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Neonectria cankers on trees

– Abstract book, SNS/NKJ network meeting, Ås 06.02.2018

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Content

Preface

"Neonectria cankers on trees – meeting of changed climatic conditions and increased problems in Scandinavian horticulture and forest production by interdisciplinary networking" is a Nordic network-project financed by Nordic Forest Research (SNS) and Joint Committee for Agricultural and Food Research (NKJ). The network period is 2017 and 2018 and the first meeting is organized by NIBIO at Ås, Norway. Abstracts submitted from the speakers are published in this book which will be available online. A promised outcome of the network was a review article on the *Neonectria* species occurring on trees in the Nordic countries. Thus, this book of abstracts is an attempt to collect all available information for the review paper and therefore each participating country has submitted an extended reference list together with the abstracts.

The three *Neonectria* species that are most commonly occurring on Nordic trees are *Neonectria ditissima*, *Neonectria fuckeliana* and *Neonectria macrospora*. They cause damage on a range of trees in forestry and horticulture. A cooperation covering both forestry and horticulture is new, and we hope the *Neonectria* network will be fruitful for the research within this subject and thereby benefit the industries.

Lofthus/Ås 31.01.2018

Jorunn Børve Venche Talgø

Tuesday 6th Feburary

10:20 **Welcome,** Dan Aamlid, NIBIO

10:30-12:30 *Neonectria ditissima*

10:30-11:00 **Guest lecture:** Roland Weber, Esteburg, Germany

11:00-11:20 **Denmark:** Iben Thomsen, Copenhagen University/Magnus Gammelgård, Aarhus University

11:20-11:50 **Finland:** Fredrik Bjørklund, Pro Agria/ Tuuli Haikonen,Natural Resources Institute/Marika Pylkäinen, Finnish Tree Care Association

11:50-12:10 **Norway:** Jorunn Børve, Norwegian Institute of Bioeconomy Research

12:10-12:30 **Sweden:** Larissa Gustavsson, Swedish University of Agricultural Sciences

12:30-13:15 **Lunch with discussion**

13:15-14:30 *Neonectria neomacrospora*

13:15-13:45 **Guest lecture:** Ana Perez- Sierra, Forest Research, UK

13:45-14:05 **Denmark:** Knud Nor Nielsen, Copenhagen University

14:05-14:25 **Norway:** Venche Talgø, Norwegian Institute of Bioeconomy Research
Discussion

14:30-16:10 *Neonectria fuckeliana*

14:30-15:00 Guest lecture: Richard O'Hanlon, Agri-Food and Biosciences Institute (AFBI), Northern Ireland

15:00-15:10 Denmark: Iben M. Thomsen, Copenhagen University

15:10-15:30 Finland: Anne Uimari, Natural Resources Institute

15:30-15:50 Norway: Halvor Solheim/ Venche Talgø, Norwegian Institute of Bioeconomy Research

15:50-16:10 Sweden: Martin Pettersson, Swedish University of Agricultural Sciences

16:10 **Discussion – future plans, knowledge gaps and potential usefulness of the network**

17:00 **(or later if needed) Closing of the meeting**

Apple canker caused by *Neonectria ditissima* in Northwestern Europe

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Next to apple scab caused by *Venturia inaequalis*, canker due to *Neonectria ditissima* is the most important disease of apple trees in the cool maritime climate of Northwestern Europe. An internationally accepted body of knowledge on *V. inaequalis* is in contrast to regional and sometimes contradictory opinions on *N. ditissima*. Co-ordinated research and knowledge exchange between Scandinavian institutions would be of great benefit to fruit farmers.

Biology

Apple and pear as well as other broad-leaved trees can become infected. The taxonomic distinction between fruit tree isolates (*N. galligena*) and forest tree isolates (*N. ditissima*) has recently been abandoned, but the issue of specific races has not yet been fully resolved. On apple trees, infections take place *via* natural openings (fruit or leaf scars) or wounds in the bark of twigs, branches or trunks. Following a latency period of some weeks, months or even years, necrotic bark lesions become visible and gradually enlarge in the course of several months (Figure 1). In Northwestern European commercial orchards, infections at leaf fall in autumn are the most important entry route, and these typically become visible in the following spring from the flowering period onwards. Within a few weeks of appearance of a canker, macroconidia are produced on superficial sporodochia. These spread the infection within the affected plant by water splash, giving rise to a heterogeneous distribution in which trees bearing multiple cankers are interspersed with healthy ones. Infections may penetrate from a bark canker into the underlying woody tissue where colonisation becomes visible as dark brown longitudinal streaks inside the previous year's growth ring. The epidemiological role of xylem growth is uncertain. In Northwestern Europe, perithecia begin to be formed at the end of the first season of a canker. Ascospores are released explosively and become airborne, enabling the fungus to reach new orchards within a downwind radius of several hundred metres. In Northwestern Europe, there is evidence that the peak of ascospore release is in autumn, although a second peak may appear in spring.

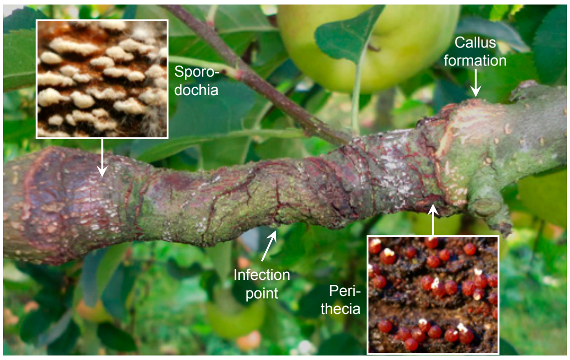

Figure 1. Features of a perennial canker on the robust apple cultivar 'Topaz'.

At the margin of a canker, callus formation indicates a defence reaction by the tree. Although no monogenetic resistance to canker has as yet been described, apple varieties differ strongly in their susceptibility. Age and growth vigour of the trees as well as infection conditions and inoculum availability also have an effect on canker susceptibility. In robust varieties callus formation may be sufficient to contain and ultimately kill off the infection. Conversely, highly susceptible varieties such as 'Nicoter' (Kanzi) or 'Civni' (Rubens) cannot be grown in Northwestern Europe unless all available measures are taken to protect the trees from infections.

Nursery tree infections

Latent *N. ditissima* infections may break out after planting of trees in the production orchard in spring. Such undetected nursery infections are particularly dangerous for two reasons. Firstly they sabotage the farmer's efforts at canker control through hygiene, and secondly they typically affect the trunk, leaving little choice but to uproot the tree. With susceptible varieties, such infections may affect up to 20% of young trees during their first growing season in a commercial orchard. In less susceptible varieties longer durations of latency are possible, giving rise to a gradual loss of trees over several years by 'covered canker' surrounding the entire lower trunk. Even rootstocks may be affected in this way (Figure 2).

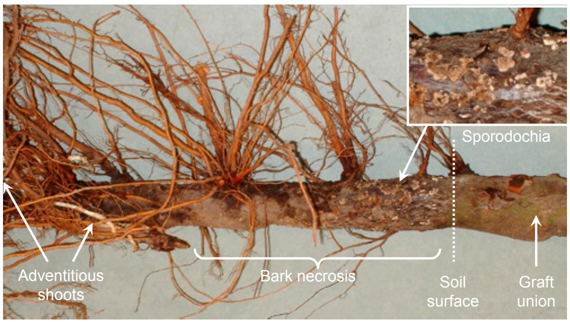

Figure 2. Features of a covered canker developing on the M9 rootstock of a two-year-old 'Jonagold' tree.

Fruit infections due to *N. ditissima*

Floral infections give rise to a blossom-end rot in the course of the growing season, whereas infections of the maturing fruit become visible as a post-harvest rot in storage. There is a correlation between the severity of canker on individual trees and the incidence of fruit rots, suggesting that these infections are caused chiefly by conidia, not ascospores.

Control of apple canker

The two principal control options are pruning (=hygiene) and fungicide use. Pruning should be conducted from flowering time onwards until the end of July, and again in winter. All visible canker lesions should be cut off from twigs or excised from trunks. Larger pieces of infected wood should be removed from the orchard in order to prevent the continued release of ascospores from dead infected wood. The intensity of canker pruning is dependent on susceptibility of the trees. Given that most scab fungicides sprayed during the growing season also have an effect against canker, specific chemical control of apple canker should focus on the period of leaf fall because of the high number of susceptible leaf scars and because no regular sprays against other pathogens are made at that time. In Northwestern Europe, fungicides should possess a high rainfastness in order to avoid a repetition of sprays at short intervals. In Northern Germany, 1-3 sprays with copper fungicides in autumn are indispensible for canker control in susceptible varieties.

An integrated approach to canker control should also include indirect measures. Excessive nitrogen fertilisation of young trees promotes rapid vegetative growth which renders the tree more susceptible to canker. Susceptible cultivars should not be planted downwind of heavily cankered old orchards. Pruning should not be conducted during periods of wet and mild weather. Nursery trees should be carefully inspected for cankers prior to planting, and repeatedly during the first growing season. Young trees with trunk canker should be removed immediately.

Perspective for further research

There is a need for a better understanding of the dynamics of *N. ditissima* development (perithecium development, ascospore discharge) at the end of the growing season. In particular, it would be desirable to predict the likely severity of autumnal canker infections ahead of fungicide sprays at leaf fall. The unique efficacy of copper salts in canker control has been documented, but the registration of such fungicides is difficult in Scandinavia. Therefore, strategies to reduce the copper input into the orchard should be developed. Further, a standardised evaluation of canker susceptibility of cultivars relevant to Scandinavian growers should be conducted. The issue of nursery tree infections also deserves further research: How do these infections arise, and how can they be detected prior to explanting of the trees? The above issues are being addressed in the 'KreftKamp' project which is a Scandinavian research effort under Norwegian leadership.

Literature

Weber, R.W.S. 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. Erwerbs-Obstbau 56:95-107.

Neonectria on broadleaves in Denmark

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Neonectria causes problems in a wide range of tree species and productions in Denmark. The main challenges are apples (*N. ditissima*) and Christmas trees (*N. neomacrospora*), but cankers and top dying caused by *N. ditissima*, *N. fuckeliana* and *N. neomacrospora* can also be seen in forest production species, and occasionally in amenity trees.

N. ditissima has been known in Denmark for more than a century due to the fungus causing cankers on broadleaved trees, mainly beech, ash and fruit trees. The impact in forests has varied depending on predisposing factors such as climate, insects and forest management. At present, beech stands in the vulnerable age span (0-40 years) constitute about 3% of the forest area in Denmark, equal to 20% of the beech stands. A few cases of severe but local outbreaks of beech bark canker have been reported, both in the past and in the last 15 years where high precipitation has been common. The main management recommendation is thinning and removal of trees with symptoms.

The apple production area in Denmark was 1,380 ha in 2017 and pears 300 ha. There has been a small decline in the apple area, while pear production has increased. *Neonectria ditissima* (syn *N. galligena*) and *Venturia inaequalis* are the two main fungal diseases causing economic losses in the fruit production. *N. ditissima* infection happens via wounds in early autumn by conidia and later with ascospores. The reverse order occurs in spring. The infection period is long, and it's difficult to protect the trees the whole time, especially in humid conditions.

Figure 1. *Neonectria ditissima* canker and fruit bodies on apple and 10 year old canker on beech from severely affected young forest stand.
Photos: M. Gammelgaard Nielsen and IM Thomsen

Extended literature list on *Neonectria ditissima* from Denmark

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Neonectria ditissima in Finland – epidemiology and disease management in the almost peripheral regions of pathogen distribution?

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Neonectria ditissima is the causal agent of canker disease in apple (*Malus* sp.) and other broad-leaved tree species. In Finland, the commercial apple production is mainly located in the semi-maritime regions of the southern coastal areas and archipelago. Many of the largest towns with their street trees are also located in the southern part of Finland. The climatic conditions in southern and southwestern Finland are conducive for *Neonectria ditissima*, and severe apple canker can be observed in the main apple production regions. The climate in the rest of the Finland is subarctic (Köppen climate classification), however the mildest south-eastern lake area is expected to gradually become climatically suitable for commercial apple production by 2040 (Kaukoranta *et al.*, 2010).

Apple canker has seen as a relevant disease in Finland for over 100 years (Gauffin *et al.*, 1906). A recent increase in disease severity is believed to be due to latent infections in newly established orchards, the intensified cultivation technique with less vigorous rootstocks, the choice of susceptible cultivars and rootstocks, and the changing, more maritime climate (Gabrielsson, 2014). Similar problems with latent infections and subsequent spread of canker have been reported in Norway, and the importance of knowledge transfer for higher vigilance and informed decision-making in disease management in orchards is acknowledged (Børve *et al.*, 2015; Gabrielsson, 2014; Haikonen, 2017).

The most common apple cultivar in Finland has traditionally been the Canadian cultivar Lobo, which often shows high disease prevalence and severe symptoms in the field. Of the other common cultivars, Discovery, Summerred, Åkerö and Rajka are often severely diseased. Cultivars with field tolerance to canker in the Finnish conditions are at least Aroma/Amorosa, Santana, and, to some degree, Rubinola (Gabrielsson, 2014). Plant trials with several cultivars grown in Finland are being conducted to understand the role of genetic canker tolerance in cultivar performance (Haikonen *et al.*, 2017).

Several broad-leaved street tree species are known hosts for *N. ditissima*. Of the severely affected tree species, especially lindens (*Tilia* spp.) are common street trees in the Finnish cities. In *Tilia* trees, closed cankers in young trees and open cankers in the old trees are often observed. Canker wounds are also known to predispose street trees for the action of saprotrophic fungi, making the secondary effects of the disease more severe than the primary effects are. In practical tree care, the causal agents of the canker diseases are usually not identified, but tree care professionals have a working knowledge on canker management. Instead of an immediate removal, the condition of cankered trees is being followed. Visual tree assessment (VTA) supplied with drill resistance measurement or sonic tomography are the main tools. Branch cankers may be removed by pruning.

The infections in imported apple plantlets appear common, but field observations have additionally suggested local, airborne infections originating from near heavily infested orchards. The field dispersal occurs via the release of fungal conidia and ascospores, which in more southern climates may be present year-round (Weber, 2014). Analysis on the amounts of the latent infections originating from nurseries and the new airborne infections in the orchards are lacking. Preliminary air sampling results in the springtime show that the ascospores can be airborne in the time of apple bud-burst, flowering and petal dehiscence (Haikonen *et al.* unpublished results).

Modelling climatic risk in Finland confirmed that the coastal and archipelago regions of Southern and Central Finland are at high climatic risk for disease development (Haikonen *et al*. unpublished results). Additionally, the southern lake area appears a moderate risk region. The modelling results revealed a clear year-to-year variation in the risk level and time of disease conducive conditions. The highest risk for apple canker disease progression was calculated in the Septembers, the main apple harvest time, and the second highest in the Junes, the apple bloom time. Practical validation of the modelling results and their relevance for canker management in fruit orchards and street tree care needs to be discussed.

Figure 1. *Neonectria* canker symptoms on apple (*Malus domestica*). A young lesion (top). A cracking canker wound on a trunk of a young apple tree (bottom left). An open canker on a trunk of an old apple tree (bottom right). Photos: Tuuli Haikonen

Figure 2. Apple canker threatens the fruit-forming wood. Left: Young infections resulting in the deaths of a lateral bud and a fruit spur, and expanding lesions on the twig. Right: Stem canker inflicts blossom wilt and dieback on an attached branch. Photos: Pernilla Gabrielsson

Literature

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Figure 3. An open *Neonectria* target canker on a trunk of *Tilia* sp. Photo: Tuuli Haikonen

Extended literature list on *Neonectria ditissima* from Finland

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Neonectria ditissima in Norway

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European canker caused by *Neonectria ditissima*, is a serious disease in Norwegian apple (*Malus domestica*) production (Figure 1). It used to be a predominantly coastal disease, but over the recent decades, it has become important in all apple producing areas of the country. New cultivars, milder winters and increased precipitation in inland regions may be factors leading to increased damage by the fungus. *Neonectria ditissima* has been found in a number of deciduous tree species in Norway, including genera like *Acer*, *Alnus*, *Cornus*, *Fagus*, *Fraxinus*, *Populus*, *Prunus*, *Sorbus* and *Tilia*, as well as in the evergreen broad leaf tree *Ilex aquifolium* (Figure 1). However, the economic losses are minor in these tree species.

Figure 1. Canker wounds with numerous fruiting bodies of *Neonectria ditissima* on apple (top) and holly (*Ilex aquifolium*) (bottom). Photos: Vence Talgø

In fruit growing, we have focused on identifying critical phases during the tree production where infections may occur. Investigations have shown that rootstocks are susceptible during propagation (Kolltveit, 2015; Børve *et al.,* 2018), and rootstock B9 was more susceptible than M9 (Børve *et al.,* 2018). Furthermore, it was documented that budwood can carry *N. ditissima* that may initiate infections in young trees (Børve *et al.,* 2017a). Inoculated buds on T-budded trees developed disease from the time of budding until next spring when only a few buds had survived (about 10%). On grafted trees, most of the inoculated buds started to grow, but the trees developed disease during the first or second season (Figure 2) (Børve & Stensvand, 2017).

Recently, a species-specific Taqman real-time PCR assay was developed at NIBIO for rapid identification and quantification of *N. ditissima*, e.g in buds and rootstocks (Pettersson *et al.* 2017).

Existing knowledge about the pathogen's biology and potential treatments has been communicated to the industry during field tours, grower meetings and in trade journals (Weber, 2014; 2016). Photos of symptoms and signs of the disease/pathogen on apple cultivars grown in Norway were collected and presented in different languages in a manual for pruning personnel (Børve & Myren, 2016) and in trade journals (Børve *et al.,* 2016).

Figure 2. Canker caused by *Neonectria ditissima* in the section between rootstock and budwood on a grafted apple tree (top), and fungal staining of the wood above an infection of *N. ditissima* on a young apple tree (bottom). Photos: Jorunn Børve

Extended literature list on *Neonectria ditissima* from Norway

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Research on European canker at SLU, Sweden: knowledge gained, tools developed, lessons learned

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European canker is the most serious disease in apple production in Sweden. The disease is caused by a necrotrophic fungus, *Neonectria ditissima*. Spores of the fungus are present in the orchards all year around and infect trees and fruit through natural and man-made wounds. The disease damages branches, the main trunk and fruit in storage. The trees may become infected during propagation. The disease is mainly controlled by removing and burning of cankered wood. The chemical and biological control is very limited. Breeding new cultivars with high level of resistance is an urgent need.

Canker research at the Department of Plant Breeding at SLU in Sweden has been conducted since 2010 in collaboration with national and international partners. The research focus has been on development of plant resistance tests, evaluation of canker resistance in genetically diverse germplasm, understanding molecular mechanisms underlying resistance, dissection of genetic basis of canker resistance, genetic diversity of the fungus, disease diagnostics. The main outcome:

a) Efficient and reliable spore inoculation-based plant resistance tests have been developed and tested in two plant models, cut shoots and young trees (Garkava-Gustavsson *et al.*, 2013; Ghasemkhani *et al.*, 2015a; Garkava-Gustavsson *et al.*, 2016; Garkava-Gustavsson *et al.*, in prep.);

b) These plant resistance tests were successfully applied in germplasm screenings (Garkava-Gustavsson *et al.*, 2016; Garkava-Gustavsson *et al.*, in prep.) and in other studies (Ghasemkhani *et al.*, 2016a);

c) A Real-time PCR assay has been developed and applied for detection and quantification of the fungus in infected trees (Ghasemkhani *et al.*, 2016a; Ghasemkhani *et al.*, 2016b);

d) Differentially expressed genes have been identified in an RNA-seq study on the partially resistant cultivar 'Jonathan' and the highly susceptible cultivar 'Prima' of which several co-localized with previously identified QTL intervals (Ghasemkhani *et al.*, 2015b; Garkava-Gustavsson *et al.*, in prep.);

e) Putative QTL for infection percentage have been detected;

f) Genetic diversity of the pathogen has been investigated with SSR and AFLP markers (Ghasemkhani *et al.*, 2016c)

Our ongoing research is aimed at penetrating into molecular mechanisms of resistance. We evaluate the tempo-spatial differences in resistance in a set of partially resistant and susceptible cultivars by conducting transcriptomic, proteomic, metabolomic and lipidomic analyses with the aim to get an integrated view on differences in cultivar responses under *N. ditissima* attack. We are also working on mapping of virulence loci in the fungus by GWAS (genome wide association study). All this research aims to come to a better understanding of defense mechanisms and resistance components as well as generate insights in the plant-pathogen interaction and possible co-evolution.

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Update on *Neonectria neomacrospora* on *Abies* spp. in Britain

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Neonectria canker of fir trees was first described from grafted white fir (*Abies concolor*) in a German nursery more than 100 years ago and was reported in other European countries without causing high levels of damage. However, it was in 2008 that Norway reported severe Neonectria canker symptoms on white fir, in 2011 was reported on subalpine fir (*A. lasiocarpa*) in Denmark, in 2015 the disease was detected on white fir in Sweden, and in 2017 it was reported in Belgium on grand fir (*A. grandis*). In Britain, there were sporadic reports of the disease associated with severe twig canker and dieback in Scotland on Greek fir (*Abies cephalonica*) and in England on white fir from the 1960s, and causing dieback and cankers on noble fir (*A. procera*) in Wales from the 1990s. Nevertheless, in 2015 the death of Taiwan fir (*A. kawakamii*) was reported on a fir collection in England and the cause was identified as *N. neomacrospora*. This situation prompted the survey of other firs in tree collections and plantations in England and Wales. Since then the number of affected trees has increased and the disease has been detected in a private garden, in a pinetum/arboretum and in at least 24 forest sites. Although Noble fir is the most severely affected species in forest plantations, the disease has been detected on 15 different fir species in the different tree collections.

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Neonectria on conifers in Denmark

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Neonectria causes problems in a wide range of tree species and productions in Denmark. The main challenges are apples (*N. ditissima*) and Christmas trees (*N. neomacrospora*), but cankers and top dying caused by *N. ditissima*, *N. fuckeliana* and *N. neomacrospora* can also be seen in forest production species, and occasionally in amenity trees.

Neonectria neomacrospora

Approximately 22.000 ha, 3.6 % of the Danish forest area or 0.5 % of Denmark, is used for production of Christmas trees and greenery. The main species are *Abies nordmanniana* (age 0-20 years) and *A. procera* (age 0-40 years). Several Abies species are used for timber production, mainly *A. alba* and *A. grandis*. In total there are 57.184 ha of firs in Denmark, equal to 19 % of conifers and 9 % of the total forest area.

Since 2011 many cases of severe twig blight and dieback in species of fir caused by *N. neomacrospora* have been reported in Denmark. The pathogen has most likely been present for some years prior to this outbreak, as symptoms were noticed at least since 2003. However, no known reports identifying the fungi, or describing its symptoms on fir in Denmark, exist prior to 2011, even though *N. neomacrospora* was first described in Schleswig, North Germany, in 1913.

N. neomacrospora disperse by micro- and macroconidia, as well as ascospores. Where the former most likely are splash-dispersed locally within the individual tree, the ascospores are mainly dispersed by wind. Possible seasonality of dispersal is not yet described, but sporodochia carrying macroconidia have been observed all year round, and ascospores have been shown to disperse down to subzero degrees (unpublished data).

Figure 1. *Neonectria neomacrospora* symptoms and fruitbodies on *Abies*. Photos: IM Thomsen and K Nor Nielsen

Sanitation of infected stands will lower inoculation loads on the remaining trees. Perithecia often form abundantly after trees have been cut and can often be found in log stacks, even from apparently healthy stands. Susceptibility differs both among and within species, and breeding for resistance may be a long term solution to *Neonectria* fir canker.

Currently research aim to describe the population genetic structure and migration history of the pathogen in Europe and North America. By assessing population sizes, gene flow and admixture, we can give a risk assessment based on the adaptive potential of the pathogen.

Neonectria fuckeliana

Bark necroses and topdying caused by the related species *N. fuckeliana* are probably more common in Denmark than foresters realize, mainly on spruce (*Picea abies* and *P. sitchensis*). Symptoms have been observed by pathologists for more than a century, but no surveys or in-depth studies have been carried out. At risk are spruces age 0-20, which constitute 6.2 % of conifers, in relation to topdying. In addition, *P. abies* age 20-40 in relation to wounds caused by red deer and machines which may be used by the fungus to enter the bark and the outer sapwood. Based on forest inventory data, an average of 6.8% of Norway spruce has bark damage on stems due to deer and 1.2 % has damage due to machines.

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Neonectria neomacrospora in Norway

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In June 2008, a disease outbreak caused by *Neonectria neomacrospora* was discovered on white fir (*Abies concolor*) in several counties in southern Norway. Later, damage by this pathogen was also found on subalpine fir (*A. lasiocarpa*), Siberian fir (*A. sibirica*), European silver fir (*A. alba*), Greek fir (*A. cephalonica*), Korean fir (*A. koreana*), Noble fir (*A. procera*), Nordmann fir (*A. nordmanniana*), Norway spruce (*Picea abies*) (on one occasion only below a stand of Siberian fir infected by *N. neomacrospora*) and hemlock (*Tsuga heterophylla*). Prior to these findings, Neonectria canker was reported on fir in Norway by Jørstad & Roll-Hansen (1943) and Robak (1951), and confirmed as *N. neomacrospora* by Roll-Hansen (1962).

White fir and subalpine fir are the most severely damaged species in Norway. On those two hosts, attacks often result in mortality. On all other host species, shoot and twig dieback is dominating. White fir is mainly used in landscape plantings as solitary trees. Since 2008, the majority of white firs planted in southern Norway have been severely damaged or died because of *N. neomacrospora*. On subalpine fir, the pathogen has been found in several forest stands planted in the 1960's, a seed orchard, a provenance trial, several landscape plantings and Christmas tree fields. Since subalpine fir is a very important species for the Norwegian Christmas tree industry, it has been the main focus in our investigations.

Inoculation tests confirmed that *N. neomacrospora* may kill subalpine fir (Figure 1).

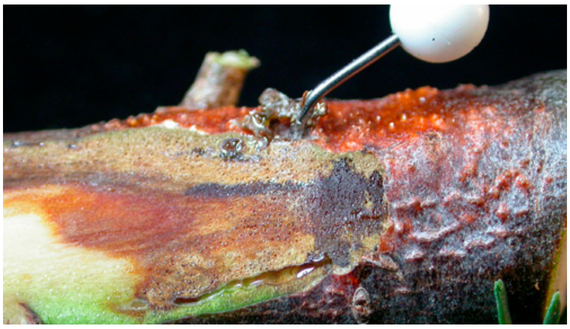

Figure 1. The leading edge of a canker wound on subalpine fir (*Abies lasiocarpa*) three months after inoculation with *Neonectria neomacrospora*. Photo: Venche Talgø

We found *N. neomacrospora* to be seed borne, sometimes in rather high frequencies. To reduce the damages in nurseries and production fields, and to limit the risk of long distance spread of the pathogen via seed and transplant trade, we have recommended regular surveys of seed plantations and seed testing. For testing seed and other tissue samples for *N. neomacrospora*, a species-specific Taqman real-time PCR assay for rapid identification and quantification of the fungus was developed.

In general, disease severity has been less prominent on subalpine fir Christmas trees than in other type of plantings. This is likely due to the use of fungicides in Christmas trees during bud break to avoid needle cast by *Rhizosphaera kalkhoffii* and other foliar fungi. In laboratory tests of several fungicides on mycelial growth (Figure 2) and spore germination, we found that copper oxide (Nordox 75WG), commonly used by the growers, has high efficacy against *N. neomacrospora.*

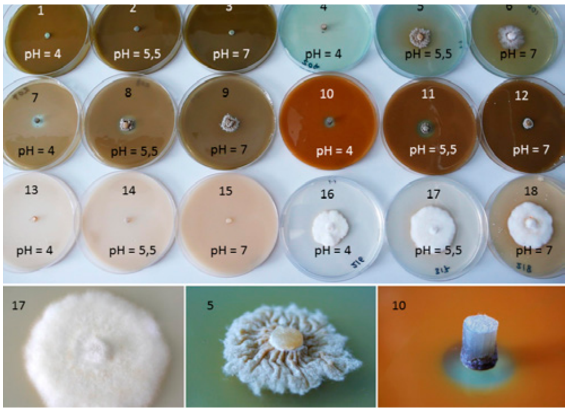

Figure 2. Growth of *Neonectria neomacrospora* on artificial medium (PDA) mixed with fungicides and adjusted to different pH values; 1-3: Nordox 75WG (copper oxide) 1.5 gram/litre (g/l), 4-6: Nordox EXP 0.5 g/l, 7-9: Nordox EXP 1.0 g/l, 10-12: Nordox EXP 1.5 g/l, 13-15: Topsin WG (thiophanate-metyl) 1.5 g/l, and 16-18: sterile water (negative control). The close-up images in the lower panel show examples of different growth patterns of the fungal colonies depending on treatment; 17 normal growth, 5 rugose and darker than normal, 10 sparse growth at the top of the agar plug. Photos: Venche Talgø

In a field trial, we investigated if different nutrient contents in disease free transplants affected the degree of damage caused by N. neomacrospora. The transplants, which had received high or low levels of nitrogen, potassium, calcium and magnesium, were placed under severely infected subalpine fir in a forest stand. After nearly two years, 53% of the trees were dead and only 2.5 % were still appearing healthy. This clearly shows the potential of transferring the disease from older, infected trees to newly planted trees in the vicinity. Nutrient status of the plants at time of planting did not seem to influence susceptibility.

In 1997, no disease was observed in a remote stand of subalpine fir planted in Rogaland county (Hjelmeland) in southwestern Norway in 1967, however, in 2007 numerous trees were dead or dying due to *N. neomacrospora* (Figure 3, top and middle). In 2007, damage by *N. neomacrospora* was also found on white fir and subalpine fir in several locations in the same county, indicating an epidemic. Due to airborne ascospores, *N. neomacrospora* is capable of spreading relatively fast over larger distances. Both temperature and precipitation were above normal prior to the outbreak observed in Hjelmeland (Figure 3, bottom), and may have driven the epidemic.

Figure 3. A forest stand of subalpine fir (*Abies lasiocarpa*) in Hjelmeland appearing healthy in 1997 (top) and severly damaged by *Neonectria neomacrospora* in 2007 (middle). The precipitation and temperature (monthly average) in the region were above normal in the period between 1997 and 2007 when the disease developed. The black horizontal line shows the 30-year normal precipitation. Scale for temperature to the left (°C) and annual mean precipitation (mm) to the right. Photos: Terje Pundsnes (top) and Venche Talgø (middle)

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Neonectria fuckeliana as a pathogen of Sitka spruce in Northern Ireland

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In Northern Ireland, mainly mature Sitka spruce (*Picea sitchensis*) trees have been affected by *N. fuckeliana*, in groups of several dozen at each site. Since 2012, aerial surveys have been picking up dead trees in several sites, with a gradual greying of foliage also noted. Symptoms include multiple resinous lesions on the main stem, typically along its length, but at one site more prevalent near the base of the stems. *Neonectria fuckeliana* has been detected by q-PCR in 29 locations, in all six counties of Northern Ireland. Typical perithecia have been observed at 3 sites. The pathogen (recorded as Nectria cucurbitula; *Nectria fuckeliana*) have previously been found in Northern Ireland as far back as 1884, and was recorded causing resinous lesions on Sitka spruce and Norway spruce in Co Wicklow (Ireland) in 1983. The widespread distribution and historic records of the organism indicate that it may be native to the island of Ireland. In several of the affected sites other stressing factors have been noted- bad drainage, close planting, mechanical wounds, and the presence of Armillaria and/or Collybia has been recorded. Overall, the disease is very worrying for the forestry industry as almost 70% of the tree species composition of forests in Ireland and Northern Ireland are composed of Sitka spruce. Many of these trees may have been planted on unsuitable sites, and so may be already stressed by one or more of the factors listed above. Samples from tree nurseries have not been tested, so the presence of the organism in planted saplings is unknown.

Figure 1. Deas Sitka spruce (Picea sitchensis) trees found to be infected with Neonectria fuckeliana in Northern Ireland.

Figure 2. Typical stem resinous lesions associated with *Neonectria fuckeliana* infection in *Picea sitchensis*.

Neonectria canker on Norway spruce in Finland

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The ascomycete fungus *Neonectria fuckeliana* causes a canker disease on conifers. The pathogen induces formation of resin flowing stem wounds reducing tree growth, wood strength and timber quality. Necrotic tree parts and occasionally tree death are also consequences of the disease.

In Finland the symptoms resembling Neonectria canker have been described in 1930s, however, not until 2000s the disease became a serious health threat for Norway spruce (*Picea abies*). Occurrence of *N. fuckeliana* has increased in recent years and infected stands have been observed in different parts of the country. Interestingly, the disease has not yet been encountered in North Finland. The age of the evaluated spruce stands has ranged from five to over sixty years old. However, the fungus has caused the worst damaged including tree death in spruces less than 25 years-old which often have been planted in former agricultural land. On the bark of the damaged trees, the reddish perithecia, the fruiting bodies of sexual reproduction, have been commonly found. The perithecia-produced ascospores have been considered as the main source of infection and dispersal of the spores is believed to happen mainly by splashing water. Interestingly, infestation by *Cydia pactolana* moth has been observed in several N. fuckeliana-infected stands in Finland and the putative interaction between the pathogen and the pest needs further clarification.

Over twenty isolates of *N. fuckeliana* have been pure-cultured from the infected Finnish spruce stands. They have highly homologous ITS, EF-1alpha and -tubulin barcoding sequences compared to each other. However, the morphology of isolate cultures on PDA medium varies (e.g. culture colour, intensity of sporulation and the amount of aerial hyphae). In PDA, all isolates have *acremonium*-like anamorphs producing only microconidia and the fungal cultures prefer the growth temperature around 24°C. In the green house inoculation tests, all studied isolates infected Norway spruce seedlings but were not able to induce canker formation on the seedlings of *Pinus sylvestris* or *Abies* species.

Norway spruce is the second most cultivated tree in Finland and thus, has a high ecological and economic value. The intense spruce cultivation increasing the supply of *N. fuckeliana* host as well as a changing climate possibly offering better living and spreading conditions for the fungus may have affected on the increased occurrence of the disease.

Figure 1. *Neonectria fuckeliana* infection kills Norway spruce tops (top left), resin flowing cankers and stem wounds caused by *Neonectria fuckeliana* (top right) and co-occurrence of *Neonectria fuckeliana* (red perithecia, arrows) and *Cydia pactolana* moth (larval resin mixed frass piles from a previous season, arrowheads) on Norway spruce stem (bottom).

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Neonectria fuckeliana on spruce in Norway

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Ninety years ago, the Norwegian mycologist Ivar Jørstad (1887-1967) reported *Nectria* infection on spruce (*Picea abies*) for the first time in Norway (Jørstad 1928). He described it as a typical wound parasite on stem and branches, and that it caused mortality and/or top dieback of young trees. Jørstad estimated that the very first finding in Norway probably dated back to 1840 in the Oslo region. Although Jørstad did not described the pathogen to species level, it is reasonable to assume that the report concerned *Neonectria fuckeliana*, since this species was later found on spruce with identical symptoms (e.g. Roll-Hansen & Roll-Hansen 1979, Huse 1981).

Like Jørstad, Roll-Hansen & Roll-Hansen (1980) also described *N. fuckeliana* as a wound pathogen based on wounding experiments in Norway spruce. They also isolated the fungus from «sound-looking wood» (Roll-Hansen & Roll-Hansen 1979). The same was observed by Huse (1981), who found *N. fuckeliana* to be very common inside the heartwood of Norway spruce at all heights, but most abundant from 2-5 m above ground. *Neonectria fuckeliana* was also isolated later in other wounding experiments in Norway spruce; artificial wounding (Solheim & Selås 1986) or wounds made by deer (Veiberg & Solheim 2000).

In contrast to the notion of *N. fuckeliana* as a wound pathogen, we have several times during the last decade, isolated the fungus from black canker wounds that did not seem to be related to wounds caused by animals, thinning operations or other biotic or abiotic damaging agents (Talgø 2009, Talgø *et al.* 2015). The canker wounds often occurred at the base of dead twigs or small branches. Sometimes there were red fruiting bodies (perithecia) in and/or around such wounds, but more frequently, perithecia occurred on the bark of dying, older trees together with heavy resin flow (Talgø *et al.* 2017). In most cases, the dieback seemed to start from the top, even on older trees.

Over a time span of 16 months, we followed the development of perithecia from *N. fuckeliana* under field conditions. The perithecia appeared in clusters, and we observed that a cluster normally concisted of fruiting bodies at different development stages; varying from yellow/orange (young) to dark red (old) in colour (Figure 1). This enabels windborne spread of mature ascospores throughout the growing season given sufficient humidity.

Neonectria fuckeliana has proven pathogenic in recent inoculation tests on Norway spruce (Krokene *et al.* 2001, Talgø *et al.* 2015), but far from as aggressive as the related species *N. neomacrospora* on firs (*Abies* spp.) (Talgø, unpublished data).

At mortality stage, older trees in southeastern Norway have often been found to be attacked by bark beetles (*Ips typographus*) in addition to *N. fuckeliana*, but we have also found *N. fuckeliana* on damaged spruce in western Norway where the beetle is absent. Thus, even if the beetle is able to spread inoculum from *N. fuckeliana*, it may be concluded that the fungus is not dependent on the beetle to disperse its spores. More likely, trees weaken by *N. fuckeliana*, or other fungi, attract the bark beetles (Økland, pers. comm.). Both Armillaria root rot (*Armillaria* spp.) and annosus root rot (*Heterobasidion annosum*) are commonly found on trees with *N. fuckeliana* symptoms and signs, however, that is not always the case. Therefore, we belive *N. fuckeliana* can be a primary pathogen (Talgø *et al.* 2017).

Recent increase in amount and intensity of rainfall may have triggered formation and extensive release of ascospores, causing epidemic-like outbreaks of cankers on spruce. This has cleraly been the case with *N. neomacrospora* on fir in the same regions and during the same timeframe (Talgø, unpublished data).

A species-specific Taqman real-time PCR assay was developed for rapid identification and quantification of *N. fuckeliana* from plant tissue. The primer will be used in seed tests, to trace latent infections etc.

Figure 1. Clusters of fruiting bodies (perithecia) from *Neonectria fuckeliana* on spruce (*Picea abies*) in Norway in May 2016 (top) and August 2017 (bottom). Photos: Venche Talgø

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Neonectria canker – a potential threat to production of spruce in Sweden

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In May 2015, a disease survey in Swedish Christmas tree fields was conducted. The fungus *Neonectria fuckeliana* was isolated from six Norway spruce (*Picea abies*) trees with top-dieback. The trees with top-dieback had dead top-shoots and approximately three-four dead branch whorls (Figure 1). This top-dieback was one of the more common problems we

found on the Norway spruce Christmas trees.
During summer 2016, a Norway spruce Christmas tree field was selected and 1000 trees were investigated for top-dieback. Top-dieback was found in 2% of the trees. However, the farmer had already pruned and removed several dead-tops during the spring in 2016, so the top-dieback was likely higher than 2%. A more thorough investigation is needed to get a reliable top-dieback severity estimate in the Christmas tree production.

Inoculation trials using microconidial suspension of *N. fuckeliana* were completed in 2016-2017. Norway spruce rooted cuttings were wounded and inoculated. Our experiments showed that shearing wounds are sufficient for establishment of *N. fuckeliana* infections, however, the disease development was slow. During the experiments' 8-11 months' timeframe, only minor necrotic cankers occurred, but the fungus was readily reisolated. No fruiting bodies were observed.

The observed top-dieback appear above a girdling point on the upper part of the stem. From the inoculation experiments, one could expect a gradual branch dieback on some of the many shearing wounds created annually by tree shaping activities. However, gradual symptom development such as progressive branch die-back has not yet been observed in Christmas tree fields.

Christmas tree grower's need efficient management strategies to avoid top-dieback. However, more knowledge is needed about the life-cycle and factors influencing successful infection by *N. fuckeliana*. For example, we need to know if the disease pressure is high during the time of shearing and if older wounds can also become infected.

The related species *N. neomacrospora* was reported for the first time in Sweden in the 2015 disease survey of Swedish Christmas trees. *Neonectria neomacrospora* was isolated from dead shoots from two Nordmann fir (*Abies nordmanniana*) Christmas trees and one older Nordmann fir tree in an ornamental planting. None of the three trees had severe dieback as described in recent research from Denmark and Norway. However, it is important to keep an eye on *N. neomacrospora* in Sweden as it has shown to be an aggressive fungus on fir trees in neighboring countries.

Figure 1. Top-dieback of Norway spruce (*Picea abies*) in Swedish Christmas tree fields in 2015. The fungus *Neonectria fuckeliana* was isolated from the margin between dead and alive tissues. Photos: Martin Pettersson

Extended literature list on *Neonectria fuckeliana* in Sweden

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NIBIO
NORWEGIAN INSTITUTE OF
BIOECONOMY RESEARCH

Norsk institutt for bioøkonomi (NIBIO) ble opprettet 1. juli 2015 som en fusjon av Bioforsk, Norsk institutt for landbruksøkonomisk forskning (NILF) og Norsk institutt for skog og landskap.

Bioøkonomi baserer seg på utnyttelse og forvaltning av biologiske ressurser fra jord og hav, fremfor en fossil økonomi som er basert på kull, olje og gass. NIBIO skal være nasjonalt ledende for utvikling av kunnskap om bioøkonomi.

Gjennom forskning og kunnskapsproduksjon skal instituttet bidra til matsikkerhet, bærekraftig ressursforvaltning, innovasjon og verdiskaping innenfor verdikjedene for mat, skog og andre biobaserte næringer. Instituttet skal levere forskning, forvaltningsstøtte og kunnskap til anvendelse i nasjonal beredskap, forvaltning, næringsliv og samfunnet for øvrig.

NIBIO er eid av Landbruks- og matdepartementet som et forvaltningsorgan med særskilte fullmakter og eget styre. Hovedkontoret er på Ås. Instituttet har flere regionale enheter og et avdelingskontor i Oslo.