



PTDW 2011

Nordic Baltic Potato Tuber-Disease Workshop,
Hamar, Norway, 16. – 19. November 2011

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Book of abstracts

Editors:

Arne Hermansen and Merete Wiken Dees

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Preface

Bioforsk has the pleasure to welcome you to the Nordic Baltic Potato Tuber-Disease Workshop 2011 (PTDW 2011) at Hamar, Norway 16-18 November 2011. This Workshop is mainly directed towards potato advisors, including the potato industry, and scientists from the Nordic and Baltic countries. In addition, we also have participants that are plant breeders, students and other people interested in potato quality. In total there are about 60 participants at the workshop from the Nordic countries, UK, Switzerland, USA and China.

The increase in sale of potatoes as a washed and pre-packed product has meant that skin blemish diseases have become economically important, as they affect appearance and therefore the quality. There are several pathogens, including bacteria and fungi, that can cause skin blemish diseases, including *Streptomyces* spp. (common scab), *Spongospora subterranea* f. sp. *subtereanea* (powdery scab), *Helminthosporium solani* (silver scurf), *Rhizoctonia solani* (black scurf), *Polyscytalum pustulans* (skin spot) and *Colletotrichum coccodes* (black dot). In addition, there are also free living nematodes (*Pratylenchus penetrans*) that might cause lesions on potatoes resembling common scab.

For Norwegian potato growers losses due to scab and scurf are estimated to approx. NOK 9 mill yearly. For fresh market potatoes, scab and scurf are a more severe problem because of the importance of appearance, and all kinds of skin blemishes are important, particularly silver scurf. For the fresh market potato business in Norway, the cost of skin blemish diseases is yearly approx. NOK 28 mill.

This Workshop is an activity in Bioforsk project: "Improved potato quality by reduced skin blemish diseases (scab and scurf) in Norwegian potato production" (2008-2012). This project is financed by grants from the Research Council of Norway, the Foundation for Research Levy on Agricultural Products, the Agricultural Agreement Research Fund, and Norwegian

potato growers and food industries; Gartnerhallen, Bama, ICA-Norge, NF-Grønt, KiMs and Maarud.

The foreign experts attached to this project, Alison Lees (UK), Leslie Wanner (USA) and Jari Valkonen (Finland), are contributors in the workshop. In addition invited speakers are Lv Dianqiu from China and Ueli Merz from Switzerland.

The workshop has 5 different sections, in which the 3 first have presentations from the project:

1. Occurrence of skin blemish diseases in the Nordic and Baltic countries
2. Diagnosis and biology of different skin blemish pathogens
3. Control of skin blemish diseases
4. Research activities on other potato tuber diseases in Nordic and Baltic countries
5. Future challenges

In the table of contents, the abstracts are presented in the same order as found in the program.

The scientific workshop committee consists of Jari Valkonen (Finland), Björn Andersson (Sweden), Bent J. Nielsen (Denmark) and Arne Hermansen (Norway). The local organizing committee consists of Erling Fløistad, Kari Munthe and Arne Hermansen from Bioforsk, Plant Health and Plant Protection Division, Ås (Norway).

We wish to thank our workshop sponsors Research Council of Norway and Bioforsk. In addition, we are also thankful to the other financial contributors to the Bioforsk project mentioned above.

We hope this workshop will be an interesting meeting point for scientists, advisors and other participants from the different countries to exchange results and experiences and discuss future challenges regarding potato tuber diseases, with the main focus on skin blemish diseases.

Arne Hermansen
Organizing committee chair

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Survey of skin blemish diseases in Norwegian potato production in 2008 and 2009

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The increase in the sale of potatoes as a washed and pre-packed product has meant that skin blemish diseases have become economically important, as they affect appearance and therefore the quality. The main objective of this survey was to obtain an overview of the occurrence of the different skin blemish diseases (scab and scurf) in Norwegian potato production.

A survey of incidence of scab and scurf after the growing seasons 2008 and 2009 was carried out on 241 potato lots representing different cultivars and regions. From each season potatoes were sampled by potato companies during two 14 days periods late January and early February, and late March and early April.

All potato lots coming to the companies in the sampling periods were assessed by the company staff. If more than one potato lot arrived from the same cultivar and grower during these periods, only one of the potato lots was examined. Each potato lot could vary in size from 7.5 tons to 578 tons, but the samples included in the survey represented approx one subplot of 30 tons from the larger lots.

Regular potato samples for analyses (approx 10 kg) was washed in tap water and placed in a barrel (approx 35 cm in diameter on top). Tubers visible from the top of the barrel were scored for different scab and scurf diseases using a percentage scale. Six different disease categories were used; common scab, powdery scab, black scurf, silver scurf/black dot, skin spot, others. A sub-sample of 20 tubers with typical symptoms of the skin blemish symptoms of each of the lots was sent to Bioforsk for further examination.

The staff at Bioforsk scored the 20 tubers for different skin blemish diseases in three rough categories for each disease; “a lot”, “some” and “not detected”. Ten of the tubers were further examined using a modified “plug test”. Two sectors (each divided in three parts) from each tuber surface were cut, placed in a 9 cm Petri dish, and incubated humid at 15 °C for 8 days. Then the sectors were examined by using a stereo microscope for the presence of black scurf (*Rhizoctonia solani* (mycelium)), silver scurf (*Helminthosporium solani* (conidia and conidiophores)), skin spot (*Polyscytalum pustulans* (conidia and conidiophores)), and black dot (*Colletotrichum coccodes* (microsclerotia and/or acervuli)).

The occurrence of the nematodes *Pratylenchus* spp., was examined in tubers that had symptoms resembling common scab. Samples from scabby lesions of 5 tubers were boiled in 0.05 % acid fuchsin and lactoglycerol. For each tuber two 1 cm² areas of skin, situated on opposite sides of the tuber, were removed using a cork borer and mounted on an objective slide for examination in light microscope.

The results from the subsamples sent to Bioforsk showed that *H. solani* was present in all lots. *P. pustulans* and *R. solani* (mycelium) was found in 80 % of the lots while *Streptomyces* spp. and *C. coccodes* was present in 50 - 70 % of the lots. *S. subterranea* was found in 25-50 % of the lots. *Pratylenchus* spp. was present in 60 % of examined subsamples with common scab symptoms. The relationship between root lesion nematodes and *Streptomyces* need further studies. There were some variations between years and between cultivars regarding incidence for the different scab and scurf diseases.

Identification and characterization of *Streptomyces* species causing common scab in Norway

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Common scab is a serious disease of potatoes reducing crop value and causing yield losses. The disease is caused by soil-borne, gram-positive, filamentous bacteria of the genus *Streptomyces*. Relatively few of the several hundred species in the genus are plant-pathogenic. The plant pathogenic species are a relatively diverse group. Plant pathogenicity in the genus is based on production of the phytotoxin thaxtomin which has an impact on cellulose biosynthesis. *Streptomyces scabies*, *S. turgidiscabies*, *S. acidiscabies* and *S. europaeiscabiei* are among the most well known common scab causing pathogens worldwide.

Our survey was conducted to clarify which *Streptomyces* species cause common scab in Norway. Bacteria were isolated from scab lesions on tubers sampled in two years from different locations in 15 counties of Norway spanning ~1400 km from south to north. Symptoms on tubers were diverse, ranging from superficial to deep-pitted lesions, and in some cases raised lesions. After DNA extraction from pure cultures, the primer pair txtAB1/txtAB2 was used to detect putative pathogenic strains and probable non-pathogenic strains.

Pathogenicity of selected strains was tested on radish seedlings and potato. The ability of the bacterial strains to infect potato or radish was consistent with the presence of the *txtAB* operon, the pathogenicity determinant.

Microarray-based comparative hybridization was conducted to identify a selection of the pathogenic strains of *Streptomyces* obtained from potato scab lesions and to compare genetic differences between them. Species determination was done by PCR based on the variable regions in the 16S rRNA gene.

The Norwegian strains of *Streptomyces* were assigned to *S. europaeiscabiei* (69 %) and *S. turgidiscabies* (31 %) based on the 16S rRNA gene and microarray analysis. Surprisingly, *S. scabies* was not found in Norway in our survey.

Occurrence of potato tuber diseases in China and control strategies

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China is the greatest potato production country in the world, with potato planting area of 4.75 million ha, output of 69 million tons (FAO, 2009), accounting for 25.93 % of the world's growing area, and 20.95 % of total output, but the average yield is less than 1 ton/ha. The serious occurrence of diseases is one key reason affecting China's potato yield and quality. Among them, the potato tuber diseases, such as common scab, black scurf, dry rot and others, have great impact on tuber quality.

With the development of China's potato industry, the planting area of potato is increasing year by year, so crop rotation is difficult to be achieved for limited area in some potato growing regions, which resulted in soil-borne diseases occur more serious. For example the potato black scurf disease, the incidence rate is about 4 % to 6 % in some potato producing areas of Heilongjiang, Liaoning, Jilin and Hebei. Some serious plots can reach 20 % or more. As a major skin disease, common scab affecting commercial quality of potato, is most common in China, such as in Heilongjiang, Inner Mongolia, Hebei, Shandong, Shanxi, Shaanxi, Guizhou, Gansu, Sichuan, Hunan, Hubei, Yunnan and other provinces, which nearly covers all the main potato producing areas in China. In the arid climate, alkaline soil, even for continuous cropping areas have a higher prevalence. In particular, the production of minitubers are affected seriously by the disease, and the incidence rate of common scab for minitubers has reached 30 % to 60 % in North of China, resulting in significant economic losses.

Potato dry rot induced by *Fusarium* (*Fusarium* spp.) is also a very important fungal disease occurring in potato storage period in China. General incidence rate is 10-30 %, and storage loss rate can reach 60 % in some severe cases.

Control strategies:

1. disease-resistant varieties breeding. Screening and breeding disease-resistant potato varieties are fundamental and effective ways to reduce the occurrence of diseases. But at present, apart from some general resistant varieties, no special varieties anti-scab, dry rot or black scurf disease.
2. growing disease-free seed is an important and effective means to prevent disease epidemics.
3. taking different agricultural technical measures aim to different diseases, such as rotation, irrigation etc.
4. using chemical control.

Challenges of a common pathogen with an uncommon plant response: Potato Common Scab

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Potatoes are the fourth most important staple food crop, and are grown worldwide. However, potatoes are affected by numerous pests and diseases, seriously impacting production costs and sustainability. Common scab (CS) is an important disease for which there is no adequate control. CS decreases potato quality and marketability because of wart-like, russet or pitted lesions on the potato skin. Effective control of CS depends on greater understanding of both the host and the pathogen. Traditional cultural control strategies like irrigation and reduced soil pH are insufficient and often fail. There is no evidence for resistance genes or for a classic defense response against *Streptomyces*. Recent research is focused in two areas that should help in controlling CS: (1) Research-based knowledge of the plant pathogenic

soil bacteria in the genus *Streptomyces* that cause the disease has enabled improved detection of the pathogen and its distribution, helped clarify under what circumstances the disease is more severe, and helped refine our understanding of mechanisms of pathogenicity; and (2) Development of reliable disease-resistant cultivars. Factors that hamper development of CS-resistant potato cultivars include variable effects of environmental conditions, need for better sources of resistance, genetic variation in pathogen populations, and environmental variability in CS severity from year to year and location to location. Though no single measure is sufficient for managing CS, integrated use of cultural methods together with planting cultivars with the best CS resistance for a region is currently the best option for control.

Diagnostics, epidemiology and control of potato blemish diseases - Focus on black dot

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Black dot, caused by *Colletotrichum coccodes*, is an important constraint to the pre-pack potato industry in the UK due to development of unsightly brown necrotic lesions on the tuber surface. In order to implement effective disease-management strategies for black dot it is first important to understand the relative contribution of different inoculum sources in causing disease.

Previous studies investigated the role of seed- and soil-borne inocula, but the potential of naturally occurring levels of inoculum to cause disease under specific environmental and management conditions was not known due to difficulties in accurate quantification of seed- and soil-borne inocula.

In the work described here, seed-tuber and soil-borne inocula of *C. coccodes* were quantified using an existing real-time PCR assay and related to subsequent incidence and severity of disease. In four field trials, a controlled-environment experiment and through the monitoring of 122 commercial crops, seed-tuber inoculum was found to be relatively less important than soil-borne inoculum in causing black dot, and

the level of seed-tuber inoculum did not significantly affect either the incidence or severity of disease or the percentage of progeny tubers deemed unmarketable. By contrast, soil-borne inoculum had the potential to result in high levels of disease and the level of *C. coccodes* soil infestation (pg DNA g⁻¹ soil) was found to have a significant effect. At soil infestation levels below 100 pg DNA *C. coccodes* g⁻¹ soil, 7 % of commercial crops had an incidence of black dot greater than 20 %, increasing to 40 % and 57 % of crops at levels of 100-1000 pg g⁻¹ and >1000 pg g⁻¹ soil, respectively.

Interpretation of disease risk based on inoculum levels must, in the future, be informed by agronomic variables and potential control strategies. The results of field and controlled environment trials to investigate the effects of other factors including fungicide application, irrigation, host resistance and crop duration, all of which may act in combination with soil-borne inoculum levels, on the development of disease, will be discussed. An overview of how risk factors and control measures can be managed to provide control of the disease will be given.

Occurrence, biology and epidemiology of *Spongospora subterranea* f. sp. *subterranea*, the cause of powdery scab of potato

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The importance of this disease, caused by the zoosporic pathogen *Spongospora subterranea* f. sp. *subterranea* (Sss), has increased worldwide. In some regions it is one of the most important problems in potato production. Infected seed lots may be rejected depending on the tolerance limit (mostly zero to low) or extra grading is needed. Similarly, supermarket quality standards may require additional grading labor for ware potatoes and infected potatoes for processing produce more waste and less profit. Sss is also capable of vectoring a plant pathogenic virus, *Potato mop-top virus*, which causes internal symptoms on tubers, a quality problem especially for processing. Furthermore, root infection (galling) seems to reduce yield.

Sss belongs to the order of *Plasmodiophorida*, commonly called the Plasmodiophorids, and is an obligate, non-culturable parasite, making research difficult. It has a wide host range, but the resting spore stage has only been observed on solanaceous plants. The pathogen survives as resting spores (sporosori) in the soil for many years. It is assumed that the spores need a specific stimulus to release the zoospores. The

nature of host recognition is unknown, but all the Plasmodiophorids have a unique mechanical penetration method. The post-infection development is again unclear and we do not know e.g. which factor(s) determine if a plasmodium either develops into a zoosporangium or a gall in roots. With true fungi, a resting structure is mostly related to recombination but this relationship has not yet been found with Plasmodiophorids.

It is commonly assumed that powdery scab prefers cool and wet conditions and heavy soils. Optimal temperature for tuber infection is 12 - 13 °C and for root galling around 17 °C. Soil water is essential for the spread of the zoospores thus stopping irrigation around tuber initiation (susceptible stage) can prevent infection. A wet-dry-wet soil moisture pattern seems to favor the disease. This might explain the fact that powdery scab occurs also in hot and dry regions where soil temperatures can be as high as 40 °C in average and irrigation is applied. Heavy soils with high water retention capacities are thought to encourage the disease but sandy soils seem to be the worst.

Diagnostics, epidemiology and control of potato blemish diseases - Focus on *Rhizoctonia*

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Rhizoctonia solani is a soil-borne pathogen that comprises several groups which are pathogenic to different host species. In potato, infection of shoots by *R. solani* soon after planting causes stem canker and can significantly delay plant emergence. Stolon infection can influence the number and size distribution of harvested tubers, and in severe cases of disease total yield can be reduced. Sclerotia of *R. solani* can develop on progeny tubers, resulting in the tuber blemish disease black scurf, thus affecting tuber quality and acting as an inoculum source for subsequent crops. Isolates of *R. solani* causing potato disease have been ascribed mainly to anastomosis group 3 (AG-3), although several other AG groups, including AG-4, AG-5 and AG-9, can also infect potato.

Soil-borne *Rhizoctonia solani* can be quantified accurately in artificially inoculated soils using real-time PCR assays. However, current sampling strategies and diagnostic tests appear not to accurately represent field populations of *R. solani*. Our studies to try to determine an improved sampling strategy that would adequately reflect field inoculum levels are described.

Due to a lack of knowledge of how *R. solani* survives, is transmitted and causes disease, it has been difficult to confidently predict that a simple soil diagnos-

tic assay will be appropriate for a risk assessment of stem canker or black scurf or to make disease control recommendations. To underpin any interpretation of a diagnostic test, the relative importance of soil- and seed-borne inoculum levels in causing disease, and how these relationships are affected by factors such as soil type, environment, agronomic practices, anastomosis group and control options is being studied.

R. solani can exist in two forms in the soil, as sclerotia and as saprophytic hyphae. The relative frequency of these two types of inoculum and their relative impact on disease development also needs to be determined as the correlation between DNA levels in sclerotia and hyphae with that of disease development is very likely to be different and therefore the epidemiological importance of each propagule type is important in establishing the link between inoculum and disease.

Using controlled environment experiments and field trials we have investigated how seed and soil inoculum affect disease development and their interactions with a number of environmental and soil factors. Results will be presented and ongoing work described.

Decision making in potato skin blemish diseases control using real-time diagnostic methods; Norwegian results from 2009 and 2010

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Real-time PCR assays developed at SCRI was used to study the relationship between the levels of scab and scurf pathogens in the seed and soil and potential disease risk in 80 commercial potato crops in Norway in 2009 and 2010. The Norwegian Agricultural Advisory Service took out samples of both seed tubers before planting and progeny tubers after harvest and soil samples in spring and in the middle of August.

Black dot (*Colletotrichum coccodes*)

There were a wide range of soil inoculum levels in the fields. There were also a wide range of inoculum levels in the seed tubers. The amount of black dot in progeny tubers increased to some extent with the level of inoculum in soil and seed tubers (R^2 (adj)=43.2%).

Skin spot (*Polyscytalum pustulans*)

Generally, the level of soil inoculum in spring and in autumn was low. There were large variations between crops in the level of seed tuber inoculum and this correlated well (R^2 (adj)=45.2 %) with the amount of skin spot inoculum in progeny tubers.

Common scab (*Streptomyces* spp.)

There were generally low levels of inoculum in soils both in the spring and in the autumn as well as on seed tubers. Relatively low levels of common scab developed in the yield. Correlation analysis of the inoculum level in soils and seed tubers with the incidence of common scab in progeny tubers did not explain much.

Silver scurf (*Helminthosporium solani*)

There were low levels of inoculum in soils in the spring and in the autumn. All crops had relatively high level of inoculum in seed tubers. There was a wide range in the level of inoculum in progeny tubers, but no correlation to the inoculum levels in seed tubers or soils.

Black scurf (*Rhizoctonia solani*)

There were low levels of inoculum in soils in the spring and in the autumn. There was a good correlation between the amount of black scurf hyphae found in the plug test and the inoculum level found using the PCR test on tubers. The correlation between sclerotia and inoculum level found using PCR on tubers was less good. The amount of inoculum in seed tubers or soils did not explain much of the inoculum level in progeny tubers.

Powdery scab (*Spongospora subterranea* f. sp. *subterranea*)

There were a wide range of soil inoculum levels in the examined fields. The correlation between the percentage of the surface covered with symptoms and the inoculum level found using PCR was not good. This is probably caused by the patchy nature of the symptoms and that a large part of the spore powder was washed away from some seed lots. The inoculum level in seed tubers correlated to some extent to the inoculum level in progeny tubers (R^2 (adj)=34.6 %) and it was slightly improved when we included the soil inoculum level in the autumn (R^2 (adj)=36.9 %).

Conclusion

For skin spot, powdery scab and black dot the disease incidence increased with the amount of inoculum on the seed tubers. For black dot and powdery scab the disease incidence also increased with soil inoculum. For common scab, silver scurf and black scurf other factors than inoculum level were more important for disease incidence in the progeny tubers. Our results indicate that real time PCR diagnostic tools can be used to select seed lots and soils with low inoculum levels to reduce the problem with skin spot, black dot and powdery scab.

Integrated control of *Spongospora subterranea* f. sp. *subterranea*, the cause of powdery scab of potato

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The soil-borne pathogen *Spongospora subterranea* f. sp. *subterranea* produces many resting spores which can remain dormant for long periods being highly resistant to environmental stresses and spread the disease on seed potatoes and in contaminated soil. Despite many years of research, no efficient and economically sound control method is available for powdery scab. A disease management strategy which integrates several components is the best long term approach to powdery scab control. 'Plant clean seed into clean soil' is a simple advice but yet very difficult to be fully applied.

Planting clean seed prevents spread of the disease especially over long distances. This requires implementation of effective and internationally standardized certification rules. Visual inspection has to be optimized to avoid misidentification of scab symptoms, supported by immunotest, ELISA and molecular markers. In situations where infected seed has to be planted, seed treatment might help but only when tuber infection level is less than moderate. A potential way of short distance dissemination is the spread of effluent of animals fed with diseased potatoes.

Different tools and methods have been developed to detect and quantify soil inoculum. Producers want to minimize the risk for crop infection. Professional

producers have their own approach to solve the problem: every year they look for virgin soils. Traditional farms do not have much choice beside crop rotation. Reliable information about the contamination level of their fields could be an important part of their powdery scab management. Bioassay, immunology and molecular marker are all able to detect the pathogen in the soil, with different sensitivity. Unfortunately, none of them allow to clearly identify a healthy soil and, except for the bioassay, they detect also non-virulent inoculum. In those cases where the use of contaminated soil is inevitable, chemicals, biofumigation, trap crops or biocontrol may help to reduce the initial inoculum.

The core measure of a long-term powdery scab control strategy is host resistance. Numerous field screenings of cultivars and breeding lines showed substantial differences in susceptibility to tuber infection. Susceptibility to root infection should also be assessed as sporosori produced in root galls contribute to a large part of the soil inoculum. Powdery scab has not been included in potato breeding programs until recently. There is a need for better understanding of the genetic nature of the pathogen, as successful disease resistance breeding relies on detailed knowledge of the genetic background of pathogen populations.

Effect of soil humidity and pH on common scab severity - controlled climate experiments

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The current study aimed at finding how soil humidity and pH affected the severity of two common scab pathogen species (*Streptomyces turgidiscabies* and *S. europaeiscabiei*) in potatoes. The work was performed in the growth seasons of 2009-2011 in climate chambers at the University of Tromsø, northern Norway (69° 40'N). The chambers had natural light conditions and a temperature of 18 °C in 2009 and 18/12 °C (day/night) in 2010-2011. The standard soil had a pH of 4.8, and a high level (pH 7.2) was attained by adding limestone meal. Plants of the susceptible potato cv. Gullauge, were grown in 12 litre pots filled with a mixture of soil, fertilizer, lime and inoculum of the two *Streptomyces* species produced at Bioforsk Plant Health and Plant Protection Division. Soil humidity was altered during a four weeks period from the beginning of tuber formation by keeping pots at three humidity levels (dry, normal and wet). The water content was measured to 8, 15 and 22 percent, respectively, during this period. At harvest the coverage of scab lesions on tubers (% of the tubers surface area) was graded visually using an assessment key. In addition, several yield parameters were registered.

The results varied between years. In 2009, only plants inoculated with *S. turgidiscabies* showed significant damage (32 % coverage of tuber lesions)

compared to control plants (0 %). Tubers inoculated with *S. europaeiscabiei* showed only minor damage (7 %) this year. The scab severity was not affected by soil humidity for any of the two *Streptomyces* species. In 2010, the two common scab pathogen species showed similar damage and similar reactions to humidity. There were significantly less scab lesions on tubers at normal and wet conditions (19 and 13 % coverage, respectively) than at dry conditions (35 %). In 2011, *S. europaeiscabiei* caused more severe damage on tubers than *S. turgidiscabies* (18 and 11 % lesions, respectively) and there were significantly less scab lesions at wet conditions (9 %) as compared to dry and normal conditions (19 and 16 %, respectively).

The yields were not affected significantly by inoculation with any of the species, except for 2009 when *S. turgidiscabies* reduced total yield with 20 percent. In conclusion it seems like the common scab damage caused by both *S. turgidiscabies* and *S. europaeiscabiei* may be reduced by wet soil conditions during the period of tuber formation. The two pH levels did not affect scab severity significantly in any of the two years of experiments (studied only in 2010-2011).

Effect of cultivation techniques on scurf and scabs - Norwegian results from 2009-2010

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The effects of soil conditions and cultural control methods on development of skin blemish diseases were studied in three different field experiments during two years. All studies included the cultivars Saturna and/or Asterix, which are susceptible to different skin blemish diseases. The seed tubers had various skin blemish pathogens present, but were as clean as possible regarding *Streptomyces* spp. causing common scab. In experiment 1 and 2, inoculum of *S. europeiscabiei* and *S. turgidiscabies* were added at planting (except in control plots). However there were some problems with the quality of the inoculums the first year of experiment. All field experiments had 4 replicates each year. At harvest some tubers were sampled for 'plug test' where sectors of the tuber were incubated at 15 °C for 8 days before examination for skin blemish diseases using stereomicroscope. In addition, samples of 8 kg were stored at 12 °C for two weeks and then at 6 °C for 5 months to allow development of skin blemish diseases. Percent coverage of diseases on tubers was visually determined.

Experiment 1. Effects of fertilizers and soil compaction

This study included two field plots each year (Apelsvoll and Solør) in the variety Saturna, inoculated by both *Streptomyces* species. Soil compaction after planting was studied, together with five fertilizing strategies with potential to reduce skin blemish diseases. The strategies tested were FullgjødselR 11-5-18 (control), FullgjødselR 6-5-20 + 2*Nitrabor, shrimp shell meal and additions of elementary sulphur (Brimstone), Mantrac Optiflo sprayed 4 times, and FZB24 (spore forming *Bacillus*) combined with Phosphorus given at planting.

Both seasons were fairly wet after tuber initiation and the levels of common scab, as well as other skin blemish diseases, were generally low. In most experiments inoculation with *S. turgidiscabies* resulted in the highest incidences of common scab. There were

no significant differences in skin blemish diseases between the different fertilizer strategies tested.

Experiment 2. Effects of soil humidity

The effect of 8 different periods of drought and irrigation on various skin blemish diseases was studied at Apelsvoll in cultivars Asterix and Saturna. Movable plastic roofs were used to simulate five drought periods (mostly 20 day periods) from planting to harvest.

Asterix had minor common scab infections, while Saturna got higher levels of common scab after dry periods. In 2009 the dry period of 10-30 July caused most common scab. In 2010 different periods (from late June to early August) gave elevated levels of this disease. *S. turgidiscabies* resulted in the highest incidences of common scab. In 2010 powdery scab (*Spongospora subterranea* f. sp. *subterranea*) in Asterix decreased with drought late July and increased when the soil was kept wet.

Experiment 3. Effects of harvest, curing and storage strategies

The cultivar Asterix was used in field trials at Apelsvoll, using split split plot design. Nine combinations of early and late harvest together with haulm killing 22, 11 or 0 days were tested. Soil moisture was high at all harvest dates. After harvest samples were split in two curing strategies (forced ventilation/moderate %RH + keeping high %RH) and after two weeks split again in two temperature regimes (directly to 4 °C + lowering to 4 °C over two months).

Dry conditions during curing reduced occurrence of silver scurf (*Helminthosporium solani*) significantly, while lowering of temperatures directly after curing resulted in less powdery scab. Powdery scab also increased by late harvest, while the highest occurrence of black scurf sclerotia was found when the crop was harvested 22 days after vine killing.

Alternative methods for control of potato common scab

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Potato common scab is caused by a number of *Streptomyces* species. In Finland, the causal agents are *Streptomyces scabies* (Thaxter) Lambert & Loria and *S. turgidiscabies* Takeuchi. The scab-causing *Streptomyces* spp. are well-adapted, successful plant pathogens that survive in soil also as saprophytes. Control of these pathogens has proved to be difficult. Most of the methods used to manage potato common scab are aimed at controlling *S. scabies*, the most common of the scab-causing pathogens. Our studies investigated *S. scabies* and *S. turgidiscabies* as causal organisms of common scab and explored new approaches for control of common scab that would be effective against both species.

S. scabies and *S. turgidiscabies* are known to co-occur in the same fields and in the same tuber lesions in Finland. In our studies both these pathogens caused similar symptoms on potato tubers, and the types of symptoms varied depending on cultivar rather than the pathogen species. Pathogenic strains of *S. turgidiscabies* were antagonistic to *S. scabies* *in vitro* indicating that these two species may be competing for the same ecological niche. In addition, strains of *S. turgidiscabies* tolerated lower pH than those of *S. scabies* and were highly virulent on potato. Taken together, these findings suggest that *S. turgidiscabies* is a major contributor to the common scab problem in Finland.

The bacterial phytotoxins, thaxtomins, are produced by the scab-causing *Streptomyces* spp. and are es-

sential for the induction of scab symptoms. In this study, thaxtomins were produced *in vitro* and four thaxtomin compounds isolated and characterized. All four thaxtomins induced similar symptoms of reduced root and shoot growth, root swelling or necrosis on micro-propagated potato seedlings. The main phytotoxin, thaxtomin A, was used as a selective agent in a bioassay *in vitro* to screen F1 potato progeny from a single cross. Tolerance to thaxtomin A *in vitro* and scab resistance in the field were correlated indicating that the *in vitro* bioassay could be used in the early stages of a resistance breeding program to discard scab-susceptible genotypes and elevate the overall levels of common scab resistance in potato breeding populations.

The potential for biological control of *S. scabies* and *S. turgidiscabies* using a non-pathogenic *Streptomyces* strain (346) isolated from a scab lesion and *S. griseoviridis* strain (K61) from a commercially available biocontrol product was studied. Both strains showed antagonistic activity against *S. scabies* and *S. turgidiscabies* *in vitro* and suppressed the development of common scab disease caused by *S. turgidiscabies* in the glasshouse. Furthermore, strain 346 reduced the incidence of *S. turgidiscabies* in scab lesions on potato tubers in the field. These results demonstrated for the first time, the potential for biological control of *S. turgidiscabies* in the glasshouse and under field conditions and may be applied to enhance control of common scab in the future.

Field evaluation of bacterial antagonists and arbuscular mycorrhizal fungi against black scurf

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In intensive potato farming with narrow crop rotations, soil-borne fungal pathogens such as *Rhizoctonia solani*, *Helminthosporium solani*, *Colletotrichum coccodes*, the causal agents of different kinds of skin blemish diseases, are an increasing threat to the profitability of the potato production in Sweden. After late blight (*Phytophthora infestans*), *R. solani* ranks as the second most important reason for economic losses. *R. solani* can survive on plant residues in soil for a long time and role of soil inoculum is not well understood. At present it is hard to achieve satisfactory control of *R. solani* by available cultural and plant protection practices. The use of antagonistic micro-organisms can be an attractive alternative to manage plant diseases that are difficult to control by other means.

We have studied effects of selected antagonistic bacteria and compared this with effects of commercial preparations Root Professional, Biorize and Mykym (based on *Glomus* spp.) and Proradix (based on *Pseudomonas* sp) on scurf in field trials conducted

on naturally infested soils. Reduced disease incidence was consistently recorded for two bacterial isolates (belonging to *Serratia* sp.) during two years of experimentation with one cultivar, one dose and one application as soil inoculum at planting. An increase in total tuber yield by 19 % was recorded. An increase in yield due to upgrading of tuber quality was not estimated. The bio-control effects depended on the micro-organism involved and the level of pathogen inoculum in the soil. Pathogenic fungi colonising the tuber surfaces were identified and estimated by cultivation technique and qPCR respectively. Analysis of peels from infected tubers confirmed dominance of *R. solani* as the main pathogen but also revealed occurrence of both *C. coccodes* and *H. solani*. Some results from these studies will be presented.

Soil and seed treatments with fungicides against skin blemish diseases

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Seed treatment trials were carried out with three to four different seed lots planted at two locations each year during 2005 to 2008. The fungicides Maxim100FS (fludioxonil 100g/l) and Rizolex 50 FW (tolclofosmethyl 500g/l) were compared including different application methods. These trials showed that there were large variations between locations in development of skin blemish diseases in harvested tubers originating from the same seed lot. We tested three different application methods, spray-table or dipping some weeks prior to planting or spraying of tubers at planting. No significant differences between the different application methods were found. Both Maxim100FS and Rizolex 50FW reduced the amount of black scurf (*Rhizoctonia solani*) sclerotia and the frequency of *R. solani* hyphae on the progeny tubers. The effect of the treatments on the progeny tubers were measured in the autumn. At that time not much silver scurf (*Helminthosporium solani*) symptoms had developed. Therefore the effect on silver scurf was measured using the “plug test”. In the plug test three sectors from the tuber surface were cut, placed in a 9 cm Petri dish, and incubated at 15 °C for 8 days before they were examined using a stereo microscope. Only Maxim 100FS reduced the frequency of *H. solani* sporulation on the progeny tubers.

Seed treatment trials were carried out with three different seed lots planted at two locations each year during 2008-2010. Emesto (penflufen 50g/l), Amistar (azoxystrobin 250g/l) and Rizolex 50FW were tested. Amistar was applied in furrow at planting spraying 3l/ha as a narrow band (10 cm wide) under the seed tubers, and the Emesto and the Rizolex were applied prior to planting by dipping. As in the experiments

mentioned above there were large variations between locations in development of skin blemish diseases. All three treatments reduced the amount of *R. solani* sclerotia and hypha on the progeny tubers. Amistar and Emesto also reduced the frequency of *H. solani* sporulation on incubated progeny tubers (plug-test). In 2008 and 2010 there were attack of black dot (*Colletotrichum coccodes*) at one location and in these trials there were less black dot in the Amistar treatment.

Fungicide sensitivity tests were carried out for *R. solani*, *H. solani*, *C. coccodes* and the skin spot pathogen *Polyscytalum pustulans* with seven different fungicides. Mycelial growth of ten isolates of each fungal species was measured on fungicide amended PDA media. All isolates of *R. solani* were sensitive to pencycuron, penflufen, protiokonazol, and to some degree to imazalil. One of the *R. solani* isolates had reduced sensitivity to tolclofosmethyl and fludioxonil. The sensitivity to azoxystrobin varied a lot between the *R. solani* isolates. Pencycuron and tolclofosmethyl had no or low effect on the mycelia growth of *C. coccodes*, *H. solani* or *P. pustulans*. All isolates of *C. coccodes*, *H. solani* and *P. pustulans* were sensitive to imazalil and protiokonazol, except one *P. pustulans* isolate with low sensitivity to protiokonazol. All isolates of *H. solani* were sensitive to fludioxonil and penflufen, but there were a large variation in the sensitivity to azoxystrobin. All isolates of *C. coccodes* were sensitive to fludioxonil and to azoxystrobin and to some extent to penflufen. All isolates of *P. pustulans* were sensitive to azoxystrobin and penflufen and to some extent to fludioxonil.

Field damage in potato by lesion nematode *Pratylenchus penetrans*, its association with tuber symptoms and its survival in storage

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Nematodes, commonly known as round worms, are the most common multicellular animals on planet Earth. After 1000 million years of evolution members of the phylum Nematoda have a high bionomic diversity. As habitants of the soil and rhizosphere nematodes are involved in energy fluxes, and affect carbon and nutrient cycles. As plant parasites, either alone or in synergism with other pathogens, nematodes are responsible for plant disease complexes and major crop losses.

A growth depression in a field of potato (*Solanum tuberosum*) cv. Saturna [resistant to pathotype Ro1 of potato cyst nematode (PCN) *Globodera rostochiensis*], suggestive of potato cyst nematode damage, was detected in Grue, eastern Norway. Analyses of soil samples did not detect PCN, but demonstrated the occurrence of a large number of lesion nematodes *Pratylenchus penetrans*. Tubers from the depressed part of the field had severe symptoms similar to those caused by the common scab bacterium *Streptomyces scabies*. Potato yield was reduced by 50 % in the affected area of the field. Transect-sampling showed plant growth to be negatively correlated with densities of *P. penetrans* and suggested a damage threshold of potato to the nematode of 100 specimens per 250 g of soil.

Common scab (*Streptomyces scabies*) occurred frequently in the affected area. *P. penetrans* was present in roots, underground stems, stolons and tubers. Tubers from the depressed part of the field had severe symptoms similar to those caused by the common scab bacterium. In tubers, nematodes were detected inside cross-lesions typical symptoms of common scab, and occurred also in the outermost 0.5 mm tissue associated with such lesions. In pots with sterile sand, micro-tubers of potato cv. Saturna,

produced from meristems, were grown in a greenhouse infected with, *P. penetrans*, *S. scabies*, and a combination of *P. penetrans* and, *S. scabies*. *P. penetrans* alone induced tuber lesions similar to those of common scab. Also, the combined inoculation of the bacterium and the nematode seemed to enhance symptom expression. Similar scab symptoms, in connection with lesion nematode infections, have been observed on potato tubers cv. Oleva, which also is relatively tolerant to common scab.

Symptomatic tubers cv. Saturna first stored at 4 °C for 20 weeks were transferred to pots with sterile sand and grown for 3 months in the greenhouse. In these cultures *P. penetrans* was first detected in soil 8 weeks after planting. Examination at harvest of soil, roots, stolons, tubers demonstrated symptoms typical of *P. penetrans*. Interestingly, *P. penetrans* survives storage of potatoes, from which new infections may develop. Hence, potato tubers do appear to be an important means for the spread of *P. penetrans* to new areas. The fact that the symptoms induced by this nematode may be mistaken for symptoms of common scab suggests that the frequency of *S. scabies* might have been overestimated in regular surveys. Infections by *P. penetrans* have important implications for scab control. This pertains in particular to recommended maintenance of high soil moisture at and during 4-9 weeks after tuber set. If symptoms are related to nematode infection rather than to the scab bacterium, this recommendation would allow for a rapid build-up of lesion nematodes resulting in a decrease in both yield and marketability of the tubers. Further studies are needed to investigate the extent of this problem.

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Fusarium dry rot on potatoes in Norway: Identification of pathogenic *Fusarium* spp. and implementation of molecular diagnostic methods

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Fusarium dry rot is one of the most important postharvest diseases on potato. We are studying the importance of this disease in the project “Improved quality of Norwegian fruits, potatoes and vegetables after long and short-term storage” (2010-2014).

Fusarium dry rot is caused by several species of *Fusarium*. In a survey in Norway in 1978 *F. coeruleum* was the most common *Fusarium* species isolated from potato. The disease has been more common in Norway in the last decade.

One of the main tasks of our study is to find out which *Fusarium* spp. currently causes dry rot problems in Norway. Knowledge about which species we have can provide us with better understanding of the *Fusarium* dry rot problem and thereby make basis for a better control strategy towards the disease.

In three growing seasons from 2010-2013 a survey on *Fusarium* spp. in potato samples collected from different regions in Norway will be carried out. The identification of the species will be based on morphological and molecular methods.

In the season 2010/2011 five species of *Fusarium* were identified:

- *F. coeruleum*
- *F. avenaceum*
- *F. culmorum*
- *F. cerealis*
- *F. sambucinum*

Another important task in the project is to develop a multiplex real-time PCR detection assay to detect the most important *Fusarium* species as well as another important storage pathogen *Boeremia foveata* (gangrene). In the survey in 2010/2011 the most important *Fusarium* species were *F. coeruleum*, *F. avenaceum* and *F. culmorum*. Therefore the multiplexing is currently concentrated on these species. The multiplex testing will be particularly useful for predicting disease development in storage at the time of harvest.

Prediction of storability and biological control for preventing spoilage and quality loss in stored potatoes - A new NKJ-project on post-harvest potato diseases

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Rotting of vegetables and fruit in storage by microbes is a world-wide problem that causes big losses in agriculture, industry and trade. The goal of this project is to use potato as a model to develop diagnostic test for quality control and to apply biological control for prevention of post harvest diseases of potato. Interaction of potato tubers with fungal pathogens causing dry rot (*Fusarium*), potato blight (*Phytophthora*) and silver scurf (*Helminthosporium*), and soft rot (*Pectobacterium* and *Dickeya*) will be used as model systems. The gene expression in the host and the pathogens will be studied with deep sequencing in two conditions; in high humidity that promote the diseases and in low humidity suppressing them. The gene expression profiling of interesting genes in the

host and in the pathogens during interactions in different conditions will be verified with real-time PCR. The goal is to understand, how the various processes in the host and the pathogens are affected by humidity, and identify processes which can be linked to the onset of rotting. Based on this information, realtime PCR or ELISA test will be developed for the identification of the lots that have the highest risk of rotting. Furthermore, biological control organisms will be evaluated for the ability to prevent rotting and skin blemishes in harvested potato tubers. The interaction of the control organisms with the pathogens will be evaluated and studied in the conditions either promoting or suppressing the post-harvest diseases.

'*Dickeya solani*', a new form of blackleg in potato - occurrence and importance in Sweden

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Earlier studies in Sweden showed that blackleg was caused by the bacterial genus *Pectobacterium atrosepticum*. A more aggressive form of blackleg appeared in the Netherlands in 2005. Analyses with PCR-techniques of infected seed potatoes showed that it was a new species or sub-group within the genus *Dickeya* spp, which was named '*Dickeya solani*'. The bacteria were spread across Europe with seed potatoes. A large proportion of seed potatoes used in Sweden is imported. A study from the last two growing seasons confirms that infections caused by '*Dickeya solani*' also occurs in Sweden.

A total of 93 analyses from the 2010 harvest were included in our inventory of which 79 of the samples were from seed lots. Analyses of about 40 samples have been funded within our project. The remaining data were made available by different companies. The 93 samples included in the study consisted of 44 different varieties, both table and starch potato varieties. Samples were sent to the laboratory NAK, the Netherlands, via the Swedish Board of Agriculture and the laboratory MTT in Finland, where they were tested with PCR-technique for a variety of bacteria that can cause blackleg. The samples were analyzed for the occurrence of the bacteria *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya* spp. NAK also did an analy-

sis to determine if *Dickeya* spp was '*Dickeya solani*', *Dickeya dianthicola* or another *Dickeya* species.

In 20 of the 93 samples included in the study bacteria of the genus *Dickeya* spp were confirmed. When further analysis was done, '*Dickeya solani*' was the most common species, even if *Dickeya dianthicola* and *Dickeya* spp also were found. *Pectobacterium atrosepticum*, causing the type of blackleg we are used to, was found in a total of 44 samples and *Pectobacterium carotovorum* subsp. *carotovorum* in a total of 74 samples. Nine of the 93 samples did not contain any of the different bacteria that were analyzed. In 42 samples two or more types of bacteria were found. *Dickeya* spp was found in ten of the 44 varieties tested and was proved to be '*Dickeya solani*' in seven of these varieties. One of these varieties was a starch potato variety. '*Dickeya solani*' were found in five varieties of Dutch origin (one or more generations in the past) and in one sample where the origin is identified as Swedish and in one variety imported from Finland, where it is unsure of the origin is Dutch or Finnish. We cannot draw conclusions about how widespread '*Dickeya solani*' is in Sweden, because the samples are not sufficiently representative. But the study confirmed that we have *Dickeya* spp. and '*Dickeya solani*' in Sweden.

Potato blackleg and soft rot caused by new, highly virulent pathogens

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Potato blackleg and soft rot are bacterial diseases of potato. Symptoms include dark discoloration of the stem base and aerial stem rot. Damaged vascular tissue leads to wilting of the affected plants. Soft rotting of tubers also occurs. Latent infections are common and the diseases are mainly spread by contaminated seed potatoes. In recent years potato blackleg has been responsible for most of the cases where seed potatoes were rejected or downgraded by the regulatory bodies in northern Europe. Also producers of ware potatoes may experience large crop losses (up to 40 %) as a result of the disease.

There are several bacteria that can cause the described symptoms. They have previously been considered to belong to the genus *Erwinia*: *Erwinia carotovora* subspecies *carotovora*, *Erwinia carotovora* subspecies *atroseptica* and *Erwinia chrysanthemi*. Phylogenetic classification with new DNA technology has led to regrouping of many plant pathogenic bacteria in recent years. The new current names for the above mentioned bacteria are: *Pectobacterium carotovorum*, *Pectobacterium atrosepticum* and *Dickeya chrysanthemi*. In the genus *Dickeya*, not only *D. chrysanthemi* has potato among its host plants, but also *D. dadanthi*, *D. zeae* and especially *D. dianthicola* have been shown to be the cause of damage in potato production. *D. dianthicola* was first reported as an aggressive potato pathogen from the Netherlands in the early 1970's. In the following years similar detections were made in Denmark, Finland, France, Hungary, Poland, Slovenia, Spain and Switzerland. In 2005 a new, more aggressive *Dickeya* was detected in the Netherlands, and in the following years also in Belgium, Finland, France, Israel and Poland. In 2010, the bacterium has been detected in several locations in the United Kingdom. This bacterium,

which has "appeared" quite recently, has become the dominant cause of blackleg in the Netherlands and some other European countries within 5 years, with a five-fold increase of the related annual economic losses (about 25 million EURO). It is reported that the bacterium is more aggressive than both *P. atrosepticum* and *D. dianthicola*, can attack in a much wider range of climatic conditions, needs lower bacterial concentrations to cause disease and spreads faster and more efficiently in the potato field. The name '*Dickeya solani*' has been proposed for this harmful organism.

In Norway, '*D. solani*' has not yet been detected. Bioforsk Plantehelse has received potato samples with unusual blackleg symptoms in the past two years, some of which proved to be infected by virulent strains of *Pectobacterium wasabiae*. Currently, a small, initial *Dickeya*-survey is carried out at the institute. More than hundred isolates have been collected from 17 arbitrarily gathered samples of symptomatic potato plants. These are in the process of being characterized with realtime PCR assays. In 2012, a systematic survey will be carried out, funded by the Norwegian Food Safety Authority, targeting mainly potato varieties of foreign origin which have entered the country in recent years.

Summary

Bacterial populations are changing and new, highly virulent pathogens appear. '*Dickeya solani*' is now being held responsible for the majority of blackleg cases in Northern Europe. The bacterium spreads quickly, but has so far not been detected in Norway. The risk of introduction of the bacterium increases each time imported seed potatoes are planted in this country.