



**NIBIO**  
NORWEGIAN INSTITUTE OF  
BIOECONOMY RESEARCH



NIBIO BOOK | VOL. 3 NR 6 2017

# Proceedings from The 12<sup>th</sup> International Christmas Tree Research and Extension Conference

Honne, Norway 6<sup>th</sup> – 11<sup>th</sup> September 2015

Venche Talgø & Inger Sundheim Fløistad (eds.)



Scientific editors: Venche Talgø & Inger Sundheim Fløistad

The publisher of NIBIO BOOK is:  
NIBIO, P.O.Box 115, N-1431 Ås  
Norway

Editor-in-Chief: Per Stålnacke  
Associate editor: Marianne Stenrød  
Cover photo: Venche Talgø

ISBN: 978-82-17-01907-7  
ISSN: 2464-1189

Production: [www.xide.no](http://www.xide.no)

**Proceedings from The 12<sup>th</sup> International  
Christmas Tree Research and Extension  
Conference**

**Honne, Norway 6<sup>th</sup> – 11<sup>th</sup> September 2015**

# Foreword

On September 6th – 11th in 2015, the Norwegian Institute of Bioeconomy Research (NIBIO) organized The 12th International Christmas Tree Research and Extension Conference (CTREC) at Honne, Norway.

Around 40 participants from Australia, Austria, Canada, Denmark, France, Greece, Hungary, Iceland, Norway, UK, and USA gathered to share skills and recent research related to Christmas tree production and marketing.

Nearly 50 presentations (oral and poster) were given during the conference covering the following topics; Breeding & genetic, Insects, Tree health, Physiology, Growth conditions & integrated pest management, Postharvest, and Market & economy. Abstracts, extended abstracts or papers from all presentations are available in this proceedings. In addition, the following ten research articles and a short communication were published in a special issue of Scandinavian Journal of Forest Research after the conference (Special Issue: Christmas Tree, VOL. 32, NO.5, 2017):

- Landgren, C., Nielsen, U.B. & Chastagner, G. 2017. Comparison of noble fir progeny from US Pacific Northwest collection regions and Denmark for Christmas tree traits. *Scandinavian Journal of Forest Research* 32(5):366–375.
- Fløistad, I.S., Nyeggen, H. & Skage, J.-O. 2017. Field trial with *Abies lasiocarpa* progenies from plus trees and seed orchard clones for Christmas tree production in Norway. *Scandinavian Journal of Forest Research* 32(5):376–383.
- Crain, B.A. & Cregg, B.M. 2017. Using irrigation and mulch to control cone production in Fraser fir (*Abies fraseri*). *Scandinavian Journal of Forest Research* 32(5):384–390.
- Crain, B.A. & Cregg, B.M. 2017. Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research* 32(5):391–396.
- Bucholz, E., Frampton, J., Jetton, R., Tilotta, D. & Lucia, L. 2017. Effect of different headspace

concentrations of bornyl acetate on fecundity of green peach aphid and balsam woolly adelgid. *Scandinavian Journal of Forest Research* 32(5):397–405.

- Kohlway, W.H., Whetten, R.W., Benson, D.M. & Frampton, J. 2017. Response of Turkish and Trojan Fir to *Phytophthora cinnamomi* and *P. cryptogea*. *Scandinavian Journal of Forest Research* 32(5):406–411.
- Pettersson, J.M., Frampton, J., Rönnberg, J., Shew, D., Benson, D.M., Kohlway, W.H., Escanferla, M.E. & Cubeta, M.A. 2017. Increased diversity of *Phytophthora* species in Fraser fir Christmas tree plantations in the Southern Appalachians. *Scandinavian Journal of Forest Research* 32(5):412–420.
- Nielsen, U.B., Xu, J., Nielsen, K.N., Talgø, V., Hansen, O.K. & Thomsen, I.M. 2017. Species variation in susceptibility to the fungus *Neonectria neomacrospora* in the genus *Abies*. *Scandinavian Journal of Forest Research* 32(5):421–431.
- Chastagner, G.A., Riley, K., Coats, K.P., Eikemo, H. & Talgø, V. 2017. Delphinella shoot blight and Grovesiella canker on *Abies lasiocarpa* in western USA. *Scandinavian Journal of Forest Research* 32(5):432–437.
- MacDonald, M.T., Lada, R.R. & Veitch, R.S. 2017. Seasonal changes in balsam fir needle abscission patterns and links to environmental factors. *Scandinavian Journal of Forest Research* 32(5):438–445.
- Pappaspryopoulos, K. & Grigoriadis, N. 2017. Introducing CHO.C.O. (Choose, Cut, Offset) farms in the Christmas tree market: a first year implementation results. *Scandinavian Journal of Forest Research* 32(5):446–449.

Several field trips, organized by The Norwegian Christmas Tree Grower Association (Norsk Juletre), were also included in the conference program.

We want to thank a number of people for valuable support before and/or during the conference:

- Nils Vagstad, Arne Hermansen, Kari Margrethe Munthe, and Erling Fløistad at NIBIO
- Gary A. Chastagner, the coordinator of our IUFRO group (unit 2.02.09 - Christmas trees)
- Knut Huse at The Forestry Extension Institute at Honne for guiding us through the arboretum
- John-Anders Strande and Gorden Håland from The Norwegian Christmas Tree Grower Association for organizing field tours
- Eleonora Høst for guiding us through the forest nursery at Biri (Skogplanter østnorge)

- Knut Helset and Karl Henrik Hals, Christmas tree growers in the regions of Hamar and Hokksund, respectively, for letting us visit their farms
- Ragnar Johnskås at The Norwegian Forest Seed Center (Skogfrøverket), Hamar, for guiding us through seed orchards and provenance trials
- Heidi R. Bye and Jan Ulitzsch at Skogfrøverket for welcoming us to an orientation about their production

**Venche Talgø and Inger Sundheim Fløistad**  
Organizing committee



Photo: E. Fløistad

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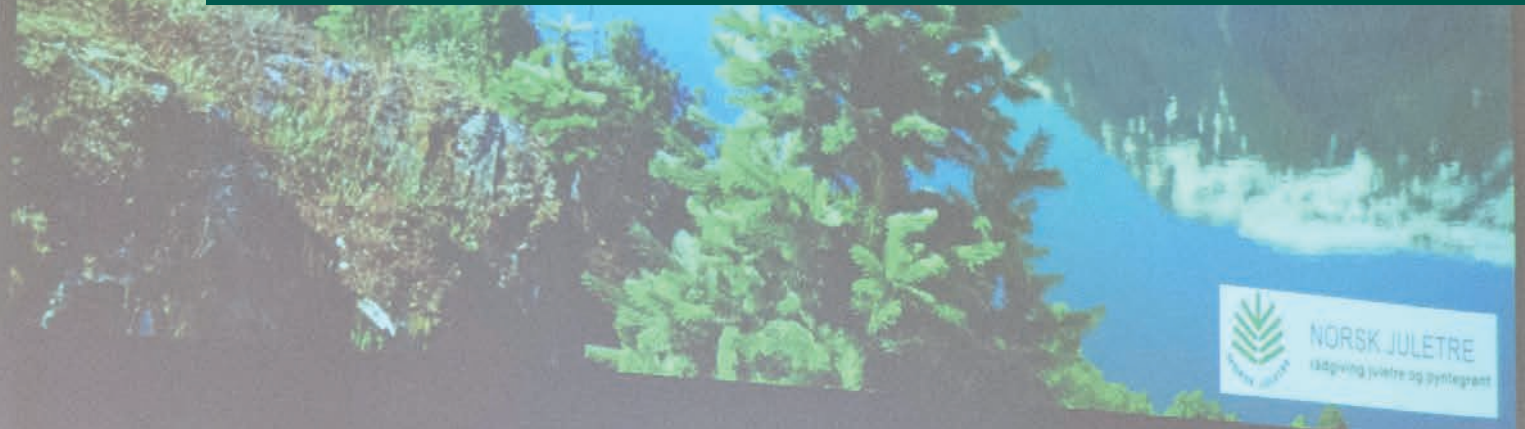
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## ORAL PRESENTATIONS



## OPENING SESSION

# Producing Christmas trees in “The land of the midnight sun”

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The Norwegian Christmas tree grower association (Norsk Juletre) has 470 members and 7 local associations. The association is a member of CTGCE (Christmas Tree Grower Council Europe).

Christmas tree production is a relatively new agricultural concept in Norway. After the tradition of Christmas trees was introduced in Norway around 1825 and until the nineteen eighties, trees were traditionally taken from the forests as a thinning early in the rotation. Although this still goes on today, fewer seem to find the time for such activities.

Christmas tree production became popular towards the end of the nineteen nineties, when less suitable land for agriculture was planted with Christmas trees. Many growers who planted during this period were under the impression that the job was done when the plants were in the ground. Thus, many of these plantations, which lacked the demanded quality, were sold off on export after the millennium and reclaimed for pastures or other activities. Some of them are today dense forests. This resulted in skepticism from agricultural authorities towards accepting planting of Christmas trees on agricultural land, and even skepticism from landowners towards commitment to produce Christmas trees.

The first professional producers orientated themselves towards Denmark to gain knowledge. Therefore, Nordmann fir (*Abies nordmanniana*) became a popular tree, especially in the south-west where the climate allowed this production. Later, subalpine fir (*A. lasiocarpa*) became popular on the market and now constitutes about 50% of the fir

production. Although the south-west is still the most important area for fir Christmas tree production in Norway, subalpine fir also allows production of fir in areas with harsher climate.

Annual consumption and import of natural trees in Norway are 1.900.000 and 325.000 trees (+ unregistered import), respectively. Number of trees taken from conventional forestry every year are 400.000, and the annual professional production is 1.100.000 trees. The annual national market of natural trees consists of 60% fir (hereof 50% Nordmann fir and 50% subalpine fir) and 40% spruce, mainly Norway spruce (*Picea abies*). Totally, 20% of the consumers say they use an artificial Christmas tree (numbers from 2012 and 2013). Prices from 2014 were on average NOK 450 (EUR 54, USD 60) for spruce and NOK 600 (EUR 72, USD 80) for fir.

## BREEDING AND GENETIC

# Environmental control of cone production in Fraser fir (*Abies fraseri*) Christmas trees

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Due to its beautiful form, strong branches, and exceptional needle-retention, Fraser fir is rapidly gaining in popularity as a Christmas tree in Michigan and elsewhere in the United States. Michigan plantation-grown Fraser fir trees often produce heavy cone crops at a much younger age than those in natural stands. Cones must be removed by hand at considerable expense, and cone production alters the tree structure, decreasing the value as a Christmas tree.

In true firs, cone buds differentiate during the summer, but do not open until the following spring. Thus, environmental conditions during summer bud differentiation may regulate cone production for the following year. Fraser fir is endemic to a small region of cool temperate rain forest in the southeastern Appalachians. Annual precipitation is high—twice that of central Michigan—and mean summer temperatures are below 16°C, which is much cooler than Michigan. It seems likely that these climatic differences play a significant role in heavy coning in Michigan. This is consistent with what is known about conifer reproduction in general, much of which comes from research to promote cone production in seed orchards: Tree age, size, hormonal interactions, water and nutrient availability, and temperature are key drivers of cone development (Owens & Blake 1985; Owens 1995). In addition, we have observed dramatic variation in coning within individual fields of singular seed source and planting date (Crain *et al.* 2012). This suggests that highly localized environmental signaling regulates cone development.

In 2011, we established multiyear observational studies to understand the environmental factors regulating early cone development. Using multiple regression on data collected from 10 farms over 4 years, we are developing models to predict cone crop yield based on weather conditions during the preceding summer. This may provide advance warning to growers, giving them time to procure extra workers to remove cones in particularly heavy coning years.

In 2013, we established designed experiments at multiple locations to examine the effects of environmental variables (water, temperature, nutrition) on cone production. In our mulching and irrigation studies, results suggest that drought and heat stress increase coning, but that neither irrigation nor mulching sufficiently reduce stress to control coning.

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# Comparing noble fir progeny from collection regions in the Pacific northwest and Denmark

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Noble fir (*Abies procera* Rehd.) is the leading Christmas tree produced in the states of Oregon and Washington (USA) with yearly plantings of over 4 million trees. In Denmark, noble fir is primarily utilized for greenery, though Christmas tree production is expanding. Progeny and provenance testing utilizing commercial plantations has been ongoing since mid-1970's. Testing since 1996 has included 215 families (primarily 1/2 sibling families) from across the natural range of noble fir and includes families from Danish imported seed. Traits investigated for Christmas trees include height, grade, color and incidence of Current Season Needle Necrosis (CSNN). The 6 regions that are compared and the number of observed progeny are:

- Oregon Cascades (936 observed trees) – From Mt. Hood in the north to the McKenzie River in the south, noble is fairly continuous in distribution beginning at elevations above 3 500 feet.
- Oregon Coast (13 178 observed trees) – The distribution is scattered on isolated peaks above 2 000 feet.
- S. Oregon Cascades (1 412 observed trees) – This is where noble mixes with Shasta fir (*A. magnifica* var. *shastensis*) in the area of the McKenzie River at elevations above 3 000 feet.
- Washington Cascades (1 695 observed trees) – The distribution begins at Larch Mountain in the south and extends to Stevens Pass where the natural distribution ends.

- Willapa Hills, Washington (564 observed trees) – Many suggest this area is an extension of the noble fir distribution of the Oregon Coast. Noble fir is found only on the upper elevations of a few mountain peaks, notably BawFaw/ Boistfort.
- Danish Collections (1 199 observed trees) – All of the Danish collections originated from the Pacific Northwest (PNW). Likely, collection sites are in on Mt. Hood and perhaps in the Washington Cascades out of Fort Vancouver. Selection and breeding have focused on traits for greenery over many years.

Evaluations suggest the 1/2 sibling sources from the coastal mountains in Oregon, consistently are among the top for Oregon and Washington producers based on tree value. Selections from the Oregon and Washington Cascade mountains consistently rank lower for value. Sources from the southern limit of noble fir in the Cascades, are consistently slower growing with an open growth habit and share traits with Shasta fir. The tested Danish sources share many traits with the Cascade mountain sources with consistently high evaluations for superior color and low CSNN incidence.

# The use of plant growth regulators for coning and height control in *Abies* and *Picea*

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Early efforts to increase cone production in seed orchards relied on cultural treatments, such as girdling, root pruning, fertilization, and induced drought (Puritch 1972). Results were highly variable, and occasionally detrimental. Subsequent work with plant growth regulators (PGRs) — particularly exogenous application of gibberellins (GAs) — resulted in greatly enhanced cone production, especially when combined with cultural treatments (Puritch 1979). Little research has been directed toward the use of PGRs to regulate cone production in *Abies*, but trunk-injection of GA<sub>4/7</sub> combined with girdling and tenting does increase cone production in Pacific silver fir (*Abies amabilis*) (Owens *et al.* 2001).

In Michigan plantations, Fraser fir (*A. fraseri*) Christmas trees frequently produce heavy cone crops, which are expensive to remove and may reduce the value of the tree. Since GAs are often used to enhance coning in conifer seed orchards, it seems reasonable that GA-inhibitors may reduce cone production. GAs are also involved in stem elongation, so GA-inhibitors should reduce vegetative growth and may decrease the need for shearing. In 2013, we established four-year studies at four locations to evaluate the use of PGR treatments for coning and height control in Fraser fir. Twenty trees were randomly assigned to one of five treatments: 1) water control; 2) GA<sub>4/7</sub> (positive control); 3) Cycocel (chlormequat); 4) Trimtect (paclobutrazol); 5) Cambistat (paclobutrazol). Treatments 3–5 are GA-biosynthesis inhibitors. Treatment 5 was applied one time in early spring by soil injection. All other treatments were applied 3 times at 10 day intervals by foliar drench, during the period of cone bud

initiation and differentiation. In the first year, GA inhibitors reduced cone production by 15–70%, and GA doubled cone production. However, results were not consistent across sites.

We also established studies at multiple locations to evaluate the use of PGRs in height control in blue spruce (*Picea pungens*) and Norway spruce (*P. abies*). In spring 2013, 20 randomly selected trees were treated once with Cambistat by soil injection, and 20 trees were selected as untreated controls. Trees were scored each fall for bud density and height and lateral growth. Height control was highly significant in 2013 and 2014. In 2014, results from one site in central Michigan were typical, with average leader growth of 24.3 cm for treated small blue spruce, compared with 36.7 cm for the control – a difference of 40.5%.

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# Field trial with *Abies lasiocarpa* progenies for Christmas tree production in Norway

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*Abies lasiocarpa* (Hook.) Nutt is together with *A. nordmanniana* (Steven) Spach the most common exotic species for Christmas tree production in Norway. While *A. nordmanniana* is used mostly in coastal areas, *A. lasiocarpa* can also be grown in the interior part of the country where the climate generally is too harsh for growing *A. nordmanniana*.

The wide natural range of *A. lasiocarpa* in western North America, from Alaska and Yukon in the north to Arizona and New Mexico in the south, represents great differences among populations in growth, morphology, and phenology. Provenance field trials in Norway for Christmas tree production have previously been performed by Hansen *et al.* (2004) and Skage *et al.* (2012). However, progenies of *A. lasiocarpa* from seed orchards and progenies of seeds collected from plus trees have never been compared in field trials in Norway. Two experimental plots were established in the southern part of Norway for testing seed sources with potentially high Christmas tree value.

The experiment included progenies from 17 selected plus trees with superior morphological traits from 11 populations in the USA and Canada (Stavrum & Johnskås 1995, Stavrum & Gislerud 1996), and progenies from 6 seed orchard clones in Kaupanger in the western part of Norway. One experimental site was established in the spring of 2000, at Luster municipality, Sogn og Fjordane County in western Norway. The other site was established in the spring of 2001 at Stange municipality, Hedmark County in eastern Norway.

The average survival percentage was 71% in Luster and 74% in Stange, with the highest mortality during the first three years. Due to early bud break in *A. lasiocarpa* selection for late bud break, and planting sites with minimal risks for spring frost is important.

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# Genomic technologies and applied tree breeding

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The past ten years have seen phenomenal progress in the development of tools for detecting and analyzing genetic and biochemical variation. These tools have generally been developed and first applied in biomedical research, and have then spread to other fields as diverse as agriculture, ecology, population biology, and forestry. These tools are sometimes referred to as genomic technologies, because they allow analysis of many, if not all, genes or gene products in an organism in parallel. Such methods have been applied in research projects on forest trees over the past decade, and the question naturally arises of when they might find application in practical breeding programs working with Christmas tree species. This presentation will provide an overview of genomic technologies, including high-

throughput methods for discovery and analysis of genetic variation as well as methods for detection of regulatory interactions among genes or between genes and environmental signals. Some examples of research projects underway using these methods will be described, and future prospects for integration of these methods into applied breeding programs will be discussed. A key question is how to decide when these technologies are ready to move into application in practical breeding programs, and a reasonable approach is to prioritize the opportunities based on the probability of return on investment and the opportunity cost of failing to apply tools as they become available.

## INSECTS

# Risk of *Adelges (Dreyfusia) nordmannianae* surviving on cut Nordmann fir Christmas trees and boughs

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In Europe, where Nordmann fir [*Abies nordmanniana* (Steven) Spach] is widely grown for Christmas trees and boughs, the silver fir woolly adelgid [*Adelges (Dreyfusia) nordmannianae*] is a serious pest on this host. Although not common, this pest has been observed on Nordmann fir trees at several locations in western Washington. During the past few years, data has been collected on its rate of spread and life cycle in plantings at Puyallup. Information about host susceptibility and the effectiveness of insecticide treatments in controlling this pest have also been collected.

In an effort to determine the risk that adelgids could be spread from one location to another via the movement of infested cut Christmas trees or boughs, experiments were done in 2013 and 2014 to examine the potential for adelgids to survive on harvested boughs. Branches from five heavily-infested Nordmann fir trees were utilized during this test. Three sets of branches, consisting of a single branch from each tree, were harvested in December/January. One set was stored in ventilated plastic crates outdoors. The remaining two sets were displayed indoors at 20°C for about 5 weeks. One set of the displayed branches was displayed with their bases in water and the other set was displayed dry. Following the indoor display period, both sets of the displayed branches were placed in ventilated plastic crates and stored outdoors with the other branches. Checks consisted of branches that were tagged, but not harvested from the tree. The effect of these different display and storage conditions on adelgid

survival was determined by periodically examining the branches to determine the viability and life stages of the adelgids through early April.

There was no evidence of mortality of the overwintering adelgids on the unharvested branches on the trees. They started laying eggs in March and crawlers were evident by early April, which was about 3 weeks prior to bud break. In 2013, the adelgids on the harvested branches that were displayed indoors in water laid eggs which hatched, producing crawlers during the indoor display period. By the end of the display period, there was no evidence of live stem mother adelgids, eggs or crawlers on any of the branches that were displayed dry. No eggs were ever found on the branches that were originally cut and stored outdoors. By mid-March to early April, there were no surviving adelgids on any of the harvested branches, suggesting that there is virtually no risk of spreading the silver fir woolly adelgid from one area to another via cut trees or boughs.



# Effect of bornyl acetate on reproduction of the green peach aphid and balsam woolly adelgid

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Fraser fir [*Abies fraseri* (Pursch) Poir.] Christmas trees are an important crop in the Southern Appalachian region of the United States with an annual revenue exceeding \$US 100 million in North Carolina alone. Although most growers in the region utilize Integrated Pest Management (IPM) practices, the exotic balsam woolly adelgid (BWA, *Adelges piceae* Ratzeburg) forces growers to employ expensive insecticidal treatments at a cost of over \$US 1.5 million annually to maintain the marketability of their crop. An understanding of the chemical basis for BWA resistance is therefore essential to accelerate development and deployment of resistant planting stock and mitigate the impact of this destructive pest.

Although fir species are known to vary in their resistance to BWA, there is little evidence to suggest why some species such as Fraser fir are very susceptible, while others such as Veitch fir (*A. veitchii* Lindl.) from Japan are highly resistant. Our profile comparisons of extractives (acetone-soluble) from the stem of Veitch and Fraser fir tissue via gas chromatography coupled with mass spectroscopy (GS-MS) have consistently shown markedly higher amounts of bornyl acetate (BA) in Veitch fir. As a first step toward evaluating the effect, if any, of BA on the life cycle of BWA, the objective of this study is to determine the effect of various BA concentrations in the headspace above egg masses and adults on egg eclosion in BWA and vivipary (live birth) of a surrogate, the green peach aphid (*Myzus persicae* Sulzer).

We have developed a very simple protocol to vary the volatile BA concentration in the headspace of vessels that consists of diluting BA in silicone oil. Concentrations are measured by solid phase micro-extraction (SPME) fiber collection followed by GC-MS injection and analysis. In separate trials, adults of green peach aphid or eggs of BWA are placed into vessels with 5 different concentrations of BA (bracketing those measured for Veitch and Fraser fir) in addition to control vessels containing only silicone oil or water. After a week, samples are frozen and eggs and insects examined microscopically. The effect of BA on reproduction was presented.

# Evaluating beneficial insects for aphid control in Christmas trees

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For many Christmas tree growers in Western Oregon, the 2013 growing season exhibited some of the worst aphid infestations and consequently the worst damage to crops seen during the last decade. In an effort to assist growers with alternatives to insecticide use, an observational study of aphid control in the Willamette Valley was conducted during the summer of 2014. Our goal was to determine if we could limit aphid damage in Christmas trees by releasing and/or attracting beneficial insects. Three natural enemies of aphids for field release and one attractant were used in the study. The treatments investigated were: *Aphidoletes aphidimyza* (predatory midge), *Aphidius matricariae* (small parasitic wasp), *Chrysoperla rufilabris* (green lacewing), and Methyl salicylate (Predalure™).

Natural enemies were released at eight Christmas tree sites in 3 stages early May, mid-May and finally in early June. Evaluation sites varied from 14 acres in size and were planted with noble and grand fir species. Evaluations began in early June and ended by midAugust with examinations at each site on 2 week intervals. Evaluations ranked live aphid presence on trunk and needles, presence of aphid mummies and visual aphid damage. Beneficial insects were observed at all eight sites throughout the study. The three most common aphid predators identified at all sites were adult ladybugs, hoverflies and green lacewings, respectively. Our observational trial highlighted the inherent challenges of releasing beneficial insects into an open field environment. Evaluating impacts of released aphid predators proved to be difficult.

Although many aphid predators were identified, it is uncertain whether those insects were naturally occurring or a result of our releases. Aphid presence was low across the region in 2014, yet on three study sites aphid population increase was followed by an increase in predator counts.

# Review of new insecticides examined in Denmark for control of silver fir woolly aphid (*Dreyfusia nordmanniana*) in Christmas trees

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## Introduction

In Denmark, as well as most other EU-countries (European Union), there is increased concern about the environmental effect caused by use of pesticides. Especially insecticides that are very harmful for water living organisms make politicians and others concerned. Since 2012, the traditionally used insecticides for Christmas trees, synthetic pyrethroids, were more or less banned in Denmark for use in Christmas tree production.

At present, only a reduced dose of lambda-cyhalothrin (trade name Karate) is allowed in Christmas trees, but the dose is too low to give certainty for sufficient aphid control. Finally, a dramatic increase in taxing was put on pyrethroids, so pine weevil control in forestry and other insect control in agriculture are now very costly. Therefore, a lot of new insecticides have been tested in Denmark during the last years.

One of them has now been registered via a minor use registration for use against various aphid species (Fig. 1 and 2). Some of the new insecticides have been tested with addition of additives, but this did not give better effect on aphids, while it significantly increased the risk of discoloration of the needles when applied after bud break. Among newly tested insecticides, only acetamiprid (trade name Mospilan) has thus far been registered for use in Christmas trees in Denmark.

## Acetamiprid

Acetamiprid (trade name Mospilan) is one of several neonicotinoides, which has, as most other newly tested insecticides, a very good effect on aphids (Fig. 3), just like the traditionally used pyrethroids.

Acetamiprid has been reported to be systemic in some agricultural crops, but this effect could not



Figure 1. Silver fir woolly aphid (*Dreyfusia nordmanniana*) totally covering the underside of Nordmann fir (*Abies nordmanniana*) needles (left). The aphids may destroy the trees and make the needles curl and turn yellow (right).



Figure 2. Severely damaged small Nordmann fir (*Abies nordmanniana*) with yellow and curled needled due to aphids.

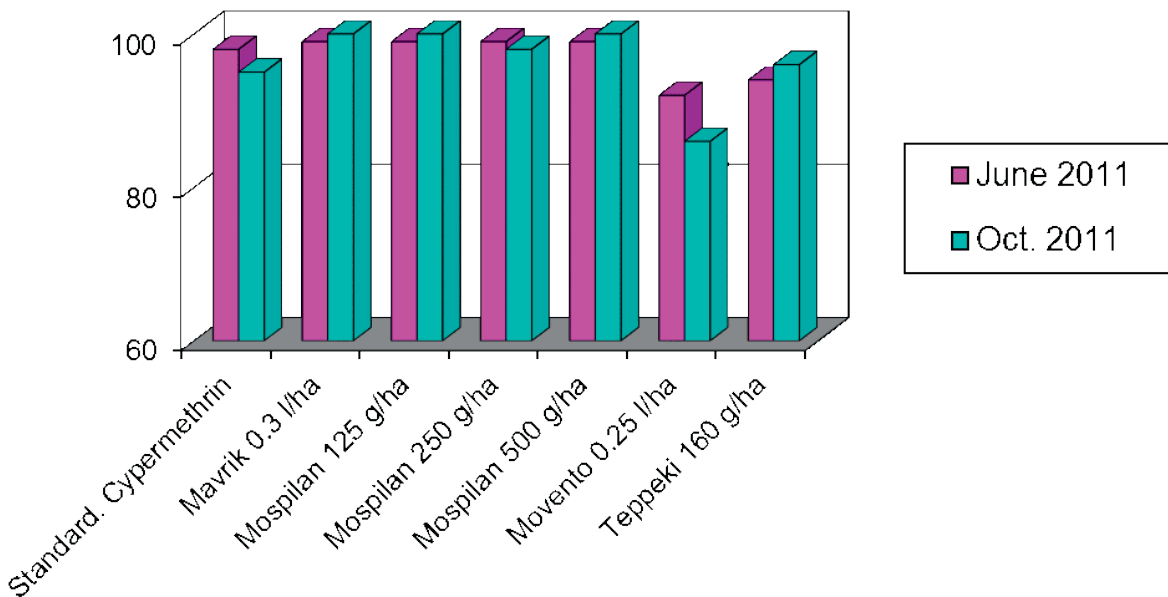


Figure 3. Aphid control (% efficiency) with some newly tested insecticides 2011.

be observed in coniferous tree species. Acetamiprid has not caused discoloration of Nordmann fir (*Abies nordmanniana*), even if the buds had broken at time of applicaion.

It seems to be a never ending work to test and have registered new insecticides and other pesticides ready for replacing pesticides banned by the authorities.

# Insects causing plant protection problems in Christmas tree plantations in Hungary

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## Introduction

Hungary is a kind of frontier concerning cultivation of Christmas trees. Only a small part of the country is suitable for growing Christmas trees, which is where the precipitation and the humidity are the highest in the country.

The ratio of the different species cultivated in Christmas tree plantations changed at the beginning of the 2000s, and at the same time the most important harmful insect species were discovered.

Integrated plant protection plays a dominant role in effective and economical Christmas tree cultivation. The susceptibility of Christmas trees, the presence of some invasive species, furthermore the spread and propagation of these species due to the climate change, may cause serious problems in plantations. The timing of the chemical treatments is difficult. This production can be characterised by a lack of lures and trapping methods.

We investigated the following: 1) What kind of insect species dominate different Christmas tree species? 2) What is the ratio of the invader and native species on Christmas trees? 3) How do the different host plants and the cultivation methods influence the infestation of the insects?

## Material and Methods

The main cultivation areas in eight plantations were visited every month from April to September during two years. Fifty trees were randomly chosen and examined from top to base. The galls were counted, and the infestation of aphids was estimated based on a five-grade scale. The presence and absence of



Figure 1. *Physokermes piceae*

the damage caused by *Cydia pactolana* and *Epinotia* spp. were examined. Based on the results four categories were created considering the frequency of the insects.

## Results

Nine to ten species of four coniferous tree genera are cultivated in the Hungarian Christmas tree plantations nowadays. The most harmful insects in Christmas tree plantations in Hungary belong to the suborder Sternorrhyncha (Table 1).

Regarding the Lepidoptera species, *Epinotia* spp. and *Cydia pactolana* may become dangerous in Christmas tree plantations. They can also support the secondary infection of fungi. As a special case, the invasive harlequin ladybird can be mentioned, which would like to overwinter in the Christmas trees. They get sprayed in autumn because they cause inconveniences to the customers.

**Table 1.** The most frequent Sternorrhyncha species in the examined Christmas tree plantations in Hungary (see abbreviations below the table)

Christmas tree spp./ Sternorrhyncha spp.	<i>Abies nord.</i>	<i>Abies lasioc.</i>	<i>Picea abies</i>	<i>Picea omor.</i>	<i>Picea pung.</i>	<i>Pseud. men.</i>	<i>Pinus nigra</i>	<i>Pinus sylv.</i>
<i>Adelges laricis</i>			✓					
<i>Adelges tardus</i>					✓			
<i>Adelges prelli</i>				✓				
<i>Sacchiphantes viridis</i>			✓					
<i>Sacchiphantes abietis</i>			✓					
<i>Dreyfusia nord.*</i>	✓			✓				
<i>Dreyfusia merkeri</i>	✓							
<i>Gilletteella cooleyi</i>						✓		
<i>Pineus pini</i>								✓
<i>Cinara spp.</i>	✓	✓	✓	✓	✓	✓	✓	✓
<i>Elatobium abietinum</i>	✓	✓	✓					
<i>Mindarus abietinus</i>	✓	✓						
<i>Physokermes inopinatus</i>			✓		✓			
<i>Physokermes piceae</i>			✓		✓			

Abbreviations: *nord.* = *nordmanniana*/\**nordmanniana*, *lasioc.* = *lasiocarpa*, *omor.* = *omorika*, *pung.* = *pungens*, *Pseud. men.* = *Pseudotsuga menziesii*, *sylv.* = *sylvestris*, *inopinatus* = *inopinatus*

In total, 33.3% of the insects which were found on Christmas trees were invasive species, the origin of 5.6% of the insects were unknown and 61.1% of the species were native. Using higher portions of nitrogen fertiliser significantly increased ( $p=0.0013$ ) the frequency of aphids and adelgids. Sunny, dry locations significantly raised the risk of infestation of *Dreyfusia nordmanniana* ( $p=0.0037$ ), *Sacchiphantes* spp. ( $p=0.0042$ ), *Physokermes* spp. ( $p=0.0001$ ) (Fig. 1-2), and *Cydia pactolana* ( $p=0.0028$ ).

### Discussion

*Dreyfusia merkeri* was identified in the Hungarian fauna during this examination.

*Dreyfusia nordmanniana* is not a host-alternating species in Hungary. The phenology of the adelgids differs from data given in literature. The protection against the above mentioned species is difficult due to their morphology, life-cycle and their shelters. The right timing to spray is very important because the gallicolae is non-dispersal, so a lot of galls may develop and they can severely affect the growth of young coniferous trees. Some species of *Adelges* have host alternating populations, and non-host alternating populations as well. The ratio of the invasive insects is high in Christmas tree plantations. Black pine has a good resistance against insects. The different colour Douglas firs have



Figure 2. *Physokermes inopinatus*.

different resistance against the woolly aphids. The green Douglas fir is infested severely while the blue one is almost resistant. The grey one has a moderate resistance. Considering the cultivation methods using higher portions of nitrogen fertiliser increases the risk of the sedentation and multiplication of aphids and adelgids. Sunny, dry locations enhance the risk of infestation of several harmful insects.

## TREE HEALTH

# Increased number of *Phytophthora* species found in Fraser fir Christmas tree plantations in the Southern Appalachians

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Fraser fir (*Abies fraseri*) is an economically important species cultivated for Christmas tree production in the Southern Appalachian Mountains of the United States. Annual sales average about 100 million USD in North Carolina alone. Root rot and mortality caused by *Phytophthora cinnamomi* result in significant economic losses to the Fraser fir Christmas tree industry. In previous surveys conducted in 1972 and 1997–98 in North Carolina, the incidence of *Phytophthora* root rot was 9% and *P. cinnamomi* was the predominant species isolated from roots of Fraser fir. We hypothesized that the *Phytophthora* species composition in the Southern Appalachian Fraser fir production region has changed since the previous surveys because the industry has drastically increased importation of planting stock from outside of the region.

During 2014, a survey was conducted on Fraser fir Christmas tree plantations in the Southern Appalachians (NC, TN, and VA) to enumerate the *Phytophthora* species present. Using a weighted sampling strategy based on Christmas tree acreage in 14 counties, symptomatic Fraser fir roots were collected from trees in 103 commercial production

fields. In total, six species of *Phytophthora* were isolated from 82 sites in 13 counties. *P. cinnamomi* accounted for 71% of the isolates, *P. cryptogea* for 23% and collectively, *P. citrophthora*, *P. europaea*, *P. pini* and *P. sansomeana* accounted for 6%. *P. citrophthora*, *P. europaea*, *P. pini* and *P. sansomeana* have not been identified in previously published Fraser fir surveys conducted in the region. While *P. cinnamomi* was still the predominant species isolated from infected Fraser fir roots, *P. cryptogea* appears to have become an important pathogen contributing to losses to the Christmas tree industry in the Southern Appalachian Mountains.

# Response of Turkish and Trojan fir seedlings to *Phytophthora cinnamomi* and *P. cryptogea*

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Phytophthora root rot, primarily caused by the oomycete *Phytophthora cinnamomi* Rands, is a large problem for the Christmas tree industry in North Carolina, leading to more than \$US 6 million in losses annually. Fraser fir [*Abies fraseri* (Pursch) Poir.], one of the most desirable Christmas tree species in the United States, has no known innate resistance to this disease while some exotic fir species, such as Trojan (*A. equi-trojani* Aschers. & Sint) and Turkish (*A. bornmuelleriana* Mattf.) fir display varying amounts of resistance.

A large *Phytophthora*-resistance screening trial was completed using 1600 seedlings from 12 Turkish and Trojan fir families with Fraser and momi fir (*A. firma* Sieb. & Zucc.) seedlings included as susceptible and resistant controls, respectively. Each family (or species) was inoculated with each of eight *Phytophthora* isolates, six *P. cinnamomi* and two *P. cryptogea*. The isolates were collected from a number of different diseased plant hosts (*Abies*, *Camellia*, and *Juniperus* spp.) within North Carolina. Plants were grown in Cone-tainer tubes under 55% shade with daily irrigation at a research nursery in Raleigh. Mortality was assessed as percent shoot necrosis bi-weekly for 16 weeks with a final observation the following year after bud break.

Overall, fir species resistance rankings confirmed previously reported results; momi fir was the most resistant, followed by Turkish and Trojan fir with Fraser fir being most susceptible. *P. cinnamomi* isolates were generally more aggressive on all fir species than *P. cryptogea* isolates. There was a significant interaction between host fir species and *Phytophthora* isolates although the relative resistance rankings of fir species was consistent across *Phytophthora* isolates. *P. cryptogea* has recently become more prevalent in Fraser fir Christmas tree plantations in the Southern Appalachian region. The two *P. cryptogea* isolates were originally isolated from Fraser fir and resulted in 50% and 100% mortality on Fraser fir in this study. Turkish and Trojan fir families appear to possess quantitative resistance to *Phytophthora* species common in North Carolina.



# Mapping *Phytophthora* root rot resistance in fir species through genotyping by sequencing

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The oomycete, *Phytophthora cinnamomi* Rands, causes root rot disease on a broad range of fir and pine species used as Christmas trees. One of the most valuable Christmas tree species, Fraser fir [*Abies fraseri* (Pursch) Poir.] has no innate immunity to *Phytophthora*, and *Phytophthora*-related damages in the Christmas tree industry add up to more than \$US 6 million annually. However an exotic fir species, Trojan (*Abies equi-trojani* Aschers. & Sint) fir has previously shown varying amounts of resistance to *Phytophthora* root rot.

DNA was extracted from foliage of progeny in an open pollinated Trojan fir family (n=161), which was then screened for root rot resistance against *Phytophthora cinnamomi* with an overall mortality of 71%. Libraries were prepared for Genotyping by Sequencing (GBS) to identify genetic marker loci. A small subset of individuals from other inoculated Turkish, Trojan, Fraser, and momi fir families were also genotyped for comparison. The DNA libraries were sequenced on 2 Illumina HiSeq lanes, returning 342 million reads. The resulting sequence was filtered to 413,000 unique tags via the Tassel pipeline, 117,000 of which segregate within the selected Trojan fir family. The segregating tags were tested for association with the disease resistance phenotype, and significance was determined by a permutation test. 205 tags were identified as significantly associated with root rot resistance. The tags were mapped to a draft genome assembly of loblolly pine (*Pinus taeda*), to help identify putative markers, and significant tags were also blasted against the NCBI database to identify genes with known function.

Using the 205 significant genetic markers associated with resistance, we hope to identify the genetic basis of the disease resistant phenotype. The markers associated with disease resistance in the large Trojan fir family can then be compared to the markers identified in the other fir species and families to look for consistent association of specific markers with disease resistance. The understanding of the genetic basis of *Phytophthora* root rot resistance obtained from this study will guide future breeding efforts to develop resistant planting stock suitable for use on *Phytophthora* infested land.

# Screening *Abies* for resistance to *Phytophthora* root rot

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*Phytophthora* root rot (PRR) causes significant losses in bare-root conifer nurseries and Christmas tree plantations. True fir trees (*Abies*) are common hosts of *Phytophthora*, and popular Christmas tree species such as noble fir (*A. procera*) and Fraser fir (*A. fraseri*) are particularly susceptible. A complex of *Phytophthora* species are collectively recognized as causal agents of PRR, and vary regionally among U.S. production regions. There are limited methods available to growers for controlling PRR, but efficacy varies depending on geographic region, host species, field topography, and prior land uses. For these reasons, efforts to identify fir trees that display resistance to PRR under variable environmental conditions are justified to alleviate losses.

A large-scale greenhouse resistance screening study challenged one-year-old seedlings of 7 species of fir with 3 virulent genotypes from each of 4 species of *Phytophthora*. The *Phytophthora* isolates employed in the study were collected from fir roots during a nationwide sampling effort of tree plantations in 5 major U.S. Christmas tree production regions. In order to adequately test host performance over a range of environmental conditions, the experiment was conducted simultaneously in two greenhouses set to two different temperatures. The cool weather greenhouse was maintained at a temperature range of 15 - 21°C to replicate prevailing conditions in temperate regions such as the Pacific Northwest (PNW). The warm weather greenhouse was sustained in the 26 - 32°C range to simulate the southeastern U.S. and California. Although species such as Nordmann fir (*A. nordmanniana*) and Turkish fir (*A. bornmuelleriana*) are traditionally considered to be more tolerant to PRR than the highly susceptible noble and Fraser firs

In the PNW, evidence has shown that these species are apt to fail in other growing regions with different environmental conditions and *Phytophthora* communities. The design of this study intended to address these anomalies.

Plant material was randomized into each greenhouse in a split-split block design and inoculated by inserting colonized rice grains into the growing media. Mortality was rated weekly, and at 13 weeks all surviving seedlings were re-inoculated in the same manner. The experiment is expected to continue for an additional 5 weeks; at which time, root rot ratings and moisture content calculations should provide insight as to which seedlings qualify to be considered on the spectrum of resistance.

Tissue from resistant trees will yield genetic material appropriate for genomics testing in pursuit of molecular markers associated with resistance. It is also intended that individual trees will be conserved for future breeding applications. The goals of this study were to supplement established knowledge regarding *Phytophthora* species virulence and *Abies* sensitivities, and to enhance crop productivity by providing growers with resistant planting stock.

# Fungicide resistant *Botrytis* strains are present in forest nurseries in Norway

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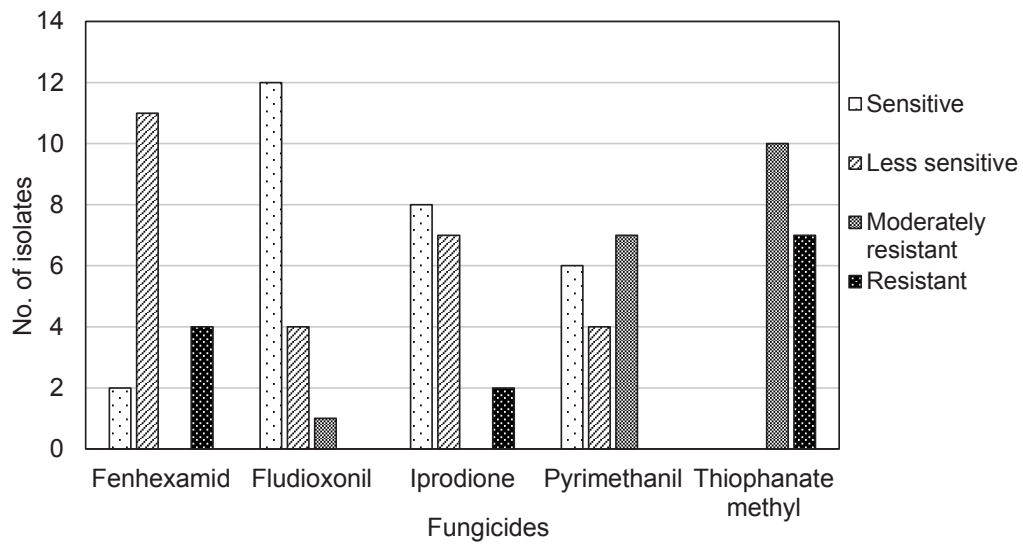
Fungal diseases are among the main challenges in the seedling production of Norway spruce (*Picea abies*) in Norway, and grey mould caused by one or several *Botrytis* species is considered the most problematic (Fig. 1). Control is mainly based on use of fungicides containing the active ingredient thiophanate methyl, fenhexamid or iprodione. A project was started in 2014 aiming to improve control of fungal diseases on spruce in forest nurseries. Due to the use of fungicides to which resistance have been reported in a number of horticultural crops in many countries, including Norway, we are currently investigating the presence of resistant *Botrytis* strains in forest nurseries. In an initial experiment, we examined 18 *Botrytis* isolates for resistance to fenhexamid by measuring radial growth on artificial medium amended with discriminatory dosages of the fungicide. Five isolates



Figur 1. Grey mould, caused by *Botrytis* sp. on second year seedlings of Norway spruce (*Picea abies*). Photo: G. M. Strømeng.

showed high resistance. In a second experiment, 17 isolates were examined for resistance towards fenhexamid, fludioxonil, iprodione, pyrimethanil, and thiophanate methyl by measuring germ tube growth on artificial media amended with discriminatory dosages of the respective fungicides. Because a fourth fungicide consisting of two active ingredients (cyprodinil and fludioxonil) has been included in the spraying programme in several of the nurseries over the past few years, fludioxonil and pyrimethanil (has cross resistance with cyprodinil) were included in the test as well. Seven out of 17 isolates were resistant to thiophanate methyl, while the remaining ten showed moderate resistance (Fig. 2). Four isolates were resistant to fenhexamid, while two were resistant to iprodione.

One and seven isolates showed moderate resistance to fludioxonil and pyrimethanil, respectively, while no isolates showed full resistance to these two active ingredients (Fig. 2). It was alarming that four isolates were resistant to two fungicides, i.e. had developed multiple drug resistance (MDR). Two MDR isolates were resistant to fenhexamid and thiophanate methyl, while the other two were resistant to fenhexamid and iprodione. Moreover, these MDR isolates also showed moderate resistance to one, two or three of the other fungicides. Thus far, isolates from seven nurseries have been examined, of which we found MDR in two nurseries, strains resistant to one fungicide in one nursery, and moderate resistance towards at least one of the fungicides in all but one nursery (isolates from the latter were only included in the first experiment). Our preliminary conclusion is that use of thiophanate methyl should be avoided in the future, and resistance development to fenhexamid should be carefully monitored.



Figur 2. Sensitivity to five fungicides in 17 isolates of *Botrytis* sp. collected from second year seedlings from seven forest nurseries in Norway.

# Identification and pathogenicity of *Phomopsis* isolates associated with spruce decline in Christmas and landscape tree settings in Michigan, USA

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Michigan is a major nursery producing state, with spruce (*Picea* spp.) being important species for both the landscape and Christmas tree industries. In the early 2000s, mature spruce in Michigan began to show combined symptoms of needle loss and branch dieback, which we term spruce decline. These symptoms have been reported on nursery and landscape spruce in Wisconsin as curling and necrosis of tips with stem cankers (Sanderson & Worf 1986), and later reported on nursery and tree farm settings in Michigan as cankers (Igoe *et al.* 1995), but never

before on mature spruce in Michigan. While most conspicuous on Colorado blue spruce (*Picea pungens*), spruce decline symptoms are also found on white (*P. glauca*) and Norway (*P. abies*) spruce throughout Michigan, as well as other states. Rarely observed are indentations or resinous exudates typical of canker diseases like *Cytospora* (Sinclair & Lyon 2005). Only when the bark layer is removed are numerous brown cankers with occasional resinous streaking seen in the phloem and cambium of a single branch (Fig. 1). Isolates from over 100 cankers from symptomatic



Figure 1. Colorado blue spruce branch collected from a landscape tree displaying spruce decline symptoms. Photo inserts of scraped areas revealing numerous *Phomopsis* cankers throughout the branch.

spruce trees in landscapes and tree farms throughout the state were used to identify the pathogen. ITS1F and ITS4 sequencing revealed *Phomopsis* taxa as the most common fungal organism in the canker. BLAST analysis demonstrated 95% identity to *Phomopsis occulta*, supporting previous literature (Igoe *et al.* 1995; Sanderson & Worf 1986), and 99% identity to *Diaporthe eres*. However, *Phomopsis* isolates were variable, forming 5 groups that differed across 11 different base pair positions. Pathogenicity tests using a representative isolate from each *Phomopsis* group were carried out on Colorado blue, Norway, and white spruce. Using 15 replicates for each spruce species by *Phomopsis* group combination, inoculations were performed by creating a 0.1 x 0.1 x 0.1 cm hole approximately 3 cm from the soil line and inserting an agar plug of fungal tissue. An agar plug with no fungal tissue was used as a control. The bark was scraped back nine weeks post-inoculation and canker area was measured using the longest and widest points. The largest cankers formed on Colorado blue spruce for each *Phomopsis* group, followed by white, then Norway spruce, while controls had no canker expansion. Additionally, not all *Phomopsis* groups were equally virulent. Isolates from groups 2, 4 & 5 were most virulent on Colorado blue spruce, moderately virulent on white

spruce and had lower virulence on Norway spruce. In contrast, the two isolates from group 3 had very low virulence levels for all spruce species tested. Group 1 showed moderate virulence on Colorado blue spruce and very low virulence levels for white and Norway spruce. Recovered *Phomopsis* cultures from cankers on inoculated spruce trees were genetically identical to inoculated isolates. Since *Phomopsis* isolates vary genetically and in virulence, research is focused on using multiple genes to clarify the taxonomy of *Phomopsis* involved in spruce decline. As with other tree declines, we recognize that many other factors like needlecast diseases and insects could also play important roles in disease initiation and/or progression. To our knowledge, this is the first report of *Phomopsis* causing cankers on mature spruce in Michigan.

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# *Neonectria neomacrospora* has caused severe damage on true fir (*Abies* spp.) in Denmark

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In 2011, typical red fruiting bodies from a *Neonectria* sp. were found on subalpine fir (*Abies lasiocarpa*) in a provenance trial in Denmark (Talgø *et al.* 2012). Isolates obtained were identical to Norwegian isolates from white fir (*A. concolor*). Later the isolates were identified to *Neonectria neomacrospora* by ITS sequencing of the rDNA. The fungus was first described by Wollenweber in 1931 under the name

*Nectria cucurbitula* (TODE) Fr. v. *macrospora* Wr. n. v. It has been present in Norway and North America for decades, and the imperfect stage was reported on fir elsewhere in Europe, but the detection on subalpine fir was the first time the fungus was found in Denmark. Typical symptoms and signs were flagging (dead branches) and heavy resin flow. Red fruiting bodies (perithecia) were found



Figure 1. Symptoms (dead branches) and signs (red fruiting bodies) of *Neonectria neomacrospora* on Nordmann fir (*Abies nordmanniana*) Christmas trees in Denmark, where this aggressive pathogen recently caused an epidemic on several fir species. Photos: V. Talgø

on several diseased trees. No perithecia were seen on current year dieback or branches that obviously had been dead for a longer period. Perithecia were only present on branches that had died the previous year (brown needles still attached), and especial abundant where dead needles had accumulated on lateral branches. This was likely due to preservation of humidity after rain- and dewfall, creating ideal conditions for fungal growth. Koch's postulates were fulfilled on subalpine fir seedlings. Since the first finding in Denmark in 2011, the fungus has caused an epidemic and great losses on many fir species in Danish Christmas tree fields (Fig. 1), forest stands, seed orchards and ornamental plantings. It has also been found to be seed borne and occurring in nurseries.

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# Development and application of a PCR based test for the identification of *Neonectria neomacrospora* damaging *Abies* species

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In Norway, true firs (*Abies* spp.) are very important for the Christmas tree production, especially Nordmann fir (*A. nordmanniana*) and subalpine fir (*A. lasiocarpa*). In June 2008, a severe disease outbreak was discovered on white fir (*A. concolor*) in several counties in southern Norway, and identical symptoms were observed on white fir in areas of Sweden bordering southeastern Norway. A *Neonectria* sp. was isolated from the diseased trees, and sequencing of the internal transcribed regions (ITS) of ribosomal DNA showed that all isolates were identical and most similar to *N. ditissima*, a damaging pathogen in fruit orchards. The cultures obtained showed up to 99% similarity to *N. ditissima* in the ITS region, and as low as 96% similarity to *N. fuckeliana*, which has been known for decades on Norway spruce (*Picea abies*) in the Nordic countries. By the end of 2012, some new reports to the GenBank matched the

sequences from our isolates from true fir, revealing that the causal organism of the epidemic on fir was *N. neomacrospora*.

In the last few years, *N. neomacrospora* has been detected and identified on many new fir species; in total 19 species and subspecies of *Abies* (Talgø & Thomsen 2015). In addition, *N. neomacrospora* has been isolated in a single case from Norway spruce (*Picea abies*) and recently also from western hemlock (*Tsuga heterophylla*) (Talgø & Brurberg, 2015).

Due to the increasing problems with *N. neomacrospora*, we have developed a Taqman real-time PCR assay specific for this fungus, for rapid identification and detection. The real-time PCR assay was optimised with various concentrations of primers and probe. The optimal concentrations gave standard curves



Figure 1. Mycelial growth of *Neonectria neomacrospora* emerging from a canker wound of an incubated branch from subalpine fir (*Abies lasiocarpa*). Photo: V. Talgø

with high correlation coefficient, indicating a reproducible linear response in detection of increasing concentrations of *N. neomacrospora* DNA. The assay was validated for specificity to *N. neomacrospora* by testing several isolates of *N. neomacrospora*, *N. ditissima* and *N. fuckeliana*. The latter two gave none or very weak signals. The assay was also tested on symptomatic plant samples from the forest and spore catches from a branch with a canker wound of sporulating *N. neomacrospora*. The assay successfully detected airborne spores of *N. neomacrospora* as well as the fungus in plant samples (Fig. 1), and hence will be a valuable tool for identification, detection and epidemiological studies of the pathogen.

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# Inoculation experiments with *Neonectria neomacrospora* on *Abies nordmanniana*

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Nordmann fir (*Abies nordmanniana*) is a widely used Christmas tree species in northern Europe. Since 2009, the bark parasite *Neonectria neomacrospora* has caused a canker epidemic on true firs (*Abies* spp.) in Norway and Denmark, including Nordmann fir Christmas tree stands (Talgø *et al.* 2013). Typical symptoms and signs are dead branches, canker wounds with dead tissue below the bark, heavy resin flow, and the presence of fungal structures: cream coloured spore pustules with the conidial stage *Cylindrocarpon cylindroides* and red fruiting bodies (perithecia) with ascospores (Fig. 1).

The fungus has also been found in Canada as a pathogen on balsam fir (*A. balsamea*) around 1960 (Ouellette & Bard 1966), and more recently on various fir species in the Pacific Northwest (USA)

(Chastagner *et al.* 2015). The anamorph stage was first described from grafted white fir (*A. concolor*) in a German nursery more than 100 years ago (Wollenweber 1913).

The susceptibility varies both within and amongst species, with subalpine fir (*A. lasiocarpa*), white fir, and Spanish fir (*A. pinsapo*) amongst the most susceptible both in field assessments and inoculation experiments on detached shoots (Fig. 2). However, there also seems to be large within-species variation in susceptibility, in some cases relatable to subspecies or provenances, but also on a single tree level (clones). Testing for differences in susceptibility can be done via artificial inoculation on cut shoots placed in water.



Figure 1. Nordmann fir (*Abies nordmanniana*) stand with symptoms of *Neonectria neomacrospora* damage. Red fruiting bodies were found on dead branches (inserted photo). Photos: I. M. Thomsen

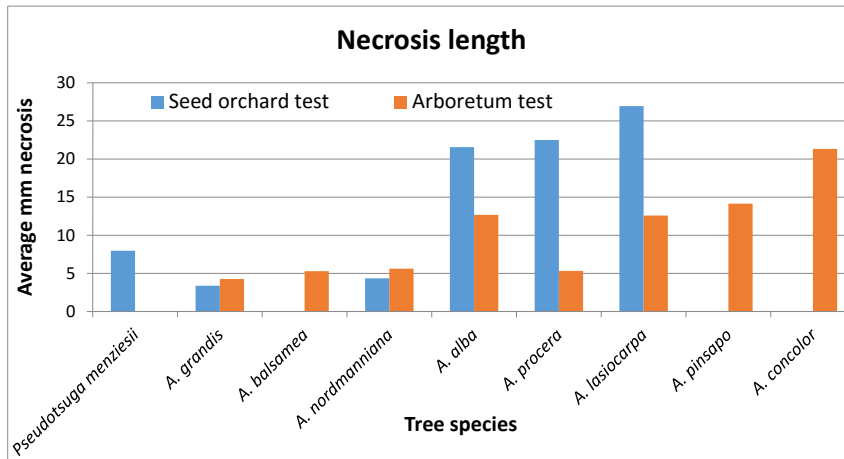


Figure 2. Results from two different inoculation experiments with *N. neomacrospora* on firs (*Abies* spp.) and Douglas fir (*Pseudotsuga menziesii*). One included twigs from different seed orchards, and one was done on twigs from the collection of true firs at the Arboretum in Hoersholm. There was high variation, but Nordmann fir (*A. nordmanniana*) and grand fir (*A. grandis*) seemed to be amongst the less susceptible.

Various methods for inoculation were tested on Nordmann fir, and the most effective was creating an entry point by removing a needle from the shoot, and placing a 0.5 mm plug from a *N. neomacrospora* culture upside down on the wound (Fig. 3). This method was also successful when used on other fir species. However, in general the infection success is so high, even on fully mature shoots, that it may obscure the variation in susceptibility, at least within a species. Inoculation with ascospores or conidia would imitate natural infection more closely. This is difficult to handle due to the need for freshly prepared spore suspensions, since mature perithecia cannot be stored over time for lab experiments, and macroconidia do not form readily on agar.

Various factors may influence the result of inoculation, such as shoot size, inoculum age, duration of experiment, humidity and temperature, wound area, and development stage of shoots. Further development of a reliable inoculation method which mirrors natural infection and pathogen–host interactions is needed in order to study the genetic variation in susceptibility of firs to *N. neomacrospora*. The intention is to identify highly resistant genotypes for use in breeding programs.

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Figure 3. Inoculation experimental set-up after the method by Venche Talgø with removal of needle (arrow) before placing mycelial plug of *Neonectria neomacrospora* on needle scar and incubating in boxes (with water) for three weeks. Extensive needle loss and necrosis under the bark on a subalpine fir (*Abies lasiocarpa*) shoot indicates successful infection by the pathogen. Photos: I. M. Thomsen

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# *Neonectria* – an update on genetic variation in tree susceptibility based on ocular field evaluations

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In Denmark, the fungus *Neonectria neomacrospora* was firstly identified in spring 2011 in a provenance trial of subalpine fir (*Abies lasiocarpa*) and a Nordmann fir (*A. nordmanniana*) grafted clonal seed orchard.

This newly identified fungus has caused severe problems in a number of species from the genus *Abies*, including Christmas tree stands, stands for timber production and seed orchards throughout Denmark, and also in the collection at Hørsholm Arboretum. During 2012 and 2013, a series of reports on damages were recorded, and the presumably virulence of the new damaging agent has caused serious concern in the Christmas tree industry. A number of field registrations have been carried out. For all evaluations the same scale from 0–10 has been used for describing the total tree damage; no damage (0), weak (1–3), moderate (4–5), severe damage (7–9), and dead (10).

In Nordmann fir clonal seed orchards significant differences were seen between clones. Some genotype site interaction were found, with indications of a needle and shoot sucking aphid (*Adelgids dreifuscia*) having a role in increased fungal attack (based on a statistical correlation).

The Arboretum in Hørsholm comprises 33 different *Abies* species and subspecies, unevenly distributed. In total, 360 individuals are scattered across the Arboretum collection, not randomized and uneven aged. However, despite the lack of statistical experimental design, this collection offers an interesting opportunity to evaluate the species susceptibility in a nearly even environment. A very large variation in the damage score for species was seen. The group of the most damaged species included white fir (*A. concolor*) and subalpine fir.

# Seed borne fungi on Christmas trees

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Christmas tree production has increased substantially in Norway during recent years. Traditionally, Norway spruce (*Picea abies*) was the main Christmas tree. However, during the last two decades species of fir (*Abies* spp.), mainly Nordmann fir (*A. nordmanniana*) and subalpine fir (*A. lasiocarpa*), have become the most important Christmas tree species. Noble fir (*A. procera*) is an important species for bough production.

Several fungi have been reported to be associated with conifer seeds. Some are saprophytes and have no effect on seed quality, others are causing decay and reduction of germination of stored seeds. Some seed borne fungi may also attack germinating seedlings before emergence or needles and shoots of seedlings after emergence. Infected nursery stock may contribute to infections in production fields (Prochazkova & Sutherland 1997).

The presence of fungi on fir seed lots have been investigated in Norway during the last 10 years. In 2005, samples from twelve seed lots originating

from Norway (three noble fir, two subalpine fir), Georgia (three Nordmann fir), Canada (two subalpine fir), Austria (one Nordmann fir), and Russia (one Nordmann fir) were tested using agar plate (PDA and WA) methods (Talgø *et al.* 2010a). Some fungi were identified to species level based on sequencing of ITS regions of rDNA. The most important finding was that *Sydowia polyspora* (Fig. 1) was present on seed from all countries; ten samples were infected, in frequencies of 0.5–87% infected seeds. Previously, this fungus had only been reported to be seed borne on Scots pine (*Pinus sylvestris*) in Britain (Whittle 1977). This fungus has been found to be associated with current season needle necrosis (CSNN) and *Sclerophoma* shoot dieback, both diseases commonly observed in forest nurseries and Christmas tree plantations, especially on Nordmann fir (Talgø *et al.* 2010b). *Sirococcus conigenus*, causing shoot blight of several conifer species, was found in one Norwegian *A. procera* seed lot, where 31% of the seeds were infected (Talgø *et al.* 2010a). *Caloscypha fulgens*, the seed or cold fungus, was detected at low levels on subalpine fir seed from Canada (Talgø *et*

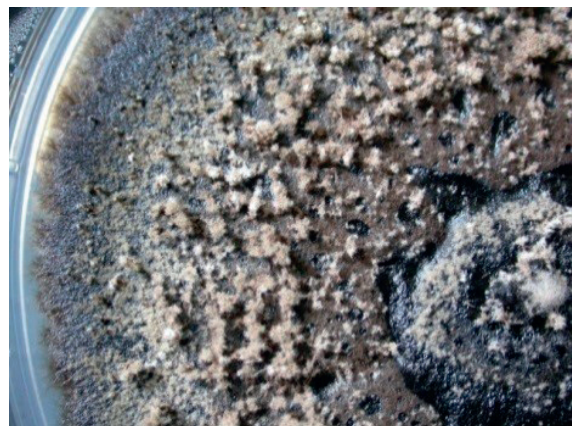


Figure 1. Growth of *Sydowia polyspora* on agar. Young culture (8 days) to the left, 6 months old culture to the right. Photos: V. Talgø

al. 2010a). In addition, the following fungal genera were recorded: *Acremoniella*, *Acremonium*, *Alternaria*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Dictyopolyschema*, *Epicoccum*, *Fusarium*, *Genicularia*, *Mucor*, *Neonectria*, *Penicillium*, *Phoma*, *Rhizopus*, *Sordaria*, *Trichoderma* and *Trichothecium* (Talgø et al. 2010a). Species within some of these fungal genera are known pathogens in nurseries and production fields.

In 2009, *S. polyspora* was detected in samples of pine and Norway spruce seedlings during germination tests at the Norwegian Forest Seed Center, indicating that *S. polyspora* also was seed borne on spruce. In a seed test to investigate how widespread it might be on conifer seeds, we detected *S. polyspora* in 28 out of 44 seed lots tested, representing different species within seven out of eight genera tested; *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Thuja* and *Tsuga* (not on *Chamaecyparis*). In an inoculation experiment, we found that *S. polyspora* may strongly reduce the emergence of noble fir seeds. In a seed treatment experiment to control *S. polyspora* on conifer seeds, using samples from two naturally infected seed lots (*Pinus mugo* var. *rotunda* and *A. procera*), a fungicide containing boskalid+pyraclostrobin (Signum) was effective against the fungus without influencing the germination capacity.

Testing of seeds from diseased Nordmann fir and subalpine fir has revealed infection by *Neonectria neomacrospora*, sometimes in rather high frequencies. This fungus can cause dead shoots, dead branches, canker wounds with heavy resin flow, and in severe cases death of trees. Serious damage has been observed in Christmas tree fields, forest stands, and seed orchards.

To reduce the damages in nurseries and production fields, and to limit the risk of long distance spread of important pathogens via seed and transplant trade, surveys of seed plantations and seed health testing is recommended.

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# Does the severity of current season needle necrosis decrease on older stands of noble fir?

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Current season needle necrosis (CSNN) is a poorly understood disease on true firs (*Abies* spp.) grown for Christmas trees in Europe and North America. Early research suggested that CSNN was likely a physiological disorder that was associated with calcium deficiency and environmental stress. However, recent research in Norway has found that the endophyte, *Sydowia polyspora* may play a role in the development of this disease. In the U.S. Pacific Northwest (Oregon, Washington, and Idaho) and British Columbia, CSNN is most commonly seen on noble (*A. procera* Rehd.) and grand fir [*A. grandis* (Douglas ex D. Don) Lindl.] grown at low elevation sites. Similar needle damage has also been observed on white fir [*A. concolor* (Gord. & Glend.) Lindl. ex Hildebr.], Nordmann fir [*A. nordmanniana* (Steven) Spach] and Turkish fir (*A. bornmuelleriana* Mattf.). In Europe, CSNN has also been observed on grand and noble firs, but the greatest economic impact has been on Nordmann fir, the dominant Christmas tree species in Denmark and Norway.

Since 2004, the yearly severity of CSNN has been evaluated on noble fir trees in a series of genetic trials planted at WSU-Puyallup. This is a low elevation (10 to 30m) site that is very conducive to the development of CSNN and has provided an opportunity to examine yearly variation in development of CSNN and determine the variation in resistance to this disease among the different sources of trees in these trials. Unlike most trials where data was only collected for a few years, data were collected over an 8-year and 10-year period for trials that were established in 2002 and 2004, respectively. These 2002 and 2004 replicated plantings contained 25 trees from 35 and 53 sources, respectively. Starting 2 years after

planting, the severity of CSNN on each tree was rated annually on a scale of 0 to 10, where 0 = no CSNN, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, ..., and 10 = 91-100% of the current season foliage damaged by CSNN.

Data from these longer-term evaluations indicated that there was significant year-to-year variation in CSNN in both plantings. There was a trend of reduced damage as trees aged in the 2002 trial and there was a significant negative correlation between the age of trees and the severity of CSNN in the 2004 trial. While the reasons for this decrease are unclear, its implications for our understanding of this disease was discussed.



## PHYSIOLOGY

# Does shoot temperature regulate cone production in *Abies fraseri*?

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### Abstract

The ability to influence strobilus (cone) formation in *Abies* is important both for Christmas tree growers who want to reduce costs associated with undesired cone production and for seed orchard managers who require consistent seed production. Heat and drought are important environmental regulators of reproduction in mast-seeding species, and have been used to increase cone production in conifers, including Pacific silver fir (*Abies amabilis*). In Fraser fir (*Abies fraseri*), we tested the effects of temperature on cone formation using overhead misting for evaporative cooling and polyethylene tenting for solar heating throughout the period of active growth, including the window of reproductive bud initiation and differentiation. Misting reduced average daily temperatures of lateral shoots where cones develop by 2.4°C and daily maximum temperatures by 5.0°C, while also increasing soil moisture by 20%. Tenting increased average daily shoot temperatures by 1.4°C and maximum temperatures by 3.8°C, while increasing soil moisture by 12%. Neither misting nor tenting affected cone production, suggesting that cone formation in Fraser fir is regulated by factors other than temperature at the developing bud.

Keywords: *Abies*, cone induction, seed cone, heat, drought, seed orchard

### Introduction

Many conifers species are mast-seeding, with seed production highly variable across years, yet synchronized across a population (Kelly 1994). The

mechanisms governing mast-seeding are not well understood, but weather conditions at the time of cone initiation and differentiation are important drivers (Roland *et al.* 2014). In particular, high temperatures and dry conditions during the summer increase subsequent cone production in many conifers, including pine, spruce, and fir (Owens & Blake 1985; Solberg 2004; Messaoud *et al.* 2007). Tenting (covering a tree with polyethylene to trap solar heat) has been used in seed orchards as one of many cultural and chemical treatments designed to increase cone initiation, particularly under non-inductive conditions (e.g., cool, wet summers) (Owens & Blake 1985). Although cultural treatments are generally most effective in combination with GAs, tenting alone may increase cone production in some conifers, such as Sitka spruce (Tompsett 1977). To our knowledge the effects of heat treatment have been reported for only one *Abies* species. Tenting Pacific silver fir [*Abies amabilis* (Douglas ex Loudon) Douglas ex Forbes] significantly increased the effectiveness of other treatments (GA<sub>4</sub>/7 + fertilizer + girdling), although tenting alone was not tested (Owens *et al.* 2001).

While seed orchard managers want to increase cone production, Christmas tree growers in the United States want to reduce cone production. Fraser fir [*Abies fraseri* (Pursh) Poir.] is an important Christmas tree species in the Midwest and in the southern Appalachians, where annual sales exceed 100 million USD (Pettersson *et al.* 2015). Grown outside of its native environment, such as in plantations in Midwestern states, young Fraser fir trees produce

abundant, intermittent cone crops (Cregg *et al.* 2003). Developing cones consume photosynthates at the expense of vegetative growth, and leave behind unsightly stalks and resinous scales upon disintegration in early fall (Crain *et al.* 2012). To produce saleable trees growers must remove cones by hand, which is labor-intensive (Cregg *et al.* 2003). Even in plantations in the southern Appalachians within the native range of Fraser fir, heavy cone production is a growing problem on larger trees. In that market, supply of Fraser fir trees has outpaced demand for several years, allowing many trees to grow large enough to begin producing cones, particularly following periods of heat and drought. In both Michigan and North Carolina (Owen 2015), some producers report cone removal to be their most expensive cultural management practice, when considered on a per-tree basis.

Since warming trees by tenting increases cone production in Pacific silver fir (Owens *et al.* 2001), it seems likely that cooling of the tree crown might decrease cone production in Fraser fir. Evaporative cooling by overhead misting has been used effectively in conifer seed orchards to delay budbreak for frost protection and reduction of outside-pollen contamination (Fashler & El-Kassaby 1987). Mist-cooling has also been used for frost protection in tree fruits, and may delay bloom by 7 – 10 days (Rijal *et al.* 2014). This delay is highly localized, with branches that evade mist—such as those growing above the reach of the sprayers—breaking bud in synchrony with control trees. This indicates that timing of budbreak is regulated by temperature at (or near) the developing bud, consistent with findings that meristem temperature, rather than air temperature, regulates phenology in plants (Savvides *et al.* 2013).

The purpose of the current study was to test whether the temperature at the lateral bud locally regulates cone initiation and differentiation in Fraser fir. Two methods were used, one that has been used in seed orchards to increase cone production, and another that could be used by Christmas tree growers to reduce cone production. Tenting was used to increase temperature in the upper crown by solar heating, and a misting system was used to decrease temperature in the upper crown by evaporative cooling.

## Materials and Methods

In 2014, the study was established at Michigan

State University's Southwest Michigan Research and Extension Center (Benton Harbor, Michigan). The site (42°05'17.2"N 86°21'28.1"W) had been planted for a study in 2006 in four 8-row blocks of alternating sets of spruce and fir trees on 2.1 m x 2.1 m spacing (Cregg *et al.* 2009). In mid-May 2014, Fraser fir trees were scored for current-year and prior-year seed cone production, and assigned a value of 0 for no cones, 1 for fewer than 20 cones, and 2 for 20 or more cones. Prior-year score was based on the number of cone stalks remaining from previous years. Since trees with high reproductive output were desired for the study, trees with an average current- and prior-year score of one or below were excluded. By late May, all cones had emerged and were counted on each tree and 54 trees were selected for study. The mean height ( $\pm$  SE) of selected trees was 2.43 ( $\pm$  0.042) m. Trees were randomly assigned to one of three treatments: mist-cooling, tenting, and control. Twenty-one trees were selected for mist-cooling, 12 for tenting, and 21 were left untreated as controls. Fewer trees were selected for tenting because construction of additional tents would have exceeded available labour constraints. At least two buffer trees separated the trees that were misted from trees that were tented or control to prevent overspray by the misting system.

### Mist-cooling treatment

Water was provided from an onsite well, run through a sediment filter, and regulated to 210 – 240 kPa before being delivered to trees using 18-



Figure 1. Micro-sprayer delivering mist for evaporative cooling of upper tree canopy.

mm polyethylene tubing and micro-sprayers (35 l/h nozzle, flat static spreader; NaanDanJain Irrigation Ltd., Israel). A micro-sprayer was attached a few cm below the top buds on the main stem of each treated tree, and connected to the leader using zip-ties or grafting bands painted with castor oil for UV resistance (Fig. 1). Polyethylene tubing (4-mm) connected the sprayer to the 18-mm polyethylene supply lines. Misting was controlled by an evaporative cooling application running on a datalogger (CR1000, Campbell Scientific, Logan, UT, USA) as part of a system developed in Michigan for delay of budbreak in cherry and apple (Rijal *et al.* 2014). The datalogger activated the system by powering a solenoid on the main water supply line. Misting was based on evaporation rates calculated as a function of ambient air temperature and relative humidity (RH) measured at 1-min intervals at upper-canopy height using a combination temperature + RH sensor (HMP60, Vaisala Oyj, Helsinki, Finland). This sensor was housed in a multi-plate radiation shield (R. M. Young Co., Traverse City, MI) on a weather station at the edge of the field. The misting system remained active while temperatures were above 15°C and RH was less than or equal to 90%. Misting was activated for 105 s, followed by a delay that varied from 750 s at 15 – 20°C and 75 – 90% RH to 210 s at > 24°C and 0 – 50% RH. The end result was that mist was reapplied just frequently enough to maintain some moisture on needle surfaces. Misting commenced on May 29 for 16 trees. Because of labour and time constraints, misting commenced on June 6 for the remaining 5 trees. Misting was discontinued on Aug. 15.

#### Tent-heating treatment

Four-sided, trapezoidal frames were constructed from 38 mm x 38 mm pine boards, overlaid with 0.15 mm polyethylene film, and placed over trees selected for tent-heating treatment (Fig. 2). The polyethylene film enclosed only the upper third to half of each tree canopy, leaving the lower canopy uncovered and the top of the tree open to prevent excessive heat build-up. Tents were installed on June 6 and removed on August 15.

#### Data collection

Shoot temperature (as a proxy for bud temperature) was measured in three randomly selected mist-cooled trees, and in two tented and control trees using thermistor temperature probes installed June 28. The temperature probe was built using a 10 K



Figure 2. Polyethylene tent used for solar heating of upper tree canopy.

thermistor (NTCLE413, Vishay Intertechnology, Malvern, PA, USA) in a DC half-bridge configuration with a 10 K reference resistor (MFP-25BRD52-10K, Yageo America, San Jose, CA, USA). The thermistor tip was left exposed, and the remaining circuitry was encased in high adhesive flow heat shrink tubing (NSPA-HST540C-48, National Standard Parts Associates, Inc., Pensacola, FL, USA). Temperature was calculated by fitting a polynomial to the calibration curve as  $T = - (y - 2200) / 27.5$ , where T is temperature in degrees C and y is the output from the probe measured in mV. Thermistor probe accuracy was  $\pm 1\%$ , yielding a temperature accuracy range from  $\pm 0.9$  °C at 0 °C to  $\pm 0.4$  °C at 40 °C. Temperature probes were embedded (1 per tree) into 1.5 x 2 mm holes drilled into 1-yr-old, lateral branches just below current-season growth, in the upper few whorls on the south side of the tree, but shaded from the sun. Soil moisture was also measured under these same trees. On July 2, volumetric moisture probes (10HS, Decagon Devices, Inc., Pullman, WA, USA) were installed at a depth of 30 cm just outside the tree dripline. Soil moisture and stem temperature readings were taken every 60 s, and the mean was recorded every hour throughout the remainder of the treatment period by a data logger (Em5b, Decagon Devices, Inc., Pullman, WA, USA).

Six trees in each treatment were randomly selected to track growth. For each tree, the length of the terminal leader and one lateral shoot in the upper third of the crown on the south side of the tree were measured periodically from late June to early August. At the end of the growing season, leader length, crown radius, lateral shoot length, and bud density (buds/cm) were measured for all trees. In early October, we collected soil samples randomly from 10 locations within each treatment. The soil cores were extracted from the surface of the mineral soil to a depth of 20 cm using a 1.3 cm soil recovery probe. Soil cores were separated into five replicates per treatment and analyzed for pH and EC.

We collected needle samples for stable carbon isotope analysis to determine the effects of misting and tenting on tree moisture and heat stress. In October 2015, we collected one lateral shoot that included needles from 2014 and 2015 from an unshaded upper branch on the south side of 12 – 15 randomly selected trees within each treatment. We divided the shoots into five replicate samples per treatment per year, and dried them for three days at 70°C. Needle tissue was separated from stems, ground to 40 mesh (0.420 mm), and packed in tin capsules. The Center for Stable Isotope Biogeochemistry at the University of California–Berkeley used an IsoPrime 100 mass spectrometer (Isoprime Ltd., Stockport, UK) to determine the ratio of <sup>13</sup>C to <sup>12</sup>C ( $\delta^{13}\text{C}$ ) in needle samples. From this ratio, we calculated carbon isotope discrimination ( $\Delta^{13}\text{C}$ )—which serves as an integrated measure of moisture stress during growth—following the equations summarized in Gregg and Zhang (2000). Needles samples were also pooled by treatment and sent to a commercial analytical laboratory (Waters Agricultural Laboratories, Inc., Camilla, GA) for nutrient analysis. Because needles from misted trees were coated in a flakey residue, care was taken to remove the residue, and an additional sample was prepared (washed in DI water), to compare results. In May 2015, coning frequency (% of trees coning) and density (number of cones/tree) were recorded for all trees.

### Data analysis

Effects of treatments on growth were tested by analysis of variance based on a completely randomized design using PROC MIXED in SAS v9.4 (SAS Institute, Chicago, IL, USA). Means separation was accomplished using Tukey's honestly significant differences (HSD). The non-parametric Kruskal – Wallis test (PROC NPAR1WAY) was used to test differences in cone production because transformation failed to satisfy parametric assumptions. The effects of treatments on soil temperature, moisture content, and  $\Delta^{13}\text{C}$  were tested using a two-tailed Dunnett's test. All differences were tested at  $\alpha = 0.05$ .

## Results

### Cone production

All trees in the trial produced cones in 2015, with the exception of a single control tree. Mean cone density (average number of cones/tree) did not vary among treatments. Comparison of cone production against a pre-treatment baseline for each tree showed no treatment effect (Table 1).

### Shoot temperature and soil moisture

Evaporative cooling reduced daily mean temperature of misted shoots by 2.4°C (max 3.6°C, min 0.1°C) (Fig. 3) and midday (maximum) temperature by an average of 5.0°C (max 8.4°C, min -1.7°C) (Fig. 4) compared to shoots on control trees during the period covered by environmental sensor data.

Solar heating of tented trees increased daily mean temperature by 1.4°C (max 2.6°C, min -0.1°C) and midday temperature by an average of 3.8°C (maximum 8.2°C, minimum -2.4°C) compared to control trees. Night (minimum) temperatures were similar in all treatments. All treatments affected the shape of the temperature response curve (Fig. 5). Temperatures of tented trees increased much more rapidly, and earlier in the day. Temperatures increased much more slowly in misted trees, yielding a much flatter response curve, even during cool,

**Table 1. Treatment effects on cone production in *Abies fraseri*, Benton Harbor, MI, 2014.**

Treatment	n	Coning frequency (%) <sup>a</sup>	Cone density <sup>b</sup>	Cone difference <sup>c</sup>
Control	25	96	81.2	61.7
Spray	17	100	80.8	63.1
Tent	11	100	73.8	55.3

a. Percent of trees producing cones; b. average number of cones per tree; c. difference between cones produced in 2015 and 2014 (pre-treatment baseline). Means did not differ among treatments at  $\alpha = 0.05$  (Kruskal – Wallis test).

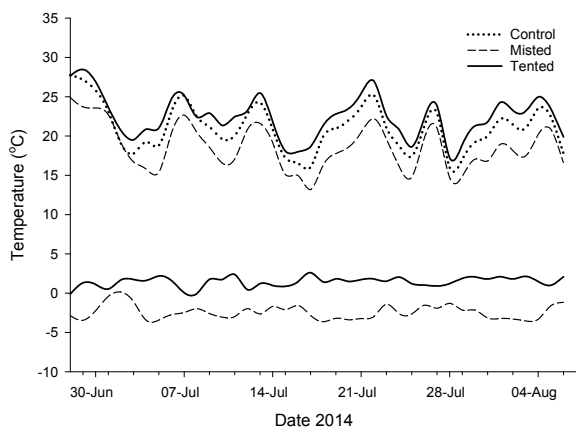


Figure 3. Average daily temperatures of shoots of Fraser fir trees that were tented, misted, or control. Bottom set of lines represents differences between temperatures of tented or misted shoots and control.

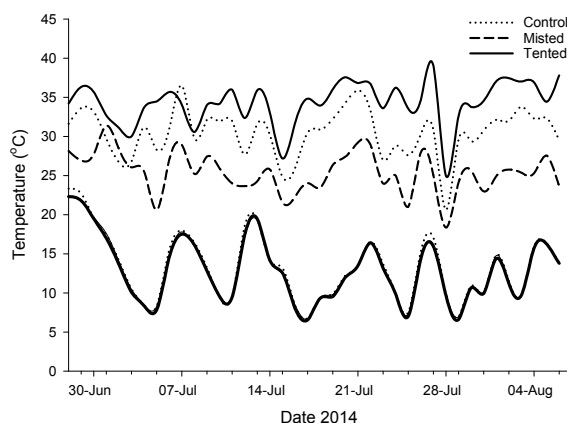


Figure 4. Daily maximum and minimum temperatures of shoots of Fraser fir trees that were tented, misted, or control. Minimum temperatures overlap for all treatments.

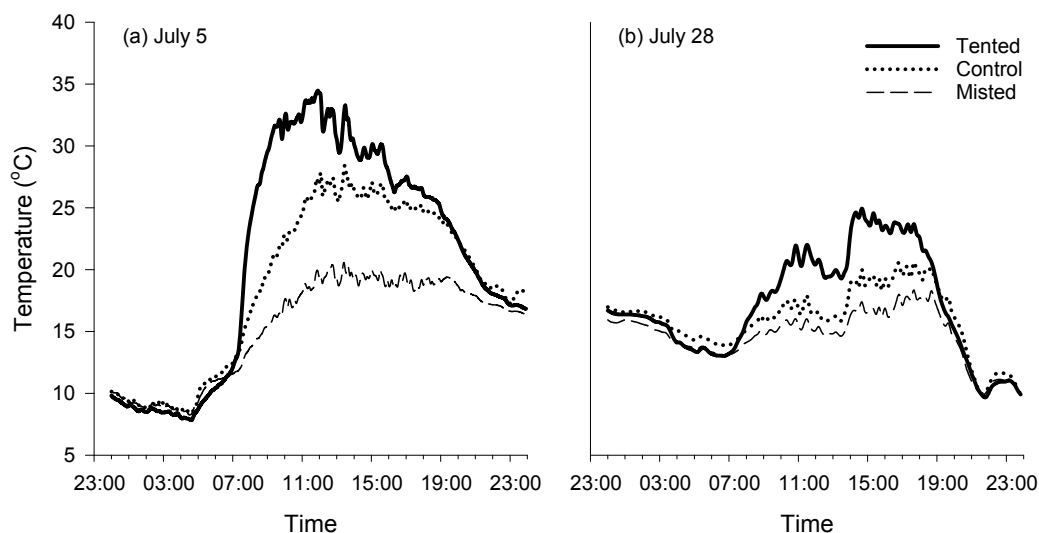


Figure 5. Temperatures of shoots of Fraser fir trees that were tented, misted, or control on (a) warm, sunny (July 5); and (b) cool, cloudy (July 28) days in 2014.

cloudy weather. Volumetric soil moisture content was higher under both misted trees (mean 0.30, max 0.31, min 0.28) and tented trees (mean 0.28, max 0.31, min 0.18) than control (mean 0.25, max 0.28, min 0.17). In general, moisture content in the soil under tented trees remained several percentage points higher than under control trees, but the shape of the response curve over time was similar (Fig. 6), with moisture content trending down through the summer. Moisture content in soil under misted trees was much higher than under tented or control trees, and remained near field capacity throughout the summer.

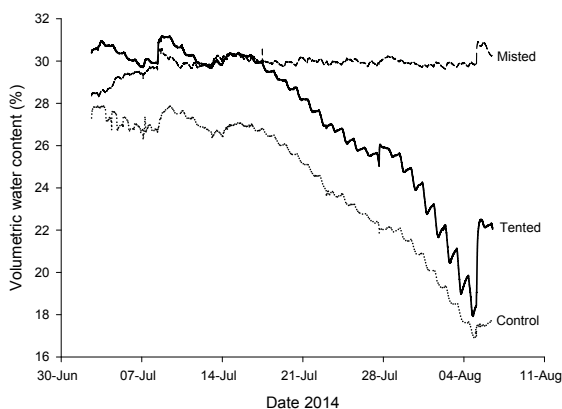


Figure 6. Soil moisture under tented, misted, or control Fraser fir trees, 2014.

**Table 2.** *Abies fraseri* growth (mean ± SE) by treatment, Benton Harbor, MI, 2014.

Treatment	n	Leader length (cm)	Lateral length (cm)	Crown radius (cm)	Bud density (no./cm)
Control	25	48.0 ± 2.7	20.9 ± 1.2	86.4 ± 2.0	12.2 ± 0.57
Spray	21	42.0 ± 3.0	23.4 ± 1.3	87.6 ± 2.2	10.9 ± 0.62
Tent	12	42.3 ± 4.0	21.2 ± 1.7	83.3 ± 2.9	11.1 ± 0.82

Means did not differ significantly among treatments at  $\alpha = 0.05$  (ANOVA).

### Vegetative growth

Leader growth was similar in all treatments, and was largely complete by mid-July, but slow growth continued until late July. Growth of lateral shoots varied among treatments. By July 10, lateral growth had ceased in tented trees and had reached > 95% completion in all other trees. By July 17, lateral growth was complete in control trees, but very slow growth (< 1 cm) continued into late July in misted trees. Growth rates were highly variable within and among treatments, and there were no differences in any of the final growth parameters (Table 2).

### Foliar and soil analyses

$\Delta^{13}\text{C}$  was the same in needles of misted trees ( $21.45 \pm 0.14\text{‰}$ ), control trees ( $21.47 \pm 0.14\text{‰}$ ), and tented trees ( $21.06 \pm 0.12\text{‰}$ ), indicating that treatments had no effect on stomatal conductance. Nutrient concentrations and content in needle samples were similar for tented and control trees and within the expected range for Fraser fir trees grown in Michigan plantations (Shelton 1997; Rothstein and Lisuzzo 2006). However, Fe, Ca, and Mg were much higher in misted trees (139.7 ppm, 1.71%, and 0.12%, respectively) than control (55.7 ppm, 0.47%, and 0.09%), in both washed and unwashed needles. Misting over two seasons increased soil pH from  $5.02 \pm 0.09$  to  $5.6 \pm 0.09$ , compared to control. Tenting did not affect pH ( $4.86 \pm 0.09$ ). EC did not vary among treatments, and averaged  $0.06 \pm 0.01 \mu\text{S}/\text{cm}$ .

### Tree injury

A few damaged or dead shoots were observed in the upper crown of tented trees during the treatment period; insect damage was apparent in some cases. Damage was more severe in misted trees, four of which died in the fall following treatment (Fig. 7). No other tree mortality was evident in the field. Needle surfaces of misted trees were covered by a white, flaky residue. In trees that died, misted needles turned chlorotic, then reddish-brown, generally progressing from the interior of the tree



Figure 7. Fraser fir tree damage due to intolerance to salts present in well water used for the misting treatment.

outward over a period of several weeks, and buds on the affected branches died. Non-misted branches lower in the tree remained green for at least a few months, but it seemed unlikely that the tree would recover. Plant materials in various stages of decline—including one whole tree—were examined by MSU Diagnostic Services (East Lansing, MI). No association was found with pathogens that could explain the observed decline.

## Discussion

### Shoot temperature and soil moisture

Although environmental sensors were not installed until the end of bud differentiation, they provide insight into the effectiveness of the treatments. Shoot temperature was consistently higher during the day for tented trees, and lower for misted trees, as intended. However, soil moisture content also changed in response to treatments, and it was not possible for us to separate the effects of changes in soil moisture from the effects of changes in temperature. Soil moisture remained near field capacity under misted trees, suggesting that efficiency of the water delivery system may be improved. However, this also indicates that no additional irrigation is required when this misting system is in use, and no increase in soil-pathogen activity (e.g. *Phytophthora* root rot) was observed. Soil moisture remained somewhat higher under tented trees than control trees, despite the increase in temperatures within the tents. Condensation was consistently observed on the inside of the polyethylene tent material, and dripping of this condensation may explain the higher soil moisture levels.

### Growth

Growth of lateral shoots was affected by treatments, with incremental growth continuing in misted trees until around the time that leader growth was complete, a few weeks after growth had ceased in other treatments. This persistent growth was likely the result of lower temperatures, since phenology is regulated by thermal time (Trudgill *et al.* 2005; Cleland *et al.* 2007). However, cessation of leader growth was similar in all treatments, suggesting that growth cessation and entrance into dormancy are more likely under the control of photoperiod than temperature, as in other species (Ekberg *et al.* 1979; Rossi *et al.* 2006).

### Tree stress

$^{12}\text{C}$  is preferentially incorporated into plant tissue at the expense of the heavier  $^{13}\text{C}$ , which is discriminated against during photosynthesis. Stress factors that reduce stomatal conductance, such as drought and heat, decrease the ratio of  $^{12}\text{C}$  to  $^{13}\text{C}$  in plant tissue (Farquhar 1989). Stable carbon isotope analysis uses that ratio to provide a single measure of discrimination,  $\Delta^{13}\text{C}$ , which integrates information on cumulative tree stress throughout the growing season. In this study, neither misting nor tenting affected  $\Delta^{13}\text{C}$ , which remained at levels associated

with lower stress in other studies (e.g. Cregg 2005; Taylor *et al.* 2013). This lack of treatment effect is surprising, and indicates that temperatures and soil moisture were not limiting to photosynthesis in 2014, even under tented conditions.

### Cone production

It was surprising that treatments had no effect on cone production, particularly given the dramatic enhancement in cone production under nearly identical conditions in tented Pacific silver fir (Owens *et al.* 2001). However, cultural treatments are rarely effective for cone enhancement unless combined with GA, and tenting alone was not tested in Pacific silver fir (Owens *et al.* 2001). Timing is also critical, and treatments may have been applied too early. For example, in spruce, heat does not promote cone production when initiated during early, rapid shoot elongation, but is promotive when applied toward the end of lateral shoot expansion, during reproductive bud initiation (Ross 1985). This promotive effect may be due to heat-induced changes in GA metabolism (Chalupka *et al.* 1982). In the Pinaceae, less-polar GAs are involved in reproductive determination (Owens 1995). In Norway spruce, these less-polar GAs increase after one day of tenting, and remain elevated for 2 to 3 weeks (Chalupka *et al.* 1982). In spruce and fir, reproductive bud initiation occurs during late shoot elongation, followed by anatomical differentiation over the next 2 – 3 weeks (Owens & Blake 1985). Therefore, to maintain elevated levels of promotive GAs throughout bud differentiation, heat treatments should be timed to coincide as closely as possible with bud initiation during late shoot elongation. When applied early in the season, the elevated levels of endogenous GAs may have reverted to baseline prior to reproductive differentiation. In the case of Pacific silver fir (Owens *et al.* 2001), tenting was applied at vegetative budbreak at a high elevation site with much cooler temperatures than in this study. At our site, reproductive bud initiation likely occurred in mid-June in control trees, earlier in tented trees, and later in misted trees, based on shoot phenology. Treatments were set in place two to three weeks prior to strobilus initiation. If tenting increased the levels of promotive GAs, they would likely have returned to baseline prior to reproductive initiation.

### Tree injury

Although the problem was not widespread, some shoots were damaged in the upper crown of tented

trees. Most damage appeared to be the direct result of high temperatures within the tent, although tents did provide cover for insects, and high temperatures and humidity that could promote disease. Damage to shoots may account for a portion of the reduction in cone production observed in tented trees.

Damage to misted trees was more severe, resulting in the death of 4 of the 21 trees. In the early stages of tree decline, needle and bud necrosis was limited to misted branches, indicating that the eventual mortality was the direct or indirect result of misting. Although needles turned reddish-brown (Fig. 7), the temporal pattern was not consistent with *Phytophthora* root rot, and a thorough examination of plant material indicated that pathogens were not involved. The water used for misting was very hard, 193 mg/l as CaCO<sub>3</sub>. Fe, Ca, and Mg concentration were much higher in needles of misted trees, reflecting the quality of the water used for misting. The white, flakey material observed on misted needles was probably a buildup of Ca and Mg salts deposited throughout the summer as water evaporated from the needles. Symptoms were similar to those observed in conifers exposed to deicing salts from nearby roadways (Barrick 1979). Mortality was likely a direct result of salt toxicity.

### Conclusions

Misting increased soil moisture content and reduced lateral shoot temperatures in Fraser fir. However, it had no effect on cone production, and cannot be recommended as a cultural practice to reduce heavy cone production in plantation-grown Christmas trees. Tenting of trees increased both lateral shoot temperature and soil moisture content, but likewise had no effect on cone production. It is possible that combining misting with GA inhibitors may result in reduced cone production, and likely that combining tenting with GA will increase cone production, as in Pacific silver fir (Owens *et al.* 2001). In summary, neither mist-cooling nor warming by tenting affected cone formation, suggesting that cone formation in Fraser fir is regulated by factors other than temperature.

### Funding

We gratefully acknowledge funding from the Michigan Christmas Tree Association, Michigan's plant agriculture initiative at Michigan State University (Project GREEN). This work was

supported by the USDA National Institute of Food and Agriculture, Hatch project MICL01978.

### Acknowledgements

We thank Michigan State University Southwest Research and Extension Center for use of their research facilities, Dr. James Flore for use of his mist-cooling system, and Ishara Rijah for assistance with programming the datalogger.

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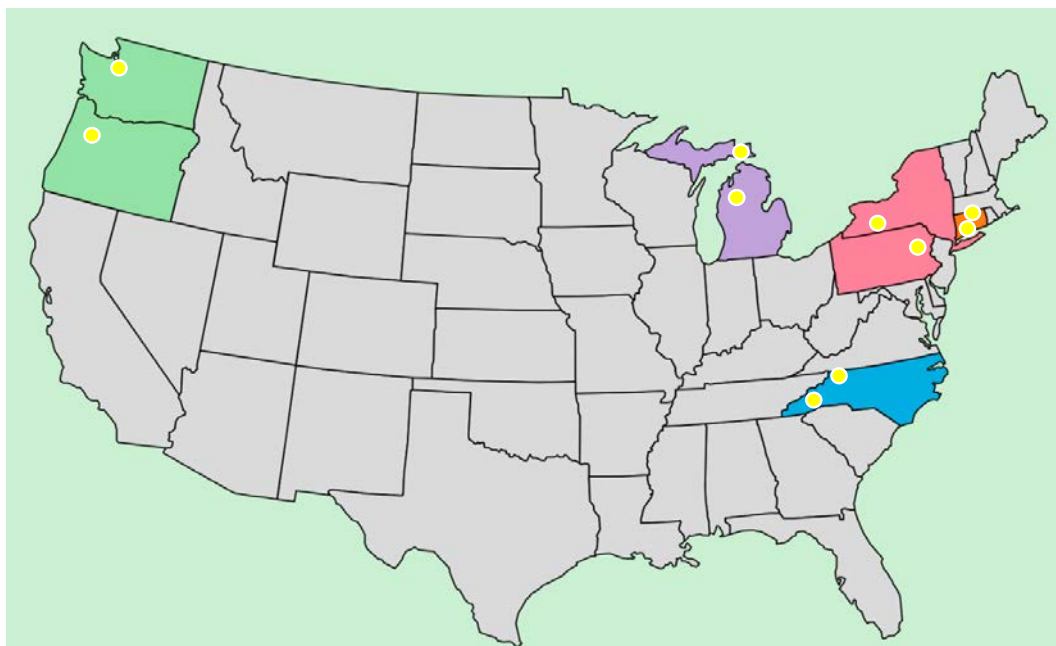
# Variation in survival and bud break of Turkish and Trojan fir in the United States

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Turkish (*Abies bornmulleriana* Mattf.) and Trojan [*Abies equi-trojani* (Aschers. et. Sint. ex Boiss) Mattf.] fir are endemic species to the Republic of Turkey. Both species have become increasingly used as

Christmas trees in Europe and North America in part due to their resistance to some common diseases and insect pests. Also, both species are better adapted to warm and dry climates than native fir species and



Number	Region	County	Latitude	Longitude	Elevation (m)	Date Measured (2014)
1	Connecticut	New Haven, CT	41° 24' 29" N	72° 54' 29" W	40	19-May
2	Conecticut	Hartford, CT	41° 57' 44" N	72° 31' 35" W	37	---
3	Michigan	Ingham, MI	46° 58' 23" N	84° 27' 57" W	262	6-May
4	Michigan	Wexford, MI	44° 24' 03" N	85°38' 06" W	341	30-May
5	North Carolina	Haywood, NC	35° 29' 23" N	82° 58' 08" W	831	13 May
6	North Carolina	Ashe, NC	36° 23' 59" N	81° 19' 04" W	899	14-May
7	Pacific Northwest	Benton, OR	44° 50' 04" N	122° 42' 44" W	294	15 April
8	Pacific Northwest	Thurston, WA	47° 02' 28" N	122° 41' 07" W	152	21-22 April
9	PA/NY	Carbon, PA	40° 53' 24" N	75° 36' 04" W	300	10-19 May
10	PA/NY	Steuben, NY	---	---	---	June

Figure 1. Location of the U.S. field trials of the Collaborative Fir Germplasm Evaluation (CoFirGE) Project.

have been accepted by Christmas tree consumers. In 2010, the Collaborative Fir Germplasm Evaluation (CoFirGE) Project was organized as a partnership of university research and extension faculty and Christmas tree grower associations in five production regions of the United States (Connecticut, Michigan, North Carolina, Pennsylvania–New York, and the Pacific Northwest) and Denmark with the goal of evaluating both species for use in the Christmas tree industry. Cones were collected from 20 mother trees each of three Turkish and two Trojan fir provenances in the fall of 2010. The seeds were germinated and seedlings were grown in a greenhouse in Oregon and in 2013, two-year old seedlings were planted into two field trials in each of five production regions in the United States (Figure 1). The field trials are being cultured according to regional Christmas tree practices.

First year survival and second year budbreak were recorded at nine sites (n=23,560 trees) and eight sites (n=22,518 trees), respectively. Budbreak at each was measured on a scale from 0 (tight bud with no swelling) to 6 (shoot fully elongated) on the dates

shown in Figure 1. Overall survival was excellent (>90%) except at two sites (3 and 10, Figure 1) with no differences among provenances. The two Trojan fir provenances broke bud earlier than the three Turkish fir provenances. The overall heritability estimate for budbreak on an individual tree basis was low (0.06) but modest on a family means basis (0.59). Both varied considerably across regions (0.04–0.47 and 0.34–0.79, respectively). The latitude, longitude and elevation of the mother tree were significantly correlated with the survival (0.29, 0.30 and 0.30, respectively) and budbreak (–0.75, –0.78 and –0.78, respectively) of its progeny. While these early results are generally encouraging, some northern studies have encountered damages from extreme winter temperatures and/or late spring frosts. Evaluation of these studies will continue through age eight years and generate considerable knowledge of how these species are adapted to the major Christmas tree production regions of the United States. Ultimately, material will be selected from these trials and grafted into regional seed orchards to provide tested material for growers.

# Using herbicides to interrupt cone development on Fraser fir

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When subjected to heat and moisture stress, Fraser fir can produce heavy cone crops on immature Christmas trees. For some North Carolina Fraser fir growers, hand-pulling cones is their most labor-intensive and expensive cultural practice on a per-tree basis. There is a treatment window in spring after cones break bud and before foliage emerges when it is possible to spray cones without damaging new growth. Starting in 2012, treatments have been made during this window using different herbicidal materials that have the potential to kill immature cones. By killing cones early in their development, foliage grows more normally and hand-pulling is unnecessary. The 2012 treatments included herbicides and agricultural adjuvants that had a history of damaging immature foliage without harming mature foliage. Household cleaning products that are used as herbicides by some organic farmers were also tested. Materials were applied to the tops of trees with cones using a solo backpack sprayer with an extended wand. Goal 2xl (oxyfluorfen) provided the first limited success in killing emerging cones without damaging mature foliage. Additional conventional herbicides were tested in 2013 and 2014. Labeled organic herbicides and tobacco sucker control products that burn foliage on contact were added to the treatment list in 2014.

In 2015, six of the most promising products were used: five organic herbicides and a tobacco sucker control product. Treatments included several rates of Scythe (fatty acid), Off Shoot-T (fatty alcohol), Axxe (ammoniated pelargonic salts), Avenger (citrus oil), WeedZap (clove and cinnamon oil), and industrial 20% vinegar. Seventeen treatments were applied to trees with cones at three different farms. High rates of Axxe, Scythe, Avenger, and Off Shoot-T killed more than 60% of cones at one location. When spray results at one farm were adjusted for windy conditions by omitting values for missed cones (those exhibiting no sign of any spray damage), Axxe and Scythe treatments killed more than 90% of treated cones and Avenger and WeedZap treatments killed more than 70% of treated cones. Extensive foliage injury was associated with 20% vinegar. Light foliage injury was associated with the highest rate (10% solution) of Scythe on some trees. For these potential cone treatments to be adopted by growers, they will need to work when applied by mechanized sprayers. In 2016 the best products will be tested using backpack, mistblower, and hydraulic sprayers.

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# GROWTH CONDITIONS AND INTEGRATED PEST MANAGEMENT

## Evaluating nitrogen source and application timing for optimal nitrogen uptake

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Application of commercial fertilizer materials has become an essential part of plantation management for many Christmas tree producers in Michigan. Most nitrogen applications are surface applied in the form of urea or ammonium sulfate. With surface applications of nitrogen there is potential for nitrogen loss from volatilization, leaching and denitrification. Some growers have begun to add nitrogen stabilizers due to concerns over the loss of nitrogen, hoping to reduce nitrogen loss and optimize plant uptake. The addition of nitrogen stabilizers can add an additional \$70.00+ per ton. We established trials in the fall of 2013 to determine if timing of nitrogen application or the choice of nitrogen fertilizer products influenced growth or foliar nitrogen values.

The nitrogen fertilizer sources that were applied were urea, stabilized nitrogen (SuperU®) and ammonium sulfate. The stabilized nitrogen source is designed to slow nitrate-N loss by including urease and nitrification inhibitors. These products were applied as split applications fall/spring or just as a spring application. In the fall of 2015 leader and lateral length and foliage samples were collected. Our initial results indicate that shoot growth did not differ among any of the treatments, including the unfertilized control. Fertilization increased foliar nitrogen levels compared to unfertilized controls but there was no difference in foliar nitrogen among fertilizer treatments.

# Integrated pest management education for the Christmas tree industry in Oregon, USA

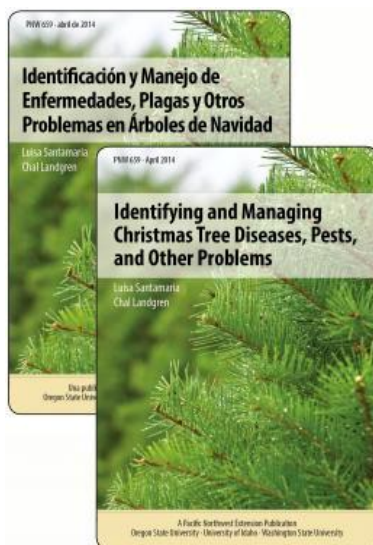
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The Pacific Northwest is the country's largest Christmas tree producer. Oregon has 63,000 acres of Christmas trees, with a sales value of \$110 million in 2013. In addition to the ongoing applied research, education for workforce and grower awareness about export threats are priority areas for Extension and outreach at the NWREC. Two new bilingual (English and Spanish) publications have been produced in the last three years to support these educational objectives. In 2012, with grant support, the field guide *Identifying and Managing Christmas Tree Diseases, Pests, and Other Problems* (Fig. 1), was developed to help Christmas tree growers and field workers implement pest management activities. It features descriptions of diseases, pests, disorders, and damage affecting Christmas trees in the Pacific Northwest, and describes how to identify and manage these problems. It includes management calendars,

susceptibility scales, over 100 color photos, and a glossary of terms. It was designed in a pocket-size, flip book format on waterproof paper for field use. Three hundred copies were produced and sold out the following year. This first edition was revised and published by Extension & Experiment Station Communications (EESC) at Oregon State University in April 2014 (PNW659). It was accepted as a Pacific Northwest Publication (Idaho, Oregon and Washington) because of the interest for the entire region.

A second field guide, *Best Management Practices for Christmas Tree Export* (Fig. 2), was developed to provide information on identifying and managing pests of concern to export trading partners. It features best management practices to help minimize the presence of pests at harvest and describes how to identify



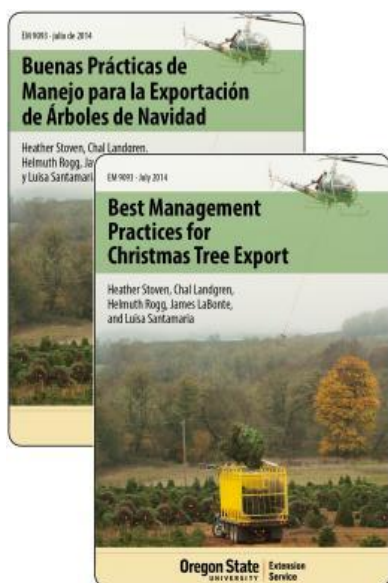
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these problems. It includes management calendars, pest quarantine information, legal considerations for exporting, and options for monitoring and trapping. It was also published by EESC in July 2014 (EM 9093). These two publications won the 2015 Silver Award in the diversity category from the Association for Communication Excellence, an organization that recognizes professional work and service in agriculture.

These two guides are intended to facilitate better communication between English and Spanish speakers and help ensure successful harvests. They have been an excellent resource to support regional IPM trainings for the Christmas tree industry, particularly targeting crew leaders and workers that only speak Spanish. Topics covered in these trainings included field scouting, pest identification, best management practices for export, use of digital microscopes, and sampling protocols. Follow-up evaluations found a 75% improvement in worker knowledge associated with scouting of Christmas tree problems after trainings.

# Management of diseases in Norwegian Christmas tree fields

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To control disease problems in Christmas trees, good knowledge about culturing methods, plant material (e.g. species and provenances), climatically conditions, soil- and nutrient parameters, biological and chemical control methods etc. is required. The best management practice is often a holistic, environmental friendly approach or so-called integrated pest management (IPM). In IPM, the goal is not to eradicate all damaging agents, but to keep the impact below a trash hold level.

Healthy transplants are of vital importance to give the production a good start and to avoid introduction of new diseases that may follow the nursery stock. Furthermore, correct identification of disease

causing agents is crucial for implementing proper management. Thus, it is useful for growers to have adequate literature available. In many Christmas tree producing countries, handbooks about diseases, pests, disorders and weed problems have been produced, recently also in Norway (Talgø and Fløistad 2015).

Management of air- and soilborne diseases requires different approaches. In general, airborne diseases may be kept at a low level by decreasing air humidity in and around the trees by planting parallel to the most predominating wind direction and not too dense. Good weed control and pruning off lower branches (making of handles) will also add to more



Figure 1. Grinding of stumps before planting a new generation of Christmas trees. Norway 2012. Photo: V. Talgø



rapid drying of foliage after precipitation. Some herbaceous weed and broad leaved trees serve as alternate hosts to rust fungi, e.g. rosebay willow herb (*Chamerion angustifolium*) and *Epilobium* spp. for silver fir needle rust (*Pucciniastrum epilobii*), bird cherry (*Prunus padus*) for cone rust (*Thekopsora areolate*) and willow (*Salix caprea*) for fir-willow rust (*Melampsora abietis-capraearum*). The rusts may be managed by controlling the alternate hosts, mechanically or by herbicides, inside and in the vicinity of Christmas tree fields. To minimize the use of pesticides (herbicides, fungicides and insecticides), application to individual trees should be considered, especially if the problem is not yet widespread. To avoid problems getting out of hand, regular monitoring for potential damaging agents is vital. The most vulnerable stage concerning both biotic and abiotic damages is during shoot elongation. That is the period when fungicide application may be necessary to control fungi like *Delphinella* shoot blight (*Delphinella abietis*) and *Neonectria* canker (*Neonectria neomacrospora*), especially in humid, coastal regions. In general, to keep the disease pressure low, pruning of diseased shoots, branches and in severe cases removal of whole trees is advisable.

Soilborne problems are mainly caused by the diseases *Armillaria* root rot (*Armillaria* spp.), annosus root rot (*Heterobasidion annosum*) and *Phytophthora* root rot (*Phytophthora* spp.). They can all do considerable

harm, but due to survival in soil for decades even without the preferred host, *Phytophthora* root rot is considered the most devastating of the three pathogens. For the former two diseases, stump removal before replanting is a good management strategy. Against *Phytophthora* root rot, well drained soil may reduce the impact, but in wet areas on heavy soil *Phytophthora* spp. will have enough moisture to thrive however well the soil is drained. Selection for more resistant hostplants is probably the best approach for the future.

Concerning both air- and soilborne diseases, we advise to clear-cut a field before planting a new generation of Christmas trees, including stump removal if necessary. By such sanitation, the disease pressure will be reduced. Equipment's for stump removal or grinding (Fig. 1) are often not affordable by growers, thus, hiring of such machinery is common in Norway. The worry concerning such practice is that *Phytophthora* spp. may spread to new areas (between farms) via infested soil. Thus, thoroughly cleaning of machinery before entering new areas is of utmost importance.

Talgø, V. & Fløistad, I. 2015. Skader i juletrerefelt – biotiske og abiotiske årsaker [Damages in Christmas tree plantations – biotic and abiotic causal agents]. *Bioforsk Fokus*: 10(5). 144 pp.

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## POSTHARVEST, MARKET AND ECONOMY

# Postharvest moisture status and quality of trees displayed in tenon-type Christmas tree stands

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Displaying Christmas trees in water holding stands has been shown to be an effective way of maintaining tree freshness, minimizing needle loss and reducing fire hazards associated with displayed trees. Water uptake during display is influenced by a number of factors, including tree species, the moisture content of the tree when it is set up, the temperature and relative humidity of the display area, how long it has been since the base of the tree was cut, the water-holding capacity of the stand, and the care the tree receives during display.

During the past few years, there has been an increased use of tenon-types of water-holding stands to display table-top trees in the United States. These stands have been used in Europe for a number of years and the concept behind them is to use a commonly available cutter to shave the end of the stem down to a uniform sized tenon that varies in length and diameter depending on the cutter that is used. The tenon is then inserted into a receptacle in the stand. In the U.S., table-top trees are sold already attached to the stand. Consumers select a tree, take it home and add water to the stand.

During the past two years, we have conducted postharvest display trials with noble (*Abies procera* Rehd.), Fraser [*A. fraseri* (Pursh) Poir], and Nordmann fir [*A. nordmanniana* (Steven) Spach] table-top trees to determine what effect tenon stands have on their freshness and quality. Trees with freshly-cut bases that were displayed directly in water maintained high moisture level and quality ratings throughout the 10 to 14 day trials. However, trees that were displayed in the water-filled tenon stands had similar moisture levels and quality ratings to trees that were displayed without water. These trees dried rapidly and by 7 to 10 days, they had dried to the point that they posed a fire hazard. Results from these trials indicated that displaying trees in water-holding, tenon-type stands was a very ineffective way of maintaining the freshness and quality of displayed trees.

# Inferring gene networks regulating needle abscission in Fraser fir through RNA-seq data

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Needle abscission (NA) is a plant physiological process that involves a few layers of cells in specific sites called abscission zones (AZ) where needles are shed. This process is triggered by endogenous factors combined with a variety of environmental signals and stresses. Fraser fir (*Abies fraseri*) has been ranked as one of the most popular Christmas tree species sold in North America and represents over 90% of all the trees grown in North Carolina as Christmas trees. Although postharvest needle retention has a prominent role in the Christmas tree industry's competitiveness with artificial trees, the physiology of the process is currently vaguely defined, and the underlying control mechanisms and gene regulatory networks are completely unclear. Fir species vary considerably in needle holding ability. Needle loss data show a high level of tree-to-tree variation among populations and individuals, and a high correlation from year-to-year within individual, suggesting a strong genetic component of this phenotypic variation. Next-generation sequencing (NGS) technologies are defining new breeding strategies for plants and animals. NGS of

RNAs (RNA-Seq) is a powerful approach to determine the relationship between the coded information in a genome, its expression and phenotypic variation. To identify key regulatory genes, we constructed cDNA libraries using AZs from Fraser fir trees that exhibit good and poor needle retention based on three years of previous phenotypic data. Differentially expressed genes that characterized trees with good or poor needle retention will be clustered into functional groups and used to reconstruct novel gene regulatory networks controlling needle abscission in Fraser fir. We are currently testing new cluster approaches and including data from other firs that exhibit extreme needle loss. Our main goal is to predict the needle retention behavior of a tree based on the expression of a pool of genes. Once a proven genetic marker system has been developed, a modeling analysis will be carried out to compare the cost and time savings of the new system relative to current assessment methods.

# Seasonal changes in balsam fir needle abscission patterns and links to environmental factors

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Several studies have suggested that postharvest needle retention increases in autumn, likely due to cold acclimation. But some of the dynamics of the phenomenon have yet to be studied. The objectives of this study were to (1) describe seasonal changes in postharvest abscission, water uptake, fluorescence, and moisture content of balsam fir (2) determine the relationship of needle abscission with water uptake, fluorescence, and moisture content, and (3) link the postharvest changes to certain environmental factors. Branches were collected from 18 trees each month and needle abscission, fluorescence (fv/fm), water uptake, and water content were monitored for 12 weeks. Fluorescence, water uptake, and water content were all correlated with needle abscission throughout the study, which added to their value as strong indicators of postharvest quality.

Further, the above 4 factors were all improved in autumn months compared to spring or summer months and strongly related to changes in

photoperiod. It is suggested that photoperiod has the most influence on seasonal changes in postharvest quality, with only little improvement attributed to temperature. Average needle abscission commencement can be described as a function of photoperiod using  $y = 109.3 - 5.7x$  ( $R^2 = 78\%$ ) and average needle retention duration can be described as a function of photoperiod using  $y = 108.3 - 4.9x$  ( $R^2 = 92\%$ ). Since each function describes a strong, negative, linear relationship it can be suggested that the shorter the photoperiod during the harvest of Nova Scotia grown balsam fir, then longer it will take for postharvest abscission to commence and complete. This implies that the ideal date for harvest would be December 21st and superior needle retention would be found in trees harvested close to this date.

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# The European Christmas tree industry – aspects of markets and production

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The European Christmas tree industry is organized in the Christmas Tree Grower Council of Europe (CTGCE) which was founded in 1989 for individual members but in 2002 was changed to an organization for national associations. Today, 13 countries are members of CTGCE – see [www.ctgce.com](http://www.ctgce.com) for further information.

The statistics for Christmas tree production in Europe are difficult to estimate since no Europe-wide report system is in place. Therefore, overall figures for the production are based upon national reports and these figures can be variable due to Christmas trees being either forest or agriculture.

We estimate that Christmas trees are produced on some 115,000 ha in Europe resulting in an annual production of approximately 75 million Christmas trees. The production is mainly focused on fir with Nordmann fir as the most dominant of these. Among

the spruces, Norway spruce and blue spruce are most commonly used. Germany has the largest production of Christmas trees in Europe whereas Denmark is the second largest and the biggest exporting country for Christmas trees in Europe.

Production cost for growing Christmas trees is highly dependent on labor costs. However, even with high Danish labor costs it can be feasible to grow Christmas trees in comparison with many agricultural crops.

# The challenges of establishing a niche market for CHO.C.O. (CHOose, Cut, Offset) Christmas tree farms in Greece

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## Introduction and objective of the research

Greece used to have a small – but quite significant for some of its semi-mountainous areas – real Christmas trees market. The producers of these cultivated trees (mostly fir, *Abies borissi-regis*) achieved a good income by selling their product in Athens and Thessaloniki, the two major cities in Greece, and the consumers were satisfied that they had the chance to have a real Greek tree for the Christmas time. However, due to several reasons, the last twenty years there is a significant decay in the market, which every year becomes worse and worse, as Figure 1 shows.

The reasons for having reached a quantity of only 40 thousand trees harvested in 2013, compared to 140 thousand trees twenty years ago, may be summed up to the following:

- the lack of suitable marketing
- competition with artificial trees
- financial crisis
- producers getting older
- low tree quality and high price

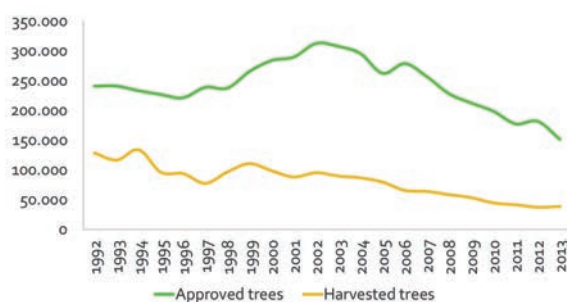


Figure 1. Approved for harvest and finally harvested trees by producers in Greece (Source of data: Greek Ministry of Environment. Figure: the authors)

On the other hand, research on real Christmas trees in Greece, if any, has been very limited, and has not given any alternatives to the producers, or the consumers.

This year (2015) is the first time that there is a fund for conducting a research in the Forest Research Institute of Thessaloniki on how to offer a new product for both the supply and demand side. The fund, named AgroETAK, a word that represents the words Innovation, Technological Development and Research in the Agricultural and Forest environment, funds almost 200 proposals for the improvement of primary sector. The finance comes both from the

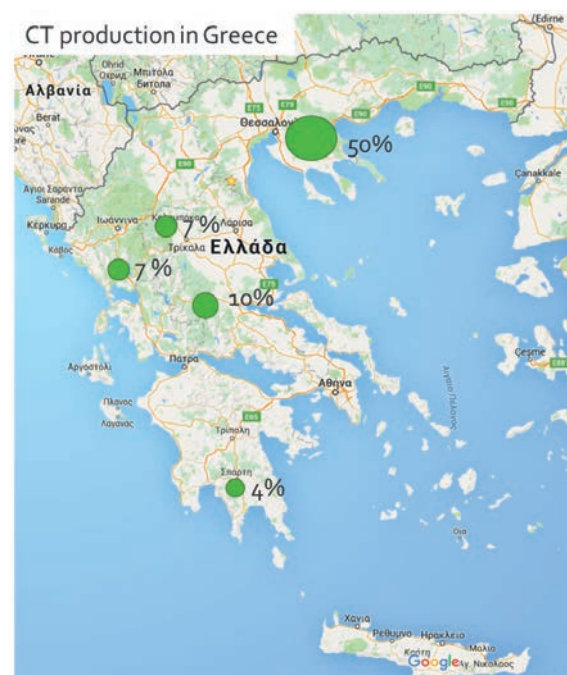


Figure 2. Christmas trees production in Greece

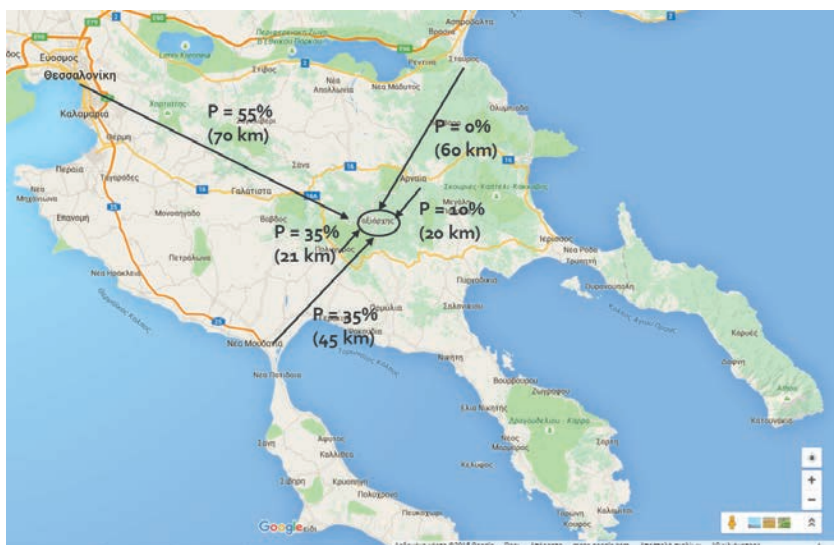


Figure 3. Distance from possible CHOCO farms (circle) and the probability P that the producers give for a customer to come to the farm

European Union and national resources. Among others, AgroETAK funds the whole program from where this research came. The program is called CHOCO project, an acronym for the words Choose, Cut, Offset.

The aim is to produce guidelines on how to implement the Choose & Cut farms for real Christmas trees in Greece, a product which is very popular in the United States of America, and Canada; how to enhance this experience by offering the opportunity to producers and consumers to offset the negative environmental impact that is produced by the activity (mainly from the added kilometers and their corresponding emissions for reaching the farm); and how to certify that the whole activity is carbon neutral. The main objective of the research project CHO.C.O. (CHOose, Cut, Offset) is to establish a niche market where some of the producers who are located close to urban areas will use a part of their Christmas tree cultivation to offer the experience of CHO.C.O. farms to a part of consumers who want to live such an experience, instead of just buying their tree in the city. The research program lasts until November 2015 and among its deliverables there is the production of a handbook for the producers and for the Public Forest Service, which supervise the harvest and transportation of the real Christmas trees, and the dissemination of the research, mainly to the producers.

In this paper we present the preliminary results of the research program, and we try to give some guidelines for the adoption of the CHO.C.O. farms by the more experienced in real Christmas trees countries.

## Methodology

The project has researched the supply (producers) and demand side (customers) for understanding if there is an interest in Greece for CHOCO farms. The research area for the supply side is the village Taxiarchis, on mountain Cholomontas in Chalkidiki peninsula in Greece, where more than 50% of the production takes place annually and the best quality trees are delivered to the markets. Figure 2 shows the map of Greece and the five main Christmas trees production areas. The research area for the demand side was the city of Thessaloniki, Greece, which is located 70 km west from Taxiarchis.

Other research included the offsetting and certification for the carbon neutrality issue of the farms, the guidelines for producing annual sustainability reports for the operation of CHOCO farms and their impacts on environment and society, and the dissemination of the work.



Figure 4. Possible CHOCO farm in the Taxiarchis region (Photo: G. Gerasimidis, CHOCO project)

## Results and guidelines

The supply side seems to like the idea of the CHOCO farms. However, there are some problems to be solved first. Most of the existing cultivations, part of which will be used as CHOCO farms, are far from satisfying road network and they may be unreachable during December, when there is a lot of rain in the area. Additionally the village is far from Thessaloniki, where the farmers give  $P = 55\%$  probability for the customers to come to the farm, as Figure 3 confirms.

The demand side favors the idea of visiting a CHOCO farm, however, they are ready to do a return trip not more than 40 kilometers, which is what the customers do worldwide as literature shows. They like that they will visit a carbon neutral farm and receive a certificate for that. Eighty percent of the customers that in the past had never bought a real Christmas tree, are ready to visit a CHOCO farm (Figure 4).

The farmers could make their CHOCO farm carbon neutral if they purchased carbon offsets from a domestic or international voluntary carbon market. The rationale for this is that the kilometers and corresponding emissions that the customers make in order to visit such a farm, would not have existed if the producers did not attract them to the farm. Therefore, CHOCO project suggests that the farmers internalize this external environmental cost and purchase carbon offsets to neutralize it. The certificate can be one from the Ministry of Environment, or the one that the Voluntary Carbon Organization gives when one purchases offsets. Finally, the CHOCO farmers could collectively publish an annual sustainability report after the Christmas period showing some of the following indicators:

- Emissions due to CHOCO farms operation
- Carbon offsets purchased
- Customers visiting the farms
- Customers visiting other local business due to CHOCO farm
- Additional workers in the farm due to CHOCO
- Other indicators that the farmers would find relevant

Some of the above findings may be relevant to international producers and CHOCO farms may become popular internationally where the pressures for carbon neutrality increase.

## Acknowledgments

This research project was funded under the Action “Research & Technology Development Innovation projects (AgroETAK)”, MIS 453350, in the framework of the Operational Program “Human Resources Development”. It was co-funded by the European Social Fund and by National Resources through the National Strategic Reference Framework 2007–2013 (NSRF 2007–2013) coordinated by the Hellenic Agricultural Organisation “DEMETER” (Forest Research Institute of Thessaloniki / Scientific supervisor: Dr Nikolaos Grigoriadis).



# Fjordtree

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The Norwegian Christmas tree grower association applied in 2012, together with the two biggest wholesalers, for grants to finance the “Fjordtree”-project. The objective of the project was to build “Fjordtree” as a brand for Norwegian grown subalpine fir (*Abies lasiocarpa*) in the premium segment of the export market. Grants and internal funding gave the project a total budget of NOK 4,500,000 (EUR 560,000).

Norway spruce (*Picea abies*) has traditionally been the Christmas tree of choice in Norway. Then Nordmann fir (*A. nordmanniana*) became popular, but in 2012 it was anticipated that a new tree would dominate the market in the future. One of the candidates was subalpine fir, which already had been planted in a relatively large scale in Norway. It was therefore a necessity to secure a market for this tree. It was also expected that the scale of the planting previous years would exceed the national market, and export would be a crucial outlet the following years.

According to the prognoses we should see the rapidly growing supply of subalpine fir already now (2015), but it seems to be absent. We are currently investigating if this is because the trees are coming later on the market due to earlier climatic problems and/or consecutive hard shearing, or that large portions of the plantings have been taken out of production due to other reasons.

We conducted the first export of 1.500 trees of the Fjordtree brand to Germany in 2014. The outlet price in Germany was approximately NOK 1200 (EUR 150). The reception was extremely uplifting and it is already demand for more trees then we can deliver in 2015.

The brand is currently owned by:

- Norsk Juletre (The Norwegian Christmas tree grower association)
- Norsk Juletre Service AS (wholesaler)
- Ligos KS (wholesaler)





# POSTER PRESENTATIONS

# Variation in postharvest needle retention characteristics of Turkish and Trojan fir populations from Turkey

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Postharvest needle retention is an important attribute of Christmas trees. Previous studies with Nordmann fir have shown that needle retention is under strong genetic control and that progeny from open-pollinated trees with superior retention also tend to exhibit the same characteristic. In 2010, cones and branches were collected in Turkey from three Turkish fir (*Abies bornmuelleriana* Mattf.) populations (Adapazarı-Akyazı, Bolu-Alada and Karabiik-Keltepe) and two Trojan fir [*A. equi-trojani* (Aschers. & Sint. ex Boiss) Mattf.] populations (Çanakale-Çan and Balıkesir-Kazdaı) as part of the international Collaborative Fir Germplasm Evaluation (CoFirGE) Project. Collections were made from 20 different trees, representing a range of elevations within each population, during the first week of October. As much as possible, cone-bearing trees showing good Christmas tree form and growth traits and spaced at least 100 meters from one another were selected to reduce relatedness. In addition to collecting cones and making a number of measurements on each of the mother trees at the time of cone collection, 4 branches were collected from each tree. The branches were collected from the upper third of the crown where each had good exposure to sunlight. To assess differences in needle retention among the trees, subtending lateral branches (“tongues”) were harvested from each branch. These were transported to Ankara and displayed without water in a room that was maintained at about 20 °C. After 10 days, the branches were gently rubbed between fingers three times and the severity of needle loss for each age class of needles (2009 and 2010) was rated

according to the following scale: 0 = no needle loss, 1 = < 1%, 2 = 1-5%, 3 = 6-15%, 4 = 16-33%, 5 = 34-66%, 6 = 67-90% and 7 = 91-100% needle loss.

Needle loss ratings among the individual trees from the Adapazarı-Akyazı, Bolu-Alada and Karabiik-Keltepe Turkish fir populations ranged from 0 - 6.8, 0 - 6.3, and 0 - 5.6, respectively. The ratings for the Çanakale-Çan and Balıkesir-Kazdaı Trojan fir populations ranged from 0 - 5.6 and 0 - 3.6, respectively. The percentage of trees within each population that had needle loss ratings <1 ranged from 35 to 50.5%. There was no difference between elevation and needle loss ratings among any of the populations of trees. This baseline data will be compared with future needle loss data collected from the progeny growing in U.S. and Denmark in common garden studies.

# Growth and postharvest needle retention characteristics of balsam fir grown in western Washington

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In 2008, a replicated common garden field trial was established at the Washington State University Research and Extension Center in Puyallup, WA to evaluate the growth and postharvest characteristics of 26 provenances of balsam fir [*Abies balsamea* (L.) Mill.] and eight progeny collections of 'bracted' balsam fir [*A. balsamea* var. *phanerolepis* (L.) Mill. var. *phanerolepis* Fernald]. A single source of Fraser fir [*A. fraseri* (Pursh) Poir] was included in the trial as a standard. Seed was obtained from the Canadian Forest Service's National Tree Seed Center (NTSC) and P+2 seedlings were outplanted in February of 2008 in a 0.44 ha plot at 1.8 m x 1.8 m spacing. The plot design was a randomized complete block with five blocks. Five trees of each source were planted in a row within each block. To obtain information on adaptability to growing conditions in western Washington, data were collected on growth, bud break growing-degree days (GDD), and color. Tree form and commercial grade were assessed in 2014, and were used to estimate the wholesale value of each tree. During fall 2012 and 2014, two branches were harvested from each tree and displayed dry to determine the postharvest needle retention characteristics of each tree. Needle loss was rated on a scale of 0 (none) to 7 (91–100% loss).

All of the balsam sources broke bud prior to Fraser fir and there was a significant difference in bud break GDD among the balsam sources. In 2014, tree heights ranged from 1.5 to 2.1 m and there was no significant difference in foliage color. Seed source had a significant effect on the estimated commercial value of trees. Average values by seed source ranged

from \$14.74 to \$27.34. There was considerable variability in value within regional seed sources. Four of the five highest value seed sources and four of the five lowest value fir seed sources were from New Brunswick. The average 2012 and 2014 needle loss ratings for the seed sources ranged from 1.4 to 4.0. Although trees from the NTSC No. 20021377 seed source were among the top five when rated for value, this source from Fairview, New Brunswick had the highest needle loss rating.

Even though WSU Puyallup is outside of the natural range of balsam and "bracted" balsam fir this study indicates that there are sources of these species that are well adapted for the production of Christmas trees in western Washington. Given that a seed source with a high tree value did not always have acceptable postharvest needle retention, care needs to be taken when selecting seed sources in order to insure the best tree quality as well as profitability.

# Effectiveness of hot water dips to eliminate slugs on exported Christmas trees

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In the United States, the Pacific Northwest (PNW) region leads the nation in the production of Christmas trees. Over 90% of the trees produced are either shipped throughout the U.S. or exported to a number of foreign countries. For example, in 2012 the Oregon Department of Agriculture and Washington State Department of Agriculture issued 2 349 and 66 federal phytosanitary certificates, respectively. Trees were shipped to 17 countries, with the bulk going to: Mexico (2 243), Canada (42), Hong Kong (41), Japan (17), and Singapore (18). A total of 283 container loads were also shipped to Hawaii.

Although most exported trees are mechanically shaken prior to shipping to reduce the risk of certain “hitchhikers” such as yellowjackets and slugs, the presence of slugs on exported trees has become a major issue in Mexico and Hawaii. In addition to mechanical shaking of unbaled trees, there has been some interest in using a “hot water shower” treatment that was developed to treat potted plants that are infested by an invasive coqui frog (*Eleutherodactylus coqui*) to rid trees of slugs. In 2012, 25% of the 67 quarantined containers that were treated in Hawaii were given a “hot water shower” at 47.7°C for 8 minutes. While this hot water treatment appeared promising, the system was very labor intensive and costly. In 2013 and

2014, a series of trials were conducted to examine the effectiveness of hot water dips in killing slugs on Christmas trees. Slugs were immersed in water that was heated to temperatures between 34.4 to 51.1°C for periods between 15 seconds and 12 minutes. Checks consisted of slugs immersed in water at 12.8°C. Noble fir (*Abies procera* Rehd.) and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] branches were also included in these tests to determine if the treatments had any adverse effects on the foliage. The shortest exposure duration/temperature that resulted in 100% mortality of all the slugs was 30 seconds at 47.7°C. A 2 minute exposure was required at 41.1°C to kill all of the slugs. Damage was only observed on branches that were exposed to >44.4°C for more than 2 minutes. These data indicated that short-duration hot water dips could be used to reduce the risk of spreading slugs on exported Christmas trees.

# Delphinella shoot blight and Grovesiella canker on *Abies lasiocarpa* in western USA

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Delphinella shoot blight, caused by the fungus *Delphinella abietis*, attacks several species of true fir (*Abies* spp.) in North America and Europe. The fungus kills current year needles (Fig. 1), and in severe cases entire shoots, and dead needles become covered with numerous, black pseudothecia. Grovesiella canker (*Grovesiella abieticola*) results in dead shoots and branches on fir and can eventually kill whole trees (Fig. 2). In Europe, the fungus has only been found in Poland (Sieber & Kowalski 1993). In 2013, in a provenance trial of subalpine fir (*Abies lasiocarpa*) and corkbark fir (*A. lasiocarpa* var. *arizonica*) at Sandpoint, Idaho, disease estimates for both pathogens were carried out on a scale from 0 to 3 (0 = no damage, 1 = minor damage, 2 = medium

damage, 3 = severe damage). Some trees were dead or missing. No attempt was made to identify the cause of mortality since most of them had already been dead for a long period. In previously published material from the provenance trial at Sandpoint, the disease described as a *Phoma*-type blight (Barney *et al.* 2013) was most likely *D. abietis* and *G. abieticola* was not mentioned. The seed sources originated from the Rocky Mountain states of Colorado, Utah, and New Mexico and from the mountains of Arizona. The fir trial was established in 2001 with a total of 960 trees [3 replicates (blocks) of 16 randomly distributed subplots (seed sources) with 20 trees in each]. The 16 seed sources included six corkbark fir (Apache-Sitgreaves, Cibola, Coconino, Coronado, Gila and



Figure 1. *Delphinella abietis* on subalpine fir (*Abies lasiocarpa*) at Sandpoint, Idaho, USA 2013. Photo: V. Talgø



Figure 2. Subalpine fir (*Abies lasiocarpa*) with dead branches due to *Grovesiella abieticola* at Sandpoint, Idaho, USA 2013. Photo: V. Talgø

Santa Fe) and ten subalpine fir (Arapaho, Carson, Cibola, Dixie, Kaibab, Manti-La Sal, Rio Grande, San Isabel, San Juan, Uncompahgre). Corkbark firgrows in native stands between 8000 – 12000 feet (2438 – 3658 m), while subalpine fir is found between 2000 – 11000 feet (610 – 3353 m). Significant differences between provenances in susceptibility to *D. abietis* and *G. abieticola* were observed. In general, subalpine fir was more susceptible to both diseases than corkbark fir. This was also reported by Barney *et al.* (2013) for the *Phoma*-type blight, and corresponds well with results from a provenance trial in Norway, where the general outcome was that susceptibility to *D. abietis* decreased with increasing altitude of the seed source and increased with the latitude (less blue/waxy varieties) (Talgø *et al.* 2015). The four subalpine fir provenances of Uncompahgre, Manti-La Sal, Dixie and Arapaho were more susceptible to both diseases. In 2013, *D. abietis* was also found on subalpine fir in the lowland of Washington State, but neither disease was detected in native stands at Mt. Rainier, Mt. Spokane, Sherman Pass or Frazer Creek.

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# Utilizing webinars to increase the adoption of integrated pest management

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In October 2013, Michigan State University Extension launched an online, on-demand series of webinars focused on increasing grower and educator awareness of IPM (integrated pest management) resources, practices, history and implications. From December 2013–December 2014, available webinars include; Introduction to Integrated Pest Management, Integrated Pest Management Resources, Entomology 101, Plant Pathology 101, Soil Science 101, Plant Science 101 and Insect Scouting in Fruit Crops. Webinar viewing was incentivized by partnering with the Michigan Department of Agriculture and Rural Development to provide continuing education credits for certified pesticide applicators. This approach to content delivery proved popular and allowed MSU Extension to access traditionally underserved audiences in Michigan as well as new national and international participants. The program was evaluated using an online pre- and post- survey of viewers. During the first ten months,

there were 1,663 webinars viewed. An approximate 430 viewers reported an acreage impact of 1.2 million acres. Approximately 30% identified as growers, 20% landscapers, 19% recreational gardeners, 13% crop consultants, 10% agriculture educators, 8% general public, 5% pesticide distributors, 3% students, and 0.4% policy makers. Based on the preliminary evaluation of the MSU IPM Webinar Series, prerecorded and on-demand webinars offer an affordable and accessible way for stakeholders to access University resources and an efficient means for garnering a wider audience for those resources and increasing the adoption of IPM practices.

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# A summary of BCTGA UK trials in Christmas trees 2013 to 2015

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With many UK Nordmann Fir plantations now entering their third rotation, pests, diseases and weeds have become an increasing problem for growers. To improve the knowledge base in the UK the BCTGA have conducted a development programme to study these problems and identify potential solutions.

Initial studies have included trials on Current Season Needle Necrosis, Silver Fir Woolly Aphid (*Dreyfusia nordmanniana*), improved crop establishment, glyphosate rainfastness, crop tolerance to post emergent herbicides, Nordmann Fir leader control and optimum Nordmann Fir provenance selection.

The BCTGA wishes to thank the many agrochemical companies who provided products for these trials, and to Maitland who provided the plants for the provenance studies. Particularly thanks go to Agrovista, Bayer Environmental Science, Nufarm UK and Nutrel UK who also sponsored some of this work.



## **British Christmas Tree Growers Association**

### **Summary of Field Development Trials, 2013 to 2015**

(Typically 1 or 2 trials per study with 3 or 4 random replicates of 10 to 20 trees)

2013

#### **1. Crop Establishment Enhancers - Year 1.**

Outline: Three year trial to assess effectiveness of fertilisers, seaweed extracts, mycorrhiza, bio-stimulents & sterilants applied at planting, or monthly April to September to encourage increased growth.

Result: *No improvement to year 1 growth was recorded, but the slow release fertiliser did minimise a botrytis attack.*

#### **2. Leader Control in Nordmann Fir - Year 1**

Outline: To compare the effectiveness, and potential crop effects of 1-naphthylacetic acid, ethephon, prohexadione, Topstop pliers & the Rapstrap for the control of Nordmann Fir leader length.

Result: *All treatments reduced leader length, with 1-naphthylacetic acid having the greatest effect, but with some needle damage.*

#### **3. Control of Silver Fir Woolly Aphid - Year 1**

Outline: To evaluate the effectiveness of pirimicarb, deltamethrin, acetamiprid, flonicamid and spirotetramat on *D.nordmannianae*.

Result: *Deltamethrin controlled 81% of the adelgid in early April. Acetamiprid was the most effective treatment applied at mid flush.*

#### **4. Control of Current Season Needle Necrosis - Year 1**

Outline: To assess the effects of multiple applications of a range of fungicides including triazoles, strobilurins, dithianon, kresoxin-methyl, boscalid, and phosphite applied in the autumn and the spring.

Result: *No fungicide treatment was effective & many damaged the crop, particularly EC & SC triazoles applied at bud burst.*

2014

#### **1. Crop Establishment Enhancers in Nordmann Fir - Year 2**

Outline: As 2013.

Result: *No improved growth from bio-stimulants. All granular fertilisers resulted in 6-10% increase in leader growth.*

Monthly phosphite applications resulted in a 40% increase in leader length.

#### **2. Leader Control in Nordmann Fir - Year 2**

Outline: As 2013 but excluding prohexadione and adding half rate ethephon.

Result: *In unusually good growing conditions only 1-naphthylacetic acid was effective.*

#### **3. Control of Silver Fir Woolly Aphid in Nordmann Fir - Year 2**

Outline: As 2013 + imidacloprid & thiacloprid. Also basal treatments of thiacloprid and flonicamid plus soil injection of imidacloprid.

Result: *All treatments were effective, with deltamethrin applied early April providing 99% control & imidacloprid soil injection 95%.*

#### **4. Control of Current Season Needle Necrosis in Nordmann Fir - Year 2**

Outline: As 2013 with damaging fungicides replaced by WDG formulations, and the addition of two biostimulants and phosphite.

Result: *No fungicide treatment was effective & many damaged the crop, including some of the WDG formulations.*

#### **5. The Tolerance of Nordmann Fir and Norway Spruce to Post Emergence Herbicides applied x1 and x3.**

Outline: A range of herbicides were applied September, pre flushing and at full flush. Treatments included glyphosate +/- oil, diflufenican +/- glyphosate, flazasulfuron +/- glyphosate, foramsulfuron + iodosulfuron +/- oil, amidosulfuron, metsulfuron methyl, flumioxazin, fluroxypyr, metamuron, propyzamide+pendimethalin, rimsulfuron, tribenuron-methyl, sulfosulfuron, prosulfocarb.

Result: *With the exception of glyphosate + oil, all September treatments were tolerated by the crop. No unacceptable crops effects were noted from the pre flushing application at single or treble rate. Full flush applications of pendimethalin, metamitron and propyzamide produced no crop damage, and amidosulfuron was moderately tolerated. All other post flush treatments were damaging.*

2015

#### **1. Crop Establishment Enhancers in Nordmann Fir - Year 3**

Outline: As 2013.

Result: *All granular fertilisers, and particularly nitrogen improved growth as did the materials phosphite & Lysaplant. Agroblen, Microfem, Smartgrass, Hydro-complex and Optimin2412 + Nitro 30 all produced in excess of a 15% improvement in plant density.*

#### **2. Leader Control in Nordmann Fir - Year 3**

Outline: Multiple applications of 1-naphthylacetic acid, ethephon were studied alone, and following the use of Topstop pliers.

Result: *Topstop pliers followed by 1-naphthylacetic acid was the most effective treatment, but the Topstop / ethephon treatment was also effective. All treatments of ethephon alone reduced the number of >35cm leaders.*

#### **3. Control of Silver Fir Woolly Aphid in Nordmann Fir - Year 3**

Outline: A comparison of April treatments plus a mid summer treatment to control the adelgids hatching in August.

Result: *Deltamethrin outperformed thiacloprid & flonicamid at the April timing. August flonicamid treatment was also effective.*

#### **4. Control of Current Season Needle Necrosis in Nordmann Fir - Year 3**

Outline: With fungicide applications proving unsuccessful, the use of calcium to improve cell wall strength to protect against fungal attack was studied. CaNo3 was applied as a granular fertiliser or as multiple foliar feeds with or without additives.

Result: *Initial foliar analysis suggest that no treatments was successful in raising Ca levels, although Silicon additives may assist.*

#### **5. The Influence of Bud Selection on subsequent leader straightness when Cutting Long Leaders of Nordmann Fir.**

Outline: Long leaders were cut in March above large buds, small buds & double buds, & compared with total leader removal.

Result: *No differences in leader straightness were noted from bud selection. Total leader removal produced the most straight leaders.*

#### **6. The Influence of Formulation and added Adjuvant to the Rainfastness of Glyphosate**

Outline: Earlier work indicated that the addition of adjuvants made glyphosate rainfast in 1 hour of heavy rain. This trial used 2.5l/ha 360g/l glyphosate & studied the influence of formulation and additives when light rainfall fell between 10 minutes and 1 hour.

Result: *Glyphosate alone controlled most grasses when light rain fell after 10 minutes. Perennial broadleaves were not controlled after light rain, but weed control was improved by the addition of adjuvants.*

#### **7. Identifying Optimum Provenances on a variety of Soil Types and Locations in England & Scotland**

Outline: Five trials have been established to compare 5 wild seed provenances with 5 from seed orchards on various soils & locations.

Result: *This is an 8 year trial, with first assessments on flushing date & plant form to commence in year three.*

# UK trials 2013 to 2015 for the control of Current Season Needle Necrosis (CSNN) in Nordmann fir using fungicides and calcium treatments

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CSNN has been a minor problem in the UK for many years, but since 2011 many UK plantations have been severely affected, with particularly bad attacks in 2012 and 2013. Previous work has identified that the associated pathogen *Sydowia polyspora* can be controlled by some fungicides in the laboratory, but effectiveness of fungicides has not been confirmed in the field. UK Field trials were conducted in 2013 and 2014 using multiple applications of a wide variety of fungicides from a diverse range of fungicide groups including triazoles, strobilurins and mixtures at autumn and spring applications. The work confirmed that field applications of fungicides at these timings and rates did not appear to control the condition. However, severe crop scorch was recorded from a number of applications, particularly EC and SC formulations of triazoles including cypraconazole and tebuconazole applied at bud swelling.

In 2015, work was predicated on reducing pathogen access by using soil and foliar applications of calcium nitrate, with foliar applications applied with and without additives claimed to have been successfully used in other horticultural sectors. The results from these applications are currently being analysed.



## British Christmas Tree Growers Association

### UK trials 2013 to 2015 for the control of Current Season Needle Necrosis (CSNN) in Nordmann Fir using fungicides and calcium treatments.

\* CSNN has been a minor problem in the UK for many years.

From 2011 many UK plantations have been severely affected, with particularly bad attacks in 2012 and 2013.

\* Previous work in Scandinavia has identified that the associated pathogen *Sydowia polyspora* can be controlled by some fungicides in the laboratory, but effectiveness of fungicides has not been confirmed in the field.

\* Other work has suggested that calcium is moved from the new needles in June to develop root structure. It is further suggested that infection may be reduced or avoided if calcium levels can be retained during this period to avoid weakening cell walls and increasing the risk of damage from infection.

\*Field trials in the UK in 2013 / 2014 were undertaken to further explore the role of fungicides, and in 2015 to identify potential means of increasing calcium levels in the new needles.

#### 2013 UK Field Trials

Infection level (untreated trees): 14% of trees in August 2012. 1.2% infection in July 2013.

Application T1 - T3: Every 14 days - 30th August to 28th September, T4 = bud swelling, T5 = early flush, T6 = full flush

No assessment of effect on CSNN was possible due to the low rate of infection in 2013, however, crop effects from fungicide treatments from the application (300l/ha) made at bud swelling were as follows:

Fungicide	Rate/ha	Scorch	Fungicide	Rate/ha	Scorch
0. epiconazole + pyraclostrobin + fluxapyroxide	3.0l/ha	High	7. prochloraz	1.0l/ha	Low
1. cyproconazole	0.5l/ha	Severe	8. kresoxin - methyl	200g/ha	Low
2. tebuconazole + trifloxystrobin	1.0l/ha	Severe	9. dithianon	750g/ha	Low
3. fluoxystrobin + prothioconazole	1.5l/ha	High	10. boscalid + pyraclostrobin	800g/ha	Low
4. tebuconazole	0.5l/ha	High	11. thiophanate methyl	1100g/ha	Low
5. cyproconazole+picoxystrobin	1.0l/ha	Severe	12. phosphite	9.0l/ha	Low
6. cyproconazole+azoxystrobin	1.0l/ha	Severe	13. fenamidone + fosetyl aluminium	30kg	Very low
C1. Unsprayed control		Very low	C2. Unsprayed control		Very low

#### 2014 UK Field Trials

This trial predominantly used Water Dispersible Granule formulations in an attempt to minimise any crop scorch.

Infection level: 38% of trees in 2013. 48% of trees in 2014.

Application: T1 = bud swelling, T2 = early flush, T3 = full flush. Application volume: 150l/ha.

Scoring: 1 - 9. 1 = no necrosis, 9 = all 2014 needles lost. Mean infection of CSNN on 30 trees:

Fungicide	Rate/ha	CSNN Score	Fungicide	Rate/ha	CSNN Score
1. Unsprayed	-	5.6	7. boscalid + pyraclostrobin	800g/ha	6.0
2. copper oxychloride	6 l/ha	5.8	8. phosphite	5.0l/ha	5.3
3. tebuconazole	500ml/ha	Not assessed	9. phosphite + Lysaplant	" +100ml/ha	4.7
4. tebuconazole+trifloxystrobin	400g/ha	5.3	10. fosetyl + propamocarb	2.5l/ha	7.5
5. kresoxin - methyl	200g/ha	4.5	11. cyprodinil + fludioxonil	1kg/ha	6.0
6. dithianon	750g/ha	5.5			

**Discussion:** These results demonstrate that none of the fungicide treatments tested protected the trees from infection. Severe crop effects were noted from the application of tebuconazole +/- trifloxystrobin, resulting in abandoning the tebuconazole plots.

#### 2015 UK Field Trials

Two trials examined the effect on calcium levels of soil applied granular fertilisers, and foliar applications applied alone & with and with additives claimed to have been successfully used to improve cell wall structure in other horticultural crops.

Granular fertilisers were applied on March 28th 2015, and foliar applications made every 14 days from April to early July.

CSNN infection level on the trial sites: 30 - 40% of trees in 2014. <10% of trees in 2015.

Preliminary Assesments	CSNN*		% Ca**			CSNN*		% Ca**	
	MC	St	MC	St		MC	St	MC	St
1. Everris Ca + N Controlled Release	2	2	0.54	0.47	8. Yara Nitrobor (N15.5% Ca26.3%+Bo)	1	3	0.30	0.52
2. Everris Agroleaf Power 11-5-19 + 9CaO	3	5	0.46	0.50	9. Yara Liva Calcinit 15%N+19%Ca + Boron	5	4	0.33	0.45
3. Everris Ca+N ContRel./Agroleaf Power	1	2	0.28	0.38	10. Yara Nitrobor / Yara Liva Calcinit	1	0	0.36	0.52
4. " / " + phophite	0	3	0.46	0.40	11. " / " / Ametros	3	2	0.37	0.50
5. " / " + Ametros	4	4	0.50	0.48	12. " / " / Cal-Silic	0	3	0.46	0.53
6. " / " + Megafol	3	2	0.34	0.44	15. Cal-Silicate (Compo)	1	1	0.42	0.42
7. " / " + Cal-Silicate	0	1	0.57	0.47	16. Ametros (Agrovista)	2	2	0.41	0.41
					18. Untreated	4	3	0.35	0.43

\* No of trees (out of 30). \*\*Foliar analysis taken end August 2015.

**Discussion:** None of the materials tested have consistently increased Ca levels in the crop, although many treatments did assist the uptake of other nutrients, particularly Mn & Mg. None of the treatments prevented the occurrence of CSNN in the treated trees.

# UK trials 2013 to 2015 for the control of silver fir woolly aphid (*Dreyfusia nordmanniana*)

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Past control of the silver fir woolly aphid (*Dreyfusia nordmanniana*) in the UK has largely been through the use of synthetic pyrethroids in January / February or at full flush. These control strategies were felt to be sub optimal, and likely to reduce the populations of beneficial predatory insects. Field trials were designed to suggest effective control through better selection of insecticide and accurate timing.

In 2013, trials showed that deltamethrin in early April provided excellent control of the pest, while the neonicotinoid acetamiprid was highly effective post flushing, with less effect of predatory insects expected.

In 2014 & 2015, trials confirmed the deltamethrin effectiveness and timing, and found thiacloprid to be the superior neonicotinoid for use during flushing. The 2014 trials also assessed the effectiveness of basal sprays and soil injection of systemic insecticides, used to minimise effect on non target insects. Both techniques provided acceptable results, but tended to be less effective than foliar sprays.

The 2015 field trials also included August applications of acetamiprid and flonicamid applied to the late summer generation prior to wax formation. Results from these applications are currently being assessed.



## **British Christmas Tree Growers Association (BCTGA)**

### **UK trials 2013 to 2015 for the control of Silver Fir Woolly Aphid *Dreyfusia nordmanniana***

Control of the Silver Fir Woolly Aphid *Dreyfusia nordmanniana* in the UK has largely been through the use of synthetic pyrethroids in January / February, or at full flush. These control strategies were felt to be sub optimal, and likely to reduce the populations of beneficial predatory insects.

Field trials were designed to suggest effective control through informed selection of insecticide and accurate timing.

#### **2013 UK Field Trial**

This trial compared the effectiveness of insecticides applied alone at bud burst (20 May) and at early flush (21 June) or following deltamethrin pre bud burst (5th April). The untreated area had received an unsuccessful deltamethrin in January. The untreated trees had a mean of 3.56 adelgids per 10 shoots per tree.

Treatments applied 20/5 & 12/6	Rate /ha	No deltamethrin 5 April		Following deltamethrin 5 April		
		Aphids*	% control	Aphids*	% control	
None	-	356	0%	28	81.1%	
deltamethrin	300ml	-	-	12	96.6%	
flonicamid	140g	190	47%	2	99.4%	
acetamiprid	250g	14	86%	0.7	99.8%	
pirimicarb	480g	360	0%	14	96.1%	
spirotetramat	500ml	196	46%	1.2	99.6%	* Number of aphids per 10 trees
imidacloprid**	178.5	633	0%	32	91.0%	** As a soil drench

**Discussion.** The pre bud burst application of Decis removed over 80% of the adelgids, facilitating all of the post flushing sprays to reduce the remainder of the aphids. Acetamiprid was the only product in this trial to successfully control the adelgid in the absence of the pre bud burst deltamethrin. The lack of control from January applied deltamethrin is noted.

#### **2014 UK Field Trials**

These trials were undertaken to confirm the 2013 results and to assess the effectiveness of basal sprays and soil injection of systemic insecticides as a means of minimising effects on non target insects. A second trial studied the effectiveness of controlling an existing heavy infestation when no early treatment had been applied.

Application Site 1: T1: End February, T2: Mid April (egg hatch) , T3 Mid Flush. Application Site 2: 21st May (mid flush)  
Application volume (foliar) 300l/ha.

Infestation: Site 1: Mean 22.6 adelgids on three laterals per tree. Site 2: 42% of trees had moderate to severe infestations. Five branches per tree were assessed through a 1 - 9 scoring system.

Treatment	Rate	Timing	Application	Site 1		After April deltamethrin		Site 2
				Aphids	% control.	Aphids	% control.	% control.
0. Unsprayed	-			104	0%	-	-	0%
1. deltamethrin	300ml	T1	Foliar	99	4.8%	-	-	-
14. acetamiprid	250g	T3	Stem	28.6	72.5%	-	-	31%
13. imidacloprid	178.5ml	T3	Stem	25.4	75.6%	-	-	55%
3. imidacloprid	178.5ml	T3	Foliar	25.0	76.0%	0.3	99.7%	49%
7. flonicamid	140g	T3	Foliar	12.3	88.2%	1.4	98.7%	-
4. spirotetramat	500ml	T3	Foliar	10.4	90.0%	0.8	99.2%	31%
6. acetamiprid	250g	T3	Foliar	7.9	92.4%	0.1	99.9%	41%
15. imidacloprid	178.5g	T2	Soil Inject.	5.1	95.1%	-	-	42%
5. thiacloprid	375ml	T3	Foliar	3.7	96.4%	0.6	99.4%	80%
2. deltamethrin	300ml	T2	Foliar	1.3	98.7%	-	-	-

**Discussion:** The importance of deltamethrin at egg hatch was confirmed. Basal sprays and soil injection provided acceptable results where infestations were relatively light (Site 1), but tended to be less effective than foliar sprays. Thiacloprid was the only material tested to provide control on Site 2 under severe infection pressure.

#### **2015 UK Field Trials**

In order to study the use of insecticides less likely to control beneficial insects, flonicamid and thiacloprid were tested as alternatives to deltamethrin at the early April timing. A second set of treatments studied the use of flonicamid and acetamiprid to control the second August generation of adelgid.

Infestation pressure: Mean of 45% of trees had adelgid infestations. Application volume: 280l/ha

April 10th Treatment	Rate	% control	August 11th Treatment	Rate/ha	% Control
1. deltamethrin	300ml	93%	5. flonicamid	140g	82% (adults) 86% (egg sacs)
2. flonicamid	140g	0%	6. acetamiprid	250g	87% ** 94% **
2. thiacloprid	375ml	75%	7. Unsprayed	-	-
4. Unsprayed	-	-			

**Discussion:** This study suggests that deltamethrin remains the preferred April treatment for adelgid control.

Poor control by April applied flonicamid reflects inadequate systemic activity at this timing.

Applications in August of both flonicamid and acetamiprid were effective in controlling adults and egg production.

# Baiting for *Phytophthora* in waterways associated with Christmas tree production in Norway, Belgium and Denmark

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## Impact of *Phytophthora* spp. in Christmas tree fields

*Phytophthora* root rot on true firs (*Abies* spp.) caused by several *Phytophthora* spp. is a serious disease in the Christmas tree and bough production in USA, but there are very few reports from Europe (Talgø & Chastagner 2012). In Norway, we found that *P. cambivora* killed noble fir (*A. procera*) intended for bough production (Talgø *et al.* 2006) and *P. megasperma* and a *P. inundata*-like species killed subalpine fir (*A. lasiocarpa*) and Nordmann fir (*A. nordmanniana*) Christmas trees, respectively (Talgø *et al.* 2007).

## Pathways for introduction and spread of *Phytophthora* spp.

Most *Phytophthora* spp. are soil borne and may spread locally by infested soil on equipment's, vehicles, footwear etc. Over longer distances, *Phytophthora* spp. are often spread as stowaways on traded, rooted plants. In the Norwegian case where the *P. inundata*-like species killed Nordmann fir, there were clear indications that the pathogen had arrived by imported nursery stocks. In Europe, there is an extensive trade with fir seedlings, thus, *Phytophthora* spp. may also become a problem for the Christmas tree industry in other European countries.

## Baiting method and locations

A simple way to survey for *Phytophthora* spp. in an area is by baiting in waterways. Different leaves may be used as bait, but for surveys in the Christmas tree

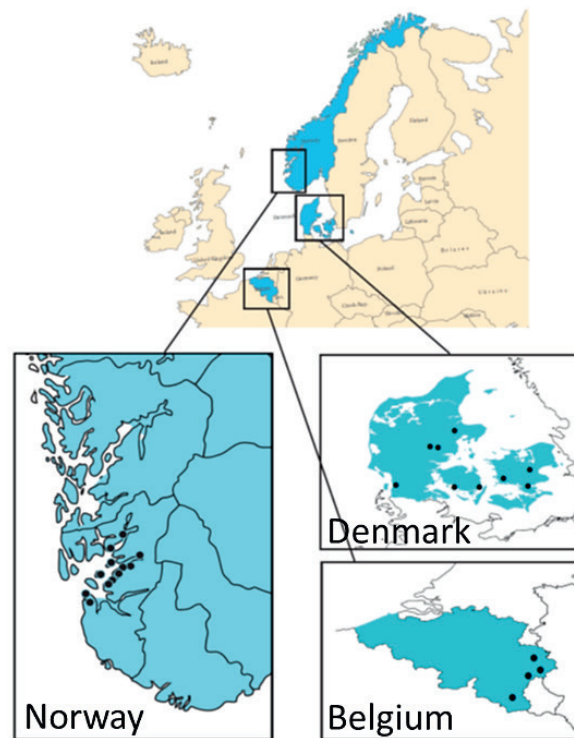


Figure 1. Maps indicating where baiting for *Phytophthora* spp. was carried out in Norwegian, Belgian and Danish Christmas tree plantations (black dots).

fields in Norway, Belgium and Denmark, mature leaves from *Rhododendron* 'Cunningham's White' (not surface sterilized) were used. The leaves were placed in net bags and left in ditches, small streams or drains for one week. Each bag had a styrofoam floating device to keep the baits near the surface and the bags were anchored to the shore. After baiting, the leaves were rinsed gently under tap water. Black





Figure 2. Nordmann fir (*Abies nordmanniana*) in a Christmas tree field where *Phytophthora inundata* was isolated from infected tissue on cankered stems. The pathogen was also detected by baiting water in a ditch nearby. Norway 2012. Photo: V. Talgø

or water soaked spots on the *Rhododendron* leaves indicated *Phytophthora* infections. Sections from the leading edges of such spots were dissected and used for isolation. Locations where baiting took place in the respective countries are indicated in Fig. 1 and further described below.

### Baiting in Norway

The baiting was carried out in streams, ditches or lakes in 12 locations in Rogaland County in southwestern Norway in 2012. All selected waterways for baiting were within, or directly fed by runoff from, Christmas tree plantations. No surface sterilization was carried out before plating on *Phytophthora* selective medium (P<sub>10</sub>ARPH - 17 g corn meal agar, 1000 ml distilled water, 0.4 ml pimaricin, 0.25 g ampicillin, 0.01 g rifampicin, 1.0 ml dimethylsulfoxide, 0.1 g pentachloronitrobenzene, and 0.035 g hymexazol). The plates were kept under laboratory conditions (room temperature and daylight). This resulted in isolation of several *Phytophthora* spp., some from multiple locations: *P. plurivora*, *P. gonapodyidis*, *P. inundata*, *P. cryptogea* and some isolates that we were not able to identify to species level. Where *P. inundata* was found, the pathogen was also isolated from diseased Nordmann fir (Fig. 2).



Figure 3. Method used to bait for *Phytophthora* from water in Belgium. Photo: S. Schmitz

### Baiting in Belgium

In 2015, baiting was carried out in streams running in the lower parts of Christmas tree fields of Nordmann fir in Southern Belgium (Wallonia). Four baiting sites were selected. At each baiting site, three bags (Fig. 3) were set at a distance of 5–10 meters from each other. Pieces from leaves showing spots, were disinfected in bleach for 30 sec, rinsed in sterile water and placed onto medium (PARP) at 20°C in the dark. Hyphal growth resembling *Phytophthora* were sub-cultured and hyphal tip cultures were produced. *Phytophthora* spp. were isolated from two of the four baiting sites. Two species of *Phytophthora* were identified: *P. cambivora* and *P. gonapodyides*.

## Baiting in Denmark

The baiting in Denmark took place in 10 locations in 2015. The same procedure as in Norway was followed. From infected *Rhododendron* leaves (Fig. 4) the following *Phytophthora* spp. were isolated; *P. gonapodyides*, *P. lacustris*, *P. plurivora* and *P. syringae*.

## Identification of isolates

All isolates were identified to species level by sequencing their ribosomal Internal Transcribed Spacer (ITS) regions. Briefly, DNA was extracted from mycelium on V8 (300 ml filtered tomato juice, Carrefour Belgium diluted in 1.2 l demineralized water with 5.6 g CaCO<sub>3</sub> and 15 g bacto-agar) (Belgium) and PDA (potato dextrose agar) (Norway, Denmark) plates using a commercial kit. PCR was carried out with the primers ITS4 and ITS5 (Denmark, Norway) or primers ITS2 and ITS5 (Belgium), and amplification products were sequenced. A BLAST search was carried out to compare the sequences to ITS sequences available in GenBank.

## Conclusion

*Phytophthora* spp. were detected in several samples from water in Christmas tree fields in Norway, Denmark and Belgium. It is important to bear in mind that *Phytophthora* spp. may be present in an area without causing mortality of fir, especially on well-drained soil. However, if infested water is used for irrigation or floods occur, the infection level may increase. Impact also depends on how resistant the host plants are, and the aggressiveness of the *Phytophthora* spp. in question.



Figure 4. Dark spots and patches on *Rhododendron* leaves after baiting in water in a Danish Christmas tree field in 2015.  
Photo: V. Talgø

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# Scleroderris canker found on Nordmann fir in Norway

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In 2013, scleroderris canker caused by *Gremmeniella abietina* was found on Nordmann fir (*Abies nordmanniana*) in a Christmas tree fir in Vest-Agder County in southern Norway. *G. abietina* has never been reported on this host in Norway or elsewhere. Pycnidia with conidial spores of the *Brunchorstia* stage of the fungus were detected around dead buds (Fig. 1). The bark of the diseased area had resin droplets and was slightly sunken and cracked. There was a sharp margin between dead and living tissue, from where the fungus was isolated. The fungus was also isolated from spore mass. The internal transcribed spacer region (ITS) sequence of the nuclear ribosomal DNA from the Norwegian isolate from Nordmann fir was identical (100%) to several *G. abietina* reported to GenBank, but different from *G. abietina* var. *balsamea* (less than 97% similarity). *G. abietina* attacks a number of conifer hosts in Europe and North America, especially pine (*Pinus* spp.). In Norway, the latest epidemic by this fungus occurred on Scotch pine (*P. sylvestris*) in 2001 in the southeastern part of the country. The following year, Norway spruce (*Picea abies*) seedlings damaged by *G. abietina* were found in forest nurseries in the same area (Talgø & Stensvand 2003). *G. abietina* var. *balsamea* has been reported on balsam fir (*A. balsamea*) in North America and on Sachalin fir (*A. sachalinensis*) in Japan (EPPO 2009). Infections by *G. abietina* may occur via wounds throughout the growing season, but primarily new needles become infected in June–July in Norway. From the needles, mycelia may grow into the shoots and cause canker



Figure 1 Dead shoot of Nordmann fir (*Abies nordmanniana*) with a pycnidium of *Gremmeniella abietina* that had cracked open and exposed the whitish conidial spore mass. Photo: V. Talgø

wounds and sometimes girdling. The development of disease primarily takes place at low temperatures, and the fungus may grow down to 0 °C. Thus, symptoms may not be prominent until the next spring, and therefore infected seedlings may become marketed with latent infections.

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Talgø, V. & Stensvand, A. 2003. *Gremmeniella abietina* (furusas knopp og greintørke). Grønn kunnskap e 7(101):2 pp.

# Characterization of postharvest quality of two French Christmas trees: Nordmann fir (*Abies nordmanniana*) and Norway spruce (*Picea abies*)

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Currently, 90% of all Christmas trees sold in France are cut trees. Consumers buy their firs earlier and earlier, but they want them still “fresh” until the beginning of January. The “freshness” notion covers criteria such as needle density, colour and brightness. The needle losses after consumer purchase is a major concern to the industry. Therefore, it is important to better understand the factors involved in maintaining the quality, upstream and downstream the sale of the Christmas Tree. The objective of this study was to characterize the “freshness” as to find criteria for warrant the quality, on the two majors species grown and sold in France: *Abies nordmanniana* and *Picea abies*. Three provenances with contrasted climate, from oceanic to continental (Finist re, Beauce and Morvan), were compared to evaluate the environment effect on trees postharvest quality. Biophysical and physiological criteria such as needle water content, xylem pressure potential, needle colour, biochemical content, were evaluated periodically throughout the harvest, distribution and consumption phases. Photosynthetic activity as measured just before the harvest. Temperature and hygrometry were continuously monitored during the postharvest phases. Global esthetical aspect of trees was also evaluated after the consumption phase. Preliminary results showed that needle water content and colour were maintained until the trees were placed indoors (3 weeks after harvest), only xylem pressure potential decrease previously. The two species differed in particular on their colour characteristics. The *A. nordmanniana* were greener at harvest and turned less yellow than *P. abies* during the consumption phase. Moreover, *P. abies* had a poor needle retention compared to *A. nordmanniana*. In

the same way, the colour characteristics also enabled to distinguish provenance: trees from Finist re were more yellow than others. In conclusion, it was clear that the critical phase was when trees were placed in drying conditions (consumption phase) and it might be possible to slow down this degradation. The next step will be to test improved process such as hydration, to maintain much longer the postharvest quality of Christmas trees.



## POSTHARVEST QUALITY OF CHRISTMAS TREES IN FRANCE

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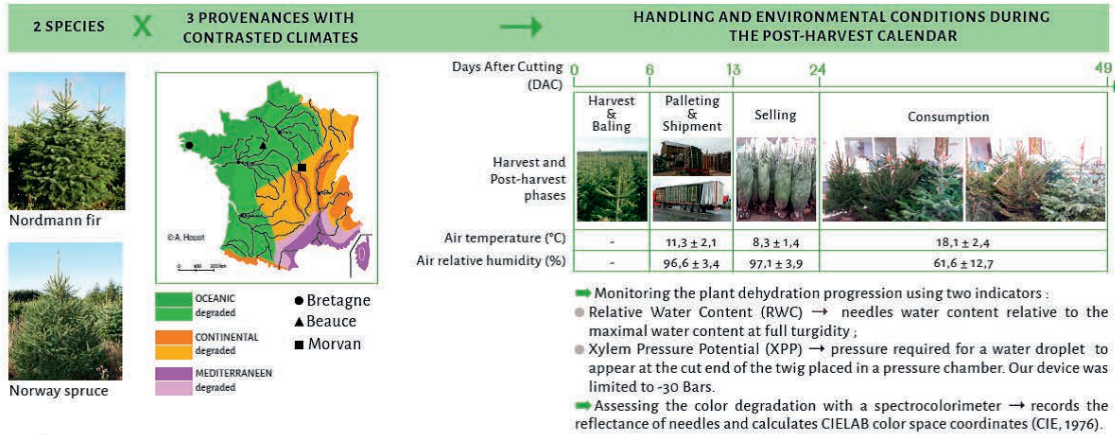
### Introduction and objectives

Nordmann fir (*Abies nordmanniana*) and Norway spruce (*Picea abies*) are the main Christmas tree species sold in France. These two species differ in shape, fragrance, color and needle retention. This latter criterion is important as 90 % of all Christmas trees sold in France are cut trees. Moreover, consumers buy their firs earlier and earlier (beginning of December), but they want them still "fresh" until the beginning of January. In this context, producers and retailers need an

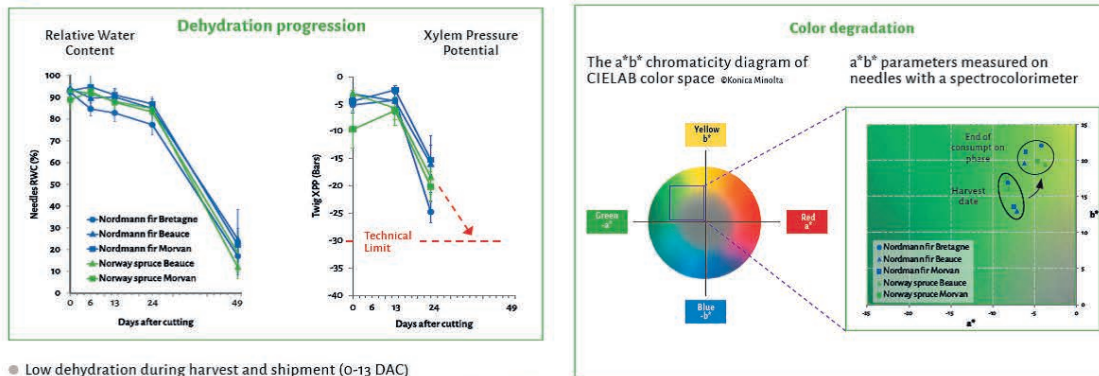
easy and quick procedure to determine the degree of freshness and tools to improve it, or at least maintain it. Upon their request, a research program on post-harvest quality improvement was initiated, whose first main objectives were :

- to find criteria to assess the freshness of trees from cutting until consumers' use ;
- to characterize the impact of storage and handling phases on post-harvest quality.

### Material and methods



### First results



- Low dehydration during harvest and shipment (0-13 DAC)
- High water loss during consumption phase : RWC dropped about 70 % at 49 DAC
- XPP in twigs was an earlier dehydration indicator than RWC of needles : XPP dropped by four fold during celling phase.

The a\*b\* parameters measurement highlighted the yellowing of needles objectively. This color degradation occurred during the consumption phase.

### Conclusion and prospects

- Christmas trees freshness declined when they were placed in drying conditions (consumption phase).
- XPP measurement and spectrophotometry could be interesting tools for characterizing the freshness of the trees during the post-harvest. But these methods should be adapted to a quality approach such as « Label Rouge » certification.
- Moreover, new freshness indicators will be tested, based on gaz exchange and/or biochemical analyses.
- One of the next strategic objectives of this research program will be to test methods to slow down the freshness degradation, for example christmas tree rehydration.

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Crédits photos : ASTREDHOR, AFSN

Partenaire financier : FranceAgriMer

# Lipid and fatty acid changes linked to postharvest needle abscission in balsam fir

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Needle loss after harvest is a major problem for Atlantic Canada's balsam fir Christmas tree and greenery industry. Lipid and fatty acids constitute membrane integrity and any change in membrane integrity is reflective of the changes in lipid and fatty acid composition triggering physiological dysfunction in cellular trafficking. It is hypothesized that lipids and fatty acid compositional changes trigger and/or modulate postharvest needle abscission in balsam fir. Balsam fir branches were collected from a clonal orchard in Debert, NS, and kept hydrated in the lab at an average temperature of 20–24°C and a light intensity of 85–95  $\mu\text{mol m}^{-2}\text{s}^{-1}$  supplied by incandescent and fluorescent lights. Parameters including needle loss and water use were measured initially and three times a week for 11 weeks. Membrane injury (MII) was measured initially and once a week. In addition, needles were sampled on site, and at the start of abscission and again at peak abscission postharvest and analyzed for polar lipids and fatty acids (FAs). Peak abscission was estimated at 11 weeks postharvest. During this time water use decreased by 67%, and MII increased by 134%. Total polar lipids significantly decreased

from 53.3  $\text{nmol mg}^{-1}$  DW to 6.6  $\text{nmol mg}^{-1}$  DW ( $p = 0.00$ ). This could be due to degradation, reutilization or membrane remodeling, and/or inhibition of lipid biosynthesis. Galactolipids found primarily in the chloroplasts, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), showed the greatest decline; 17.6 to 0.8, and 14.7 to 0.9  $\text{nmol mg}^{-1}$  DW, respectively, suggesting predominantly chloroplast membrane breakdown. MGDG and DGDG composed 33% and 27.5%, respectively, of total polar lipids in fresh needles. This dropped postharvest to 11.5% and 13%, respectively, at peak abscission. There was a significant decrease in  $\alpha$ -linolenic acid ( $p = 0.000$ ), the main FA comprising MGDG and DGDG.  $\alpha$ -linolenic acid ( $\text{nmol mg}^{-1}$  DW) correlated inversely with needle loss ( $r_p = -0.910$ ). Unsaturated:saturated fatty acid ratios changed significantly ( $p = 0.000$ ) from 4:1 to 1:1 from initial sampling to peak abscission. There was also an inverse correlation between the ratio of unsaturated:saturated fatty acids and needle loss ( $r_p = -0.843$ ).



# EXCURSIONS

# Honne arboretum

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Honne arboretum was established in 1981 and occupies an area of about 20 ha. The Arboretum at Honne exhibits more than 150 species of Norwegian and foreign tree species and shrubs. Tree species with relevance to forestry and related fields dominate the collection, mainly conifers. The Arboretum is designed for educational purposes but also to serve as a recreational area. Fig. 1 is from a guided tour for the CTREC group. Most of the species are planted in small stands and the species in one genera are normally established next to each other as indicated in the map.

The Arboretum is located at Honne, which originally was a farm with roots back to the Middle Ages. The Forestry Extension Institute has since 1972 been operating their activities from Honne. The institute is a nationwide training and extension institution in forestry and related fields. Honne Hotel and Conference Center is a subsidiary of the institute and responsible for operating the conference center.

The local growing conditions at Honne are good, especially with regard to soil conditions. The bedrock consists of limestone and slate which creates favorable soils. Climatically the location of Honne creates stable winters and reasonably hot summers together with an annual precipitation of around 800 mm contributes to ecological conditions that gives good conditions for a number of species.



Figure 1. Knut J. Huse guided the CTREC participants through the arboretum at Honne. Photo: I.S. Fløistad



# Biri forest nursery

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## Some background facts

- New production facilities built in 2008–2010 after a fire in 2008
- 10 acres of greenhouses, 8 acres used for cultivation today (Fig. 1)
- Access for mechanization like transplanting, sorting and packing
- Capacity to produce at least 12 to 15 million plants per year
- Heated by biofuel
- 750 square meters cold storage

## Production in 2015

- About 8 million plants in production for forestry, about 7.5 million Norway spruce, and the rest Scots pine, larch, deciduous trees and some subalpine fir for Christmas trees.
- About 1.5–2 million one-year-old plants (M95) in production (for 2016). The rest are two-year production; about one million M60 and the rest (about 5 million) is M95.

## Seeds

- All commercial seed comes from The Norwegian Forest Seed Center in Hamar.
- Many different provenances of spruce, pine and fir. Important to use seeds that are adapted to the area to be planted on, with great emphasis on altitude. Seeds come from seed orchards and populations. Seeds from orchards are more expensive, but increases the production.

## Sowing

- A new sowing machine installed in May 2015 changed our production to germination in microtrays (828 cells per tray). Sowing takes place in a vacuum drum seeder. Seeding is faster than conventional sowing. The micro trays takes four times less space than ordinary M95 trays and six times less space than the M60 trays. Number of micro plants per square meter is 3,300 plants. The micro-plants are grown in the greenhouse for 2–3 months and then transplanted into the ordinary trays, and transported into the field, or placed in cold storage in the winter (in



Figure 1. Biri forest nursery. Photos: O. Dæhlen

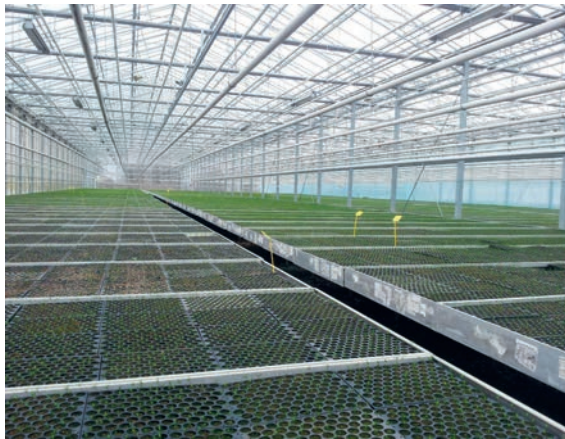


Figure 2. Spruce seedling production at Biri nursery. Photos: E. Høst

mikrotrays). They are sown in three different trays depending on the type of product, and the timing of sales. One-year-olds are sown in February / March, M60 sown in May / June and M95 sown in July / August. The last batch that are sown goes into the freezer at  $-2\text{ }^{\circ}\text{C}$  for the winter.

- Scots pine are sown in Jiffy plugs, then put for cultivation in a greenhouse. After a few weeks, they are transported outdoors to grow one season. In the autumn they get packed before storage (freezer).
- Birch sowing is still the old way in the ordinary trays (M60). After the germination we remove excess plants. It is an expensive production. In 2016, we will try to sow with a seeder. Alder are sown by a seeder, but the seeds are bigger.

### Transplanting

The 2–3 months old micro-plants are transplanted mechanically from the micro trays into the ordinary trays with 95 or 60 holes. Then we put the transplanted trays for further cultivation in the greenhouse or out in field depending on the time of the year. M60 plants grow two seasons out in the field, while M95 are grown one season. The advantage is space saving; exploiting the space in the trays by avoiding too many empty holes, and we will have more uniform plants (Fig. 2).

### Cultivation in the field

- Less challenges with weeds, since we avoid one season out in the field. However, we have challenges with controlling the weeds, especially



Figure 3. Waxed plant. Photo: E. Høst

those established from seeds. We are controlling the weeds both mechanically and chemically.

- Got problems with liverwort in the trays when the only chemical for managing it, Mogeton (quinoclamine), was not approved in the growing season 2015. We tried alternative solutions, with variable results.
- Dense program for spraying against fungal diseases. Problems with botrytis blight in cultures. Cooperating with Nibio to find good solutions.
- Insects are sometimes a problem in the cultures.

## Packing

- Packaging for sale or cold storage is done manually. The plant quality is checked, where the staff determine whether the plants cover the requirements as forest plants. The plants are packed in bundles.
- Before the bundles get loaded into crates they are treated chemically against large pine weevil with chemicals in a spray tunnel. Customers who do not want chemical treatment are choosing wax protection (Fig. 3). Wax is a mechanical barrier against the large pine weevil, it cracks and falls off the plants after two growing seasons, therefore a longer-acting protection.
- The plants are packed in chip boxes, 300 pieces in boxes for M60 and 500 pieces of M95.

## Excursions near Hamar, Hedmark county

R. Johnskås<sup>1</sup> & V. Talgø<sup>2</sup>

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### Christmas tree farm

At the farm of Knut Helset (Fig. 1), two field trials for Christmas tree breeding were recently established by the Norwegian Forest Seed Center (Skogfrøverket), one with subalpine fir (*Abies lasiocarpa*) and the other Norway spruce (*Picea abies*).

The subalpine fir trial was established in 2015 as one of six locations. There are a total of 35 seedlots represented per site; eleven from selected clones at Biri seed orchard, most of them being cork bark fir (*A. lasiocarpa* var. *arizonica*), seven from selected individual trees in a subalpine fir stand in the municipality of Gjøvik (Oppland county), and the remaining seventeen provenances known from the Christmas tree production in Norway. In addition to gaining new knowledge for the Christmas tree production, it will enable for selection of the most interesting provenances and families for future breeding programs. A similar trial with other families and provenances was established in 2010. Some of

the best known provenances are represented in both trials.

The Norway spruce trial was planted in spring 2014 (Fig. 2). Parallel trials were established in seven other locations in southern Norway.

The primary goal is to test both old and new seed sources under different climatic conditions to enable selection of the best Christmas trees for the area in question. Among the well-known seed sources are Stein seed orchard, which is currently used a lot in Buskerud county, seed lot no. 4178 - L2 which is generally used a lot in south-eastern Norway, and clones mainly originating from Stange seed orchard near Hamar. In the trial, a total of 30 seedlots are represented, hereof five from controlled crossings, fourteen from open pollinated clones and eleven provenances.



Figure 1. The Christmas tree farm of Knut Helset with part of the subalpine fir (*Abies lasiocarpa*) field trial in the foreground. Photo: V. Talgø



Figure 2. Participants of the international Christmas tree research and extension conference at Honne in 2015 visiting the Christmas tree field trials at the farm of Knut Helset, here the Norway spruce (*Picea abies*). Photo: V. Talgø



Figure 3. The subalpine fir (*Abies lasiocarpa*) provenance Grassie Mountain grafted on Spring Mountain. Photo: G. Tyczynski

### Seed orchard at Braset

This is a newly established 1.2 ha seed orchard for subalpine fir in Vangsåsen in the municipality of Hamar. The location is situated at an altitude of approximately 400 m above sea level. In 2010, around 1500 three year old rootstock from the provenance Spring Mountain was planted in the seed orchard. Distance between the trees are currently 4x2 meter, but will be thinned to 4x4 meter in the future. In



Figure 4. Successful grafting of the subalpine fir (*Abies lasiocarpa*) provenances Grassie Mountain onto spring Mountain. Photo: A. Besemer

2013, a two-meter high fence was installed to keep mouse, deer and other bigger animals out of the planting.

The provenance Spring Mountain was chosen as rootstock because it was one of the most promising provenances for Christmas tree production in 2010, and also because plants were available in a nursery at the time. The initial plan was to select grafting material from Spring Mountain from the highest quality individuals in Christmas tree fields. It was logic to use rootstock and grafting material from the same provenance, because if some of the grafting points failed, a seed producing tree could be established from buds/branches on the rootstock. However, after the rootstock trees had been planted, the preference by the Christmas tree growers was to graft Grassie Mountain onto the established Spring Mountain rootstocks (Fig. 3). This was due to Grassie Mountain becoming a highly demanded provenance in Norway, especially in Rogaland county in southwestern Norway, and difficulties getting enough seeds. During the winter 2012/2013 grafting material from the best trees from Grassie Mountain was collected in three Christmas tree fields in western Norway, i.e. two fields in Hjelmeland municipality in Rogaland county and one in Bergen municipality in Hordaland county. Material for a total of ten grafting's from each of 34 trees were collected.

During the winter 2014/2015, grafting material from 29 trees (clones) of Grassie Mountain was collected at Jønsberg. This time approximately 15 from each clone. Thus, there are now 63 different clones (34 + 29) in the seed orchard.



Figure 5. Controlled crossing of subalpine fir (*Abies lasiocarpa*) at Jønsberg in 2015. Here female flowers are protected by bags from pollen from other trees in the field trial at Jønsberg. Photo: R. Johnskås



Figure 6. Female flowers of subalpine fir (*Abies lasiocarpa*).  
Photo: R. Johnskås



Figure 7. Male flowers of subalpine fir (*Abies lasiocarpa*).  
Photo: R. Johnskås

All grafting has thus far taken place under field conditions (in the seed orchard) and the survival rate has been satisfactory. By fall 2014, nearly 90 % had survived, that is close to 700 trees (Fig. 4). In 2015, the ones that did not succeed the previous year, was grafted again. In the period 2016–2020, the remaining 200 rootstocks will be grafted.

Despite that some cones were produced already the year after grafting, it will still take many years before the seed production will undertake some volume. All grafting material was collected on trees that were from 10 – 18 year old, thus, based on experience, it will take at least 10–18 years before any production of interest is possible.

### Provenance trial of subalpine fir at Jønsberg

The trial was established in 1999 with 76 provenances from Arizona/New Mexico in the south to Alaska in the north. The field was one out of eight trials scattered around southern Norway. The primary goal was to select provenances for Christmas trees under different climatic conditions (Skage *et al.* 2012, Fløistad *et al.* 2015). Later, it was decided to keep the provenance trial at Jønsberg as a tree archive (the only one out of the eight) for future grafting material etc. Today most of the field trial has been thinned, but all provenances are still represented with approximately 30 trees per provenance (initially 90).

At Jønsberg the fungal disease *Delphinella* shoot blight (*Delphinella abietis*) has, as presented on the excursion during the CTREC, caused severe damage, especially on provenances with green foliage. Provenances with blue foliage are generally relatively resistant. Luckily, that is also the case for

the important Christmas tree provenance Grassie Mountain (Talgø *et al.* 2015).

In the provenance trial at Jønsberg, series of controlled crossings were carried out in 2015 (Fig. 5). According to the temperature in the growing season 2014, flowering and subsequent cone production was expected in 2015. Plans were made for controlled crossing with each other of some selected trees of Grassie Mountain, and also to cross some with individuals from other provenances of interest for the Christmas tree production. Unfortunately, flowering was less than expected. Only a few trees had female flowers (Fig. 6) and even fewer had male flowers/pollen (Fig. 7). Concerning individuals of interest for a breeding program from Grassie Mountain, only five trees had female flowers and non produced pollen. However, in total 62 controlled crossings were successful. Some pollen was brought in from Biri seed orchard, and some from individual trees of unknown origin, but known to give Christmas trees of high quality. The goal was to find one or more seed sources with unique characteristics concerning Christmas tree production.

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# Christmas tree farm in Buskerud county

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The Christmas tree production at the farm started in 1995 with Norway spruce (*Picea abies*) and subalpine fir (*Abies lasiocarpa*). In 2010, all cultivated land had been planted with Christmas trees, a total of 21 ha. In recent years they have reclaimed some old pastures and hayfields from forestation. When all become planted, the production area will increase to 30 ha.

Today (2015) the production is divided between:

- 15 ha *Abies nordmanniana* (provenance Aspheronsk) (Fig. 1)
- 5 ha *Picea abies* of a local provenance suitable for Christmas tree production (Fig. 2)
- 2 ha of *Abies lasiocarpa*

The two latter species will be used on the reclaimed area.

## Frost

In 2014, there were problems with frost damage of buds (top/leaders), probably caused by a combination of wind and cold temperatures, although the lowest temperature in the area was recorded to only  $-17^{\circ}\text{C}$ .

## Sales

Today, they only sell around 3.000 Norway spruce on their own stand in the nearby city of Drammen and some wholesale. However, a large increase is expected the coming years when more of the production becomes ready for marketing.



Figure 1. Karl Henrik Hals in one of his Nordmann fir fields. Photo: I.S. Fløistad



Figure 2. Karl Henrik Hals in a Norway spruce field. Photo: I.S. Fløistad

# The Norwegian forest seed center in Hamar – 120 years in tree seed trade

Ø.M. Edvardsen

The Norwegian Forest Seed Center (Skogfrøverket), Hamar, Norway  
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The Norwegian forest seed center is a foundation with national responsibility for seed trade, tree breeding, orchard management and seed testing in the forestry sector, which includes Christmas tree growers. The Seed Center was first established as a state funded regional kiln in 1895. The building and kilns that are in use today were established in 1953 after a fire, and the new kiln became one of the largest of its kind in the world. The Seed Center is now private but was owned by the Ministry of Agriculture until 1995. In 2014, the seed center had 14 employees and an approx. 14 million NOK turnover.

The main tasks are:

- Collection, kilning of cones and procurement of seeds
- Longtime storage (seed bank) and seed sales to nurseries and forest owners
- Seed improvement and testing of seeds and cones
- Seed orchard management (mainly *Picea abies*, but also *Abies lasiocarpa*, *Pinus sylvestris*, *Picea sitchensis*, *Pinus contorta*, *Alnus glutinosa*, and *Betula pendula*)
- Tree breeding (mainly *Picea abies* and *Abies lasiocarpa*)
- Certification of seeds
- Hardiness testing of plants
- General information and advisory to customers, forestry and public

Tree breeding, seed orchard management and seed testing are funded by the Ministry of Agriculture. The breeding tasks are carried out in cooperation with NIBIO. About 90% of the seed sales are Norway spruce (*Picea abies*). Scots pine (*Pinus sylvestris*), although an important timber species, is



Figure 1. Heidi Røsok Bye giving an orientation about Skogfrøverket. Photo: I.S. Fløistad



Figure 2. Conference participants visiting Skogfrøverket. Photo: I.S. Fløistad

mainly regenerated in seed stands. Norway spruce and subalpine fir (*Abies lasiocarpa*) are the main Christmas tree species. Norway spruce seeds are mainly collected in seed orchards (75%). Seeds of subalpine fir are mainly purchased from the US or Canada or collected in Norwegian stands established in the 1960's. New seed orchards with subalpine fir are established.



## Participants at the CTREC 2015

Last Name	First Name	Institution/Company
Braham	Anne Margaret	North Carolina State University, USA
Brodal	Guro	Norwegian Institute of Bioeconomy Research, Norway
Bruberg	May Bente	Norwegian Institute of Bioeconomy Research, Norway
Bucholz	Ethan	North Carolina State University, USA
Chastagner	Gary	Washington State University, USA
Christensen	Paul	PC-Consult, Denmark
Christensen	Claus Jerram	Danish Christmas tree association, Denmark
Crain	Brent	Michigan State University, USA
Demasi	Leonardo	Dural Christmas Tree Farm, Australia
Fløistad	Inger Sundheim	Norwegian Institute of Bioeconomy Research, Norway
Frampton	John	North Carolina State University, USA
Haaland	Gorden	Norwegian Christmas tree grower association, Norway
Kohlway	Will	North Carolina State University, USA
Kowalski	Judy	Oregon State University, USA
Lada	Rajasekaran	Dalhousie University, Canada
Landgren	Chal	Oregon State University, USA
Li	Shiyou	Natural resources Canada, Canada
Lizotte	Erin	Michigan State University, USA
MacDonald	Mason	Dalhousie University, Canada
Macri	Lynette	Dural Christmas Tree Farm, Australia
Matallana	Lilian	Maxwell, USA
McKeever	Kathleen	Washington State University, USA
McTavish	Christine	Michigan State University, USA
Møller	Else	Forest Farmers Union of Iceland, Iceland
Nielsen	Ulrik Bräuner	University of Copenhagen, Denmark
Nielsen	Knud Nor	University of Copenhagen, Denmark
O'Donnell	Jill	Michigan State University, USA
Owen	Jeffrey	North Carolina State University, USA
Palmer	Colin	British Christmas Tree Growers Association, UK
Papaspyropoulos	Konstantinos	Forest Research Institute, Greece

Last Name	First Name	Institution/Company
Santamaria	Luisa	Oregon State University, USA
Schuster	Karl	Chamber of Agriculture, Austria
Skage	Jan Ole	Norwegian Institute of Bioeconomy Research, Norway
Strande	John-Anders	Norwegian Christmas tree grower association, Norway
Strømeng	Gunn Mari	Norwegian Institute of Bioeconomy Research, Norway
Talgø	Venche	Norwegian Institute of Bioeconomy Research, Norway
Thomsen	Iben Margrete	University of Copenhagen, Denmark
Tuba	Katalin	University of West-Hungary, Hungary
Ulitzsch	Jan	The Norwegian Forest Seed Center, Norway
West	Rachel	Dalhousie University, Canada
Whetten	Ross	North Carolina State University, USA
Wilmot	Nancy	Astredhor Grand Est, France





# NIBIO

NORWEGIAN INSTITUTE OF  
BIOECONOMY RESEARCH

NIBIO - Norwegian Institute of Bioeconomy Research was established July 1 2015 as a merger between the Norwegian Institute for Agricultural and Environmental Research, the Norwegian Agricultural Economics Research Institute and Norwegian Forest and Landscape Institute.

The basis of bioeconomics is the utilisation and management of fresh photosynthesis, rather than a fossile economy based on preserved photosynthesis (oil). NIBIO is to become the leading national centre for development of knowledge in bioeconomics. The goal of the Institute is to contribute to food security, sustainable resource management, innovation and value creation through research and knowledge production within food, forestry and other biobased industries. The Institute will deliver research, managerial support and knowledge for use in national preparedness, as well as for businesses and the society at large.

NIBIO is owned by the Ministry of Agriculture and Food as an administrative agency with special authorization and its own board. The main office is located at Ås. The Institute has several regional divisions and a branch office in Oslo.

