trial design. Biological Invasions 8: 1159-1178.
- Griffith, S.M. 2000. Changes in dry matter, carbohydrate and seed yield resulting from lodging in three temperate grass species. Annals of Botany 85: 675-680.
- Griffiths, D.J. 1965. Breeding for higher seed yields from herbage varieties. Journal of the National Institute of Agricultural Botany 10: 320-331.
- Herrmann, D., Boller, B., Studer, B., Widmer, F. & Kölliker, R. 2006. QTL analysis of seed yield components in red clover (*Trifolium pratense* L.). Theoretical and Applied Genetics 111: 536-545.
- Jensen, L.B., Andersen, J.R., Frei, U., Xing, Y., Taylor, C., Holm, P.B. & Lübberstedt, T. 2005. QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat VRN1. Theoretical and Applied Genetics 110: 527-536.
- Konishi, S., Izawa, T., Lin, S.Y., Ebana, K., Fukuta, Y., Sasaki, T. & Yano, M. 2006. An SNP caused loss of seed shattering during rice domestication. Science 312: 1392-1396.
- Lee, D. & Natesan, E. 2006. Evaluating genetic containment strategies for transgenic plants. Trends in Biotechnology 24: 109-114.
- Luo, H., Hu, Q., Nelson, K., Longo, C. & Krausch, A.P. 2004. Controlling transgene escape in genetically modified grasses. In: Hopkins, A., Wang, Z.Y., Mian, R. Sledge, M. & Barker, R.E. (eds.). Molecular Breeding of Forage and Turf. Developments in Plant Breeding 11: 245-254.
- Marshall, A.H. & Wilkins, P.W. 2003. Improved seed yield in perennial ryegrass (*Lolium perenne* L.) from two generations of phenotypic selection. Euphytica 133: 233-241.
- Nurminiemi, M., Tufto, J., Nilsson, N-O. & Rognli, O.A. 1998. Spatial models of pollen dispersal in the forage grass meadow fescue. Evolutionary Ecology 12: 487-502.
- Quarrie, S.A., Pekic Quarrie, S., Radosevic, R., Rancic, D., Kaminska, A., Barnes, J.D., Leverington, M., Ceoloni, C. & Dodi, D. 2006. Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57: 2627-2637.
- Rognli, O.A. 1987. Genetic variation in arctic populations of timothy (*Phleum pratense* L.). I. Seed production characters. Hereditas 107: 27-54.
- Rognli, O.A., Tomiuk, J., Tufto, J., Nurminiemi, M. & Jørgensen, R.B. 1999. Gene flow from transgenic crop plants to wild populations. NT Technical Report - Nordtest 409. 42 pp. Espoo, Finland. ISSN 0283-7234.

- Rognli, O.A., Nurminiemi, M. & Nilsson, N-O. 2000. Effects of distance and pollen competition on gene flow in the wind-pollinated grass *Festuca pratensis* Huds. *Heredity* 85: 550-560.
- Ruf, S., Karcher, D. & Bock, R. 2007. Determining the transgene containment level provided by chloroplast transformation. *PNAS Early edition*. www.pnas.org/cgi/doi/10.1073/pnas.0700008104
- Saski, C., Lee, S.B., Fjellheim, S., Guda, C., Jansen, R.K., Luo, H., Tomkins, J., Rognli, O.A., Daniell, H. & Clarke, J.L. 2007. Complete chloroplast genome sequences of *Hordeum vulgare*, *Sorghum bicolor* and *Agrostis stolonifera*, and comparative analyses with other grass genomes. *Theoretical and Applied Genetics (In press)*
- Song, X-J., Huang, W., Shi, M., Zhu, M-Z. & Lin, H-X. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genetics* 39: 623-630.
- Wang, Z-Y., Hopkins, A., Lawrence, R, Bell, J. & Scott, M. 2004. Field evaluation and risk assessment of transgenic tall fescue (*Festuca arundinacea*) plants. *In: Hopkins, A., Wang, Z.Y., Mian, R. Sledge, M. & Barker, R.E. (eds.). Molecular Breeding of Forage and Turf. Developments in Plant Breeding* 11: 367-379.
- Watrud, L.S., Lee, E.H., Fairbrother, A., Burdick, C., Reichman, J.R., Bollman, M., Storm, M., King, G. & van de Water, P.K. 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *PNAS* 101: 14533-14538.

Strategies to combine improved reproductive and agronomic traits in forage plant breeding programmes

Athole H. Marshall, Michael T. Abberton, Daniel Thorogood, Ian P. Armstead & Terence P.T. Michaelson-Yeates

The Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, UK, SY233EB

athole.marshall@bbsrc.ac.uk

ABSTRACT

The emphasis of the forage grass and legume breeding programmes at IGER is changing to incorporate selection for environmental traits that reduce the environmental footprint of grassland agriculture. The ability to produce adequate seed yields is essential to ensure the success of commercial variety development. In perennial ryegrass progress has been made in selecting for improved seedset which has increase seed yield without increasing the density of reproductive tillers which will impact on forage quality. Developments in genetic mapping are increasing our knowledge of the genetic control of this trait. In white clover, increased seed yields have been obtained by selecting for peduncle strength. Introgression of reproductive traits from *T. nigrescens* is an additional strategy. Molecular approaches are being developed to increase the speed and precision of the introgression of these traits and knowledge is being obtained to enable marker assisted selection for key reproductive traits.

Key words: environmental traits, forage legumes, forage production, forage quality, grasses seed yield

INTRODUCTION

Forage species are integral components of sustainable livestock production systems. The forage grasses are routinely grown in mixtures with legumes such as white clover (*Trifolium repens* L.) that fix atmospheric N which is then available to the companion grass and also provide a source of safe, home-grown, traceable protein (Frame and Newbould 1986). Perennial ryegrass (*Lolium perenne* L.) breeding at IGER has made significant progress in the development of high yielding grass varieties with good forage quality (Humphreys 2005) while white clover (*Trifolium repens* L.) breeding has made significant advances in developing varieties with enhanced forage yield, persistence and tolerance of fertiliser N (Abberton & Marshall 2005). Reform of the Common Agricultural Policy (CAP), concerns about diffuse pollution of N and P in the context of the EU Water Framework Directive

(WFD) and the impact of climate change have resulted in an increasing emphasis on multi-functional grassland systems, and a greater focus on the role of grassland in delivering ecosystem services. At IGER targets for both grass and legume breeding programme are changing to accommodate selection for “environmental traits” that will reduce the environmental footprint of grassland agriculture. However, irrespective of the changing focus of the breeding programmes, the ability of varieties of forage grasses and legumes to produce adequate quantities of seed is important to ensure the success of commercial variety development and that the varieties that arise from these plant breeding programmes are available to the grassland farmer. This paper outlines some of the strategies that have been adopted in the plant breeding programmes to maintain reproductive potential whilst incorporating improved agronomic and environmental traits.

IMPROVING SEED YIELD OF PERENNIAL RYERASSES

In perennial ryegrass, seed yields are variable and often low and many of the characteristics associated with high seed yield are negatively correlated with agronomic performance (van Wijk 1980). Seed yield is a complex trait with a low heritability (Elgersma 1990). Perennial ryegrass breeding at IGER has produced varieties with improved dry matter yield and dry matter digestibility however this has been achieved by selection for high tiller density and reduced flowering intensity which has led to lower than acceptable seed yields (Wilkins 1997). Selection for seed production traits that are dependent on preferable allocation of resources from vegetative to reproductive organs will inevitably be detrimental to agronomic performance (Wilkins 1991). However, seed set (i.e. the proportion of florets that produce a seed, *sensu strictu* caryopsis) and seed retention are two reproductive independent of vegetative growth performance traits (Wilkins 1991). In perennial ryegrass considerable variation for seed set has been observed in nominally fertile crosses with

Elgersma & Sniezko (1988) observing ranges from 8 % to 73 %. We have therefore focused our genetic analyses of seed production on seed set.

In our studies two cycles of phenotypic selection for seed yield per tiller were carried out to improve seed production. This resulted in more seeds per reproductive tiller and a greater proportion of florets forming seeds (greater seedset) in selected material (25 % greater in the selected variety AberDart than AberElan, and 18 % greater than the control variety Talbot). This was confirmed in field experiments which showed that cv. AberDart had the greatest proportion of florets forming seeds (% seedset), and the greatest number of seeds per tiller of the 19 varieties examined, despite having only average numbers of reproductive tillers (Table 1). Selection for seed yield per tiller would therefore appear to be a successful strategy to maintain seed yield of perennial ryegrass and compensate for reduced numbers of reproductive tillers, enabling the development of improved varieties which combine good forage characteristics and acceptable seed yields.

Table 1. Seed yield components of the selected variety AberDart and the mean of 18 commercial varieties and selection lines of perennial ryegrass grown in the field.

Variety	Reproductive tillers/0.05 m ²	Spikelets/tiller	Seeds per spikelet	% seedset	Seeds per tiller
Range	69-116	18.3-27.0	0.7-4.5	17.7-62.9	15.5-103.5
Mean (n=19)	98.6	23.0	2.8	40.9	65.5
AberDart (ranking)	97 (11)	26.3 (3)	3.9 (3)	62.9 (1)	103.5 (1)

IMPROVING SEED YIELD OF WHITE CLOVER

In white clover, seed yields are unreliable and often low due to the lack of insect pollinators and adverse weather conditions during seed crop development. Moderate moisture stress can increase the number of reproductive buds (Bissuel-Belaygue *et al.* 2002) but excessive rainfall during flowering, pollination and harvesting can reduce seed yields. Strategies to increase seed yields include the development of improved seed crop

management systems (Hollington *et al.* 1989) and harvesting techniques and equipment. Plant breeding approaches have been hampered by the fact that although genetic variation for the components of seed yield exists (Hill *et al.* 1989) scope for further progress within the white clover gene pool appears limited. Selection for enhanced peduncle (flower stalk) strength has increased seed yields by ensuring that a higher proportion of the earliest formed inflorescences remain intact through to harvest (Marshall 1995).

An alternative approach has been the development of interspecific hybrids between white clover and the annual, profusely flowering ball clover (*T. nigrescens* Viv.), using conventional crossing techniques, with the objective of introgressing reproductive traits into white clover (Marshall *et al.*, 2002a). Advanced backcrosses with increased inflorescence production have been developed by increasing the proportion of nodes on the stolon that are reproductive with no detrimental effect on forage yield or quality. The increase in inflorescence production and seed yield of the backcross hybrid (BC₃) hybrid in comparison with the control variety Menna has also been achieved without reducing persistence. The first potential variety to be developed from this programme is now in official trials in the UK. Selections from the BC₃

were made in the field on the basis of leaf size, yield, persistence and reproductive potential. Three groups of plants, representing the small, medium and large leaf size were polycrossed separately to produce the BC₃ (F₁)_s, BC₃ (F₁)_m and BC₃(F₁)_L selection lines. The medium and large leaved selection lines had a significantly greater inflorescence production and potential seed yield than the control varieties of comparable leaf size in plot experiments (Table 3). The small leaved selection line was comparable with the control variety AberDale which was selected on the basis of high seed yields. This confirms the potential of this approach as a strategy to improve seed yield of white clover without compromising agronomic performance and this material will form the basis of future varieties with good seed yield.

Table 3. Inflorescence production and potential seed yield (kg ha⁻¹) of the BC₃ hybrid of different leaf size with control varieties. Data is a mean of two harvest years. Potential seed yield = ripe inflorescences/m² x seed wt./inflorescence

Hybrid/variety	Inflorescences		Potential seed yield
	Total	Ripe	(kg ha ⁻¹)
BC ₃ (F ₁) _s	855	421	361
AberDale	956	440	429
BC ₃ (F ₁) _m	862	508	453
Menna	678	330	334
BC ₃ (F ₁) _L	983	522	597
Olwen	561	199	275
s.e.d.	100.1	57.2	89.6
Significance	***	***	***

MOLECULAR APPROACHES - LEGUMES

Progress in forage legume breeding is constrained by the time required to evaluate material in the field particularly for complex traits such as seed yield. The use of molecular markers has the potential to significantly alleviate these limitations through incorporation of marker assisted selection (MAS) into plant breeding programmes. As the first step in using this approach, IGER, with The Plant Biotechnology Centre, Victoria have

developed the first *T. repens* map and quantitative trait loci (QTL) for agronomically important characters, including seed yield, in single plants have been located across sites and years (Cogan *et al.* 2006). Molecular approaches are also being developed to increase the speed and precision of the introgression programmes between *T. repens* and *T. nigrescens*, reducing the problems of linkage drag and the number of cycles of selection required.

GENETIC MAPPING APPROACHES - PERENNIAL RYEGRASS

Current research has concentrated on QTL analysis of specific mapping families of perennial ryegrass to provide information on the genetic control of seed set and also to determine whether there is scope for targeted selection of genes to improve seed setting ability in ryegrass. By comparing orthologous regions in rice, we wish to identify putative orthologues of genes underlying these QTL in order to develop gene specific markers for screening allelic variation in available germplasm and to incorporate favourable

alleles into elite breeding lines by introgression.

Initial QTL analyses of seed setting in two unrelated mapping populations (one F2 and one BC1 family) have revealed a QTL in the same region of linkage group 7 (Fig. 1). This QTL coincides with a major flowering date QTL (Armstead *et al.* 2004, 2005). It was found to explain 18 and 21 % of the total variance for seed set in the F2 and BC1 families respectively which translated into a four fold and 1.4 fold difference in seed set per spikelet, measured as number of seeds set per spikelet (Table 4). In both mapping populations, low seed set was associated with marker genotypes with a later mean heading date phenotype (Table 4).

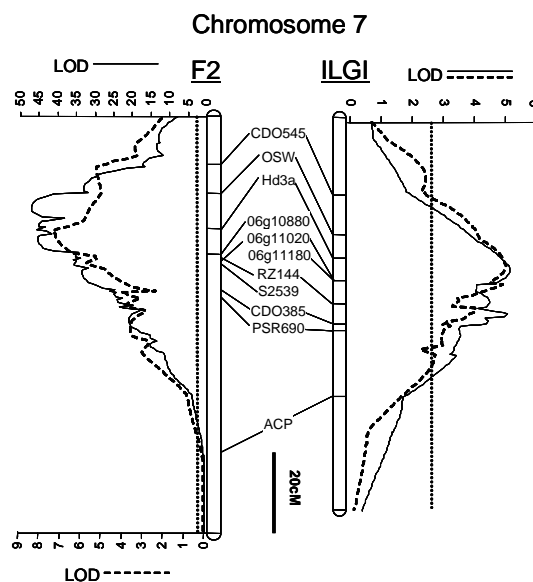


Fig 1. Interval mapping LOD profiles for heading date (solid line) and seeds/tiller (dashed line) for chromosome 7 of the F2 and BC1 families. Genome wide significance level is indicated by the dotted line.

Table 4. Mean heading date and seed setting scores for segregating genotypes at *hd3* locus derived markers.

	F2						BC1					
	Mean heading date (days after 1 st April)			Mean seedset (\log_{10} seeds tiller ⁻¹ + 1)			Mean heading date (days after 1 st April)			Mean seedet (seeds spikelet ⁻¹)		
marker	<i>aa</i>	<i>ab</i>	<i>Bb</i>	<i>aa</i>	<i>ab</i>	<i>Bb</i>	marker	<i>aa</i>	<i>ab</i>	<i>bb</i>	<i>Aa</i>	<i>ab</i>
<i>Hd3agt</i>	32.4	45.7	59.1	0.69	0.44	0.18	<i>Hd3a(Ld)</i>	58.1	55	59.1	1.55	2.20

One promising candidate gene underlying the seed setting QTL is the *S5* gene identified on

chromosome 6 in rice which is known to be largely syntenic with *Lolium* Chromosome 7.

The *S5* or 'wide-fertility' or 'embryo-sac fertility' locus (Yanagihara *et al.* 1995, Qiu *et al.*, 2005, Ji *et al.* 2005) has certain allele combinations that resulted in fertility of around 50 % as opposed to 100 % or very close to 100% observed in fully fertile crosses. These phenotypes would appear to be analogous to those found associated with our ryegrass QTL that result in only partial loss of fertility. Although the gene has not been cloned its identification has been narrowed down to no more than five Open Reading Frames spanning a region of 56kb (Qiu *et al.* 2005, Ji *et al.* 2005). We have developed two EST markers (6g10880 and 6g11180) which are polymorphic in the two mapping families that immediately flank this region and a third (6g11020) that lies within this region and they map to a region that coincides with the seed setting QTL (Fig. 1). This region falls between the two heading date loci *hd3a* and *hd1* that underlie the coincident heading date QTL.

Late heading date is often associated with low seed yield in perennial ryegrass and it has been assumed that this is due to seed maturation occurring late in the growing season during relatively unfavourable growing and harvesting

conditions. Our mapping results suggest that linkage of seed set genes to heading date genes may, at least partly, be responsible for the association, and the development of gene specific markers for *hd3a* and *hd1* and the ryegrass equivalent of the *S5* locus has given us the opportunity to identify and use recombinants in which the association is broken.

Undoubtedly other loci that effect seed yield will be identified but manipulation of this particular region of the genome on linkage group 7 will have significant impact on stabilising seed yield in ryegrass without unfavourable effects on forage yield and forage and turf quality.

ACKNOWLEDGEMENTS

This work was supported by the BBSRC (Biotechnology and Biological Sciences Research Council) and Defra (Department for the Environment, Food and Rural Affairs). IGER bred grass and clover varieties are marketed by Germinal Holdings Ltd.

LITERATURE

- Abberton, M.T. & Marshall, A.H. 2005. Progress in breeding perennial clovers for temperate agriculture. *Journal of Agricultural Science* 143: 117-135.
- Armstead, I.P., Turner, L.B., Farrell, M. et al. 2004. Synteny between a major heading-date QTL in perennial ryegrass (*Lolium perenne* L.) and the Hd3 heading-date locus in rice. *Theoretical and Applied Genetics* 108 (5): 822-828.
- Armstead I.P., Skot, L. & Turner L.B., 2005. Identification of perennial ryegrass (*Lolium perenne* (L.)) and meadow fescue (*Festuca pratensis* Huds.) candidate orthologous sequences to the rice Hd1(Se1) and barley HvCO1 CONSTANS-like genes through comparative mapping and microsynteny *New Phytologist* 167 (1): 239-247.
- Bissuel-Belaygue, C., Cowan, A.A., Marshall, A.H. & Wery, J. 2002. Reproductive development of white clover (*Trifolium repens* L.) is not impaired by a moderate water deficit that reduces vegetative growth. II. Inflorescence, floret and ovule production. *Crop Science* 42: 406-414.
- Cogan, N.O.I., Abberton, M.T., Smith, K.F., Kearney, G., Marshall, A.H., Williams, A, Michaelson-Yeates, T.P.T., Bowen, C, Jones, E.S., Vecchies, A.C. & Forster, J.W. 2006: Individual and multi-environment combined analyses identify QTLs for morphogenetic and reproductive development traits in white clover (*Trifolium repens* L.). *Theoretical and Applied Genetics* 112 (8): 1401-1415.
- Elgersma, A. 1990 Spaced-plant traits related to seed yield in plots of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 51: 151-161.
- Frame, J. & Newbould, P. 1986. Agronomy of white clover. *Advances in Agronomy* 40: 1-88.
- Hill, J., Norris, I.B. & Michaelson-Yeates, T.P.T. 1989: The inheritance of floral characters in white clover (*Trifolium repens* L.). *Annals of Applied Biology* 115: 101-103.

- Hollington, P.A., Marshall, A.H. & Hides, D.H. 1989: Effect of seed crop management on potential seed yield of contrasting white clover varieties. II Seed yield components and potential seed yield. *Grass and Forage Science* 44: 189-193.
- Humphreys, M.O. 2005. Genetic improvement of forage crops-past, present and future. *Journal of Agricultural Science* 143: 441-448.
- Ji, Q., Lu, J.F., Chao, Q., Chao, Q., Gu, M.H. & Xu M.L. 2005. Delimiting a rice wide-compatibility gene S-5(n) supercript stop to a 50 kb region. *Theoretical and Applied Genetics* 111: 1495-1503.
- Marshall, A.H. 1995. Peduncle characteristics, inflorescence survival and reproductive growth of white clover (*Trifolium repens* L.). *Grass and Forage Science* 50: 324-330.
- Marshall, A.H., Michaelson-Yeates, T.P.T., Abberton, M.T., Williams, T.A. & Powell, H.G. 2002. Variation for reproductive and agronomic traits among *T. repens* x *T. nigrescens* third generation backcross hybrids in the field. *Euphytica* 126: 95-201.
- Qiu, S.Q. Liu, K.D. & Jiang J.X. 2005. Delimitation of the rice wide compatibility gene S5(n) to a 40-kb DNA fragment. *Theoretical and Applied Genetics* 111 (6): 1080-1086.
- Wijk, A.J.P. van 1980. Breeding for improved herbage and seed yield in *Setaria sphacelata* (Schumach.) Stapf and Hubbard ex Moss. *Agric. Res. Rep.900*, Pudoc, Wageningen.
- Wilkins P.W. 1991. Breeding perennial ryegrass for agriculture. *Euphytica* 52 (3): 201-214.
- Wilkins, P.W. 1997. Useful variation in in vitro digestibility within perennial ryegrass *Eupytica* 93: 249-255.
- Yanagihara, S., McCouch, S.R. & Ishikawa, K. et al., 1995. Molecular analysis of the inheritance of the S-5 locus, conferring wide compatibility in India-Japonica hybrids of rice (*Oryza sativa* L.) *Theoretical and Applied Genetics* 90 (2): 182-188.

Relationship among seed yield and seed components using path analysis in *Poa angustifolia* collected from Zanjan, Iran

P.Moradi¹⁾, A.A.Jafari²⁾ & H.M. Nadoushan³⁾

¹⁾Agricultural and natural resources research center of Zanjan, Iran

²⁾Scientific Board of Research Institute of Forests and Rangelands, Tehran, Iran

³⁾Scientific Board of Seed and Plant Improvement Institute Karaj, Iran

Parvizmoradi@yahoo.com

ABSTRACT

Poa angustifolia is an important perennial grass species that belong to the *Poa* genus. It naturally grows in Zagros and Alborz mountains rangelands in the west and north of Iran. It has early growth in spring with good quality for animal productivity. In order to evaluate relationships among seed yield, and its components, nine accessions of *Poa angustifolia* were collected from different parts of the Zanjan rangelands, in the northwestern part of Iran. An experiment was conducted using randomized complete block design with 4 replications during 2004-2005. Data were collected for plant height, panicle length, peduncle length, flag leaf length, panicle emergence, panicle number, DM yield, seed yield, thousand grain weight, harvest index, seed weight per panicle and seed number per panicle. Data were collected and analyzed across two years. Results of analysis of variance showed significant differences among populations for all of the characters, indicating the existence of genetic variation. Estimates of broad-sense heritability (h^2_b) were moderate to high for all of traits except peduncle length, thousand grain weights and harvest index. Seed yield had positive and significant correlation with DM yield, panicle number, harvest index, thousand grain weight, seed weight per panicle and seed number per panicle. DM yield had positive and significant correlation with panicle length, leaf size, panicle emergence date, panicle number per plant, thousand grain weight and seed yield. Based on results of regression analysis four traits as panicle number, plant height, thousand grain weight and seed number per panicle were included in regression equation. The results derived from path analysis indicated that panicle number and seed number per panicle had the highest direct effect values on seed production. In conclusion, these two traits will be effective as selection indices for improving seed yield in *Poa angustifolia*.

Key words: forage yield, genetic variation, path analysis, *Poa*, seed components, seed yield

INTRODUCTION

Poa angustifolia (narrow-leaf meadow grass) is an important perennial grass species that belong to the *Poa* genus. It naturally grows in Zagros and Alborz mountains rangelands in the west and north of Iran. It is being used for grazing and hay production and consumed by livestock. *Poa angustifolia* grows in areas with 750 m to 2900 m altitude (Rechinger 1970). It has early growth in spring, good quality for

animal productivity, and good adaptability to a vast range of severe conditions all over the country. In recent years, higher grazing pressure and unpalatable weed invasion had led to increasing soil erosion and consequently decreasing population of this species. Therefore, revegetating those areas using new improved grass varieties is the most economical and possible means of recovery.

In most forage crops, including *Poa angustifolia*, yield has two distinct commodities: forage and seed. These two commodities are inextricably interdependent. To improve grass varieties for both seed and herbage yield, knowledge of genetic parameters is important for choosing an efficient selection strategy (Dudley 1997). In addition, knowledge of correlations between traits of interest is also useful in designing an effective breeding program for a crop. Since selection for seed or DM yield alone may not be the best criterion to improve seed or/and DM production, it is important to study genetic correlations between agronomic traits, which may have high heritability, and yield, which has low heritability (Falconer & Mackay 1996). *Poa angustifolia* has an important role in grassland productivity in Iran. Little breeding work has been done on this species under Iranian climatic conditions. Therefore, this research project was conducted to determine variation for seed yield and morphological traits in Iranian *Poa angustifolia* germplasm, to examine relationships among seed yield and reproductive traits, and to identify the most important traits governing seed yield through a multivariate approach.

MATERIALS AND METHODS

The germplasm utilized in this study were nine population of narrow-leaf meadow grass (*Poa angustifolia* L.) which were collected from different part of Zanjan province during 2000-2001. From each accession, eighteen seedlings were established on compost. The vegetative seedlings were transplanted to the field in April 2002. A randomized complete block design with 4 replications was established in rows 40 cm apart, with 40 cm spacing within rows. Non-experimental spaced plants were planted in two border rows surrounding the experimental area. The first harvest of 2004 and 2005 data were assessed for seed production and seed components. The following morphological characters were measured on individual plants in each plot:

1. Panicle emergence date as the number of days from 21 March to the stage at which three flowering shoots were visible.
2. Plant height (cm) from the soil surface to the tip of tallest panicle just prior to seed harvest.
3. Peduncle length (cm) from the base of panicles to flag leaf node just prior to seed harvest.
4. Panicle number per plant as the number of fertile panicles per plant.
5. Panicle length (cm): the length of five developed panicles was averaged from peduncle to tip of panicles at anthesis date.
6. Flag leaf length (cm): the length of five developed flag leaf were measured and averaged
7. Thousand-grain weight: a sample of 200 clean seeds was weighed and multiplied by 5.
8. DM yield: each individual plant was harvested, allowed to air dry, and dry weight was expressed in g plant⁻¹. Thus, this represented the above-ground biological yield.
9. Seed weight per panicle as the ratio of seed yield to number of panicles.
10. Seed number per panicle as the ratio of seed weight per panicle to the average weight of one seed.
11. Harvest index as the ratio of seed yield to total biological yield
12. Seed yield: dried plants were threshed and cleaned. The clean seed was weighed and yield expressed in g plant⁻¹.

Collected data were subjected to a combined analysis of variance across years using a split-plot-in-time design with years as sub-plots (Steel & Torri 1980). Phenotypic correlations among characteristics were determined for all pair-wise combinations on means of each genotype over two years. Stepwise regression analysis was used for seed yield as dependent variables. Using path analysis, correlation of those traits that were included in the equation was partitioned into direct and indirect effects. All statistical analyses were conducted by SAS V.8.0 (SAS Inst. 2001).

RESULTS AND DISCUSSION

Descriptive statistics and broad sense heritability for seed yield and morphological traits derived from analysis across years are summarized in Table 1. All characters under observation varied greatly. Estimates of broad-sense heritability (h^2_b) for peduncle length, thousand grain weights and harvest index were

low (0.0 to 0.21) and for other traits were moderate to high. Estimate h^2_b for seed yield ($h^2_b=0.40$) was lower than the estimates of

Jafari *et al.* (2006) in tall fescue. Thus, to improve seed yield, recurrent selection based on progeny testing should be effective.

Table 1. Descriptive statistics on average values of 12 traits over 2 years in 9 accessions of narrow leaf meadow grass grown as spaced plants

Traits	Mean	Minimum	Maximum	SE Mean	St Dev	CV %	H^2_b
Plant height (cm)	56.2	16.5	79.8	3.2	13.8	24.5	0.82
Panicle length (cm)	7.26	5.73	9.50	0.27	1.12	15.5	0.48
Peduncle length (cm)	21.5	11.8	36.3	1.7	7.2	33.6	0.00
Leaf size (cm)	3.84	1.68	11.00	0.67	2.84	74.0	0.72
Panicle emergence (day)	34.0	20.0	44.5	1.6	6.8	20.0	0.50
Panicle number	87.3	36.3	132.5	7.5	31.8	36.4	0.77
DM yield (g)	33.2	17.1	69.5	3.0	12.9	38.8	0.58
Seed yield (g)	4.38	1.47	8.95	0.43	1.82	41.5	0.40
Thousand grain weight (g)	0.28	0.18	0.33	0.01	0.04	14.4	0.14
Harvest index	0.15	0.05	0.27	0.01	0.06	39.3	0.21
Seed weight/ panicle (g)	0.06	0.03	0.10	0.01	0.02	43.6	0.61
Seed number/ panicle	199.0	87.7	334.5	19.5	82.7	41.5	0.67

The results of combined analysis across years showed significant differences among accessions for all traits (Table 2). Genotype by year Interaction effect were significant for all of traits except DM yield, suggesting that more than one environment should be used to assess the breeding material. The estimates of phenotypic correlations are summarized in Table 3. The correlation between seed yield and DM yield was strongly positive, which indicated that correlated response to selection for one trait should improve the other traits. Jafari *et al.* (2006) in tall fescue found the same conclusions. Seed yield had positive and significant correlation with panicle number, harvest index, thousand grain weight, seed weight per panicle and seed number per panicle. According to results, selection for higher seed yield by increasing these traits seemed possible. DM yield had positive and significant correlation with panicle length, leaf size, panicle emergence date, panicle number per plant, thousand grain weight and seed yield (Table 3). These results suggest that selection for these traits would increase DM yield in *Poa angustifolia*.

Relationship between panicle emergence date and DM yield was positive. This is similar to the finding of Mohammadi *et al.* (2005) in *Bromus*

inermis. But in contrast with Jafari *et al.* (2006) in tall fescue. These results suggested that selection for late flowering accessions would increase DM yield in *Poa angustifolia*. However, in severe summer drought in Mediterranean climate such as that in Iran, this relationship has no benefit for farmers and dry land farming.

Prior to path analysis, the important traits were defined by a stepwise regression equation for seed yield as dependant and other traits as independent variables (Table 4). Based on results of regression analysis four traits as panicle number, plant height, thousand grain weight and seed number per panicle were included in regression equation. With exception of plant height, for the other traits the regression results was similar to correlation analyses in Table 3.

Path analysis is a method to explain cause and causation among traits and identify more effective traits (Wright 1921). In this study, seed yield were used as dependent and the four other traits that were included in regression equation as independent variables. The correlation coefficients were partitioned to direct and indirect effects using path coefficients (Dewey & Lu 1959).

Table 2. Combined analysis of variance for 12 traits in 9 accessions of narrow leaf meadow grass grown as spaced plants.

Source	DF	Plant Height (cm)	Panicle length (day)	Peduncle length (cm)	Leaf size (cm)	Panicle Emergence (day)	Panicle number	DM yield (g)	Seed yield (g)	Thousand grain weight (g)	Harvest index	Seed weight/ panicle	Seed number/ panicle
Treat	8	1173**	5.41**	44.13*	38.4**	134.3**	5972**	799**	9.4*	0.34**	0.67**	0.26**	33760**
Rep	3	67.88	3.25	3.89	0.52	12.7**	99	410*	1.95	0.21	0.20	0.06	2612
Error 1	24	43.68	1.22	16.58	0.75	2.10	324	122.1	1.06	0.10	0.15	0.03	3465
Year	1	550**	14.2**	1948**	80.6**	1449**	650	3460**	34.8**	2.8**	0.70*	0.19**	1922
Treat × Year	8	367**	3.55**	154**	20.1**	77.8**	2529**	171.7	14.4**	0.71**	1.96**	0.22**	24107**
Error 2	27	31.00	0.81	16.17	1.13	3.25	250	87.0	1.05	0.14	0.13	0.02	1294

*, ** = Significant at 5 %, 1 %, respectively.

Table 3 Phenotypic correlations among seed yield and morphological traits estimates over two years.

Traits	Plant height (cm)	Panicle length (day)	Peduncle length (cm)	Leaf Size (cm)	Panicle emergence (day)	Panicle number	DM yield (g)	Thousand grain weight (g)	Harvest index	Seed weight/ panicle (g)	Seed number/ panicle
Panicle length (day)	0.46**										
Peduncle length (cm)	0.37*	-0.19									
Leaf size (cm)	0.55**	0.35*	0.72**								
Panicle emergence (day)	0.21	0.03	0.47**	0.43**							
Panicle number	0.19	-0.21	0.04	0.01	-0.23						
DM yield (g)	0.22	0.10	0.53**	0.41**	0.32*	0.55**					
Thousand grain weight (g)	-0.24	-0.35*	0.38*	0.00	0.09	-0.01	0.39**				
Harvest index	-0.07	-0.20	-0.45**	-0.25	-0.36*	0.11	-0.51**	0.02			
Seed weight/ panicle (g)	0.06	0.23	-0.06	0.17	0.42**	-0.57**	0.01	0.27	0.45**		
Seed number/ panicle	0.21	0.42**	-0.17	0.22	0.41**	-0.61**	-0.16	-0.21	0.43**	0.95**	
Seed yield (g)	0.06	-0.08	0.03	0.09	0.22	0.52**	0.53**	0.37*	0.57**	0.59**	0.41**

*, ** = Significant at 5 %, 1 %, respectively.

Table 4. Equation components extracted from stepwise regression

Equation components	Unstandardized coefficients	Standardized coefficients	t-values	P-values
Constant	-7.87			.155
Panicle number	0.054	0.924	15.29	.000
Plant height (cm)	-0.023	-0.166	-3.12	.000
Thousand grain weight (g)	17.20	0.432	8.43	.000
Seed number/ panicle	0.020	0.914	14.94	.000

R square= 0.85 R=0.92

Table 5. Partitioning correlation coefficients to direct and indirect effects for seed yield in *Poa angustifolia*

Trait	Direct effect	Indirect effect through				Correlation Coefficients (r)
		Panicle number	Plant height (cm)	Thousand Grain Weight (g)	Seed number/ panicle	
Panicle number	1.567	---	-0.08	-0.01	-0.96	0.52
Plant height (cm)	-0.425	0.30	---	-0.14	0.33	0.06
Thousand grain weight (g)	0.598	-0.02	0.10	---	-0.32	0.37
Seed number/ panicle	1.575	-0.96	-0.09	-0.12	---	0.41

According to Table 5, panicle number and seed number per panicle had the highest direct effects values with the same sign as correlation coefficient, suggested that these traits had genetic relationships with seed yield and selection for panicle number and

seed number per panicle lead to improve seed yield. It was concluded that for improving seed yield in *Poa angustifolia*, using these two traits will be effective as selection indices. These indices could be applied for selection of genotypes with higher seed yield.

LITERATURE

- Dewey, D. R. & Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheatgrass seed production. *Agronomy Journal* 51: 515 -518.
- Dudley, J.W. 1997. Quantitative genetics and plant breeding. *Advances in Agronomy* 59: 1-23.
- Falconer, D.S. & Mackay, T.F.C. 1996. Introduction to quantitative genetics (fourth edition). 464 pp. Longman Group Ltd. London.
- Jafari, A.A., Setavarz H. & Alizadeh, M.A. 2006. Genetic variation for and correlations among seed yield and seed components in tall fescue, *Journal of New Seeds* 8: in press
- Mohammadi, R., Mirlohi, A.F., Khayyam-Nekouei, M. & Razmjoo, K. 2005. Evaluation of genetic diversity, heritability and relationships among the traits in *Bromus inermis* Leyss. (In Persian). Proc. First National Forage Crops Congress of Iran. Karaj, Iran.
- Rechinger, K.H. 1970. *Flora Iranica*. No. 70. Graz, Austria.
- Steel, R.G.D. & Torrie, J.H. 1980. Principles and procedures of statistics: A biometrical approach. Second edition, 633 pp. Mc Graw Hill book company, London.
- SAS Institute Inc.2001. SAS/STAT User's guide. Vers.8. SAS Institute Inc. Cay.N.C.
- Wright, S.1921. Correlation and causation. *Journal of Agricultural Research* 20: 557-55.

Seed and herbage yield in *Bromus tomentellus* Boiss. grown under optimal and drought stress conditions

A. Jafari¹⁾, A. Bafandeh Rouzbahani²⁾ & E. Rahmani³⁾

¹⁾Research Institute of Forests and Rangelands, Tehran, Iran

²⁾Former postgraduate student of Islamic Azad University, Brojed branch, Iran

³⁾Agricultural and natural resource research centre of Lorestan province, Iran

aajafari@rifr-ac.ir

ABSTRACT

In order to study variation and correlations among seed yield and its components (phenological traits, plant height, panicle number, panicle length, thousand grain weight, peduncle length, spikelet per spike, DM yield, seed number and seed weight per panicle, seed yield and harvest index) in *Bromus tomentellus* Boiss, 11 genotypes were sown in two separate experiments under optimum and drought stress condition using randomized complete block designs with three replications in Brojerd, Iran during 2004-2006. Seed yield means in optimum (Y_p) and stress (Y_s) condition were used to estimate five drought resistance indices as: tolerance index (TOL), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI). The data subsequently were used in principal components analysis. Based on the first two principal component scores genotypes were classified into four groups. Genotype 3752M with average values of 265 kg ha^{-1} seed yield in drought stress recognized as the best genotype for seed production in dry land farming. 587P10, 587P6 and 2296M with average values of 600 to 697 kg ha^{-1} , had higher seed yield in optimum condition. For drought condition 587M, 2854M and 587P7 had relatively higher seed production. The results of phenotypic correlation analysis showed that seed yield had positive and significant correlation with panicle number, plant height, panicle length, DM yield, seed weight and seed number per panicles in optimum condition. For drought stress, seed yield had a positive correlation with panicle number, thousand grains weight, seed weight per panicles and harvest index. The results of stepwise regression analysis for seed as dependant variables showed significant effects of panicle number, seed weight per panicles and harvest index in seed production in both environments. The results derived from path analysis indicated that the same traits had higher direct effect on seed production in both conditions. In addition, in drought condition, DM yield and anthesis date positively and negatively had direct effects on seed production, respectively. We conclude that there was significant variation for most traits in the *Bromus tomentellus* populations evaluated to improve seed and herbage yield. Selection in dry land farming system should focus on increased DM yield, panicle density and seed weight per panicles coupled with early flowering.

Key words: *Bromus tomentellus* Boiss, drought resistance indices, path analysis, regression, seed yield

INTRODUCTION

Russian brome grass (*Bromus tomentellus* Boiss.) grows in Zagros and Alborz mountains rangelands in the west and north of Iran in areas with 1600 m to 3000 m altitude having more than 300 mm annual participation (Rechinger 1970). It is being used for sheep grazing and hay production. In recent years, higher grazing pressure and unpalatable weed invasion had led to increasing soil erosion and

consequently decreasing population of palatable species. Therefore, re-vegetating of those areas by new improved grass varieties is the most economical and possible means of recovery. The preferred method for rehabilitation of this area is to use native forage species including *Bromus tomentellus*, that they are important components in Iran's rangeland ecosystems. However, many of

native plant materials have poor seedling vigor and are difficult to establish and they do not compete as well with weedy grasses such as *Bromus tectorum*. For improvement of the adapted native plant materials for both seed and herbage yield, one have to evaluate them under persist seasonal climatic variations, frequent droughts and low precipitation. Selection of populations under environmental stress conditions is one of the main tasks of plant breeders for exploiting the genetic variations to improve the stress tolerant cultivars. Drought indices as: tolerance index (TOL), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI) had been used for screening drought-tolerant genotypes (Fernandez 1992). In addition, knowledge of correlations between traits of interest is also useful in designing an effective breeding program for a crop. Since, selection for seed or DM yield alone may not be the best criterion to improve seed or/and DM production. Hence, it is also important to study correlations between agronomic traits, which may have high heritability, and yield, which has low heritability (Falconer & Mackay 1996).

Bromus tomentellus has an important role in grassland productivity in Iran. Little breeding work has been done on this species. Therefore, this research project was conducted to 1) assess the selection criteria for identifying drought tolerant and high-yielding genotypes in drought stress and non-stress field conditions and 2) determine the phenotypic correlations between characters and the contribution of each component to seed yield.

MATERIALS AND METHODS

The germplasm utilized in this study were 11 populations of Russian brome grass (*Bromus tomentellus* Boiss) which collected from Zagros mountain rangelands in west and south west of Iran. Accessions were sown in two separate experiments under optimum and drought stress condition using randomized complete block designs with three replications in Brojerd, Iran in 2004. In the first harvest of 2005 and 2006, data were assessed for seed yield and seed components. The following traits were measured in each plot.

1. Phenological stages (panicle emergence, anthesis date, seed milky date and maturity date) were measured as the number of days from 21 March to 10 percent visible of each phenological stage.
2. Panicle number: was recorded as the number of fertile panicles per m²
3. Plant height (cm) was recorded from the soil surface to the tip of tallest panicle.
4. Peduncle length (cm) was measured from the base of panicles to flag leaf.
5. Spikelet per spike were counted as mean of spikelets in 10 fertile spikes
6. Panicle length (cm): the length of five developed panicles was averaged from peduncle to tip of panicles at anthesis date.
7. Thousand-grain weight: a sample of 250 clean seeds was weighed and multiplied by 4.
8. Seed weight per panicle was calculated as the ratio of individual plant seed yield to number of panicles.
9. Seed number per panicle was calculated as the ratio of seed weight per panicle to average weight of one seed.
10. DM yield: all of plants in plots were harvested and weighted as kg ha⁻¹.
11. Harvest index was calculated as the ratio of seed yield to total DM yield
12. Seed yield: dried plants were threshed, cleaned and seed yield weighed as kg ha⁻¹.

Collected data were analyzed for each environment and combined over two environments. Seed yield means in two optimum (Y_p) and stress (Y_s) condition were used to estimate five drought resistance indices as: tolerance index (TOL), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI). The data were analyzed to principal components. Using by-plot graph based on the first two components, genotypes were classified into four groups (Fernandez 1992). Phenotypic correlations among characteristics were determined for all pair-wise combinations for each environment. Two separate stepwise regression equation were developed for seed as dependant variable and other traits as independent variables for each experiment. Finally, the correlation coefficients of those traits that were included in regression equation were partitioned to their direct and indirect effects using path analysis. All statistical analyses were conducted by SAS V.9.0 (SAS Inst. 2004).

RESULTS AND DISCUSSION

The results showed significant differences among genotypes for all of traits except seeds weight per panicle. Genotype by environment Interaction effects were significant for all of traits, suggesting that more than one environment should be used to assess the breeding material (data not shown). Using by-plot graph based on principal components analysis over drought tolerance indices (Yp, Ys, TOL, SSI, MP, GMP and STI), the genotypes were classified into four groups (Fig. 1). Genotype 3752M with average values of 265

kg ha⁻¹ seed yield under drought stress recognized as the best genotype for this environment. 587P10, 587P6 and 2296M with average values of 600 to 697 kg ha⁻¹, had higher seed production in optimum condition. 587M, 2854M and 587P7 were ranked as average for seed production in drought condition (Table 1). For DM yield 587P10, 587P6 with average values of 3.6 to 3.7 ton ha⁻¹ DM production were identified for irrigation condition. 587P12, 587P3 587P7, 587M had higher herbage mean values for both conditions (data not shown).

Table 1. Mean values of seed yield and five drought tolerance indices in *Bromus tomentellus* grown under optimum and drought stress conditions over two years.

Name	Seed yield (kg ha ⁻¹)						
	Irrigated	Drought	TOL	MP	SSI	STI	GMP
1690M	481	104	377	293	1.14	0.67	224
2296M	600	159	442	380	1.07	1.26	309
2309	376	123	252	250	0.98	0.61	215
2854M	279	176	104	228	0.54	0.65	222
3752M	476	265	211	371	0.65	1.67	355
587M	399	155	245	277	0.89	0.82	249
587P10	697	152	546	425	1.14	1.40	325
587P12	434	134	300	284	1.01	0.77	241
587P3	536	109	426	322	1.16	0.78	242
587P6	664	150	514	407	1.13	1.32	315
587P7	444	167	277	305	0.91	0.98	272
Mean	489.6	154.0					

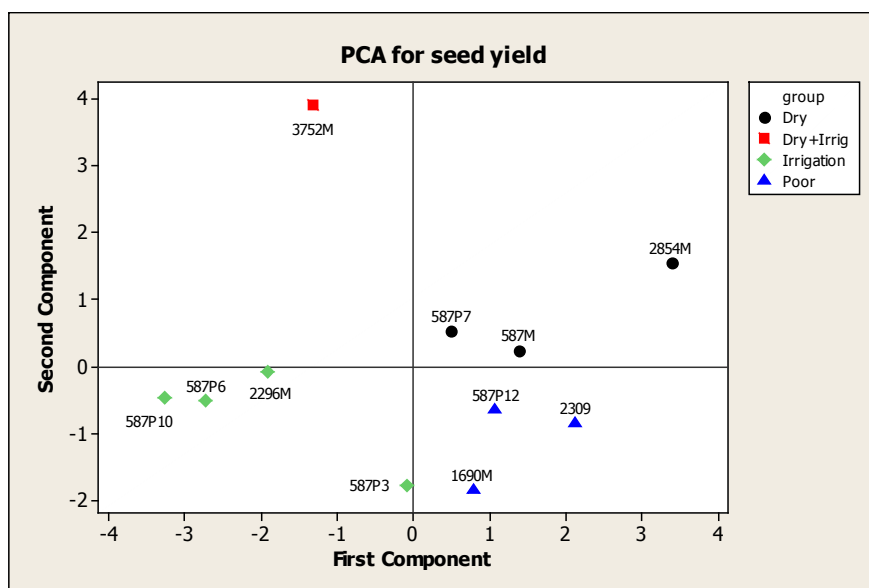


Fig. 1. Scatter plot of 11 accessions of *Bromus tomentellus* from principal components analysis for seed yield and five drought tolerance indices.

Table 2. Phenotypic correlation among seed yield and morphological traits in 11 genotypes of *Bromus tomentellus* grown in optimum (below the diagonal) and drought stress (above the diagonal) based on means of data for two years.

Traits	Panicle emerg. (day)	Anthe. date (day)	Seed milky date	Matur. date (day)	Panicle no. per m ²	Plant height (cm)	Pedunc. length (cm)	Spikel. no. per spike	1000 grain weight	Panicle length (cm)	Seed Weight/ panicle	Seed no. per panicle	DM Yield, kg ha ⁻¹	Harv. index	Seed Yield, kg ha ⁻¹
Panicle emergence		0.87**	0.86**	0.53**	-0.34*	0.01	-0.62**	0.91**	-0.74**	0.69**	0.43*	0.88**	0.80**	-0.64**	-0.11
Anthesis date (day)	0.86**		0.95**	0.79**	-0.17	0.31	-0.33*	0.79**	-0.49**	0.77**	0.44**	0.76**	0.70**	-0.49**	0.00
Seed milky date (day)	0.84**	0.90**		0.83**	-0.17	0.38*	-0.28	0.79**	-0.54**	0.73**	0.39*	0.77**	0.73**	-0.55**	-0.01
Maturity date(day)	0.78**	0.82**	0.82**		-0.01	0.60**	0.15	0.43**	-0.26	0.55**	0.15	0.42*	0.43**	-0.33*	-0.01
Panicle no. per m ²	0.55**	0.45**	0.55**	0.46**		0.21	0.34*	-0.28	0.56**	-0.26	-0.06	-0.34*	-0.03	0.31	0.63**
Plant height (cm)	0.49**	0.62**	0.60**	0.42*	0.35**		0.54**	0.04	0.03	0.29	-0.20	-0.05	0.10	-0.14	0.00
Peduncle length (cm)	-0.36*	-0.19	-0.14	-0.16	-0.30	0.05		-0.64**	0.49**	-0.33*	-0.47**	-0.63**	-0.40*	0.23	0.02
Spikelet no. per spike	0.83**	0.75**	0.74**	0.61**	0.39*	0.53**	-0.46**		-0.70**	0.72**	0.60**	0.95**	0.76**	-0.55**	0.07
1000 grain weight (g)	-0.77**	-0.65**	-0.65**	-0.62**	-0.44**	-0.18	0.51**	-0.75**		-0.47**	0.00	-0.67**	-0.54**	0.72**	0.50**
Panicle length (cm)	0.77**	0.70**	0.70**	0.63**	0.41*	0.66**	-0.36*	0.81**	-0.65**		0.50**	0.73**	0.62**	-0.38*	0.05
Seed weight/panic	-0.23	-0.13	-0.09	-0.26	-0.10	0.42*	0.33*	-0.03	0.49**	0.02		0.70**	0.37*	0.11	0.60**
Seed no. per panicle	0.70**	0.65**	0.59**	0.52**	0.37*	0.61**	-0.36*	0.83**	-0.64**	0.79**	0.21		0.71**	-0.48**	0.10
DM yield (ha ⁻¹)	0.76**	0.67**	0.79**	0.58**	0.81**	0.51**	-0.28	0.71**	-0.65**	0.67**	-0.08	0.59**		-0.74**	0.07
Harvest index	-0.59**	-0.49**	-0.58**	-0.44**	-0.47**	0.05	0.39*	-0.51**	0.71**	-0.40*	0.60**	-0.21	-0.66**		0.53**
Seed yield (kg ha ⁻¹)	0.21	0.20	0.28	0.17	0.61**	0.54**	0.00	0.27	0.09	0.34*	0.59**	0.38*	0.48**	0.17	

*, ** = Coefficients of correlation are significant at 5 %, 1 %, respectively.

The estimates of phenotypic correlations in both environments are summarized in Table 2. The results showed that seed yield had positive and significant correlation with panicle number, plant height, panicle length, DM yield, seed weight and seed number per panicles in optimum condition. These results were in agreement with Jafari *et al.* (2006) in tall fescue. In drought environment, seed yield had a positive correlation with panicle number, thousand grains weight, seed weight per panicles and harvest index. Relationship between phenological traits and DM yield

generally was positive. These results suggested that selection for late flowering accessions might increase DM yield in *Bromus tomentellus*.

The results of stepwise regression analysis for seed as dependant variables under optimum condition showed significant effects of panicle number, seed weight per panicles, harvest index, spikelet per spike and thousand grain weight (Table 3). In dry condition, panicle number, seed weigh per panicles, harvest index, DM yield, anthesis date and milky date were included in regression equation (Table 3).

Table 3. Results of stepwise regression analysis for seed yield as dependent variables and other traits as independent variables in *Bromus tomentellus* grown under both optimum and drought stress conditions

Irrigation			Drought stress		
Equation	Un-standard	Standard	Equation	Un-standard	Standard
Constant	-336	0.00	Constant	-102	0.00
Panicle no. per m ²	0.39	0.77	Panicle no. per m ²	0.16	0.46
Seed weight per panicle (mg)	477	0.34	Seed weight/panic (mg)	617	0.47
Harvest index	220	0.28	Harvest index	430	0.63
Spikelet no. per spike	7.6	0.38	DM yield (Kg ha ⁻¹)	0.04	0.44
1000 grain weight (g)	26.0	0.35	Anthesis date (day)	-5.2	-0.56
			seed milky date (day)	3.6	0.45
R-Sq	85	85	R-Sq	93	93

Table 4. Partitioning phenotypic correlations among seed yield and seed components to direct and indirect effects in 11 genotypes of *Bromus tomentellus* grown in optimum condition

Traits	Direct Effect	Indirect effects through					Total corr.
		Panicle per m ²	Seed weight per panicle	Harvest Index	Spikelet per spike	1000 grain weight (g)	
Panicle number m ⁻²	0.78		-0.03	-0.13	0.15	-0.16	0.61
Seed weight/panic (mg)	0.33	-0.08		0.17	-0.01	0.18	0.59
Harvest index	0.28	-0.37	0.20		-0.20	0.26	0.17
Spikelet no. spike ⁻¹	0.39	0.31	-0.01	-0.14		-0.28	0.27
1000 grain weight (g)	0.37	-0.35	0.16	0.20	-0.30		0.09

Residual effects=0.37

Table 5. Partitioning phenotypic correlations among seed yield and seed components to direct and indirect effects in 11 genotypes of *Bromus tomentellus* grown in drought stress condition.

Traits	Direct effect	Indirect effect through					Total Corr.	
		Panicle per m ²	Seed weight per panicle	Harvest index	DM yield (kg ha ⁻¹)	Anthesis date (day)		Milky date
Panicle no. per m ²	0.45		-0.03	0.20	-0.02	0.07	-0.05	0.63
Seed weight per Panicle (mg)	0.44	-0.03		0.07	0.18	-0.19	0.12	0.60
Harvest index	0.66	0.14	0.05		-0.35	0.21	-0.17	0.53
DM yield (kg ha ⁻¹)	0.48	-0.01	0.16	-0.49		-0.30	0.23	0.07
Anthesis date (day)	-0.43	-0.08	0.20	-0.32	0.33		0.30	0.00
Milky date (day)	0.32	-0.08	0.17	-0.36	0.35	-0.41		-0.01

Residual effects=0.27

The results derived from path analysis indicated that panicle number; seed weight per panicles and harvest index had significant direct effect on seed production in both conditions (Tables 4 and 5). In addition, in drought condition, DM yield and anthesis date positively and negatively had direct effect on seed production, respectively (Table 5). The negative relationship between seed yield and anthesis date that are in agreement with Jafari *et al.* (2006) in tall fescue, indicating that selection of early flowering accessions led to

increasing seed yield in this species. Thousand grain weight had direct effect on seed production under optimum condition suggested that improvement of seed yield was possible by selection of thousand grain weight in this environment. We concluded that there was significant variation for most traits in the *Bromus tomentellus* populations evaluated to improve seed and herbage yield. Selection in drought condition should focus on increased DM yield, panicle density and seed weight per panicles coupled with early flowering.

LITERATURE

- Falconer, D.S. & Mackay, T.F.C. 1996. Introduction to quantitative genetics (fourth edition). 464 pp. Longman Group Ltd, London, UK.
- Fernandez, G.C.J. 1992. Effective selection criteria for assessing stress tolerance. In: Proceedings of the International Symposium on Adaptation of vegetables and other food crops in temperature and water stress. AVRDC Publication. Taiwan.
- Jafari, A.A., Setavarz, H. & Alizadeh M.A. 2006. Genetic variation for and correlations among seed yield and seed components in tall fescue, *Journal of New Seeds* 8: in press
- Rechinger, K.H. 1970. *Flora Iranica*. (F Iran) No 70. Graz, Austria.
- SAS Institute. 2004. SAS/STAT Software: Version 9. SAS Institute Inc. Cary, NC.

Evaluation of seed yield and seed components in tall fescue (*Festuca arundinacea* Schreb.) through correlation, regression and path analysis

S. Afkar¹⁾, A. Jafari²⁾ & G. Karimzadeh³⁾

¹ Post graduate student of Tarbiat Modaress University, Tehran, Iran.

² Scientific Board of Research Institute of Forests and Rangelands, Tehran, Iran

³ Scientific Board of Tarbiat Modaress University, Tehran, Iran

aajafari@rifr-ac.ir

ABSTRACT

Tall fescue (*Festuca arundinacea* Scrab.) is one of the important perennial forage grasses that naturally grow in Iranian pasture and rangelands. In order to evaluate relationships among seed yield, and its components, twenty four accessions and varieties were sown as spaced plants using a complete block design with three replications at Alborz research station, Karaj, Iran, during 2003-2005. The data were collected and analyzed for panicles emergence date, anthesis date, panicle number, plant height, panicle length, thousand grain weight, seed number and seed weight per panicle, seed yield, dry matter yield and plant stand, germination percent, speed of germination and vigor index. The results of correlation analyses showed that seed yield had positive and significant correlation with plant height, panicle number, dry matter yield, plant stand, panicle length, seed weight and seed number per panicles. Forage DM yield had positive and significant relationships with plant height, panicle number, panicle length, seed yield and plant stand. Panicle emergence date was negatively correlated with both seed yield and DM yield. Using stepwise regression analysis with seed yield as dependant variable, the results showed significant effects of panicle number, seed weight per panicle, DM yield, anthesis date and plant stand ($R^2=0.97$). The results derived from path analysis indicated that panicle number and seed weight per panicles had significant direct effects on seed production. DM yield had low direct effect on seed production, but indirectly increased seed yield through panicle number. For DM yield as dependent variable, seed yield, panicle length and plant stand were positively and seed number per panicle and panicle number negatively the most important traits. It is concluded that there is significant variation in the tall fescue populations to improve seed and herbage yield. Selection should focus on increased DM yield, panicle weight and panicle density coupled with early flowering.

Keywords: *Festuca arundinacea*, tall fescue, seed yield, correlation, regression and path analysis

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb) is one of the main perennial grasses that naturally grow in temperate pasture and rangelands in northern and western Iran. Tall fescue has an important role in grassland productivity and any improvement in its herbage and seed production would be very beneficial for animal productivity. To improve grass varieties for both seed and herbage yield, knowledge of genetic variation and correlations between traits of interest is useful in designing an effective breeding program. Selection for seed or DM yield alone may not be the best criterion to improve seed

or/and DM production. Hence, it is also important to study genetic correlations between agronomic traits, which may have high heritability, and yield, which has low heritability (Falconer & Mackay 1996). The extent to which various characters are correlated in forage grasses has been studied by a number of investigators. Some positive and significant correlations between seed yield and both DM yield and plant height (Veronesi & Falcinelli 1988, Jafari *et al.* 2006) has been reported in tall fescue. Martiniello (1998) and Jafari *et al.* (2006) found in tall fescue panicle emergence date to be negatively correlated with seed yield. In contrast, Nguyen & Sleper (1983) reported positive relationship between maturity score

and seed yield. The objective of this study was to examine relationships among seed yield, DM yield and reproductive traits through, correlation, regression and path analysis.

MATERIALS AND METHODS

The tall fescue genotypes used in this study were domestic and 20 foreign accessions from Ireland, Russia, Italy, Belgium, Australia and USA. Twenty-four accessions were evaluated as spaced plants during 2003-2006 in Research Institute of Forests and Rangelands, Karaj, Iran. From each accession, 18 seedlings were established in compost in April 2004. After growing in the glasshouse, the seedlings were transplanted to the field. An experiment was established using a randomized complete block design with three replications. In each plot 6 genotypes of each accession were allocated in 50×50 cm row spacing. The first harvest of each year was assessed for seed production and seed components. The following morphological characters were measured on individual plants in each plot in 2004 and 2005:

1. Panicle emergence date was measured as the number of days from 21 March to the stage at which three flowering shoots were visible.
2. Anthesis date was measured as the number of days from 21 March to the stage at which three flowering shoots were pollinating.
3. Plant height (cm) from the soil surface to the tip of tallest panicle was recorded just prior to seed harvest.
4. Panicle number per plant was recorded as the number of fertile panicles per plant.
5. Panicle length: the length of five developed panicles was averaged from peduncle to tip of panicles at anthesis date.
6. Plant stand: were estimated visually as 1=poor stand to 5=high stand.
7. Thousand-grain weight: a sample of 200 clean seeds was weighed and multiplied by 5.
8. DM yield: each individual plant was harvested, allowed to air dry, and dry weight was expressed in gr/plant. Thus, this represented the above-ground biological yield.
9. Seed number per panicle was calculated as the ratio of seed weight

per panicle to average weight of one seed.

10. Seed weight per panicle was calculated as the ratio of seed yield to number of panicles.
11. Seed yield: dried plants were threshed and cleaned. The clean seed was weighed and yield expressed in gr/plant.
12. Percent and speed of germination: A subsample of pure seed was sown in petry dishes. Germinated seeds were recorded at 3, 6, 9, 12 and 15 days. In accordance with Maguire (1962), the speed of germination was calculated by the following equation:

$$G.S = \frac{\sum n}{\sum n(n \times DN)} \times 100$$

Where n= is the number of seed germinated on days, DN=being the number of days after sowing corresponding to n, and the highest G.S. is the fastest speed.

13. The vigour index was calculated by following equation:

$$Vi = \frac{\%Gr \times MSH}{100}$$

Where: VI=vigor index, %Gr=final germination percentage and MSH=mean seedling height

The collected data for seed yield and reproductive characters were subjected to a combined analysis of variance across two years using a split-plot-in-time design with years as sub-plots (ANOVA and means of genotypes were not shown). Phenotypic correlations among characteristics were determined for all pair-wise combinations on means of each genotype over two years. Two separate stepwise regression analysis were used for seed yield and forage DM yield (as dependant variables) and finally, the correlation coefficients between those traits that were included in regression equation, were partitioned into direct and indirect effects on seed and DM yield. All statistical analyses were conducted using SAS V9.0 (SAS Inst. 2004).

RESULTS AND DISCUSSION

The results of correlation analyses showed a positive and significant correlation ($P \leq 0.01$) between seed yield with plant height, panicle number, dry matter yield, plant stand, panicle length, seed weight and seed number per panicles (Table 1).

Table 1. Phenotypic correlations among seed yield and morphological traits estimates from mean of two years analysis of 24 accessions of tall fescue

	Panicle emerg. (days)	Anth. date (days)	Plant Height (cm)	Panicle no. /plant	Panicle length (cm)	1000 grain weight (gr.)	Germ- ination per- cent	Vigor index	Plant Stand (1-5)	DM yield (g p ⁻¹)	Seed no. /pan- icle	Speed of germin ation	Seed weight /panicle (mg)
Anthesis date (days)	0.86**												
Plant height (cm)	-0.40*	-0.44*											
Panicle No. plant ⁻¹	-0.33	-0.28	0.79**										
Panicle length (cm)	-0.10	-0.14	0.72**	0.68**									
1000 grain weight (g)	-0.45*	-0.47*	-0.10	-0.25	-0.42*								
Germination percent	-0.49*	-0.59**	0.20	0.12	0.07	0.38*							
Seed vigor index	-0.43*	-0.56**	0.22	0.07	0.07	0.37	0.94**						
Plant stand (1-5)	-0.54**	-0.37	0.50**	0.66**	0.51**	-0.18	0.26	0.14					
DM yield (g plant ⁻¹)	-0.42*	-0.30	0.82**	0.78**	0.74**	-0.02	0.08	0.09	0.60**				
Seed No. panicle ⁻¹	0.08	0.05	-0.01	0.11	0.26	-0.42*	-0.10	-0.17	0.55**	0.01			
Speed of germination	-0.35	-0.52**	0.29	0.33	0.24	0.20	0.85**	0.88**	0.22	0.15	-0.06		
Seed weig. panicle ⁻¹ (mg)	-0.04	-0.07	-0.08	-0.03	0.12	-0.09	-0.01	-0.08	0.49*	-0.02	0.93**	-0.04	
Seed yield (g plant ⁻¹)	-0.39*	-0.39*	0.73**	0.87**	0.69**	-0.15	0.13	0.09	0.75**	0.76**	0.45*	0.29	0.40*

*, ** = Significant at 5%, 1%, respectively.

These results were in agreement with previously published reports on tall fescue (Nguyen & Sleper 1983, Veronesi & Falcinelli 1988, Jafari *et al.* 2006). The correlations between panicles emergence date and both DM yield and seed yield were negative ($P \leq 0.05$) (Table 1). This is similar to the finding of Martiniello (1998) who suggested that selection for early flowering accessions would increase both DM and seed yield in tall fescue. Correlation between DM yield and seed yield were strongly positive, suggesting that selection for one would improve the other. Similar results were reported by Veronesi & Falcinelli (1988) and Jafari *et al.* (2006). DM yield had positive and significant relationships with panicle number, panicle length and plant height. This result was expected because these traits are yield components and they are included in aerial biomass. Using stepwise regression analysis for seed yield as dependant variable, the results showed a significant effects of panicle number, seed weight per panicle, DM yield, anthesis date and plant stand on seed yield production ($R^2=0.97$)

(Table 2). Therefore, it was suggested that selection for those characters would lead to increasing tall fescue seed yield. The results derived from path analysis indicated that panicle number and seed weight per panicles had directly significant effects on seed production. DM yield had low direct effects on seed production, but it indirectly increased seed yield by increasing panicle number (Table 3). For DM yield as dependent variable, seed yield, panicle length and plant stand were positively and seed number per panicle and panicle number negatively entered into the regression equation as the most important traits (Table 4). Among them seed yield, panicle length and plant stand had positively direct and indirect effects on DM yield production (Table 5) suggesting that selection for those traits will increase tall fescue DM yield. It was concluded that there was significant variation in the tall fescue populations evaluated to improve seed and herbage yield. Selection should focus on increased DM yield, panicle weight and panicle density coupled with early flowering.

Table 2. Results of stepwise regression analysis with seed yield as dependant variable and other traits as independent variables

Traits	Steps 1	Steps 2	Steps 3	Steps 4	Steps 5
Constant	-0.05	-1.80	-2.55	-1.29	-1.32
Panicle No. per plant	0.84**	0.85**	0.71**	0.69**	0.81**
Seed weight per panicle (mg)		3.41**	3.41**	3.34**	4.33**
DM yield (g plant ⁻¹)			0.21*	0.18*	0.23**
Anthesis date (days)				-0.02*	-0.02**
Plant stand (1-5)					-0.29**
R ² (%)	75	93	94	95	97

*, ** = Regression coefficients are significant at 5%, 1%, respectively.

Table 3. Results of path analysis for partitioning of total correlation between seed yield and other important traits into direct and indirect effects

Traits	Direct	Indirect					total Correlation
		Panicle no plant ⁻¹	Seed weight panicle ⁻¹ (mg)	DM yield (g plant ⁻¹)	Anthesis date (days)	Plant stand (1-5)	
Panicle No./plant	0.84		-0.02	0.18	0.04	-0.18	0.87
Seed weight panicle ⁻¹ (mg)	0.55	-0.03		-0.01	0.01	-0.13	0.40
DM yield (g plant ⁻¹)	0.23	0.66	-0.01		0.04	-0.16	0.76
Anthesis date (days)	-0.15	-0.24	-0.04	-0.07		0.10	-0.39
Plant stand (1-5)	-0.27	0.56	0.27	0.14	0.05		0.75

Residual error =0.123

Table 4. Results of stepwise regression with DM yield as dependant variable and other traits as independent variables.

Traits	Steps 1	Steps 2	Steps 3	Steps 4	Steps 5	Steps 6
Constant	3.63	5.03	3.92	4.19	4.84	5.93
Seed yield	0.32*	0.55*	0.50*	0.45**	0.51**	0.86**
Seed weight/panic (mg)		-0.24	-0.30*	-0.47**	-0.52**	-0.70**
Panicle length (cm)			0.13*	0.16**	0.16**	0.19**
Plant stand (1-5)				0.35*	0.38*	0.49**
Speed of germination					-0.64*	-0.66*
Panicle No./plant						-0.42*
R ² (%)	72	75	79	82	85	88

*, ** = Regression coefficients are significant at 5%, 1%, respectively.

Table 5. Results of path analysis for partitioning of total correlation effects between DM yield and other important traits into direct and indirect effects.

	Direct		Indirect				total Correlation
	Seed yield	Seed No. /panicle	Panicle length (cm)	Plant stand (1-5)	Speed of germination	Panicle No./plant	
Seed yield	0.86	-0.31	0.30	0.32	-0.06	-0.35	0.76
Seed No./panicle	-0.69	0.39	0.11	0.23	0.01	-0.04	0.01
Panicle length (cm)	0.43	0.60	-0.18	0.21	-0.05	-0.27	0.74
Plant stand (1-5)	0.42	0.65	-0.38	0.22	-0.05	-0.26	0.60
Speed of germination	-0.21	0.25	0.04	0.10	0.09	-0.13	0.15
Panicle No./plant	-0.40	0.76	-0.08	0.29	0.28	-0.07	0.78

Residual Errors =0.347

LITERATURE

- Falconer, D.S. & Mackay, T.F.C. 1996. Introduction to quantitative genetics, Fourth edition. Longman Group Ltd. London. 464 pp.
- Jafari, A.A., Setavarz, H. & Alizadeh, M.A. 2006. Genetic variation for and correlations among seed yield and seed components in tall fescue, Journal of New Seeds 8: in press
- Maguire, J.D. 1962. Speed of germination in selection and evaluation for seedling vigor. Crop Science 2: 176-177.
- Martiniello, P. 1998. Influence of agronomic factors on the relationship between forage production and seed yield in perennial forage grasses and legumes in a Mediterranean environment. Agronomie 18: 591-601.
- Nguyen, H.T. & Sleper, D.A. 1983. Genetic variability of seed yield and reproductive characters in tall fescue. Crop Science 23: 621-626.
- Veronesi, F. & Falcinelli, M. 1988. Evaluation of an Italian gemplasm collection of *Festuca arundinacea* Schreb through a multivariate analysis. Euphytica 38: 211-220.

Evaluation for seed yield and seed components among Iranian accessions of *Bromus persicus* Boiss. ex Steud.

A. Jafari¹⁾, A. Bafandeh²⁾, E. Rahmani³⁾ & H. Panahpour¹⁾

¹⁾Research Institute of Forests and Rangelands, Tehran, Iran.

²⁾Former postgraduate student of Islamic Azad University, Brojed branch, Iran

³⁾Agricultural and natural resource research centre of Lorestan province, Iran

aajafari@rifr-ac.ir

ABSTRACT

In order to study variation and correlation of seed yield and seed components, 12 genotypes of *Bromus persicus* were sown under irrigated condition using a randomized complete block designs with three replications in Brojerd, Iran during 2004-2006. The data were collected and analysed for panicle emergence date, anthesis date, seed milky, date maturity date panicle number, plant height, panicle length, peduncle length, spikelet per spike, thousand grain weight, spike length, seeds per panicle, seed weight per panicle, DM yield, harvest index and seed yield. There were significant ($P \leq 0.05$) and no significant difference among genotypes for DM yield and seed yield, respectively. Genotypes 68P11 and 119M with average values 950 kg/h had higher seed production. For DM yield 172M, 116M and 617M with average values of 2.7 to 2.8 Ton/h produced higher total annual DM yield. The results of phenotypic correlation analysis showed that seed yield had positive and significant correlation with spikelet per spike, thousand grain weight, spike length, harvest index, seeds per panicle and seed weight per panicle. DM yield was positively correlated with both panicle number and plant height and negatively with maturity date, seeds per panicle and harvest index. The results of stepwise regression analysis for seed as dependant and other traits as independent variables showed significant effects of spikelet per spike, panicle number, seed weight per panicle and thousand grain weight on seed yield. Among them, spikelet per spike, panicle number and thousand grain weight had positive and direct effect on seed yield. The results of PCA analysis showed that the first three components determined 79 % of the total variation. Seed yield and seed components in the first and phenological traits in the second components were identified as important traits for cluster grouping. Using Ward cluster method the genotypes were grouped into 3 clusters. The genotypes in cluster 1 and 3 had higher average values for herbage and seed yield, respectively. We concluded that improvement of *Bromus persicus* for seed yield in irrigated condition should possible by selecting many spikelets per spike, dens panicle and higher thousand grain weight.

Key words: *Bromus persicus*, cluster analysis, DM yield, path analysis, PCA, regression, seed

INTRODUCTION

The botanical composition of rangelands is variable in Iran. *Bromus persicus* Boiss. ex Steud, synonym of *Bromus tomentosus* Trin is belonging to the genus of *Bromus*, subgenus of *Festucoides* and family of *Poaceae*. It is native in Iran, Afghanistan, Iraq, Turkey and Caucasus (Nasir & Ali 1970). *Bromus tomentosus* naturally grows in temperate pasture and rangelands in northern and western Iran in areas with 1700 m to 3000 m having more than 300 mm annual participation (Rechinger 1970). It is being used for sheep grazing and hay production.

In grass breeding programs for improved DM yield, it is very important the knowledge of new varieties seed production and its relationship with forage yield. Knowledge of correlations between traits of interest is useful in designing an effective breeding program for a crop. Complex plant characters, such as yield, are quantitatively inherited and influenced by genetic as well as genotype by environment interaction effects. Therefore, selection for seed or DM yield alone may not be the best criterion to improve seed or/and DM production. Hence, it is also important to study correlations between agronomic traits, which may have high heritability, and yield, which has low heritability (Falconer & Mackay

1996). In addition, assessment of divergence genetic materials is also vital to success of designed to exploit gene recombination. Strong positive relationships have been found between genetic distance and heterosis in broad range of crop species (Humphreys 1991). Measure of genetic distance should have more value to breeding when based on a broad range of traits relevant to breeding objectives.

Bromus persicus has an important role in grassland productivity in Iran. Little breeding work has been done on this species. Therefore, this research project was conducted to 1) determine the phenotypic correlations between characters and the contribution of each component for seed yield and 2) determine pattern of variation for seed yield and seed components to identify groups of accessions through a multivariate approach.

MATERIALS AND METHODS

The germplasm utilized in this study were 12 populations of *Bromus persicus* Boiss which were collected from Alborz mountain rangelands in northern Iran. Accessions were sown in irrigated condition using randomized complete block designs with three replications in Brojerd, Iran in 2004. In the first harvest of 2005 and 2006, data were assessed for seed yield and seed components. The following traits were measured in each plot.

1. Phenological stages (panicle emergence, anthesis date, seed milky date and maturity date) were measured as the number of days from 21 March to 10 percent visible of each phenological stage.
2. Panicle number: was recorded as the number of fertile panicles per square meter.
3. Plant height (cm) was recorded from the soil surface to the tip of tallest panicle.
4. Peduncle length (cm) was measured from the base of panicles to flag leaf.
5. Spike length (cm): averaged length of developed spike in 10 plants was averaged at anthesis date
6. Spikelet per spike were counted as mean of spikelets in 10 fertile spikes
7. Panicle length (cm): the length of five developed panicles was averaged from peduncle to tip of panicles at anthesis date.

8. Thousand-grain weight (TGW): a sample of 250 clean seeds was weighed and multiplied by 4.
9. Seed weight per panicle was estimated as the ratio of individual plant seed yield to number of panicles.
10. Seeds per panicle were calculated as the ratio of seed weight per panicle to average weight of one seed.
11. DM yield: all of plants in plots were harvested and dry weight was expressed as kg ha^{-1} .
12. Harvest index was calculated as the ratio of seed yield to total DM yield
13. Seed yield: harvested plants were dried, threshed, cleaned and seed yield was expressed as kg ha^{-1} .

Collected data were analyzed for each year and combined across years using a split plot in time with years as sub plots. Phenotypic correlations among characteristics were determined for all pair-wise combinations over two years. Stepwise regression analysis was used for seed yield as dependant and other traits as independent variables. Using path analysis, correlation coefficients of those traits that were included in regression equation were partitioned into direct and indirect effects. Finally, all variables were used in principal components and cluster analysis. All statistical analyses were conducted by MINITAB 14.1 (2003). A distance coefficient of 6.0 was arbitrarily chosen to separate the accessions into three cluster groups in a dendrogram.

RESULTS AND DISCUSSION

The results showed significant differences among genotypes for panicle number, seeds per panicle, seeds weight per panicle, maturity date, spikelet per spike seed yield and harvest index (Table 1). Genotypes 68P11 and 119M with average values of 950 kg ha^{-2} had higher seed production. For DM yield 172M, 116M and 617M with average values of 2.7 to 2.8 Ton ha^{-1} produced higher total annual DM yield.

The estimates of phenotypic correlations are summarized in Tables 2. The results showed that seed yield had positive and significant correlation with spikelet per spike, thousand grain weight, spike length, harvest index, seeds per panicle and seed weight per panicle.

Table 1. Mean of seed yield and morphological traits in 12 accessions of *Bromus persicus* grown as sward under irrigation condition

Name	Panicle emergence	Anthesis Date	Milky Date	Maturity Date	Plant Height (cm)	Peduncle Length (cm)	Spikelet no./ Spike	TGW (g)	Spike Length (cm)	Seed Weight/ Panicle (g)	Panicle no./ M ²	Seeds Per Panicle	Dm Yield (kg ha ⁻¹)	Harvest Index	Seed Yield (kg ha ⁻¹)
116M	45.7	57.0	73.7	90.0	68.1	18.4	8.0	3.4	12.7	0.10	1225	29.7	2712	0.23	621
119M	45.7	57.0	73.7	96.7	72.1	21.5	11.7	3.8	15.0	0.19	773	51.0	2330	0.43	949
138M	46.3	57.0	73.7	98.3	75.0	21.3	9.0	3.7	14.9	0.14	820	36.3	2678	0.28	661
172M	45.7	57.0	73.7	90.0	69.1	22.9	7.0	3.2	13.0	0.09	1237	29.3	2847	0.22	588
357M	45.7	56.0	73.0	96.7	72.4	21.3	11.0	3.4	13.0	0.15	1178	43.0	2399	0.41	913
617M	43.3	56.0	73.0	91.7	72.9	20.9	7.0	3.4	12.5	0.10	1260	28.3	2716	0.23	624
68M	46.3	56.0	73.0	96.7	67.7	23.5	9.7	3.5	14.1	0.15	945	41.3	2353	0.37	791
68P1	45.7	56.0	73.0	96.7	67.4	18.9	10.7	3.6	14.5	0.18	602	51.0	2504	0.35	777
68P10	46.3	56.0	73.0	96.7	70.9	23.9	11.0	3.8	14.5	0.15	1108	40.0	2480	0.39	895
68P11	44.0	55.0	72.3	93.3	72.7	23.7	12.7	3.7	15.0	0.22	682	59.7	2117	0.53	953
68P14	45.7	55.0	72.3	95.0	72.1	24.5	10.3	3.6	14.3	0.17	756	47.3	1458	0.55	798
68P15	45.7	57.0	73.0	98.3	72.9	22.5	10.7	3.5	15.5	0.18	672	50.3	1781	0.45	797
Mean	45.5	56.3	73.1	95.0	71.1	22.0	9.9	3.5	14.1	0.15	938	42.3	2365	0.37	781
SE	0.96	0.87	0.61	2.07	3.48	1.69	1.38	0.17	1.01	0.03	175.6	6.95	481.2	0.09	120.9

Table 2. Phenotypic correlations among seed yield and morphological traits from analysis of 12 accessions of *Bromus persicus* grown as sward.

	Panicle emergence	Anthesis date	Milky date	Maturity date	Panicle no. m ⁻²	Plant height (cm)	Pedunc. length (cm)	Spikelet no./ spike	TGW (g)	Spike length (cm)	Seed weight/ panicle (g)	Seeds Per Panicle	DM Yield (kg ha ⁻¹)	Harvest Index
Anthesis date	0.26													
Milky date	0.26	0.95**												
Maturity date	0.48**	0.16	0.15											
Panicle no. m ⁻²	-0.19	0.02	0.05	-0.61**										
Plant height (cm)	0.10	-0.15	-0.22	-0.08	0.18									
Peduncle length (cm)	0.11	-0.19	-0.19	0.12	0.09	0.39*								
Spikelet no./spike	-0.06	-0.11	-0.20	0.17	-0.32*	0.15	0.07							
1000 grain weight (g)	-0.05	-0.14	-0.11	0.32*	-0.23	0.17	0.12	0.25						
Spike length (cm)	0.02	-0.11	-0.22	0.11	-0.11	0.45**	0.33*	0.69**	0.24					
Seed weight/panicle (g)	-0.02	-0.21	-0.25	0.33*	-0.60**	0.01	-0.02	0.85**	0.42**	0.55**				
Seeds per panicle	-0.04	-0.18	-0.24	0.27	-0.60**	-0.03	-0.06	0.86**	0.18	0.53**	0.97**			
DM yield (kg ha ⁻¹)	-0.20	-0.21	-0.16	-0.46**	0.64**	0.33*	0.06	-0.10	-0.01	0.23	-0.28	-0.32*		
Harvest index	0.08	0.00	-0.05	0.39*	-0.60**	-0.17	-0.04	0.64**	0.22	0.17	0.76**	0.77**	-0.75**	
Seed yield (kg ha ⁻¹)	-0.23	-0.26	-0.32*	-0.03	0.04	0.17	0.09	0.84**	0.38*	0.63**	0.75**	0.71**	0.19	0.44**

*, ** = Coefficients of correlation are significant at 5 %, 1 %, respectively.

Table 3. Results of stepwise regression analysis for seed yield as dependant variables and other traits as independent variables extracted from original and standardized data.

Equation components	Un-standardized			Standardized		
	Coefficients	T-values	P-values	Coefficients	T-values	P-values
Constant	-725			0.00		
Spikelet no spike ⁻¹	18.1	2.71	0.011	0.21	2.71	0.01
Panicle no m ⁻²	0.24	14.17	0.000	0.70	14.17	0.00
Seed weight/Panicle (g)	4338	10.11	0.000	0.97	10.11	0.00
1000 grain weight (g)	61.0	2.31	0.028	0.09	2.31	0.03
R square	96			96		

Table 4. Results of path analysis for partitioning of total correlation between seed yield and other important traits into direct and indirect effects.

Traits	Direct Effect	Indirect effects through				Total Correlation
		Spikelet per spike	Panicle no. per m ²	Seed weight per Panicle (g)	Maturity Date	
Spikelet no spike ⁻¹	0.31		-0.22	0.03	0.72	0.84**
Panicle no m ⁻²	0.67	-0.10		-0.02	-0.51	0.04
Seed Weight/Panicle (g)	0.10	0.08	-0.16		0.36	0.38*
1000 Grain Weight (g)	0.85	0.26	-0.40	0.04		0.75**

Residual error = 0.20

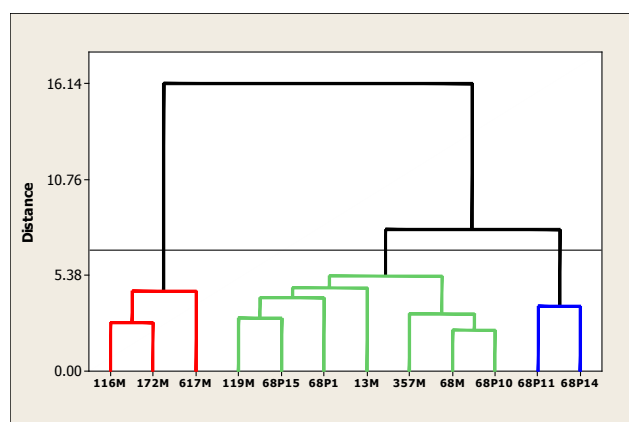


Fig. 1. Dendrogram of 12 accessions *Bromus persicus* using ward cluster analysis method.

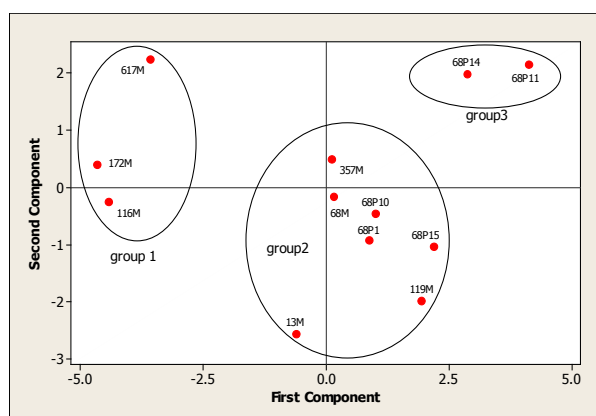


Fig. 2. Scatter plot of 12 accessions *Bromus persicus* for the first two principal components.

DM yield was positively correlated with panicle number and plant height and negatively with maturity date, seeds per panicle and harvest index. The relationship between DM yield and maturity date and between seed yield and milky date were generally negative (Table 2). This result was in agreement with Jafari *et al.* (2006) in tall fescue, indicating that selection of early flowering accessions led to increasing both seed yield and DM yield in *Bromus persicus*.

The results of stepwise regression analysis for seed as dependant and other traits as independent variables showed significant effects of spikelet per spike, panicle number, seed weight per panicle and thousand grain weight on seed yield (Table 3). Among them, spikelet per spike, panicle number and thousand grain weight had direct and positive effect on seed yield (Table 4). We concluded that improvement of seed yield in irrigated

condition should be possible by selecting many spikelets per spike, dens panicle and higher thousand grain weight. Using principal component analysis, the first three components determined 79 % of the total variation (data not shown). The relative magnitude of eigenvectors from the first principal component axis indicates that seed yield and seed components were the most important traits for classifying accessions into clusters. From the second principal component axis, four phenological stages were important. Plant height and peduncle length had high eigenvectors in the third principal component axis. Based on the first two principal component scores genotypes were scattered in Fig. 2. Using ward clustering method the

genotypes were grouped into 3 clusters (Fig. 1). The genotypes in cluster 1 and 3 had higher average values for herbage and seed yield, respectively. Distribution of accessions based on the first two principal component scores was in agreement with cluster analysis (Fig. 2).

ACKNOWLEDGEMENTS

The authors thank Dr M. H. Assareh, Head of Research Institute of Forests and Rangelands that funded this research and Dr H. M. Arefi, Head of Natural Resource Gene Bank that provided seed samples.

LITERATURE

- Falconer, D. S. & Mackay, T. F. C. 1996. Introduction to quantitative genetics (fourth edition), 464 pages. Longman Group Ltd, London, UK.
- Humphreys, M. O. 1991. A genetic approach to the multivariate differentiation of perennial ryegrass (*Lolium perenne* L.) populations. *Heredity* 66: 437-443.
- Jafari, A. A., Setavarz, H. & Alizadeh M. A. 2006. Genetic variation for and correlations among seed yield and seed components in tall fescue, *Journal of New Seeds* 8. In press.
- MINITAB 14.1. 2003. Statistical software. Minitab Inc.
- Nasir, E. & Ali, S. I. (eds.). 1970. Flora of [West] Pakistan. (F Pak) [Lists as B. p. Boiss.].
- Rechinger, K.H. 1970. Flora Iranica. F Iran. No 70. Graz, Austria

Effect of self incompatibility on seed yield in a perennial ryegrass F₂ population

Bruno Studer, Louise Bach Jensen & Thomas Lübberstedt

University of Aarhus, Faculty of Agricultural Sciences,
Research Centre Flakkebjerg, Department of Genetics and Biotechnology,
Forsøgsvej 1, DK-4200 Slagelse

bruno.studer@agrsci.dk

ABSTRACT

Seed yield is of major interest for the key grassland species *Lolium perenne* L. This is particularly true for Denmark, where large amounts of forage seeds are produced. Although seed yield is a complex trait and affected by agricultural practices and environment, several studies revealed reasonable genetic variation for seed yield and seed yield components, which promises good prospects for improvement by selection. In this study, an F₂ mapping population of perennial ryegrass (VrnA) was assessed in the glasshouse for genetic variation of seed yield and its components based on lattice designs with four replications over two years. The traits seed yield per panicle (SYPA) and seed yield per plant (SYP) revealed repeatabilities ranging from 65 and 63 %, respectively. The adjusted entry means of the lattice analysis and a genetic linkage map consisting of 31 SSR, 50 AFLP and 17 CAPS markers were used for QTL analysis. Two QTL on linkage group (LG) 1 and LG 2 each explaining 25 % of the phenotypic variation (Vp) for the trait SYP co-locate with QTL found for SYPA. Here we report on QTL mapping of seed yield in perennial ryegrass and discuss the interference of gametophytic self incompatibility with seed production in the investigated F₂ mapping family.

Key words: *Lolium perenne* L, QTL analysis, seed yield, seed yield components, self incompatibility

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grass species of temperate grassland. For seed production, the ability to achieve a reasonable seed yield is of outmost importance for both forage and turf varieties. Although being affected by agricultural practices and environment, several studies in forage grasses revealed reasonable genetic variation for seed yield (Bugge 1987, Elgersma 1990, Elgersma *et al.* 1994). However, breeding progress for seed yield may be a trade-off to forage production and thus requires detailed characterisation of its underlying genetic components. Genetic linkage mapping and QTL analysis have the potential to unravel such complex traits, but require characteristic population designs based on biparental crosses. Therefore, this

study aimed (1) to identify the genetic variability for seed yield within a F₂ perennial ryegrass mapping population, (2) to identify QTL affecting seed yield and (3) to discuss the interference of gametophytic self incompatibility with seed production in the investigated F₂ mapping family.

MATERIALS AND METHODS

An F₂ mapping population of perennial ryegrass recently characterised for vernalisation response (VrnA; Jensen *et al.* 2005) was assessed in the glasshouse for genetic variation of seed yield. The 184 F₂ individuals were supplemented with the mapping parents and grandparents, which were used to represent seed yield capacity of parental genotypes.

The traits SYPA (Seed Yield per PANicle) and SYP (Seed Yield per Plant) were assessed in the glasshouse on single spaced plants based on a 12 x 8 lattice design with four clonal replications. For SYPA, three selected panicles representative for the respective genotype were separately harvested and threshed. Their seeds were separated and cleaned from glumes and weighed (g/panicle). The same cleaning procedure was applied to assess the trait SYP (g/plant). Lattice analysis using the PLABSTAT software, version 2 P (Utz 2000) was performed in order to estimate quantitative genetic parameters. Repeatability was calculated by dividing the genotypic variance component σ_g^2 with the sum of σ_g^2 and the effective mean square of the error.

For QTL analysis, the linkage map consisting of 31 SSR, 50 AFLP and 17 CAPS markers (Jensen *et al.* 2005) and the MapQTL software (van Ooijen and Maliapaard 1996) were used. QTL analysis was based on a multiple QTL model (MQM) and automatic cofactor

selection (backward elimination, $P < 0.02$) was used for the detection of significantly associated markers as cofactors. LOD significance threshold levels were determined using 200 permutations.

RESULTS

The assessed traits SYPA and SYP showed considerable variation within the *VrnA* mapping population. Lattice analysis revealed highly significant ($P < 0.01$) genotypic variance components for both traits. Repeatabilities of 0.65 and 0.63 supported that the observed phenotypic variation (V_p) in this population was caused by genetic effects (Table 1). The mean value for SYPA was 0.052 g per panicle with a maximum of 0.196, whereas total seed yield per plant revealed an average of 1.68 g per plant with the highest value of 6.54 g. Both traits were highly correlated (0.73, $P < 0.01$).

Table 1. Key values of the phenotypic characterisation of seed yield per panicle (SYPA) and total seed yield per plant (SYP) of 184 F_2 genotypes from the *VrnA* mapping population. Traits were assessed in a glasshouse experiment based on a lattice design with four replicates per genotype.

Trait	SYPA (g/panicle)	SYP (g/plant)
Genotypic variance ^a	1.56 ^{**} (189)	1.54 ^{**} (190)
Repeatability	0.65	0.63
Average yield	0.052	1.68
Maximum yield	0.196	6.54
LSD ^b	0.041	1.87

^{**} $P < 0.01$

^aVariance components, degree of freedom is given in parenthesis

^bLeast significant difference at $P < 0.05$

QTL for SYPA and SYP exceeding the genome wide significance LOD threshold of 3.9 were observed on LG 1 and LG 2. QTL for both traits co-located at position 20 centiMorgan (cM) on LG 1 and 28 cM on LG 2, respectively. For SYPA, a high proportion (29.1 %) of observed V_p was explained by the QTL on LG

1, revealing the highest LOD value at the map position of the SSR marker PR37. The QTL on LG 2 explained 13.7 % of V_p and was closely linked to the CAPS marker LpRGA7 with a distance of 2.8 cM. Similar results were found for SYP QTL with 24.5 % explained V_p on both LGs.

Table 2. Detailed description of QTL for seed yield per panicle (SYPA) and total seed yield per plant (SYP) observed in the F₂ mapping population Vr_nA. Results are based on multiple QTL model (MQM) mapping using MapQTL and a genetic linkage map based on 31 SSR, 50 AFLP and 17 CAPS markers.

Trait	LG	Cofactor	Position	Lod	% expl Vp	Closest marker
SYPA	1	PR37 (20.1)	20.1	21.02	29.1	PR37!
	2	LpRGA7 (31.0)	28.2	3.46	13.7	LpRGA7 (2.8)
SYP	1	PR37 (20.1)	20.1	11.8	24.5	PR37!
	2	LpRGA7 (31.0)	28.2	5.17	24.5	LpRGA7 (2.8)

DISCUSSION

Phenotypic variation of SYPA and SYP within the F₂ mapping population Vr_nA was largely explained by two QTL on LG 1 and LG 2. Their co-location for both traits as well as highly correlated phenotypic data indicated, that SYPA, i.e. seed set, is an important component affecting SYP. Similar results were found in the closely related grass species *Festuca pratensis* Huds, where path coefficient analysis identified panicle fertility to be a major trait contributing to SYP (Fang *et al.* 2004). However, the magnitude of the explained Vp for both QTL was surprisingly high, especially since SYP is known to be a complex trait influenced by a number of other traits, e.g. flowering time, plant height, 1000-seed weight, and number of florets per panicle. Therefore, a large number of QTL with small effects was expected instead of only two regions on LG 1 and LG 2, where large regions of marker segregation distortion were observed. The markers most closely linked to the respective QTL were highly distorted ($P < 0.0001$), some allele combinations under- (or even not) represented in the investigated F₂ population. Such significant segregation distortion ratios are indicative for gametophytic incompatibility (SI) mediated by two loci in

ryegrass (Cornish *et al.* 1979, Fearon *et al.* 1983) and have been used to map alleles involved in SI of rye (Fuong *et al.* 1993). The map positions of the S and Z loci in ryegrass have been reported to map on similar regions on LG 1 and LG 2 (Thorogood *et al.* 2002). Furthermore, the large proportions of explained Vp of the described QTL for SYPA and SYP are caused by negative effects of F₂ individuals heterozygous for their most closely linked markers. Such individuals may share the maximal number of different SI alleles and, therefore, showed a significantly ($P < 0.01$) reduced seed set, even if the whole population was used as pollinator. This study shows the effect of SI on SYPA and SYP in an F₂ population and the necessity of the selection of favourable combinations of incompatibility alleles when designing populations. Even if synthetics are used for the production of commercial forage grass cultivars, selection for traits that are linked to S and Z may seriously affect the ability to produce a reasonable seed set.

ACKNOWLEDGEMENTS

This project was kindly supported through the Directorate for Food, Fisheries and Agri Business (DFFE), Denmark.

LITERATURE

- Bugge, G. 1987. Selection for seed yield in *Lolium perenne* L. Plant Breeding 98: 149-155.
- Cornish, M.A., Hayward, M.D. & Lawrence, M.J. 1979. Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. Heredity 43: 95-106.
- Elgersma, A. 1990. Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). Plant Breeding 105: 117-125.

- Elgersma, A., Winkelhorst, G.D. & Nijs, A.P.M. 1994. The relationship between progeny seed yield in drilled plots and maternal spaced-plant traits in perennial ryegrass (*Lolium perenne* L.). *Plant Breeding* 112: 209-214
- Fang, C., Aamlid, T.S., Jørgensen Ø. & Rognli, O.A. 2004. Phenotypic and genotypic variation in seed production traits within a full-sib family of meadow fescue. *Plant Breeding* 123: 241-246.
- Fearon, C.H., Hayward, M.D. & Lawrence, M.J. 1983. Self-incompatibility in ryegrass. 5. Genetic control, linkage and seed set in diploid *Lolium multiflorum* Lam. *Heredity* 50: 35-45.
- Fuong, F.T., Voylokov, A.V. & Smirnov, V.G. 1993. Genetic studies of self-fertility in rye (*Secale cereale* L.). 2. The search for molecular marker genes linked to self-incompatibility loci. *Theoretical and Applied Genetics* 87: 619-623.
- Jensen, L.B., Andersen, J.R., Frei, U., Xing, Y., Taylor, C., Holm, P.B. & Lübberstedt, T. 2005. QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat *VRN1*. *Theoretical and Applied Genetics* 110: 527-536.
- van Ooijen J.W. & Maliepaard, C. 1996. MapQTL™ version 3.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen
- Thorogood, D., Kaiser, W.J., Jones, J.G. & Armstead, I. 2002. Self-incompatibility in ryegrass. 12. Genotyping and mapping the S and Z loci of *Lolium perenne* L. *Heredity* 88: 385-390
- Utz, H.F. 2000. PLABSTAT: a computer program for statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Seed yield and seed yield components in winter cultivars of common vetch (*Vicia sativa* L.)

Vojislav Mihailović, Đura Karagić, Aleksandar Mikić, Slobodan Katić, Dragan Milić & Vukašin Draganović

Institute of Field and Vegetable Crops, Forage Crops Department, Maksima Gorkog 30, 21000 Novi Sad, Serbia

vojamih@ifvcns.ns.ac.yu

ABSTRACT

Common vetch (*Vicia sativa* L.) is a traditional crop and the most widely distributed of the vetch species in Serbia. The seed production of winter cultivars of common vetch is often faced with many difficulties, such as lodging, large portion of empty pods, and heavy harvest losses, especially in the years with excessive rainfall, resulting in both high costs and insufficient production for the market. The breeding programme on winter cultivars of common vetch in the Institute of Field and Vegetable Crops in Novi Sad and the development of new cultivars are aimed at high, quality and stable forage yields and reliable seed yields. A small-plot trial was carried out at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops from the autumn 2004 to the summer 2006. It included seven Novi Sad winter cultivars of common vetch, namely NS Sirmium, Novosadska Domaća, Novosadska 624, Neoplanta, Tara, Morava and Kadmos NS. All seven cultivars were sown at a crop density of 120 plants m⁻² and were harvested in the stage of full maturity of the first pods. The greatest number of plants before the harvest was in Novosadska Domaća (96 plants m⁻²). The cultivar Novosadska 624 had the greatest plant height (133 cm). The greatest numbers of stems, fertile nodes, pods and seeds were in Neoplanta (6.0 stems plant⁻¹), Kadmos NS (24.0 fertile nodes plant⁻¹), Kadmos NS (39.7 pods plant⁻¹) and Tara (84.3 seeds plant⁻¹). The largest thousand seed mass was in Novosadska 624 (67 g). The largest plant mass was in NS Sirmium (17.41 g) and the highest harvest index was in Novosadska 624 (0.40). The highest seed yields were in Tara and Kadmos NS (4.19 g plant⁻¹ and 1717 kg ha⁻¹).

Key words: common vetch, low temperature tolerance, seed yield, seed yield components, winter cultivars

INTRODUCTION

As important components in the improvement of natural grasslands and valuable forage field crops, vetches (*Vicia* spp.) represent one of the most quality solutions for a constant demand for plant protein in animal husbandry (Mišković 1986). They are utilised in the form of green forage, hay, forage meal, silage and haylage, either alone or in mixtures with small grains (Mihailović *et al.* 2004). Vetches are also traditional forage crops in Serbia and are regarded as having been cultivated longer than other annual legumes such as forage pea (Đorđević 1942). The most widely distributed vetch species in Serbia is common vetch (*V. sativa* L.), while hairy vetch (*V. villosa* Roth)

and Hungarian vetch (*V. pannonica* Crantz) are cultivated sporadically (Vučković 1999). It is estimated that vetches in Serbia are grown on between 3,000 and 7,000 ha without significant changes in the area throughout the decades (Mikić *et al.* 2006).

According to a rather widespread opinion, the development of winter cultivars of diverse annual legumes could significantly increase the cultivation area of these crops, especially in the temperate regions (Duc 1997). It is often determined that winter cultivars of common vetch often have higher yields than spring cultivars (Mihailović *et al.* 2006b). Moreover, winter cultivars of common and other vetches represent one of the most

appreciated cover crops in contemporary systems such as organic farming and sustainable agriculture (Ćupina *et al.* 2004). On the other hand, the seed production of winter cultivars of common vetch is faced with many difficulties, such as lodging, large portion of empty pods and heavy harvest losses, especially in the years with excessive precipitations, resulting in both high costs and insufficient production for the market. Together with high, quality and stable yields of green forage and hay and an improved tolerance to low temperatures, one of the major goals of breeding winter cultivars of common and other vetches has always been an increased seed yield (Mišić 1966), in order to provide a reliable seed production for the market.

The study was aimed at assessing the potential of winter cultivars of common vetch for seed yield and seed yield components.

MATERIALS AND METHODS

A small-plot trial was carried out at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops from the autumn 2004 to the summer 2006. It included seven winter cultivars of common vetch, all of which were developed in the

Institute of Field and Vegetable Crops, namely NS Sirmium, Novosadska Domaća, Novosadska 624, Neoplanta, Tara, Morava and Kadmos NS. All cultivars were sown at a crop density of 120 plants m⁻² and were harvested in the stage of full maturity of the first pods (Mihailović *et al.* 2005a).

There were monitored main seed yield components, such as number of plants m⁻² before the harvest, plant height, number of stems plant⁻¹, number of fertile nodes plant⁻¹, number of pods plant⁻¹, number of seeds plant⁻¹ and thousand seeds mass (g), as well as plant mass (g), seed yield (g plant⁻¹ and kg ha⁻¹) and harvest index.

The results were processed by analysis of variance (ANOVA) with the Least Significant Difference (LSD) test applied, using the computer software MSTAT-C.

RESULTS

With significant differences between some of the nine examined cultivars at both levels of 0.05 and 0.01, the average number of plants before harvest varied from 59 plants m⁻² in the cultivar Novosadska 624 to 96 plants m⁻² in the cultivar Novosadska Domaća (Table 1).

Table 1. Average values of seed yield components in winter cultivars of common vetch at Rimski Šančevi for the period years of 2004/05 and 2005/06

Cultivar	Number of plants before harvest (m ⁻²)	Plant height (cm)	Number of stems (plant ⁻¹)	Number of fertile nodes (plant ⁻¹)	Number of pods (plant ⁻¹)	Number of seeds (plant ⁻¹)	Thousand seed mass (g)
NS Sirmium	87	86	6.0	21.0	22.3	56.0	50
Novosadska Domaća	96	92	3.7	15.7	18.7	53.7	57
Novosadska 624	59	133	1.7	8.3	10.0	46.3	67
Neoplanta	78	108	6.0	10.0	19.3	48.3	51
Tara	62	105	4.7	14.0	18.3	84.3	48
Morava	84	73	4.7	17.0	19.0	48.7	50
Kadmos NS	93	112	5.7	24.0	39.7	78.7	50
<i>LSD</i> _{0.05}	26	14	2.0	4.3	4.0	8.6	9
<i>LSD</i> _{0.01}	34	20	2.4	5.8	5.4	11.5	11

Table 2. Average values of plant mass, seed yield and harvest index in winter cultivars of common vetch at Rimski Šančevi for the period years of 2004/05 and 2005/06

Cultivar	Plant mass (g)	Seed yield (g plant ⁻¹)	Seed yield (kg ha ⁻¹)	Harvest index
NS Sirmium	17.41	2.88	1317	0.14
Novosadska Domaća	11.31	3.40	1575	0.30
Novosadska 624	7.85	3.16	1650	0.40
Neoplanta	13.18	2.49	1527	0.18
Tara	13.36	4.19	1533	0.29
Morava	11.24	2.63	1583	0.23
Kadmos NS	10.75	3.93	1717	0.36
<i>LSD</i> _{0.05}	4.49	1.58	308	0.15
<i>LSD</i> _{0.01}	6.35	2.64	394	0.26

The greatest average plant height was in the cultivar Novosadska 624 (133 cm), while the smallest average plant height was in the cultivar Morava (73 cm). A difference in the average number of stems between the cultivars Novosadska 624 (1.7 plant⁻¹) and NS Sirmium and Neoplanta (both 6.0 plant⁻¹) was significant at both levels of 0.05 and 0.01. The cultivar Kadmos NS had the greatest number of fertile nodes (24.0 plant⁻¹), while the cultivar Novosadska 624 had the smallest number of fertile nodes (8.3 plant⁻¹). With significant differences between some of the nine examined cultivars at both levels of 0.05 and 0.01, the average number of pods ranged between 10.0 plant⁻¹ in the cultivar Novosadska 624 and 39.7 plant⁻¹ in the cultivar Kadmos NS. The greatest average number of seeds was in the cultivar Tara (84.3 plant⁻¹), while the smallest average number of seeds was in the cultivar Novosadska 624 (46.3 plant⁻¹). There were significant differences in the average thousand seeds mass between the cultivar Novosadska 624 (1.7 plant⁻¹) and all other examined cultivars.

There were significant differences between the examined cultivars in the average plant mass, such as between Novosadska 624 (7.85 g) and NS Sirmium (17.41 g). The highest seed yield per plant was in the cultivar Tara (4.19 g plant⁻¹), while the lowest seed yield per plant was in the cultivar Neoplanta (2.49 g plant⁻¹). As a result of all seed yield components, the average seed yield per area unit in the nine examined winter cultivars of common vetch varied between 1317 kg ha⁻¹ in the cultivar NS Sirmium and 1717 kg ha⁻¹ in the cultivar Kadmos NS (Table 2). The cultivar NS Sirmium had the lowest average harvest

index (0.14), while the cultivars Morava and Novosadska 624 had the highest average harvest index (0.40).

DISCUSSION

There is a great variability of all agronomic characteristics related to seed yield in common vetch. Certain cultivars, such as Novosadska Domaća and Kadmos NS are rather tolerant to low temperatures and have a considerably high proportion of the plants surviving winter. Others, such as Novosadska 624 and Tara, have a number of the plants that reach the harvest being nearly one half of the initial stand density, but also show a good ability to neutralise a negative impact of the reduced plant number by optimising the relationship between other yield components (Mihailović *et al.* 2005b). Cultivars such as Kadmos NS have moderate plant height and thousand seed mass and owe much of their potential for seed yield to increased numbers of fertile nodes and pods (Mihailović *et al.* 2006a).

In conclusion, this research confirms that increasing the seed yield in winter cultivars of common vetch is possible by assessing and combining the desirable values for each seed yield component without decreasing its potential for high and stable forage yields. In the light of a raising significance of stand stability and uniformity, advanced winter cultivars of common vetch should have an obvious advantage in comparison to local or traditional landraces, mostly due to non-reduced stands after the winter and homogenous growth and development.

ACKNOWLEDGEMENTS

The research was carried out within the Project 6847 *Breeding, Growing Technology*

and Utilisation of Annual Forage Crops of the Ministry of Science and Environmental Protection of the Republic of Serbia (2005-2007).

LITERATURE

- Ćupina, B., Erić, P., Krstić, Đ. & Vučković, S. 2004. Forage catch crops in sustainable agriculture and organing farming. *Acta Agriculturae Serbica*, IX, 17, 451-459.
- Duc, G. 1997. Faba bean (*Vicia faba* L.). *Field Crops Research* 53: 99-109.
- Đorđević, V. 1942. Gajenje biljaka za stočnu hranu. Srpsko poljoprivredno društvo, 100 pp, Belgrade, Serbia.
- Mihailović, V., Erić, P. & Mikić A. 2004. Growing peas and vetches for forage in Serbia and Montenegro. *Grassland Science in Europe*, 9: 457-459.
- Mihailović, V., Matić, R., Mikić, A. & Ćupina B. 2005a. Possibilities for utilization of spring vetch for grain. *Contemporary Agriculture*, LIV, 3-4: 318-322.
- Mihailović, V., Mikić, A., Ćupina, B., Vasiljević, S. & Milić, D. 2005b. Agronomic characteristics of Novi Sad winter vetch cultivars. Offered Papers of the XX International Grassland Congress Grasslands - a Global Resource, Dublin, Ireland, 26 June - 1 July 2005: 89.
- Mihailović, V., Mikić, A., Karagić, Đ., Katić, S., Pataki, I. & Matić, R. 2006a. Seed yield and seed yield components in winter cultivars of four vetch (*Vicia* L) species. Abstract Book of the XXVI EUCARPIA Fodder Crops and Amenity Grasses Section and XVI *Medicago* spp. Group Joint Meeting Breeding and Seed Production for Conventional and Organic Agriculture, Perugia, Italy, 3-7 September 2006: 2.25.
- Mihailović, V., Mikić, A., Matić, R., Ćupina, B., Katić, S., Karagić, Đ., Erić, P. & Krstić, Đ. 2006b. A comparative study on the forage yield in winter and spring cultivars of pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.). Abstracts of the COST Action 852 Final Meeting Quality Legume-Based Forage Systems for Contrasting Environments, Gumpenstein, Austria, 30 August - 3 September 2006: 51.
- Mikić, A., Ćupina, B., Katić, S. & Karagić, Đ. 2006. Importance of annual forage legumes in supplying plant proteins. *A Periodical of Scientific Research on Field and Vegetable Crops*, Institute of Field and Vegetable Crops, Novi Sad, Serbia, 42, 1: 91-103.
- Mišić, T. 1966. Some results in the breeding of winter vetch (*Vicia sativa* L.) for the north-eastern regions of Yugoslavia. *A Periodical of Scientific Research on Field and Vegetable Crops*, Institute of Field and Vegetable Crops, Novi Sad, Serbia, 4: 111-121.
- Mišković, B. 1986. Krmno bilje. Naučna knjiga, Beograd, Serbia, 508 pp.
- Vučković, S. M. 1999. Krmno bilje. Institut za istraživanja u poljoprivredi Srbija, 553 pp., Beograd, Bonart, Nova Pazova, Serbia.

Development of Kentucky bluegrass for non-burn seed production

William J. Johnston¹⁾, Richard C. Johnson²⁾, Charles T. Golob¹⁾, Mathew C. Nelson³⁾, Karine Paré¹⁾ & Kathleen L. Dodson¹⁾

¹⁾Washington State University, Department of Crop & Soil Sciences, Pullman, WA, USA

²⁾USDA-ARS Western Regional Plant Introduction Station, Pullman, WA, USA

³⁾United States Golf Association Greens Section, Northwest Region, Twin Falls, ID, USA

wjohnston@wsu.edu

ABSTRACT

A ban on burning of post-harvest grass seed residue has been implemented in Washington and Idaho and restrictions are in place in Oregon, USA. Without residue burning, Kentucky bluegrass (*Poa pratensis* L.) seed yield decreases over time. Growers have implemented yearly mechanical residue removal (raking and baling) and shorter rotations to maintain adequate seed yield; however, this practice is economically less viable to growers and is potentially harmful to the environment. The goal of this study is to develop turf-type bluegrasses that will maintain seed yield over several years without post-harvest burning. This long-term study began in 1994 at Pullman, WA with the evaluation of 228 Plant Introduction (PI) accessions from the USDA-ARS Kentucky bluegrass germplasm collection and the development of a core collection based on 17 agronomic characteristics using Ward's cluster analysis. In a 3-year residue management and turfgrass quality study of the core, PI accessions that had both high seed yield without burning and good turf quality were identified. This germplasm underwent further selection for two years in a space-plant nursery to identify within accession variation. Seed was obtained from four plants of each accession with the highest seed yield, seed weight, seed per panicle, and panicles per unit area. Seed from the original population (USDA-ARS germplasm collection) were also included. Seed were grown in flats in a greenhouse and plants were transplanted into a seed increase nursery in September 2004, at Central Ferry, WA. This material (eight accessions and two commercial cultivars x five selection parameters x 100 plants per parameter) was harvested June 2006 and is currently being evaluated in on-farm seed production and turfgrass trials at several locations in eastern Washington. Ultimately, turf-type Kentucky bluegrasses that can be grown for several years without burning will be released.

Key words: open-field burning, *Poa pratensis* L., post-harvest residue removal, turfgrass

INTRODUCTION

A ban on open-field burning of post-harvest residue of grass grown for forage or turfgrass seed has been implemented in Washington State and Idaho and restrictions are in place in Oregon, USA. Previous research has shown that without post-harvest residue burning, Kentucky bluegrass seed yield decreased over time (Johnson *et al.* 2003). This has forced growers to use mechanical post-harvest

residue removal (raking and baling) and shorter rotations to maintain economically viable seed yields. Also, the practice of shorter rotations is environmentally unsound compared to long-term cropping, as it requires increased fertilizer and pesticide inputs and causes soil erosion.

The objectives of this research were to: 1) evaluate the phenotypic diversity in Kentucky bluegrass germplasm with a wide genetic

base, 2) determine seed production capacity of diverse germplasm in burn and alternative residue management systems while evaluating for turfgrass potential, 3) assess the within variation in agronomic attributes of selected PI accessions and then select individual plants of each accession for high seed weight, seed per panicle, panicles per unit area, and overall seed yield, and 4) determine the selection response by testing the resulting selections for seed yield and turfgrass quality (current ongoing objective).

MATERIALS AND METHODS

Diversity evaluation of the USDA-ARS Kentucky bluegrass collection

In 1994, at the Turfgrass Research Area at Pullman, WA, a Kentucky bluegrass nursery was planted using 228 PI accessions from the USDA-ARS Western Regional Plant Introduction Station Kentucky bluegrass collection and 17 commercial cultivar “checks”. Bluegrass was planted in 1-m rows in a randomized complete-block (RCB) experimental design with three replications. The accessions were characterized for 17 agronomic parameters during 1994 and 1995. PI accessions were differentiated using an agronomic core according to Ward’s method as executed by PROC CLUSTER, option Ward’s (cluster analysis using unweighted pair-group method using arithmetic averages) (SAS Institute 1985).

Utilization of the agronomic core in residue management and turf trials

In September 1996, the agronomic core of 20 PI accessions, 16 “free picks” (non-core accessions that had high seed yield and appeared to have turf potential), and nine commercial cultivars were established. The plots were 1.2 m x 6.4 m in a RCB with three replications. Also, in September 1996, separate adjacent turfgrass evaluation plots, 1.2 m x 1.5 m, were established at 25 g plot⁻¹ in a RCB with three replications. Turf plots were managed and evaluated according to criteria developed by the National Turfgrass Evaluation Program (NTEP). In fall 1997, seed production plots were subdivided by residue management treatments of open-field burning, raking and baling, and no residue removal. Residue management treatments were again applied fall 1998 and 1999 and seed were harvested in 1998, 1999, and 2000.

Selection within accessions for diversity in seed yield components.

Based on the above research, core accessions having high seed yield without burning (raking and baling) and good turfgrass quality were selected for further study. A space-plant nursery was planted with a total of 10 entries (eight PI accessions and two commercial cultivars); 28 plants per entry in a RCB with three replications. Individual space plants were harvested in 2002 and 2003. Panicles were counted, threshed, seed was cleaned, counted, and the weight recorded. Based on the data, 100 seed were obtained from four selected plants of each accession. The selection parameters were: 1) plant with highest seed yield, 2) plant with highest seed weight, 3) plant with highest seed per panicle, 4) plant with highest panicles per unit area, and 5) remnant seed from the USDA-ARS Kentucky bluegrass collection.

Seed increase nursery

One hundred seed of each selected plant and 100 seed from the original USDA-ARS Kentucky bluegrass population for each accession were germinated in vermiculite and individual plants of each accession x selection parameter were established in flats in a greenhouse. In October 2004, the plants were established in a seed increase nursery at Central Ferry, WA. The seed increase nursery consisted of 5000 plants (eight accessions and two commercial cultivars x five selection parameters x 100 plants). The 100 plants of each accession or commercial cultivar x selection parameter were planted in two, 15.2-m rows in a non-replicated block design. In June 2006, plots were swathed, material was placed into cloth bags, air dried, threshed, cleaned with a M2-B seed cleaner, seed was debarbed, and weighed.

Seed production and turfgrass trials

To evaluate turfgrass quality, in August 2006, a NTEP-type trial was established with the 50 entries in a RCB with three replications at the Turfgrass and Agronomy Research Area at Pullman, WA. Individual plot size was 1.5 m x 1.5 m seeded at 11 g m⁻². Seed production plots were established in April 2007 at two sites in eastern Washington. Fifty entries were planted in a RCB with three replications. Individual plots consisted of seven, 2-m rows with 35-cm-row spacing. Seeding rate was 7 kg ha⁻¹.

RESULTS

Diversity evaluation of the USDA-ARS Kentucky bluegrass collection

A core collection subset was generated by cluster analysis of the agronomic data from all 245 genotypes (Johnston *et al.* 1997, Nelson 1996). Twenty-two clusters were developed (two were later eliminated due to poor seed yield) and one representative accession from each cluster was chosen at random to constitute the core collection. This core represents seven countries and approximately 10 % of the 228 accessions studied. Core collections of 5 to 10 % of the accessions in a collection have been suggested as sufficient to represent the majority of genetic diversity in the entire collection (Casler 1995, Kouame & Quesenberry 1993). The core collection, nine commercial cultivars checks from diverse morphological grouping (Murphy 1990), and 17 non-core selections based on turfgrass

potential and seed yield were chosen for further study.

Utilization of the agronomic core in residue management and turf trials

Compared with burn treatments, yield was reduced 27 % when residue was mechanically removed from plots and 63 % when residue was retained (Johnson *et al.* 2003). Higher yield was promoted by a long heading-to-anthesis period, a relatively short anthesis-to-harvest period, and an early harvest date (maturity). Although both seed per panicle and panicles m⁻² were positively correlated with yield, lower yield with non-burn residue management was closely associated with panicles m⁻² (Table 1). Turf quality was negatively correlated with yield. However, panicles m⁻² were not significantly correlated with turf quality, so indirect selection for yield in genotypes with high panicles m⁻² should have minimal impact on turf quality.

Table 1. Correlation between turf quality and seed yield factors at Pullman, WA during 1998 and 1999.

	Turf quality	Biomass	Yield	Harvest index	Weight seed ⁻¹	Seed panicle ⁻¹	Panicles m ⁻²
Texture ¹	-0.33*	0.32*	0.37*	-0.12ns	-0.20ns	0.30ns	0.25ns
Color ²	0.67**	-0.56**	-0.40*	0.43**	0.23ns	-0.56**	-0.17ns
Quality ³	-----	-0.53**	-0.48**	0.22ns	0.15ns	-0.55**	-0.26ns
Yield	-0.48**	0.84**	-----	0.12ns	0.05ns	0.76**	0.66**

¹Leaf texture was rated 1 to 9; 9 = fine.

²Genetic color was rated 1 to 9; 9 = dark green.

³Turfgrass quality was rated 1 to 9; 9 = excellent.

Selection within accessions for diversity in seed yield components

To determine the within accession variability, seed yield components and seed yield data were obtained on 840 space plants at Pullman, WA [10 entries (eight PI accessions and two commercial cultivars), 28 plants per entry with three replications] harvested in 2002 and 2003 (Johnston 2004). The data were analyzed for 1000 seed weight, seed per panicle, panicles m², and seed yield (g cm⁻²). There was considerable variation among and within accessions and we were able to identify the highest contributing single plant within each accession or check for each parameter.

2004. In June 2006, plots were evaluated for seed head height, blade texture, color, uniformity of heads, turf potential, and date of harvest (non-replicated data not presented). Seed was harvested in June 2006 and ample clean seed was obtained for field trials for seed production and turfgrass quality.

Seed production and turfgrass trials

A turfgrass evaluation trial was established at Pullman, WA in August 2006. Seed production trials were established April 2007 at two on-farm sites in eastern Washington. Currently, limited data is available.

Seed increase nursery

Seed production at Central Ferry, WA was poor in 2005 due to the late fall planting in

SUMMARY

The goal of this long-term project, begun in 1994, is to develop Kentucky bluegrasses that can produce acceptable seed yield over several harvests without open-field burning of post-harvest residue. To accomplish this goal, the USDA-ARS Kentucky bluegrass collection of 228 PI accessions (along with 17 commercial cultivars) was evaluated for diversity based on 17 agronomic factors and a core collection was developed using Ward's cluster analysis. The core collection, 16 non-core accessions chosen for high seed yield and good turfgrass potential, and nine commercial cultivars were evaluated for several years in residue management and turf trials. Germplasm with high seed yield without burning (residue removed by raking and baling) and good turf quality was identified. This germplasm was evaluated for two years in a space-plant nursery and variation within accessions was identified for seed yield

components (seed weight, seed per panicle, panicles m⁻², and seed yield). So, the potential exists for plant selection and enhancement in Kentucky bluegrass germplasm. Seed increase was completed on the selected germplasm to obtain sufficient seed for on-farm seed production and turfgrass trials, which were established in April 2007 and August 2006, respectfully. This research is expected to lead to Kentucky bluegrasses cultivars that do not require post-harvest open-field burning to maintain productivity.

ACKNOWLEDGEMENTS

The authors wish to thank the USDA-ARS Grass Seed Cropping System for a Sustainable Agriculture, the Washington State Department of Ecology, and grower cooperators for their partial support of this research.

LITERATURE

- Casler, M.D. 1995. Patterns of variation in a collection of perennial ryegrass accessions. *Crop Science* 35: 278-282.
- Kouame, C.N. & Quesenberry, K.H. 1993. Cluster analysis of a world collection of red clover germplasm. *Genetic Resources and Crop Evolution* 40: 39-47.
- Johnson, R.C., Johnston, W.J. & Golob, C.T. 2003. Residue management, seed production, crop development, and turf quality in diverse Kentucky bluegrass germplasm. *Crop Science* 43: 1091-1099.
- Johnston, W.J. 2005. Development of high yielding Kentucky bluegrass for non-thermal seed production. USDA-ARS Grass Seed Cropping Systems for a Sustainable Agriculture. FY2005 Progress Reports: 13-16.
- Johnston, W.J. 2004. Development of high yielding Kentucky bluegrass for non- thermal seed production. USDA-ARS Grass Seed Cropping Systems for a Sustainable Agriculture. FY2005 Progress Reports: 20-23.
- Johnston, W.J. & Johnson, R.C. 2000. Washington State and USDA work to preserve and clarify the rich diversity of Kentucky bluegrass. *Diversity*, 16, number 1&2: 30-32.
- Johnston, W.J., Nelson, M.C., Johnson, R.C., & Golob, C.T. 1997. Phenotypic evaluation of *Poa pratensis* L.: USDA/ARS plant introduction germplasm collection. *International Turfgrass Research Journal* 8: 305-311.
- Murphy, J.A. 1990. Seed standards for sod certification. Rutgers Cooperative Extension Bulletin FS738, Rutgers University, Rutgers, NJ.
- Nelson, M.C. 1996. Development of a Kentucky bluegrass core collection: Phenotypic evaluation of the USDA/ARS plant introduction germplasm collection. M.S. thesis, Washington State University, Pullman, WA.
- SAS Institute, Inc. 1985. SAS user's guide: Statistics 5th edition. N.C. State University Press, Cary, NC.

Improved seed production in a new Italian cultivar of lucerne (*Medicago sativa* L.)

Renzo Torricelli, Nicola Colesanti & Mario Falcinelli

Plant biology, Agro-environmental and Animal Biotechnology Department
University of Perugia, Italy

torricel@unipg.it

ABSTRACT

Increasing forage yield is the most important objective in lucerne breeding programmes. The commercial success of newly selected cultivars depends not only on their forage attributes, but also on their ability to produce seed. In lucerne breeding programmes, genetic variability is exploited to develop cultivars with high quality and quantity forage production, but in general, seed yield aptitude has not been an important criterion. Numerous studies have reported that it should be possible to increase seed yield in lucerne without negatively affecting forage yield. In this study genetic variation in seed yield components was investigated in a new *Medicago sativa* L. cultivar named 'Casalina'. This variety was developed from a Central Italian ecotype. It is characterised by good adaptability, but has an irregular seed yield. The trial was conducted for 3 years (2002, 2003 and 2004). In the third year, seed yield components were analysed on 469 selected spaced plants. There was large variation in seed yield. Seed yield was highly correlated with dry matter yield, number of stems and number of pods per raceme. In September 2004, sixty-five plants, characterised by high seed yield, high forage production and resistance to diseases, were selected. These genotypes will be used to obtain a new adapted synthetic variety which will be more productive in both seed and forage.

Key words: genetic variation, forage production, seed yield components.

INTRODUCTION

Lucerne (*Medicago sativa* L.) is an autotetraploid ($2n=4x=32$), open-pollinated species with polysomic inheritance and characterised by high levels of polymorphism within and among populations. *M. sativa* is one of the most important forage crops in temperate climates, and is grown on more than 32 million ha throughout the world (Bouton 2001). Its importance is due to its great ability to produce both of high quality and quantity forage yields. Furthermore lucerne is even more important for restoring soil fertility and can grow with low inputs of fertiliser, herbicides and pesticides. In the last years there has been particular interest in using lucerne in the expanding sector of organic agriculture. In Italy, the most cultivated areas are located in the Northern (Po valley) and Central regions, where it is cultivated on about 800,000 ha. Recently, the

number of new lucerne varieties has increased in Italy, as well as throughout Europe. Breeding strategies mainly take into consideration: yield stability, persistence, and forage quality; in fact, dry matter yield (DMY) is still the most important breeding target for lucerne and therefore deserves particular attention (Veronesi *et al.* 2006). However the ability to give a high seed yield ensures the success of a newly selected cultivar and determines its effective distribution to farmers at reasonable prices (Lorenzetti 1981). The theoretical seed yield of lucerne, calculated from the number of flowers and ovules, is 12 tons per hectare (Lorenzetti 1993), but the actual seed yield achieved under the most favourable conditions reaches only 4% of the potential yield (Bolaños-Aguillar *et al.* 2000). The causes of the large gap between potential and true seed yields remain unclear, despite the fact that such knowledge is highly desirable as a basis for the development of improved

management practices and the adoption of suitable breeding strategies aimed at increasing seed production (Falcinelli 1999). In lucerne, a significant positive phenotypic correlation between seed and forage yield has been observed (Melton 1969, Boçsa & Buglos 1983, Veronesi & Falcinelli 1987).

The present research was carried out to evaluate the possibility of increasing seed yield in a new cultivar named 'Casalina'. This variety, developed from a Central Italian ecotype, is characterised by good adaptability, but has an irregular seed yield.

MATERIALS AND METHODS

On February 9, 2002, 2000 seeds of cv. 'Casalina' were germinated in Petri dishes placed in a growth chamber at 24 °C. The seedlings were then planted in a greenhouse. After two months in the greenhouse, the plants (988 random plants) were clipped and transplanted (spaced plant) in the experimental field of the University of Perugia (central Italy, 176 m a.s.l., 43° 2'N, 12° 24'E). In the three consecutive years, 2002, 2003 and 2004, the following characteristics were recorded per plant: vegetative regrowth (VR) evaluated on a scale of 1-9 (1=min; 9=max) on October 14, 2002; following winter dormancy (FWD) evaluated on a scale of 1-9 (1=dormant plant; 9=non dormant plant) on December 27, 2002; spring regrowth (SR) evaluated on a scale of 1-9 (1=min; 9=max) on March 18, 2003; plant height (PH, cm) recorded on May 13, 2003; dry matter yield/plant (DMY, g) recorded on May 16, 2003; flowering date (FD) recorded in days after June 1, 2003; stem number (SN) recorded on June 6, 2003; growth habit (GH) evaluated on a scale of 1-4 (1=erect; 4=prostrate) on June 20, 2003; raceme number (RN) recorded on the longest stem in July 2004; legume number per raceme (LNR); legume number per stem (LNS) recorded on the longest stem in July 2004; seed

yield/plant (SYP, g) recorded in July 2004. In September 2004, on the basis of information obtained, sixty-five plants, characterised by high seed yield, high forage production and resistance to diseases, were selected.

Statistical analysis

Data from the 469 plants still alive at the end of second year were analysed. Some plants were eliminated because they flowered too early or too late, others did not conform to the ideotype, and a few plants were diseased. Statistical analyses of data were carried out using procedures from the SAS program (SAS 1990). Correlation coefficients were calculated using CORR procedure on the data of 469 plants. Phenotypic selection was made on 469 plants and UNIVARIATE analysis and selection differentials (S) were carried out. The structure of genetic variation among the 469 plants based on all traits was analysed using Principal Component Analysis (PCA). From the correlation matrix eigenvalue and eigenvector matrices were obtained.

RESULTS

Correlation analysis

Correlation coefficients for all traits evaluated are given in Table 1. Seed yield per plant (SYP) showed a significant and positive correlation with dry matter yield (DMY) ($r=0.40^{**}$), stem number (SN) ($r=0.27^{**}$), spring regrowth (SR) ($r=0.19^{**}$), legume number per raceme (LNR) ($r=0.19^{**}$) and legume number per stem (LNS) ($r=0.15^{**}$), while seed yield per plant showed a negative correlation with flowering date (FD) ($r=-0.20^{**}$). Within seed yield components, legume number per stem (LNS) showed a strong and positive correlation with raceme number recorded on the longest stem (RN) ($r=0.83^{**}$) and with legume number per raceme (LNR) ($r=0.36^{**}$). Raceme number (RN) had a significantly negative correlation with legume number per raceme (LNR) ($r=-0.16^{**}$).

Table 1. Correlation coefficient among all evaluated traits: seed yield/plant (SYP); vegetative regrowth (VR); following winter dormancy (FWD); spring regrowth (SR); plant height (PH); dry matter yield (DMY); flowering date (FD); stem number (SN); growth habit (GH); raceme number (RN); legume number per raceme (LNR); legume number per stem (LNS).

	SYP	VR	FWD	SR	PH	DMY	FD	SN	GH	RN	LNR
VR	0.10	-									
FWD	0.06 ^{ns}	-0.02 ^{ns}	-								
SR	0.19 ^{**}	0.44 ^{**}	0.11 [*]	-							
PH	0.18 ^{**}	0.02 ^{ns}	0.17 ^{**}	0.15 ^{**}	-						
DMY	0.40 ^{**}	0.27 ^{**}	0.19 ^{**}	0.45 ^{**}	0.39 ^{**}	-					
FD	-0.20 ^{**}	-0.06 ^{ns}	-0.01 ^{ns}	-0.20 ^{**}	-0.10 [*]	-0.18 ^{**}	-				
SN	0.27 ^{**}	0.28 ^{**}	-0.01 ^{ns}	0.35 ^{**}	0.05 ^{ns}	0.46 ^{**}	-0.14 ^{**}	-			
GH	0.11 [*]	0.15 ^{**}	0.03 ^{ns}	0.20 ^{**}	0.05 ^{ns}	0.19 ^{**}	-0.20 ^{**}	0.17 ^{**}	-		
RN	0.06 ^{ns}	-0.01 ^{ns}	0.09 [*]	0.01 ^{ns}	0.01 ^{ns}	0.07 ^{ns}	-0.05 ^{ns}	-0.03 ^{ns}	-0.07 ^{ns}	-	
LNR	0.19 ^{**}	0.09 ^{ns}	0.01 ^{ns}	0.01 ^{ns}	0.01 ^{ns}	0.06 ^{ns}	-0.01 ^{ns}	-0.04 ^{ns}	0.02 ^{ns}	-0.16 ^{**}	-
LNS	0.15 ^{**}	0.03 ^{ns}	0.08 ^{ns}	0.01 ^{ns}	-0.01 ^{ns}	0.08 ^{ns}	-0.05 ^{ns}	-0.01 ^{ns}	-0.06 ^{ns}	0.83 ^{**}	0.36 ^{**}

ns: not significant; *, ** significant at the 0.05, 0.01 level, respectively

Table 2. Dry matter yield/plant (DMY) and seed yield/plant (SYP) of selected plants in relation to the initial population and selection differentials (S).

Traits	Initial population			Selected plants		S
	Mean±es	Range	C.V.	Mean±es	C.V.	
DMY	125.45±1.69	25.00-255.00	0.29	162.81±3.45	0.17	37.36
SYP	10.01±0.27	0.36-32.53	0.57	15.954±0.50	0.26	5.94

Phenotypic selection

On the basis of the correlation analysis, in September 2004, 65 plants, characterised by high seed yield and high forage production were selected. The effects of the different intensities of selection to seed yield and dry matter yield are reported in Table 2. The selection responses obtained for dry matter yield/plant and seed yield/plant were remarkable, $S=37.36$ (about 30%) and 5.94 (about 57%), respectively. The variation range (among 469 genotypes) was from 25 to 255 g for dry matter yield (DMY) and from 0.36 to 32.53 g for seed yield (SY). With reference to the coefficient of variability (C.V.), seed yield/plant (SYP) was higher than that of dry matter yield (DMY), both in the initial population and in the selected group.

Principal Component Analysis

The Principal Component Analysis (PCA) (Fig. 1 and Tables 3,4) showed that the first three components accounted for 22.3%, 15.8% and

10.3% of the variance, respectively, and that their cumulative variance was 48.3% (Table 3). A scatter diagram representing these three components (Fig. 1 a,b) shows the distribution of the 469 plants and of the 65 selected ones, respectively. On the basis of the eigenvector values (Table 4), the four traits responsible for maximum separation along the first component (Prin1) were, as expected, dry matter yield and seed yield/plant, followed by spring regrowth and stem number. Legume number per raceme and raceme number allowed genotypes to be discriminated on the second component (Prin 2). On the third component (Prin 3) the greater contribution was represented by plant height and following winter dormancy. According to eigenvector values of the second component, the multivariate analysis indicated that the seed yield components, raceme number and legumes per raceme could clearly discriminate the selected plant group.

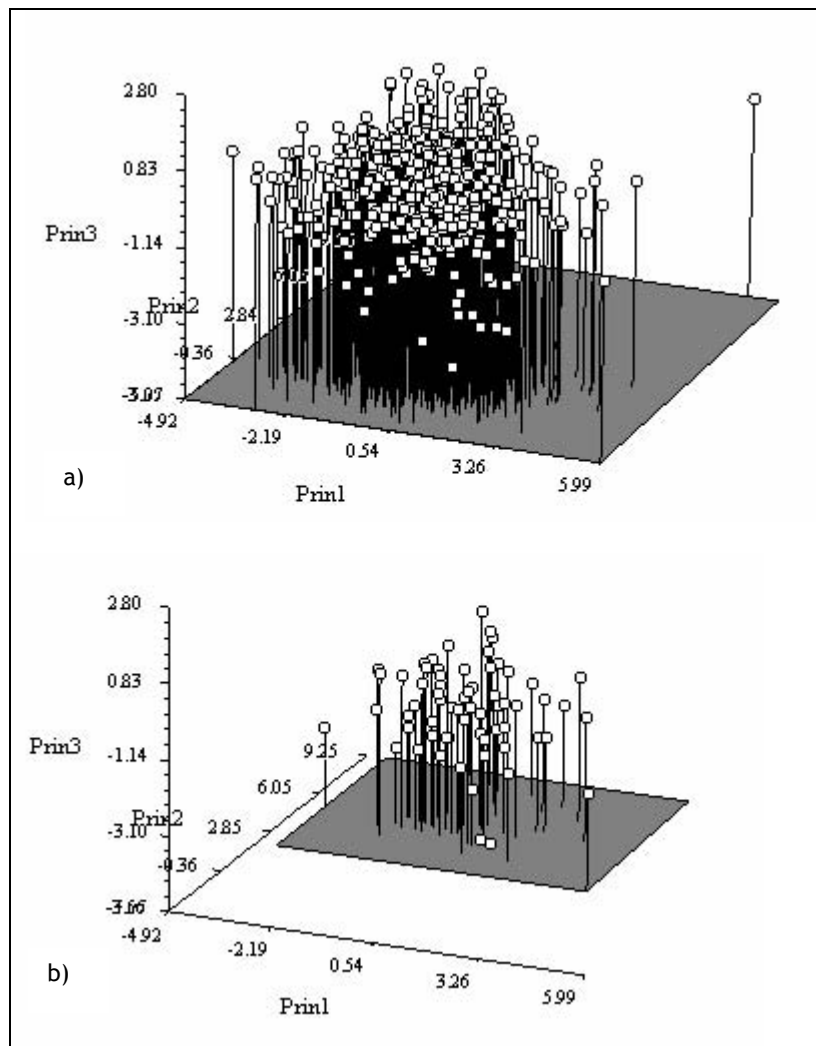


Figure 1. Scatter diagrams of 469 initial plants (a) and of 65 selected plants(b) obtained from the first three principal components after Principal Component Analysis (PCA) of the data matrix.

Table 3. Eigenvalues, percentages and cumulative variance in Principal Component Analysis of data matrix.

Component	Eigenvalue	% Variance	Cumulative variance
First	2.670	0.223	0.223
Second	1.891	0.158	0.380
Third	1.239	0.103	0.483
Fourth	1.157	0.097	0.578
Fifth	1.040	0.087	0.666
Sixth	0.914	0.076	0.743
Seventh	0.791	0.066	0.809
Eighth	0.768	0.064	0.873
Ninth	0.620	0.052	0.924
Tenth	0.507	0.042	0.966
Eleventh	0.380	0.032	0.998
Twelfth	0.024	0.002	1.000

Table 4. Eigenvector value on the first three axes in Principal Component Analysis of data matrix.

Traits	1 st component	2 nd component	3 rd component
Dry matter yield	0.488	-0.038	0.203
Spring regrowth	0.420	-0.128	-0.088
Stem number	0.384	-0.139	-0.194
Seed yield/plant	0.346	0.071	0.022
Legume number per raceme	0.134	0.687	-0.159
Raceme number	0.089	0.644	0.051
Plant height	0.239	-0.022	0.568
Following winter dormancy	0.128	0.103	0.529
Vegetative regrowth	0.314	-0.107	-0.370
Flowering date	-0.237	0.009	0.002
Growth habit	0.228	-0.163	-0.111
Legume number	0.108	0.151	-0.366

DISCUSSION

One of the main goals of many forage breeders is the development of cultivars with the potential to produce high yields of good forage. Since this objective is achieved without genetic capacity to attain economic levels of seed production, evaluation of seed yield of genetic materials is an important step in lucerne improvement. Successful lucerne seed production is also favoured by stand establishment and climatic conditions that promote good flowering and contribute to bee pollination (Rincker *et al.* 1988). In Italy, breeding of lucerne is still at an early stage compared with other species such as maize or wheat. Many improved cultivars of lucerne are synthetics which are still characterised by a wide genetic base. Thus, there is sufficient variation in lucerne varieties for seed yield to be increased substantially by selection and breeding. The large variation for seed yield may have very diverse origins such as differences in male fertility, female fertility and ease of tripping (Balaños- Aguillar *et al.* 2000). Moreover, several studies carried out in outcrossing forage species have shown that the genetic load can cause post-fertilisation abortion of developing embryos. This is the result of the presence of recessive lethal alleles maintained in a heterozygous condition which are expressed when recombination or inbreeding occurs (Falcinelli 1999).

The evaluation of our genetic materials showed a large variation in seed yield corresponding to the values reported in the literature; Balaños- Aguillar *et al.* (2000) reported a range from 0.30 to 30.75g per plant in 214 genotypes, and Raush (1964)

reported a range from 0 to 30.75g of seed per plant measured on 1301 individual plants. In general, seed yield per plant had a positive correlation with stem number per plant, spring regrowth, legume number per raceme, legume number per stem and dry matter yield per plant. This result confirms the possibility of selecting for seed yield/plant without negative responses affecting dry matter yield in lucerne. Concerning the seed yield components, legume number per stem showed a high and positive correlation with raceme number and legume number per raceme. On the contrary, negative correlations were found for raceme number per plant and legume number per raceme. The PCA analysis emphasized that the seed yield components, in particular legume number per raceme and raceme number, are useful to discriminate the best plants for forage and seed production. This result suggests that these characteristics are an interesting target to take into consideration when breeding for seed yield in lucerne. Phenotypic selection of spaced plants allowed genotypes characterised by high seed yield, high forage production and resistance to diseases to be chosen. These genotypes will be used to obtain a new adapted synthetic variety which will be more productive in forage and with stable seed production, and this is fundamental for the commercial development of lucerne varieties.

ACKNOWLEDGEMENTS

This research was funded by the Project PRIS2 Azioni di Innovazioni e Ricerca a Supporto del Piano Sementiero.

LITERATURE

- Balaños-Aguillar, E. D., Huyghe, C., Julier, B. & Ecalle, C. 2000. Genetic variation for seed yield and its components in alfalfa (*Medicago sativa* L.) populations. *Agronomie* 20: 333-345. INRA, EDP Sciences.
- Boçsa, I. & Buglos, J. 1983. Seed yield and some factors influencing seed setting at the variety level in lucerne. *Zeitschrift für Pflanzenzuchtung* 90: 172-176.
- Bouton, J.H. 2001. Alfalfa. *In: Gomide J.A, Mattos, W.R.S. & da Silva, S.C. (eds.). Proceedings XIX International Grassland Congress, Sao Pedro, Sao Paulo, Brazil, 11-21 February 2001.* pp 545-547.
- Falcinelli, M. 1999. Temperate forage seed production: Conventional and potential breeding strategies. *Journal of New Seeds* 1: 1
- Lorenzetti, F. 1981. Relationship between dry matter and seed yield in leguminous forage plants. *In: van Bogaert, G. (ed.). Breeding high yielding forage varieties combined with high seed yield. Meeting of the Eucarpia Fodder Crops Section, Merelbeke, Belgium.* pp. 57-74.
- Lorenzetti, F. 1993. Achieving potential herbage seed yields in species for temperate regions. *In: Baker, M.J., Crush, J.R. & Humphreys L.R. (eds.). Proceedings of the XVII International Grassland Congress.* pp. 1621-1628.
- Melton, B. 1969. Comparative seed and forage yield in crosses of selected alfalfa clones as compared to polycross progeny. *Crop Science* 9: 253-255.
- Rausch, H. 1964. The causes of infertility in lucerne (*M. media* Pers). Investigations on correlations between factors determining seed yield, *Zeitschrift für Pflanzenzuchtung* 51: 141-166.
- Rincker, C.M., Marble, V.L., Brown, D.E. & Johansen, C.A. 1988. Seed production practices. *In: Hanson, A.A. Barnes, D.K. & Hill Jr., R.R. (eds.). Alfalfa and Alfalfa Improvement. Agronomy No. 29.* pp. 985-1021. ASA, CSSA, SSSA, Madison, Wisconsin, USA. .
- SAS 1990. SAS/STAT User's Guide. Version 6. SAS Institute, Cary, North Carolina, USA.
- Veronesi, F. & Falcinelli, M. 1987. Seed yield selection in *Medicago sativa* L. and correlation responses affecting dry matter yield. *Plant Breeding* 99: 77-79.
- Veronesi, F., Huyghe, C. & Delgado, I. 2006. Lucerne breeding in Europe: Results and research strategies for future developments. *In: Lioveras, J. et al. (eds.). Sustainable Grassland Productivity - Proceedings of 21th General Meeting of the Grassland Federation, Badjaz, Spain 3-6 April 2006.*

Experience of perennial ryegrass seed production in Finland of cultivars from central Europe

Markku Niskanen¹⁾ & Oiva Niemeläinen²⁾

¹⁾MTT Agrifood Research Finland, Plant Production Research, Alapääntie 104, FI-61400 Ylistaro, Finland

²⁾MTT Agrifood Research Finland, Plant Production Research, Building E, FI-31600 Jokioinen, Finland

markku.niskanen@mtt.fi

ABSTRACT

Seed production characteristics of six perennial ryegrass (*Lolium perenne*) cultivars were tested in field experiments in Finland where the main objective was to study the suitability of cultivars from central European origin for seed production. Four diploid and two tetraploid cultivars were included in the study. Two of the studied cultivars were of the amenity type. Tested cultivars were Aubisque (4n, fodder & amenity type, The Netherlands (NL)), Barrage (2n, amenity type, NL), Cheops (4n, fodder type, NL), Danilo (2n, amenity type, Denmark), Mongita (2n, fodder type, NL), Riikka (2n, fodder type, Finland). The trials were established in 2003 in a barley cover crop at Jokioinen (latitude N 60°49') and at Ylistaro (latitude N 62°57'). Seed yield was harvested in 2004. Tiofanat methyl (Topsim M) spray against snow mold just prior to winter was included as main plot treatment in the split plot experimental design with cultivars in the sub plot. In winter 2003/2004 winter damages were relatively small with an average of 21 % damage in the untreated control at both sites. The spraying with Topsim M against snow mold decreased winter damage percentages slightly in treated plots to 19 % at Jokioinen and 15 % at Ylistaro. Finnish cv. Riikka had the least winter damage and cv. Mongito most winter damages at both trial sites. Cv. Barrage was the earliest cultivar reaching ear emergence and cv. Cheops was the latest reaching ear emergence seven days later. Cv. Barrage produced the highest number of fertile tillers per m². The tetraploid cultivars Aubisque and Cheops had naturally the highest seed weight. At Jokioinen the trial was harvested early; the cultivars Aubisque, Danilo and Riikka were harvested on 12.8.2004, cultivars Barrage and Mongita on 14.8.2004 and cv. Cheops on 17.8.2004. The latter part of the growing season 2004 was exceptionally rainy which disturbed harvesting at Ylistaro. Early cultivars may have suffered from seed shattering due to delayed harvesting although all the cultivars lodged completely which might have reduced seed shattering. At Ylistaro the trial was combine harvested on 7.9.2004 (all cultivars on the same date). The treatment against snow mold did not have significant effect on seed yield at either trial site in this experiment. Differences in the seed yield were large among the studied cultivars. Cultivars seed yield (average of the snow mold treatments) ranged in Jokioinen from 674 kg ha⁻¹ (cv. Danilo) to 1066 kg ha⁻¹ (cv. Riikka). Seed yield of cv. Cheops was 942 kg ha⁻¹ and 817 kg ha⁻¹ of cv. Barrage. At Ylistaro cultivars' seed yield ranged from 398 kg ha⁻¹ (cv. Danilo) to 1011 kg ha⁻¹ (cv. Cheops). Seed yield of cv. Riikka was only 485 kg ha⁻¹, which is considered to be due to severe seed shattering losses. Seed yield of cv. Barrage was 767 kg ha⁻¹. All of the cultivars studied were early enough in Finnish growing conditions. The results encourage further study of the biological and economical feasibility of seed multiplication in Finland. This study will focus on seed multiplication of amenity type cultivars for the domestic market.

Key words: *Lolium perenne*, seed yield, winter damage

Forage and seed yield of sulla (*Hedysarum coronarium* L.) varieties and landraces in a semi-arid Mediterranean environment

Gaetano Amato, Dario Giambalvo, Dario Graziano & Paolo Ruisi

Università di Palermo, Dipartimento di Agronomia ambientale e territoriale,
Viale delle Scienze, 90128 Palermo, Italy

amato@unipa.it

ABSTRACT

Sulla (*Hedysarum coronarium* L.) is an important forage crop in some Mediterranean rainfed agricultural systems. The species plays a key role in improving sustainability of cereal-based cropping systems of such areas. Usually farmers grown local landraces, but, due to the increasing interest for the species, breeding activities have been developed and new varieties recently released. A field trial was carried out during 2002/03 and 2003/04 seasons in a semi-arid, hilly area of Sicily (S. Stefano Q.) to evaluate six varieties and two landraces in terms of forage and seed yield. The experiment was set up as a randomized block design with four replications. In both years, half plot was utilized to assess forage production and the second half for seed yield. A large variation was observed among the studied varieties/landraces for most of the recorded traits. Furthermore, the entries ranked differently for forage and seed yield as well as for the performance at the first and at the second year of the cycle.

Key words: biennial forage crop, landrace, variety

INTRODUCTION

Sulla is a short-lived perennial forage crop (usually biennial) grown for fodder in many semi-arid Mediterranean environments. The crop plays a key role in improving sustainability of cereal-based cropping systems of such areas mainly because of its contribution for nitrogen supply and maintenance of soil organic matter. In Italy it is cultivated on 88.000 hectares mainly in the southern regions (ISTAT 2006). In Sicily, commercial varieties and, mostly, local populations are grown on 44.000 hectares; however, the species has a larger diffusion considering that wild or naturalized populations are spread over the permanent and occasional pastures particularly on the clayey and calcareous soils in the hilly inner area. Seed production is often obtained as a secondary product from stands established to produce forage. Usually seed is harvested from second year crops and from selected areas within the crop where more vigorous

plants and less weeds are present (Stringi & Amato 1998).

Recently, a new interest has arisen for this crop, also in non traditional cultivation areas, due mainly to the very good adaptation to marginal environments, to the versatility for agricultural and non-agricultural uses (pasture, green forage, silage, hay, cover crop, honey production, landscape architecture) (Talamucci 1998), and also to the peculiar chemical composition, particularly the presence of condensed tannins (CT) in its leaf and stem tissues. Due to the increasing interest for the species, breeding activities have been developed mainly in areas where the crop was not traditionally grown and new varieties have been recently released. The aim of this research was to evaluate, in a semi-arid Mediterranean environment, the performance, both for forage and seed yield, of some new varieties also in comparison with old varieties and landraces.

MATERIALS AND METHODS

The trial was carried out during 2002/03 and 2003/04 seasons in a semi-arid, hilly area of Sicily (S. Stefano Q., AG, 37° 30'N; 13° 31'E; 178 m a.s.l.) on a deep, clayey, well structured soil with wheat as previous crop. Six commercial varieties ('Carmen', 'Corona', 'Grimaldi', 'Irpinia' and 'S. Omero' selected in central Italy and 'Sparacia' selected in Sicily) and two Sicilian landraces ('Gangi' and 'Resuttano') were arranged in a randomized block design with four replications and plot size of 5.0 x 1.5 m. Sowing was done on 21 December 2002 in rows 25 cm apart at 500 germinable seeds m⁻². Half of each plot was used to evaluate forage production (FP) and the other half to evaluate seed production (SP). At the first year FP plots were cut at full flowering (May) whereas SP were left undisturbed until seed maturity. At the second year, both subplots (FP and SP) were cut for forage production at mid-autumn (28 November) and the regrowth was treated as at the 1st year.

In the FP subplots at each cut and in the SP at the autumn cut, plants were cut at 5 cm stubble height and total fresh matter (FM) was recorded. A random sample (approx. 1 kg FM) was immediately separated into sulla and weeds and the fractions were dried at 105 °C to estimate dry matter (DM) yield. Another

sample (approx. 0.5 kg FM) of pure sulla was separated into leaves, stems, inflorescences and dead material and dried at 105 °C. Only at the first year a further sample of forage was harvested for condensed tannin (CT) determination and suddenly hand-separated into botanical fractions, packed in ice, transported to the laboratory and stored at -20 °C. Freeze-dried samples were ground to pass through a 1 mm diameter sieve, mixed and extracted with 70 % aqueous acetone in duplicate, and quantified by a spectrophotometric method following the butanol-HCl-Fe³⁺ assay (Porter *et al.* 1986) using delphinidin as the reference standard; absorbance was read at 550 nm.

In the SP subplots, date of first open flowers, full flowering and flowering closure were recorded. At maturity, plant lodging, plant height, and 1st raceme height were recorded. Total plot biomass was separated into sulla and weeds, dried and weighted. 30 racemes per plot were randomly sampled and number and weight of loment, segments and seeds were recorded. Sulla plants were passed through a plot thresher; the unhulled seed was then passed through a laboratory hulling machine to assess seed yield.

The analysis of variance was performed according to the experimental design and means separation was calculated using LSD values if F-test was significant at $P \leq 0.05$.

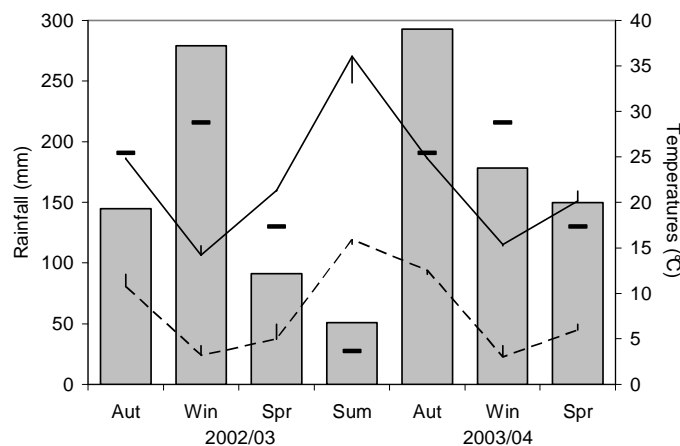


Fig. 1. Seasonal precipitation (histograms), mean maximum temperatures (solid line) and mean minimum temperature (dotted line) at Pietranera farm during the trial period. Horizontal segments indicate mean long-term period rainfalls (27 years) and vertical segments indicate differences from long term temperatures.

Weather

Temperature and precipitation data were collected at the experimental site (Fig. 1). The 2002/03 season (August-July) had a total rainfall of 533 mm, similar to the long-term mean of 563 mm, but with a wetter winter and a drier spring. The 2003/04 season was particularly favourable for the crop regrowth after the summer stasis mainly due to a wet autumn and a total precipitation 23 % higher than normal.

RESULTS AND DISCUSSION

At each cut, weed incidence was always less than 10 % of total DM production. As no significant interaction between weed growth

and variety/landrace was found, only sulla biomass was considered. Mean annual DM production varied significantly among the eight varieties/landraces. 'Carmen' and 'Resuttano' had the highest yield (on average, 9.0 t DM ha⁻¹ per year), whereas 'Irpinia' and 'Gangi' gave the lowest productions (on average, 6.9 t DM ha⁻¹ per year) (Table 1). However, the varieties/landraces showed different response in the two growing seasons (significant interaction growing season x variety/landrace, $P < 0.0001$). 'S.Omero' produced in the first year about 60 % of the total biennial forage yield and landrace 'Resuttano' only 27 %; the varieties 'Corona' and 'Grimaldi' and the landrace 'Gangi' showed a quite well-balanced forage yield in the two growing seasons.

Table 1. Mean annual dry matter yield (DMY), and contribution of 1st year on biennial production, leaf proportion on total biomass at the 1st and 2nd year and content of condensed tannin (CT) in leaves and whole plant at 1st year in eight varieties / landraces of sulla at flowering.

Variety/landrace	DMY		Leaf proportion		Condensed tannin	
	Mean t ha ⁻¹ year ⁻¹	1 st year %	1 st year %	2 nd year %	leaves g kg ⁻¹ DM	plant g kg ⁻¹ DM
Carmen	9.07	43.8	44.7	14.2	20	14
Corona	7.99	49.2	32.3	14.3	21	12
Gangi	6.85	48.6	24.3	20.3	27	17
Grimaldi	7.99	48.5	34.0	15.0	24	15
Irpinia	6.90	35.1	51.5	16.2	14	8
Resuttano	8.86	27.3	54.4	14.3	30	20
S. Omero	7.17	59.4	33.7	14.5	18	11
Sparacia	7.75	35.7	48.5	16.6	25	19
LSD _{0.05}	1.41	13.1	8.4	3.7	2.5	1.9
Mean	7.82	43.4	40.4	15.6	22	15

Varieties/landraces differed strongly for the proportion of plant fractions. At the first year, forage of 'Corona', 'Grimaldi' and 'S. Omero' was composed of approximately one third of leaves. Forage of 'Gangi' showed the lowest proportion of leaves (24.3 %); on the contrary, 'Resuttano', 'Irpinia' and 'Sparacia' (that were also the less productive in the first year) had the highest leaf contribution to forage yield (on average 51.5 %). In the second growing season the contribution of leaf at full flowering was markedly lower than in the first year and small differences among the studied entries were detected.

The three Sicilian populations (cv. Sparacia and landraces 'Gangi' and 'Resuttano') had the highest CT content (on average 27.3 and 18.7 g kg⁻¹ DM respectively in the leaves and in the whole plant). 'Irpinia' was characterized by the lowest concentrations. The CT concentration of leaves of the variety 'Grimaldi' was similar to that reported for the same variety and the same growth stage by Piluzza *et al.* (2000) in a Sardinian environment. No significant relationships were found between DM yield and whole plant or single fraction CT content suggesting that selection should be effective in producing cultivars with high herbage yield and,

alternatively, with low or high content of condensed tannin (Amato *et al.* 2005).

After the summer growth stasis, the regrowth produced on average 3.9 t DM ha⁻¹ (November cut). The different use of the crop at the first year (forage or seed) did not affect the DM yield whereas significant differences were observed among populations. The landrace 'Resuttano' had the faster regrowth after stasis yielding 5.4 t DM ha⁻¹ and 'Irpinia' gave the lowest DM yield (2.9 t ha⁻¹); the remaining entries gave statistically similar productions (range 3.5-4.1 t DM ha⁻¹). The differences among varieties/landraces for the distribution of forage production both between and within

the two growing seasons, according to Sulas *et al.* (1997), suggest that it's possible to extend the grazing period by means of the use of different varieties in the forage system.

On average, mean annual seed production was 277 kg ha⁻¹, and significant differences between the two growing seasons were observed (351 and 202 kg ha⁻¹, respectively in the 1st and 2nd year; $P < 0.0001$). The relatively low seed production at the 2nd growing season seems due to the large vegetative growth (supported by the peculiar environmental conditions) leading to a high intraspecific competition with negative effects on the reproductive phase.

Table 2. Mean annual seed yield and 1st year contribution on biennial production, 1000-seed weight, number of laments raceme⁻¹ and segments loment⁻¹, and percentage of segments without seed (means of 2 years).

Variety / landrace	Seed yield		1000-seed weight g	Laments raceme ⁻¹	Segments loment ⁻¹	Empty segments %
	Mean kg ha ⁻¹	1 st year %				
Carmen	340	55.1	5.52	24.9	2.59	25.9
Corona	304	73.5	5.33	23.2	2.56	27.0
Gangi	386	58.6	6.00	26.9	2.67	24.4
Grimaldi	308	70.4	5.16	24.4	2.63	26.9
Irpinia	246	65.6	5.19	26.8	2.69	23.3
Resuttano	90	26.3	5.43	23.8	2.69	36.3
S. Omero	324	72.3	5.19	23.8	2.65	27.6
Sparacia	214	74.0	5.49	25.1	2.64	35.2
LSD _{0.05}	83	13.7	0.18	ns	ns	7.2
Mean	277	62.0	5.41	24.9	2.64	28.3

Varieties and landraces had an unstable response in the two growing seasons. The highest yielding populations ('Gangi' and 'Carmen') gave similar yields in the two years; the varieties 'Corona', 'Grimaldi' and 'S. Omero' produced about three-fold at first year compared to the second. The landrace 'Resuttano' was characterized by the lowest seed yield particularly at the first year cycle.

The studied varieties and local populations showed different survival strategies. In fact the low productivity both of biomass and seed of 'Resuttano' at the first year, associated with a faster autumn regrowth and a high vegetative production at the second year, let us suppose that this population at the first year of the cycle, prefer redirect the photo-assimilates towards reserve organs (mainly tap root) rather than towards the gamic reproductive organs. On the contrary the 'Gangi' landrace showed a clear preference in

the use of photo-assimilates towards the reproductive process rather than vegetative activities. The other entries expressed intermediate behaviour.

The 1000 seed weight was not statistically different in the two growing seasons and the entries had a similar ranking in the two years (not significant interaction growing season x variety/landrace); the landrace Gangi had the heaviest seeds (6.00 g per 1000 seeds) whereas the varieties Grimaldi, Irpinia and S. Omero had the lightest seeds (on average, 5.18 g). No significant differences were observed among the varieties and landraces for the number of laments per raceme which, however varied between the two growing seasons (22.2 and 27.5, respectively in 1st and 2nd year; $P < 0.0001$). Nevertheless, in the second year a greater incidence of segments without seed was measured (44.5 vs. 12.6 %; $P < 0.0001$). Significant differences were also

observed among entries; the percentage of empty segments was higher in the 'Resuttano' landrace and in cv. Sparacia. The number of segments per loment was unaffected by growing season and population.

ACKNOWLEDGEMENTS

The research was funded by the University of Palermo. The authors are grateful to V. Cannella and F. Labruzzo for technical support.

LITERATURE

- Amato, G., Di Miceli, G., Giambalvo, D., Scarpello, C. & Stringi, L. 2005. Condensed tannins content in sulla (*Hedysarum coronarium* L.) as affected by environment, genotype and growth stage. *In*: Bioactive compounds in pasture species for phytotherapy and animal welfare. Cnr-Ispaam, Sassari (I): 41-54.
- ISTAT 2006. Dati congiunturali sulle coltivazioni. www.istat.it
- Piluzza, G., Bullitta, S., Deroma, M. & Odoardi, M. 2000. The accumulation of condensed tannins in local population of sulla. *Cahiers-Options-Mediterraneennes* 45: 199-202.
- Porter, L. J., Hrstich, L. N. & Chan, B. G. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 1: 223-230.
- SAS 1996. SAS/STAT User's Guide. Version 6. SAS Institute, Cary, NC, USA.
- Stringi, L. & Amato, G. 1998. La sulla nell'ambiente siciliano: utilizzazione e prospettive di valorizzazione. *Quaderni dei Georgofili* 45: 29-51.
- Sulas, L., Re, G.A., Ledda, L. & Caredda, S. 1997. The effect of utilization frequency on sulla (*Hedysarum coronarium* L.) forage production and its components. *Italian Journal of Agronomy* 1: 89-94.
- Talamucci P. 1998. Il ruolo della sulla nell'attuale contesto della foraggicoltura italiana. *Quaderni dei Georgofili* 45: 7-27.

Modeling pollen dispersal between white clover fields relevant to co-existence with GM-white clover

Christina L. Løjtnant¹⁾, Christian F. Damgaard²⁾ & Rikke B. Jørgensen¹⁾

¹⁾Risø National Laboratory, DK-4000 Roskilde,

²⁾DMU, DK-8600 Silkeborg

christina.loejtnant@risoe.dk

ABSTRACT

As the importance of genetically modified crops increases, so do the need to know more about, how we can secure that EU thresholds of GM in NON-GM products and seeds are not exceeded. There are many ways genes can be dispersed and in this study we apply a mathematical model (developed by Cresswell et. al. 2002, *Oikos* 96: 375-384), which models the amount of gene flow between fields caused by pollinators. The model output provides information for assessments of gene dispersal from transgenic varieties.

The model is based on three parameters, which can relatively easy be estimated in the field. The parameters are: E foraging pattern of the pollinator, b average number of pollinator visits in a field, and ψ paternity shadow. It is the first time the model is applied on white clover (*Trifolium repens*), where *Apis mellifera* is the predominant pollinator. The foraging behavior of *Apis* has necessitated some changes of the model, which will be discussed.

Key words: gene flow, insect pollination

Plant breeding and seed production of apomictic tropical forage grasses

John W. Miles¹⁾ & Michael D. Hare²⁾

¹⁾ Centro Internacional de Agricultura Tropical (CIAT), A.A. 6713, Cali, Colombia

²⁾ Faculty of Agriculture, Ubon Ratchathani University, Thailand

j.miles@cgiar.org

ABSTRACT

Since apomictic reproduction (asexual reproduction by seed) circumvents female meiosis it ought to ensure good seed set even in meiotically unstable hybrids. However, this seems not generally to be the case. Improvement of apomictic brachiariagrasses in Colombia began with hybridization using a tetraploidized, sexual ruzigrass biotype developed in Belgium. Commercial seed production of the first two hybrid-derived apomictic cultivars from the CIAT breeding program are described. The first release, cv. Mulato, yields only about one-tenth as much seed as conventional brachiariagrass cultivars, which are derived directly from apomictic germplasm accessions collected in Africa. Low seed yield in Mulato is owing to poor seed set, rather than poor flowering. A second released cultivar, Mulato II, has better seed yield than Mulato even though it has lower inflorescence density. It still yields less than conventional cultivars. The brachiariagrass breeding scheme currently employed at CIAT is briefly described and the issues involved in applying effective selection pressure to achieve cumulative genetic gains in the seed yield of apomictic hybrids are discussed.

Key words: apomixis, brachiariagrasses, cultivar, seed set, seed yield.

INTRODUCTION

Ruminant livestock production in the Tropics and Subtropics relies to a large extent on grazing of C₄ grasses. While the main C₄ crop species (e.g., maize [*Zea mays* L.], sorghum [*Sorghum bicolor* (L.) Moench], pearl millet [*Pennisetum glaucum* (L.) R. Br.], and sugarcane [*Saccharum officinarum* L.]) reproduce sexually, many of the economically important C₄ forage grasses reproduce by apomixis, i.e., asexual reproduction by seeds (Asker & Jerling 1992). Apomicts are among the most economically important warm-season forage grass species, and include buffelgrass [*Pennisetum ciliare* (L.) Link (syn *Cenchrus ciliaris* L.)], guineagrass (*Panicum maximum* Jacq.), bahiagrass (*Paspalum notatum* Flügge), and three of the four commercial species of brachiariagrasses [*Brachiaria brizantha* (A. Rich.) Stapf (palisadegrass); *B. decumbens* Stapf (signalgrass); and *B. humidicola* (Rendle) Schweick. (koroniviagrass)]. (The commercial *B. ruziziensis* (ruzigrass), which is closely

related to palisadegrass and signalgrass, is a naturally sexual diploid.)

Apomixis offers a unique means directly and faithfully to propagate even highly heterozygous genotypes through the convenient vehicle of seed. In fact, among the known apomictic crop plants, the only group where commercial multiplication combines asexual reproduction with seed propagation are the tropical forage grasses. Other domesticates where apomictic reproduction is known are fruit species where commercial propagation is by vegetative means (cuttings or grafting), rather than seeds.

Two types of apomixis are found in the warm-season grasses: diplospory and apospory. These differ by the origin of the unreduced cell that gives rise to the female gametophyte: the diplosporous gametophyte originates by mitosis from an unreduced megaspore mother cell following a failure of meiosis; the aposporous gametophyte

originates directly from a somatic, nucellar cell. Apospory is the type of apomixis found in the most important of the commercial warm-season forage grasses (e.g., the brachiariagrasses, guineagrass, buffelgrass, and bahiagrass).

To date, the vast majority of commercial warm-season forage grass cultivars are direct selections from collections of natural germplasm, commonly known as "ecotype selections" (Vogel & Burson 2004). Breeding programs based on deliberate hybridization and selection exist (buffel-, guinea-, brachiaria-, and bahiagrass), but their practical success to date has been very limited. One of the constraints on plant breeding of apomicts is the need to modify and adapt breeding procedures to take into account the peculiarities of apomictic reproduction and effectively and efficiently harness the advantages of asexual reproduction to exploit heterosis. Where cross compatible sexual germplasm exists, as it generally does, to allow genetic recombination in an apomictic species, then apomixis offers many advantages in a plant breeding program (Vogel & Burson 2004).

It is commonly supposed that apomictic reproduction, in and of itself and owing to the fact that it bypasses female meiosis, should lead to improved reproductive fertility, and by extension, higher seed yields, particularly in apomicts of hybrid origin (e.g., Vogel & Burson 2004). An often repeated statement by Darlington (1939, cited in Asker & Jerling 1992) asserts that "apomixis is an escape from sterility...". However, greater seed fertility for apomicts is not inevitable, particularly in pseudogamous apomicts where fertile pollen is required for the development of endosperm. A recent report from Brazil (Risso-Pascotto *et al.* 2005) suggests that genetic incompatibilities in an aposporous interspecific hybrid (*Brachiaria ruziziensis* x *B. brizantha*) resulted in up to 65% sterile pollen grains. Since apomictic brachiariagrasses are pseudogamous, the authors suggest that pollen sterility may be an important cause of poor seed fill in interspecific hybrids. And Asker & Jerling (1992) observe that "In *Poa pratensis*, sexual aberrants may have better seed-setting than original, facultatively apomictic types."

The present paper aims to:

1. Describe breeding procedures currently employed in CIAT's brachiariagrass breeding program and how these have evolved since the first experimental hybrids were made in 1988.
2. Outline the difficulties that have been encountered in commercial seed production of the first hybrid brachiariagrass cultivar (cv. Mulato) and their probable causes.
3. Describe seed production of a second brachiaria hybrid cultivar release (Mulato II).
4. Describe possible plant breeding approaches aimed at improving seed production characteristics of future brachiariagrass hybrid cultivars.

BRACHIARIAGRASS BREEDING PROGRAM AT CIAT

All four commercial *Brachiaria* species are of African origin. These commercial species (with the exception of ruzigrass) are polyploid, aposporous apomicts, i.e., they reproduce asexually by seed. Ruzigrass is a diploid, allogamous sexual species (Ferguson & Crowder 1974). Taken together, the apomictic polyploid species (signalgrass, palisadegrass, and koroniviagrass) are by far the most important sown forage grasses in the neo-Tropics (Santos Filho 1996). Ruzigrass is commercialized only to a limited extent in tropical America owing to its extreme susceptibility to spittlebugs and poor edaphic adaptation. It is much more important commercially where spittlebugs are not a production limitation, e.g., in Thailand (Hare & Phaikew 1999).

The early brachiariagrass cultivars (Basilisk signalgrass; Marandu, La Libertad, and Toledo palisadegrass; and Tully and Llanero koroniviagrass) are "ecotype selections", apomictically reproducing clones selected from ex situ germplasm collections, either in northern Australia (Basilisk, Tully) or in tropical America (Marandu, in Brazil; La Libertad, Toledo, and Llanero, in Colombia). Kennedy ruzigrass is a sexual population released in Australia.

Interest in brachiariagrass breeding was motivated by the perceived deficiencies of

existing cultivars (Miles & Valle 1996): spittlebug susceptibility in Basilisk and Kennedy; lack of adaptation to the very acid, infertile soils common in the American savannas in Marandu, La Libertad, and Toledo; poor feed quality and excessive seed dormancy in Tully and Llanero.

Signalgrass, palisadegrass, and ruzigrass are closely related, while koroniviagrass is phylogenetically more distant (Renvoize *et al.* 1996). Genetic recombination even within groups was, until the mid-1980s, restricted by intra- and interspecific ploidy differences, and especially by apomictic reproduction, which precludes direct hybridization between apomictic genotypes even at the same ploidy level.

An important breakthrough came with the development, in Belgium, of a tetraploidized, sexual ruzigrass (Swenne *et al.* 1981). Preliminary results of crossing studies (Ndikumana 1985) suggested that apomixis was simply inherited as a monogenic dominant, with sexual genotypes being homozygous recessive at the apomixis locus, as had previously been reported for guineagrass (Savidan 1975, 1983). These preliminary results, which were subsequently fully confirmed by C.B. do Valle and collaborators (Valle *et al.* 1994, Valle & Savidan 1996), suggested that reproductive mode might readily be manipulated in a plant breeding program (Miles & Valle 1996).

Experimental hybridization at CIAT in the signal-/palisade-/ruzigrass group began with the acquisition, in 1988, from Dr. Cacilda B. do Valle of Embrapa's Beef Cattle Center, of tetraploid, sexual ruzigrass germplasm derived directly from the Belgian material. The basic procedure for achieving genetic recombination was to use the cross-compatible tetraploid, sexual germplasm as female, and pollinate with the normal pollen produced by tetraploid apomicts. Such crosses were expected to "release" abundant genetic variation (Vogel & Burson 2004), given presumed genetic heterozygosity in both parents, but particularly in the natural tetraploid apomicts.

Most of the early proposals of breeding schemes for apomicts (summarized in Savidan 2000, Vogel & Burson 2004) were based either on incomplete or erroneous information on inheritance of reproductive mode (e.g., Taliaferro & Bashaw 1966, for buffelgrass;

Burton 1992, for bahiagrass), or had only a short-term perspective (e.g., Taliaferro & Bashaw 1966; Bashaw 1980b; Bashaw & Funk 1987). Only the breeding scheme proposed for guineagrass by Pernès *et al.* (1975), explicitly contemplated the accumulation of genetic gain over recurrent cycles of selection and recombination. Although many authors note the advantages of apomictic (asexual) reproduction faithfully to reproduce heterozygous genotypes, and hence to exploit heterosis (e.g. Vogel & Burson 2004), almost none of the proposed breeding schemes describes a mechanism that would accumulate the non-additive, heterotic effects contributing to heterosis (However, see Miles 1995).

The brachiariagrass breeding program at CIAT began with the assumption that repeated cycles of hybridization and selection would be needed to achieve the combination of characters and levels of character expression needed in improved cultivars (Miles & Valle 1996). The available sexual germplasm, derived from the Belgian tetraploidized sexual ruzigrass, has a very narrow genetic base [as evidenced by low polymorphism of molecular genetic markers (J. Tohme unpublished data)]. Further, this germplasm is highly susceptible to spittlebugs (Cardona *et al.* 1999), and has poor edaphic adaptation (Rao *et al.* 1998). The characters to be combined (spittlebug resistance and edaphic adaptation) were available in separate apomictic accessions. Hence, the initial plan was:

1. Cross different apomicts, each having one or another of the desired characters, to the sexual tetraploid ruzigrass.
2. Identify superior first cycle sexual hybrids (homozygous recessive at the apomixis locus) with expression of the desired characters.
3. Recombine selected hybrid sexual clones by open pollination in an isolated crossing block to synthesize a sexually reproducing, tetraploid breeding population with a broad genetic base, i.e., including genes from accessions of signalgrass and palisadegrass as well as ruzigrass.

Since sexuality in brachiariagrass is conditioned by the homozygous recessive genotype (aaaa) at a single "apomixis locus" (Valle & Savidan 1996), the synthetic sexual

population will remain fully sexual as long as it is not contaminated by pollen from cross-compatible apomicts (genotype Aaaa). Hence, the synthetic brachiariagrass breeding population, like any allogamous sexual crop species (e.g. maize), can be subjected to recurrent cycles of selection and hybridization to improve the levels of expression of desired characters.

Given the obvious advantages of apomictic reproduction in maintaining cultivar purity (no cross-pollination) and faithfully and easily propagating heterozygous (and heterotic) genotypes, the intention was to release only apomictic genotypes as commercial cultivars. Thus, in order to capture genetic gain in the synthetic sexual breeding population, elite sexual clones derived from the population are crossed (as females) with selected apomicts (as pollen parents) to produce hybrid populations segregating for reproductive mode (Miles *et al.* 2004). Following the identification of apomictic segregants in such hybrid populations, either by cytological examination of embryo sacs, by progeny test, or by molecular markers, the best apomictic hybrids can be multiplied directly and evaluated for eventual commercial release.

Six 2-yr cycles of intrapopulation selection have been conducted in the tetraploid sexual breeding population since it was synthesized, with heavy selection pressure on spittlebug resistance. Resistance has responded steadily to selection, so that we now routinely isolate sexual clones that are more resistant to spittlebugs than any accession in the brachiariagrass germplasm collection (Miles *et al.* 2006). Recent results show that this resistance is inherited by a significant proportion of sexual-by-apomictic hybrids, even if the apomictic parent is highly susceptible (e.g. Basilisk signalgrass) (C. Cardona unpublished data).

Recently, a cyclic breeding scheme -- recurrent selection on specific combining ability (Hull 1945, Miles 1997) -- designed to accumulate non-additive genetic effects contributing to heterosis has been implemented (Miles *in press*). In this scheme, selection in the synthetic sexual breeding population is based not on performance of sexual clones per se, but on the performance of their hybrid progeny. Briefly, a series of sexual clones are crossed (as females) with a single (male) "tester" genotype to produce a series of hybrid, testcross families. These

testcross progenies, rather than the sexual clones per se, are evaluated to determine which of the sexual clones will be intercrossed to reconstitute the (improved) sexual population. The breeding scheme automatically generates a cohort of apomictic hybrid clones each breeding cycle. The best apomictic individuals in the best testcross progenies are potential cultivars.

SEED PRODUCTION AND SEED SET

In spite of expectations that apomictic reproduction will circumvent potential reproductive sterility problems, empirical results to date suggest that other factors are operating. The first hybrid brachiariagrass cultivar released, cv. Mulato, has disappointingly low seed yields, in spite of prolific, well-synchronized flowering induced by short daylength. Low seed yields are associated with poor seed set (Hare *et al.* 2007a), rather than low spikelet density. While conventional cultivars (e.g. Basilisk signalgrass, Marandu, or Toledo palisadegrass) routinely produce commercial pure seed yields in the range of 500 to 1,000 kg ha⁻¹ (Hopkinson *et al.* 1996, Souza 1999), average commercial yields of Mulato do not exceed 100 kg ha⁻¹, with maximum yields rarely passing 200 kg ha⁻¹.

Seed yields of Mulato II, under both experimental and commercial conditions, have consistently exceeded those of its predecessor, cv. Mulato. In one trial, the highest seed yield of Mulato II (258 kg ha⁻¹) was 60% higher than the highest seed yield of Mulato (161 kg ha⁻¹) (Hare *et al.* 2007b). In another trial, pure seed yield of Mulato II reached 500 kg ha⁻¹ (Hare *et al.* 2007c). Several smallholder farmers in Thailand regularly hand harvest over 600 kg ha⁻¹.

While the published literature is scanty, poor seed yields, whether owing to poor seed set or shy flowering, seem to be a common defect of newly formed apomictic forage grass hybrids. The first buffelgrass hybrids released from the Texas A&M breeding program (Bashaw 1968, 1980a) were never widely adopted in spite of their improved forage characteristics, and this failure was apparently associated with erratic and often poor seed yields (M.A. Hussey personal communication 2006) and consequently scarce supplies of commercial seed and high

seed prices. A more recent buffelgrass release, cv. Frio, which arose as a spontaneous outcross in the open-pollinated progeny of a facultative buffelgrass plant introduction, is reported to have lower seed set than common buffelgrass (Hussey & Burson 2005). Likewise, a promising, hybrid-derived apomictic bahiagrass clone, Tifton 7, was never released owing to concerns about low seed yields (W.W. Hanna personal communication 2007). Published data on commercial seed yields of guineagrass cultivars (Souza 1999) suggests lower yield for newly synthesized hybrid cultivars (e.g. 130-145 kg ha⁻¹ for ground swept seed of cvs. Centenário and Vencedor) than for cultivars selected directly from collections of natural apomictic germplasm (e.g. 180-200 kg ha⁻¹ for cvs. Tanzânia-1 and Mombaça).

We hypothesize that the low seed yield of new hybrids is owing to insufficiently rigorous selection being applied on seed production characteristics, combined possibly with a negative association between seed yield and vegetative vigor or forage yield. The relatively high seed yields of conventional cultivars selected from natural germplasm is probably attributable mainly to the effect of natural selection of genotypes with prolific seed set from populations of natural hybrids.

In order to achieve progress in the genetic improvement of seed yields, we will need a scheme to select cyclically on this trait. However, there is an important unknown in this regard. We know, for instance, that selection directly on phenotype in our synthetic sexual tetraploid population for spittlebug resistance gives good genetic gain in the population (Miles *et al.* 2006). Further, this gain in resistance is recovered in apomictic hybrids that are candidates for commercial release (CIAT 2007). For seed yield, the feasibility of capturing genetic gains achieved in the sexual population has yet to be demonstrated and is not so obvious. It is possible that the genes that contribute importantly to seed yield - particularly genes contributing to high seed set - in sexually reproducing plants are not the same as the genes contributing to seed set in apomicts, owing to the differences in gametophyte formation, fertilization, and early embryo and endosperm development between the two reproductive types. If this is true, then genetic gain for seed set in the synthetic sexual population may not be fully recovered in apomictic hybrids formed with sexual

clones improved for seed set. This question could be answered experimentally, but this would be a fairly major project involving a comparison of the magnitudes of the correlation of seed set between relatives (e.g. between parent and offspring or between siblings) of the same or of different reproductive modes.

In any case, and until quantitative data are available, it is probably safe to assume that any difference in magnitude of correlations of seed set between relatives of different reproductive modes is unlikely to be so great as to change the sign of the correlation, i.e., that selection on seed set of sexuals will produce a negative correlated response in the resulting apomictic hybrids. Hence, it should be possible to capture, in apomictic hybrids, at least a portion of the genetic response to selection on seed set in the synthetic sexual population.

It ought to be even more effective to select on seed set in testcross progenies, and perhaps better yet to select on seed set of only the apomictic individuals in testcross progenies. However, this would require phenotyping large numbers of individual testcross hybrids for reproductive mode at an early stage of testing to identify the apomictic individuals in testcross progenies. This would compromise efficiency unless there is a very large discrepancy in the genes controlling seed set as between sexual or apomictic genotypes. Such large-scale phenotyping for reproductive mode will be very difficult until a reliable molecular genetic marker of apomixis is developed.

Hence, the current strategy is simply to cull on low seed set at two stages in the recurrent selection cycle: firstly, on differences in seed set among sexual clones per se when they are exposed to pollination by the apomictic tester, and secondly, among sexual clones based on mean seed set of their testcross progenies. The first cycle of selection on testcross performance has not been completed. Hence, the viability of our strategy is as yet untested.

In the meantime, new sexual-by-apomictic hybrids that are candidates for eventual commercialization are now being culled rigorously on seed set at several points in their development, beginning at an early stage. We use the criterion of seed set, rather than seed yield, owing to the fact that

flowering response at our very low-latitude testing sites in Colombia (3° to 4° N) does not necessarily reflect flowering at latitudes more typical of commercial seed production areas (15° to 20°, N or S). We assume that caryopsis formation, or seed set, is largely or entirely independent of latitude.

In a first stage, seed resulting from open-pollination of promising individual plants in unreplicated, space-planted field nurseries of new hybrids is recovered by enclosing mature inflorescences in mesh bags. Crude seed is weighed and "full" seeds (with caryopsis) are separated with a laboratory seed blower. "Percent seed set" is expressed as weight of full seeds divided by total weight of the harvested seed sample. In the most recent data set, these percent seed set values ranged between 0 and 57% for a set of 353 hybrids of unknown reproductive mode (cf. 49% for cv. Basilisk or 41% for cv. Marandu). Fully reliable data on seed yield will require proper confirmation in replicated trials conducted in relevant seed production environments. The preliminary data were used to cull 54% of the pre-selected new hybrids.

The open pollinated seed harvested from space planted hybrids in field nurseries is used to establish progeny trials to assess reproductive mode. A uniform progeny indicates an apomictic hybrid, while segregation within the progeny indicates a sexual hybrid, or a highly sexual, facultative apomict. When the apomictic hybrids have been identified, their seed is harvested by bagging inflorescences. Percent seed set, by

weight, is determined for each apomictic progeny. Hybrids with low seed set are again culled at this stage before promising apomictic hybrids are distributed for further agronomic testing.

Ample variation among our brachiariagrass hybrids has been demonstrated. During 2006, 28 promising new hybrids were identified as apomicts in a progeny test conducted at the main CIAT experiment station. Progenies were replicated from one to four times in 5-plant plots, in a completely randomized design. Open-pollinated seed was harvested by enclosing inflorescences in mesh bags. "Percent seed set", estimated by dividing the weight of full seed by the total weight of harvested seed, ranged from 0 to 41% (cf. 16% for cv. Marandu) ($P < 0.01$).

Culling on seed set in sexual-by-apomictic hybrid populations will help ensure elimination of hybrids with potentially poor seed yield. However, any gain in seed set in a particular hybrid population will not be cumulative over breeding cycles.

Seed yield in apomictic forage grasses should be at least as responsive to selection as grain yield in annual crops and perhaps even more responsive, given the almost complete absence of prior artificial selection on seed yield. Hence, we remain optimistic that, with time, it will be possible to breed cultivars of apomictic tropical forage grasses with high yield of good quality forage combined with high commercial seed yields.

LITERATURE

- Asker, S.E. & Jerling, L. 1992. Apomixis in plants. CRC Press, Inc., Boca Raton, FL, USA.
- Bashaw, E.C. 1968. Registration of Higgins buffelgrass. *Crop Science* 8: 397-398.
- Bashaw, E.C. 1980a. Registration of Nueces and Llano buffelgrass. *Crop Science* 20: 112.
- Bashaw, E.C. 1980b. Apomixis and crop improvement. pp. 45-63. *In*: Fehr, W.R. & Hadley H.H. (eds.). *Hybridization of crop plants*. ASA, CSSA, Madison, WI, USA.
- Bashaw, E.C. & Funk, C.R. 1987. Apomictic grasses. pp. 40-82. *In*: Fehr, W.R. (ed.). *Principles of cultivar development: Vol. 2, crop species*. Macmillan: New York, NY.
- Burton, G.W. 1992. Manipulating apomixis in Paspalum. p. 16-19. *In*: Elgin, J.H. & J.P. Miksche (eds.). *ARS Research Bull. 104*. USDA-ARS: Washington DC.
- Cardona, C., Miles, J.W., & Sotelo, G. 1999. An improved methodology for massive screening of *Brachiaria* spp. genotypes for resistance to *Aeneolamia varia* (Homoptera: Cercopidae). *Journal of Economic Entomology* 92: 490-496.
- CIAT 2007. Annual report 2006. Project IP-5: Tropical grasses and legumes: optimizing genetic diversity for multipurpose use. CIAT, Cali, Colombia. *In press*.

- Ferguson, J.E. & Crowder, L.V. 1974. Cytology and breeding behavior of *Brachiaria ruziziensis* Germain et Evrard. *Crop Science* 14: 893-895.
- Hare, M.D. & Phaikaew, C. 1999. Forage seed production in northeast Thailand. pp. 435-443. *In*: D.S. Loch & Ferguson, J.E. (eds.) Forage seed production. Vol. 2: Tropical and subtropical species. CABI Publishing, Wallingford, UK.
- Hare, M.D., Tatsapong, P. & Saiprasert, K. 2007a. Seed production of two brachiaria hybrid cultivars in north-east Thailand. 1. Method and time of planting. *Tropical Grasslands* 41: 26-34.
- Hare, M.D., Tatsapong, P. & Saiprasert, K. 2007b. Seed production of two *brachiaria* hybrid cultivars in north-east Thailand. 2. Closing date. *Tropical Grasslands* 41: 35-42.
- Hare, M.D., Tatsapong, P. & Saiprasert, K. 2007c. Seed production of two *brachiaria* hybrid cultivars in north-east Thailand. 3. Harvesting method. *Tropical Grasslands* 41: 43-49.
- Hopkinson, J.M., Souza, F.H.D. de, Diulgheroff, S., Ortiz, A., & Sánchez, M. 1996. *In*: Miles, J.W., Maass, B.L., & Valle, C.B. do (eds.) *Brachiaria: Biology, agronomy, and improvement*. pp. 124-140. CIAT, Cali, Colombia and CNPGC/EMBRAPA, Campo Grande, MS, Brazil.
- Hull, F.H. 1945. Recurrent selection for specific combining ability in corn. *Journal American Society of Agronomy* 37: 134-145.
- Hussey, M.A. & Burson, B.L. 2005. Registration of 'Frio' buffelgrass. *Crop Science* 45: 411-412.
- Miles, J.W. 1995. Application of recurrent selection for specific combining ability to the improvement of apomictic *Brachiaria*. *In*: *Harnessing Apomixis, An International Conference*. 25-27 September 1995. College Station, TX, USA. p.64. [Abstract].
- Miles, J.W. 1997. A breeding scheme to exploit heterosis in apomicts. *In*: CIMMYT. 'Book of abstracts. The genetics and exploitation of heterosis in crops; an international symposium'. Mexico, DF, Mexico. pp. 182-183. [Abstract].
- Miles, J.W. (In press). Apomixis for cultivar development in tropical forage grasses. *International Plant Breeding Symposium, Second*. 20-25 August 2006. Mexico City, Mexico.
- Miles, J.W., Cardona, C. & Sotelo, G. 2006. Recurrent selection in a synthetic brachiariagrass population improves resistance to three spittlebug species. *Crop Science* 46: 1088-1093.
- Miles, J.W., & Valle, C.B. do 1996. Manipulation of apomixis in *Brachiaria* breeding. *In*: Miles, J.W., Maass, B.L. & Valle, C.B. do (eds.). *Brachiaria: Biology, agronomy, and improvement*. pp. 164-177. CIAT, Cali, Colombia, and CNPGC/EMBRAPA, Campo Grande, MS, Brazil.
- Miles, J.W., Valle, C.B. do, Rao, I.M. & Euclides, V.P.B. 2004. *Brachiariagrasses*. *In*: L.E. Moser, L.E., Burson, B.L. & Sollenberger, L.E. (eds.) *Warm-season (C₄) grasses*. *Agronomy Monograph* 45. pp. 745-783. ASA, CSSA, SSSA, Madison, WI, USA.
- Ndikumana, J. 1985. Etude de l'hybridation entre espèce apomictiques et sexuées dans le genre *Brachiaria*. Ph.D. Dissertation. Université Catholique de Louvain, Louvain-La-Neuve, Belgium.
- Pernès, J., René-Chaume, R., René, J., & Savidan, Y. 1975. Schéma d'amélioration génétique des complexes agamiques du type *Panicum*. *Cah. ORSTOM, Sér. Biol.* 10: 67-75.
- Rao, I.M., Miles, J.W., & Granobles, J.C. 1998. Differences in tolerance to infertile acid soil stress among germplasm accessions and genetic recombinants of the tropical forage grass genus, *Brachiaria*. *Field Crops Research* 59: 43-52.
- Renvoize, S.A., Clayton, W.D. & Kabuye, C.H.S. 1996. Morphology, taxonomy, and natural distribution of *Brachiaria* (Trin.) Griseb. *In*: Miles, J.W., Maass, B.L., & Valle, C.B. do (eds.). *Brachiaria: Biology, agronomy, and improvement*. pp. 1-15. CIAT, Cali, Colombia, and CNPGC/EMBRAPA, Campo Grande, MS, Brazil.
- Risso-Pascotto, C., Pagliarini, M.S. & Valle, C. B. do. 2005. Meiotic behavior in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (*Poaceae*). *Euphytica* 145: 155-159.
- Santos Filho, L.F. 1996. Seed production: perspective from the Brazilian private sector. *In*: Miles, J.W., Maass, B.L., & Valle, C.B. do (eds.). *Brachiaria: Biology, agronomy, and improvement*. pp. 141-146. CIAT, Cali, Colombia, and CNPGC/EMBRAPA, Campo Grande, MS, Brazil.
- Savidan, Y. 1975. Hérité de l'apomixie. Contribution à l'étude de l'hérité de l'apomixie sur *Panicum maximum* Jacq. (analyse des sacs embryonnaires). *Cah. ORSTOM, Sér. Biol.* 10(2): 91-95.
- Savidan, Y.H. 1983. Genetics and utilization of apomixis for the improvement of guineagrass (*Panicum maximum* Jacq.). *In*: Smith, J.A. & Hays, V.W. (eds.). *Proceedings of the 14th International Grassland Congress*, Lexington, Kentucky, 15-24 June 1981. pp. 182-184. Westview Press, Boulder, CO, USA.
- Savidan, Y. 2000. Apomixis: genetics and breeding. *Plant breeding Reviews* 18: 13-86.
- Souza, F.H.D. de. 1999. *Brachiaria* spp. in Brazil. *In*: Loch, D.S. & Ferguson, J.E. (eds.). Forage seed production. Vol. 2: Tropical and subtropical species. pp. 371-379. CABI Publishing, Wallingford, UK.

- Swenne, A., Louant, B.-P., & Dujardin, M. 1981. Induction par la colchicine de formes autotétraploïdes chez *Brachiaria ruziziensis* Germain et Evrard (Graminée). *Agronomie Tropical* 36: 134-141.
- Taliaferro, C. M. & Bashaw, E.C. 1966. Inheritance and control of obligate apomixis in breeding buffelgrass, *Pennisetum ciliare*. *Crop Science* 6: 473-476.
- Valle, C. B. do, Glienke, C., & Leguizamon, G.O.C. 1994. Inheritance of apomixis in *Brachiaria*, a tropical forage grass. *Apomixis Newsletter* 7: 42-43.
- Valle, C.B. do, & Savidan, Y.H. 1996. Genetics, cytogenetics and reproductive biology of *Brachiaria*. *In: Miles, J.W., Maass, B.L., & Valle, C.B. do (eds.). Brachiaria: Biology, agronomy, and improvement.* pp. 147-163. CIAT, Cali, Colombia, and CNPQC/EMBRAPA, Campo Grande, MS, Brazil.
- Vogel, K.P., & Burson, B.L. 2004. Breeding and genetics. p. 51-94. *In: Moser, L.E., Burson, B.L., & Sollenberger, L.E. (eds.). Warm-season (C₄) grasses.* Agronomy Monograph 45. ASA, CSSA, SSSA, Madison, WI, USA.

Sources of new tropical forage cultivars: Past, present and future

Donald S. Loch

35 Hilltop Crescent, Alexandra Hills, Q 4161, Australia

lochd@bigpond.com

ABSTRACT

The continuing release of new cultivars is important for forage/pasture seed industries worldwide because of its positive impact on the demand for seed. Prior to the 1960s, new cultivars came mainly from African countries. From the 1960s to the late 1990s, Australia became the major developer of new forage cultivars, but declined in importance as public research funding was reduced. Since the early 1980s, CIAT and South American countries have become increasingly important sources of new cultivars. The major source of new cultivars has been superior natural ecotypes. Since the 1960s, conventional plant breeding has also played a minor role, limited by the ready availability of good natural ecotypes in germplasm banks and the predominance of apomixis (asexual reproduction) among tropical grasses. Novel breeding strategies have recently been developed to unlock genetic variation in apomictic grasses. Private funding to develop proprietary cultivars (mainly from major species, particularly grasses, because of market size considerations) is increasing. Implications for developing countries - historically dependent on developed countries for their forage cultivars - are also explored.

Key words: pasture, plant breeding, seed marketing, research funding

INTRODUCTION

The continuing release of new cultivars is important for forage/pasture seed industries worldwide because of its positive impact on the demand for seed. In a recent overview of tropical forage seed production, Loch & Ferguson (1999) listed some 490 tropical forage cultivars from more than 65 grass and 50 legume taxa (species, sub-species, botanical varieties) that had been made available to commerce as formal or informal releases at some time in the past >100 years (but mostly in the past 50 years or so). Of these, more than 70% were grasses; and approximately one-third of the grasses and half of the legumes were represented by a single cultivar. By comparison, seed crops of >550 cultivars from just 8 grass species were at that time being grown in Oregon (USA) - more than the total number of tropical cultivars, current and obsolete, listed by Loch & Ferguson (1999).

The emphasis on developing new commercial species rather than new cultivars of existing

commercial species reflects to some extent the relatively short history of grassland science in the tropics, and its earlier stage of development compared with temperate areas. More importantly, though, it also reflects the great diversity of environment and of farming systems in the tropics. Forage use ranges from extensive pure-grass or grass-legume pastures in Australia and Brazil to small intensively managed areas (mostly less than 0.5 ha) in southern and South-East Asia. Vegetative planting of grasses (particularly *Cynodon* and *Pennisetum* spp.) is common in many countries under better rainfall and/or irrigation. However, the great majority of tropical forages are propagated by seed.

This paper explores the development of tropical forage species and cultivars to date. It looks at changes in the factors, the countries and the methods that have contributed to the development and release of new forages. Finally, it looks at current trends and their likely future impact on new releases in an increasingly globalised market.

HISTORICAL DEVELOPMENT OF TROPICAL FORAGE CULTIVARS

Pre-1930

The first cultivars of some of what later became major tropical grass species were available in various countries by the early 1900s. These included *Cenchrus ciliaris* (syn. *Pennisetum ciliare* - buffel grass), *Chloris gayana* (Rhodes grass), *Panicum maximum* (Guinea grass), *Paspalum dilatatum* (paspalum/Dallisgrass) and *Pennisetum purpureum* (elephant grass/Napier grass). While seed (or in some cases vegetative planting material) of these species was mainly produced to meet local demand as agricultural and pastoral activities expanded into new regions, significant international export markets also developed for species like *C. gayana* and *P. dilatatum*.

In contrast, there were very few tropical legumes commercially available during this era. Notable exceptions were the annuals *Stylosanthes humilis* (Townsville stylo) and *Macroptilium lathyroides* (phasey bean) in Australia and *Alysicarpus vaginalis* (alyce clover) in USA.

1930-59

Africa. The modern era of forage species/cultivar development began in the 1930s in colonial Africa. The main activities here were centred in East African countries (mainly Kenya and Tanzania) and Zimbabwe, with additional contributions from South Africa and other African countries. A number of new cultivars were added to existing economic species, particularly *C. gayana*, *C. ciliaris*, *P. maximum* and *P. purpureum*. Cultivars were also developed from several species new to modern agriculture, mostly grasses; these included the grasses *Digitaria eriantha* subsp. *eriantha* (digit grass), *Eragrostis curvula* (weeping lovegrass), *Panicum coloratum* (coloured Guinea/Makarikari grass) and *Setaria sphacelata* (setaria), and the legumes *Lablab purpureus* (lablab), *Neonotonia wightii* (glycine) and *Trifolium semipilosum* (Kenya white clover). While many of these early cultivars have long since become obsolete and are no longer grown commercially, others like *L. purpureus* 'Rongai' in Kenya, *C. gayana* 'Katambora' in Zimbabwe and *P. coloratum* 'Bambatsi' in South Africa (both collected by Dr Oliver West), and *D. eriantha* 'Irene' in

South Africa are still extensively grown today, albeit with some inevitable genetic drift in the last three outbreeding grass cultivars following uncontrolled multiplication in different countries and/or regions for >50 years.

Prominent during this golden age of pasture research in Africa was A.V. Bogdan, who worked as Pasture Research Officer in Kenya for 19 years during the 1950s and 1960s. In addition to the numerous cultivars and new economic species he developed, Bogdan conducted pioneering research on a variety of diverse topics, including flowering behaviour and breeding system, pollen movement as related to isolation distance, stolon and seed morphology, and sowing depth.

Australia. In Australia, the development of grass-legume pastures became a major goal as tropical pasture research by the Commonwealth Scientific and Industrial Organization (CSIRO) and the Queensland Department of Primary Industries (QDPI) in particular intensified during the late 1940s and 1950s (Eyles *et al.* 1985). The impact of this research was seen first in the increasing number of new grass cultivars developed during the 1950s, particularly *C. ciliaris*. In turn, the release of new cultivars coincided with increasing mechanisation of seed harvesting and emerging large markets for seed of *C. ciliaris*, *C. gayana*, *P. maximum* var. *trichoglume* (green panic) and *Sorghum almum* to sow new pastures, particularly in the Brigalow region.

USA. Vegetative propagation is extensively used for certain forages in southern USA, particularly under high rainfall and/or with irrigation. This situation first emerged during the 1940s with the release of the initial *Cynodon* hybrids from Tifton (Georgia) and the development of effective machinery for planting vegetative material of them (Burton 1954). The widely used *Paspalum notatum* (bahiagrass) cultivars Pensacola and Argentine were also first released during the 1940s, and now account for large tonnages of seed still produced mainly by opportunist harvesting in Florida.

1960-79

Australia. From 1960 onwards, new legume releases also contributed to the expanding range of Australian-developed cultivars as the so-called "tropical pasture revolution" gathered pace. An important landmark and

catalyst for further research was the release of the *Macroptilium atropurpureum* cultivar 'Siratro' in 1960. This was bred by Dr Mark Hutton from a cross between two parent lines of the species collected in Mexico (Hutton 1962, Oram, 1990). Following selection of superior recombinants in the F2 population, the F3 and F4 families were grown in mixed pasture plots with *C. gayana* and subjected to heavy intermittent grazing with cattle. Three families, selected under this system, were combined in equal proportions to form 'Siratro'.

During this 20-year period, 29 new grass cultivars from 18 different species were released. The 31 new legume cultivars released during the same period came from 20 different species.

Africa. In Kenya, releases from Bogdan's program continued through to about the mid-1960s, and the last of Dr Jos Boonman's bred varieties were released in the mid-1970s. Some work also continued in Zimbabwe. However, the loss of experienced scientists together with the necessary research funding and supporting infrastructure effectively ended local exploitation of Africa's rich forage germplasm resources.

1980-present

Latin America. Until the mid-1970s, many of the forage cultivars used in tropical Latin America were imported along with the seed, mainly from Australia. Around that time, local seed production began, particularly in Brazil the cost of imported seed stimulated. From about the early 1980s, the Centro Internacional de Agricultura Tropical (CIAT) and the national pasture programs in Brazil and other South American countries also became increasingly important sources of new cultivars. Strong domestic seed markets quickly emerged, driven both by new pasture plantings (firstly in the Amazon region and later in the Cerrados in Brazil) and by the need to re-plant pastures after a cropping phase. During the 1990s, however, funding of CIAT's pasture research program and the scientific staff to implement it have been progressively and drastically cut back, thereby greatly restricting future new cultivar releases from this source.

The pattern of forage use (and hence seed production) has changed radically since the 1970s. In Brazil, the market was initially dominated by *Hyparrhenia rufa* (jaragua),

Melinis minutiflora (molasses grass) and *P. maximum*. Production throughout South America is now dominated by the *Brachiaria* species, together with much smaller amounts of *Andropogon gayanus* (gamba grass). While much of the basic work was done by CIAT, new cultivars of *B. decumbens* (signal grass), *B. humidicola* (Koronivia grass), *B. brizantha* and *A. gayanus* were progressively released through the national programs. The new grasses were complemented by a number of new legume releases, of which *Stylosanthes guianensis* (stylo) 'Pucallpa' has been the most widely successful; nevertheless, local markets are still dominated by grass seeds. Export markets, mainly for grass seeds, have also developed to other parts of Latin America and to some Asian countries.

Australia. Many of new grass cultivars developed during the 1960s and 1970s could be broadly classed as coming from "high fertility" species, such as *C. gayana*, *P. maximum* and *Pennisetum clandestinum* (kikuyu grass). In part, this reflects the more fertile areas then being developed for forage use. From about 1980 onwards, greater emphasis was given to medium and lower fertility grasses, such as *Bothriochloa*, *Dichanthium* and *Digitaria* species. Similarly, many of the early legumes were bulky twining or sprawling species like *M atropurpureum* and the *Desmodium* species (*D. intortum* and *D. uncinatum*); these produced high forage yields under experimental cutting regimes, but failed to persist commercially under constant grazing. Again the focus moved from about the late 1970s, this time to less fertility demanding and more persistent, grazing tolerant legumes such as the *Aeschynomene* and *Arachis* spp., *Chamaecrista rotundifolia* (round-leafed cassia) and *Vigna parkeri* (creeping vigna).

In the 2 decades following 1980, 38 new grass cultivars from 20 species were released, together with 27 new legume cultivars from 20 species. Although better adapted to lower soil fertility and heavier grazing pressure than their predecessors, many of these have yet to make their mark in extensive pasture plantings. Beginning in the 1980s and accelerating through to the late 1990s, public and industry research funding was reduced to the point where there can be little further development of new cultivars through publicly-funded research.

In Australia, the major tropical forage grass species (*C. ciliaris*, *C. gayana*, *P. coloratum*, 'Silk' *Sorghum*) are adapted to medium and lower rainfall areas, which are the major markets for forage seed. Similarly, the main perennial legumes are the *Stylosanthes* spp., *S. hamata* (Caribbean stylo) and *S. scabra* (shrubby stylo), which are adapted to the extensive areas of low fertility soils in these same districts. Apart from the main species used, another significant difference relative to other tropical countries is the minimal use that has been made of vegetatively propagated species and cultivars in Australia. This reflects both the relatively dry environment in northern Australia, and the underlying philosophy that dissemination of improved forage material as seed is more rapid and effective.

GERMPLASM EVALUATION VS. PLANT BREEDING

The overwhelming source of new tropical forage cultivars to date has been natural ecotypes, whose superiority is determined by through germplasm evaluation. Since the early 1960s, conventional plant breeding has also played a minor role, but the ready availability of good natural ecotypes of many species of interest in germplasm banks means that this has become the preferred (and easier and cheaper) route in most cases. Both Ferguson & Loch (1999) and Miles (2001) have commented on the limited success of plant breeding with tropical forage species thus far and highlighted the main reasons for lack of successful bred cultivars.

The release of *M. atropurpureum* 'Siratro' in 1960 triggered a substantial investment in plant breeding by CSIRO for the next 2 decades (with a maximum of 9 plant breeders employed in 1969 - Miles, 2001) before recognising the very limited commercial success generated by this effort and progressively withdrawing these resources during the 1980s and 1990s. Firstly, breeding efforts were concentrated on legumes, with only one breeder dedicated to grass work (but not on any of the major economic species at that time). In general, plant breeders employed by private seed companies focus their breeding efforts on the major economic species before considering minor or niche species. Secondly, plant breeding in many cases was premature. For species new to

agriculture, evaluation of existing ecotypes (germplasm accessions) is the logical starting point from which to generate the first cultivar(s) and through commercial use develop information as to where future plant breeding efforts might be usefully directed. As the natural variation within an established species becomes exhausted by cultivar development, selection within existing or hybridised populations becomes increasingly attractive. Thirdly, plant breeders need to develop good contact with the commercial sector (seed companies and farmers) to ensure that their breeding objectives, strategies and outcomes are relevant. No matter how academically elegant the breeding strategy, the final bred cultivar must meet real, not imagined, industry needs if it is to be adopted. The first and third comments are equally applicable to germplasm evaluation.

The predominance of apomixis (asexual reproduction) among tropical grasses is frequently cited as an obstacle to plant breeders. However, while hybridisation of the numerous apomictic tropical grasses is more difficult than among outbreeding genotypes, it is not impossible as shown by Miles *et al.* (2004) with *Brachiaria* species where they have been able to use a tetraploidised sexual *B. ruziziensis* (Ruzi grass) line (developed by doubling the chromosomes in normal sexual diploid material) to make inter-specific crosses, which later segregate to give some stable apomictic lines for evaluation.

FUTURE TRENDS

The withdrawal of funding from the major pasture research programs that were developing new cultivars in Australia and at CIAT in South America has now slowed new cultivar releases to a trickle; resources have also been cut in pasture programs in the southern states of the US. At the same time, this affects developing countries, which have been dependent on those major programs for cultivars and experimental material to trial under their conditions. Even in Thailand where tropical forage use is now well-established (Hare 1993), the main cultivars in use have come from Australia (*B. ruziziensis* 'Kennedy' and *S. hamata* 'Verano') and South America (*S. guianensis* 'Pucallpa'). For future germplasm evaluation activities, access to the major tropical international germplasm

collections held in Australia and at CIAT and the International Livestock Research Institute (ILRI) is vital. Information on promising accessions as well as released cultivars collated by Cook *et al.* (2005) should provide welcome assistance in the future for scientists in developing countries in lieu of personal guidance from experienced scientists previously employed in the developed country programs.

While niche cultivars from minor species may still have a place for special purposes in developing countries, the developed countries are more likely to see a consolidation of the tropical forage species used, with new cultivars being developed from fewer species of greater economic importance. The advent of Plant Breeder's Rights (PBR) in an increasing number of tropical countries enables the intellectual property embodied in new bred cultivars to be protected, both on domestic markets and on selected export markets. This may provide renewed impetus for plant breeding programs, this time funded privately by commercial seed companies rather than relying on public or industry

funds. At the same time, the use of biotechnology as a plant improvement tool is effectively precluded by its high cost relative to the small size of most tropical forage seed markets. Environmental concerns about the potential weediness of certain tropical forage species (e.g. Humphries *et al.* 1991, Low 1997) could again shift the emphasis in plant improvement programs towards a more limited number of already commercial species.

Private funding of future tropical forage breeding programs (irrespective of whether these are conducted by employing private breeders or by way of contract with public research institutions) should ensure a more commercial focus than previously; but will be restricted to more profitable major species as shown by Frey (1996) with temperate species. The role of producing improved cultivars of minor species will remain with public research agencies; but it is not clear where (or, indeed, if) public and/or industry funding will become available for this purpose in the future.

LITERATURE

- Burton, G.W. 1954. Coastal Bermuda grass for pasture, hay and silage. Georgia Agricultural Experiment Stations Bulletin NS 2.
- Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. & Schultze-Kraft, R. 2005. Tropical Forages: An Interactive Selection Tool. (CD-ROM). CSIRO, DPI&F (Qld), CIAT and ILRI, Brisbane, Australia.
- Eyles, A.G., Cameron, D.G. & Hacker, J.B. 1985. Pasture Research in Northern Australia - Its History, Achievements and Future Emphasis. Research Report No. 4, CSIRO Division of Tropical Crops and Pastures, Brisbane, Australia.
- Ferguson, J.E. & Loch, D.S. 1999. Tropical and subtropical forage seed production: looking back and to future horizons. In Loch, D.S. & Ferguson, J.E. (eds.), Forage Seed Production. 2. Tropical and Subtropical Species pp. 445-457. CAB International.
- Frey, K.J. 1996. National plant breeding study - I. Human and financial resources devoted to plant breeding research and development in the United States in 1994. Iowa Agriculture and Home Economics Experiment Station Special Report 98.
- Hare, M.D. 1993. Development of tropical pasture seed production in Northeast Thailand - two decades of progress. *Journal of Applied Seed Production* 11: 93-96.
- Humphries, S.E., Groves, R.H. & Mitchell, D.S. 1991. Plant invasions of Australian ecosystems: a status review and management directions. *Kowari* 2: 1-127.
- Hutton, E.M. 1962. Siratro - a tropical pasture legume bred from *Phaseolus* DC. *Australian Journal of Experimental Agriculture & Animal Husbandry* 2: 117-125.
- Loch, D.S. & Ferguson, J.E. 1999. Tropical and subtropical forage seed production: an overview. In: Loch, D.S. & Ferguson, J.E. (eds.). Forage Seed Production. 2. Tropical and Subtropical Species pp. 1-40. CAB International.
- Low, T. 1997. Tropical pasture plants as weeds. *Tropical Grasslands* 31: 337-343.
- Miles, J.W. 2001. Achievements and perspectives in the breeding of tropical grasses and legumes, pp. 509-515. Proceedings of the XIX International Grassland Congress.

Miles, J.W., Do Valle, C.B., Rao, I.M. & Euclides, V.P.B. 2004. Brachiariagrasses. In Moser, L.E., Burson, B.L. & Sollenberger, L.E. (eds) Warm-season (C_4) grasses. pp. 745-783. Agronomy Monograph 45. ASA, CSSA & SSSA, Madison, Wisconsin, USA.

Oram, R.N. 1990. Register of Australian Herbage Plant Cultivars (third Edition). Australian Herbage Plant Registration Authority, CSIRO Division of Plant Industry, Melbourne, Australia. (Cited 1 May 2007. Also available through <http://www.pi.csiro.au/ahpc/>).

Seed and seedling response to salinity effects in two species of *Atriplex halimus* and *Atriplex semibaccata*

F. Arab¹⁾, A. Jafari²⁾, M. Jafari³⁾, M.H. Assareh²⁾ & A.Tavili³⁾

¹⁾Postgraduate student of Faculty of Natural Resource, University of Tehran, Iran

²⁾Scientific Boards of Research Institute of Frosts and Rangelands, Tehran, Iran

³⁾Scientific Boards of Faculty of Natural Resource, University of Tehran, Iran

arab_fahimeh@yahoo.com

ABSTRACT

Atriplex sp. with many species, have quick growing and good compatibility to different areas of desert environments in central rangelands of Iran. It is being used for grazing by livestock for its higher forage nutritive values and sufficient protein content. This research was conducted to determine the more salinity tolerant *Atriplex* among two species of *Atriplex halimus* and *Atriplex semibaccata*. For this propose, for each species a factorial experiment was conducted using five levels of 0, 100, 200, 300 and 400 mM NaCl and CaCl₂ on seed characteristics. Seeds were sown on laboratory and data were recorded and analyzed for percent and speed of germination, length of root and shoot and vigor index. The results showed significant differences between the two species for germination percent and speed of germination ($P \leq 0.01$). The higher values were obtained for *Atriplex halimus* (Fig. 1). The main effects of salinity were significant for all traits in both species ($P \leq 0.01$) and the salinity by species interactions were significant for germination percent and speed of germination ($P \leq 0.01$). All traits were generally decreased by increasing salt concentration. For germination percent there was no significant difference between the control and 200 mM salt for *Atriplex halimus* and between the control and 100 mM salt for *Atriplex semibaccata*. For vigor index, the higher values were obtained for *Atriplex halimus* in 200 mM salt concentration, suggesting that this species is more tolerant to salinity than *Atriplex semibaccata*. The regression equation between salinity level and seed germination were quadratic and linear for *Atriplex halimus* and *Atriplex semibaccata*, respectively. The regression equations were quadratic and linear for speed of germination and vigor index in the both species, respectively. Using probit analyses LD₅₀ were estimated as 224 and 164 mM salt concentration for *Atriplex halimus* and *Atriplex semibaccata*, respectively. The results indicated that *Atriplex halimus* was more tolerant than *Atriplex semibaccata* to salinity. *Atriplex halimus* can be used for cultivation in moderately saline rangelands.

Key words: *Atriplex halimus*, *Atriplex semibaccata*, germination percent, salinity, seed vigor, speed of germination.

INTRODUCTION

Salt problems are of great concern in arid and semiarid regions, where soil salt content is naturally high and precipitation is insufficient for leaching. Although some crops are moderately tolerant of saline conditions, many crops are adversely affected by even low levels of salt (Jeannette *et al.* 2002). The genus *Atriplex* includes important species with diverse ecological adaptations. It is

being used for grazing and soil conservation. It grows naturally in most of the central parts of Iran and known as a quick-growing plant for the production of usable forage. It is important in arid and salty lands of Iran as a valuable food resource in the winter when there is no green forage or a decrease in the mean nutritional components. For rangeland establishment in salinity area, one of the most effective methods is planting of salt-tolerant species in salt stressed situation. To

develop highly salt-tolerant *Atriplex* sp. the present study was undertaken with *Atriplex halimus* and *Atriplex semibaccata* during seed germination and early seedling growth. The aim of this study was to examine the range of salinity tolerance among two *Atriplex* species and to confirm the reproducibility of the germination and seedling growth performance.

MATERIALS AND METHODS

A factorial experiment based on completely randomized design was conducted in Gene Bank division in Research Institute of Forests and Rangelands, Tehran, Iran, in December 2005. For each species two accessions were collected. The normal ISTA (1985) laboratory germination test procedure was used at five levels of salinity: 0, 100, 200, 300 and 400 mM of Sodium and Calcium chloride in a ratio of 1:1. For each accession 100 pure seeds were sterilized with 70% ethyl alcohol for five minutes and washed with distilled water. Four replicates (25 seeds per replicate) of sterilized seed were placed in Petri dishes on double Whatman papers (TP). For protection against molds, the water used to moisten the seed samples and substrata contained 0.002 % benomyl fungicide. The samples were immediately transferred into a germinator at $20\pm 4^\circ\text{C}$ with 1000 lux light for 15 days.

The percent and speed of germination were recorded at 3, 6, 9, 12 and 15 days. In accordance with Maguire (1962), the speed of germination was calculated by the following equation:

$$G.S = \frac{\sum n}{\sum n(n \times DN)} \times 100$$

where n= is the number of seed germinated on days, DN= the number of days after sowing

Table 1. Analysis of variance of germination percent, speed of germination, seedling length (mm) and vigor index in *Atriplex halimus* and *Atriplex semibaccata* species.

Source of variances	Degree of freedom	Germination %	Speed of germination	Seedling length (mm)	Vigor index
Species	1	4961 **	67.5 **	13.9	189.7
Salt	4	23981 **	60.6 **	4369 **	5050 **
Species x salt	4	877 **	11.5 **	83.5	48.6
Error	30	158.4	0.42	80	63
CV%		24	28	31	6

, ** = mean of squares are significant at 5% and 1%, respectively.

corresponding to n, and the highest G.S. is the fastest speed.

After growth of seedlings for 15 days, the length of roots and shoots of 10 randomly-selected seedlings from each replicate were measured. The vigour index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time (Abdul-Baki & Anderson 1973). It is calculated by following equation:

$$Vi = \frac{\%Gr \times MSH}{100}$$

where: VI = vigour index, %Gr. = final germination percentage, and MSH = mean seedling height.

Salinity and species effects were determined by analysis of variance (SAS Inst. 2004, Table 1). Treatment means were compared using the Duncan method. Regression analysis was performed to define linear relationships between each variable and the salinity level. LD₅₀ values were estimated using probit analyses.

RESULTS AND DISCUSSION

The results showed significant differences between the two species for germination percent and speed of germination ($P \leq 0.01$). The higher values were obtained for *Atriplex halimus* at all salinity levels (Fig. 1). The main effects of salinity were significant for all traits in both species ($P \leq 0.01$), values generally decreasing with increasing salt concentration. For germination percent there was no significant difference between control and 200 mM salt for *Atriplex halimus* and between control and 100 mM salt for *Atriplex semibaccata* (Fig. 1). The results indicate that the former species is more salt tolerant.

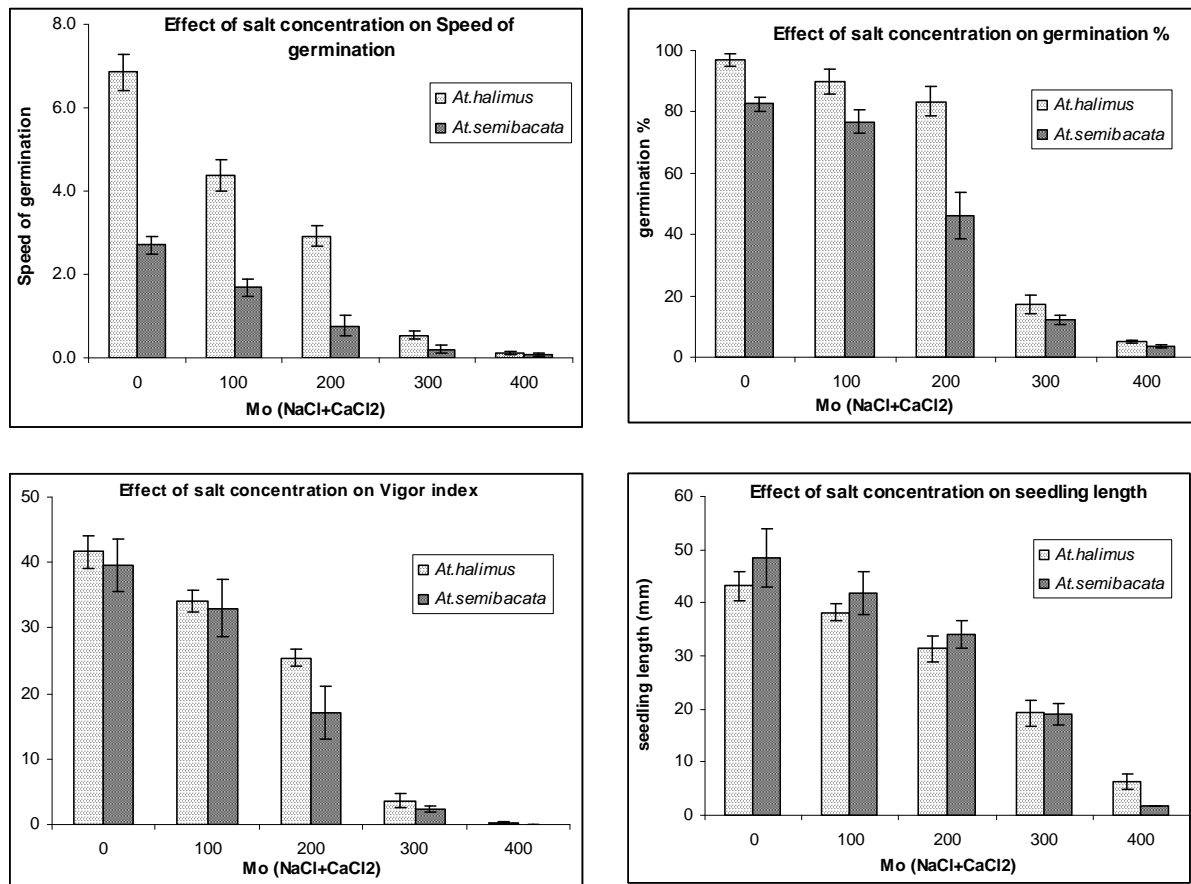


Fig. 1. Effect of salt concentration on germination percent, speed of germination, seedling length and vigor index in two *Atriplex* species

Table 2. Results of probit analysis for determination of lethal dose (LD₅₀ and LD₉₅) for seeds/seedlings of *Atriplex halimus* and *Atriplex semibaccata*.

Species	LD ₅₀	LD ₉₅
<i>Atriplex halimus</i>	224	479
<i>Atriplex semibaccata</i>	164	416

The salinity by species interaction was significant for germination percent and speed of germination ($P \leq 0.01$). For vigor index, the higher values were obtained for *Atriplex halimus* in 200 mM salt concentration, also suggesting that it was more tolerant to salinity than *Atriplex semibaccata* (Fig. 1).

The result of this study verifies Munns & Termaat's (1986) findings that salinity decreases length of seedlings. Iqbal *et al.* (1998) reported that plants will use more vital energy to absorb water as soil salinity increases. Because seedlings can't consume all their initial energy to overcome the osmotic pressure of soil solution, they will use part of their initial energy to absorb water from the soil and the remaining part to perform their metabolic activeness. Under such conditions seedling growth will be limited.

Regression analysis was performed to define relationships between each variable and the salinity level. The relationships between salinity level and seed germination percent were quadratic and linear for *Atriplex halimus* and *Atriplex semibaccata*, respectively (Fig. 2). In both species, the regression equations for speed of germination and seedling length were quadratic, while those for vigor index were linear.

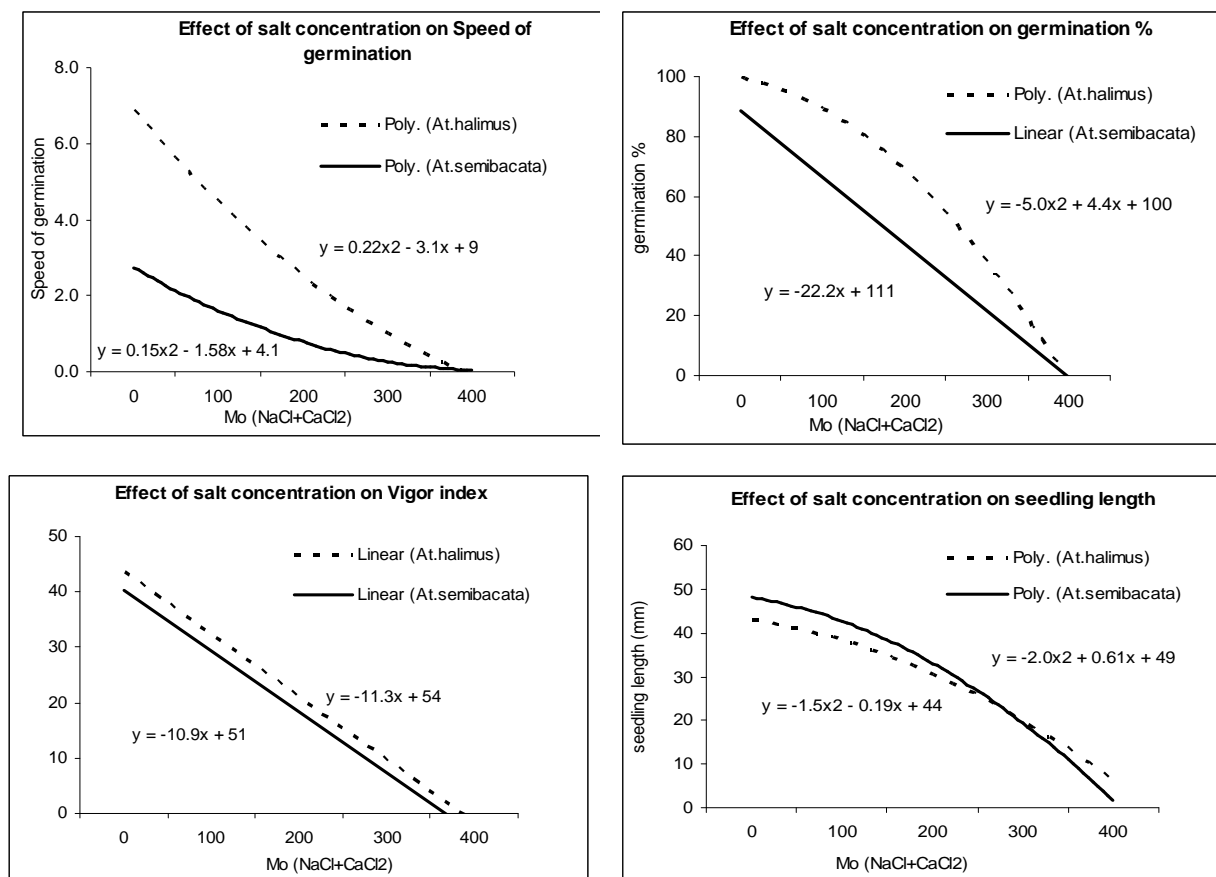


Fig. 2. Salinity curves and regression equations for germination percent, speed of germination, seedling length and vigor index in two *Atriplex* species

The lethal doses for 50 % and 95 % death of seeds / seedlings (LD₅₀ and LD₉₅) were higher in *Atriplex halimus* than in *Atriplex semibaccata*. Along with the other results presented in this paper, this suggested that *Atriplex halimus* is more salt-tolerant than *Atriplex semibaccata* and can be used for cultivation in moderate saline rangelands.

ACKNOWLEDGEMENTS

The authors thank Dr M.H. Arefi, Head of Iranian Natural Resource Gene Bank, that provided seed samples.

LITERATURE

- Iqbal N., Ashraf, M., Javad F., Iqbal Z. & Shah G.H. 1998. Effect of salinity on germination and seedling growth of wheat (*Triticum aestivum* L.). Pakistan Journal of Biological Sciences 1: 226-227.
- Munns R. & Termaat A. 1986. Whole plant responses to salinity. Australian Journal of Plant Physiology 13: 143-160.
- Jeannette S., Bayuelo-Jiménez, R. & Jonathan P. 2002. Salinity tolerance of *Phaseolus* species during germination and early seedling growth. Crop Science 42: 1584-1594.
- Abdul-Baki A.A. & Anderson, J.D. 1973. Vigor determination in soybean seed by multiplication. Crop Science 3: 630-633.
- International Seed Testing Association 1985. International Rules for Seed Testing. Seed Science and Technology 13: 365-513.
- Maguire, J.D. 1962. Speed of germination in selection and evaluation for seedling vigor. Crop Science 2: 176-177.

Development of new *Chloris gayana* cultivars with improved salt tolerance from 'Finecut' and 'Topcut'

Margaret Zorin¹⁾ & Donald S. Loch²⁾

¹⁾Selected Seeds Pty Ltd, 167 Collingwood Road, Birkdale, Q 4159, Australia

²⁾35 Hilltop Crescent, Alexandra Hills, Q 4161, Australia

margaret_zorin@primusonline.com.au

ABSTRACT

Chloris gayana (Rhodes grass) is an outbreeding species with both diploid and tetraploid genotypes. In the work reported, 4 generations of mass selection for improved salt tolerance and forage quality were applied to 3 breeding populations derived from the diploid cultivars 'Finecut' and 'Topcut'. First generation seedlings of 'Finecut' population were divided into 2 groups (earlier flowering, more erect genotypes primarily for hay use and later flowering, more spreading genotypes primarily for grazing use), while selection within the single 'Topcut' population focused on the more erect genotypes primarily for hay use. Within each generation, selection for the desired attributes was applied in 3 stages: (i) germination under saline conditions; (ii) growth and survival under saline conditions; and (iii) agronomic attributes under non-saline conditions. Three new synthetic cultivars of Rhodes grass, each based on elite single-plant clones selected from the final generation, have been produced from the three breeding populations. 'Gulf' (12 clones, hay use) and 'Reclaimer' (15 clones, grazing use) were derived from 'Finecut', and 'Salcut' (15 clones, hay use) was derived from 'Topcut'.

Key words: agronomic attributes, breeding, germination, rhodes grass, salt glands, survival,

INTRODUCTION

Rhodes grass (*Chloris gayana*) is one of the more salt-tolerant C4 forage grasses, and is widely sown as a major forage species, both in Australia and overseas (Loch *et al.* 2004). In subtropical and warm temperate eastern Australia where Rhodes grass has been grown for >100 years, it is well adapted to a wide range of medium-textured soils under moderate rainfalls (c. 600-1200 mm annual average). It is still used most widely in grazed pastures and short-term pasture leys, but is increasingly being grown for hay or silage production and is highly regarded for soil conservation and remediation purposes.

Dryland salinity in Australia is increasing (mainly from south-western Australia through to northern NSW and southern Queensland), so much so that the Australian government introduced an A\$1.4 billion National Action Plan in Nov. 2000 to tackle salinity and water quality problems across the nation. The primary cause is the clearing of vegetation

from recharge areas, leading to rising water tables in discharge areas at the lower end of the landscape. During the past decade or so, Rhodes grass has been introduced to Western Australia where it provides a year-round grass cover rather than the seasonal (autumn-spring) cover provided by the more traditional temperate pasture species. Excellent Rhodes grass pastures have been established, usually down to the edge of discharge areas where salinity becomes more concentrated through water movement and where the level of salt tolerance in the current varieties is insufficient to cope with the levels of salinity present. Varieties that are more salt-tolerant would overcome this current limitation.

Rhodes grass is one of the few out-breeding subtropical forage grasses, and shows variation in salt tolerance and other agronomic attributes in unselected populations and cultivars. The present work follows an earlier breeding program (also funded by Selected Seeds Pty Ltd), which led to the release of 'Finecut' and 'Topcut'.

These improved cultivars, 'Finecut' in particular, have captured a significant part of the Middle East market for Rhodes grass seed, in addition to strong domestic sales. The new breeding program was primarily directed at increasing salt tolerance levels in 'Finecut' and 'Topcut', while at the same time seeking to enhance their hay production and grazing performance for both domestic and export markets.

MATERIALS AND METHODS

Breeding Strategy

Mass selection was applied to four generations of seedlings derived from 'Finecut' and 'Topcut' Rhodes grass (2001-05) with the aim of improving both the level of salt tolerance and the agronomic attributes across each of three breeding populations. Based on first generation performance, the 'Finecut' population was divided into earlier flowering, more upright genotypes (primarily for hay) and later-flowering, more spreading genotypes (primarily for grazing). Selection within the 'Topcut' population remained focused throughout on the more upright genotypes (primarily for hay).

In the first generation, selection for salt tolerance related only to plant growth and survival under high salinity, and was followed by selection for improved agronomic characters. Selection for germination under saline conditions was added from the second generation onwards, giving the same three-stage selection process for generations 2, 3 and 4:

1. Germination under saline conditions;
2. Growth and survival under saline conditions; and
3. Agronomic performance under non-saline conditions.

Agronomic selection within the 2 populations aimed primarily at hay production was directed towards early-flowering, fine-stemmed plants with a dense, leafy, upright growth habit. Selection within the third population aimed primarily at grazing use was directed instead at later-flowering plants with a more spreading habit of growth, but still with dense, leafy growth.

Breeding Procedures

First generation seedlings (2000 each from Breeder's seed of 'Finecut' and 'Topcut') were germinated in water and established individually in a soil-free peat-vermiculite mix in 5 cm square tubes. As the first of these seedlings started to tiller, they were transferred to trays with the lower one-third of the tube immersed in a salt (NaCl) solution, the concentration of which was progressively raised by 0.2M every 2-3 days to 0.7M NaCl (based on Malkin & Waisel (1986)). These were held at the final 0.7M NaCl level for approximately 2 months by which time approximately 85-90% of the plants had died. The remaining plants (300 'Finecut', 168 'Topcut') were removed, washed, re-potted in fresh peat-vermiculite mix, and later transplanted into the field as spaced plants on a 3 m x 3 m grid. Date of first flowering (defined as 3 fully exerted inflorescences per plant) was recorded at weekly intervals. Mature plants were assessed for leafiness, density, habit, and fineness of leaf and stem.

Based on these data, 18 plants from 'Finecut' and 8 from 'Topcut' were selected as superior hay types (viz., dense, leafy, erect habit, fine leaf and stem, and early flowering). A further 12 plants from 'Finecut' were selected as superior grazing types (viz., dense, leafy, spreading stoloniferous habit, and late flowering). The selected plants were re-established in 30 cm pots, and each of the three groups grown in isolation to produce seed to initiate the next generation.

Selection for salt tolerance during germination was introduced in the second generation. Germination was conducted in sand bottom-watered with a nutrient solution containing 0.2M NaCl (determined from preliminary trials with seeds of two Rhodes grass cultivars and solutions with 0-0.5M NaCl). At the start of generations 2-4, seed from the previous generation was sown and lightly covered by sand in a "flood-and-drain" hydroponic system with 0.2 M NaCl plus complete plant nutrients. Despite heavy seeding rates, numbers of germinated seedlings were initially quite small, indicating very heavy selection pressure for germination under saline conditions. Identity of the maternal parents from the previous generation was maintained up to this point, and the performance of these individual lines confirmed that the progeny of some parent plants were less salt tolerant during germination than other parent plants. The

germinated seedlings were then subjected to bottom watering with saline water increasing to levels of 0.7-1.0M NaCl. The final surviving plants (<5% of the number that could have potentially germinated in the absence of salt) were then grown to maturity as spaced plants under non-saline conditions in the field near Brisbane (Queensland, Australia) to assess their agronomic performance as described for the first generation. The individual plants were fertilised with 100 kg N/ha applied to the surrounding 2m X 2m area. In each case, plants not selected were sprayed out with glyphosate, leaving only the selected plants to produce seed for the next generation.

RESULTS AND DISCUSSION

Following 4 cycles of selection, 3 new synthetic Rhodes grass cultivars (each based on a number of elite clones selected from the final generation of the three breeding populations) have been constituted as shown in Table 1. In each case, the selected single-plant clones have been divided vegetatively and established as balanced polycross blocks for seed multiplication in isolation from all other Rhodes grass plants in north Queensland. The aim is to ensure that seed marketed will be no more than two generations of multiplication removed from the selected clones from which each cultivar has been derived.

Table 1. Origin, agronomic selection criteria, and numbers of selected clones used to constitute new Rhodes grass cultivars following 4 cycles of mass selection.

Cultivar	No. of clones	Origin	Agronomic selection criteria
Gulf	12	Finecut	Early flowering, dense, leafy, erect habit with fine leaf and stem
Salcut	15	Topcut	Early flowering, dense, leafy, erect habit with fine leaf and stem
Reclaimer	15	Finecut	Late flowering with a dense, leafy, stoloniferous (spreading) growth habit

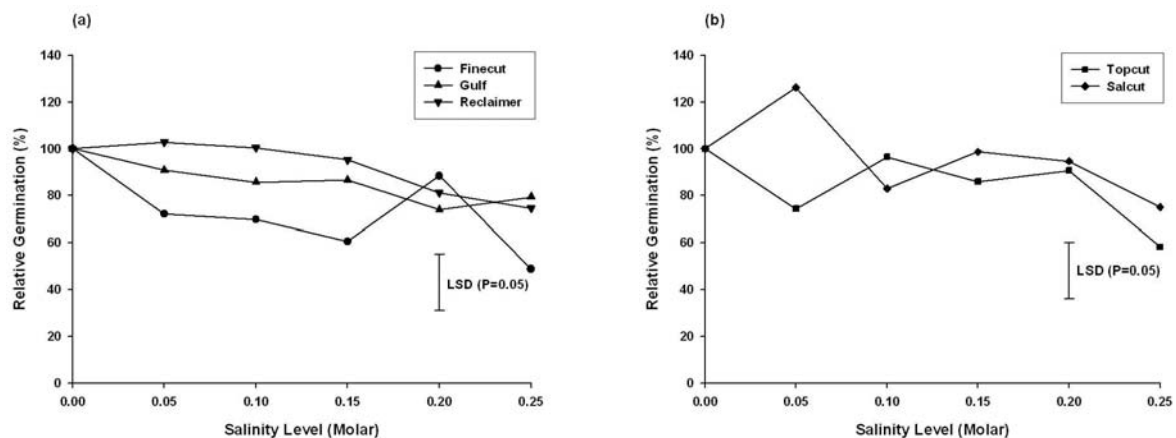


Fig. 1. Relative seed germination (pure water = 100%) after 3 weeks under varying levels of salt in the substrate for 3 Rhodes grass cultivars (Gulf, Reclaimer and Salcut) selected for salt tolerance compared with the original unselected cultivars (Finecut and Topcut).

Further work is being conducted to support the commercialisation of these 3 cultivars. Firstly, Selected Seeds intends to register these cultivars for Plant Breeder's Rights, thereby protecting the intellectual property generated through its investment in the current breeding program. This requires comparative growing trials to document and describe their characteristics for Distinctness,

Uniformity and Stability (DUS). Secondly, the levels of salt tolerance actually achieved in the finished varieties are being documented, both under controlled glasshouse and laboratory conditions and in the field. In this respect, the gains in salt tolerance demonstrated by Malkin & Waisel (1986) over 5 generations of mass selection from 'Pioneer' Rhodes grass are encouraging.

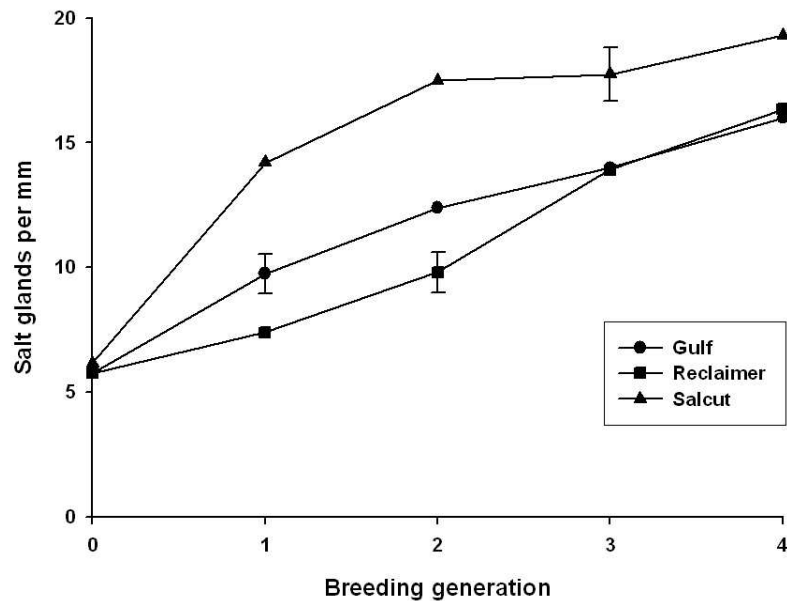


Fig. 2. Changes in salt gland density in 3 Rhodes grass breeding populations over 4 generations of selection for salt tolerance. Vertical bars show LSD values ($P=0.05$) for each cultivar.

Plants vary in their tolerance to salinity at different developmental stages. To succeed in the commercial world, a salt tolerant cultivar needs not only to grow under saline conditions; it must also be able to germinate in that same saline environment (albeit perhaps with some help from management practices to minimise the effects of salinity at that stage). However, the available evidence from other plants (e.g. Mano & Takeda 1997) suggests that plants capable of tolerating saline conditions during germination may not necessarily show a comparable level of salt tolerance during growth, and vice versa. We must therefore assume that salt tolerance at different stages of growth is essentially unrelated, hence the two-stage selection process for salt tolerance developed and applied from the second generation onwards. Other breeders have independently adopted similar approaches when selecting for salt tolerance (e.g. Rose-Fricke & Wipff 2001, Rose-Fricke *et al.* 2003).

A randomised block experiment (5 cultivars, 6 salinity levels, 4 replicates) was conducted under standard germination testing conditions on top of absorbent paper substrate under an alternating temperature regime (20/30°C night/day) with light during the day as recommended for Rhodes grass by ISTA

(2003). Due to osmotic stress, germination of Rhodes grass seed in salt is typically delayed by about 1 week, hence the 3-week final count in this experiment. Analysis of variance showed highly significant improvements ($P=0.01$) in the germination of the 3 improved cultivars across all salt levels and significant Cultivar x Salinity interactions ($P=0.05$), indicating an improved ability to germinate at higher salinity (data for main effects not presented). Germination data for the individual cultivars are shown in Fig. 1. However, levels of germination here were much higher than seen when sown in sand and irrigated hydroponically with saline water, suggesting that the applied salt may not remain evenly distributed through the paper substrate following subsequent irrigations with water to maintain adequate moisture for germination.

Further trials on the effects of salinity on germination and growth in sand are planned, in which pots can be bottom-watered hydroponically. With reliable data obtained under defined salinity levels in homogeneous conditions, the interpretation of responses from complementary field trials (which must inevitably be conducted under heterogeneous conditions, both spatially and temporally) should be easier.

In conclusion, it is interesting to speculate as to the possible mechanism(s) involved in improving salinity tolerance of Rhodes grass. While a number of possible sources of salt tolerance have been identified (Loch *et al.* 2004), the density of salt glands may well be an important factor as suggested by de Luca *et al.* (2001). This is supported by counts of

salt gland density made on the selected plants retained from each of the 5 breeding generations, showing that the density of salt glands in each case had been increased 3-fold by selection for salt tolerance (Fig. 2). Nevertheless, more detailed studies of this and other salt tolerance mechanisms in Rhodes grass will obviously remain a fruitful avenue for other researchers in the future.

LITERATURE

- de Luca, M., García Seffino, L., Grunberg, K., Salgado, M., Córdoba, A., C. Luna, C., Ortega, L., Rodríguez, A., Castagnaro, A. & Taleisnik, E. 2001. Physiological causes for decreased productivity under high salinity in Boma, a tetraploid *Chloris gayana* cultivar. *Australian Journal of Agricultural Research* 52: 903-910.
- ISTA 2003. International Rules for Seed Testing. Edition 2003. International Seed Testing Association, Bassersdorf, Switzerland.
- Loch, D.S., Rethman, N.F.G. & van Niekerk, W.A. 2004. Rhodesgrass. *In*: Moser, L.E., Burson B.L. & Sollenberger, L.E. (eds.), Warm-season (C_4) grasses, pp. 833-872. *Agronomy Monograph* 45. ASA, CSSA & SSSA. Madison, Wisconsin, USA.
- Malkin, E. & Waisel, Y. 1986. Mass selection for salt resistance in Rhodes grass (*Chloris gayana*). *Physiologia Plantarum* 66: 443-446.
- Mano, Y. & Takeda, K. 1997. Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica* 94: 263-272.
- Rose-Fricker, C. & Wipff, J.K. 2001. Breeding for salt tolerance in cool-season turfgrasses. *International Turfgrass Society Research Journal* 9: 206-212.
- Rose-Fricker, C.A., Fraser, M.L & Wipff, J.K. 2001. Registration of 'Brightstar SLT' perennial ryegrass. *Crop Science* 43: 2309-2310.

Development of a Texas bluegrass hybrid for turf usage

Jay B. Burr

The Scotts Company, 7644 Keene Rd. NE, Gervais, Oregon 97301, USA

Jay.Burr@Scotts.com

ABSTRACT

An interspecies turfgrass breeding program was initiated to address the issue of heat and drought tolerance in cool season turfgrasses. Texas bluegrass (*Poa arachnifera* Torr.) is a forage species that possesses traits including natural heat tolerance, drought tolerance, aggressive rhizomes and disease resistance. Kentucky bluegrass (*Poa pratensis* L.) has a long history of excellent turf performance and good seed production. Crosses between Texas Bluegrass and Kentucky bluegrass were generated to produce diverse breeding progeny material. Progeny from this cross resulted in male plants, female plants and plants with perfect flowers. The progeny also demonstrated of variation in seed yield, germination, disease resistance, heat and drought tolerance. A screening program to evaluate for these characteristics in the hybrid plants was initiated to identify progeny with the best combination of positive characteristics. In 2004, a plant patent was filed for a hybrid with the experimental name of HB 129. The variety HB 129 was then registered with the crop variety name of 'Thermal Blue' with the USDA. After an extensive evaluation program, Thermal Blue exhibits not only excellent turf characteristics but also improved heat tolerance and disease resistance. Another added and unexpected turf performance trait identified was shade tolerance. Seed production characteristics were also impressive. With drought tolerance, it is well suited for dryland seed production and does not require field burning to produce seed each year. University testing indicated this product was best suited for the transition zone of the southern US, where tall fescue (*Festuca arundinacea*) is the common turfgrass. The Scotts marketing group felt that a product that mixes tall fescue and Thermal Blue was needed and this has resulted in one of the most successful seed product sales programs in Scott's current history.

Key words: disease resistance, drought tolerance, dryland seed production, heat tolerance, Kentucky bluegrass, seed production, Thermal Blue, transition zone.

PLANT BREEDING

Scotts heat tolerant bluegrass hybrid breeding program began in 1993. The goal was to capture genetic diversity from Texas bluegrass, a native species, and incorporate it into a turfgrass cultivar. Some positive turf traits of Texas bluegrass include its natural heat tolerance, drought tolerance, its aggressive rhizomes, winter color and disease resistance. The concept of using Texas Bluegrass in a breeding program is not a new idea. The United States Department of Agriculture (USDA) in their Agricultural yearbook of 1936 contains pictures of Texas

bluegrass X Kentucky bluegrass hybrid progeny that were taken in 1909. The USDA conducted this breeding program. The issue that kept these programs from producing viable cultivars was mainly poor seed production and recovery of viable seed. Some of the Texas bluegrass hybrid progeny contained negative traits of Texas bluegrass such as its dioecious nature, coarse leave texture, open canopy, upright growth habit, very poor seed production, poor seed germination and light green color.

Texas bluegrass plants are dioecious, therefore if any seeds form when a Texas

bluegrass female plant is pollinated with Kentucky bluegrass pollen, they are a hybrid cross. Dr. John Hardison had made some initial Texas bluegrass by Kentucky bluegrass crosses in 1942 to 1944 while working as a plant pathologist for the USDA in Oklahoma. After moving to Oregon in 1945 he tabled this project until his retirement. In 1990 he started to make additional crosses and in 1993 joined forces with Scotts to further explore these crosses. Extensive screening and testing was required to sort out the progeny to identify which ones had positive turf characteristics with acceptable seed production. In 2003 Scotts came out with the first high quality Texas bluegrass hybrid called "Thermal Blue".

The USDA recognized that Thermal Blue was developed from a cross of Texas bluegrass by Kentucky bluegrass. Because selection pressure was towards plants that resembled Kentucky bluegrass approval was given to call this a variety of Kentucky bluegrass. There were several previous interspecies crosses involving Kentucky bluegrass that were also classified as Kentucky bluegrass.

SEED PRODUCTION

Economic seed yield had been the key issue that has prevented previous plant breeding programs from being successful. Scotts choose to focus on this issue and applied initial screening pressure to such issues as progeny with perfect flowers, low amount of basal hair or "cotton" on the seed, heavy seed weight and high percentage of apomixis. The high amount of cotton, light weight of seeds and low seedling vigor were the most difficult traits to overcome. Exploring many different parental combinations lead to the resolution of these issues and opened the door to commercial viability of this product.

During the production of breeder seed at The Scotts Company's Gervais, Oregon research station it was noticed that the Texas bluegrass hybrids did not recover quickly from burning after harvest. The following year the plots were not burned and there was little or no reduction in seed head numbers. The state of Washington banned all burning of grass seed crops in 1997. Trial production fields were planted in the non irrigated areas of eastern Washington. This area produces large acreages of common or public varieties of

Kentucky bluegrass seed. Initial results were promising enough that a 40.5-hectare field of the Texas bluegrass hybrid was established near the town of Rockford, Washington. This field produced an average of near 1,120 kilograms per hectare each year over three years of harvest. An average seed yield in Kentucky bluegrass in this area is about 670 kilograms per hectare. It was not long before growers were asking for the "good grass" that they had heard about in the coffee shops. Production was also established under a center pivot irrigation system on sandy soil to supply high quality seed production required by sod growers. Seed yields were good in this area also but the most notable comment was from the growers was that the grass used less water than conventional Kentucky bluegrass. Growers in this area monitor the moisture level of the soil on a daily basis and they have reported always using less water when growing Thermal Blue seed crops.

TURF PERFORMANCE

Extensive testing was conducted at University sites across the United States to help define the potential market for this product. A photograph from Auburn University in Alabama accelerated the marketing of this variety. It showed turf plots with only a few plots having healthy green turf and the other plots are dead or damaged. All of the green turf were either Scotts Texas bluegrass hybrids or warm season turf species. Auburn also identified the shade tolerance of Thermal Blue. North Carolina State University confirmed the Brown patch resistance and showed that the product worked well in blends with tall fescue (*Festuca arundinacea*). The data from these Universities and others indicated that this variety could perform well in climates that conventional Kentucky bluegrass struggles to survive. Conventional Kentucky bluegrass has not been successful in this region as the turf performance was hindered by heat, moisture stress and disease.

Since the release of Thermal blue, three additional heat tolerant bluegrasses have been released, Dura Blue, Solar Green, and Thermal Blue Blaze. Plant patents have been filed for each of these varieties. Thermal Blue and Solar Green have similar turf characteristics in that they establish quickly, are medium green in color, medium fine

texture, brown patch tolerance, aggressive underground spreading rhizome system, wear tolerance and mix well with tall fescue. Dura Blue and Thermal Blue Blaze are dark green in color, medium leaf texture, exceptional brown patch and dollarspot disease tolerance, have aggressive rhizomes, outstanding recovery from drought stress, superior shade performance, active winter green color in the transition area of the US, plus mix well with tall fescue. Unfortunately these latter two varieties have about half the seed yield potential of Thermal Blue and Solar Green.

MARKETING SUCCESS

The transition zone of the US is an area of the mid west and east coast where the cool season turf species struggle to survive due to warmer summer temperatures and high humidity. The climate is not warm enough for the warm season turf species to perform well. Homeowners in this area usually plant tall fescue, a cool season grass, and overseed additional tall fescue each fall to compensate

for the thinning of the stand due to disease and hot temperatures throughout the summer. The mixture of tall fescue and Thermal Blue Kentucky bluegrass offer the homeowner an option to the annual overseeding of their lawn. The mixture is 90% tall fescue and 10% Thermal blue, which would give it close to a 50-50 mixture on a seed count basis. The Thermal Blue will spread and fill in as disease and heat thins the stand. The sale of the Scotts consumer lawn division's Pure Premium Heat Tolerant Blue Brand seed mixture was released in 2005 with an aggressive Television and Radio advertising campaign. This product was an outstanding success in the tall fescue transition region, overselling the forecasted pounds several times over. The product continues to sell well and additional uses are developing as other turf options are explored. Additional retail product mixtures of tall fescue/Thermal Blue have been released in 2007 to maximize turf performance for density and traffic tolerance in the transition zone.

LITERATURE

United States Department of Agriculture 1937. Yearbook of Agriculture. 75th Congress, 1st Session. House Document No. 28. p. 1060

Achieving forage ryegrass seed yields of 3000 kg ha⁻¹ and limitations to higher yields

Phil Rolston¹⁾, Jason Trethewey¹⁾, Bede McCloy²⁾ & Richard Chynoweth³⁾

¹⁾AgResearch, Private Bag 4749, Christchurch New Zealand

²⁾NZ Arable, PO Box 16-101 Christchurch New Zealand

³⁾Foundation for Arable Research, PO Box 80, Lincoln, New Zealand

phil.rolston@agresearch.co.nz

ABSTRACT

Some New Zealand growers have achieved ryegrass seed yields of 3000 kg ha⁻¹. Factors limiting high seed yields are discussed. They include the failure of 50 to 70 % of fertile florets in the last two weeks before harvest to develop into saleable seeds, suggesting higher seed yields can be achieved. Harvest losses contribute to this loss, with up to 20 % of seed shattering and lost during harvest. To understand and manage fertile floret loss requires a better understanding in three areas (i) the relationship between the plant growth regulator trinexapac-ethyl, lodging, light interception and photosynthesis in carbohydrate (CHO) production and allocation; (ii) the relationships between nitrogen, soil moisture, lodging and growth of basal vegetative tillers during seed fill; (iii) hormonal and genetic regulation of seed head CHO resource allocation, seed size, seed number and seed shattering.

Key words: harvest loss, light interception, *Lolium*, nitrogen, plant growth regulator, seed development .

INTRODUCTION

In the early 1990's forage ryegrass (*Lolium perenne*, *L.x bocucheanum*) seed yields of 2000 kg ha⁻¹ were only occasionally achieved (Rolston 1995). A decade later 2000 kg ha⁻¹ is the seed yield that many New Zealand seed growers target there inputs and budgets around and a yield that is regularly, although inconsistently, achieved. A survey of 14 forage ryegrass growers from the South Canterbury region in 2006 found that 50 % had at least one field that produced >2000 kg ha⁻¹ and 28 % of fields had produced seed yields of >2000 kg ha⁻¹ (Rolston & Chynoweth 2006). In recent years forage ryegrass seed yields of 3000 kg ha⁻¹ have occasionally been reported. In this paper we examine inputs and identify key factors required to achieve 3000 kg ha⁻¹ seed yields.

Climate from anthesis to harvest, especially cool but sunny conditions, appear to favor seed yield, associated with increases in thousand seed weight (TSW); perhaps the

result of lower respiration loss, higher photosynthetic rates and reduced evapotranspiration stress. High seed yields in the 2006/07 harvest were associated with these conditions and had high TSW (in diploids 2.5 to 2.8 g compared to 2.0 to 2.2 g in average years). Seed yields tend to be higher on deeper soils with good water holding capacity. Plant breeders of forage grasses generally don't select for seed yield beyond ensuring that poor seed yielding parents are removed. We observe that some tetraploids have better seed yields than diploids; but a wide range of early-mid flowering diploid cultivars have achieved high seed yields.

Beyond these three factors management is a key driver of seed yield. Often seed yields vary widely in the same season despite being managed with similar inputs, either between fields on the same farm or for the same cultivar between farms in the same district. This suggests that timing of inputs may be more critical than imagined. Our work suggests that one set of factors is achieving a balance of inputs especially nitrogen (N) and

Plant Growth Regulators (PGRs) to ensure the crop remains standing with a high green area (especially in the seed head), during anthesis until seed is physiologically mature.

Floret fertility and reproductive efficiency

In the past some researchers have focused on floret site utilization (FSU), which is often less than 20 %, as a measure of reproductive efficiency (Elgersma 1990). Given that there is a great surplus of florets we determined the fate of fertile florets two weeks before harvest. Fertile florets were defined as

developing seeds that could be identified visually without magnification. Fertile floret numbers were generally high (>5/spikelet), suggesting that pollination was not a limitation to seed yield, even in overcast damp conditions where no obvious pollen clouds were seen (Table 1). However, less than 50 % of these fertile florets were recovered as saleable seed (Table 2). The percentage of fertile florets that produced saleable seeds was less when high nitrogen inputs were used, so as seed yield increased the efficiency of using fertile florets declined.

Table 1. Fertile, sterile and total florets per spikelet (\pm standard deviation) and percent fertile florets in cv. Hillary 2006/07.

	fertile	sterile	total	% fertile
dryland	6.2 \pm 0.7	2.4 \pm 0.6	8.6 \pm 0.8	73 \pm 6
irrigated	5.4 \pm 0.5	2.2 \pm 0.4	7.7 \pm 0.7	71 \pm 4

Table 2. Seed yield (SY) and Fertile floret site utilization (FFSU) for three nitrogen rates (applied + mineral N of 30 kg ha⁻¹).

N (kg ha ⁻¹)	SY (kg ha ⁻¹)	FFSU (%)
30	1013	48
200	1640	32
350	1536	29
LSD 5%	356	12
Sign.	1%	5%

Floret position, anthesis and seed maturity

A seed crop consists of a population of mixed aged reproductive tillers, formed in autumn, early winter and early spring. There is typically 10 to 14+ day spread between the emergence of the early seed heads and emergence of the later seed heads. Within an inflorescence spikelets at the tip flower first while the basal spikelets may flower 4 to 7 days later. Thus, close to harvest the top spikelets have a seed moisture content that is 5 % less than the basal spikelets (Table 3). Within the spikelet the basal floret flowers

first and the tip last. The effect of this is, as harvest is approached, there are seeds of different maturity stages (immature to fully mature and shattering) and seeds of different seed weights. At harvest there is a three fold variation in seed weight when seeds from the basal floret are compared with seeds from the tip floret of a spikelet (Fig. 1).

Table 3. Seed moisture content (SMC) and standard error (SE) of the means for spikelets in three positions in the head.

Spikelet position	SMC, %	SE mean
Basal	55.4	\pm 1.7
Mid	51.8	\pm 1.4
Top	50.7	\pm 1.4

These findings and the fact that occasional crops of 3000 kg ha⁻¹ seed yields were being achieved led us to examine what is limiting seed yields, to identify causes of fertile floret failure and to determine factors critical for achieving high seed yields.

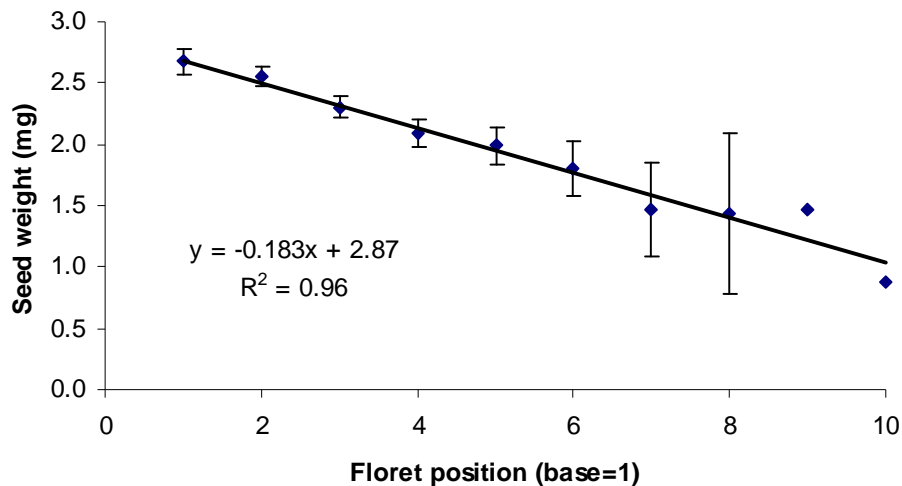


Fig. 1. Relationship between floret position in the spikelet (1= basal floret) and seed weight at physiological maturity adjusted to 12% SMC.

MANAGEMENT INPUTS

Seed yields between fields within the same farm and between farms in the same district vary widely even when similar levels of inputs are used. This suggests that not only is the rate of input important, but the timing of the input and the interaction of the input with weather, soil type and other crop management inputs must also be critical.

Nitrogen

Nitrogen (N) rate trials demonstrated that many NZ growers were using high N rates that were reducing seed yields. A series of N rate trials have suggested that optimum seed yields are achieved from total N inputs (applied and soil available N) of between 180 and 210 kg N ha⁻¹ (Fig 2.). In 2002/03 a survey of seed growers showed average applied N inputs of 221 kg N ha⁻¹. Three years later this had declined to 154 kg N ha⁻¹ (Rolston & Chynoweth 2006). Our trials show a strong relationship between high N inputs and early lodging (Fig. 3); and between high N inputs and increased growth of vegetative tillers during seed fill (Fig. 4).

Systems trials

Seed production is a multi input system with interactions occurring between inputs in the

system. Many agronomic seed production trials focus on variable rates or timing of one input. System trials comparing variable inputs of the three most expensive inputs used by NZ growers; N, fungicides (F) and plant growth regulators (PGR) showed that yields are more influenced by PGR ≥ F > N; depending on the level of stem rust (*Puccinia graminis*) and other leaf diseases.

Plant growth regulators (PGR)

There is a strong inter-relationship between lodging, light interception, and nitrogen rate with PGR usage. In a trial that compared N rates with either 300 g ha⁻¹ trinexapac ethyl (TE) applied as 1.2 l/ha Moddus (25 % TE) or nil PGR, there was a large seed yield difference, 2000 vs. 1200 kg ha⁻¹, due to PGR. The PGR yield advantage was associated with a delay in the onset of lodging (Fig. 3) and higher photosynthetic active radiation (PAR) at the flag leaf (Fig. 5). Of interest is the low level of PAR that reaches the flag leaf in crops that are standing or just starting to tip over (5-25% lodging). This reinforces our thoughts that the rachis, spikelets and glumes may be critically important photosynthetic organs that possibly have more influence on seed yield than flag leaf tissue.

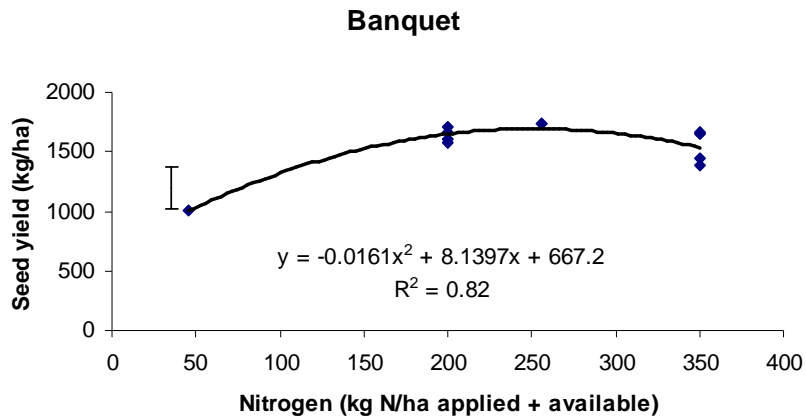


Fig. 2. Relationship between applied nitrogen and seed yield in perennial ryegrass cv Banquet.

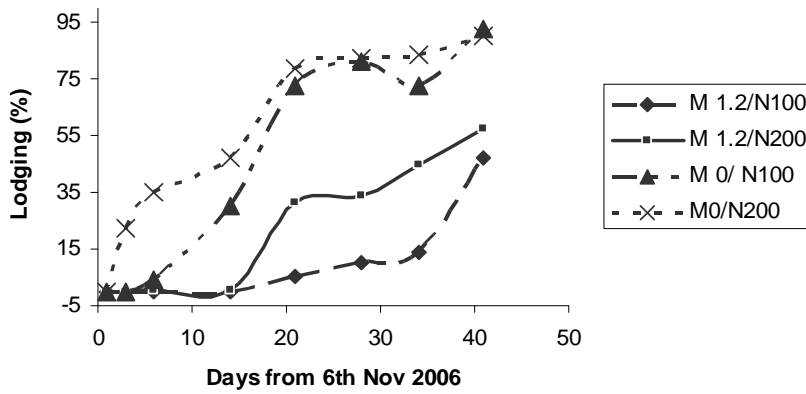


Fig. 3. Lodging time course for treatments with 300 g a.i. ha⁻¹ trinexapac ethyl (1.2 L ha⁻¹ Moddus (M1.2) or nil Moddus (M 0) and 100 kg N ha⁻¹ (applied + mineral N 0-30 cm (N100) or 200 kg N ha⁻¹ (N200). Anthesis was from day 1.

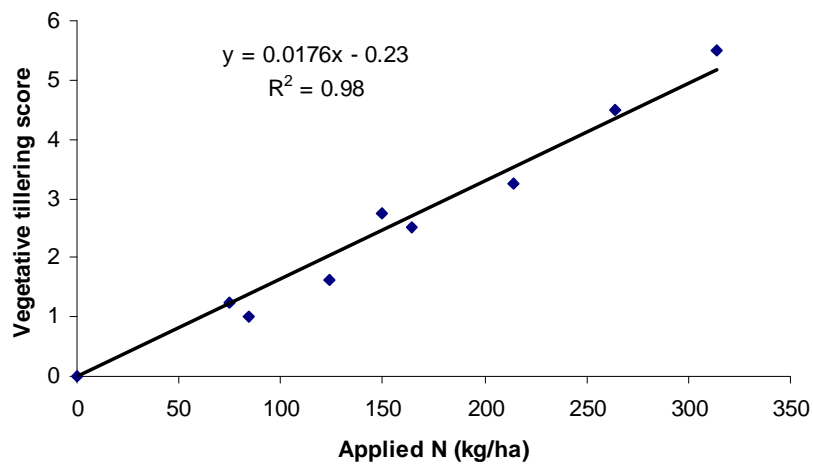


Fig. 4. Relationship between applied nitrogen (N) and secondary vegetative growth in late seed fill and seed yield in perennial ryegrass cv. Impact 2005/06.

Lodging and secondary growth

Lodging during seed fill is associated with the appearance of secondary growth in the form of new vegetative tillers. There is a strong negative relationship between the amount of secondary growth and seed yield (Fig. 6). It is postulated that secondary growth is a strong sink that requires carbohydrates from the reproductive mother tiller.

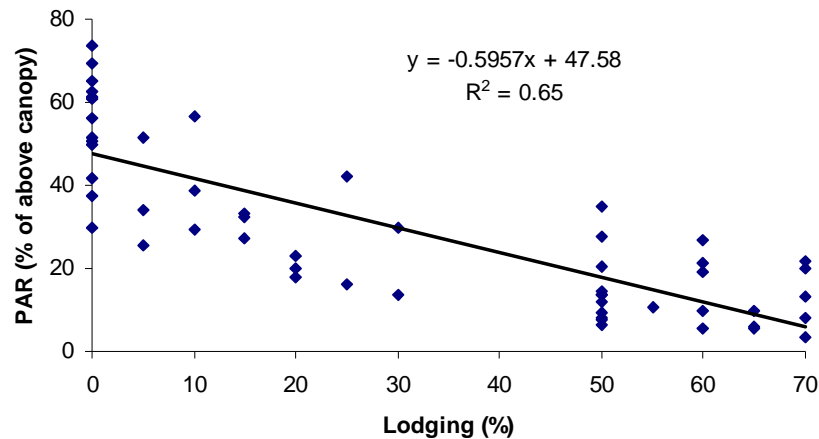


Fig. 5. Relationship between interception of photosynthetic active radiation (PAR) at mid canopy and lodging at mid seed fill in cv. Hillary (mean of two measurements 24th and 28th December 2006.) Above canopy PAR = 1010 $\mu\text{E s}^{-1} \text{m}^{-2}$.

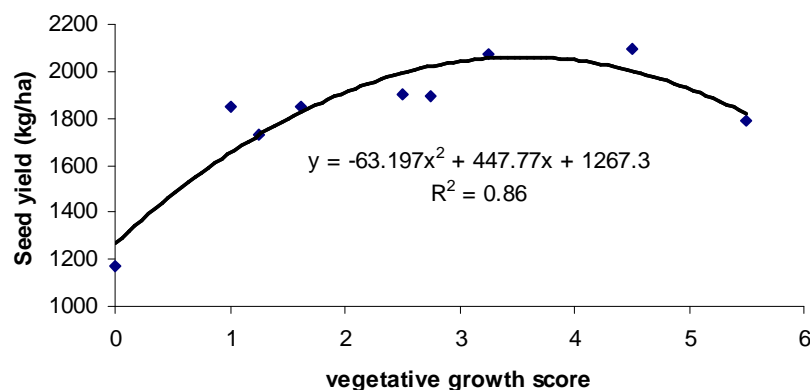


Fig. 6. Relationship between secondary vegetative growth in late seed fill and seed yield in perennial ryegrass cv. Impact 2005/06.

We suggest that to achieve high seed yields, ryegrass crops should be managed to remain standing through anthesis and seed filling, thus ensuring that PAR is at high levels in the upper canopy and to ensure that secondary vegetative tiller growth is not stimulated.

Carbohydrates

Seeds are approximately 80% carbohydrate and other carbon based products; with the balance being $\text{H}_2\text{O} = 12\%$; $\text{N} = 2\%$; minerals (ash) = 6%. Carbohydrate and carbon based

products (lipids and the carbon component of proteins) in seeds are the products of current photosynthesis and possibly some pre-anthesis photosynthesis. The distribution of water soluble carbohydrates (WSC) in a high seed yielding ryegrass crop is described by Trethewey & Rolston (2007). Maximizing photosynthesis and optimising WSC translocation to the seed during seed development is assumed to be the primary driver of seed yield.

Green Area

Historically the focus on green leaf area (GLA) has been the role of the flag leaf.

Relationships between flag GLA and seed yield occur (Fig. 7); although in the absence of stem rust the reduction in seed yield from a reduced flag leaf GLA can be small. The flag leaf is only one green tissue and the flag leaf area is smaller than the area of the rachis, spikelets and glumes or the leaf sheath of the flag leaf. When we maintain flag GLA, are we also maintaining green area in other important photosynthesizing tissue?

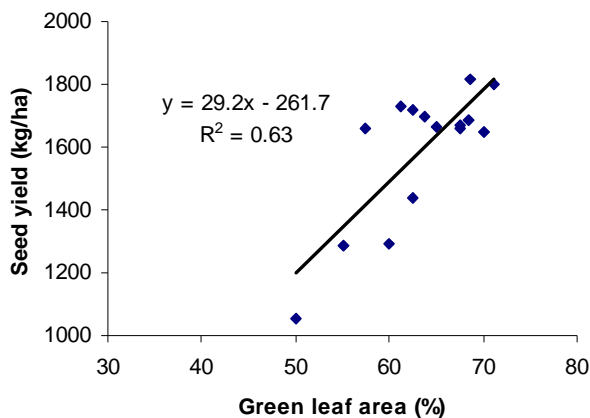


Fig. 7. Relationship between flag leaf green area and seed yield in perennial ryegrass 2000/01 Methven district.

Harvest loss and shattering

In high yielding crops large harvest losses (19-20%) occurred in two out of three crops assessed as having a harvest potential at cutting of $>3000 \text{ kg ha}^{-1}$. A component of this loss is the result of differential head emergence and flowering within the head resulting in different seed maturity within the head (Table 3) and between heads that emerged at different times. In the green house our attempts to reduce shattering using the ethylene inhibitor aminoethoxyvinylglycine (AVG; ReTain™) to delay abscission layer development have been unsuccessful. Breeding for reduced shattering

is an objective that grass breeders should consider.

DISCUSSION

High seed yields $>3000 \text{ kg ha}^{-1}$ are now occasionally being achieved by New Zealand perennial ryegrass seed growers. There are favourable and unfavourable weather events that the grower has no control over; however there are important crop management objectives to achieve these high seed yields. These are:

- keeping the crop standing through anthesis and seed development by the use of an appropriate amount of PGR;
- ensuring there is a high green surface area through seed development; noting that the seed head may be more important than the flag leaf; by using appropriate fungicide inputs;
- the above two parameters will ensure that a high PAR interception in the upper canopy and especially in the seed head to flag leaf region is achieved;
- ensuring that there is no secondary tillering by not oversupplying N fertiliser or over irrigating the crop;
- minimizing harvest losses by first managing the crop to achieve a compact flowering period and at harvest reducing the time between cutting and combine harvest;
- the timing of inputs (and the interaction with weather and soil type) is possibly more critical than the input rate applied, demonstrated by the wide variation within and between farms that broadly apply the same rates of inputs.

CONCLUSION

In the last 12 years our expectation of the highest yields that perennial ryegrass seed growers could achieve have risen from $>2000 \text{ kg ha}^{-1}$ to $>3000 \text{ kg ha}^{-1}$.

LITERATURE

- Elgersma, A. 1990. Spaced-plant traits related to seed yield in plots of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 51: 151-161.
- Rolston, M.P. 1995. Ryegrass 2000. Yield and Quality in Herbage Seed Production. *In: Proceedings of the 3rd International Herbage Seed Conference, Halle (Saale), Germany.* pp. 296-297.
- Rolston, M.P. & Chynoweth, R. 2006. Benchmarking: South Canterbury Seed Growers 2005/06. Report to Foundation of Arable Research FAR H05/16. 13pp.
- Trethewey, J.A.K & Rolston, M.P. 2007. Carbohydrates and seed yield limits in forage ryegrass. *In: Proceedings of the 6th International Herbage Seed Conference, Norway.* pp. 107-112.

Carbohydrates and seed yield limits in forage ryegrass

Jason A. K. Trethewey & Phil Rolston

AgResearch Limited, Lincoln Research Centre, Private Bag 4749,
Christchurch, New Zealand

jason.trethewey@agresearch.co.nz

ABSTRACT

Forage grass seed yields are often low and variable with only 10-20% of florets producing saleable seed. Seed yield can be influenced directly or indirectly by agronomic components such as plant height, leaf area, dry-matter yield, and lodging resistance. All of these components can be affected by the amount and composition of carbohydrates available. Information regarding the storage and mobilisation of carbohydrates during reproductive development in forage grasses is limited. To identify whether carbohydrate maybe limiting seed yield the pattern of accumulation of water-soluble carbohydrates (WSC) in the stem and their remobilisation was investigated in the field. Proportionately large concentrations of WSC were found in the stems of *Lolium perenne* post anthesis. These concentrations continued to increase during seed fill through to harvest. In a ryegrass crop that yielded 2950 kg/ha there was 2200 kg/ha of WSC remaining in the internodes. Although the proportions of high molecular weight (HMW) storage WSC increased in all internodes during reproductive development, low-molecular weight (LMW) mobile WSC were always highest in upper internodes. Understanding the mechanisms that underlie carbohydrate partitioning to the seed could in future result in significantly higher yields.

Key words: floret site utilisation, fructan, harvest index, reproductive efficiency, seed fill, seed production

INTRODUCTION

While perennial ryegrass (*Lolium perenne*) is the most widely sown perennial forage grass in New Zealand, seed yields measured as harvest index and reproductive efficiency (actual seed yield compared to potential seed yield) are low and variable with only 10-20% of the above ground matter being harvested as seed and 10 to 30 % of available florets producing saleable seeds (Elgersma 1990, Rolston *et al.* 2005). Seed yield is ultimately determined by the amount of carbohydrate transported to the seed. Therefore, an understanding of the different types and amounts of carbohydrates that are synthesised and transported to the seed is essential for a better understanding of seed yield.

In seeds and grains of forage grasses and cereals, starch is the major storage non-structural carbohydrate. However, for much of the growing season starch is often only a minor component of the total reserve carbohydrate in the plant. Instead, vegetative tissues of cereals and temperate forage grasses such as perennial ryegrass and tall fescue (*Festuca arundinacea*) accumulate water-soluble carbohydrates (WSC) often in high concentrations (Chatterton *et al.* 1989). These carbohydrates, stored in leaves and stems, are mainly in the form low molecular-weight (LMW) sucrose and high molecular-weight (HMW) sucrose-derived polymers of fructose (fructans) (Borrell *et al.* 1989). Whereas simple linear (2 \rightarrow 1)- β -linked fructans (inulins) are common in dicotyledons, temperate forage grasses store a more complex mixed-type of fructan where

fructose residues are predominantly linked (2 α 6)- β .

Many studies of fructan accumulation reflect the general concept that fructans are accumulated in grasses and cereals when photosynthesis (source activity) is greater than consumption (sink activity) of carbohydrates for growth. For example, fructans are accumulated in leaves and leaf sheaths of plants experiencing cool conditions (Schnyder & de Visser 1999), in barley and wheat stems just prior to anthesis and after anthesis during the early part of seed-filling (Borrell *et al.* 1989, Bonnett & Incoll 1993) and in well illuminated excised leaves of *Lolium temulentum* (Cairns & Pollock 1988). However, most studies of fructan metabolism in grasses have been carried out using model systems in which leaf tissue is induced to favour accumulation of fructans or by exposing young plants to continuous light in order to increase source activity while cooling roots and leaf meristems to reduce sink activity.

In contrast, hydrolysis of fructans from tissues in which they have accumulated occurs when demand from sinks is greater than the supply of assimilates from current photosynthesis. For example, defoliation through grazing or mowing reduces the leaf area and, thereby, the supply of current photosynthate to the leaf meristems. Therefore, the carbohydrate supply to leaf meristems after defoliation depends transiently on reserve carbohydrates. During vegetative growth, leaf bases are the major storage site and can accumulate up to 70% of all fructans (Volenc 1986, Morvan-Bertrand *et al.* 1999). Defoliation induces a strong decrease in fructan contents and evidence suggests that new leaf production in perennial ryegrass is strongly influenced by the levels of fructans in the tissue prior to defoliation (Turner *et al.* 2001). A positive correlation between plant WSC concentrations before defoliation and subsequent regrowth from stubble left behind after defoliation has been obtained for perennial ryegrass (Donaghy & Fulkerson 1998) and tall fescue (Volenc 1986).

While much is known about the distribution of fructans and other WSC and their function during vegetative growth of grasses under conditions that limit photosynthesis, such as defoliation, the mobilisation of WSC from vegetative organs to the seed during reproductive development is not well

understood. As in the leaf and sheath the predominant carbohydrates accumulated in the stems of grasses and cereals are fructans and although WSC are remobilised from the stem during seed filling, the reported contribution varies. Also, the amount of stored carbohydrate contributed by vegetative organs to the seed has generally been inferred from the relationship between decreasing levels of leaf and stem total WSC and increasing dry mass of the seed head. The pattern of accumulation, remobilisation and significance of individual carbohydrates in specific organs during reproductive growth is not known. The objective of this research was to investigate the accumulation and mobilisation of WSC in the stem of perennial ryegrass during reproductive development.

MATERIALS AND METHODS

Plant material

A trial was positioned in an established perennial tetraploid ryegrass (cv. Grasslands Sterling) crop on a local farm in Canterbury, New Zealand (43° 36'S, 172° 24'E) from September 2005 to January 2006. Treatments were replicated four times in a randomised block design. Spring nitrogen (N) 150 kg total N ha⁻¹ was applied during September and October. Moddus plant growth regulator (1.3 L ha⁻¹) was applied at GS 32 (during October) and broad leaf weeds and diseases controlled by spraying. Plots were irrigated when necessary to avoid water stress.

Sampling procedure

At the start of stem elongation 0.25 m² quadrats were collected from early September 2005 to early January 2006. At each sampling date the developmental stage of the tillers were recorded using the Zadoks scale. A group of comparable tillers were frozen, freeze-dried and dissected into various vegetative and reproductive fractions for chemical analysis. A sub-sample of material was weighed and dried (80°C, 18 h) to obtain fresh and dry weights. Following anthesis, a sample (0.25m²) without the Moddus treatment was also harvested. Daily temperatures (minimum and maximum) were recorded and converted into growing degree days (GDD).

Extraction of water-soluble carbohydrates
Freeze-dried internodes were ground to powder in a modified coffee grinder. Water-

soluble carbohydrates were extracted from ground freeze-dried samples as follows. Samples (20 mg) were extracted with 2 x 1 mL of 80% (v/v) aqueous ethanol (85 °C, 30 min) to extract low-molecular weight components and subsequently with 2 x 1 mL distilled water (65 °C, 30 min) to extract polymeric and higher-molecular weight carbohydrates. Extracts were combined, centrifuged and reduced under vacuum to remove pigments. Residues were then resuspended in water (2 mL). Aliquots were removed and WSC quantified using a colorimetric anthrone assay (Jermyn 1956).

RESULTS

There was a significant increase in the amount of HMW storage WSC in internodes from early head emergence through seed fill to harvest (Fig. 1).

At harvest, 2000 kg ha⁻¹ of HMW storage WSC remained in the internodes. In contrast, the concentration of LMW mobile WSC steadily decreased over the same period. By comparison, both mobile and storage WSC in all leaf blades and all leaf sheaths steadily declined from early head emergence through to harvest. At harvest the leaf blades and leaf sheaths combined contained 50 kg ha⁻¹ WSC (data not shown). In reproductive heads, mobile WSC increased substantially during seed fill and subsequently declined to harvest. In the same tissue storage WSC decreased.

The amount of storage WSC in individual internodes increased during reproductive development with the majority of WSC accumulated in the two lower most internodes (internodes three and four below the head) (Fig. 2). Mobile WSC in all internodes decreased over the same time period (data not shown).

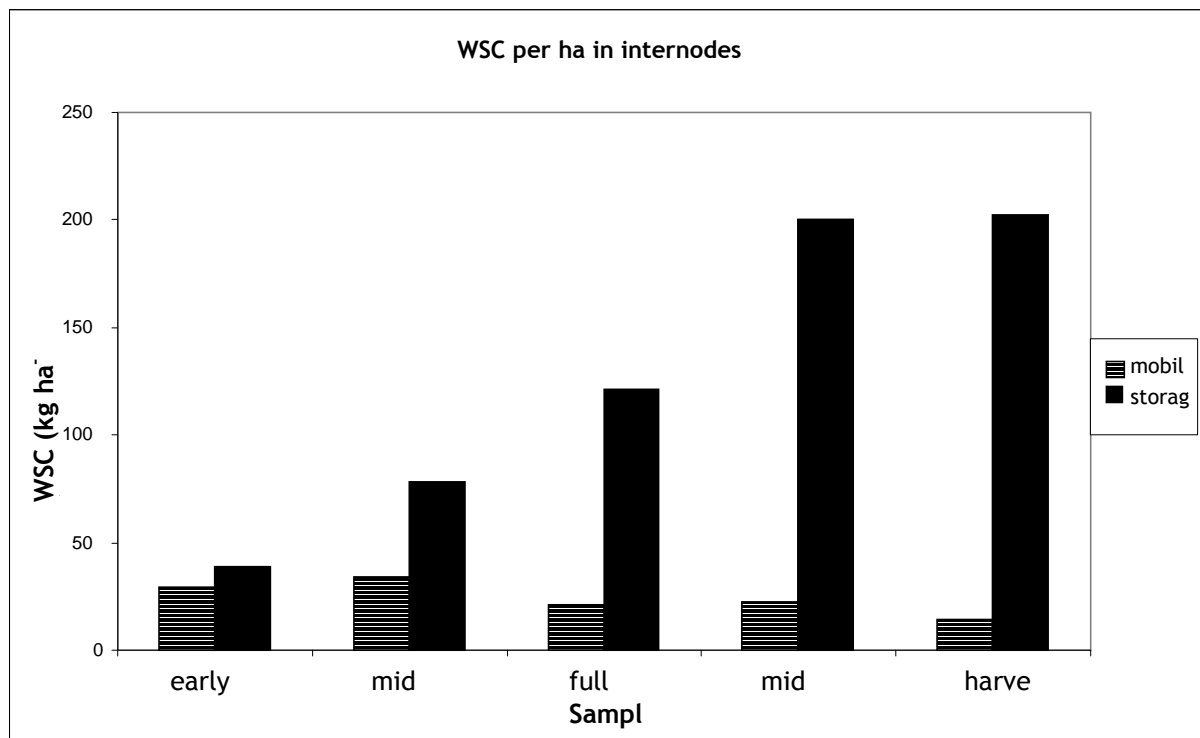


Fig. 1. Total amount of water-soluble carbohydrates in internodes of perennial ryegrass during reproductive development.

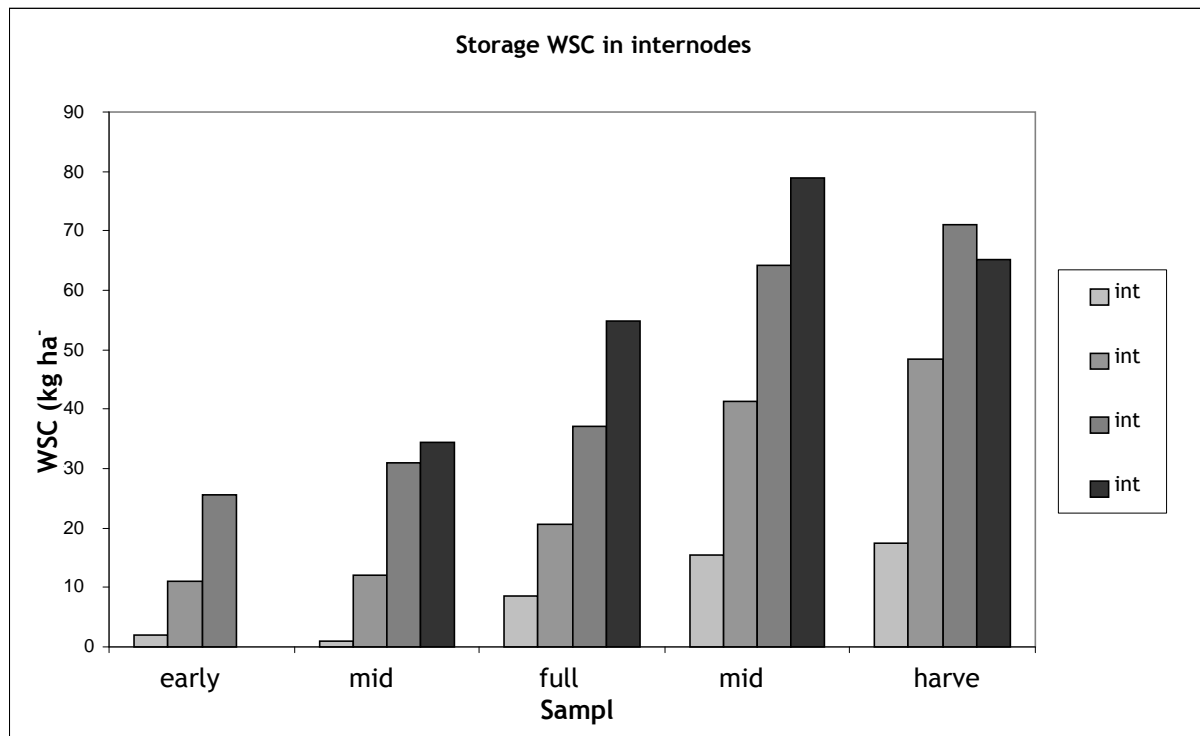


Fig. 2. Concentration of HMW storage water-soluble carbohydrates in individual internodes of perennial ryegrass during reproductive development. Internodes are numbered basipetally: 1= first internode below head (upper most internode); 4= fourth internode below head (lower most internode).

DISCUSSION

In perennial ryegrass the difference between potential seed yield and actual seed yield is large and variable (Elgersma 1990, Rolston *et al.* 2007). The results of this study suggest that the production of assimilate following anthesis is not limiting seed yield. Following anthesis and during seed fill large amounts of high molecular-weight (HMW) storage WSC accumulated in the basal internodes of perennial ryegrass. Similarly, Warringa & Marinissen (1997) found that in perennial ryegrass at final harvest WSC, measured as total reducing sugars, accounted for 25% (dry weight) of the stem. Also, Griffith (1992) showed that in Italian ryegrass (*Lolium multiflorum* Lam.) at anthesis the greatest concentration of storage WSC was found in the lower two thirds of the stem.

While the total amount of assimilate in the tiller is not limiting seed yield, the distribution and remobilisation of available WSC to the seed is an important factor limiting seed yield. In perennial ryegrass reducing light intensity by 76% following anthesis reduced stem WSC but had virtually

no affect on the relative contribution of seeds to total tiller weight (Warringa & Marinissen 1996). In contrast, in cereals the importance of the flag leaf and stem in supplying carbohydrate during grain filling is well established and it is generally accepted that stems contribute a significant amount of assimilates to the developing grain (Borell *et al.* 1989). In winter barley (*Hordeum sativum*) storage WSC comprise approximately 30% (dry weight) of the stem at the time of maximum stem mass (Bonnett & Incoll 1993) and in wheat, severe shading during seed fill was shown to reduce stem dry weight and grain yield (Kiniry 1993). Assimilation of WSC and their remobilisation within different organs of perennial ryegrass in a variety of stressful conditions needs to be examined in more detail.

Previous reports suggest that younger daughter tillers may compete with seeds for carbohydrate during reproductive development. In perennial ryegrass Clemence & Hebblethwaite (1984) found that allocation of ^{14}C to younger tillers increased to 24% during seed development. However, in the present study the large amounts of high

molecular-weight (HMW) storage WSC found in the stem during reproductive development suggest there is no competition for assimilate. Furthermore, the results of Warringa & Marinissen (1997) indicate that after anthesis there is little net exchange of ^{13}C between main tillers and subtending tillers. Similarly, Matthew (2002) labelled flowering tillers of perennial ryegrass with ^{14}C and found stems and seed heads contained the largest amount of radiocarbon whereas translocation into daughter tillers only accounted for 3.5% of ^{14}C recovered. The amount of carbon assimilated into various structural and non-structural carbohydrates in different tissues during reproductive development and their remobilisation into the seed requires further investigation.

As well as sucrose and sucrose-derived fructans, other WSC have been detected in vegetative and reproductive organs of grasses. Loliose is a trisaccharide containing galactose attached (1 \rightarrow 3) to sucrose, similar in structure to raffinose. Interestingly, in perennial ryegrass, loliose is reported to account for approximately 2% of the dry-weight of the seed and 80% of all WSC in the seed (Amiard *et al.* 2003), however the role of loliose in the seed is unknown. Variable amounts of (1 \rightarrow 3),(1 \rightarrow 4)- β -glucans are also found in vegetative and reproductive organs of grasses and cereals (Bacic *et al.* 1988, Trethewey & Harris 2002). This WSC is the major component of the endosperm cell wall comprising 50-75% (dry weight), which is subsequently degraded and used as a

carbohydrate source for the germinating seedling (Stone & Clarke 1992). During elongation of vegetative tissues there is an increase in the concentration of (1 \rightarrow 3),(1 \rightarrow 4)- β -glucans, followed by a steady decline once elongation has stopped. The contribution of cell-wall (1 \rightarrow 3),(1 \rightarrow 4)- β -glucan that is degraded following elongation of vegetative tissues such as leaves and stems and redistributed during seed fill in forage grasses is unknown.

Breeding for increased seed yield through improved source-sink relationships in the leaf, stem and inflorescence can be effective only when the factors that determine the priority for allocation of assimilates to each sink during reproductive development are understood, along with the contribution of carbohydrate from each organ. Different carbohydrates are likely to be remobilised and transported to developing seed at different rates. Knowing these patterns will enable crop managers and breeders to identify the most important carbohydrates. The significance of stored carbohydrates to seed yield can then be established and this information used for evaluating and enhancing seed yield in forage grasses in the future

ACKNOWLEDGEMENTS

This research was funded by the Foundation for Arable Research, Lincoln, New Zealand.

LITERATURE

- Amiard, V., Morvan-Bertrand, A., Billard, J.P., Huault, C., Keller, F. & Prud'homme, M.P. 2003. Fructans, but not the sucrosyl-galactosides, raffinose and loliose, are affected by drought stress in perennial ryegrass. *Plant Physiology* 132: 2218-2229.
- Bacic, A., Harris, P.J. & Stone, B.A. 1988. Structure and function of plant cell walls. In: Preiss, J. (ed.), *The Biochemistry of Plants, Volume 14, Carbohydrates*, pp. 297-371. Academic Press, San Diego, California, USA.
- Bonnett, G.B. & Incoll, L.D. 1993. Effects on the stem of winter barley of manipulating the source and sink during grain-filling. II. Changes in the composition of water-soluble carbohydrates of internodes. *Journal of Experimental Botany* 44: 83-91.
- Borrell, A.K., Incoll, L.D., Simpson, R.L. & Dalling, M.J. 1989. Partitioning of dry matter and the deposition and use of stem reserves in semi-dwarf wheat crop. *Annals of Botany* 63: 527-539.
- Cairns, A.J. & Pollock, C.J. 1988. Fructan biosynthesis in excised leaves of *Lolium temulentum* L. I. Chromatographic characterisation of oligofructans and their labelling patterns following $^{14}\text{CO}_2$ feeding. *New Phytologist* 109: 399-405.

- Chatterton, N.J., Harrison, P.A., Bennett, J.A. & Asay, K.H. 1989. Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. *Journal of Plant Physiology* 134: 169-179.
- Clemence, T.G.A. & Hebblethwaite, P.D. 1984. An appraisal of ear, leaf and stem ^{14}C assimilation, ^{14}C -assimilate distribution and growth in reproductive seed crop of amenity *Lolium perenne*. *Annals of Applied Botany* 105: 319-327.
- Donaghy, D.J. & Fulkerson, W.J. 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and Forage Science* 53: 211-218.
- Elgersma, A. 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.
- Griffith, S.M. 1992. Changes in post-anthesis assimilates in stem and spike components of Italian ryegrass (*Lolium multiflorum* Lam.). I. Water soluble carbohydrates. *Annals of Botany* 69: 243-248.
- Jermyn, M.A. 1956. A new method for determining ketohexoses in the presence of aldohexoses. *Nature* 177: 38-39.
- Kiniry, J.R. 1993. Nonstructural carbohydrates of wheat stems as influenced by sink-source manipulations. *Journal of Plant Physiology* 134: 243-250.
- Matthew, C. 2002. Translocation from flowering to daughter tillers in perennial ryegrass (*Lolium perenne* L.). *Australian Journal of Agricultural Research* 53: 21-28.
- Morvan-Bertrand A., Boucaud, J. & Prud'homme M. 1999. Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. *Journal of Experimental Botany* 50: 1817-1826.
- Rolston, M.P., Archie, W.J. & Rumball W. 2005. Branched inflorescence perennial ryegrass (*Lolium perenne* L.) - seed yield evaluated in field trials and response to nitrogen and trinexapac-ethyl plant growth regulator. *New Zealand Journal of Agricultural Research* 48: 87-92.
- Rolston, M.P., Trethewey, J.A.K., McCloy, B. & Chynoweth, R. 2007. Achieving forage ryegrass seed yields of 3000 kg/ha and limitations to higher yields. *Proceedings of the 6th International Herbage Seed Conference, Norway*.
- Schnyder, H. & de Visser, R. 1999. Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. *Plant Physiology* 119: 1423-1436.
- Stone, B.A. & Clarke A.E. 1992. *Chemistry and biology of (1 \rightarrow 3)- β -glucans*. La Trobe University Press, Melbourne, Australia.
- Trethewey J.A.K. & Harris P.J. 2002. Location of (1 \rightarrow 3)- and (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucans in vegetative cell walls of barley (*Hordeum vulgare*) using immunolabelling. *New Phytologist* 154: 347-358.
- Turner L.B., Humphreys, M.O., Cairns, A. & Pollock, C.J. (2001). Comparison of growth and carbohydrate accumulation in seedlings of two varieties of *Lolium perenne*. *Journal of Plant Physiology*, 158, 891-897.
- Volenc, J.J. 1986. Nonstructural carbohydrates in stem base components of tall fescue during regrowth. *Crop Science* 26: 122-127.
- Warringa, J.W. & Marinissen, M.J. 1996. The effect of light intensity after anthesis on dry matter distribution and seed yield of *Lolium perenne* L. *Grass and Forage Science* 51: 103-110.
- Warringa, J.W. & Marinissen, M.J. 1997. Sink-source and sink-sink relations during reproductive development in *Lolium perenne* L. *Netherlands Journal of Agricultural Science* 45: 505-520.

Translocation of nitrogen (N) to the seeds of perennial ryegrass in relation to the availability of N

René Gislum & Birte Boelt

Faculty of Agricultural Sciences, University of Aarhus, 4200 Slagelse, Denmark.

rene.gislum@agrsci.dk

ABSTRACT

In the present study perennial ryegrass cv. Linn was vernalised and grown in small containers in a growth chamber. Nutrients were applied in two treatments (N2.1 and N4.1) using a decreasing supply model to simulate soil conditions where the availability of N decreases throughout the growing season. At flowering an additional N application was introduced to half of the plants at each N concentration (N2.1+ and (N4.1+). The objective was to evaluate the effect of an additional N application during the experimental period on 1) the fresh weight of the plants, 2) the number of seeds and 3) the N accumulation in seeds. There was a clear effect of the two N treatments on the fresh weight and a further effect of an additional N application at plants with a low N status. There was a clear effect of the N2.1+ treatment compared to the N2.1 treatment on the number of seeds. The N accumulation in the seeds was clearly divided according to the N treatment. The highest N accumulation was achieved at the N4.1+ treatment and the lowest N accumulation was achieved at the N2.1 treatment. We conclude that the total availability of N has an effect on the fresh weight of the plants, the number of seeds and the N accumulation in the seeds, and that the effect of an additional N application is dependent on the current plant N status.

Key words: nitrogen, seed yield, translocation

INTRODUCTION

Nitrogen (N) translocated from the different plant organs to the developing seeds is the main N source during seed filling. This source-sink relationship is therefore heavily dependent on the amount of N accumulated in the plant at the start of seed filling. There is reason to believe that an additional N application at flowering could have a positive effect on the seed weight or the number of seeds harvested. However, there is also the risk that an additional N application will only contribute to vegetative growth of the plant. The effect of an additional N application will most likely depend on the current N status of the plant.

The objective was to evaluate the effect of an additional N application during the experimental period on 1) the fresh weight of the plants, 2) the number of seeds and 3) the N accumulation in seeds.

MATERIALS AND METHODS

Seeds of perennial ryegrass (*Lolium perenne* L. cv. Linn) were sown in 278 ml black plastic pots, and after germination the pots were thinned to one seedling per pot. The pots were filled with a commercial soil mix (Sunshine 400 series, McConkey Co., Sumner, WA, USA). The plants were vernalised in growth chambers for 9 weeks at a day/night temperature of 7 °C and an irradiance of 250 to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the spectral range 400 to 700 nm. After vernalisation the plants were placed one week in an unheated glasshouse for acclimation. After acclimation 100 plants were randomly divided into five groups and each group was placed in a container (length*width = 0.3*0.6 m). The plants were afterwards moved to growth chambers with a 16/8 h light/dark regime at 18/12 °C (defined as the start of the experimental period, day 0). Tungsten bulbs gave a photon flux density of 250 to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The five containers with 20 plants each were placed randomly in the climate chamber.

At day -27 (start of the experiment was at day 0) each container received a nutrient solution of the following composition (macronutrients): 1 mol m⁻³ CaCl₂, 1 mol m⁻³ MgSO₄, 1 mol m⁻³ K₂SO₄, 0.1 mol m⁻³ KH₂PO₄ and (micronutrients) 50 mmol m⁻³ KCL, 2 mmol m⁻³ MnSO₄, 2 mmol m⁻³ ZnSO₄, 0.5 mmol m⁻³ CuSO₄, 25 mmol m⁻³ H₃BO₃, 0.5 mmol m⁻³ Na₂MoO₄, 40 mmol m⁻³ FeEDTA. With the change of the nutrient solution every third day the N concentration in each container was added at decreasing rates in order to lower N availability during plant development. Thus, the concentration of N (NO₃:NH₄⁺, 1:1) at each change of nutrient solution was calculated from equation 1:

$$N_t = \frac{N_0}{1 + e^{\frac{t-t_0}{b}}} \quad [1]$$

where N_t and N₀ denotes N concentration at day t and day zero, respectively. t₀ and b were constants that were preset at 10.2 and -2.8, respectively. The basis for calculating t₀ and b was a stepwise decrease in N concentration as follows: day -21, 4 %; day -15, 10 %; day -9, -6 %; day -3, 37 %; day 3, 55 %. Two different N treatments were used with the following start concentration: 2.1 mM N (N2.1) and 4.1 mM N (N4.1) (Fig. 1). At day -6 the plants in each of the two N treatments were divided into two groups. One of the groups in the two N treatments continued to receive the decreasing N concentration. The other group in the two N treatments received an additional N concentration at this day (N2.1+ and N4.1+). At day -3 (three days before the start of the experiment) the N concentration of the four N treatments was: 2.1 mM N (4.66 mM), 2.1+ mM N (7.41 mM),

4.1 mM N (9.09 mM) and 4.1+ mM N (11.84 mM). In the following the four N treatments are denoted as N2.1, N2.1+, N4.1 and N4.1+. The start of the experiment was at day 0.

Four randomly chosen plants (n=4) of each N rate (a total of 20 plants) were harvested at 6, 15, 21, 27 and 30 days after start of the experiment. At harvest occasions, the fresh weight of the plants (plant and root) and the number of seeds were recorded before the material was dried at 80 °C. Plant material was ground using a Tecator Cyclotec 1093 sample mill and analyzed for total N using a Perkin Elmer 2400 Series II CHNS/O analyzer.

RESULTS

There was a clear effect of the two N treatments (N2.1 and N4.1) on the fresh weight (Fig. 2). For the N2.1+ treatment the fresh weight seems to have reached a plateau on day no. 15, whereas this plateau was not reached before day number 30 for the N2.1 treatment. An additional N application from N4.1 to N4.1+ had no clear effect on the fresh weight of the plants (Fig. 2).

There was a clear effect of the N2.1+ treatment compared to the N2.1 treatment on the number of seeds (Fig. 3). When an additional N application was made (N4.1 to N4.1+) the number of seeds increased, however, there was no mutual difference in the number of seeds between the N4.1 and N4.1+ treatments.

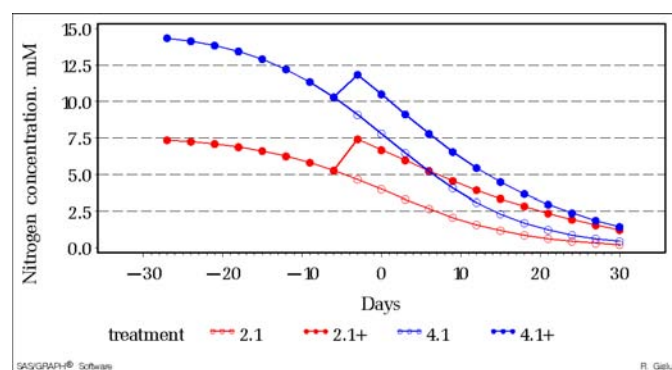


Fig. 1. Nitrogen (N) application scheme with 2.1 mmol N (red circle), 2.1+ mmol N (red dot), 4.1 mmol N (blue circle) and 4.1+ mmol N (blue dot).

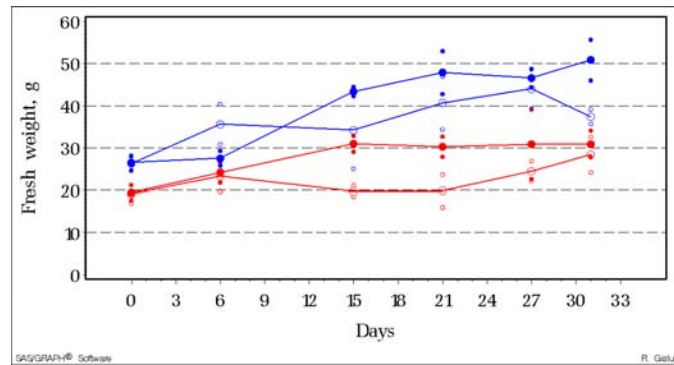


Fig. 2. Fresh weight of 20 plants at four different nitrogen (N) treatments (N2.1 mmol N (red circle), N2.1+ mmol N (red dot), N4.1 mmol N (blue circle) and N4.1+ mmol N (blue dot)). Standard deviations are shown with same but smaller symbols (n=4).

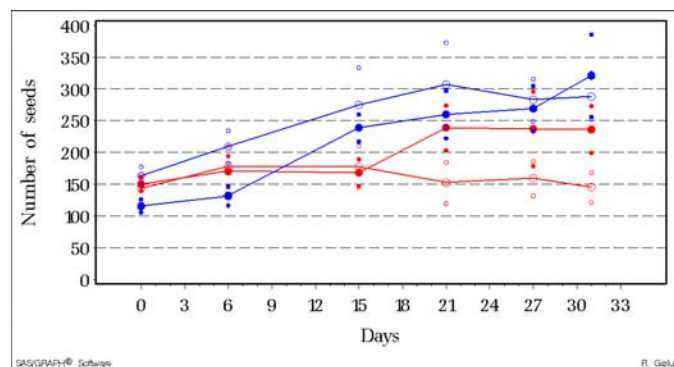


Fig. 3. Number of seeds in 20 plants at four different nitrogen (N) treatments (N2.1 mmol N (red circle), N2.1+ mmol N (red dot), N4.1 mmol N (blue circle) and N4.1+ mmol N (blue dot)). Standard deviations are shown with same but smaller symbols (n=4).

The N accumulation in the seeds was clearly divided according to the N treatment (Fig. 4). The highest N accumulation was achieved at the N4.1+ treatment and the lowest N accumulation was achieved at the N2.1 treatment (Fig. 4). There was an inexplicable

merging in the N accumulation at day 27 in the N2.1 and N2.1+ treatments. Furthermore there was a higher N accumulation at the N4.1 treatment compared to the N4.1+ at day number 6.

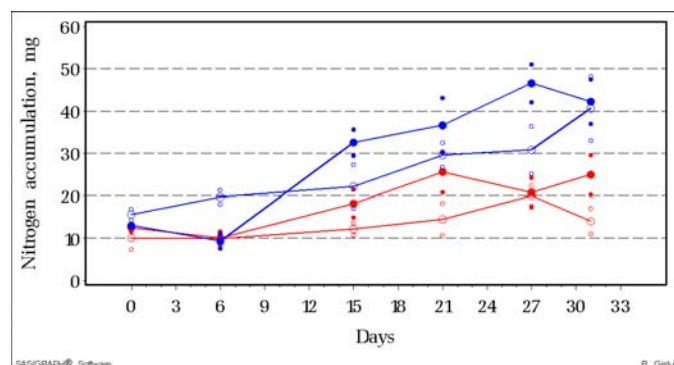


Fig. 4. Nitrogen accumulation (mg per 20 plants) in seeds at four different nitrogen (N) treatments (N2.1 mmol N (red circle), N2.1+ mmol N (red dot), N4.1 mmol N (blue circle) and N4.1+ mmol N (blue dot)). Standard deviations are shown with same but smaller symbols (n=4).

DISCUSSION

Elgersma (1990) found that seed yield in perennial ryegrass was more correlated with the number of seeds per unit area than with seed weight. Warringa (1996) stated that only 55 % of the total number of florets in perennial ryegrass was harvested as seeds, but not necessarily recorded after the cleaning process. The light seeds, defined as seeds that are too light to pass through the cleaning process, accounted for 35 %, which left only 20 % of the seeds recorded after cleaning. The light seeds were not recorded after harvest due to poor seed filling or because they were immature at harvest (Warringa 1996). This shows that increasing seed weight will have a pronounced positive effect on seed yield

The low seed weight in the harvested seeds is a result of a low dry matter accumulation, which is due to a low growth rate and/or a

short duration of growth. In both cases carbon and N are very important constituents. The amount of N translocated to the seeds is determined by the source-sink conditions and a possible competition between the different sinks. Warringa and Marinissen (1997) concluded that N was largely redistributed from the stem and leaves to the seeds and that 59 % of the N in the flowering tiller were located in the seeds. The present results showed that it is possible to increase the amount of N translocated to the seeds and that there is an unused potential in seeds and there is a positive correlation between the number of seeds and N in the seeds.

We conclude that the total availability of N has an effect on the fresh weight of the plants, the number of seeds and the N accumulation in the seeds, and that the effect of an additional N application is dependent on the current plant N status.

LITERATURE

- Elgersma, A. 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* 49: 141-154.
- Warringa, J.W. 1996. Physiological constraints to seed growth in perennial ryegrass (*Lolium perenne* L.). Ph.d.-thesis, Landbouwniversiteit Wageningen, ISBN 90-5485-633-5.
- Warringa, J.W. & Marinissen, M.J. 1997. Sink-source and sink-sink relations during reproductive development in *Lolium perenne* L. *Netherlands Journal of Agricultural Science* 45 (4): 505-520.

Seed production in the northern light: Implications of temperature and daylength for flower induction and seed yield

Ola M. Heide

Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P. O. Box 5003, NO-1432 Ås, Norway

ola.heide@umb.no

ABSTRACT

Flowering is a necessary although not an adequate prerequisite for seed production. A fundamental understanding of the flowering physiology of the various species is therefore important for establishing a favourable potential for seed yield. This is particularly important in the marginal northern environment. Temperate grasses can be classified into two main categories according to their environmental control of flowering: 1) Species with a regular long day (LD) induction of flowering and 2) Species with a dual induction requirement. The former group includes the temperate annual grasses and a few perennial species such as *Phleum pratense* and *Poa nemoralis*. However, most temperate perennial grasses have a dual induction requirement for flowering, a primary induction that is brought about by low temperature and /or short days (SD), and a secondary induction which requires a transition to LD and is enhanced by moderately high temperatures. In most dual induction species SD and low temperature are interchangeable and independently able to fulfil the primary induction requirement. Yet, the two environmental factors are highly interactive in the process. At low temperature (0-6 °C) induction takes place in both SD and LD, whereas at higher temperatures induction becomes increasingly dependent on SD, until a critical temperature of about 12-18 °C is reached, above which primary induction cannot take place regardless of photoperiodic conditions. Critical temperatures and photoperiods as well as the necessary exposure time vary greatly among the species. In some species, especially arctic-alpine species, initiation of inflorescence primordia takes place during SD primary induction in the autumn, an important adaptation to the arctic-alpine environment. However, in most grass species initiation requires a transition from SD to LD and accordingly it does not take place until the spring. Critical photoperiods for secondary induction vary from 9-10 h in Mediterranean ecotypes to more than 16 h in sub-arctic ones. The critical number of LD cycles varies from four to eight, whereas 12-16 cycles are required for the full stem elongation and flowering response. The dual floral induction system provides an ideal mechanism for synchronizing the life cycle of the grass species with the dramatic seasonal changes of the northern environment. The paper gives a number of examples of how research-based knowledge of the mechanisms and responses controlling these reactions have been successfully utilized for selection of favourable locations of production and choice of proper production agronomy for high and stable commercial seed yield.

Key words: climatic environment, dual floral induction, floral initiation, location of seed production, photoperiod, temperate grasses.

INTRODUCTION

Successful seed production at high northern latitudes represents a particular challenge because of the very special temperature and

light climate prevailing in these regions. Not only is temperature during the growing season sub-optimal for flowering and seed maturation, the extreme seasonal photoperiodic changes represent an additional constraint on floral

initiation and development for many species. The fact that most temperate perennial grasses and other herbage species have a dual induction requirement for floral initiation and development represents a special obstacle for the establishment of a high seed yield potential at high latitudes. Under such marginal conditions it is particularly important to have a clear understanding of the flowering physiology involved and the climatic requirements for floral initiation and development of the various species in order to select the best geographic locations and the best management strategies for seed production of the various species. In the following, this paper presents examples of how strategic flowering physiology research can be instrumental in helping this process.

Being a small country situated at the outskirts of the World, Norway is a small seed-producing country. However, because of the need for local cultivars adapted to the local Norwegian climatic conditions, it has always been a national strategy to cover most of the national herbage seed demand by local production. In terms of flowering physiology, the presented results complement the pioneering research by workers such as H.A. Allard, M.W. Evans, and W.E. Loomis in the USA, J.P. Cooper in UK, D. Bommer in Germany, and L.T. Evans in Australia.

Temperate perennial grasses have been shown to represent two principally different categories with respect to life history and flowering physiology strategies:

Species with single induction requirement

Relatively few species are represented in this group. An important example is *Phleum pratense* (timothy). *Poa nemoralis* is another example of little commercial interest.

Species with dual induction requirements (primary and secondary induction):

This group comprises most temperate perennial grasses such as
Bromus inermis
Dactylis glomerata
Festuca pratensis, *F. rubra* and other
Festuca spp.
Lolium perenne
Phleum alpinum
Poa pratensis and several other *Poa* spp.

As we shall see, these differences have important implications for seed production agronomy and selection of geographic locations

for seed production of the various species. It is also evident that there is no direct connection between taxonomy and flowering physiology of the various species, as closely related species often fall into different flowering response categories.

RESULTS AND DISCUSSION

Most of the research presented here has been conducted in controlled environments. Plants have been grown in natural spring and/or summer daylight in phytotron compartments with full control of temperature and humidity. Photoperiodic manipulation has been performed by moving the plants over night into adjacent growth rooms with low-intensity incandescent light or darkness. In this way, the total daily light integral has been kept at almost the same level regardless of the daylength conditions.

Single induction species

Unlike most other temperate perennial grasses, timothy (*Phleum pratense*) has no winter requirement for flowering (Langer 1955, Cooper 1958, Ryle and Langer 1963). Thus, first year seedlings readily form flower heads after spring sowing, and flowering is also common in the aftermath following early summer cutting. Flower initiation and internode elongation was early shown to be an obligatory long day (LD) response (Evans & Allard 1934, Allard & Evans 1941). As in many other perennial grasses, the flowering response of various timothy cultivars and ecotypes is also shown to be related to the latitudinal origin of the plant material, northern types demanding longer photoperiods for flowering than more southern ones (Evans & Allard 1934, Cooper 1958). This was very clearly demonstrated in an experiment with four Norwegian and two British timothy cultivars (Heide 1982). Cultivars with sub-arctic origin in Norway (69° N and 67° 30' N) had a critical daylength for flowering of 16.5 h and 16 h, respectively, whereas south Norwegian (59° N) and British cultivars (52° 30' N) had critical daylengths of about 14 h.

Despite the LD induction requirement, Langer (1956) found that tillers produced after the middle of April in Britain rarely produce heads, and Cooper (1958) demonstrated that this was an effect of high spring and early summer temperature. Heide (1982) found that cultivars of high-latitude origin are

It should be noticed, however, that the species, and also cultivars within species, vary as to whether they actually initiate inflorescences in the primary induction environment or, whether this occurs only after the transition from SD to LD conditions. Whereas arctic cultivars of *Poa pratensis* and *Alopecurus pratensis* readily form fully differentiated inflorescences under SD conditions, species such as *Lolium perenne*, *Bromus inermis*, *Dactylis glomerata*, *Festuca pratensis* and *F. rubra* as well as *Phalaris arundinacea* all require a transition from SD to LD before any morphological changes take place at the apical meristem (Heide 1994). Thus, although the environmental requirements for flowering are similar, the actual control point in the differentiation cycle is different in the two groups of grasses. Such differences can have important implications for seed production performance at various locations. The critical photoperiods for secondary induction range from 9-10 h in Mediterranean to more than 16 h in high-latitude ecotypes. Similarly, the critical number of inductive LD cycles vary from a minimum of 4-8 to 12-16 cycles for a full flowering response.

In most dual induction grasses SD and low temperature are interchangeable and independently able to fulfil the primary induction requirement. Yet, they are highly interactive in this process. Most species become day neutral at low temperature (0-6 °C) and primary induction can take place in both SD and LD. Primary induction is then identical with the process known as vernalization. At higher temperatures induction

becomes increasingly dependent on SD, until a critical temperature is reached at 12-18 °C, above which primary induction is not possible in either daylength. An intriguing question is the specificity of the primary induction processes in SD and LD. Since vernalization is known to act directly on the apical meristem, while photoperiodic induction is sensed by the leaves, it is likely that the two means of primary induction may work through principally different transduction pathways. Still, the final outcome is the same, namely the ability of the plant to undergo secondary induction.

Critical temperatures and photoperiods as well as critical exposure times for primary induction vary greatly among species, as well as among ecotypes of different geographical origin within a species. *Bromus inermis* and sub-arctic ecotypes of *Poa pratensis* and *Alopecurus pratensis* will initiate flowers with as little as 4-6 weeks of exposure to inductive conditions, while *Festuca pratensis* and *Deschampsia* species may need as much as 20 or more weeks of exposure for saturation of the primary induction requirement. Species such as *Dactylis glomerata* and *Lolium perenne* have intermediate requirements. Primary induction in cultivars of meadow fescue (*Festuca pratensis*) takes place in SD at temperatures up to 12 °C (optimum at 9 °C), but may also take place in LD (even in continuous light), although this requires temperatures as low as 3-6 °C (Heide 1988). However, the number of panicles per plant was twice as high in SD at the optimal temperature of 9 °C than in LD at low temperature (Fig. 2).

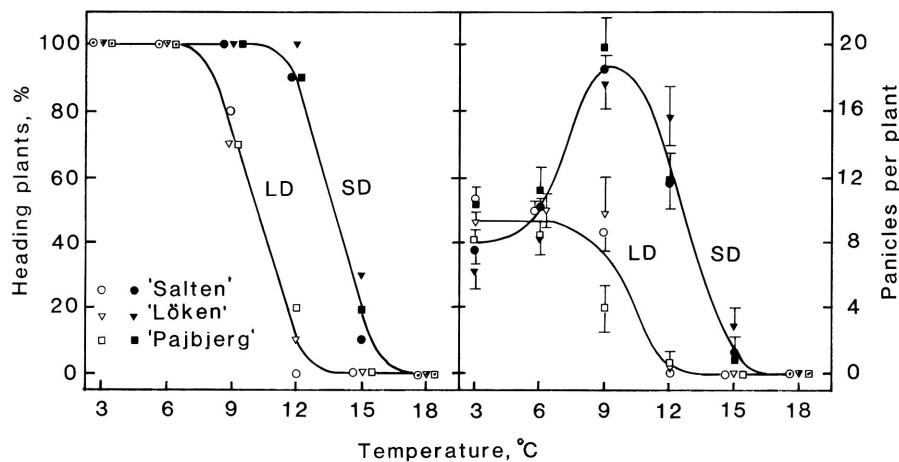


Fig. 2. Effects of photo-period and temperature during 20 weeks of primary induction on subsequent flowering in LD at 21 °C in three cultivars of *Festuca pratensis* (Heide 1988).

The requirement for such specific environmental conditions over extended time periods of 20 or more weeks can only be fulfilled in regions with relatively mild and snow-free autumns and winters. Such conditions are not very prevalent in Norway where the temperature usually drops rather quickly after the transition to SD at fall equinox, and where snowfall often comes early. Differences in winter climate are clearly reflected in seed yields at various locations. This has prompted Norwegian seed growers to locate meadow fescue seed production to southern and coastal regions with relatively mild winters and short snow-cover. Since the potential for rich flowering and high seed yield of such a species to a very large extent is determined by the number of inducible tillers in the autumn, the situation has also had important implications for choice of seed production agronomy and practices, such as use of cover crop, time of sowing, nitrogen inputs and removal of stubble and regrowth in early autumn. Although such measures are of general

importance, they have proved to be particularly critical under the marginal climatic conditions in Norway.

Smooth brome grass (*Bromus inermis*) is another temperate perennial grass species in which specific flower induction requirements have had important implications for Norwegian seed production strategies. This is a short-long-day plant that requires a transition from SD to LD in order to initiate floral primordia (Heide 1984). In this species low temperature has no inducible effect, on the contrary, the optimum temperature for the SD primary induction is 15-18°C, and even temperatures as high as 24°C are fully inductive in SD conditions. Temperatures below 12°C on the other hand, were only marginally effective for SD induction (Table 2), while vernalization at 3°C for up to 16 weeks was without effect on flowering.

Table 1. Effects of photoperiod and temperature on flowering (percent flowering plants) of smooth brome grass (*Bromus inermis*). The plants were exposed to the indicated conditions for 10 weeks followed by secondary induction at 21° and LD. The data are means of three experiments with two cultivars. (After Heide 1984).

Temp. °C	Photoperiod, h						
	8	10	12	14	16	18	24
6	-	55	-	-	-	-	0
9	-	85	-	-	-	-	0
12	100	100	100	55	10	0	0
15	100	100	-	-	-	-	0
18	100	100	100	25	0	0	0
21	100	100	-	-	-	-	0
24	100	100	75	20	0	0	0

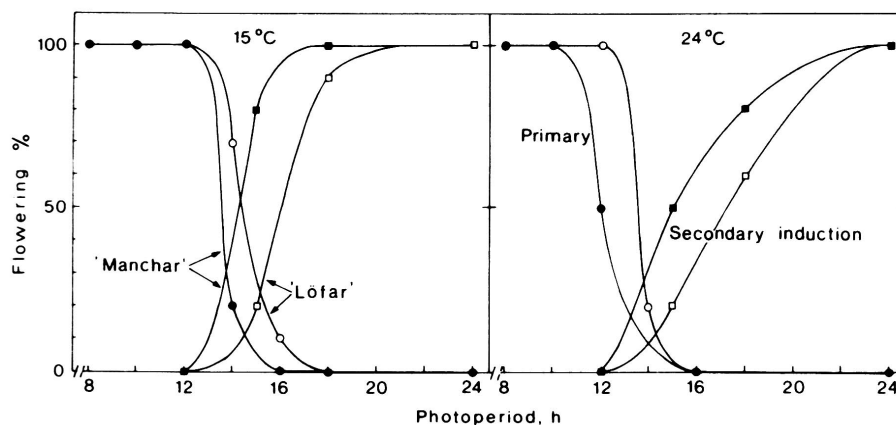


Fig. 3. Summary of photoperiod and temperature effects in primary and secondary induction of flowering in two cultivars of smooth brome grass (*Bromus inermis*). (Heide 1984).

Primary and secondary induction had contrasting critical photoperiod profiles, although they were over-lapping at a photoperiod of about 14 h (Fig. 3). Critical photoperiods for primary induction were longer at intermediate than at high temperature, while the critical photoperiod for secondary induction increased with increasing temperature. Also, the critical photoperiods for both primary and secondary induction were longer for the Norwegian cv. Løfar than for the American cv. Manchar.

Although the critical exposure time is only 4-6 weeks, the demand for a combination of SD and relatively high (intermediate) temperature is difficult to meet in the Norwegian environment. The period with favourable temperatures for SD induction of smooth bromegrass is thus marginally short in inland areas of South Norway and this has resulted in low seed yields in comparison with coastal and more southern locations. Accordingly, the production has successfully been located to southern and coastal areas with the best autumn temperature conditions.

A special feature of smooth bromegrass is that the plants develop elongated vegetative culms in response to LD in the sowing year. This effect is mediated by endogenous gibberellin (GA) which is inhibitory to primary induction (Heide, Bush & Evans 1987). It was therefore expected that artificial lowering of the endogenous GA level by application of GA biosynthesis inhibitors such as CCC in late summer and autumn would improve primary induction in this species. This proved to be the case and a single spray in August was shown to increase seed yield by up to 50 % (Aamlid *et al.* 1995). However, spring application gave as

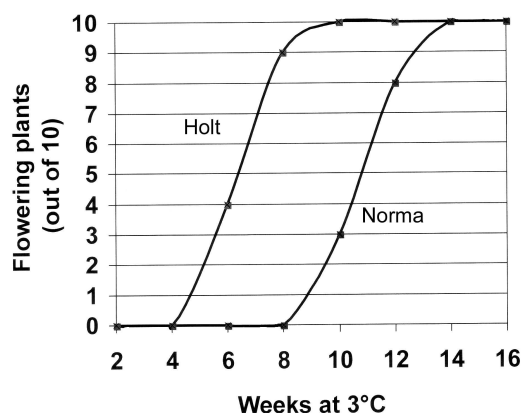


Fig. 4. Response to increasing length of primary induction at 3°C in LD in the *Poa pratensis* cultivars Holt (sub-arctic) and Norma (temperate, DK). (After Heide 1980).

good or even better results, and this has become standard practice in Norwegian smooth bromegrass seed production, while efficient post-harvest mowing of regrowth is used to knock down GA levels in autumn. As a result of such improved management practices and strategic geographic location, the average Norwegian seed yields of smooth bromegrass increased nearly four-fold over a twenty-year period.

A final example is *Poa pratensis* (smooth meadowgrass or Kentucky bluegrass) in which seed production has been rather tricky because of large ecotypic variation and strong genotype x environment interactions. Norwegian agriculture needs cultivars that are adapted to a wide range of light and temperature conditions, and such cultivars have proved to vary widely also in flowering responses and seed yield performance. While arctic cultivars such as 'Holt' and 'Lavang' from 68°45'N are very responsive to both SD and low temperature and initiate floral primordia after exposure to as little as 6-8 weeks of optimal conditions, cultivars of more southern origin require more than 10-12 weeks of exposure for the same response (Fig. 4). Another important difference is that high-latitude ecotypes initiate fully differentiated inflorescence primordia in the autumn, while most southern ecotypes require a transition from SD to LD conditions for this process to take place, and therefore, do not initiate inflorescence primordia until in spring. Furthermore, the high-latitude cultivars do not have an obligatory requirement for LD conditions for secondary induction but are able to develop short and stunted culms even under 8-h SD conditions. These factors have far-reaching consequences for flowering and seed yield.

Formation of inflorescence primordia in autumn is an important adaptation to the short growing season in the North as it facilitates an early and rapid flower development in spring and allows seed maturation in a short growing season. However, when such northern *Poa pratensis* cultivars are transferred to lower latitudes in South Norway, they experience a long period with optimal conditions for primary induction and form advanced inflorescences in the autumn. Such advanced inflorescence primordia are vulnerable to various kinds of damage, and in spite of an established seed potential in autumn, seed yields are often disappointingly poor.

There seems to be a number of reasons for the poor and unstable seed yields of the arctic *Poa pratensis* cvs. Holt and Lavang in south-eastern Norway (Håbjørg 1978, Heide 1980, Aamlid 1996):

- Frost damage of the differentiated inflorescences
- Insect damage of the differentiated inflorescences
- Over-induction, resulting in abortion of inflorescences
- Little re-growth after first seed harvest - 'second year seed yield depression'
- Sub-optimal daylength for secondary induction in spring.

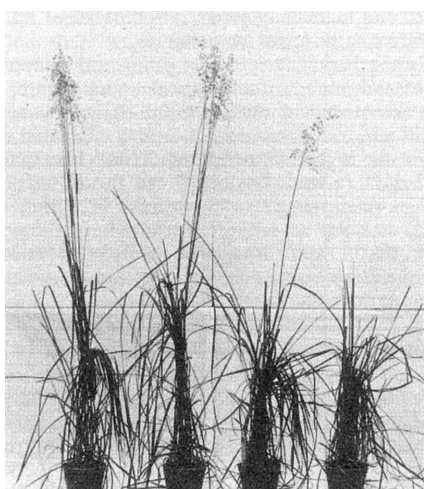


Fig. 5. Frost kill of inflorescence primordia of primary induced *Poa pratensis* cv. Holt plants after exposure to temperatures of 0, -5, -10 and -15°C (left to right) for 4 days (Heide 1980).

Freezing experiments by Heide (1980) demonstrated that plants of cv. Holt with advanced inflorescence primordia are very vulnerable to frost damage. When plants which had been exposed to 3°C/12 h photoperiod for 12 weeks were subjected to controlled freezing (cooling at 5°C per day) to temperatures of 0, -5, -10 and -15°C for two or more days, the

number of surviving panicles was progressively reduced and at -15°C all inflorescences were killed (Fig. 5). It was noticeable that all plants survived in good condition and except for the killing of inflorescence primordia, there was no damage to the plants. The succulent and nutritious inflorescence primordia are also attractive targets for certain insect larvae such as the frit fly (*Oscinella frit*), and severe attacks of this and *Javacella spp.* were reported by Håbjørg (1979). Another negative phenomenon is over-induction which is shown to occur also in other species after extended exposure to primary induction conditions.

It was suggested that all these constraints can be avoided in areas with early and stable snow-cover and late snowmelt in the spring (Heide 1980). Such conditions will stop flower development at a relatively early stage in autumn, allow for good over-wintering conditions, and prevent exposure to short photoperiods and moderate temperatures in spring. Under such conditions good and stable seed yields can be obtained, and the Norwegian seed production of high-latitude cultivars of *Poa pratensis* have therefore, been located to inland areas where such conditions are the normal. With the predicted and ongoing global warming it is likely, however, that this production must be removed to areas with even more continental climates and greater winter stability.

The arctic *Poa pratensis* cultivars have now been replaced by a more broadly adapted new cultivar with better seed yield potential and more stable seed yield. As a result, Norwegian seed yields of smooth meadowgrass have increased substantially. Although plant breeding thus have been the solution in this case, the sub-arctic *Poa pratensis* cultivars provide an excellent example of the far-reaching implications the climatic environment may have for flowering and seed yield in cultivars in which performance is dominated by strong genotype x environment interactions.

LITERATURE

- Aamlid, T.S. 1996. Second year yield depression in seed production of subarctic *Poa pratensis* in South Norway. *Norwegian Journal of Agricultural Sciences* 10: 113-124
- Aamlid, T.S. 2000. Varm april gir store timoteifrøavlinger. *Norsk frøavlsnytt*, 5 (5): 6.
- Aamlid, T.S., Skuterud, R., Heide, O.M. & Torskenes, E. 1995. Autumn application of chlormequat chloride (CCC) in seed production of *Bromus inermis*. *Norwegian Journal of Agricultural Sciences*, 9: 271-279.

- Allard, H.A. & Evans, M.W. 1941. Growth and flowering of some tame and wild grasses in response to different photoperiods. *Journal of Agricultural Research* 62: 193-228.
- Cooper, J.P. 1958. The effect of temperature and photoperiod on inflorescence development in strains of timothy (*Phleum* spp.). *Journal of the British Grassland Society* 13: 81-91.
- Evans, M. W. & Allard, H.A. 1934. Relation of length of day to growth of timothy. *Journal of Agricultural Research* 48: 571-586.
- Håbjørg, A. 1978. Climatic control of floral differentiation and development in selected latitudinal and altitudinal ecotypes of *Poa pratensis*. *Meldinger fra Norges landbrukshøgskole* 55 (16): 1-26.
- Håbjørg, A. 1979. Floral differentiation and development of selected ecotypes of *Poa pratensis* L. cultivated at six localities in Norway. *Meldinger fra Norges Landbrukshøgskole* 58 (4): 1-19.
- Heide, O.M. 1980. Studies on flowering in *Poa pratensis* L. ecotypes and cultivars. *Meldinger fra Norges Landbrukshøgskole* 59 (14): 1-27.
- Heide, O.M. 1982. Effects of photoperiod and temperature on growth and flowering in Norwegian and British timothy cultivars (*Phleum pratense* L.). *Acta Agriculturae Scandinavica* 32: 241-252.
- Heide O.M. 1984. Flowering requirement in *Bromus inermis*, a short-long-day plant. *Physiologia Plantarum* 62: 59-64.
- Heide, O.M. 1988. Flowering requirement of Scandinavian *Festuca pratensis*. *Physiologia Plantarum* 74: 487-492.
- Heide, O.M. 1994. Control of flowering and reproduction in temperate grasses. *New Phytologist* 128: 347-362.
- Heide, O.M., Bush, M.G. & Evans, L.T. 1987. Inhibitory and promotive effects of gibberellic acid on floral initiation and development in *Poa pratensis* and *Bromus inermis*. *Physiologia Plantarum* 69: 342-350.
- Langer, R.H.M. 1955. Ear formation in timothy grass (*Phleum pratense*) following vernalization and short-day treatments. *Nature* 176: 263.
- Langer, R.H.M. 1956. Growth and nutrition of timothy (*Phleum pratense*) I. The life history of individual tillers. *Annals of Applied Biology* 44: 166-187.
- Ryle, G.J.A. & Langer, R.H.M. 1963. Studies on the physiology of flowering in timothy (*Phleum pratense* L.). I. Influence of daylength and temperature on initiation and differentiation of the inflorescence. *Annals of Botany* 27: 213-231.

Seed yield contribution in smooth brome grass (*Bromus inermis* Leyss.) and meadow fescue (*Festuca pratensis* Huds.) established on different dates and with different plant densities

Trygve S. Aamlid & Lars T. Havstad

The Norwegian Institute for Agricultural and Environmental Research
Bioforsk Øst Landvik, N-4886 Grimstad, Norway

trygve.aamlid@bioforsk.no

ABSTRACT

Tiller demography and contribution to seed yield were studied in first year seed crops of smooth brome grass (SB, *Bromus inermis* 'Løfar') and meadow fescue (MF, *Festuca pratensis* 'Salten') planted on different dates and with increasing plant densities (A: 15 Jun. / 11 plants m⁻², B: 15 or 30 July / 44 plants m⁻², C: 15 August or 10 September / 178 plants m⁻²) in field trials at Landvik, SE Norway. While the total tiller population in most crops increased until seed harvest, it decreased during panicle elongation in crops of SB and MF that had reached 2000 and 3500 tillers m⁻² in early spring, respectively. Except for the fact that many of the primary tillers of SB died after producing barren stems, tillers formed in August and September had the greatest chance of becoming reproductive and produced the heaviest inflorescences in both species. Most tillers produced in winter or early spring either remained vegetative or died, but spring-emerging tillers contributed up to 30% of the total seed yield in early-established, low-density crops of MF. It is concluded that spring-emerged tillers contribute more to seed yield in MF than in SB and more in seed crops established early at low plant density than in crops established late at higher density.

Key words: flower induction, seed production, sowing time, sowing rate, tiller demography

INTRODUCTION

Both grasses with intravaginal tillering and a bunch-type growth habit, such as meadow fescue (MF), and grasses with a rhizomatous growth habit, such as smooth brome grass (SB), require low plant densities to maximise seed yields. Nordestgaard (1975) found 50-100 plants per m² to be optimal for seed production of MF. For SB, we have seen examples of excellent seed yields from crops planted to only 10 plants per m².

Both SB and MF have dual requirements for flower induction (Heide 1994). While the primary induction requirement in SB is met by 4-6 weeks of short-day exposure with no response to classical vernalization treatments, MF shows limited response to photoperiod, but requires an extended period at 0-10°C. First year seed yields of both

species have been shown to decline if sowing is delayed after mid July (Jonassen & Hillestad 1990, Jonassen 1994).

With the possible exception of red fescue (*Festuca rubra* L., (Aamlid 2005)), individual tillers of perennial grasses have no juvenile stage where they are non-receptive to primary induction stimuli. At least in perennial ryegrass (*Lolium perenne* L.) new tillers may be vernalised as buds or while still in the leaf sheaths of their mother tillers (Kleinendorst 1974). Alternatively, new tillers may receive primary induction stimuli indirectly through their vascular connections with parent tillers (Havstad *et al.* 2003). A consequence of indirect induction is that tillers emerging in spring/early summer may be able to produce seed in the same year.

In the present experiments we established seed crops of MF and SB on three different dates during summer, with a low plant density on the earliest date, an intermediate plant density on the intermediate date and a high plant density on the latest date. By doing so, we intended to produce crops with different tiller structures during primary induction in autumn. Our objective was to study the reproductive behaviour of tillers formed during consecutive periods, especially those formed in spring.

MATERIALS AND METHODS

Field trials were established on silt loam soils at Bioforsk Øst Landvik (58°N) in 1998 and 1999. Plants of MF 'Salten' (origin 67°N) and SB 'Løfar' (origin 61°N) were raised from seed on three occasions and grown at 20°C and continuous light for four weeks after seedling emergence. The plants were transplanted into 1.5m x 1.5m plots as shown for Crops A, B and C in Table 1. Within a central 30 cm x 30 cm subplot of each plot, each newly emerged tiller was tagged with coloured wire-rings at one to two month intervals (no tagging during the winter 1998/99 due to frost/snow). All plots received 50 kg N ha⁻¹ after the last

planting and in the following spring. On 1 Nov 1998 and 10 Nov. 1999, at the end of the establishment seasons, aboveground plant material was cut to a height of 5 cm, dried, weighed and used to calculate shoot dry weight per tiller. In the seed harvest year, heading date was recorded for all reproductive tillers and panicles harvested with a pair of scissors at seed maturity. Within each plot panicles were grouped according to emergence period before seed threshing and cleaning.

Analyses of variance were conducted for each species in each year according to a split plot model with planting date / plant density as the main factor and tiller emergence periods as subgroups. This allowed for a good statistical test of tiller emergence period and the interaction planting date / plant density x tiller emergence period, but comparisons of planting dates / plant densities were hampered by few degrees of freedom. The relationships between plant age and tiller density at the end of the establishment season and panicle production from tillers produced after the November registration (in the following referred to as late-formed tillers) were calculated by simple correlation analysis.

Table 1. Planting dates and plant spacings / densities used for crops A, B and C; and tiller densities and mean shoot dry weights per tiller at the end of the establishment year 1998.

Species	Crop	Planting date	Plant spacing cm	Plant density m ⁻²	1 Nov. 1998	
					Tiller density m ⁻²	Shoot dry weight per tiller (g)
<i>Bromus inermis</i>	A	15 Jun.	30 x 30	11	1256 ab ¹⁾	0.47 a
	B	15 Jul.	15 x 15	44	926 b	0.27 a
	C	15 Aug.	7.5 x 7.5	178	1741 a	0.09 b
<i>Festuca pratensis</i>	A	15 Jun.	30 x 30	11	1070 c	0.63 a
	B	15 Jul.	15 x 15	44	1815 b	0.18 b
	C	15 Aug.	7.5 x 7.5	178	2515 a	0.09 c

¹⁾ In this and the following tables, means followed by the same letter are not significantly different according to the Student Neuman Keul multiple comparison test at $P < 0.05$.

RESULTS

Because of the space limits for this paper, tables will be presented only for the 1998/99 trial. A few comments will be made to the 1999/2000 trial which gave mostly very similar results.

Total tiller population

Significant differences occurred in tiller density by the end of the establishment season. In SB the intermediate crop produced the lowest tiller number in 1998/99 (Table 1) but the highest tiller number in 1999/00. In MF, the two late-established crops were always denser than the early-established crop. The average shoot dry weight per tiller

at the end of the growing season was always higher in crops established early at low density than in crops established later at higher densities (Table 1).

The most vigorous tillering of SB occurred in late August and September in both years (data not shown). In 1998, this was the case also for MF, but in 1999, crops A and B of this

species had their main tillering period before 10 Sep. Tillering was generally slow after 10 Oct. but resumed in early spring in both species. Seed crops of SB with densities higher than 2000 tillers m⁻², and seed crops of MF with densities higher than 3500 tillers m⁻² in early spring showed a net decline in tiller number during panicle elongation (data not shown).

Table 2. Reproductive tillers per m² formed during consecutive periods in seed crops of a) *Bromus inermis* and b) *Festuca pratensis* in 1998-99. Percentage of total fertile tiller population is given in parentheses. (Crop A: Planted on 15 June at 11 plants m⁻²; Crop B planted on 15 July at 44 plants m⁻²; Crop C planted on 15 Aug at 178 plants m⁻²).

a) <i>B. inermis</i>	Period of tiller emergence					Total (<i>P</i> <0.05)
	Before 15 Aug	15 Aug - 2 Oct	2 Oct - 1 Nov	1 Nov - 3 May	3 May - Seed harvest	
Crop A	15 (3%)	381 (66%)	67 (12%)	107 (18%)	4 (1%)	574 ab
Crop B	41 (8%)	307 (61%)	93 (18%)	59 (12%)	4 (1%)	504 b
Crop C	26 (4%) ¹⁾	515 (71%)	148 (20%)	33 (5%)	0 (0%)	722 a
Mean (<i>P</i> <0.001)	27 cd (5%)	401 a (67%)	103 b (16%)	66 bc (11%)	3 d (1%)	600
Significance of interaction: <i>P</i> <0.01						

b) <i>F. pratensis</i>	Period of tiller emergence					Total (NS)
	Before 15 Aug	15 Aug - 2 Oct	2 Oct - 1 Nov	1 Nov - 3 May	3 May - Seed harvest	
Crop A	256 (27%)	300 (31%)	78 (8%)	311 (32%)	18 (2%)	963 a
Crop B	196 (14%)	722 (52%)	96 (7%)	359 (26%)	21 (1%)	1396 a
Crop C	233 (16%) ¹⁾	967 (65%)	63 (4%)	200 (14%)	19 (1%)	1482 a
Mean (<i>P</i> <0.001)	228 b (18%)	663 a (51%)	79 c (6%)	290 b (23%)	20 c (2%)	1280
Significance of interaction: <i>P</i> <0.001						

Reproductive tillers

The contribution of tillers formed during consecutive periods to the final panicle population is shown in Table 2. Reproductive tillers were produced earlier in the establishment year in MF than in SB, but, on the other hand, MF also had a greater percentage of winter/early spring-produced tillers within its panicle population. As indicated by the significant planting date / plant density x tiller age interactions, the relative contribution of late-formed tillers to the panicle population decreased with later planting / higher planting density for both species in 1998/99 (Table 2) and for MF in 1999/00. For SB, the situation in 1999/00 was reversed, most likely due to a low tiller density of crop C in this year. On average for both experimental years and all planting

dates / plant densities, 88 and 76 % of the reproductive tillers had emerged before early November in SB and MF, respectively.

Days to heading

In both species and both years, the difference in heading date between the earliest and the latest-formed tillers was about five days (data not shown).

Seed weight per inflorescence

The heaviest inflorescences in 1999/2000 were produced by tillers formed before 30 Jul in MF and between 10 Sep. and 10 Oct. in SB. As compared with these very productive tiller groups, panicles from spring-emerging tillers were 62% lighter in MF and 76% lighter in SB (data not shown). Similar results were produced in 1998/99.

Seed yield

Regardless of planting date / plant density, most of the seed yield of SB in 1999 (Table 3) was produced on tillers that had emerged in

late August and September. In both years, seed yields of MF were distributed among several age groups in crop A, but concentrated on tillers produced in August and September in crop B and C.

Table 3. Seed yield (kg ha⁻¹) from tillers formed during consecutive periods in seed crops of a) *Bromus inermis* and b) *Festuca pratensis* in 1998-1999. Percentage of total seed yield is given in parentheses. (Crop A: Planted on 15 June at 11 plants m⁻²; Crop B planted on 15 July at 44 plants m⁻²; Crop C planted on 15 Aug at 178 plants m⁻²).

a) <i>B. inermis</i> Planting date / density (m ⁻²)	Period of tiller emergence					Total (NS)
	Before 15 Aug	15 Aug - 2 Oct	2 Oct - 1 Nov	1 Nov - 3 May	3 May - Seed harvest	
Crop A	7 (1%)	941 (77%)	90 (7%)	182 (15%)	1 (0%)	1221 a
Crop B	168 (13%)	767 (62%)	151 (12%)	149 (12%)	9 (1%)	1244 a
Crop C	66 (6%)	787 (74%)	151 (14%)	60 (6%)	0 (0%)	1064 a
Mean (<i>P</i> <0.001)	80 (7%) b	832 (71%) a	131 (11%) b	130 (11%) b	3 (0%) b	1170
Significance of interaction: NS						

b) <i>F. pratensis</i> Planting date / density (m ⁻²)	Period of tiller emergence					Total (NS)
	Before 15 Aug	15 Aug - 2 Oct	2 Oct - 1 Nov	1 Nov - 3 May	3 May - Seed harvest	
Crop A	209 (26%)	286 (35%)	66 (8%)	250 (31%)	2 (0%)	813 a
Crop B	131 (17%)	450 (58%)	36 (5%)	149 (19%)	9 (1%)	775 a
Crop C	125 (20%)	433 (69%)	13 (2%)	39 (6%)	16 (3%)	626 a
Mean (<i>P</i> <0.1)	155 (21%) b	390 (53%) a	38 (5%) bc	146 (20%) b	9 (1%) c	738
Significance of interaction: NS						

Table 4. Simple correlation coefficients between plant age and tiller density as determined on 1 Nov 1998 and 10 Nov 1999 and panicles numbers emanating from tillers produced after these dates. Lower left (in italics): *Bromus inermis*; upper right: *Festuca pratensis*. n=15.

	Plant age as of 1 / 10 Nov	Tiller density as of 1 / 10 Nov	Panicles from late- emerging tillers
Plant age as of 1 / 10 Nov	-	-0.83 (<i>P</i> <0.1)	0.51 (<i>P</i> <5)
Tiller density as of 1 / 10 Nov	<i>-0.23 (NS)</i>	-	-0.30 (NS)
Panicles from late-emerging tillers	<i>0.24 (NS)</i>	<i>-0.52 (P<5)</i>	-

Correlations

While inflorescence production from late-formed tillers in SB was negatively correlated with plant density the previous fall (Table 4), plant age exerted minor influence on both panicle number and seed yield in this species. In *Festuca pratensis*, plant age and tiller density in November were mutually correlated and their relationships with seed yield from late-formed tillers were therefore of the same magnitude. Plant age was, however, more influential than tiller density

on the number of panicles emanating from spring-formed tillers.

DISCUSSION

In both species most reproductive tillers had been formed the previous autumn. This is confirmed earlier studies in MF (Langer & Lambert 1959, Havstad 1998) and SB (Lamp 1952). However, as much as one third of the panicles in the early-established / low-density crop A of MF emanated from tillers that had

emerged after November (Table 2). In agreement with Fang *et al.* (2004) and Aamlid *et al.* (2005), this suggests that the primary induction requirement in MF is not as extreme as indicated by Heide (1994); at least the requirement applies only to seedlings and not to individual tillers of the species.

The effect of plant age on panicle development from late-formed tillers is confounded with the effect of tiller density and, thus, competition level within the various crops. It is, however, interesting that tiller density, but not plant age, was significantly correlated with panicles emanating from late-formed tillers in SB, whilst it was the other way round in MF (Table 4). This suggests that vascular connections to parent tillers is more important for the reproductive behaviour of young tillers in MF than in SB.

Besides determining whether a tiller became reproductive or not, tiller emergence period had a major impact on seed yield per inflorescence. This is in agreement with Ryle & Langer (1963) who explained inflorescence size as a function of meristem size at the time of spikelet initiation. As a result of more seeds per inflorescence, tillers formed early made a greater relative contribution to seed yield (Table 3) than to total panicle population (Table 2).

When not using a cover crop, it is usually recommended to establish Norwegian seed crops of SB and MF at a seeding rate of about 5 kg ha⁻¹ during the first two weeks of July. On the condition that this gives about 100 plants m⁻² Fig. 1 presents a generalized chart of the contribution to seed yield that can be expected from tillers formed during consecutive periods in such crops.

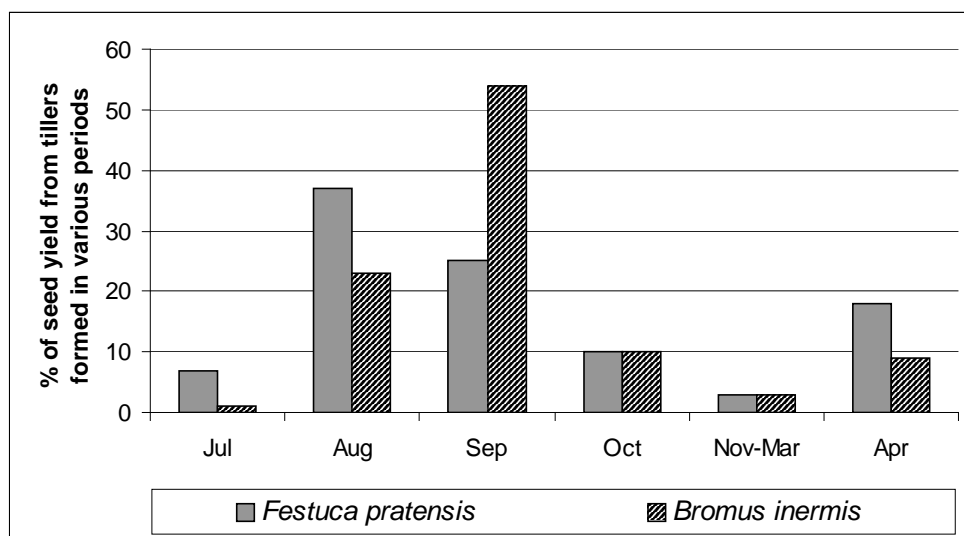


Fig. 1. Generalized pattern of the contribution to seed yield of tillers formed during various periods in seed crops of *Festuca pratensis* and *Bromus inermis* sown without cover crop during the first two weeks of July.

LITERATURE

- Aamlid, T.S. 2005. A comparison between primary induction requirements in vegetatively propagated ramets and seedlings of *Festuca pratensis* and *Festuca rubra*. *Acta Agriculturae Scandinavica* 55: 299-303.
- Fang, C., Aamlid, T.S., Jørgensen, Ø. & Rognli, O.A. 2004. Phenotypic and genotypic variation in seed production traits within a full-sib family of meadow fescue (*Festuca pratensis* Huds.). *Plant Breeding* 123: 241-246.
- Havstad, L. 1998. Seed yield of meadow fescue (*Festuca pratensis* Huds.) in Norway and Denmark: The effects of locations, cultivars and autumn management. *Acta Agriculturae Scandinavica* 48: 144-158.

- Havstad, L.T., Aamlid, T.S., Heide, O.M., & Junntila, O. 2003. Transfer of florigenic stimuli between tillers in photoperiodically split plants of *Dactylis glomerata* and *Bromus inermis*. *Physiologia Plantarum* 118: 270-277.
- Heide, O.M. 1994. Control of flowering and reproduction in temperate grasses. *New Phytologist* 128: 347-362.
- Jonassen, G.H. 1994. Establishment of smooth brome grass (*Bromus inermis* Leyss.) seed crops with or without cover crop, and on different dates without cover crop. *Norsk landbruksforskning* 9: 51-57. (In Norwegian, with English abstract).
- Jonassen, G.H. & Hillestad, R. 1990. Etablering av frøeng uten dekkvekst. *In: Frøavl. NJF seminarium nr 173. Tune Landboskole, Denmark June 18-20 1990. pp. 84-93.* (In Norwegian).
- Nordestgaard, A. 1975. Seed rates of meadow fescue (*Festuca pratensis* Huds.) for seed production. *Tidsskrift for Planteavl* 79: 417-428. (In Danish, with English abstract).
- Kleinendorst, A. 1974. Some effects of vernalization on the reproductive capacity of *Lolium perenne* L. *Netherlands Journal of Agricultural Science* 22: 6-21.
- Lamp, F. 1952. Reproductive activity in *Bromus inermis* in relation to phases of tiller development. *Botanical Gazette* 113: 413-438.
- Langer, R.H.M. & Lambert, D.A. 1959. Ear-bearing capacity of tillers arising at different times in herbage grasses grown for seed. *Journal of the British Grassland Society* 14: 137-140.
- Ryle, G.J.A. & Langer, R.H.M. 1963. Studies on the physiology of flowering of timothy (*Phleum pratense* L.). II. Influence of temperature and daylength on the size of the inflorescence. *Annals of Botany* 27: 233-244.

The effect of barley ratio (*Hordeum vulgare* L.) as a companion Crop on seed yield and yield components of common vetch (*Vicia sativa* L.) sown at different rates

Huseyin Ozpinar¹⁾, Hikmet Soya²⁾ & Zeki Acar³⁾

¹⁾Aegean Agricultural Research Institute, P.O. Box 9, Menemen-Izmir, Turkey

²⁾Aegean University Agricultural Faculty, Bornova- Izmir, Turkey

³⁾Ondokuz Mayıs University Agricultural Faculty, Samsun, Turkey

huseyin_ozpinar@hotmail.com

ABSTRACT

The objective of this study was to determine the optimum seeding rates of common vetch of when grown for seed in combination with various ratios of barley as a companion crop. Experiments were carried out in a completely randomised block design with four replicates at Aegean Agricultural Research Institute, Izmir, Turkey in the 1995/96 and 1996/97 growing seasons. Materials used in this study were early flowering common vetch cultivar 'Kubilay-82 and the barley cultivar 'Gem Arpa'. Common vetch densities were 100, 150, and 200 vetch plants/m². The ratios for barley were 0, 10, 20 and 30 %. In this study, vetch plant height, pod number, seed number per pod, vetch seed yield, 1000-seed weight, days to maturity, vetch seed water content, and vetch seed germination were observed. In general, vetch plant densities did not significantly affect measured characters. Companion crop significantly reduced common vetch seed yield, the number of pod per unit area and significantly increased common vetch plant height.

Key words: Common vetch, *Vicia sativa*, barley, *Hordeum vulgare*, mixture, seed yield.

INTRODUCTION

Although Turkey has suitable climatic and soil conditions for agriculture, the country is not self-supplied with animal products. There are two main reasons: Firstly, low-yielding local animal populations account for most of the livestock, and secondly, good quality forage is not produced in sufficient amounts.

Forage crops accounts for only 10 % of the cultivated field crop area in Turkey. In addition to this, high-yielding cultivars are not commonly used, preventing sufficient forage production. The area dedicated to common vetch cultivation, which has an important share of forage crops, is 260 000 ha. The average annual vetch seed production is 165 000 ton, green hay production 298 255 ton, and hay production 326 857 ton. Vetch cultivation area accounts for 24 % of the forage crop area with 13.5 % green hay, and 17 % hay production (Anonymous 1994).

The fact that there is 500 000 ha cotton production area available to intercropping, makes it possible to increase the forage crop cultivation area. Vetch, vetch plus oats or vetch plus barley mixtures could be sown in those areas (Soya & Avcioğlu 1991).

Common vetch growth is semi-erect but becomes prostrate as the crop matures. This kind of growth makes machine harvest difficult. In order to solve these constraints sowing vetch in mixtures with barley or oat produces positive results as stated by many researchers (Algren 1956).

The common vetch cultivar, Kubilay 82, which can be sown in mixtures of barley or oat, occupies 5 % of the vetch cultivation area in the Aegean Region and has tended to increase rapidly (Dizdaroğlu & Sabancı 1993).

The objective of this study was to determine the optimum seeding rate for seed yield of common vetch cv. Kubilay 82 sown with barley at various ratios.

MATERIALS AND METHODS

This study was carried out at the Aegean Agricultural Research Institute in the 1995-1996 and 1996-1997 growing seasons. The location of the institute is 38°01' N and 27°01' E, at an elevation of 12 m above sea level.

Rainfall during the growing periods, (459.3 mm in 1996 and 441.7 mm in 1997) were lower than the long term average of 528.6 mm). But rainfall during the spring months of the second year was higher than that during the first year. The mean temperatures, after March in second year, were lower compared to those in the first year and the long-term average. Maximum temperatures for both years were lower than long-term average and maximum temperatures of second year were lower than the first year.

The soil type of experimental site was typic Ustorhent. Physical and chemical characteristics of the 0-20 cm soil layer were 36,4 % sand, 16,0 % clay, 47,6 % silt, pH 7,7, total salt 0.046, pH 4,7 %, organic material 1,8 %, available P₂O₅ 420 kg ha⁻¹ and K₂O 783 kg ha⁻¹.

Materials used in the experiment were early common vetch (*Vicia sativa* L) cv. Kubilay 82, and barley (*Hordeum vulgare*) cv. Gem. both of which have been developed and registered by the Aegean Agricultural Research Institute.

The experiment had two factors as the vetch seeding rate was targeted at a plant number of was 100, 150, or 200 plant m⁻² and the companion crop ratios were 0, 10, 20, or 30 %. The experiment was arranged in a randomized block design with four replicates. Plot dimensions were 2.4 m by 5 m, giving a 12 m² plot area. Sowing was carried out using as 12 rot plot drill at 20 cm row spacing. Fertilizers applied before sowing were 70 kg ha⁻¹ P₂O₅ as triple super phosphate (Urem 1985) and 30 kg ha⁻¹ N as ammonium sulphate (Ulgen & Alemdar 1979). Weed control was performed by hand. During the experiment,

serious pest and diseases were not encountered.

Sowing and harvest dates were 11 January 1995 and 18 June 1996 for the first year, and 20 November 1996 and 10 June 1997 for the second year, respectively.

Characters observed were emergence, maturation time (day), vetch plant height (cm), pod number m⁻², the number of seed per pod, vetch seed yield (kg ha⁻¹), 1000-seed weight (g), seed water content (%) and germination (%).

All data were statistically analysed using analysis of variance.

RESULTS AND DISCUSSION

Plant numbers per m² were close to the target plant number in both years. Physiological maturity times were 190 day for the first year and 202 day for the second year. None of the experimental factors affected physiological maturity time.

Apart from vetch plant height, vetch seeding rate did not have significant effects on the characters examined (data not shown)

In mixtures, barley ratios had a significant effect on vetch seed yield. Pure vetch sowings gave higher seed yield. As barley ratios increased, vetch seed yield decreased (Table 1). Decrease in vetch seed yield in mixture might have stemmed from two reasons. Firstly, competition between vetch and barley plants in favour of barley, secondly, decrease in vetch plant number per unit area. The fact that seed yield of mixture in the second year was lower than that in the first year may be attributed to the second year's weather conditions which accentuated competition between barley and vetch. Being cooler than the first year and long term average, the second year promoted vetch vegetative growth thus decreasing generative development. Under these circumstances, barley seed yield increased. These results are in agreement with the findings that climatic conditions had significant effect on competition of vetch with companion crop (van der Kamp 1966).

Table 1. Effect of barley ratios in vetch+barley mixtures on vetch yield characteristics

Year	Barley Ratios (%)	Seed Yield kg ha ⁻¹	Pod number m ⁻²	Seed number per pod	1000-seed weight (g)	Vetch Seed water content (%)	Germination (%)	Plant height (cm)
96	0	2040 a	2530 a	7.8	54.0 b	3.3 b	92.4	22.9 b
	10	1960 ab	2010 ab	7.7	56.2 a	3.8 b	90.8	75.0 a
	20	1760 b	1705 b	7.7	55.8 a	4.6 a	93.0	76.0 a
	30	1760 b	1640 b	7.6	56.6 a	4.0 ab	93.4	74.5 a
	LSD (5%)	220	650	NS	1.4	0.7	NS	4.3
97	0	1960 a	1150	7.9	48.1 b	8.0 b	96.7	20.1 b
	10	1690 b	1145	8.0	50.7 a	8.7 ab	97.0	76.7 b
	20	1640 b	1110	8.0	51.2 a	9.0 a	97.5	79.2 a
	30	1560 b	935	8.0	50.9 a	9.2 a	97.1	80.0 a
	LSD (5%)	220	NS	NS	2.2	0.8	NS	3.9
Mean	0	2000 a	1840 a	7.9	51.1 b	5.6 c	94.5	21.5 b
	10	1820 b	1575 ab	7.9	53.5 a	6.2 b	93.9	75.8 a
	20	1700 bc	1410 b	7.8	53.5 a	6.8 a	95.2	77.9 a
	30	1660 c	1290 b	7.8	53.8 a	6.6 ab	95.2	77.3 a
	LSD (5%)	150	364	NS	1.9	0.5	NS	2.9

In general, it was determined that pure vetch sowing gave higher yields than mixture sowings (Stoimenov 1959, Grienko 1960, van der Kamp 1966). By contrast, Soya (1994) stated that a 85 % vetch plus 15 % barley mixture gave the highest vetch seed yield. While this may be due to vetch cultivars responding differently to mixture ratios and seeding rates, the same researchers stated that vetch seed rate decreased with increased barley ratios. Therefore, results of this study are in agreement with findings of various studies showing that it is necessary that companion crop ratios be lower or between 10 and 20 % (Melzer 1964, van der Kamp 1966, Soya 1994). But there are some studies which gave the ideal mixture ratios of 30 % of companion crop (Berkner 1954, Gulcan 1989, Kotecki 1990).

The effect of barley ratios on the number of pod per m² was found to be significant. Pure vetch sowing gave the highest pod number. As barley ratios increased, a decrease was observed in pod number. The year effect was found to be significant for pod number; first year gave more pods (Table 1). Weather conditions in the second year gave more favourable conditions to barley crop resulting in decrease in the pod number of vetch plants.

The effect of barley ratios on the number of seeds per pod was not significant in either

year. But year effect was important and there was an increase in the number of seed per pod in the second year. This indicates that the number of seed per pod was not affected by plant number per unit area and by competition between and within plants. But, an increase in seeds per pod under unfavourable conditions may stem from a decrease in pod number.

In this study, findings about the number of seed per pod are in agreement with some previous work (Saglamtimur *et al.* 1990; Cakmakci and Acikgoz, 1994). However, fewer seeds per pod were reported from some studies in which different cultivars were tested under different climatic conditions (Aydogdu & Acikgoz 1995, Martiniello & Ciola 1995). An increase in the number of seed per pod as stated by Soya (1994) was not observed in this study.

The effect of barley ratios on 1000-seed weight was found to be significant. Pure vetch sowings gave lower 1000-seed weights. Year effect was also observed, the first year having greater 1000-seed weight. The fact that the greater number of pods was in pure vetch sowing led to more competition within and between vetch plants, giving rise to a reduction in seed filling. In contrast to this, the fewer vetch plants and competition with barley in mixture sowing reduced the number of pods per unit area, thus assimilates were

evenly distributed between seeds and pods. Similar results were also observed by Soya (1994) who stated that 1000-seed weight increased in mixture sowings but decreased in pure sowings. Findings of this study are in agreement with results of Saglamtimur *et al.* (1990), Cakmakci & Acikgoz (1994), Martiniello & Ciola (1995), and Siddique & Loss (1996).

Barley ratios had significant effects on vetch seed water content at harvest (Table 1). In general, vetch seed water content of mixtures was higher than pure stands. But it was not too high to create any problem for the harvest or post harvest process. Year effect was also important as the second year had higher seed water contents..

Table 2. Effect of vetch seed rates in vetch+barley mixtures on vetch plant height (cm).

Vetch density (plants/m ²)	Year		
	1996	1997	Mean
100	63.8	67.8 a	65.8 a
150	62.7	61.7 b	62.2 b
200	60.3	62.5 b	61.4 b
LSD (5%)	NS	3.4	2.5

There was not any effect of barley ratios on the germination of vetch seed. Germination was always over 90 %.

Vetch plant height was significantly affected by both vetch seed rates and barley ratios

(Tables 1 and 2). There was an interaction between year and barley ratios.

While the highest vetch plant height was obtained from the 20 % barley mixture in the first year, it was from the 30 % mixture in the second year. The lowest vetch plant height was observed in pure sowings in both years. Lower vetch seed rates in mixture favours more upright vetch plant growth. However, all mixture sowings gave satisfactory upright growth for machine harvest. Algren (1956), Kotecki (1990), Saglamtimur *et al.* (1990) stated similar results in relation to having higher vetch plant height with mixture sowings. Soya (1994) also observed that increased barley ratios gave higher vetch plant height.

Sowing vetch with barley in mixtures gives more upright vetch growth, resulting in much easier machine harvest and reduced harvest losses compared with pure vetch sowing. But there are some points that should be taken into consideration when mixture sowing is practised. Developing stages of companion crop and vetch plants should be similar. Vetch varieties to be used in mixture should be early. Early varieties such as cv. Kubilay 82 generally have large seeds which are easier to separate from barely seeds. Although lodging of barley plants in mixture occurs at all plant densities, it happens more frequently vetch seeding rates and barley ratios are increased.

LITERATURE

- Algren, H.G., 1956, Forage Crops. McGraw-Hill Book Company, Inc. Newyork.
- Anonymous, 1994. Tarımsal Yapı ve Üretim 1992. Dev. İst. Ens.Yay. Nr.1544. Ankara.
- Aydoğdu, L., and E. Açıkgoz, 1995: Effect of seeding rate on seed and hay yield in common vetch (*Vicia sativa* L.). *J. Agronomy and Crop Science* 174, 181-187, Berlin.
- Berkner, F., 1954. Pflanzenzüchterische studien an winter-u. sommerwicken. zeitschrift f. *Planzenzüchtung* 33 (2): 157-68. Paul Parey in Berlin.
- Çakmakçı, S., and E. Açıkgoz, 1994: Component of seed and straw yield in common vetch (*Vicia sativa* L.). *Plant Breeding* 113, 71-74, Berlin.
- Dizdaroğlu, T., ve C.O. Sabancı, 1993: Ege ve Guneş Marmara Bolgelerinde fig üretimini sınırlayan faktörler. *Anadolu Ege Tar. Ara. Ens. Dergisi* 3(1):13-39.
- Gülcan, H., 1989. Yem Bitkileri. Çukurova Üni. Zir. Fak. Ders Kit. No. 1. Adana.
- Kotecki, A., 1990. Effect of pure and mixed culture on the seed yield of summer vetch (*Vicia sativa* L.). *Herb. Abs.* 60(1): 13. Nr.92.
- Martiniello, P., and A. Ciola, 1995. Dry matter and seed yield mediterranean annual legume species. *Agronomy Journal*. Vol. 87 (5), 985-993.

- Melzer, H., 1964. Zur anbaumethodik von sommerwicken im Norden der DDR. Das Saat-u. Pflanzgut. Nr. 2. P.30-32.
- Siddique, K.H.M., and S.P. Loss, 1996. Growth and seed yield of vetches (*Vicia* spp.) in South-westren Australia. Australian Journal of Experimental Agriculture **36**, 587-93.
- Soya, H., 1994. The effects of barley ratios (*Hordeum vulgare* L.) as campanion crop and row spacings on seed yield and yield components of common vetch (*Vicia sativa* L.). Anadolu J. Aegean Agricultural Research Institute **4**(1): 8-18.
- Soya, H., ve R. Avcıođlu, 1991. Outlook of leguminous forage crops and possibilities of increasing production areas in Turkey. The Proceedings of Second Forage and Pasture Crops Congress p. 409-415 Bornova, İzmir.
- Stoimenov, S., 1959. Verbesserte Production von Wickensaatgut. Bull. Wiss.-techn. Inform. Landwirt. Forschung-Ins.-Knesha. Nr. 1: 24-26
- Ülgen, N., ve N. Alemdar., 1979: Azotlu Gübrelerin Çeşitli Kültür Bitkilerinin Verimlerine Olan Etkilerinin Karşılaştırılması. I. Orta Anadolu Bölgesi. Köyışleri ve Kooperatifler Bakanlığı, Topraksu Gen. Müd. Top. ve Güb. Ara. Ens. Yay. Nr. 82
- Ürem, A., 1985. Türkiye'de Önemli Yem Bitkilerinin Üretimi, Yetiştirilmesi ve Bazı Tescilli Çeşitlerin Özellikleri ile Tohumluk Sorunları. Ege Böl.Zir.Ara.Ens.Yay. Nr.55.
- van der Kamp, T.C., 1966. Fodder vetch seed production field trial results in 1959, 1960 and 1961. Herb.Abs. **36** (3), Nr.1334.

Winter wheat: its effect on ryegrass seed yield and control options

Richard Chynoweth¹⁾, Nick Pyke¹⁾ & Phil Rolston²⁾

¹⁾Foundation for Arable Research, P.O. Box 80, Lincoln, New Zealand

²⁾AgResearch, Private Bag 4749, Christchurch, New Zealand

chynowethr@far.org.nz

ABSTRACT

Both ryegrass and wheat are important crops in the rotation of arable farming within New Zealand. Ryegrass crops commonly follow cereal crops in our rotation. Worldwide, much work has been completed looking at the impact of weeds on the grain yield of cereals. For example, wild oats and Italian ryegrass have been shown to reduce wheat yields at low plant populations and by up to 60 % at higher populations. However, limited work has been completed on the effect of cereals on the seed yield of ryegrass. Some estimates of grain losses during the harvesting of cereal crops put potential losses at between 40 and 470 seeds m⁻² (approx. losses of 20 - 235 kg ha⁻¹). Volunteer cereals can therefore be a major weed in autumn sown herbage seed crops. The presence of cereals is of concern due to a number of factors, (i) direct competition with the crop species, (ii) seed contamination (export issues) and (iii) disease carryover (e.g. take all). The objectives of these studies were to (i) quantify the effects of cereal contamination on harvested seed yield and (ii) to investigate control measures for cereal contamination. Results from a combination of three trials (two wheat and one barley) suggest no yield penalty ($P < 0.05$) in perennial ryegrass until cereal populations reach approximately 20 plants m⁻². This is followed by a rapid decline in ryegrass seed yield as cereal population increases. Above 20 plants m⁻² competition had a large effect on relative seed yield, with an average drop off of 1.5 % per cereal plant, e.g. for a 2000 kg ha⁻¹ crop this would equate to a 30 kg ha⁻¹ loss for each cereal plant. Various herbicide treatments were successfully trialled for the control of both winter wheat and barley contamination in ryegrass seed crops.

Key words: barley, cereal, contamination, glyphosate, herbicide, *Lolium*, weed competition.

INTRODUCTION

On arable farms within Canterbury, New Zealand (NZ), ryegrass seed crops are often sown in rotation following cereals (Foundation for Arable Research (FAR) unpublished data). This can result in significant amounts of volunteer cereals competing with perennial ryegrass (*Lolium perenne* L.) seed crops. Later flowering cultivars and/or Italian ryegrass (*Lolium multiflorum* Lam.) seed crops can be grazed later when the cereal species has become reproductive and commenced stem elongation. As stem extension occurs livestock have the opportunity to remove the growing point (apex). In many cases this results in the cereal not recovering or suppresses it enough not to cause major competitive problems

towards the ryegrass seed crop. However, in early flowering ryegrass cultivars, which get grazed and closed earlier, volunteer cereals are a greater problem. A stale seedbed or ploughing can reduce the problem in some cases. However, most growers prefer to plant immediately without any extra costs (ploughing vs. minimal cultivation vs. chemical control) or delay. Recent work undertaken by FAR suggests that any delay in sowing date reduces income from both grazing and seed yield by up to \$NZ 10 ha⁻¹ day⁻¹ (FAR 2006). Trial work undertaken by FAR demonstrated the competitive effects of wild oats (*Avena fatua* L) in ryegrass and their control (FAR 1998). Work has been carried out on the competitive effects of wild oats and

grasses on cereals but limited work has been carried out on the effects of cereals on ryegrass seed crops.

This paper attempts to summarise a number of field trials where the effects of cereal contamination and cereal control were investigated. The main objectives of these trials were (i) to investigate the effects of cereal competition on the seed yield of a mid season forage perennial ryegrass cultivar and (ii) to provide growers with practical tools for the reduction of cereals in ryegrass seed crops.

MATERIALS AND METHODS

Winter wheat competition

Trials investigating the effects of winter wheat (*Triticum aestivum* L.) on seed yield were planted in March and April 2003 and 2004, respectively, at Lincoln (43° S latitude), New Zealand. Both trials were sown with cultivar Aries HD, a mid season diploid perennial ryegrass at a sowing rate of 12 and 8 kg/ha in 2003 and 2004, respectively. In 2003, wheat was mixed with the ryegrass seed before sowing and drilled at the same time as the ryegrass. In 2004, wheat was surface applied to the plots and cultivated in using the drill at ryegrass planting. Target wheat populations were 0, 5, 10, 20 and 40 plants/m² (cultivar Claire) and 0, 5, 10, 20, 40 and 80 plants/m² (cultivar Amarok) in 2003 and 2004, respectively. Both trials received 1.2 L ha⁻¹ Moddus[®] (a.i. 250 g L⁻¹ trinexapac-ethyl) and 181 kg N ha⁻¹ in the form of urea.

Winter wheat control

Perennial ryegrass, cultivar Banquet (a late season tetraploid, long rotation ryegrass) and wheat (cultivar Torlesse) were sown 11 May 2005. Ryegrass sowing rate was 12 kg ha⁻¹ while a target wheat population was 50 plant m⁻². Three chemicals were evaluated, Teedal[™] (7 kg ha⁻¹) (a.i. 630 g kg⁻¹ TCA plus 110 g kg⁻¹ 2,2 dichloropropionic acid), Moddus[®] (1 & 2 L ha⁻¹) and glyphosate (250 ml ha⁻¹, a.i. 360 g L⁻¹ glyphosate). Applications of Teedal[™] and glyphosate took place on 7 September 2005 while Moddus[®] was applied 22 November 2005. Cereal contamination was 36 plants m⁻² in late winter which equated to approximately 39 ears m⁻² in January 2006 (Table 1). Plots were machine windrowed, harvested and seed cleaned to a 1st generation standard.

Barley competition and control

Barley (*Hordeum vulgare* L.) (cultivar Triumph) was applied pre ryegrass sowing at 500 kg ha⁻¹ on 17 March 1999 and incorporated to 50 mm sowing depth. Perennial ryegrass (cultivar Nui) was sown at two dates on 7 and 30 April respectively at 10 kg ha⁻¹ in a split plot design (sowing date equals split plot treatment). Pre emergence glyphosate at 2 L ha⁻¹ (720 g ha⁻¹ a.i.) was applied to all plots, except the untreated control, when 1 % of the ryegrass had emerged. Post emergence applications included Teedal[™] (7.5 kg ha⁻¹), glyphosate (250 ml ha⁻¹), Kerb[®] (750 ml ha⁻¹ (a.i. 500 g L⁻¹ propyzamide and 42 g L⁻¹ ethylene glycol) and glyphosate wick wiped. Two quadrants of 1 m × 3 rows were hand harvest at 42-45 % seed moisture content and threshed when dry. The trial area received 92 kg N ha⁻¹. It was not treated with Moddus[®].

Individual trials were subjected to separate ANOVA, the combined trial data was subjected to regression analysis only.

Table 1. Cereal population effects in seed yield (kg/ha) of perennial ryegrass (*Lolium perenne* L.) in three separate trials, 1999-2004, Lincoln, New Zealand.

Trial	Cereal population (plants m ⁻²)	Seed Yield (kg ha ⁻¹)
Wheat 2003	0	1650
	7	1630
	15	1730
	24	1580
	37	1590
	Sign. (<i>P</i> <0.05)	NS
Wheat 2004	0	1800
	4	1810
	8	1890
	14	1880
	22	1740
	36	1580
Sign. (<i>P</i> <0.05)	NS	
Barley 1999	0	1540
	1	1450
Note: Only 7 th April sowing data presented.	21	1430
	25	1430
	40	1180
	61	510
Sign. (<i>P</i> <0.05)	LSD = 490	

RESULTS

Winter wheat: its effect on ryegrass seed yield

Ryegrass seed yield decreased at higher cereal populations in all trial seasons. Statistical differences in seed yield between contamination levels were present only in the barley trial in 1999 (table 1). There was a general trend for a slight increase in seed yield at low cereal populations. Note; only data from 7th April sowing is shown as

preemergence glyphosate removed the majority of cereal contamination in the 30 April 1999 sowing i.e. cereal had been sown for 44 days before glyphosate application.

Results from a combination of three trials (two wheat and one barley) suggest no yield penalty until a cereal population of approximately 20 plants/m². This was followed by a rapid decline in ryegrass seed yield as cereal population increased (Fig. 1).

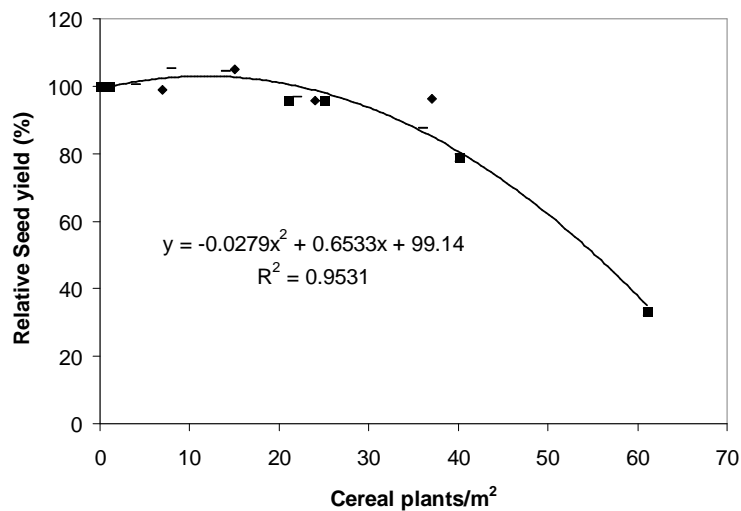


Fig. 1: Relative seed yield of perennial ryegrass when contaminated with winter cereal (◻= barley, 1999; ◻= wheat 2003; ◻ = wheat 2004) (wheat and barley combined). Results from three New Zealand trials (1999-2006)

Cereal control

In wheat the application of TeedalTM controlled all volunteers, while glyphosate showed high levels of control ($P < 0.05$) compared to the untreated area (Table 1). Moddus[®] gave no control of wheat ($P < 0.05$). Despite variations in cereal contamination, no difference ($P < 0.05$) was found in harvested seed yield.

Table 1: Trial results for chemical control options of winter wheat, FAR arable site, dryland, 2006 harvest.

Treatment	Moddus [®] rate (L ha ⁻¹)	Wheat (heads m ⁻²)	Seed Yield (kg ha ⁻¹)
Ryegrass only control	-	0	1120
Ryegrass + wheat	-	39	1210
Moddus [®] 1.0 L ha ⁻¹	1.0	39	1310
Moddus [®] 2.0 L ha ⁻¹	2.0	34	1250
Glyphosate (360) 0.3 L ha ⁻¹	-	0	1210
Glyphosate (360) 0.3 L ha ⁻¹	1.0	1	1140
Teedal TM 7.0 kg ha ⁻¹	-	0	1080
Teedal TM 7.0 kg ha ⁻¹	1.0	0	1120
LSD _{0.05}		12	NS

Winter barley control

The use of a stale seed bed and delayed sowing, with the use of glyphosate pre-crop emergence, was very effective at controlling barley volunteers (data not shown). The application of Teedal™ (7 kg ha⁻¹) and glyphosate (250 ml ha⁻¹) were also effective at controlling barley post emergence (1 plant m⁻²). Kerb® (750 ml ha⁻¹) gave adequate control but not significantly less than Teedal™ and glyphosate. All treatments increased seed yield over the control (61 plants m⁻²).

DISCUSSION

A critical population of 20 cereal plants m⁻² was identified before ryegrass seed yield declined. Above 20 cereal plants m⁻², competition had a large effect on relative seed yield, with an average drop off of 1.5 % per cereal plant, e.g. for a 2000 kg ha⁻¹ crop this would equate to a 30 kg ha⁻¹ loss for each cereal plant above 20 plants m⁻². This result is consistent with wild oat studies as reported in FAR (1998). Wright & Hebblethwaite (1983) found a reduction of 1 % per 10 wheat plants under UK growing conditions. These results suggest that at low cereal populations, ryegrass is a strong competitor during the reproductive phases of growth, while wheat is possibly more competitive during vegetative growth.

Although populations of cereals below approximately 20 plants m⁻² did not reduce seed yield, they can reduce saleability of the

seed line and carry over disease. When cereals survive as a volunteer they effectively act as a host for all cereal diseases, removing the aim of most rotations, e.g. carryover of 'take all' disease (*Gaeumannomyces graminis* var. *tritici*). However, it should also be noted that most grasses, including ryegrass, can be a host for 'take all'. Any ryegrass crop for export to Australia must contain very low levels of cereal contamination to meet quarantine regulations.

For the control of winter wheat there was no statistical difference between treatment yields. Control of both wheat and barley was achieved by the application of Teedal™ or glyphosate. When using glyphosate, extreme care must be taken when considering rate and timing. Crops must have adequate tiller numbers as growth will be reduced by up to 50 %. Teedal™ will also reduce growth but to a lesser extent, approximately 16 % (Rolston & Archie 1999). Grazing can be used on most late cultivars (including Italian ryegrass) to effectively remove the growing point of most cereal volunteers. However this does not necessarily give 100 % control, which may be required.

ACKNOWLEDGEMENTS

FAR would like to thank AgResearch, NZArable and PGGWrightson who carried out this Research.

LITERATURE

- FAR 1998. Wild oat control in ryegrass seed crops. (ed.) Rolston, P. and Archie, B. Foundation for Arable Research, Arable Update, Herbage, No. 12. New Zealand.
- FAR 2006. Sowing date in perennial and perennial type hybrid ryegrasses. Foundation for Arable Research, Arable Update, Herbage, No. 46. New Zealand.
- Rolston, M.P. & Archie, W. 1999. Volunteer cereal control in ryegrass. Report to Foundation for Arable Research, New Zealand.
- Wright D. & Hebblethwaite P.D. 1983. Volunteer winter wheat: its effects and control in ryegrass seed production. Weed Research 23: 273-281.

Vacuum seed planter to plant ryegrass seed in space plant nursery

Lloyd R. Nelson

Texas Agricultural Experiment Station, Texas A&M University Agricultural Research and Extension Center, P.O. Box 200, Overton, TX 75684, USA

lr-nelson@tamu.edu

ABSTRACT

A single row push vacuum seed planter (Seed Ace) is marketed by Sutton Agricultural Enterprises, Inc., Salinas, CA, USA (www.suttonag.com/SeedAce.html). Nozzles (pencil size) are inserted into a 10 cm diameter vacuum (powered by a chargeable battery) wheel. The vacuum wheel turns by a ground driven drive wheel. As the vacuum wheel turns, the nozzles pass into a seed tank (reservoir) and suction causes seed to stick to a small hole in each nozzle. As the wheel turns around, it passes over a seed hopper, plastic brushes knock the seed off from the nozzle and seed drops into a tube and is planted. The distance between seed drops can be adjusted from about 5 cm to 60 cm. The depth of planting is adjustable and works well in a prepared seedbed. The cost of the machine is approximately \$1800 or 1400E. The planter has potential to be a useful tool in plant breeding programs where labor is limited. Advantages are ease to operate, little time required for planting, eliminates need for greenhouse space, labor and supplies. Disadvantages include more than one seed being picked up about 10% of time requiring seedlings to be thinned which is time consuming. Planter does not work as well with small seeded grasses, and requires a minimum of 45 g of seed. Volunteer grass seedlings (weeds) can result in a loss of that nursery.

Key words: grass breeding, *Lolium multiflorum*, ryegrass breeding

INTRODUCTION

The objective of this research was to compare two methods of planting a space planted ryegrass (*Lolium multiflorum* Lam.) nursery. In our forage and turfgrass ryegrass breeding program we are always short of personnel to conduct work involved in the program. Only one technician is available for day to day operations. In addition, we often ask the question of how many space plants do we need to maintain genetic variability and limit inbreeding depression, especially with the forage program. Our space plants are started by planting single seed in peat cups in the greenhouse where they are grown for 4 to 6 weeks. They are then transplanted to the field utilizing a transplanter pulled behind a tractor. Each plant must be picked up and dropped into cups on a rotating wheel, which places the plant into the prepared soil in a row with spacing about 60 cm between plants. This method has been successful;

however, it required much manual labor and greenhouse space. When dry weather occurred, we either delayed transplanting or increased risk of plant loss in the field. We have cone planters available; however, they do not separate seed sufficiently for a "space" planting. In 2005, we became aware that a push-type vacuum planter was available and was used for planting vegetable seed. Most of the vegetable seeds are round and are not long, thin and very light in weight as are most grass seed. After some investigation, we purchased a unit and have been testing it for two growing seasons.

MATERIALS AND METHODS

The planter (Seed Ace) is marketed by Sutton Agricultural Enterprises, Inc., Salinas, CA, USA (www.suttonag.com/SeedAce.html). A single row, push vacuum seed planter weighs 18 kg with battery, so it can be transported quite

easily. The planter was designed to sow seed effectively and accurately in order to save labor thinning plants. Nozzles (5 cm long by 7 mm width) are inserted into a 10 cm diameter vacuum (powered by a chargeable battery) wheel. The vacuum wheel turns by a ground driven (chain) drive wheel. As the vacuum wheel turns, the nozzles pass into a seed tank and suction causes seed to stick to a small hole in each nozzle. As the wheel turns around, nozzle tips pass over a seed hopper, plastic brushes knock the seed off the nozzle, and seed drops into a tube and is planted. The nozzle then rotates around to pick up the next seed. The distance between seed drops can be adjusted from about 5 to 60 cm. The depth of planting is adjustable and works well in a prepared seedbed. The planter has small double disk openers, a drag chain, followed by a wide (14 cm) packer wheel which leaves a firm seedbed. The cost of the machine is approximately \$1800 or 1400E. The planter was easy to operate and we used a string staked at each end to plant along side to insure rows were straight.

RESULTS AND DISCUSSION

We calibrated the planter utilizing ryegrass seed in our lab on a white floor to count seed and measure spacing as seed were dropped. Seed spacing as specified by the manual can be from about 5 to 60 cm, or any desired distance between these measurements. This is accomplished by changing the number of nozzles and/or sprocket wheel size on the drive chain. The planter seems to be very reliable and has worked well in almost all planting situations. The system is not perfect by any standards in our opinion. It required a sizable amount of seed before the nozzles will pass through the seed tank, or approximately 45 g of ryegrass seed. Seed must be very clean, without any trash, or seed parts will be

picked up in place of seed. Upon completion of each nursery, the seed tank is not easily cleaned out, as a small door must be removed, which allows the seed to flow out of the bottom of the tank. This requires a large pan, and still some seed usually spills out, especially if there is any wind. We had one occurrence where we evidently did not closed the small door properly and had seed dropping out while we were planting. This resulted in many seedlings appearing which were not in the intended row. Approximately 10% of the time, a nozzle will pick up two seed, so a double plant occurs; however, they can be thinned in the field with a close examination. We believe this is a more serious problem with smaller seed, such as we have with turf-type annual ryegrass seed. This would likely also be the case with perennial ryegrass seed. In comparison with transplanted plants, seeded plants are much smaller and slower growing for the first 2- to 3-months. This makes it more difficult to inspect the rows, and plants, especially if there are volunteer plants in the nursery. We spray our nurseries with a 2,4-D product to control broadleaf weeds; however, we also have grassy weeds, so they must be controlled by hoeing. Thinning of plants is required; however, this process can be completed later in the season, thereby allowing the breeder to make selections for desired characters. In conclusion, the space planter is a good tool which should be useful in many grass breeding programs. It should result in less labor during the planting process, more rapid planting of numerous space planted nurseries, and eliminates the need for growing space plants in the greenhouse. The space planter does require fields with minimum volunteer grassy weeds, and probably more labor (thinning and weed control) later in the season. We will continue to utilize the planter in our plant breeding program.

Effect of cutting schedule on seed yield in alfalfa

Dura Karagić, Slobodan Katić, Sanja Vasiljević & Dragan Milić

Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Serbia

djura@ifvcns.ns.ac.yu

ABSTRACT

To ensure high and stable seed yields in regions with semi humid climate, it is necessary to prevent the luxuriant growth of alfalfa plants and their subsequent lodging. The most effective method of controlling rank growth in alfalfa is cutting. Effect of cutting schedule on alfalfa seed yield was studied in four-year experiments (2001-2004). Four cutting schedules with variable dates of initial cutting were tested: first cutting for hay was carried out on May 5 (budding) in the c_1 schedule, on May 15 (start of flowering) in the c_2 schedule, on May 25 (full flowering) in the c_3 schedule, and on May 5 and June 5 in the c_4 schedule. Second regrowth was used for seed production in the c_1 - c_3 schedules, and third regrowth in the c_4 schedule. The study involved the seven most widely grown alfalfa cultivars in Serbia, six domestic and one French. The single late cut (c_3 schedule) achieved the best balance among the yield components and thus the highest seed yield (468 kg ha^{-1}). Significant positive correlations were found between seed yield on one side and the number of fertile shoots per unit area and the number of pods per inflorescence on the other, $r = 0.608$ and $r = 0.837$, respectively.

Key words: lodging, *Medicago sativa* L., pod, pollination, seed production, stem.

INTRODUCTION

In regions with semi humid climate, weather conditions in the year of growing are the chief source of variation in alfalfa seed yield. In years with high rainfall, alfalfa plants are lush and they lodge easily. Lodged plants are not suitable for pollination and low seed yields are consequently produced. To ensure high and stable seed yields, it is necessary to prevent the luxuriant growth of alfalfa plants and their subsequent lodging. Cutting is the most effective practice to limit the luxuriant growth. Also, cutting date is used for timing the beginning and duration of flowering period in seed crops, with the intention of synchronizing flowering period with maximum activity of pollinating insects.

The climatic conditions of Serbia favor the production of alfalfa seed from the second regrowth. The first regrowth is used for production of hay. With early first and second cuts, seed may be also produced from the third regrowth.

MATERIALS AND METHODS

Effect of cutting schedule on alfalfa seed yield and yield components was studied in four-year experiments (2001-2004). The experimental site was located in northern Serbia, at $45^{\circ}20' \text{ N}$, $19^{\circ}51' \text{ E}$, at 80 m above sea level. This area has a continental semi arid to semi humid climate, a mean monthly air temperature of 11.0°C , an annual sum of precipitation of around 600 mm, and a highly uneven distribution of precipitation. Table 1 shows the mean monthly air temperatures and monthly sums of precipitation for the period March-September. The trial was established according to a randomized block design with four replicates. Alfalfas were sown on April 8, 2000 at a row-to-row spacing of 25 cm and with a seeding rate of 15 kg ha^{-1} seed. The size elementary plot unit was 10 m^2 ($2 \times 5 \text{ m}$) with eight rows per plot.

Four cutting schedules with variable dates of initial cutting were tested: first cutting for hay was carried out on May 5 (budding) in the

c₁ schedule, on May 15 (start of flowering) in the c₂ schedule, on May 25 (full flowering) in the c₃ schedule, and on May 5 and June 5 in the c₄ schedule. Second regrowth was used for seed production in the c₁-c₃ schedules, and third regrowth in the c₄ schedule. The study involved the seven most widely grown alfalfa cultivars in Serbia, six domestic and one French (Table 6). The cultivars were tested for seed yield, total number of shoots per unit area, number of fertile shoots, plant height, number of pods per inflorescence and the number of seeds per pod.

Alfalfa seed was harvested in a single passage of a Hege harvester, following desiccation with *diquat* performed when about 70 % of pods on normally developed plants were at the stage of physiological maturity. Seed yield was calculated on the basis of measurements of processed seed per elementary unit. The results were statistically processed by analysis of variance. Significant differences between mean values were identified by the LSD test.

Table 1. Basic climatic parameters for the period 2001-2004

Parametar	Year	Month							Sum/ Average
		March	April	May	June	July	August	September	
Precipitation (mm)	2001	73	127	75	233	56	30	162	756
	2002	11	26	87	27	33	55	46	285
	2003	9	8	23	31	60	30	84	245
	2004	16	112	89	97	63	39	42	458
Mean air temperature (°C)	2001	10.9	11.2	17.8	18.2	22.3	22.7	16.1	17.0
	2002	8.9	11.7	19.1	21.7	23.6	22.2	17.0	17.7
	2003	6.0	10.9	20.6	24.0	22.6	24.6	17.2	18.0
	2004	9.0	14.8	15.5	20.7	21.9	20.4	13.9	16.6

RESULTS AND DISCUSSION

The average seed yield in the 2001-2004 period was 343 kg ha⁻¹, significantly higher than the Serbian average of 250 kg ha⁻¹. Maximum variation in seed yield level was caused by weather conditions in the year of growing. In 2002, which had favorable ecological conditions, the yield of seed was

4.3 times higher than in 2001, which had highly unfavorable conditions. Numerous authors (Žarinov & Ključ 1990, Stjepanović 1998) agree that variation in alfalfa seed yield is primarily due to weather conditions in the year of growing. Among them, the total amount and distribution of rainfall were most important. However, yield level may be stabilized to a certain degree by adjusting cutting schedule.

Table 2. Seed yield (kg ha⁻¹) as affected by year and cutting schedule.

Cutting schedule (C)	Year (Y)				Average	LSD for comparison of cutting treatments
	2001	2002	2003	2004		
c ₁	163	573	372	234	335	LSD 5% = 20 LSD 1% = 26
c ₂	167	589	203	297	314	
c ₃	225	1041	192	415	468	
c ₄	108	656	57	199	255	
Average	166	715	206	286	343	
LSD for comparison of years	LSD 5% = 21 / LSD 1% = 28					
LSD for cutting treatment x year interaction: LSD 5% = 39 / LSD 1% = 53						

The highest seed yield, on average 468 kg ha⁻¹, was achieved with the system of late cutting (C₃). This yield was highly significant (higher by 28-46 %) in relation to the other systems. When the first cutting is performed later in the season, plant regeneration will coincide with the time of year characterized by higher air temperatures and lower soil moisture, speeding up plant development (Kalu & Fick 1981). The late cutting system ensured a low crop density, i.e. a reduced number of shoots per unit area. Simultaneously, this system produced the largest number of productive shoots (Table 3) and the largest portion of productive shoots of the total number of shoots. Regrowth rate was significantly faster in relation to the other systems, but the plants were shorter and they had a reduced number of internodes (data not shown). The dry matter content in the stem was significantly higher in relation to the early and medium cutting systems (Karagić 2004). Owing to these

characteristics, plant sensitivity to lodging was reduced and conditions for alfalfa flowering and activity of pollinating insects improved, resulting in highest number of pods per raceme (Table 4). Significant positive correlations were found between seed yield and the number of pods per raceme, $r = 0.837$.

The early and medium schedules produced lower yields (335 kg ha⁻¹ and 314 kg ha⁻¹, respectively; Table 2). In these schedules, the regrowth of the seed crop coincided with a period of high soil moisture and low air temperatures, resulting in the tallest plants (Table 5) with lush and dense growth which is prone to lodging. The results obtained in the 2003, which was extremely dry, were significantly different from the average values. In this year, the highest yield was achieved with the early cutting schedule (372 kg ha⁻¹) and the seed yield was positively correlated with plant height ($r = 0.668$).

Table 3. Number of fertile stems per m² as affected by year and cutting schedule.

Cutting schedule (C)	Year (Y)				Average	LSD for comparison of cutting treatments
	2001	2002	2003	2004		
C ₁	167	186	380	257	248	LSD 5% = 18 LSD 1% = 23
C ₂	222	303	390	192	277	
C ₃	242	344	267	208	265	
C ₄	161	242	227	277	227	
Average	198	269	316	234	254	
LSD for comparison of years		LSD 5% = 19/ LSD 1% = 28				
LSD for cutting treatment x year interaction: LSD 5% = 35/ LSD 1% = 50						

Table 4. Number of pods per raceme as affected by year and cutting schedule.

Cutting schedule (C)	Year (Y)				Average	LSD for comparison of cutting treatments
	2001	2002	2003	2004		
C ₁	5.19	13.01	10.61	10.44	9.81	LSD 5% = 0.53 LSD 1% = 0.70
C ₂	4.90	13.29	8.50	10.96	9.41	
C ₃	7.29	15.64	10.11	12.37	11.35	
C ₄	5.31	13.97	6.68	10.34	9.07	
Average	5.67	13.98	8.98	11.03	9.91	
LSD for comparison of years		LSD 5% = 0.71/ LSD 1% = 0.91				
LSD for cutting treatment x year interaction: LSD 5% = 1.27/ LSD 1% = 1.51						

Table 5. Plant height at anthesis as affected by by year and cutting schedule.

Cutting schedule (C)	Year (Y)				Average	LSD for comparison of cutting treatments
	2001	2002	2003	2004		
C ₁	111	77	54	89	83	LSD 5% = 2 LSD 1% = 3
C ₂	112	90	55	97	88	
C ₃	92	81	46	102	80	
C ₄	88	63	38	60	62	
Average	101	78	48	87	79	
LSD for comparison of years		LSD 5% = 2 / LSD 1% = 3				
LSD for cutting treatment x year interaction: LSD 5% = 4 / LSD 1% = 6						

Table 6. Seed yield depending on cutting schedule and variety in 2001-2004 (kg ha⁻¹)

Variety	Cutting schedule				Average	LSD for comparison of varieties
	C ₁	C ₂	C ₃	C ₄		
NS Banat ZMS II	307	264	429	287	322	LSD 5% = 26 LSD 1% = 34
Kruševačka 22	340	296	423	261	330	
Novosađanka H-11	263	264	428	242	299	
Zaječarska 83	346	370	469	272	364	
NS Slavija	359	336	532	228	364	
NS Mediana ZMS V	298	273	423	273	317	
Europe Desprez	433	396	574	224	407	
Average	335	314	468	255	343	
LSD for comparison of varieties		LSD 5% = 20 / LSD 1% = 26				
LSD for cutting treatment x variety interaction: LSD 5% = 52 / LSD 1% = 68						

On average for years, the lowest seed yield, 255 kg ha⁻¹, was obtained with the c₄ schedule. Two cuttings for hay preceding seed production from the third regrowth (c₄) tend to reduce plant growth vigor. Somehow, two cuts at the beginning of budding exhausted the plants for reserves (Erić 1988). Additionally, shortage of available soil water in the period of regrowth, intensive growth and budding of the third cut affected negatively the forming of alfalfa generative parts. Compared with optimum water supply, dry conditions reduced the number of fertile stems two times, which significantly reduces seed yield (Goloborodko & Bodnarčuk 1998).

All varieties in the study produced maximum seed yields in the system of late cutting (c₃) (Table 3). The highest yields were achieved of cvs. Europe and Slavija in the c₃ cutting treatment (574 and 532 kg ha⁻¹, respectively), the varieties least susceptible to lodging. On the other hand, the seed yields of these varieties were very sensitive to drought and

to more intensive cutting schedule in the c₄ treatment (224 and 228 kg ha⁻¹, respectively).

The lowest seed yield was obtained with Novosađanka (299 kg ha⁻¹), due to the presence of the yellow alfalfa (*M. falcata* L.) genes incorporated during breeding (*M. sativa* L. x *M. falcata* L.). The yellow alfalfa is susceptible to lodging and its seed yield capacity is low (Galilov 1988).

In conclusion, weather conditions in the year of growing exhibit most pronounced effects on alfalfa seed yield and yield components. Among these conditions, the total amount and distribution of rainfall play the decisive role. In this study, the annual mean values ranged from 166 to 715 kg ha⁻¹.

Variations in alfalfa seed yield level may be controlled to some extent by the cutting schedule. The late cutting at full flowering ensures a reduced stand density and maximum number of productive shoots. Also,

plant height is reduced and number of pods per raceme significantly increased in relation to the systems of early and medium cutting. Consequently, plant sensitivity to lodging is considerably reduced while conditions for alfalfa flowering and activity of pollinating insects are improved, all that resulting in increased seed yield.

The effect of variety on alfalfa seed yield was significant. The highest seed yields were achieved by the varieties Europe and NS Slavija. The lowest seed yield was achieved

by the variety Novosađanka. Genotype sensitivity to lodging was closely associated with seed yield - the lower the lodging rate, the higher the seed yield.

ACKNOWLEDGEMENTS

This research was supported by Ministry of Science, Technology and Development of the Republic of Serbia (Project No. 0412).

LITERATURE

- Erić, P. 1988. Uticaj načina gustine setve na prinosa i kvalitet semena lucerke (*Medicago sativa* L.) sorte NS-Banat ZMS II na zemljištu tipa černozem. Doktorska disertacija, 176 pp. Univerz. u Novom Sadu, Polj. Fakultet.
- Galilov. A.A. 1988. Semenaja produktivnost ljucerni želtaj v zavisimosti od sposoba poseva i normi viseva semjan. Nekatorie aspekti semenovodstva lucerni. Selekcija i semenovodstvo br 3: 51-53.
- Goloborodko, S.P. & Bodnarčuk, L.I. 1998. Semenovodstvo ljucerni, 172 pp. Fitocentr, Kiev.
- Kalu, B.A. & Fick, G.W. 1981. Quantifying morphological development of alfalfa for studies of herbage quality. *Crop Science* 21: 267-271.
- Karagić, Đ. 2004. Komponente prinosa, prinosa i kvalitet semena lucerke u zavisnosti od sistema kosidbe. Doktorska disertacija, 174 pp. Poljoprivredni fakultet, Univerzitet u Novom Sadu.
- Stjepanović, M. 1998. Lucerna, 145 pp. Nova zemlja, Osijek.
- Žarinov & Ključ 1990. Ljucerna, 2. Izdanje, pererabotano i dopolnenoe, 238 pp. Urožaj, Kiev.

Weed regulation and establishment of organic seed crops of *Trifolium pratense* L., *Phleum pratense* L. and *Festuca pratensis* Huds.

Ann-Charlotte Wallenhammar¹⁾ & Per Ståhl²⁾

¹⁾Rural Economy and Agricultural Society, P.O. Box 271, SE- 701 45 Örebro, Sweden

²⁾Rural Economy and Agricultural Society, P.O. Box 275, SE-581 02 Linköping, Sweden

ac.wallenhammar@hush.se

ABSTRACT

Results presented here show the effects of weed regulation on weed occurrence and seed yield in first year seed crops of red clover (*Trifolium pratense* L.), timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.). Twelve cm row spacing / high-intensity weed harrowing and 36 cm row spacing / inter-row hoeing were compared with a control treatment (12 cm row spacing / no mechanical weed control) in six field trials in red clover, one trial in timothy, and two trials in meadow fescue harvested in 2003 and 2004. The experiments also included a treatment in which grass or clover seed and seed of the cereal cover crop were mixed and drilled simultaneously at 24 cm row spacing, enabling row hoeing after crop emergence. As compared with the control treatment, seed yield of red clover increased, albeit not significantly, by all weed regulation methods investigated. Weed harrowing in the spring of the seed harvest year was acceptable in all species and increased seed yield of meadow fescue significantly by 26 %. In both grasses, the seed yield on plots where cover crop seed and grass seed had been drilled simultaneously at 24 cm row spacing was comparable to the control treatment. The lowest yield in both grass seed crops was recorded at 36 cm row spacing / row hoeing. The purity analyses emphasised the difficulties in cleaning alsike clover (*Trifolium hybridum* L.) from timothy seed lots and white clover (*Trifolium repens* L.) and *Tripleurospermum perforatum* from meadow fescue seeds lots.

Key words: drilling, hoeing, inter-row, meadow fescue, red clover, simultaneous, timothy, *Tripleurospermum perforatum*, weed harrowing.

INTRODUCTION

The production of organic herbage seed has increased significantly in Sweden during the last few years. Seed production was initially restricted due to a poor market (Wallenhammar *et al.* 2005), but by the introduction of partly organic seed mixtures in 2003, where at least 35 % of the components red clover, timothy and meadow fescue are organic seed, the market increased. The acreage increased from 104 hectares in 2000 to 2943 hectares in 2006, which implies that Sweden is by far the largest organic seed producer of timothy, meadow fescue and red clover in the world (Pedersen, 2006). This rapid increase means an increasing demand for the development of

cultivation techniques. The experiences of the farmers were documented during 2002 and 2003, and different cultivation techniques as weed regulation, harvesting methods for red clover, and support of plant nutrition were demonstrated at field sites (Ståhl *et al.* 2004).

Results presented here show the effects of weed regulation on weed occurrence and seed yield in organic seed crops of red clover, timothy and meadow fescue.

MATERIALS AND METHODS

Nine field experiments were established in 2002 and 2003 and harvested in 2003 and

2004, respectively. The experiments were performed by the Field Experimental Divisions at the Rural Economy and Agricultural Societies, partly as on-farm trials and partly on experimental farms in districts with certified organic seed production.

A randomized block design with four replications was used for each experiment. Weed biomass was determined before seed harvest. In treatments with 12 cm row distance all weeds were collected in 4 subplots of 0.25 m². For row distances >12 cm, weed was collected along a distance of 1 meter and covering half of the width of the row distance in 4 different rows. The weeds were counted, their fresh weight determined, and the three most common species shown separately. The plots were harvested directly without prior swathing. Plots yields were cleaned by the Rural Economy and Agricultural Society at Borrbj, and per cent cleanouts determined. Purity analysis was performed at the Seed Testing Station of Central Sweden Ltd,  rebro, on a composite sample from each treatment in each trial, according to the Swedish Board of Agriculture instructions for certified seed. The seed number of other species was determined in samples of 10 g of cleaned seed for timothy and 50 g of cleaned seed of red clover and meadow fescue, and per cent purity in samples of 1 and 5 g, respectively. The highest contamination of other species accepted in certified seed of the investigated species is, on total, 1.5 weight-%, and of one individual species 1.0 weight-%.

Six field experiments were carried out in red clover, one in timothy, and two in meadow fescue.

In red clover, the following treatments were compared:

- A. Seed crop row distance 12 cm. No mechanical treatment (control).
- B. Seed crop row distance 12 cm, high-intensity weed harrowing after harvest of cover crop and in spring.

- C. Seed crop row distance 36 cm, row hoeing after harvest of cover crop and in spring.
- D. Seed crop row distance 24 cm, cover crop and clover seed mixed and drilled simultaneously, row hoeing after emergence, after harvest of cover crop, and in spring.

In timothy and meadow fescue, the trials included an additional treatment E with 12 cm row distance and high-intensity weed harrowing only in the spring of the seed harvest year.

Except in treatment D, the undersown seed crop was established directly after the cereal cover crop. The cover crop was drilled at 90 % of the normal seeding rate when cover crop and seed crop were drilled separately and 70 % of the normal seeding rate when cover crop and seed crop were mixed before drilling. Depending on row distance, the following seeding rates of red clover (tetraploid) were used; 12 cm: 5 kg ha⁻¹, 24 cm: 4 kg ha⁻¹, 36 cm: 3 kg ha⁻¹. Of timothy the following seeding rates were used; 12 cm: 8 kg ha⁻¹, 24 cm: 5 kg ha⁻¹, 36 cm: 5 kg ha⁻¹; and of meadow fescue; 12 cm: 12 kg ha⁻¹, 24 cm: 8 kg ha⁻¹, 36 cm: 8 kg ha⁻¹.

Statistical analysis was conducted according to SAS Mixed model.

RESULTS

Red clover

Although differences were not significant, there was, on average for six trials, a 4-8 % increase in seed yield over the control treatment by the weed regulation methods investigated (Table 1). Drilling at 12 cm combined with high-intensity weed harrowing after cover crop harvest and in spring tended to give the highest seed yield and the lowest cleanouts. Determination of the biomass of *Tripleurospermum perforatum* in the field also confirmed the advantage of high-intensity weed harrowing compared to the other treatments.

Table 1. Weed regulation in red clover. Seed yield, % cleanout at seed cleaning, and biomass of the weed *Tripleurospermum perforatum* in the field. Average of six field experiments in south and central Sweden, 2003 and 2004

Treatment	Seed yield kg ha ⁻¹ (rel.)	% clean- out	¹ <i>T.perforatum</i> fresh weight, g m ⁻²
A. Row distance 12 cm No mechanical treatment (control)	220 (100)	12.8	367
B. Row distance 12 cm, weed harrowing after harvest of cover crop and in spring	240 (108)	8.6	128
C. Row distance 36 cm, row hoeing after harvest of cover crop and in spring	230 (104)	12.0	335
D. Row distance 24 cm, cover crop and clover seed mixed before drilling, row hoeing after emergence, after harvest of cover crop and in spring	230 (105)	9.9	169
Prob.	Ns	ns	ns

¹ Average of three experiments

Timothy and meadow fescue

Weed harrowing in spring only had a significantly positive influence on seed yield of meadow fescue (Table 3), but not of timothy (Table 2). The lowest yield of both species was recorded at 36 cm row spacing. The purity analyses reflected the difficulties in cleaning seeds of alsike clover (*Trifolium hybridum*) from timothy and seeds of white clover (*Trifolium repens*) from meadow fescue (data not shown). Apart from these species, *Tripleurospermum perforatum* was the dominating weed in the field, especially in the row-hoed treatment in meadow fescue

(Table 3). However, for this weed, there appeared to be no relationship between field occurrence and seed number found after cleaning.

According to the purity analysis (results not shown here) the seed of meadow fescue passed the requirements for certified seed on one of the experimental sites, whereas the prevalence of *T. perforatum* and white clover exceeds the limits on the other site. The timothy seed did not pass the requirements due to a high content of *T. hybridum*.

Table 2. Weed regulation in timothy. Yield, % cleanout at seed cleaning, and biomass of the weed *Tripleurospermum perforatum* in the field. Results from one experiment.

Treatment	Seed yield, kg ha ⁻¹ (rel.)	% cleanout	<i>T.perforatum</i> fresh weight, g m ⁻²
A. Row distance 12 cm No mechanical treatment (control)	314 (100)	23.5	295
B. Row distance 12 cm, weed harrowing after harvest of cover crop and in spring	289 (92)	25.0	250
C. Row distance 36 cm, row hoeing after harvest of cover crop and in spring	199 (63)	28.1	260
D. Row distance 24 cm, cover crop and timothy seed mixed before drilling, row hoeing after emergence, after harvest of cover crop and in spring	297 (95)	20.0	200
E. Row distance 12 cm, weed harrowing in spring only	324 (103)	20.0	260
Prob.	ns	ns	ns

Table 3. Weed regulation in meadow fescue. Yield, % cleanout at seed cleaning, biomass of the weed *Tripleurospermum perforatum* in the field, and seed number of *Tripleurospermum perforatum* in 50 g of cleaned seed. Average of two field experiments in central Sweden 2003-2004

Treatment	Seed yield kg ha ⁻¹ (rel)	% clean- out	<i>Tripleuro- spermum perforataum</i> fresh weight g m ⁻²	Seed number of <i>T. perforatum</i> in 50 g cleaned seed.
A. Row distance 12 cm No mechanical treatment (control)	310 (100)	19.6	153	202
B. Row distance 12 cm, weed harrowing after harvest of cover crop and in spring	370 (117)	16.5	176	104
C. Row distance 36 cm, row hoeing after harvest of cover crop and in spring	280 (89)	15.8	478	300
D. Row distance 24 cm, seed of cover crop and meadow fescue mixed before drilling, row hoeing after emergence, after harvest of cover crop, and in spring	310 (99)	16.0	527	111
E. Row distance 12 cm, weed harrowing in spring only	390 (126)	10.7	134	152
Prob.	0.03	ns	ns	ns
LSD	60			

DISCUSSION

The purity and germination requirements for certified organic seed are the same as for conventionally produced seed, implying large demands of efficient weed control. Weed control might be improved by different establishment techniques. Today most farmers drill at 12 cm's (Stahl *et al.* 2004), and weed problems are obvious.

Our results in red clover showed that seed yield did not decrease for any of the methods investigated (Table 1). Increased yield by increasing row distance to 24 cm in conventional red clover) was reported by Larsson (2002). Weed control tended to be improved by weed harrowing at 12 cm row distance and by row hoeing at 24 cm row distance, whereas the weed biomass at 36 cm row distance / row hoeing was similar to that of the control. In the grass seed crops the lowest yield was recorded when drilling at 36 cm, which is in line with older experiences (Nilsson & Leissner 1950). Weed harrowing early in the spring of the seed harvest year increased yield in meadow fescue by 26 % (Table 3), whereas weed harrowing in autumn and spring increased yield by 17 %. Harrowing

in autumn might have reduced the crop stand, while the harrowing in spring most probably contributed to increased nitrogen mineralization. In timothy, with a fairly late start of growth in spring, yield decreased 8 % after harrowing both in autumn and spring, whereas the corresponding yield increase on plots harrowed only in spring was 3 %. Simultaneous drilling showed a tendency to increased yield only in red clover, but in an earlier experiment this technique was successful also in timothy (Wallenhammar 2004). This technique was also recommended to red clover seed growers during the 1940s (Hertzman *et al.* 1940).

In conclusion, the experimental data show that weed harrowing in the spring of the seed harvest year had a positive influence on yield and/or weed contamination in all of the species investigated. This is a measure to be taken in organic seed crops as growth and nitrogen mineralization will be stimulated. The purity analysis points at the difficulties to clean alsike clover from timothy (Aamlid 1997, Rabaek-Pedersen 2006), and to clean large amounts of *T. perforatum* and whitel clover from meadow fescue. Row hoeing may be a solution on fields where white clover and alsike clover are expected to become troublesome.

ACKNOWLEDGEMENT

The investigations are part of the project
“Cultivation techniques in organic ley seed

production” financed by the organic research
program, Ekoforsk, at SLU 2002-2004.

LITERATURE

- Aamlid, T. S. 1997. Økologisk Frøavl: en forutsetning for videre vekst av økologisk landbruk. Hummelposten 2: 20-22. (In Norwegian)
- Hertzman, N., Nilsson-Leissner, G., Schwanbom, N & Aronson, L. 1940. Rödklöver. *In: Lantbrukets Fröodling* (pp. 59-78). Nordisk Rotogravyr, Stockholm, Sweden. (In Swedish).
- Larsson, G. 2002. Rödklöver (*Trifolium pratense*) till frö. Radavstånd- utsädesmängd. *In: Nordisk Jordbruksforskarens Förening. Seminarium vallfröodling 24-26 juni 2002.* 341. pp. 99-104. (In Swedish, with English abstract)
- Nilsson- Leissner, G., Weibull, W. & Nordén, F. 1950. Timotej. *In: Fröodling av Lantbruksväxter.* (pp.138-142). LT:s Förlag, Stockholm, Sweden. (In Swedish).
- Rhaebeck Pedersen, T. 2006. Ekologisk vallfröodling- en grundlig genomgång. *In: Ekologisk vallfröodling. Jordbruksverket. Jordbruksinformation 21: 3-11.* (In Swedish).
- Ståhl, P., Pettersson, B. & Wallenhammar, A-C. 2004. Ekologisk vallfröodling 2002-2003. ([http:// www.hush.se/t/filarkiv/Ekologisk%20vallfröodling%20slutdokument.pdf](http://www.hush.se/t/filarkiv/Ekologisk%20vallfröodling%20slutdokument.pdf))
- Wallenhammar, A-C. 2004. Ekologisk vallfröodling 2003. From [www.evp.slu.se/ekoforsk/projekt/resultat2003/vallfro_03. htm](http://www.evp.slu.se/ekoforsk/projekt/resultat2003/vallfro_03.htm). 14 maj 20067.
- Wallenhammar, A-C., Ståhl, P. & Pettersson, B. 2005. Ekologisk vallfröodling utvecklas starkt. *Svensk Frötidning 2: 13-15.* (In Swedish).

Seed production of various timothy (*Phleum pratense* L.) cultivars as affected by seeding rates

Ann-Charlotte Wallenhammar & Lars Eric Anderson

Rural Economy and Agricultural Society, P.O. Box 271, S-701 45, Örebro, Sweden

ac.wallenhammar@hush.se

ABSTRACT

Seed yields of new timothy (*Phleum pratense*) cultivars, recently introduced in Swedish seed production, have been lower and more variable than those of older cultivars. In this study we investigated the effect of seeding rate on seed yield of four current cultivars in the first harvest year. Five field trials were carried out in 2000-2003 in Örebro county, central Sweden. The cultivars SW 'Alexander', 'Grindstad' (SW), SW 'Ragnar' and 'Lischka' (SSd) were sown at seeding rates 3, 6 and 9 kg ha⁻¹, with spring barley as cover crop. On average for all locations, the highest yield was obtained in 'Lischka'; 500 kg ha⁻¹, followed by 'Alexander'; 452 kg ha⁻¹, 'Grindstad'; 448 kg ha⁻¹ and 'Ragnar'; 378 kg ha⁻¹. On average for trials there were only small differences in seed yield at various seeding rates; 459, 448, 427 kg ha⁻¹ was obtained at rate 3, 6 and 9 kg ha⁻¹, respectively. Seed yields of 'Alexander' were independent of the seeding rates studied, whereas those of 'Ragnar' and 'Lischka' tended to decrease with increasing seeding rate. In 'Grindstad', the highest yield was recorded at 6 kg ha⁻¹. The number of shoots in spring increased with increasing seeding rate in all cultivars, from an average of 924 m⁻² at 3 kg ha⁻¹ to 1175 and 1316 m⁻² at 6 and 9 kg ha⁻¹, respectively. However, high numbers of shoots did not correspond to high numbers of ears, as the records were 583, 641 and 663 ears m⁻², respectively. The results show that a low seeding rate can be recommended in all cultivars tested.

Key words: cultivars, density, ears, seed yield, shoots, tillers

INTRODUCTION

In recent years there has been an increasing interest for timothy seed production followed by a demand for know-how on growing techniques. The latter has become even more important since the special model for EG-subsidiaries was altered and hence profitability decreased compared to other crops. In Sweden, recently introduced timothy cultivars often give lower seed yields compared to older ones, and seed yields also vary widely among growers. Thalbitzer (1999), investigating 120 cultivars of red fescue (*Festuca rubra*) in Denmark, showed that seed production can vary to a great extent. Is there a need for different growing techniques concerning newer cultivars?

Investigations 1997-2001 showed that timothy seed yield depends strongly on nitrogen status during different stages of development (Wallenhammar 1998, Wallenhammar, 1999, Wallenhammar & Anderson 2002). N-fertilizing as early as at growth start in spring increased yield in early cultivars such as 'Grindstad' (SW), whereas SW 'Ragnar' was little affected.

A number of current cultivars have strong vegetative growth, which could negatively affect seed production. The aim of our study was to find which seeding rates are optimal for establishment, development and seed production in four current cultivars of timothy.

MATERIALS AND METHODS

Field experiments were established in 1999-2002 in Örebro county, central Sweden. The investigation focused on the first year of seed production with a total of five experiments harvested. Four cultivars; SW 'Alexander', 'Grindstad' (SW), SW 'Ragnar' and 'Lischka' (SSD) were compared at three seeding rates; 3, 6 and 9 kg ha⁻¹, respectively. The cover crop spring barley was sown in advance, perpendicularly to the seeding direction for timothy. Cultivars were sown at a row distance of 12 cm. Rolling was performed before and/or after seeding depending on local conditions. Straw was removed after harvest.

A randomized block design with three replications was used for each experiment. Shoots and ears were counted along 2 x 1.0 m row per plot. Numbers of shoots were recorded in October and in late May; ears in July. The plots were harvested without prior swathing and yield was determined from 20 m². Experiments were fertilized with N, P, K and S. Nitrogen applied was 30 kg ha⁻¹ in September and 60 kg ha⁻¹ in the end of April.

Statistical analysis was conducted using SAS GLM procedure/ Duncan's multiple range test.

RESULTS

On average for all experiments and seeding rates, the highest yield was obtained in 'Lischka' (500 kg ha⁻¹) followed by 'Alexander' (452 kg ha⁻¹), 'Grindstad' (448 kg ha⁻¹), and 'Ragnar' (378 kg ha⁻¹) (Fig. 1). On

average for cultivars there were only small differences in seed yield depending on the seeding rate; 459, 448 and 427 kg ha⁻¹ were obtained after seeding 3, 6 and 9 kg ha⁻¹, respectively.

Looking at each cultivar (Fig. 2), the yield of 'Alexander' was independent of seeding rate, whereas decreased yields were obtained with increasing seeding rates in 'Ragnar' and 'Lischka'. The highest yield of 'Grindstad' was recorded at 6 kg ha⁻¹. However, these differences were not statistically significant in any of the cultivars.

The number of shoots in late May increased, although not always significantly, with increasing seeding rate in all cultivars (Fig. 3). Shoots per m² in spring were, on average, 924, 1175 and 1316 in crops seeded at 3, 6 and 9 kg ha⁻¹, respectively (Fig. 5). However, high numbers of shoots did not correspond to high numbers of ears (Fig. 6), as the records were 583, 641 and 663 ears m⁻², respectively.

The numbers of shoots recorded in spring were, on average higher than those recorded in autumn (Figs. 4 and 5). Tillering decreased with increasing seeding rate and was, in spring, somewhat lower in 'Lischka' compared to other cultivars (Fig. 5).

On three locations the seeding rate 3 kg ha⁻¹ showed highest yield for all cultivars on average, whereas 6 kg and 9 kg ha⁻¹ top-yielded at one location each (results not shown). In the latter case poor germination was indicated by a proportionally low number of shoots.

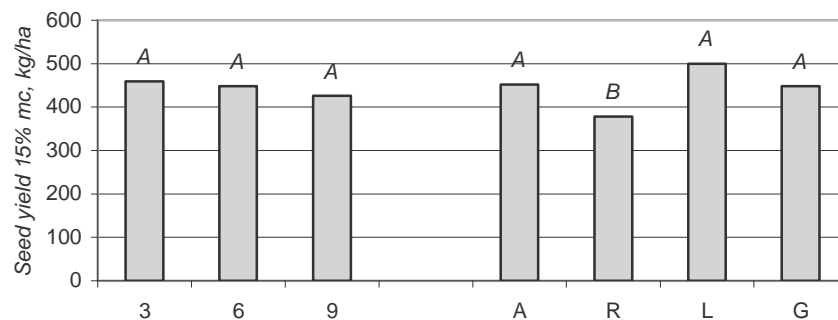


Fig. 1. Main effects of seeding rate and cultivar on seed yield. (3/6/9 = seeding rate in kg ha⁻¹, A='Alexander', R='Ragnar', L='Lischka', G='Grindstad'). Average of five trials. Different letters over the bars show significant differences according to Duncan's multiple range test at $P < 0.05$.

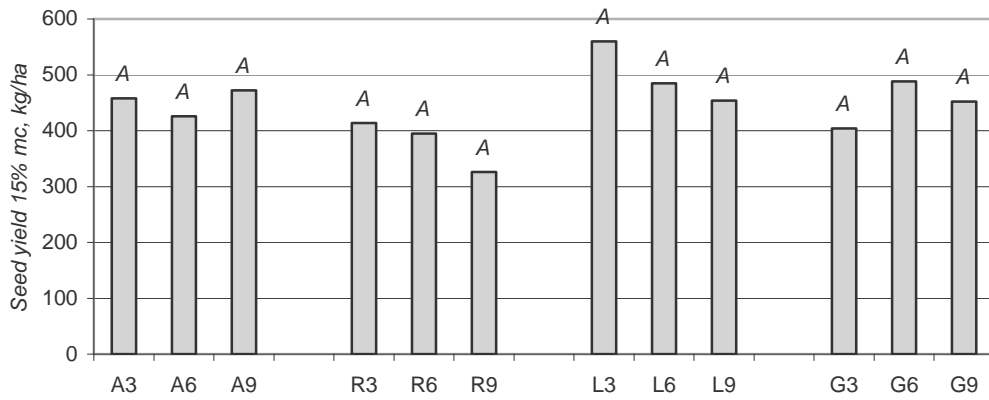


Fig. 2. Seed yield for each combination of cultivar and seeding rate (A='Alexander', R='Ragnar', L='Lischka', G='Grindstad', 3/6/9= seeding rate in kg ha⁻¹). Average of five trials. Within each cultivar, lack of significance according to Duncan's multiple range test at $P<0.05$, is indicated by the same letter over the bars.

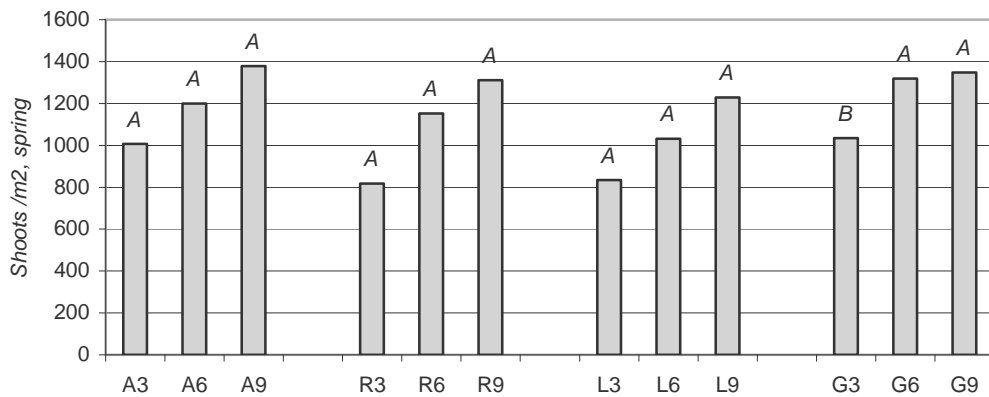


Fig. 3. Number of shoots m² in spring for each combination of cultivar and seeding rate. (legends as in Fig. 2). Average of four trials. Within each cultivar, different letters over the bars show significant differences according to Duncan's multiple range test at $P<0.05$.

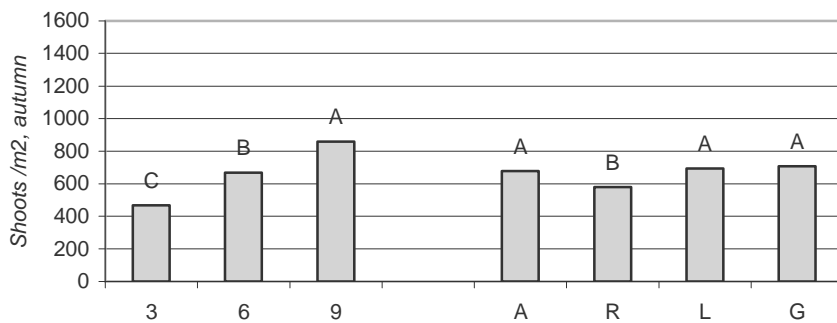


Fig. 4. Main effects of seeding rate and cultivar on number of shoots m² in October, (legends as in Fig. 1). Average of five trials. Different letters over the bars show significant differences according to Duncan's multiple range test at $P<0.05$.

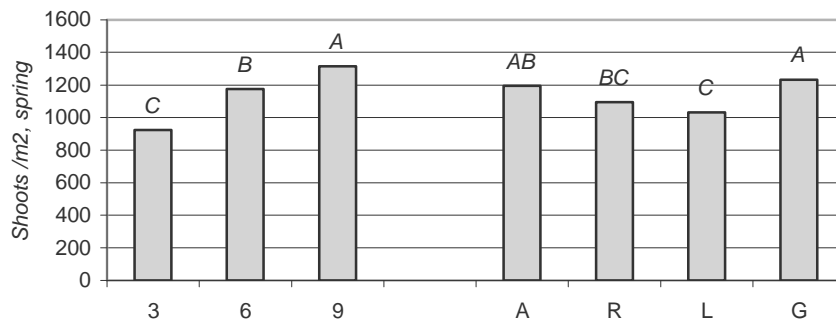


Fig. 5. Main effects of seeding rate and cultivar on number of shoots m⁻² in late May, (legends as in Fig. 1). Average of four trials. Different letters over the bars show significant differences according to Duncan's multiple range test at $P < 0.05$.

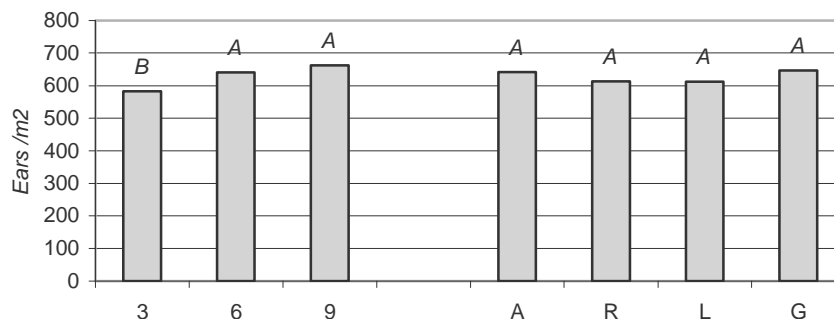


Fig. 6. Main effects of seeding rate and cultivar on number of ears m⁻², (legends as in Fig. 1). Average of four trials. Different letters over the bars show significant differences according to Duncan's multiple range test at $P < 0.05$.

DISCUSSION

Our results show that a low seeding rate, $< 6 \text{ kg ha}^{-1}$, can be recommended for all cultivars provided conditions for field emergence are favourable. There is no need for high numbers of shoots as a reduction will take place resulting in approximately the same numbers of ears regardless of seeding rate. The results show, although not statistically significant, a yield depression at high seeding rate in 'Ragnar' and 'Lischka', suggesting that low

seeding rate may be most favourable in these cultivars. Our results also point to the relatively lower tillering capacity of 'Lischka' in spring.

ACKNOWLEDGEMENTS

The investigation was financed by the Royal Swedish Academy of Agriculture and Forestry and the Rural Economy and Agricultural Society of Örebro county.

LITERATURE

- Thalbitzer, F. 1999. Rødsvingelsorternas avkast i 1997. Dansk Frøavl 2: 98 (In Danish).
- Wallenhammar, A-C. 1998. Kvävegödsling till timotejfrövall (*Phleum pratense* L.). In: Seed Production. The Nordic Association of Agricultural Scientists, Report no. 121. pp. 83-90. (In Swedish, with English abstract)
- Wallenhammar, A-C. 1999. Kvävegödsling och beståndsutveckling i timotejfrövall (*Phleum pratense* L.). Slutrapport. Kungliga Skogs- och Lantbruksakademien. 8 pp. (In Swedish)
- Wallenhammar, A-C & Anderson, L. E. 2002. Kvävestrategier i timotejfrövall (*Phleum pratense* L.) In: Seed Production. The Nordic Association of Agricultural Scientists, Report no. 342. pp. 129-136. (In Swedish, with English abstract)

Weed regulation by cutting in organic seed crops of *Trifolium pratense* L. and *Trifolium repens* L.

Ann-Charlotte Wallenhammar¹⁾, Per Ståhl²⁾, Bo Christiansson³⁾ & Lars Andersson⁴⁾

¹⁾Rural Economy and Agricultural Society, Box 271, SE-701 45 Örebro, Sweden

²⁾Rural Economy and Agricultural Society, Box 275, SE-581 02 Linköping, Sweden

³⁾Rural Economy and Agricultural Society, Box 9084, SE- 291 09 Kristianstad, Sweden

⁴⁾Swedish University of Agricultural Sciences, Dept of Crop Production Ecology, Box 7043, SE 750 07 Uppsala, Sweden

ac.wallenhammar@hush.se

ABSTRACT

The effects of weed regulation by cutting on different dates and development stages on weed occurrence and seed yield of white clover (*Trifolium repens*) and red clover (*Trifolium pratense*) were investigated in field trials in south and central Sweden in 2005 and 2006. In white clover cutting (b) at the budding stage, (c) at 1-2 flowers m⁻², and (d) in mid June were compared with an uncut control (a). Cutting at the budding stage increased seed yield by an average of 28 %, whereas seed yield decreased significantly by 62 % after the latest cutting treatment. The number of flowers tended to increase after the two early cutting treatments, and the biomass of the dominating weeds *Geranium molle*, *Capsella bursa pastoris* and *Tripleurospermum perforatum* decreased significantly. The per cent of cleanout was lower for all cutting treatments, while the number of weed seeds detected in the purity analyses was highest in the control treatment. In red clover, mild (20 cm stubble height) and hard (5-8 cm stubble height) cutting at stem elongation in late May, and the same treatments at 40 cm crop height in mid June, were compared with an uncut control treatment. Hard cutting in June decreased seed yield by 31 %, delayed seed maturation by 10 days and decreased the number of flowers significantly, whereas the other cutting treatments showed a slight yield increase compared with the control treatment. The biomass of *T. perforatum* was reduced less efficiently by mild cutting in mid June than by the other cutting treatments. No clear differences in percentage of pure seed in uncleaned seed lots were distinguished between treatments. It is concluded that cutting at the early budding stage in white clover and at stem elongation in red clover will reduce weed problems in both species. Cutting will also synchronise flowering, with an increasing prevalence of pollinators, and thus increase seed yield.

Key words: budding, cutting heights, stem elongation, red clover, white clover

INTRODUCTION

The production of organic herbage seed has increased significantly in Sweden during the last few years. Organic seed production was initially restricted due to a poor market (Wallenhammar *et al.* 2005), but by the introduction of partly organic seed mixtures in 2003, where at least 35 % of the components red clover, timothy and meadow fescue are organic seed, the market increased. The acreage increased from 104 hectares in 2000 to 2943 hectares in 2006, which implies that

Sweden is by far the largest organic seed producer of timothy, meadow fescue and red clover in the world (Pedersen 2006). The production of white clover seed has hitherto been limited, but from 2004 to 2006 the acreage increased by 70 % to 418 hectares. White clover seed is now contracted in areas in central Sweden where this crop was not grown earlier, and there is a great demand for knowledge about production techniques.

Weed regulation by cutting the crop in the seed production year has been tested by farmers, but in Sweden this method has not

earlier been investigated experimentally using modern equipment. In order to investigate the effects of cutting at different development stages on weed occurrence and seed production, field trials were started in 2005. Results from 2005 and 2006 are presented here.

MATERIALS AND METHODS

Nine field experiments, four in white clover and five in red clover, were conducted in 2005 and 2006. The field experiments were performed by the Field Experimental Divisions of the Rural Economy and Agricultural Societies, partly as on-farm trials and partly on experimental farms in districts with certified organic seed production. A randomized block design with four replications was used for each experiment. The cutting was performed using a pasture mower; either a mulcher with hammers or a knife mower, and the green plant material was removed from the field. Weed biomass was determined three weeks after the last cutting. All weeds were collected in 4 subplots of 0.25 m². The fresh weight was determined and three most common species were shown separately. The plots were swathed prior to seed harvest. Plot yields were cleaned by the Rural Economy and Agricultural Society at Borrby, and percent cleanouts determined. Purity analysis was performed by the Seed Testing Station of Central Sweden Ltd, Örebro, on a composite sample from each treatment in each trial, conducted according to the Swedish Board of Agriculture instructions for certified seed. The seed number of other species was determined in samples of 20 and 50 g of cleaned seed for

white clover and red clover, respectively, and per cent purity in samples of 2 and 5 g, respectively. The highest contamination of other species accepted in certified seed of the investigated species is, on total, 1.5 weight-%, and of one individual species 1.0 weight-%. Statistical analysis was conducted according to SAS Mixed Model.

RESULTS

White clover

In white clover cutting was performed at three points of time according to the following plan:

- A: no cutting (control)
- B: cutting at budding stage (no flowers visible but buds visible at the bottom of the stand (31 May - 6 June)
- C: Cutting 14 days later than treatment B, 1-2 white flowers m⁻² visible (31 May - 13 June)
- D: Cutting in flowering in mid June (14-21 June)

The highest yield was obtained with the earliest cutting time, treatment B (Table 1). The content of pure seed and the fraction of other species (weeds) decreased after all treatments, except for at one experimental site where *Rumex spp.* was prevalent (results not shown). The latest cutting reduced the number of weeds to a larger extent, but it also reduced seed yield significantly. Rainfall after cutting was low in both years, hence, the crop stand was lower the later cutting was performed, implying problems at seed harvest. At the experimental sites in southern Sweden a yield increase was obtained also in treatment C, while the yield in this treatment was severely reduced in central Sweden. Percent cleanouts were reduced for all treatments (results not shown here).

Table 1. Seed yield, number of flowers, biomass of *Tripleurospermum perforatum* in the field, and number of other species in purity analyses in white clover cv. SW Sonja after cutting at different developmental stages. Average of four field experiments in south and central Sweden in 2005 and 2006.

Treatment	Seed Yield, kg ha ⁻¹	Relative Yield	¹ Number of flowers m ⁻²	<i>T. perforatum</i> biomass fresh weight, g m ⁻²	Seed number of other species in 20 g cleaned seed
A. No cutting	223	100	541	1527	120
B. Cutting at budding stage	286	128	753	419	153
C. Cutting at 1-2 flowers m ⁻²	217	97	640	82	94
D. Cutting in mid June	114	58	524	21	48
Prob.	0.02	-	ns	ns	ns
LSD	89				

¹Average of two experiments

Table 2. Seed yield, number of flowers, biomass of *Tripleurospermum perforatum* in the field, and number of other species in purity analyses in red clover cvs. SW Bjursele, SW Sara and SW Betty after cutting at different developmental stages. Average of five field experiments in south and central Sweden in 2005 and 2006.

Treatment	Seed yield kg ha ⁻¹	Relative yield	¹ Number of flowers m ⁻²	<i>T. perforatum</i> biomass fresh weight, g m ⁻²	Seed number of other species in 50 g cleaned seed
A. No cutting	156	100	919	223	100
B. Mild cutting at stem elongation	169	108	984	87	46
C. Hard cutting at stem elongation	173	111	747	87	44
D. Mild cutting at 40 cm crop height	182	117	991	145	61
E. Hard cutting, at 40 cm crop height	108	69	522	65	93
Prob.	ns	-	0.036	0.005	Ns
LSD			357	98	

¹Average of four experiments

Red clover

The experimental plan in red clover comprised five treatments:

- A: No cutting (control)
- B: Mild cutting to 20 cm (above the growing point) at stem elongation (20-31 May)
- C: Hard cutting to 5-6 cm at stem elongation (20-31 May)
- D: Mild cutting to 20 cm when crop was 40 cm high (7-12 June)
- E: Hard cutting to 5-6 cm when crop was 40 cm high (7-12 June)

The cutting treatments, except hard cutting in June, increased seed yield by 8, 11 and 17 % respectively (Table 2). The number of flowers tended to increase after mild cutting, whereas hard cutting in June decreased the numbers of flowers significantly compared to the control treatment. The latter treatment also reduced seed yield by 31 % (Table 2) and delayed maturation by 10 days (not shown). Rainfall was favourable at all experimental sites after the first cutting, but the season was dry in the southern part of Sweden after the second cutting, thus affecting seed yield. The biomass of *T. perforatum* was significantly reduced by cutting except by the mild treatment at 40 cm crop height (Table 2). No significant difference in the seed number of other species in cleaned seed could be distinguished among treatments. Mild cutting did not affect seed maturation date.

DISCUSSION

In Denmark, organic seed production of white clover is often regarded as a hazardous crop, but regulation of crop stand, control of insect pests, and favourable conditions for pollination can contribute to increased and

stabilized yield (Boelt 2002). In order to get an even development of flowers, it is important that the crop is not too dense. The crop density can be regulated by cutting, which implies that many new leaves and flower buds will develop and, hence, flowering will be synchronised.

Our results show that cutting had a positive influence on the development of flowers as well as on seed yield in white clover (Table 1). The optimal cutting time was determined to be the early budding stage, which is in line with the Danish experiences (Boelt 2002). Weed biomass and prevalence of other species in the seed was reduced most efficiently by early cutting (Table 1).

Experiences from USA (Steiner *et al.* 1995), France (Bouet & Sicard 1997) and Denmark (Boelt 2002) suggest that red clover can be cut in late spring as a means to synchronise flowering with the activity of pollinators such as bumblebees (*Bombus* spp.) and honeybees (*Apis* spp.). In our experiments cutting increased seed yields except when hard cutting was accomplished late. Cutting has been used as a weed regulation method by many organic seed growers in Sweden during 2005 and 2006, and with the yield increases demonstrated in this study, we strongly recommend this method, not only as a means to weed control, but also to improve pollination and seed set.

The weed species dominating in both white clover and red clover in these trials were fairly easy to clean out since only a low number of other species were found in cleaned seed. However, *Geranium molle*, an abundant weed in one of the white clover experiments, was difficult to separate (data not shown). In conventional seed production, this weed was known to cause trouble before herbicides were introduced (Nilsson-Leissner *et al.* 1950b).

Cutting in early spring while the buds are just above the soil surface, about 1 June in the southern part of Sweden, was a well-known method to regulate weeds and synchronise flowering prior to the era of herbicides (Nilsson *et al.* 1950a). In early varieties of red clover, taking an early forage harvest was recommended in order to synchronize flowering with the activity of bumble bees. Evidently, the importance of bumble bees for pollination of red clover was well known 100 years ago (Elofson 1913), but somehow, this knowledge was lost. We welcome all seed producers, organic as well as conventional, to adopt and apply this knowledge and to provide for actions that are beneficial for

bumble bees and hence, pollination, as a ecosystem service (Risberg 2004).

The Swedish seed company Svalöf Weibull AB, contracting a major part of the seed production in the country, report on a larger proportion of rejected seed lots in organic compared to conventional production due to a high level of other species which are difficult to clean out (Bertil Bertilsson, pers comm. 2007). Our investigations show that cutting will not only reduce weed biomass and improve seed lot purity, but also have a positive influence on seed yield. The investigations continue in 2007.

ACKNOWLEDGEMENT

These investigations are part of the project 'Techniques for establishment and weed regulation in organic seed crops of white clover, red clover and grass' financed by the organic research program, Ekoforsk, at the Swedish University of Agricultural Sciences 2005-2007.

LITERATURE

- Boelt, B. 2002. Legume seed production & research in Europe. *Forage Seed* 9 (1): 33-34.
- Bouet, S. & Sicard, G. 1997. *Trifolium pratense* L. (red clover) in France. *In: Fairey, D.T. & Hampton, J.G. (eds.). Forage Seed Production. I. Temperate Species*, pp. 377-384. CAB International.
- Elofson, A. 1913. Rödklöver. *In: Fröodling av klöver och timotej*. pp. 40-85. C.E. Fritzes Bokförlags Aktiebolag, Stockholm. (In Swedish).
- Nilsson- Leissner, G., Weibull, W. & Nordén, F. 1950a. Rödklöver. *In: Fröodling av Lantbruksväxter*, pp.120-134. LT:s Förlag, Stockholm. (In Swedish).
- Nilsson- Leissner, G., Weibull, W. & Nordén, F. 1950b. Vitklöver. *In: Fröodling av Lantbruksväxter*, pp.138-142. LT:s Förlag, Stockholm. (In Swedish).
- Pedersen, T. 2006. Ekologisk vallfröodling- en grundlig genomgång. *In: Ekologisk vallfröodling*. pp. 3-10. Statens Jordbruksinformation no 21. (In Swedish).
- Risberg, J. 2004. Humlor (*Bombus*) på ekologiska och konventionella gårdar- odlingssystemets och landskapets betydelse för en ekologisk nyckelresurs. Svenska Vildbiprojektet vid ArtDatabanken, SLU och Avdelningen för Växtekologi, Uppsala Universitet. Examensarbeten/Seminarieuppsatser, 69. Department of Crop and Plant Ecology, SLU, Uppsala, Sweden. 47 pp. (In Swedish, with English abstract).
- Steiner, J.J., Leffel J.A., Gingrich, G. & Aldrich-Markham, S. 1995. Red clover seed production: III. Effect of hay harvest time under varying environments. *Crop Science* 35:1667-1675.
- Wallenhammar, A-C., Ståhl, P. & Pettersson, B. 2005. Ekologisk vallfröodling utvecklas starkt. *Svensk Frötidning* 2: 13-15. (In Swedish).

Cover crops and weed control in establishment of organic seed crops of meadow fescue (*Festuca pratensis* Huds.)

Kari Bysveen¹⁾, Trygve S. Aamlid²⁾ & Svein Ø.Solberg³⁾

¹⁾Fabio Extension Service Group for Organic Farming
Gjennestadtunet 83, N-3160 Stokke, Norway

²⁾Norwegian Institute for Agricultural and Environmental Research
Bioforsk Øst Landvik, Reddalsveien 215, N-4886 Grimstad, Norway

³⁾The Nordic Gene Bank, P.O.Box 41, SE-230 53 Alnarp, Sweden

kari.bysveen@lfr.no

ABSTRACT

During 2002-2005, Norwegian seed yields of meadow fescue (*Festuca pratensis* Huds.) were 52% lower in organic than in conventional seed production. The difference was most conspicuous in ley year 1, suggesting that seed crop establishment is a limiting factor in organic seed production. Meadow fescue is usually underseeded in a cover crop of spring wheat or spring barley, and many organic growers delay the underseeding until after harrowing for weed control. Our objectives were (1) to determine whether organic seed crops of meadow fescue should be underseeded in the same operation as, or immediately after, the cover crop (implying no harrowing for weed control); or if they should be underseeded in conjunction with, or just after, weed harrowing (on average nine days after the cover crop); and (2) to compare the establishment of meadow fescue seed crops without cover crop or in cover crops of spring barley, spring wheat, peas or green fodder (barley + peas). On average for all cover crops and four trials, first year's seed yields of meadow fescue were 11 % lower for crops seeded after weed harrowing than for crop seeded immediately after the cover crop. Weed harrowing combined with delayed seeding also resulted in more scentless mayweed (*Tripleurospermum inodórum*) and other weeds in ley year 1. Despite less plant-available nitrogen in spring, seed crops underseeded just after the cover crop were more lodged than crops underseeded after weed harrowing. On average for the two seeding dates, the highest seed yield were produced from crops seeded in pure stand and from crops that had been underseeded in green fodder and received an extra 30 kg N ha⁻¹ in manure shortly after green fodder harvest in late July. The results suggest that seed crops of meadow fescue need ample supply of light, water and nutrients in August to produce a good seed yield in ley year 1.

Key words: green fodder, light penetration, nitrogen, pea, seeding time, spring barley, spring wheat, weed harrowing

INTRODUCTION

Seed production statistics for the period 2002-2005 showed that average seed yields of red clover (*Trifolium pratense* L., cv. Nordi), timothy (*Phleum pratense* L., cv. Grindstad), and meadow fescue (*Festuca pratensis* Huds., cv. Fure) were, in turn, 31, 40 and 52% lower in organic than in conventional farming (The Norwegian Food Safety Authority, personal communication). As meadow fescue is less

competitive than red clover and timothy, it is important to reduce the weed pressure as much as possible before the establishment of meadow fescue seed crops. Particularly important is that couch grass (*Elymus repens*), whose seed may be difficult to clean out of meadow fescue seed, and other perennial grasses are eliminated before seed crop establishment. As the yield gap between conventional and organic farming is usually greater in ley year 1 than in ley year 2, the

establishment year seems to be very critical in organic seed production of meadow fescue.

In Norway, meadow fescue seed crops are usually underseeded in a cover crop of spring barley or spring wheat. Spring barley is less open and more competitive during initial growth stages than spring wheat, but this may be offset by a two to three weeks earlier harvest time for spring barley, thus allowing the seed crop a longer growing period in autumn. Some growers have speculated that alternative cover crops such as green fodder or peas might be useful for organic seed crops establishment.

Instead of underseeding the seed crop in the same operation as, or immediately after, the cover crop, many organic farmers have invested in weed harrows with air-seeders. Pre-emergence harrowing, or harrowing when the cover crop is at the first leaf stage, is usually recommended in organic production of cereals; however, this implies that the seed crop has to be seeded 1-2 weeks later than the cover crop. Furthermore, given the low seeding rates used for seed crop establishment, air-seeders may not distribute the seeds uniformly enough to secure even seed crop emergence. This is especially the case for meadow fescue, which is normally established from a lower seed number per square metre than timothy or red clover. A third dilemma is that rolling that is often carried out after underseeding seed crops may counteract the effect of weed harrowing.

The objectives of the present research were (1) to determine whether organic seed crops of meadow fescue should be underseeded in the same operation as, or immediately after the cover crop (implying no harrowing for weed control), or in conjunction with weed harrowing 1-2 weeks later than the cover crop; and (2) to compare pure stand establishment of meadow fescue seed crops with establishment in various cover crops.

MATERIALS AND METHODS

This research was planned, seed yields cleaned and statistical analyses performed by The Norwegian Institute for Agricultural and Environmental Research, Bioforsk Øst Landvik. Two trials were carried out at Bioforsk Øst Landvik and two on-farm trials by the Farmers Extension Service Groups in Hedmark and Buskerud, respectively.

The field trials were established according to a split plot design with two replicates, weed harrowing / sowing time on main plots, and cover crops on subplots. Subplot size was 8.0 m x 1.5 m. The treatments were:

Factor 1: Weed harrowing / sowing time

- A. Meadow fescue seeded immediately after cover crop. No harrowing for weed control.
- B. Meadow fescue seeded after weed harrowing, on average 9 days after the cover crop.

Factor 2: Cover crop / extra manure after cover crop harvest

1. No cover crop. Meadow fescue cut twice; 30 kg N ha⁻¹ in manure after last cut.
2. Spring barley cv. Annabell (2 row) seeded at a rate 160 kg ha⁻¹. 30 kg N ha⁻¹ in manure shortly after cover crop harvest.
3. Spring wheat cv. Zebra seeded at a rate 190 kg ha⁻¹. 30 kg N ha⁻¹ in manure shortly after cover crop harvest.
4. Green fodder (mixture of spring barley (6 row) and pea), seeded at a rate 160 kg ha⁻¹. No manure after cover crop harvest.
5. As 4 but with 30 kg N ha⁻¹ in manure shortly after cover crop harvest.
6. Semi-leafless pea for seed maturation, cv. Integra, seeded at a rate 235 kg ha⁻¹. No manure after cover crop harvest.
7. As 6 but with 30 kg N ha⁻¹ in manure shortly after cover crop harvest.

Meadow fescue cv. Fure was always seeded at a rate 7 kg ha⁻¹ in rows perpendicularly to the cover crops. On average for the four trials, harvest dates in the sowing year were: No cover crop (treatment 1): 8 July and 23 Aug.; spring barley (treatment 2): 23 Aug.; spring wheat (treatment 3): 5 Sep.; green fodder (treatments 4 and 5): 27 July; and peas (treatments 6 and 7): 2 Sep. In treatment 1 the cut material was not removed, but returned to the field. In treatments 2, 3, 6 and 7, the cover crop straw was chopped with a flail chopper and returned.

In the ley years, all crops received manure from chicken or cows corresponding to 50 kg total N ha⁻¹. Recordings included light penetration (Landvik only) and cover crops

yields in the sowing year; soil mineral nitrogen to 20 cm depth in the spring of ley year 1; and seed yield, lodging and per cent weed infestation in ley years 1 and 2. As of April 2007, results are available from the sowing year and two ley years for the two trials established in 2003, but only from the sowing year and ley year 1 for the two trials established in 2004.

RESULTS AND DISCUSSION

As the statistical analyses did not indicate significant interactions between the two experimental factors, only main effects will be presented.

Effects of seeding time / harrowing

On average for four trials, grain yields of barley, spring wheat and peas were 3605, 3300 and 2910 kg ha⁻¹, respectively (data not shown). These yields were not affected by weed harrowing / time of underseeding meadow fescue. The dry matter yields of green fodder tended ($P=0.09$) to be higher on plots which had not been harrowed (5380 kg ha⁻¹) than on plots with had been harrowed for weed control (4830 kg ha⁻¹).

An average nine-day delay in the seeding time for meadow fescue to allow for weed harrowing resulted in an 11 % seed yield reduction in the first year of as compared with crops that had been seeded immediately after the cover crop (Table 1). Surprisingly, the seed yield reduction was even more accentuated in the second ley year (18 %), although this result is not final as two trials remain to be harvested in 2007. Despite the fact that soil mineral nitrogen in ley year 1 was higher on weed-harrowed than on non-harrowed plots, seed crops seeded just after the cover crop tended to be more vigorous and therefore had slightly, but significantly higher lodging scores than crops seeded after harrowing.

Scentless mayweed (*Tripleurospermum inodórum*, syn. *Matricaria inodora*) is one of the most common weeds in Norwegian seed production. Although seeds of this species are simpler to clean out of meadow fescue than out of the timothy and red clover seed lots, it

is often so abundant that it causes a significant seed yield reduction in ley year 1. In these trials, scentless mayweed was a problem only at Hedmark, and Table 1 shows that it was more abundant on weed-harrowed than on non-harrowed plots. Scentless mayweed used to be classified as biennial (Korsmo *et al.* 1981), but it has also been shown that most plants germinating in early spring will die after flowering and seed production in the establishment (Skuterud 2002). The present results corroborate these findings, suggesting that the later plants of scentless mayweed germinate, the greater risk that they will survive and produce seed in ley year 1. Even with respect to the total weed population, our results suggest that harrowing for weed control was contraproductive as delayed seeding reduced the competitive ability of the seed crop. This effect had probably been even more accentuated had the seed of meadow fescue been broadcast with an air-seeder mounted on the weed harrow instead of placed in drills with an ordinary sowing machine as in the present trials.

Effects of cover crop / extra manure after cover crop harvest

During the first part of the establishment year, more light penetrated to the underseeded meadow fescue in cover crops of peas or spring wheat than in cover crop of spring barley or green fodder (Fig. 1). After about, 1 July, this situation was reversed, especially for the peas which were often heavily lodged during the latter part of the growing season.

The main effect of cover crops on seed yields varied among the four trials, but on average, yields in ley year 1 were higher on plots established without cover crop (treatment 1) or in the green fodder crop with extra nitrogen (treatment 5) than in the other cover crops. This result highlights the importance of an early harvest of the cover crop and of readily available nitrogen in the late summer and early fall. The result is in good agreement with Heide (1994) who documented that plants of meadow fescue have a long requirement for primary induction, and with Havstad (1998) who found that seed crops of meadow fescue ought to receive its input of autumn nitrogen as early as 1 August.

Table.1 Seed yields, weed population, lodging, and soil mineral N in organic seed crops of meadow fescue in ley year 1 and 2 as affected by seeding time / weed harrowing and use of cover crops.

	Ley year 1					Ley year 2				
	Seed yield kg ha ⁻¹	Rel	Weed total	% of pop. Scent -less may- weed	Lodg- ing at seed harv. %	Soil min N, kg ha ⁻¹	Seed yield kg ha ⁻¹	Rel	Total weed popu- lation %	Lodg- ing at seed harv. %
Number of trials	4	4	4	1	4	3	2	2	2	2
Main effect of seeding time of meadow fescue / weed harrowing										
A. Meadow fescue seeded just after cover crop. No weed harrowing.	618	100	20	22	66	7,0	712	100	17	58
B. M.f. seeded after weed harrowing, ~9 days after A.	550	89	25	30	51	11,4	584	82	15	45
P-value	<0.05	-	<0.05	<0.05	<0.01	<0.0 5	<0.0 1	-	>0.20	<0.01
Main effect of cover crops / extra manure after cover crop harvest										
1. No cover crop	688	100	21	14	59	9,6	603	100	21	52
2. Spring barley	571	83	24	33	59	9,0	658	109	18	45
3. Spring wheat	526	76	23	26	58	9,4	637	106	19	52
4. Green fodder	544	79	27	38	56	12,0	722	120	12	56
5. Green fodder + N	647	94	21	28	57	7,8	712	118	11	54
6. Peas	559	81	21	20	59	8,7	581	96	18	54
7 Peas + N	553	80	24	28	68	8,1	622	103	14	48
P-value	<0.05	-	>0.20	>0.20	>0.20	>0.2 0	0.13	-	0.15	>0.20
LSD (P<0.05)	96	-	-	-	-	-	-	-	-	-

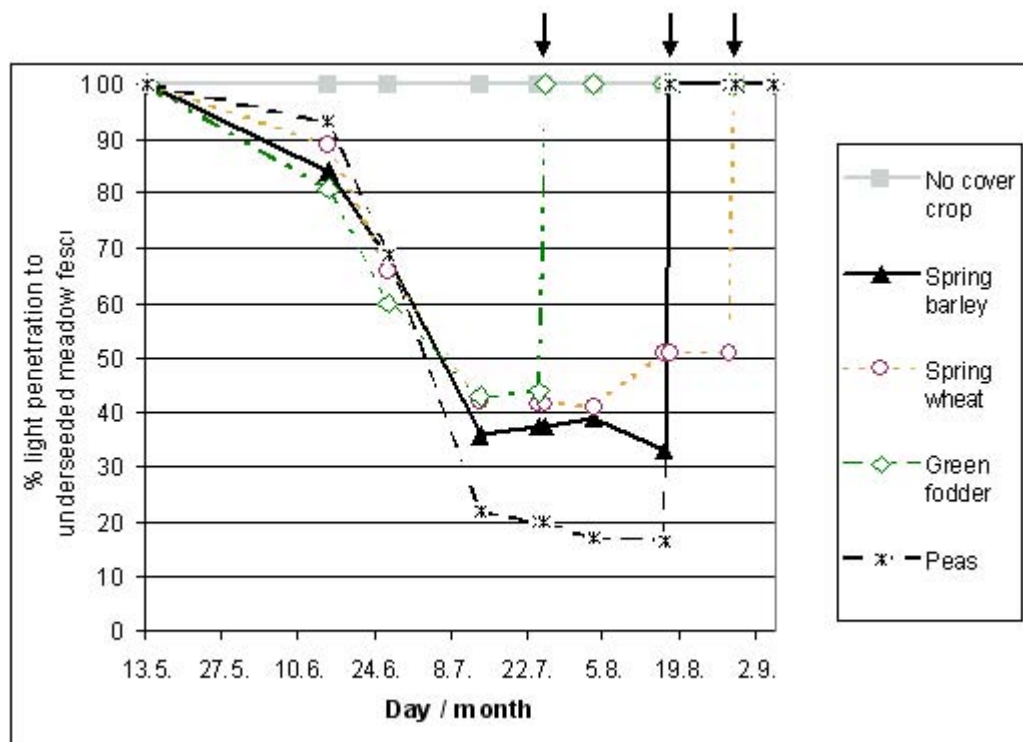


Fig. 1. Per cent light penetration to underseeded meadow fescue in cover crops of spring barley, spring wheat, green fodder and peas as compared with meadow fescue established in pure stand (=100%). Arrows from left to right indicate harvest date for green fodder, spring barley / pea, and spring wheat, respectively. Data from trial established at Landvik in 2004.

Somewhat unexpectedly, seed yields were not higher after peas than after spring barley. While other workers have found peas to be a good cover crop for slowly-establishing grasses such as smooth-stalked meadow grass (*Poa pratensis*; Boelt 1997) and red fescue (*Festuca rubra*; Aamlid 1996), the present results are not surprising given the poor light penetration in lodged crops of peas (Fig. 1). Unlike spring cereals, semi-leafless peas will be more prone to lodging if established at low seeding rates, and in the present trials, pea emergence was often poor due to birds picking germinating seeds. Boelt (1997) also found seed yields of meadow fescue to be similar after establishment in spring barley or peas.

The highest seed yields in the first ley year were harvested from plots seeded without cover crop. Many organic farmers might be reluctant to such a practise because of weed problems, but in our trials, two times mowing was sufficient to control annual weeds such as *Chenopodium album*, *Spergula arvensis*, *Sonchus asper* and *Galium aparine*. Had the weed population been dominated by lower

species such as *Stellaria media*, it would perhaps be better to use cover crop, because it would be difficult to control the small weeds properly by cutting.

CONCLUSIONS

1. Rather than taking time for weed harrowing, organic seed crops of meadow fescue should to be underseeded at the same time as the cover crops.
2. As compared to underseeding in spring barley or spring wheat, first year seed yields of meadow fescue can be increased by establishing the seed crop without cover crop or in a green fodder crop which is harvested not later than 1 August. The first method should only be practiced where the flora is dominated by tall-growing weed species that are easy to control by two cuttings in the establishment year. Our data confirm that that ample supply of light, water and nutrients in August is important for first year seed yields of meadow fescue.

LITERATURE

- Aamlid, T.S. 1996. Seed production of Norwegian breeding lines of red fescue (*Festuca rubra* L.) established with or without field peas as cover crops. Norsk landbruksforskning 10: 315-330. (In Norwegian, with English abstract).
- Boelt, B. 1997. Undersowing *Poa pratensis* L., *Fesutca rubra* L., *Festuca pratensis* Huds., *Dactylis glomerata* L. and *Lolium perenne* L. for seed production in five cover crops. I. The yield of the cover crops and the seed yield of the undersown grasses. Journal of Applied Seed Production 15: 41-48.
- Havstad, L.T. 1998. Seed yield of meadow fescue in Norway and Denmark: The effects of locations, cultivars and autumn management. Acta Agriculturæ Scandinavica 48: 144-158.
- Heide, O.M. 1994. Control of flowering and reproduction in temperate grasses. New Phytologist 128: 347-362.
- Korsmo, E. Vidme, T. & Fykse. H. 1981. Korsmos ugrasplansjer. Landbruksforlaget, Oslo. (In Norwegian).
- Skuterud, R. 2002. Growth rhythm of *Matricaria perforata* Merat. In: Grass- and clover seed production. NJF-report no 341. pp.127-128. (In Norwegian, with English abstract).

Subterranean clover (*Trifolium subterraneum*) as a companion crop during establishment of organic seed crops of timothy

Svein Ø. Solberg¹⁾, Kari Bysveen²⁾ & Trygve S. Aamlid³⁾

¹⁾ The Nordic Gene Bank, P.O.Box 41, SE-230 53 Alnarp, Sweden

²⁾ Fabio Extension Services, Gjennestadtunet, N-3160 Stokke, Norway

³⁾ The Norwegian Institute for Agricultural and Environmental Research, Bioforsk Øst Landvik, Reddalsveien 215, N-4886 Grimstad, Norway

svein.solberg@nordgen.org

ABSTRACT

Subterranean clover (*Trifolium subterraneum*) as a companion crop during establishment of organic timothy (*Phleum pratense*) seed crops was examined in four field trials studies in SE Norway from 2001 to 2004. Clover and timothy were either mixed before sowing or seeded in every other row with the possibility of removing winter-surviving plants of clover in the spring of ley year 1. *Trifolium alexandrinum*, *T. resupinatum*, *T. incarnatum*, *Melilotus officinalis*, *Lotus corniculatus* and *Melicago lupulina* were also included in the study, in addition to control plots with no legume companion crop. No manure was applied except to the spring wheat cover crop in the year of establishment. The results showed that subterranean clover was the most promising legume, resulting in a 20 % increase in timothy seed yield in the first seed harvest year. The result could partly be explained by less competition from weeds during the establishment of the seed crop. In autumn of the sowing year, after the wheat had been harvested, subterranean clover covered up to 70 % of the soil surface, leaving less space for weeds. During the following winter, subterranean clover died, leaving more space to the first year timothy seed crop. The positive results with subterranean clover as a companion crop were confirmed by on-farm studies at three different sites. A seed mixture of 0.5-0.7 kg ha⁻¹ timothy and 1.0-1.2 kg ha⁻¹ subterranean clover is now recommended when establishing organic timothy seed crops on farms with no or limited access to manure.

Key words: ecology, green manure, intercropping, mixed cropping, organic farming, seed production, weeds.

INTRODUCTION

Reduced yields due to weeds (including voluntary clovers) and lack of nutrients are known to be the major challenges for organic grass seed production in Norway. Various strategies to overcome these problems have been suggested. Application of more manure is one possibility, but the agricultural policy over the past decades has resulted in few animals in the areas where grain and grass seeds are produced. Application of conventional pig or chicken manure is also restricted by the official rules for

certification of organic crops. Mixed cropping with legumes is a known strategy in organic production worldwide. In earlier times this was also used in timothy (*Phleum pratense*) seed production, where red clover (*Trifolium pratense*) seed and timothy seed were produced together. Today the OECD certification standards do not allow seed production of two species in mixed stands.

Experiments from 1994 to 2002 showed that organic seed yields of timothy could be favoured by mixed cropping with white clover (*Trifolium repens*) or alsike clover (*Trifolium*

hybridum) (Aamlid 2002, 2003). The main problem, however, was that the seed size of these clovers made them difficult to separate from timothy during the seed cleaning processes. This was especially seen in the first year of harvest, but for white clover even in the second year.

Experiments and practical experience have shown that proper seed yields can be obtained in the second year of organic grass seed production. This is true for both timothy and meadow fescue (*Festuca pratensis*), which are the major grass species used in Norway. The challenge is therefore to increase seed yields in the first year. In 2001 new experiments were set up to investigate various legume crops in mixed cultivation with organic grass seed crops. Subterranean clover (*Trifolium subterraneum*) was one of the species to be examined. Both the size of the seed (clearly bigger) and the annual life cycle made this clover worth further studies.

MATERIALS AND METHODS

Field trials

A total of seven legumes were studied in four field trials in timothy (*Phleum pratense*) 'Grindstad' at two different sites in SE Norway (Gjennestad and Landvik) from 2001 to 2004. The study was also carried out in meadow fescue, but the results from that species are not included in this paper. The following companion crops were compared with a control treatment without companion crop: *Trifolium subterraneum*, *T. alexandrinum*, *T. resupinatum*, *T. incarnatum*, *Melilotus officinalis*, *Lotus corniculatus* and *Melicago lupulina*. The legume seed was either mixed with the timothy seed before sowing or legumes and timothy were seeded in every second row 15 cm apart, allowing surviving legumes to be removed by row cultivation in the first ley year. Six hundred timothy seeds and one hundred legume seeds were seeded per m². Cow manure corresponding to 80 kg total-N ha⁻¹ was applied before seeding, but not used subsequently in the experimental period.

Wheat was used as cover crop and the straw was removed after harvesting.

On farm studies

A total of three farms starting organic timothy seed production in 2003 or 2004 were selected. One part of each seed crop (at least 0.5 ha) was seeded with a mixture of *T. subterraneum* and timothy. The rest was seeded with timothy alone. The study was carried out in cooperation with the local extension services.

RESULTS AND DISCUSSION

Field trials

On average for the four trials, *Trifolium subterraneum* as a companion crop resulted in a 19 % increase in the first year seed yield of timothy (Table 1). *T. subterraneum* covered an average of 69 % of the soil surface in the autumn of the sowing year, but it disappeared completely during the first winter. Also *Trifolium alexandrinum* tended to have positive effect on seed yields (13 % increase; Table 1, while the other annual legumes had less impact. (data not shown).

Seeding biannual and perennial legumes (*Melilotus officinalis*, *Lotus corniculatus* and *Melicago lupulina*) resulted in reduced yield and a weed problem the first seed year of timothy, but *Melicago lupulina* mostly died and gave a significant yield increase the second year (Table 1). It seems obvious that the first year crop must be cut for green fodder if such species are to be included.

On farm studies

The positive effect of *Trifolium subterraneum* on timothy seed production was confirmed in the on-farm studies (Table 2). On average for three locations, a 22 % increase in the first seed yield was found. The method can be recommended for organic timothy seed production on farms without manure. A mixture of 5-7 kg timothy and 10-12 kg *Trifolium subterraneum* per hectare can be recommended under such conditions.

Table 1. Effects of selected legumes in the first and second seed harvest year of timothy. Mean of four trials.

	Timothy seed yield		% coverage		Purity analysis		
	Kg ha ⁻¹	relative	seeded legume	other species	Seeded Timothy	legumes	Others
First ley year							
No legume control	287	100	0	29	89.5	0	7.3
<i>T. subterraneum</i>	341	119	0	27	93.4	0	4.5
<i>T. alexandrinum</i>	323	113	0	25	95.0	0	3.3
<i>Medicago lupulina</i>	220	77	33	18	90.5	0.8	6.3
LSD 5 %	92	-	23	15	ns	ns	ns
Second ley year							
No legume control	616	100	0	14	98.2	0	1.5
<i>T. subterraneum</i>	617	100	1	9	98.2	0	1.0
<i>T. alexandrinum</i>	603	98	0	12	98.5	0	1.0
<i>Medicago lupulina</i>	739	120	7	5	99.2	0	0.7
LSD 5 %	79	-	11	10	ns	ns	ns

Table 2. Seed yield of timothy (kg ha⁻¹) seeded with and without *Trifolium subterraneum*. Results from the on-farm studies

	Seed yield, kg ha ⁻¹			
	Farm 1	Farm 2	Farm 3	Average
No legume)	650	370	440	490
<i>T. subterraneum</i>	730	490	570	600

CONCLUSION

In conclusion, this research shows that a 20 % yield increase in timothy is possible in the first seed year by using *Trifolium subterraneum* as a green manure companion crop. *Medicago lupulina* in another interesting legume that warrant further investigation in a system with green fodder in

the first ley year and seed production in the second and subsequent ley years.

ACKNOWLEDGEMENTS

We would like to thank Felleskjøpet Agri, Strand Brænderi and Vestfold Farmers Union for their support. The study was founded by the Norwegian Research Council and the Norwegian Agricultural Authorities.

LITERATURE

- Aamlid, T.S. 2002. Yield and quality of mixed seed crops of timothy (*Phleum pratense*) and clovers (*Trifolium* spp.) in an organic cropping system. Acta Agriculturae Scandinavica, Section B. Soil and PlantScience 52: 18-24.
- Aamlid, T.S. 2003. Animal manure or companion cropping with herbage legumes as nitrogen sources in organic seed production of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). In: Herbage Seeds in the New Millenium - New Markeds, New Products, New Opportunities. Proceedings of the Fifth International Herbage Seed Conference, Gatton, Australia 23-26 November 2003: 53-58.

Alternating forage and seed production as a method to improve feasibility of tall fescue seed production at northern latitudes

Oiva Niemeläinen¹⁾ & Markku Niskanen²⁾

¹⁾MTT Agrifood Research Finland, Plant Production Research, Building E, FI-31600 Jokioinen, Finland

²⁾MTT Agrifood Research Finland, Plant Production Research, Alapääntie 104, FI-61400 Ylistaro, Finland

oiva.niemelainen@mtt.fi

ABSTRACT

Problems have been encountered in seed production of tall fescue (*Festuca arundinacea*) in Finland although it is a highly productive species in forage production. Two trials were conducted alternating forage and seed production. In the trials, which located at Jokioinen (latitude N 60°49') and Ylistaro (latitude N 62°57'), tall fescue (cv. Retu) was harvested either for seed (2 stubble treatments), hay (2 cuts) or silage (2 and 3 cuts) in the first year after establishment in barley. Seed yields ranged from 120 to 150 kg ha⁻¹ at the both trial sites and forage DM yields ranged from 10030 kg ha⁻¹ to 11140 kg ha⁻¹ in Jokioinen and from 6570 to 8340 kg ha⁻¹ in Ylistaro. Regrowth after seed harvest was 4110 kg DM ha⁻¹ in Jokioinen. In the second year all treatments were harvested for seed. Seed yield ranged from 640 to 790 kg ha⁻¹ in Jokioinen and from 410 to 660 kg ha⁻¹ in Ylistaro. In Jokioinen three silage cuts, two hay cuts and seed production including regrowth removal resulted in highest seed yield and were nearly equally successful. In Ylistaro harvesting twice for hay and seed production including regrowth removal were most successful. It is concluded that alternating forage and seed production in tall fescue might facilitate benefits to sole seed production in Finland.

Key words: crop management, *Festuca arundinacea*, hay production, production systems, regrowth management, silage production, seed yield

Prediction of herbicide selectivity in grass seed crops from pot experiments

Solvejg K. Mathiassen, Mette Rabølle & Per Kudsk

University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Flakkebjerg, DK-4200 Slagelse, Denmark

Solvejg.mathiassen@agrsci.dk

ABSTRACT

The selectivity on *Festuca pratensis* and *Lolium perenne* seedlings of herbicides with various modes of action was compared in field and pot experiments. Visual assessments and measurement of the relative vegetation index in field experiments were compared to biomass reductions in pot experiments carried out simultaneously with the field tests. In the pot experiments we also studied the influence of parameters assumed to affect crop selectivity in the field. In general, selectivity was higher in the field experiment compared to the pot experiments, probably because the field experiments were carried out in a period with dry soils. Additional comparative experiments are required to reveal whether it is possible to predict variations in field performance from pot experiments.

Key words: diflufenican, fenoxaprop-P-ethyl, iodosulfuron, logarithmic spraying, pendimethalin, sulfonylureas, tribenuron,

INTRODUCTION

The interest of agrochemical companies in developing herbicides for seed crops has declined as the potential profit in minor crops is too low compared to the high costs of registration trials. Considering herbicides that already are registered in other crops, efficacy trials are not required in Denmark, however, it is still necessary to prove selectivity in grass seed crops. The overall objective of the present study is to develop a model that can predict herbicide selectivity in seed crops. Our hypothesis is that field selectivity at the seedling stage can be predicted using a specific set-up of pot experiments. The set-up of pot experiments depends on the inherent properties of the herbicides and the intended use pattern. This paper reports the results from experiments in *F. pratensis* and *L. perenne* in 2006 in which the selectivity of a number of herbicides with different modes of action was compared in field and pot experiments carried out simultaneously. The experiments are repeated this year and additional experiments are being carried out in *Dactylis glomerata*, *Poa pratensis*, *Festuca rubra* and *Festuca arundinacea*.

MATERIALS AND METHODS

A selection of herbicides with different modes of action was used in the study (Table 1). All herbicides are registered in spring barley.

Field experiment

F. pratensis (cv. Laura) and *L. perenne* (cv. Lasso) were grown in the field at Research Centre Flakkebjerg. The herbicides were applied at the 1- to 2-leaf stage or at the 3- to 4-leaf stage using a logarithmic sprayer. The plot size was 2.5 m x 25 m and the dose was reduced by a factor 2 for each 7.5 m, consequently each plot represented a dose interval from 1 N to 1/10 N. Each treatment was replicated twice without a cover crop in addition to one replicate with spring barley as a cover crop. The activity was assessed by visual assessments 5 and 10 weeks after application. The relative vegetation index was measured 10 weeks after application as plant canopy reflectance in the red and the near-infrared spectrum.

Table 1. Herbicides included in the selectivity test in *Festuca pratensis* and *Lolium perenne*. Maximum doses (1N) and growth stages at application in the field experiment are shown.

Herbicide	Active ingredient	Maximum dose (1 N) (g a.i./ha)	Growth stage (number of leaves)
Stomp	Pendimethalin	1600	1 - 2
DFF	Diflufenican	125	1 - 2
DFF + Oxitril	Diflufenican + ioxynil + bromoxynil	125 + 200	1 - 2
Hussar OD*	Iodosulfuron	12.5	3 - 4
Ally ST	Metsulfuron	4	3 - 4
Express ST**	Tribenuron	15	3 - 4
Harmony**	Thifensulfuron	15	3 - 4
Gratil**	Amidosulfuron	30	3 - 4
Primera S***	Fenoxaprop-P-ethyl	110	3 - 4

*Herbicides applied in mixture with 0.5 L ha⁻¹ Renol (methylated vegetable oil)

0.1% Contact (non-ionic surfactant), *0.2% Isoblette (an-ionic surfactant)

Pot experiments

F. pratensis (cv. Laura) and *L. perenne* (cv. Lasso) were sown in 1 L pots in field soil on the same day as the field experiments. The pots were placed outdoor and herbicide applications were carried out on the same dates as in the field using a laboratory pot sprayer. Each herbicide was applied at 5 doses. In additional pot experiments different parameters expected to affect herbicide performances were varied. For all herbicides the influence of growth stage was examined applying the herbicides at the 1- to 2-leaf stage and at the 3- to 4-leaf stage. For diflufenican and pendimethalin the influence of sowing depth and soil moisture was examined and for tribenuron and iodosulfuron the influence of adjuvants was determined. Foliar and soil uptake was examined for pendimethalin, iodosulfuron and tribenuron. Foliar uptake was obtained by covering the soil surface with vermiculite during spraying and subsequently removing this inactive material after the spray deposits had dried. Soil uptake was assessed as overall efficacy minus foliar efficacy.

The plants were harvested 4 weeks after application and foliar fresh and dry weights were recorded. The results were analysed using a log-logistic dose-response model. The doses resulting in 50% biomass reduction (ED₅₀) were estimated.

RESULTS AND DISCUSSION

The maximum doses of fenoxaprop-P-ethyl, pendimethalin, diflufenican and diflufenican

+ ioxynil + bromoxynil did not affect growth of *F. pratensis* and *L. perenne* in the field experiment. The ED₅₀ doses of the sulfonylurea herbicides were estimated from the relative vegetation index measurements. On *L. perenne* the ED₅₀ doses of tribenuron, thifensulfuron and amidosulfuron were 160-200% higher than the recommended doses in spring barley indicating that treatments can be expected to be selective. The ED₅₀ doses of metsulfuron and iodosulfuron corresponded to 60% and 20% of the recommended doses in spring barley and consequently, there is a risk applying these herbicides in the same doses as recommended in spring barley. In accordance with the findings in previous studies (Mathiassen *et al.* 1999), *F. pratensis* was more susceptible to herbicide treatments than *L. perenne*, and treatments with the sulfonylurea herbicides at the recommended doses in spring barley can not be done in *F. pratensis* without a risk of growth reduction.

The pot experiments showed a high selectivity of diflufenican on both species while pendimethalin reduced biomass by up to 50% when applied at the 1- to 2-leaf stage but did not affect growth at the 3- to 4-leaf stage. Additional trials with different sowing depths showed that damage was related to a direct contact between seed and herbicide and could be avoided by sowing at a depth of 2 cm. Comparison of foliar and total activity revealed that the activity of pendimethalin was solely related to soil activity. Surprisingly, 1 Lha⁻¹ fenoxaprop-P reduced biomass of *F. pratensis* by 40% in the pot experiments.

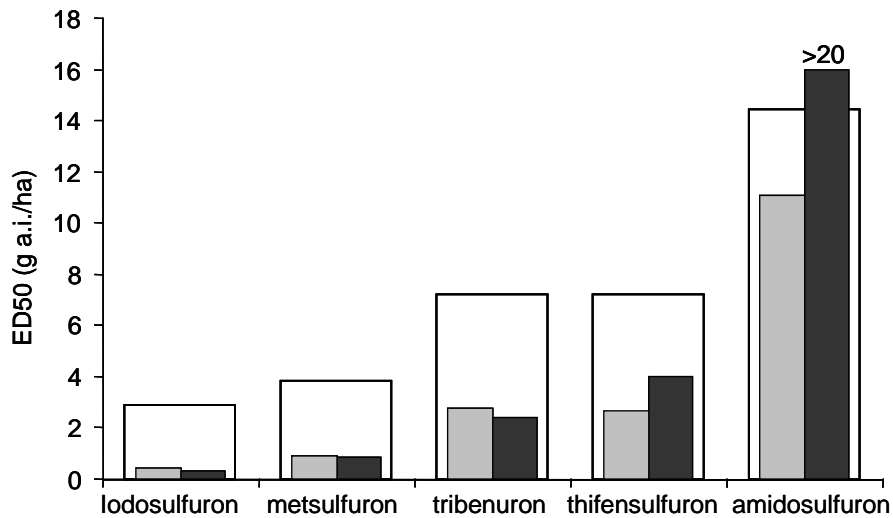


Fig. 1. ED₅₀ doses of sulfonylurea herbicides on *F. pratensis* in field and pot experiments and recommended doses in spring barley.

The susceptibility of *F. pratensis* to the sulfonylureas was similar in the field and pot experiments except for amidosulfuron (Fig. 1), whereas *L. perenne* was more tolerant in the field. Low soil moisture in the field at the time of herbicide application may have reduced soil activity of the sulfonylurea herbicide. The results of other pot experiments showed that soil activity is an important component in overall activity of iodosulfuron and metsulfuron at early growth stages whereas the activity of tribenuron is mainly related to foliar activity.

CONCLUSION

The results from the first year's comparative pot and field trials showed that *F. pratensis* and *L. perenne* were generally more tolerant

in field experiments than in pot experiments. Consequently, herbicides may be assumed to be non-selective based on results from pot experiments although selective in the field (false negative), which of course is better than the opposite situation (false positive). Additional trials will reveal whether field performance under contrasting conditions can be better predicted by varying parameters affecting herbicide performance in the pot experiments.

ACKNOWLEDGEMENTS

We thank all the technicians who have been involved in the experiments for their excellent work.

LITERATURE

Mathiassen S.K., Kudsk P. & Jensen P.E. 1999. Seedling tolerance of different grass species to sulfonylurea herbicides. *Journal of Applied Seed Production* 17: 7-14.

Selectivity of some herbicides in eight grass species grown for seed in the conditions of Central Europe - preliminary results

Radek Macháč & Bohumír Cagaš

OSEVA PRO Ltd., Grassland Research Station at Zubří, Hamerská 698,
756 54 Zubří, Czech Republic

machac@oseva.cz

ABSTRACT

In small plot field trials conducted for several years the selectivity of some herbicides in 8 grass species in the temperate zone was studied. In the experiments basic and double rates of herbicides were tested. Evaluations were made of potential phytotoxicity of herbicides in the respective grass species and their influence on the number of fertile stems and seed yield and seed quality (1000-seed weight, germination capacity). Preliminary results (1 harvest year) suggested the possibility of using the herbicides ARRAT (tritosulfuron 250 g kg⁻¹, dicamba 500 g ha⁻¹) and AURORA SUPER SG (carfentrazone-ethyl 1.5 %, MCPP-P 60 %) in all grass species under study: Kentucky bluegrass (KBG), cocksfoot, meadow fescue, red fescue, timothy, perennial ryegrass, and loloid and festucoid types of festulolium. In some species (KBG, cocksfoot, perennial ryegrass), however, there was a decrease in yield when a double rate was applied. A combination of ARKEM (metsulfuron 200 g kg⁻¹) + CZ-600 (mecoprop-P 600 g l⁻¹) proved to be good only in the loloid type of festulolium and timothy. ESTERON (2,4-D EHE 850 g l⁻¹) showed good results in both types of festulolium, meadow fescue and timothy. The preparation HUSAR (iodosulfuron 50 g kg⁻¹ + mefenpyr-diethyl 150 g kg⁻¹) applied at a basic rate was selective in Kentucky bluegrass, cocksfoot and timothy; at a double rate only in timothy. In meadow fescue this preparation was highly phytotoxic and when used at a double rate it practically damaged the stand.

Key words: grasses, herbicide, seed production, selectivity

INTRODUCTION

Grass seed production has a long tradition in the Czech Republic and export of grass seeds in an important part of agribusiness. The weak point is, however, the yield of grasses for seed which falls behind the average of the EU. One of the causes is non-recognition of seed production stands as a result of high weed infestation or non-recognition of seeds containing weed seeds. In the Czech Republic there are several herbicides that are registered for use in grasses for seed (MCPA, fluroxypyr, clopyralid, mecoprop, dichlorprop, ethofumesate, chlorsulfuron, triasulfuron + dicamba, amidosulfuron, florasulam and others). Unfortunately, the changing spectrum of weeds and the

existence of resistant weed populations require new and more effective herbicides.

MATERIALS AND METHODS

Tests of the selectivity of herbicides were performed in small-plot trials in Zubří (North Moravia, 360 m a. s. l., cambisols, average temperature 7.6 °C, rainfall 903 mm). The size of plots was 10 m². For each herbicide, the base rate and the double rate (= 2 times the base rate, i.e. simulation of spray overlap) was tested in two replicates. The trials were established in the year 2005 and in the year 2006 one cycle of herbicide tests was completed. Fertilizer application of experiment: autumn 45 kg N ha⁻¹, phosphorus and potash according to soil supply (40 kg P₂O₅ha⁻¹, 60 kg K₂O ha⁻¹). Depending on the

species, grasses were given additional fertilization at a rate of 60-80 kg N ha⁻¹ in spring. Herbicides were applied in 300 litres

of water per ha at the stage of maximum tillering of grasses by a precise plot sprayer. The rates applied are given in Table 1.

Table 1 Trade name, active substances, doses (base rates) and tested grass species

Trade name	Active substance	dose a. s. per ha	KBG	cocksfoot	Hykor	Lofa	timothy	meadow fescue	red fescue	perennial ryegrass
Arrat	tritosulfuron	50 g								
	dicamba	100 g	x	x	X	x	x	x	x	X
Aurora super	carfentrazone-ethyl	15 g	x	x	X	x	x	x	x	X
	MCPP-P	600 g								
Arkem	metsulfuron-methyl	6 g	x	x	X	x	x	x		
+ CZ 600	mecoprop-P	900 g								
Esteron	2,4 D EHE	1275g	x	x	X	x	x	x		
Callisto 480 SC	Mesotrione	144 g			X	x	x	x		
Husar	iodosulfuron	3 g								
	mefenpyr-diethyl	30 g	x	x						
Atlantis	mesosulfuron-ethyl	4.5 g								
	iodosulfuron-methyl	0.9 g	x	x						
	mefenpyr-diethyl	13.5 g								

At regular intervals (14, 21 and 28 days after treatment) phytotoxicity of grasses to herbicides was evaluated. Before harvest plant samples for analysis were taken. At harvest, grasses were cut by a small-plot combine harvester Wintersteiger Elite, each plot being harvested separately. The threshed seed was completely dried and purified using a laboratory cleaner Kamas. Determinations were made of grass seed yield, purity, germinating capacity, thousand-seed weight and number of fertile culms. The results were statistically analyzed using ANOVA and Tukey's Post Hoc test.

RESULTS

Phytotoxic to Kentucky bluegrass (growth retardation) were predominantly Arkem + CZ 600, Atlantis and Husar. Minor symptoms of phytotoxicity appeared after a double rate of Aurora Super SG. Seed yields in most treatments were comparable to the control,

In an intergeneric hybrid of festucoid type cv. Hykor minor symptoms of phytotoxicity were reported in stands treated with Aurora super SG and when double rates of Arrat and Callisto (chlorosis) were applied, especially on the first two dates of evaluation (14, 21 days after treatment). Growth retardation and colour change were observed in the stand on plots treated with Arkem + CZ 600. Seed

only with Atlantis and a double rate there was an insignificant (but important) yield reduction. When a double rate of Atlantis was applied, thousand seed weight was highly significantly (p=99 %) reduced. When double rates of Atlantis and Husar were applied, there was a highly significant reduction in the number of fertile culms. The results concerning Atlantis may, however, be affected by a later application of this preparation. Detailed results are given in Table 2.

As far as cocksfoot is concerned, minor symptoms of phytotoxicity were observed in stands treated with Aurora super SG (chloroses), especially on the first two dates of evaluation (14, 21 days after treatment). Growth retardation was observed in cocksfoot treated with Arkem + CZ 600, Atlantis and Husar. In cocksfoot treated with Atlantis reduced heading was observed. The results are shown in Table 3.

yields in all treatments were almost comparable with the control, only when double rates of Arrat, Arkem + CZ 600 and Callisto were applied, a yield reduction was recorded. A similar situation was evident in yield characteristics. With these treatments a smaller number of fertile culms and a smaller number of seeds per panicle were observed (Table 4).

In an intergeneric hybrid of loloid type cv. Lofa minor symptoms of phytotoxicity (growth retardation) were reported in stands treated with a double rate of Arrat, especially on the first two dates of evaluation (14, 21 days after treatment). A higher level of growth retardation was observed in stands treated

with Arkem + CZ 600. On plots treated with a double rate of Callisto chloroses developed. Seed yields in all test treatments (besides the treatment with a double rate of Aurora Super) were insignificantly higher compared with the standard-treated control. The results are shown in Table 5.

Table 2. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in Kentucky bluegrass cv. Slezanka in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.		Sign.	no.m ⁻²	Sign.
untreated		0	0	0	303.6	x	0.2599	x	605	X
Arrat	base	0	0	0	319.0	ns ¹⁾	0.2440	ns	570	ns
	2x	0	0	0	359.2	ns	0.2502	ns	583	ns
Aurora Super	base	0	0	0	305.3	ns	0.2566	ns	573	ns
	2x	0	8	10	325.4	ns	0.2420	ns	583	ns
Arkem + CZ 600	base	0	10	10	337.1	ns	0.2554	ns	590	ns
	2x	0	10	13	330.3	ns	0.2658	ns	566	ns
Atlantis	base	0	15	28	281.8	ns	0.2668	ns	533	ns
	2x	0	25	25	265.6	ns	0.2207	-- ²⁾	486	--
Husar	base	5	10	0	303.3	ns	0.2603	ns	536	ns
	2x	20	20	20	242.1	ns	0.2619	ns	476	--
Esteron	base	0	0	0	373.4	ns	0.2650	ns	600	ns
	2x	0	0	0	313.6	ns	0.2493	ns	570	ns

¹⁾ Not significantly different from control treatment. ²⁾ Significantly different from control treatment

Table 3. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in cocksfoot cv. Dana in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.		Sign.	no.m ⁻²	Sign.
untreated		0	0	0	601.5	x	1.1422	X	317	X
Arrat	base	0	0	0	605.6	ns	1.1206	ns	344	ns
	2x	0	0	0	484.6	ns	1.1856	ns	244	ns
Aurora Super	base	10	5	0	664.0	ns	1.1119	ns	335	ns
	2x	18	15	10	477.8	ns	1.1359	ns	274	ns
Arkem + CZ 600	base	0	5	5	687.5	ns	1.0994	ns	376	ns
	2x	13	8	5	418.5	ns	1.1306	ns	218	ns
Atlantis	base	0	15	20	600.3	ns	1.0310	-	337	ns
	2x	0	35	35	214.6	--	0.9123	--	136	--
Husar	base	10	10	10	649.7	ns	1.1200	ns	349	ns
	2x	20	20	20	395.9	ns	1.0569	ns	220	ns
Esteron	base	0	0	0	624.8	ns	1.1916	ns	341	ns
	2x	0	0	0	389.1	ns	1.1937	ns	214	ns

Table 4. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in festulolium cv. Hykor in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
untreated		0	0	0	901.6	X	2.6596	x	487	X
Arrat	base	0	0	0	911.4	Ns	2.6998	ns	479	ns
	2x	3	0	0	798.7	Ns	2.6495	ns	415	ns
Aurora Super	base	10	3	0	982.5	Ns	2.5498	ns	501	ns
	2x	18	10	0	901.6	Ns	2.7196	ns	449	ns
Arkem + CZ 600	base	10	13	20	916.3	Ns	2.7359	ns	484	ns
	2x	20	30	40	673.8	-	2.6399	ns	357	ns
Callisto	base	0	0	0	879.6	Ns	2.6739	ns	455	ns
	2x	13	10	0	776.7	Ns	2.6492	ns	414	ns
Esteron	base	0	0	0	889.4	Ns	2.6581	ns	475	ns
	2x	0	0	0	987.4	Ns	2.6551	ns	493	ns

Table 5. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in festulolium cv. Lofa in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
untreated		0	0	0	911.6	X	3.5730	x	842	x
Arrat	base	0	0	0	1056.4	Ns	3.5473	ns	763	ns
	2x	5	5	0	945.2	Ns	3.6549	ns	813	ns
Aurora Super	base	0	0	0	998.1	Ns	3.6276	ns	907	ns
	2x	0	0	0	815.9	Ns	3.5798	ns	790	ns
Arkem + CZ 600	base	5	13	10	1007.9	Ns	3.6990	ns	787	ns
	2x	5	13	20	1065.3	Ns	3.6617	ns	873	ns
Callisto	base	0	0	0	1134.8	Ns	3.6180	ns	807	ns
	2x	15	10	5	1065.8	Ns	3.4744	ns	793	ns
Esteron	base	0	0	0	921.2	Ns	3.6644	ns	810	ns
	2x	0	0	0	927.6	Ns	3.6148	ns	890	ns

In timothy grass phytotoxicity was observed when Arkem + CZ 600 (growth retardation), Callisto (chloroses), Husar (growth retardation) and Esteron („wilting“) were applied. As for yield, all the test preparations at a basic rate insignificantly surpassed the control. When a double rate of Aurora Super In meadow fescue phytotoxic were Arkem+CZ 600 and Husar. Phytotoxicity was manifested by growth retardation and reduced heading (a double rate of Husar resulted in complete destruction of the stand). Seed yield was comparable with the control when Arrat, Aurora Super SG and Esteron were applied. A lower yield depression was evident after the application of Callisto (especially a double rate). A significant seed yield decrease was recorded when a combination of Arkem+CZ 600 and Husar was applied. With a double

SG and Esteron were applied, an insignificant reduction in seed yield was reported. After the application of Esteron there was a highly significant reduction in the number of fertile culms which was compensated for by insignificantly greater thousand-seed weight and by the number of seeds per ear. The results are given in Table 6. rate of these preparations there was also a considerable decrease in the number of fertile tillers. (Table 7).

Red fescue did not display any symptoms of phytotoxicity after the application of the test herbicides. There were no significant deviations from the control in seed yield and yield characteristics either. The highest seed yield was obtained after treatment with Aurora super SG (Table 8).

Table 6. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in timothy cv. Sobol in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
untreated		0	0	0	779.3	x	0.4342	X	842	x
Arrat	base	0	0	0	850.6	ns	0.4323	Ns	677	ns
	2x	0	0	0	841.8	ns	0.4483	Ns	717	ns
Aurora Super	base	0	0	0	697.3	ns	0.4149	Ns	783	ns
	2x	10	10	5	854.6	ns	0.4063	Ns	710	ns
Arkem + CZ 600	base	18	13	10	893.8	ns	0.4288	Ns	690	ns
	2x	15	13	5	818.3	ns	0.4187	Ns	697	ns
Callisto	base	23	13	10	827.1	ns	0.3983	Ns	773	ns
	2x	8	5	5	850.2	ns	0.4250	Ns	893	ns
Husar	base	25	28	15	860.4	ns	0.4020	Ns	937	ns
	2x	5	8	8	869.8	ns	0.4115	Ns	740	ns
Esteron	base	5	8	8	869.8	ns	0.4838	*	623	--
	2x	10	13	10	740.9	ns	0.4771	Ns	633	--

Table 7. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in meadow fescue cv. Rožnovská in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
untreated		0	0	0	898.2	x	2.4765	X	666.7	X
Arrat	base	0	0	0	917.8	ns	2.3948	Ns	653.3	ns
	2x	0	0	0	916.3	ns	2.4655	Ns	663.3	ns
Aurora Super	base	0	0	0	943.3	ns	2.4641	Ns	856.7	ns
	2x	0	0	0	926.1	ns	2.2623	Ns	786.7	ns
Arkem + CZ 600	base	20	25	25	752.2	ns	2.5691	Ns	836.7	ns
	2x	28	55	75	225.4	--	2.3985	Ns	310.0	--
Callisto	base	0	0	0	857.5	ns	2.2408	-	880.0	ns
	2x	0	0	0	732.6	ns	2.3805	Ns	796.7	ns
Husar	base	23	45	60	580.7	ns	2.5806	Ns	593.3	ns
	2x	35	50	90	0.0	--	0.0000	--	0.0	--
Esteron	base	0	0	0	994.7	ns	2.4558	Ns	956.7	*
	2x	0	0	0	1037.8	ns	3.5168	Ns	726.7	ns

Table 8. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in red fescue cv. Tagera in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
untreated		0	0	0	973.6	x	1.2888	X	1274	X
Arrat	base	0	0	0	999.1	ns	1.3359	ns	1095	ns
	2x	0	0	0	915.3	ns	1.2868	ns	1105	ns
Aurora Super	base	0	0	0	1025.6	ns	1.2927	ns	1067	ns
	2x	0	0	0	1131.9	ns	1.3256	ns	1110	ns

Table 9. Effect of selected herbicides on injury, seed yield, thousand seed weight and number of fertile tillers in perennial ryegrass cv. Olaf in the first harvest year.

Treatment	dose	injury in %			seed yield		TSW		fertile tillers	
		14 d	21 d	28 d	kg ha ⁻¹	Sign.	Sign.	no.m ⁻²	Sign.	
Untreated		0	0	0	866,8	x	2.0449	X	1528	X
Arrat	base	10	0	0	963,3	ns	2.0323	ns	1747	ns
	2x	0	0	0	851,9	ns	1.9481	ns	1663	ns
Aurora Super	base	0	0	0	804,9	ns	2.0149	ns	1600	ns
	2x	0	0	0	838,0	ns	1.9889	ns	1907	ns

The phytotoxicity of the test herbicides in perennial ryegrass was almost zero, only with a double rate of Arrat slight chloroses were observed within 14 days of treatment. After that time all symptoms disappeared. However, compared with the control, seed yield was insignificantly lower. Yield characteristics did not show any deviations from the control (Table 9).

DISCUSSION

Good protection of grass seed stands against weeds as a necessary prerequisite for stand certification and satisfactory grass seed yields require broad-spectrum herbicides with selectivity to particular grass species. According to the preliminary results reasonably good herbicides that might be applied to grasses for seed are Arrat, Aurora super SG and Esteron, which showed very good selectivity in all test grass species. Similar results about the selectivity of carfentrazone-ethyl were obtained by Affeldt *et al.* (2003), who reported low phytotoxicity of the active substance alone and in combination with some other herbicidal substances especially when applied at the stage of tillering. Relatively good results were also produced by Husar and Atlantis in Kentucky bluegrass where they are very

important for the control of annual bluegrass. Higher phytotoxicity of these preparations was found in cocksfoot. The preparation Husar showed also good results in timothy grass but very high phytotoxicity to meadow fescue.

Despite some symptoms of phytotoxicity, quite promising seems to be Callisto 480 SC in intergeneric hybrids and timothy grass. Besides the spectrum of dicotyledonous weeds it controls some troublesome warm-loving grassy weeds.

One-year tests of selectivity of herbicides in grasses grown for seed produced some positive results and prospect of controlling weeds which have become quite widespread in grass seed stands in the Czech Republic in recent years (Cagaš *et al.* 2006). To confirm these results it is necessary to verify testing of selectivity in the following years with the aim of excluding the effect of the year of cultivation.

ACKNOWLEDGEMENTS

These experiments were carried out only thanks to support provided by the Association of Grass and Clover Seed Growers.

LITERATURE

- Affeldt, R.P., Cole, C.M., Colquhoun, J.B., Mallory-Smith, C.A. & Brewster, B.D. 2003. Tolerance of fall-planted seedling grasses to carfentrazone tank-mixes. *In*: W. C. Young III (ed.) Seed production research at Oregon State University USDA-Ars cooperating. Department of Crop and Soil Science Ext/CrS 123, 3/04: 31-36.
- Cagaš, B., Macháč, J., Frydrych, J. & Macháč R. 2006. Occurrence of biotic harmful agents in Czech grass seed production (1995 - 2004). *Plant Protection Science* 42(2): 58-65.

Control of grass weeds in seed production of *Phleum pratense*, *Poa pratensis* and *Festuca rubra*

Kirsten S. Tørresen¹⁾, John Ingar Øverland²⁾ & Trygve S. Aamlid³⁾

¹⁾The Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, NO-1432 Ås, Norway

²⁾Vestfold Extension Group, Gjennestadtunet, NO-3160 Stokke, Norway

³⁾The Norwegian Institute for Agricultural and Environmental Research, Arable Crops Division, Landvik, Reddalsveien 215, NO-4886 Grimstad, Norway

kirsten.torresen@bioforsk.no

ABSTRACT

The control of *Alopecurus geniculatus*, *Poa annua* and *Poa trivialis* using iodosulfuron (Hussar/Hussar OD) was investigated in field experiments in the seed harvest year in timothy (*Phleum pratense*), and in the sowing year and seed harvest year in smooth meadow-grass (*Poa pratensis*) and red fescue (*Festuca rubra*). Iodosulfuron (10 g a.i. ha⁻¹) usually had good effect on *Poa trivialis* and *Alopecurus geniculatus*. As for *Poa annua* the effect on seed contamination was better than on weed coverage in the field. Early application improved weed control in the seed harvest years, and iodosulfuron was shown to perform well at low temperatures. The herbicide often delayed timothy development, but caused seed yield reduction only in two out of eight experiments, both with moist soil at treatment. The visual damage increased with increasing rates and use of additives (alcoholetoxyolate or rape oil). A questionnaire investigation among timothy growers in 2004 showed that farmers using Hussar had 43% lower contamination of *P. trivialis* in cleaned seed yield and 20% lower yield than the farmers not using Hussar. In some trials in timothy and smooth meadow-grass, the new formulation Hussar OD gave slightly more damage than the old formulation Hussar. While well-established timothy crops seem to tolerate some visual damage without seed yield reduction, the risk of yield reduction in first year crops can usually be avoided by splitting the application into 5 g a.i. ha⁻¹ at 14 days intervals. In smooth meadow-grass and red fescue established without cover crop, repeated applications of 5 g a.i. ha⁻¹ in the sowing year resulted in better control of *P. annua* and significant seed yield improvements compared to application only in the seed harvest year. Both Hussar and Hussar OD have off-label approvals for members of the Norwegian Seed Growers Association.

Key words: *Alopecurus geniculatus*, iodosulfuron, *Poa annua*, *Poa trivialis*, red fescue, smooth meadow-grass, timothy.

INTRODUCTION

The grass weeds species *Poa trivialis* (rough meadow-grass), *Poa annua* (annual meadow-grass) and *Alopecurus geniculatus* (marsh foxtail) can cause problems in grass seed production in the year of sowing and in the harvest year(s) by competing with the crop for light, water and nutrients, and by contaminating the seed yield. Problems with contamination occur especially in smooth meadow-grass (*Poa pratensis*) where these weeds are difficult to clean out.

We have investigated the efficacy of Hussar (50 g iodosulfuron kg⁻¹) and the newer formulation Hussar OD (100 g iodosulfuron L⁻¹) against grass weeds, and the selectivity of these products in timothy (*Phleum pratense*), smooth meadow-grass, red fescue (*Festuca rubra*), sheep's fescue (*Festuca ovina*) and smooth brome grass (*Bromus inermis*). Results of grass weed control in timothy, smooth meadow-grass and red fescue are presented here. Both Hussar and Hussar OD are approved off-label in grass seed production in Norway.

In 2004 Hussar was used on dispensation in the seed harvest year in timothy and smooth meadow-grass. As many farmers experienced damage in timothy, a questionnaire was sent to timothy seed growers in Vestfold county. Some results from this survey are also presented.

MATERIALS AND METHODS

Hussar was tested in the seed harvest year in timothy, smooth meadow-grass and red fescue from 2001 to 2006. Hussar OD was included in 2005 and 2006. The products were tested at various rates (5-20 g a.i. ha⁻¹) in early spring when growth had started and/or 14 days later, with or without additives. An intermediate spraying time was included in timothy in 2005 and 2006. In smooth meadow-grass and red fescue, the products were also tested in the sowing year (no cover crop), either in one application with a large dose or in split application at 2 or 4 weeks interval. In some trials, the products were sprayed both in the sowing year and the harvest year.

The field trials were established according to randomised complete block designs with three replicates. The trials were laid out by the farmers extension service groups in the seed production districts and by Bioforsk Landvik. All treatments were performed with the Nor-sprayer (a knapsack sprayer driven by compressed air), XR TeeJet 11002 nozzles, a pressure of 1.5-2 bar and a spraying volume of 250 L ha⁻¹. Temperature and relative humidity were monitored in the crop, and soil moisture assessed (1=very dry, 5=very moist) at 0-2 and 2-10 cm soil depth at the time of spraying. Per cent cover of seed crop and weeds was recorded, and visual crop damage assessed relatively to untreated control plots (no damage=0%). The trials were combined directly with plot combines. Plot yields were cleaned lightly in order not to eliminate differences among treatments. The content of grass weeds in the yield was analysed by Kimen Sävarelaboratoriet AS for timothy and smooth meadow-grass and Bioforsk Landvik for red fescue. All seed yields presented in this paper from the field trials have been corrected to 100 % purity and 12 % seed moisture content.

The results were analysed by ANOVA, and LSD_{5%} calculated to detect differences between treatments. The trials in timothy were examined closely by correlation

analyses, to find explanations for variations in crop damage, efficacy against weeds, and yield results. The independent variables studied in these analyses were: temperature, relative humidity, and soil moisture at treatment; mean temperature and sum precipitation 7 d before and 7 d after treatment (taken from the nearest weather station); and time of application (expressed as days after 1 April). All statistical analyses were performed by SAS (SAS Institute Inc., 1988).

The questionnaire investigation was performed in cooperation with Vestfold Extension Group and Bioforsk Landvik in 2004. One hundred timothy growers in Vestfold were asked for conditions around spraying with Hussar, use of other pesticides, cropping practice and yield level. Seventy-five of the farmers replied. Data on seed yield and weed contamination were supplied by the seed companies Felleskjøpet Agri and Strand Brænderi AS.

RESULTS AND DISCUSSION

Timothy

While Hussar also controlled a number of broadleaved species such as *Tripleurospermum inodorum*, focus in this paper will be on *Poa trivialis* which is the most important grass weed in timothy seed production in Norway. The trials from 2001 to 2004 showed better control of this weed, but also more damage to the seed crop, with increasing rates of Hussar and with the addition of alcohotoxylate. There was also a tendency for early applications to control *P. trivialis* better than later ones (data not shown).

In 2005, one trial was carried out in a first year crop, and one trial in a third year crop of the same variety and on the same farm (Table 1). Both crops had 7-10 % *P. trivialis*, but unlike in the previous trials, there was no consistent effect of application rate, additive (in this case Renol) or delayed application on weed control. Visual damage to the timothy stand was more evident in the first than in the third year crop, but this was not correlated with seed yield which was generally higher on sprayed than on non-sprayed plots. This means that timothy can tolerate some damage without a loss in seed yield. The yield data in Table 1 may suggest

that Hussar ought to be applied earlier in old than in newly established seed crops.

In 2006, two trials were conducted, both in first year crops with virtually no *P. trivialis*. The data in Table 1 confirm that such crops may benefit from a one week delay in the application of Hussar. In these trials, Hussar OD seemed to be slightly tougher to the timothy than the old formulation Hussar given at the same rate and with or without the same additive.

By mistake, split application was not included in the first year crop in 2005. In the third year

crop in 2005, split application at reduced rates gave slightly poorer control of *P. trivialis*, slightly more damage to the timothy seed crop, and exactly the same yield as giving the full rate early. On average for the two trials in first year crops in 2006, damage to the timothy stand was the same, but seed yield slightly higher after split application. All taken together, split application may be a safer alternative than spraying the full rate early in first year crops. Then the second application can be cancelled if the first application shows a good effect.

Table 1. Effect of Hussar and Hussar OD applied in the seed harvest years 2005 and 2006 on % cover of *P. trivialis*, % damage to the timothy seed crop and seed yield. Spraying time: A=spring, timothy 5-10 cm, B=7 d after A, C=14 d after A. "-"=missing values.

2005 Herbicide g a.i. ha ⁻¹	Ley year	Un- treated	Hussar			Hussar OD			Hussar			LSD _{5%}
			0	10	10 ¹⁾	5	10	10 ¹⁾	15	10 ¹⁾	10 ¹⁾	
Spraying time		-	A	A	A	A	A	A	B	C	A + C	
% cover of <i>P. trivialis</i>	1	7	1	2	-	1	3	2	5	0	-	5.0
	3	10	3	3	3	2	3	2	7	3	5	5.4
% damage to timothy crop	1	0	5	15	-	10	10	17	40	25	-	9.3
	3	0	0	3	0	3	4	7	10	10	10	5.1
Seed yield, kg ha ⁻¹	1	597	688	573	-	633	595	659	701	820	-	125
	3	568	585	691	524	660	728	674	610	608	691	139

2006 Herbicide g a.i. ha ⁻¹	No. of trials	Un- treated	Hussar			Hussar OD			Hussar			LSD _{5%}
			0	10	10 ¹⁾	5	10	10 ¹⁾	15	10 ¹⁾	10 ¹⁾	
Spraying time		-	A	A	A	A	A	A	B	C	A + C	
% damage to timothy crop	1	0	2	7	0	5	15	12	7	35	15	-
Seed yield, kg ha ⁻¹	2	787	738	769	779	711	750	748	921	808	800	160

¹⁾Added Renol (rape oil), 0.5 L ha⁻¹.

Analyses of correlation based on eight trials with timothy from 2001 to 2006 showed that the seed yield on unsprayed control plots decreased significantly with increasing infestation of *P. trivialis* ($r=-0.543$, $p=0.01$, $n=21$). Conversely, there was a significant positive correlation between *P. trivialis* infestation on unsprayed plots and the relative seed yield increase due to spraying with Hussar / Hussar OD ($r=0.490$, $p=0.02$, $n=21$). On the other hand, no significant correlation could be established between the visual damage to the timothy

stand and seed yield on sprayed relative to unsprayed plots.

Among the eight trials, seed yield was reduced after the early application of Hussar only in two trials. In those trials, the soil was characterised as moist (4 on a scale from 1 to 5). Correlation analyses including all trials also showed a tendency to negative impact of soil moisture at treatment on the seed yield on sprayed relative to unsprayed plots ($r=-0.37$ - -0.42 , $p=0.05$ - 0.09 , $n=21$). This indicates that if the soil is moist and growing conditions good at spraying, a reduced rate

(5 g a.i ha⁻¹) should be used, or the application should be split into two applications at reduced rates. Hussar is acting through the leaves, but can have a small soil action. On the label of Hussar it is stated that Hussar should not be used when the soil is waterlogged.

The efficacy of Hussar/Hussar OD against *P. trivialis* (when present) was negatively correlated both with the temperature at spraying ($r=-0.79$, $p=0.004$, $n=11$, shown as regression in Fig. 1) and the temperature seven days before spraying ($r=-0.63$, $p=0.04$, $n=11$). This means that cold weather prior to and at spraying enhanced the effect of Hussar/Hussar OD against *P. trivialis*. As the temperature at spraying was not related to

spraying time given as days after 1 April, this seems to be a true temperature effect, and not an effect of growth stage at spraying.

The questionnaire investigation in 2004 showed that growers using Hussar, on average, had lower seed yield than those not using Hussar (Table 2). While Hussar (7.5-10 g a.i. ha⁻¹) reduced the seed lot contamination of *P. trivialis* and *A. geniculatus*, the number of *P. annua* seeds increased. As *Poa annua* normally encroaches open areas, this was probably due to less competition, primarily for light, from the damaged timothy seed crop. Other trials have also shown Hussar / Hussar OD to be less efficient against *P. annua* than against *P. trivialis* and *A. geniculatus*.

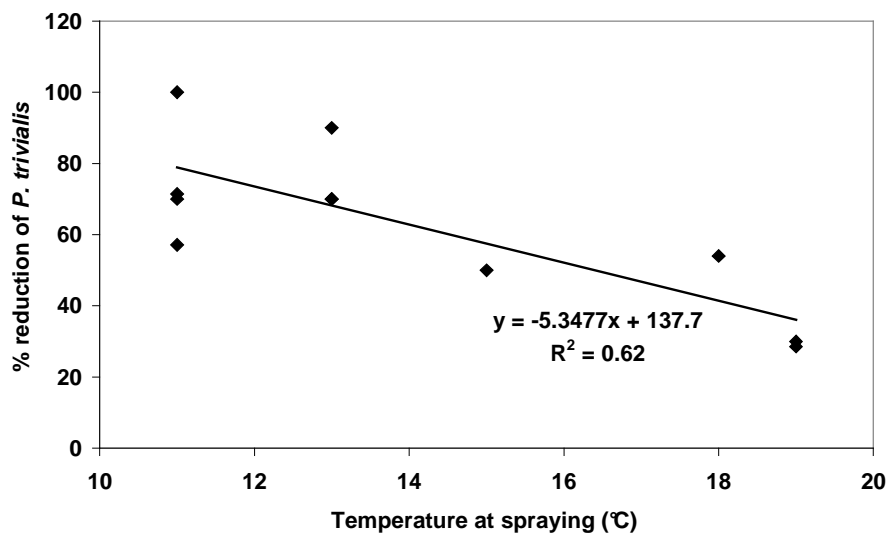


Fig. 1. Effect of by temperature at spraying on per cent reduction in *Poa trivialis* coverage after application of Hussar or Hussar OD (10 g iodosulfuron ha⁻¹ + additive).

Table 2. Average seed yield and weed contamination of sprayed and non-sprayed timothy seed crops in Vestfold County in 2004. Number of seed lots indicated in parentheses.

Treatment with Hussar	2004			
	Yield (kg ha ⁻¹)	No. of seeds per kg cleaned seed yield		
		<i>P. trivialis</i>	<i>P. annua</i>	<i>A. geniculatus</i>
No	905 (32)	516 (31)	129 (31)	290 (31)
Yes	726 (28)	293 (28)	286 (28)	186 (28)

Smooth meadow-grass and red fescue
Minimal differences between Hussar-formulations were found in seed crops of smooth meadow-grass and red fescue after spraying in the seed harvest year. Hussar and

Hussar OD were both effective against *A. geniculatus*, while the effect on *P. annua* was variable with respect to field coverage, but better with respect to seed purity (data not shown).

The trials with application of Hussar both in the sowing year and the seed harvest year demonstrated the importance of *Poa annua* control in the sowing year for first year seed yields of smooth meadow grass (Table 3) and red fescue (Table 4). In both species the best seed purity was obtained after repeated applications of 5 g a.i. ha⁻¹ in the sowing year combined with 10 g a.i. ha⁻¹ in the harvest year. Adding alcholetoxylate or Renol to the

old Hussar formulation made little difference in smooth meadow grass (Table 3), and replacement of Hussar with Hussar OD made little difference in red fescue (Table 4). We have, however, recent data showing that Hussar or Hussar OD should not be applied to smooth meadow grass in the sowing year unless field emergence is even and seedlings have reached the two-leaf stage.

Table 3. Effect of Hussar in the sowing year and seed harvest year on seed yield and % contamination of grass weeds in lightly cleaned seed yield of smooth meadow-grass 2004-2005. Ariane S (clopyralid + fluoxypyr + MCPA) was used to control broadleaved weeds. Spraying times: A=broadleaved weeds 2-4 leaves, crop 2-3 leaves; B=1 month after A, C=1 month after B; and D=in the spring the seed harvest year, crop height 5-8 cm.

Herbicide g a.i. ha ⁻¹	No. of trials	Ariane S	Ariane S + Hussar	Hussar		
		500	500 + 10-15 ¹⁾	5 ¹⁾ + 5 ¹⁾ + 5 ¹⁾	5 ²⁾ + 5 ²⁾ + 5 ²⁾	5 ¹⁾ + 5 ¹⁾ + 5 ¹⁾ + 10 ¹⁾
Spraying time		A	A+D	A+B+C	A+B+C	A+B+C+D
Seed yield, kg ha ⁻¹	2	519	438	882	863	841
% <i>A. geniculatus</i>	1	4.5	1.4	0.2	0	0.1
% <i>P. annua</i>	2	2.7	1.0	0.9	1.1	0.4
% <i>P. trivialis</i>	1	1	0,3	0.2	0.2	0.2

¹⁾ Added alcholetoxylate, 0.05% of spraying volume.

²⁾ Added Renol (rape oil), 0.5 L ha⁻¹.

Table 4. Effect of Hussar and Hussar OD in the sowing year and first seed harvest year on seed yield and % contamination of grass weeds in lightly cleaned seed yield of red fescue 2005-2006. Ariane S was used to control broadleaved weeds. Spraying times: A=1 month after sowing, B=2 weeks after A, C=spring in the seed harvest year, crop height 5-8 cm.

Herbicide, g a.i. ha ⁻¹	No. of trials	Ariane S	Hussar	Hussar OD				Ariane S + Hussar OD	LSD 5%
		500	10 g ¹⁾	10	10 ¹⁾	5 + 5	5 + 5 + 10	500 + 10	
Spraying time		A	A	A	A	A + B	A + B + C	A + C	
Seed yield, kg ha ⁻¹	1	265	746	797	810	707	860	324	141
% <i>P. Annua</i>	1	19.7	8.6	7.9	5.5	6.0	5.0	13.9	-
% <i>A. Geniculatus</i>	1	1.7	0.0	0.4	0.0	0.5	0.0	4.7	-

¹⁾ Added Renol, 0.5 L ha⁻¹

ACKNOWLEDGEMENTS

These trials were partly performed on assignment from the Norwegian Food Safety Authority. The trials were funded also by the

Norwegian Seed Growers Association, the Farmers' Extension Groups and The Norwegian Institute for Agricultural and Environmental Research.

LITERATURE

SAS Institute Inc. 1988. SAS/STAT User's guide. Release 6.03 Edition. SAS Institute Inc., Cary, NC.

Seed production of *Koeleria gracilis* Pers.

Ants Bender & Rene Aavola

Jõgeva Plant Breeding Institute, 48 309, Jõgeva, Estonia

ants.bender@jpbi.ee

ABSTRACT

Crested hairgrass (*Koeleria gracilis* Pers., syn. *K. macrantha* (Ledeb.) J. A. Schultes) is a tussocky, undersized winter grass with greyish narrow leaves. The species is used for lawns in Estonia. The variety 'Ilo' was released in 1997. Agrotechnology of its seed production was studied for 7 years. It is reasonable to sow a seed field without a cover crop at the first chance in spring (May). Drill space can be either 45 (seeding rate 2.5 kg ha⁻¹) or 15 cm (5.0 kg ha⁻¹). Crested hairgrass has slow initial development; it takes 10-20 days from sowing to emergence. In the seeding year, the plants can cover the drill space of 45 cm during two months. In crop years it takes a month from the outset of growth in spring. *Koeleria* is unable to completely cover a 60 cm drill space leaving the field open to weeds for the entire season. The species has a good lodging resistance. Height of reproductive tillers ranges between 48-62 and 71-91 cm in a droughty and rainy summer, respectively. Nitrogen applications of 35 kg ha⁻¹ in late summer after removal of residue and 70 kg ha⁻¹ in spring are sufficient. The plants bloom in late June, the seeds mature a month later. A seed yield of 600 kg ha⁻¹ can be obtained in the first crop year. The weight of 1000 seeds ranges from 0.230 to 0.281 g. The following sieves are appropriate: with round holes of 2.5 mm for preliminary seed cleaning, upper and lower sieve both with oblong holes of 0.65 and 0.45 mm, respectively. The machine must be equipped with an aspiration system.

Key words: drill space, nitrogen fertiliser, yield, sowing rate, sowing time

INTRODUCTION

Crested hairgrass (*Koeleria gracilis* Pers., syn. *K. macrantha* (Ledeb.) J. A. Schultes) is a tussocky, undersized winter grass with greyish narrow leaves. It is spread in Australia, Eurasia and North America (Dixon 2000). The northern edge of its area of adaptation hardly stretches till south-east Estonia (Eesti 1979). Jõgeva Plant Breeding Institute (PBI) has initiated the cultivation of this species for lawns and released a cv. 'Ilo' (Bender 1995). Breeding programs of crested hairgrass have been started in the Netherlands and Canada. Dutch cultivars 'Barkoel' and 'Barleria' are recommended for turf (Barenbrug 2000), Canadian cv. 'ARC Mountain' for forage (Woosaree *et al.* 2004). The species has been cultivated for a limited period of time. To our knowledge, seed production agrotechnics of the species have not been investigated before.

MATERIALS AND METHODS

Seed production agrotechnics of crested hairgrass cv. 'Ilo' was investigated in field trials at Jõgeva PBI in 2001-2006. Two series of trials were sown in a split-plot design in 2001 and 2003. We studied the impacts of sowing time and rate, drill space, timing and application rate of nitrogenous fertilizer on the seed yield. The trials were established to a fallow without a cover crop, in wide (45 cm) drill space, except for the trial to study that factor. Plots of 11.25 m² in size were replicated 4 times. Seed yield was measured from 6 m². The experiments were laid on calcareous cambisol (K₀), with pH_{KCl} 6.1, humus concentration 2.1 %, total nitrogen (N) 0.13 %, P 230, K 229, Ca 1550 and Mg 77 mg kg⁻¹ soil. The amounts of N, P₂O₅ and K₂O applied before sowing were 70, 44 and 80 kg ha⁻¹, respectively. P and K were applied with the complex fertilizer Skalsa, N as ammonium nitrate. In the crop years, 70 kg N ha⁻¹ was applied after the outset of plant growth in

spring, unless other treatments were foreseen. A sowing rate of 5 kg ha⁻¹ was regarded as a basic value.

The following machinery was used in the process of experimentation: Seed drill Hege 90-1 (wide drill space) and Hege 80 (narrow space), mineral fertiliser distributor Hege 33, sprayer Hege Tecnomat for pest control. The seeds were directly harvested at full maturity with a combine Hege 125 C. The harvested mass was ventilated at a dryer and cleaned with a laboratory seed cleaner of Kamas-Westrup company. Seed quality was analysed in the lab of Agricultural Research Centre. Various treatments did not affect the purity and germination of seeds. Even if these differences occurred in the field, the seeds were homogenized by harvesting and subsequent cleaning, when rising airflow and sieves removed lighter and smaller seeds and chaff. Statistical data analyses were performed using ANOVA procedure.

RESULTS AND DISCUSSION

Sowing time

Seed fields of low grasses with small seeds and slow initial development are established in Estonia before summer without a cover crop. Our experimental data (Table 1) affirm the same for crested hairgrass. Sowing at the first chance in spring produced the highest seed yield. In the droughty year 2001 later sowings (24 May and 10 June) formed stands where the number of vegetative shoots at the end of season equalled those of the first sowing time. However, the number of reproductive tillers was markedly less which significantly reduced the seed yield compared

with the earlier sowing. Sowing on 24 June 2001 suffered from drought and formed a thin stand, which did not produce considerable seed yield in the first crop year. However, it significantly outyielded the other treatments in the two subsequent years. Treatments that were more productive in the extraordinarily droughty year 2002 formed less reproductive shoots next year and the seed yields were inversely proportional to the ones obtained in the previous year. Spring 2003 was rainy, and the sowings did not suffer from drought. However, the plots seeded in June also produced significantly lower seed yields next year in this trial compared with the plots seeded earlier.

As a mean of two experiments and 8 crop years, the plots seeded on 24 May produced the most seed. The superiority was mainly caused by the higher seed yield in 2003. The gain in the seed yield (4.9%) in relation to the first sowing time was not significant.

Sowing rate

The first experiment to investigate the proper sowing rates was sown in late June 2001. The field suffered from drought. All seeding rates formed even, but very thin stands that did not yield considerable seed in the first crop year (Table 2). As a result of abundant tillering, the density of the stands had improved by the second crop year and they produced superior, quite uniform seed yields. Another trial to examine the sowing rates was seeded on 15 June 2003. A variant with even more reduced sowing rate (2.5 kg ha⁻¹) was added to this experiment. After favorable and humid season, this treatment turned out to be productive in the subsequent year.

Table 1. The impact of sowing time on the seed yield of crested hairgrass, kg ha⁻¹.

Sowing date	Expt. seeded in 2001					Expt. seeded in 2003			Total	Relative, %
	2002	2003	2004	2005	2006	2004	2005	2006		
10 May	300.5	157.2	247.6	215.7	135.3	437.7	151.8	183.9	1694.4	100.0
24 May	270.4	254.7	205.4	228.3	117.0	437.5	223.6	156.7	1776.6	104.9
10 June	253.0	324.5	203.0	178.3	89.5	362.6	210.1	123.9	1655.4	97.7
24 June	27.0	390.1	272.4	189.4	80.9	317.4	164.6	116.6	1477.5	87.2
LSD 5%	21.1	30.4	45.2	11.4	11.0	39.5	14.5	18.1	95.9	

Table 2. The impact of sowing rate on the seed yield of crested hairgrass, kg ha⁻¹.

Sowing rate, kg ha ⁻¹	Expt. seeded in 2001					Expt. seeded in 2003			Total	Relative, %
	2002	2003	2004	2005	2006	2004	2005	2006		
2.50	-	-	-	-	-	486.8	205.3	191.3	692.1	
3.75	11.3	545.8	391.4	163.3	146.3	482.0	180.4	189.8	1964.0	100.0
5.00	10.4	530.4	376.4	226.6	211.7	464.7	177.9	177.2	1963.6	100.0
6.25	12.2	545.0	341.9	260.1	171.2	446.3	182.0	152.4	1939.9	98.8
LSD 5%	6.5	78.4	29.3	14.1	15.7	68.3	14.1	12.6	131.6	

Table 3. The impact of drill space on the seed yield of crested hairgrass, kg ha⁻¹.

Drill space, cm	Expt. seeded in 2001					Expt. seeded in 2003			Total	Relative, %
	2002	2003	2004	2005	2006	2004	2005	2006		
15	11.8	574.6	326.1	190.1	114.7	517.6	174.8	129.5	1924.5	110.9
45	10.5	435.8	381.7	163.8	139.3	388.2	159.4	195.7	1735.1	100.0
60	9.7	322.5	289.2	160.6	133.9	255.3	177.8	138.3	1353.4	78.0
LSD 5%	4.2	64.7	44.2	18.9	21.4	39.2	9.2	16.1	124.6	

The data from two trials and eight harvest years indicate that the sowing rate has minor impact on the seed yield. At early sowing the plants of crested hairgrass conform the density of stands through tillering, provided that nutrient and humidity levels are adequate.

Drill space

Seed fields of low grasses in Estonia are usually established with wide drill space of 45 cm. In our experiments it was also the standard with which we compared narrow (15 cm) and wider (60 cm) drill space. In a trial seeded in late June 2001, like before mentioned, the stands did not produce considerable seed yield in the first crop year (Table 3). Seed yield differences caused by various drill spaces appeared since the second crop year, when narrow space with a sowing rate of 8 kg ha⁻¹ produced a higher yield and greater number of reproductive shoots per surface unit than stands with a wide drill space. The second sowing in 2003 affirmed the superiority of narrow drill space already in the first crop year. The data summarized over two trials and eight harvest years

indicate that seed yield produced with 15 cm drill space was, on average, 10.9 % higher than with the standard 45 cm space. The advantage of narrow space was greater in the first crop years and declined later.

Nitrogen fertiliser

Crested hairgrass is a winter- type species. Its seed yield is dependent on tillering in late summer, and this tillering should be favoured by fertilisation. Spring dressing influences the seed yield through the number of seeds per panicle and 1000 seed weight. The aim of our experiment was to explore, whether nitrogenous fertiliser equal to 35 kg N ha⁻¹ added after residue removal in late summer and 70 kg N ha⁻¹ applied in spring are sufficient for crested hairgrass to attain its maximum seed yield. Nitrogen rates exceeding standard applications for spring and summer did not increase the seed yield in our trials (Table 4). Single dressing of 105 kg N ha⁻¹ in spring caused sporadic lodging in rainy seasons. This led to seed loss during harvesting, which should be avoided. Increasing the fertiliser rate from 35 to 70 in late summer increased seed yield only in the droughty year 2006.

Table 4. The impact of the rate and application time of nitrogenous fertilizer on the seed yield of crested hairgrass, kg ha⁻¹.

N, kg ha ⁻¹ in late summer + spring	Expt. seeded in 2001					Expt. seeded in 2003			Total	Relative, %
	2002	2003	2004	2005	2006	2004	2005	2006		
35+70	5.7	619.5	291.6	189.5	127.0	495.8	189.4	132.9	1924.4	100.0
70+70	8.6	575.6	293.2	170.3	185.5	486.1	186.7	197.2	1917.7	99.7
35+105	7.3	555.5	286.5	153.3	146.5	459.4	192.8	138.3	1793.1	93.2
0+105	6.7	528.1	263.8	180.4	116.3	438.4	203.7	97.3	1718.4	89.3
LSD 5%	3.0	20.1	15.6	11.4	19.4	43.8	8.1	10.2	80.2	

CONCLUSION

Our data collected during eight harvest years suggest that seed field of crested hairgrass must be sown on the first chance in spring. This assures maximum yield in the first crop year. When sown in late June or later, the seed yield of the first crop year will be poor. However, in such cropless years the stand of crested hairgrass does not tiller above critical density and gives normal seed yield next year. Because of extensive care and input need in cropless year, late sowing can not be recommended. Hence control of rooty weeds must be done a year before sowing a new field.

In case of favourable growing conditions and if a precise seed drill is used, 2.5 kg ha⁻¹ of crested hairgrass seed of 100% sowing value suffices for sowing a field with wide drill space. Enhanced sowing rate will not assure higher yield, on the contrary - could even decrease it. Sufficient sowing rate in case of narrow drill space is 5 kg ha⁻¹.

Seed field can be established with wide (45 cm) or narrow (15 cm) drill space. The latter way guarantees higher seed yield in the first years, but the grower will face weed control problems. There is a trend to restrict pesticide use. Interest has been taken in mechanical weed control methods that require wider drill space (Lund-Kristensen *et al.* 2000, Boelt *et al.* 2002, Boelt 2003). In organic seed production, only wide drill space is applicable. Drill space of 45 cm turned out to be proper in our trials. This enables the use of a tractor with lesser traction for mechanical weed control without treading the stand. Wider drill space (60 cm) enables the use of more powerful tractors for weed

control between the drills, but there are two concurrent adverse phenomena: 1) plant foliage can not cover the drill space and risk of weed infestation remains throughout the summer; 2) in case of lodging the foliage does not uphold reproductive shoots and the panicles sink to the ground between the drills. In the event of rain the seeds germinate in the field if the panicles are in contact with soil. The combine harvester does not pick them up and yield loss is considerable.

Crested hairgrass is a species with rather good lodging resistance. Length of its reproductive shoots ranged between 48-62 cm in droughty and between 71-91 cm in rainy summers. Sufficient N rates for the fertilisation of a seed field are 70 kg ha⁻¹ in spring and 35 kg ha⁻¹ in summer after removal of the stubble.

Powdery mildew (*Erysiphe graminis* DC.) infects crested hairgrass (Dixon 2000). Our data show that the seed yield was more dependent on agrotechnics and weather conditions than on negligible infection of the leaves with powdery mildew (Sooväli & Bender 2006), therefore fungicide treatments were not required. Yet, it is appropriate to pay attention to pest control especially with respect to silver top in the second and subsequent crop years.

Crested hairgrass has slow initial development: the seedlings emerge 10-20 days after sowing. In the sowing year the foliage covers, during two months after emergence, the ground between drills lying 45 cm apart. In the crop year it takes one month from the outset of growth in spring. The stand blooms in the second half of June, seed ripens in the second half of July or the first days of August. Seed yield reaches up to

600 kg ha⁻¹ in the first crop year, 1000 seed weight remains between 0.230-0.281 g, and germination ranges from 80 to 98 %. In our experiments the number of reproductive shoots per surface unit influenced the 1000 seed weight - the more reproductive shoots per unit area the higher the seed yield, but the lower the 1000 seed weight.

The profitability of a stand is maintained for 2-3 years. Proper sieves for the final seed cleaning are the following: a sieve with round holes of 2.5 mm for pre-cleaning, both upper and under sieves with oblong holes of 0.65 mm and 0.45 mm, respectively. The machine

must be equipped with an aspiration system that can be adjusted according to visual survey. The germination of the seeds of crested hairgrass has not dropped below 80 % during the course of a 4 year period in an unconditioned warehouse.

ACKNOWLEDGEMENTS

The authors thank Estonian Science Foundation for allocating a grant no. 4733 for financing this study.

LITERATURE

- Barenbrug. 2000. Range of varieties 2000. Barenbrug, Oosterhout, Netherlands.
- Bender, A. 1995. Breeding and improvement of grass and legume varieties after 1944 at Jõgeva. Transactions of Jõgeva Plant Breeding Institute 'Plant breeding and seed production' 7: 206-216. (In Estonian, with English abstract).
- Boelt, B. 2003. Organic forage seed production. *In: Proceedings of fifth International Herbage Seed Conference, 23-26 November 2003, Gotton, Australia.* pp. 43-47.
- Boelt, B., Deleuran L. & Gislum R. 2002. Organic seed production in Denmark. Newsletter of the International Herbage Seed Production Research Group 34: 3-5.
- Dixon, J.M. 2000. *Koeleria macrantha* (Ledeb.) Schultes (*K. alpigena* Domin, *K. cristata* (L.) Pers. Pro parte, *K. gracilis* Pers., *K. albescens* auct. Non DC.). *Journal of Ecology* 88: 709-726.
- Eesti NSV flora XI. 1979. Tallinn, 567 pp.
- Lund-Kristensen, J., Jensen, M.T. & Grønbæk, O. 2000. Organic production of grass and clover seed in Denmark - a new challenge to the seed industry. *In: Proceedings of the 18th General Meeting of the European Grassland Federation, Denmark.* pp. 539-541.
- Sooväli, P. & Bender, A. 2006. The occurrence of powdery mildew on crested hairgrass in different growing conditions. *Agronomy Research* 4: 385-388.
- Woosaree, J., Acharya, S.N. & Darroch, B.A. 2004. ARC Mountain View June Grass. *Canadian Journal of Plant Science* 84: 245-247.

Importance of Ecological Conditions for Herbage Seed Production in Turkey

Ilknur Ayan¹⁾, Zeki Mut²⁾ & Zeki Acar¹⁾

¹⁾Ondokuz Mayıs University, Faculty of Agriculture, Department of Field Crops, 55139 Kurupelit - Samsun, Turkey

²⁾Ondokuz Mayıs University, Vocational School of Bafra, Samsun, Turkey

ilknuray@omu.edu.tr

ABSTRACT

A serious problem exists within seed production in Turkey. Especially for herbage seed, there is big gap between production and demand. However, most of the cultivated herbage crops origin is Anatolia. Furthermore, most of the regions except Black Sea Coastal Region are suitable for herbage seed production. Besides of cultivated herbage crops, seed is needed for amelioration of pasture, re-establishing green areas and using for other places. Requirement of various herbage crops has significantly increased and it is continuously increasing. Recently, in the national agriculture which was dragged to a bottle neck, herbage seed production may provide new opportunities especially for small farmers. However, numerous problems exist in herbage seed production, for example lack of knowledge, low level of mechanization and lack of varieties for different ecological areas. After solving these problems herbage seed production may be increased and resolve in a profitable sector. Turkey could be a significant seed production centre in future.

Key words: ecological conditions, herbage, problems, seed production, Turkey

INTRODUCTION

At least 20-30% of the arable field area should be assigned to forage production for sustainable and productive farming (Acar & Ayan 2000). This ratio is only 3.5-4% in Turkey (Anon. 2002). One of the most significant reasons for inadequacy of forage production is lack of seed.

Because improved varieties are highly sensitive to environmental conditions (Manga 1991), it is essential to use seeds of varieties which can raise their genetic capacities to maximum level under the environmental conditions in the region where they are grown (Acar 1994). Under normal conditions 20-30 % increase in herbage yield is possible by using high quality seeds. This increase can be as much as 3 to 4 folds with the use of hybrid varieties (Sehirali 2002).

A huge amount of seeds of various forage species and varieties will be needed in the future for different aims. The need for seeds of various forage species will increase due to

an increase in planting areas of forages. Furthermore, herbage seeds will be needed in the future to prevent erosion and to build parks, gardens and recreational areas.

Turkey, which has 15 different climatic regions with different ecological characteristics, is the gene centre for most culture plants. Turkey can be considered as the leading seed production centre due to its climatic characteristics and its insect population. Production of high quality and disease-free seeds is possible due to the low relative humidity during the seed maturation periods for winter and summer plants. The objective of this study is to highlight the ecological conditions in Turkey with regard to seed production.

CLIMATIC TYPES IN TURKEY AND THEIR RELATIONSHIPS WITH HERBAGE SEED PRODUCTION

Turkey, surrounded by sea on three sides, is characterised with broad plateaus, mountains and deep valleys. Turkey is located between temperate and subtropical climatic zones. For this reason, different seasons can occur in various regions during the same period (Anon. 2007).

1. Mediterranean climate

This climate type is generally seen in Mediterranean and Aegean regions. It is characterised by hot, dry summers and mild, wet winters. Average annual temperature can reach up to 20 °C at the East Mediterranean coasts. In coastal areas snow and frost events are rarely seen, and there is a dry and hot period from late spring and to mid fall. The coastal area is not appropriate for seed production of many plants. In the interior parts of the Aegean and Mediterranean regions there are suitable relative humidity and temperature values and rich insect population during pollination and fertilization periods of herbage. These conditions increase the importance of this region in terms of seed production. Watering is needed in case rainfall is low during the growing period.

2. Black-Sea Climate

All the seasons are rainy throughout the Black-Sea coasts. Average annual temperature is 8 - 12 °C. The total annual precipitation is above 1000 mm on east and west coasts of the region. The amount of rainfall can reach up to 2200 mm in Rize and Hopa on the far east coasts. The interior areas of the region are quite convenient for seed production of many crops.

3. Semi-moist Marmara Climate:

The whole Marmara region, except for Black Sea coasts, has this climate. In the warmest month (July) the average temperature is 23-24 °C, and in the coldest month (January) the average temperature is 3-5 °C. The highest amount of precipitation is registered in winter. Annual average precipitation and relative humidity are 500-700 mm and 69.6-73%, respectively. Snow and frost are rare in this climate. This climate is appropriate for seed production for most cold and warm season plants.

4. Steppe Climate:

Middle-Anatolia and surroundings, Goller Region, Middle-East Anatolia, West of the East Anatolia and South-East Anatolia show the characteristics of this semi-dry climate type. The most prominent characteristic of this climate type is the existence of large heat differences between daytime and night-time. Summer months are appropriate for seed production. However, some problems may arise in seed production of warm-season plants due to the extreme hot days during summer.

5. Terrestrial East Anatolian Climate:

Average winter temperature is below 0 °C. In the coldest month (January), average temperature ranges from -10 to -8 °C. The average temperature of the warmest month does not exceed 20 °C. Average annual precipitation is above 500 mm and its regime is partly regular. The region is covered by snow during winter. Average annual relative humidity is 60.2%. This region is suitable for seed production of cold-tolerant forages. Especially sainfoin and alfalfa seeds are produced in this region.

General

Daylight period and intensity are latitude-dependent parameters. The daylight period and intensity are higher in the south of Turkey. Daylight period are 6 hours per day in March and April months, 9 hours per day in May, 10 hours per day in June, July and August, 9 hours per day in September and 6 hours per day in October. Daylight period and intensity appears to be suitable for forage seed production.

For most herbage seed crops, low relative humidity and high temperature values are necessary during seed maturation for high quality production. Temperature requirements during generative period are 20-25 °C for cold season plants and 25-35 °C for warm season plants. These values indicate that most of the climatic regions in Turkey are appropriate for seed production.

The regions in which high quality forage seeds can be produced are shown on Fig. 1.

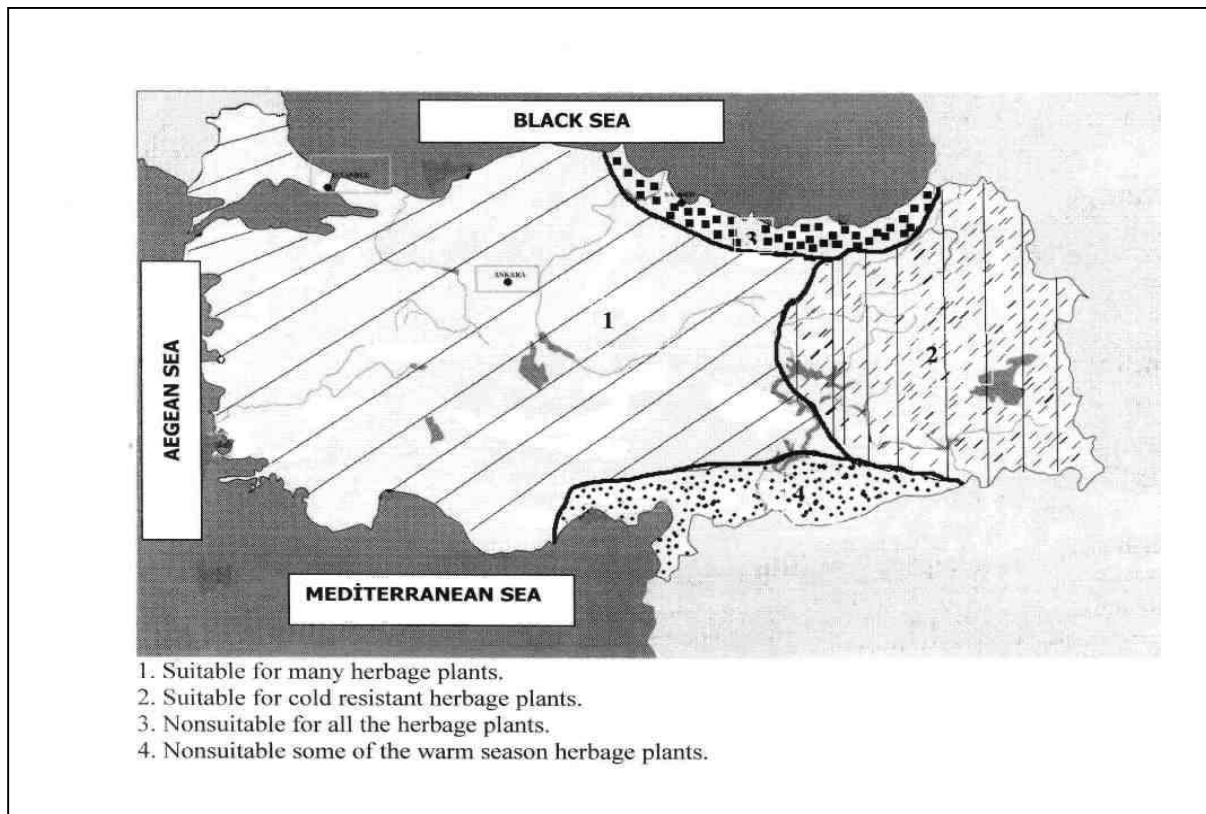


Fig. 1. Distribution of the suitable/unsuitable regions for herbage seed production in Turkey

Table 1. Land usage in Turkey (Anon. 2005a)

Land usage type	Area (million ha)	Ratio (%)
AGRICULTURE AREA	26.593	34.09
Field area	18.110	23.22
Horticulture (Vineyard, Fruit and Vegetable)	3.527	4.52
Fallowing area	4.956	6.35
PASTURE AND RANGES	21.500	27.56
FOREST VE SHRUBBERY	21.189	27.17
OTHER	8.718	11.18
TOTAL	78.00	
DRY LAND AREA	17.250	
IRRIGATED AREA	4.900	
IRRIGATION TARGET	8.500	

LAND USE, FORAGE SEED PRODUCTION IN TURKEY AND IMPORT

Agricultural, range and pasture areas are 26.6 and 21.5 million hectare, respectively. Field crops are grown on 18 million hectare, approximately. Almost 5 million hectare have irrigation, but 8.5 million hectare areas could be irrigated in the next years.

The seed production and distribution system in Turkey has been based on certain principles. The seeds produced according to the 308-numbered law or imported from abroad are distributed to the farmers via public and private sector in coordination of the Ministry of Agriculture and Rural Affairs (MARA) and the Agricultural Production and Development Directory (APDD). Private seed enterprises distribute seeds in the system of sales outlet. The number of private seed

enterprises increased from 5 to 134 in the period 1963-2005. Public seed enterprises carry out their activities via distributor enterprises commissioned by MARA (Genctan et al. 2005). The 'Seed Production Law' came into force in 2006 with rule number 5553, and its practice instructions are being prepared. Produced amounts of various forage seeds in 2006 are given in Table 2. As it can be seen, only alfalfa, sainfoin and vetch seeds are produced by the public sector. Private sector produces some grass seed for forage in addition to sainfoin, alfalfa and vetch. A large

amount of the domestic forage seed requirement is met by import. However, neither domestic production nor imported seed is not enough to meet the demand. For example, while seed produced in Turkey met 19.5 % of the alfalfa seed requirement, seed imported from abroad met 16 % of the requirement in 2005. Almost 64.5 % of alfalfa seeds used by farmers is uncontrolled. While 37 % of seed demand for vetch (*Vicia sativa* L., *Vicia pannonica* Crantz.) could be met by domestic certified seed production, only 1.5 % could be met by imports in the same year.

Table 2. Certified forage seed production and import in 2004 (tonnes) (Anon 2005b).

Species	Domestic production		Import	Species	Domestic production		Import
	Public	Private			Public	Private	
<i>L. corniculatus</i>	-	-	5	<i>D. glomerata</i>	-	15	11
<i>M. sativa</i>	143	136	230	<i>F. arundinacea</i>	-	35	162
<i>O. sativa</i>	632	50	140	<i>F. ovina</i>	-	8	42
<i>T. repens</i>	-	-	23	<i>F. pratensis</i>	-	-	3
<i>T. pratense</i>	-	-	3	<i>F. rubra com</i>	-	-	79
<i>T. alexandrinum</i>	-	-	1	<i>F. rubra rubra</i>	-	18	405
<i>T. subterraneum</i>	-	-	2	<i>F. rubra trich.</i>	-	-	4
<i>T. incarnatum</i>	-	-	1	<i>L. multiflorum</i>	-	30	10
<i>V. pannonica</i>	760	40	60	<i>L. perene</i>	-	191	456
<i>V. sativa</i>	369	389	-	<i>P. pratense</i>	-	-	7
<i>A. cristatum</i>	-	15	7	<i>P. pratensis</i>	-	25	175
<i>A. tenuis</i>	-	-	12	<i>P. trivialis</i>	-	-	1
<i>A. capillaries</i>	-	-	3	Grasses	12	-	-
<i>A. stolonifera</i>	-	-	1	Various mixture	-	-	273
<i>A. elatius</i>	-	-	1	Sorghum x sudan grass hybrids	-	51	151
<i>B. inermis</i>	-	10	10	<i>S. sudanense</i>	-	5	-
<i>C. dactylon</i>	-	-	194	<i>S. bicolour</i>	-	-	32

Requirement for seeds of different forage species and varieties will be increased in future due to the range improvement and increase in forage production. Among the forage species which can be used in the range improvement in Turkey are alfalfa, sainfoin, white clover, red clover, subterranean clover, hybrids clover, birdsfoot trefoil, wheat grasses, smooth brome, orchard grass, fescue grasses, burnet etc. Furthermore, seed requirements will be increased due to supports which are given to farmers by the government to stimulate forage production. Perennial ryegrass, bentgrass, bluegrass, fescue grasses, Bermuda grass and white clover are needed for landscaping of sloped places near roads, surroundings of dams, airports, etc. (Avcioglu et al. 1997).

PROBLEMS IN FORAGE SEED PRODUCTION

Most of the producers except for large firms have no adequate information on forage production, especially seed production. They can not easily access new information and technologies. Among the most significant problems encountered by alfalfa producers in the Aegean region are fertilization, cutting technique, irrigation, establishment and determination of harvest time (Dizdaroglu et al. 1992). Vetch producers in the same region reported mechanisation of sowing and harvest, determination of suitable mixing ratios, soil preparation, information and equipment deficiency in fertilization as the most significant problems (Dizdaroglu & Sabanci 1992). Most of the equipments used in forage production are different from those

used in production of other crops. Domestic production of equipment used in forage production is not adequate due to the fact that forage production was not widespread in our country. Imported equipments can not be purchased by producers due to their high prices (Acar & Ayan 2000). Forage seed production in broad areas is impossible without using machine power. There are severe problems in seed harvest and threshing of small-seeded forages. Mechanisation problem should be solved in order to improve forage seed production.

The number of highly productive varieties of forage species (except for alfalfa), which are adapted to the climate of the 15 main regions and most sub-regions in Turkey, is very limited. However, our country is the gene centre of most forage species (Ozgen *et al.* 1995). Suitable varieties of species which are adapted to the ecological conditions of different regions should be improved by breeding programmes. On the other hand, forage varieties which are bred in foreign countries and adapted to the ecological conditions of our country should also be identified. Supports for the producers who used certified seeds should be increased.

Required regulations in marketing and purchasing of seeds should be made and problems should be solved. Seed producers should sell the seed with reasonable price. On the other hand, forage producer should buy seed at a reasonable price. This problem should be solved by using regulations such as bourse and/or cooperative.

CONCLUSION

Ecological conditions in Turkey are very suitable for forage seed production. However, there are some technical and administrative problems. If these problems can be solved, our country can produce forage seeds in adequate amount and also can be an important seed production centre.

ACKNOWLEDGEMENTS

We thank to "ALTIN Tarim Ltd Company", "ATAKOL Tarim Tohum ve Hayvan Sanayi Ltd. Company" and Bend Medical Ltd Company" for supports.

LITERATURE

- Acar, Z. & Ayan, I. 2000. Forages culture OMU Agri.Faculty press, Book No:2, p.153. Samsun.
- Anonymous 2002. Agricultural Structure and Production in Turkey. DİE Pub., Ankara.
- Anonymous 2005a. Crop Production Statistics. www.tuik.gov.tr/VeriBilgi.do (17.04.2007).
- Anonymous 2005b. MARA , APDD, National Seed Providing, Distribution and Production Program. Ankara.
- Anonymous 2007. Climatologic classification. *In*: www.meteor.gov.tr (12.04.2007)
- Acar, Z. 1994. The possibilities of benefit from 'Apomiksis' to solve seedling problem. *In*: Standard year 33, number: 386, pp: 29 - 32.
- Avcioglu, R., Soya, H., Geren, H. & Cevheri, A. 1997. Investigation on the performances of some turf grasses under Bornova ecological conditions. *In*: II.Field Crops Congress. pp. 509-513. Samsun.
- Dizdaroglu, T. & Sabanci, C.O. 1992. Technical and economical characters and, main problems of Vetch (*Vicia sp*) culture in Aegean and South Marmara Regions. Turkish Ministry of Agriculture and Rural Affairs, Agricultural Researches General Directors. Result reports, Menemen-Izmir.
- Dizdaroglu, T., Sabanci C.O. & Eginlioglu, G. 1992. Alfalfa (*Medicago sativa* L.) cultivation and its cost, main technical and economical problems in Aegean and South Marmara Regions. MARA, Agricultural Researches General Directors, Result reports, Menemen-Izmir.
- Genctan, T., Tugay, M.E., Gecit, H.H., Bozkurt, B., Ergun, E., Ekiz, H., Yalvac, K., Gevrek, M.N., Elci, A. & Balkan, A. 2005. Seed, seedling and sapling production and using. *In*: www.zmo.org.tr/etkinlikler/6tk05/040temelgenctan.pdf
- Manga, I. 1991. Problems of seed production. II. *In*: Rangeland and Forage Congress, 28-31 May, Izmir, p. 472.
- Ozgen, M., Adak, M.S., Karagoz, A. & Ulukan, H. 1995. Conservation and utilizing of plant genetic resources. *In*: IV. Technical Congress of Turkish Agriculture Engineering. TR Agriculture Bank, Culture press No: 26, Ankara, p. 309.
- Shirali S. 2002. Seed and Seed Technology. Faculties press, Istanbul.

Economic damage thresholds for the clover seed weevil (*Apion fulvipes* Geoff.) and the lesser clover leaf weevil (*Hypera nigrirostris* Fab.) attacking white clover (*Trifolium repens* L.) seed crops

Lars Monrad Hansen & Birte Boelt

University of Aarhus, Faculty of Agricultural Sciences,
Forsøgsvej 1, DK - 4200 Slagelse.

LarsM.Hansen@agrsci.dk

ABSTRACT

White clover (*Trifolium repens*) is a very important crop in Denmark. A satisfactory yield is dependent of a sufficient pollination but a severe reduction in the white clover seed yield can be due to insect pests. The two most important insect pests are the clover seed weevil *Apion fulvipes* and the lesser clover leaf weevil *Hypera nigrirostris*, which can reduce the seed yield by more than 50 %. In 2002 - 2005 four field experiments with two treatments (untreated and insecticide treated) were carried out including the white clover variety 'Milo'. Damage caused by the weevils was calculated as the difference in number of weevils and the difference in yield between the averages of insecticide treated and untreated plots. The yield loss is expressed as a percentage of the treated plots, which allows comparison between years when yields in insecticide treated plots varied. Danish white clover crops for seed production are sprayed with an insecticide 2-4 times every year to ensure that insect pests will not cause yield reduction. However, probably some of the sprayings are unnecessarily in most years. In present research trials have been carried out to produce data to construct an economic damage threshold model. In organic grown white clover defoliation is a method to remove weevils with consequently lesser damage from the weevils. The later the defoliation the better effect against the weevils. However, late defoliation other things being equal gives lower yield

Key words: Defoliation, field trials, insecticide application, organic grown clover

INTRODUCTION

White clover (*Trifolium repens* L.) is a very important crop in Denmark. White clover is used in a mixed sward with grasses at about 180.000 ha and in seed production at about 5000 ha. 70 % of the white clover seed yield is used for export to countries all over the world. In the period 1995-2005 the average seed yield was $452 \pm 85 \text{ kg ha}^{-1}$ (Pedersen 2005, Brancheudvalget årsberetning 2006).

A satisfactory yield is dependent of a sufficient pollination but a severe reduction in the white clover seed yield can be due to insect pests. The two most important insect pests are the clover seed weevil *Apion*

fulvipes Geoff. and the lesser clover leaf weevil *Hypera nigrirostris* Fabr. They are both serious pests and can reduce the seed yield by more than 50 % (Hansen 2002, Weiss & Gillott 1993).

The clover seed weevil is about 2 mm long, elongate, strongly convex and all tibiae are yellowish. It is widely distributed and very common throughout the Palaearctic Region, and the most common and abundant species of the genus *Apion* in the Denmark. The main crop is white clover (*Trifolium repens* L.) Adults migrate to hibernate in uncropped area away from the white clover fields, under dead leaves in hedges, edges of wood etc. In early spring the adults fly to the host plants and,

after feeding until maturity, begin oviposition and continue throughout the summer (Bovien & Jørgensen 1934, Dieckmann 1977, Gønget 1997).

The lesser clover leaf weevil is about 4 mm long. The general colour is brown or black and the black variety sometimes show a greenish or greyish tinge. It is worldwide distributed and the primary host plants are true clovers, *Trifolium* spp.

Adults overwinter mainly in the clover crops but also in ditches, fence rows and wood lots from where they in the spring mainly crawl to the nearest clover crop. Oviposition occurs in spring and early summer. (Detwiler 1923, Markkula & Tinnilä 1956, Metcalf *et al.* 1962, Sechriest & Treece 1963, Weiss & Gillot 1993)

Danish white clover crops for seed production are sprayed with an insecticide 2-4 times every year to ensure that insect pests will not cause yield reduction. However, no economic damage threshold has until now been carried out.

In organic grown white clover defoliation is a method to remove weevils with consequently lesser damage from the weevils. The later the defoliation the better effect against the weevils. However, late defoliation other things being equal gives lower yield.

MATERIALS AND METHODS

In 2002 - 2005 every year a field experiment was carried out including the white clover variety 'Milo'. Two treatments were carried out: 1. Untreated and 2. Insecticide treated. The applied insecticides were esfenvalerate, lambda-cyhalothrin and tau-fluvalinate all used in Danish standard doses (Jensen *et al.* 2006). They were applied every other week from defoliation and 6-8 weeks forward with reference to keep the treated plots free of weevils. The experimental layout was a randomised block design with 4 replicates and plots size 8 x 7.5 m where only the central 8 x 2.5 m was harvested. The seed yield was calculated to 100 % purity and 12 % water content.

To estimate the number of weevil larvae in the flower heads and stalks 6 or 12 flower heads and stalks per plot were sampled.

Damage caused by the weevils was calculated as the difference in number of weevils and the difference in yield between the average of insecticide treated and untreated plots. The yield loss is expressed as a percentage of the treated plots, which allows comparison between years when yields in insecticide treated plots varied.

To ensure an adequate pollination beehives including honey-bees (*Apis mellifera mellifera*) were placed closed to the experiments.

A multiple regression approach was taken, in which the yield loss (YL) was the response variable and the weevil's *A. fulvipes* (A) and *H. nigrirostris* (H) the independent variables. The SAS REG procedure was used for all analyses (SAS Institute 1998).

An experimental layout as mentioned above was carried out with reference to investigate how successful defoliation is as a method to remove weevils with consequently lesser damage from the weevils. This method is appropriate for organic grown white clover. Four treatments were included: 1. No defoliation; 2. Defoliation 28 May; 3. Defoliation 28 May + 2 weeks; 4. Defoliation 28 May + 4 weeks.

RESULTS AND DISCUSSION

Economic damage threshold

The results from the economic damage field experiments are shown in Table 1. The numbers of weevils shown are the average number for the whole sampling period. A GLM-test (Duncan) shows that there are no significant differences in the number of weevils for the separate plots during the sampling time at 3-4 weeks. The numbers of the clover seed weevil and the lesser clover leaf weevil fluctuates significant between the separate years. An attempt to relate these fluctuations to meteorological parameters was unsuccessful.

Table 1. Results from field experiments 2002-2005

Year	Weevils/flower head					Yield loss		Yield treated	
	<i>A. fulvipes</i>		<i>H. nigrirostris</i>		N	kg ha ⁻¹	%	Kg ha ⁻¹	
2002	6.2	A	0.9	A	97	180	43.9	410	A
2003	3.1	B	1.2	B	96	119	42.2	282	B
2004	3.5	B	0.4	C	168	167	29.7	562	C
2005	11.9	C	1.2	B	72	451	39.8	1133	D

Figures in the same column with same letter are not significant different

Table 2. *A. fulvipes* / flower head

	2001	2002	2003	2004	2005	Mean
Untreated	7.8	8.0	7.8	4.0	14.6	8.4
Defoliation May 28	8.7	7.1	6.7	5.1	11.9	7.9
Defoliation May 28 + 2 weeks	7.4	4.9	0.4	3.1	15.3	6.2
Defoliation May 28 + 4 weeks	2.0	1.9	0.3	4.3	12.3	4.2

Also the yield in untreated plots fluctuates significant between the separate years. Many factors have influence on the final yield. In addition to the weevils also defoliation, pollination and harvest are important factors.

Influence from the *A. fulvipes* and *H. nigrirostris* on seed yield loss is significant. A regression model constructed on data from Table 1 result in:

$YL=10.6 \times \ln(A+1) + 19.3 \times H$; where YL is pct. seed yield loss, A is number of *A. fulvipes* per flower head and H is number of *H. nigrirostris* per flower head ($r^2=0.97$; $F=31.0$ and $P=0.03$).

The highest number of larvae we have seen in present investigation was less than 12 weevils which is equal to a yield loss about 25 %. In our monitoring we have not seen incidence of *A. fulvipes* more than 16 larvae/head.

If the incidence of *A. fulvipes* is set to zero the damage from *H. nigrirostris* can be calculated and it can be seen that about 5 larvae per head eats approximately 100 % of the yield. However, in our monitoring we have not seen incidence of *H. nigrirostris* more than 2 larvae/head equal to about 40 % yield loss.

When you are able to count the number of weevil larvae in the white clover crop most of the damage have been done by the larvae. It is more interesting to forecast the damage by

the number of adults attacking the crop in spring and early summer. It is possible to convert the damage threshold model for that purpose.

It is known that *Apion* lay about 100 egg/female, *Hypera* about the double and the number of white clover flower head per m² are about 900. The threshold model can be converted to:

$YL=10.6 \times \log(0,06A+1) + 4.3 \times H$; where YL is pct. yield loss, A is number of *A. fulvipes* per m² and H is number of *H. nigrirostris* per m². Insecticide application cost about 135 DKR and the price of white clover is 18 DKR kg⁻¹. An insecticide application is equivalent to about 8 kg white clover seed. If the potential yield is 500 kg ha⁻¹ 8 kg will be 1.6 % of the yield and 7 *A. flavipes* m⁻² or 0.4 *H. nigrirostris* m⁻² can cause that.

Because of the relatively long egg laying period probably more than one insecticide treatment is appropriate. In most years it is possible to find more than 7 *A. fulvipes* per m² and/or 0.4 *H. nigrirostris* per m². Therefore, the problem is not to avoid spraying when weevils are present but to keep the number of sprayings low. One-year field experiment shows that there is significant effect for 2 insecticide applications but not for 3 and 4.

Defoliation

In some years late defoliation results in a significant reduction in the number of both *A.*

fulvipes and *H. nigrirostris* as shown in Table 2 and 3. However, late defoliation also means lower yield (Table 4).

Table 3. *H. nigrirostris* / flower head

	2001	2002	2003	2004	2005	Mean
Untreated	0.1	0.6	1.0	0.6	1.3	0.7
Defoliation May 28	0.1	0.6	1.3	0.9	1.2	0.8
Defoliation May 28 + 2 weeks	0.1	0.3	0.1	0.1	0.8	0.3
Defoliation May 28 + 4 weeks	0.1	0.2	0.1	0.2	0.6	0.2

Table 4. Yield (kg ha⁻¹) from defoliation experiments

	2001	2002	2003	2004	2005	Mean
Untreated	62	31	223	384	619	409
Defoliation May 28	67	21	110	234	692	345
Defoliation May 28 + 2 weeks	35	13	191	220	734	382
Defoliation May 28 + 4 weeks	36	12	134	131	305	190

LITERATURE

- Bovien, P. & Jørgensen, M. 1934. Orienterende undersøgelser over angreb af snudebiller (*Apion*) i kløverhoveder. Tidsskrift for Planteavl 41: 337-353.
- Brancheudvalgets årsberetning 2005 .2006. 44pp. Brancheudvalget for frø, København
- Detwiler, J. D. 1923. Three little known clover insects, 28 pp. Cornell University Agricultural Experiment Station, Ithaca, New York, Bulletin 420.
- Dieckmann, L. 1977. Beiträge zur Insectenfauna der DDR: Coleoptera - Curculionidae (Apioninae). Beiträge zur Entomologie 27: 7-143.
- Gønget, H. 1997. The Brentidae (Coleoptera) of Northern Europe. Fauna Entomologica Scandinavica 34: 119-121, Brill, New York.
- Hansen, L. M., 2002. Organic white clover (*Trifolium repens* L.) production. Importance of the white clover seed weevil (*Apion fulvipes* G.) and possibilities for control. In: NJF-seed Conference, 24 - 26 June, 2002: 87-93.
- Jensen, P. K., Jørgensen, L. N., Nielsen, G. C., Nielsen, S. F., Petersen, P.H. & Paaske, K. 2006. Vejledning i planteværn 2006. 323 pp. Landbrugsforlaget, Aarhus.
- Markkula, M. & Tinnilä, A. 1956. Studies on the biology of the lesser clover leaf weevil, *Phytonomus nigrirostris* Fabr. (Col. Curculionidae), 66 pp. The Finnish State Agricultural Research Board, Helsinki 152.
- Metcalf, C. L., Flint, W. P. & Metcalf, R. L. 1962. Destructive and useful insects: Their habits and control (fourth edition), 1087 pp. McGraw Hill Book Company Inc. New York.
- Pedersen, C. A. 2005. Oversigt over landsforsøgene 2005, 14pp. Dansk Landbrugsrådgivning, Århus.
- SAS Institute. 1998. SAS/STAT® User's Guide. SAS Institute, Cary, North Carolina.
- Weiss, R. M. and Gillot, C. 1993. The biology of the lesser clover leaf weevil, *Hypera nigrirostris* (Fabr.) (Coleoptera: Curculionidae), on red clover, *Trifolium pratense* L. The Canadian Entomologist 125: 831-837.

Factors affecting the activity of Moddus M in red fescue

Solvejg K. Mathiassen, Mette Rabølle, Birte Boelt & Per Kudsk

University of Aarhus, Faculty of Agricultural Sciences,
Department of Integrated Pest Management, Flakkebjerg, DK-4200 Slagelse,
Denmark

Solvejg.Mathiassen@agrsci.dk

ABSTRACT

The influence of growth stage and climatic conditions on the efficacy of Moddus M (trinexapac-ethyl) in red fescue (*cv. Maxima*) was examined in climate simulators and in a field experiment. Plant samples were collected in the field and placed in climate simulators running 3 different climatic scenarios representative of the month of May. Four doses of Moddus M (0.4, 0.8, 1.2 and 1.6 L/ha) were applied one day later and the plant samples were replanted in the field trial 6 days after application. The applications were made at BBCH growth stages 31-33, 49-51 and 55-57. Significant responses on straw length were found only with 1.2 L/ha and 1.6 L/ha Moddus M. Applications in the field experiment were made at the same growth stages as in the pot experiment. The temperature varied from 10 to 20°C at the time of application. The highest yields were obtained at high doses and no differences were observed between growth stages. The results showed that doses, growth stages as well as climatic conditions affected the activity of Moddus M. Conclusions concerning the ranking of importance of the factors could not be drawn on the basis of the results due to a strong interactions between the factors.

Key words: climatic condition, growth stage, seed yield, straw length, temperature, trinexapac-ethyl

INTRODUCTION

Moddus M (trinexapac-ethyl, 260 g a.i. L⁻¹) has an off-label registration in *Festuca rubra* (red fescue), *Lolium perenne* (perennial ryegrass), *Dactylis glomerata* (cocksfoot) and *Festuca arundinacea* (tall fescue) in Denmark. The efficacy in red fescue and perennial ryegrass is variable and there is a need for more knowledge concerning relevant crop factors, the mode of action of Moddus M, and factors affecting the activity for a better identification of the fields and growth conditions where treatment is profitable.

Generally, yield increases in Denmark following the use of Moddus M are lower than reported from other countries. It is assumed that growth stage (GS), N-level and climatic factors influence the activity of Moddus M, but it is unclear which factors are most important and how they interact. The objective of our study was to examine the influence of growth stage and climatic factors on the activity of Moddus M in red fescue. It is difficult to separate these effects in field experiments as the climatic conditions may vary at the different growth stages. In the present study a combined design of field and climate simulator experiments was used.

MATERIALS AND METHODS

A field experiment in red fescue cv. Maxima was established in spring barley in 2005. The crop was fertilised with 70 kg N ha⁻¹ in the autumn supplemented with 50 kg N ha⁻¹ in the spring of 2006. Five doses of Moddus M (0, 0.4, 0.8, 1.2 and 1.6 L ha⁻¹, corresponding to 0, 100, 200, 300 and 400 g a.i. ha⁻¹) were applied at 3 different growth stages (BBCH 31-33, 49-51 and 53-57). In addition split applications of 2 x 0.8 L/ha were carried out for all combinations of growth stages. Each treatment was replicated 4 times. Prior to harvest plant samples were collected in each plot. The length of straw, panicle, and internodes were measured on 15 shoots from each plot. The field experiment was harvested and seed yield recorded.

Plant samples (15 cm in diameter) were collected in the field experiment in the early spring using a specially designed soil drill that enables maintaining a large part of the root section. The samples were left in the drilling holes until BBCH 31-33, 49-51 and 52-57 and then transferred to the climate simulators. Application of Moddus M was carried out one day later. Three different climatic scenarios with natural fluctuation in temperature and air humidity were run in the climate simulators (Table 1).

Table 1. Climatic scenarios in the climate simulator experiment.

Clim-ate	Mean temperature (minimum and maximum temperature in brackets)	Relative humidity
1	8 ^o C (5-11)	80%
2	20 ^o C (12-28)	60%
3	20 ^o C (16-24)	80%

The plant samples remained in the climate simulators until 6 days after application and were subsequently replanted in the field. The field plots with the plant samples were irrigated during the summer to ensure reestablishment. At harvest the plant samples were cut at the soil surface. The length of straws, panicle and internodes of 15 shoots were measured from each sample.

RESULTS AND DISCUSSION

The seed yield of the untreated plots in the field experiment was 1480 kg ha⁻¹. No lodging was observed at the flowering stage. Seed yield was significantly increased by all treatments except 0.4 L ha⁻¹ ha at GS 31-33 and 0.8 L ha⁻¹ at GS 53-57. The performances of 1.6 ha⁻¹ ha Moddus M applied as a single application and 2 x 0.8 L ha⁻¹ applied as split applications were similar irrespective of growth stages. Growth stage at application had no significant impact on the seed yield while seed yield was increased with increasing dose (Fig. 1). Straw length was not affected by low doses of Moddus M while high doses tended to reduce the straw length. The maximum reduction of straw length was 4 cm (7% reduction of untreated). No significant differences in internode length were identified. However, not only the growth stage but also the climatic conditions varied at the three application timings in the field. At the first application the maximum temperature was 20^o C while it was 15^o C at the second application and 10^o C at the third application time. Consequently, it was not possible to decide whether the observed effects were due to growth stage, climatic condition or interactions between these parameters.

In the climate simulator experiment the activity of Moddus M was studied at 3 different climates for each growth stage. The activity was assessed as the effect on straw length. It was not possible to determine seed production in the plant samples due to an uneven number of plants per sample. There was a strong interaction between growth stage and temperature while air humidity was of minor importance (data not shown). High temperatures increased the efficacy at BBCH 31-33 and 49-51 but had no influence at BBCH 53-57 (Fig. 2). At low temperature the efficacy was unaffected by growth stage. The highest efficacy was obtained at BBCH 49-51 and high temperature. The effects on straw length were more pronounced in the climate simulator experiment (up to 40% reduction of untreated) compared to the field experiment. The statistical analysis did not reveal significant differences between the length of specific internodes depending on the growth stage at application.

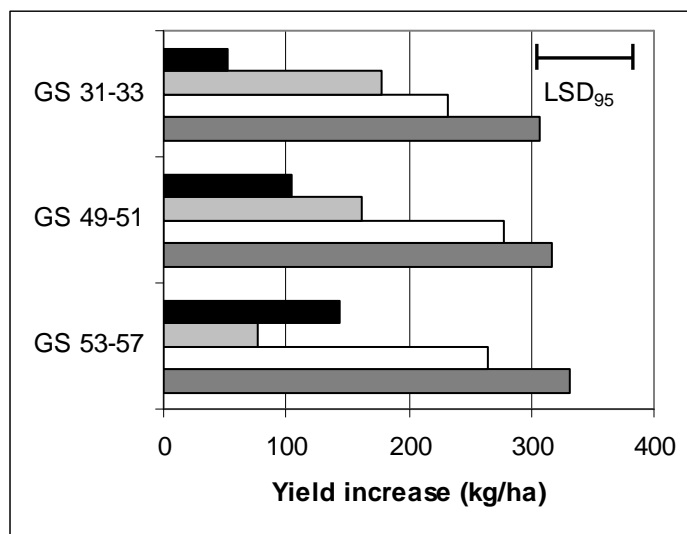


Fig. 1. Effect on seed yield (kg ha^{-1}) of Moddus M applied at different growth stages in the field experiment. ■ 0.4 L ha^{-1} , □ 0.8 L ha^{-1} , □ 1.2 L ha^{-1} , and ■ 1.6 L ha^{-1} .

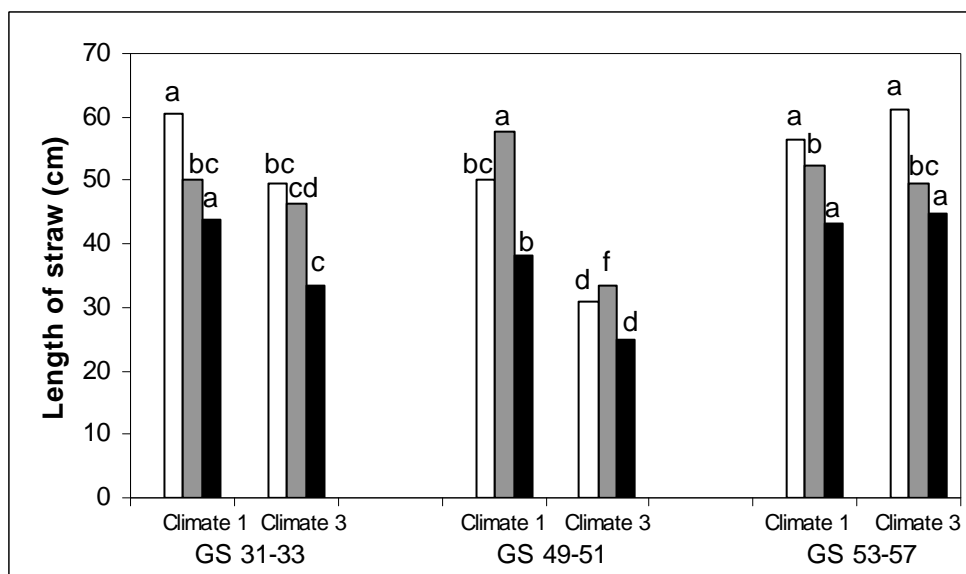


Fig. 2. Influence of growth stage and temperature on the efficacy of Moddus M on straw length in the climate simulator experiment. Climatic parameters are shown in Table 1. The straw length of untreated plants was 60.4 cm.

■ Control, □ 0.4 L ha^{-1} , □ 0.8 L ha^{-1} and ■ 1.6 L ha^{-1} . Within each dose bars designated different letters are significant different according to Duncan's test.

Although in the field experiment, optimal conditions were present only at BBCH 31-33, the responses to Moddus M were not significantly different from the responses at the two other application times. Despite the lack of significant effects on straw length, significant seed yield increments were obtained indicating that Moddus M had an influence on physiological processes affecting yield.

CONCLUSION

The results show that dose, growth stage and climatic conditions affect the activity of Moddus M. Significant yield responses can be obtained at early as well as at late application. It is not possible to draw conclusions concerning the relative importance of growth stage and climatic

conditions as they seem to interact. High temperature increases the efficacy of Moddus M at early growth stages but is of minor importance at late applications. Air humidity has a low impact on efficacy. Besides the effect on straw length that indirectly can influence seed yield, Moddus M seems to influence other physiological processes affecting seed yield. Further field- and climate simulator experiments conducted simultaneously will hopefully provide a better understanding of the parameters affecting the performance of Moddus M.

ACKNOWLEDGEMENTS

We thank all the technicians who have been involved in the experiments for their excellent work.

Response of creeping red fescue (*Festuca rubra* L.) and perennial ryegrass (*Lolium perenne* L.) to spring nitrogen fertility and plant growth regulator applications in Oregon

William C. Young III¹⁾, Tom B. Silberstein²⁾, Tom G. Chastain¹⁾ & Carol J. Garbacik¹⁾

¹⁾Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR USA
97331

²⁾Oregon State University Extension Service, 3180 Center St NE, Salem, OR USA
97301

william.c.young@oregonstate.edu

ABSTRACT

Recently registered plant growth regulators (PGR) for use in creeping red fescue and perennial ryegrass for control of excessive growth and lodging were evaluated in conjunction with varied rates of spring-applied nitrogen (N) to determine if seed yield can be increased with higher than recommended spring N rates. In our studies there was no seed yield advantage from nitrogen rates over 56 kg N ha⁻¹ in creeping red fescue or 160 kg N ha⁻¹ in perennial ryegrass, and the yield advantage from PGR application also levelled off at about the same point. Thus, the spring N rate for optimum seed production in creeping red fescue and perennial ryegrass is the same with or without the use of PGR.

Key words: seed production, lodging, N, PGR, seed yield, yield components

INTRODUCTION

The objective of this paper is to summarize recent trials investigating the influences of nitrogen (N) fertilizer and plant growth regulator use in the management of cool-season grasses grown for seed in Oregon, USA. Nitrogen has been recognized as the most critical mineral element limiting seed yield of grasses, and improvement of N fertilizer management is recognized as one of the most promising practices to increase seed yield in cool-season grasses (Rolston *et al.* 1985). It is also clear that seed yield in grasses depends on both the establishment and utilization of yield potential (Hebblethwaite *et al.* 1980), and that spring applied N may affect different yield components (Hebblethwaite & Ivins 1977).

Most cool-season species were developed primarily for forage qualities (Griffiths *et al.*

1980), and more recently for turf characteristics - both type cultivars show rapid growth response to available N, making them even more vulnerable to lodging when grown at high fertility rates used for seed production. Lodging results when fertile tillers have insufficient stem strength to support their own weight, and can impede both pollination and seed development (Hebblethwaite *et al.* 1978). Cultural practices to control lodging, such as altering the amount and time of N application (Hebblethwaite 1977), grazing, and defoliation (Green & Evans 1956, Roberts 1965) have shown inconsistent results.

Studies investigating plant growth regulators (PGRs) in Oregon began in the mid-1980s with research on the effects of paclobutrazol. In grass species, this sterol biosynthesis inhibitor taken up by the roots reduced levels of

endogenous gibberellins resulting in internode compression. Results from these trials on perennial ryegrass (*Lolium perenne* L.) (Young *et al.* 1995), Chewing's fescue (*Festuca rubra* var. *commutata* Gaudin), tall fescue (*Festuca arundinacea* Schreber), and orchardgrass (*Dactylis glomerata* L.) (Young *et al.* 1999) have been published. Paclobutrazol was briefly marketed in Oregon as Parlay[®], but its registration was cancelled in the late 1980s due to soil persistence and resulting deleterious effects on non-grass rotation crops.

MATERIALS AND METHODS

Recently (in the late 1990s), a new generation of foliar applied plant growth regulators became available, which were evaluated in Oregon (and elsewhere). The two products currently registered for use in Oregon being trinexapac-ethyl (marketed as Palisade[®]) and prohexadione-calcium (marketed as Apogee[®]). These products reduce the level of biologically active gibberellins (GA₁) through inhibition of the later steps in GA biosynthesis. Both have given excellent control of stem elongation resulting in reduced lodging and have significantly increased seed yields (Silberstein *et al.* 2000a, 2000b, 2001a, 2001b, 2002a, 2002b). More recently, Oregon researchers have investigated the response of spring N fertility with these foliar plant growth regulators in creeping red fescue (*Festuca rubra* L.) (Gingrich *et al.* 2002) and perennial ryegrass (Silberstein *et al.* 2003). The objective of these trials was to confirm earlier results of an absence of a N x PGR interaction, which was our experience with the previous generation of older, soil-applied compounds.

In 2001 and 2002, two large-scale, on-farm trials were conducted in a 'Shademark' creeping red fescue field near Silverton, Oregon. The 2000-01 crop year was third seed harvest; plots were again situated in the same field for the fourth crop year (2001-02). All cultural management was provided by the cooperating grower except the N applications. Spring N treatments were applied in a single application at: 0, 34, 56, 78, 101, 123, and 157 kg N ha⁻¹. At early heading stage of growth 210 g a.i. ha⁻¹ trinexapac-ethyl (1.5 pints/ acres as Palisade[®]) was applied to half of each plot. Plots were 7 m x 185 m and treatments were replicated three times in a

split block design. Plots were managed the same in the 2001-02 crop year except a lower rate (140 g a.i. ha⁻¹ trinexapac-ethyl) of Palisade[®] was applied. Grower equipment was used for swathing and harvest of the plots. A weigh wagon was used to determine seed yields. Sub-samples collected at harvest were used to determine clean-out, 1000 seed weight and calculate clean seed yields.

A similar experiment was conducted in an established stand of 'Cutter' perennial ryegrass in the fourth crop year at Hyslop Crop Science Research Farm near Corvallis, Oregon during the 2001-02 crop year. Plots size was 3 m x 15 m and treatments were applied in a factorial design with N rates (four rates) and PGR (Apogee[®], Palisade[®] and untreated) as main factors. The resulting 12 treatments (4 N rates x 3 PGRs) were replicated four times. Spring nitrogen treatments were split-applied with total N treatments at 100, 160, 200 and 250 kg ha⁻¹ to cover a range of rates from less than optimum (100 kg ha⁻¹) to above optimum, (250 kg ha⁻¹). PGR treatments were applied at a single rate of 400 g a.i. ha⁻¹ at the 2nd node stage of plant development. Plots were sampled during anthesis to determine fertile tiller density, stem length, and above ground biomass. Inflorescences were also randomly sampled for yield component analysis and spike length measurements. Harvesting was done using a 2 m wide swather for windrowing and a Hege 180 small plot combine for harvest. Combined-harvested seed samples were cleaned using a M2-B clipper cleaner for final cleanout; sub-samples of combined seed were taken for 1000 seed weights.

RESULTS

A summary of the 2001 creeping red fescue data is presented in Table 1. Seed yield is the only variable with a significant N x PGR interaction. The seed yield interaction is best observed graphically (Fig. 1). The optimum spring N rate to crops not being treated with PGR was at 34 kg N ha⁻¹ as opposed to 56 kg N ha⁻¹ to crops that had been sprayed with trinexapac-ethyl; N rates above these levels did not result in additional seed yield. Normal spring N rates used by Oregon fine fescue seed growers is 56 kg N ha⁻¹ as recommended by OSU Extension (Gingrich *et al.* 2003).

Table 1. Statistical summary of harvest component responses to spring-applied N and PGR in 'Shademark' creeping red fescue, 2001.

Main Factors	Seed yield	1000 seed weight	Spikelets per infl.	Florets per spikelet	Spike length
N rate	*** ¹	NS	*	NS	***
PGR	***	*	NS	(*)	NS
N rate x PGR	**	NS	NS	NS	NS

¹ NS = not significant, (*) = P value ≤ 0.10 , * = P value ≤ 0.05 , ** = P value ≤ 0.01 , *** = P value ≤ 0.001

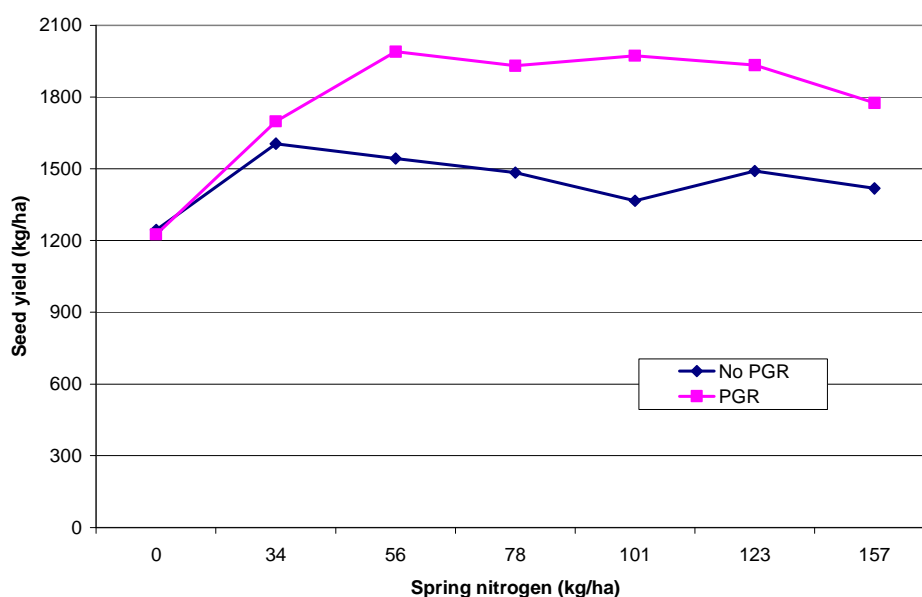


Fig. 1. 'Shademark' creeping red fescue seed yield response to spring-applied N and PGR, 2001.

Similarly in 2002, there was no significant interaction observed for any variable beside seed yield. Neither total biomass, harvest index, 1000 seed weight nor plant height were affected by PGR (Table 2). Additionally,

yield component responses were measured in 2002; no evidence of a N x PGR interaction was observed (Table 3). Nitrogen significantly affected all variables measured except florets per spikelet; PGR affected only seed yield.

Table 2. Statistical summary of harvest component responses to spring-applied N and PGR in 'Shademark' creeping red fescue, 2002.

Main Factors	Seed yield	Total biomass	Harvest index	1000 seed weight	Plant height
N rate	*** ¹	***	***	*	**
PGR	(*)	NS	NS	NS	NS
N rate x PGR	**	NS	NS	NS	NS

¹ NS = not significant, (*) = P value ≤ 0.10 , * = P value ≤ 0.05 , ** = P value ≤ 0.01 , *** = P value ≤ 0.001

Table 3. Statistical summary of yield component responses to spring-applied N and PGR in 'Shademark' creeping red fescue, 2002.

Main Factors	Spikelets per infl.	Florets per spikelet	Spike length	Fertile tillers		Actual seed number	Potential seed number	Floret site utilization
				density	specific dry wgt.			
N rate	*** ¹	NS	***	**	***	***	***	**
PGR	NS	NS	NS	NS	NS	NS	NS	NS
N rate x PGR	NS	NS	NS	NS	NS	NS	NS	NS

¹ NS = not significant, ** = P value ≤ 0.01 , *** = P value ≤ 0.001

Table 4. Statistical summary of harvest component responses to spring-applied N and PGR in 'Cutter' perennial ryegrass, 2002.

Main Factors	Seed yield	Total biomass	Harvest index	1000 seed weight	Plant height	Lodging score
N rate	*** ¹	NS	NS	NS	NS	***
PGR	***	NS	***	NS	***	***
N rate x PGR	NS	NS	NS	NS	NS	(*)

¹ NS = not significant, (*) = P value ≤ 0.10 , *** = P value ≤ 0.001

Table 5. Statistical summary of yield component responses to spring-applied N and PGR in 'Cutter' perennial ryegrass, 2002.

Main Factors	Spikelets per infl.	Florets per spikelet	Spike length	Fertile tillers		Actual seed number	Potential seed number	Floret site utilization
				density	specific dry wgt.			
N rate	NS ¹	***	*	NS	NS	***	*	NS
PGR	NS	**	***	NS	NS	***	NS	*
N rate x PGR	NS	NS	NS	NS	NS	NS	NS	NS

¹ NS = not significant, * = P value ≤ 0.05 , ** = P value ≤ 0.01 , *** = P value ≤ 0.001

In perennial ryegrass there was no significant N x PGR interaction for any variable other than lodging (Tables 4 and 5). Seed yield increased as N rates increased from 100 to 160 kg ha⁻¹, but did not statistically increase as the N rate went above 160 kg ha⁻¹ (data not shown). Seed yield from PGR treated plots averaged 459 kg ha⁻¹ above the untreated. However, seed yield leveled off at the 160 kg N ha⁻¹ rate with or without PGR applications similar to the fine fescue response in Figure 1. Seed size (1000 seed weight) was not affected by any of the treatments.

SUMMARY

Seed growers have speculated that N rates higher than those recommended by the Oregon State University Extension Service may provide a yield advantage where rapid stem elongation and subsequent delay in crop lodging is achieved with plant growth regulator use. Data presented here for creeping red fescue and perennial ryegrass suggest otherwise. The use of plant growth regulators does not justify producers changing their normal spring-applied N rates.

LITERATURE

Gingrich, G.A., Silberstein, T.B. & Young, W.C. III 2002. Effect of spring nitrogen rates and Palisade application in seed yield in red fescue. *In*: Young, W.C. III (ed.). 2001 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 121

(4/02): 17-18.

- Gingrich, G.A., Hart, J.M., Horneck, D.A., Young W.C. & Silberstein, T.B. 2003. Fine Fescue Seed (Western Oregon- West of the Cascades). FG 6-E. Oregon State University Extension Service.
- Green, J.O. & Evans, T.A. 1956. Manuring and grazing for seed production in S.37 cocksfoot and S.215 meadow fescue. *Journal of the British Grassland Society* 11: 165-173.
- Griffiths, D.J., Lewis, J. & Bean, E.W. 1980. Problems of breeding for seed production in grasses. *In: Hebblethwaite, P.D. (ed.). Seed production. Proceeding of the Easter School in Agricultural Science, 28th, University of Nottingham.* pp. 37-50. Butterworths, London.
- Hebblethwaite, P.D. 1977. Irrigation and nitrogen studies in S-23 ryegrass grown for seed. I. Growth, development, seed yield components and seed yield. *Journal of Agricultural Science (Cambridge)* 88: 605-614.
- Hebblethwaite, P.D. & Ivins, J.D. 1977. Nitrogen studies in *Lolium perenne* L. grown for seed: I. Level of application. *Journal of the British Grassland Society* 32: 195-204.
- Hebblethwaite, P.D., Burbidge, A. & Wright, D. 1978. Lodging studies in *Lolium perenne* grown for seed. 1. Seed yield and yield components. *Journal of Agricultural Science (Cambridge)* 90: 261-267.
- Hebblethwaite, P.D., Wright, D. & Noble, A. 1980. Some physiological aspects of seed yield in *Lolium perenne* L. (perennial ryegrass). *In: Hebblethwaite, P.D. (ed.). Seed production. Proceeding of the Easter School in Agricultural Science, 28th, University of Nottingham.* pp. 71-90. Butterworths, London.
- Roberts, H.M. 1965. The effect of defoliation on the seed-producing capacity of bred varieties of grasses. III. Varieties of perennial ryegrass, cocksfoot, meadow fescue and timothy. *Journal of the British Grassland Society* 13: 255-261.
- Rolston, M.P., Brown, K.R., Hare, M.D. & Young, K.A. 1985. Grass seed production: Weed, herbicides and fertilizers. *In: Hare, M.D. & Brock, J.L. (eds.) Producing herbage seeds. Grassland Research Practice Series 2.* pp. 15-22. New Zealand Grassland Association, Palmerton North.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2000a. Response of cool season grasses to foliar applications of Palisade (trinexapac-ethyl) plant growth regulator, 1999. *In: Young, W.C. III (ed.). 1999 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 114 (4/00):* 35-39.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2000b. Response of cool season grasses to foliar applications of Apogee (prohexadione-calcium) plant growth regulator, 1999. *In: Young, W.C. III (ed.). 1999 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 114 (4/00):* 40-44.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2001a. Response of cool season grasses to foliar applications of Palisade (trinexapac-ethyl) plant growth regulator, 2000. *In: Young, W.C. III (ed.). 2000 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 115 (4/01):* 24-30.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2001b. Response of cool season grasses to foliar applications of Apogee (prohexadione-calcium) plant growth regulator, 2000. *In: Young, W.C. III (ed.). 2000 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 115 (4/01):* 33-36.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2002a. Response of cool season grasses to foliar applications of Palisade (trinexapac-ethyl) plant growth regulator, 2001. *In: Young, W.C. III (ed.). 2001 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 121 (4/02):* 9-14.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2002b. Response of cool season grasses to foliar applications of Apogee (prohexadione-calcium) plant growth regulator, 2001. *In: Young, W.C. III (ed.). 2001 Seed production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/Crs 121 (4/02):* 18-21.
- Silberstein, T.B., Young, W.C. III, Chastain, T.G. & Garbacik, C.J. 2003. Response of perennial ryegrass to spring nitrogen fertility and plant growth regulator applications. *In: Proceedings of the Fifth International Herbage Seed Conference, Gatton, Australia.* Pp. 147-151.
- Young, W.C. III, Chilcote, D.O. & Youngberg, H.W. 1995. Seed yield response of perennial ryegrass to spring applied nitrogen at different rates of paclobutrazol. *Journal of Applied Seed Production* 13: 10-15.
- Young, W.C. III, Chilcote, D.O. & Youngberg, H.W. 1999. Chemical dwarfing and the response of cool-season grass seed crops to spring applied nitrogen. *Agronomy Journal* 91: 344-350.

Economical optimal nitrogen (ECO-N) application rate is all that matters for the growers

René Gislum¹⁾, Phil Rolston²⁾, John M. Hart³⁾, Richard Chynoweth⁴⁾, Bede McCloy⁵⁾ & William C. Young III³⁾

¹⁾Faculty of Agricultural Sciences, University of Aarhus, 4200 Slagelse, Denmark.

²⁾AgResearch, Private Bag 4749, Christchurch, New Zealand

³⁾Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR USA 97331

⁴⁾Foundation for Arable Research, PO Box 80, Lincoln, New Zealand

⁵⁾NZ Arable, PO Box 16-101, Christchurch, New Zealand

rene.gislum@agrsci.dk

ABSTRACT

The EConomical Optimal Nitrogen (ECO-N) application rate has been calculated in several local field experiments and the purpose is to enhance the profit for seed growers and minimise the environmental impact associated with the use of nitrogen (N). However, only very limited results are available on national or international response models. The objectives of this study were to 1) compare and evaluate different response models, 2) calculate ECO-N and the effect of fluctuation in seed and N prices, 3) define the criteria for using ECO-N and the difference between a local and a global ECO-N and 4) discuss how to make ECO-N results easily available and useful as a management tool for seed growers.

Seed yield data were collected from 126 field experiments with perennial ryegrass (*Lolium perenne* L.) receiving different N application rates, using different cultivars and performed in different years in Oregon, New Zealand and Denmark. Three yield response models (quadratic, quadratic + plateau and linear + plateau) were fitted to the N application rates and seed yield and the best response model was defined based on Akaike information criterion, root mean square error of prediction and adjusted coefficients of determination (R^2) values.

We conclude that 1) the quadratic and the quadratic + plateau response models seem to fit the data from NZ, OR and DK about equally well, 2) the prize of seed and N only has a limited effect on the ECO-N application rate, 3) the use of ECO-N should be based on a sound model and that there should be a selecting of which experimental results should be in the model, furthermore we conclude that ECO-N should be calculated on local national experimental results and 4) a possible way to improve the knowledge transfer could be through the internet.

Key words: economy, models, nitrogen, seed yield

INTRODUCTION

The purpose for calculating the EConomical Optimal Nitrogen (ECO-N) application rates is to enhance the profit for seed growers and minimise the environmental impact associated with the use of nitrogen (N). Both

issues have global attention in the seed producing areas and it is expected that this interest will increase in the future. Hence, several local response models, defined as the effect of different nitrogen applications rates on the seed yield, using data from local field experiments, have been developed and ECO-N has been calculated. However, only very

limited results are available on national or international response models.

The objectives of this study were to 1) compare and evaluate different response models where the evaluation on the individual models is based on adjusted correlation coefficients, the Akaike information criterion and Root Mean Square Error of Prediction, 2) calculate ECO-N and the effect of fluctuation in seed and N prices, 3) define the criteria for using ECO-N and the difference between a local and a global ECO-N and 4) discuss how to make ECO-N results easily available and useful as a management tool for seed growers.

MATERIALS AND METHODS

Seed yield (kg ha^{-1}) data were collected from field experiments with perennial ryegrass (*Lolium perenne* L.) in Oregon, New Zealand and Denmark (Table 1). In Denmark and in Oregon the N application rate in the individual plots was used whereas in New Zealand the N application rate plus 0-30 cm mineral N in the soil at the initiation of the spring growing season was used in the yield response models.

Table 1. Information describing the experiments in each country/state.

Country/ state	No. of data- sets	No. of plots	Years	Range of N	No. of culti- vars
Oregon (OR)	8	167	1998- 2000	0-275	3
New Zealand (NZ)	9	323	2003- 2005	29- 351	8
Denmark (DK)	109	1378	1973- 2004	0-200	19
Global	126	1870	1973- 2005	0-351	30

Three yield response models (quadratic, quadratic + plateau and linear + plateau) were fitted to the data using NLIN in the SAS Statistical Analysis System (SAS 1999). For the three statistical models, *yield* is the seed yield in kg ha^{-1} , *N* is the nitrogen application rate/soil and applied N in kg ha^{-1} , *a* is the intercept, *b* is the linear coefficient and *c* is the quadratic coefficient parameters to be estimated.

The quadratic model is: $Yield = a + bN + cN^2$ [1]

The quadratic + plateau model is:

$$Yield = a + bN + cN^2 \quad \text{if } N < C \text{ [2]}$$

$$Yield = P \quad \text{if } N \geq C \text{ [3]}$$

where *C* is the N application rate or the soil N and applied N, which occurs at the intersection of the quadratic response and the plateau line. *P* is the plateau yield. Both *C* and *P* parameters are obtained by fitting the model to the data.

The linear + plateau model is:

$$Yield = a + bN \quad \text{if } N < C \text{ [4]}$$

$$Yield = P \quad \text{if } N \geq C \text{ [5]}$$

Akaike Information criterion (AIC) (Akaike 1969), Root Mean Square Error of Prediction (RMSEP) (Esbensen 2000) and adjusted coefficients of determination (R^2) values were used to compare the ability of the three different models used to describe the seed yield (Table 2).

The AIC was calculated as:

$$AIC = n * \ln\left(\frac{\sum (y_i - \hat{y}_i)^2}{n}\right) + 2 * p \quad [6]$$

where y_i is the actual seed yield and \hat{y}_i is the predicted seed yield, *n* is the number of observations and *p* is the number of parameters used in the model. In the quadratic models $p=3$ and in the linear models $p=2$. The prediction ability is best when the predictive error (RMSEP) is at its lowest. The descriptive AIC predictive power of the model is higher the lower the value of the AIC.

The Root Mean Square Error of Prediction (RMSEP) is a measure of the average difference between predicted and measured yield expressed as seed yield (kg ha^{-1}):

$$RMSEP = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n}} \quad [7]$$

The prediction ability is best when the predictive error (RMSEP) is at its lowest. Adjusted coefficients of determination (R^2) values were calculated using equation [8].

$$R^2 = 1 - \frac{SS_{Residual}}{SS_{Corrected}} \quad [8]$$

where $SS_{Residual}$ is the residual sum of squares and $SS_{Corrected}$ is the corrected total sum of squares for the model. The higher R^2 values the better description of the data using the model.

For the quadratic + plateau and the linear + plateau the AIC, RMSEP and adjusted R^2 are only based on the data before the plateau is reached.

For the quadratic + plateau and the linear + plateau the maximum seed yield is the intersection between the quadratic/linear model and the plateau. When the price relation between the seed yield and the nitrogen is > 1 the ECO-N is closer to the maximum seed yield than when the price relation is < 1 . The ECO-N was calculated using the price relation and the estimated parameters from the individual models using IML in the SAS Statistical Analysis System (SAS 1999). In the ECO-N calculations two different prices (low and high) for seed and N were used (Table 3). All prices are in the monetary system from the respective country e.g. the prices from Oregon are in US\$.

RESULTS

The average seed yield in Denmark was considerable lower than in Oregon and New Zealand which showed no mutual difference (Fig. 1). Of the total number of data, almost 74 % were from Danish field experiments.

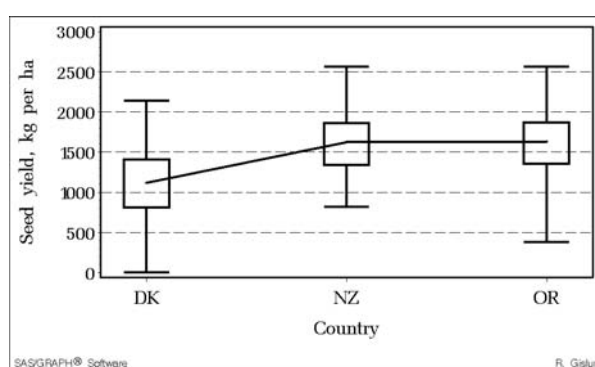


Fig. 1. Box plot of seed yields from Denmark (DK), New Zealand (NZ) and Oregon (OR). The middle of the box plot shows the average seed yield, the upper whisker is the 75 % percentile and the lower whisker the 25 % percentile. The upper horizontal line is the maximum seed yield and the lower horizontal line is the minimum seed yield.

The fact that a high proportion of the data were from DK with a lower seed yield had a significant effect on the global model (Fig. 2).

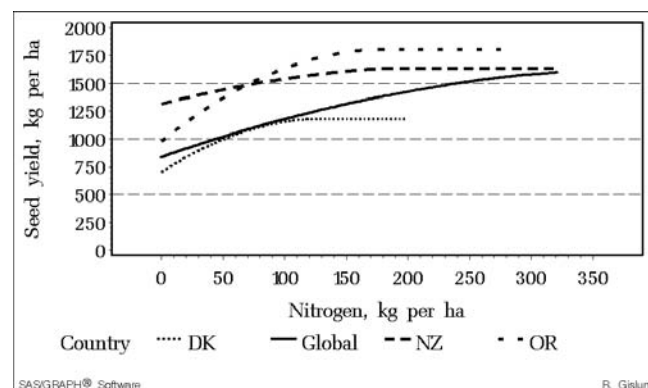


Fig. 2. The best models developed in Denmark (DK), New Zealand (NZ), Oregon (OR) and globally. In DK, NZ and in OR the seed yield could best be described using a quadratic + plateau model, whereas the global seed yields are described using a quadratic model.

The most characteristic results from the test of the three models to describe the seed yield were the significantly lower R^2 values in the results from New Zealand compared to results from Oregon and Denmark (Table 2). Of the three models tested on the NZ, OR and DK results, the quadratic + plateau was slightly better than the quadratic and the linear + plateau (Table 2).

The highest ECO-N was calculated on the results from NZ followed by results from OR and finally from DK (Table 3). There was only a limited effect on varying prices on seeds and N (Table 3).

Table 2. Adjusted R², AIC and RMSEP values for the three models developed to describe the relation between seed yield and nitrogen in New Zealand, Oregon, Denmark and globally.

	Quadratic			Quadratic + plateau			Linear + plateau		
	R ²	AIC	RMSEP, kg seed ha ⁻¹	R ²	AIC	RMSEP, kg seed ha ⁻¹	R ²	AIC	RMSEP, kg seed ha ⁻¹
Oregon	0.49	1932	309	0.48	1380	307	0.38	1958	337
New Zealand	0.03	3761	328	0.05	750	48	0.01	3767	334
Denmark	0.22	16401	388	0.11	11198	373	0.06	16488	402
Global	0.18	22518	415	0.18	22518	415	0.18	22518	415

Table 3. Economical optimum nitrogen (N) application rates at low and high prices using the best models from the different countries. All prices are in the monetary system from the respective countries. Both prices on seeds and N are per kg.

	N prices		Seed prices				
		US\$ 0.56	US\$ 0.84	NZ\$ 1.28	NZ\$ 1.92	DKK 5.60	DKK. 8.40
Oregon	US\$ 0.56	163	170				
Oregon	US\$ 0.84	153	163				
New Zealand	NZ\$ 0.84			183	202		
New Zealand	NZ\$ 1.26			156	183		
Denmark	DK kr. 3.6					119	123
Denmark	DK kr. 5.4					113	119

DISCUSSION

The two response models (quadratic and quadratic + plateau) seem to fit the data from NZ, OR and DK about equally well. Some results from the Danish experiments are notably low and it should be considered to remove them from the model. The reason that the global response model was better described using a quadratic model is a result of the variation in seed yield in NZ, OR and DK. From an agronomical point of view, the main issue must be to decide if data above the maximum seed yield should be described using a linear or a quadratic model. If a linear response model is chosen above the maximum seed yield, the economical loss from the application of N above ECO-N is limited to the expenses from the N and the application of it. If a quadratic response model is selected there is a decrease in the seed yield after the maximum seed yield has been reached. Besides the expenses from the N, and the application of it there is a lower seed yield and the economical consequences from excess application of N are substantial higher. The choice of response model after the maximum seed yield is therefore extremely

important. Furthermore, the choice of results used to develop the response model is very important. Results from field experiments where other N sources, besides the applied N and a 'natural' mineralization of N to a plant available form is present e.g. manure or clover in previous years, should be avoided or the effect of the N should be taken into consideration when the ECO-N application rate is calculated. Results from field experiments with notably low seed yields, which was the case in some Danish field experiments should be removed from the model.

The use of soil N plus applied N in the response models from the NZ experiments should in theory describe the yield responses better than the use of only the applied N which is the case in the experiments from DK and OR. However, this was not the case in the present study. The reason is most probably that the yield response in a large number of the NZ experiments could best be described by a flat yield response curve.

The minor effect of high and low prices of seeds and N on the ECO-N was not surprising

and is a result of the low slope in the response models when the maximum yield is reached. No ECO-N application rates were calculated for the global response model due to the different prices on seeds and N in NZ, OR and DK.

An important issue in applied science is 'knowledge transfer'. Results from the experiments should be communicated fast and clearly to the main users, which in this case, is the herbage seed growers. The use of the internet as a method to transfer knowledge to the farmers is indisputable. In the present example with ECO-N a possible way to communicate the results to the herbage seed growers could be to develop a robust response model and make it possible for the herbage seed growers to enter in their actual or expected seed and N prices. On

basis of the response models and the price relation between the seeds and the N the ECO-N is calculated. By typing different prices on the seeds and N it is possible for the herbage seed grower to follow the differences in ECO-N.

We conclude that 1) the quadratic and the quadratic + plateau response models seem to fit the data from NZ, OR and DK about equally well, 2) the price of seed and N only has a limited effect on the ECO-N application rate, 3) the use of ECO-N should be based on a sound model and that there should be a selecting of which experimental results should be in the model, furthermore we conclude that ECO-N should be calculated on local national experimental results and 4) a possible way to improve the knowledge transfer could be through the internet.

LITERATURE

- Akaike, H. 1969. Fitting Autoregressive Models for Prediction. *Annals of the Institute of Statistical Mathematics* 21: 243-247.
- Esbensen, K.H. 2000. *Multivariate Data Analysis-In Practice*, 4th ed. CAMO, Oslo, 598 pp.
- SAS. 1999. *SAS Online Doc. Version 8*. SAS Institute, Cary, NC, USA

Growth regulation, fungicides and nitrogen interaction in seed crop production

Christian Haldrup

Danish Agricultural Advisory Service, National Centre, Crop Production
Udkaersvej 15, Skejby, DK-8200 Aarhus N, Denmark

crh@landscentret.dk

ABSTRACT

Chemical plant growth regulators should only be used in healthy seed crops, during good growing conditions. The crop must not be in shortage of water or be stressed in any other way. Growth regulation should be conducted when there is 'growing weather', with day temperatures over 12 °C and night temperatures over 5 °C. Treatment should be carried out from beginning of stem elongation to beginning of earing, growth stage 32-50.

Key words: *Dactylis glomerata*, *Festuca arundinacea*, *Festuca pratensis*, *Festuca rubra*, *Lolium perenne*, *Poa pratensis*

SUMMARY OF DANISH FIELD TRIALS

Perennial ryegrass (*Lolium perenne*)

Where an additional 50 kg N was applied per ha, a yield response was obtained from growth regulation in early and tetraploid varieties by adding 0.4 L Moddus M per ha. Without additional nitrogen, no yield response was obtained. The results are shown in Fig. 1. In both 2005 and 2006 the highest yield response and net yield response was reached, where extra nitrogen was applied in addition to growth regulation with Moddus M and fungicides. In late varieties growth regulation did not result in higher yield response, neither did application of additional nitrogen. The results are shown in Fig. 2.

During recent years many trials have been conducted with growth regulation of rye grass (*Lolium perenne*) with and without additional application of nitrogen. The conclusion is that growth regulators should be applied in early and tetraploid varieties if the amount of fertilizer is more than 150 kg nitrogen per ha. It cannot be recommended, irrespective of nitrogen amount, to use growth regulation in late varieties of perennial rye grass.

Red fescue (*Festuca rubra*)

A yield response of 217 kg seed per ha has been obtained for 30 kg nitrogen extra per ha, applied in spring, and growth regulation with a mixture of 0.4 L Moddus M + 1.25 L CCC 750. For 30 kg nitrogen per ha alone the result was a yield response of 58 kg seed per ha. A yield response of 73 kg seed per ha was obtained for growth regulation, where no extra nitrogen was applied.

Cocksfoot (*Dactylis glomerata*)

Trials with growth regulators in cocksfoot have shown profitable yield responses. In 2004 and 2006 when the weather was reasonably humid at the end of May and the beginning of June yield responses were obtained through fungicide application.

Tall fescue (*Festuca arundinacea*)

Yield responses are obtained with growth regulation with Moddus M and a mixture of 0.4 L Moddus M + 1.25 L CCC 750 per ha. In most trials the highest yield response has been obtained where this mixture was used, but in some trials the highest yield response was obtained with 0.8 L Moddus M per ha. In 2006 control of fungus diseases and pests contributed to positive yield responses.

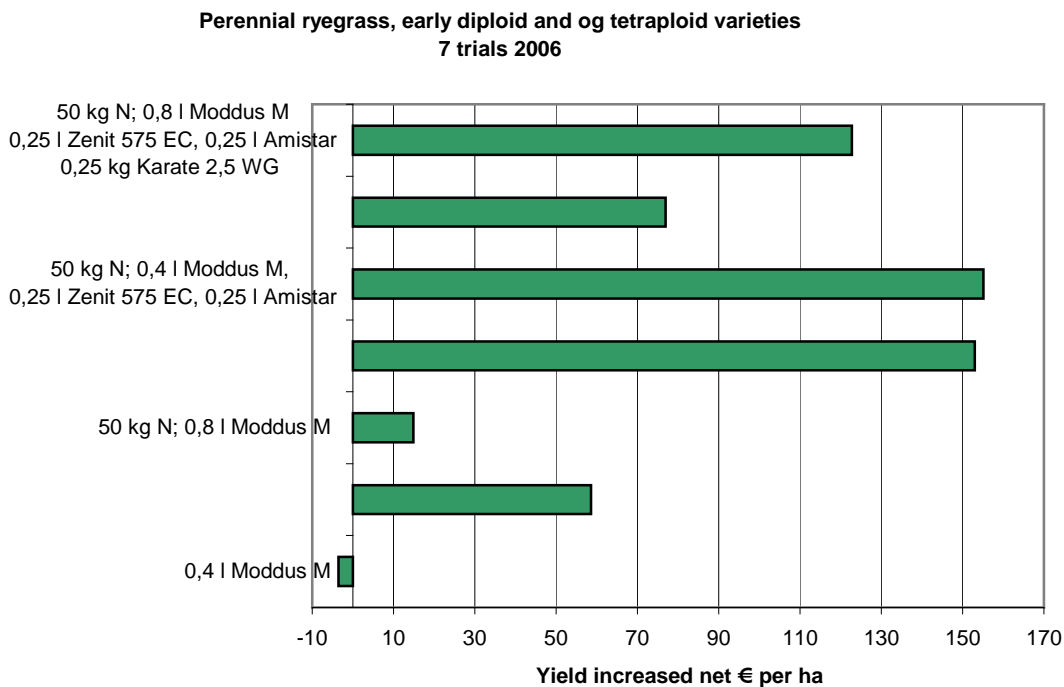


Fig. 1. Net yield response for additional nitrogen, growth regulation, disease and pest control in early and tetraploid varieties of perennial rye grass. Average of 7 trials 2006.

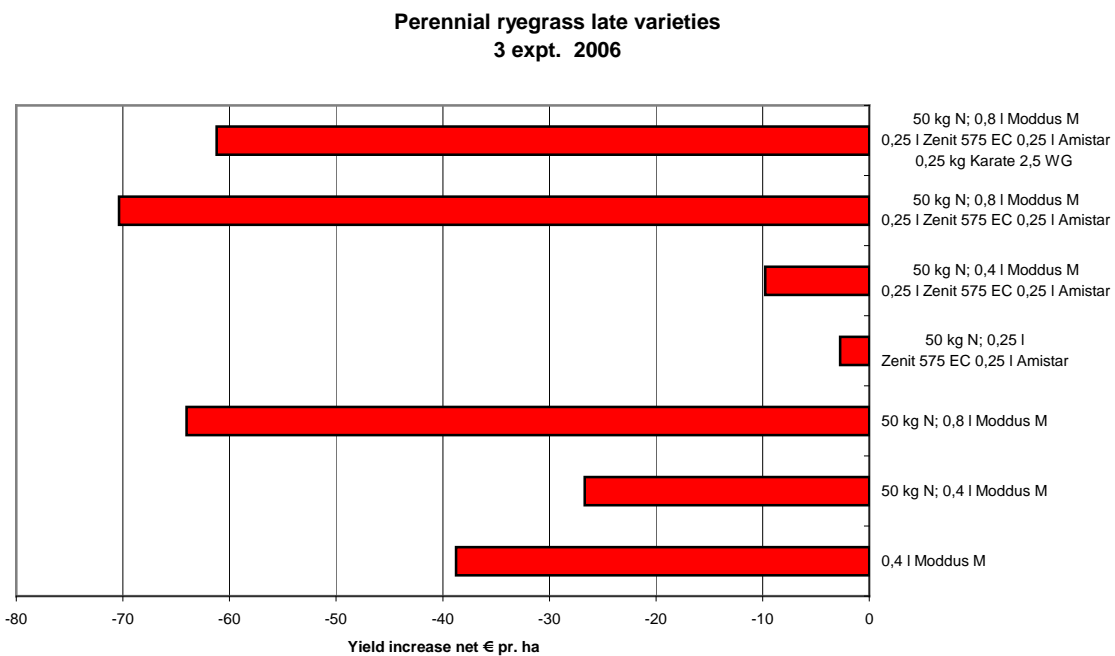


Fig. 2. Net yield response for additional nitrogen, growth regulation, disease and pest control in late varieties of perennial rye grass. Average of 3 trials 2006.

Tall fescue (*Festuca arundinacea*)

Yield responses are obtained with growth regulation with Moddus M and a mixture of 0.4 L Moddus M + 1.25 L CCC 750 per ha. In most trials the highest yield response has been obtained where this mixture was used, but in some trials the highest yield response was obtained with 0.8 L Moddus M per ha. In 2006 control of fungus diseases and pests contributed to positive yield responses.

Meadow fescue (*Festuca pratensis*)

In fescue grass yield responses have been obtained with control of diseases and in some trials with pest control. Yield responses have not been sufficiently high to cover the costs for growth regulators.

Smooth-stalked meadow grass (*Poa pratensis*)

In meadow grass for fodder yield responses of 160 kg seed per ha were obtained with a combination of a high nitrogen amount (190 kg nitrogen per ha in all) and growth regulation with a mixture of 0.4 L Moddus M and 1.25 L CCC 750 per ha.

No yield response was reached in lawn grass varieties, short straw and semi-long varieties. 70 kg nitrogen per ha was applied in autumn, followed by 30-120 kg nitrogen per ha in spring. The growth regulation was conducted during good growth conditions from beginning of elongation to beginning of earing. In 2005 and 2006 a trial plot was established with and without growth regulation, including application of 190 kg nitrogen per ha in all.

White clover - growing it in competition

Nick Pyke¹⁾, Tabitha Armour¹⁾, Richard Chynoweth¹⁾ & Bede McCloy²⁾

¹⁾Foundation for Arable Research, PO Box 80, Lincoln, New Zealand

²⁾New Zealand Arable, PO Box 16101, Hornby, Christchurch, New Zealand

pyken@far.org.nz

ABSTRACT

White clover (*Trifolium repens* L.) seed yields are extremely variable and the management practices used by growers are differ markedly. Thus when farmers achieve good yields they often are unable to determine the management practices which contributed to these good yields. For two seasons FAR has run a competition between groups of growers to produce a plot of white clover using the groups agreed crop management strategy. In year one each of the five groups had an irrigated and dryland white clover plot at the same site. In the second year each of the four groups grew an irrigated plot of white clover each. The inputs (costs and timings) are assessed and crop yields and net returns per hectare were determined. Yields, costs of growing the crop and returns per hectare varied markedly between the grower groups. In year one dryland yields ranged from 250 to 550kg/ha and irrigated yields ranged from 760 to 930kg/ha. Margins ranged from \$220 NZ to \$3700 NZ per ha. This project has been an excellent method for discussing white clover production and management with growers.

Key words: herbicides, irrigation, profitability seed yield, *Trifolium repens*

INTRODUCTION

White clover (*Trifolium repens* L.) is an important seed crop in New Zealand's arable production. The seed produced is essential for the New Zealand pastoral industry and New Zealand also exports NZ\$20 million per annum of clover seed. In addition clover is valuable in arable crop rotations through its restorative properties and providing valuable grazing on mixed cropping farms. Although white clover is a very important seed crop, most of the research has focussed on pasture production and white clover in a pasture sward.

Research on seed production has concentrated on the influence of plant growth regulators (Budhianto *et al.* 1995), nutrient requirements of the crop (Clifford & Rolston 1990), change of cultivar (Clifford *et al.* 1995), insect pests (Schroeder & Chapman 1995), and physiological impacts on seed set (Thomas 1996, Thomas & Pasumarty 1996). More recently industry funded research has concentrated on weed control in white clover (FAR 1997, 1999, 2001, 2002). The influence

of water deficit on seed production in white clover has also been evaluated by a number of researchers (Oliva *et al.* 1994, Bissuel-Belaygue *et al.* 2002, Martin *et al.* 2003). This research has been interpreted to provide irrigation guidelines for farmers (FAR 2003).

Significant yield benefits have been achieved in this research but when farmers implement these practices in the field, results are variable or are not cumulative. This project was undertaken to better define in-field responses from different management approaches and increase farmer uptake of new information through grower groups.

MATERIALS AND METHODS

Teams of growers were invited to grow plots of white clover (cultivar Tribute) at a common site (FAR Arable Site, Chertsey, New Zealand) in each of the 2005/06 (year 1) and 2006/07 (year 2) seasons. In year 1 there were five teams (Darfield, Eiffelton, Ellesmere, FAR, Methven) and each team grew an irrigated and a dryland plot of white

clover. In year 2 the four teams (Darfield, Ellesmere, FAR, Methven) grew a plot of irrigated white clover only. In each year the teams took over the white clover plots after sowing in late March / early April and were responsible for imposing their own management of the plots to achieve the best economic outcome possible. The crop was drilled in 30 cm rows into a silt loam soil to create plots that were 40m x 14.5m in size. The major difference in management practices were in the herbicides used and the timings of herbicide applications. There were some differences in the nutrients applied and the timing of nutrient applications. The site has significant weed pressure mostly from broad leaf weeds such as field pansy (*Viola arvensis*), wireweed (*Polygonum aviculare*), fumitory (*Fumaria* spp.), cornbind (*Polygonum convolvulus*) and shepherds purse (*Capsella bursa-pastoris*). In year 1 irrigated plots were irrigated twice and received 61 mm (25 mm in December and 36 mm in early January) of water at \$2.50 per mm.

All plots were sprayed with a desiccant; 2 - 3L ha⁻¹ of diquat in year 1 (irrigated plots received a higher rate) and 2-2.5 L ha⁻¹ of MCPA followed by 2.5 - 3.5 L ha⁻¹ of diquat in year 2, direct harvested on the same day each year and machine dressed. Margins were calculated using a seed price of NZ\$5.50 kg⁻¹. A standard value for harvesting of \$250 ha⁻¹ was used and a machine dressing and testing rate of \$0.50 kg⁻¹ was used for all teams.

The weather conditions differed markedly between the two years. The year 1 season was dry through the spring and summer period including through flowering and seed fill.

In year 2 there was a significant snowfall in early June which reduced crop development. This was followed by a particularly cold spell where frost heave markedly reduced the plant populations. The summer was dull and cool with regular rainfall events and no reliance on irrigation. The harvest was very late but good conditions prevailed through harvest.

This project was designed as a technology transfer project and thus there was no replication of plots and no statistical analysis of data was undertaken.

RESULTS

The management of plots varied markedly between the teams in each year. In year 1 the plots established well and increased crop vegetative growth in the irrigated plots resulted in one team electing to top the plot to manage vegetative growth. In year 2, although it was damp, crop growth was slow and, due partly to frost heave, the vegetative growth was less and no teams used topping to manage crop vegetative matter.

The major difference in management and input costs in both years was the use of and expenditure on herbicides by the teams. In year 1 there was a marked difference in the number and timing of herbicide applications, the products used (Table 1) and the expenditure on herbicides. In year 2 there were even greater differences in the number and timing of herbicide applications (Table 2). There was a tendency for the teams to use cheaper herbicide options in year 2.

Table 1: Herbicide and fertiliser inputs to white clover plots in year 1

ELLESMERE	DARFIELD	EIFFLETON	METHVEN	FAR
24-May 65g/ha Preside	1-July 1.5L/ha Jaguar	24 May 1.0L/ha Jaguar	14-Jun Inter-row spray 2.0L/ha Glyphosate + 7g/ha Escort	14-Jun Inter-row spray 1.0L/ha Glyphosate + 1.0L/ha Jaguar
15-Aug 1.5L/ha Jaguar		4-Aug 1.5L/ha Jaguar + 2 L/ha 24-D amine		24-Aug 1.5L/ha Jaguar + 0.375L/ha Gallant
	15-Sep 125kg/ha sulphur super + 10kg/ha boron	24-Aug 125kg/ha sulphur super, 10kg/ha boron, 100g/ha molybdenum	15-Sep 100kg/ha ammonium sulphate + 10kg/ha boron	
	11-Oct 65g/ha Preside + 4L/ha Tropotox			
27-Oct Inter-row: 4L/ha Buster Over row: 1.0L/ha Gallant + 0.4L/ha Spinnaker	27-Oct Inter-row: 4L/ha Buster + Contact Over row: 0.5L/ha Gallant	27-Oct Inter-row: 4L/ha Buster + Contact		28-Oct Inter-row: 4L/ha Buster Over row: 5.0L/ha Pulsar
9-Nov Topped		7-Nov 5L/ha Pulsar + 0.5L/ha Gallant		

Table 2: Herbicide and fertiliser inputs to white clover plots in year 2

ELLESMERE	DARFIELD	METHVEN	FAR
9 Jun 40 g Preside + oil		9- Jun Inter-row spray 2.0L/ha Glyphosate + 7g/ha Escort	
12-Sep 400ml Spinnaker + oil	12-Sep 1.5L/ha Jaguar		12-Sep 1.5L/ha Jaguar
1-Sep 20kg/ha Nitrogen	27 Oct 100kg/ha ammonium sulphate +10kg boron		1-Sep 125kg/ha ammonium sulphate
6-Oct 200ml Quantum + sticker		30-Oct 1.6l/ha paraquat 250	
13-Nov 1.4l/ha paraquat 250			
28-Nov Inter-row: 2L/ha Buster + Contact	28-Nov Inter-row: 4L/ha Buster + Contact Over row: 400ml Spinnaker + Contact		28-Nov Inter-row: 3L/ha Buster + Contact Over row: 400ml Spinnaker + Contact

Preside = flumetsulam; Jaguar = diflufenican + bromoxynil; Buster = glufonisate ammonium; Tropotox = MCPA +MCPB; Gallant = haloxyfop; Escort = metsulfuron-methyl; Pulsar = bentazone + MCPB; Spinnaker = imazethapyr; Quantum = diflufenican.

Table 3: Expenditure on inputs (\$ ha⁻¹)

	ELLESMERE	DARFIELD	EIFFELTON	METHVEN	FAR
Year 1 dryland	\$1214	\$1266	\$1035	\$989	\$1086
Year 1 irrigated	\$1623	\$1600	\$1480	\$1437	\$1535
Year 2 irrigated	\$1239	\$1182	-	\$1075	\$1156

Table 4. Yield (kg ha⁻¹) for different plots

	ELLESMERE	DARFIELD	EIFFELTON	METHVEN	FAR
Year 1 dryland	478	547	234	395	322
Year 1 irrigated	938	859	766	934	862
Year 2 irrigated	603	594	-	716	633

Table 5. Margins (\$ ha⁻¹) for different plots

	ELLESMERE	DARFIELD	EIFFELTON	METHVEN	FAR
Year 1 dryland	1400	1725	225	1190	640
Year 1 irrigated	3550	3200	2700	3650	3220
Year 2 irrigated	2080	2086	-	2861	2325

The differences in herbicide usage and fertiliser applied resulted in large differences in expenditure on the crop (Table 3). In year 1 expenditure on the irrigated crop was 28% more than on the dryland crop and in year 2 expenditure was 25% less than for an irrigated crop in year 1, some of this was due to not needing irrigation. The Methven team had the lowest expenditure in both the dryland and irrigated plots because they used cheaper herbicide options and used one less herbicide than most teams each year. The different herbicide treatments resulted in different weed species and weed populations being present and most teams needed to use a late herbicide. These were often applied interrow, to ensure weeds were not competitive late in the season and to control interrow volunteer clover. In some crops (particularly Eiffelton in year 1) weed competition was expected to impact negatively on yield.

Yields differed markedly between the different teams but more so between irrigated and dryland in year 1 (Table 4).

Apart from large yield differences between irrigated and dryland plots in year 1, the major contributor to difference in yield appeared to relate to weed competition and possibly to the influence of herbicides directly on white clover seed yield. In the dryland plots the low yielding plot was only 42% of the highest yield. However, the differences in yield between the low yielding plots and the highest yielding plots was smaller in the irrigated plots than the dryland plots in each year.

The differences in yield and expenditure on the crop resulted in large differences in the margins for the different teams growing white clover at the same site (Table 5). The Methven team had the best margin in the irrigated plots in both years while the Darfield team achieved the best result in the dryland situation.

DISCUSSION

Yields in year 2 were less than the irrigated plots in year 1. Cool conditions through crop establishment delayed crop growth and development. These were followed by frost heave which reduced plant populations and cool damp conditions through flowering that reduced bee activity and pollination.

There were very significant differences in the management practices of the teams of growers, all trying to grow the most profitable crop at the same site. These differences were mainly in the number and timing of herbicides used. The competition showed it is possible to get very effective control of the broad range of weed species with a number of herbicide programmes which range markedly in price. The Methven team achieved very effective weed control with just two herbicide applications, both at a relatively low cost.

There were large differences in yield between the different crops. Within the irrigated or dryland plots in any one year these differences appeared to be largely due to weed competition although some of the herbicides applied late in the season did cause minor crop damage which may have reduced yields. If growers do not have adequate weed control early in the season then they are faced with needing to use expensive late season herbicides which can cause crop damage. The reductions in seed yield in white clover due to different weed densities is not clear (FAR 2001, 2002). Therefore farmers often need to make decisions in relation to herbicide application with limited knowledge of the potential yield benefits.

Previous research (FAR 1997, 1999, 2001, 2002) has shown significant differences in yield between different herbicide active ingredients and products used in white clover crops. However, no studies have investigated the profitability of different herbicide programmes over the whole season. This extension project indicates low cost herbicide options (glyphosate + metsulfuron-methyl) applied interrow in mid-winter were a very cost effective option for the two years at this site.

The use of irrigation in year 1 markedly increased seed yields. The average yield increase of 477 kg ha⁻¹ following two irrigations was greater than the yield increase achieved by Martin *et al.* (2003) from no irrigation to highest yield which was from application every three weeks at a third of the rate required to replace the soil moisture deficit. The in-field seed yield response in this project from keeping the crop stressed through careful management of irrigation at key times supports research data (Oliva *et al.* 1994, Martin *et al.* 2003, Bissuel-Belaygue *et al.* 2002). Across the 5 plots the average yield increase was 477kg ha⁻¹ which at \$5.50/kg⁻¹ is an extra income of \$2623 ha⁻¹ for expenditure of \$152 on water (17:1 ROI).

This competition has given farmers valuable new information on profitable clover production and has also identified the need for further information on the impact of weed density on white clover seed yield, particularly late in the season, if farmers are to improve the return on investment in late season weed control.

ACKNOWLEDGEMENTS

We are grateful for the input from the teams of farmers into the competition and to FAR for funding this project.

LITERATURE

- Bissuel-Belaygue, C., Cowan, A.A., Marshall, A.H. & Wery, J. 2002. Reproductive development of white clover (*Trifolium repens* L.) is not impaired by a moderate water deficit that reduces vegetative growth. *Crop Science* 42: 414-422.
- Budhianto, B., Hampton, J.G., Hill, M.J. & Thomas, R.G. 1995. Effect of time of harvest and paclobutrazol on seed yield in white clover cv. Grasslands Pitau. *New Zealand Journal of Agricultural Research* 38: 1-6.

- Clifford, P.T.P. & Rolston, M.P. 1990. Mineral nutrient requirements for white clover seed production. *Journal of Applied Seed Production* 8: 54-58.
- Clifford, P.T.P., Sparks, G.A. & Woodfield, D.R. 1995. The intensifying requirements for white clover cultivar change. *In: Woodfield, D.R. (ed.). White clover: New Zealand's competitive edge. Agronomy Society of New Zealand special publication 11; Grassland research and practice series 6: 19-24.*
- FAR Arable Update Herbage No. 5 1997. Weed management in white clover.
- FAR Arable Update Herbage No.17 1999. Herbicides for 2nd year white clover.
- FAR Arable Update Herbage No.26 2001. Weed management in white clover.
- FAR Arable Update Herbage No.31 2002. Glyphosate for white clover management.
- FAR Arable Update Herbage No.36 2003. Irrigation of white cover seed crops.
- Martin, R.J., Gillespie, R.N., Maley, S.& Robson, M. 2003. Effect of timing and intensity of drought on the yield of white clover (*Trifolium repens* L.). *In: Proceedings 11th Australian Agronomy Society, Geelong. www.regional.org.au/au/asa/2003/c/14/martin.htm*
- Oliva, R.N., Steiner, J.J.& Young, W.C. III 1994. White clover seed production: II. Soil and plant water status on yield and yield components. *Crop Science* 34: 768-774.
- Schroeder, N. & Chapman, R.B. 1995. The impact of two insecticides on hemipteran pests and beneficial arthropods in a white clover seed crop. *In: Proceedings of the 48th New Zealand Plant Protection Conference* 48: 170-174.
- Thomas, R.G. 1996. Limitations to seed set in white clover (*Trifolium repens* L.). I. Preliminary observations. *Journal of Applied Seed Production* 14: 59-66.
- Thomas, R.G. & Pasumarty, S.V. 1996. Limitations to seed set in white clover (*Trifolium repens* L.). II. The influence of light intensity on floral development. *Journal of Applied Seed Production* 14: 67-72.

Fungicides in Czech grass seed production

Bohumír Cagaš & Radek Macháč

OSEVA PRO s.r.o., Výzkumná stanice travinářská Rožnov-Zubří, Hamerská 698,
756 54 Zubří, Česká republika

cagas@oseva.cz

ABSTRACT

The investigation into the impact of three fungicides (Amistar - azoxystrobin, Archer Top 400 EC - fenpropidin + propiconazole, and Juwel Top - epoxiconazole + fenpropimorph + kresoximmethyl) on the occurrence of fungal leaf diseases and some parameters of seed yield in 15 grass species (*Festuca rubra*, *Festuca pratensis*, *Dactylis glomerata*, *Festulolium*, *Poa pratensis*, *Phleum pratense*, *Lolium perenne*, *Trisetum flavescens*, *Arrhenatherum elatius*, *Cynosurus cristatus*, *Hoculus lanatus*, *Poa compressa*, *Poa nemoralis*, *Poa palustris* and *Agrostis gigantea*) started in the Grassland Research Station Rožnov-Zubří in 2006. The grasses were treated with fungicides at a rate of 1 L ha⁻¹ at developmental stages BBCH 51-55. The results from the first year (2006) showed that only Juwel Top decreased the attack of leaf diseases in *Dactylis glomerata* and *Poa pratensis*. Higher seed yield and higher weight of seeds per panicle were observed in *Dactylis glomerata* and *Cynosurus cristatus* after treatment with all the fungicides tested. When Amistar, Archer 400 EC and Juwel Top were applied, higher thousand-seed weight (TSW) was reported in *Festuca rubra*, *Dactylis glomerata*, *Arrhenatherum elatius* and *Poa compressa*.

Key words: fungicides, germination, grasses, seed yield, thousand-seed weight (TSW)

INTRODUCTION

Biotic stress, particularly virus and fungal diseases, is an important factor which has a negative effect on seed yield and quality in grasses grown for seed in the Czech Republic. Long-term monitoring of pest and disease occurrence in grass seed stands indicates that the most important diseases, besides parasitic *Fusarium* ear disease, are powdery mildew, rusts, ergot, leaf spots and choke mould. (Cagaš *et al.* 2006). Unfortunately, the range of registered fungicides that can be used in grasses grown for seed is very limited. After the substance based on triadimefon has been excluded, growers may use only the preparations Tilt 250 EC and Bumper 25 EC (propiconazole), which are registered as effective against ergot of grasses. In the year 2006, when verification experiments had been conducted for several years, azoxystrobin (Heritage) was registered but only for use in lawns against winterkill. After the year 2000 there was a high incidence of black rust of grasses in the Czech Republic, which in some varieties of perennial ryegrass considerably affected seed yield and quality. With respect

to the need to register an effective fungicide, field trials with the aim to verify the efficacy of several promising preparations in major grass species were established in the year 2005 with support of the Association of Grass and Clover Seed Growers. These preparations were also used to treat some other species grown under the program of maintenance breeding.

MATERIALS AND METHODS

The efficacy of the fungicides Amistar (azoxystrobin, 250 g a.i. L⁻¹), Archer Top 400 EC (fenpropidin + propiconazole, 275 + 125 g a.i. L⁻¹), and Juwel Top (epoxiconazole + fenpropimorph + kresoxim-methyl, 125 + 150 + 125 g a.i. L⁻¹) for use against leaf diseases and their potential effects on yield and yield components were tested in 15 grass species. The special trials with pesticides on the plots of the Grassland Research Station at Zubří involved eight grass species in the first harvest year - Kentucky bluegrass (*Poa pratensis* L.) 'Slezanka', orchard grass (*Dactylis glomerata* L.) 'Dana', intergeneric

hybrid (*Festulolium* spp.) of loloid type 'Lofa', intergeneric hybrid of festucoid type 'Hykor', red fescue (*Festuca rubra* L.) 'Tagera', timothy (*Phleum pratense* L.) 'Sobol', meadow fescue (*Festuca pratensis* L.) 'Rožnovská', and perennial ryegrass (*Lolium perenne* L.) 'Olaf'. Treatments were also made in nine local grass species grown in the maintenance breeding programme (second cropping year). The species were yellow oat grass (*Trisetum flavescens* L.) 'Rožnovský', tall meadow oat (*Arrhenatherum elatius* /L./ P. Beauv. ex J.S. et K.B. Presl) 'Rožnovský', crested dog's tail (*Cynosurus cristatus* L.) 'Rožnovská', false redtop (*Poa palustris* L.) 'Rožnovská', wood meadow grass (*Poa nemoralis* L.) 'Dekora', Canada blue grass (*Poa compressa* L.) 'Dekora', common bent grass (*Agrostis gigantea* Roth) 'Rožnovský', perennial ryegrass (*Lolium perenne* L.) 'Linar', and velvet grass (*Holcus lanatus* L.) 'Hola'. All treatments were replicated three times in 20 m² plots, the rate of all fungicides being 1 L ha⁻¹ at developmental stages BBCH 51 - 55. The control plots remained untreated. Before seed harvest the rate of infection by leaf diseases was assessed on the 20 leaves per plot) using a scale of 1 to 9 (1 equals 100 % leaf area covered with chloroses and necroses, 9 equals no infection). At the time of seed harvest, samples were taken from each treatment to determine seed yield (per sample and per plot after cutting with a small-plot harvester), seed weight and number per panicle, thousand seed weight and their germinating capacity. The differences between treatments were evaluated using analysis of variance (ANOVA).

RESULTS

Seed yield

After treatment with all the fungicides tested significant increases in seed yield were only recorded in orchard grass and crested dog's tail. After Amistar was applied, insignificant increase in yield was found in red fescue, *Festulolia* 'Hykor', yellow oat grass, velvet grass, perennial ryegrass 'Linar', Canada blue grass, and false redtop. After treatment with Archer Top 400 EC insignificantly higher seed yield was reported in red fescue, *Festulolia* 'Hykor', yellow oat grass, velvet grass and false redtop. Similarly, insignificant increase

in seed yield was evident after treatment with Juwel Top in red fescue, *Festulolia* 'Hykor', yellow oat grass, tall meadow oat, velvet grass, perennial ryegrass 'Linar', Canada blue grass, wood meadow grass, and common bent grass (Tables 1 and 2).

Seed weight per panicle (ear)

Like seed yield, significantly higher seed weight per inflorescence was reported in orchard grass and crested dog's tail after treatment with all the three fungicides tested. After Amistar was applied, insignificant increase in this parameter was recorded in red fescue, *Festulolia* 'Hykor', yellow oat grass, velvet grass, perennial ryegrass 'Linar', Canada blue grass and false redtop. After the application of Archer Top 400 EC insignificant increase in seed weight per inflorescence was recorded in red fescue, *Festulolia* 'Hykor', yellow oat grass and velvet grass. The application of the fungicide Juwel Top resulted in insignificant increase in seed weight per inflorescence in red fescue, *Festulolia* 'Hykor', yellow oat grass, tall meadow oat, velvet grass, perennial ryegrass 'Linar', Canada blue grass and common bent grass (Tables 1 and 2).

Thousand seed weight

After treatment with the three fungicides significant increase in thousand-seed weight was recorded in orchard grass. Treatment with Amistar resulted in significant increase in this parameter in red fescue. Similarly, after treatment with Archer Top 400 EC thousand seed weight increased in Canada blue grass and after treatment with Juwel Top in tall meadow oat. Significant decrease in thousand-seed weight was reported in velvet grass after treatment with Archer Top 400 EC (Tables 1 and 2).

Germinating capacity

In most species germinating capacity was not affected by fungicides, only in creeping bent was there a significant decrease in this parameter after the preparations Archer 400 EC and Juwel Top had been applied. On the contrary, increased germinating capacity was observed in false redtop after treatment with Archer 400 EC. In Canada bluegrass extremely low germinating capacity was recorded in all treatments, including the untreated control.

Table 1. Effect of selected fungicides on seed yield (kg ha^{-1}), seed yield per panicle (g), thousand seed weight (TSW, g) and germinating capacity (%) in red fescue cv. Tagera, orchard grass cv. Dana, meadow fescue cv. Rožnovská, perennial ryegrass cv. Olaf, timothy grass cv. Sobol, Kentucky bluegrass cv. Slezanka, Festulolia cv. Hykor and Festulolia cv. Lofa in the first harvest year.

Grass species	Parameter	Fungicide			Untreated	LSD
		Amistar	Archer Top 400 EC	Juwel Top		
<i>Festuca rubra</i> Tagera	seed yield	1364.7	1078.4	1349.6	1261.7	ns
	seeds/panicle	0.093	0.073	0.092	0.086	ns
	TSW	1.3727*	1.3224	1.3430	1.2851	0.0658
	germinating capacity	95.0	91.5	93.0	91.5	ns
<i>Festuca pratensis</i> Rožnovská	seed yield	1086.0	1194.5	1135.8	1153.0	ns
	seeds/panicle	0.126	0.139	0.132	0.134	ns
	TSW	2.3745	2.4140	2.5125	2.4765	ns
	germinating capacity	95.5	93.5	92.0	97.8	ns
<i>Dactylis glomerata</i> Dana	seed yield	1541.7**	1345.6**	1264.9**	848.8	341.5
	seeds/panicle	0.300**	0.262**	0.242**	0.165	0.066
	TSW	1.2281**	1.1435	1.2434**	1.1422	0.0786
	germinating capacity	95.0	91.5	93.0	93.0	ns
<i>Festulolium</i> Hykor	seed yield	1621.8	1499.2	1680.3	1356.3	ns
	seeds/panicle	0.311	0.288	0.323	0.260	ns
	TSW	2.7490	2.5195	2.6170	2.6596	ns
	germinating capacity	95.0	95.3	92.0	96.3	ns
<i>Festulolium</i> Lofa	seed yield	968.3	1101.1	1096.9	1251.8	ns
	seeds/panicle	0.106	0.121	0.120	0.137	ns
	TSW	3.5720	3.5568	3.5079	3.5730	ns
	germinating capacity	95.0	95.3	92.0	94.3	ns
<i>Poa pratensis</i> Slezanka	seed yield	415.3	331.1	419.8	504.6	ns
	seeds/panicle	0.056	0.044	0.056	0.068	ns
	TSW	0.2660	0.2610	0.2617	0.2599	ns
	germinating capacity	17.0	13.8	20.0	14.3	ns
<i>Phleum pratense</i> Sobol	seed yield	776.4	606.2	760.5	827.3	ns
	seeds/panicle	0.111	0.086	0.108	0.118	ns
	TSW	0.4142	0.4112	0.4293	0.4342	ns
	germinating capacity	93.3	93.3	91.5	96.5	ns
<i>Lolium perenne</i> Olaf	seed yield	612.6	461.2	441.3	656.8	ns
	seeds/panicle	0.046	0.035	0.033	0.049	ns
	TSW	2.1014	2.0813	2.0762	2.0449	ns
	germinating capacity	97.5	98.3	97.0	96.0	ns

Table 2. Effect of selected fungicides on seed yield (kg ha^{-1}), seed weight per panicle (g), thousand seed weight (g) and germinating capacity (%) in yellow oat grass cv. Rožnovský, tall meadow oat cv. Rožnovský, crested dog's tail cv. Rožnovská, velvet grass cv. Hola, perennial ryegrass cv. Linar, Canada bluegrass cv. Razula, wood meadow grass cv. Dekora, false redtop cv. Rožnovská and common bent-grass cv. Rožnovský in the second harvest year.

Grass species	Parameter	Fungicide			Untreated	LSD
		Amistar	Archer Top 400 EC	Juwel Top		
<i>Trisetum flavescens</i> Rožnovský	seed yield	409.0	454.1	410.4	367.4	ns
	seeds per panicle	0.033	0.037	0.033	0.030	ns
	TSW	0.2815	0.2463	0.2515	0.2346	ns
	germinating capacity	95.3	93.0	87.3	94.3	ns
<i>Arrhenatherum elatius</i> Rožnovský	seed yield	633.7	681.1	790.0	775.5	ns
	seeds per panicle	0.109	0.117	0.136	0.134	ns
	TSW	3.6015	3.7179	4.1669**	3.7284	0.4304
	germinating capacity	96.3	95.0	95.8	96.5	ns
<i>Cynosurus cristatus</i> Rožnovská	seed yield	450.4*	478.1*	478.4*	318.8	127.2
	seeds per panicle	0.033*	0.035*	0.035*	0.023	0.009
	TSW	0.4606	0.4429	0.4353	0.4356	ns
	germinating capacity	94.0	90.5	90.0	89.8	ns
<i>Holcus lanatus</i> Hola	seed yield	90.6	94.7	104.7	82.8	ns
	seeds per panicle	0.037	0.038	0.042	0.034	ns
	TSW	0.3328	0.3064**	0.3449	0.3485	0.0286
	germinating capacity	74.0**	91.8	91.3	86.5	7.8
<i>Lolium perenne</i> Linar	seed yield	576.8	464.4	501.4	467.5	ns
	seeds per panicle	0.044	0.036	0.038	0.036	ns
	TSW	1.4543	1.3573	1.4866	1.4403	ns
	germinating capacity	97.5	95.8	96.3	96.3	ns
<i>Poa compressa</i> Razula	seed yield	725.3	530.6	641.6	630.1	ns
	seeds per panicle	0.027	0.020	0.024	0.023	ns
	TSW	0.1478	0.1618*	0.1499	0.1460	0.0
	germinating capacity	1.3	0.5	3.0	1.0	ns
<i>Poa nemoralis</i> Dekora	seed yield	131.5	120.8	135.7	134.2	ns
	seeds per panicle	0.017	0.015	0.017	0.017	ns
	TSW	0.2569	0.2416	0.2366	0.2447	ns
	germinating capacity	89.8	81.0	79.3	85.0	ns
<i>Poa palustris</i> Rožnovská	seed yield	140.0	76.8	80.7	87.2	ns
	seeds per panicle	0.030	0.016	0.017	0.019	ns
	TSW	0.1596	0.1433	0.1456	0.1536	ns
	germinating capacity	32.3	51.5*	28.0	35.3	14.9
<i>Agrostis gigantea</i> Rožnovský	seed yield	185.7	166.1	261.9	196.9	ns
	seeds per panicle	0.018	0.016	0.025	0.019	ns
	TSW	0.1057	0.0982	0.1059	0.1053	ns
	germinating capacity	45.3	34.8*	38.8*	56.5	17.4

Table 3. Effect of selected fungicides on the rate of infection by mycoses (1-9, where 1 equals 100 % of leaf area covered with chloroses and necroses and 9 equals no infection) in red fescue cv. Tagera, orchard grass cv. Dana, meadow grass cv. Rožnovská, perennial ryegrass cv. Olaf, timothy grass cv. Sobol, Kentucky bluegrass cv. Slezanka, Festulolia cv. Hykor and Festulolia cv. Lofa in the first harvest year.

Grass species	Fungicide			Untreated	LSD
	Amistar	Archer Top 400 EC	Juwel Top		
Festuca rubra	5.43	4.75	5.45	3.90	ns
Tagera					
<i>Festuca pratensis</i>	6.85	6.63	5.90	6.25	ns
Rožnovská					
<i>Dactylis glomerata</i> Dana	5.48	4.83	6.90**	4.40	1.60
Festulolium					
Hykor	6.40	6.30	6.48	6.03	ns
<i>Festulolium</i>					
Lofa	7.58	7.15	7.58	7.15	ns
<i>Poa pratensis</i> Slezanka	4.48	4.80	6.55**	4.45	2.04
Phleum pratense					
Sobol	7.03	6.58	7.30	7.05	ns
Lolium perenne					
Olaf	3.55	4.15	4.28	3.15	ns

Diseases

A significantly lower rate of leaf spot infection was found in orchard grass and Kentucky bluegrass after treatment with Juwel Top. Insignificant effects of the other two fungicides were reported in eight grass species (Table 3).

DISCUSSION

The registration of one or more effective fungicides in the Czech grass seed production is very urgent. The main reason is a greater incidence of *Puccinia graminis* subsp. *graminicola* Urban in recent years and also annual occurrence of various leaf spots (*Drechslera* spp., *Mastigosporium* spp., *Pyrenophora* spp., *Rhynchosporium* spp., *Scolecotrichum* spp.). As evident from the results obtained in the year 2006 when there was a high incidence of black rust of grasses in several localities, the harmfulness of this pathogen seems to be very high. For example, in cv. Bača yield decreased by 42 % and the thousand-seed weight dropped by 1.61 g. Therefore, searching for an appropriate preparation and an optimum date of treatment is now quite common in many countries (Gingrich & Mellbye 2004). However, similar data on damage from leaf spots caused by different pathogens are missing. This often leads to underestimation of the danger and consequently to little

concern of chemical companies to register fungicides which would be effective in grass seed production. Moreover, there are no data on the effect of fungicides on seed yield and its parameters under the conditions of slight infection pressure or even in the absence of the disease.

The results from the tests of three different fungicides in 15 grass species in the year 2006 when there was no incidence of black rust of grasses in the locality Zubří showed that, especially in some grass species, fungicide treatments are absolutely necessary. Treatment with Juwel Top significantly affected the rate of occurrence of leaf spots in orchard grass and Kentucky bluegrass (Table 3). The results suggest that the leaf disease and consequently the loss of leaf functionality in orchard grass have a strong effect on seed yield and seed weight per inflorescence. In this grass species the application of all the preparations tested increased yield, seed weight per inflorescence and also thousand-seed weight. The same applies to crested dog's tail. There was no marked incidence of leaf spots, but violet coloration of leaves and stems of unknown origin was observed (Tables 1 and 2). Fluctuations in germinating capacity cannot be seriously commented now. Insignificant increase in yield after fungicide treatment was also observed in other grass species. After one-year tests it is, however, too early to reach an unequivocal conclusion.

The first year of tests of fungicides on grasses grown for seed showed especially in some grass species that treatment with these preparations will be desirable (orchard grass), even in years when there is no considerable infection pressure of diseases. A high incidence of black rust of grasses in perennial ryegrass in different localities of the Czech Republic also stresses the need for fungicide

treatments. Tests of fungicides will continue in the next two years.

ACKNOWLEDGEMENTS

These experiments were carried out only thanks to support provided by the Association of Grass and Clover Seed Growers.

LITERATURE

- Cagaš B., Macháč J., Frydrych J. & Macháč R. 2006. Occurrence of Biotic Harmful Agents in Czech Grass Seed Production (1995 - 2004). *Plant Protection Science* 42 (2): 58 - 65.
- Gingrich G. A. & Mellbye M. E. 2004. The effect of fungicides on seed yield and economic returns in perennial ryegrass. In: Young, W.C. III (ed.). *Seed production research at Oregon State University USDA-ARS cooperating. Department of Crop and Soil Science Ext/CrS 124, 4/05, 7 - 9.*

Plant growth regulators and insect control in seed production of red clover (*Trifolium pratense*)

John Ingar Øverland¹⁾ & Trygve S. Aamlid²⁾

¹⁾ Vestfold Agricultural Extension Group, Gjennestadtunet 83, N-3160 Stokke, Norway

²⁾ The Norwegian Institute for Agricultural and Environmental Research, Bioforsk Øst Landvik, Reddalsveien 215, N-4886 Grimstad, Norway

john.ingar.overland@lfr.no

ABSTRACT

The application of plant growth regulators (PGRs) and insecticides, either alone or in tank mixture, was tested in nine on-farm trials laid out according to three different experimental plans in SE Norway (59-61°N) from 2002 to 2006. The first plan compared the PGRs trinexapac-ethyl (125 or 250 g a.i. ha⁻¹), chlormequat chloride (2000 g a.i. ha⁻¹) and ethephon (240 g a.i. ha⁻¹) applied on two dates and compared with an untreated check. On average for three trials, seed yield of the diploid cv. Nordi (origin 61°N) increased 15 % by application of trinexapac-ethyl (250 g a.i. ha⁻¹) at stem elongation, decreased 18 % by application of ethephone at flower bud emergence, and was not significantly affected by any of the other treatments. The diploid cv. Bjursele (two trials) and the tetraploid cv. Betty (one trial) showed no response to any of the PGRs. In the second experimental plan, laid out in two seed crops of the diploid cv. Lea in 2005, trinexapac-ethyl (125 or 250 g a.i. ha⁻¹) was applied alone or in tank mixture with the insecticides alpha-cypermethrin (20 g a.i. ha⁻¹) or dimethoate (500 g a.i. ha⁻¹) on two dates. These trials verified the positive effect of trinexapac-ethyl, but the insecticides had no significant effect, which is not surprising as no harmful insects were detected. The third experimental plan compared insecticides at two developmental stages in a seed crop of cv. Lea with a high occurrence of *Apion* seed weevil and the lesser clover leaf weevil *Hypera nigrirostris*. In this trial, alpha-cypermethrin increased seed yield by 14 % when applied at flower bud emergence. Effective from 2004, Moddus 250 EC (trinexapac-ethyl) was approved in Norwegian seed production of selected cultivars of red clover. Research is still underway to evaluate the need for control of various weevils.

Key words: Alpha-cypermethrin, *Apion* sp., chlormequat chloride, dimethoate, ethephon, *Hypera nigrirostris*, trinexapac-ethyl, weevils.

INTRODUCTION

With an annual acreage of 3-400 ha, red clover (*Trifolium pratense*) is the most important legume in Norwegian herbage seed production. While the use of plant growth regulators (PGRs) has become common practice in seed production of most grasses, there has been little research into the use of growth regulators in clover seed production. Unlike the situation in Denmark and many other countries, control of weevils and other insects has also not been recommended as a

standard practise in our country. As the optimal application time may well coincide, this paper reports results from field trials with PGRs and insecticides in Norwegian seed crops of red clover.

MATERIALS AND METHODS

A total of nine on-farm field trials were carried out from 2002 to 2006 according to Good Experimental Practise (GEP) standard by the Farmers' Extension Service groups in SE

Norway. Experimental plans were developed, seed yields cleaned, and statistical analyses performed by The Norwegian Institute for Agricultural and Environmental Research, Landvik.

RESULTS

Experimental plan 1: Plant growth regulators (PGRs)

The experimental plan used in 2002 and 2003 included three different PGRs but no insecticides. The diploid cv. Nordi responded

with 15 and 9 % seed yield increase when trinexapac-ethyl (Moddus 250 EC) was applied at 250 g a.i. ha⁻¹ at stem elongation or flower bud emergence, respectively (Table 1). The seed yield increase was accompanied by a reduction in thousand seed weight and plant height at flowering. Ethephon (Cerone) had a negative impact on seed yield, especially when applied at bud emergence. The diploid cv. Bjursele (two trials) and the tetraploid cv. Betty (one trial) showed no plant height or seed yield response to any of the PGRs (data not shown).

Table 1. Seed yield (corrected to 100% purity and 12% seed water content), plant height at flower buds emergence and flowering, and thousand seed weight as affected by various PGRs. Mean of three trials in cv. Nordi, 2002-2003.

PGR	Rate, g a.i. ha ⁻¹	Time of application	Seed yield, mean of three trials		Plant height at bud emergence cm	Plant height at flowering cm	1000 seed weight mg
			kg ha ⁻¹	Rel.			
1 Untreated	-	-	528	100	76	119	1697
2 Chlormequat-chloride	2000	Stem elongation	509	96	75	110	1690
3 Ethephon	240	(BBCH	487	92	72	106	1691
4 Trinexapac-ethyl	125	32-33)	549	104	69	112	1665
5 Trinexapac-ethyl	250		607	115	63	100	1656
6 Chlormequat-chloride	2000	Bud emergence	542	103	76	114	1739
7 Ethephon	240	(BBCH	435	82	77	106	1633
8 Trinexapac-ethyl	125	50-51)	548	104	76	111	1679
9 Trinexapac-ethyl	250		578	109	77	99	1571
<i>P</i> -value			<0.01	-	<0.001	<0.05	<0.05
LSD (<i>P</i> <0.05)			66	-	4	12	76

Experimental plan 2: Trinexapac-ethyl in combination with insecticides

Trinexapac-ethyl was combined with the pyrethroid alpha-cypermethrin or the organophosphate dimethoate in two trials in the diploid cv. Lea in 2005. The early application of trinexapac-ethyl increased seed yield significantly in the trial at Hedmark and showed a similar tendency (*P*=0.14) in the trial at Vestfold (Table 2).

A tank-mixture of trinexapac-ethyl and insecticide showed no significant seed yield increase compared to the PGR applied alone. This is not surprising as no harmful insects was discovered at the time of spraying, nor were there any damages on the leaves or the seeds which could indicate the presence of insect pests (data not shown in table).

Table 2. Effect of trinexapac-ethyl used alone or in tank mixture with insecticides, on seed yield (corrected to 100% purity and 12% seed water content) and plant height at bud emergence in two trials in cv. Lea, 2005.

PGR / insecticide	Rate, g a.i. ha ⁻¹	Time of application	Location: Vestfold kg ha ⁻¹	Seed yield, kg ha ⁻¹		Rel.	Plant height at bud emergence, cm
				Location: Hedmark kg ha ⁻¹	Mean of two trials kg ha ⁻¹		
1 Untreated	-	-	540	211	376	100	80
2 Trinexapac-ethyl	125	Stem elongation	616	262	439	117	71
3 Trinexapac-ethyl	250	elongation	636	369	503	134	65
4 Trinexapac-ethyl + alpha-cypermethrin	250 + 20	(BBCH 32-33)	607	338	472	126	66
5 Trinexapac-ethyl + dimethoate	250 + 500		685	360	523	139	66
6 Trinexapac-ethyl	125	Bud emergence	624	305	465	124	77
7 Trinexapac-ethyl	250	emergence	685	230	458	122	79
8 Trinexapac-ethyl + alpha-cypermethrin	250 + 20	(BBCH 50-51)	724	208	466	124	78
9 Trinexapac-ethyl + dimethoate	250 + 500		717	270	493	131	78
<i>P</i> -value			0.14	<0.01	>0.20		<0.05
LSD (<i>P</i> <0.05)			-	82	-		9

Experimental plan 3: Insecticides

In one trial conducted in cv. Lea in 2006, both the red clover seed weevil (*Apion* sp.) and the lesser clover leaf weevil (*Hypera nigrirostris*) could easily be found by the time of stem elongation. Cleaning of seed from the 6.6 x 1.5 = 9.9 m² experimental plots showed a significant seed yield increase from the late

application of alpha-cypermethrin, and this tended to be confirmed (*P*=0.13) by an independent determination of seed in samples of 50 flower heads collected randomly in each plot before harvest (Table 3). However, an assessment of the proportion of cleaned seed with visible insect damage showed no significant difference among treatments.

Table 3. Seed yield (corrected to 100% purity and 12% seed water content), average seed yield per flower head as determined independently in 50 heads, and percentage of cleaned seeds with visible insect damage. Results from one trial in cv. Lea, 2006.

Insecticide	Rate, g a.i. ha ⁻¹	Time of application	Seed yield		Seed yield pr head, mg	Seeds with insect damage, weight %
			Kg ha ⁻¹	Relative		
1 Untreated	-	-	927	100	154	1.3
2 Alpha-cypermethrin	20	Stem elongation (BBCH 32-33)	988	107	155	0.3
3 Dimethoate	500		865	93	149	0.3
4 Alpha-cypermethrin	20	Bud emergence (BBCH 50-51)	1056	114	164	1.0
5 Dimethoate	500		898	98	146	0.7
<i>P</i> -value			<0.01		13	>20
LSD (<i>P</i> <0.05)			89		-	-

DISCUSSION

Stem elongation and flowering of northern cultivars of red clover are long-day responses (Lunnan 1989). The first of these processes is directly mediated by the plant hormone gibberellic acid (GA). GA is also important for flowering control, although its exact role is not completely understood (Heide 1994). Trinexapac-ethyl is a plant growth regulator inhibiting the last step in GA biosynthesis, i.e. the conversion of inactive GA₂₀ to the bioactive form GA₁, and its positive effect on red clover plant height and seed yield is in line with results obtained with most grasses originating at high latitudes (Aamlid 2003). On average for five trials in cvs. Nordi and Lea, trinexapac-ethyl applied at 250 g a.i. ha⁻¹ at stem elongation produced a significant ($P < 0.01$) seed yield increase of 21% (weighed mean of Tables 1 and 2). As the costs for application of trinexapac-ethyl at 250 g a.i. ha⁻¹ is less than the price of 2 kg seed of cvs. Nordi and Lea, there is no question about the profitability of using trinexapac-ethyl routinely in seed production of these red clover cultivars. Based on these trials, red clover seed crops were included on the Norwegian label for Moddus 250 EC in 2004.

Cvs. Nordi and Lea are both diploid, highly productive cultivars, mostly selected from germplasm collected in SE Norway (60-61° N). It is surprising that the effect of trinexapac-ethyl on stem elongation or seed production in these cultivars was not replicated in cv. Bjursele or its chromosome-doubled counterpart cv. Betty which both originate in Northern Sweden (65° N) and are used in seed mixtures for higher latitudes and altitudes in Scandinavia. One explanation for this result might be that the endogenous level of GA is lower in cvs. Bjursele and Betty than in cvs. Nordi and Lea when grown for seed at 16-17 hour photoperiod in Southern Scandinavia. In this line, Lunnan (1989) documented that shoot growth declined more rapidly in cv. Bjursele than in cv. Molstad (predecessor of cvs. Nordi and Lea) when the cultivars were transferred from continuous light to 8 h photoperiod.

Chlormequat-chloride is also a gibberellic acid biosynthesis inhibitor that has been documented to have a small positive effect on red clover seed yields when applied at early flower emergence (Niemelainen 1987).

However, unlike trinexapac-ethyl, chlormequat-chloride blocks an early step in GA biosynthesis, and its effect is therefore less specific. In our trials with cvs. Nordi and Lea, seed yield increases after application of chlormequat-chloride were not consistent, and this PGR has therefore never been labelled for red clover seed crops in Norway. As for ethephon, the negative impact of on red clover seed yield (Table 1) is in agreement with Niemelainen (1987) who also documented later flowering and reduced seed yields after application of this compound.

Although no systematic attempt was made to determine seed yield components, the significantly lower thousand seed weight on plots sprayed with trinexapac-ethyl is probably an indication of more developing seeds and thus a higher competition for limited resources on sprayed than on unsprayed plots. This might be due to more flower heads and/or more florets per head, but it is perhaps more likely that trinexapac-ethyl had a positive effect on seed set. This could, among other reasons, be due to a reduction in corolla tube length and thus easier pollination by insects, as documented for the PGR daminozide (e.g. Puri & Laidlaw 1983).

Experimental plan 2 and 3 examined the use of insecticides for pest control. In Norway the red clover seed weevil (*Apion apricans* and perhaps other *Apion* species) might cause some damage, but so far, it has not been considered a serious problem. The same applies to the southernmost part of Sweden where Folkesson (2006) recently documented severe invasion of *Apion* sp. in only 10% of the red clover seed crops as opposed to 80 % of the white clover (*Trifolium repens* L.) seed crops.

At present, insect control in red clover seed crops is focusing on the lesser clover leaf weevil (*Hypera nigrirostris*), of which cocoons (parasited pupae) were found in many Norwegian seed lots from 2005 and 2006. Danish studies indicate that one larva of *Hypera nigrirostris* will cause the same reduction in white clover seed yield as ten larvae of *Apion* sp. (L.M. Hansen, pers. comm., Hansen & Boelt 2006), and this may well be correct also for red clover.

The reason why no weevils were found in the combined PGR / insecticide trials in 2005, but both insects in the insecticide trial in 2006 might be that the mean temperature for May-July was about 1.5°C higher in 2006 than in 2005. Many seed growers are now speculating whether the climate change, with warmer and dryer summers and milder and wetter winters, may cause more invasion of red clover seed weevils and especially the lesser clover leaf weevil in SE Norway. Traditionally,

most growers have been reluctant to spray their seed crops with insecticides shortly before flowering as this may repel pollinating insects, but the results from 2006 shows this effect will be more than compensated by the control of weevils and other harmful insects. Research is currently underway to predict the invasion of the red clover seed weevil and the lesser clover leaf weevil in Norwegian seed crops of red clover.

LITERATURE

- Aamlid T.S. 2003. Effects of trinexapac-ethyl (Moddus) in seed production of eight temperate grasses. *In: Herbage Seeds in the New Millennium - Fifth International Herbage Seed Conference, Gatton, Australia 23-26 November 2003.* pp. 170-175.
- Folkesson Ø. 2006. Monitoring and control of clover seed weevil (*Apion* spp.) in white clover and red clover seed in southern Skåne. *In: NJF Seminar 395. Herbage Seed Production.* Contribution no 2.5G (Unpag.).
- Hansen L.M. & Boelt, B. 2006. Så er kløversnudebillerne her igjen. *Frøavlren 89 (6): 4.* (In Danish).
- Heide, O.M. 1994. Control of flowering and reproduction in temperate grasses. *New Phytologist 128: 347-362.*
- Lunnan T. 1989. Effects of photoperiod, temperature and vernalization on flowering and growth in high-latitude populations of red clover. *Norwegian Journal of Agricultural Sciences 3: 201-210.*
- Niemelainen O. 1987. The effect of some growth regulators on the seed yield of tetraploid clovers. *Journal of Applied Seed Production 5: 67 (Abstract).*
- Puri K.P & Laidlaw A.S. 1983. The effect of cutting in spring and application of Alar on red clover (*Trifolium pratense* L.) seed production. *Journal of Applied Seed Production 1: 12-18.*

Effects of trinexapac-ethyl (Moddus) in seed crops of Italian ryegrass and timothy

Georges Rijckaert

Institute for Agricultural and Fisheries Research (ILVO)
Applied Genetics and Breeding (PGV), Caritasstraat 21, B-9090 Melle, Belgium

georges.rijckaert@ilvo.vlaanderen.be

ABSTRACT

During the period 2000-2004, Italian ryegrass (3 trials) and timothy (2 trials) were the subject of plant growth regulation in seed production research in Belgium (51 °N), in order to obtain higher and more stable seed yields over the years. Relatively wet and variable weather during flowering and/or seed ripening is disadvantageous for seed yield. A more upright crop, which becomes lodged close to harvest, would be beneficial. In 2 Italian ryegrass trials (2000 and 2001), the plant growth regulator Moddus was applied at 4 rates (0-100-200-300 g a.i. ha⁻¹) and at 2 application dates corresponding to growth stages GS 31-32 and GS 33-34. In a third trial, the same doses were used alone or in combination with a fungicide. On average, the optimal dose of Moddus was 100-200 g a.i. ha⁻¹, depending on growing conditions. Over a rather wide growth stage i.e. GS 31-34, the mean optimal seed yield increased by 17 % (192 kg ha⁻¹). The higher dose (300 g ha⁻¹) was irrelevant, because of heavy crop inhibition and higher crop heterogeneity that made crop evaluation very difficult at field crop inspection.

Two similar trials (2001 and 2004) with timothy indicated an optimal dose of 100-300 g a.i. ha⁻¹, preferably applied at the growth stage GS 32-33. Mean optimal seed yield increased by 20 % (208 kg ha⁻¹). Based on the above trials (Italian ryegrass) and the results from UK and Holland, Moddus has been authorised for growth regulation in C1-seed crops of all ryegrass species in Belgium since 2002.

Key words: growth regulation, lodging, seed production

INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam.) is the most important grass species grown for seed in Belgium. Over the last two years (2005 and 2006) Italian ryegrass seed crops represented on average 2097 ha, i.e. 54 % of the total grass seed area. Italian ryegrass seed crops are primarily managed as a combined forage-seed production system, with a forage cut taken during May, and after regrowth, a second cut taken for seed. This intensive production system, mostly concentrated on dairy farms and mixed farms in Belgium, together with a large input of manure, could still be more intensified in favour of the seed yield potential by the use of synthetic, foliar plant growth regulators (PGR).

Around the turn of the century a lot of growth regulation research on various grass species was carried out throughout the world (Borm 2000, Silberstein *et al.* 2001, Aamlid 2003, Chastain *et al.* 2003). However, Italian ryegrass was somewhat neglected in this respect. Indeed, plant growth regulators have a great potential of improving herbage seed yields by reducing lodging (at flowering) and secondary regrowth (at harvest). This may result in a higher floret site utilization and thousand seed weight (TSW). However, the simple extrapolation of results on perennial ryegrass (*Lolium perenne* L.) from abroad to Italian ryegrass would be very doubtful and not applicable because of the large differences between the two species; e.g. Italian ryegrass is an extremely fast growing, very leafy and bulky crop; secondary regrowth is also very common under the rather humid Belgian climatic conditions.

Furthermore, in 1999, the registration of trinexapac-ethyl (Moddus) was pending for use in grass seed crops in NW-Europe (UK, NL...), and Novartis Benelux (now Syngenta) was looking for more detailed research on Italian ryegrass in order to obtain an authorization or extension of the use of Moddus to all ryegrass species in Belgium. On the other hand, timothy basic seed of RvP-cultivars became a new, alternative seed crop in Belgium, however, with varying seed yields in the beginning. Timothy is very prone to lodging and to seed losses at harvest, but at the same time lodging has an extreme, negative impact on seed yield, especially under humid conditions. Therefore, a more upright crop with postponed lodging and even easier harvesting would be beneficial to stabilize the seed yield level over years.

The objectives of the present research were to determine the optimal rate and application time (growth stage) of the plant growth regulator trinexapac-ethyl (Moddus) for seed yield and related characters in Italian ryegrass and timothy.

MATERIALS AND METHODS

Five growth-regulation experiments were conducted on a sandy-loam soil at ILVO - Unit Plant Sciences - Applied Genetics and Breeding, Melle. Namely, 2 trials with Italian ryegrass and 2 trials with timothy were grown as 1st year seed crops and were sown during the previous autumn, using a self-driven precision drill (Øyord system). The fifth trial

concerned a 2nd year seed crop of Italian ryegrass that followed the trial of 2000 on the same plots. Factorial designs with application rate and date as main factors were used in the above 1st year seed crop trials, while in the 2nd year trial (2001) main factors were application rate (Moddus) and fungicide (0 or 1 L ha⁻¹ Amistar; azoxystrobin, 250 g a.i. L⁻¹). Four replications were used in all trials.

Treatments were applied using a 2.5 m wide boom sprayer (compressed air) with 5 flat nozzles - XR 11002VS; spray volume: 250 L ha⁻¹; pressure: 1.85 kg cm⁻². Details of crop management and treatments for both species are summarized in Tables 1 and 2. Treatment dates were selected to coincide with planned growth stages. Gross plot size was 2.5 x 11 m = 27.5 m² and net plot was reduced to 1.5 x 10 m = 25 m² in order to avoid border effects and possible drift effects from the applied chemicals.

When appropriate, the following parameters were evaluated: lodging, crop inhibition, height of stem and ear, ear density (0.3 m²), secondary growth at harvest and residual action of PGR treatments in the biomass. Finally, seed was harvested by cutting with a forage plot harvester (1.5 m wide) designed for efficient bagging. The harvested swath was air dried in jute bags and during the winter it was threshed and cleaned. Subsamples were tested for thousand seed weight and germination. Straw yield was also determined to give an additional evaluation for crop inhibition.

Table 1. Details of Italian ryegrass trials; cv. Bellem (2X)

	Trial n° 1 1 st year seed crop harvest 2000		Trial n° 2 1 st year seed crop harvest 2001		Trial n° 3 2 nd year seed crop harvest 2001	
<i>Time of defoliation</i>	24/05/00		23/05/01		15/05/01	
Harvest date + moisture content	9/08/00 34.8%		2/08/01 40.8%		27/07/01 33.2%	
Treatments	T1	T2	T1	T2	FO ⁽¹⁾	F1 ⁽²⁾
- planned growth stage	GS 31-32	GS 33-34	GS 31-32	GS 33-34	GS 33-34	idem
- application date	8/06/00	16/06/00	11/06/01	21/06/01	11/06/06	idem
- average n° of nodes	1.4	3.2	1.9	3.0	3.0	idem
- crop height (cm)	38.9	70.6	38.0	62.6	54	idem
PGR treatments Moddus	0 - 100 - 200 - 300 g a.i./ha					

(1) no fungicide (2) 1 L/ha Amistar = azoxystrobin 250 g/l)

Table 2. Details of timothy trials

	Trial n° 4 cv. Tibor 1 st year seed crop harvest 2001		Trial n° 5 cv. Comer 1 st year seed crop harvest 2004	
Harvest date + moisture content	30/07/01	27.0%	6/08/04	21.7%
Treatments	T1	T2	T1	T2
- planned growth stage	GS 31-32	GS 33-34	GS 31-32	GS 33-34
- application date	14/05/01	21/05/01	12/05/04	21/05/04
- average n°. of nodes	1.6	2.6	2.2	3.5
- crop height (cm)	46.1	65.4	38.0	91.0
PGR treatments Moddus	0 - 100 - 200 - 300 g a.i./ha			

Table 3. Effect of Moddus on seed yield and related characters in Italian ryegrass, 2000 (trial n° 1)

Main factor treatments	Seed yield (kg/ ha)	Straw yield DM (ton/ha)	Seeds/m ² calculated (x 1000)	TSW (mg)	Culm length ³ (cm)	Ear length ³ (cm)	Lodging ⁴ 19/07/00 (0 - 10)	Secondary growth ⁵ (1 - 5)
Rate ¹								
- 0	981 c	5.9 a	45.5 c	2157	104.8 a	20.5 a	6.8 a	5.0 a
- 100	1055 b	5.6 b	49.4 b	2140	96.0 b	18.9 ab	6.1 b	4.2 b
- 200	1158 a	5.5 b	53.9 a	2151	94.8 b	18.6 ab	5.7 c	2.9 c
- 300	1153 a	5.5 b	53.6 a	2154	89.3 c	18.4 b	5.1 d	2.5 d
Date								
- T1	1076	5.7	50.5	2135	97.2	18.8	6.0	3.9 a
- T2	1097	5.7	50.6	2166	95.2	19.4	5.8	3.4 b
LSD ²								
Rate	57	0.2	2.9	NS	4.6	1.6	0.4	0.3
Date	NS	NS	NS	NS	NS	NS	NS	0.2
Rate x date	NS	NS	NS	NS	NS	NS	0.6	0.5

¹ rate in g a.i./ha

³ just before harvest

⁵ just before harvest: 1 = very little; 5 = very much

² LSD at P<0.05

⁴ lodging score at anthesis: 0 = erect; 10 = completely lodged

Weather conditions are particularly important in the case of grass seed production, and the response to growth regulation was very dependent on the yearly growing season. The 3 seasons concerned were characterized as follows:

- 2000: growing weather in June, very wet in July with 234 mm rainfall instead of 75.1 mm (average).
- 2001: very dry during May and June with 21.4 mm and 28.4 mm, respectively (average of 60.5 mm and 67.5 mm); drought stress after treatment applications; wet during first 3 weeks of July.
- 2004: below average rainfall in April, May and June, and rather wet in July with many rainy days during the first 3 weeks.

RESULTS AND DISCUSSION

Italian ryegrass

Moddus significantly increased seed yield in all 3 trials, with optimal increases of 18% - 15.9% and 8.3% against the untreated controls, for the respective rates of 200 - 100 and 100 g a.i./ha (Tables 3 and 4). The optimal application time was less critical and the application window between GS 31 and GS 34 was appropriate. The lower seed yield (-9.3%) at T2 (trial n° 2) could be explained by the heavy drought stress after the 2nd application. In trial n° 3 (data not shown), the fungicide effect on seed yield was +10.1% (197 kg/ha) against no fungicide, without any interaction with the 4 Moddus rates.

Seed yield increases could be explained by reduced values for straw yield (except in trial n°2), for culm length, lodging and secondary growth at harvest. All parameters resulted in a more favourable micro-environment for good pollination, seed setting, seed filling, ripening and harvesting, with consequently a higher seed number per unit area (Tables 1 and 2). TSW was less affected, except in trial n° 2, where the T2-treatments had a suboptimal flowering (postponed bloom and rain), which provoked lower seed yield and higher TSW as a compensation factor. In both

trials, the anti-lodging response was most pronounced at the higher rates after the 2nd date (interaction). Despite many influencing factors for seed yield, secondary growth was the most critical parameter under Belgian climatic conditions. Linear regression analysis showed that secondary growth accounted for 40% and 34% for the variance in seed yield. Finally, there was a tendency for reduced ear length at the highest rate of Moddus. This could be a limiting factor for the potential seed yield.

Table 4. Effect of Moddus on seed yield and related characters in Italian ryegrass, 2001 (trial n°2)

Main factor treatments	Seed yield (kg/ha)	Straw yield DM (ton/ha)	Seeds/m ² calculated (x 1000)	TSW (mg)	Culm length (cm)	Ear length (cm)	Lodging 24/07/01 (0 - 10)	Secondary growth 1/08/01 (1 - 5)
Rate								
- 0	1295 c	5.98	62.9 b	2059 c	85.4 a	20.5 a	8.7 a	3.3 a
- 100	1501 b	6.02	70.9 a	2119 b	78.3 b	18.8 b	8.6 a	2.4 b
- 200	1514 b	5.76	72.6 a	2093 bc	74.9 b	18.3 b	8.2 b	1.4 c
- 300	1617 a	5.76	73.3 a	2210 a	75.6 b	17.7 b	7.8 c	1.0 c
Date								
- T1	1554 a	5.94	74.4 a	2089 b	80.2 a	19.0	8.5 a	2.0
- T2	1409 b	5.82	65.5 b	2152 a	76.9 b	18.6	8.1 b	2.1
LSD								
Rate	81	NS	3.8	47	3.6	1.2	0.2	0.7
Date	58	NS	2.7	33	2.6	NS	0.2	NS
Rate x date	NS	NS	5.3	NS	4.5	NS	0.3	NS

Table 5. Effect of Moddus on seed yield and related characters in timothy, 2001(trial n°4)

Main factor treatments	Seed yield (kg/ha)	Straw yield DM (ton/ha)	TSW (mg)	Culm length 5/07/01 (cm)	Ear length 5/07/01 (cm)	Lodging 25/07/01 (0 - 10)
Rate ¹						
- 0	1032 c	11.0	439 b	126.0 c	10.7	6.4 a
- 100	1064 bc	11.0	447 b	127.8 bc	10.6	5.6 b
- 200	1141 ab	11.3	454 b	130.2 ab	10.4	4.0 c
- 300	1218 a	11.3	473 a	132.6 a	10.3	2.6 d
Date						
- T1	1067 b	11.0 b	446 b	127.7 b	10.6	5.5 a
- T2	1161 a	11.3 a	461a	130.6 a	10.4	3.8 b
LSD						
Rate	80	NS	14	3.5	NS	0.67
Date	56	0.26	10	2.5	NS	0.48
Rate x date	112	0.53	19	NS	NS	0.95

Table 6. Effect of Moddus on seed yield and related characters in timothy, 2004 (trial n°5)

Main factor treatments	Seed yield (kg/ha)	Straw yield DM (ton/ha)	TSW (mg)	Culm length 3/08/04 (cm)	Ear length 3/08/04 (cm)	Lodging 19/07/04 (0 - 10)	Secondary growth 4/08/04 (1 - 5)
Rate ¹							
- 0	1037 c	10.8	439 b	108.3	13.4	6.9 a	4.4
- 100	1130 b	11.0	437 b	109.5	13.0	6.2 a	3.6
- 200	1141 b	10.9	438 b	112.6	12.8	5.4 c	2.6
- 300	1250 a	11.0	462 a	107.4	12.6	3.1 d	1.5
Date							
- T1	1165 a	10.9	449 a	110.2	12.8	4.8 b	2.7
- T2	1114 b	10.9	439 b	108.6	13.1	6.0 a	3.4
LSD ²							
Rate	54	NS	14	NS	NS	0.2	-
Date	38	NS	10	NS	NS	0.2	-
Rate x date	NS	NS	NS	NS	NS	0.3	-

Timothy.

In trial n° 4, seed yield in timothy was significantly increased only at the highest rate for date T1 and the 3 highest rates for date T2 (interaction - data not shown). These treatments gave an average seed yield increase of about 17% (179 kg/ha) when compared with the untreated control (Table 5). The optimal treatment was 0.4 l Moddus (100 g a.i./ha) applied at date T2 (GS 32-33). This was probably due to drought stress. In trial n° 5 (Table 6), Moddus significantly increased seed yield with increasing application rate and especially at date T1. However, the application date T1 coincided with application date T2 from trial n° 4; i.e. GS 32-33. The optimal application conditions were 1.2 l Moddus (300 g a.i./ha) applied at GS 32-33. The higher rate needed in this trial could be explained by the rather wet weather during July 2004. Other factors affected by Moddus treatments are shown in Tables 5 and 6.

Crop lodging was the most influenced parameter. The growth stage GS 32-33 was the most responsive in controlling lodging (interaction rate x date in both trials). Also the reduction of secondary growth at harvest was striking (trial n° 5). Culm length (end of anthesis - trial n° 4) was surprisingly prolonged by the use of Moddus. This could be attributed to a temporary inhibition followed by a so-called postponed cell elongation. The same phenomenon occurred for straw yield (Table 5). Ear length was not affected in any of the trials.

In conclusion, considering the very contrasting growing conditions in the research years and the limited number of trials, further seed production research will be necessary in order to establish better economic and agronomic effects for optimizing the growth regulator inputs.

LITERATURE

- Aamlid, T.S. 2003. Effects of trinexapac-ethyl (Moddus) in seed production of eight temperate grasses. In: Proc. 5th Intern. Herbage Seed Conference, Gatton, Australia: 170-175.
- Borm, G. 2004. Groeiregulator Moddus nu in alle graszaadgewassen toegelaten. Praktijkonderzoek Plant & Omgeving. <http://www.kennisakker.nl/>
- Chastain, T.G., Young, W.C. III, Garbacik, C.J. & Silberstein, T.B. 2003. Seed partitioning and yield responses to trinexapac-ethyl in perennial ryegrass. In: Proc. 5th Intern. Herbage Seed Conference, Gatton, Australia: 104-108.
- Silberstein, T.B., Young III, Chastain, T.G., & Garbacik, C.J. 2001. Responses of cool season grasses to foliar applications of Palisade (trinexapac-ethyl) plant growth regulator. In: W.C. Young III (Ed), Seed Production Research at Oregon Univ. USDA-ARS Cooperating. Depart. of Crop and Soil Science Ext/Crs, 121: 17-21.

Use of plant growth regulators on annual ryegrass: The Oregon experience

Mark E. Mellbye¹⁾, Gale A. Gingrich²⁾ & Tom B. Silberstein³⁾

¹⁾Oregon State University Extension Service, P.O. Box 765 Albany, OR, USA.

²⁾Marion Ag Service, 7746 St. Paul Hwy, St. Paul, OR USA 97137

³⁾Oregon State University Extension Service, 3180 Center St NE, Salem, OR, USA.

mark.mellbye@orst.edu

ABSTRACT

On-farm field trials were conducted to evaluate the response of annual ryegrass (*Lolium multiflorum*) to plant growth regulator application (trinexapac-ethyl and prohexadione-calcium). Rates of application and timings were comparable to usage on perennial grass seed species. Across four years of field testing, the average increase in seed yield was 10%. The response was not as consistent as that observed on perennial seed crops in Oregon.

Key words: Apogee, prohexadione-calcium, Palisade, seed production, trinexapac-ethyl

INTRODUCTION

The use of synthetic plant growth regulators (PGR) has become an accepted crop production program for many seed producers in Oregon, USA. Two products are currently registered for use on grass seed crops and are applied to fields to reduce crop lodging, facilitate swathing, and to increase seed yields. Products used are Palisade[®] (trinexapac-ethyl) and Apogee[®] (prohexadione-calcium), both foliar applied plant growth regulators with similar modes of action in plant species. During the past ten years Oregon State University (OSU) and private researchers have conducted many experimental trials with PGR products on perennial grass seed species (See OSU Seed Production Research Reports beginning in 1997 through 2004). Significant and fairly consistent results were obtained from PGR applications to perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*), and fine fescue (*Festuca rubra* var. *rubra*). Plant growth regulator (PGR) products used in the trials were either prohexadione-calcium, a dry flowable formulation (Apogee in the USA) or trinexapac-ethyl, an EC formulation (Palisade in the USA, same active ingredient as in Moddus). Applications were made using an ATV sprayer mounted with a 6.1 m boom

species with yield responses ranging from 15% to 40%. Limited work was conducted on annual ryegrass (*Lolium multiflorum*). In this article we summarize the results from seven on-farm OSU Extension Service trials designed to measure the effect of Apogee and Palisade on annual ryegrass seed yield over a range of different management conditions and years, and how responses compare with that observed in the regions perennial grass seed species.

MATERIALS AND METHODS

Large scale, on-farm seed yield trials were established on commercial annual ryegrass seed fields in Linn County between 1999 and 2006. Each trial was arranged in a randomized complete block design with three replications with the exception of the 2005 trial, which was established as a strip plot design. Individual plots were 7 m wide by 70 m to 90 m long. equipped with TeeJet 11002 VS nozzles. Application was at standard spray pressures and application volumes used in the industry (140 L ha⁻¹ and 200 k Pa).

All prohexadione-calcium treatments included methylated seed oil surfactant at 0.25 % and

the liquid nitrogen (N) at 0.5 % by spray volume. No surfactant or liquid N was applied with the trinexapac-ethyl treatments. Applications were made in mid-April, corresponding to the 2-node to early flag leaf emergence growth stages. All tests, except one 2005 field, were conducted on ‘Gulf’ annual ryegrass, the most commonly grown diploid annual ryegrass cultivar in Oregon.

Plots were swathed and combined using grower equipment to obtain a full 4.3 m swath (the common commercial windrower size) down the center of each plot. A weigh wagon was used to measure combine yields. Sub-samples of the harvested seed were collected to determine 1000 seed weight, percent cleanout, and calculate total clean seed weight.

lodging on all the annual ryegrass fields tested. In comparison to perennial species though, the effect was not as pronounced and did not carry through visually to harvest except at high rates (e.g. over 200 g a.i. ha⁻¹ trinexapac-ethyl). Seed yield response ranged from zero to 18 % over the untreated checks (Table 1). Over seven site-years, no response was observed 30 % of the time. The average seed yield across years was 2520 kg ha⁻¹ without PGR and 2800 kg ha⁻¹ with PGR with an average seed yield increase of about 10 % (250 kg ha⁻¹).

The effect on seed yield in annual ryegrass was less consistent and on average lower in comparison to perennial ryegrass, where the average response in Oregon research plots has commonly been 20% or higher.

RESULTS AND DISCUSSION

Across years, use of both PGR products provided some reduction in plant height and

Table 1. The effect of plant growth regulators on seed yield of annual ryegrass across years in trials conducted in Oregon, USA, by the Oregon State University Extension Service, 1999-2006.

Products	Rate	Year, variety, establishment, and % increase above check plot							Average
		1999 Gulf (+11%)	2002 Gulf (zero)	2002 Gulf (+15%)	2005 Tetraploid (zero)	2005 Gulf (+14%)	2006 Gulf (+18%)	2006 Gulf (+5%)	
	(g a.i. ha ⁻¹)	----- (kg ha ⁻¹) -----							
Check	0,00	2720	2670	2380	2210	1820	2650	3175	2520
Trinexapac-Ethyl (Palisade/Moddus)	106	-	-	-	-	2070	2550	3210	-
	140	-	2700	2740	-	-	-	-	-
	210	3000	-	-	-	2170	3460	3340	-
	560	-	-	-	-	-	-	3625	-
Prohexadione-Calcium (Apogee)	135	-	-	-	-	2015	2970	3412	-
	190	-	-	-	2280	-	-	-	-
	270	-	-	-	-	2070	3570	3400	-
Average of all pgr treatments over years									2810
LSD (0.05)		245	N.S.	270	N.S.	220	510	220	

In 2006, clean seed yields well above the average for the region were obtained from both annual ryegrass seed fields used in this study (over 3400 kg ha⁻¹). Under these conditions, which favored good spring growth along with significant crop lodging, the response to PGR depended on the rate of application. Interestingly, the highest seed yield was from the 560 g a.i. ha⁻¹ rate of application of trinexapac-ethyl. While not an economical treatment for farmers, the response to this high rate demonstrated crop tolerance and shed some light on why low rates of application (less than 200 g a.i. ha⁻¹) have not been very consistent on annual ryegrass: low rates may simply not provide enough effect physiologically to consistently increase seed yield or control lodging in a rapidly growing annual ryegrass seed crop.

In general, differences in percent cleanout and 1000 seed weights have been small and non-significant except at very high PGR application rates.

Applications of foliar PGR to commercial seed fields of cool season grasses grown in Oregon can result in significant increases in seed yields. There is a fairly wide variation in the amount of yield increase obtained from the application of PGR on annual ryegrass, but an average response of about 10% appears reasonable based on work conducted to date. Further evaluation of the rate of application may be needed along with testing of later timings to help improve consistency of response from PGR application on this annual grass seed species.

LITERATURE

Young, W.C. III (ed.). (1997-2004). Various Authors and reports on the response of perennial grass seed species to plant growth regulators. In: Seed Production Research at Oregon State University - USDA-ARS Cooperating. Department of Crop and Soil Science, Ext/Crs 111-123. <http://cropandsoil.oregonstate.edu/seed-ext>.

Comparison of soil N tests for prediction of spring N rate in perennial ryegrass seed production

John M. Hart¹⁾, Phil Rolston²⁾, Mark E. Mellbye³⁾, Tom B. Silberstein⁴⁾, William C. Young III¹⁾, Bede L. McCloy⁵⁾, Gale A. Gingrich⁶⁾, Neil W. Christensen¹⁾ & René Gislum⁷⁾

¹⁾Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR USA 97331

²⁾AgResearch, Private Bag 4749, Christchurch, New Zealand

³⁾Oregon State University Extension Service, PO Box 765, Albany, OR USA 97321

⁴⁾Oregon State University Extension Service, 3180 Center St NE, Salem, OR USA 97301

⁵⁾NZ Arable, PO Box 16-101, Christchurch, New Zealand

⁶⁾Marion Ag Service, 7746 St. Paul Hwy, St. Paul, OR USA 97137

⁷⁾University of Århus, Flakkebjerg, Denmark

john.hart@oregonstate.edu

ABSTRACT

Soil N supply usually requires supplementation with fertilizer to produce optimum perennial ryegrass seed yield. Spring N rate can be estimated from long-term N rate seed yield experiments, soil N measurements, tissue N measurements, or a combination of these approaches. Predicting N requirements from soil N tests is not universally successful nor are measured parameters standard. Differences in cropping sequence, weather pattern, mineral and mineralizable N between perennial ryegrass production areas create a challenge for predictive soil N measurement.

Key words: mineralizable N, nitrogen uptake, plant available N, Oregon, New Zealand

INTRODUCTION

Soil N supply usually requires supplementation with fertilizer to produce optimum perennial ryegrass seed yield. Production in Oregon, New Zealand, and northern Europe is characterized by relatively mild winters and sufficient rainfall to leach some or most all available or nitrate-N from the soil. Since winter precipitation will leach fall applied N application, most of the perennial ryegrass N requirement is added in the spring. Spring N rate can be estimated from long-term N rate seed yield experiments, measurement of soil N, measurement of tissue N, or a combination of these approaches. Predicting perennial ryegrass spring N requirement from soil N tests is not universally successful.

A useful soil test must predict the amount of nutrient needed from a rapid, easily reproducible analytical procedure and simple

sampling protocol. Most soil tests measure an extractable nutrient form that is related to the biologically available nutrient pool. Most soil tests are not a quantitative measure of nutrient content, rather an index of ability to supply nutrients.

Cations such as potassium, calcium, and magnesium are easily measured after displacement from soil colloids. Relatively insoluble nutrients such as phosphorus are difficult to measure as the soluble or available P in soil solution is replenished constantly during the growing season. Testing for nitrogen is also complex since less than 5 % of the total amount is available; soil N pools are dynamic, mediated by microbial, physical, and chemical factors.

Soil testing to predict nitrogen rate was practical only for arid and semi-arid environments until recently, when an in-

season test for nitrate in moist cool environments was developed in the eastern US (Magdoff *et al.* 1990).

Assessment of nitrogen status for western Oregon cool season crops such as wheat needed a different approach than the in-season nitrate test developed in the eastern US. The work of Christensen and others provides wheat growers a test to refine spring nitrogen rate. From a soil sample in January, the nitrogen mineralization soil test has the ability to accurately predict nitrogen fertilizer needs in spring (Christensen *et al.* 2004, Christensen & Mellbye 2006). Some growers have successfully reduced spring nitrogen rates by 50% using the test.

Grass seed growers in western Oregon and New Zealand desire a method to predict site and season specific crop N rates, especially the amount of spring N to apply. No soil test method has been able to predict spring N rate in Oregon. This paper will discuss N in perennial ryegrass grass seed production and experiences with soil N tests to predict spring N application rate in Oregon and New Zealand.

RESULTS AND DISCUSSION

Perennial ryegrass seed production N system components and amounts are presented in Table 1. Only a fraction, 1 to 5 %, of the total amount of N in the top 15 cm of soil is available to the crop in a season. Total N in the top 15 cm of western Oregon soil used for perennial ryegrass production is approximately 2600 to 3400 kg ha⁻¹. Almost double the average amount of total N found

in Oregon soil is present in New Zealand perennial ryegrass fields, 5400 kg ha⁻¹.

Using the general guideline that 1 to 5 % of the total N is available for crop production, then, 1 % N would provide 26 to 34 kg N ha⁻¹ in Oregon fields and 42 to 89 kg N ha⁻¹ in New Zealand fields. If 5 % was available then 130 to 170 kg N ha⁻¹ would be provided to the crop in Oregon and between 200 and 540 kg N ha⁻¹ in New Zealand. The higher amount is not measured in western Oregon or New Zealand perennial ryegrass fields as shown in Table 1. For Oregon, nitrogen measured in perennial ryegrass when no spring N is added varies from 23 kg N ha⁻¹ to 110 kg N ha⁻¹, with an average of 62 kg N ha⁻¹. New Zealand perennial ryegrass obtains a similar range of N from the soil as the grass does in Oregon, but the average soil contribution, 97 kg N ha⁻¹, is higher in New Zealand than Oregon. The percentage of total N for the average N supplied to perennial ryegrass is similar in NZ and OR, between 1.8 and 1.9 %.

The highest amount of soil N provided to perennial ryegrass is 5 to 8 times greater than the lowest amount. Growers would like a measure or indication of the N supply so the increasingly expensive spring N could be adjusted to take advantage of soil N supply.

Total N is not a suitable predictor for spring N need as it varies little from field to field and does not change seasonally. In addition, the spring N rate to produce optimum economic yield changes seasonally for a field. A measurement that reflects a changing soil N supply is needed.

Table 1. N system amounts or components from 22 Oregon perennial ryegrass seed fields from 10 years (27 site years) and 19 New Zealand site years.

Component	Oregon		New Zealand	
	Average -- kg ha ⁻¹ --	Range -- kg ha ⁻¹ --	Average -- kg ha ⁻¹ --	Range -- kg ha ⁻¹ --
N in aboveground biomass ¹	180	100 to 225	168	115 to 182
From soil ²	62	23 to 110	97	25 to 140
Total soil N ³	3315	2630 to 3370	5400	4190 to 8900
Available or mineral soil N ⁴				
Nitrate-N	19	5 to 36	36	17 to 112
Ammonium-N	11	7 to 40	16	4 to 46
Total	30	12 to 76	52	30 to 122

1- In aboveground biomass (straw and seed) after anthesis and before harvest at optimum yield.

2- As measured in aboveground biomass of plots not receiving N in the spring.

3- 15 cm depth (20 cm NZ). Concentration the same for 0 to 30 cm sample depth, range 0.13 to 0.2%.

4- 30 cm sample depth, range for NO₃-N 1 to 9 mg/kg and for NH₄-N 2 to 10 mg/kg.

The opposite of measuring total N is to measure only available N or mineral N, ammonium and nitrate forms. This approach is not suitable for production areas with substantial winter rainfall as little nitrate or ammonium-N is measured in spring in western Oregon where average November through February rainfall is 170 mm. Some Oregon fields will have as little as 5 kg nitrate-N ha⁻¹ in the spring (Table 1). In contrast, New Zealand winter precipitation averages 50 mm per month and the four year average mineral N was 52 kg N ha⁻¹ (Table 1).

All the ammonium reported in a soil test is not expected to be available. Estimates used in Oregon are 5 to 10 kg ammonium-N ha⁻¹ measured in a soil test not available to perennial ryegrass seed crop.

Mineral or available N is not a complete indicator of soil N supply as these forms supply less N than measured in the crop (Table 1). The average N content in aboveground biomass is 62 kg N ha⁻¹ (Oregon) and 97 kg N ha⁻¹ (NZ). This amount is double the average available or mineral N, 30 kg N ha⁻¹ (Oregon) and 52 kg N ha⁻¹ (NZ), showing mineralization provides approximately half the N from soil during season.

The remaining soil N test to be evaluated for prediction of spring N rate in perennial ryegrass is the nitrogen mineralization test that is quite successful in predicting spring N rate for soft white winter wheat in the same area as perennial ryegrass seed production for Oregon. Nitrogen mineralization is measured by incubating at 40 °C a mixture of 20 grams soil and 25 ml water for 7 days. Ammonium-N produced in this time is measured and subtracted from initial soil ammonium-N to provide amount of N mineralized.

The relationship between N mineralized during incubation and nitrogen uptake in plots with no N added is presented in Fig. 1. Oregon perennial ryegrass N content is independent of N mineralized as measured by the seven day incubation. N from soil is mineralized slowly, for a long time, or at a different rate than measured by the seven day incubation test. Curtain *et al.* (2006) report a 28 day soil incubation test at 20 °C is an excellent indicator of plant available N. Unfortunately, the length of time required for this test is not practical for production agriculture.

In contrast to the lack of relationship between mineralized N and perennial ryegrass N uptake in Oregon, mineralizable N in New Zealand increased linearly as plant N increased, Fig. 1. The slope of the line fitted to the New Zealand data supports the earlier statement that the source of approximately half the plant available N from plots with no N is N mineralized during the growing season.

The relationship of both mineral and mineralized N to plant N uptake in New Zealand allows use of these tests for prediction of spring N rate in perennial ryegrass seed production. New Zealand producers are told to aim for total spring N input of 200 kg N ha⁻¹. They should measure mineral N in top 30 cm of soil during early spring and subtract the amount measured from 200 for the spring N rate. In addition, the wheat calculator model "Sirius" is being adapted for prediction of N application in perennial ryegrass seed production. The model has been used to predict spring N requirements in several trials with reasonable results, especially by not over recommending N.

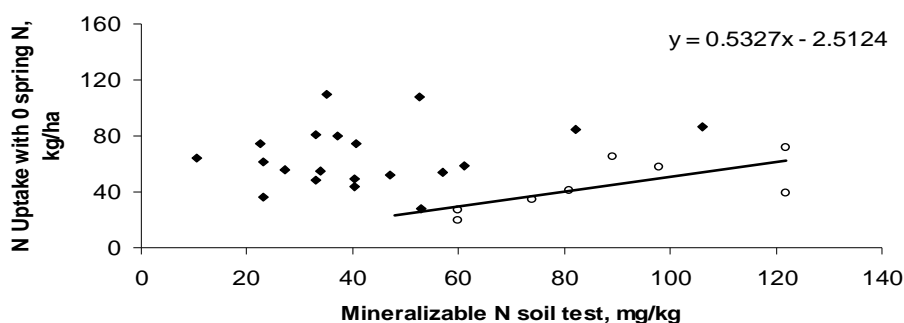


Fig. 1. N in aboveground plant material (nitrogen uptake) at anthesis as influenced by Nmin soil test from 17 Oregon sites (□) during 5 years and 11 sites from 3 years in New Zealand (◊). Regression equation and line for New Zealand data.

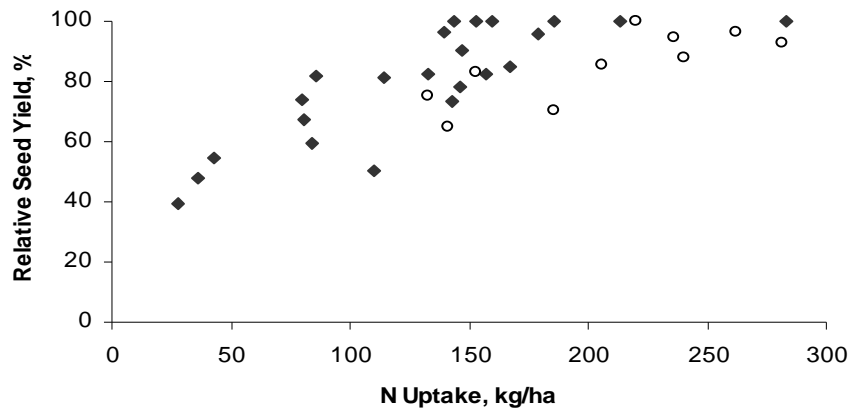


Fig. 2. The relationship of N uptake and relative seed yield of perennial ryegrass from Oregon in 2006 (\blacklozenge) and New Zealand 2003 to 2005 (\square).

A procedure that integrates available soil N and N that will become available during the growing season is not apparent for Oregon. Plants perform the integration, so use of plant N as a predictor of spring N need was pursued. In Oregon during 2006, relative seed yield increased linearly until perennial ryegrass accumulated approximately 150 kg N ha⁻¹ in the aboveground biomass, Fig. 2.

A similar relationship is found in New Zealand, but slightly more N is required, approximately 168 kg ha⁻¹ in the aboveground biomass, to attain maximum yield. The data in Fig. 2 is from a limited data set. A larger set from Oregon perennial ryegrass fields accumulated approximately 200 kg N ha⁻¹ to produce maximum yield, Fig. 3.

Since N uptake and seed yield are consistently related across sites and years, measuring

plant N during the growing season is an approach that will hopefully allow prediction of spring N addition. Rowarth *et al.* (1998) recommend tissue N of concentration of 3% be present two weeks after spikelet initiation for perennial ryegrass to have 80% relative yield. In 2006, perennial ryegrass fields were sampled about May 1. Tissue N and relative yield are shown in Fig. 4.

A tissue N of approximately 2.5% was necessary for maximum relative yield. This value is lower than tissue N concentration reported by Rowarth *et al.* (1998) as they sampled at spikelet initiation, which is at least two weeks if not a month before our sampling date in 2006. Sampling tissue in early May is probably too late to practically add N if the tissue N is not sufficient, as N uptake is highest during early stem elongation (Sullivan *et al.* 1999).

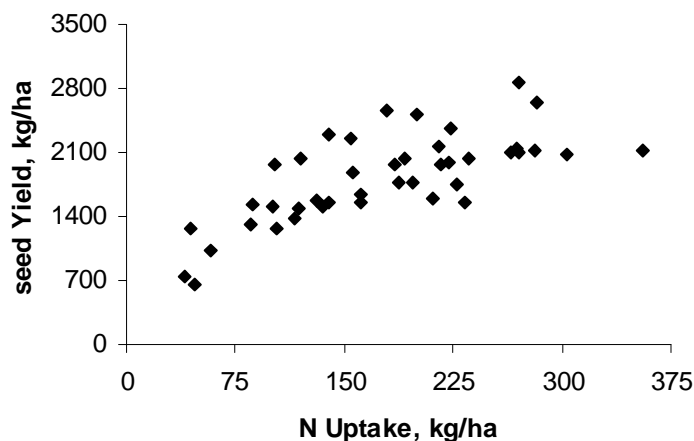


Fig. 3. The relationship of N uptake and perennial ryegrass seed yield from multiple sites for five years.

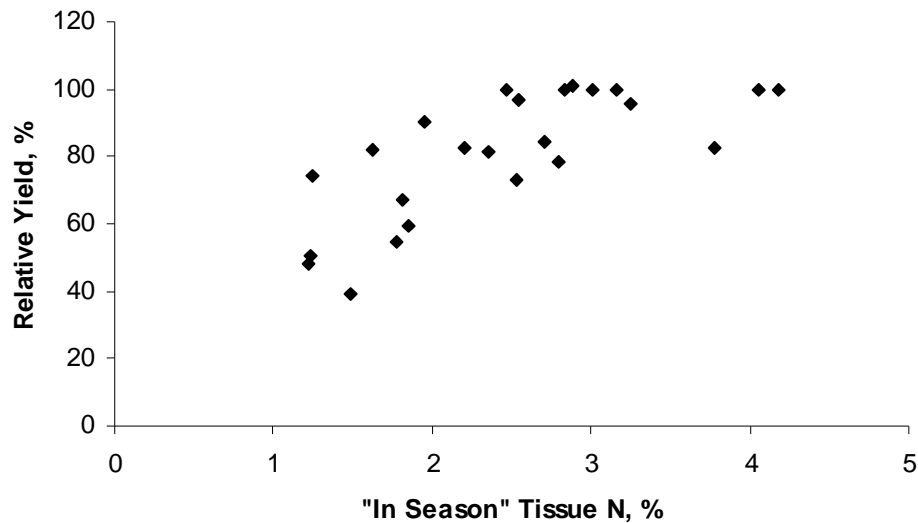


Fig. 4. Perennial ryegrass tissue N from May 1 sample and relative seed yield from the 2006 season.

The current approach in Oregon is to investigate adding spring N rate that is low but possibly adequate, check plant N status, and add N if tissue N is not sufficient. We are pursuing measurements to obtain N content early in the growing season so N can be added if necessary. A similar approach is being considered in New Zealand. Our hope is to measure N content using rapid, non-destructive imagery. If N content cannot be measured nondestructively, a destructive estimation of biomass with tissue N measurement will provide N content.

SUMMARY

Soil N tests have not adequately or accurately predicted perennial ryegrass N supply or seed yield in Oregon. The relationship between tissue N content and seed yield is promising in our measurements and the literature. Efforts in New Zealand and Oregon are pursuing tissue N measurement as indicator of spring N need for perennial ryegrass production.

LITERATURE

- Christensen N.W., Fery, M.L., Mellbye, M.E. & Silberstein, T.B. 2004. Use of the Nmin soil test to predict N fertilizer needs for direct-seeded winter wheat following grass seed crops in the Willamette Valley. *In*: Young W.C. III (ed.). 2003 Seed Production Research at Oregon State University USDA-ARS Cooperating, Department of Crop and Soil Science Ext/CrS 123, 3/04, Corvallis, OR.
- Christensen N.W. & Mellbye, M.E. 2006. Validation and recalibration of a soil test for mineralizable nitrogen. *Communications in Soil Science and Plant Analysis* 37: 2199-2211.
- Curtin, D., Wright, C.E., Beare, M.H. & McCallum, F.M. 2006. Hot water-extractable nitrogen as an indicator of soil nitrogen availability. *Soil Science Society of America Journal* 70: 1512-1521.
- Magdoff F.R., Jokela, W.E., Fox, R.H. & Griffen, G.F. 1990. A soil test for nitrogen availability in the northeastern United States. *Communications in Soil Science and Plant Analysis* 21: 1103-1115.
- Rowarth J.S., Boelt, B., Hampton, J.G., Marshall, A.H., Rolston, M.P., Sicard, G., Silberstein, T.B., Sedcole, J.R. & Young, W.C. III 1998. The relationship between applied nitrogen, nitrogen concentration in herbage and seed yield in perennial ryegrass (*Lolium perenne* L.). I. Cv. Grasslands Nui at five sites around the globe. *Journal of Applied Seed Production* 16: 105-114.
- Sullivan D.M., Hart, J.M. & Christensen, N.W. 1999. Nitrogen uptake and utilization by Pacific Northwest crops. Oregon State University Extension Service, PNW 513, Corvallis, OR.

Nitrogen fertilization of Italian ryegrass (*Lolium multiflorum*) for seed production: Requirement evaluation for N supply calculation

Marie-Laure Casals¹), Francois Deneufbourg¹), Georges Rijckaert²) & Georges Sicard¹)

²)FNAMS, Impasse du verger - 49800 Brain/Authion - France

²)ILVO, Caritasstraat 21 - B-9090 - Melle - Belgium

marie-laure.casals@fnams.fr

ABSTRACT

The effects of different levels of nitrogen on seed yield and yield components of Italian ryegrass were assessed in three locations (in South and Northwest of France, and in Belgium), during three growing seasons (1998 to 2000). Different nitrogen supplies were compared (from 40 to 130 kg N/ha), with a reference treatment (without nitrogen) in order to estimate N soil contribution. N uptake determination was obtained for each treatment at full ear emergence. Results show a good relationship between N level uptake at ear emergence and seed yield, with a maximum yield obtained at about 110 kg/ha N uptake (aerial part of plant and root system). Soil born nitrogen, measured by N uptake at ear emergence on non-fertilized treatments, varied from 25 to 75 kg/ha between trials. These results show that the estimation of soil born nitrogen is necessary for the calculation of N supply. Seeds of Italian ryegrass are mainly produced in livestock farming where organic fertilization generally occurs. In these situations, soil born nitrogen contribution can be important, so nitrogen supply must be reduced to satisfy plant requirement.

Key words: Italian ryegrass, nitrogen fertilization, N uptake, seed production

INTRODUCTION

The nitrogen (N) fertilization of Italian ryegrass is based upon an empiric skill, achieved in an economic and environmental context. Many seed growers routinely apply the same amount of nitrogen. In most experiments, the authors looked for a direct relationship between fertilizer rate and yield response, but did not give any indication about the amount of nitrogen taken up by the plant.

Italian ryegrass is grown in different areas in France, on contrasting soils and agricultural environments. In addition, most of the Italian ryegrass seeds crops are located in stock farming areas, classified as 'sensitive' by the European Nitrate guideline (91/676/EEC, Dec. 1991). On such farms, soil N mineralization can be very important. This is the reason why

it is more difficult to use the same amount of nitrogen each time, because soil residual N could be so different and most important.

We thought that the assessment of N uptake could be a good tool to explain differences observed between trials and to adapt the recommendations to local situations. In this paper, we tried to define the optimum N uptake for seed yield of Italian ryegrass under different agroclimatic conditions. Control plots (N=0) were always used to determine the soil-born N available for the crop. The N uptake was determined at the end of ear emergence, because at this stage, the yield potential is achieved and the maximum nitrogen uptake occurs before and during stem elongation.

MATERIALS AND METHODS

From 1998 until 2000, field experiments were conducted in three different production areas: Lavaur in the south of France, Angers (Brain sur l'Authion) in the northwest, and Melle in Belgium. In all experiments, an early application of 100 units of nitrate was conducted at the end of winter (February - March) before the forage cut (generally at the end of April). Afterwards, four rates of spring

nitrogen application (2nd application) were compared: 0, 40, 70, 100 and 130 kg N ha⁻¹ (Table 1). Two varieties were used: Fastyl in France and Bellem in Belgium.

In all trials, plant samples were clipped at the end of ear emergence or at the beginning of flowering, dried and weighted. A subsample was analysed for N content, and the N uptake by Italian ryegrass (kg N ha⁻¹) calculated.

Table 1. Date of nitrate application for the production of forage (1st application), dry matter yield in first cut, and date of nitrogen application and for seed production (2nd application).

Trials	1 st application (100 N) (1 or 2 times)	Dry matter forage cut (t ha ⁻¹)	2 nd application
Melle	20/02/98	5.7	29/04/98
	01/04/99	4.5	06/05/99
	06/03/00	5.6	28/04/00
Lavaur	17/02 and 02/04/98	8.4	23/04/98
	08/02 and 13/04/98	5.0	23/04/99
Brain sur l'Authion	11/02 and 03/03/98	4.3	21/04/98
	02/02 and 12/03/99	7.0	29/04/98
	21/02 and 15/03/00	4.5	03/05/00

RESULTS

Relationship between N uptake and seed yield

Seed yields are expressed in percentage of the maximum seed yield obtained in each trial. These maximum seed yields varied widely, from 500 to 1730 kg ha⁻¹.

Fig. 1 shows the relationship between nitrogen uptake at the end of ear emergence and relative seed yield of all trials. The response curve can be divided in two parts:

- (1) Until 80 kg N ha⁻¹ of uptake, seed yield increased rapidly in all trials.
- (2) Above 80 kg N ha⁻¹, the increase in Nitrogen uptake had no effect on seed yield.

From this curve, we consider that the optimum amount of N uptake of Italian

ryegrass is, on average, 80 kg ha⁻¹, and 30 % more if we take into account the nitrogen uptake by roots and the lowest tiller parts which are not cut. Thus, the total nitrogen requirement of Italian ryegrass is almost 110 kg ha⁻¹. Two varieties (Fastyl and Bellem) seem to have the same optimum level of N uptake.

The data show the small quantity of nitrogen uptake by Italian ryegrass in seed production after the forage cut in spring. Only 80 kg N ha⁻¹ absorbed until the flowering by the aerial part of the plant, is necessary to optimise seed yield. If the nitrogen need is not reached, we can note a strong decline in seed yield. At the opposite, if there is too much nitrogen uptake, the plant continues to absorb it - luxury consumption - without any impact on yield, but with a possible stronger lodging of the seed crop.

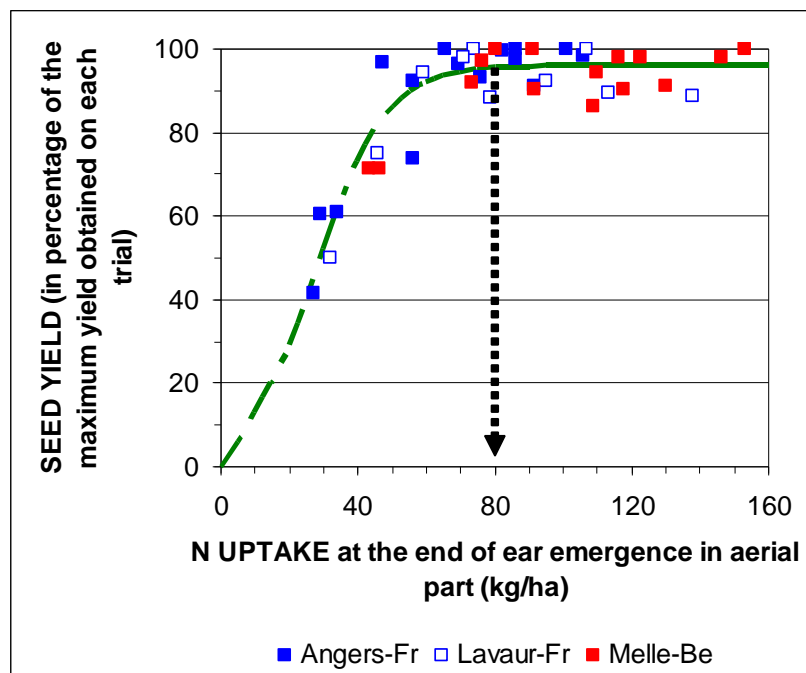


Fig. 1. Seed yield response to nitrogen uptake in Italian ryegrass, obtained in 8 trials at 3 locations (2 in France and 1 in Belgium) and in 3 years (from 1998 to 2000).

Table 2. Nitrogen uptake at ear emergence in the non fertilized treatment.

	Amount of N taken up (kg ha ⁻¹)			
	< 30	30 to 50	50 to 70	> 70
Number of situations	2	4	0	2

Table 3. Soil contribution (N uptake without any additional N supply), N supply and N uptake to reach the maximum yield (kg/ha) in all trials.

Years Trials	1998			1999			2000	
	Angers	Lavour	Melle	Angers	Lavour	Melle	Angers	Melle
Soil contribution, kg N ha ⁻¹	29	32	73	34	71	43	27	46
N fertilizer input to reach max. yield kg N ha ⁻¹	40	70	0	70	0	40	70	40
N uptake at the maximum yield	56	86	73	66	71	80	76	76
Yield (kg ha ⁻¹)	1040	1126	1026	1472	1635	1610	1536	1674

Nitrogen uptake in control treatment (Nup0):

This parameter gives the contribution of the soil-borne nitrogen to crop growth. Nup0 appeared extremely variable in the different trials, ranging from 25 to 75 kg ha⁻¹, with an average of 44 kg ha⁻¹ (Table 2).

This variability is not surprising, considering the differences in soils, previous crops (linseed, wheat, maize) and weather conditions in all trials. The results underline how important is the nitrogen high-grade of the soil. Soil-borne nitrogen can contribute a large proportion to the total amount needed by Italian ryegrass for a maximum seed yield.

This must be taken into account to calculate the adequate fertilizer rate. To give an example, in some trials (Melle in 1998, Lavaur in 1999; Table 3), soil-born nitrogen amounted to the optimum nitrogen uptake without any additional N supply.

CONCLUSION

The results of these trials show that the maximum seed yield of Italian ryegrass was reached at about 110 kg ha⁻¹ of total nitrogen uptake at the end of ear emergence (80 kg ha⁻¹ absorbed by aerial part of plant and 30 kg ha⁻¹ by roots; more nitrogen uptake could affect the seed yield.

The soil contribution to the total N requirement of the crop was variable and often high.

Savings of mineral nitrogen supplies are possible with a better prediction of soil born nitrogen. By such predictions, we could generate a significant cut in the production costs and decrease excessive N leaching. A better knowledge of nitrogen uptake to obtain the maximum yield would allow building a method of forecast, which is already done with other species for seed production.

ACKNOWLEDGEMENTS

M. L. Casals would like to thank co-authors G. Rijckaert, F. Deneufbourg and G. Sicard. Thanks are also expressed to J.L. Pelissou and C. Etourneau who carried out the field trials in France.

Correlation between seed yield and biomass-nitrogen - a case study using multiple field experiments

René Gislum & Birte Boelt

Faculty of Agricultural Sciences, University of Aarhus, 4200 Slagelse, Denmark.

rene.gislum@agrsci.dk

ABSTRACT

Several strategies for future nutrient managements systems describe the need to apply e.g. nitrogen (N) on a 'plant demand' basis. Before such management systems can be introduced and implemented in herbage seed production there is an urgent need to increase knowledge in the area of plant N concentration and the plant N accumulation in relation to seed yield. Among several relevant issues two seems of key importance 1) definition of the optimum sampling time to measure the plant N concentration and/or the plant N accumulation and 2) which correlation coefficients between the plant N concentration and/or the plant N accumulation and the seed yield can be expected at this sampling time.

A total of 82 datasets were made from 7 different field experiments in red fescue (*Festuca rubra* L.) and perennial ryegrass (*Lolium perenne* L.) using a total of 6 different cultivars in the period from 1995 to 2005. A dataset was made at each sampling date. In all experiments plant samples were cut at ground level and analysed for total N. Plant N concentration and plant N accumulation was recorded. Seed yields from first year seed harvest were recorded.

The coefficients of determination and the akaike information criterion were used to describe the correlation between plant N concentration and the seed yield and the correlation between the plant N accumulation and the seed yield at each plant sampling time. The correlation was described as a linear (one parameter) or a quadratic (two parameters) relationship. The optimum number of parameters (one or two) used to describe the correlation was determined using R^2 , AIC, scatter plots and residual plots.

We conclude that it was not possible to define a time during the growing season where the correlation coefficients between plant N concentration and seed yield and between plant N accumulation and seed yield are always/generally high.

Key words: correlation coefficients, models, plant nitrogen accumulation, plant nitrogen concentration, seed yield.

INTRODUCTION

Several strategies for future nutrient managements systems describe the need to apply e.g. nitrogen (N) on a 'plant demand' basis. The strategies are based on measurements of the plant N concentration and/or the plant N accumulation and apply N accordingly in order to achieve the maximum yield. Before such management systems can be introduced and implemented in herbage seed production there is an urgent need to increase knowledge in the area of plant N

concentration and the plant N accumulation in relation to seed yield. Among several relevant issues two seems very important 1) definition of the optimum sampling time to measure the plant N concentration and/or the plant N accumulation and 2) which correlation coefficients between the plant N concentration and/or the plant N accumulation and the seed yield can be expected at this sampling time.

The objectives were to define the sampling time during the growing season where the highest correlation between plant N accumulation and the seed yield and the

correlation between the plant N concentration and the seed yield could be achieved.

MATERIALS AND METHODS

A total of 82 datasets were made from 7 different field experiments in red fescue (*Festuca rubra* L.) and perennial ryegrass (*Lolium perenne* L.) using a total of 6 different cultivars in the period from 1995 to 2005. A dataset was made at each sampling date. The purpose of all the experiments was to define the optimum N application rate or the optimum N application strategy to achieve the highest seed yield. In all experiments plant samples were cut at ground level with 3 cuts per plot. Each cut was 0.0625 m². Fresh weight was measured in all samples before they were oven-dried at 80 °C for at least 24 h and pooled to one sample per plot. Shoot biomass (t ha⁻¹) was calculated. The samples were ground and an aliquot of each sample was analysed for total N using the Kjeldahl method (wet digestion in H₂SO₄-H₂O₂) or the Dumas method (flash combustion with automatic N analyser). Before shedding the grass seed crop was harvested directly with a trial combiner, and seeds were dried to 12 % moisture. Seed samples were cleaned using an air-screen seed cleaner by passing them through 0.5 cm sieves. After seed drying and cleaning, one sample from each N application rate in each dataset was analysed for purity using international standardised methodology (ISTA, 1996) and seed yield expressed as 100 % clean seed was calculated. Only seed yields from first year seed harvest were used.

The coefficients of determination (R²) and the Akaike Information criterion (AIC) (Akaike, 1969) were used to describe the correlation between plant N concentration and the seed yield and the correlation between the plant N accumulation and the seed yield at each plant sampling time. The correlation was described as a linear (one parameter) or a quadratic (two parameters) relationship. The optimum number of parameters (one or two) used to describe the correlation was determined using R², AIC, scatter plots and residual plots.

Coefficients of determination (R²) values were calculated using equation [1].

$$R^2 = \frac{SS_R}{SS_T} \quad [1]$$

Where SS_R is the regression sum of squares and SS_T is the total sum of squares. The higher R² values the better description of the data using the model.

The AIC was calculated as:

$$AIC = n * \ln\left(\frac{\sum (y_i - \hat{y}_i)^2}{n}\right) + 2 * P \quad [2]$$

where y_i is the actual seed yield and \hat{y}_i is the predicted seed yield, n is the number of observations and p is the number of parameters used in the model. The descriptive AIC predictive power of the model is higher the lower the value of the AIC.

Cumulative Growing Degree-Days (GDD) were calculated each year using the formula

$$GDD = \sum_i^N T_{average_i} \quad [3]$$

where $T_{average_i}$ represents average air temperature (°C) at day i . Only days with $T_{average} > 0$ was used. GDD was computed from 1st January in each year.

All analyses were performed using the procedures PROC REG module within the Statistical Analysis System version 8.2, software package (SAS 1999).

RESULTS

The correlation coefficients were in the range from 0.01 to 0.94. The highest correlation coefficients between plant N concentration and seed yield were at approximately 500 GDD, but at 1200 GDD the correlation coefficients were also high at approximately 0.8 (Fig. 1 left). There was a tendency that the correlation coefficient increased when an additional parameter was added to the model (Fig. 1 left).

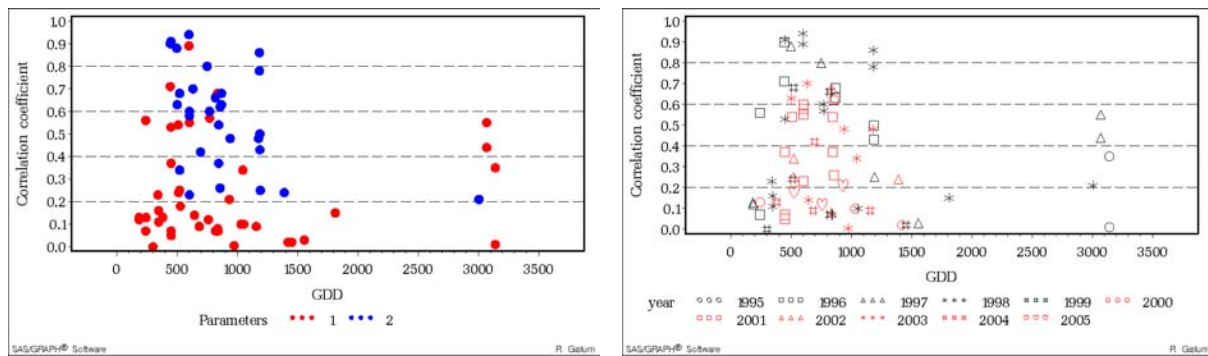


Fig. 1. Left - Plot of correlation coefficients at different growing degree days (GDD). The correlation coefficients describe the correlation between plant nitrogen status (% N) and seed yield. Results are colored in accordance with the number of parameters used to describe the correlation where red dots show a linear correlation and blue show a quadratic correlation. Right - Plot of correlation coefficients at different GDD. Results are shown with different symbols according to year.

The highest correlation coefficients between plant N accumulation and seed yield were from approximately 500 GDD to approximately 1500 GDD (Fig. 2). There was a tendency that the correlation coefficient increased when an additional parameter was added to the model (Fig. 2 left). There was a tendency that the correlation coefficients

were higher in 1996, 1997 and 1998 compared to the period from 2000 to 2005 (Fig. 2 right).

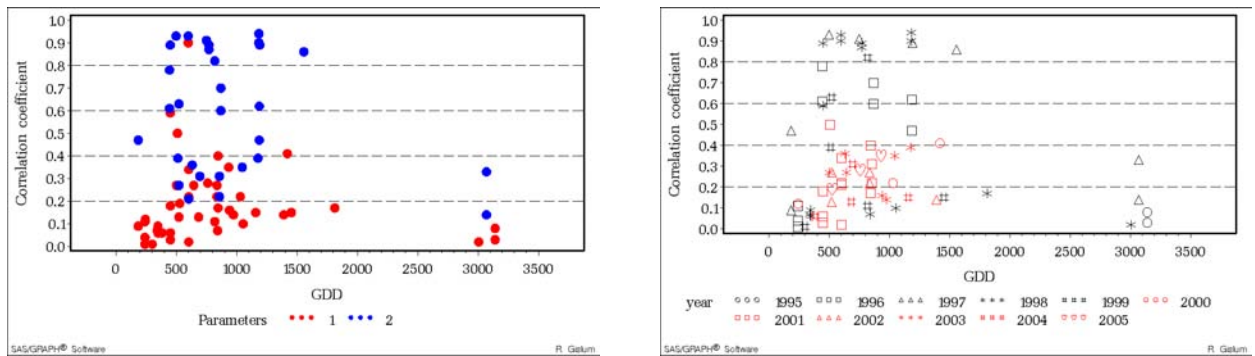


Fig. 2. Left - Plot of correlation coefficients at different growing degree days (GDD). The correlation coefficients describe the correlation between plant nitrogen accumulation (kg N ha^{-1}) and seed yield. Results are colored according to the number of parameters used to describe the correlation where red dots show a linear correlation and blue show a quadratic correlation. Right - Plot of correlation coefficients at different GDD. Results are shown with different symbols according to year.

There was no clear evidence that the effect of year had a significant effect on the correlation coefficient between plant N accumulation and seed yield (Fig. 2 right).

The AIC values were at their highest in the period from 2001 to 2005 for the correlation between plant N concentration (Fig. 3 left) and for the correlation between plant N accumulation and seed yield (Fig. 3 right).

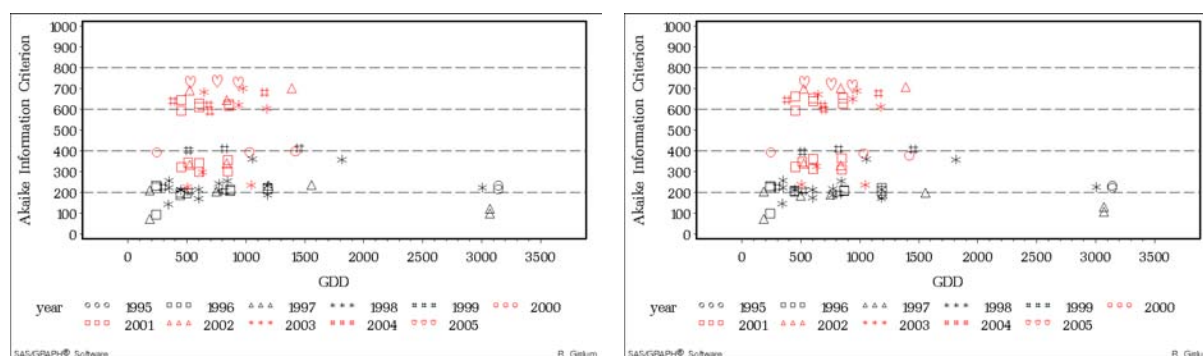


Fig. 3. Left - Plot of akaike information criterion (AIC) at different growing degree days (GDD). The AIC is a measure of the goodness of fit of the estimated statistical model used to describe the correlation. Results are shown with different symbols according to year. Right - Plot of AIC at different GDD. Results are shown with different symbols according to year.

DISCUSSION

The correlation between plant N concentration and seed yield and/or the correlation between plant N accumulation and seed yield is the foundation for developing N application strategies in herbage grass seed production. From an herbage seed growers point of view the most important issue is to achieve the highest profit from the grass seed crop and this involve optimum N fertilisation with regard to time, rate and form. An optimum N fertilisation strategy involves a possible additional N application if necessary. Currently, no method to quantify an additional N application has been implemented in herbage seed production and the first step would be to define an optimum time for this future developed method. The fact that it is impossible to define a time during the spring growing season where it is possible to ensure high correlation coefficients ($R^2 > 0.8$) in the present experiment is therefore discouraging. There are several possible theories for the low correlation coefficients achieved during the spring growing season. The most obvious is that N was not a limiting factor for the seed yield in these experiments. This is supported by the fact that the highest correlation coefficients were achieved when two parameters were used to describe the

correlation between plant concentration and seed yield and plant N accumulation and seed yield, in this case the maximum effect of the applied N was reached, which is not the case in a linear model.

The utilisation of N interact with several factors among them climate. The idea of dividing correlation coefficients within years was therefore to test if one or more years tended to be outliers e.g. if one year was especially dry. The fact that AIC values were higher from 2001 to 2005 for both plant N concentration and plant N accumulation is most probably not attributed to climate. A possible explanation for the higher AIC values could be that the experimental design in 2001 to 2005 was a latin square design with eight replicates whereas the experimental designs before 2001 were all factor designs with four replicates.

Based on the present work it was not possible to define a time during the growing season where the correlation coefficients between plant N concentration and seed yield and between plant N accumulation and seed yield is always/generally high.

LITERATURE

- Akaike, H. 1969. Fitting Autoregressive Models for Prediction. *Annals of the Institute of Statistical Mathematics* 21: 243-247.
 SAS. 1999. SAS Online Doc. Version 8, SAS Institute, Cary, NC, USA

Sulphur fertilization in seed crops of *Lolium perenne*

Jan Rinze van der Schoot, Gerard Borm & Wim van Dijk

Applied Plant Research, Wageningen UR
Edelhertweg 1, 8219 PH Lelystad, Netherlands

janrinze.vanderschoot@wur.nl

ABSTRACT

Due to environmental regulations deposition of sulphur has decreased last decades in the Netherlands. Especially on light soils far from industrial areas a lack of sulphur has been observed in several crops (a.o. winter wheat). In field trials with seed crops of *Lolium perenne* harvested in 2004, 2005 and 2006, levels from 0 to 72 kg S ha⁻¹ were applied. Fertilization with nitrogen was at the same level for all the treatments. The availability of sulphur from mineralization, deposition and upwards transport from ground water were estimated. Seed yield, above ground dry matter yield and S- and N-uptake were measured. The results showed that S-deficiency gives a slower growth in the early spring and a less dark green colour. On light clay soils in the north of the Netherlands with a low level of S-availability in two of the six trials an amount of 20 kg S ha⁻¹ had a significant positive effect on seed yield. It can be concluded that in seed crops of *Lolium perenne* total S-availability from mineralization, deposition, upwards transport from ground water, and fertilization should be about 40 kg S ha⁻¹. Under conditions of sufficient sulphur availability uptake in seed and straw was about 15 kg S ha⁻¹.

Key words: perennial ryegrass, seed production, sulphur content, sulphur uptake

INTRODUCTION

Due to environmental regulations deposition of sulphur has decreased last decades in the Netherlands. Especially on light soils far from industrial areas a lack of sulphur has been observed in several crops (a.o. winter wheat). In these crops sulphur fertilization led to higher yields. In grass seed crops, especially in crops for a second harvest, we have the impression that spring growth is slower due to an insufficient availability of sulphur. Therefore field trials were carried out to investigate effects of S fertilization for both first and second harvest seed crops of *Lolium perenne* in the North of the Netherlands, as strongest effects are expected in this region because of low S deposition levels (< 10 kg S ha⁻¹).

MATERIALS AND METHODS

In 2004, 2005 and 2006 six trials have been carried out on sites in the North of the Netherlands (Table 1). The N fertilization was

held at the same level for all treatments within a trial. S fertilization levels varied from 0-86 kg S ha⁻¹ in 2004 (Table 2). As no significant response was observed above 20 kg S ha⁻¹, rates were decreased in 2005-2006 (0-54 kg S ha⁻¹). Total amounts of nitrogen and sulphur were applied in March with different mixtures of calcium ammonium nitrate (CAN, 27% N and 0% SO₃) and ammonium sulphate (AS, 26% N and 35% SO₃). Applied S rates varied between years, as combined N/S-fertilizers were used and the recommended N rates varied between years. In 2006 the fertilization was higher because of the new N-advice for first harvest seed crops (220 kg N ha⁻¹ minus N-mineral).

Besides fertilization sulphur is also becoming available from mineralization, deposition and upward transport from ground water (hereafter called S-supply). Based on soil analysis by the Dutch laboratory Blgg. S-mineralization during the growing season in the soil layer 0-30 cm in 2004 was estimated to 4-5 kg S ha⁻¹ (2004), 12-13 kg S ha⁻¹ (2005) and 4-21 kg S ha⁻¹ (2006) (Table 2). For

upwards transport from ground water a fixed level of about 20 kg S ha⁻¹ is used (Blgg). S deposition was about 8 kg ha⁻¹).

Consequently, the S-supply was estimated to 32 kg S ha⁻¹ (2004), 40 kg S ha⁻¹ (2005) and 32-49 kg S ha⁻¹ (2006). In order to measure total aboveground dry matter yield and N/S uptake, just before harvest a crop sample was

taken by cutting the aboveground crop material within a frame of 0.5 m x 0.5 m.

The statistical analysis was carried out with the general purpose statistical package Genstat 5. The Least Significant Differences (LSDs) between treatments are based on probabilities of 95% ($\alpha=0.05$).

Table 1. Field characteristics of experimental sites.

Trial	Year	1e- or 2e-harvest	Type	silt (50µm-2µm) (%)	Organic matter (%)	N mineral (kg ha ⁻¹)	N fertilization (kg ha ⁻¹)
4-1	2004	first	amenity	14	1.6	51	134
4-2	2004	second	amenity	12	1.3	15	160
5-1	2005	first	amenity	15	2.2	51	134
5-2	2005	second	late fodder	17	1.8	0	160
6-1	2006	first	amenity	14	3	45	175
6-2	2006	second	amenity	17	2.2	11	200

Table 2. Sulphur fertilization in the experiments.

Trial	Year	S-mineralization	S-supply	Sulphur fertilization (kg S ha ⁻¹)			
				S0	S1	S2	S4
4-1	2004	5	33	0	18	36	72
4-2	2004	4	32	0	22	43	86
5-1	2005	13	41	0	9	18	36
5-2	2005	12	40	0	11	22	43
6-1	2006	4	32	0	12	24	47
6-2	2006	21	49	0	13	27	54

RESULTS

Table 3 shows some characteristics averaged for trials with a low and high S-mineralization. At low S-mineralization levels (4-1, 4-2 and 6-1) the growth in April and May was weaker in the treatments without S-fertilization (S0) than in the treatments with S-fertilization (S1, S2 and S4). The S0-treatments also had a lighter green colour. The differences between the treatments in the trials with a higher S-mineralization were small and not significant. In June in most trials no visual differences were observed between the treatments with and without S-fertilization. The biomass production of the S0 measured just before harvest was lower in trials with a low S-mineralization level.

Averaged over the trials the seed yield of the treatments without S-fertilization was lower than for the treatments with S-fertilization (Table 4). However, differences between the treatments with S-application (S1, S2 and S4) were small. In the trials of 2004 (4-1 and 4-2) seed yield of the treatment without S-fertilization was 200 and 100 kg ha⁻¹ lower for the first and second seed harvest, respectively. Only in the trial 4-1 this effect was significant. In 2005 no significant effects of S-application on the seed yield were observed. In 2006 in trial 6-1 the yield of the treatment S0 was lower than the yield of treatment S1, but the S2 had a similar yield as the S0. In the trial with the second harvest crop (6-2) the S2 had the highest yield, but this difference with S0 was not significant.

Table 3. Visual estimation of the biomass in the period April-May and measured dry matter yield just before harvest in July.

Trial	Biomass in April and May (visual estimation)		Dry matter biomass at harvest (ton ha ⁻¹)	
	Low S- mineralization	High S- mineralization	Low S- mineralization	High S- mineralization
S0	6.8	7.5	10.4	10.7
S1	7.5	7.7	10.9	10.6
S2	7.5	7.8	11.3	11.3
S4	7.8	7.8	11.0	11.0
F-prob	< 0.001	0.555	0.079	0.043
LSD 5%	0.4	0.4	0.6	0.5

Table 4. Seed yield (kg ha⁻¹) and statistical analysis

Trial	F-prob	LSD 5%	S-supply	S0	S1	S2	S4
4-1	0.012	140	33	1580	1800	1780	1820
4-2	0.552	200	32	1220	1320	1340	1300
5-1	0.427	155	41	1125	1180	1200	1100
5-2	0.750	125	40	1165	1140	1195	1170
6-1	0.751	200	32	1750	1790	1740	1830
6-2	0.421	240	49	1960	1930	2095	2000
Average	0.032	64		1470	1535	1560	1545

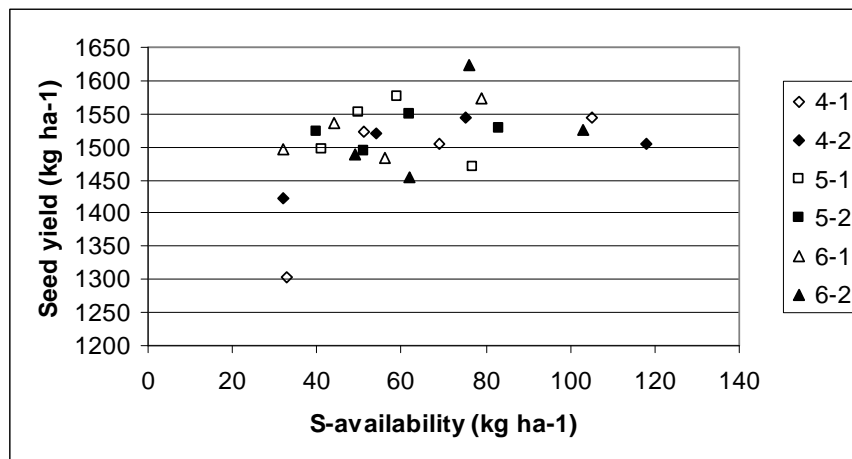


Fig. 1. Relationship between S-availability (sulphur from mineralization, deposition, upwards transport from groundwater and fertilization) and seed yield of grass seed crops.

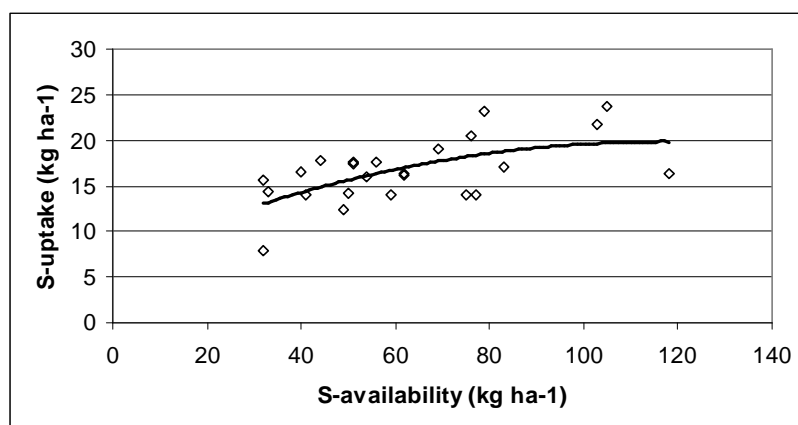


Fig. 2. Relationship between S-availability and S-uptake in seed and straw from trials harvested in 2004, 2005 and 2006 with different levels of S-application.

In Fig. 1 the relationship between total S-availability (defined as S-supply plus S-fertilization) and seed yield is shown. A correction is made for yield differences between trials. The figure shows that hardly any yield increase is observed for S-availability exceeding 40 kg S ha⁻¹. Regression analyses in order to estimate the optimal S rate, didn't give good results, because of the poor relationship between S-availability and seed yield.

The lowest S content in the above ground dry matter of the treatment without S-application was 0.9 gram S per kg dm. With S-fertilization the S content increased until the maximum of 1.9 gram S per kg dm. With a sufficient availability the S-content had an average of 1.4 gram S per kg dm. The aboveground S-uptake of the treatments with a sufficient S availability was approximately 15 kg S ha⁻¹ (Fig. 2). At higher S-availabilities the S-uptake increased to 25 kg S ha⁻¹. For the lowest S-applications (10-25 kg S ha⁻¹) about 20% (0-37%) of the applied sulphur was found in aboveground plant parts. The S recovery at the higher S rates applications was about 10%.

DISCUSSION

The estimated S-supply (excluding fertilization) at the experimental sites varied between 32 and 41 kg S ha⁻¹. This amount includes the supply from upwards transport from ground water estimated as a fixed level being the same for all trials. Therefore, possible differences between trials can affect the effects of S-fertilization.

In two of the six trials an amount of 20 kg S ha⁻¹ had a positive effect on the seed yield. This effect was significant in one trial only. As S rates lower than 20 kg S ha⁻¹ were not studied, it cannot be assessed whether lower S rates (e.g. 10 kg S ha⁻¹) would have had the same effect. In trials on sites with a S-supply of 40 kg S ha⁻¹ no effect of S-fertilization was observed. It can be concluded that a S supply of 40 kg ha⁻¹ is sufficient for optimal seed production. At lower S-supply a fertilization of 10-20 kg S ha⁻¹ can be recommended.

In order to prevent S leaching unnecessary high fertilizations should be avoided as the recovery of the applied sulphur was low.

LITERATURE

- van der Schoot, J.R. & Borm, G.E.L. 2005. Zwavelbemesting graszaad Engels raaigras. Effect van zwavelbemesting op opbrengst en kwaliteit van Engels raaigras bestemd voor zaadproductie, oogst 2004. PPO-projectrapport nr. 510251.
- van der Schoot, J.R. & Borm, G.E.L. 2006. Zwavelbemesting graszaad Engels raaigras. Effect van zwavelbemesting op opbrengst en kwaliteit van Engels raaigras bestemd voor zaadproductie, oogst 2005. PPO-projectrapport nr. 510251.
- van der Schoot, J.R. & Borm, G.E.L. 2007. Zwavelbemesting graszaad Engels raaigras. Effect van zwavelbemesting op opbrengst en kwaliteit van Engels raaigras bestemd voor zaadproductie, oogst 2005 en meerjarenanalyse. PPO-projectrapport nr. 32510251.

Implications of correlation between yield from the same plot from year to year

Kristian Kristensen¹⁾ & Lise C. Deleuran²⁾

¹⁾University of Aarhus, Faculty of Agricultural Sciences, Department of Genetics and Biotechnology, Blichers Allé 20, DK-8830 Tjele, Denmark

²⁾University of Aarhus, Faculty of Agricultural Sciences, Department of Genetics and Biotechnology, Forsøgsvej 1, DK-4200 Slagelse, Denmark

Kristian.Kristensen@agrsci.dk

ABSTRACT

The statistical analyses of trials with more than one year of harvest propose some additional complications as compared to a number of one-year trials. The complications are partly due to the fact that the same plot is harvested several times and therefore the recording from the same plot in successive years may be correlated. In this paper three different methods for modelling the correlations in red fescue are presented and the results show that all three models fit much better to the data than a model, which ignores the correlations between harvest years. The correlation structure for the correlations between different harvests of the same plots is shown for the three methods of modelling. It is shown that the test of significance for testing the hypothesis of no effects depends strongly on whether the model takes the correlations into account or not. Also the criteria for making pair-wise comparisons between estimated effects are shown and that they are highly dependent on whether the correlations are ignored or not.

Keywords: analysis of variance, harvest years, mixed model, pair-wise comparisons, red fescue, test of effects.

INTRODUCTION

The statistical analyses of trials with more than one year of harvest propose some additional complications as compared to analysing only one trial or a number of one-year trials. The complications are partly due to the fact that the same plot is harvested several times and therefore the recording from the same plot in successive years may be correlated. This correlation may depend on the time interval between the recordings. When the same trial is laid out in more than one year, the analysis of more trials over more years also raises the problem of effects of calendar year. The recordings might be expected to be affected by the growing conditions in both the recording year and the year of sowing (and the years between).

The objective of this paper is to describe the complications in perennial plots and to advise

on and discuss some methods of taking these into account in the statistical analyses.

MATERIALS AND METHODS

Data from a series of experiments with red fescue will be used to illustrate the analyses. The trial was established in the years 1997, 1998 and 1999:

A randomised complete block design with 2 treatment factors:

1. Three varieties (Pernille, Suzette and Tamara)
2. Four row spacings (12, 24, 36 and 48 cm). The total amount of seeds per ha was uniform in all four spacings.

There were four replicates (complete blocks), i.e. 48 plots in the trial. For more information on the trial see Deleuran *et al.* (2002).

In each trial the plots were harvested in six consecutive years (only five harvest years will be used here). These years will be named harvest years and identified with the numbers 1, 2, 3, 4 and 5, as opposed to the calendar year in which the harvest took place.

The data were analysed in a linear mixed model, which included the fixed effect of: variety, row distance, harvest year, their 2-way interactions and their 3-way interaction. The model also included the random effect of the harvest (combination of calendar year of sowing and calendar year of harvest), random

variety effect from harvest to harvest, random effect of row distance from harvest to harvest, random effect of the interaction between variety and row distance from harvest to harvest, random effect of replicate within harvest and random effect of plot within harvest. The simplest form of such a model is obtained, when all pair-wise correlations from harvest year to harvest year are assumed to be the same. This model (and more complex models allowing for different correlations between harvest years) may be written as the sum of the following terms:

$$Y_{sbvdh} = \mu + \alpha_v + \beta_d + (\alpha\beta)_{vd} + \gamma_h + (\alpha\gamma)_{vh} + (\beta\gamma)_{dh} + (\alpha\beta\gamma)_{vdh} + A_{sh} + B_{svh} + C_{sdh} + D_{svdh} + E_{bh(s)} + F_{sbvdh}$$

where

Y_{sbvdh} is the yield in harvest year h for variety v at row spacing d in block b of the trial sown in year s

μ is the yield level in the trials

α_v , β_d , γ_h , $(\alpha\beta)_{vd}$, $(\alpha\gamma)_{vh}$, $(\beta\gamma)_{dh}$ og $(\alpha\beta\gamma)_{vdh}$ are the fixed effects of variety, row spacing, harvest year and interactions between these three factors

A_{sh} is the random effect of harvest years h sown in year s

B_{svh} is the random effect of variety v for harvest year h sown in year s

C_{sdh} is the random effect of row spacing d for harvest year h sown in year s

D_{svdh} is the random effect of the interaction between variety v and row spacing d for harvest year h sown in year s

$E_{bh(s)}$ is the random effect of block b in harvest year h sown in year s

F_{sbvdh} is the random effect of the plot with variety v at row spacing d in block b for harvest year h sown in year s

A_{sh} , B_{svh} , C_{sdh} , D_{svdh} , $E_{bh(s)}$ and F_{sbvdh} are all assumed to be normally distributed with mean zero and variance-covariance matrices Σ_A^2 , Σ_B^2 , Σ_C^2 , Σ_D^2 , Σ_E^2 and Σ_F^2 , respectively

For all variance-covariance matrices the covariances are zero except for covariances between harvesting year (i.e. different values of h for same value of the other indices). The variances in the diagonal of these matrices are assumed to be equal.

Such model allows the correlation to be modelled in many different ways. Here we will look at three different patterns of correlations and as a reference also one where the possible correlations are not taken into account. In the models 2, 3 and 4, correlations between observations from the same harvest are taken into account. The applied models are:

1. No correlations, i.e. all correlations between harvest years are zero (obtained by leaving out all random effects except for the ones between combinations of calendar year of sowing and calendar year of harvest).
2. Equal correlations, i.e. the correlations between harvest years are the same and

independent of the number of years between observations (also called Spherical or Compound Symmetry).

3. Dependent on the difference in years, i.e. the correlation between harvest years depends on the number of years between observations, so for five harvest years there will be four different correlations, ¹⁾ for two successive years, ²⁾ for observations made with two years' difference, ³⁾ for observations made with three years' difference and ⁴⁾ for observations made with four years' difference (also called Toeplitz).
4. Autoregressive, the correlations between harvest years decrease with the distance in years according to the formula $r_d = r^d$,

where d is the number of years between the observations and r is the correlation between successive years, i.e. when d is one (also called AR(1)).

In order to find the model that best describes the random effect, Akaike's information criterion (Akaike, 1974) was used. This criterion is based on the likelihood of the data for the given model and is calculated as $-2 \times \log(\text{Likelihood}) + 2 \times p$, where p is the effective number of parameters in the model. By adding the value $2 \times p$ to this criterion, the criterion will penalize for the fact that $-2 \times \log(\text{Likelihood})$ will decrease as more parameters are used for modelling the random effects and also give some preference to models using few parameters (the value of $-2 \times \log(\text{Likelihood})$ will on average decrease by 1 unit for each additional, unnecessary parameter that is added).

For more information on mixed models see e.g. McCulloch and Searle (2001). All statistical analyses were conducted using the procedure MIXED of SAS (SAS 1999).

RESULTS

It was clear that the three models, which take correlations among harvest years into account, fit better than the one without correlations (table 1). The three models, which include correlations between harvest years, were relatively alike, but the one with

equal correlations, model 1, was judged to be the best one as it had the smallest Akaike's information criterion value.

Model 3 (Toeplitz) described the correlation between the different harvest years using four different correlations. In this model the highest correlation was - as expected - found for succeeding years and the lowest when there was the maximum of four years between the recordings (table 2). The correlations between recordings made with two and three years' difference were intermediate and almost equal but with the unexpected result that three years' difference gave a slightly larger correlation than two years' difference. This model fitted the data slightly better than the one with a common correlation, as the $-2 \times \log(\text{Likelihood})$ for model 3 was smaller than that of model 2 (table 1). However, the penalization for the additional parameters needed for this model meant that model 2 was the best one when using Akaike's information criterion. The weighted sum of the four correlations in model 3 was 0.155, which was approximately the same as the common correlation estimated for model 2. The autoregressive model, model 4, did not fit the data as well as the model with equal correlations, model 2, and the model with unequal correlations, model 3, - most probably because the correlations did not decrease as quickly as implied by the autoregressive model.

Table 1. Fit statistics for comparing four different models (small values are better than larger values)

Model for correlations	-2 log Likelihood	Akaike Information Criterion
1. No correlations	10897	10899
2. Equal correlations (Spherical, CS)	9427	9449
3. Dependent on year difference (Toeplitz)	9407	9465
4. Autoregressive, AR(1)	9437	9457

Table 2. Estimated correlations between observations from the same plot for each of the four correlation models

Model for correlations	Number of years between harvests			
	1	2	3	4
1. No correlations	0	0	0	0
2. Equal correlations (Spherical, CS)	0.162	0.162	0.162	0.162
3. Dependent on year difference (Toeplitz)	0.219	0.123	0.142	0.017
4. Autoregressive, AR(1)	0.216	0.046	0.010	0.002

Table 3. Test of significance in two different models P-values for testing the hypothesis of no effect for each of the factors and their interaction

Effect	1. No correlations	2. Equal correlations
Variety	<.0001	0.0840
Row spacing	<.0001	0.0078
Harvest year	<.0001	0.0422
Variety × Row spacing	0.5415	0.2986
Variety × Harvest year	<.0001	0.7268
Row spacing × Harvest year	0.8570	0.2626
Variety × Row spacing × Harvest year	0.8355	0.0024

Table 4. Calculated LSD-values for comparing the means in the main effect and 2-way interaction tables

Effect	1. No correlations	2. Equal correlations
Variety	19	74
Row spacing	22	25
Harvest year	54, 96, 141, 186 ¹	117, 158, 224, 316 ¹
Variety × Row spacing	37	33/78/79 ²
Variety × Harvest year	64, 102, 145, 189/42 ³	179, 201, 241, 298/164 ³
Row spacing × Harvest year	68, 105, 147, 191/49 ⁴	121, 160, 224, 314/37 ⁴

¹) For differences of 1, 2, 3 or 4 years

²) For same variety/same row spacing/other

³) For differences of 1, 2, 3 or 4 years of same variety/same harvest year

⁴) For differences of 1, 2, 3 or 4 years of same row spacing/same harvest year

The results of testing the hypothesis of no effect for each of the main factors, their 2-way interactions and the 3-way interaction are shown in table 3 for two models (the ones judged to be the best and the worst using Akaike's information criterion. In most cases, the hypotheses were rejected at a far too high level of significance when the worst model, model 1, was used (model 1 did not take any correlation into account). The only noticeable reverse situation was the test of the 3-way interaction, which was significant using model 2, but not when using model 1.

One of the possible methods for making pair-wise comparisons of the effects for each of the main effects and their 2-way interaction tables, the LSD value, is shown in table 4. In some cases, the LSD-values became far too small when the model did not take the correlations into account. For example, when the correlations were ignored, the LSD-value for comparing varieties was only about ¼ of the value obtained when using the model, which took correlations into account.

DISCUSSION AND CONCLUSION

The calculations showed that it was important to take correlations between harvest times

into account when analysing data from different harvest years. This conclusion may most probably be generalised to any situation in which the same variable is recorded more than once and analysed statistically in a model, which includes observations from more than one recording time.

In conclusion, these calculations showed that the tests for testing the hypothesis may be wrong, if the correlations are ignored. In that case, also the criterion for making pair-wise comparisons may be much too small (or large).

ACKNOWLEDGEMENTS

We thank the technical staff at Aarhus University, Crop Ecology and Product Quality for their very skilled assistance.

LITERATURE

- Akaike, H. 1974. A new look on the statistical model identification. *IEEE Transaction on Automatic Control* AIC-19: 716-623
- Deleuran, L. C., Jensen, A.M.D., Boelt, B. and Gislum, R. 2002. Row spacing of perennial ryegrass (*Lolium Perenne* L.) and red fescue (*Festuca Rubra* L.) grown for seed in perennial fields. NJF-report no. 341. Grass and clover seed production. Sweden 24-26 June 2002. pp. 13-19.
- McCulloch, C. E. and Searle, S.R. 2001. Generalized, Linear and Mixed Models. John Wiley & sons, Inc. New York, 325 pp
- SAS Institute. 1999. SAS OnlineDoc, Version Eight. SAS Institute Inc., Cary NC, USA (<http://v8doc.sas.com/sashtml/>)

Straw residue management in seed production of meadow fescue (*Festuca pratense* Huds.) and timothy (*Phleum pratense* L.)

Lars T. Havstad

The Norwegian Institute for Agricultural and Environmental Research
Bioforsk Øst Landvik, Reddalsveien 215, N-4886 Grimstad, Norway

lars.havstad@bioforsk.no

ABSTRACT

Different methods of straw residue management were evaluated in field trials with seed crops of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) in SE Norway during 2000-2005. Compared to straw removal, which up to now has been the most common straw management practice in seed production of the two species, straw chopping and spreading at the back of the combiner during seed harvest did not reduce seed yield in the following year when stubble height was kept at a low level (preferably less than 10 cm). However, in order for newly developed tillers to rapidly penetrate the straw layer in autumn, the chopped straw had to be spread uniformly in the field. The experiments did not provide any support for an extra input of nitrogen in autumn, either in timothy or meadow fescue, when the straw was chopped rather than removed. In both species, also burning of straw and stubble soon after seed harvest was an efficient and fast clean-up method in the field after harvest. However, due to problems with smoke emission, especially near traffic roads and populated areas, field burning is not recommended as a preferable straw management method.

Key words: burning, flail-chopping, nitrogen, residue removal, seed yield, stubble height.

INTRODUCTION

Timothy and meadow fescue are the two main species in Norwegian herbage seed production, covering an annual harvesting area of about 2000 and 600 ha, respectively. A majority of growers of both species bale and remove the straw from the field after seed harvest. However, the demand for straw to animal feed or bedding has declined and many seed growers have difficulties with straw disposal. Straw removal also takes away valuable nutrients from soil.

The most efficient alternatives to straw removal are either to chop the straw at the back of the combiner during combining, or to burn the straw on the field soon after seed harvest. Alternatively, straw and stubble can be flail-chopped after harvest. No straw management experiments have earlier been carried out in these two species under Norwegian conditions.

When straw is chopped, the organic material that is added to the soil has to be microbially degraded. During the degradation process a temporary shortage for nitrogen in soil may occur. On fields where straw removal has been practiced, an N-application rate of 0 or 20-30 kg N ha⁻¹ in autumn has been recommended to timothy and meadow fescue, respectively.

The intention of this project was to evaluate various straw management methods in order to find alternatives for straw removal in timothy and meadow fescue. An additional aim was to examine whether straw chopping rather than straw removal implies an altered optimal N-fertilization strategy in autumn in the two species.

MATERIALS AND METHODS

Two different experimental series were carried out in seed crops of timothy and meadow fescue during 2000-2005 at various locations in Southeast Norway. The different straw management treatments were usually performed after harvest of first year seed crops.

Experimental series I:

In the first series a total of ten different straw management treatments, including straw chopping and open field burning, as further described in Table 1, was evaluated in six on-farm trials in timothy cv. Grindstad and one trial in meadow fescue cv. Fure. Each trial had three replicates. In the meadow fescue trial, stubble height was 7 cm and the DM straw yield at seed harvest was 5.8 tonnes ha⁻¹. In the six timothy trials, stubble height varied from 10 to 25 cm and the DM straw yield from 2.6 to 7.0 tonnes ha⁻¹.

Experimental series II:

In the second series, three trials in timothy cv. Grindstad and five trials in meadow fescue cv. Fure were carried out during 2003-2005. Stubble height at harvest in the various trials varied from 5 to 20 cm in both species. On average for the trials in timothy and meadow fescue, the DM straw yield that was chopped / removed at harvest was 5.2 tonnes ha⁻¹ and 4.2 tonnes ha⁻¹, respectively. The average N-concentrations in straw dry matter of timothy and meadow fescue were 0.8 and 0.9 %, respectively.

The four different straw management treatments during or soon after seed harvest were combined with autumn nitrogen inputs of 0 or 30 kg ha⁻¹ in timothy (Table 2) and 0, 20 or 40 kg ha⁻¹ in meadow fescue (Table 3). The nitrogen was applied as calciumnitrate soon after seed harvest/straw management.

In meadow fescue, stubble, straw and wilted leaves were burned in early spring, before any growth had occurred (two replicates),

while the remaining replicates were left non-burned.

Except for the experimental treatments, crops in both series were managed in accordance with Norwegian practice. In timothy, this included N-fertilization of 75 - 100 kg N ha⁻¹ in spring and growth regulation with chlormequat chloride (CCC, 2 kg a.i. ha⁻¹) at the early tiller elongation stage (Z 31). In meadow fescue, 80-90 kg N ha⁻¹ was applied in early spring and crops received trinexapacethyl (Moddus, 0.15 kg a.i. ha⁻¹) at Z 31.

RESULTS AND DISCUSSION

In both timothy and meadow fescue, experimental series I and II showed several straw management methods to be acceptable alternatives to straw removal (Tables 1-3). For most seed growers, the most effective and less laborious method will be to chop the straw at the back of the combiner during seed harvest. Straw chopping as a high-yielding alternative to straw removal was also demonstrated under Danish conditions in perennial ryegrass and in wide-spaced established seed crops of tall fescue (Boelt 2006). In experiments conducted in Oregon (USA), straw chopping never negatively affected seed yield in orchardgrass and in some cultivars of perennial ryegrass and tall fescue, but reduced seed yield in red fescue, colonial bentgrass and smooth-stalked meadow grass (Chastain *et al.* 1996, 1997).

Stubble height at combining was not an experimental factor in these trials. However, only fields with long stubble (higher than 15 cm) responded positively, with regard to seed yield, on treatments where stubble was removed (flail chopped or burned) after harvest. This indicates that stubble height should be kept at a low level (preferable less than 10 cm) during combining / straw chopping. When a longer stubble is left, stubble height should be reduced after harvest, e.g. by a flail chopper, in order to improve light penetration.

Table 1. The effect of various straw and stubble treatments on seed yield (kg ha⁻¹) in timothy (means of six trials) and meadow fescue (one trial). Experimental series I.

Treatments	Seed yield			
	Timothy		Meadow fescue	
	kg ha ⁻¹	Rel.	kg ha ⁻¹	Rel.
1. Straw removed after harvest	772	100	812	100
2. Similar to treatment 1 + stubble chopped soon after harvest	862	112	901	111
3. Straw chopped and spread during the combining process	777	101	882	109
4. Similar to treatment 3 + straw/stubble burned after seed harvest	886	115	972	120
5. Similar to tr. 3 + straw, stubble and wilted grass burned in early spring	748	97	929	114
6. Similar to treatment 3 + straw and stubble flail-chopped after harvest	837	108	913	112
7. Straw / stubble not chopped or burned (untreated straw in windrows)	572	74	705	87
8. Similar to treatment 7 + straw and stubble burned after seed harvest	844	109	913	112
9. Similar to treatment 7 + straw, stubble and wilted grass spring burned	630	82	917	113
10. Similar to treatment 7 + straw and stubble flail-chopped after harvest	866	112	840	103
P %	<0.1		>20	
LSD 5 %	84		-	

In both timothy and meadow fescue, straw chopping at the back of the combiner was carried out either directly on straw from standing crops or on straw that had been dried in windrows for 4 to 7 days. Although dried straw was easier to chop, the chopping technique functioned well on both straw types. Irrespective of straw dryness, a more important factor in order to obtain a successful result was to spread out the straw material evenly on the field so that newly developed tillers were able to easily penetrate the covering layer of chopped straw.

As also demonstrated in other seed crops, such as red fescue, perennial ryegrass and orchardgrass (Chilcote *et al.* 1981), field burning soon after harvest was a fast and efficient method to remove straw and stubble from the field, both in timothy and meadow fescue, without any negative impact on the following years seed yield (Table 1). However, due to problems with smoke emission, especially near traffic roads and populated areas, open field burning after harvest is not recommended as a preferable straw management method.

Table 2. Main effects of straw management and N-input in autumn on panicle number m⁻² and seed yield (kg ha⁻¹) of timothy. Mean of three trials. Experimental series II.

	Reproductive tillers m ⁻²	Seed yield	
		kg ha ⁻¹	rel.
Straw / stubble management			
Straw removed after harvest (control)	616	617	100
2. Straw chopped and spread during the combining process	587	655	106
3. Straw and stubble chopped by a tractor driven chopper	661	674	109
4. Straw chopped twice (first with the combine straw chopper and again after harvest with a tractor driven chopper)	608	673	109
P %	>20	>20	
N application in autumn (soon after seed harvest)			
A. 0 kg N ha ⁻¹	604	623	100
B. 30 kg N ha ⁻¹	632	687	110
P %	>20	>20	

Table 3. Main effects of straw management, N-input in autumn and spring burning on panicle number m⁻² and seed yield (kg ha⁻¹) of meadow fescue. Mean of five trials. Experimental series II.

	Panicles m ⁻²	Seed yield	
		kg ha ⁻¹	rel.
Straw / stubble management			
Straw removed after harvest	775	734	100
2. Straw chopped and spread during the combining process	802	733	100
3. Straw and stubble chopped by a tractor driven chopper	886	751	102
4. Straw chopped twice (first during combining and again with a tractor mounted flail chopper soon after harvest)	848	758	103
P %	>20	>20	
N application in autumn (soon after seed harvest)			
A. 0 kg N ha ⁻¹	804	742	100
B. 20 kg N ha ⁻¹	846	748	101
C. 40 kg N ha ⁻¹	833	743	100
P %	20	>20	
Spring burning			
X. No burning in spring	792	672	100
Y. Stubble and wilted grass burned before onset of growth	863	816	121
P %	>20	<1	

In timothy, burning of straw, stubble and wilted leaves in early spring in experiment I was an uncertain straw management method that normally reduced seed yield (Table 1). Especially the strong heat emission evolving from burning windrows of straw (treatment 9) caused stand injury and a subsequent decline in seed yield. Meadow fescue was apparently more tolerant to spring burning than timothy (Table 1). In experimental series II, the average seed yield of meadow fescue was 21 % higher on burned than on non-burned plots in early spring (Table 3). This is in agreement with earlier studies which have shown spring burning to be an efficient and yield-beneficial method to remove autumn regrowth in meadow fescue (Havstad 2002).

When straw is chopped and spread on the field, nutrients are recycled. As calculated from the average N-concentration found in straw and the straw yield, the supply of organic N was about 38 kg ha⁻¹ in meadow fescue and 42 kg ha⁻¹ in timothy. In a Danish study, where chopped straw of three temperate grasses was placed on top of soil, about 30-40 per cent of the herbage N-content was released during autumn. However, it was uncertain whether the released N was taken directly up by the crop or temporarily incorporated into soil microbes (Clausen 2002).

In these experiments, nitrogen application in autumn did not significantly increase seed yield in either timothy or meadow fescue (Tables 2 and 3). However, in one of the three timothy trials, which was located on a sandy soil and suffered from severe drought in spring, the highest seed yield was obtained on plots receiving nitrogen (30 kg ha⁻¹) in autumn. The beneficial effect of autumn nitrogen in this trial was probably due to the poor availability of nitrogen in spring caused by the dry conditions. In meadow fescue, optimal N level in autumn was usually dependent on whether the field was burned in spring or not (data not shown). On plots where spring burning was not practiced, nitrogen application in autumn actually had a negative effect on seed yield. This was probably related to a higher production of tillers in autumn on fertilized than on non-fertilized plots. When the wilted biomass on fertilized plots was not burned in spring, generative development was hampered due to shading. All in all, the experiments did not provide any support for an extra input of nitrogen in autumn, either in timothy or meadow fescue, when straw was chopped rather than removed.

ACKNOWLEDGEMENT

Thanks are extended to the staff at the Bioforsk Øst Landvik and the Farmers Experimental and Advisory groups in Vestfold,

Aust-Agder, Østfold and Buskerud for excellent management of the experiments, and to the Research Council of Norway and the Norwegian Seed Growers Association for economical support of the project.

LITERATURE

- Boelt, B. 2006. Frøgræshalm er en betydelig N-ressource. Frøavlaren 6:6-7. (In Danish.)
- Clausen, D. 2002. Kvælstoffrigivelse fra frøgræshalm fra engrapgræs, strandsvingel og Alm. rajgræs. Specialeprosjekt, 87 pp. Institut for Jordbrugsvidenskab. Den Kgl. Veterinær- og Landbohøjskole. (In Danish, with English abstract).
- Chastain, T.G., W.C. Young, C.J. Garbacik, B.M. Quebbeman, G.A. Gingrich, M.E. Mellbye & S. Aldrich-Markham. 1996. Residue management options for Willamette Valley grass seed crops. In: Young, W (ed). 1995. Seed Production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/CrS 106, 3/96: 1-5.
- Chastain, T.G., W.C. Young, C.J. Garbacik, T.B. Silberstein & M.E. Mellbye. 1997. Full straw management: Effects of species stand age, technique and location on grass seed crop performance. In: Young, W (ed). 1996. Seed Production Research at Oregon State University USDA-ARS Cooperating. Department of Crop and Soil Science Ext/CrS 110, 4/97: 14-16.
- Chilcote, D.O, H.W. Youngberg & W.C. Young. 1981. Postharvest residue burning as a management tool in grass-seed production. In: Smith, S. (ed). Proceedings of the 16th International Grassland Congress: 254-257.
- Havstad, L.T. 2002. Behandling av stubb og gjenvekst i frøeng av engsvingel (*Festuca pratensis* Huds.). In: Vallfrøodling/ Grass and clover seed production. NJF-rapport nr 341:93-98 (in Norwegian, with English abstract).

Post-harvest treatments in smooth-stalked meadow grass (*Poa pratensis* L.) - effect on carbohydrates and tiller development

Birte Boelt

Faculty of Agricultural Sciences, University of Aarhus, 4200 Slagelse, Denmark

Birte.Boelt@agrsci.dk

ABSTRACT

Temperate grass species require a period of short days/low temperature to respond to flower induction stimuli. The same environmental conditions stimulate the increase in carbohydrate concentration in aboveground biomass and the accumulation of reserve carbohydrates in the basal plant parts. The present investigation was initiated to investigate the effect of post-harvest treatments on dry matter production in autumn, carbohydrate content, the number of reproductive tillers and seed yield in a turf-type cultivar 'Conni' of smooth-stalked meadow grass. The results show that post-harvest treatments influence seed yield and yield components with the general tendency that defoliation increases the number of reproductive tillers, whereas chopping of straw results in fewer reproductive tillers and a reduced seed yield. This effect was eliminated if the crop was defoliated approximately six weeks after harvest and all residues removed. The results from plant samples in autumn indicate that decreasing aboveground biomass production leads to a higher carbohydrate concentration which may stimulate the reproductive development in smooth-stalked meadow grass.

Key words: Straw, residue management, defoliation, open field burning.

INTRODUCTION

Temperate grasses have a seasonal variation in the carbohydrate content and in general, the carbohydrate content is found to increase and accumulate especially in the basal parts with the cessation of growth due to decreasing temperature and day length (Smith & Grotelueschen 1965, Pollock & Jones 1979, Solhaug 1991). It is conditions similar to these that induce the reproductive development in a number of temperate grass species with a dual induction requirement for flowering (Heide 1994). In seed production experiments it has been found that the number of reproductive tillers has a pronounced, positive effect on seed yield in smooth-stalked meadow grass - especially in first-year seed crops (Boelt 1997, Meijer & Vreeke, 1988), and that post-harvest treatments have an effect on the number of reproductive tillers and hence seed yield in second year and older crops.

In the period 2001-2005 experiments were carried out to investigate carbohydrate levels in autumn in smooth-stalked meadow grass exposed to different post-harvest treatments and to monitor the effect of these on seed yield and yield components.

MATERIALS AND METHODS

The effect of post-harvest treatments on seed yield, the number of reproductive tillers and biomass carbohydrate levels in smooth-stalked meadow grass (*Poa pratensis* L.) cv. Conni was analysed in a field experiment at the Faculty of Agricultural Sciences, Research Centre Flakkebjerg. A smooth-stalked meadow grass seed crop was established in three consecutive years (2001, 2002 and 2003) on a sandy loam soil in the cover crop field pea. No experimental treatments were performed during the first seed production

year, but immediately after the first seed harvest, six post-harvest treatments were performed (Table 1). Except for in treatment 3 and 4 straw was removed immediately after seed harvest and the stubble height was cut

to approximately 6 cm. Nitrogen was applied at a rate of 90 kg ha⁻¹ in autumn and 60 kg ha⁻¹ in spring applied as calcium ammonium nitrate.

Table 1. Post-harvest treatments

Post-harvest treatment	
1.	Control
2.	Open field burning immediately after seed harvest
3.	Chopping of straw and stubble immediately after seed harvest
4.	As treatment 3 but 6 weeks later. Chopped material removed.
5.	Stubble cut to 2-3 cm immediately after harvest. Cut material removed.
6.	As treatment 3 but 6 weeks later. Cut material removed.

In the autumn, in the first week of October, all plots were defoliated to a 10-12 cm stubble height prior to N-application. Twice during autumn and at the onset of spring growth plant samples were collected from an area of 0.13 cm⁻² and at a soil depth of approximately 10 cm. After cleaning from soil, the plant samples were divided into a top and root fraction. These samples were frozen in liquid nitrogen and freeze-dried for 24 hours. Total carbohydrates were extracted using a 0.1 M sodium acetate buffer and hydrolysed with 0.037 M sulphuric acid. Quantification of glucose, sucrose, fructose, fructan and total sugar was performed using a coupled enzymatic assay procedure (Larsson & Bengtsson 1983, Knudsen 1997).

The experimental design was a randomised complete block with four replications. The net plot size was 8 m x 2.5 m. Prior to seed harvest one sample was cut within an area of 0.25 m², and the number of reproductive tillers was counted. Before shedding the grass seed crop was swathed and after 8-12 days of windrowing the crop was threshed with a trial combine harvester, and seeds were dried to 12 % moisture. After seed drying and cleaning, samples were analysed for purity using international standardized

methodology, and the seed yield expressed as 100 % clean seed was calculated.

Data were analysed as a one-factorial design. Analysis of variance (PROC GLM, SAS 1999) was performed for each harvest. Means of main effects of post-harvest treatments were declared different at $P < 0.05$ according to Student Newman Keuls multiple comparison test.

RESULTS

Seed yields varied significantly between years with the highest seed yield recorded in 2005 followed by 2003 and 2004 with the yields of 1359, 990 and 863 kg ha⁻¹ respectively. The effect of post-harvest treatments was significant ($P=0.02$), and the highest and lowest yield were obtained in the treatments 'untreated + defoliation 6 weeks after harvest and all residue removed' and 'chopping of straw' respectively (Fig. 1a). Similarly, post-harvest treatments had an effect on reproductive development ($P=0.001$), where the 3 defoliated treatments had a significantly higher number of reproductive tillers than 'chopping of straw'.

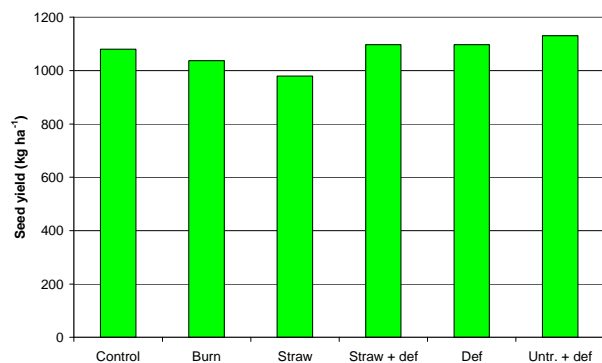


Fig. 1a. Effect of post-harvest treatments on seed yield (average 2003-2005).

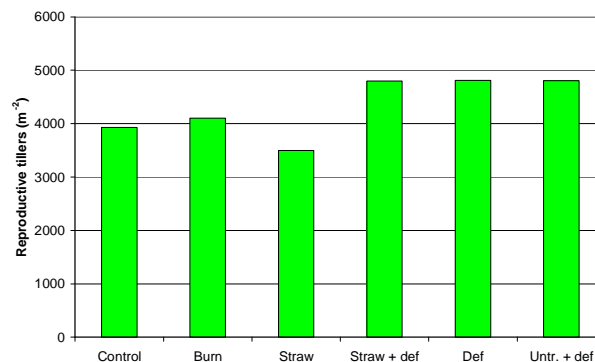


Fig. 1b. Effect of post-harvest treatments on the number of reproductive tillers (average 2003-2005).

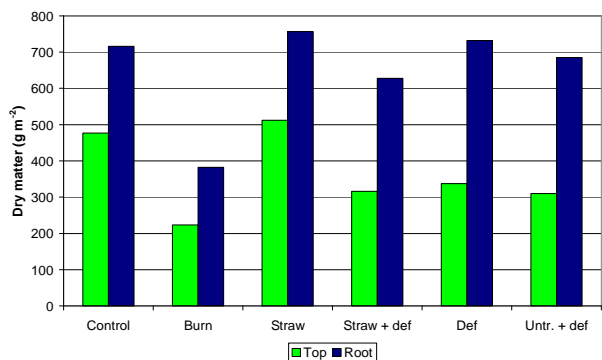


Fig. 2a. Effect of post-harvest treatments on top and root dry matter in autumn (average 2003-2005).

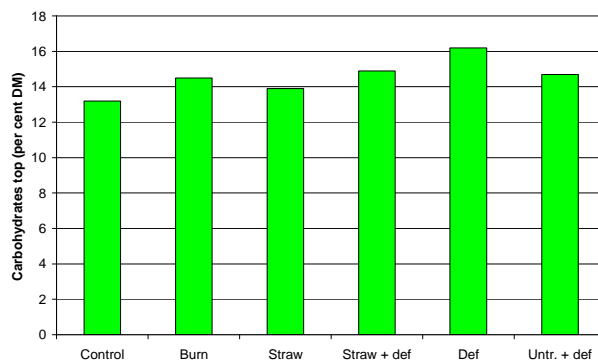


Fig. 2b. Effect of post-harvest treatments on the percentage of carbohydrates in top (average 2003-2005).

Post-harvest treatments also influenced dry matter production in both top and root fractions (Top: $P=0.02$; Root: $P=0.002$) (fig. 2a). Burning of straw clearly decreased both top and root dry matter production compared to other treatments, whereas the 3 defoliated treatments decreased only the top dry matter production. The percentage of carbohydrates in autumn was higher in the 3 defoliated treatments compared to 'control' (fig. 2b). Post-harvest treatments had an overall significant effect on carbohydrate content in autumn ($P=0.02$).

DISCUSSION

The results are in accordance with earlier investigations stating that the number of reproductive tillers has a pronounced positive effect on seed yield in smooth-stalked meadow grass - both in first and second-year seed crops (Boelt 1997, Meijer & Vreeke 1988). Chopping of straw decreased the development of reproductive tillers and

hence seed yield was decreased in the smooth-stalked meadow grass cultivar 'Conni', however defoliation and removal of all residues six weeks after harvest eliminated this negative effect on seed yield. This procedure has become relevant in areas where nitrogen application levels are restricted due to the release of nutrients from straw during autumn rains (Clausen & Boelt 2002). Earlier findings by Nordestgaard (1988) showed that defoliation was an alternative to open field burning, especially in turf cultivars of smooth-stalked meadow grass. In addition, the results showed that higher yields were obtained if post-harvest treatments were performed approximately 6 weeks after harvest. The present results confirm this finding, which may indicate that the requirements for the regrowth period are fewer in smooth-stalked meadow grass than found in red fescue (*Festuca rubra* L.). Similarly Aamlid (1993) found that especially older stands (second year) were favoured by autumn cutting in late August/early September in Norway.

Apart from chopping of straw the performed post-harvest treatments tended to reduce the amount of dry matter, especially in top fractions, and the results indicate a correlation between a reduced amount of dry matter and an increased carbohydrate content expressed in per cent of dry matter. Heide (1994) proposed that accumulation of photosynthates may be essential for the competence of seedlings to respond to flower inductive stimuli. Chastain & Young (1998) found that tiller height in autumn at the end of the regrowth period strongly correlated to reproductive development and seed yield in smooth-stalked meadow grass - the shorter the tiller the higher seed yield. In 45 smooth-stalked meadow grass genotypes with contrasting turf quality, Johnston *et al.* (2003) found that a high turf quality was negatively correlated with seed yield and seeds per panicle, however, a high number of reproductive tillers were not associated with

poor turf quality. This indicates reproductive tiller development as an important component in the breeding process for combining high-quality turf characteristics with high seed yield. In addition, the present investigation shows that cultivation techniques such as post-harvest treatments may be performed to stimulate the reproductive growth in the turf cultivar of smooth-stalked meadow grass 'Conni' and that this correlates to the carbohydrate status of the plant.

ACKNOWLEDGEMENTS

The author wishes to thank the technical staff at the Faculty of Agricultural Sciences, Research Centre Flakkebjerg for their skilful assistance.

LITERATURE

- Aamlid, T.S. 1993. Autumn treatment in smooth meadowgrass (*Poa pratensis* L.) grown for seed. Norsk landbruksforskning 7: 117-138. (In Norwegian with English abstract).
- Boelt, B. 1997. Undersowing *Poa pratensis* L., *Festuca rubra* L., *Festuca pratensis* Huds., *Dactylis glomerata* L. and *Lolium perenne* L. for seed Production in five cover crops. II. Effect of the cover crop plant density on the seed yield of the undersown grasses. Journal of Applied Seed Production 15: 49-53.
- Chastain, T.G. & Young III, W.C. 1998. Vegetative plant development and seed production in cool-season perennial grasses. Seed Science Research, 8(2): 295-301. (In Danish, with English abstract).
- Clausen, D. & Boelt, B. 2002. Chopping of grass seed straw in smooth staked meadow grass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). NJF-seminar report no 341: 22-29.
- Heide, O. 1994. Control of flowering and reproduction in temperate grasses. New Phytologist 128: 347-362.
- Johnson, R.C., Johnston, W.J. & Golob, C.T. 2003. Residue management, seed Production, crop development, and turf quality in diverse Kentucky bluegrass germplasm. Crop Science 43: 1091-1099.
- Knudsen, K.E.B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science Technology 67: 319-338.
- Larsson, K. & Bengtsson, S. 1983. Bestemmelse af lavmolekylære sukre ved kolorimetri. Metodebeskrivning nr. 22. Statens Landbrukskemiske laboratorium, Uppsala, Sverige.
- Meijer, W.J.M. & Vreeke, S. 1988. The influence of autumn cutting treatments on canopy structure and seed production of first-year crops of *Poa pratensis* L. and *Festuca rubra* L. Netherlands Journal of Agricultural Science 36: 315-325.
- Nordestgaard, A. 1988. Alternatives to burning the seed-grass straw. Tidsskrift for Planteavl 92: 103-114.
- Pollock, C.J. & Jones, T. 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytologist 83: 9-15.
- SAS 1999. SAS Online Doc. Version 8. SAS Institute, Cary, NC, USA.
- Smith, D. & Grotelueschen, R.D. 1965. Carbohydrates in Grasses. I: Sugar and fructosan composition of the stem bases of several northern-adapted grasses at seed maturity. Crop Science 6: 263-266.
- Solhaug, K.A. 1991. Effects of photoperiod and temperature on sugars and fructans in leaf blades, leaf sheaths and stems, and roots in relation to growth of *Poa pratensis*. Physiologia Plantarum 82: 171-178.

Using seed moisture to determine optimum swathing time in annual ryegrass (*Lolium multiflorum* Lam.) seed production

Tom B. Silberstein¹⁾, Mark E. Mellbye²⁾, William C. Young III³⁾ & Tom G. Chastain³⁾

- ¹⁾ Oregon State University Extension Service, Marion County Extension Office, 3180 Center St NE, Salem, OR, USA.
²⁾ Oregon State University Extension Service, Linn County Extension Office, P.O. Box 765 Albany, OR, USA.
³⁾ Dept of Crop and Soil Science, Crop Science 109, Oregon State University, Corvallis OR 97331

tom.silberstein@oregonstate.edu

ABSTRACT

Grass seed crops in Oregon are usually harvested in a two step process in order to minimize seed shattering losses - swathing to allow air drying for about a week, then combining. Since grass seed crops do not pollinate and mature over a uniform time period, there is a wide range of seed maturity within any crop stand. Determining the best time to swath grass seed crops is a balance between cutting too early and too late since both of these extremes can have an impact on seed quality as well as seed yield. Seed moisture content is considered the best indicator of the physiological maturity in grass seed crops for determining when to swath. Previous work in the United Kingdom has identified the range of moisture contents for optimizing harvested seed yield in direct combined annual ryegrass. Research on other temperate climate grasses was also done in the Willamette Valley of Oregon in the late 1960's. Though there are currently about 50,000 ha of annual ryegrass in seed production, very little information on what would be the best swathing time for maximizing seed yield and seed quality exists. Lack of any regional data on annual ryegrass as well as recent significant improvements in equipment used to harvest grass seed crops were the primary factors in conducting this two year study. Results from this research determined that swathing annual ryegrass at approximately 45 percent seed moisture optimizes seed yield.

Key words: physiological maturity, grass seed

INTRODUCTION

Seed moisture content is probably the best indicator of the physiological maturity in grass seed crops for determining when swathing (windrowing) is to be done for harvesting seed. Since grass seed crops do not pollinate and mature over a uniform time period, there is a wide range of seed maturity within a crop stand. In order to optimize the time to swath grass seed crops, there is a balance between cutting too early and too late. Cutting too early at high moisture content shortens the seed fill period and can cause reduced seed

size and increase the number of immature seed. Cutting too late at low moisture content can decrease yield through losses due to seed shattering (Klein & Harmond 1971, Andersen & Andersen 1980). Both of these extremes can have an impact on seed quality as well as seed yield. For annual ryegrass, there is very little information relating to the practices in Oregon's Willamette Valley on what would be the best cutting time for maximizing seed yield and seed quality. Previous work in the U.K. has identified the range of moisture contents for optimizing harvested seed yield in direct combined annual ryegrass (Hides *et al.* 1993). Research

was also done in the Willamette Valley on tall fescue (Andrade *et al.* 1994) as well as perennial ryegrass, orchardgrass, and fine fescues (Klein & Harmond 1971). There is also some evidence in research by Andersen and Andersen (1980) on several grass species that the crop is able to continue development as it dries in the windrow. How much this post swathing continued development benefits seed yield is not well known.

In addition to using seed moisture content as a factor to determine when to swath, many growers cut their crops under high humidity conditions either at night or early morning to take advantage of dew on the crop as a means to reduce seed shatter. Swathing under high dew conditions lets the grower delay swathing to allow more time for the later maturing portion of the crop to continue seed fill and hopefully increase harvested seed yield. Though this is a common practice in some areas of seed production in the Willamette Valley, there is little applied research available that quantifies any beneficial effect that swathing during high dew conditions may have on seed yield. This two year trial was designed to compare harvested seed yields at different seed moisture contents and verify recommendations previously available. This also provided an opportunity to compare the efficacy of more modern harvest equipment than was used in previous studies by Klein & Harmond over 30 years ago.

Two years of research were conducted with annual ryegrass at the Hyslop Research Farm in Corvallis, Oregon, to:

- 1) Determine the optimum seed moisture content swathing annual ryegrass and
- 2) Measure the effect of early dew at swathing time.

This information will be used to provide annual ryegrass growers previously unavailable guidelines to help determine the best times to begin harvest.

MATERIALS AND METHODS

Research plots were planted at Hyslop Research Farm in the Winter of 2004 by no-till drilling annual ryegrass into a previous crop of meadowfoam (*Limnanthes alba* Benth.). Following the meadowfoam harvest in 2003, the field was sprayed with 2.2 kg a.i. ha⁻¹ glyphosate (Buccaneer Plus[®]) on August 8, 2003 to control volunteer sprout and

weeds. Additional control of seedling meadowfoam and other seedling sprout weeds was done with an application of 104 g a.i. ha⁻¹ clopyralid + 550 g a.i. ha⁻¹ 2,4-D amine (Curtil[®]) and 1.1 kg a.i. ha⁻¹ glyphosate (Buccaneer Plus[®]) on November 3. Pre-plant applications of 2.2 kg a.i. ha⁻¹ glyphosate (Buccaneer Plus[®]) and 28 g a.i. ha⁻¹ carfentrazone (Aim[®]) were done on February 10, 2004. On February 12, the field was no-till drilled with annual ryegrass at a seeding rate of 28 kg ha⁻¹ using a John Deere power drill. The new planting was fertilized with 336 kg ha⁻¹ of 16-16-16 (56 kg/ha N/a) the following day. Additional fertilizer was applied on March 29 (110 kg N ha⁻¹ as urea) and April 12 (34 kg ha⁻¹ as urea) for a total N application of 200 kg ha⁻¹. The field also received an application of 430 g a.i. ha⁻¹ bromoxynil + 430 g a.i. ha⁻¹ MCPA (Bronate[®]) and 14 g a.i. ha⁻¹ carfentrazone 35 (Aim[®]) on April 7. For the second year (2005) the annual ryegrass was planted in November, 2004 using conventional tillage (plow, disc, and harrow). The new planting was fertilized with 308 kg ha⁻¹ of 16-16-16 (50 kg N ha⁻¹) pre-plant incorporation. Spring fertilizer was applied on March 17 for a total N application of 153 kg ha⁻¹ plus 34 kg ha⁻¹ sulfur. Experimental design was set up as a 5 x 2 factorial with seed moisture content and dew as the two main factors replicated four times. There were five seed moisture contents (50, 45, 40, 35, and 30%) at two dew levels (dew present - early am, and no dew present - early pm) for a total of 10 treatments. Analysis was done using Statistix[®] statistical software. Plots were swathed using a modified John Deere 2280 swather and combined with a Hege 180 plot combine. Cleanout was determined by using an M2-B clipper cleaner, seed size was measured by taking 1000 seed weights from combine run samples and germination tests were done according to ISTA rules.

RESULTS

Seed moisture and dew

Annual ryegrass plots were swathed at the five different moisture contents listed in Table 1. Clean seed yield was highest at the 45 % seed moisture content. Swathing at 40 % seed moisture and lower caused rapid declines in seed yield. In addition, there were seed moisture x dew interactions in both years. The interactions, presented in Table

2, show the benefit of swathing with humidity in the crop high when seed moisture is less than optimum. At 45-50 % moisture, there was little difference in yield due to the presence or absence of dew on the crop. However, at lower seed moistures (40 % and

below), yield was reduced by an average of 373 kg ha⁻¹, equal to an average loss of 22 % just by delaying swathing until later in the day (6 hours later) after the dew had dried off of the crop.

Table 1. Seed yield, 1000 seed weight, and germination in annual ryegrass swathed at different seed moisture contents and dew levels, Hyslop Research Farm, 2004-05.

Treatment	Seed Yield (kg ha ⁻¹)		1000 Seed Wt. (g)		Seed Germination (%)	
	2004	2005	2004	2005	2004	2005
<u>Seed moisture %</u>						
50	2886	1367 a	2.51 d ³	3.22 b	98.0	98.7 a
45	3127	1391 a	2.76 c	3.22 b	97.9	98.4 a
40	2908	1074 b	2.87 b	3.40 a	97.9	97.4 b
33	2674	867 c	2.93 b	3.41 a	98.3	98.0 ab
28	2137	744 c	3.05 a	3.40 a	97.7	96.9 b
LSD 0.05(0.10)	* ¹	134 ²	0.07	0.08	NS	(1.1)
<u>Time of day</u>						
Dew ⁴	2877	1192 a	2.82	3.35	---	---
No dew	2615	985 b	2.83	3.34	---	---
LSD 0.05	* ¹	85 ²	NS	NS		

¹ Significant Seed Moisture X Time of day interaction at P<0.05.

² Significant Seed Moisture X Time of day interaction at P between 0.05 and 0.10

³ Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values.

⁴ Early morning (~6 am) for dew, Early afternoon (~1pm) for no dew.

Table 2. Seed yield interaction in annual ryegrass swathed at different seed moisture contents and time of day at Hyslop Research Farm, 2004-05.

Seed Moisture (%)	2004		Difference (dew-no dew)		2005		Difference (dew-no dew)	
	Dew ¹	No Dew	(dew-no dew)	(%)	Dew	No Dew	(dew-no dew)	(%)
	-----	kg ha ⁻¹ -----		(%)	-----	kg ha ⁻¹ -----		(%)
50	2871 a ²	2901 ab	(30)	1	1367 a ²	1368 a	(1)	0
45	3062 a	3192 a	(130)	4	1526 a	1255 a	271	-18
40	3146 a	2669 bc	477	-14	1131 b	1018 b	113	-10
33	2921 a	2426 c	495	-17	1010 bc	725 c	285	-28
28	2386 b	1888 d	498	-21	927 c	560 d	367	-40
LSD 0.05	-----	292 -----			-----	158 -----		

¹ Early morning, - with dew present; early afternoon - no dew present.

² Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values.

Table 3. Annual ryegrass seed yield comparison between 2004 and 2005, Hyslop Farm.

	Seed yield (kg ha ⁻¹)		
	2004	2005	2 year average
Seed moisture at swathing, %			
50	2886	1367	2127
45	3127	1391	2259
40	2908	1074	1991
33	2674	867	1771
28	2137	744	1441
Time of day			
Dew present ¹⁾	2877	1192	2035
No dew present	2615	985	1800

¹⁾ Early morning, - with dew present; early afternoon - no dew present.

Seed yields were very good in 2004 even with the later planting in February. In contrast, yields in 2005 were much lower than normal due to heavy damage from significant vole populations (Table 3). There were a lot of seed heads missing or chewed off. However, even with vole impact, the relationships of the treatment effects were almost identical in both years as shown in Table 2. Shattering was visually evident when the plots were swathed under the drier conditions. Seed size (1000 seed weight) increased significantly as

the crop was harvested at lower seed moistures. Factors that may contribute to this are the loss of the smaller seed at the distal end of the spikelets, as they tend to shatter first, and maybe continued fill of seeds that did not shatter. The largest seed are typically at the base (proximal) of the spikelet and do not shatter as readily, thus increasing the portion of seed that is larger and potentially increasing 1000 seed weights. Germination tended to decrease at the lower seed moisture ($P < 0.10$) but it is unclear as to the cause and the difference was rather small at less than 2 %. Germination averaged from 96.9 to 98.7 percent, well above the 90% required for certified seed.

DISCUSSION

Seed moisture content is a useful tool in determining the range of maturity for maximizing yield in grass harvested for seed. Looking at the 2 year average for annual ryegrass, the optimum seed moisture content was around 45 %. Yield declined as moisture content approached 40 % and below. However, some flexibility in delaying swathing time can be utilized if there is dew present on the crop.

LITERATURE

- Andersen, S. & Andersen, K. 1980. The relationship between seed maturation and seed yield in grasses. *In*: Hebblethwaite, P.D. (ed.). Seed Production. pp. 151-172. Butterworth Publishers, London.
- Andrade, R.P., Grabe, D.F. & Ehrensing, D. 1994. Seed Maturation and harvest timing in turf type tall fescue. *Journal of Applied Seed Production* 12: 34-46.
- Hides, D.H., Kute, C.A. & Marshall, A.H. 1993. Seed development and seed yield potential of Italian ryegrass (*Lolium multiflorum* Lam.) populations. *Grass and Forage Science* 48: 181-188.
- Klein, M.K. & Harmond, J.E. 1971. Seed Moisture - A harvest timing index for maximum yields. *Transactions of the American Society of Agricultural Engineers* 14: 124-126.

Optimum harvest time of tall wheatgrass seed

Leandro M. Cardoso¹⁾, Sara I. Alonso²⁾, Andrea M. Clausen¹⁾ & Jorge Castaño¹⁾

¹⁾Estación Experimental Agropecuaria Balcarce INTA, CC 276, 7620 Balcarce, Argentina.

²⁾Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, CC 276, 7620 Balcarce, Argentina

lcardoso@balcarce.inta.gov.ar

ABSTRACT

Tall wheatgrass (*Thinopyrum ponticum*) is a forage species of uneven maturity in the Buenos Aires Province, Argentina. It is difficult to determine the optimum harvest time (OHT) to secure high seed quality with low seed shattering. Natural shattering and seed quality, measured by thousand seed weight, pure seeds percentage, vigour, % germination after 5 and 24 days, and germination velocity, were analyzed to establish OHT. The characters were measured in 3 varieties, using caryopses located at low, medium and top sections of spikes, and for 4 visually determined maturity categories: stem and rachis green (GG); stem green with rachis partially brown (G1/2B); stem green with rachis completely brown (GB); and both stem and rachis brown (BB). Independent of varieties, ANOVA ($\alpha = 0.05$) detected that seed quality 1) increased with spike maturity from GG to BB, and 2) was lower in seeds from the lower spike section compared with seed from the upper sections. Shattering affected 44 % of spikes at GG, and 86, 91 and 93 % of spikes at G1/2B, GB and BB, respectively. The OHT of tall wheatgrass is the stage when the majority of the spikes have green stems and rachis completely brown.

Key words: harvest date, maturity, seed loss, purity, *Thinopyrum ponticum*, viability.

INTRODUCTION

Tall wheatgrass (*Thinopyrum ponticum*) is a late, cool season perennial forage grass, cultivated on alkaline flooded soils. In the southeast of the Buenos Aires Province, Argentina, the seed harvest is carried out from mid February to March (Castaño 1995). The harvest is indirect and carried out mechanically, including cutting, windrowing and collecting. Plants of tall wheatgrass have a high number of tillers of different order, and therefore maturity is not uniform, either between or within spikes (Ferrari & Maddaloni 2001). Consequently, it is difficult to establish the optimum harvest time (OHT) to maximize both seed yield and quality. Delays in the swathing of the crop, and/or collection of the swaths, can reduce seed yield and quality by shattering, predation and deterioration. On the other hand,

early harvest can affect viability, vigour and purity, also resulting in poor seed quality (Hebblethwaite 1983, Dell'Agostino 2001). Seed lots of low quality do not meet the standards of commercialization imposed by the National Institute of Seeds (INASE 1988) and their use can affect the successful implantation of pastures. According to Castaño (1995), the seeds of tall wheatgrass can be considered mature when the spikes present the rachis brown or straw-coloured and the stem green. Nevertheless it is not known how the seed quality parameters are affected, nor the intensity of shattering at the maturity stage or at earlier or later states of maturation. The objective of this work was to establish the optimal harvest time in tall wheatgrass through determination of viability, vigour, purity and shattering in spikes of different state of maturity, defined by the colour of rachis and stem.

MATERIALS AND METODS

Spikes of three experimental varieties (SA, SM and SJ) and four maturity categories were harvested by hand at the end of the summer at Balcarce (37°45' S, 58°18' W) in the southeast of the Buenos Aires Province, Argentina. Maturity categories were established as a function of stem (S) and rachis (R) colours: GG, when S and R were green; G1/2B, when S was green but R was partially brown or straw-coloured; GB, when S was green but R was completely brown; and BB, when both S and R were brown. Stems and spikes of each variety and maturity stage were cut into three sections: low = L, medium = M and top = T. Seed weight, percentage of pure seeds related to sample weight and number of spikelet with loss of seeds by natural shattering were determined on 30 spikes for each combination variety x maturity stage x spike section. Caryopses from 70 spikes were used to carry out a germination test with four replicates, the replicates were put at different levels in the incubator cabinet. Fifty seeds were arranged on the top of paper inside plastic boxes and incubated for 24 days at 8 hours at 30°C and light + 16 hours at 20°C and dark (Ellis *et al.* 1985, ISTA 2004). Percentage of germination at days 5 (first count) and 24 (final count) as well as germination velocity ($V = \{\sum(G_i \cdot D_i) / N\}$, where: G_i = % of seeds germinated between counts i and $i-1$; D_i = days from incubation to count i ; N = final germination) were registered. Analysis of variance within

each variety was performed according to a RCB design, with 12 treatments (maturity stage x spike section) and four replicates for germination variables. Mean treatment comparisons were performed with the LSD test ($\alpha=0.05$). Due to a procedure mistake, the treatments GB x L and GB x M for the variety SJ had to be discarded from the analysis.

RESULTS

The variety SJ had higher initial and final germination counts than the varieties SM and SA (Fig. 1 and Tables 1 and 2). Except for the SA variety that did not present significant differences between maturity stages, the germination after 5 and 24 days of incubation and germination velocity were significantly lower in spikes of the GG maturity class than in spikes with the rachis completely brown, (Fig. 1 and Table 1).

Thousand seed weight differed among the three varieties, with mean values of 6.6, 6.9 and 7.2 g for SM, SJ and SA, respectively. Within each variety, the interaction maturity stage x spike section was significant. Generally, the seeds from the lower section and from the GG maturity class had lower thousand seed weight than seeds from other maturity classes or spike sections. However, these differences were not significant in all varieties (data not shown).

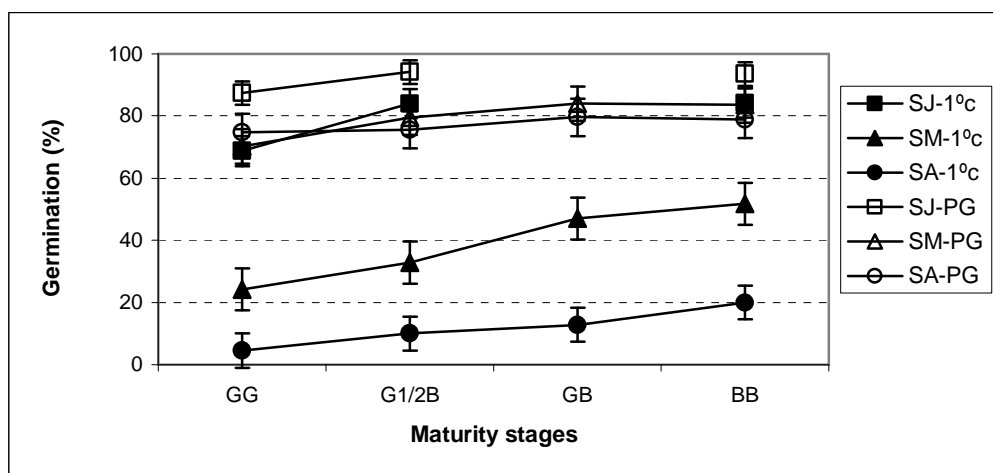


Fig. 1. Germination percentages at days 5 (1° count) and 24 (final count) for three varieties (SJ, SM, SA) and four maturity stages.

Table 1. Germination velocity (days) in three varieties and at four spikes maturity stages. Duncan critical range (DCRange) among means of treatments in columns ($\alpha=0.05$).

Varieties			
Maturity	SA	SM	SJ
GG	11.8	13.8	7.2
G1/2B	11.5	13.0	6.1
GB	10.6	11.2	-
BB	10.5	10.5	5.9
<i>DCRange</i>	0.4	2.2	0.8

Among varieties, the proportion of spikelets with natural shattering in the upper section was 71% (SM), 81% (SA) and 84% (SJ), while the percentage of pure seeds varied from 63% (SA) to 69% (SJ). Independently of the variety, the losses due to shattering were less in spikes harvested at the GG stage than in those harvested at later stages. Conversely, the percentage of pure seeds remaining on the spike was larger at G1/2B and GB than at the other maturity stages, and larger in the middle and inferior sectors (66.7 and 67.5%) in relation to the superior (62%) (Fig. 2).

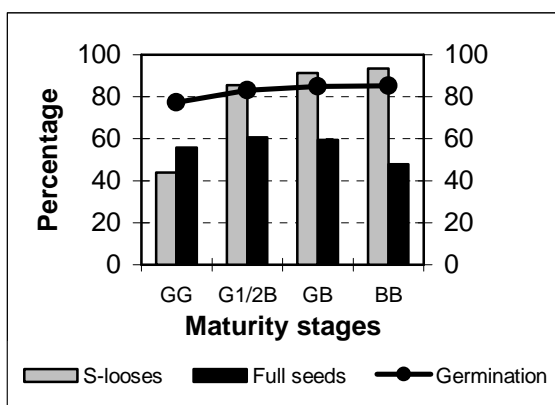


Fig. 2. Means of percentages of full seeds, spikelet with seed lost and germination at 24 days, for spikes of different maturity stages.

Table 2. Germination at days 5 (1° count) and 24 (final count, PG) and germination velocity of seeds from three varieties and three spikelet positions. Duncan critical range (DCRange) among treatments in columns ($\alpha=0.05$).

Varieties			
Location	SA	SM	SJ
1° count (%)			
Top	13.5	38.8	81.0
Medium	12.8	43.5	80.6
Low	9.2	34.6	75.6
<i>DCRange</i>	4.6	5.8	4.8
PG (%)			
Top	78.4	79.2	91.5
Medium	80.4	81.6	93.5
Low	72.0	77.0	89.4
<i>DCRange</i>	5.2	4.8	3.8
Velocity (days)			
Top	11.0	12.0	6.2
Medium	10.7	11.7	6.3
Low	11.7	12.6	6.6
<i>DCRange</i>	1.05	0.72	0.44

DISCUSSION

For the commercialisation of seeds of tall wheatgrass of the highest quality category ('original, registered or certified') 80 % germination and 90 % purity are required, while for the seed category 'identified', 75 % and 85 % are required, respectively (INASE 1988). The seeds of the variety SJ reached the maximum germination standard independently of the maturity stages of the spikes, while SA never reached the 80 % germination criterion and SM only reached this requirement for the more mature spikes.

Although seeds of SJ had the highest purity, none of the varieties reached the purity standards required. Analyzing the seed lots of tall wheatgrass in the region, Peretti & Escuder (1990) found that a high percentage of the seeds presented percentages of purity smaller than the ones required by the INASE. In this work the low values of purity could be the result of the inclusion of immature spikes with a high number of empty seeds, and the fact that after the harvest, the seed lots were

not air cleaned. The variety SJ had the best seed quality (germination, purity and germination velocity), while SA appeared to be the variety with the lowest seed quality.

In spikes with green rachis (GG), the quality of the seed was low as they had a lower initial germination percentage than those from spikes G1/2B, and a lower final germination percentage and germination velocity than those from spikes with the rachis completely brown (GB and BB). Furthermore, the GG spikes presented a low purity due to the presence of empty seeds.

At the maturity stages GB and BB, the seeds had high values for viability and vigour, indicating that these are the best maturity stages for harvest, although a high percentages of spikelets had been shattering. Among these stages, BB spikes had lower purity probably due to the fact that the quantity of seed losses per spikelet was larger than for GB spikes. Consequently, GB it is the recommended stage for harvest.

Within the spike, the seeds from the lower sector presented lower values of germination, germination velocity and 1000 seed weight, but with a larger proportion of pure seeds. This means that the seeds in this sector were less mature than in the middle and upper sector, and this is coincident with Ferrari & Maddaloni (2001) who established that in tall wheatgrass the maturity begins in the superior part of the spike.

In this study, we found that OHT for tall wheatgrass is when the largest number of spikes present green stems with rachis completely brown (GB). Harvest at later stages would increase the losses due to shattering and would reduce the percentage of pure seeds. On the other hand, harvest at earlier would result in a high quantity of seeds but with less viability and vigour.

ACKNOWLEDGEMENTS

Authors thank Universidad Nacional de Mar del Plata (Project 15/208) and ANPCyT (PICTR 00109) for financial support.

LITERATURE

- Castaño, J. 1995. Cap. 6: Agropiro Alargado. *In*: Producción de semillas de gramíneas forrajeras en el sudeste bonaerense. INTA, CERBAS, EEA Balcarce. pp. 65-70.
- Dell'Agostino, E. 2001. Producción de semillas de especies forrajeras templadas. *In*: Maddaloni, J. & Ferrari, L. (ed.). Forrajeras and Pasturas del ecosistema templado húmedo de la Argentina. INTA- U.N. Lomas de Zamora, Fac. Cs. Agr. pp. 407-456.
- Ellis, R. H., Hong T. D, and Roberts E. H. 1985. Handbooks for Genebanks: N° 2. Handbook of Seed Technology for Genebanks. Vol.1, IBPGR, Rome.
- Ferrari, L. & Maddaloni, J. 2001. 5. Agropiro alargado. *In*: Maddaloni, J. & Ferrari, L. (ed.). Forrajeras and Pasturas del ecosistema templado húmedo de la Argentina. INTA- U.N. Lomas de Zamora, Fac. Cs. Agr. pp. 125-134.
- Hebblethwaite, P.D. 1983. Producción moderna de semillas. Ed Hemisferio Sur. Tomo I and II. 783 pp.
- ISTA 2004. Internacional Rules for Seed Testing. ISTA, Suiza.
- INASE 1988. Disposición N° 12. SAGyP, Ministerio de Economía. 6/1988. Argentina.
- Peretti, A. & Escuder, C.J. 1990. Evaluación de la calidad de semillas forrajeras en el sudeste bonaerense. *Revista Argentina de Producción Animal* 10: 331-344.

Effects of harvest date on seed yield and quality of *Scorpiurus muricatus* L.

Giuseppe Di Giorgio, Paolo Ruisi, Dario Graziano, Dario Giambalvo & Gaetano Amato

Università di Palermo, Dipartimento di Agronomia ambientale e territoriale,
Viale delle Scienze, 90128 Palermo, Italy

paoloruisi@unipa.it

ABSTRACT

Scorpiurus muricatus L. is an annual legume, widely distributed in the Mediterranean basin, appreciated by farmers for palatability, nutritive value and productivity. Despite its potential as forage crop, for improving poor pastures or as cover crop, the species is not well studied, particularly as seed production is concerned. As many other pasture legumes, the species is characterized by a long duration of flowering and ripening as well as by pod-shedding. The objective of this research was to acquire knowledge useful to identify harvest techniques able to limit seed losses and ensuring a good seed quality. Therefore the effects of harvesting at four different maturity stages on seed yield and quality were studied. The field trial was carried out in a semi-arid, hilly area of Sicily (southern Italy) using a randomized block design with four replications. First harvest was done when first pods were shed and the last when all the pods were mature. Harvesting too early resulted in low yield and poor seed quality whereas harvesting too late gave the highest seed production, seed weight and germination but resulted in a total pod-shedding.

Key word: Germination, Mediterranean semi-arid environment, pasture legume, pod shedding, Prickly scorpion's tail, seed losses

INTRODUCTION

Scorpiurus muricatus L. is an annual legume widely distributed as spontaneous in pastures of Mediterranean basin, particularly in Morocco, Algeria, South France and South Italy (Bensalem *et al.* 1990, Beale *et al.* 1991). The species is broadly tolerant of soil type and soil pH (Ehrman & Cocks, 1990) and highly drought tolerant (El Shaer 1995). It produces a conspicuous biomass (Di Giorgio *et al.* 2007) with a high protein content (Licitra *et al.* 1997). In some areas of Sicily (particularly in the Hyblean plateau) farmers ascribe to *Scorpiurus muricatus* a very high galactogogue effect and palatability so that its abundance increases the value of the pasture. Despite its potential as forage crop and for pasture improvement, the species is not well studied so far and there is a lack of knowledge about agronomical aspects, particularly concerning seed production.

S. muricatus is a prostrate plant characterized by a long duration of flowering and ripening (Di Giorgio *et al.* 2007). Furthermore the species is characterized by pod-shedding and therefore, during the reproductive stage, it is possible, at the same time, to observe flowers, green and brown pods in the standing plants, and shed pods on the soil surface. In a seed crop, these characteristics cause high seed losses and could reduce seed harvest efficiency in terms of seed yield and quality. This work aimed to evaluate patterns of change in seed yield and seed viability with maturity in *Scorpiurus muricatus* in a semi-arid Mediterranean environment.

MATERIALS AND METHODS

The trial was carried out during the 2004/05 growing season in a semi-arid, hilly area of Sicily (S. Stefano Q., AG, 37° 30'N; 13° 31'E; 178 m a.s.l.) on a deep, clayey, well structured soil with wheat as previous crop. Treatments consisted in four seed harvest dates. First harvest was carried out when first pods were shedding (13 June) and the last harvest when all pods were mature (26 July). The other two harvests were carried out at intermediate dates (23 June and 5 July). The treatments were arranged in a completely randomized block design with four replications. Seeds of a population of *S. muricatus* (collected in a natural pasture of northern Sicily, 38° 01'N, 13° 58'E, 122 m a.s.l.) were sown on 12 January 2005 at 250 viable seeds m⁻². Plot size was 2 x 3 m (10 rows 3 m long and 0.20 m apart). No fertilizer was applied and the trial was carried out under rainfed conditions. Weeds were manually removed after germination and during the growing season.

At each harvest date, stands were manually cut and shed pods were gathered from the ground by hand. Total cut fresh biomass and pods from the ground were weighted. A sample of fresh biomass (approx. 0.5 kg) was separated in leaves, stems and pods and the botanical fractions were weighted and oven-dried at 105 °C for 24 h to determine moisture content. The same procedure was applied to a sample of pods gathered from the ground. Remaining biomass was air-dried when necessary and passed through a plot thresher to separate pods; the pods were then passed through a laboratory hulling machine to assess seed yield. Pods collected from the ground were threshed as well and seeds were weighted. For each treatment, a germination test was carried out in November 2005 on samples from stands and from ground using 400 seeds (100 seeds x 4 replicates) placed on moist filter paper into Petri dishes and put in a germinator for 14 days at 20 °C in darkness. Germinated seeds were scored and removed from the tests on a daily basis. The mean germination time (MGT) was computed as $MGT = \frac{\sum[(t_x)(n_x)]}{\sum(n_x)}$ where n_x = the number of newly germinated seed at time t_x and $\sum(n_x)$ = the total number of germinated seeds. The analysis of variance was performed according to the experimental design and least significant difference (LSD) values were calculated at the 0.05 level of

probability. Data in percentages were not transformed.

Weather

The site of evaluation has a typical Mediterranean climate with an average rainfall of 551 mm, mostly concentrated during the autumn-winter period (74 %) and in a lesser amount in spring (18 %). Means of minimum and maximum temperatures are 10.0 and 23.3 °C. Total rainfall during the 2004/2005 growing season was 842 mm (53 % higher than the long-term average); the abundant spring rainfall was particularly favourable to crop growth.

RESULTS AND DISCUSSION

When the first pods started to shed, plants were still green (including most of the pods) and the moisture of standing biomass (stems, leaves, flowers and pods) was about 71 %. At the two following harvest dates, moisture content went down to 64 and 39 %, respectively, and when all the pods were mature it was about 10 % (Fig. 1).

The moisture content of pods shed from the standing crop at first harvest was quite high (about 43 %) but it declined rapidly to about 20% in the following harvest; at the two last harvest dates, pods were dried (moisture content of about 12%).

Total seed yield increased significantly with harvest date from 97 g m⁻² at the first harvest to 145 g m⁻² at third. Differences in yield between the second and fourth harvests were not significant (Fig. 2). Seed production was similar to the values reported by Bouazza & Abdelguerfi (1995) and Abbate *et al.* (1999) in field trials carried out in Algeria and in the Hyblean plateau (Sicily), respectively.

Despite the harvest was carried out manually, some pod-shedding occurred during the harvest operations. At the first harvest the seed losses were 26% of the total yield and standing seed yield was 72 g m⁻². After 13 days, the proportion of shed pods rapidly increased and the seed losses were 71% of total; at complete maturity pod-shedding reached 100%. Therefore, as observed in many annual medics, *S. muricatus* also shows pod-shedding as soon as pods ripe.

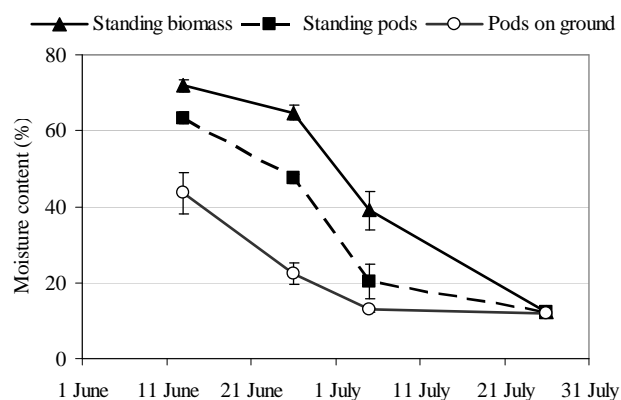


Fig. 1. Moisture content (%) of standing whole biomass, pods, and of pods on ground at four harvest dates. The bars indicate standard errors (n=4).

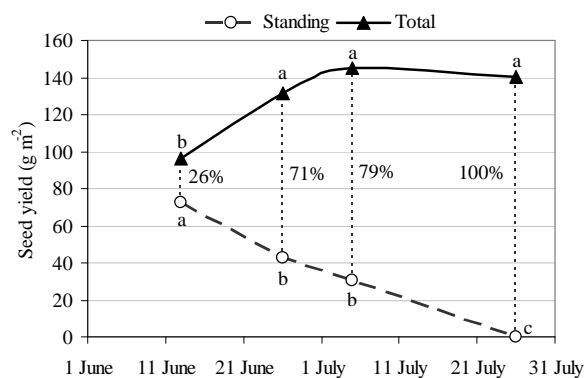


Fig. 2. Standing and total seed yield from the beginning of natural shedding (13 June) to complete maturity (26 July). Different letters indicate significant differences for $P \leq 0.05$. Percentage values indicate seed losses of total yield.

Table 1. Germination, 1000 seed weight and mean germination time (MGT) of seeds from standing pods or from soil surface (ground) at four harvest dates.

		13 June	26 June	5 July	26 July	$LSD_{0.05}$
Germination (%)	Standing	12,8 ^{***}	63,7 [*]	93,0 ^{ns}		17.2
	Ground	65,5	90,3	98,0	98,0	14.6
1000-seed weight (g)	Standing	9,2 [*]	10,7 ^{ns}	11,0 ^{ns}		0.8
	Ground	10,4	11,1	11,0	10,9	ns
MGT (days)	Standing	3,87 ^{***}	2,61 ^{ns}	2,25 ^{ns}		1.06
	Ground	2,70	2,51	2,12	2,11	0.36

^{*}, ^{***}, ^{ns}, differences with "ground" significant at $P=0.05$, 0.001 and not significant, respectively

For all harvest dates, germination tests showed very low incidence of fresh seeds (1-2%) and of hard seeds (1-4%); in particular the lack of hard seeds was due to the hulling machine used which determined an efficient seed scarification.

The germination level of the seeds of standing pods increased from 12.8 to 93.0 % delaying harvest time from 13 June to 5 July due to the decreasing incidence of immature seeds (dead during germination test) (Table 1). At first harvest, the seeds of standing pods had the lowest 1000-seed weight, indicating that reserve accumulation was still in progress. Furthermore the germinated seeds had poor vigour as clearly demonstrated by the highest mean germination time (MGT).

Even the seeds of the pods collected early on the soil surface (shed naturally or in consequence of the gently manual harvest) showed a germination significantly lower than at the subsequent harvests (Table 1), despite the seeds had gained their final dry weight (variations of 1000-seed weight not significant among harvest dates). Furthermore, the MGT significantly decreased from early harvest to maturity. The low germination and vigour observed for seed from the ground at the first harvest date were likely affected by the high contribution of pods detached from stand during harvest operations (not completely mature) to the total pods collected on soil surface; probably, this contribution decreased with harvest time due to the rapid increase of pods naturally shed (mature).

The standing viable seed yield, calculated from production and germination rate, at each harvest time, never exceeded 30 g m^{-2} (Fig. 3) or 30% of total viable seed yield. At first harvest the standing viable seed yield was reduced by poor germination level and low seed weight whereas later by progressive pod-shedding.

The results showed that direct combining of *Scorpiurus muricatus* could be very uncertain due to the high moisture content of standing biomass and to the shedding of ripe pods. On the other hand, it should be noted that the seed remaining on soil surface could regenerate a pasture stand in the subsequent growing season. However, to maximize commercial seed production, alternative harvesting methods (i.e. windrowing and subsequent combine harvest or desiccation and combining) need to be investigated

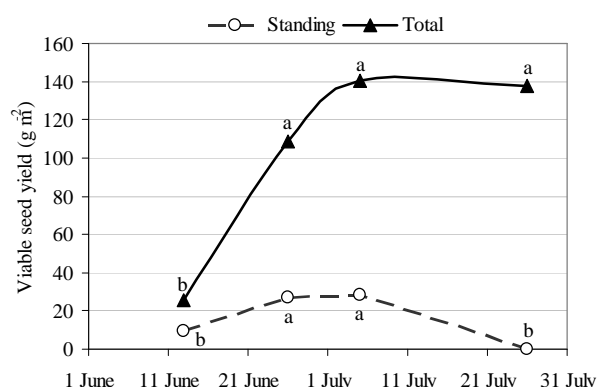


Fig. 3. Standing and total viable seed yield. Different letters indicate significant differences for $P \leq 0.05$

ACKNOWLEDGMENTS

The research was funded by the University of Palermo, Doctorate in Mediterranean Agro-ecosystems. The authors would like to thank A. Cannella and F. Labruzzo for technical support.

LITERATURE

- Abbate, V., Patané, C., Sortino, O. & Cammarata, M. 1999. Seed production of *Scorpiurus subvillosus* L. in Sicily. First results on crop management. In: Proceedings of the 4th International Herbage Seed Conference, Perugia (I), May 23-27, 1999. pp.142-146.
- Beale, P.E., Lahlou, A. & Bounejmate, M. 1991. Distribution of wild annual legume species in Marocco and relationship with soil and climatic factors. Australian Journal of Agricultural Research 42 (7): 1217-1230 .
- Bensalem, K., Abdelguerfi, A., Abdelguerfi-Berrekia, R. 1990. Relation du genre *Scorpiurus* L., avec certains facteurs du milieu en Algérie. Fourrages 124: 407-419.
- Bouazza, L. & Abdelguerfi, A. 1995. De la production de semences d'ecotypes locaux aux essais de charge en zones marginales. Cahiers Option Mediterraneennes 12: 67-69
- Di Giorgio, G., Ruisi, P. & Giambalvo D. 2007. Characterization and agronomical evaluation of *Scorpiurus muricatus* L. populations collected in Sicily. In: Proceedings of XXVI EUCARPIA 'Breeding and seed production for conventional and organic agriculture, Faculty of Agriculture, 3-7 Sept. 2006, Perugia, Italy. pp. 249-252.
- Ehrman, T. & Cocks, P.S. 1990. Ecogeographic survey of wild annual legumes in Syria: distribution patterns. Journal of Applied Ecology 27: 578-591.
- El Shaer, H.M. 1995. Potential use of cultivated range plants as animal feed in the Mediterranean coastal zone of Egypt. In: Proc. the Meeting of FAO working group on Mediterranean pastures and fodder crops. Sylvopastoral System, Environmental, Agricultural and Economic Sustainability, Avignon, France, 29 May - 2 June, 1995.
- Licitra, G., Carpino, S., Schadt I., Avondo, M. & Barresi, S. 1997. Forage quality of native pastures in a Mediterranean area. Animal Feed Science and Technology 69 (4): 315-328.

Harvest time, harvest method and germination losses during storage of fodder galega (*Galega orientalis* Lam.) seed

Jonas Slepetyš

Department of Plant Nutrition and Agroecology, Lithuanian Institute of Agriculture, Akademija, LT 58344, Kedainiai district, Lithuania

jonas.slepetyš@lzi.lt

ABSTRACT

The objectives of this work were 1) to identify optimal harvest time and harvest method for fodder galega seed crops; 2) to study changes in seed germination during storage. Experiments were carried out during 1996-2006. Seed harvest was studied in the first to fourth harvest year of a seed crop established in 1995. Fodder galega seed was harvested using either swath or desiccation + direct combining when 60 %, 80 %, and 90 % of pods had matured and 14 days after 90 % maturity. In 1999 the harvested seed was put in paper bags and seed quality changes studied for seven years in a heated and unheated store. The highest seed yield was obtained when fodder galega was harvested using direct combining following desiccation when 90 % of pods had matured. In drier weather the seed could be harvested using the swath harvesting method. Due to the low shattering potential, seed harvest of fodder galega should not be hastened; it is better to wait for more favourable weather conditions. In the dry year of 1999 seed germination was 92-98% regardless of harvest time and harvest method. After five years' storage in a heated store, germination had changed little to 82-89 %, but in an unheated storage, it had declined to 19-40 %. In the unheated store, seeds maintained germination better when completely mature at harvest. In conclusion, fodder galega seeds can be stored securely for only two years in an unheated store as opposed to five to six years in a heated store.

Key words: desiccation, *Galega orientalis*, seed longevity, seed moisture content, seed storage, seed yield, swath

INTRODUCTION

In Lithuania the total area with perennial grasses was 1.33 million hectares in 2006. The most important legumes are white clover, red clover and lucerne, which are commonly grown with grasses. Farmers are looking for new legumes, characterised by persistence and a high adaptability to growing conditions. Fodder galega (*Galega orientalis* Lam.) is one of the most persistent plants in swards, which is capable of fixing atmospheric nitrogen and notable for a high productivity (Virkajarvi & Varis 1991, Raig 1994, Adamovich 2000). Attempts have been made to grow fodder galega on extensively used or conserved soils, used-up quarries and dug-off peatbogs.

Fodder galega cultivation protects the soil from erosion and prevents the spread of weeds (Vavilov & Kondratjev 1975). Galega can be grown on various soils if they are not acid and waterlogged (Raig & Nommsalu 2001). According to soil requirements, fodder galega is similar to lucerne and sainfoin, and it can produce very high yields. Experimental data show that this long-lived plant survives in pure stand for 15-19 years (Drikis 1995, Adamovich 2000). However, fodder galega also has some disadvantages, such as very slow development in the sowing year and in the first year of use and sensitivity to frequent cutting or grazing (Raig 1994, Lillak & Laidna 1999, Moller *et al.* 1997). Compared with other legumes (clover, lucerne) little

research has been done on its cultivation. Research evidence on fodder galega cultivation for seed is even scarcer. During the recent years the demand for galega seed has been on a steady increase, however, the amounts produced still cannot meet the demand. As a result, it is very important to increase galega seed yield. The experimental objectives were as follows: 1) to identify optimal harvest time and harvest method for fodder galega seed; 2) to study changes in seed germination during storage.

MATERIALS AND METHODS

Experiments were carried out during 1996-2006. Four harvesting experiments were carried out on a sod gleyic loam soil (Epicalcari - Endohypogleic Cambisol in the central part of Lithuania (55°23' N, 23° 51' E). The soil contained on average 2.63 % humus, 0.17 % total-N, 104 mg (kg dry soil)⁻¹ of P, 125 mg (kg dry soil)⁻¹ of K. The soil pH was 7.0. Fodder galega 'Gale' was sown at a seed rate of 10 kg ha⁻¹ and a row spacing of 23 cm with a cover crop of spring barley. The experiment was designed as a randomized complete block with 4 replications and a plot size of 2.5m × 14.0m. Seed harvesting was investigated for four consecutive years, i.e. in the the first to fourth year of use. Fodder galega seed was harvested using swathing or direct combine harvesting after 60±5, 80±5 and 90±5 % of pods had matured and 14 days after the last term. The analysis of galega pod maturity was made twice a week starting at the fifth - sixth week from the beginning of flowering. For this purpose, 4-6 samples, each from a 0.25m² plot, were cut from representative places of the stand. The pods were divided into 4 groups of maturity: green, yellowish - green, yellow - semibrown and dark brown. Seed harvesting time was determined according to the number of brown and yellow - semibrown pods, expressed in per cent. Prior to direct harvesting, crops were desiccated using diquate 1 L ha⁻¹ (5 L ha⁻¹ Reglone). Then the crop were threshed using a combine (Sampo 500) after 5-7 days. Crops cut into thin swaths were dried on 20-30 cm high stubble for the same number of days. before being threshed using the same combine. The seed was dried, cleaned and seed quality determined. Seed yield data were corrected to 100% purity and 13% moisture content.

The seed harvested in 1999 was used for a storage experiment. The seed was stored for seven years in 1 kg paper bags at two different places; a winter-heated room (laboratory), and an unheated wooden store (barn). The air temperature in the heated room varied from 20° to 25°C and the relative humidity from 45 to 60 %. In the wooden store air temperature varied from -25° to 30°C, and the humidity from 35% to 85%.

RESULTS AND DISCUSSION

The fodder galega crop flowered for 24-48 days irrespective of weather conditions. Due to the lengthy flowering, seed ripening was uneven. Even when most of the pods had turned brown, part of the pods were still green, underdeveloped, and part of the inflorescences were still flowering. Having labelled individual inflorescences, we determined seed quality for the various combinations of ripeness classes and harvesting methods (Table 1). When fodder galega pods were green, the seeds were underdeveloped regardless of harvesting method; the 1000 seed weight ranged from 4.11 to 5.31 g. Seed germination was low (52-72 %) and did not meet the standard of 80 %. In more mature yellow or semibrown pods the seed had reached their maximum weight, but germination still showed a tendency to increase. The quality of the seed of this pod maturity group was similar to the quality of fully ripe seed. Consequently, the optimal harvest time of fodder galega seed crops can be determined according to the percentage of yellow and brown pods in the crop.

Fodder galega seed yield is generally higher than that of other legumes and depends on the age of the crop and the weather conditions (Vavilov & Kondratjev 1975, Raig 1994). During our experimental period the highest rate of precipitation fell in 1998. The driest growing season was 1999. The lowest seed yield was produced by fodder galega in the first year of use (Table 2).

Due to the drought and frosts in May the seed yield in the fourth year of use (1999) was more than halved compared with the seed yield in the second and third year of use. Averaged data from four years indicate that the highest seed yield, 521 kg ha⁻¹, was obtained when fodder galega was direct-harvested when 90 % of pods had ripened.

Comparison of swath harvesting and direct harvesting after desiccation shows that in two years significantly higher seed yield were obtained in the direct harvesting treatment, in the other two years the seed yields were similar for both harvesting methods. When the crop was combined directly without desiccation, seed yields were significantly lower. Only in the very dry year 1999 the seed yields were similar for direct combining with and without desiccation. Most researchers consider direct combining as the preferred method and recommend harvesting fodder galega when 70-80 % of the pods have

matured (Vavilov & Kondratjev 1975, Spruogis 1999). Early harvesting when only 60 % of pods had ripened was not justified in our trials, as lower seed yields were obtained in all years. A two-week delay in harvesting after 90 % pod maturity resulted in a seed yield reduction, however, this decline was not significant every year. Consequently, fodder galega harvesting should not be hastened, and one can adjust to the weather conditions. In drier weather one can use swath harvesting, while in wetter years one should use direct harvesting with prior desiccation of the crop.

Table 1. Fodder galega seed quality as affected by maturation at harvest and harvesting method (Mean of four trials, 1996-1999).

Harvesting method	Green pods, green seed		Yellow-green pods, green seed		Yellow-semibrown pods, light yellow seed		Brown pods, yellow seed	
	1000 seed weight, g	germination, %	1000 seed weight, g	germination, %	1000 seed weight, g	germination, %	1000 seed weight, g	germination, %
Swathing	4.11	62	6.32	81	7.19	87	6.70	90
Direct after desiccation	4.24	52	6.02	81	6.89	82	6.88	92
Direct without desiccation	5.31	72	7.10	88	6.95	82	6.69	89
LSD _{0.05}	1.01	29	0.54	5	0.54	8	0.46	6

Table 2. Fodder galega seed yield, harvested by different methods and at different times

Method of harvesting	Mature pods %	Seed yield kg ha ⁻¹				Average
		1996	1997	1998	1999	
Swath	60±5	107	532	493	184	329
Swath	80±5	144	643	703	239	432
Direct	80±5	187	639	748	272	462
Swath	90±5	192	737	728	282	485
Direct	90±5	235	758	775	315	521
Direct ^x	90±5	183	662	685	316	462
Swath ^a		177	650	714	272	453
Direct ^a		202	655	709	315	470
Direct ^{ax}		168	603	673	332	444
LSD _{0.05}		14	56	80	33	26

a - harvesting 14 days after last term

x - direct harvesting without desiccation

Although the moisture content of pure seed was low (11.1-14.7 %) in all treatments, the highest moisture content of threshed seed and chaff was identified when galega had been threshed directly without desiccation. The lowest 1000 seed weight was recorded when the seed was harvested at the stage when 60 % of pods had matured. With delayed harvesting after complete maturity, the seed weight consistently declined.

The seed harvested at 60% pod maturity germinated 86% on average for the four years, and that germination was below 80 % only in 1996. Although the highest germination was achieved when 90 % of pods had ripened, i.e. when 66 days had elapsed from the start of flowering, Harvesting method and delay of harvesting did not have any significant effect on seed germination.

In 1999 germination of the seed that was going to be used for the storage experiment was 92-98 % (Table 3). The average content of hard seed was 26 % (16-41 %) and the seed moisture 8.5-9.6 %.

Table 3. Variation of fodder galega seed germination (%) harvested in 1999 and stored in a heated (ht) and an unheated (un) storage over seven years

Method of harvesting	Matured pods %	Germination %										
		1999	2001		2002		2004		2005		2006	
			ht	Un	Ht	un	Ht	un	ht	un	ht	un
Swath	60±5	95	84	86	91	49	82	22	74	22	46	11
Swath	80±5	96	89	81	90	62	86	29	70	21	52	12
Direct	80±5	95	86	85	84	60	84	19	83	20	58	16
Swath	90±5	98	88	83	91	64	87	33	78	36	76	18
Direct	90±5	95	89	84	91	59	86	40	80	29	61	17
Direct ^x	90±5	97	91	86	92	64	85	37	82	22	57	21
Swath ^a		95	93	89	89	55	85	31	79	24	50	20
Direct ^a		92	91	82	82	53	89	23	84	21	38	11
Direct ^{ax}		95	89	87	87	59	86	22	82	24	49	13
For trial		3	8		7		5		5		7	
LSD _{0.05}												
Mean		95	89	85	89	58	79	32	79	24	54	15
For storage		3	3		2		2		2		2	
LSD _{0.05}												

a - harvesting 14 days after last term, x - direct harvesting without defoliation

A sharp and significant decline (up to 49-64 per cent units) in the germination of seed stored in the unheated storage occurred in the third year of storage. In the heated storage the corresponding seed germination varied insignificantly and totalled 84-91%. In the fifth storage year in the unheated storage better germination was exhibited by the seed harvested when matured pods accounted for 90%. The germination of seed stored in the heated room differed inappreciably and met the standard. In the sixth year of storage (2005) seed germination declined to 70-84 %, even in the heated storage. The greatest reduction occurred when the seed had been swath - harvested immature at the first harvesting dates. This leads to the conclusion that when immature seed is harvested, it loses germination more rapidly during storage compared with the harvested at complete maturity. The germination of seed stored in the heated storage declined sharply to 54 % (38-76 %) in the seventh year of storage (2006) while the germination of seed stored in the unheated storage was extremely low 11-21 % after this extended storage period. In order to preserve seed germination (viability) for a longer period, it should be stored in heated storage.

Conclusions

The best seed harvesting time of fodder galega is when 90% of pods have ripened. Due to the strong attachment of pods to stems it is not expedient to hasten harvesting. Harvesting time and method can be chosen subject to the weather conditions. In rainy weather it is better to use direct harvesting with a prior dessication. After three years in an unheated storage seed germination dramatically declines and does not meet the standard. In an unheated storage in paper bags the seed can be securely stored for only two years, as opposed to five -six years in a heated storage

ACKNOWLEDGEMENTS

This work (as part of study No T- 78/07) was supported by the Lithuanian State Foundation of Science and Studies.

LITERATURE

- Adamovich, A. 2000. Productive longevity of eastern galega (*Galega orientalis* Lam.) / grass sward. *In: Soeggard, K. et al. Grassland farming. Grassland Science in Europe 5: 100-103.*
- Drikis, J. 1995. Eastern galega and alfaalfa grown in pure stands and in mixtures, their yielding capacity and yield quality. Jelgava, 84 .
- Lillak, R. & Laidna, T. 1999. Productivity of long - term forage legumes depending on growing conditions. *In: Bartkaite, O. et al. (eds). Horticulture and vegetable growing. Bابتai 19(3)-2: 62-72.*
- Moller, E., Hostrup, S.B. & Boelt, B. 1997. Yield and quality of fodder galega (*Galega orientalis* Lam.) at different harvest managements compared with lucerne (*Medicago sativa* L.) *Acta Agricultural Scandinavica. Soil and Plant Science 47: 89-97.*
- Raig, H. 1994. Advances in the research of the new fodder crop *Galega orientalis* Lam. *In: Nommsalu, H.(ed.) Fodder Galega (Galega orientalis Lam.) research in Estonia. The Estonian Research Institute of Agriculture, Saku: 5-24.*
- Raig, H. & Nommsalu, H. 2001. Biological characterisation of fodder galega. *In: Nommsalu H. (ed.) Fodder galega. Saku: 15-22.*
- Spruogis, V. 1999 Technology of growing fodder galega for forage and seed. Kaunas, 70.
- Vavilov, P.P. & Kondratev, A.A. 1975. *In: New fodder crops. pp. 227-247. Moscow,*
- Virkajarvi, P. & Varis, E. 1991. The effect of cutting times on goat's rue. (*Galega orientalis* Lam.) leys. *Journal of Agricultural Science in Finland 63: 391-402.*

Influence of harvest timing and storage location on the longevity of timothy (*Phleum pratense* L.) seed

Jon Atle Repstad¹⁾, Trygve S. Aamlid²⁾ & Lars T. Havstad²⁾

¹⁾ Felleskjøpet Agri, P.O.Box 469 Sentrum, N-0105 Oslo, Norway

²⁾ The Norwegian Institute for Agricultural and Environmental Research
Bioforsk Øst Landvik, Reddalsveien 215, N-4886 Grimstad, Norway

jon.repstad@felleskjopet.no

ABSTRACT

Norwegian agriculture is totally dependent on a safe supply of seed of winter-hardy timothy varieties. The annual seed consumption varies depending on the extent of winter damages, particularly in northern Norway, and the average seed yield varies with weather and harvest conditions in the seed-producing districts in the southeastern and central part of the country. To buffer these variations, seed companies always keep stocks corresponding to 50-100 % of the average annual seed consumption. Such large stocks are risky to maintain as seed lots will lose germination over time. Our objective was to elucidate the effect of seed harvest time and seed storage location on the longevity of timothy seed. In 2003, timothy 'Grindstad' was combined directly on 2, 5 or 8 August corresponding to a seed moisture content (SMC) of 34, 27 and 20 %, respectively. After harvest the seed was dried to 10-11 % SMC. Germination analyses were accomplished 3, 15, 26 and 38 months after seed harvest; the three latter after splitting each seed lot into four sub-lots that were stored either in a conditioned seed store (4°C, 30% RH), or in unconditioned warehouses at three climatically different locations. While seed harvest time had no effect on germination three months after harvest, differences became increasingly evident as time went by. After 38 months' storage, seed stored in the conditioned store or in the warehouse at the continental location Tynset germinated, on average for harvest times, 15-16 units better than seed stored in the warehouse at the coastal location Vaksdal; and seed lots harvested at 20 % SMC germinated, on average for storage locations, 24 units better than seed harvested at 37 % SMC. While it has long been documented that direct combining at high SMC may damage seed germination, there has been less awareness that this damage may not manifest itself until after a certain storage period.

Key words: direct combining, germination, seed production, seed vigour

INTRODUCTION

Timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.) and red clover (*Trifolium pratense* L.) are the major species in herbage seed production in Norway. The annual production of about 1500 tonnes is enough to cover the Norwegian demand for seed of these species. The timothy variety 'Grindstad' (origin 59°N) constitutes more than 1/3 of this production. From 1991 to 2004 the average timothy seed yield in Norway varied from 460 to 760 kg ha⁻¹.

Grassland winter damage is a common problem in Norway, but damages vary from year to year and from district to district. As varieties from Denmark, England and countries further south are not considered winter-hardy enough for the Norwegian climate, seed companies prefer to keep large stocks of Norwegian varieties rather than having to import timothy seed from abroad. The stocks should never be less than 50 % of the average annual consumption of 'Grindstad' and 'Vega (origin 65°N), the mostly used timothy varieties, and less than

100 % of the average annual consumption of 'Noreng', the variety with the most northerly adaptation (69° N). Such comprehensive stocks are expensive to maintain, and there is always a risk that seed lots will lose their germination capacity. To avoid storage losses it is important to choose which seed lots to store, and to store them under the right conditions. Working in the Pacific northwestern part of USA, Hafenrichter *et al.* (1965) documented that grass seed maintained germination much longer when stored in a warehouse in the continental climate of Idaho than when stored in the humid climate of coastal Washington.

Unlike the situation in countries with more stable weather conditions during the seed harvest period, Norwegian grass seed crops are almost always combined directly. Despite the early warnings from Arnold & Lake (1965) that directly combined timothy seed will never attain the same quality as seed recovered after swath and windrow curing, Norwegian timothy seed growers typically combine their crops gently already when the seed moisture content (SMC) is around 30%. At this early combining, the drum periphery speed is typically set to around 15 m s⁻¹ the concave clearance to 30 mm in the front and 20 mm in the rear. Weather permitting, the windrows will often be rethreshed after three to five days to save the 20-30% of the total seed yield that was not recovered in the first combining. The practice is based on seed harvest trials carried out in the 1960ies, showing average seed yields (direct combining + recombining) of 376 + 95 = 471 kg ha⁻¹ when the first combining was carried out at 36-39 % SMC, versus 400 + 51 = 451 kg ha⁻¹ when the first combining was delayed for one week until SMC had dropped to 26-28 % (Time and Hillestad 1975). Provided gentle adjustment of the combiner, the authors reported that seed lots combined directly at 36 % SMC germinated 86 %, as opposed to 92 % for seed lots had been combined directly at 26 % SMC. The OECD germination limit for certification of timothy seed lots is 80 %, but since 2002, Norwegian timothy prices have been based on a standard germination capacity of 92 %, the seed grower being deducted if the seed germinates less than 90 %. In 2004 and 2005, the germination capacity of Norwegian timothy seed lots averaged 92.6 and 91.1 %, respectively (The Norwegian Food Authority, personal communication).

Besides the fact that seed yields are much higher now than 30-40 years ago, one of the shortcomings of the early work by Time and Hillestad (1975) is that all germination analyses were conducted within a few months from seed harvest. Compared to swath and windrow curing, direct combining at high SMC may well have implications for seed longevity beyond those reported by the initial germination analysis. Thus, the objective of the present investigation was to study long-term effects of seed harvest time on the longevity of timothy seed under various storage conditions.

MATERIALS AND METHODS

In the summer 2003 seed of timothy 'Grindstad' was harvested in a variety trial at Bioforsk Øst Landvik. Plots were harvested at three day intervals; on 2, 5 and 8 Aug., always with the same Wintersteiger plot combiner adjusted to a drum periphery speed of 23.8 m s⁻¹ and a concave clearance 6 mm in the front and 3 mm in rear. The weather during the harvesting period was warm, stable, and without any rainfall; hence, the seed water content (SWC), as determined by drying the seed to constant weight on a Mettler scale at 120°C, dropped more than 2 per cent units per day, from an average of 34 % at the first harvest to 27 and 20 % and the second and third harvest, respectively. After harvest, seed lots were always dried with unheated air to 10-11 % SWC before storage. The plots were combined only once.

The seed was cleaned in November 2003 at an average SWC of 10.3 %. After cleaning, the lots were analysed for purity, thousand seed weight and per cent dehulled seed in the seed laboratory at Bioforsk Øst Landvik. Germination was determined on germination tables set to a day/night temperature of 25/20°C (daily 8 h/16 h alterations), with seedlings being counted after 7 and 14 days. A seed was considered germinated when seedling height had reached 5 mm. Two parallel samples of 100 seeds were analysed per seed lot.

After the initial germination analysis, each seed lot was split into four and stored in paper bags at the following locations:

- Conditioned storeroom at Bioforsk Landvik, 4°C and 30 % RH (control).
- Unconditioned warehouse at Felleskjøpet Vaksdal (60° 28'N, 5° 44'E)
- Unconditioned warehouse, Felleskjøpet Tynset (62° 16'N, 10° 46'E)
- Unconditioned warehouse at Felleskjøpet Holstad (59° 40'N, 10° 48'E)

The unconditioned stores at Felleskjøpet represent three different climates. Vaksdal has a typical coastal climate, Tynset a typical continental climate, and Holstad is somewhere between the two others. Holstad is the main seed store Felleskjøpet Agri.

New samples for germination analyses were taken in November 2004, 2005 and 2006 (15, 26 and 38 months after seed harvest, respectively). The germination data were arsin-transformed to ensure normality and analysed by traditional analyses of variance (PROC ANOVA / PROC GLM, SAS 1990). Main effects of harvest time, storage location and their interaction were tested against a pooled error comprising all interactions with germination sample number, which was considered the only random variable in the experiment. Significant differences were identified using the Student Neuman Keul multiple comparison test at $P < 0.05$.

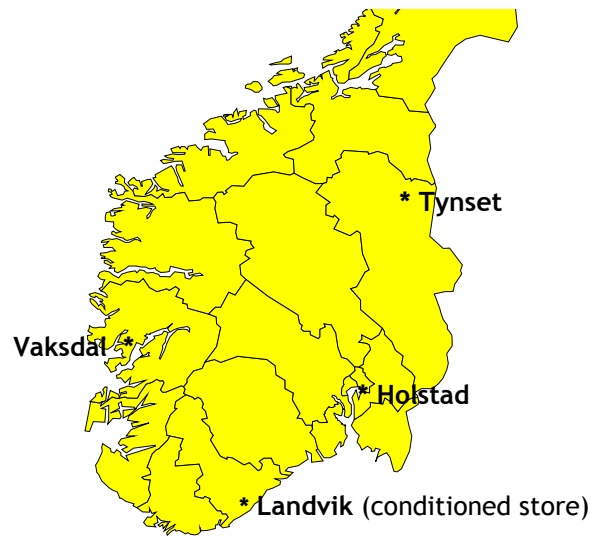


Fig. 1. Map of South Norway showing storage locations.

RESULTS AND DISCUSSION

Seed yields at the first, second and third harvest were 818, 832 and 770 kg ha⁻¹, respectively. As only one plot was harvested on each date, these differences could not be tested statistically. The results are, however, in general agreement with Time & Hillestad (1975). There was no difference in thousand seed weight between the harvest dates; this indicates both that the seed had attained physiological maturity at the first harvest date, and that no severe seed shedding had occurred before the last harvest.

Table 1. Germination of 'Grindstad' timothy 3, 15, 26 and 38 month after harvest, measured as normal seedlings after 7 and 14 days. Data for 2004, 2005 and 2006 are means of seed stored in unconditioned warehouses at Vaksdal, Holstad and Tynset.

Seed harvet date in 2003	Seed moisture content at harvest	November 2003		November 2004		October 2005		October 2006	
		7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
2 Aug.	34	76a	86a	79c	85c	72c	76c	62c	70c
5 Aug.	27	79a	88a	87b	90b	82b	85b	77b	84b
8 Aug.	20	80a	87a	95a	96a	90a	92a	91a	94a

Within each column, means followed by the same letter are not significantly different according to Student Neuman Keul multiple comparison test at $P < 0.05$.

Three months after harvest, seed germination was not significantly affected by harvest date. Especially when counted after 7 days, germination was rather low, even in the lot harvested at 20 % SMC. This probably reflects some kind of fresh-seed dormancy, even as late as three months after harvest. By comparison, Hill and Watkin (1975) found timothy seed combined at 30-40% SWC to be dormant immediately after harvest, but not after three months' storage. In their study, seed combined at 25% or lower SWC showed no dormancy even immediately after harvest. While the authors explained this as a need for afterripening only in early-harvested seed lots, the present results show that timothy seed may be dormant for several months regardless of SWC at harvest. As a precaution against such dormant conditions, the ISTA rules prescribes freshly timothy seed to be prechilled before germination tests; however, this was not accomplished in the present experiments.

Significant effects of harvest time started to show up at the second germination test in November 2004, after fifteen months of storage (Table 1). On this occasion, the seed that had been combined at 20 % SWC germinated 11 per cent units better than the seed that had been combined at 34 % SWC. One and two years later, in October 2005 and November 2006, respectively, this difference had increased to 16 and 24 units, respectively. On the latter occasion, i.e. after three years of storage, the germination of the early-most harvested seed lot had fallen below the OECD 80 % certification criterion. Differences in the number of seedlings counted after 7 days, which is in many ways a seed vigour test, increased even more, from 16 units in 2004 and 18 units in 2005, to 29 units in 2006.

The results show very clearly the importance of harvest time for timothy seed longevity. Small damages to membranes or other structure in the seed may not show up at the initial germination a few weeks or months after seed harvest, but they will manifest themselves as time goes by in the seed stores. In this respect, the Norwegian practise of combining timothy seed directly at a relatively high SMC seems to put seed storability at a certain risk, although it should be noted that the drum speed and concave clearance used in this experiment was much tougher than what is recommended during the

first combining of timothy seed in Norway. New experiments are now underway to elucidate the effect of combine setting on the storability of timothy seed.

On average for harvest times, seed stores had significant effect on germination after 15 month's storage (Table 2). Differences after 26 months storage were also mostly unclear. However, in October 2006, after almost three years in the warehouses, a relatively clear pattern showed up. As expected, the seed that had been stored under cool and dry conditions in the seed storage room at Landvik had retained the highest germination capacity, but the difference from seed stored in the unconditioned warehouse in the typically continental climate at Tynset was not significant. Seed stored in Felleskjøpets main store at Holstad differed from the seed stored at Tynset and Landvik with respect to the number of seedlings after seven, but not after fourteen days. By contrast, seed stored in the maritime climate at Vaksdal had dropped so much in germination that it would have been discarded based on current OECD rules. These results are in good agreement with those of Hafenrichter *et al.* (1965) and emphasize the importance of selecting warehouses in continental climates for long-term seed storage.

Table 2. The effect of storage location on germination after 7 and 14 days of timothy 'Grindstad', analysed 38 month after seed harvest.

Storage	November 2006	
	7 days	14 days
Landvik, conditioned store	85a	90a
Vaksdal, warehouse	68c	74b
Holstad, warehouse	78b	86a
Tynset, warehouse	84a	89a

The present investigation will continue with sampling of the seed lots for germination analysis every year in October or November. Gorecki & Mierzejewska (1989) reported that the greatest fall in viability of timothy seed occurred between the fourth and fifth year of storage, but this will of course depend on storage conditions. So far, the interactions between seed harvest time and storage condition has not been significant in our study, but such interactions may well show up over the next couple of years. Starting this year, we have also initiated a new project

trying to predict the storability of timothy seed using different seed vigour tests.

Holstad and Tynset, and Anne A. Steensohn and Ove Hetland, Bioforsk Øst, for germination analyses.

ACKNOWLEDGEMENTS

We thank Jan Anders Otterstad, Felleskjøpet Agri, for organizing seed storage at Vaksdal,

LITERATURE

- Arnold R.E. & Lake J.R. 1965. Direct, indirect or coule threshing in herbage seed production. I: S.48 timothy. *Journal of Agricultural Engineering Research* 10: 204-211.
- Gorecki R.J & Mierzejwska D. 1989. The vigour of timothy (*Phleum pratense* L.) seed store up to 5 years. *Acta Societatis Botanicorum Polonae* 58: 263-272.
- Hafenrichter A.L., Foster R.B. & Schwendiman, J.L. 1965. Effect of storage at four locations in the west on longevity of forage seeds. *Agronomy Journal* 57: 143-147.
- Hill M.J. & Watkin B.R. 1975. Seed production studies on perennial ryegrass, timothy and prairie grass. 2. Changes in physiological components during seed development and method of harvesting for maximum seed yield. *Journal of the British Grassland Society* 30: 131-140.
- Time K. & Hillestad R. 1975. Harvesting of timothy seed. *Forskning og forsøk i landbruket* 26 (4): 1-61 (Supplement). (In Norwegian, with English abstract).

Hardseededness and patterns of seed softening in burr medic (*Medicago polymorpha* L.)

Dario Graziano, Paolo Ruisi & Gaetano Amato

Università di Palermo - Dipartimento di Agronomia ambientale e territoriale,
Viale delle Scienze, 90128, Palermo (Italy)

paoloruisi@unipa.it

ABSTRACT

In Mediterranean-type environments, hardseededness (seed coat impermeability) ensures the persistence of annual pasture legumes as it protects the seed from germination at inopportune times (false breaks of season). The aim of this work was to study the dynamics of seed softening (loss of impermeability) in 6 Sicilian natural populations and 2 cultivars (Cavalier and Anglona) of *Medicago polymorpha* L. The accessions under investigation were selected within a Sicilian germplasm collection to represent a range of morpho-phenological traits but all were characterized by good forage aptitudes. Seeds used in the experiment were produced in the spring 2006 at Pietranera Farm, S. Stefano Q., in a hilly area of the Sicilian inland. After testing for initial hard seed levels, pod lots of each accession were placed on the soil surface in the field on 13 July 2006 into pockets of fiberglass flywire. Four replicates for each entry were removed during the summer-autumn period at 60, 96 and 138 days after field placement. At each sampling date, the number of residual hard seeds was determined. Significant differences in the time and rate of seed softening were observed among accessions.

Key words: forage legume, natural populations, seed dormancy, semi-arid Mediterranean environment, Sicily.

INTRODUCTION

In Mediterranean-type environments, hardseededness (due to the impermeability of the testa) is of a great importance for the persistence of annual pasture legumes in various farming systems (continuous pasture, ley farming, phase farming). In fact, this innate mechanism of seed dormancy avoids seed germination when out-of-season rains occurs (false breaks of season) and to germinate when conditions are really suitable for seedling establishment and subsequent plant development (Taylor 1993). *Medicago polymorpha* L. is one of the most widespread spontaneous annual legumes in Mediterranean basin and several authors underline its considerable potential to improve degraded pastures in areas with Mediterranean-type climate. Sicily (island in the middle of Mediterranean sea) has high variability for climatic and pedological conditions that have

produced a large variability for growing period, morphological traits and agronomical aptitudes in burr medic (Graziano *et al.* 2007).

The aim of this work was to study the effects of environmental conditions on the degree and patterns of seed softening in field conditions as well as the variability among genotypes for hardseededness. To this end the study included six Sicilian populations of burr medic, all characterized by good agronomical aptitudes and selected to represent the range of morpho-phenological traits previously observed and the cultivars Anglona (selected from a Sardinian population) and Cavalier (a cross-bred selected in Australia).

MATERIALS AND METHODS

Field trial was carried out during 2006 at Pietranera Farm (37° 30'N, 13° 31'E, 178 m a.s.l.) on a deep clayey soil. The site of evaluation, a hilly area of the Sicilian inland, has a typical Mediterranean climate with an average annual rainfall of 550 mm and a hot and dry summer (24.3 °C and 27 mm rainfall, on average). Pods of each accession were collected at maturity (last week of June 2006) from fifteen plants sown on 28 November 2005 and grown as spaced. 200 pods per accession were manually threshed and average number of seeds per pod was calculated. A second set of pods was mechanically threshed. A germination test was carried out on 29 June using 400 seeds for each accession (100 seeds x 4 replicates) and each threshing treatment. Seeds were placed on moist filter paper in Petri dishes (diam. 14 cm), closed tight with a self-sealing flexible film and put in a germinator at 20 °C in the dark. After 14 days, germinated, fresh, dead and hard seeds were counted. A set of pods for each entries was stored at room temperature. Pod lots were placed in fibreglass flywire envelopes (10 by 15 cm) that were joined together in strips containing one sample of each accession randomly allocated within each strip. Individual envelopes contained sufficient pods to furnish about 100 seeds. On 13 July, all strips were placed on the soil surface in a randomised block design with 4 replicates. Temperatures of bare soil surface were recorded every 30 min using probes (iButton, Dallas Semiconductor Inc.) covered with 2 mm of soil. Strips were removed on 11 September (60 days after placement - DAP), 17 October (96 DAP) and 28 November (138 DAP). At each sampling date, seeds were carefully removed from pods by hand. Seedlings, germinated seeds, seeds which had previously imbibed and dried out before germinating (as indicated by disruption of their seed coats) and intact seeds were counted. Intact seeds were used for a germination test as previously indicated. Numbers of germinated, fresh, dead and hard seeds were converted in percentages. Analysis of variance was used to analyze data which were not transformed.

RESULTS AND DISCUSSION

Total rainfall between placement in the field (13 July) and first sampling date (11 September) was 37 mm (30 mm in the last two days before sampling) (Fig. 1); mean daily maximum and minimum soil surface temperatures were 49.2 and 18.6 °C, with peaks of 56 and 13 °C, respectively. At the time of the first sampling, no germinated seeds were found inside envelopes. After the first and before the second sampling date (17 October), total rainfall was 119 mm with nearly 50 % of rainy days. This allowed an abundant germination inside envelopes but some seeds imbibed and dried out before germinating. Soil surface temperatures were quite lower than the first period, with mean daily maximum and minimum of 30.7 and 14.2 °C, respectively (fig. 1). In the last period (18 October - 28 November), rainfall was 128 mm, mainly distributed at the end of October and in the last week before the third sampling date; mean daily maximum and minimum soil surface temperatures were 24.9 and 8.3 °C, respectively.

Proportions of hard seeds in the manually threshed samples at the 29 June germination test were very high, with a mean value of 98.3 % (Table 1), proving that high level of hard seed is a peculiar characteristic of burr medic. No significant differences were observed among the accessions. Mechanical threshing treatment caused effective scarification, allowing imbibition and germination. In fact, germinated seeds were on average 84.8 % and residual hard seed fell down to 9.5 %. However, variability was observed between accessions: 'S.Cristina' showed the highest percentage of germinated seeds (99 %); 'Cerami', cv. Cavalier and 'Vizzini' showed the highest percentage of residual hard seeds (21.0, 17.0 and 16.7 % respectively).

'Quattrocchi' had the lowest value of germinated seeds due to the high rate of dead seed. This accession was characterized by the lowest 1000 seed weight and probably this could be the cause of embryo damages during threshing treatment. Fresh seeds incidence was on average very low, but 'Quattrocchi' and cv Cavalier showed values higher than other accession (Table 1).

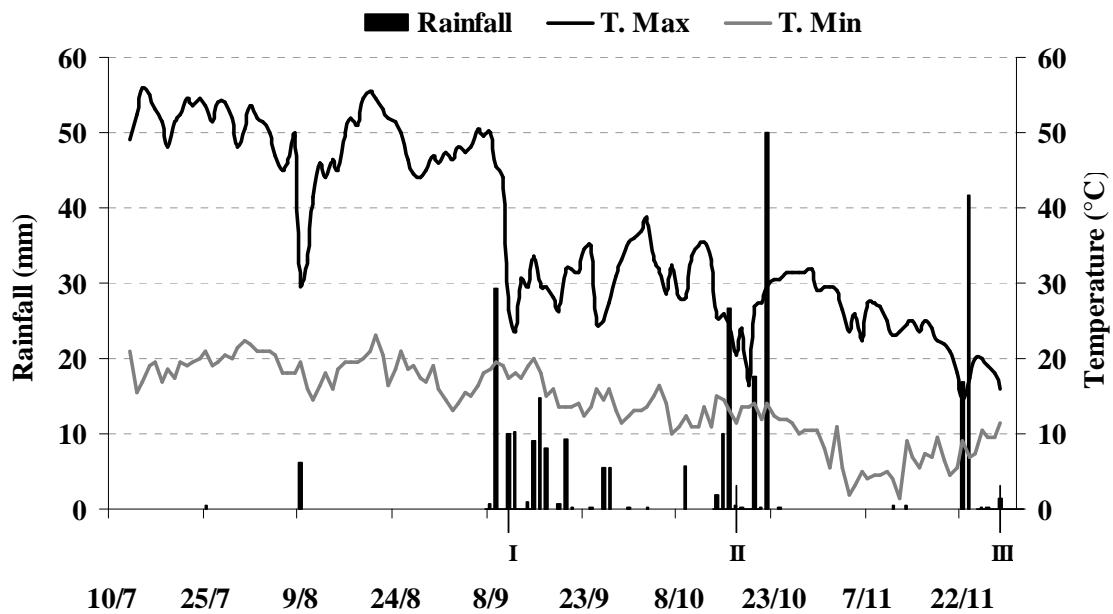


Fig. 1. Daily maximum and minimum soil surface temperatures and rainfall (columns) at experimental site during summer-autumn 2006. Roman numerals indicate sampling dates.

Tab. 1. Germination of seeds of *M. polymorpha* accessions (%) at harvest time (29 June) after manual or mechanical threshing.

Accessions	Percentage germination							
	Manual				Mechanical			
	Germinated	Hard	Fresh	Dead	Germinated	Hard	Fresh	Dead
Cerami	0.5	99.5	0.0	0.0	78.3	21.0	0.3	0.3
Quattrocchi	0.0	99.0	0.0	1.0	68.7	4.3	5.3	21.7
S. Cristina	2.6	96.9	0.5	0.0	99.0	0.0	0.0	1.0
Vizzini	0.5	99.0	0.0	0.5	77.0	16.7	2.0	4.3
Favara	1.0	99.0	0.0	0.0	94.7	3.7	1.3	0.3
Pizzolungo	2.6	97.4	0.0	0.0	91.3	7.0	0.7	1.0
Cavalier	1.6	98.4	0.0	0.0	78.7	17.0	3.7	0.7
Anglona	2.5	97.0	0.0	0.5	91.0	6.7	1.7	0.7
Mean	1.3	98.3	0.1	0.3	84.8	9.5	1.9	3.8
LSD _{0.05}	ns	ns	Ns	ns	9.0	6.2	3.1	4.9

The proportion of hard seeds decreased for all accessions during exposure of pods on the soil surface (Fig. 2). Summer seed softening (60 DAP) was low, ranging between 18.4 % and 7.7 %, and no significant differences were observed among accessions. At the second and third field sampling (96 and 138 DAP) the seed softening increased, on average to 20.6 and 31.2 % respectively, as well as variation among accessions. At 138 DAP, 'Cerami' and cv. Anglona were characterized by the highest proportion of residual hard seeds (80.3 and 77.5 % respectively), whilst 'Favara' and 'Quattrocchi' showed the highest percentage

of softening with a proportion of residual hard seeds of 49 and 58.2 % respectively. Estimations of hardseededness after 138 DAP in seed lots kept under different conditions (field or room) allow us to evaluate the main components causing hard seed breakdown: conservation condition or seed ageing (Fig. 3). For seven genotypes the latter components had a small or null effect: in fact, germination test carried out at the end of November on seeds kept under room condition showed a high rate of hardseededness. Reduction of hardseededness was high only in 'Favara' (45.7 %): in this case

hard seed breakdown was due to seed ageing and the effects of field condition were smaller. This population was also characterized for a high proportion of fresh seeds at the 28 November test in samples kept under room conditions: percentage of fresh seeds was on average 34.7, whereas mean of other accessions was just 1.7. Taylor (1996a) already observed this phenomenon in

M. polymorpha, showing genotypic differences among different accessions: seeds of cv. Serena needed at least 14 days to germinate, while all seeds of cv. Santiago germinated after 5 days. So it could be that high rate of fresh seeds in 'Favara' is due to a mechanism of slow imbibition and 14 days were not enough to allow germination.

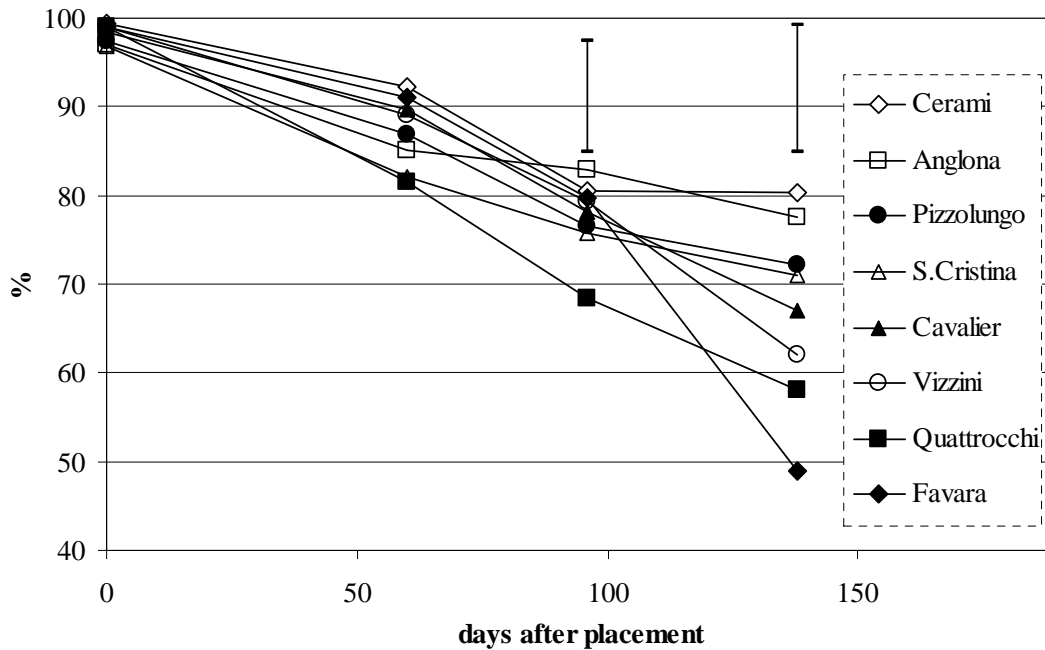


Fig. 2. Patterns of hard seed breakdown in eight accessions of *M. polyhorpha* under field conditions. Vertical bars represent LSD for $P < 0.05$.

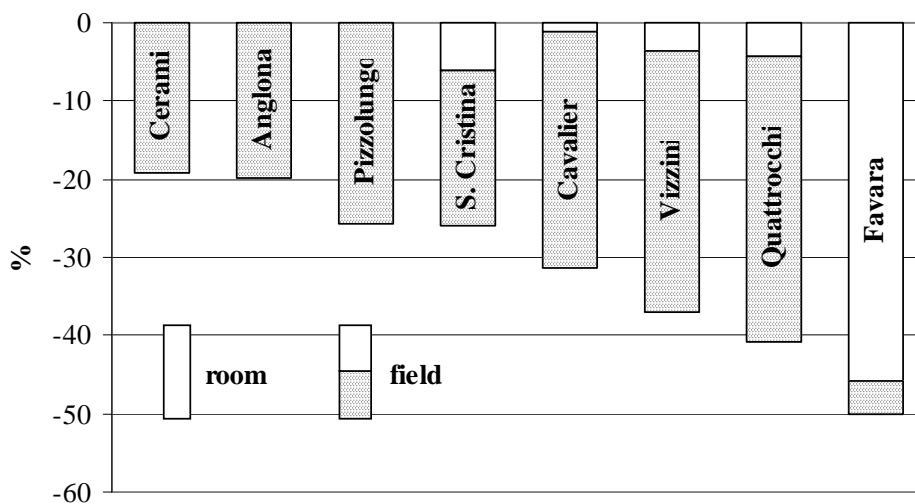


Fig. 3. Reduction of hard seeds (% of initial level) at 138 DAP of samples kept under room or field conditions.

CONCLUSIONS

These experiments confirm that high hard seeds level after seed maturity is characteristic of *Medicago polymorpha* L. In fact, accessions under investigation showed a low rate of germinated seeds just after harvest with an average proportion of hard seeds of 98 %. Exposure of pods on the soil surface from maturity till the end of November (about five months) produced a reduction of hard seeds (on average 31.2 %), but the level varied largely among accessions. In fact, the residual hard seeds ranged between 80 % ('Cerami') and 49% ('Favara'). Taylor (1996b) also showed variability among

burr medic accessions with values ranging between 30 and 93 % after 6 months under field conditions. The different behaviour of 'Favara' population compared to the other accessions, particularly for the high proportion of fresh seeds after 5 months from maturity, need to be further investigated.

ACKNOWLEDGEMENTS

The research was funded by the University of Palermo, Doctorate in Mediterranean Agro-ecosystems. We are grateful to F. Cannella and A. Cannella for technical support.

LITERATURE

- Graziano, D., Di Giorgio, G. & Amato G. 2007. Genetic diversity and potential agronomic value of *Medicago polymorpha* L. populations collected in Sicily. *In: Proceedings of XXVI EUCARPIA Fodder Crops and Amenity Grass Section and XVI Medicago sp. Group joint Meeting on 'Breeding and seed production for conventional and organic agriculture'*, Perugia (Italy), 3-7 September 2006. pp. 240-243
- Taylor, G.B. 1993. Effect of some characteristics of diurnal temperature fluctuations on the softening of hard seeds of *Medicago polymorpha*. *In: Baker, M. (ed.). Proceedings of the XVII International Grassland Congress, Palmerston North. pp. 256-257. New Zealand Grassland Association.*
- Taylor, G.B. 1996a. Effect of the environment in which seeds are grown and softened on the incidence of autumn seed softening in two species of annual medics. *Australian Journal of Agricultural Research* 47: 141-159.
- Taylor, G.B. 1996b. Incidence and measurement of autumn seed softening within *Medicago polymorpha* L. *Australian Journal of Agricultural Research* 47: 575-586.

Patterns of hardseed breakdown within *Scorpiurus muricatus* L. in a semi-arid Mediterranean environment

Giuseppe Di Giorgio, Paolo Ruisi & Dario Giambalvo

Università di Palermo, Dipartimento di Agronomia ambientale e territoriale,
Viale delle Scienze, 90128, Palermo (Italy)

paoloruisi@unipa.it

ABSTRACT

Scorpiurus muricatus L. is an annual legume widespread in natural pastures of the Mediterranean area and it is appreciated by farmers for palatability and nutritive value. Despite its potential for pastures improvement, the species is not well studied, so far. This work is a part of a project aimed to improve knowledge about the species. The evolution of hardseededness (seed coat impermeability) was investigated in 6 natural populations of *S. muricatus*. The accessions under investigation, all characterized by good agronomical aptitudes, were selected within 30 Sicilian populations to represent the variability for the observed morpho-phenological traits. The seed used in the trial was produced in the spring 2006 at Pietranera Farm, S. Stefano Q. After testing for initial hard seed levels, pod lots of each accession were placed on the soil surface in the field on 13 July 2006 into pockets of fiberglass flywire. Three replicates for each entry were removed 138 days after field placement. Number of residual hard seeds was determined. The experiment confirmed that high level of hardseededness after seed maturity is characteristic of *Scorpiurus muricatus*. In fact, populations under investigation displayed a low rate of germinated seeds after harvest with an average proportion of hard seeds close to 95 %. Exposure of the loment on the soil surface during the summer-autumn period ensuing seed maturity caused a considerable reduction of hard seed level (46 %, on average), with significant differences among accessions.

Key words: pasture legume, natural populations, residual hard seeds, seed dormancy, Sicily.

INTRODUCTION

Self-reseeding annual legumes play an important role in the Mediterranean natural pastures due to their considerable production, good quality of forage and nitrogen fixation capacity that contributes to improve soil fertility. Persistence of these species in Mediterranean-type environments is ensured by hardseededness. This primary mechanism of seed dormancy due to the impermeability of the testa allows seed to escape summer drought and false break of season and to germinate when conditions are really favourable (Taylor 1993).

Scorpiurus muricatus L. (prickly scorpion's tail) belongs to this group of species and is widely distributed in the Mediterranean area, particularly in Morocco, Algeria, South

France, and South Italy. Generally farmers appreciate palatability, nutritive value and productivity of the species. In some areas of Sicily (particularly in the Hyblean plateau) farmers ascribe to *Scorpiurus muricatus* a very high galactogogue effect, and its abundance increases the value (also economic) of the pasture. Despite *Scorpiurus muricatus* could represent a very interesting species for the Mediterranean areas, the use of this annual legume in agricultural systems as forage crop is not well documented and only some studies have been carried out to improve degraded pastures using the species (Ghassali *et al.* 1998, Osman *et al.* 1999). It is evident that there are still large gaps in the knowledge of features of the species. The aim of this work was to investigate the variability among 6 Sicilian natural populations of *S. muricatus* for initial hardseededness and the

effects of environmental conditions in the summer-autumn period ensuing seed production on seed softening process (loss of impermeability).

MATERIALS AND METHODS

The trial was conducted during summer-autumn 2006 in a typical hilly area of the Sicilian inland with mean annual rainfall of

550 mm (Pietranera Farm, S. Stefano Q., 37° 30'N, 13° 31'E, 178 m a.s.l.) and a hot and dry summer (24.3 °C and 27 mm rainfall, on average). The accessions under investigation, all characterized by good agronomical aptitudes, were selected within 30 Sicilian populations collected in 2005 (Di Giorgio *et al.* 2007) to represent the variability for the observed morpho-phenological traits. In Table 1 we report the list of populations and details of sites of collection.

Table 1. Collection site information of the accessions of *S. muricatus* under investigation

Collection sites	Lat (°N)	Long (°E)	Altitude (m a.s.l.)	Mean Annual Rain (mm)	Mean Annual Temp (°C)	pH
Buonfornello	37°58'	13°50'	7	550	18,5	8,1
Fiume Tusa	38°00'	14°16'	12	750	18,5	8,9
Grotte Mazzamuto	38°01'	13°33'	122	550	16,5	7,9
Montevago	37°42'	12°55'	133	650	17,5	8,7
Pietranera	37°33'	13°30'	428	650	15,5	8,4
Polizzi	37°50'	13°58'	594	900	16,5	8,0

Loments of each accession were collected at maturity (28 June 2006) from 15 plants sown on 30 November 2005 and grown as spaced. 200 legumes per accession were manually threshed and the average number of seeds per loment was determined. A second set of loments was mechanically threshed. A germination test was performed with manually threshed seeds, starting on 29 June, using 400 seeds (100 seeds x 4 replicates) for each entry. Seeds were placed on moist filter paper into Petri dishes that were closed tight with a self-sealing flexible film and put in a germinator for 14 days at 20 °C in the dark. Germinated, fresh, dead and hard seeds were counted at the end of the test. A set of loments of each population was stored in paper bags at room temperature. For each accession, twenty intact loments (sufficient to furnish 100 seeds at least) were placed into fiberglass flywire pockets (10 by 15 cm) joined together in strips (60 by 15 cm). Populations were randomly allocated within each strip. On 13 July, all strips were placed on the bare soil surface and held down with tent pegs. A randomized block design with 3 replicates was adopted. On 28 November (138 days after field placement - DAP), strips were removed from the ground and seeds carefully extracted by hand. Seedlings, germinated

seeds, seeds which had previously imbibed and dried out before germinating and intact seeds were counted. Intact seeds were used for a germination test as previously indicated, together with two samples of seeds (manually and mechanically threshed) for each accession stored meanwhile at room temperature. Soil surface temperatures (daily minimum and maximum) were recorded every 30 min throughout the period of the experiment using probes (iButton, Dallas Semiconductor Inc.) covered with 2 mm of soil. Numbers of germinated, fresh, dead and hard seeds were converted in percentages. Analysis of variance was used to analyse the data which were not transformed.

RESULTS AND DISCUSSION

Rainfall between the placement of the loments in the field and the drawing date (13 July-28 November, 138 days) was 285 mm (Fig.1). Mean daily maximum and minimum soil surface temperatures were 36.8 and 14.3 °C, with peaks of 56.0 and 1.5 °C, respectively. During the summer period (between the placement in the field and the first week of September, 57 days), maximum temperatures exceeded 50 °C in 31 days and

45 °C in 51 days. In the central period of the trial (8 September-22 October, 45 days), total rainfall was 217 mm (25 rainy days) and an abundant germination was observed. Finally, in the last period (23 October-28 November,

37 days), rainfall was quite lower (61 mm in the last days of the period) and soil surface temperatures rarely exceeded 30 °C and frequently were lower than 10 °C.

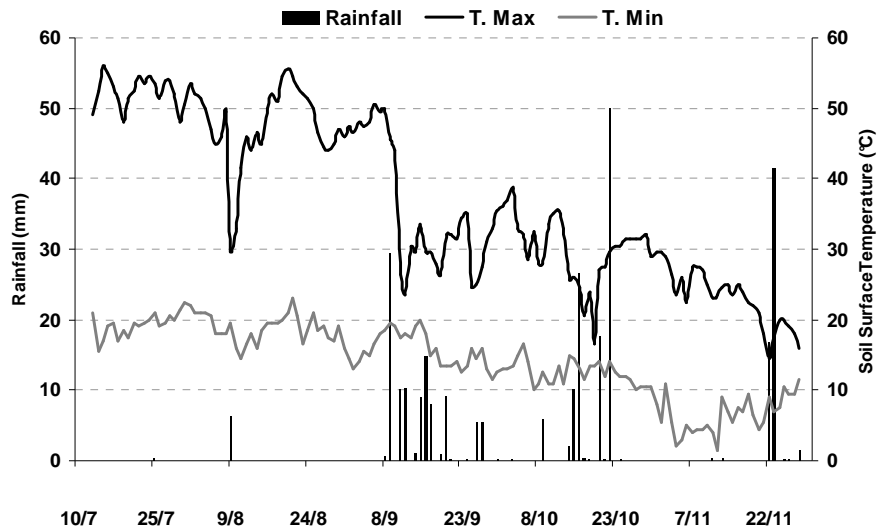


Fig. 1. Daily maximum and minimum soil surface temperatures, and rainfall (columns) at experimental site.

Germination test carried out after harvest (29 June) on seeds from manually threshed lomentos showed for all the entries very high proportions of hardseededness, with a mean value of 94.7 %. This is a peculiar characteristic of the species as observed by Patanè (1998). No significant differences were found among the accessions and the range was between 98.5 % of the 'Polizzi' entry and 90.5 % of 'Montevago' (Fig. 2). Levels of hard seed decreased for all accessions during the exposure period of the lomentos in the field, with 48.6 % of residual hard seed, on average. 'Mazzamuto' accession was the slowest in the seed softening process, with a final proportion of hard seed equal to 67 %. The other populations had hard seed levels between 37 % ('Montevago') and 49.7 % ('Fiume Tusa'), statistically not different (Fig. 2).

Estimations of hardseededness carried out by germination test after 138 DAP in seed lots kept under different conditions (field or

room), allowed us to evaluate the main components causing hard seed breakdown: conservation condition or seed ageing. In particular, the seed ageing component had a small (or null) effect for 3 genotypes ('Buonfornello', 'Mazzamuto' and 'Montevago') (Fig. 3). 'Pietranera', 'Polizzi', and 'Fiume Tusa' entries showed a low reduction of hard seed level due to seed ageing (equal to 7.4 %, on average). Therefore, for all accessions, the main component causing the reduction of hardseededness was the exposure on the soil surface under daily temperature and moisture fluctuations. In particular, 'Pietranera' and 'Montevago' populations displayed the highest reduction (-55.4 % and -52.7 %, respectively) of hard seed level due to the permanence of the lomentos in the field but, in the former the influence of seed ageing was higher than in the latter (-9.5 % and -0.8 %, respectively) (Fig. 3).

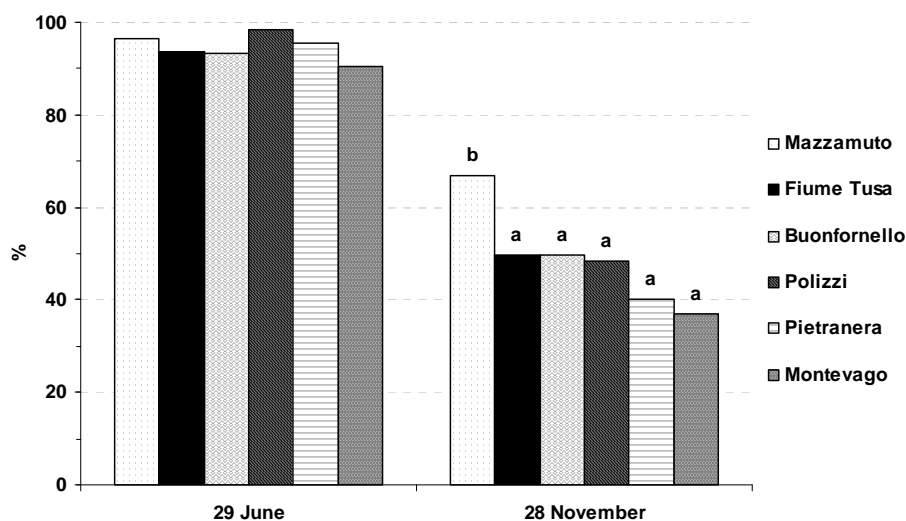


Fig. 2. Hard seed levels (%) of *S. muricatus* accessions at harvest (29 June) and after 138 DAP of exposure of laments on the soil surface (28 November). Columns with the same letter do not differ significantly ($P < 0.05$).

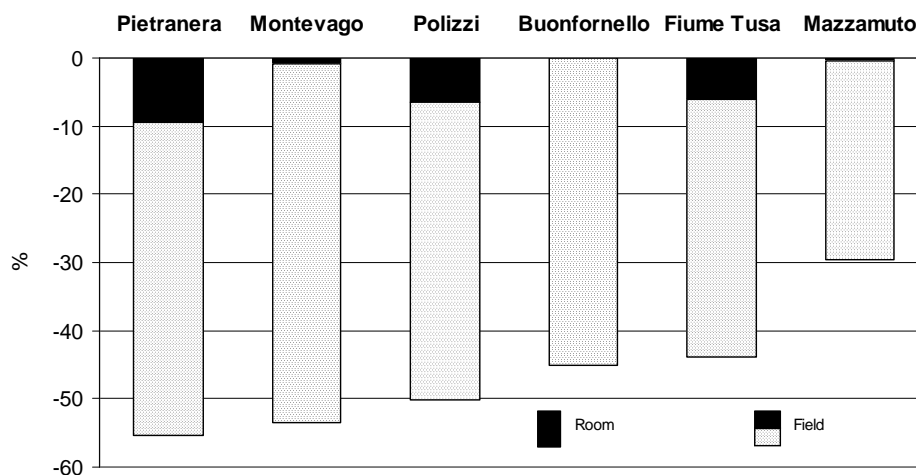


Fig. 3. Reduction (%) of hard seed levels of *S. muricatus* accessions after 138 days (28 November) of conservation under room or field conditions.

Mechanical threshing treatment had an important effect on variation of hard seed level for all the accessions under investigation. In fact, mechanical threshing caused a partial scarification of seeds, allowing imbibition and germination. Germination test carried out on seed mechanically threshed after 152 days of storage under room conditions showed a mean hard seed level of 51.7 %, with a reduction of 40 % compared with manually threshed seeds (differences significant at $P < 0.0001$). 'Mazzamuto' entry displayed the highest level of hardseededness (60.7 %), but differences

among populations were not statistically significant (Fig. 4). Mechanical threshing of the laments had an appreciable effect on hard seed level reduction, but probably this effect can differ largely depending on threshing machine and modalities adopted.

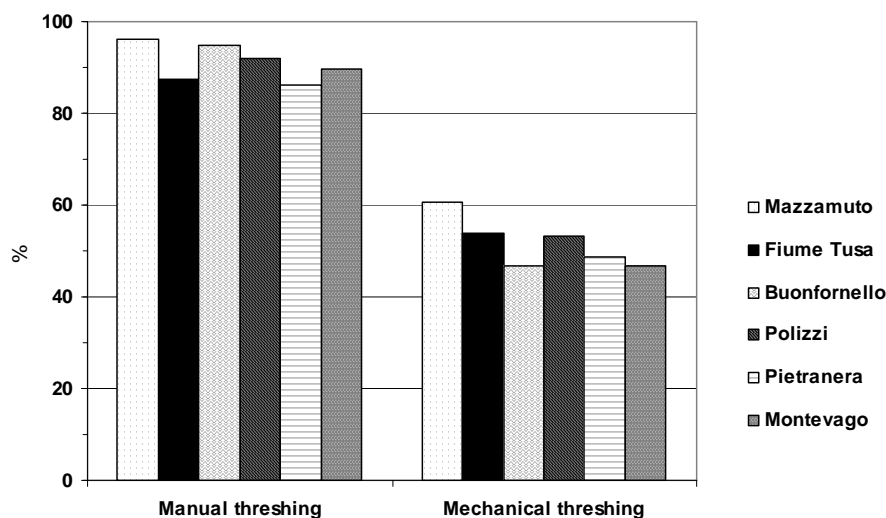


Fig. 4. Hard seed levels (%) of *S. muricatus* accessions at the end of a conservation period (29 June-28 November) under room conditions after manual or mechanical threshing.

ACKNOWLEDGEMENT

The research was funded by the University of Palermo, Doctorate in Mediterranean agro-

ecosystems. The authors are grateful to F. Cannella and V. Cannella for technical support.

LITERATURE

- Di Giorgio G., Ruisi P. & Giambalvo, D. 2007. Characterization and agronomical evaluation of *Scorpiurus muricatus* L. populations collected in Sicily. *In: Proc. XXVI EUCARPIA 'Breeding and seed production for conventional and organic agriculture'*, Perugia, 3-7 Sept. 2006 pp. 249-252.
- Ghassali, F., Osman, A.E. & Cocks, P.S. 1998. Rehabilitation of degraded grasslands in North Syria: the use of awassi sheep to disperse the seeds of annual pasture legumes. *Experimental Agriculture* 34: 391-405.
- Osman, A.E., Nassar, A. & Hassan, S.H. 1999. Grassland improvement by reseeding native legumes and protection from grazing in the Bekaa Valley, Lebanon. *Dynamics and sustainability of Mediterranean pastoral systems. Zaragoza: CIHEAM-IAMZ* 39: 147-151.
- Patanè, C. 1998. Effetto di alcuni pretrattamenti sulle caratteristiche germinative dei semi duri di *Scorpiurus subvillosus* L. *Sementi elette* 6: 43-46.
- Taylor, G.B. 1993. Effect of some characteristics of diurnal temperature fluctuations on the softening of hard seeds of *Medicago polymorpha*. *In: Proceedings of the XVII International Grassland Congress, Palmerston North, New Zealand.* pp. 256-257.

Longevity of seeds of *Poa trivialis* and *Vulpia bromoides* under field conditions as related to burial depth and straw cover

Peter Kryger Jensen

University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

PeterK.Jensen@agrsci.dk

ABSTRACT

The survival of seeds of *Poa trivialis* and *Vulpia bromoides* was followed under field conditions. Small samples of seeds of the two species were subjected to different treatments under field conditions simulating the distribution of seeds following different tillage implements and strategies. Immediately after being collected, the seeds were cleaned and counted and small samples with a known number was prepared and placed under field conditions again. The influence of different burial depths including placement directly on the soil surface for different intervals was investigated. For seeds placed at the soil surface the influence of straw cover was included to investigate the influence of straw disposal technique. After a time interval the seed samples with a small soil layer was collected and a germination test was carried out to investigate the seed survival according to the practice of straw handling at harvest. Seed survival at the soil surface was generally very low, whereas incorporation into the soil increased seed longevity.

Key words: *Poa trivialis*, *Vulpia bromoides* seed survival, straw management, soil tillage strategy

INTRODUCTION

Poa trivialis and *Vulpia bromoides* constitute serious problems in the seed production of some grasses in Denmark. The area infested with the two species has been increasing in the last decade, and control in grass seed crops is very difficult or impossible. In this situation preventive methods constitute important tools in a strategy to reduce the contamination with such weeds (Jensen 2006). Very few investigations on the fate of the seeds of the two species under field conditions exist. Persistence of seeds of *Poa trivialis* under field conditions however is longer than for many other grass species (Lewis 1973, Roberts 1986). Longevity of seeds in the soil is an important characteristic for many troublesome weed species. However seed longevity is often strongly influenced by incorporation depth in the soil (Roberts & Feast 1972, Moss 1985, Jensen 1999) and agronomic practices therefore has a large influence on contamination level. In this

study the influence of timing of incorporation of seeds and incorporation depth on the seed survival of the two species was investigated. The study also included an examination of the influence of straw cover on survival of seeds placed at the soil surface.

MATERIALS AND METHODS

Seeds of the weed species included in the investigation were collected from populations in fields at Flakkebjerg or close to this location. The seeds were collected from plants near maturity when the seeds shattered from the plants when gently shaken. Immediately after being collected, the seeds were cleaned and counted and small samples with a known number was prepared. Two different experimental series was carried out in the period 2005-2006. In the first series, the longevity of seeds in different soil depths was investigated. Samples of 400

seeds were counted and mixed with 0.2 l sterilised soil and placed in fabric mesh bags. The bags were then buried in the field in different depths in the plough layer simulating incorporation by soil cultivation. Seeds were also placed directly on the soil surface simulating no tillage. These seed samples were not mixed with soil but were covered with a piece of wire netting in order to avoid bird damage. The samples were placed in two litres plastic pots that were buried leaving only 1 cm of the edge above soil level. The pots were first filled with soil to approximately 2 cm from the top. Then followed a layer of felt and finally 2 dl sterilised soil on which the seed sample was placed. This means that the seed samples were placed at the same level as the surrounding field but 1 cm below the edge of the pot, in order to keep the sample together. The samples were normally placed in the field in August. After a year, in the following autumn the seed samples were collected and a germination test was carried out.

The seed survival at the soil surface was followed in other experiments where seed samples were placed at the soil surface in small plots. These were either left uncovered or covered with straw, simulating different straw disposal possibilities. The pots were prepared the same way as described earlier and were covered with a piece of wire netting in order to avoid bird damage. These samples were also typically placed in the field in August. The samples were then collected in the end of September, corresponding to the normal time for establishing winter cereals, and a germination test was carried out.

A small-scale field experiment was carried out investigating the influence of stubble treatments on emergence of the two species in the period from August to establishment of winter cereals in mid-September. Seed samples of 500 seeds of each species was dispersed in small plots and subjected to either no tillage or a superficial harrowing in 1-2cm depths. The two tillage treatments were combined with either removal of the straw or seeds covered with chopped straw corresponding to 5 t per ha⁻¹. After assessment in the beginning of September the entire field was sprayed with glyphosate followed by a shallow seedbed preparation in 1-2 cm depth a few days after the herbicide treatment. Late autumn and in the following spring establishment of the two species was assessed. All experiments included 4 replicates. Generally in both types of investigation, the time from seed harvest to burial in the soil under field conditions was kept to a few days in order to limit changes in dormancy status of the seeds during this period.

RESULTS

Two years results investigating the emergence of plants from seed samples kept at or close to the soil surface from seed shedding in August to the end of September the same year is shown in Table 1. Except for *Vulpia* in 2005 very few plants emerged from the samples placed at the soil surface whether they were covered with straw or not. Incorporation to just 2 cm depth increased emergence from the samples strongly indicating a much higher longevity.

Table 1. Number of *Poa trivialis* and *Vulpia bromoides* plants established from seed samples kept at different soil depths in the field from August to the end of September the same year. The samples originally contained 100 seeds.

Soil depth (cm)	<i>Poa trivialis</i>		<i>Vulpia bromoides</i>	
	2005	2006	2005	2006
0, no cover	1.5	0	9.5	0
0, straw cover	0	0	4	0
2	22.5	24	32	19
LSD ($P=0.05$)	8.6	2.4	4.6	2.0

Table 2. Number of *Poa trivialis* and *Vulpia bromoides* plants established from seed samples kept at different soil depths in the field from August 2005 to the end of September the following year. The samples originally contained 400 seeds.

Soil depth (cm)	<i>Poa trivialis</i>	<i>Vulpia bromoides</i>
0, no cover	0	1
0, straw cover	0	1
2	31	0
5	28	1
10	60	22
25	129	23
LSD ($P=0.05$)	44	Ns

The first year's results of a trial series investigating the emergence of plants from seed samples kept at different depths in the field from seed shedding in August to September the following year is showed in Table 2. With *Poa trivialis* there is a clear gradient reflecting increasing viability of seeds with increasing depth of the seed sample in the soil. The emergence of *Poa* from samples kept at 25 cm depth corresponds to approximately 1/3 of the original seed content still able to germinate after 13 month. The results with *Vulpia* show the same order although there are no significant differences due to a large variation in emergence rate of plants from samples kept at 10 and 25 cm.

Germination and establishment of plants of the two species were also followed in small-scale field experiments (Table 3). Seed

samples were placed at the soil surface in a stubble field and were subjected to four different treatments in August. The emergence was then followed in the beginning of September. Following this assessment, the entire experiment was sprayed with glyphosate and a few days after followed by the preparation of a seedbed with shallow harrowing. Late in the autumn and the following spring, emergence of plants following this treatment was assessed. For both species there was minor and non-significant differences in emergence of plants following the 4 different treatments in the stubble. After removal of the first emerged plants by glyphosate and the seedbed preparation there was a very limited emergence of plants of both species in all 4 treatments reflecting a very limited survival of seeds.

Table 3. Number of *Poa trivialis* and *Vulpia bromoides* plants established from seed samples placed at the soil surface in August and then subjected to the 4 different treatments. Following the assessment of plants on 9 September the entire trial was sprayed with glyphosate and a seedbed was prepared with a shallow harrowing a few days after the glyphosate treatment. Emerged plants late autumn and the following spring were assessed following the seedbed preparation. The seed samples originally contained 500 seeds.

Treatment in August	<i>Poa trivialis</i> plants			<i>Vulpia bromoides</i> plants		
	9 Sep.	7 Nov.	28 Apr.	9 Sep.	7 Nov.	28 Apr.
No tillage, no straw	4.8	1.3	1.0	36.5	4.5	6.5
Tillage 1-2 cm, no straw	4.8	2.5	0.3	18.8	3.8	6.3
No tillage, straw cover	6.5	1.0	1.5	26.8	1.3	3.8
Tillage 1-2 cm, straw cover	8.5	1.8	1.5	28.5	2.5	3.8
LSD ($P=0.05$)	Ns	Ns	Ns	Ns	Ns	ns

DISCUSSION

The investigation has shown a very limited emergence of plants of the two species from seeds kept at the soil surface for a period of approximately one month. This is supposed to reflect that most seeds of the two species disappear quickly if left on the soil surface for just a short period. The factors involved in depleting the seed samples for viable seeds were not investigated. However, emergence of plants from the samples placed at the soil surface only accounts for a minor proportion of the total seed sample, as this fraction was assessed in a number of experiments. The influence of other factors such as fatal germination, predation and destruction by fungi attacks were not determined. The shallow tillage treatment to 1-2 cm depth has the primary effect to contaminate the seeds with soil. This will prolong the period where seeds are kept moist after a rainfall and it will improve the chance of a seed being destroyed by fungi. A shallow tillage on the other hand only has a limited effect on the

vertical placement of the seeds (Moss 1988, Grundy *et al.* 1999) that are expected only to a very limited extent to be incorporated to the tillage depth. If the seeds were incorporated in the soil, survival of the seeds was strongly improved. This was especially the case for *Poa trivialis*, which showed a clear increase in viability of seeds the deeper incorporated the seeds were. However with both species a much larger proportion was viable if the seeds were incorporated to just 2 cm depths. The study emphasizes the conclusions seen with other grass species that agronomic practices that leaves the newly produced seeds at the soil surface as long as possible before incorporation in the soil minimizes the seed bank and reduces the problems in the following crops.

ACKNOWLEDGMENTS

Thanks to the Danish Seed Council for financial support to the investigation

LITERATURE

- Grundy, A.C. Mead, A. & Burston, S. 1999. Modelling the effect of cultivation on seed movement with application to the prediction of weed seedling emergence. *Journal of Applied Ecology* 36: 663-678.
- Jensen, P.K. 1999. Soil tillage strategies that reduces the survival of volunteer grass seeds. Proc 4th International Herbage Seed Conference, Perugia: 228-231
- Jensen, P.K. 2006. Longevity of seeds of *Apera spica-venti*, *Alopecurus myosuroides* and *Bromus* species under field conditions as related to burial depth and straw cover. In: Proceedings NJF seminar 378, Tillage systems for the benefit of agriculture and the environment, NJF report vol 2, nr 4, p.46.
- Lewis, J. 1973. Longevity of crop and weed seeds: Survival after 20 years in soil. *Weed Research* 13: 179-191.
- Moss, S.R. 1985. The survival of *Alopecurus myosuroides* Huds. Seeds in soil. *Weed Research*, 25: 201-211.
- Moss, S.R. 1988. Influence of cultivations on the vertical distribution of weed seeds in the soil. In: Proceedings VIII^e Colloque International sur la biologie, L'ecologie et la systematique des mauvaises herbes: 71-80.
- Roberts, H.A. 1986. Persistence of seeds of some grass species in cultivated soil. *Grass and Forage Science* 41: 273-276.
- Roberts, H. A. & Feast, P.M. 1972. Fate of seeds of some annual weeds in different depths of cultivated and undisturbed soil. *Weed Research* 12: 316-324.

APPENDIX:

- **Tables 1-32. Seed production acreage (ha) of various grasses and clovers in the world and in EU countries, 1993-2007**
- **Tables 33-64. Total seed production (tons) of various grasses and clovers in the world and in EU countries 1993-2006**

ACKNOWLEDGEMENT:

**The information in this appendix was kindly provided
by the Danish Seed Council**

Table 1. Total world seed production area of grasses and clovers, 1993-2006, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	193 430	31 901	-	29 797	860	167 506	40 976	208 482	464 470
1994	202 180	32 388	-	37 920	1 884	143 465	39 634	183 099	457 471
1995	207 336	26 862	-	29 717	2 672	158 725	46 541	205 266	471 853
1996	211 920	31 803	-	27 723	3 297	164 136	49 633	213 769	488 512
1997	224 768	33 851	-	30 932	3 438	182 038	53 039	235 077	528 065
1998	252 891	35 057	-	31 497	3 137	217 938	54 191	272 129	594 711
1999	265 915	35 995	-	35 695	3 333	201 540	57 978	259 518	600 456
2000	274 802	32 617	-	44 442	2 743	194 444	54 373	248 817	603 421
2001	272 814	24 084	-	42 592	2 580	196 337	46 186	242 523	584 593
2002	263 047	20 251	-	30 824	2 289	169 329	32 316	201 645	518 056
2003	249 642	25 829	-	24 905	1 579	192 748	27 533	220 281	523 239
2004	257 322	30 199	10 794	30 187	2 239	211 943	34 659	246 602	577 180
2005	271 170	32 459	13 813	38 306	2 914	219 462	45 267	264 729	622 763
2006	273 393	30 803	16 740	41 654	2 930	223 252	54 528	277 780	641 258
<u>Average</u>									
1996/05	254 429	30 215		33 710	2 755	194 992	45 518	240 509	564 100
<u>Average</u>									
2001/05	262 799	26 564		33 363	2 320	197 964	37 192	235 156	565 166

Table 2. Total seed production area in some EU countries, 1993-2006, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	56 159	26 462	36 951	23 162	12 195	4 634	3 049	18 381	11 192	8 134
1994	54 825	18 671	29 149	17 175	10 446	5 326	3 998	15 059	10 757	4 798
1995	62 128	21 071	28 822	17 943	10 145	6 580	4 927	17 355	12 376	6 979
1996	60 890	21 296	30 234	20 347	10 738	7 427	6 189	19 187	12 861	10 122
1997	64 396	25 483	35 340	20 235	11 035	7 563	6 664	25 735	13 155	8 239
1998	83 556	30 728	37 694	26 084	11 523	8 085	6 487	23 403	12 062	13 502
1999	80 092	21 885	35 400	28 388	8 572	7 663	7 516	23 502	14 680	14 275
2000	78 698	22 210	30 894	27 586	7 314	9 005	7 655	25 827	11 498	12 160
2001	82 724	19 782	29 613	23 775	6 601	10 958	9 681	21 817	10 141	9 112
2002	68 305	18 056	26 909	19 320	6 060	12 032	10 142	13 343	7 333	6 476
2003	83 644	21 674	28 370	21 697	6 936	11 863	9 538	12 159	6 270	4 669
2004	87 825	24 946	34 083	25 742	6 220	11 759	7 876	16 733	8 493	3 911
2005	89 995	26 925	36 727	29 303	6 005	11 067	7 403	20 243	11 102	6 811
2006	92 894	25 329	35 493	28 563	6 039	12 703	8 980	23 319	15 490	6 754
<u>Average</u>										
1996/05	78 013	23 299	32 526	24 248	8 100	9 742	7 915	20 195	10 760	8 928
<u>Average</u>										
2001/05	82 499	22 277	31 140	23 967	6 364	11 536	8 928	16 859	8 668	6 196

Table 3. Total world seed production area of perennial ryegrass, 1993-2006, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	47 447	8 485		66		59 779	5 402	65 181	121 179
1994	53 113	8 989		224		42 872	4 395	47 267	109 593
1995	55 747	8 559		202		56 814	6 040	62 854	127 362
1996	54 768	10 951		450		66 619	7 208	73 827	139 996
1997	60 391	11 016		184		74 900	6 737	81 637	153 228
1998	70 064	13 904		1 176		87 272	5 582	92 854	177 998
1999	75 478	16 631		1 639		66 004	8 088	74 092	167 840
2000	73 611	15 033		5 085		64 741	8 537	73 278	167 007
2001	69 418	10 127		3 543		61 163	7 751	68 914	152 002
2002	61 191	5 872		2 066		51 797	5 010	56 807	125 936
2003	67 638	7 687		3 168		69 364	4 071	73 435	151 928
2004	71 887	10 434	2 489	6 345		78 661	6 268	84 929	176 084
2005	78 087	11 914	3 775	9 738	3	80 605	8 203	88 808	192 325
2006	69 030	10 704	3 639	14 391	3	79 369	10 114	89 483	187 250
2007		9 543							
<u>Average</u>									
1996/05	68 253	11 357		3 339		70 113	6 746	76 858	160 434
<u>Average</u>									
2001/05	69 644	9 207		4 972		68 318	6 261	74 579	159 655

Table 4. Total seed production area of perennial ryegrass in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	23 453	13 195	9 503	4 623	8 396	86	9	1 410	3 320	658
1994	18 144	6 005	7 900	2 838	7 369	257	43	956	3 202	119
1995	24 248	10 984	8 578	4 265	7 450	270	42	1 133	4 040	810
1996	29 017	13 093	8 917	5 465	8 092	330	38	1 105	4 681	1 183
1997	30 639	16 794	9 989	6 014	8 549	346	62	1 244	4 351	811
1998	38 410	19 449	10 678	7 192	8 285	391	70	1 036	3 739	675
1999	30 585	10 840	10 304	6 710	5 623	440	85	1 367	5 743	740
2000	25 970	13 366	12 270	6 639	4 992	419	96	2 148	4 066	1 966
2001	27 003	11 533	7 972	6 179	4 863	462	68	2 314	3 140	1 934
2002	25 638	9 191	6 202	5 262	4 451	537	79	1 158	1 934	1 519
2003	35 262	13 119	7 675	7 289	5 268	562	86	979	1 786	1 050
2004	36 786	16 745	9 656	8 652	4 739	814	75	1 471	3 453	856
2005	36 380	18 980	9 343	9 227	4 550	818	75	1 409	4 694	1 160
2006	37 558	17 579	9 446	8 376	4 612	817	47	1 750	6 058	1 281
2007	30 970	13 617		7 010		562				
<u>Average</u>										
1996/05	31 569	14 311	9 301	6 863	5 941	512	73	1 423	3 759	1 189
<u>Average</u>										
2001/05	32 214	13 914	8 170	7 322	4 774	639	77	1 466	3 001	1 304

Table 5. Total world seed production area of Italian ryegrass, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	45 407	3 007				30 474	5 786	36 260	84 674
1994	49 900	2 344		61		20 556	5 106	25 662	77 967
1995	51 761	1 278		32		22 447	6 828	29 275	82 346
1996	50 911	3 070		337		25 100	9 468	34 568	88 886
1997	48 180	4 031		2 821		35 714	12 831	48 545	103 577
1998	51 478	3 064		2 875		43 154	17 553	60 707	118 124
1999	51 972	3 222		1 700		36 977	11 731	48 708	105 602
2000	51 700	2 459		138		22 000	6 716	28 716	83 013
2001	49 960	2 103		279		22 531	4 891	27 422	79 764
2002	48 350	3 061		402		21 277	4 952	26 229	78 042
2003	47 450	4 787		1 135		26 967	6 778	33 745	87 117
2004	50 543	5 727	2 118	1 685		34 781	9 474	44 255	104 328
2005	50 749	6 257	2 800	1 694		33 374	11 696	45 070	106 570
2006	53 339	4 039	2 847	2 086		29 591	10 663	40 254	102 565
2007		2 294							
<u>Average</u>									
1996/05	50 129	3 778		1 307		30 188	9 609	39 797	95 502
<u>Average</u>									
2001/05	49 410	4 387		1 039		27 786	7 558	35 344	91 164

Table 6. Total seed production area of Italian ryegrass in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	3 891	2 688	14 211	3 987	1 627			2 772	1 265	1 573
1994	2 601	2 573	8 797	1 883	1 314		7	2 308	925	1 676
1995	2 428	2 595	9 871	2 717	1 034		20	3 503	1 436	1 651
1996	2 037	2 438	10 483	4 374	1 128		8	4 052	1 327	3 700
1997	3 759	3 649	14 782	4 945	1 119		32	6 851	1 896	3 206
1998	5 484	4 869	15 735	5 900	1 437		57	9 160	2 169	5 429
1999	4 104	3 992	13 632	5 133	1 079		28	6 247	840	4 140
2000	2 816	2 063	5 506	2 500	625		7	2 951	416	2 920
2001	1 970	1 686	8 056	1 857	372		7	1 576	341	2 702
2002	1 596	2 165	8 493	2 105	352		7	1 713	462	2 054
2003	2 251	2 753	10 299	3 162	412		7	3 273	857	1 775
2004	2 636	2 546	14 533	4 434	278		-	5 922	1 125	1 460
2005	2 573	2 159	15 460	4 356	188		5	6 581	1 538	2 290
2006	2 275	1 495	12 478	3 058	183		5	5 112	2 168	2 028
2007	1 703	1 567		2 367						
<u>Average</u>										
1996/05	2 923	2 832	11 698	3 877	699		16	4 833	1 097	2 968
<u>Average</u>										
2001/05	2 205	2 262	11 368	3 183	320		5	3 813	865	2 056

Table 7. Total world seed production area of hybrid ryegrass, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993		764				2 508	243	2 751	3 515
1994		1 149				1 247	13	1 260	2 409
1995		1 192				1 619	74	1 693	2 885
1996		1 137				2 098	118	2 216	3 353
1997		1 366				3 016	259	3 275	4 641
1998		1 984				4 656	257	4 913	6 897
1999		2 078				5 263	483	5 746	7 824
2000		2 010				3 405	648	4 053	6 063
2001		1 383				1 926	162	2 088	3 471
2002		1 668				1 509	154	1 663	3 331
2003		2 517				2 235	195	2 430	4 947
2004		2 846	620			3 060	750	3 810	7 276
2005		2 642	1 108		11	4 155	1 477	5 632	9 393
2006		2 510	857		18	4 382	1 686	6 068	9 453
2007		1 652						-	
<u>Average</u> 1996/05		1 963				3 132	450	3 583	5 720
<u>Average</u> 2001/05		2 211				2 577	548	3 125	5 684

Table 8. Total seed production area of hybrid ryegrass in EU, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	1 111	73	396	714	380	-		1	38	70
1994	455	93	143	379	350	-		-	13	
1995	555	77	93	342	446	18		1	73	
1996	444	63	139	755	520	45		1	41	
1997	933	99	234	941	586	21		24	44	69
1998	1 681	140	363	1 488	1 012	22		13	68	107
1999	1 523	172	368	2 150	1 019	18		48	63	227
2000	840	298	351	1 060	790	40		102	73	280
2001	373	98	168	672	692	27		9	45	70
2002	246	57	54	720	511	38		27	56	55
2003	673	19	114	812	532	5		45	66	40
2004	1 050	63	143	1 188	494	16		492	52	155
2005	1 437	154	429	1 459	529	-		652	123	567
2006	1 403	263	412	1 821	409	-		570	185	798
2007	1 303	396								
<u>Average</u>										
1996/05	920	116	236	1 125	669	23		141	63	157
<u>Average</u>										
2001/05	756	78	182	970	552	17		245	68	177

Table 9. Total world seed production area of red fescue, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	10 729			9 508	38	28 652	4 729	33 381	53 656
1994	9 462			11 678	27	28 119	3 687	31 806	52 973
1995	7 094			6 048	5	23 667	3 976	27 643	40 790
1996	5 759			5 653	-	18 924	3 969	22 893	34 305
1997	5 363			8 588	21	21 532	3 452	24 984	38 956
1998	5 756			7 072	33	31 229	4 441	35 670	48 531
1999	6 963			6 635	30	36 136	6 057	42 193	55 821
2000	8 543			8 024	29	38 788	7 871	46 659	63 255
2001	8 252			9 050	35	42 380	8 243	50 623	67 960
2002	7 126			6 248	71	26 923	6 120	33 043	46 488
2003	4 982			2 856	89	29 613	3 631	33 244	41 171
2004	4 868			6 383	89	31 368	3 264	34 632	45 972
2005	5 617			9 479	64	35 258	5 376	40 634	55 794
2006	6 249			6 386	59	37 027	7 628	44 655	57 349
2007								-	-
<u>Average</u> 1996/05	6 323			6 999	46	31 215	5 242	36 458	49 825
<u>Average</u> 2001/05	6 169			6 803	70	33 108	5 327	38 435	51 477

Table 10. Total seed production area of red fescue in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	15 257	5 067	3 331	2 978	954	906	13	738	1 822	2 169
1994	15 575	4 806	3 157	2 726	745	1 109	25	715	1 904	1 068
1995	14 739	2 545	1 779	1 675	624	887	7	535	2 181	1 260
1996	13 129	1 620	1 751	1 227	530	796	1	550	1 934	1 485
1997	15 473	1 570	1 916	1 382	314	786	4	906	1 643	903
1998	22 494	2 822	2 441	2 114	339	818	5	934	1 680	1 827
1999	26 033	3 276	2 696	2 484	400	921	5	1 148	2 872	2 037
2000	27 317	2 750	3 075	3 808	412	1 006	5	2 224	3 527	2 120
2001	29 676	2 701	3 724	4 210	247	1 256	9	2 602	3 956	1 685
2002	16 073	2 562	3 343	3 078	299	1 533	9	1 687	2 878	1 555
2003	21 490	1 962	2 706	1 852	334	1 410	5	692	1 800	1 139
2004	23 593	1 920	2 098	1 980	351	1 249	-	610	1 782	751
2005	25 670	2 278	2 518	2 938	310	1 317	-	1 200	2 275	1 650
2006	26 195	2 613	2 819	3 225	372	1 584	-	2 310	3 567	1 391
2007	21 257	2 105				1 672				
<u>Average</u>										
1996/05	22 095	2 346	2 627	2 507	354	1 109	4	1 255	2 435	1 515
<u>Average</u>										
2001/05	23 300	2 285	2 878	2 812	308	1 353	5	1 358	2 538	1 356

Table 11. Total world seed production area of meadow fescue, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993				665	469	4 600	2 749	7 349	8 483
1994				1 070	696	7 001	2 611	9 612	11 378
1995				1 623	651	8 336	2 950	11 286	13 560
1996				2 060	557	8 047	3 522	11 569	14 186
1997				2 175	590	7 090	3 472	10 562	13 327
1998				1 762	702	6 819	3 098	9 917	12 381
1999				1 671	985	6 808	3 000	9 808	12 464
2000				1 691	724	7 925	3 470	11 395	13 810
2001				1 377	687	8 097	3 440	11 537	13 601
2002				699	693	7 442	2 835	10 277	11 669
2003				284	490	6 860	2 334	9 194	9 968
2004				287	262	6 171	2 345	8 516	9 065
2005				688	565	7 322	2 702	10 024	11 277
2006				1 163	643	8 576	3 767	12 343	14 149
2007									-
<u>Average</u> 1996/05				1 269	625	7 258	3 022	10 280	12 175
<u>Average</u> 2001/05				667	539	7 178	2 731	9 910	11 116

Table 12. Total seed production area of meadow fescue in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	931	53	2 140	8	18	788	662	1 640	605	261
1994	1 890	106	3 009	10	12	984	984	1 417	484	103
1995	2 608	204	3 427	10	13	1 023	1 005	1 342	746	362
1996	2 044	109	3 613	97	9	1 199	960	1 583	1 125	294
1997	1 457	30	3 216	104	13	1 271	1 012	1 853	1 007	235
1998	1 309	28	2 723	131	9	1 246	1 331	1 630	892	293
1999	1 075	25	2 741	117	6	1 296	1 541	1 554	812	220
2000	1 628	29	2 998	63	6	1 793	1 392	2 061	467	461
2001	1 596	15	2 609	68	13	2 130	1 649	2 231	376	292
2002	1 144	19	2 420	57	6	2 113	1 668	1 726	232	306
2003	927	22	2 364	46	-	1 756	1 736	1 429	232	186
2004	775	11	2 148	52	-	1 560	1 595	1 446	276	68
2005	984	26	2 819	80	-	1 774	1 590	1 659	252	40
2006	1 138	17	3 595	52	-	2 221	1 510	2 255	515	43
2007	1 307	9				2 009				
<u>Average</u>										
1996/05	22 095	2 346	2 627	2 507	354	1 109	4	1 717	567	240
<u>Average</u>										
2001/05	23 300	2 285	2 878	2 812	308	1 353	5	1 698	274	178

Table 13. Total world seed production area of sheep's fescue, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993				90		3 717	125	3 842	3 932
1994				238		3 179	153	3 332	3 570
1995	1 174			242		2 054	177	2 231	3 647
1996	842			81		1 432	172	1 604	2 527
1997	676			55		1 240	80	1 320	2 051
1998	700			229		2 221	97	2 318	3 247
1999	1 236			842		2 974	167	3 141	5 219
2000	3 166			1 468		4 350	270	4 620	9 254
2001	2 564			1 575		4 789	338	5 127	9 266
2002	1 242			542		3 363	220	3 583	5 367
2003	556			96		1 552	204	1 756	2 408
2004	518			87		2 117	198	2 315	2 920
2005	526			110	1	2 750	268	3 018	3 655
2006	579			343	7	3 426	316	3 742	4 671
2007								-	-
<u>Average</u>									
1996/05	1 203			509		2 679	201	2 880	4 591
<u>Average</u>									
2001/05	1 081			482		2 914	246	3 160	4 723

Table 14. Total seed production area of sheep's fescue in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	713	253	2 366	219	9	-		23		75
1994	973	377	1 517	203	27	-		50		75
1995	867	144	851	149	14	-		45		75
1996	541	51	684	105	13	-		18		75
1997	392	63	609	127	13	-		27		
1998	602	62	1 327	182	-	-		42		20
1999	1 115	164	1 306	273	-	5		58		48
2000	1 735	378	1 698	347	-	5		127		116
2001	1 852	259	2 125	414	9	10		199		92
2002	1 028	153	1 829	197	21	42		164		11
2003	641	177	525	191	21	30		132		40
2004	909	124	886	107	-	37		132		44
2005	1 036	149	1 364	106	-	38		204		57
2006	1 398	232	1 655	83	-	10		152		46
2007	1 527	291				15				
<u>Average</u> 1996/05	985	158	1 235	205	8	17		110		50
<u>Average</u> 2001/05	1 093	172	1 346	203	10	31		166		49

Table 15. Total world seed production area of tall fescue, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	32 218	919		68		4 113	268	4 381	37 586
1994	27 714	174		57		2 841	213	3 054	30 999
1995	30 158	143		101		2 202	153	2 355	32 757
1996	34 687	197		420		2 780	256	3 036	38 340
1997	41 361	307		1 377		3 498	281	3 779	46 824
1998	48 923	558		3 183		4 923	462	5 385	58 049
1999	52 396	780		6 690		5 653	666	6 319	66 185
2000	55 027	1 027		7 211		6 845	780	7 625	70 890
2001	63 416	1 245		3 905		7 408	1 093	8 501	77 067
2002	65 865	1 638		4 807		8 579	1 012	9 591	81 901
2003	57 059	1 549		3 312		9 572	727	10 299	73 222
2004	57 488	1 260	1 003	2 054		10 059	716	10 775	72 892
2005	58 815	1 028	1 315	1 550		9 335	1 275	10 610	73 334
2006	64 011	908	1 331	1 319		9 610	1 589	11 199	77 437
2007		954							
<u>Average</u>									
1996/05	53 504	959		3 451		6 865	727	7 592	65 870
<u>Average</u>									
2001/05	60 529	1 344		3 126		8 991	965	9 955	75 683

Table 16. Total seed production area of tall fescue in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	45	1 121	25	2 808	36	-	-	39	1	198
1994	55	437	5	2 203	33	-	-	12	4	136
1995	142	181	-	1 811	33	-	-	1	18	94
1996	216	348	-	2 107	25	-	-	2	10	184
1997	222	763	9	2 335	21	-	5	36	6	136
1998	730	958	13	3 039	16	-	8	40	11	338
1999	988	945	36	3 423	16	-	30	147	6	396
2000	1 813	1 057	154	3 778	9	-	40	191	9	505
2001	2 123	1 512	226	3 281	9	-	57	389	31	596
2002	2 408	1 910	214	3 384	9	-	58	408	88	405
2003	2 871	1 887	283	3 987	-	26	89	393	107	160
2004	3 371	1 948	244	3 946	-	12	170	438	122	154
2005	2 680	1 780	212	4 177	-	45	140	700	188	361
2006	2 869	1 776	188	4 148	-	73	128	880	337	303
2007	3 903	1 729				9				
<u>Average</u>										
1996/05	1 742	1 311	139	3 346	11	8	60	274	58	324
<u>Average</u>										
2001/05	2 691	1 807	236	3 755	4	17	103	466	107	335

Table 17. Total world seed production area of festulolium, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993							211	211	211
1994						39	84	123	123
1995						248	71	319	319
1996						149	186	335	335
1997						219	331	550	550
1998						97	441	538	538
1999						176	798	974	974
2000						368	1 562	1 930	1 930
2001		6				303	1 348	1 651	1 657
2002		125				195	889	1 084	1 209
2003		586				110	575	685	1 271
2004		706				238	815	1 053	1 759
2005		687				645	1 314	1 959	2 646
2006		908				955	1 899	2 854	3 762
2007		655							
<u>Average</u> 1996/05		211				250	826	1 076	1 287
<u>Average</u> 2001/05		422				298	988	1 286	1 708

Table 18. Total seed production area of festulolium in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993								211		
1994			39					84		
1995			248					71		
1996			149					89		
1997			219					198	2	
1998	11		86					366	6	
1999	45		131					629	34	
2000	251		109			5		1 257	62	
2001	222		65			16		1 162	51	
2002	123		56			16		684	30	
2003	77		33					483	11	
2004	160		42			36		639	54	
2005	263		63	267		52		925	174	
2006	542		167	246		-		1 267	261	
2007	912									
<u>Average</u>										
1996/05	115		95	27		13		643	42	
<u>Average</u>										
2001/05	169		52	53		24		779	64	

Table 19. Total world seed production area of Kentucky bluegrass, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	38 426			690	38	10 744	1 151	11 895	51 049
1994	42 893			1 209	31	14 209	827	15 036	59 169
1995	40 482			2 106	16	14 677	1 071	15 748	58 352
1996	43 896			1 441	-	11 404	1 153	12 557	57 894
1997	47 475			692	23	8 836	1 087	9 923	58 113
1998	52 277			360	9	8 938	916	9 854	62 500
1999	54 903			157	27	10 201	1 128	11 329	66 416
2000	60 432			209	24	10 674	1 202	11 876	72 541
2001	58 396			108	27	12 136	1 153	13 289	71 820
2002	59 583			370	33	14 128	1 108	15 236	75 222
2003	53 615			268	39	13 186	1 036	14 222	68 144
2004	54 062			196	50	12 204	960	13 164	67 472
2005	57 772			17	76	12 810	937	13 747	71 612
2006	59 619			172	89	12 941	810	13 751	73 631
2007									
<u>Average</u> 1996/05	54 241			382	31	11 452	1 068	12 520	67 173
<u>Average</u> 2001/05	56 686			192	45	12 893	1 039	13 932	70 854

Table 20. Total seed production area of Kentucky bluegrass in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	6 038	3 750	531	9		425		225	363	504
1994	8 856	4 155	737	14		461		173	339	261
1995	9 264	4 224	605	18		457		390	403	224
1996	6 885	3 429	566	35		402		430	499	194
1997	5 530	2 345	557	11		334		473	468	97
1998	5 808	2 253	511	2		302		394	390	93
1999	7 065	2 279	462	2		243		410	528	126
2000	7 817	2 051	403	14		241		490	522	153
2001	9 629	1 784	307	15		284		580	452	51
2002	11 136	1 832	290	39		542		552	436	40
2003	10 106	1 568	413	58		713		500	392	33
2004	9 335	1 354	417	5		630		410	359	23
2005	10 166	1 201	409	1		568		319	346	23
2006	10 503	1 160	296	-		502		269	334	20
2007	8 799	1 052				525				
<u>Average</u>										
1996/05	8 348	2 010	434	18		426		456	439	83
<u>Average</u>										
2001/05	10 074	1 548	367	24		547		472	397	34

Table 21. Total world seed production area of rough bluegrass, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993				51		594		594	645
1994				16		649		649	665
1995	1 275			20		537		537	1 832
1996	1 558			20		347		347	1 925
1997	1 788					259		259	2 047
1998	2 149					360		360	2 509
1999	2 149					288		288	2 437
2000	2 570					345		345	2 915
2001	1 420					400	10	410	1 830
2002	1 526					396	10	406	1 932
2003	870					263		263	1 133
2004	1 048					308	4	312	1 360
2005	979					244		244	1 223
2006	915					263		263	1 178
2007									
<u>Average</u>									
1996/05	1 606					321		323	1 931
<u>Average</u>									
2001/05	1 169					322		327	1 496

Table 23. Total world seed production area of timothy, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993				17 668	214	5 736	2 338	8 074	25 956
1994				22 273	815	6 830	3 767	10 597	33 685
1995				18 182	1 550	8 799	4 006	12 805	32 537
1996				16 658	2 011	11 132	2 844	13 976	32 645
1997				14 052	2 087	11 082	2 606	13 688	29 827
1998				13 454	2 198	10 392	2 477	12 869	28 521
1999				14 602	1 922	10 690	2 600	13 290	29 814
2000				17 372	1 450	12 140	2 860	15 000	33 822
2001				20 838	1 386	14 834	3 279	18 113	40 337
2002				14 439	878	15 748	3 255	19 003	34 320
2003				13 410	493	15 311	2 540	17 851	31 754
2004				12 479	1 445	13 951	2 645	16 596	30 520
2005				13 571	1 794	12 614	3 569	16 183	31 548
2006				13 847	1 547	14 444	4 875	19 319	34 713
2007									
<u>Average</u> 1996/05				15 088	1 566	12 789	2 868	15 657	32 311
<u>Average</u> 2001/05				14 947	1 199	14 492	3 058	17 549	33 696

Table 24. Total seed production area of timothy in EU, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	203	48	1 625	21	196	1 578	2 073	1 428	351	22
1994	412	17	1 421	1	208	1 996	2 773	1 150	419	4
1995	822	7	1 568	11	212	2 537	3 631	859	491	4
1996	761	48	2 041	8	180	3 197	4 902	881	719	7
1997	475	39	2 056	8	189	3 440	5 018	1 075	666	3
1998	293	7	1 986	10	179	3 584	4 332	979	412	67
1999	318	4	2 063	-	157	2 893	5 262	1 025	382	67
2000	552	11	2 661	-	143	3 240	5 525	1 402	289	82
2001	653	22	2 855	3	103	3 972	7 189	1 735	345	8
2002	895	28	2 742	3	100	4 238	7 680	1 795	275	5
2003	881	30	2 803	3	90	4 314	7 135	1 241	281	5
2004	856	2	2 779	3	10	4 675	5 575	1 160	352	5
2005	741	8	2 448	-	86	4 167	5 082	1 394	453	6
2006	545	10	2 251	-	64	4 880	6 638	1 840	645	6
2007	590					3 922				
<u>Average</u> 1996/05	643	20	2 443	4	124	3 772	5 770	1 269	417	26
<u>Average</u> 2001/05	805	18	2 725	2	78	4 273	6 532	1 465	341	6

Table 25. Total world seed production area of cocksfoot, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	7 649	1 015		173	2	4 929	1 018	5 947	14 786
1994	8 094	1 013		235	4	5 426	814	6 240	15 586
1995	8 357	1 000		219	23	4 778	1 222	6 000	15 599
1996	8 531	1 174		210	60	5 004	822	5 826	15 801
1997	8 300	987		274	140	5 011	1 663	6 674	16 375
1998	8 406	376		241	16	5 455	1 808	7 263	16 302
1999	6 924	454		242	125	6 190	1 787	7 977	15 722
2000	6 661	348		297	48	7 072	1 562	8 634	15 988
2001	7 268	460		215	36	6 711	1 863	8 574	16 553
2002	7 734	676		263	37	6 605	1 398	8 003	16 713
2003	7 657	559		140	13	6 021	950	6 971	15 340
2004	7 329	626	475	182	8	6 297	1 101	7 398	15 543
2005	7 030	668	644	81	5	6 012	1 038	7 050	14 834
2006	6 463	646	711	32	29	6 176	1 344	7 520	14 690
2007									
<u>Average</u> 1996/05	7 584	633		215	49	6 038	1 399	7 437	15 917
<u>Average</u> 2001/05	7 404	598		176	20	6 329	1 270	7 599	15 796

Table 26. Total seed production area of cooksfoot in EU, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	1 158	15	378	3 103	170	40	8	239	642	20
1994	1 789	3	260	3 296	143	69	30	216	485	5
1995	2 133	14	212	2 018	135	137	29	325	731	2
1996	2 538	13	203	1 853	127	151	10	102	592	2
1997	2 448	16	272	1 837	134	116	10	372	995	2
1998	2 494	18	237	2 242	132	140	11	475	991	114
1999	2 658	13	132	2 869	127	112	27	502	1 089	29
2000	3 020	15	80	3 473	138	107	25	846	266	283
2001	2 923	7	106	3 287	152	87	28	1 155	212	281
2002	3 619	11	118	2 444	160	118	20	833	214	112
2003	3 812	-	133	1 899	153	116	6	449	205	81
2004	3 545	20	169	2 096	155	118	-	591	219	98
2005	2 463	9	165	2 830	212	104	-	475	264	141
2006	2 116	21	307	3 160	284	57	-	612	318	152
2007	3 260	20				35				
<u>Average</u> 1996/05	2 952	12	162	2 483	149	117	14	580	505	114
<u>Average</u> 2001/05	3 272	9	138	2 511	166	109	11	701	223	143

Table 27. Total world seed production area of bentgrass, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	6 212	713		87	79	224	674	898	7 989
1994	5 682	226		240	64	135	339	474	6 686
1995	5 237	162		256	57	147	321	468	6 180
1996	5 298	234		129	78	115	308	423	6 162
1997	5 451	403		113	113	121	377	498	6 578
1998	4 817	493		49	128	175	359	534	6 021
1999	4 693	466		12	150	269	303	572	5 893
2000	4 638	467		1	100	374	617	991	6 197
2001	3 929	278		1	31	335	583	918	5 157
2002	3 396	371		16	54	365	457	822	4 659
2003	3 319	311		16	53	318	355	673	4 372
2004	3 185	305		-	50	404	370	774	4 314
2005	3 444	345		-	34	383	346	729	4 552
2006	3 472	387		-	69	299	317	616	4 544
2007		443							
<u>Average</u> 1996/05	4 217	367		59	79	286	408	693	5 390
<u>Average</u> 2001/05	3 455	322		7	44	361	422	783	4 611

Table 28. Total seed production area of bentgrass in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	15	184	23	-				393	113	168
1994	32	94	10	-				131	71	134
1995	38	90	14	5				122	88	109
1996	16	78	6	15				125	85	95
1997	3	112	0	3				191	91	95
1998	45	121	3	3				190	117	43
1999	89	175	0	-				163	99	36
2000	166	187	4	17				477	93	33
2001	151	162	6	16				484	65	34
2002	210	128	6	21				380	35	38
2003	169	137	8	15				286	29	40
2004	184	213	3	-				297	40	28
2005	197	181	3	-				280	40	11
2006	124	163	2	-				253	48	11
2007	125									
<u>Average</u>										
1996/05	123	149	4	9				287	69	45
<u>Average</u>										
2001/05	182	164	5	10				345	42	30

Table 29. Total world seed production area of red clover, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993	5 342	1 057		731	20	7 883	15 617	23 500	30 650
1994	5 322	1 498		619	247	6 093	16 915	23 008	30 694
1995	5 565	1 076		686	370	8 212	18 966	27 178	34 875
1996	5 245	1 127		264	591	7 659	18 784	26 443	33 670
1997	5 273	1 111		601	464	6 635	19 230	25 865	33 314
1998	7 795	832		1 096	51	8 260	16 305	24 565	34 339
1999	8 655	860		1 505	96	9 773	20 898	30 671	41 787
2000	7 847	1 268		2 946	369	11 005	17 744	28 749	41 179
2001	7 167	899		1 701	363	8 575	11 677	20 252	30 382
2002	5 779	781		972	509	6 814	4 538	11 352	19 393
2003	4 800	780		220	378	6 850	3 885	10 735	16 913
2004	4 431	594		489	297	7 608	5 423	13 031	18 842
2005	5 581	579		1 378	337	7 996	6 762	14 758	22 633
2006	6 317	666		1 915	408	9 358	9 076	18 434	27 740
2007		856							
<u>Average</u> 1996/05	6 257	883		1 117	345	8 118	12 525	20 642	29 245
<u>Average</u> 2001/05	5 552	727		952	377	7 569	6 457	14 026	21 632

Table 30. Total seed production area of red clover in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	85		1 937	4 678	48	767	284	8 923	2 641	2 415
1994	306		1 316	3 616	35	357	136	7 410	2 871	1 217
1995	488		917	4 917	30	1 157	193	8 608	2 145	2 383
1996	436		1 092	4 288	29	1 166	270	9 737	1 809	2 898
1997	552		1 103	2 510	18	1 107	521	12 018	1 901	2 677
1998	677		1 124	3 719	16	1 402	673	7 939	1 481	4 496
1999	823		1 181	5 186	45	1 481	533	10 051	2 188	6 209
2000	912		1 302	5 661	83	1 896	552	11 144	1 676	3 241
2001	435		1 104	3 571	106	2 234	668	7 151	1 093	1 367
2002	387		936	1 915	68	2 481	619	2 061	671	376
2003	478		843	2 299	60	2 465	471	2 152	494	120
2004	464		841	3 178	130	2 041	458	3 007	635	244
2005	361		1 341	3 631	42	1 513	496	4 290	739	476
2006	424		1 736	4 165	51	1 719	635	5 787	1 028	675
2007	524					2 087				
<u>Average</u> 1996/05	553		1 087	3 596	60	1 779	526	6 955	1 269	2 210
<u>Average</u> 2001/05	425		1 013	2 919	81	2 147	542	3 732	726	517

Table 31. Total world seed production area of white clover, 1993-2007, ha

	USA	New Zealand	Australia	Canada	Norway	EU-15 total	EU-10 Total	EU 25 total	World total
1993		15 941				3 553	665	4 218	20 159
1994		16 995				4 269	710	4 979	21 974
1995	486	13 452				4 188	686	4 874	18 812
1996	425	13 913				3 326	823	4 149	18 487
1997	510	14 630				2 885	633	3 518	18 658
1998	526	13 846				3 987	395	4 382	18 754
1999	546	11 504				4 138	272	4 410	16 460
2000	607	10 005				4 412	534	4 946	15 558
2001	1 024	7 583			16	4 749	355	5 104	13 727
2002	1 255	6 059			14	4 188	358	4 546	11 874
2003	1 696	7 053			25	4 526	252	4 778	13 552
2004	1 963	7 701	4 089		38	4 716	326	5 042	18 833
2005	2 570	8 339	4 171		24	5 959	304	6 263	21 367
2006	3 399	10 035	7 355		57	6 835	444	7 279	28 125
2007		8 885							
<u>Average</u>									
1996/05	1 112	10 063			12	4 289	425	4 714	16 727
<u>Average</u>									
2001/05	1 702	7 347			24	4 828	319	5 147	15 871

Table 32. Total seed production area of white clover in some EU countries, 1993-2007, ha

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	2 665		485	14	359	44	-	339	110	31
1994	3 088		838	6	210	93	-	437	198	40
1995	3 259		659	5	154	94	-	420	186	24
1996	2 479		590	18	85	141	-	511	176	39
1997	2 254		378	18	76	142	-	468	14	85
1998	3 158		467	62	95	180	-	205	36	106
1999	3 383		348	41	97	254	5	153	68	24
2000	3 516		283	226	116	253	13	407	38	32
2001	3 718		290	202	35	480	6	220	61	34
2002	3 406		206	95	83	374	2	135	150	22
2003	3 743		171	84	66	466	3	105	120	10
2004	3 853		124	101	63	571	3	114	155	2
2005	4 800		153	231	88	671	15	155	97	9
2006	5 541		141	229	62	840	17	257	138	22
2007	5 003					644				
<u>Average</u>										
1996/05	3 431		301	108	80	353	5	247	92	36
<u>Average</u>										
2001/05	3 904		189	143	67	512	6	146	117	15

Table 33. World production of grass and clover seed, 1993-2006, tons registered.

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	237710	16444	392	152349	14754	167103	421649
1994	266563	13885	980	153871	20289	174160	455588
1995	264088	15471	1507	174028	26767	200795	481861
1996	303183	21681	2102	169990	11275	181265	508231
1997	311984	22906	2056	182992	17670	200662	537609
1998	322639	23125	1680	218973	18591	237564	585009
1999	380103	23831	2056	214422	21116	235538	641528
2000	385246	25818	1449	189598	15846	205444	617957
2001	373273	19149	1539	193193	13645	206838	600799
2002	368727	12706	1045	158703	12543	171246	553724
2003	329006	25327	726	200637	11787	212424	567483
2004	392053	25128	1356	216037	17063	233100	651637
2005	361689	31480	1902	235415	18858	254273	649344
2006	393642			237074	33176	270250	
Average							
1996/05	352790	23115	1591	197996	15839	213835	591332
Average							
2001/05	364950	22758	1313	200797	14779	215576	604597

Table 34. Production of grass and clover seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	52 158	35 058	21 922	19 355	13 677	2 200	845	4 816	3 239	1 299
1994	67 269	24 250	24 429	16 361	11 441	3 759	1 627	4 615	4 441	1 240
1995	78 131	28 722	25 400	15 206	11 432	4 510	2 303	5 712	5 658	1 853
1996	64 371	30 539	24 415	18 696	12 426	3 751	2 721	4 798	3 575	1 979
1997	68 044	34 318	31 357	18 963	11 156	4 059	1 626	8 112	4 605	3 780
1998	87 592	39 382	28 448	27 970	12 818	3 071	1 556	8 006	5 471	4 082
1999	86 133	30 383	34 498	29 449	9 659	4 891	3 095	9 063	6 138	4 648
2000	87 330	29 248	21 713	25 493	7 787	4 490	2 814	6 562	4 235	4 115
2001	88 760	25 951	27 252	21 857	7 455	6 241	4 729	4 994	4 034	3 564
2002	65 228	23 654	20 915	20 993	6 433	6 882	4 134	4 036	3 594	3 629
2003	91 592	34 295	23 807	22 124	6 832	5 994	3 170	5 553	2 499	2 217
2004	89 275	36 384	31 960	28 182	6 419	5 271	1 698	9 767	3 953	1 961
2005	109 298	36 913	30 876	27 795	6 164	7 451	2 768	7 963	5 359	2 812
2006	110 668	36 810	29 041	28 151	7 131	7 301	3 844	16 416	6 019	3 720
Average										
1996/05	83 762	32 107	27 524	24 152	8 715	5 210	2 831	6 885	4 346	3 279
Average										
2001/05	88 831	31 439	26 962	24 190	6 661	6 368	3 300	6 463	3 888	2 837

Table 35. World production of perennial ryegrass seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	71 954	7 265		63 065	1 265	64 330	143 549
1994	82 634	6 294		54 127	2 460	56 587	145 515
1995	77 276	8 433		76 448	3 210	79 658	165 367
1996	88 540	12 076		83 049	2 481	85 530	186 146
1997	97 140	11 358		86 264	2 961	89 225	197 723
1998	106 982	12 680		101 785	2 658	104 443	224 105
1999	126 541	13 586		83 228	4 109	87 337	227 464
2000	120 169	15 804		72 919	2 835	75 754	211 727
2001	112 916	10 327		67 470	3 120	70 590	193 833
2002	95 992	5 766		58 844	2 807	61 651	163 409
2003	95 233	10 259		87 245	2 180	89 425	194 917
2004	116 670	11 674		96 133	3 220	99 353	227 697
2005	121 361	13 648	3	94 585	3 760	98 345	233 357
2006	109 485			103 018	6 437	109 455	
Average							
1996/05	108 154	11 718		83 152	3 013	86 165	206 038
Average							
2001/05	108 434	10 335		80 855	3 017	83 873	202 643

Table 36. Production of perennial ryegrass seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	22 457	18 968	4 927	5 090	10 609	57	7	500	764	
1994	24 674	9 049	7 596	3 207	8 774	295	30	513	1 908	
1995	34 731	17 640	7 912	4 836	9 189	334	45	756	2 230	202
1996	35 574	20 056	7 261	7 300	10 232	345	29	582	1 578	225
1997	36 140	22 771	8 376	6 973	9 044	342	27	475	1 985	440
1998	45 058	26 779	7 410	9 421	9 723	339	18	200	1 964	459
1999	38 352	16 056	10 405	9 328	6 555	528	56	429	2 991	620
2000	31 415	19 678	6 103	8 203	5 757	413	45	497	1 636	592
2001	30 731	15 930	7 471	6 955	5 982	526	44	563	1 635	824
2002	27 490	13 106	4 750	7 515	5 077	497	31	396	1 499	788
2003	41 495	22 416	6 881	9 334	5 320	577	35	401	962	633
2004	43 495	25 689	7 986	11 829	5 093	678	14	779	1 677	491
2005	42 423	26 910	7 500	10 486	4 822	982	43	375	2 441	375
2006	50 106	26 769	8 000	10 850	5 290	858	30	1 638	3 187	845
Average										
1996/05	37 217	20 939	7 414	8 734	6 761	523	34	470	1 837	545
Average										
2001/05	37 127	20 810	6 918	9 224	5 259	652	33	503	1 643	622

Table 37. World production of Italian ryegrass seed, 1993-2006, tons registered.

	USA	New Zealand	EU 15 total	EU 10 total	EU 25 total	World total
1993	81 055	2 984	32 113	4 175	36 288	120 327
1994	107 873	2 494	25 785	3 215	29 000	139 367
1995	105 314	1 199	29 515	4 773	34 288	140 801
1996	106 778	3 173	35 085	4 374	39 459	149 410
1997	100 304	4 037	45 926	7 891	53 817	158 158
1998	96 336	3 107	54 840	10 942	65 782	165 225
1999	120 474	3 117	49 326	8 454	57 780	181 371
2000	110 096	3 398	27 124	4 397	31 521	145 015
2001	95 201	2 366	28 512	2 958	31 470	129 037
2002	101 683	3 027	27 103	3 511	30 614	135 324
2003	86 369	6 765	33 354	3 919	37 273	130 407
2004	115 238	6 728	45 626	8 095	53 721	175 687
2005	83 165	9 269	40 064	8 697	48 761	141 195
2006	112 554		35 682	11 352	47 034	
Average						
1996/05	101 564	4 499	38 696	6 324	45 020	151 083
Average						
2001/05	96 331	5 631	34 932	5 436	40 368	142 330

Table 38. Production of Italian ryegrass seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	4 243	4 476	12 165	4 439	1 740			1 781	1 356	960
1994	4 158	3 996	9 484	2 689	1 459			1 258	827	1 047
1995	3 873	3 827	11 734	3 085	1 134			2 513	1 147	1 018
1996	2 593	4 291	11 900	4 683	1 234			2 323	817	1 031
1997	4 875	6 694	17 310	5 598	1 129			4 251	1 057	2 246
1998	6 431	7 525	16 829	8 010	1 702			6 140	1 764	2 724
1999	5 552	6 230	16 919	6 260	1 558			4 671	744	2 819
2000	3 864	2 788	8 529	3 016	646			1 682	430	2 142
2001	2 694	2 627	10 893	2 201	493			762	332	1 686
2002	1 909	3 241	9 667	2 920	409			1 093	331	1 709
2003	2 904	4 337	11 168	3 709	504			2 495	435	573
2004	3 098	3 900	18 357	5 689	283			5 769	1 044	811
2005	3 494	3 213	17 000	4 791	278			4 849	1 353	1 812
2006	3 340	2 230	13 500	4 808	367			6 765	1 561	937
Average 1996/05	3 741	4 485	13 857	4 688	824			3 403	831	1 755
Average 2001/05	2 820	3 464	13 417	3 862	393			2 994	699	1 318

Table 39. World production of hybrid ryegrass seed, 1993-2006, tons registered.

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993		629		2 546	33	2 579	3 208
1994		570		1 587	6	1 593	2 163
1995		967		1 888	45	1 933	2 900
1996		1 304		2 153	18	2 171	3 475
1997		1 635		3 067	133	3 200	4 835
1998		1 886		5 196	86	5 282	7 168
1999		2 144		6 253	207	6 460	8 604
2000		2 442		3 903	309	4 212	6 654
2001		1 924		2 254	125	2 379	4 303
2002		1 979		1 724	227	1 951	3 930
2003		3 128		2 344	89	2 433	5 561
2004		2 567		3 569	559	4 128	6 695
2005		3 542	14	4 548	668	5 216	8 772
2006				5 145	1 413	6 558	
Average 1996/05		2 255		3 501	242	3 743	6 000
Average 2001/05		2 628		2 888	334	3 221	5 852

Table 40. Production of hybrid ryegrass seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Czech	Poland	Hungary
1993	1 107	90	217	714	380	1	11	
1994	632	113	126	379	350	-	6	
1995	821	89	114	342	446	1	44	
1996	550	103	97	755	520	1	16	1
1997	1 096	112	216	941	586	24	6	103
1998	2 006	209	297	1 488	1 012	13	37	29
1999	2 014	241	377	2 150	1 019	48	50	75
2000	1 144	388	276	1 060	790	102	45	142
2001	466	103	197	672	692	9	44	73
2002	262	53	54	720	511	27	30	168
2003	705	22	111	812	532	45	22	17
2004	1 408	83	139	1 283	580	376	34	112
2005	1 716	196	300	1 563	620	272	61	228
2006	1 948	333	400	1 596	787	512	133	628
Average 1996/05	1 137	151	206	1 144	686	92	35	95
Average 2001/05	911	91	160	1 010	587	146	38	120

Table 41. World production of red fescue seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	6 971		17	27 514	711	28 225	35 213
1994	7 875		16	32 535	1 068	33 603	41 494
1995	5 357		5	26 064	1 582	27 646	33 008
1996	4 989		-	17 643	850	18 493	23 482
1997	5 049		21	19 028	1 410	20 438	25 508
1998	4 859		15	29 148	1 448	30 596	35 470
1999	5 930		20	35 807	1 894	37 701	43 651
2000	9 260		20	43 011	2 875	45 886	55 166
2001	8 295		20	46 825	3 111	49 936	58 251
2002	6 472		37	26 471	2 157	28 628	35 137
2003	4 776		25	32 185	1 427	33 612	38 413
2004	3 990		35	31 127	1 159	32 286	36 311
2005	5 296		37	48 089	1 499	49 588	54 921
2006	6 841			42 373	2 188	44 561	
Average 1996/05	5 892		23	32 933	1 783	34 716	40 631
Average 2001/05	5 766		31	36 939	1 871	38 810	44 606

Table 42. Production of red fescue seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	16 509	6 188	1 347	2 160	640	603	5	162	549	
1994	20 506	5 597	2 091	2 844	442	1 015	8	307	761	
1995	19 415	2 398	1 383	1 457	381	914	5	266	959	357
1996	13 298	1 723	775	1 208	194	388	3	135	329	386
1997	14 612	1 533	1 032	1 146	150	487	-	277	631	502
1998	23 149	2 115	865	2 033	178	657	2	197	766	485
1999	25 893	3 892	1 870	2 745	274	855	2	389	978	527
2000	33 007	2 959	1 677	3 801	310	907	-	821	1 469	585
2001	34 977	3 297	2 831	4 162	137	1 013	3	944	1 628	539
2002	17 259	2 246	1 817	3 170	201	1 467	1	459	1 107	591
2003	25 278	2 440	1 343	1 697	248	1 217	1	145	585	697
2004	23 921	2 377	1 359	2 080	282	966	-	134	652	339
2005	39 369	2 429	1 600	2 819	218	1 475	-	278	860	247
2006	32 750	2 970	1 700	2 973	298	1 427	-	1 266	129	651
Average										
1996/05	25 076	2 501	1 517	2 486	219	943	1	378	901	490
Average										
2001/05	28 161	2 558	1 790	2 786	217	1 228	1	392	966	483

Table 43. World production of meadow fescue seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993			217	3 148	823	3 971	4 188
1994			364	5 431	1 399	6 830	7 194
1995			354	5 850	1 200	7 050	7 404
1996			355	5 652	1 529	7 181	7 536
1997			309	4 518	1 218	5 736	6 045
1998			321	3 318	643	3 961	4 282
1999			518	4 421	939	5 360	5 878
2000			383	5 565	1 143	6 708	7 091
2001			443	6 073	1 272	7 345	7 788
2002			325	4 566	909	5 475	5 800
2003			249	4 462	1 063	5 525	5 774
2004			129	3 304	863	4 167	4 296
2005			354	5 269	912	6 181	6 535
2006				6 031	2 603	8 634	
Average 1996/05			338	4 715	1 049	5 764	6 102
Average 2001/05			300	4 735	1 004	5 739	6 038

Table 44. Production of meadow fescue seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	823	55	1 482	-	12	494	275	658	130	
1994	1 692	112	2 402	7	11	809	432	1 035	205	
1995	2 273	179	2 127	10	8	800	476	760	261	28
1996	1 624	88	2 685	99	9	775	400	1 053	338	48
1997	1 047	24	2 386	61	6	720	288	675	313	80
1998	902	12	1 426	82	-	581	355	287	206	54
1999	670	24	2 121	95	5	972	605	498	215	50
2000	1 427	29	2 498	45	6	1 125	544	860	104	99
2001	1 448	11	1 961	39	7	1 836	732	965	103	59
2002	846	12	1 658	30	4	1 548	467	660	119	15
2003	803	23	2 164	33	-	1 038	454	783	73	42
2004	404	13	1 659	33	-	860	318	636	87	22
2005	1 043	10	2 000	49	-	1 432	700	505	80	2
2006	962	17	2 700	50	-	1 514	758	1 690	201	36
Average 1996/05	1 021	25	2 056	57	4	1 089	486	692	164	47
Average 2005/01	909	14	1 888	37	2	1 343	534	710	92	28

Table 45. World production of sheep's fescue seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993				1 830	16	1 846	1 846
1994				2 367	19	2 386	2 386
1995	730			1 546	9	1 555	2 285
1996	477			745	37	782	1 259
1997	528			768	14	782	1 310
1998	399			1 179	15	1 194	1 593
1999	1 163			2 323	19	2 342	3 505
2000	4 364			3 090	38	3 128	7 492
2001	2 965			3 795	61	3 856	6 821
2002	1 655			2 528	134	2 662	4 317
2003	555			1 181	81	1 262	1 817
2004	631			1 781	40	1 821	2 452
2005	523		1	2 143	47	2 190	2 714
2006	914		5	2 264	175	2 439	
Average 1996/05	1 326			1 953	49	2 002	3 328
Average 2001/05	1 266			2 286	73	2 358	3 624

Table 46. Production of sheep's fescue seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	505	250	627	230	4			14	2	
1994	910	323	884	204	5			16	3	
1995	884	159	332	147	10			1	8	
1996	455	53	132	92	-			2	5	27
1997	343	70	235	91	8			3	9	2
1998	592	57	375	136	-			8	5	2
1999	1 078	176	781	212	-	4		13	4	2
2000	1 795	314	602	316	-	0		18	6	14
2001	1 665	197	1 421	401	1	4		55	6	-
2002	965	171	1 128	187	10	23		49	2	83
2003	690	194	106	136	10	16		25	4	52
2004	913	169	553	97	-	15		25	5	10
2005	1 086	162	700	109	-	51		28	2	17
2006	1 113	217	750	113	-	-		66	1	5
Average										
1996/05	958	156	603	178	3	11		23	5	21
Average										
2001/05	1 064	179	782	186	4	22		36	4	32

Table 47. World production of tall fescue seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	46 874	400		3 974	78	4 052	51 326
1994	33 476	47		2 917	58	2 975	36 498
1995	37 981	96		1 802	90	1 892	39 969
1996	56 347	95		2 661	59	2 720	59 162
1997	66 175	136		2 944	56	3 000	69 311
1998	68 707	249		4 959	43	5 002	73 958
1999	79 106	406		5 863	95	5 958	85 470
2000	87 621	415		7 325	155	7 480	95 516
2001	101 645	250		8 504	222	8 726	110 621
2002	114 515	578		9 361	251	9 612	124 705
2003	92 235	652		12 071	349	12 420	105 307
2004	101 517	598		11 083	360	11 443	113 558
2005	99 408	336		10 244	381	10 625	110 369
2006	108 312			11 318	1 185	12 503	
Average							
1996/05	86 728	372		7 502	197	7 699	94 798
Average							
2001/05	101 864	483		10 253	313	10 565	112 912

Table 48. Production of tall fescue seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	19	1 594	15	2 265	14			11	1	59
1994	52	587	1	2 107	18			7	2	41
1995	138	209	-	1 400	5			1	4	75
1996	208	501	-	1 874	11			1	1	34
1997	202	848	3	1 814	6		1	12	1	30
1998	690	1 119	9	2 990	2		1	3	6	27
1999	1 100	1 217	19	3 280	-		9	24	1	64
2000	2 088	1 454	89	3 450	4		15	37	1	104
2001	2 798	1 958	272	3 162	7		30	120	1	97
2002	2 622	2 838	216	3 323	1		17	122	14	115
2003	4 431	3 143	239	3 776	-	10	18	183	25	130
2004	3 530	2 839	192	4 069	-	35	42	212	37	110
2005	3 476	2 550	170	3 693	-	40	50	253	21	93
2006	4 062	2 993	180	3 800	-	-	51	633	92	406
Average 1996/05	2 115	1 847	121	3 143	3	9	18	97	11	80
Average 2001/05	3 371	2 666	218	3 605	2	17	31	178	20	109

Table 49. World production of festulolium seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993					102	102	102
1994				14	55	69	69
1995				137	30	167	167
1996				76	109	185	185
1997				100	136	236	236
1998				40	127	167	167
1999				107	417	524	524
2000				254	719	973	973
2001		3		258	616	874	877
2002		114		137	272	409	523
2003		565		97	278	375	940
2004		557		173	366	539	1 096
2005		582		643	629	1 272	1 854
2006				903	1 586	2 489	
Average 1996/05				189	367	555	738
Average 2001/05		364		262	432	694	1 058

Table 50. Production of festulolium seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993								102		
1994			14					55		
1995			137					30		
1996			76					97		
1997			100					99	1	
1998	7		33					112	3	
1999	37		70					346	11	
2000	200		53			0		647	20	
2001	212		40			5		503	20	
2002	82		42			12		231	11	
2003	87		10					242	8	
2004	145		10			18		356	1	
2005	293		30	300		20		442	58	
2006	696		80	127						
Average 1996/05	106		46					308	13	
Average 2001/05	164		26			11		355	20	

Table 51. World production of Kentucky bluegrass seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	19 456		6	7 963	147	8 110	27 572
1994	22 574		8	15 185	257	15 442	38 024
1995	22 315		2	15 571	273	15 844	38 161
1996	30 036		-	9 936	294	10 230	40 266
1997	26 623		8	8 202	283	8 485	35 116
1998	28 996		2	6 486	181	6 667	35 665
1999	30 270		8	9 601	302	9 903	40 181
2000	37 435		5	8 242	241	8 483	45 923
2001	37 063		8	10 643	215	10 858	47 929
2002	33 628		8	11 302	350	11 652	45 288
2003	37 094		10	10 465	219	10 684	47 788
2004	39 964		11	9 475	199	9 674	49 649
2005	38 486		22	12 904	257	13 161	51 669
2006	39 293			12 323	253	12 576	
Average							
1996/05	33 960		8	9 726	254	9 980	43 947
Average							
2001/05	37 247		12	10 958	248	11 206	48 464

Table 52. Production of Kentucky bluegrass seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Czech	Poland	Hungary
1993	4 247	3 344	171	4		201	21	126	
1994	10 056	4 425	312	12		392	80	177	
1995	10 590	4 115	358	8		434	66	197	8
1996	5 699	3 659	217	16		247	110	169	11
1997	5 520	2 210	231	5		186	88	179	11
1998	4 728	1 506	98	2		116	33	137	10
1999	6 607	2 464	225	1		219	73	207	15
2000	6 318	1 552	119	7		151	67	143	13
2001	8 212	1 747	177	6		215	47	142	8
2002	8 709	1 920	97	18		427	83	241	10
2003	8 228	1 649	114	38		409	120	55	16
2004	7 348	1 252	105	4		376	66	82	11
2005	10 509	1 383	150	1		483	52	148	9
2006	9 982	1 217	120			452	133	59	7
Average 1996/05	7 188	1 934	153	10	-	283	74	150	11
Average 2001/05	8 601	1 590	129	13	-	382	74	134	11

Table 53. World production of rough bluegrass seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993				456		456	456
1994				564		564	564
1995	1 214			477		477	1 691
1996	1 492			344		344	1 836
1997	1 972			265		265	2 237
1998	2 136			227		227	2 363
1999	2 136			267		267	2 403
2000	2 597			314		314	2 911
2001	1 507			342		345	1 852
2002	1 368			296		296	1 664
2003	810			235		235	1 045
2004	1 163			169		169	1 332
2005	1 044			258		258	1 302
2006	1 036			208		209	1 245
Average 1996/05	1 623			272		272	1 895
Average 2001/05	1 178			260		261	1 439

Table 55. World production of timothy seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993			141	2 051	5 319	7 370	7 511
1994			507	3 388	9 135	12 523	13 030
1995			1 102	4 800	12 808	17 608	18 710
1996			1 437	5 273	598	5 871	7 308
1997			1 367	4 328	608	4 936	6 303
1998			1 222	3 253	424	3 677	4 899
1999			1 389	5 519	794	6 313	7 702
2000			878	5 331	601	5 932	6 810
2001			933	7 823	807	8 630	9 563
2002			502	7 156	815	7 971	8 473
2003			321	6 597	745	7 342	7 663
2004			1 065	4 983	700	5 683	6 748
2005			1 365	5 634	871	6 505	7 870
2006				6 638	2 216	8 854	8 854
Average 1996/05			1 048	5 590	696	6 286	7 334
Average 2001/05			837	6 439	788	7 226	8 063

Table 56. Production of timothy seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	70	9	621	12	109	675	506	407	22	
1994	215	6	790	1	192	1 050	1 121	313	97	
1995	567	40	856	8	161	1 454	1 711	293	136	16
1996	393	26	973	5	98	1 543	2 232	245	134	14
1997	265	2	951	5	93	1 767	1 241	304	98	6
1998	142	3	769	3	71	1 131	1 135	142	64	6
1999	163	8	1 143	-	95	1 586	2 312	392	78	7
2000	367	19	1 370	-	94	1 314	2 151	287	45	18
2001	329	24	1 606	1	53	1 997	3 781	480	44	3
2002	401	12	1 127	2	60	2 004	3 540	401	87	6
2003	524	12	1 341	1	52	2 028	2 594	341	87	5
2004	319	1	1 302	2	38	1 964	1 320	387	87	-
2005	319	5	1 000	-	57	2 292	1 923	309	101	3
2006	199	7	1 100	-	48	2 273	2 961	1 086	168	3
Average 1996/05	322	11	1 158	2	71	1 763	2 223	329	83	7
Average 2001/05	378	11	1 275	1	52	2 057	2 632	384	81	4

Table 57. World production of cocksfoot seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	5 644	515	1	3 513	131	3 644	9 804
1994	7 254	396	3	5 273	123	5 396	13 049
1995	8 317	535	13	4 179	249	4 428	13 293
1996	8 914	818	45	3 882	52	3 934	13 711
1997	8 371	570	99	3 767	168	3 935	12 975
1998	7 459	248	92	4 397	185	4 582	12 381
1999	7 009	236	53	5 161	297	5 458	12 756
2000	6 476	174	24	6 604	196	6 800	13 474
2001	7 066	299	18	5 719	339	6 058	13 441
2002	7 514	233	14	5 189	307	5 496	13 257
2003	6 997	453	8	6 139	202	6 341	13 799
2004	7 237	321	1	5 351	214	5 565	13 124
2005	5 370	267	2	5 308	156	5 464	11 103
2006	6 613			5 045	539	5 584	
Average							
1996/05	7 241	362	35	5 152	212	5 363	13 002
Average							
2001/05	6 837	315	9	5 541	244	5 785	12 945

Table 58. Production of cocksfoot seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	813	9	67	2 232	136	15	1	34	94	
1994	1 687	3	84	3 305	144	42	3	33	78	
1995	1 996	18	100	1 709	72	67	6	61	178	2
1996	2 148	15	54	1 452	102	53	1	11	26	1
1997	2 198	17	94	1 155	107	46	0	54	79	11
1998	2 066	13	43	1 981	118	42	2	46	118	8
1999	2 352	11	37	2 397	139	55	1	107	167	15
2000	3 560	11	22	2 697	154	59	6	125	42	18
2001	2 898	6	38	2 599	56	59	4	252	41	26
2002	2 889	4	55	1 982	138	61	1	156	80	42
2003	4 403	-	32	1 255	151	67	0	109	53	19
2004	3 285	11	62	1 733	129	58	0	141	52	8
2005	2 832	5	60	2 087	149	43	-	50	40	2
2006	2 375	16	80	2 134	284	27	-	295	104	23
Average										
1996/05	2 863	9	50	1 934	124	54	2	105	70	15
Average										
2001/05	3 261	5	49	1 931	125	58	1	142	53	19

Table 59. World production of bentgrass seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	3 022	78	8	81	90	171	3 279
1994	2 391	78	12	56	54	110	2 591
1995	2 809	97	12	64	51	115	3 033
1996	2 924	83	22	33	43	76	3 105
1997	3 043	80	38	40	61	101	3 262
1998	2 667	109	19	61	54	115	2 910
1999	2 891	45	40	113	68	181	3 157
2000	3 024	106	16	150	82	232	3 378
2001	2 271	95	6	137	77	214	2 586
2002	2 146	20	10	131	92	223	2 399
2003	2 073	77	12	154	72	226	2 388
2004	1 919	42	9	102	58	160	2 130
2005	1 902	64	9	123	46	169	2 144
2006	2 205			115	103	218	
Average 1996/05	2 486	72	18	104	65	170	2 746
Average 2001/05	2 062	60	9	129	69	198	2 329

Table 60. Production of bentgrass seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary	
1993	7	72	2					37	15	38	
1994	18	38	2					15	9	30	
1995	18	42	3	1				10	17	23	
1996	7	22	-	4				17	13	13	
1997	1	37	-	1				33	15	13	
1998	17	44	-	-				29	15	8	
1999	49	64	-	-				28	26	14	
2000	87	56	-	7				60	7	14	
2001	77	51	2	7				50	6	21	
2002	80	51	1	8				66	9	17	
2003	89	59	1	5				60	4	8	
2004	48	50	1	2				53	1	3	
2005	70	50	1	-	1			34	9	-	
2006	70	41	1	-	1			89	8	4	
Average											
1996/05	53	48		3			-	-	43	11	11
Average											
2001/05	73	52	1	4			-	-	53	6	10

Table 61. World production of red clover seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993	2 734	113	2	2 663	1 813	4 476	7 325
1994	2 486	176	70	2 339	2 318	4 657	7 389
1995	2 571	182	19	3 543	2 384	5 927	8 699
1996	2 495	143	243	2 001	818	2 819	5 700
1997	2 579	232	215	2 365	2 703	5 068	8 094
1998	3 847	144	9	2 635	1 765	4 400	8 400
1999	4 338	187	28	4 543	3 483	8 026	12 579
2000	3 932	232	123	4 224	2 210	6 434	10 721
2001	3 772	204	109	2 776	679	3 455	7 540
2002	3 192	133	147	2 380	689	3 069	6 541
2003	2 486	198	97	2 274	1 108	3 382	6 163
2004	2 696	89	98	1 966	1 190	3 156	6 039
2005	3 406	142	92	2 974	908	3 882	7 522
2006	3 921			2 773	2 957	5 730	
Average 1996/05	3 274	170	116	2 814	1 555	4 369	7 930
Average 2001/05	3 110	153	108	2 474	915	3 389	6 761

Table 62. Production of red clover seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Estonia	Latvia	Lithuania	Poland	Hungary
1993	38	3	270	2 204	4	150	51	1 066	34	87	161	169	242
1994	170	1	361	1 605	13	113	28	901	38	251	414	363	122
1995	347	6	294	2 201	5	430	60	929	29	299	486	475	118
1996	200	2	148	1 200	5	295	48	215	8	149	45	149	187
1997	297	-	304	1 158	4	426	53	1 799	13	76	58	231	331
1998	227	-	251	1 804	3	164	19	791	11	111	71	382	266
1999	365	-	411	2 963	6	545	100	2 031	16	207	62	665	434
2000	355	-	353	2 845	13	414	53	1 336	2	127	28	282	370
2001	86	-	286	1 610	24	438	125	235	22	127	28	28	209
2002	107	-	264	1 065	13	714	68	286	15	194	30	63	74
2003	124	-	246	1 287	9	476	62	593	6	226	3	184	16
2004	79	-	220	1 333	8	266	4	819	1	51	50	194	34
2005	119	-	350	1 840	12	370	52	513	63	78	169	185	23
2006	99	-	400	1 620	25	412	44	2 156	44	75	139	370	88
Average 1996/05	196		283	1 711	10	411	58	862	16	135	54	236	194
Average 2001/05	103		273	1 427	13	453	62	489	21	135	56	131	71

Table 63. World production of white clover seed, 1993-2006, tons registered

	USA	New Zealand	Norway	EU 15 total	EU 10 total	EU 25 total	World total
1993		4 460		1 432	51	1 483	5 943
1994		3 830		2 303	123	2 426	6 256
1995	204	3 962		2 144	62	2 206	6 372
1996	191	3 989		1 457	13	1 470	5 650
1997	200	4 858		1 410	28	1 438	6 496
1998	251	4 702		1 449	21	1 470	6 423
1999	245	4 110		1 890	38	1 928	6 283
2000	272	3 247		1 542	45	1 587	5 106
2001	572	3 681	4	2 062	40	2 102	6 359
2002	562	856	2	1 515	22	1 537	2 957
2003	378	3 230	5	1 834	55	1 889	5 502
2004	1 028	2 552	9	1 195	40	1 235	4 824
2005	1 728	3 630	5	2 629	27	2 656	8 019
2006	2 468			3 238	168	3 406	
Average							
1996/05	543	3 486		1 698	33	1 731	5 762
Average							
2001/05	854	2 790	5	1 847	37	1 884	5 532

Table 64. Production of white clover seed in some EU-countries, 1993-2006, tons registered

	Denmark	Holland	Germany	France	UK	Sweden	Finland	Czech	Poland	Hungary
1993	864		11	5	29	5		22	0	
1994	1 935		282	1	33	43		83	5	
1995	2 001		50	2	21	61		24	2	6
1996	1 278		97	8	21	52		7	0	1
1997	1 183		119	15	23	65		18	0	5
1998	1 350		43	20	9	24		6	4	4
1999	1 634		120	18	8	105		14	1	6
2000	1 389		22	46	13	72		23	5	4
2001	1 825		57	42	3	115		6	4	20
2002	1 311		39	53	9	99		7	1	10
2003	1 596		51	41	6	150		11	2	9
2004	1 113		15	28	6	32		14	-	10
2005	2 291		15	57	7	258		3	-	1
2006	2 758		30	80	31	338		87	6	87
Average										
1996/05	1 497		58	33	11	97	-	11	2	7
Average										
2001/05	1 627		35	44	6	131	-	8	1	10