

13<sup>th</sup> International Christmas Tree Research and Extension Conference  
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# Abstracts

Oral Presentations

Poster Presentations

Host: The Icelandic Forest Research Mógilsá







## Oral Presentations

Abstracts in program order





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Coning and shoot growth response of Fraser fir trees to paclobutrazol application**

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Precocious coning is a frequent problem when Fraser fir (*Abies fraseri*) is grown under plantation culture for Christmas trees. Individual Fraser fir trees may produce hundreds of cones, which growers remove by hand, resulting in significant labor costs. Cone induction in firs and other conifers is affected by genetics and also driven by environmental stresses. These genetic and environmental signals are mediated through hormonal signaling, particularly gibberellins. Because of this, managers of conifer seed orchards frequently treat trees with exogenous gibberellic acid (GA) in order to promote heavier and more consistent coning in their orchards. Since coning is undesirable in Christmas tree plantations we have explored the use of GA-inhibitors to reduce cone production in Fraser fir. In a previous trial, soil applied paclobutrazol reduced cone density (number of cones per tree) two years after treatment by 33 to 54%, depending on tree size (Crain and Cregg, 2017). In the current trial we are investigating the effect of applying paclobutrazol (PBZ) on coning frequency (percent trees with cones) and coning density when treatments were applied to relatively young trees that have not yet begun to cone. Field plots were installed in spring 2016 in collaboration with four commercial Christmas tree farms in Michigan. At each farm, five treatments were assigned at random to 10-tree row plots. In addition to an untreated control, three PBZ treatments (Cambistat, Rainbow Scientific, Inc.) were applied via soil injection (100, 200, or 300 ml) and one PBZ treatment was applied as a foliar spray (TrimTect, Rainbow Scientific, Inc.). All treatments were replicated six times at each farm. We evaluated trees in 2017 to evaluate effects on coning and shoot growth. Foliar application of PBZ and the highest rate of soil-injected PBZ (300 ml per tree) significantly reduced the number of cones per tree on three of the four farms. These treatment also reduced coning but to a lesser extent on the fourth farm. PBZ applications also reduced shoot extension and increased bud density. Based on our earlier studies, the effect of soil injected-PBZ effects will likely increase over time and we will continue to monitor coning on these plots and re-apply the foliar treatments.

Crain, B. A., and Cregg, B. M. (2017). Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research*, 32(5), 391-396.

Abstract: Oral Presentation





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## **Somatic Embryogenesis in Fraser fir (*Abies fraseri*): Optimizing Levels of Abscisic Acid, Polyethylene Glycol and Maltose for Maturation**

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Somatic embryogenesis is a plant tissue culture technique used to make genetically identical copies of a single plant. This versatile technique has been applied to a variety of plant species, from foodstuffs such as corn to horticultural plants. In conifers, somatic embryogenesis is used to produce millions of seedlings per year from commercially valuable species such as loblolly pine, Douglas fir and Norway spruce. The development of a commercially viable somatic embryogenesis system could revolutionize the Christmas tree industry by 1) drastically shortening the time to produce planting stock, 2) allowing multiple desirable traits to be combined, 3) enabling control of genetic variation, *i.e.*, improving stand uniformity, 4) permitting commercial production of interspecific hybrids, 5) providing a necessary platform for genetic transformation, and 6) facilitating the development of synthetic seed technologies.

Once an embryogenic culture is established, it can proliferate into many millions of copies. In the proliferative stage, the somatic embryos (SE) are immature, lacking cotyledons and roots. In order to induce SE to mature into functional plants, chemicals such as abscisic acid (ABA), polyethylene glycol (PEG), and maltose are commonly added to the medium. However, the optimum amounts and combinations of these chemicals vary dramatically across conifer species, and even between different genotypes of the same species. In this report, we investigate the interactive effects of ABA, PEG and maltose on Fraser fir embryo maturation using a 4<sup>3</sup> factorial experimental design. The 64 treatment combinations were applied to eight clonal lines originating from seed of four open-pollinated families collected from the North Carolina Premium Fraser Fir Seed Cooperative's Big Springs Seed Orchard.

Abstract: Oral Presentation



## **‘SE fluidics system’ for plant production**

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Somatic embryogenesis (SE) is a laboratory-based method that allow for controlled production of large numbers of clonal copies of plants. In conifers, SE is the only method that can be used for large scale clonal propagation. There are also many advantages with conifer SE over cuttings production, e g the somatic embryo develops in a similar way to zygotic (seed) embryos with good connection between root and shoot and no plagiotropism in the resulting plant, virtually unlimited number of SE plants can be generated from one original starting seed, and SE cultures can be stored for prolonged time in cryostorage. Large scale production of SE plants of conifer species has however long been hampered by high demand for manual labour in the existing methods. The standard SE method depends on manual handling between different culture conditions for progression through sequential developmental steps, where the harvesting of mature embryos is the most time-consuming step. Furthermore, culturing in petri plates and other plastic consumables are both inefficient and costly. Fluidics-based technologies for automating the SE method have significant potential in clearing these hurdles. With the recent invention of an automated ‘SE fluidics system’ for multiplication, maturation, harvest, germination and planting, large scale production of conifer SE plants can be realized. Automated fluidics-based handling of the embryos for harvest, imaging and sorting provides new opportunities for creating a large database correlating the morphology and physical characteristics of embryos to the quality traits of plant formation resulting in high quality plants. Novel design of temporary immersion bioreactors allows for improved development in several steps of the SE process, and reduced handling time. The fluidics based technology has been successfully demonstrated for the full line of production of Norway spruce plants, from bioreactor to nursery and field planting. Based on our previous results from SE in firs and recent results demonstrating superior growth and development of fir SE cultures in bioreactors over petri plates, the fluidics system should be well suited for scale up production of SE plants also in firs.

Abstract: Oral Presentation





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

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Application of nitrogen fertilizer has become an essential part of plantation management for many Christmas tree producers in the United States. Most nitrogen applications are surface-applied in the form of urea or ammonium sulfate. With surface applications of nitrogen there is potential for nitrogen loss from volatilization, leaching and denitrification. Some growers have begun to use fertilizers amended nitrogen stabilizers due to concerns over the loss of nitrogen, hoping to reduce nitrogen loss and optimize plant uptake. However, the addition of nitrogen stabilizers can increase the price of fertilizer by 10 to 30 percent compared to standard urea. We established a trial in the fall of 2013 to determine if timing of nitrogen application or the choice of nitrogen fertilizer products influenced growth or foliar nitrogen values of Fraser fir. The nitrogen fertilizer sources that were applied were urea, stabilized nitrogen (SuperU®) and ammonium sulfate. The stabilized nitrogen source is designed to slow nitrate-N loss by including urease and nitrification inhibitors. Fertilizers were applied as split applications in fall and spring or just as a spring application. At the conclusion of a three year trial, results indicate that shoot growth did not differ among any of the treatments, including the unfertilized control. Fertilization increased foliar nitrogen levels compared to unfertilized controls but there was no difference in foliar nitrogen among fertilizer treatments. We also acquired hyperspectral images of the plots using an unmanned aerial vehicle (UAV or drone). We compared mean foliar nitrogen concentration with several vegetation indexes calculated from the hyperspectral images. Foliar N was correlated with RE\_NDVI and NDVI. This suggests that UAV's may provide useful information of scouting and assessing fertilizer needs in Christmas tree plantations.

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## **Magnesium deficiency and foliar fertilization in *Abies nordmanniana***

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During the last decades magnesium deficiency has become increasingly more common in Christmas tree production of *Abies nordmanniana* in Denmark. In recent years many Christmas tree growers have experienced severe symptoms of magnesium deficiency, which causes significant needle chlorosis and ultimately loss of older and necrotic needles. As a commercial product, extensive magnesium deficiency has a negative impact on the economic return of the investment.

Awareness on soil and plant nutrient status can to a large extent help prevent severe symptoms of nutrient deficiency by using a proper strategy for fertilization. However, in acute situations with severe symptoms, preventive measures are not applicable, hence requiring supplementary measures for nutrient control such as foliar applications.

The effects of foliar fertilization in *Abies nordmanniana* is a topic with limited documentation. Therefore, a range of different applications of foliar fertilization were tested on two stands with symptoms of magnesium deficiency. The main goal was to examine the effects of foliar applications to prevent needle chlorosis and loss of older needles. Three different concentrations of MgSO<sub>4</sub> (2 %, 4 % and 6 %), two commercial products (BioMagnesium60 and YaraVita Magtrac) as well MgSO<sub>4</sub> (4 %) in combination with a non-ionic additive were tested in a one-year trial.

Needle chlorosis, loss of older needles and growth parameters were evaluated and rated by visual inspection at the end of the growth season. Significant effects were found for the two commercial products, 6 % MgSO<sub>4</sub> and the treatment with 4 % MgSO<sub>4</sub> in combination with the non-ionic additive. However, the latter also provided significant scorching of current-season needle tissue. Symptoms of magnesium deficiency were reduced; however, none of the treatments eliminated the symptoms. Our results indicate that severe symptoms can be reduced by foliar applications of magnesium fertilizer during the growth season as a supplement to soil applied fertilizer.

Abstract: Oral Presentation



## Effect of different cultivation practices on the survival and growth of Icelandic Christmas trees in the early growth stage on agricultural fields

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More knowledge is required about how to efficiently produce Christmas trees on fertile soils under Icelandic conditions. A long-term research project was therefore established in West, North, and South Iceland in 2009 and 2011, on fertile, cultivated soils where shelterbelts were growing around at least part of the area. These three experiments were conducted until autumn 2012. The three most popular conifer species for Christmas trees in Iceland were compared: Norway spruce (NS; *Picea abies*), Engelmann spruce (ES; *Picea engelmannii*) and lodgepole pine (LP; *Pinus contorta*). Three different fertilizer regimes were tested: No fertilizer (control), 12 g fertilizer/plant, or 24 g /plant annually in the spring. Three different herbicide (Roundup) regimes were tested: Sprayed once in spring between plants, sprayed twice during spring and late summer between plants, sprayed once in the autumn over plants.

During the first two years after planting in West Iceland, LP showed the best growth and good survival on unfertilized plots. During the second year (winter) there was 32% mortality in NS, while the ES survived well but also had serious winter damage. In North Iceland, NS had the highest mortality (19%) after the first year, ES survived but suffered from winter damage, while LP grew best and survived well. In South Iceland, NS died completely after the first winter, ES survived but suffered from winter damage and LP had the best survival and growth rate. After four years in West Iceland, LP had the highest volume index and was both surviving and growing well, while the two spruce species were still struggling. LP was judged the best adapted species for Christmas tree production on agricultural fields in Iceland.

Both fertilizer trials gave significant negative effects on survival and growth rate in West Iceland during the first two years, especially for LP, but mostly no effect at the other sites. It did not affect the autumn nutrient content of the needles at any of the sites 2-4 years after planting. It was therefore recommended that fertile Christmas tree fields should not be fertilized during the initial two years, at least. The herbicide trials showed that spraying once between plants in the spring was not enough to keep weeds at bay, while double spraying between plants in spring and late summer was more effective. The best results were achieved by spraying in the autumn over both trees and weeds, which was the recommended method. The shelter effect was clear. The difference between the three locations was large, and the oceanic (winter) climate was found to be the main obstacle for a successful initial phase in Christmas tree production at some locations. More research is urgently needed to develop cultivation methods for Christmas trees on agricultural fields in Iceland.

The survival rate was measured again 2013 and 2014 in all sites and was severely decreasing, especially for NS. The best survival rate was in the experimental site in North Iceland far from the sea. LP had in average the best survival rate 56%, ES 51% and NS 5%. The survival rate was far lower in the West Iceland. The experimental site in South Iceland was closed 2013 due to an unacceptable low survival rate (< 20%).

**Keywords:** Iceland, Christmas trees, field production, Norway spruce, *Picea abies*, Engelmann spruce, *Picea engelmannii*, lodgepole pine, *Pinus contorta*, survival, growth, fertilizer effect, herbicide, shelter, oceanic climate.

Abstract: Oral Presentation





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## **A Study on Expansion of Christmas Tree Demand in Korea Utilizing Big Data**

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In Korea, Christmas is the most important holiday at the end of the year, regardless of people's religion. For this reason, Christmas trees have become one of the major shopping items in the Christmas season. Due to the expansion of party culture and decorative culture, nuclearization of family unit, an increase in foreign residents in Korea, fast-growing cafes, and the rising number of churches, demand for Christmas trees is expected to grow continually. However, most of the trees are artificial ones imported from China, not leading to the expansion of demand for live trees. Demand for artificial trees has grown because they are inexpensive, convenient to store and use, and can be used for several years. Nevertheless, many consumers also want live Christmas trees, considering their family's health and decoration effects.

In the Korean market for landscape trees, demand for landscape trees has sharply decreased due to the absence of large-scale landscaping projects for the past decade while the supply has maintained, which has increased landscape tree farmers' difficulties. Although a few species of trees can be used as Christmas trees, the lack of their demand has led to the farmers' difficulty. As the expansion of demand for Christmas trees seems to be very beneficial to farmers producing landscape trees, identifying its possibility will be an important study.

This research examined the current market situation of Christmas trees and potential for increasing their demand in Korea, and based on the results, studied the possibility of demand expansion for live Christmas trees. Major research methods are as follows: a field survey on the present status of the Christmas tree market; and an analysis of the demand pattern of Christmas trees and the possibility of their demand increase by utilizing big data, including unstructured data such as SNS and structured data in the media and interviews with consumers. The results of this study will contribute to the development of the industry related to Christmas trees in the landscape tree market.

Abstract: Oral Presentation



## Web blight on Christmas trees in the U.S. Pacific Northwest

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Web blight is a sporadic disease that was first observed on Christmas trees grown in the U.S. Pacific Northwest (PNW) in the 1990's. It is caused by an unnamed binucleate *Rhizoctonia* species that is closely related to *R. butinii*, which causes web blight on conifers in Europe. In the PNW this is primarily a disease of Douglas-fir in (*Pseudotsuga menziesii*) both Christmas tree and forest sites, but has also been observed on grand (*Abies grandis*) and noble fir (*A. procera*) in Christmas tree plantations and western hemlock (*Tsuga heterophylla*) and Sitka spruce (*Picea sitchensis*) in the forest. In Europe, web blight has mainly been reported on European silver fir (*A. alba*), but can also occur on Nordmann fir (*A. nordmanniana*), noble fir, Veitch fir (*A. veitchii*), Turkish fir (*A. bornmuelleriana*) and spruce (*Picea* spp.) under high disease pressure. Symptoms can appear from late fall through early spring. Initial symptoms generally consist of a browning of outer foliage in a roughly circular patch of branches. In Christmas trees, these are often in the middle to bottom third of the tree and often occur on lower branches on the north side. Affected beige to greyish needles often hang down and under moist conditions, fungal webbing may be visible, binding together clumps of dead needles. All age classes of needles can be affected. Generally, the disease does not kill the buds and shoots. When severe, the damage can involve more than 50% of the side of a tree.

The life cycle of the pathogen that causes web blight in the PNW is unclear. Basidiospores are formed on infected needles, but their role in disease spread is unknown. Local spread results from hyphae growing over needle surfaces and dislodged needles. Disease severity tends to be higher in sites with restricted air flow, close tree spacing, and dense foliage. In culture, the presence of binucleate hyphae with dolipore septa, slight constrictions at the branch points, and acute to right angle branching are diagnostic for this pathogen. Factors that affect its survival over the summer are unknown.

During 2016-2017, there was a high level of web blight in scattered Christmas tree plantations in the PNW. This appears to be associated with record high precipitation levels which occurred from October through April. Previously unreported hosts in the PNW included Nordmann and Turkish firs. Recommendations relating to management of web blight in Christmas trees include not planting near diseased stands of native forests and improving air circulation around the trees. The pathogen is sensitive to several commonly used fungicides in Christmas tree production, such as clorothalonil, but the lack of information concerning when infection occurs has limited the development of fungicide recommendations to control this disease. Since the disease usually does not affect the buds and shoots, growers often will attempt to carry over trees with limited symptoms and allow the new growth to cover up the damaged areas the next spring, enabling them to market the tree the following year. However, it is often recommended that affected trees be removed and destroyed.

Abstract: Oral Presentation



## Diseases, pests and nutrient deficiencies on Swedish Christmas trees

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In Sweden, Christmas tree production is a small but growing business. A survey aiming to determine the occurrence of various diseases, pests and nutrient deficiencies in Swedish Christmas trees, was conducted in spring 2015 to recommend optimized management methods. Another aim was to investigate if plant damaging *Phytophthora* species were present since many *Phytophthora* spp. are causing significant economic losses in Christmas tree plantations in other countries.

In southern Sweden, fields containing firs (*Abies* spp.) and/or spruces (*Picea* spp.) were surveyed at 21 farms. All the trees in one or more fields per farm were visually examined for damaging agents. Soil samples from wet areas in the fields and *Rhododendron* leaf baits in adjacent streams were used to examine the presence of *Phytophthora*.

Several potential diseases causing fungi and a *Phytophthora* sp. were found in the plantations (pathogens in alphabetic order); *Armillaria* sp. on *A. nordmanniana* and *P. pungens*, *Camarosporium* sp. on *P. pungens*, *Chrysomyxa abietis* on *P. abies*, *Delphinella abietis* on *A. nordmanniana*, *Gremmamyces picea* on *P. pungens*, *Herpotrichia juniperi* on *P. pungens*, *Lirula macrospora* on *P. abies* and *P. pungens*, *Lophoderminum piceae* on *P. pungens*, *Neonectria fuckeliana* on *P. abies*, *N. neomacrospora* on *A. nordmanniana*, *Phytophthora megasperma* on *P. abies*, *Rhizosphaera kalkhoffii* on *P. pungens*, *Sydowia polyspora* (CSNN or Schlerophoma shoot dieback) on *A. nordmanniana*, *A. procera*, and *P. abies*, and *Thekopsora areolata* on *P. abies*. Four pests (insects and mites) were found on *A. nordmanniana*: *Adelges* spp., *Dreyfusia nordmanniana*, *D. piceae*, and *Nalepella* spp. In addition, magnesium (Mg) and manganese (Mn) deficiencies were common and also wild life damages (such as rodents, hare and deer) were found on *A. nordmanniana* and *A. procera*. *Phytophthora cryptogea*, *P. gonapodyides*, *P. lacustris*, *P. megasperma*, *P. plurivora* and an unidentified species were isolated from soil and/or bait leaves.

Of the diseases found on spruce, *C. abietis*, *L. macrospora*, *N. fuckeliana*, and *R. kalkhoffii* were commonly detected and they were found on most sites. On fir, *Sydowia polyspora* dominated.

The finding of *N. neomacrospora* on a few sites was more worrying than the other diseases, pests and nutrient deficiencies, since it is an aggressive pathogen causing severe dieback in fir trees in Norway and Denmark. Magnesium deficiency and/or pest and wildlife damage was substantial at several sites. However, management measures are available for these problems. *Phytophthora* spp. have the potential to become more problematic than the other biotic and abiotic damaging agents, because they can survive in the soil for decades. In this survey, *Phytophthora megasperma* was the only

*Phytophthora* species isolated directly from roots of a Christmas tree. *Phytophthora* is thus far of minor concern for the Christmas tree growers in Sweden. However, isolation of several *Phytophthora* spp. from soil and water indicates that the presence of this agent may potentially lead to future problems. Awareness of *Phytophthora* is critically needed since *Phytophthora* damage is an emerging problem in Christmas trees in Europe and known to cause great losses for this industry in USA.

Abstract: Oral Presentation



13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## ***Heterobasidion* root rot predisposing noble fir to *Cryphalus* bark beetle attack**

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Noble fir (*Abies procera*) is among the most susceptible *Abies* species in relation to *Heterobasidion* root rot. Stump removal is a necessity if the fungus is established on sites intended for noble fir and fraser fir (*A. fraseri*). In 2013 problems with bark beetles were discovered in the noble fir greenery plantations in Denmark. The species involved (*Cryphalus piceae*) had not been recorded in Denmark before, only the closely related *C. abietis* which is considered harmless. From literature *C. piceae* has been described as problematic on several *Abies* species especially after storms, floods or other predisposing factors, and in Turkey as a secondary pest in combination with *Pityokteines curvidens*. In Denmark, fatal attacks killing whole trees seem to occur mainly as a result of *C. piceae* breeding in the bark of noble firs age 10-30 (5-10 meters tall), which are affected by *Heterobasidion*. This preference of trees infected with root rot has also been shown in the bark beetle species *Scolytus ventralis* from North America. The correlation between root rot infected trees and *C. piceae* is still being investigated. In some cases, the canker fungus *Neonectria neomacrospora* is also present in trees, where *C. piceae* is found on branches or stems, but this association may be accidental. The consequences for bow production of noble fir and for other fir species in Denmark is as yet uncertain, but so far no severe attacks have been found in the large Christmas tree production of Nordmann fir (*A. nordmanniana*).

Abstract: Oral Presentation



## Evaluating “softer” insecticides for aphid control in Oregon, USA

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Aphids (*Cinara occidentalis*, *C. abietis* and *Mindarus abietinus*) are common yearly pests of noble fir (*Abies procera*) and grand fir (*Abies grandis*) Christmas trees. The damage to the trees can range from transitory to severe deformation and or unsightly black needles. The later problems frequently result in unsaleable trees. Active aphid control strategies involve, 1) insecticide applications, 2) release of predatory insects into fields or, 3) utilizing plantings (native or introduced) that attract aphid predators. Growers, in practice, will usually segregate into one of two camps; those that rely only on insecticides and those that shun insecticide applications relying instead on some combination of points 2 and 3 above.

Part of this either/or segregation may be a function of the choice of insecticide used to control aphids. Many of the commonly used insecticides are broad-spectrum controls that eliminate beneficial insects along with controlling aphids. Another aspect of the segregation is a result of the complexity of aphid crop damage threshold determinations and the dynamics of beneficial insect populations. Growers, of necessity, are driven by economics to utilize cost effective controls that have worked in the past rather than experimenting with new, and often unproven, control strategies. In addition, newer targeted insecticide options are typically more expensive than the older broad-spectrum products and in the case of some of the “softer” insecticides, few growers have efficacy experience.

In 2016, an insecticide screening trial was established near Oregon City, with 10 products. Three products were “standard” insecticides; six were considered “softer” insecticides. The tests were conducted on grand fir Christmas trees 1.5 m (5 feet) tall with significant aphid infestations when the trial began. One general observation from the 2016 trial was that beneficial insects were plentiful in all treatment blocks after 30 days. This likely was a function of the high density of flowering Queen Ann’s lace and dandelion in the plot.

Based on 2016 results, which evaluated product application costs, aphid control efficacy and impact on beneficial insects, three of the “softer” products will be included for further testing in 2017. Those products are: M-Pede (potassium salts), Gandevo (Chromobacterium), and WE-440 (oil). Three of the “softer” products (AzaDirect, Endeavor, and Botanigard) proved to be both expensive and not especially effective. The 2017 trial will also include two systemic products, Sivanto (Flupyradifurone) and a new formulation of Spirotetramat (Movento HL).

Abstract: Oral Presentation



## **Rapid and reliable detection of three *Neonectria* species occurring in conifer and deciduous trees**

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*Neonectria ditissima*, *N. neomacrospora* and *N. fuckeliana* cause canker on apple and other broadleaf trees, true fir, and spruce species, respectively. Damage by *Neonectria* canker has seemingly increased in the Nordic countries during the past decade, presumably due to climate changes. Symptoms are canker wounds, dieback of shoots, twigs, and branches, and in some instances tree mortality. The latter is especially the case for *N. neomacrospora* on fir species. On conifers, heavy resin flow commonly occurs when attacked by *Neonectria* spp. resulting in reduced wood quality. Isolation of *Neonectria* spp. from infected plant tissues is sometimes difficult due to interference by other fast growing, secondary fungi and/or bacteria, and this isolation-to-culture practice is labor intensive and a relatively slow process. Therefore, a more rapid and accurate tool for identification of the three pathogens, based on molecular technologies, were identified as necessary for carrying out field and nursery surveys, pathogenicity experiments, screening of plant material for resistance against the fungi, and seed testing. Molecular technologies, such as PCR, can help to reduce the workload and increase detection frequencies. Real-time PCR has several advantages over conventional PCR. Products are automatically detected without post PCR processing, and most importantly, the amount of the pathogen in plant tissue samples can be quantified. In this study, three species-specific Taqman real-time PCR assays were developed to identify *N. ditissima*, *N. neomacrospora* and *N. fuckeliana* directly from infected plant tissue. Several primer sets were designed from the translation elongation factor 1-alpha (*tef*) gene for *N. ditissima* and *N. neomacrospora*, and the internal transcribed spacer (ITS) region for *N. fuckeliana*. Through several validation tests, one pair of primers per fungal species was selected. All three primer pairs proved species-specific and did not cross react with closely related fungi. Each of the Taqman assays amplified DNA from both cultures and plant samples infected by the respective fungi. Furthermore, it was possible to quantify the amount of pathogen in the infected plant tissue.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Species variation in susceptibility to the fungus *Neonectria neomacrospora* within the genus *Abies* – Danish experiments**

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During the years 2011-2014 an epidemic outbreak of the fungus *Neonectria neomacrospora* happened in Denmark. Numerous Christmas tree stands showed symptoms of dead shoot tips, necroses on branches or, in case of highly susceptible species, dieback of whole trees. A two-fold strategy was pursued to reveal knowledge about potential resistant genotypes within species and also among species especially within the genus *Abies*. The evaluation was based on 1) field observations and 2) testing by inoculating mycelium on detached branches. A number of different materials was tested, including the *Abies* collection in the Arboretum Hørsholm, which comprises 41 species and subspecies within the genus. A number of tests have been carried out on the species level, but also among clones in seed orchards, and experiments are planned among progenies. A review of the past 6 years' research is presented, and main conclusions and obstacles are presented.

Abstract: Oral Presentation



## **Inoculation of young Norway spruce rooted cuttings with *Neonectria fuckeliana***

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The fungus *Neonectria fuckeliana* causes canker disease on Norway spruce (*Picea abies*), and has become an increasing problem in Scandinavia during recent years. Lack of knowledge about pathways for the fungus, may lead to management activities promoting spread. In a greenhouse study, four different treatments of the terminal leader of three-year-old Norway spruce rooted cuttings were inoculated with a microconidial suspension using two different isolates of *N. fuckeliana*. The four treatments included different wounding techniques: 1. cutting the top shoot, 2. wounding the stem using a scalpel, 3. removal of needles, and 4. non-wounded treatment. Control plants received the same four treatments, but they were inoculated with water. Both dormant and actively growing plants were used for all treatments. Nine months after inoculation, the study was terminated and lesion length under the bark was measured. A sixth of the plants were randomly selected to test for the presence of *N. fuckeliana* using both isolation and molecular identification with real-time PCR.

Development of necrotic cankers, extensive top whorl dieback, or production of fruiting bodies of the fungus, like we observe outdoors, did not occur within the timeframe of the experiment. The lesion lengths were generally minor. However, host tissue was infected since *N. fuckeliana* was re-isolated and detected using real-time PCR from both dormant and actively growing plants.

The reaction was slow compared to a pilot study conducted 2015-2016 on five-year-old plants. Using microconidia for inoculation may be the cause of the reduced and slower symptom development. Different from macroconidia, microconidia are readily produced in culture, and therefore were chosen for this experiment. It is unknown to what extent microconidia are responsible for causing infections under natural conditions.

The weak reaction observed, where *N. fuckeliana* lived more or less asymptotically in the host tissue for 9 months, is in line with older studies describing *N. fuckeliana* as an endophyte or a weak pathogen. This contradicts the increase in top-dieback of younger trees and the large canker wounds on older trees that have recently been observed in Scandinavia. However, we believe that our field observations of increased *Neonectria* canker damages may be associated with recent climatic changes. Furthermore, the fungus is mainly spread by ascospores under natural conditions, a spore stage known to cause epidemics for several closely related *Neonectria* spp.



## Family Variation in Resistance to *Phytophthora* root rot in Eastern White Pine

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Eastern white pine (*Pinus strobus* L.) has a large natural range in eastern North America from Newfoundland southward through the Appalachian Mountains into northern Georgia, and from the Atlantic Ocean coast westward into Minnesota and southeastern Manitoba. This species is valued for wood products, landscaping and Christmas trees. In the Southern Appalachian region, Christmas tree growers often successfully plant white pine on sites where Fraser fir (*Abies fraseri* [Pursh] Poir.) has succumbed to root rot cause by *Phytophthora cinnamomi* Rands. However, on some sites in the mountains, and especially on piedmont and coastal plain sites in North Carolina, white pine is killed when *P. cinnamomi* is present in the soil.

In order to better understand genetic variation to *Phytophthora* resistance in white pine, two-year-old seedlings from open-pollinated families were inoculated twice eight weeks apart using rice grains colonized with *P. cinnamomi* during Summer 2015 and again during Summer 2016. Subsequent mortality was assessed biweekly for a period of 16 weeks following the first inoculation during both years. In the main study, 8-15 seedlings from each of 83 families were inoculated (6,050 seedlings total) with a standard *P. cinnamomi* isolate from Fraser fir. In a supplemental study, 15 seedlings from each of 20 families were inoculated with an isolate from white pine (1,500 seedlings total). One to three seedlings from each of 80 families were also included as non-inoculated controls (236 seedlings total).

Seedling mortality increased over time after inoculation and in the main study reached 18.9 and 46.1% in 2015 and 2016, respectively with final mortality for families ranging from 13.3 to 81%. Mortality in the supplemental study was higher reaching 40.0 and 58.1% in 2015 and 2016, respectively with final mortality for families ranging from 30.0 to 82.6%. Both narrow-sense individual-tree heritability ( $h_i^2$ ) and family mean heritability ( $h_f^2$ ) estimates increased from week 6 before plateauing and remaining near their maximum levels (0.49 and 0.85, respectively) during 2015. During 2016, both heritability types were lower and slightly decreased with time likely due to some mortality from other causes as the seedlings became crowded and their roots pot-bound. Similarly, 16% mortality was observed for the non-inoculated controls during 2016 while none was recorded for 2015. The relatively high amount of genetic control of disease resistance indicates that deploying resistant families will reduce losses to root rot and corroborates tree improvement efforts to further develop resistant planting stock for the Christmas tree and timber industries.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Fantastic bugs and where to find them: the biology of chalcid seed predation**

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Rapid changes in world's climate, globalization and current trade patterns are affecting forest pests and the damage they cause. These changes directly impact the development, survival, reproductive cycle and dissemination of pest species while also altering plant host susceptibility and changing the behavioral interactions among pests and other species such as natural enemies and parasitoids. Seed chalcids (*Megastigmus* spp.) are tiny wasps whose larvae destroy seeds of various plant species by consuming the megagametophyte (or endosperm) and embryo. Losses caused by *Megastigmus* have been reported in different conifers in the USA and Europe. We discovered insect larvae in immature and mature Fraser fir seeds and have been improving methods for rearing chalcid adults under lab conditions. Larval and female and male adult specimens of the insect were submitted to the NCSU Plant Disease and Insect Clinic and identified as *Megastigmus specularis* Walley. Interestingly, another wasp also emerged from these seed that we believe is a primary parasitoid of *M. specularis*. General descriptions of the life cycle and habits of seed chalcids in the Appalachian region were published in the early 1960s but no additional information has been published since. Identification of infested Fraser fir seed is particularly challenging because infestation occurs on immature cones and mature seed are small, light-weight, and frequently filled with resinous material so that their weight is similar to infested seeds. Thus, separation of infested seeds via wind columns or gravity tables is almost impossible. Our preliminary observations indicate that chalcid wasps favor certain Fraser fir families for oviposition but more data is required to confirm this hypothesis. Our main goal is to generate basic information about how the chalcid life cycle is correlated with Fraser fir seed physiology, assess clonal variation and learn more about possible natural chalcid parasites that in the long term will help us improve Christmas trees seed orchards and clone banks and to be prepared for changes in pest behaviors, outbreaks and future invasions.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## Interactions Between Root Rotting *Phytophthora*, *Abies*, and Environment

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Phytophthora Root Rot (PRR) is a serious disease of true fir (*Abies* spp.), resulting in substantial losses in the Christmas tree and conifer nursery industries. Despite the availability of select chemical alternatives and cultural control methods, practical options for reducing PRR losses in Christmas tree farms are scarce, and their utilization may be limited by expense or feasibility of execution. Extreme susceptibility of Fraser (*Abies fraseri* (Pursh) Poir.) and noble (*A. procera* Rehder) fir often exceeds the practical efficacy of existing control measures available to Christmas tree farmers and has dictated grower abandonment of *Phytophthora*-infested land for cultivation of these highly desirable species.

Defining host-pathogen interactions between species of root-rotting *Phytophthora* and *Abies* is important for tailoring management activities on a regional scale and for the potential development of molecular tools for identifying resistant host species. Classifying *Abies* species as resistant or susceptible is complicated by regional variation in abundance and aggressiveness of *Phytophthora* species and the influence of environment on symptom expression and host vigor. As previous studies performed to assess host response to PRR have focused on one or a few species of either the host or pathogen, a multifactorial experiment was conducted to assess the responses of 7 species of *Abies* challenged with 3 isolates each of 4 *Phytophthora* species under contrasting temperature conditions. Evaluation of mortality, root rot severity, and remaining root biomass after 16 weeks of exposure to the pathogen confirmed prior inferences regarding inherent variation in the resistances of various species of *Abies* and demonstrated evidence of variation in aggressiveness among different species of the pathogen as well as different isolates of the same *Phytophthora* species. The ambient temperatures at which studies were conducted had a conspicuous effect on host mortality, root rot severity, and radial growth of *Phytophthora*. Understanding how host responses differ under variable pathogen attack and ambient environment will improve efforts to control PRR using host species substitutions on infested ground and will provide parameters for researchers investigating the potential for molecular resistance marker development.

Abstract: Oral Presentation (via Skype)





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Effect of postharvest exposure to exogenous ethylene on needle loss from Christmas trees in the U.S. Pacific Northwest**

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Recent research on balsam fir (*Abies balsamea*) Christmas trees in Canada has shown that exposure to concentrations as low as 10 ppm of ethylene will significantly accelerate postharvest abscission of balsam fir needles. It is unclear what role ethylene plays in the loss of needles from other conifer species such as Douglas-fir which are more widely grown in the U.S. Pacific Northwest. It is also unclear how effective a postharvest treatment such as 1-MCP, a commonly used compound which is used to inhibit the effects of ethylene on other crops, would be in reducing needle loss and improving tree quality.

During the past three years, clonally-propagated balsam fir, Canaan fir (*A. balsamea* var. *phanerolepis*), Douglas-fir (*Pseudotsuga menziesii*), Nordmann fir (*A. nordmanniana*), and Turkish fir (*A. bornmuelleriana*) trees that are maintained at WSU Puyallup were used to determine the role of ethylene in needle loss on these four additional species of trees. These clones have been subjected to numerous needle loss tests in the past and represent a unique set of genotypes that range from clones that are predisposed to either shed or retain needles when detached branches are displayed dry. Branches harvested from the trees were used to determine what impact acute (24hr) and chronic (7-day) exposure to ethylene (0, 1, 10, 100, 500, and 1000 ppm) had on needle retention. To test the effectiveness of 1-MCP in reducing needle loss, branches were exposed to 1-MCP (0, 1, and 10 ppm) for 24 hours prior to exposure to 10 ppm ethylene for 7 days. Upon completion of the exposure periods, branches were displayed in a postharvest room maintained at 20C and the effect of the treatments on needle retention was assessed over a 2- week period of time.

During fall 2016, acute (24hr) exposure to ethylene had virtually no effect on the loss of needles by branches from either genotype of all of the conifer species tested. This confirms data collected in our previous tests and suggests that short periods of exposure to ethylene are not likely to increase the loss of needles from cut Christmas trees. Chronic (7-day) exposure of branches from genotypes of balsam, Canaan, and Douglas-fir that are predisposed to shed needles increased the severity of needle loss on these species. Pretreatment of branches from these species with 1-MCP prior to a 7-day chronic exposure to 10 ppm ethylene significantly reduced the loss of needles. Chronic exposure to ethylene did not increase needle loss on branches from genotypes of these species that are genetically predisposed to retain needles. Data on Nordmann fir was variable and very little needle loss occurred from any of the Turkish fir branches.

The implications of this research and additional research needs will be discussed.

Crain, B. A., & Cregg, B. M. (2017). Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research*, 32(5), 391-396.

Crain, B. A., & Cregg, B. M. (2017). Using irrigation and mulch to control cone production in Fraser fir (*Abies fraseri*). *Scandinavian Journal of Forest Research*, 32(5), 384-390.

Owen, J. 2015. Using Herbicides to Interrupt Cone Development on Fraser Fir. V. Talgø & I. S. Fløistad (eds.). 2015. Proceedings of the 12th International Christmas Tree Research and Extension Conference; Honne, Norway; 6-11 September 2015. p. 41.

Abstract: Oral presentation



13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Variation in the quality and keepability of wreaths made from noble fir boughs from low vs. high elevation sites in the U.S. Pacific Northwest.**

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The Mt. St. Helens area of the Cascade Mountains of Washington State represent the geographic center of noble fir (*Abies procera*) bough production for use in decorative greenery products in the U.S. Pacific Northwest (PNW). Noble fir occurs naturally at high elevation sites above 600 m and the major bough production region consists of public and private lands that were replanted with noble fir after the 1980 eruption of Mt. St. Helens. Because of the subsequent limited harvesting and replanting of noble fir in this region, the number of stands of a suitable age for bough production have been decreasing in the the Cascades. This, coupled with fluctuation in the demand for Christmas trees, has resulted in an increased conversion of former Christmas tree plantations at elevations <729 m to bough production stands. The PNW leads the U.S. in the production of Christmas trees, and noble fir represents about half of the production. In 2014, an estimated 45% of the 16 million pounds of noble fir boughs that were harvested came from former Christmas tree plantations.

Although noble fir Christmas trees and boughs are known for their excellent needle retention, some in the bough industry have been reluctant to harvest noble fir boughs from lower elevation, former Christmas tree plantations because of the perceived inferior quality and keepability of greenery products made from these boughs. To determine whether this was the case, during November and December 2016, a trial was conducted to examine the postharvest keepability of wreaths made from bough material that was collected from a total of 15 sites, which ranged from 10 to 1,297 m in elevation. We looked at the effect of elevation, wreath maker, and cold storage on the rate of moisture loss and quality of wreaths that were displayed for 15 days at 20C. There was a statistically significant difference in the amount of wastage of the boughs between the two workers ( $p = 0.003$ , t-test) who made wreaths. Although there was no significant difference in the mean elevation of sources of material used by both workers ( $p = 0.5450$ , t-test), worker 1 had more wastage than worker 2. The initial fresh weight and the total dry biomass was greater for wreaths made by worker 2 than by worker 1, who tended to cut the bough material into smaller pieces.

Cold storage at 1C for 3 weeks had no effect on the rate of drying of the displayed wreaths ( $p = 0.33$ , t-test). Overall, there was no relationship between elevation of the bough sources and drying rate. There was also no relationship of elevation with drying rate when the wreaths were grouped by worker ( $p = 0.90$ , worker 1;  $p = 0.54$ , worker 2, t-test). In the consumer survey, there was no significant difference in overall wreath quality score between workers and, overall, elevation was not correlated to consumer ratings. Although these trials need to be repeated, the data contradicts the commonly held belief in the bough industry that high elevation sources of noble fir boughs are superior to low elevation sources.

Abstract: Oral Presentation



## **Christmas tree needle separation: A marriage that ends on the carpet**

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Most conifers have the capacity to retain needles for several years losing them gradually in response to cold acclimation, a natural process referred to as needle abscission (NA). This process only involves a few layers of cells in predictable positions known as abscission zones (AZs). NA is sometimes severely affected once a tree is cut, shipped and displayed in living rooms, common practices that North American Christmas tree growers and consumers know very well. Christmas tree growers recognize post-harvest needle abscission (PNA) as a common problem but changes in weather patterns and earlier harvest practices are increasing this phenomenon and highlight the need to identify trees with better adaptation. There is a growing demand for breeding Christmas tree varieties that hold their needles for longer periods of time to encourage more consumers to buy real Christmas trees instead of artificial trees. At the same time, there is a lack of methods that allow us to predict PNA as well as a limited knowledge of the histology and gene regulatory network that controls this important trait. Although it seems likely that needle abscission in conifers has mutual putative regulators that have been described in abscission for other model and non-model plants, data have shown particular and unique features in different species. We are currently designing a system for the histological characterization of AZs in Fraser fir to complement our transcriptome data analysis to find putative control genes that play crucial roles during NA and PNA and could be used as molecular markers. The histological system includes fixation, staining and sectioning of AZs collected in the field and at different time points during indoor display followed by examination under bright-field and confocal microscopy. Using these approaches, we want to describe AZ development, identify potential anatomical differences between trees that retain their needles better and link physiological and transcriptome data in Fraser fir and other fir species.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Postharvest Christmas tree research in NC**

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Early Christmas Tree harvest to meet demands of national retailers places extreme pressure on growers to maintain adequate postharvest quality. Postharvest Christmas tree research in North Carolina has targeted several different problems that growers face including the influence of harvest date on needle retention, overheating of palletized trees, and pallet storage of trees on the retail lot. In a 2015 harvest timing needle retention study, poor needle retention was observed after early October branch harvest. Good needle retention was observed after late October and early and mid-November harvests. However, a similar study in 2016 showed poor needle retention for both early and late October harvests. Later onset of cold weather in 2016 can explain the difference in results between the two years. Results of these studies helped growers justify delayed harvest in 2016 to wait for cold temperatures. In a 2015 Christmas tree pallet heat of respiration study, temperatures in pallets approaching 32 degrees Celsius and above were associated with needle and branch necrosis. High temperatures were observed when pallets were packaged within the first four days after harvest. Delayed palletization reduced initial recorded temperatures and symptom expression. However, wet conditions at palletization may contribute to microbial activity and rising temperatures days after pallets were constructed. A forced air cooling study conducted in 2016 used large fans to pull air through rows of pallets. Temperatures in air-cooled pallets dropped 20 degrees Celsius overnight where control pallet temperatures only dropped a degree or two. By lowering initial temperatures, the potential for additional heat of respiration to damage trees may be reduced. In both 2015 and 2016, palletized Christmas trees were followed to a South Carolina retail lot through different durations of pallet storage (0, 5, 11, and 22 days on the retail lot). In dry conditions, pallet storage helped to conserve moisture and retain needles compared to loose tree storage. However, when trees were saturated with water, extended pallet storage provided conditions for microbial activity. If pallets of trees are held for extended storage, the effective limits of this storage practice must be better understood.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Postharvest Characteristics of Summer-cut *Abies procera* and *Abies nordmanniana* Christmas trees in Dependence of Watering Treatments**

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The effects of a series of watering treatments on needle loss and needle colour change were compared in *Abies nordmanniana* and *Abies procera* Christmas trees. These characteristics were measured quantitatively and qualitatively at regular intervals over 25 days and compared against stem water potential ( $\Psi$ ). The study examines the postharvest characteristics in summer in order to assess the potential use of these species as Christmas trees in the southern hemisphere. The watering treatments were designed to determine ideal postharvest treatments. They were also used to identify and better understand inflection points in drying caused by plant physiological responses, and to determine a final damage threshold after which postharvest quality declined irreparably. Cut trees of both species were set up indoors and organised into a “dry” control group and treatment groups placed in water at 0, 24, 48 and 96 hours after harvest. Decreasing stem  $\Psi$  was strongly correlated with increasing needle loss in *Abies nordmanniana* under the dry treatment ( $r = -.9565$ ). Rehydration to consistently high stem  $\Psi$  values in the watered treatments also corresponded to good postharvest characteristics across the experimental duration for *Abies nordmanniana*. Conversely, needle loss in *Abies procera* under the dry treatment was lowest and could not be correlated with water status, but overall postharvest quality declined due to wilting and eventual colour change of the current season needles. Needle loss of current season needles was also an issue in *Abies procera* treatments given water at 48 and 96 hours. Mean needle loss values between the species were not significantly different for any treatments (at  $p < 0.05$ ) except the dry *Abies nordmanniana*, which exhibited the most needle loss of any treatment. Comparison of linear correlations of stem  $\Psi$  over time in *Abies nordmanniana* under the dry treatment suggest an inflection point exists between  $-2.0$  and  $-2.45$ MPa. Both species display suitable postharvest characteristics for use as Christmas trees in summer, however postharvest processing times must be kept short before watering treatments are applied. Damage thresholds were not reached in either species in any of the watering treatments to 96 hours. Further studies are needed to accurately pinpoint damage thresholds and inflection points.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Fraser fir cone control update**

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Stress-related cones on young Fraser fir Christmas trees continue to be an expensive, laborious problem for many North Carolina growers. By treating immature cones with a chemical pruning material soon after they emerge, the need for manual removal of cones could be avoided. The purpose of this research was to identify effective rates of products that kill cones without damaging the rest of the tree. Since 2012, a range of products have been tested including conventional and organic herbicides, plant growth regulators, apple thinning agents, and chemical pruners. Of all products tested, soaps (fatty acid/alcohols) seemed to be the most effective at killing immature cones followed by several oil extracts. Two additional seasons of research build on work already reported at the previous CTRE conference. In 2016, studies were conducted using manual backpack sprayers, hydraulic hose sprayers, and a cannon mistblower. Several rates of Axxe (ammoniated pelargonic salts), Scythe (fatty acid), Off Shoot-T and Off Shoot-O (fatty alcohols), were tested using all three types of sprayers. With both backpack and hose sprayers, Axxe and Scythe continued to kill more cones than either Off Shoot product, but Axxe damaged less foliage on the tree than Scythe. Mistblower sprayer treatments failed to kill more than a few cones beyond the first row of trees. 2017 research focused on different application methods using Axxe. One study compared different sprayer treatments at 6% rates. Other studies compared conventional and overhead sprays using hydraulic hose sprayers at different rates of Axe. While 2017 treatments are still being evaluated and analyzed, preliminary results show less effective cone control than 2016 treatments. Several treatment decisions may have altered our results including nozzle selection, duration of application per tree, and how we managed residual spray material in hoses. Control may be as dependent on the amount of spray material used as on the rate of product. Additional work is needed to finalize treatment recommendations.

Abstract: Oral Presentation



## Evaluation of cone control options in Fraser fir plantations

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One of the most labor-intensive and expensive cultural practices that Christmas tree growers face is hand picking Fraser fir cones. Due to heat and moisture stress Fraser fir trees can be prolific cone producers. Implementing new practices on Christmas tree farms to help reduce the number of cones and to stop cone development can be beneficial to producers. Mulching and irrigating Fraser fir trees has shown to reduce root-zone temperatures and reduce cone formation on particularly warm sites (Crain and Cregg, 2017). Another method to reduce the number of cones is to apply plant growth regulators. Recent research in our lab has shown that application of paclobutrazol, a gibberellic acid inhibitor, reduced cone production in Fraser fir (Crain and Cregg, 2017). Dr. Jeff Owen's research at North Carolina State University has shown that applying certain herbicides on immature cones can stop their development. This research suggest that development of Fraser fir cones may be stopped with properly timed sprays of conventional or organic herbicides (Owen 2015). Based on this research we initiated a series of trials to investigate the utility of herbicides to stop development of cones once they have emerged. We established trials at five locations in 2016 and at three locations in 2017. At each location we selected 140 trees with at least 10 cones per tree. Twenty trees were assigned at random to one of seven treatments in 2016 (Avenger, Axxe, Goal, Reflex, Scythe, WeedZap, and control) and eight treatments in 2017 (Avenger-1X, Avenger-2X, Axxe-1X, Axxe-2X, Scythe-1X, Scythe-2X, mechanical cone removal, and control). The 2017 trials 2X treatments were applied approximately one week after initial (1X) applications. All treatments were applied to the upper one-third section of the tree crown with a standard backpack sprayer when cones were 3 cm long or smaller. Approximately one month after treatments were applied we evaluated each tree for the number of cones killed (brown - no evidence of additional growth), damaged (brown or partially brown with evidence of growth), and live (green and growing). We also scored trees for phytotoxicity (needle browning). Avenger, Axxe, and Scythe provided the best overall control of cones in 2016, (30% or greater cone kill) though there was some variation in results among farms. Phytotoxicity was generally low and appeared to be related to timing of application. For the 2017 season we saw that the 2X applications had the best control and Scythe-2X did the best at the majority of the farms, averaging over 90% cone kill with relatively little phytotoxicity. Mechanical cone removal was effective at removing cones but resulted in unacceptable damage to vegetative shoots. We saw better overall cone control (death) in the 2017 trials using a split application than in 2016. Chemical control of cone development appears feasible. Next key steps are developing operations systems and refining timing of application and ensuring adequate coverage.

Crain, B. A., & Cregg, B. M. (2017). Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research*, 32(5), 391-396.

Crain, B. A., & Cregg, B. M. (2017). Using irrigation and mulch to control cone production in Fraser fir (*Abies fraseri*). *Scandinavian Journal of Forest Research*, 32(5), 384-390.

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Abstract: Oral Presentation



13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Provenance variation in phenology and frost tolerance in subalpine fir (*Abies lasiocarpa*) planted in Denmark and Iceland**

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In Iceland and Denmark, there is an interest in planting subalpine fir for use as Christmas trees. To search for usable genetic material for both countries, 26 provenances of subalpine fir, covering most of its natural range, were planted in eastern Iceland and Jutland, Denmark. Flushing, bud set and survival rates were assessed. Artificial freezing of twigs, from field trials in eastern Iceland and Denmark, was done to rank the provenances for frost tolerance in the spring and autumn. The northernmost provenances showed earliest bud set, highest autumn frost tolerance and a latitudinal cline was delineated. Differences between provenances in flushing and spring frost tolerance were less than that found for bud set and autumn frost tolerance. The southernmost provenances showed earliest flushing and the most spring frost damage on buds. Mortality of single provenances in the field tests could not be attributed to low freezing tolerances in the autumn or spring. The southernmost provenances of the corkbark fir from New Mexico and Arizona showed the highest survival rate in the field trial in East Iceland, while the eastern provenances showed a low survival rate with the exception of two provenances from Utah and Wyoming. The western provenances from Washington state showed the best survival in Denmark, followed by the southernmost provenances.

Abstract: Oral Presentation



## Seasonal development of cold hardiness and needle retention in Christmas tree plantations in Michigan

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Cold hardiness and needle retention of conifers used as Christmas trees are influenced by the extent of cold exposure in the fall. Understanding the interrelationships between the development of cold acclimation and needle retention may provide insights on the environmental signals that determine needle retention and provide growers with tools to guide harvest decisions and improve post-harvest tree quality. In order to develop baseline information on cold hardiness and needle retention we initiated a survey program in cooperation with a local tree farm. We collected samples from eight trees of four species; Black hills spruce (*Picea glauca* var. *densata*), balsam fir (*Abies balsamea*), Fraser fir (*A. fraseri*), and concolor fir (*A. concolor*). Fraser fir was selected for study since it has excellent needle retention, whereas the other species studied are reported to sometimes have poor needle retention. Samples from each tree were collected on four dates; November 1, 2016; November 22, 2016; December 13, 2016, and January 11, 2017. For each date, two sets of samples were collected. One set of samples consisted of current year shoots only and were used to assess cold hardiness. The second set of samples included two-years of growth (2015 and 2016) and were used to assess needle retention. Cold hardiness was assessed by subjecting samples to progressively colder temperatures in a programmable freezer. Samples were removed the freezer at 3°C intervals as temperatures decreased from 0 to -42°C. Samples were thawed overnight in a 4°C cold room and then placed in high humidity incubation chambers at 25°C for one week to allow for the expression of cold injury symptoms. Cold injury was evaluated based on needle browning (0=none, 1=moderate, 2=severe), bud damage (0=none, 1=moderate, 2=severe), and needle chlorophyll fluorescence. Needle retention was evaluated on three shoots per tree. Shoots were placed in labeled racks and displayed dry in a minimally-heated storage building at the Michigan State University Horticulture Teaching and Research Center (mean temperature =15.5 °C, mean relative humidity = 56.8%). Needle retention was evaluated weekly for five weeks by gently rubbing the needles and rating needle loss on a 0 (no needle loss) to 7 (91-100% needle loss) scale (Nielsen and Chastagner, 2005). Cold hardiness and needle retention varied by collection date and by species. Concolor fir and balsam fir had lower cold tolerance on the first collection date than the other species and had reduced FvFm after exposure to -33°C. Cold hardiness of concolor fir was also reduced on the second collection date. There was little difference in cold hardiness among species on the remaining dates. Needle retention of concolor fir was poor on the first two collection dates, with high rates of needle-shed after one week of display. There was essentially no needle loss from concolor fir shoots collected on the third and fourth sample dates. Needle retention of balsam fir and Black hills spruce declined with increased length of display for shoots collected on first sample date. Needle retention of Fraser fir was excellent on all dates regardless of length of display. These results suggest that needle retention in concolor fir is tightly coupled to cold exposure and development of cold hardiness but the relationship is not as strong for the other species.

Nielsen, U.B. and G.A. Chastagner (2005) Genetic variation in postharvest needle retention among Nordmann fir families and grafted clones, *Scandinavian Journal of Forest Research*, 20:4, 304-312, DOI: 10.1080/02827580510008365

Abstract: Oral Presentation



13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Ensuring Christmas tree seed supply through new seed orchard development in the Pacific Northwest, USA**

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The Pacific Northwest (PNW) region of the United States has a long history of growing quality conifers for both the timber and for Christmas tree industries. Oregon and Washington State combined supply over 40% of the total US production of Christmas trees (USDA Census 2012), with Oregon leading the US in acres planted, trees harvested and number of producers.

Keeping up with the demand for quality PNW Christmas trees requires planning, innovation and sources of quality seed. Production methods have evolved through the decades. Today growers plant seedlings grown with either wild seed or from a handful of Christmas tree seed orchards. As timber companies move in the direction of orchard production for their seed, wild seed harvests have become fewer, making seed more difficult for Christmas tree growers to obtain.

Looking ahead and planning for the future, Christmas tree growers need to focus on more sustainable, tested sources of seed to meet the demand for a consistent, high quality and profitable product to offer their consumers. With this task in mind, a two-year project funded through the Oregon Department of Agriculture began in early 2016.

Oregon State University Christmas Tree Extension program working closely with the Pacific Northwest Christmas Tree Association (PNWCTA) is assisting interested producers with the establishment of new seed orchards with the latest tested selections of Douglas-fir, noble fir, Turkish fir and Nordmann fir. The project is also developing industry wide seed sharing arrangements and new management models for the on-going sustainability of these orchards.

This project takes advantage of many years of progeny testing research and utilizes top ranking trees of each species identified with superior traits. The development of new seed orchards using these superior trees is the best and least expensive method to ensure quality seed availability for the growers of the Pacific Northwest.

Abstract: Oral Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **New Danish Nordmann fir seed orchards – are they improved?**

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Nordmann fir (*Abies nordmanniana*) is the most important species for Christmas tree production in Denmark, where annually about 10 million trees are harvested. A breeding program was established in the early 1990's and more than 400 plus-trees selected. Two strategies were applied 1) Selecting in mature seed producing material and establish progeny trials, and 2) Selecting in Christmas tree stands among the best saleable trees, but due to late seed set (age 30+ years) – rely on gain by phenotypic selection. In both cases seed orchards were grafted by the Danish Nature Agency. In 2009 seed orchards based on plus-trees from the respective strategies produced seed in commercial quantities. Based on commercial bulk harvest 4 field trials were established, comprising 20 seed sources covering seed orchard progeny, un-improved seed stands and direct imports. Results after four year of testing shows large seed source variation in height growth and bud break, but also interesting differences in current season needle necrosis, a needle disorder causing reddening of this season's needles, and in needle loss of older needles presumably related to magnesium nutrient deficiency. Progress from using selected seed sources versus un-improved, their strengths and potential risks are discussed.

Abstract: Poster Presentation





## Poster Presentations

Abstracts in alphabetical order





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Alternative herbicide treatments for glyphosate-resistant weeds**

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Practitioners of Christmas tree integrated pest management in North Carolina have developed a low input weed suppression program using multiple applications of low rates of glyphosate. Annual weeds are controlled to a great extent by short-growing, perennial Dutch white clover that is itself tolerant of low rates of glyphosate. Benefits of this weed control system include soil conservation, cooler soil temperatures in the root zone of the tree, and free fixed nitrogen. However, glyphosate-resistant weeds require alternative herbicide treatments to maintain adequate weed suppression. NC growers have problems primarily with three annual weeds: horseweed or mares tail, *Conyza canadensis* L., common ragweed, *Ambrosia artemisiifolia* L., and lambsquarters, *Chenopodium album* L. Herbicides that are being evaluated include Firstrate (cloransulam-methyl), Harmony (Thifensulfuron-methyl), 2-4D amine, Weedone (2-4D ester), Butyrac (2-4DB), Garlon (tricyclopyr), and Pendelum (pendimethalin). Not only are weed control and tree injury of concern but also the herbicide tolerance of Dutch white clover. Small Christmas tree field studies in different weed populations have provided some indication of herbicide effectiveness. While Firstrate provided excellent control of horseweed and ragweed and Harmony controlled lambsquarters, the product manufacturer is unlikely to add Christmas trees to their labels. To use properly labelled herbicides, growers may have to sacrifice established Dutch white clover cover crops. Rather than identifying a single material, different herbicide choices may be required for different weed problems and management goals.

Abstract: Poster Presentation



## **Biodiversity in the open cultural landscape of the Jauerling The contribution of Christmas tree plantations, fields and meadows**

Pollheimer, M., B. Thurner, M. Hepner, N. Milasowszky, I. Schmitzberger, M. Strausz & K.P. Zulka

Poster made by Karl Schuster,  
Association of Christmas tree Growers of Lower Austria

In 2011 Frühauf et al. show that Christmas tree plantations are positive factors both for the settlement density as well as for the brute success of Wood Lark (*Lullula arborea*) and Red-backed Shrike (*Lanius collurio*) - two central protections of the Nature 2000 region Wachau-Jauerling. So the question was near to ask about the other species like insects, plants and other birds. In the present study 21 Christmas tree plantations, 19 meadows and 15 fields were examined about biodiversity.

A total of 466 different animal and plant species were found in the years 2014 and 2015 in the investigations of 55 fields, meadows and Christmas tree plantation sites at the Jauerling; 71 of these could only be found in Christmas tree plantations. A first glance shows that tree species of birds, spiders, and caterpillars, as well as vascular plants, reach high or above-average numbers of species, while the grasshoppers and butterflies are comparatively small. Meadow sites generally show high numbers of species in almost all organisms.

On average Christmas tree plantations with band spraying show higher numbers of species (10-30%) than those with full spraying.

In summary, it can be stated that Christmas tree plantations at Jauerling contain about 40-70% of the total species diversity of the habitat types of the region's open cultivated land. They thus contribute significantly to the biodiversity of the region.

About 15-25% of all animal and plant species of the open cultivated land at Jauerling are exclusively found in Christmas tree plantations.

The authors of the study give a lot of recommendations to promote the biodiversity in the region and the Association of Christmas tree Growers of Lower Austrian support their members to put this into action.

### **References**

Pollheimer, M., B. Thurner, M. Hepner, N. Milasowszky, I. Schmitzberger, M. Strausz & K.P. Zulka (2016): Biodiversity in the open cultural landscape of Jauerling. The contribution of Christmas tree plantations, fields and meadows. Final report commissioned by the Lower Austrian Christmas Tree Grower Association.

The whole reference list you get under [weihnachtsbaum@lk-noe.at](mailto:weihnachtsbaum@lk-noe.at).

Abstract: Poster Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## ***Camarographium abietis* on true fir in Norway and Denmark**

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In 2009, *Camarographium abietis* was found for the first time in Norway on white fir (*Abies concolor*) in an arboretum in south eastern Norway. In March 2015, it was found again, this time on noble fir (*Abies procera*) in a bough production field in southern Norway. A year later, it was detected on noble fir in Denmark. In all cases, the fungus was found on dead shoots and twigs. The bark on infected sections was sunken and slightly darker than in healthy areas. Black, globose pycnidia had broken through the infected bark and/or appeared in needle scars. Each pycnidium contained numerous conidial spores. The spores were fucoid with both cross and longitudinal septa and relatively large. Spores from the Norwegian noble fir sample measured on average 36.9 x 15.5 µm (n=25), while the spores from white fir were slightly bigger and measured 41.5 x 16.1 µm (n=50). The fungus was found to damage white fir in Scotland more than 90 years ago (Wilson & Anderson 1923), and in Canada, it was reported on subalpine fir (*A. lasiocarpa*) 36 years ago (Funk 1981). A test to determine pathogenicity will be carried out on noble fir in Norway in 2017.

### **References**

Funk, A. (1981). Parasitic microfungi of western trees. Canadian Forestry Service, Pacific Forest Research Centre, Victoria, BC, Canada. 190 pp.

Wilson, M & Anderson, R. B. 1923. Observations on *Camarosporium abietis* n. sp. Transactions of the British Mycological Society 9 (1–4): 144.

Abstract: Poster Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Coning and shoot growth response of Fraser fir trees to paclobutrazol application**

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Precocious coning is a frequent problem when Fraser fir (*Abies fraseri*) is grown under plantation culture for Christmas trees. Individual Fraser fir trees may produce hundreds of cones, which growers remove by hand, resulting in significant labor costs. Cone induction in firs and other conifers is affected by genetics and also driven by environmental stresses. These genetic and environmental signals are mediated through hormonal signaling, particularly gibberellins. Because of this, managers of conifer seed orchards frequently treat trees with exogenous gibberellic acid (GA) in order to promote heavier and more consistent coning in their orchards. Since coning is undesirable in Christmas tree plantations we have explored the use of GA-inhibitors to reduce cone production in Fraser fir. In a previous trial, soil applied paclobutrazol reduced cone density (number of cones per tree) two years after treatment by 33 to 54%, depending on tree size (Crain and Cregg, 2017). In the current trial we are investigating the effect of applying paclobutrazol (PBZ) on coning frequency (percent trees with cones) and coning density when treatments were applied to relatively young trees that have not yet begun to cone. Field plots were installed in spring 2016 in collaboration with four commercial Christmas tree farms in Michigan. At each farm, five treatments were assigned at random to 10-tree row plots. In addition to an untreated control, three PBZ treatments (Cambistat, Rainbow Scientific, Inc.) were applied via soil injection (100, 200, or 300 ml) and one PBZ treatment was applied as a foliar spray (TrimTect, Rainbow Scientific, Inc.). All treatments were replicated six times at each farm. We evaluated trees in 2017 to evaluate effects on coning and shoot growth. Foliar application of PBZ and the highest rate of soil-injected PBZ (300 ml per tree) significantly reduced the number of cones per tree on three of the four farms. These treatment also reduced coning but to a lesser extent on the fourth farm. PBZ applications also reduced shoot extension and increased bud density. Based on our earlier studies, the effect of soil injected-PBZ effects will likely increase over time and we will continue to monitor coning on these plots and re-apply the foliar treatments.

Crain, B. A., and Cregg, B. M. (2017). Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research*, 32(5), 391-396.

Abstract: Oral Presentation



## Day-extension with far-red light enhances growth in seedlings of subalpine fir (*Abies lasiocarpa*)

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Subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) originating from Western North America, is of considerable interest for Christmas tree production in Northern European areas such as Scandinavia. Currently 60% of the Christmas trees sold in Norway are fir, hereof 50% subalpine fir. In Norway seedlings are usually grown from seeds and cultivated in nurseries during two years in a combination of nursery greenhouses and outdoor conditions. During the greenhouse growing-phase this species commonly show undesirably early growth cessation and terminal bud formation, resulting in small plants. This will increase the production time and make the seedlings more vulnerable for stressors at the planting site.

A number of studies have reported enhanced elongation growth and delayed growth cessation and bud set in woody species in response to day extension with different light qualities. Day-extension with far-red (FR) light or increased FR proportion in the spectrum has been shown to enhance elongation growth and delay bud set in seedlings of some woody species, but such information for *Abies* is limited. Also, temperature affects timing of bud set by modulating the effect of photoperiod, but in general information about interactive light quality-temperature effects is scarce. The largest effect has been reported by use of FR light-containing treatments. Aiming at production of larger, more robust seedlings of subalpine fir for Christmas tree production, the goal of the present study was to investigate whether day-extension treatments with FR, red (R), different R:FR ratios or blue (B) light can enhance shoot elongation and delay or prevent growth cessation and bud set in subalpine fir, and whether these responses are affected by temperature.

Four experiments were conducted in growth chambers manufactured by Norwegian University of Life Science (Ås, Norway). Seeds of subalpine fir from the provenance CAN10 from 53.59°N latitude, 122.23°W longitude, 1000-1200 m a.s.l from the George Mountains in British Columbia, Canada (seed lot B13-106, Skogfrøverket, Hamar, Norway) were used. We investigated effects of day-extension with R, FR, different R:FR-ratios or B light from light emitting diodes in subalpine fir seedlings grown at different temperatures. Day extension with FR and R:FR light treatments increased shoot elongation significantly, often with more growth at 18°C than 24°C. Such treatments also delayed terminal bud development as compared to short days (SD) without day-extension, although bud set was not totally prevented. The B or R light treatments did generally not affect growth or bud development significantly as compared to SD. These results demonstrate that enhanced elongation growth in subalpine fir seedlings for Christmas tree production can be obtained by light quality and temperature management, with significantly enhanced growth in response to day-extension with FR light or R:FR-combinations, preferably under cool temperature.





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **De novo Transcriptome Assembly and molecular marker development of Balsam fir (*Abies balsamea*) with respect to post-harvest needle retention**

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Post harvest needle retention in balsam fir (*Abies balsamea*) is an important and highly desired trait, which affects the quality of the tree and profitability of the Christmas tree industry. Revealing genetic basis of needle retention is therefore instrumental for further selection and breeding programs. However, access to informative genomic regions is challenging, partially due to larger conifer genomes, thus hampers our understanding of the molecular mechanisms underlying many important biological processes. To address this, a high-throughput transcriptome sequencing technology were performed to identify genes differentially expressed between the high and low needle retainers. We also aimed to analyze single nucleotide polymorphisms (SNPs) to identify markers and genes associated with genetic variation in needle retention.

Needle loss is a consequence of various factors, of which dehydration stress is a major concern. Illumina sequencing were performed on contrasting fir varieties (high and low needle retainers) after 2, 5 and 9 days of dehydration stress. A total of 36.3 Mbp paired end clean reads were obtained and assembled into 30,647 transcripts with an average length of 1540 bp. Based on the expression profile, 1673 (18.31 %) genes were differentially expressed under 2, 5 and 9 days of dehydration stress; 160, 437 and 70 genes were up-regulated in high needle retainers and 304, 552 and 78 in low needle retainers. Analysis of transcripts using Gene ontology (GO) were revealed that about 1485 differentially expressed genes were annotated with at least one GO term. Photosynthesis, ion binding, response to stimulus, kinase and transferase activity were the most significantly enriched GO categories amongst high needle retainers. Whereas, genes predominantly expressed in low retainers were involved in phenyl propanoid biosynthesis,

flavonoid metabolic process and response to stress respectively. In addition, some unannotated genes were detected, these may provide a basis for studies of water-deficit tolerance.

The search for putative molecular markers among 30,647 coding transcripts resulted in 4,01,981 unfiltered allelic SNPs. After stringent filtering around ~2800 putative SNPs were identified to be specific for high needle retainers and a subset (n= 50) used for variability evaluation studies in fir trees. This dataset provides valuable information regarding the changes during needle abscission in response to dehydration stress and may promote identification and functional analysis of potential genes that could be used for improving needle retention in balsam fir.

Abstract: Poster Presentation



## Development of fruiting bodies produced by *Neonectria fuckeliana* on spruce

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Fruiting bodies (perithecia) of *Neonectria fuckeliana* have been observed on damaged old and young Norway spruce (*Picea abies*). They produce windborne ascospores (sexual reproductive stage) and typically form in canker wounds or in dead bark on the trunk or branches of dead or dying trees, are red in colour and appear in clusters, sometimes together with heavy resin flow. Over a timespan of 16 months, we followed the development of perithecia from *N. fuckeliana* under field conditions, revealing that a cluster normally consists of fruiting bodies at different development stages, thus, enabling windborne spread of mature ascospores throughout the growing season given sufficient humidity. The fungus also produces asexual spores (macro- and micro conidia) from sporodocia emerging from the bark. Conidia are splash dispersed during rainy periods, but this stage is not commonly observed in nature. Inoculation tests with micro-conidial spore suspension or mycelia containing microconidia proved that the fungus is pathogenic, but not very aggressive. This corresponds well with older literature, but not with recent field observations of top dieback and mortality of spruce in both Christmas tree fields and forests. At the stage of mortality, older trees have often been found to have attacks 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, it may be concluded that the beetle is not a primer vector for the disease. We rather believe that trees weakened by *N. fuckeliana* or other pathogens attract the bark beetles. 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 hypothesize that *N. fuckeliana* can be a primary pathogen. Recent increase in amount and intensity of rainfall may have triggered extensive release of ascospores, causing epidemic-like outbreaks of canker on spruce, which has clearly been the case with *N. neomacrospora* on true fir (*Abies* spp.) in the same region and during the same timeframe.

Abstract: Poster Presentation





13<sup>th</sup> International Christmas Tree Research and Extension Conference, Iceland

## **Is attack by *Neonectria neomacrospora* on subalpine fir affected by nutrient content?**

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Subalpine fir (*Abies lasiocarpa*), grown from native seed sources from western North America, are very important for the Norwegian Christmas tree production. Unfortunately, several provenances are very prone to *Neonectria neomacrospora*. We investigated if different nutrient contents in the plants affected the degree of damage caused by *N. neomacrospora*. Plug plants (2/0) from the provenance Grassie Mountain were exposed to outdoor conditions in western Norway throughout the growing season 2013. Except from the nutrients supplied to the plants from the growth medium, the plants did not receive any fertilizer. The purpose was to starve the plants in order to prepare them for nutrient experiments. After over-wintering in a freeze storage at -2°C, the plants received 9 different fertilizer treatments. In addition to control (no treatment), two nitrogen sources (NO<sub>3</sub>-N or NH<sub>4</sub> -N) each at two levels (high/h and low/l) were used. Within each of the four nitrogen treatments, two levels (h and l) of potassium (K), calcium (Ca) and magnesium (Mg) were established. The plants were used in different experiments. Here we report results from plants that were established in a forest stand of subalpine fir in southeastern Norway in spring 2014. In this forest, most of the trees, which had been planted in 1967, were dead or dying from *N. neomacrospora*. The test plants were left standing for two years before they were cut down and evaluated under a dissecting microscope. Many were dead or badly damaged and perithecia from *N. neomacrospora* had commonly developed on dead tissue. From artificial inoculation tests, *N. neomacrospora* has proven very aggressive, and preliminary results from natural infection in this nutrient experiment support that. No clear differences between treatments were obvious, but degrees of damages were rated for all test plants and statistical analyzes will be carried out.

Abstract: Poster Presentation



## ***Phytophthora* species associated with Swedish Christmas tree plantations**

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Norway spruce (*Picea abies*) and Nordmann fir (*Abies nordmanniana*) are the main Christmas tree species in Sweden, but several other fir and spruce species are also commonly grown. Almost all of the planting stock for fir species is imported as seedlings from European tree nurseries. *Phytophthora* root rot is commonly spread via nurseries and causes mortality in Christmas tree plantations throughout the world. This study aimed to determine if plant damaging *Phytophthora* species are present in or adjacent to Christmas tree fields in Southern Sweden. Fields at 14 Christmas tree farms were visually inspected and roots from symptomatic plants, soil samples from wet areas and baits (rhododendron leaves) from nearby streams and ponds were analyzed for presence of *Phytophthora*. Six species, *Phytophthora cryptogea*, *P. gonapodyides*, *P. lacustris*, *P. megasperma*, *P. plurivora* and an unidentified species were isolated from waterways and soil samples. In addition, *P. megasperma* was isolated from a diseased Norway spruce (*Picea abies*) seedling. Morphological studies, an evaluation of temperature effect on culture growth and a consecutive inoculation test of Norway spruce and Nordmann fir seedlings were carried out with one isolate from each of *P. cryptogea*, *P. megasperma*, and *P. plurivora*. None of the isolates caused extensive root rot under the experimental conditions, but they could all be re-isolated from both Norway spruce and Nordmann fir after infection. *Phytophthora* root rot has the potential to become more problematic because *Phytophthora* spp. are able to survive in the soil for decades. Therefore, once *Phytophthora* has been introduced into a field, it may become a permanent problem. *Phytophthora megasperma* was the only *Phytophthora* species isolated directly from roots of a Christmas tree, implying that *Phytophthora* is currently a minor concern for Christmas tree growers in Southern Sweden. However, the *Phytophthora* isolations from soil and water indicate the presence of this agent which may potentially lead to future problems. *Phytophthora* spp. are also known to hybridize and occasionally give rise to more aggressive *Phytophthora* spp., thus, given the damage potential, awareness of *Phytophthora* is seriously needed as it is an emerging problem on Christmas trees in Europe and known to cause substantial losses for growers in USA.

Abstract: Poster Presentation



## **Seasonal changes in lipids and fatty acids linked to needle abscission in four genotypes of balsam fir post-harvest**

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Several studies have suggested that postharvest needle retention in balsam fir, *Abies balsamea*, increases in autumn due to cold acclimation. It has been found that many changes indicative of cold acclimation occur in balsam fir during autumn months, such accumulation of ABA, raffinose, and galactose. However, the role of lipid and fatty acids in postharvest needle abscission has not been confirmed. The objective of this study was to identify if changes occur and if these changes are related to postharvest needles abscission in balsam fir.

Four genotypes of balsam fir, AB-NSD-016, AB-NSD-005, AB-NSD-140, and AB-NSD-124, were sampled (x5) in the clonal orchard owned by the Department of Natural Resources, Debert, Nova Scotia, Canada at 5 time periods between September and February. Onsite testing for capacitance, fluorescence, and membrane injury (MII) was performed, and samples were frozen in liquid nitrogen to store in a -80 °C freezer for later polar lipid and fatty acid analysis. The weather and photoperiod parameters were collected from the Environment Canada Weather Station located within a 2 km radius of the orchard. In addition, "sister" branches (x5) were collected at each sampling interval and transferred to the lab and hydrated to determine the mean needle abscission commencement (NAC), and average daily water usage (ADWU). The experiment was designed as a 4 x 5 factorial, but analyzed using repeated measures.

AB-NSD-016, AB-NSD-005, AB-NSD-140, and AB-NSD-124 had mean NACs of 42, 52, 32, and 75 days respectively. AB-NSD-005 and AB-NSD-124 had a tendency to retain longer after cold acclimation, but it was not significant. All genotypes commenced needle abscission significantly earlier in February possibly due to increasing photoperiod. ADWU improved over time.

Fluorescence, MII, and capacitance all correlated with needles loss (negatively, positively, and negatively, respectively). Fluorescence and ADWU were most closely linked with  $T_{min}$  ( $r = .881$  and  $-.751$ , respectively). With respect to polar lipids, the greatest change linked to cold acclimation was a decrease in the monogalactosyldiacylglycerol to digalactosyldiacylglycerol ratio (MGDG:DGDG) suggesting enhanced DGDG synthesis to increase chloroplast membrane stability for winter. This ratio was also directly correlated to  $T_{min}$  ( $r = .881$ ). There is also an increase in unsaturation of fatty acids related to a decrease in temperature, primarily 18:3n3 suggesting an increase in membrane fluidity. Genotype AB-NSD-124 adapted more quickly to cold acclimation than the poorer genotypes suggesting it may be more sensitive to temperature change.

Abstract: Poster Presentation



## Seasonal development of cold hardiness and needle retention in Christmas tree plantations in Michigan

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Cold hardiness and needle retention of conifers used as Christmas trees are influenced by the extent of cold exposure in the fall. Understanding the interrelationships between the development of cold acclimation and needle retention may provide insights on the environmental signals that determine needle retention and provide growers with tools to guide harvest decisions and improve post-harvest tree quality. In order to develop baseline information on cold hardiness and needle retention we initiated a survey program in cooperation with a local tree farm. We collected samples from eight trees of four species; Black hills spruce (*Picea glauca* var. *densata*), balsam fir (*Abies balsamea*), Fraser fir (*A. fraseri*), and concolor fir (*A. concolor*). Fraser fir was selected for study since it has excellent needle retention, whereas the other species studied are reported to sometimes have poor needle retention. Samples from each tree were collected on four dates; November 1, 2016; November 22, 2016; December 13, 2016, and January 11, 2017. For each date, two sets of samples were collected. One set of samples consisted of current year shoots only and were used to assess cold hardiness. The second set of samples included two-years of growth (2015 and 2016) and were used to assess needle retention. Cold hardiness was assessed by subjecting samples to progressively colder temperatures in a programmable freezer. Samples were removed the freezer at 3°C intervals as temperatures decreased from 0 to -42°C. Samples were thawed overnight in a 4°C cold room and then placed in high humidity incubation chambers at 25°C for one week to allow for the expression of cold injury symptoms. Cold injury was evaluated based on needle browning (0=none, 1=moderate, 2=severe), bud damage (0=none, 1=moderate, 2=severe), and needle chlorophyll fluorescence. Needle retention was evaluated on three shoots per tree. Shoots were placed in labeled racks and displayed dry in a minimally-heated storage building at the Michigan State University Horticulture Teaching and Research Center (mean temperature =15.5 °C, mean relative humidity = 56.8%). Needle retention was evaluated weekly for five weeks by gently rubbing the needles and rating needle loss on a 0 (no needle loss) to 7 (91-100% needle loss) scale (Nielsen and Chastagner, 2005). Cold hardiness and needle retention varied by collection date and by species. Concolor fir and balsam fir had lower cold tolerance on the first collection date than the other species and had reduced Fv/Fm after exposure to -33°C. Cold hardiness of concolor fir was also reduced on the second collection date. There was little difference in cold hardiness among species on the remaining dates. Needle retention of concolor fir was poor on the first two collection dates, with high rates of needle-shed after one week of display. There was essentially no needle loss from concolor fir shoots collected on the third and fourth sample dates. Needle retention of balsam fir and Black hills spruce declined with increased length of display for shoots collected on first sample date. Needle retention of Fraser fir was excellent on all dates regardless of length of display. These results suggest that needle retention in concolor fir is tightly coupled to cold exposure and development of cold hardiness but the relationship is not as strong for the other species.

Nielsen, U.B. and G.A. Chastagner (2005) Genetic variation in postharvest needle retention among Nordmann fir families and grafted clones, *Scandinavian Journal of Forest Research*, 20:4, 304-312, DOI: 10.1080/02827580510008365

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## Sensitivity of slugs and conifer foliage to dips in plant essential oils

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In an ongoing effort to identify postharvest treatments that are effective in eliminating slugs from exported Christmas trees, a series of experiments were conducted to determine the sensitivity of slugs to dips in plant essential oils. Initial tests indicated that *Arion rufus* slugs were very sensitive to 15 second dips in suspensions of pine oil AEF-12-01 concentrate from AEF Global (0.5, 1.0 and 5.0%). Checks consisted of slugs immersed in tap water alone at about 15C. Even at the lowest concentration tested, 80% of the slugs were killed with a 15 second dip and higher concentrations resulted in 100% mortality. In addition to three concentrations (0.5, 1.0, and 2.5%) of the AEF-12-01 pine oil that was tested previously, a single concentration (1.0%) of five additional essential oils [Pine oil (*Pinus strobus*), Fir needle oil 'Canadian' (*Abies balsamea*), Clove bud oil (*Eugenia caryophyllata*), Cedarwood oil (*Juniperus virginia*), and Cinnamon leaf oil (*Cinnamomum zeylanicum*)] were include in another slug dip trial that was conducted in November 2016. The slugs were a mixture of *Prophysaon* (most probably *P. andersoni*) and *Arion rufus*. Results from this trial indicate that there was no mortality of the slugs that were not dipped at all or the ones that were only dipped in water for 15 seconds. Increasing the length of the dip to 125 seconds resulted in 8% mortality of the slugs dipped in water alone. Dipping slugs in each of the six essential oils resulted in almost 100% mortality. Even a 15 second dip in the lowest concentration of Pine Oil AEF-12-01 Concentrate resulted in 76% mortality. These data confirm the previous data for pine oil and indicated that slugs are also very sensitive to short duration dips in a number of other essential oils.

Studies were also conducted to determine if there were any adverse postharvest effects of dipping noble fir (*Abies procera*) and Douglas-fir (*Pseudotsuga menziesii*) branches in 1% solutions of the six essential oils tested above for 125 seconds. After dipping, the branches were displayed in racks for 7 days at 20C. Dips in some of the essential oils injured the foliage of noble and Douglas-fir. Brown discoloration of the needles on some branches was evident after one or two days of display. After one week, the noble fir branches that had been dipped in both sources of pine oil, clove bud oil, and cinnamon oil had significantly lower quality ratings than the non-dipped branches or branches that were dipped in only water. No needle loss was evident on any of the noble fir branches. All of the Douglas-fir branches had very low-quality ratings after one week, because of drying and/or needle loss. Unlike the noble fir, dips in both sources of pine oil, clove bud oil, and cinnamon oil significantly increased needle loss ratings on the Douglas-fir branches compared to the non-dipped branches or branches that were dipped in only water.

Although our trials have indicated that cold water dips in all of these essential oils are very effective in killing slugs, it is unclear if the concentrations and exposure times could be reduced to the point that they are still efficacious without any risk of damage to the foliage on noble and Douglas-fir Christmas trees.

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## **Variation in bud break of Turkish and Trojan fir populations from Turkey**

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Research has shown that Nordmann (*Abies nordmanniana*), Turkish (*A. bornmulleriana*), and Trojan firs (*A. equi-trojani*) are much less susceptible to Phytophthora, Annosus root rot, spider mites, and balsam woolly adelgid than other commonly grown fir species. Turkish and Trojan fir are also much less susceptible than Nordmann fir to the silver fir adelgid that is widely distributed in Europe and has recently appeared in some areas in Washington State. Although susceptible to current season needle necrosis, Nordmann, Turkish, and Trojan firs are much less susceptible to this physiological disorder than noble fir (*A. procera*).

There is very little information relating to specific sources of these species that are suitable for use as Christmas trees in the North America. Four sources of Turkish fir were included in a recently completed project that evaluated the Christmas tree characteristic of different sources of Nordmann and Turkish fir in OR and WA. All of these sources outperformed most of the Nordmann fir sources. This research showed that Turkish fir tend to have faster growth rates than Nordmann fir and there was considerable genetic variation in the growth, quality, timing of bud break and postharvest needle retention characteristic of the different sources of Nordmann and Turkish fir.

Turkish and Trojan firs tend to have earlier bud break than Nordmann fir, which can increase the potential for damage from late spring frosts and limit their potential use in some production regions. In 2013, a series of regional common garden plots were established in the U.S. as part of the Collaborative Fir Germplasm Evaluation (CoFirGE) Project. Each plot contains progeny from 20 trees along an elevation gradient in three provenances (Adapazarı-Akyazı, Bolu-Aladağ and Karabük-Keltepe) of Turkish fir and two provenances (Çanakkale-Çan and Balıkesir-Kazdağı) of Trojan fir along with several sources of Nordmann fir and single sources of common North American Christmas tree species. Data on growth characteristics of the trees in these plantings is being collected to obtain information on the regional adaptability of these sources for use as Christmas tree. These data include a one-time evaluation of bud break during the spring.

More extensive bud break data has been collected from a 2014 plating of excess of seedlings from the CoFirGE project at WSU Puyallup. The plot includes seedlings from 10 to 11 trees from each of the three provenances of Turkish fir and 4 to 5 trees from the each of the two provenances of Trojan fir. In addition, the plot contains seedlings from three sources of Nordmann fir and one source each of noble and Fraser fir. During 2016 and 2017, bud break was evaluated weekly for the 800 trees in this replicated plot. Information will be provided on the variation in bud break among the species and sources of trees contained in this plot.

Abstract: Poster Presentation

