



Proceedings of the 9th International Christmas Tree Research & Extension Conference

September 13–18, 2009



John Hart, Chal Landgren, and Gary Chastagner (eds.)

Title

Proceedings of the 9th International Christmas Tree Research & Extension Conference
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Corvallis, Oregon and Puyallup, Washington, September 13–18, 2009

Held by Oregon State University, Washington State University, and Pacific Northwest Christmas Tree Growers' Association

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Foreword

The 9th International Christmas Tree Research and Extension Conference returned to the Pacific Northwest in 2009. OSU and WSU cohosted the conference, which was attended by 42 Christmas tree professionals representing most of the major production areas in North America and Europe.

This conference was the most recent in the following sequence:

Date	Host	Location	Country
October 1987	Washington State University	Puyallup, Washington	USA
August 1989	Oregon State University	Corvallis, Oregon	USA
October 1992	Oregon State University	Silver Falls, Oregon	USA
September 1997	British Columbia Ministry of Forests, Research Branch	Mesachie Lake, British Columbia	Canada
July 2000	Danish Forest and Landscape Research Institute	Vissenbjerg	Denmark
September 2003	North Carolina State University	Hendersonville, North Carolina	USA
October 2005	Michigan State University	Tustin, Michigan	USA
August 2007	Forest and Landscape, University of Copenhagen	Bogense	Denmark
September 2009	Oregon State University and Washington State University	Corvallis, Oregon and Puyallup, Washington	USA

Thanks are in order to a number of groups, individuals, and tree farms. Kari Summers of the Pacific Northwest Christmas Tree Association ably handled the registration, accounting, and payments. Our tour hosts at Holiday Tree Farms (Hal Schudel, Mark Arkills, Dale Stevens, and Dennis Tompkins), Silver Mountain (Jim and Shirley Heater, their families, and Bob Schaefer), Stoda Farms (Kirk Stroda, Glenn Fisher, and Brian Kerr), and Sunrise (Pat and Betty Malone) provided excellent educational tours and information.

In Washington, the Department of Natural Resources hosted our group at Mt. St. Helens on a rare “four mountain view” sunny day, while we looked at volcano impacts and noble fir bough collection. Mark and Karen Savage hosted us at their tree farm with a fantastic locally caught seafood dinner.

In addition to the tours, 27 talks and 13 posters were presented during the 5-day conference. The WSU Puyallup staff hosted a number of informal farm and visitor tours as well as a close-up view of experiment station Christmas tree research that was highlighted on Seattle evening TV news.

The 10th International Christmas Tree Research and Extension Conference will be hosted by the Christmas Tree Grower Association of Lower Austria in 2011. We look forward to a return to Europe.

Conference hosts

Chal Landgren, Oregon State University
Gary Chastagner, Washington State University

Table of Contents

Foreword	i
Table of contents	ii
Conference program	iii
List of participants	vi
Educational materials displayed	ix
List of presentations	xi
Abstracts of presentations and posters	
Breeding	1
Propagation	9
Growth conditions	25
Tree health	45
Weeds.....	70
Insect and vertebrate pests	77
Cultivation techniques	92
IUFRO business meeting	108



Conference Program

Monday, September 14

Fertility/Soils Presentations

Fertilization of greenery-producing stands in noble fir: Ecological sustainability and yield of quality branches. L.B. Pedersen and C.J. Christensen

A potential remote sensing application for nitrogen management in Christmas trees. Mike Flowers

Soil development in western Oregon. Sara Hash

Genetics/Breeding Presentations

Czech-American fir hybridization research for purposes of Christmas tree production. Jaroslav Kobliha

Nordmann fir seed orchard genetics. Ulrik Bräuner Nielsen

Evaluating Nordmann fir (*A. nordmanniana*) for Pennsylvania conditions. Ricky M. Bates

Variation in resistance to Phytophthora root rot within Turkish and Trojan fir. John Frampton

Factors affecting graft success and early growth of Fraser fir. AnneMargaret Braham

Tours

Holiday Christmas Tree Farm

State-of-the-art container nursery, wreath production facility, and Nordmann fir culture

Stroda Tree Farm

Use of ground covers, foliar fertilization, adelgid control on Fraser fir

Tuesday, September 15

Tree Health Presentations

***Sydowia polyspora* isolated from needles and seeds of true fir is associated with current-season needle necrosis (CSNN).** Venche Talgø

***Stigmina* on spruce in Michigan.** Dennis W. Fulbright

Neonectria canker on true fir and spruce in Norway. Venche Talgø

Copper-based fungicide field trials against CSNN: Results from five countries.
Iben Margrete Thomsen

Integrated Pest Management practices to achieve optimum Elongate Hemlock Scale and overall pest control in North Carolina Fraser fir. Bryan Davis

Production/Cultivation Presentations

Bud removal for tree shaping: Hormonal and growth pattern effects.
Hanne N. Rasmussen

The effect of watering and nitrogen fertilization on growth, nutrient use, and leaching in containerized Fraser fir (*Abies fraseri*). Pascal Nzokou

Growth and physiology of living Christmas trees in container production systems.
Bert Cregg

Eighteen-year research summary of Dr. Jürgen Matschke career. John Frampton

Tours

Holiday tree farm

Methods to maintain field productivity for the long term

Sunrise Tree Farm

Eco-tours for school-age children, cover crops between rows, and sustainable eco-friendly practices

Wednesday, September 16

Production/Cultivation Presentations

Growth, quality, and economic value of Fraser fir Christmas trees sheared with varying leader lengths in North Carolina, USA. Eric Hinesley

Test of various methods of application of NAA for leader length retardation on Nordmann fir. Paul Christensen

Establishment routines for *Abies nordmanniana* and *Abies lasiocarpa*.
Steinar Haugse

Weed Control Presentations

Controlling emerged weeds in actively growing conifers in Connecticut, USA.
John F. Ahrens

Test of mixtures of herbicides: Accurate mixed with diflurenican for weed control in Christmas trees. Paul Christensen

Transitioning weed suppression from Roundup Original to Roundup Powermax with backpack and mistblower sprayers. Jeff Owen

Tour

Silver Mountain Tree Farm

Types of equipment needed to harvest, load, and manage a yearly cut of hundreds of thousands of trees; private seed orchard sites; Nordmann and Turkish fir genetic trials; PNW issues relating to exports and market changes

North Willamette Research and Extension Center

Nursery and berry production, foliar fertilization of conifers in containers, experiments on minimizing water run-off from irrigation, nutrient trials for nursery production, grass breeding varieties for Christmas tree cover crops.

Thursday, September 17

Tour

Mt. St. Helens

Natural bough harvest on noble and Pacific silver fir, thinning, changes in stands following the eruption, and the future of bough harvests in the region

Friday, September 18

WSU Research Update

Approaches being used to identify trees with superior postharvest needle retention, Phytophthora root rot susceptibility trials, current-season needle necrosis

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Educational Materials Displayed

Michigan State University Christmas Tree Area of Expertise Team (AoE)

<http://www.for.msu.edu/ChristmasAOE/index.html>

The Michigan State University Christmas Tree Area of Expertise Team website was developed and is supported by MSU faculty and agents dedicated to the Christmas tree industry in Michigan. The group's goal is a profitable Christmas tree industry in Michigan that is environmentally responsible and competitive on a regional and national scale.

Michigan State University's Christmas Tree AoE is dedicated to maintaining Michigan as a major Christmas tree producing state with national visibility and stature, by conducting educational, demonstration, and research programs designed to maintain and enhance the contributions of the Christmas tree industry to Michigan's economy.

Oregon State University forest succession ownership: Ties to the Land

http://www.familybusinessonline.org/index.php?option=com_content&view=article&id=50&Itemid=51

Millions of acres of family-owned forest land will change hands in the United States within the next decade. Many transfers will happen with virtually no planning.

In Oregon, more than half of the forest landowners are over 65. The situation is similar in other states. Although most of these landowners say they want to keep the land in the family and pass it on to the next generation, few have taken the steps to do so.

The U.S. Forest Service projects that about 23.2 million acres of forest land will pass out of forest use over the next 50 years. Most of these acres will be privately owned, nonindustrial forest lands converted to residential subdivision.

Many family forest lands lie on the edges of metropolitan areas. They provide important economic, ecological, and social amenities to communities throughout Oregon and the nation. With a change of ownership comes a potential for change of use.

Succession planning is difficult at the best of times. When forest land is at stake, the differences among family members in values, goals, and critical skills can lead to disaster. Without an effective plan, the owners' intentions may not be followed. This may put the future of the land in doubt.

The fate of family forest lands is an issue not only for the families involved, but also for communities. It is an important social issue, with implications at a landscape scale.

Oregon State University Christmas Tree Nutrient Management Guide (Western Oregon and Washington), EM 8856

<http://ir.library.oregonstate.edu/jspui/bitstream/1957/12863/3/EM8856.pdf>

Current nutrient management programs must focus on three concepts for success.

- Is the management practice biologically sensible? Is it likely that fertilizing these trees at this time and with this product will produce a significant improvement in tree color or growth?
- Is the management practice economically efficient? Can I afford it based on expected results?
- Is the management practice environmentally responsible? Does it produce little or no potential negative impact on soil, water, or air quality?

When the answer to all three questions is “yes,” nutrient management practices should be used to increase Christmas tree quality and profitability.

To understand and influence plant nutritional health and performance, you need a broad knowledge of several important topics, including:

- How conifers grow
- The nutrients necessary for optimal growth
- How to assess the nutrient status of soil and plant foliage
- How to formulate a strategy for nutrient management during the rotation

These topics form the basis for this publication. This guide provides more than fertilizer and lime recommendations; you also will learn to assess a plantation’s nutritional needs based on soil and foliar analyses and rotational timing. These tools will help you design strategies for effective nutrient applications and produce high-quality trees with minimal negative environmental impact.

List of Presentations

BREEDING

- Czech-American fir hybridization research for purposes of Christmas tree production**, Jaroslav Koblíha, Jan Stejskal, and John Frampton 1
- High-throughput DNA sequencing of Fraser fir (*Abies fraseri*)**, Ross Whetten and John Frampton..... 6

PROPAGATION

- Effect of media pH and 2-(N-mirpholino) ethanesulfonic acid in Douglas-fir (*Pseudotsuga menziesii*) micropropagation systems**, Chien-Chih Chen, Eric Dice, Abdoulaye Traore, John E. Carlson, and Ricky M. Bates 9
- Douglas-fir (*Pseudotsuga menziesii*) micropropagation: Shoot multiplication of juvenile and mature genotypes**, Chien-Chih Chen, Abdoulaye Traore, John E. Carlson, and Ricky M. Bates 11
- Is there a way to the perfect Christmas tree? Clonal production of Nordmann fir by somatic embryogenesis is still problematic!** Jürgen Matschke..... 14
- Nordmann fir seed orchard genetics**, Ulrik Bräuner Nielsen and Ole K. Hansen 18
- Cutting production in Nordmann fir: Rooting, plagiotropism, and hormonal background**, Hanne N. Rasmussen, Ulrik Bräuner Nielsen, Martin Jensen, and Jens Hansen-Møller..... 20

GROWTH CONDITIONS

- Evaluating Nordmann fir (*A. nordmanniana*) for Pennsylvania conditions**, Ricky M. Bates 25
- Growth and physiology of living Christmas trees in container production systems**, Bert Cregg, Amanda Taylor, Wendy Klooster, R.T. Fernandez, and Pascal Nzokou..... 28
- Establishment routines for *Abies nordmanniana* and *Abies lasiocarpa***, Inger Sundheim Fløistad and Steinar Haugse..... 34
- Factors affecting graft success and early growth of Fraser fir**, Haley Frey, John Frampton, Eric Hinesley, Frank Blazich, and AnneMargaret Braham 36
- The effect of watering and nitrogen fertilization on growth, nutrient use, and leaching in containerized Fraser fir (*Abies fraseri*)**, Pascal Nzokou and Bert M. Cregg 37

TREE HEALTH

Variation in resistance to <i>Phytophthora</i> root rot within Turkish and Trojan fir, John Frampton, Fikret Isik, Mike Benson, and AnneMargaret Braham	45
<i>Stigmina</i> on spruce in Michigan, Dennis W. Fulbright	46
Rating needle loss of Fraser fir foliage associated with Christmas tree preservatives, Jeff Owen	50
<i>Sydowia polyspora</i> isolated from needles and seeds of true fir is associated with current-season needle necrosis (CSNN), Venche Talgø, Gary A. Chastagner, Iben M. Thomsen, Thomas Cech, Kathy Riley, Kurt Lange, Sonja S. Klemsdal, and Arne Stensvand	52
<i>Neonectria</i> canker on true fir and spruce in Norway, Venche Talgø, May Bente Brurberg, and Arne Stensvand	58
Copper-based fungicide field trials against CSNN: Results from five countries, Iben Margrete Thomsen, Venche Talgø, Gary Chastagner, Thomas Cech, Kurt Lange, Bernhard Perny, Kathy Riley, Benjamin Louis, Andrew Dobson, and Arne Stensvand	63

WEEDS

Test of mixtures of herbicides: Accurate mixed with diflufenican for weed control in Christmas trees, Paul Christensen	70
Transitioning weed suppression from Roundup Original to Roundup Powermax with backpack and mistblower sprayers, Jeff Owen, Bryan Davis, and Doug Hundley	72

INSECT AND VERTEBRATE PESTS

Elongate hemlock scale, balsam twig aphid, and balsam woolly adelgid in North Carolina Fraser fir, Bryan Davis	77
Host resistance screening for balsam woolly adelgid: Early results from 13 fir species, Leslie Newton, Fred Hain, and John Frampton	81
Predicting and timing of control for Douglas-fir needle midge in Michigan, Jill O'Donnell	84
Identifying alternatives to commercial deer repellents, Jeff Owen and Bryan Davis	87

CULTIVATION TECHNIQUES

Test of various methods of application of NAA for leader length retardation on Nordmann fir , Paul Christensen.....	92
The influence of needle location and sampling technique on nutrient concentration , John Hart, Chal Landgren, Rick Fletcher, J.T. Moody, and Mike Bondi	94
Growth, quality, and economic value of Fraser fir sheared with varying leader lengths in North Carolina, USA , Eric Hinesley.....	98
Bud removal for tree shaping: Hormonal and growth pattern effects , Hanne N. Rasmussen, Bjarke Veierskov, Jens Hansen-Møller, and Rikke Nørbæk.....	102

Czech-American fir hybridization research for purposes of Christmas tree production

Jaroslav Koblíha¹⁾, Jan Stejskal¹⁾, and John Frampton²⁾

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Introduction

European and American firs are precious coniferous species due to their production, ecological, and aesthetic significance. Their cultivation in forests supports wood production as well as the other important functions of forest land. With their high aesthetic qualities, firs increase the recreational potential of municipal forests and parks. Firs also have a major role in Christmas tree production on plantations. Christmas tree plantations hold special importance in the U.S., where they annually yield a giant economic income for farmers and land owners.

Several species, above all the European silver fir (*Abies alba* Mill.), are endangered by a long-term decline of forest stands in connection with their historical retreat (especially in central Europe). Breeding is an important tool not only for increasing production of forests, but also for improving resistance of trees and forest stands. Breeding can also improve the aesthetic quality of forest tree species dramatically, which influences Christmas trees on a large scale.

Fraser fir (*A. fraseri* [Pursh] Poir.) has gained attention as the only *Abies* native to the southeastern U.S. The systematic research of this species has been supported by its extreme economic importance. Its utilization as a major Christmas tree species brings over \$US 100 million annually to the industry in North Carolina. North Carolina is recently the second-leading Christmas tree producer in the U.S. According to Jerry Moody (2007), director of Avery County Cooperative Extension Service, Fraser fir production represents 67% of the total agricultural income of that county, with more than 1 million Fraser firs harvested annually.

A major limiting factor for the culture of true fir Christmas trees is their susceptibility to water molds of the *Phytophthora* genus. In the North Carolina Christmas tree industry alone, more than \$US 1.5 million is lost annually to Phytophthora root rot disease (caused by *Phytophthora cinnamomi* Rands). While chemical methods are available for controlling this disease in seedling and transplant beds, chemical control in plantations is stop-gap at best. Severely infested sites must be abandoned, perhaps permanently, for Fraser fir cultivation, threatening the sustainability of Christmas tree production in the region (Frampton, 2007).

One of the prominent breeding methods possibly leading to higher resistance of fir is intraspecific/interspecific hybridization. It is well known that hybrids originating from crossing species within the genus *Abies* perform extremely well in growth and vitality in comparison to the parental trees. This phenomenon is called heterosis and justifies pursuit of interspecific hybridization. Increased vitality of the interspecific hybrids is also related to their higher tolerance to changing environmental conditions. In addition, hybrids are expected to tolerate different stress factors such as air pollution or climate change consequences.

Hybrids of the second filial generation (F_2) were obtained by Koblíha (1994). An F_1 hybrid *Abies cilicica* x *Abies cephalonica* (or more precisely its fructifying graft) was featured as the mother tree.

A previous trial conducted at North Carolina State University (NCSU) inoculated seedlings of 32 *Abies* species with *P. cinnamomi* and showed that North American species are almost completely susceptible, while many Mediterranean and Asian species have some trees with resistance. Toros fir (*Abies cilicica* Carr.) from southern Turkey and Greek fir (*A. cephalonica* Loud.) were ranked fourth and eighth, respectively, for the frequency of resistant seedlings (Frampton, 2007).

The Czech University of Life Sciences (CULS) has utilized Toros and Greek fir in a long-term hybrid breeding effort aimed at developing a faster growing fir that is hardier to changing ecological conditions than the native European silver fir (*A. alba* Mill.). As a result of these efforts, seeds of F_1 , F_2 , and complex hybrids with additional fir species are available. Due in part to collaborative breeding efforts, some of these complex hybrids include Fraser fir (*A. fraseri* [Pursh] Poir.), the primary Christmas tree species in North Carolina, which is completely susceptible to *P. cinnamomi*. Screening this material for resistance to root rot may progress toward the development of resistant Christmas tree planting stock and also provide insight into the genetic control of resistance (Frampton, 2007).

Material and methods

Experimental plots

All of the Czech seed orchards were founded as biconal—grafts originated from two interspecific hybrids of the first generation F_1 *Abies cilicica* x *Abies cephalonica*. These grafts have fructified many times, which inspired Professor Koblíha in the 1980s to execute control pollination. That way, F_2 material and new interspecific hybrids were obtained. Part of this material is cultivated within the breeding station Truba, Kostelec nad Černými lesy. Owing to good experiences with coning and fertility of this material, and also outstanding growth and vitality characteristics that suggested great potential for hybridizations, it was decided to further utilize this material. Secondary grafts were taken to establish the mentioned hybridization seed orchards. These seed orchards primarily produced F_2 hybrids. Rootstocks were European silver fir.

Hybridization seed orchards with the presence of female strobili had been utilized before 2006 mainly for production of F_2 hybrids. A list of plantations below outlines their historical and present state.

Hybridization seed orchard No. 1 was established in 1994 directly at the breeding station Truba near Kostelec n.Č.l. from the material grafted in 1991 and 1992. Female coning has been observed in 2004 and 2006–2008.

Hybridization seed orchard No. 2 was established in May 1996 close to the breeding station Truba. Coning hasn't been observed so far.

Hybridization seed orchard No. 3 was established in 1997 from the material grafted in 1993 within a nursery by a village Seč near Prostějov. Female coning registered in 2003–2008.

Hybridization seed orchard No. 4 was established in May 1999 within the school enterprise Kostelec n. Č. I. This plantation began to cone in 2008.

One of the experimental plots involved in our recent hybridization trials belongs to a long-term experiment with spontaneous hybrid ancestries established in 1996. After significant mortality in the first year, new material—*A. koreana* x (*Abies cilicica* x *Abies cephalonica*) hybrids were brought in (1997) as 5-year-old seedlings. Originally there were 2 plots established with 25 trees each and without significant mortality. These hybrids began to cone in 2004, and female strobili have been observed annually ever since.

2006

Fructification in hybridization seed orchards occurred in 2006. Female strobili occurred in seed orchards No. 1 and No. 3. Seed orchard No. 3 had the highest abundance of female strobili. That led to an additional application of *Abies fraseri* pollen. This particular pollen was obtained from a single tree that is situated in the faculty arboretum in Kostelec n.Č.I.

2007

In the year 2007, the core of our activities was represented by hybridizations. We were able to import *Abies fraseri* pollen from the U.S. (a Fraser fir seed orchard located in the Appalachian Mountains of North Carolina). More specifically, we obtained frozen pollen of clones NC73, NC52, NC84, and a polymix (PC – polycross) of several clones collected in 2006. Pollen of *Abies cilicica* x *Abies cephalonica* hybrid (clones CZ1 and CZ2) was collected in Czech seed orchards. This pollen from seed orchard No. 1 had been frozen and then later shipped to the U.S. in 2008. Pollen collected in seed orchard No. 3 was used fresh for pollination at the same place.

Control pollination was performed in spring 2007 in seed orchard No. 1. Applied pollen was *Abies fraseri* (NC73, NC84). Two cones in seed orchard No. 1 originated from open pollination (F₂ Kostelec). In seed orchard No. 3, there was a similar situation—pollen of *Abies fraseri* was used (NC52, PC), plus pollen of *Abies cilicica* x *Abies cephalonica* hybrid (clones CZ1 and CZ2) was applied, thus creating F₂ Prostějov. Aside from two main seed orchards, control pollination was performed on the experimental plot in Kostelec n.Č.I. with *A. koreana* x (*Abies cilicica* x *Abies cephalonica*) hybrids. There was a great majority of *A. fraseri* pollen (NC73, PC) applied with a single exception of open pollination.

2008

During the spring of 2008, pollination took place in three of the four seed orchards (1, 3, and 4). Pollen of *Abies fraseri* was obtained from North Carolina State University. More specifically, we obtained frozen pollen of clones NC73, NC52, NC84, NC136, and polymix (PC – polycross) of various clones collected in 2007. Pollen of *Abies cilicica* x *Abies cephalonica* hybrid (clones CZ1 and CZ2) was collected in Czech seed orchards. This pollen from seed orchard No. 1 has been frozen.

In addition, in seed orchard No. 3, we tried an application of other species' pollen, specifically *Abies balsamea* and *Abies fraseri* originating from Arboretum Kostelec and *Abies koreana* from Arboretum Průhonice.

Control pollination was performed in the spring of 2008 in seed orchards No. 1, No. 3, and for the very first time also in seed orchard No. 4. Applied pollen was *A. fraseri* (NC52, NC73) with a negligible part of open-pollinated cones (F₂ Kostelec). In seed orchard No. 3, there was a similar situation—pollen of *Abies fraseri* was used (NC73, NC84, PC, NC136), plus extra *A. balsamea*, *A. koreana*, *A. fraseri*, and occasional open pollination (F₂ Prostějov).

Later this fall, cones and seed processing similar to that of 2007 is planned to be able to make conclusions about this year's pollination results. Before December, *Phytophthora* screenings are planned by the American partner.

Results

2006

Planting stock that originated from hybridizations in 2006 (1/0) is being used (saplings and seedlings). Most of the material is represented by F₂ *Abies cilicica* x *Abies cephalonica*. There is a certain percentage of (*Abies cilicica* x *Abies cephalonica*) x *Abies fraseri* saplings. Field germination assessment on this material took place in spring 2007. In Kostelec (seed orchard No. 1), we harvested 46 cones. From 9,416 seeds of F₂ *Abies cilicica* x *Abies cephalonica* collected in seed orchard No. 1, 1,360 saplings originated, which is 14.4% germination.

Seed orchard No. 3 yielded 426 cones (45 of *Abies fraseri* combination). From 111,946 F₂ *Abies cilicica* x *Abies cephalonica* seeds sown, 13,500 saplings originated (12%). From 5,730 (*Abies cilicica* x *Abies cephalonica*) x *Abies fraseri* seeds sown, 270 saplings came up (4.7%).

2007

In September 2007, mature cones of all the hybrid combinations were harvested. After the cones were measured and weighed, the seeds were extracted and examined. Seed samples of the individual combinations were then X-rayed in early October for the final share of full seed assessment. The seeds were sown during late autumn. Most of the seeds logically originated from F₂ *Abies cilicica* x *Abies cephalonica* (F₂ Prostějov). 0.6-kg samples of this origin were either shipped to the U.S. or granted to somatic embryogenesis research of our department. The U.S. side will examine this material for specific resistance to *Phytophthora cinnamomi*, which represents a serious threat to Christmas tree plantations in the U.S.

Field germination assessment took place in Kostelec in the spring of 2008.

2008

Cone harvest took place at all of the mentioned hybrid seed orchards during September 2008. Cones were measured and examined and so were the seeds. Seed samples of the individual seed lots were then X-rayed in early October for the final share of full seed assessment. Because a relatively small percentage of viable seeds was obtained by most of the samples, the sample number was later multiplied. At the end we ended up with a final sample size of 300 X-rayed seeds.

Most of the seeds were not sowed within our facilities as was the case in 2007, but were shipped to the U.S. for *Phytophthora* resistance screenings in early 2009 when just a F₂ *Abies cilicica* x *Abies cephalonica* (F₂ Prostějov) seedlot remained in the Czech Republic. A rather small amount of the open-pollinated material was again granted to somatic embryogenesis research in our department.

Discussion

As the hybridizations of 2007 have shown some promising results, we assume that the 2008 experiment could bring us a similar percentage of viable seeds. Generally, overcoming the usual 5% of viable seeds in the sample would be highly surprising (in terms of the interspecific hybrids that we work with).

However, the results of 2008 were slightly different in terms of viable seed percentage. A common trait of both seasons may be a significantly different performance of different hybrid combinations. It seems that seed orchards No. 1 and No. 3 brought different results each year, but this can be only an assumption. Seed orchard No. 3 hosted a successful hybrid combination CZ1 X NC73 (16% viable seeds!) in 2008. The cause for that result is rather unknown, and investigation of this incompatibility is beyond the scope of the project.

In the year 2008, we excluded *A. koreana* x (*Abies cilicica* x *Abies cephalonica*) in the hybridization in favor of the more promising F₁ *Abies cilicica* x *Abies cephalonica*. Also one new taxon was included—*Abies balsamea*. This idea was based on its close relationship to *Abies fraseri* so it can work as a kind of substitute when running out of *A. fraseri* pollen.

As a transport of most seeds from this year's harvest to the U.S. was organized, their sowing in our facilities was not planned. At this point, Phytophthora resistance screenings performed at NCSU are strongly preferred by both sides, for they will provide the most important results and a needed feedback to us. After completion of these tests, it will be much easier to pick the most promising hybrid combinations for our future work.

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High-throughput DNA sequencing of Fraser fir (*Abies fraseri*)

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Objectives

The objectives of this work are to identify genes expressed during normal growth and development of both the above-ground and below-ground portions of Fraser fir plants (*Abies fraseri* [Pursh.] Poir.), and to survey existing populations of Fraser fir plants for genetic variation in expressed genes.

Materials and methods

Plant materials

The first phase of this experiment used two unrelated Fraser fir seedlings (~15 cm tall). One seedling was infected with a suspension of *Phytophthora cinnamomi* Rands 4 days prior to sample collection, while the other seedling was maintained as a non-infected control. Newly expanded shoot tips and foliage were collected from the non-infected seedling, and roots (including both root tips and more mature, fully elongated primary and secondary roots) were collected from both the non-infected and the infected plants. The samples were frozen in liquid nitrogen and stored at -80°C until use.

The second phase of the experiment used foliage collected from trees grafted in a clonal archive. Foliage was collected in May from a total of 60 trees, 10 trees from each of 6 different natural populations of Fraser fir. The foliage samples were held at 4°C until pooled samples containing 3 needles from each of the 10 trees were prepared. These six pooled samples, each representing one natural population, were then frozen in liquid nitrogen and stored at -80°C until use.

Laboratory methods

Total RNA was isolated on RNeasy columns (Qiagen), using a modified lysis buffer for the first extraction step (P. Dharmawardhana, personal communication). Complementary DNA for 454 sequencing was prepared from 1-microgram samples of each of the three total RNAs, using the SMART cDNA Synthesis system (Clontech). The cDNA preparations were normalized using the duplex-specific nuclease method (Zhulidov et al, 2004) to reduce the range of variation in abundance between the most common cDNAs and the least common. Normalized cDNA samples were submitted to the University of Florida Interdisciplinary Center for Biotechnology Research service facility for DNA sequence determination using the GS-FLX reagents on a 454 instrument.

Complementary DNA samples for Illumina sequencing were prepared by the method of Mortazavi et al. (2008), using reagents purchased from Illumina. Prepared samples were submitted to the University of California Riverside Institute for Integrative Genome Biology for determination of paired-end 36-bp DNA sequences from seven samples. The first sample was a mixture of foliage and root cDNAs from the same non-infected control individual plant used for 454 sequencing, and the other six cDNA samples were prepared from the pooled foliage samples of the six natural populations of Fraser fir.

Results

DNA sequence yields

The 454 sequencing runs yielded over 1 million sequence reads with an average length of about 213 bases, giving a total yield of 221.1 million base pairs of DNA sequence information.

Assembly of these sequence reads using the Newbler assembly software (Roche) yielded a total of 75,180 contigs containing a total of 24.4 million base pairs of DNA sequences, built from 689,551 reads. Many of these contigs were small, due in part to the relatively short read lengths obtained from the GS-FLX chemistry compared to traditional Sanger sequencing technology. There were 11,814 contigs that were larger than 500 bp, and those large contigs contained a total of about 10.4 million bp of DNA sequences.

The Illumina sequencing runs yielded a total of about 59 million pairs of sequence reads from the 7 samples, or an average of 8.4 million base pairs per sample.

DNA sequence quality

The root samples were each sequenced in two half-plate regions on the 454 instrument, and the first run of each sample yielded higher quality results than the second run, as shown in the distribution of read lengths in the histograms for each of the five half-plate regions run (Figure 1). The solid lines show the distributions of DNA sequence read lengths in base pairs for the first run of each of samples S1 (Phytophthora-infested roots) and S2 (non-infested control roots). Dotted lines show the distributions of DNA sequence read lengths for the second run of S1 and S2. Sample S3 was analyzed with only one half-plate region, so only a single distribution is shown.

DNA sequence comparisons

The 454 DNA sequences were assembled into "contigs," which represent the best current estimate of fir mRNA sequences corresponding to expressed genes from foliage and roots. The fir DNA contigs were compared with the DNA sequences of pine and spruce (two other conifer genera in which DNA sequencing projects have been completed) in terms of the numbers of proteins in the Plant Reference Sequence (Ref-Seq) collection for which putative homologues have been identified and the fraction of the protein coding sequence covered by the conifer DNA sequence (Table 1).

The process of assembling the Illumina DNA sequences from the reference individual together with the 454 DNA sequences of the same plant is still underway. When that joint assembly is complete, we will identify candidate SNPs in the DNA sequences of trees from natural populations, using the

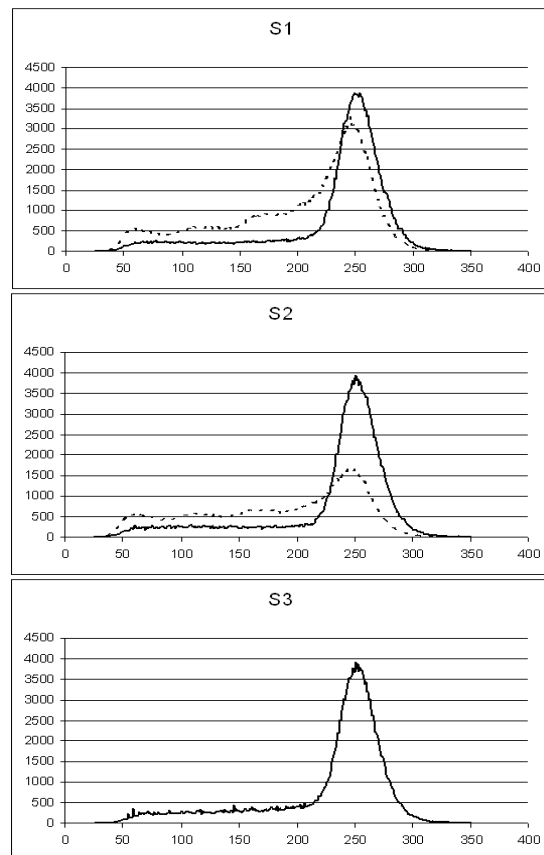


Figure 1. Distribution of read lengths for five half-plate regions. Solid lines represent first run, and dotted lines represent second run.

reference individual sequence as a guide. The candidate SNPs will then be screened in independent samples of the population to confirm polymorphism and estimate minor allele frequency.

Table 1. Plant RefSeq proteins and fraction of coding sequence.

	<i>Abies fraseri</i> (total)	<i>Abies fraseri</i> (≥500 bp)	<i>Picea glauca</i> Unigenes	<i>Picea sitchensis</i> Unigenes	<i>Pinus taeda</i> Unigenes
# Proteins	12,853	7,063	8,408	8,454	10,130
	Fraction of protein				
Median	0.316	0.592	0.450	0.558	0.500
Mean	0.413	0.583	0.493	0.582	0.529
Quartile 1	0.145	0.330	0.266	0.319	0.316
Quartile 3	0.672	0.849	0.710	0.885	0.734

Conclusions

A combination of new sequencing technologies from 454 Life Sciences and Illumina provided a very cost-effective approach to discovering genes and surveying genetic diversity in trees from six natural populations of Fraser fir. The availability of DNA sequences for Fraser fir genes allows design of oligonucleotide probes for microarray analysis of gene expression, and design of SNP genotyping assays to allow efficient high-throughput genetic analysis of both breeding populations and the threatened natural populations of Fraser fir. We are interested in using SNP markers to evaluate paternity of open-pollinated seeds of Fraser fir as a means of reducing the expense of controlled crossing, while maintaining the advantages of known pedigrees for tree improvement.

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PROPAGATION

Effect of media pH and 2-(N-morpholino) ethanesulfonic acid in Douglas-fir (*Pseudotsuga menziesii*) micropropagation systems

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Introduction

The Christmas tree industry plays an important role in Pennsylvania agriculture as well as in the nation. The goal of this micropropagation project is to develop a true-to-type clonal propagation system to alleviate the cost of tree-to-tree variation that occurs with conventional propagation. Understanding plant materials and their growing conditions may provide better assistance for later developmental stages in tissue culture.

The pH level of plant tissue culture media has been shown to be very important to many aspects of plantlet development and growth *in vitro*. Media pH level may influence nutrient uptake, micropropagation rate, rooting, and cellular growth. Media pH also can act to facilitate or inhibit ion transport through membrane ion channels. For instance, free iron is the form taken up by plants and is used for production of chlorophyll and in enzymatic reactions. With increased media pH above 6.5, iron becomes insoluble. The resulting unavailability of iron affects later plant development and leads to chlorosis. As media pH falls below 5.0, many other nutrients, such as calcium, phosphorus, and magnesium, become limited for plant uptake. Sensitivity or tolerance to changes in media pH *in vitro* varies according to specific requirements of individual species.

Plantlets grown *in vitro* may secrete secondary metabolites into the media, thereby altering media pH level. Other media components can also alter media pH, such as hydrolysis of carbohydrates and chelators with organic compounds.

The objectives of this study are to (1) determine media pH change over time under storage conditions and with the presence of plantlets, (2) evaluate the effects of media pH change on plantlet growth performance, and (3) assess the effects of adding a pH stabilizer, 2-(N-morpholino) ethanesulfonic acid (MES), to Douglas-fir micropropagation media.

Materials and methods

Spring buds from juvenile and mature donor trees were collected yearly prior to breaking dormancy. Juvenile genotypes classified as not yet producing cones were collected from donor trees at the Penn State horticulture farm at Rocksprings, PA. These trees were of Lincoln National Forest seed source and were planted 10 years earlier. Buds of mature genotypes were collected from donor trees more than 40 years old at the Penn State golf course. These elite donor trees were from a seed orchard established in a previous genetics study (Gerhold, 1984).

Explant preparation and sterilization procedures were followed as per Traore (2005). Two types of media were used, including modified DCR (Gupta and Durzan, 1985) alone and modified DCR with 2 g/L of MES. Levels of media pH were pre-adjusted to 3.6, 5.1, 5.7, 6.3, and 7.8 before adding agar, MES, and autoclaving. Control media at each media pH level without the presence of plantlets were placed in full dark and light conditions in 25°C growth chambers. Plantlets were dissected and placed into the above two types of media for incubation at 25°C under 8 hours of dark followed by 16 hours of light daily. Media pH change and plantlet growth parameters were recorded at 1, 3, 5, 7, 14, 21, 28, and 35 days of incubation time.

Results

- In general, DCR media with MES provided a more stable media pH after autoclaving, compared to pre-adjusted pH values, regardless of whether plantlets were absent or present in the media.
- All media showed a pH change after autoclaving, but DCR media with MES showed less pH fluctuation.
- Media pH fluctuated less under dark media storage conditions than under light storage conditions.
- For DCR media, pH showed a gradual decrease followed by a sharp increase. However, pH in the DCR+MES media exhibited a slower decrease followed by a convergent media pH change for all pre-adjusted pH levels.
- Plantlet weight gain over time showed an inverse relationship to media pH change, but also differed between juvenile and mature donor tree genotypes.
- After growing in media for 35 days without being subcultured, plantlets showed various growth deformities, such as chlorosis, delayed needle expansion, browning needle tips, browning bottoms of plantlets and surrounding media, vitrification, and even death.

Conclusions

- MES can be utilized as a media pH stabilizer for Douglas-fir micropropagation.
- The data suggest a 21-day subculture practice may be suitable for maintaining media freshness, media pH level, and desirable plantlet growth.
- Fluctuation of media pH can be influenced by many factors. Photo-oxidation and photolysis on light-sensitive media components may contribute to the fluctuation of media pH.
- The effect of MES and nutrient acquisition by plantlets may require further investigations on nutrient dynamics for both media and plantlets *in vitro*.
- Mature donor trees may provide bud explants better able to tolerate or adapt to pH than juvenile genotypes; Explants from mature donor tree showed continuous growth in a wider range of media pH levels.
- Explant weight gain differed between DCR and DCR+MES media.

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Douglas-fir (*Pseudotsuga menziesii*) micropropagation: Shoot multiplication of juvenile and mature genotypes

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Introduction

Pennsylvania has a very strong Christmas tree industry. According to the 2007 census of agriculture in Pennsylvania, a total of 1,599 Christmas tree farms and 34,789 acres of land were in Christmas tree production. In 2007, Christmas tree farms yielded 1,179,733 trees in Pennsylvania. These statistics ranked Pennsylvania as the fourth state in the nation for Christmas tree production. Many conifer species have been utilized for Christmas tree production and have continuously generated valuable revenues for Christmas tree growers (Gerhold, 1984). Douglas-fir (*Pseudotsuga menziesii*) has been one of the most popular Christmas tree selections.

To date, Christmas tree improvement has depended mostly on traditional breeding programs and on the creation of seed orchards by growers themselves. One of the problems that Christmas tree growers may encounter is tree-to-tree variation. Clonal propagation may provide more uniform seedlings with preselection for superior growth form, as well as insect and disease resistance. Improved uniformity would reduce the costs and losses associated with tree-to-tree variation.

Conventional micropropagation methods include five steps: culture establishment, shoot multiplication, rooting, acclimation, and field tests. Each step will influence subsequent steps. Successful shoot multiplication will generate a sufficient quantity of plantlets for use in further tests of rooting and acclimation. Our objective is to develop an efficient protocol to produce high yield and quality shoots *in vitro*.

Materials and methods

Spring buds from juvenile and mature donor trees were collected yearly prior to breaking dormancy. Juvenile genotypes classified as not yet producing cones were collected from donor trees at the Penn State horticulture farm at Rocksprings, PA. These trees were of Lincoln National Forest seed source and were planted 10 years earlier. Buds of mature genotypes were collected from donor trees more than 40 years old at the Penn State golf course. These elite donor trees were from a seed orchard established in a previous tree improvement study (Gerhold, 1984).

Explant preparation and sterilization procedures were followed as per Traore (2005). Sterilized buds were dissected and placed into a wide range of 6-Benzylaminopurine (BA) concentrations at four induction times. The growth chamber conditions were set at 25°C with 16 hours of light followed by 8 hours of dark daily. After the induction period, plantlets were transferred into plant growth regulator (PGR)-free modified DCR media (Gupta and Durzan, 1985) for incubation, followed by a 3-week subculture regime. Data were collected after 7 weeks. Survival, number of new buds produced per plantlet, and percentage of bud multiplication were evaluated. Data analyses were performed using Minitab and Statview statistical software.

Results

From the data collected to date, excised buds from juvenile and mature genotypes exhibit different responses upon receiving BA treatments *in vitro*.

- Survival rate decreased at increased BA concentrations. The best survival rates were observed with a 3-week induction time for mature genotypes and a 2-week induction time for juvenile genotypes.
- BA induction times and BA concentrations acted together to accomplish effective multiplication. For both juvenile and mature genotypes, BA concentrations for optimal multiplication ranged from 3.2 to 51.2 mg/L.
- Mature genotypes showed a trend of increasing multiplication rates with prolonged induction times. However, the 1-week induction time had an inverse correlation between multiplication percentage and BA concentration. The best multiplication percentage (31.25% at BA 51.2 mg/L) exhibited at the 3-week induction time for juvenile genotypes was a significantly higher percentage than for BA concentrations of 25.6 (13.48%), 3.2 (12.37%), and 204.8 (8.33%).
- The average number of new buds produced per explant also improved with increased BA concentrations and BA induction times.
- The effect of position of bud collection from the donor trees on multiplication percentage showed a significant difference only at 4-week induction time for juvenile genotypes. Plantlets originating from buds collected from the middle of the tree showed high multiplication percentage at low and middle BA concentrations at the 4-week induction time. Buds collected from the middle and bottom heights on the donor trees gave better multiplication than those from the tree tops.
- In addition, organogenesis occurred on explants from mature genotypes when higher concentrations of BA were applied. Juvenile genotypes did not show signs of organogenesis at any BA concentrations.

Conclusions

- Our data suggest that explants from mature genotypes are able to tolerate a wide range of BA concentrations. Not only was their survival rate following BA treatments much better than that of juvenile genotypes, but their multiplication percentage and average number of new buds produced per explants were also higher.
- A 2- to 4-week induction time with BA concentrations from 6.4 to 51.2 mg/L should be optimal for mature genotypes.
- Testing more cytokinins using buds collected from different positions on the donor trees is needed for a better understanding of multiplication and growth responses from juvenile Douglas-fir trees.
- These findings may reflect the innate abilities and limitations of Douglas-fir to adjust its growth responses to external stimuli.
- Internal auxin concentration differences in buds collected from different tree positions may play a role in regulating responses of explants to BA treatment.

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Is there a way to the perfect Christmas tree?

Clonal production of Nordmann fir by somatic embryogenesis is still problematic!

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Nordmann fir (*Abies nordmanniana*) is the most important species for the production of Christmas trees in Europe. However, obtaining imported seeds from its natural range has become increasingly problematic. Domestic seed-production plantations can provide valuable high-quality seeds. Also, valuable selections can be clonally propagated using rooted cuttings or grafting. Further, many organizations are attempting to develop somatic embryogenesis technology to produce various fir species for Christmas tree cultivation.

Imported Nordmann fir seeds from Georgia, Abkhazia, and Russia are increasingly becoming both less reliable and more expensive. In order to counter license monopolies of quality planting stock, additional seed-production plantations and clonal archives established as genetic reserves are needed. In these reserves, pollen contamination from European silver fir (*Abies alba*) must be eliminated. Particularly for Nordmann fir, the production of suitable seeds and seedlings for Christmas tree cultivation requires field testing to examine provenances, races, and/or plus trees.

Seedlings produced via somatic embryogenesis were incorporated into such field trials for the first time in 2002, but these trials have not produced the improvements expected based on the selected provenances and individual mother trees. When testing zygotic seedlings of Nordmann fir, large differences in yields, ranging from € 24,000/ha to € 70,000/ha (2007 prices), have been documented. In Germany, the most valuable provenances of Nordmann fir for Christmas tree production are North Caucasus (381.01, 382.05), Big Caucasus (163.96-B, 167.96, 216.97), and Little Caucasus (173.96 and so on) from lower elevation classes (750–1,300 m). Provenances from Turkish regions are mostly unsuitable for cultivation as Christmas trees in Germany.

Here the question arises, to what extent can somatic embryogenesis improve the yields of proven sources? The technology is still quite complex, has yet to produce valuable propagules, and still must be automated to become economically feasible.

Grafting and cuttings

Classical vegetative propagation can be used to multiply races and individual select trees and produce faithful and uniform copies of parent material. While grafting presents no real problems, rooted cutting propagation is only successful when cuttings originate from juvenile stock plants.

One cause for rooted cutting problems is the hormone status and varying enzyme pools in the stock plants that remain once the cuttings are removed. (We need a high pool of auxin oxidase in the branches of the stock plants.) An undesirable hormone/enzyme pool causes rooted cuttings and sometimes grafts to exhibit a horizontal (plagiotropic) growth habit over several years. While blue spruce, Korean fir, and corkbark fir rarely display plagiotropism, it can take Nordmann fir up to 3, and occasionally 4, years to convert to vertical (orthotropic) growth. At this point, the rooted cuttings can have less growth than even a 1-year-old seedling.

Somatic embryogenesis

Somatic embryogenesis uses somatic cells with the addition of certain hormones to produce multiple somatic embryos. These are cultured under specific conditions to produce young seedlings to plant into soil. Somatic embryogenesis is still quite a complex process to use as the initial stage for mass propagation of Christmas trees.

The procedure is divided into several stages:

- Induction of somatic embryos
- Multiplication of embryogenic cultures
- Maturation of the embryos
- Germination of mature embryos
- Acclimatization of the embryos for transfer first into the greenhouse and then into soil

Research at the Center for Horticulture Westphalia, together with Humboldt University in Berlin, developed an appropriate somatic embryogenesis process for Nordmann fir. The process was successful at multiplying select trees of proven provenances from somatic cells. Embryogenic cultures could be conserved for an unlimited period of time by storage in liquid nitrogen at -196°C .

The clones produced are presently being tested in a field trial using Christmas tree cultural practices. After 6 years in the field, somatic seedlings will require at least 2 more years to reach the current size of seedlings from similar origins. So, to improve the growth of somatic seedlings relative to similar zygotic seedlings, investigation and optimization of the following continues: (1) the relationship of hormones used during the propagation and maturation processes, (2) bud development, and (3) rooting and branch development.

Continued optimization of somatic embryogenesis procedures

Although all the steps for the production of somatic seedlings have been developed, numerous critical steps in the process require improvement. Particularly with fir species, germination, root:shoot ratios of germinants, and the development of quality terminal buds remain problematic. Further, the time-consuming manual procedures need to be automated in order to increase the efficiency of the process.

Important work procedures to be considered in the future include:

- Understanding and manipulating the *synchronous* multiplication and aging of the somatic embryos in order to prevent different developmental stages
- Optimization of cell polarization and accelerated root formation by improving hormone as well as light (intensity and quality) prescriptions
- Assuring sufficient storage of reserve materials in the embryos
- Improvement of culture shelf life by optimizing the embryo drying process
- Development of economical methods to cold-store embryos in a clone bank
- Shortening of the time period of each cultural step, above all, the phases of terminal bud development, germinant rooting, and branch development after transfer into soil
- Automation, particularly of conversion and transferring germinants from the sterile environment into soil in order to clearly reduce the cost of each plant

Currently, the somatic embryogenesis process is still much too complex and too expensive for the production of Christmas tree planting stock (Figure 1). So far, seedlings from somatic embryogenesis are at least three times more expensive than equivalent 3-year-old zygotic seedlings. This is a high price, since a genetic gain of 15 percent at most is expected from

cloning within the examined provenances, and that improvement will be realized only if somatic seedlings exhibit similar growth to equivalent zygotic seedlings.

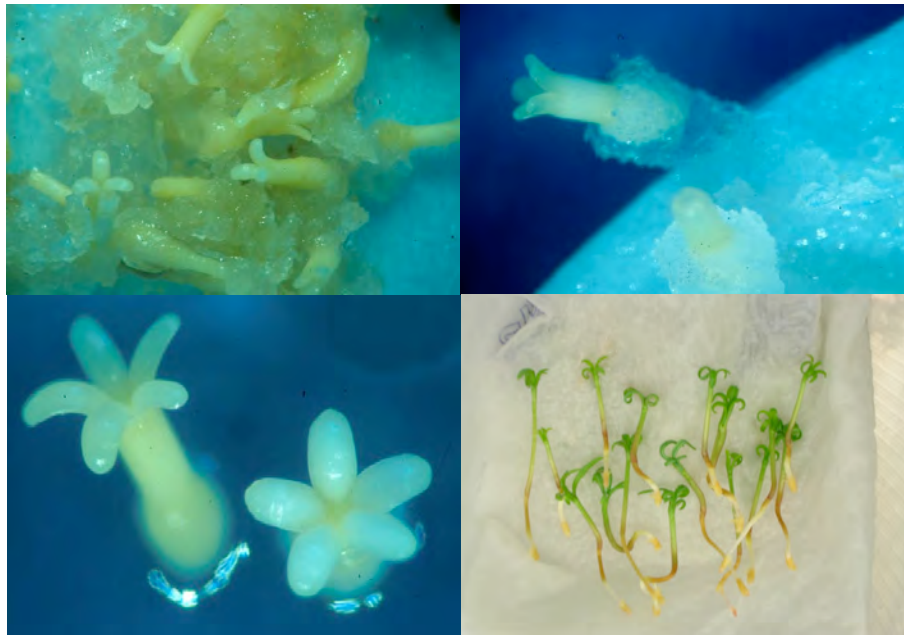


Figure 1. Clonal propagation of fir through somatic embryogenesis is a complex process requiring additional research and development before being useful to commercially propagate Christmas trees. Currently, the process often results in somatic seedlings with poor root and terminal bud development (lower right photo).

A clear improvement in the area of clonal plant production would be the use of non-fertilized female-sex cells (megagametophytic tissue) of proven select trees as the starting material. While somatic embryos possess genes from both the maternal and paternal parents, the megagametophyte has only genes from the mother plant. If the megagametophyte of proven mother trees could be used as starting material for somatic embryogenesis, the possibility exists of preferentially propagating select trees of any age.

In this case, the chromosome set of non-fertilized cells (haploid) is simple, and consequently would require doubling. In this way, pure-bred seedlings could be produced, and the portion of valuable Christmas trees in plantations would clearly increase. Unfortunately, these last steps have not yet succeeded.

Either the steps of the somatic embryogenesis process should be simplified, accelerated, and yields increased, or the current complex procedure should orient itself toward one or more of the following:

- Propagating select trees from non-fertilized megaspores
- Zygotic cell material from selected descendants of control crosses of plus trees after previous performance evaluations
- Proven hybrids with desirable characteristics from interspecific crossings (Figure 2)



Figure 2. Cloning allows the propagation of interspecific hybrids with desirable characteristics that cannot be reliably achieved through seed. Six-year-old hybrids of *A. koreana* var. *viridis* x *A. lasiocarpa* var. *arizonica* ('RD RIV') (left) and *A. koreana* 'BLU MAGIC' (ri.) x *A. lasiocarpa* var. *arizonica* ('RD RIV') (right). Photo taken 5 December 2009.

The need to test descendants of select trees from proven provenances before inclusion in an operational cloning program adds more difficulty to the process. Mass propagation through somatic embryogenesis can be justified only for clones with clearly advantageous characteristics relative to conventional seedlings. So far, no somatic clone meeting this criterion has been found. Potentially, such clones may offer extra profit by combining desirable characteristics (which is difficult to accomplish with conventional breeding), such as ideal height-to-width growth, improved needle retention, late budbreak, frost hardiness, pest resistance, drought and flood tolerance, and herbicide tolerance.

Conclusion

- Efforts were made to produce a large number of Nordmann fir through somatic embryogenesis.
- Research has been exploring the use of somatic embryos of Nordmann fir from plus trees from different stands of the Caucasian region.
- Various stages of zygotic embryos and megagametophytes were isolated for initiation of embryonic cultures. Different growth media were tested to develop a protocol for initiating somatic embryogenic lines from both immature and mature zygotic embryos.
- Genotypes of interest can be preserved during field testing in liquid nitrogen for an unlimited time period.
- Research results indicate that after 6 years in the field, somatic seedling growth is at least 2 years behind that of comparable zygotic seedlings.

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Nordmann fir seed orchard genetics

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Introduction

Nordmann fir (*Abies nordmanniana*) is the main Christmas tree species in Denmark and throughout Europe. In Denmark, provenance research was initiated in the early 1960s, and genetic research was later intensified by breeding activities and establishment of seed orchards in the 1990s and following years. The first full-rotation field testing results for Christmas tree quality, growth, and post-harvest quality of selected plus trees is now available. The majority of grafted seed orchards produced their first commercial seed crop in the autumn of 2009. To improve the genetic quality of the orchards, the poorest performing mother trees have been culled based on open-pollinated breeding values for Christmas tree and post-harvest quality.

Seed orchards link breeding to the Christmas tree industry by making the selected superior material available as seed. Seed orchards are presently the only way of multiplying genetic material on a commercial scale. Therefore, recent research has focused on the efficiency of seed orchards, and especially on some well-known potential dysfunctions of orchards.

The objective of this presentation is to summarize the state of our seed-orchard research by use of references to published studies and preliminary information from ongoing research.

Methods

The study of seed orchard dysfunctions has basically been based on two methods. Simplest and cheapest have been efforts to do ocular assessments of clone stroboli production by counting the number of female and male strobolies using a logarithmic scoring (Sirikul et al., 1991). Recently, simple sequence repeat (SSR) markers were developed for Nordmann fir (Hansen et al., 2005), and these markers have been an efficient tool to provide information on the actual parentage of seed orchard crops. Furthermore, SSR markers have been developed in a number of other *Abies* species, and some have successfully been transferred to Nordmann fir (Hansen and McKinney, in press).

Results and discussion

The use of seed orchards to multiply selected superior material from breeding activities and to link these activities to the forest or, in this case, the Christmas tree industry, is a well-known concept. However, some potential dysfunctions can undermine the expected gains, including the following.

Pollen contamination from surrounding forests

Based on SSR marker studies (Hansen and Kjær, 2006; Hansen and Nielsen, submitted; Hansen and McKinney, in press), there is only a minor influence of background pollen (3–5%) in the Danish Nordmann fir clonal seed orchards that have been studied. These orchards are isolated at least 500 meters from other Nordmann fir stands or stands of European silver fir (*Abies alba*).

Uneven clone contributions

Clonal differences in paternal reproductive success in a Nordmann fir clonal seed orchard were documented by marker studies (Hansen and Kjær, 2006), as well as by a time series of ocular evaluations (Nielsen and Hansen, submitted). A specific evaluation of parentage based on both

ocular scoring of the pollen catkins and SSR markers of the seed crop in the same year gave an interestingly good correlation (Hansen and Nielsen, submitted). The correlation was significantly improved by including differences in actual numbers of ramets of each clone. This indicates that the pollination system is fairly additive; numbers of ramets and amounts of pollen do count.

Inbreeding depression by selfing

Based on the SSR markers, there seems to be very little selfing in the tested orchards—6% in a seed crop and 1–2% in two other studies measured under field conditions (Hansen and Kjær, 2006; Hansen and Nielsen, submitted; Hansen and McKinney, in press). By use of controlled crossings, the quantitative effect of selfing on seed set and seedling growth in a nursery was evaluated. An inbreeding depression of 40% in filled seeds and 15–17% in growth traits was seen. However, a fairly large number of selfed seedlings will pass normal nursery culling procedures (Nielsen and Hansen, submitted).

Hybridization with European silver fir

From previous provenance testing of Danish sources (Nielsen and Chastagner, 2005) and practical experiences, it is well known that Nordmann fir readily hybridizes with European silver fir (*Abies alba*). The unwanted hybrid product includes fairly fast-growing and early-flushing individuals, with lower post-harvest needle retention quality. In a controlled pollination study using pollen mixtures comprising both Nordmann fir and European silver fir, it was clear that the silver fir pollen pollinated the Nordmann fir mother trees very efficiently (Hansen and Nielsen, 2008). Thus, there is no doubt that these two species readily cross.

Technical errors

Technical errors, such as grafting mistakes, labeling, and overtaken rootstocks, can be checked very efficiently by the use of SSR markers. In a recent study, 119 genotypes were found in a clonal seed orchard initially comprising only 100 clones, meaning that overgrown rootstock was a serious problem in this orchard (Hansen and McKinney, in press).

Timing and year-to-year variation

Results from ocular evaluations of clone stroboli production indicate strong year-to-year variations and also, to some extent, clone x year interactions. Although these differences are pronounced, the first evaluations seem to indicate rather good stability in the final aggregated seed orchard product for Christmas trees—despite skewed clonal contributions. Furthermore, results of clone differences in timing of pollen shedding and cone receptivity, at least in one year, indicate fairly good coordination. The study will be continued by analysis of SSR markers and filled seed counts.

Conclusion

Despite a set of potential dysfunctions, Danish Nordmann fir seed orchards are generally functioning well.

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Acknowledgments: The results are based on a cooperative effort by the State Forest Tree Improvement Station (the major Danish seed orchard owner), the Christmas tree industry, their common initiatives and financial support to the research and testing program at Forest and Landscape, Denmark.

Cutting production in Nordmann fir: Rooting, plagiotropism, and hormonal background

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The main challenges in conifer cutting propagation are rooting of the cuttings, aging of the mother plant, and plagiotropism in the offspring. Since most shoots on a Nordmann fir are inherently plagiotropic (i.e., bilaterally symmetric, with horizontal needle and bud orientation), and rooting usually is poor and declines with the age of the mother plant, vegetative propagation of this species is not currently considered a practical option. However, new research into the seasonal endogenous hormone profile enables us to identify times expected to be most favorable for rooting. The ambition is to develop a propagation protocol, primarily for breeding and experimental purposes.

Objectives

- To test rooting capacity in twigs taken in summer, when cytokinin concentration is lowest and the cytokinin:auxin ratio thus is expected to be favorable
- To examine rooting capacity and plagiotropism in relation to ortet age and to different shoot types, natural or generated after stumping

Methods and materials

Seedling trees 6 and 14 years old were stumped in April, as in Rosier et al., 2005. Natural and regrowth shoots were taken as cuttings from the young trees in August, 4 months after stumping (cohort 1), and from the older trees in July, 15 months after stumping (cohort 2). Each cutting was characterized according to origin (regrowth or natural shoot), position, size measures, and plagiotropism (Figure 1). Cutting time in July coincided with previously recorded low cytokinin-high auxin conditions in the Nordmann fir branches (Figures 2 and 3).

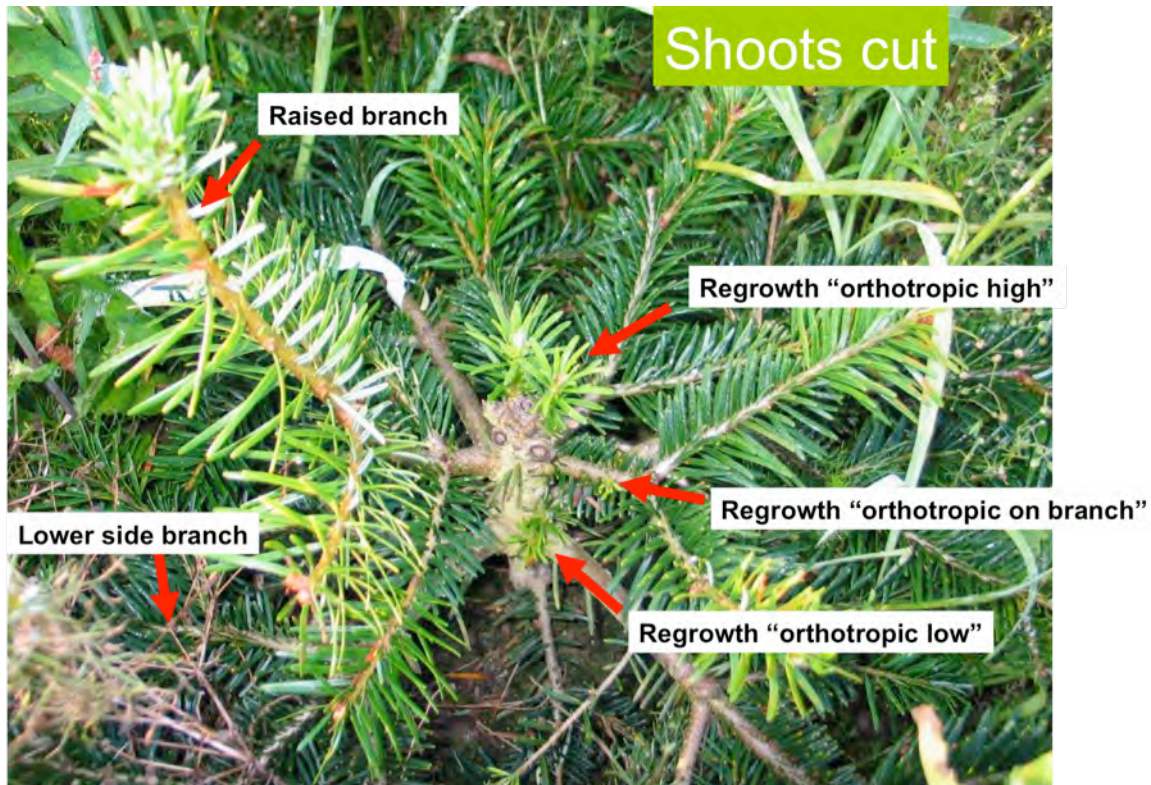


Figure 1. Shoots types, generated by stumping, and used for cuttings.

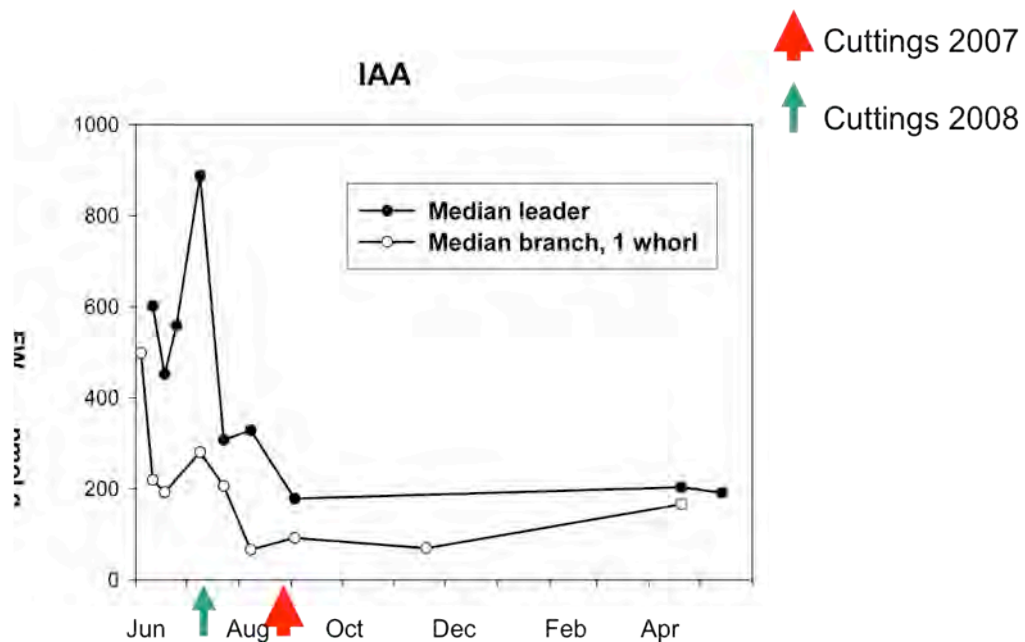


Figure 2. Auxin levels throughout the year in median part of leader, in 6-year-old intact Nordmann fir trees, and in median part of first whorl branch. Values of five pooled trees. Data from Rasmussen et al., in press.

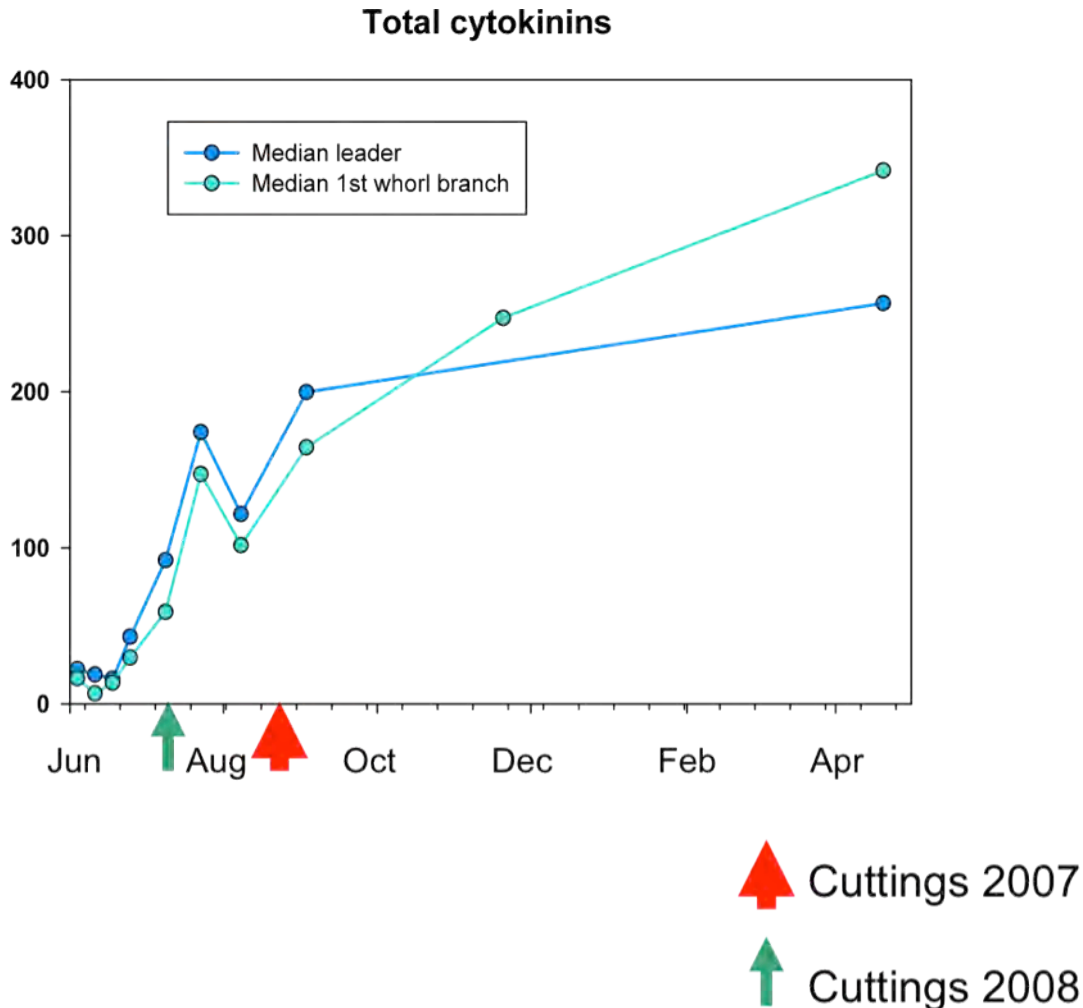


Figure 3. Cytokinin levels, as in Figure 2. Data from Rasmussen et al., 2009.

Rooting took place under semiclean greenhouse conditions. Temperature was 20°C, with supplementary light 40 W/m², 20-hour day length. There was no additional light and heating from February. Rooting was recorded during winter and spring; subsequent growth performance was recorded in August after one growing season outdoors in pots (cohort 1 only).

Results and discussion

Many of the shoots sprouting on the stem and upper side of branches after stumping were orthotropic at the outset (Figure 1). The young trees reacted faster to stumping than the old ones, yielding cutting material the first summer. Rooting occurred slowly (6–9 months after setting) and differed significantly among shoot types, with the orthotropic low rooting almost as well as the low side branches (70–80%, Figure 4). Shoots from the lower position generally rooted better than those from higher positions. Rooting in cuttings from the old trees was similar to that of the young. Application of auxin (5 mMol) to the cut end was counterproductive (Figure 4).

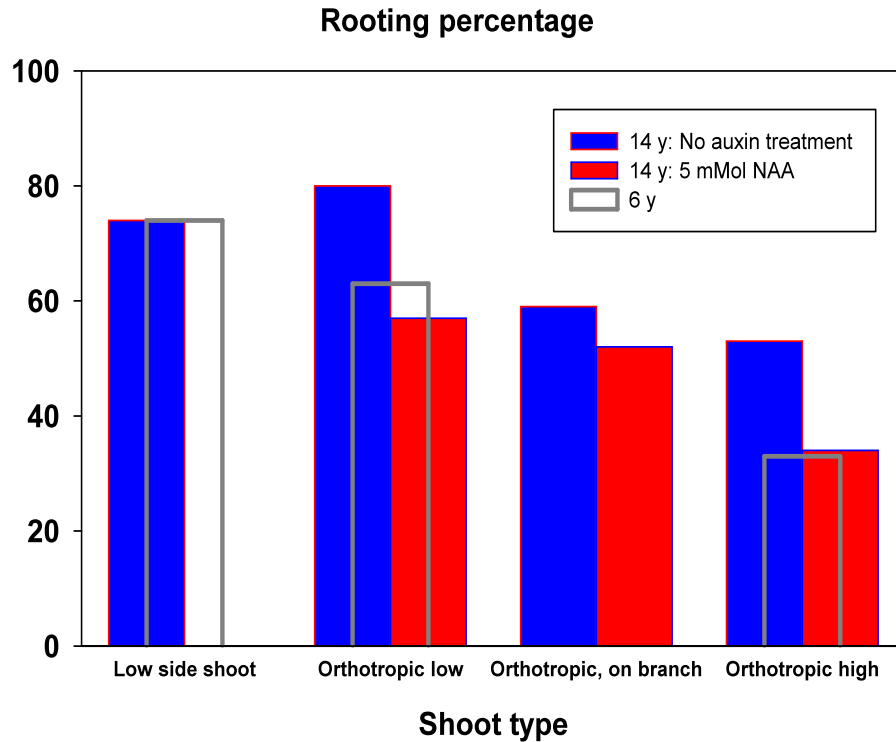


Figure 4. Rooting percentage in cuttings from 14-year-old trees, various shoot types with and without NAA pretreatment. Corresponding results in 6-year-old trees.

Cuttings from the young trees (cohort 1) were evaluated with respect to their subsequent growth pattern by a subjective scale ranging from fully orthotropic (A) to fully plagiotropic (J). Selected examples are shown in Figure 5. The scale was subsequently reduced to five color categories, as shown Figure 6.



Figure 5. Rooted cuttings with new growth showing a gradient from orthotropic (left) to plagiotropic (right) development.

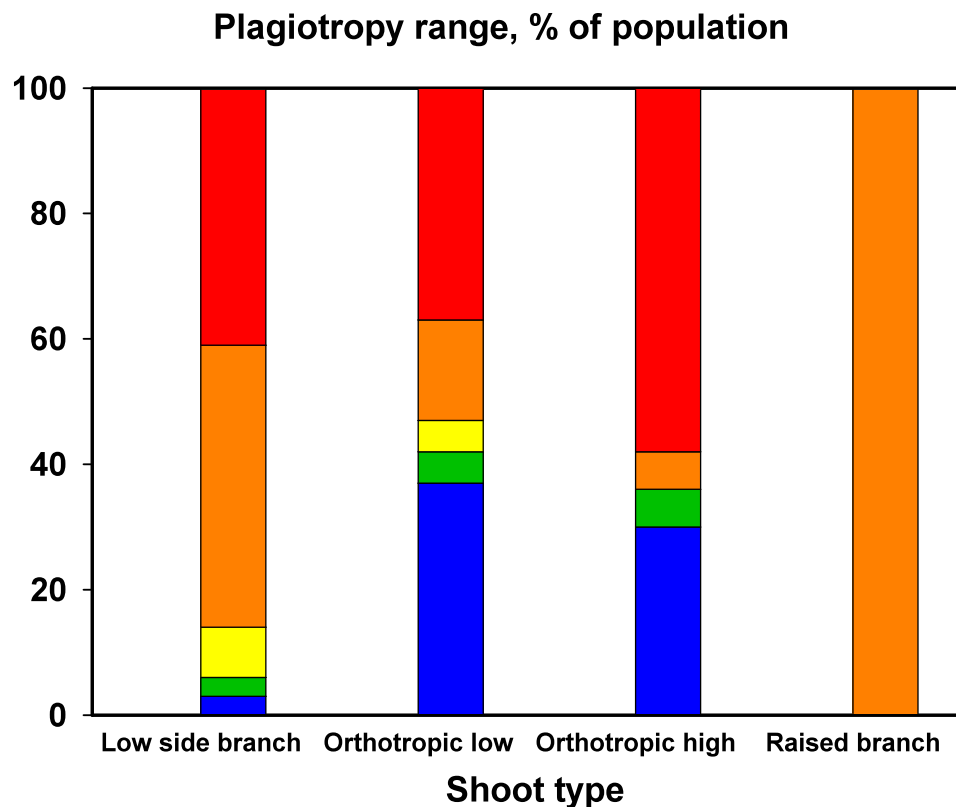


Figure 6. Growth trait distribution in rooted cuttings according to shoot type on mother tree. Blue = highly orthotropic; red = highly plagiotropic. Data from Nielsen et al. 2009.

Plantlets originating from low side branch cuttings were 96% plagiotropic, while the orthotropic low cuttings gave about 42% fully to almost fully orthotropic plantlets. Compared with orthotropic low shoots, orthotropic high shoots did not perform as well (Figure 6). Raised branch tips, in spite of upright growth on the ortet, all tended toward plagiotropy as cuttings (Figure 6). The number was small, however, since rooting was poor.

Orthotropic high and low shoots tended toward relatively low cytokinin:auxin ratio content (at cutting time) compared to plagiotropic shoots. Within cutting shoot types, those that became orthotropic after rooting appeared to have been lower in cytokinin:auxin ratio at cutting.

Preliminary conclusions

- Summer cuttings of both young (5-year) and older (14-year) trees rooted quite adequately, up to 70–80%.
- Orthotropic regrowth shoots rooted as well as regular (plagiotropic) side branches, and many of them maintained orthotropism after rooting.
- August cuttings in the first year rooted about as well (percentage and speed) as July cuttings the second. An effect of seasonal low cytokinin-high auxin conditions in the mother trees could thus not be substantiated.
- Auxin pretreatment had no positive effects.
- Shoots regenerating from lower positions on the stem performed better.

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GROWTH CONDITIONS

Evaluating Nordmann fir (*A. nordmanniana*) for Pennsylvania conditions

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Pennsylvania Christmas tree growers rely heavily upon relatively few species, with Fraser fir (*Abies fraseri*) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) comprising more than 70% of production acreage. Production costs for these species will continue to rise as pest problems intensify.

Over 95% of the Douglas-fir grown in the state is derived from seed originating in southeast New Mexico's Lincoln National Forest. Lincoln Douglas-fir is very susceptible to Rhabdochline needle cast and Swiss needle cast (Chastagner, 2001), and each year growers apply three or four fungicide sprays to control these diseases (Bates, 2005). In addition, the prevalence of Phytophthora root rot represents a major constraint to the production of Fraser fir in the eastern United States (Benson, et al., 2006). The need exists to improve the pest tolerance of these commonly used species, but there is also mounting pressure to evaluate and introduce new, potentially profitable species. Consumers of cut Christmas trees are also keen to try something

new. In recent years, there has been considerable interest in growing exotic *Abies* species among both Christmas tree growers and the landscape industry (Kelley and Bates, 2007).

No one knows when, or if, a new *Abies* species will approach the prominence of Fraser fir or Douglas-fir in the eastern U.S. Christmas tree market. However, Nordmann fir (*A. nordmanniana*) has received more attention than other exotic *Abies* species and is being widely planted across the northeast region of the U.S. While Nordmann fir holds promise due to its excellent Christmas tree characteristics and wide adaptability, testing in the eastern United States has been somewhat limited, and provenance evaluation in particular has been lacking. Knowledge of how Nordmann fir varies across differing environments in its native range can provide the basis for the selection of seed sources that are better adapted to conditions in Pennsylvania and the Mid-Atlantic states. Indeed, widespread planting of seedlings derived from untested sources is a particularly risky endeavor.

Initially, most Nordmann fir grown in the eastern U.S. came from the Ambrolauri forests of the Republic of Georgia. In our experience, trees from this seed source tend to be somewhat slow to establish and exhibit slow early-cycle growth rates. Additionally, Ambrolauri Nordmann grown on certain sites in Pennsylvania have exhibited extensive foliage bronzing, which may be an indicator of marginal hardiness. The May 2009 frost event in the eastern U.S. also revealed the susceptibility of Nordmann fir to late-spring frost damage. These experiences have caused some growers to discount Nordmann fir as a promising addition to their product mix. However, many of these early setbacks could be related to the use of seed sources that are not optimally matched to our local growing environment. This scenario has led to renewed interest in evaluating other Nordmann fir seed sources for Pennsylvania and the northeast U.S.

In 2006 we began collecting Nordmann fir stock derived from a wide range of forests in both the Greater and Minor Caucasus regions of the Republic of Georgia and the north slopes of the Greater Caucasus in Russia. Numerous evaluation sites have been established with cooperating growers in Pennsylvania. Recently, a joint project was established with Ilia Chavchavadze State University and the Tbilisi-based seed company Goni, Ltd. to collect and evaluate new seed sources that may hold potential for the northeast U.S. Plans are also underway to establish a tree evaluation site within the Republic of Georgia.

(For more information on this partnership with Georgia colleagues, visit <http://www.acdivocacoopex.org/acdivoca/PortalHub.nsf/ID/GeorgiaFtFchristmastree>).

In the fall of 2008, seed was collected in eight provenances in Georgia (Table 1, Figure 2). Cones from at least 10 trees were collected within each provenance, and several elevation ranges were represented in each provenance. Seeds were processed and delivered to University Park, PA in March 2009. Seedlings are currently being greenhouse-grown and will be used for future studies to evaluate: (1) winter hardiness and budbreak characteristics, (2) soil adaptability and tolerance to *Phytophthora* root rot disease, and (3) needle retention characteristics. Prior to 2008, collections were also made in the Arkyz and Apsheronk forests on the northern slopes of the Greater Caucasus Mountains in Russia.

Table 1. Location and average elevation of 2008 cone collection sites for Nordmann fir provenances.

2008 collections: Provenance name	Geo. coordinates N	Geo. coordinates E	Average altitude (meters)
TLUGHI (Shkhivana)	42°26'58"	43°11'53"	1,288
TLUGHI (Tskadisi)	42°27'28"	43°09'26"	1,230
TLUGHI (Jobenauri)	42°26'35"	43°07'01"	1,220
NIKORTSMINDA	42°28'14"	43°02'48"	150
DZMUISI	42°25'26"	42°58'21"	1,325
BAKHMARO	41°50'53"	42°20'54"	2,020
TADZRISI	41°43'20"	43°17'12"	1,650
AKHALDABA	41°54'53"	43°27'53"	970



Figure 1. Approximate locations of selected Nordmann fir provenances within the Republic of Georgia.

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Growth and physiology of living Christmas trees in container production systems

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In order to grow quality living Christmas trees, growers need to understand and manage container substrates, irrigation, and nutrition and fertilization. For the past 4 years, we have conducted a series of trials to examine the growth and physiological responses of four conifer species. Fraser fir (*Abies fraseri*), Colorado blue spruce (*Picea pungens*), Black hills spruce (*Picea glauca* var. *densata*), and eastern white pine (*Pinus strobus*) were grown in a Pot-in-Pot (PiP) nursery system in either 3-gallon (11.2 L) or 7-gallon (26.5 L) containers.

Container substrates made up of 80% pine bark and 20% peat moss resulted in optimal or near-optimal growth for all species. Growth for all species peaked at approximately 0.5 g of nitrogen per liter of container, although gas exchange responses suggest that growth response to fertilization may be confounded by increased leaf area and moisture stress. Preliminary results from the first year of a 2-year study indicate that cyclic irrigation (four cycles per day) can improve growth of living Christmas tree species compared to trees receiving the same daily irrigation amount applied as a standard, single irrigation cycle each day.

It is important that producers accustomed to field Christmas tree production recognize that growing trees in containers is very different than field production. Before pursuing a major investment in container production, growers need to consider site selection (especially drainage for PiP), container substrate selection, and irrigation and nutrition management. Successful management of these factors can produce high-quality living Christmas trees.

Background

Container production systems offer growers a means to produce lighter weight living Christmas trees that are easier for consumers to handle than standard balled and burlap trees. Growing Christmas trees in containers follows the general trend found in the landscape nursery market in the United States. According to a 2007 U.S. Department of Agriculture survey, container production accounted for 53% of total landscape conifer nursery production in 2006, up from 46% just 3 years earlier. At the same time, balled and burlap production dropped from 49% to 42% of the market.

In order to produce quality container-grown living Christmas trees, growers must manage three essential components of the container growing system: container substrate, fertilization, and irrigation. In this paper, we summarize the results of two trials we have conducted to improve production systems for container-grown living Christmas trees in the midwestern United States.

Experiment 1. Effect of container substrate and fertilization on growth and physiology of container-grown living Christmas trees

This experiment was conducted at Pot-in-Pot Research Nursery at the Michigan State University (MSU) Horticulture Teaching and Research Center, East Lansing, MI. In May 2006, 90 seedlings (2+2 or plug+2) each of Fraser fir (*Abies fraseri*), Black hills spruce (*Picea glauca* var. *densata*), Colorado blue spruce (*P. pungens* var. *glauca*), and eastern white pine (*Pinus strobus*) were potted in 11.2-L (#3) containers. Seedlings were potted in one of three substrate mixes selected to provide a range of physical properties. Substrate consisted of composted pine bark (B) and Canadian peat moss (PM) in ratios (vB:vPM) of either 70%:30%, 80%:20%, or 90%:10%. Thirty trees of each species were potted in each substrate mix.

Controlled-release fertilizer (Osmocote® Plus 15-9-12, 8–9 month Northern release rate; The Scotts Co., Marysville, OH) was top-dressed in the spring of 2006 and 2007. Each tree received one of three rates: low (0.25 g N L^{-1}), medium (0.5 g N L^{-1}), or high (1 g N L^{-1}). The experimental design was a split-plot in a randomized complete block design, with species as the main-plot effect and factorial combinations of fertilizer \times substrate combinations as the subplot. There were 10 blocks, each consisting of 4 rows, one for each species. Each row contained nine trees, one for each of the fertilizer \times substrate combinations. Trees were irrigated twice daily.

We measured height and caliper of all trees at the beginning and end of the 2006 and 2007 growing seasons. We measured photosynthetic gas exchange on *A. fraseri*, *P. glauca* var. *densata*, and *P. pungens glauca* with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) in July and August 2006 and May, June, July, and September 2007. On each date, measurements were taken between 9:00 a.m. and 5:00 p.m. A 0.25-L conifer chamber attachment (LI-6400-05, Li-Cor) was used to enclose a single shoot of the current season's growth on each tree. Light-saturated photosynthesis (A_{max}) and shoot conductance to water vapor (g_{wv}) were measured on shoots exposed to full sunlight, on days with photosynthetic photon flux density (PPFD) greater than $1,200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Shoots were tagged so subsequent measurements were taken on the same shoot throughout each year. We collected the tagged shoots at the end of each growing season and scanned them with a leaf area meter (LI-3000, Li-Cor) to determine projected shoot area. A portable chlorophyll fluorescence meter (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England) was used to measure the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) for individual needles from each tree. The needles were dark-acclimated for a minimum of 15 minutes before readings were taken. Dates of measurement of F_v/F_m coincided with measurements of A_{max} . We collected

approximately 5 shoots (for single-needle conifers) or 20 fascicles (for pines) from each tree on 15 August 2006 and on 12 October 2007 for foliar nutrient analyses.

Experiment 2. Effect of cyclic irrigation on growth and physiology of container-grown living Christmas trees

This study was initiated in May 2008 at the MSU Pot-in-Pot Research Nursery. Plant materials and overall experimental set-up were similar as for experiment 1. All trees were planted in a container substrate consisting of 80% pine bark and 20% peat moss. All trees were top-dressed 60 g per container controlled-release fertilizer (15-9-12; 8–0 month Northern release, Scotts, Inc.)

Trees were irrigated using pressure-compensating drip emitters or spray stakes. Trees received one of three levels of irrigation (1 cm, 2 cm, or 3 cm per day). The 1-cm and 2-cm rates were applied via drip emitters; the 3-cm rate was applied using spray stakes. Each centimeter of irrigation equaled approximately 0.5 liter per 3-gallon container. Irrigation was applied either once per day or the total amount was divided by four and applied as four cycles through the day. Tree growth, gas exchange, foliar nutrition, and variable chlorophyll fluorescence were measured as in experiment 1.

Results

Experiment 1

Fertilizer rate and species significantly affected ($P < 0.0001$) stem caliper growth in both years. Species differed in caliper growth response to fertilization, as indicated by significant interactions of species and fertilizer.

In the first season after transplanting (2006), stem caliper increased with increasing fertilizer for all species. At the highest fertilizer level, growth of *Picea glauca* plants increased at a greater rate than that of the other species in 2006.

In the second season after transplanting, caliper growth response to fertilization for all species began to plateau after 0.5 g N L^{-1} . Caliper growth of *Abies fraseri* and *Picea pungens* increased slightly from 0.5 to 1.0 g N L^{-1} , but the difference was not significant ($P > 0.05$) for either species. Addition of 0.5 g N L^{-1} resulted in a much larger increase in growth of *Pinus strobus* trees than trees of the other species, resulting in the significant species \times fertilizer interaction for caliper growth in 2007.

In contrast to caliper growth, fertilization affected height growth ($P < 0.0001$) only in 2007 (Figure 1).

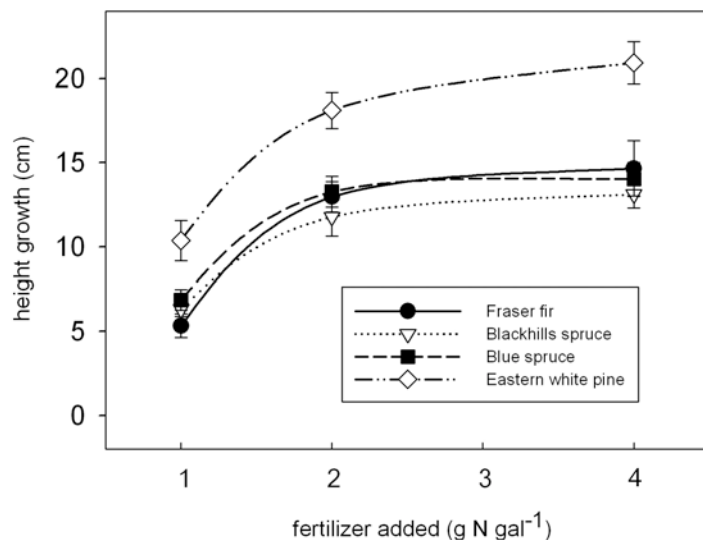


Figure 1. Height growth response of conifers grown in 12 L containers at three levels of fertilizer addition.

The effect of container substrate on height and caliper growth was smaller than the effect of fertilization. Increasing the proportion of peat moss in the container substrate increased caliper growth in 2006, whereas in 2007 caliper growth was greatest at the intermediate container mix. Height growth did not respond to substrate in 2006. In 2007, height growth increased slightly with an increased proportion of peat moss in the mix, and height growth of trees in the 70:30 mix (PB%:PM%) was greater ($P < 0.05$) than that of trees in the 90:10 mix. Across species, there were no differences ($P > 0.05$) in either growth variable between trees grown in the 80:20 or 70:30 mix.

Nitrogen concentrations of foliar samples varied ($p < 0.0001$) by species and year (Figure 2). *Picea* species had greater N concentrations compared to *A. fraseri* and *Pinus strobus* over both years. In 2007, *Picea pungens* had higher foliar N levels than *P. glauca* var. *densata*. Fertilization increased nitrogen concentration of needles for all species; however, differences between the 0.25 and 0.5 g N L⁻¹ additions were more pronounced in 2006 than in 2007 (Figure 2).

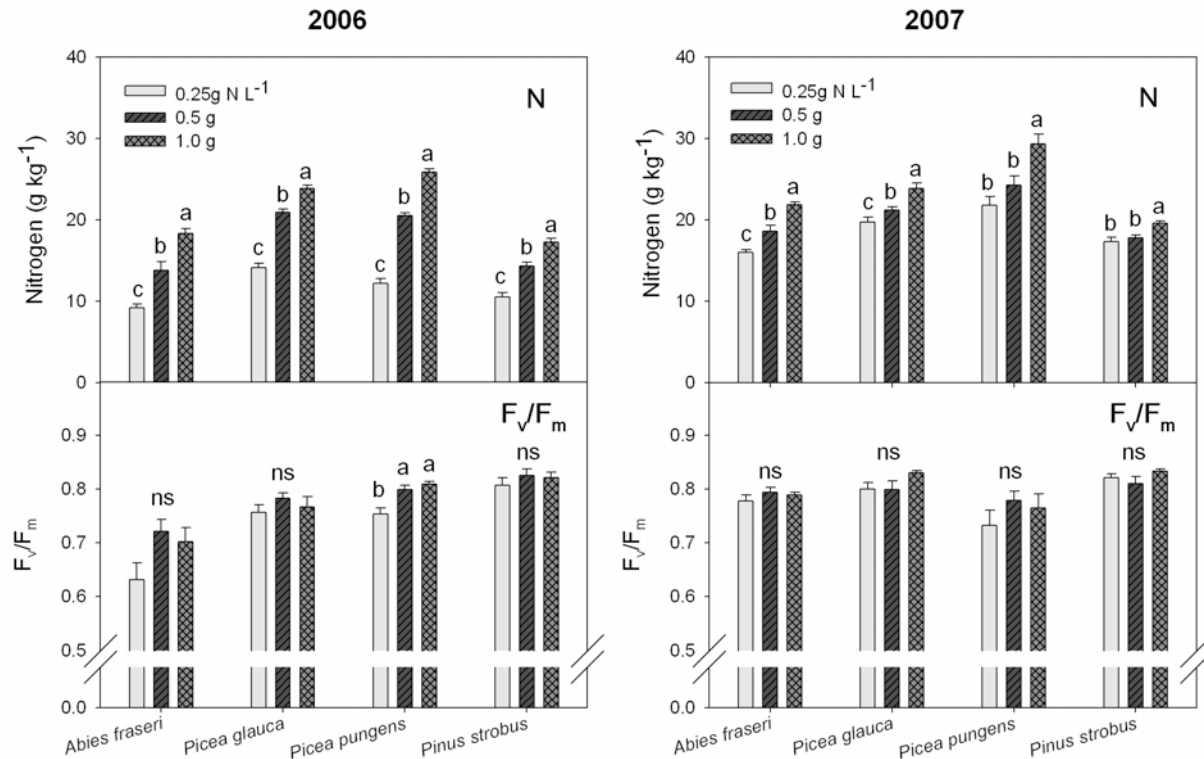


Figure 2. Foliar nitrogen concentration (top) and variable chlorophyll fluorescence of conifers grown in 12 L containers at three levels of fertilizer addition. Assignment of differing letters to the top of bars indicates a significant difference, $p < 0.0001$. NS = not significant.

Year and species affected ($p < 0.01$) chlorophyll fluorescence (F_v/F_m). Within species and years, fertilization did not affect chlorophyll fluorescence (Figure 2). Correlation analysis of F_v/F_m with foliar nutrient concentrations indicated that F_v/F_m was not consistently correlated with foliar nutrition (data not shown).

Experiment 2

Irrigation frequency had a greater effect on caliper growth of trees than irrigation rate (Figure 3). Trees receiving four cyclic pulses of irrigation per day grew more than those receiving the same amount of irrigation delivered only once per day. This trend was especially evident for the eastern white pine and Colorado blue spruce trees, which were also the fastest growing trees.

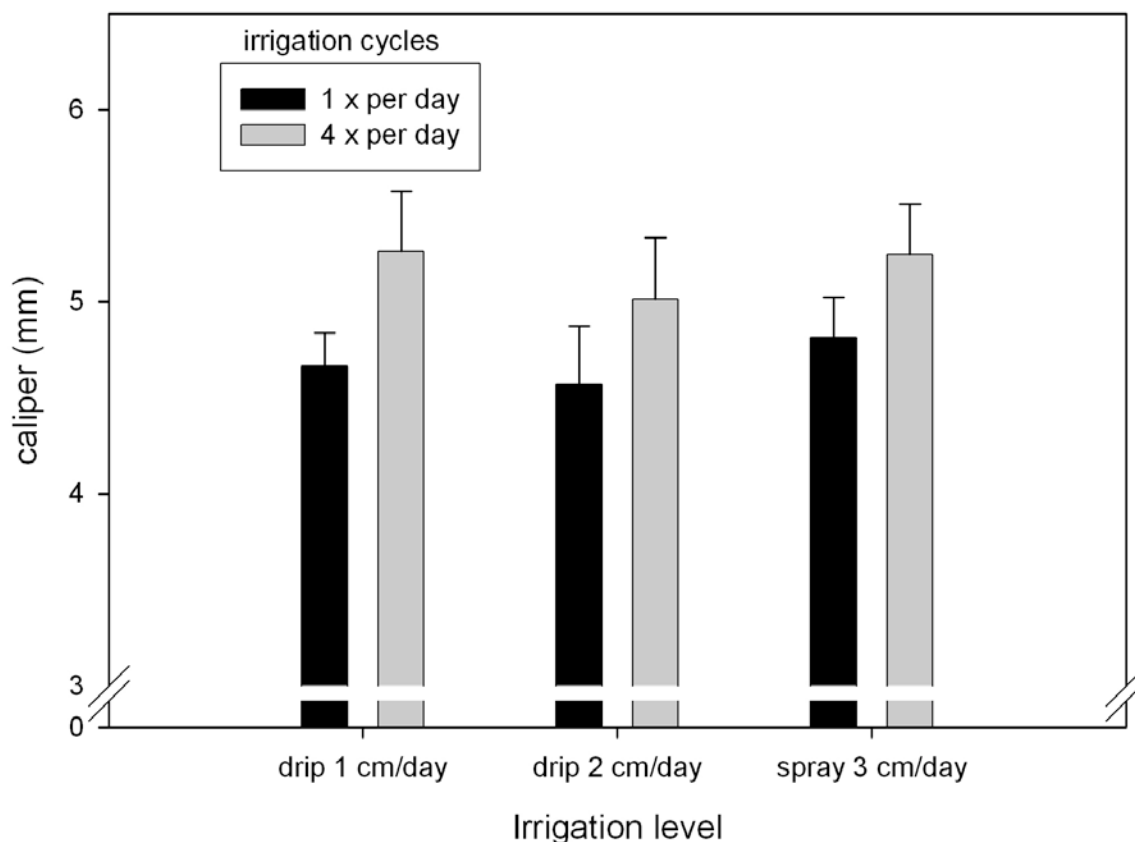


Figure 3. Caliper growth response of conifers grown in 12 L containers irrigated at three levels applied once or four times daily.

Height growth was less affected by irrigation treatments than caliper growth (data not shown). This is likely due to the fact that height growth is completed relatively early in the growing season when weather conditions are relatively mild and irrigation effects are less pronounced.

Summary

Our trials have demonstrated that *Pinus strobus*, *Picea pungens*, *Picea glauca* var. *densata*, and *Abies fraseri* can be successfully and reliably produced as container-grown living Christmas trees. Container substrates containing 80% pine bark and 20% peat moss produced maximal or near-maximal growth for all four species. Fertilizing with controlled-release fertilizer at a rate of at least 0.5 g of N per liter of container (~3 g per gallon) produced maximum growth of all species. Cyclic irrigation (four cycles per day) increased tree growth relative to trees that were given the same amount of daily irrigation as a single application.

Establishment routines for *Abies nordmanniana* and *Abies lasiocarpa*

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Introduction

The production of Christmas trees is demanding, and crucial factors are the plant material used and the establishment routines. Optimal planting dates and establishment routines will contribute to the development of a vigorous domestic Christmas tree industry.

Establishment routines that ensure a rapid growth start are important. Christmas tree seedlings typically grow slowly during the first 2 to 3 years. A dense concentration of branches on the lower part of the tree tends to allocate a lot of resources to the tree base, which grows wide and heavy. Such trees are costly to cut and transport and difficult to sell. In Norway, 9 to 10 years are usually needed from planting to harvesting Christmas trees. Decreasing the time until harvest by 1 or 2 years will increase growers' income.

Objectives

The goal of the subproject presented here is to obtain knowledge on how planting time and site conditions affect establishment in *Abies* plantations. Field experiments have been established on three locations, representing both *A. lasiocarpa* and *A. nordmanniana* in the eastern inland and in western coastal climates (Figure 1).



Figure 1. The experimental plot with *Abies nordmanniana* in Lier (eastern Norway: 59°40' N; 10°46' E).

Materials and methods

Planting was performed six times with a 3- to 4-week interval from late April to late August in 2007 and 2008. Plantings were performed with winter-stored seedlings in weeks 17, 21, and 25. Then summer plantings were completed with seedlings brought directly from nurseries in weeks 29, 32, and 35. Measurements of height and root collar diameter, as well as registration on lammas shoots, were performed following planting and following growth cessation in autumn.

In addition to field experiments, additional seedlings were planted in a controlled environment each planting week, in order to explore the seedlings' root growth capacity (RGC) on the different planting dates (Figure 2).



Figure 2. New root growth on *Abies lasiocarpa* following 3 weeks in a controlled environment.

Results and discussion

Preliminary results were promising for both early-spring planting (Figure 3, left) and planting of actively growing seedlings in July. When planting in August, there seems to be a higher risk of damage in the terminal bud, resulting in multiple tops (Figure 3, right).

Both when measuring dry weight and the length of the new roots, RGC seemed to decrease from April to late June, indicating a reduced seedling quality with prolonged chilling. When testing the growing seedlings delivered directly from the nurseries, a high RGC was present in mid-July compared with seedlings tested in early or late August.

There seems to be a clear and positive potential of increased root growth following planting of actively growing seedlings. However, care should be taken regarding late-summer planting, especially on sites where autumn frost occurs regularly.

The final revision on the field experiment will take place in 2010 when the project finalizes. More results will be available at that time.



Figure 3. *Abies nordmanniana* planted in late April (left) and early August (right).

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Factors affecting graft success and early growth of Fraser fir

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Two studies were conducted to investigate factors influencing graft success and subsequent growth of Fraser fir (*Abies fraseri* [Pursh] Poir).

In the first study, the traditional time of grafting (April) was compared with eight summer/early-fall grafting dates from mid-July through mid-October. Optimal grafting success (95%) occurred when grafts were made in April while the scions were dormant and the rootstocks were becoming active. Success of subsequent grafting dates decreased from 52% (14 July) to 0% (20 October). Shade improved summer graft success (52% with, 38% without). Irrigation did not affect graft success or growth. Grafting of stored dormant scion material in summer/early fall was not successful (< 1%).

In the second study, success and subsequent growth of Fraser fir cleft grafts were studied in relation to season of grafting (late summer vs. spring), grafter, and origin of scion material (height in the tree and lateral branch order (first vs. second). Grafting in early September yielded only 3% success compared to 70% for mid-April. Grafters had significantly different graft success (86% for Grafter 1 with 5 years experience vs. 54% for Grafter 2 with 1 year experience). First-order laterals from the upper crown yielded the best graft success and growth (except plagiotropism). First-order laterals were better than second-order laterals for all growth measurements.

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The effect of watering and nitrogen fertilization on growth, nutrient use, and leaching in containerized Fraser fir (*Abies fraseri*)

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We report on a 2-year factorial experiment investigating the effect of irrigation on tissue nutrient concentrations and nutrient leaching in *Abies fraseri*. Four-year-old 2+2 seedlings were transplanted into 10-gallon containers in the greenhouse. Four watering levels (I1= 1,930 ml/wk, I2= 1,287 ml/wk, I3= 610 ml/wk, I4= 315 ml/wk) were combined with three nitrogen fertilization treatments (F1= 6 g, F2=12 g, and F3=18 g). The nitrogen source was an ammonium sulfate formulation containing 30-0-0 and 12 sulfur applied as top-dress at the beginning of the growing season. The weekly irrigation volume for each treatment was divided into daily rations applied 5 days a week (M–F). All water leaching from a subset of specimens in each treatment was collected daily and accumulated into a weekly leaching fraction for each treatment. Growth, morphological, and biomass accumulation were evaluated for each treatment. In addition, the nutrient content in plant tissues and leaching fractions were analyzed using wet chemistry methods. All data were processed using traditional statistical methods.

Irrigation increased the height growth by 12 to 35%, depending on the fertilization treatment ($p=0.0001$). Fertilization increased height growth by 10 to 26% ($p=0.02$). A similar response was observed for Stem Diameter Growth (SDG). Total biomass accumulation increased as a result of positive response of stem and root biomass development, and foliar nitrogen content was positively affected by nitrogen fertilization and negatively affected by irrigation. Total leaching fraction was 8.15 to 23.8% in year 1 and 8.3 to 11.4% in year 2. These leaching fractions contained averages varying from 1.24 to 6.75% of the actual nitrogen applied, depending on the treatment and the year of application. The cumulative Ca, Mg, and K leached were significantly affected by the fertilization and irrigation treatments. These results suggest that nutrient addition is a strong determining factor for early development of this species. The improved growth efficiency in this study can be attributed to a combination of factors, including improved photosynthetic capacity, decreased stomatal limitations, or increased resource allocation to stems.

Introduction

Resource availability and use influence physiological processes, metabolic activities, and plant growth (Sheriff et al., 1995). Understanding key resource dynamics and how plants use them are basic to understanding plant growth, plant competition, and ecosystems functions (Sheriff et al., 1995). Among key resources, water availability and nitrogen use are critical for plant growth. Intensive cultivation of *Abies fraseri* outside its natural range is relatively new, and there is limited information available on the species' physiological response to drought stress. Water is generally considered one of the limiting factors for the numerous physiological and biochemical processes controlling plant growth and productivity. Most of the water absorbed from the soil is used in the photosynthetic process or transpired. The mechanism of response to water shortage involves stomata closure, which restricts CO₂ uptake and subsequently growth (Albaugh et al., 2008). Water availability, uptake, and utilization affect physiological processes closely associated with nutrient availability, solubility, and use. These processes include element concentrations in the soil solution, because of nutrient diffusion and mass flow to the

root surface, and then absorption of the elements by the roots to shoots, and utilization in the photosynthetic process by the foliage (Tanguilig et al., 1987; Albaugh et al., 2008). Nitrogen fertilization and uptake are critical for shoot and root growth. The capacity of plant roots to absorb water and nutrients is affected by water stress conditions (Albaugh et al., 2008). The conventional knowledge is that nutrient uptake by a crop is decreased under reduced water availability due to the decrease in the transpirational rate, which causes a reduction in the nutrient absorption capacity of roots (Tanguilig et al., 1987). Changes in soil water also affect root permeability and cause disturbance to the root metabolism (Gerakis et al., 1975). However, there are conflicting reports in the literature on plant nutrient uptake and utilization under droughty conditions. Several studies report decreased N uptake as discussed above (Tanguilig et al., 1987). Other studies indicate high N levels in plant under stress, attributed either to fast accumulation of proline (Singh et al., 1973) or fast accumulation of free amino acids that are not converted into proteins (Barnett and Naylor, 1966). The current study evaluates the hypotheses of limited nutrient uptake and high nitrogen accumulation in containerized *Abies fraseri* seedlings under various water stress levels created by irrigation treatments in a greenhouse setting.

Objectives

The goal of the study was to evaluate the effect of water stress on nutrient use and leaching in *Abies fraseri*.

Materials and methods

This 2-year greenhouse study was conducted at the Tree Research Center (TRC) at Michigan State University. Three-year-old (plug+2) Fraser fir (*Abies fraseri*) transplants were potted in 3-gallon cylindrical black plastic containers. The potting mix used was the Fafard 52 mix (Conrad Fafard, Inc.), which contains approximately 60% pine bark, along with Canadian sphagnum peat, perlite, vermiculite, dolomitic limestone, and gypsum. Fafard 52 is slightly acidic with a pH of 5.5–6.5.

Nitrogen was supplied with a granular controlled-release formulation (MESA™, Lebanon Turf) containing 30% nitrogen (30-0-0) and 12% sulfur. The fertilizer is a homogenous granule of approximately equal amounts of ammonium sulfate and methylene urea polymers.

The fertilizer (at rates of 6 g, 12 g, and 18 g of actual N per pot) was applied as top-dress in a single yearly application at the beginning of each of the two growing seasons. Water stress was generated by applying irrigation at rates of 386, 257, 122, and 63 ml/pot/day, applied daily 5 days a week (Monday through Friday) between 2 p.m. and 5 p.m. The experiment was a factorial design with four irrigation and three fertilization levels with nine containers per treatment replicated three times.

Height from the soil surface and stem diameter (root collar) were measured for each year of the study. Other morphological attributes evaluated included total terminal leader height, viable leader bud count, and terminal bud cluster. Leader height and bud count were used to calculate the leader bud density (LBD) as the total number of buds divided by the height of the leader.

Foliar tissues were collected for nutrient analyses. All leachates were collected from a subset of containers (six in 2007 and three in 2008), using plastic trays placed under the containers. Water drained naturally into the collection trays during the week and was collected from the tray twice a week. An aliquot of 100 ml was brought back to the laboratory and refrigerated until further nutrient analysis. Each week, leachate pH and EC were measured using a Horiba (model pH/EC 054). The total mass (mg) of each nutrient ion leached per week was calculated from the total volume leached and the aliquot ion concentration for each element.

The nitrogen use efficiency (NUE) was calculated as the ratio of the plant nitrogen content per unit nitrogen applied.

Morphological attributes, biomass, and nutrient content data were analyzed using the general factorial mode combining each main effect (water stress and fertilization rate) and interactions. Analysis of variance (ANOVA) was performed for a 3 x 4 factorial design with three replications on all response variables using the linear model procedure. The GLM procedure for repeated measures ($P \leq 0.05$) was used to analyze leachates' nutrient concentrations.

Results

Growth response

The data for the morphological response variables to irrigation and fertilization treatments are summarized in Table 1. Analysis of the overall effect of irrigation on growth and SDG indicated that height growth increased from 12 to 35%, while SDG increased up to 54%, depending on the fertilization treatment. The effect of fertilization on height and SDG was also positive, with height increases varying from 10 to 26%, while SDG increases varied from 4 to 32%, depending on the irrigation treatment.

Table 1. Morphological response of Fraser fir to irrigation and fertilization.

Treatments	Height Growth (cm)	Diameter Growth (mm)	Biomass accumulation			
			Stem (g)	Roots (g)	Needles (g)	Shoot/Root
I1F1	13.25 ^a	5.05 ^a	27.65	28.31	19.05	1.65
I1F2	16.52 ^b	5.83 ^{ab}	67.38	57.24	49.08	2.03
I1F3	19.19 ^c	6.90 ^b	52.05	33.54	21.11	2.18
I2F1	11.86 ^a	5.78 ^a	20.05	20.21	29.21	2.44
I2F2	14.40 ^b	5.93 ^{ab}	58.05	68.74	33.35	1.33
I2F3	16.11 ^c	6.36 ^{ab}	24.62	37.21	19.65	1.19
I3F1	10.44 ^a	3.76 ^a	19.05	28.47	18.51	1.32
I3F2	11.53 ^a	4.98 ^b	23.28	25.94	14.28	1.45
I3F3	14.58 ^b	5.14 ^b	28.28	28.67	16.38	1.56
I4F1	8.09 ^a	3.60 ^a	13.45	15.07	11.41	1.65
I4F2	9.39 ^a	3.74 ^{ab}	7.22	9.87	8.28	1.57
I4F3	10.77 ^a	4.08 ^b	18.55	14.37	13.51	2.23
Statistical analysis						
Irrigation (I)	***	**	NS	*	NS	
Fertilization (F)	*	NS	*	**	**	
I x F	NS	NS	NS	NS	NS	

Irrigation: I1=386 ml, I2=257 ml, I3=122 ml, I4=63 ml; Fertilization: F1=6 g, F2=12 g, F3=18 g

Height and diameter growth were separated, and pairwise comparison of the growth response within each irrigation treatment was performed by the LSD with Bonferroni correction at $P < 0.05$.

NS, *, **, ***: nonsignificant, or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Biomass accumulation and partitioning

Total biomass generally increased with irrigation treatments from 40 g to 110 g/tree, depending on the treatment. Higher fertilization treatments (12 g/pot and 18 g/pot) generally produced higher biomass accumulation, especially when combined with high irrigation treatments. The partition of the accumulated biomass indicates that needle biomass accumulation did not increase with higher irrigation and fertilization treatments, but roots and stem biomass accumulation were generally positively affected by both factors (Table 1).

Foliar nutrient content

Foliar N concentration was affected by irrigation in 2007 ($p=0.001$) but not in 2008 ($p=0.176$). The N concentration was significantly affected by fertilization in both 2007 ($p=0.010$) and 2008 ($p=0.006$). The total foliar N concentration increased with decreased irrigation treatment. Foliar P was not affected by both irrigation and fertilization treatments, but the N/P ratio was adequate (above 10) in both years (Figure 1).

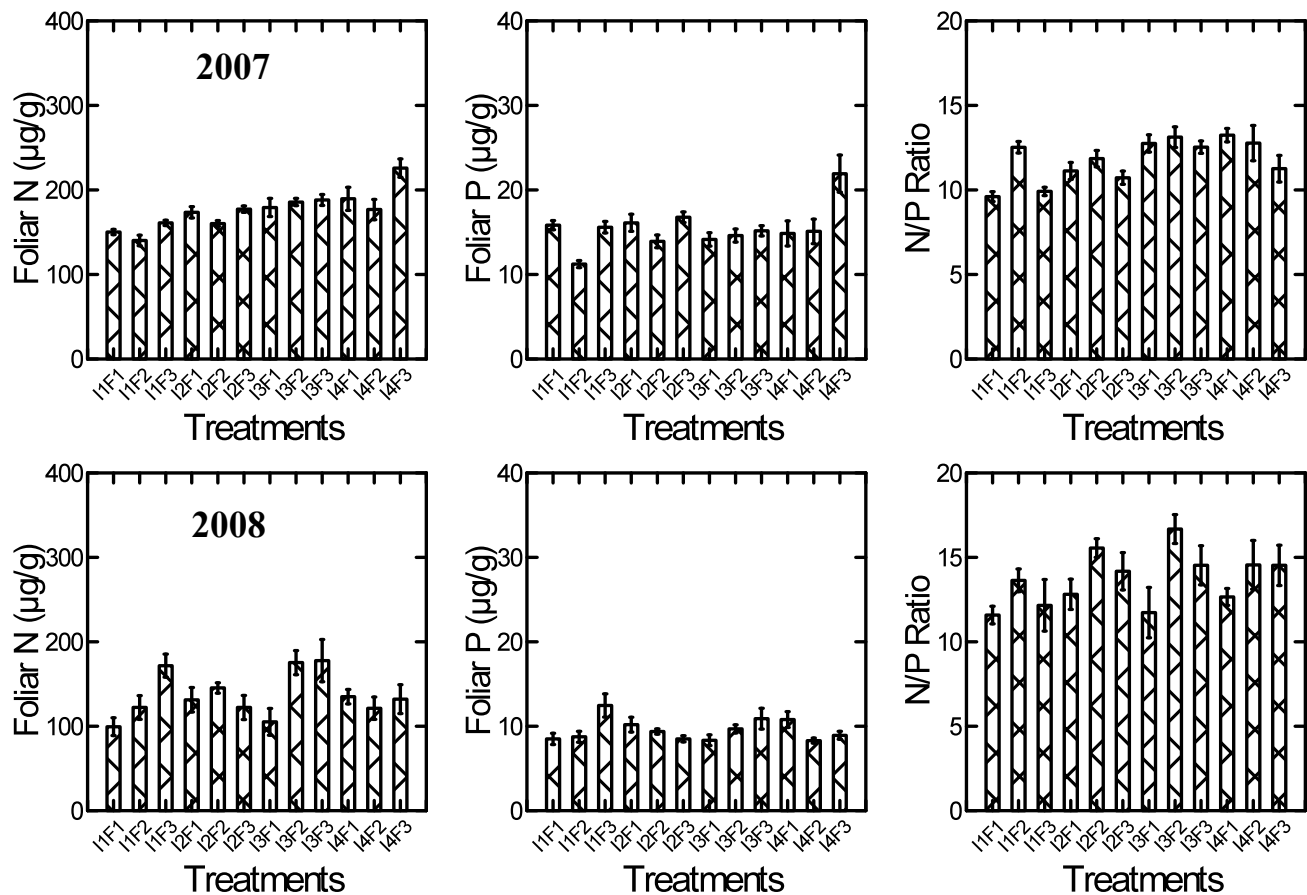


Figure 1. Change in foliar nitrogen concentration as affected by irrigation and fertilization. Foliar N content was affected by irrigation in 2007 ($p=0.001$) but not in 2008 ($p=0.176$). The N content was significantly affected by fertilization in both 2007 ($p=0.010$) and 2008 ($p=0.006$).

Irrigation: I1=386 ml, I2=257 ml, I3=122 ml, I4=63 ml; Fertilization: F1=6 g, F2=12 g, F3=18 g

Foliar Ca concentration was significantly affected by irrigation in 2007 and 2008 (Figure 2). Foliar K was not statistically significant in 2007, but increased with irrigation treatments in 2008. Foliar Mg and Mn were not affected by irrigation and fertilization treatments.

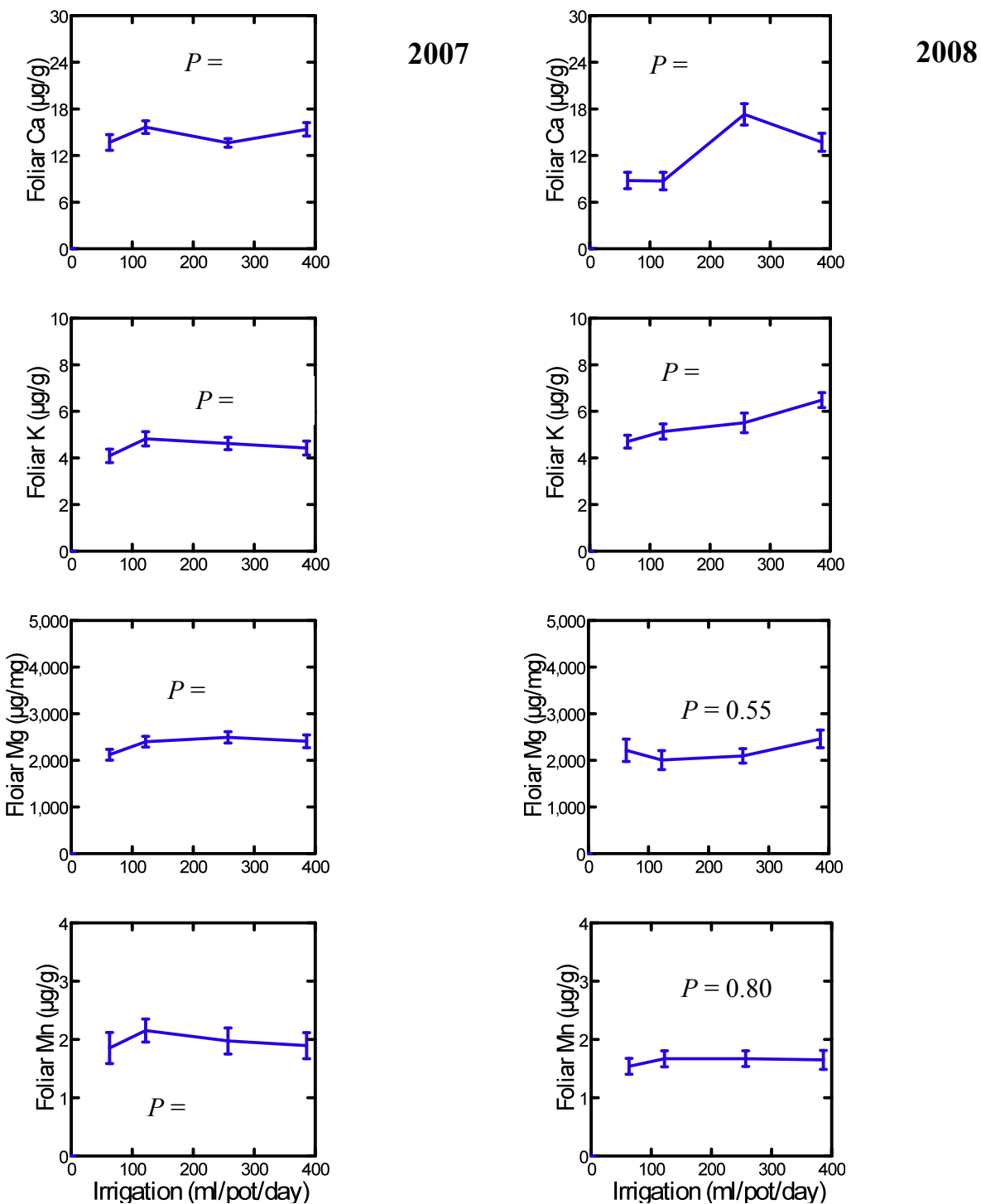


Figure 2. Change in foliar Ca, K, Mg, and Mn as affected by irrigation treatment. Ca and K were the only elements significantly affected by irrigation treatments.

Nutrient leaching

Depending on the treatment, 5 to 20% of the total water applied was collected as leaching fraction. This fraction contained 2 to 5% of the total N applied (Figure 3a). The statistical analysis of the nutrient concentration in leachates collected using the GLM ANOVA procedure for repeated measures was significant for Mg (both 2007 and 2008) and Ca (2007 only).

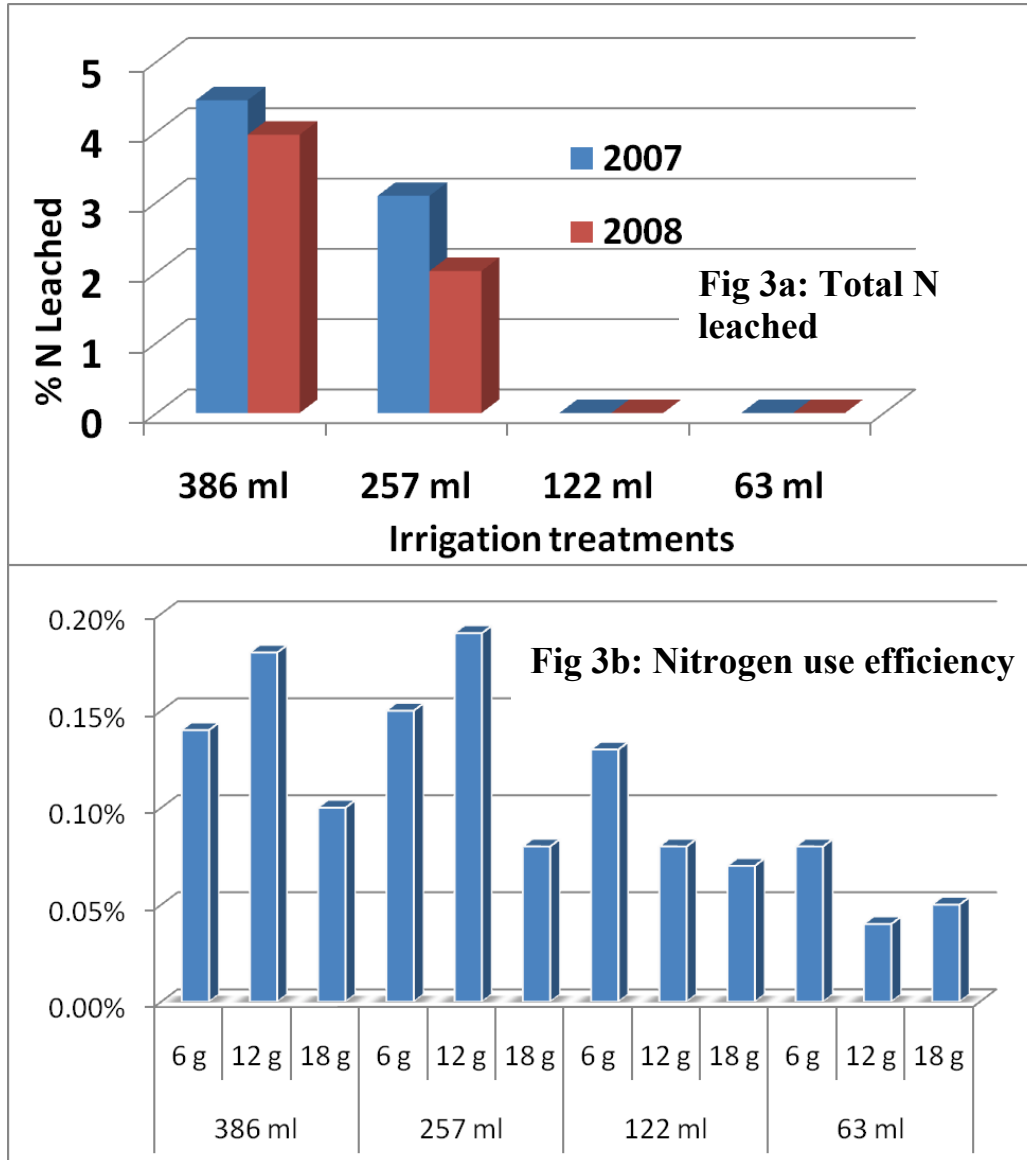


Figure 3. Total N leached (Figure 3a) and nitrogen use efficiency (Figure 3b) as affected by irrigation and fertilization treatments.

Discussion

Our results indicate that both irrigation and fertilization were limiting factors for height growth, but there was no significant interaction between the two factors. Stem diameter growth responded positively to irrigation but not to fertilization. Biomass accumulation also responded positively to irrigation applications. These conclusions are in agreement with previous studies related to growth, volume, and biomass production as related to fertilization and irrigation in various conifer species under field conditions (Snowdon and Benson, 1992; Nilsson and Orlander, 2003; Trichet et al., 2008). Maintaining high soil moisture in highly irrigated plots allows stomata to remain open longer, resulting in more volume growth (Albaugh et al., 2008). This physiological process leads to increased production of carbohydrates due to enhanced photosynthesis and explains the strong height, radial, and biomass growth response observed in this study.

Increases in accumulated biomass were the result of positive changes in stem and root biomass accumulations. We hypothesized that this was the result of a resource partitioning process favoring establishment. Our study also indicated that optimal growth can be obtained using higher irrigation and limited fertilization, underlining the importance of optimal moisture for enhanced uptake. Fertilization is several-fold more expensive than irrigation; therefore, any substantial reduction in fertilizer rate required for achieving optimal growth will result in substantial savings for growers and nursery managers. Increased resource availability also positively affected foliar nitrogen, Ca, and K content, but did not affect P, Mg, and Mn content. The strong foliar nutrient content response to fertilization suggests that nutrient addition is a strong determining factor for early development of this species.

Compared to published standards for containerized conifers (Landis et al., 1989), the foliar N and P concentrations found in this study were generally within range of nutrient sufficiency, despite a wide variation attributed to the efficiency of nutrient uptake and nutrient dynamics in the system. Increased resource availability positively affected foliar N and P. The strong response to fertilization treatments shows that nutrient addition is a determining factor for early development in production of this species. The marginal effect of irrigation and lack of interaction between foliar N and P and irrigation may have been caused by the high rate of leaching observed in high irrigation treatments. The response to the addition of water and nutrients can be variable and depends on seasonal site water balance and initial soil fertility (Trichet et al., 2008). Therefore, increased water addition through irrigation is likely to lead to soil saturation and cause a flow-through of highly mobile nutrients present in the soil solution, such as nitrates, below the root zone.

The lower foliar N concentration in higher water treatments was opposite to the higher morphological response for those treatments. High irrigation generally induced increased growth efficiency (GE) and improved nutrient use efficiency (NUE) (Figure 3b). The GE and NUE were probably caused by one or any combination of factors including improvements in the photosynthetic ability, decrease in stomatal limitations, changes in resource allocation in favor of the stem, or increases in the ratio of photosynthesis to respiration. Further physiological studies are being conducted to confirm these hypotheses.

Conclusion

The growth of container *Abies fraseri* transplant seedlings was increased by the combination of increased moisture and nitrogen fertilization. Increases in accumulated biomass were the result of positive changes in stem and root biomass accumulations. We hypothesized that this was the result of a resource partitioning process favoring establishment.

Increased water applications negatively affected foliar nitrogen, potassium, and calcium content, while phosphorus, magnesium, and manganese foliar concentrations were not affected. However, this inverse relationship is not supported by growth efficiency and nitrogen use efficiency, which was much higher under increased water applications. The foliar nutrient content response to fertilization suggests that nutrient addition is a strong determining factor for early development of this species. We suggest that improved growth and nitrogen use efficiency is caused by one or any combination of factors including improvements in the photosynthetic ability, decrease in stomatal limitations, changes in resource allocation in favor of the stem, or increases in the ratio of photosynthesis to respiration. However, these conclusions should be considered with care since seedlings grown in nutrient-rich environments can easily maintain adequate nutrient concentrations even with dilution from 2 to 3 years of growth. Further physiological studies are being conducted to confirm these hypotheses.

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TREE HEALTH

Variation in resistance to *Phytophthora* root rot within Turkish and Trojan fir

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Annually, North Carolina's Fraser fir (*Abies fraseri* [Pursh] Poir.) Christmas tree industry loses \$US 6–7 million to root rot primarily caused by *Phytophthora cinnamomi* Rands. No useful resistance has been found in Fraser fir, so, in 2003, the North Carolina State University Christmas Tree Genetics Program conducted an extensive resistance screening of 32 *Abies* species from around the world. In that trial, Turkish fir (*A. bornmuelleriana* Mattf.) and closely related Trojan fir (*A. equi-trojani* Coode et Cullen) ranked third and tenth for resistance, but mortality in these species was relatively high. These results, along with research field trial results and Christmas tree growers' experience, all indicated that Turkish fir is not uniformly resistant to *Phytophthora* root rot.

A systematic approach to understand and better use *Phytophthora* resistance within Turkish and Trojan fir was undertaken. Using seeds from a 2005 cone collection expedition to Turkey, greenhouse-grown seedlings from 105 open-pollinated families were inoculated with *P. cinnamomi*. Sixteen weeks after inoculation, overall seedling mortality was 56% for Trojan fir and 35% for Turkish fir. As a comparison, 97% of inoculated Fraser fir seedlings but only 3% of inoculated momi fir (*A. firma* Sieb. et Zucc.) seedlings died. For Turkish and Trojan fir, there was a distinct relationship between mortality and geographic origin; mortality percentage decreased from west to east (Figure 1). The western-most provenance of Kazdagi (Trojan fir) had the greatest mortality (58%), while the eastern-most provenance of Safranbolu (Turkish fir) had the least mortality (23%).

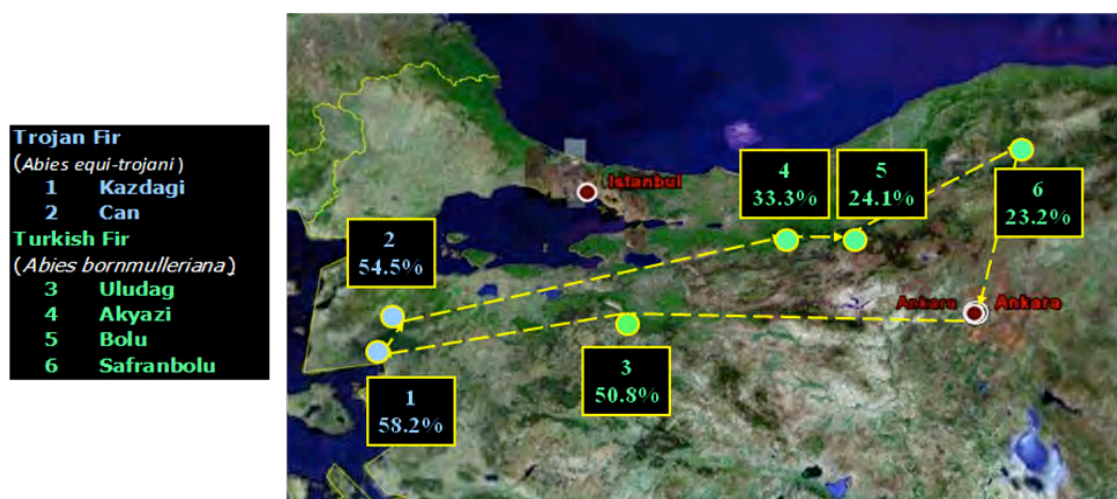


Figure 1. Location in northwestern Turkey of the provenances of Trojan (n=2) and Turkish (n=4) fir sampled. In the *Phytophthora* inoculation trial, the mortality of seedlings (percentages indicated in boxes) varied by geographic origin; mortality decreased from west to east.

Within each provenance, open-pollinated families (i.e., seedlings from the same mother tree) varied considerably. For example, seedling mortality for the Uludag provenance was 50.8%, while its most resistant family had only 20.2% mortality and its most susceptible family had 82.8% mortality. Such variation allows us to select resistant material even from generally susceptible provenances. This is fortunate since Trojan fir is generally faster growing than Turkish fir and, based on observations of young trees in natural stands, probably will produce better quality Christmas trees.

Mortality due to Phytophthora is under a large degree of genetic control. Estimates of family mean heritabilities were 0.88 for Turkish fir and 0.91 for Trojan fir. Thus, roughly 90% of the variation we observed among families for Phytophthora-induced mortality was controlled by genetics.

***Stigmina* on spruce in Michigan**

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Fungal pathogens infecting needles of conifers and causing premature needle casting are common problems in Michigan Christmas tree plantations and tree farms. Pines, Douglas-fir, true firs, and spruce all exhibit needle-casting diseases requiring some level of management. Needle-casting diseases are caused by pathogens that infect the needles at least a year in advance of the needle abscission symptom. Some symptoms indicative of infection can occur in the first year, such as chlorosis or browning of the needle, but abscission occurs 1 or more years after infection.

One of the most common diseases of spruce (*Picea*) in Michigan and other Great Lakes states is Rhizosphaera needle cast, caused by the fungus *Rhizosphaera kalkhoffii*. In Michigan, Colorado blue spruce (*Picea pungens*) is highly susceptible and can sustain severe damage. White spruce (*P. glauca*) and its variant Black Hills spruce (*P. glauca* var. *densata*) are intermediate in resistance, while Norway spruce (*P. abies*) is relatively resistant.

Infected needles produce spores during rain events in spring (May to early June), and these spores are disseminated to emerging needles, starting a new generation of infections. Infection usually begins on needles that stay wet. Therefore, needles on the inner portions of the lowest branches are more severely affected. When needles remain wet for long periods, infection and disease progress outward, leaving the tree with a thin appearance near the base of the tree. Typically, the diagnosis of Rhizosphaera needle cast is based on the presence of black fruiting structures on needles.

The first sign of infection occurs in late fall or in the spring one year after infection. At that time, the spores of the fungus emerge from the fungal fruiting bodies, which have emerged from the stomata of infected needles. These fruiting bodies are easily observed by examining needles with a hand lens (10X). The fruiting bodies harboring ascospores appear as tiny black dots in rows. The second summer after infection, symptoms appear as yellow needles that turn purplish-brown and drop from the tree. A few of these infected needles may persist on the tree

over the winter and drop off the following spring. Because of the long delay between infection in spring and needle drop the following summer, the current-year needles appear green and healthy. Branches seem to lose their needles from the trunk outward. Those branches that repeatedly lose needles for 3 or 4 years may die. Protective fungicides with the active ingredient chlorothalonil have been used to effectively manage this disease.

In 2008, a Christmas tree farm near Lake City, Michigan reported severe needle cast symptoms on blue spruce, even though chlorothalonil had been sprayed in previous years. A needle-casting disease was found in two locations on the plantation in 2007, and growers were concerned about the efficacy of fungicide applications.

Visits to the farm were made in 2008. Mature (3–4 m height) blue spruce trees exhibited needle casting and branch death at both locations on the Christmas tree plantation near Lake City (Figures 1 and 2). Many of the trees were being prepared for landscape transplanting and were rejected based on the death of lower branches. Some trees exhibited severe amounts of branch dieback from approximately 1.3 m to the ground. North sides (wet) of the trees exhibited severe needle cast and branch death when compared to the south (dry) side of the trees. Lower branches were more involved than upper branches. Some trees were disease-free.



Figure 1. Branch death around base of blue spruce on a plantation in Michigan.



Figure 2. Needle casting of 2- and 3-year-old needles on a blue spruce tree on a plantation in Michigan.

Branches were removed from trees exhibiting needle-casting symptoms and branch death symptoms and were taken back to the laboratory. Branches from healthy-looking trees were also sampled. In the laboratory, needles from branches with and without symptoms were viewed under the dissection scope at 10–40X magnifications. Needles were removed and placed on microscope slides and viewed at high power (100X).

Low-magnification (40X) dissection scope observation of the needles taken from lower branches showed black fruiting bodies erupting through the stomata of needles (Figure 3). At lower magnification (10–30X), the black dots appeared similar to *Rhizosphaera*; however, at 40X, it was clear that the fungus erupting through the stomata was not *Rhizosphaera*, but the sporodochia of *Stigmina* spp. The infection appeared similar to *Stigmina*-infected needles first observed on white spruce in Michigan in 2002. No *Rhizosphaera* fruiting bodies could be detected by microscopic observation on any needles in the plantation. Later in the summer, sporodochia were found on Black Hills spruce at another location on the same plantation (third site, same plantation).



Figure 3. Sporodochia of *Stigmina lautii* on needles from blue spruce in Michigan.

To determine how widespread this pathogen was in the state, Colorado blue spruce were surveyed from northern Lower Peninsula counties (Emmet County) to southwestern counties (Berrien County). *Stigmina* sp. was found on needles from trees from all counties examined, including Emmet, Charlevoix, Kalkaska, Wexford, Missaukee, Ingham, Clinton, and Berrien, covering the north-south length of the state.

It appears that *Stigmina* spp. is currently infecting spruce in Michigan and is already widespread in the state. In 1999, a fungus sporulating on symptomatic blue spruce and Norway spruce needles in North Carolina was identified as *Stigmina lautii*. Prior to that report, *S. lautii* had been described on black spruce and white spruce collected from various locations in British Columbia, Manitoba, and Saskatchewan, Canada. Since that time, it has been reported in Virginia, North Carolina, North Dakota, New York, Wisconsin, and Iowa. In Michigan, our first samples were found in 2002 on white spruce from a farm in Ingham County.

One problem with detecting *Stigmina* is differentiating the fruiting bodies of *S. lautii* from those of *R. kalkhoffii*, which causes Rhizosphaera needle cast disease of spruce. Both are dark and erupt through stomata of spruce needles, and both fungi appear similar with low-magnification hand lenses (10–30X). Rhizosphaera needle cast disease is commonly found in Christmas tree and landscape scenarios in Michigan, and it will be important to provide enough information for growers and extension personnel to distinguish these two fungi in order to assess the distribution and impact of *Stigmina* versus *Rhizosphaera*.

Perhaps the most telling aspect of *Stigmina* needle cast found in Michigan in 2008 was its presence in a large plantation of blue spruce that had been successfully managed for Rhizosphaera needle cast by applying chlorothalonil-based materials. Apparently, this management program had little or no effect on *Stigmina* needle cast. While no fruiting bodies of *Rhizosphaera* were found on needles in the field of the blue spruce trees in Lake City, *R. kalkhoffii* was induced from the needles by placing needles in a humidity chamber, indicating that this pathogen is still present in the stand. *Stigmina* needle cast was severe in this planting. Spray trials were begun in May 2009. Currently, it appears that *Stigmina* is replacing Rhizosphaera needle cast or that spray programs manage *R. kalkhoffii* and offer less effective management of *Stigmina*.

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Rating needle loss of Fraser fir foliage associated with Christmas tree preservatives

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Introduction

A North Carolina grower reimbursed a chain store for 30 Fraser fir trees that defoliated soon after display by consumers. The grower suspected a Christmas tree (CT) preservative sold by the store and wanted confirmation that something else induced needle loss in his trees. Previous studies by Chastagner (1990) and Hinesley and Blankenship (1991) demonstrated the harmful effects of CT preservatives, yet nearly 20 years later, the use of CT preservatives is still prevalent and a strong-selling accessory item. The product in question was different from those tested by previous researchers. Therefore, a new study of CT preservatives was conducted.

Objective

To evaluate needle retention of Fraser fir branches treated with labeled rates of both commercial Christmas tree preservatives and common ingredients of home recipes of CT preservatives.

Materials and methods

In a pilot study, branches from 10 Fraser fir trees were treated with 2 CT preservatives diluted in water according to label directions and a plain water check. Needle retention was evaluated weekly over the course of a month. A more comprehensive study followed using branches from 30 trees. Treatments included six commercial CT preservatives, two ingredients of home recipes, and tap water. The six commercial products were Keeps It Green, Tree Life, Forest Fresh, Prolong, Peters, and EZgardner. A half-rate of the EZgardner treatment used in the pilot study was added to moderate apparently excessive label rates (as indicated by heavy needle loss in the pilot study). The home recipes included bleach plus corn syrup and corn syrup alone.

Needle loss rating

- 1 < 1%
- 2 = 1–3%
- 3 = 3–5%
- 4 = 6–10%
- 5 = 11–20%
- 6 = 21–50%
- 7 = 51%+

Branches from each of the 30 trees were sorted into clean plastic buckets holding water treated with CT preservative. Buckets of branches were stored in a heated basement room and were provided 9 hours of artificial light. Relative humidity ranged between 58 and 68%. Needle loss was rated on a 7-point scale after 2 and 4 weeks. Gaps between needles were visible on branches at ratings of 4 and above.

Results

All branches in both the pilot and main studies treated with CT preservatives exhibited more needle loss than those treated with tap water. As shown in Figure 1, both CT preservatives used in the pilot study induced more needle loss than the water check. The EZgardner product induced almost complete needle loss from treated branches by the fourth week. The Peters product induced less extensive but still very noticeable needle loss. At 4 weeks, the remaining foliage of branches treated with CT preservatives was noticeably a discolored brown, as compared to the more natural green of branches treated with water alone.

After only 2 weeks, most treatments in the main study were holding their needles, as can be observed in the average needle loss ratings shown in Figure 2. Five treatments still exhibited similar needle retention as the water check. Except for the full rate of EZgardner, treatments ranged between ratings of 1 and 2, a very acceptable level of needle retention. Only the full rate of EZgardner stood out with a higher average needle loss rating at week 2.

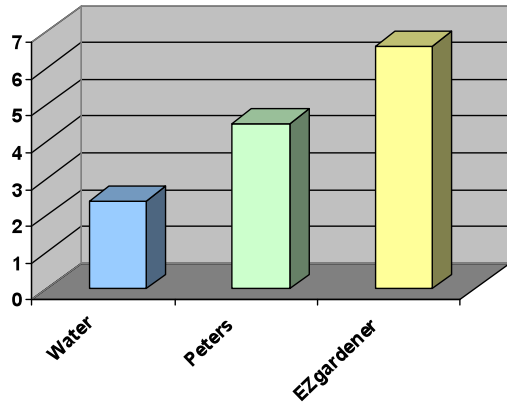


Figure 1. Average needle loss in pilot study.

Average needle loss ratings taken at week 4 are shown in Figure 3. Ratings nearly doubled from week 2 to week 4. Even branches treated with water were exhibiting 1–3% needle loss. Only one CT preservative, Forest Fresh, was still statistically similar to the check after 4 weeks. Several other treatments, including Keeps It Green, TreeLife, syrup and bleach, Prolong, and Peters were statistically similar to Forest Fresh despite higher ratings. Forest Fresh and Keeps it Green were statistically different from syrup and from both EZgardner treatments. Both rates of EZgardner preservative exhibited more needle loss than other treatments. Incurring the greatest needle loss, the full rate of EZgardner was the only preservative significantly different from other CT preservative treatments.

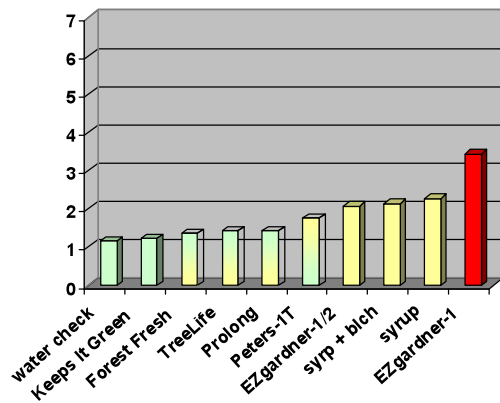


Figure 2. Average needle loss after 2 weeks.

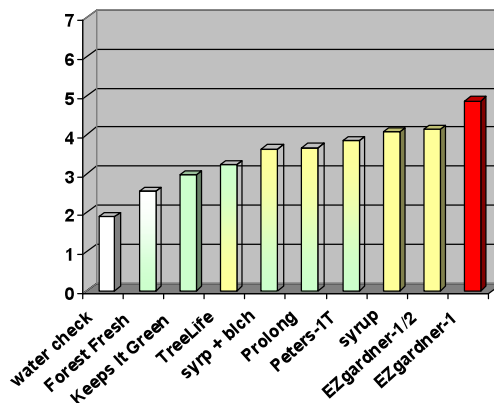


Figure 3. Average needle loss after 4 weeks.

Discussion

While these results showed some CT preservatives to be relatively harmless, some are potentially injurious to Fraser fir foliage if used at labeled rates. Since no preservative performed better than plain tap water, their use can be discouraged categorically.

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***Sydowia polyspora* isolated from needles and seeds of true fir is associated with current-season needle necrosis (CSNN)**

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Abstract

For several decades, current-season needle necrosis (CSNN) has been a serious foliage disorder on noble fir (*Abies procera*) and Nordmann fir (*A. nordmanniana*) in European and North American Christmas tree and bough plantations. Randomly distributed needles in the new foliage develop chlorotic spots or bands that later may turn necrotic and cause heavy needle cast. We isolated *Sydowia polyspora* from symptomatic Nordmann fir needles from Austria, Denmark, Germany, Norway, Slovakia, and the U.S., and from Nordmann fir seeds produced in Austria, Denmark, Georgia, and Russia. We also isolated the fungus from noble, Turkish (*A. bornmulleriana*), grand (*A. grandis*), and subalpine (*A. lasiocarpa*) fir needles, and from noble and subalpine fir seeds. Furthermore, *S. polyspora* was isolated from needles with a missing wax layer around the stomata. Inoculation tests with *S. polyspora* produced CSNN symptoms on Nordmann fir seedlings and transplants. CSNN spread from tree to tree in a trial in a plastic tunnel in Norway.

Introduction

For more than 25 years, current-season needle necrosis (CSNN) has been a poorly understood disorder on true fir (*Abies* spp.). Initial symptoms appear on new needles within 2 to 4 weeks after budbreak. CSNN symptoms consist of randomly distributed chlorotic bands and/or spots, which turn necrotic during the summer (Figure 1). Symptoms are commonly seen on Nordmann fir (*A. nordmanniana*), noble fir (*A. procera*), and grand fir (*A. grandis*) both in Europe and the U.S.



Figure 1. Current-season needle necrosis (CSNN) on Nordmann fir (*Abies nordmanniana*). Photos: Venche Talgø

In Germany, the fungus *Kabatina abietis* was isolated from grand fir needles with CSNN symptoms (Butin and Pehl, 1993). It was also associated with CSNN in Austria (Perny et al., 2002) and Norway (Talgø et al., 2007). CSNN-like symptoms were reported on Douglas-fir (*Pseudotsuga menziesii*) and grand fir in British Columbia, Canada, and the fungus *Hormonema merioides* was identified as the cause of the disease (Funk et al., 1985).

Sequencing of internal transcribed regions (ITS) of ribosomal DNA of *K. abietis*-like cultures, which included the *K. abietis* culture that Butin and Pehl (1993) had delivered to Centraalbureau voor Schimmelcultures in the Netherlands (CBS 248.93), revealed that they all belonged to *Sydowia polyspora* (Sutton, 1970), which has *Hormonema dematioides* as its conidial stage. Consequently, we suggested *K. abietis* as a synonym to *S. polyspora* (Talgø, 2009).

Objectives

Our objectives were to determine whether: (1) *S. polyspora* may be isolated from symptomatic needles of Nordmann fir and subsequently cause CSNN if inoculated onto young needles of this fir species, (2) *S. polyspora* may be associated with CSNN-symptomatic needles on other fir species, (3) seed lots of Nordmann fir may contain *S. polyspora* and thus be an important source of inoculum (previous observations), (4) fir needles with missing wax layers may contain *S. polyspora*, and (5) CSNN may spread from diseased to healthy plants.

Materials and methods (six studies)

(1) In 2007, isolations were carried out from needles with CSNN symptoms collected in Austria, Denmark, Germany, Norway, and the U.S. Needles were incubated for about 1 week on moist filter paper in 9-cm Petri dishes. The incubated needles were checked regularly for emergence of microbial growth. Sporodochia resembling *S. polyspora* were picked with a sterile needle and transferred to potato dextrose agar (PDA). The PDA was acidified with lactic acid to a pH of 5.6 to avoid bacterial growth.

(2) In 2008, isolations were carried out from needle samples collected from Nordmann fir container-grown transplants with CSNN symptoms from western Norway and from symptomatic grand fir from southeastern Norway. Furthermore, Nordmann fir from Nitra, Slovakia, and subalpine fir from Norway were tested for *S. polyspora* in 2009 (Figure 2).



Figure 2. Current-season needle necrosis (CSNN) on subalpine fir (*A. lasiocarpa*). Sporodochia are visible in the stomata lines (left). Partly missing wax layer around the stomata on Nordmann fir (*A. nordmanniana*) (right). Photos: Venche Talgø

(3) In 2009, two seed lots produced in Denmark were tested for *S. polyspora*. One had been harvested in 2006 (water content 9%) and the other in 2008 (water content 28%). We conducted two trials with the seeds. In the first, 140 seeds were tested per sample; 70 seeds were surface sterilized (10 seconds in 70% ethanol + 90 seconds in 0.5% NaOCl) and 70 seeds were untreated. Seven seeds were distributed in each of 20 Petri dishes with acidified PDA. Due to extensive growth of *Penicillium* spp. and *Trichoderma* spp. in the first trial (especially on the untreated seeds), all seeds were surface sterilized in the second trial, but this time for 20 seconds in 70% ethanol and 120 seconds in 0.5% NaOCl. A total of 210 seeds were used per sample. After the seeds were surface sterilized, they were cut in half with a scalpel; one half was placed on acidified PDA and the other on water agar (WA). (4) In 2007, Nordmann fir seeds harvested in Turkey were stratified and sown in container trays. Seedlings were inoculated with a Norwegian isolate of *S. polyspora* as soon as they germinated. Inoculation took place with culture plugs or by applying a spore suspension to the needles with a soft paintbrush.

In spring 2008, container-grown Nordmann fir transplants (approximately 15 cm high) were inoculated (with a paintbrush) with isolates from Nordmann fir samples collected in Austria, Denmark, Germany, Norway, and the U.S. (24 plants per country).

(5) In 2008, it was observed that the wax layer was missing or partially destroyed around the stomata on transplants that had been inoculated with *S. polyspora*, but no CSNN symptoms were present. Similar symptoms were found on needles without CSNN symptoms under field conditions in fields in western Norway in winter/spring 2009 (Figure 2), and samples were collected for isolation.

(6) In 2008, a test was conducted to determine the potential for CSNN to spread from one plant to another. Danish-produced container-grown Nordmann fir plants with severe CSNN symptoms served as sources of inoculum. The plants were placed among healthy Nordmann fir plants in a high plastic tunnel in Norway. One symptomatic plant was surrounded by 60 symptomless Nordmann fir plants. All of the disease-free plants were less than 50 cm away from the symptomatic plants. The symptomless plants had been in the tunnel for 2 years without showing CSNN symptoms. There were four replications. Sixty plants were kept as controls. The plants were automatically watered by overhead sprinklers four times a day (not at night) throughout the growing season. All collected isolates were identified morphologically according to Butin and Pehl (1993) and by ITS sequencing of the ribosomal DNA.

Results

(1) *S. polyspora* (Figure 3) was isolated from Nordmann fir needles from Austria, Denmark, Germany, Norway, and the U.S., and from grand, noble, and Turkish fir needles from the U.S. One isolate per country from Nordmann fir was chosen and identified by ITS sequencing. The isolates from Norway and the U.S. were identical. The isolates from Austria, Denmark, and Germany were identical, but differed from the Norwegian and American isolates by only two base pairs, and thus all five isolates were considered to be *S. polyspora*.



Figure 3. Culture of *Sydowia polyspora* isolated from Nordmann fir (*Abies nordmanniana*) needles (left) and from Nordmann fir seeds (right). On the picture from the seed test, the dark *S. polyspora* colonies are seen from the reverse side of the Petri dish. Photos: Venche Talgø

(2) Also, isolates from the grand fir sample and Nordmann fir transplants in Norway in 2008 and the Slovakian Nordmann fir and Norwegian subalpine fir in 2009 were identified as *S. polyspora* by morphological identification and ITS sequencing of the ribosomal DNA.

(3) *S. polyspora* was isolated from both of the Danish seed lots. In the seed lot from 2006, the fungus was found on less than 1% of the seeds. In the 2008 seed lot, *S. polyspora* was found on 30% of the whole seeds, and on 43% of the split seeds (Figure 3). An isolate from the latter was confirmed by ITS sequencing to be *S. polyspora*.

(4) Inoculation of seedlings (2007) and transplants (2008) with *S. polyspora* gave CSNN symptoms after approximately 1 month. In total, 24 out of the 36 inoculated seedlings had chlorotic or necrotic portions on the needles (Figure 4). The fungus was readily reisolated. Symptoms appeared on the inoculated transplants within 28 days (Figure 4). Instead of chlorotic areas turning necrotic, the chlorotic spots faded and disappeared with time as the needles developed a wax layer and became darker green. *S. polyspora* was reisolated from symptomatic needles inoculated with isolates from all five countries. *S. polyspora* was also isolated from one uninoculated control plant that had weak symptoms on a few needles.

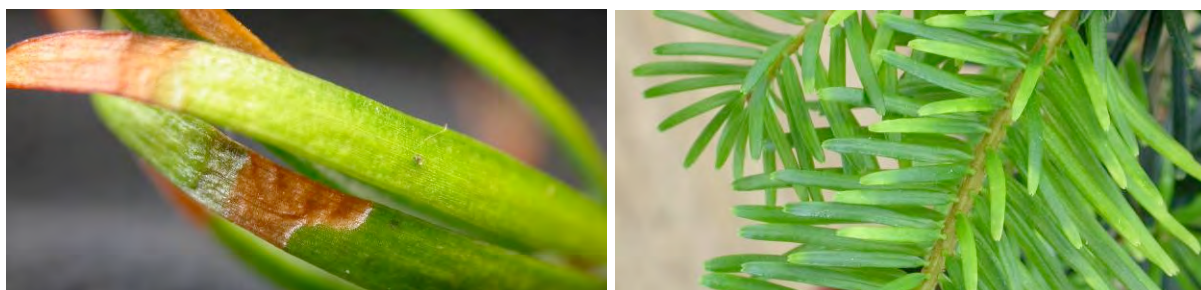


Figure 4. Current-season needle necrosis (CSNN) symptoms on a Nordmann fir (*Abies nordmanniana*) seedling (left) and a Nordmann fir transplant (right) after inoculation with *Sydowia polyspora*. Photos: Venche Talgø



Figure 5. Current-season needle necrosis (CSNN) symptoms spread from the tallest Nordmann fir (*Abies nordmanniana*) tree to the surrounding smaller trees. Control trees had no symptoms. Photo: Venche Talgø

(5) *S. polyspora* was readily isolated from the needles with missing wax that had been collected in Rogaland in 2009.

(6) A total of 26 of the 240 Nordmann fir plants (11%) in the tree-to-tree spread test developed typical CSNN symptoms (Figure 5). Symptomatic plants were distributed among all four replicates. No pattern suggested that plants closer to the inoculum-source plants were more severely attacked. Symptoms also developed on the new growth on the inoculum-source plants. We did not succeed in isolating *S. polyspora* from symptomatic needles.

Discussion

Our inoculation tests resulted in typical CSNN symptoms. The results of these pathogenicity tests and the reisolation of *S. polyspora* from symptomatic needles clearly indicate that *S. polyspora* is involved in the development of CSNN. Symptoms developed on one of the transplant control plants. This may have resulted from a latent infection from the nursery, or the plant may have become infected during handling (e.g., watering, fertilizing, or moving). The disappearance of the faint chlorotic symptoms indicates that the severity of symptom development is dependent on some unknown biotic or abiotic factors.

Several factors support a biotic cause for CSNN development. The tree-to-tree spread test indicates that the disease may spread from plant to plant. The fact that diseased needles were randomly distributed in the foliage further supports an infectious cause. Abiotic causes, such as nutrient deficiencies, give much more uniform symptoms (Talgø et al., 2005). A biotic cause is also supported by observations in Denmark that CSNN occurs much more frequently in years with outbreaks of the rust fungus *Pucciniastrum epilobii*, and that both CSNN damage and rust epidemics are correlated with wet weather during shoot elongation (Thomsen, 2008).

Further research relating to the biology, epidemiology, and management of CSNN is clearly needed.

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Neonectria canker on true fir and spruce in Norway

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Abstract

In 2008, severe outbreaks of *Neonectria* canker were found on white fir (*Abies concolor*) in southern Norway, and a *Neonectria* sp. was isolated from two counties in southwestern Norway and four counties in southeastern Norway. Both old and young trees were dead or dying. The same *Neonectria* sp. was also isolated from Siberian fir (*A. sibirica*), subalpine fir (*A. lasiocarpa*), and Norway spruce (*Picea abies*) in southeastern Norway. Previously *N. fuckeliana* had been reported on spruce species in Norway and on spruce and fir species in other countries.

Sequencing of the internal transcribed regions (ITS) of ribosomal DNA showed that all of the isolates from 2008 were identical and were more similar to *N. ditissima* (syn. *N. galligena*) than to *N. fuckeliana*. The isolates were five base pairs different from *N. ditissima*, and they may in the future be considered a new species. The perithecia were dark around the ostiole. This morphological characteristic is known from *N. ditissima*, but not from *N. fuckeliana*.

Inoculation tests were carried out on subalpine fir, white fir, and Norway spruce, and the fungus was pathogenic on all inoculated species. Thiophanate-methyl proved very effective against *Neonectria* sp. in laboratory fungicide trials.

Introduction

Neonectria canker caused by *Neonectria fuckeliana* is well known on conifers and is commonly associated with dieback of white fir (*Abies concolor*) in Europe and western North America (Callan, 1997; Schultz and Parmeter, 1990). In Canada, *N. fuckeliana* caused dieback of subalpine fir (*A. lasiocarpa*) (Funk, 1981), and in New Zealand it was reported on Monterey pine/radiata pine (*Pinus radiata*) (Dick, 2007). *N. fuckeliana* is also known to occur on spruce. In Norway, it is not regarded to be problematic on Norway spruce, but the disease can be severe on Sitka spruce (*P. sitchensis*) (Solheim, 2009).

In Norway in 2008, a *Neonectria* sp. different from *N. fuckeliana* caused extensive dieback on white fir (Figure 1), Siberian fir (*A. sibirica*), subalpine fir, and Norway spruce. Symptoms included dead, young shoots, canker wounds on branches (Figure 2) and/or the main stem, heavy resin flow, and in a few cases formation of red perithecia (Figure 2) (Talgø et al., 2008a and b).



Figure 1. Dieback caused by *Neonectria* sp. on white fir (*Abies concolor*) in Akershus County in 2008. Photos: Venche Talgø.



Figure 2. Canker wound on white fir (*Abies concolor*) (left) and red fruiting bodies (perithecia) on subalpine fir (*Abies lasiocarpa*) (right) after attack by *Neonectria* sp. Photos: Venche Talgø.

Objectives

The main objective of our work was to reveal the cause of the canker dieback. We wanted to collect isolates from canker wounds, identify them morphologically and by ITS sequencing, fulfill Koch's postulates by inoculating fir and spruce with the isolated fungus, record symptom development and reisolate, and finally screen for effective fungicides in laboratory tests.

Materials and methods

Small pieces (approximately 1 cm³) were excised from the leading edge of canker wounds. After surface sterilization (10 seconds in 70% ethanol + 90 seconds in 0.5% NaOCl), the pieces were air-dried for 1 minute, cut into smaller pieces, and placed on potato dextrose agar (PDA). For subalpine fir, we also performed isolations directly from perithecia. The branches with perithecia were surface sterilized by spraying them with 70% ethanol prior to transferring single perithecia, or spore masses exuding from them, to PDA with a sterile needle.

In addition to isolations, all plant samples from canker wounds were incubated in 100% relative humidity at room temperature.

Given the importance of subalpine fir as a Christmas tree in Norway, we inoculated this particular fir species with cultures from all three fir species and Norway spruce. Map pins (16 mm) were used to effectively inoculate and easily trace the inoculation points. The pins were

autoclaved and placed on PDA together with an agar plug (0.5 mm in diameter) from the *Neonectria* culture to be tested. After approximately 1 week at room temperature, the pins were covered with mycelia, and the needle tips were inserted into the bark or dormant buds and left there. (Three pins or more were inserted per plant, depending on the size and shape of the plant.) The plants varied in height from 23 to 85 cm. Control plants were wounded by sterile map pins. After inoculation, a plastic cover was kept on for 24 hours. The plants were given 18 hours day length, and average temperature and RH was 15.4°C and 80.7%, respectively. We also carried out pathogenesis tests on white fir and Norway spruce with cultures obtained from the respective hosts.

To select potential fungicides for further field testing against *Neonectria* sp., laboratory tests were carried out during the winter of 2008–2009. The effect of the following eight fungicides at 1, 10, and 100% concentrations of maximum dosage provided by the manufacturers were tested against mycelial growth and spore germination on Petri dishes containing PDA: copper oxide (Nordox 75WG), kresoxim-methyl (Candit[®]), sulphur (Thiovit[®] Jet), penconazole (Topas[®] 100 EC), mancozeb (Dithane NewTec), thiophanate-methyl (Topsin[®] WG), dithianon (Delan[®] WG), and copper oxychloride (Kopperkalk Bayer).

Results

Neonectria sp. was easily isolated from canker wounds on all four conifer species (Figure 3), and also from perithecia on subalpine fir.

When incubating samples from the different host plants, a whitish mycelia typically appeared from the cankered areas, and macro- and microconidia of *Neonectria* sp. were readily produced. Microconidia (*Cephalosporium* sp.) were also commonly produced on PDA, resulting in a powdery appearance of the cultures. Macroconidia (Figure 3) formed only in cultures on the less rich SNA (Spezieller Nährstoffarmer Agar).

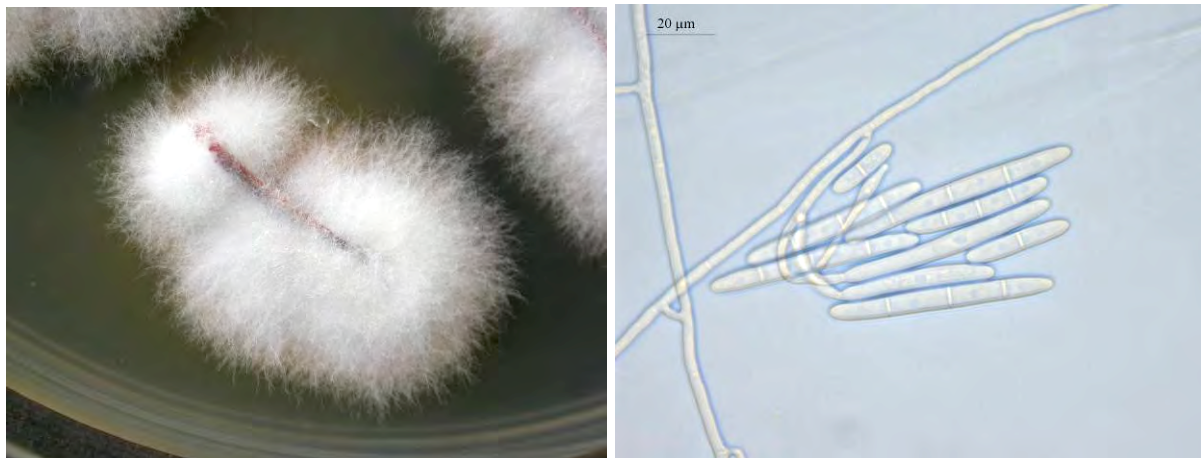


Figure 3. *Neonectria* sp. on white fir (*Abies concolor*): white, floccose culture growing from surface-sterilized wood pieces from the leading edge of a canker wound (left), and macrospores (*Cylindrocarpon* sp.) (right). Photos: Venche Talgø and Jafar Razzaghian

Unlike what is known about *N. fuckeliana*, the *Neonectria* sp. we found was dark (brownish) around the ostiole of the perithecia, which is characteristic for *N. galligena* (Ellis and Ellis, 1997).

The ITS sequences of isolates obtained from all three fir species and the spruce were identical, and they were most similar to different isolates of *N. ditissima* reported to the GenBank on *Malus*. In total, 5 bp were different in the ITS regions of the Norwegian conifer isolates, compared to the most similar *N. ditissima* isolates in the GenBank.

All inoculated trees developed foliage dieback and/or canker wounds (Figure 4), and the infected tissues inside buds, shoots, and stems were discolored (Figure 5). *Neonectria* sp. was successfully reisolated from canker wounds. Pathogenicity was also proven on white fir and Norway spruce. On control trees pierced with sterile needles, no disease symptoms were detected.

Four fungicides were effective at 100% concentrations: thiophanate-methyl, copper oxide, mancozeb, and penconazole. Thiophanate-methyl also completely suppressed all mycelial growth at 10 and 1%.

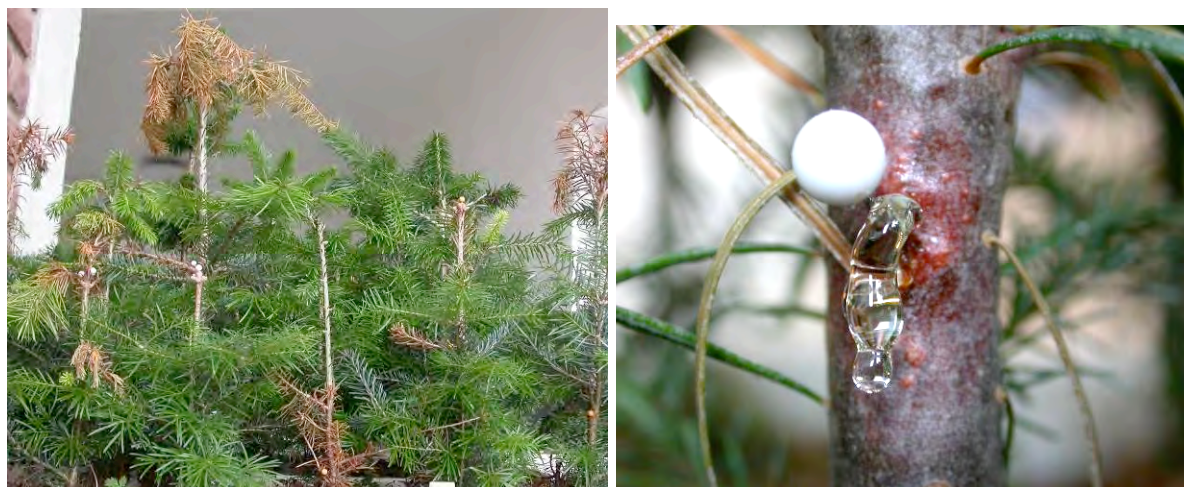


Figure 4. Shoot dieback (left) and canker wound (right) on subalpine fir (*Abies lasiocarpa*) after inoculation with *Neonectria* sp. Photos: Venche Talgø



Figure 5. Discolored tissues inside a shoot and bud (left) and stem (right) after inoculating subalpine fir (*Abies lasiocarpa*) with *Neonectria* sp. Photos: Venche Talgø

Discussion

To our knowledge, this is the first time a *Neonectria* sp. similar to *N. ditissima* has been found on conifers in Norway.

The inoculation test clearly demonstrated that the isolated *Neonectria* sp. was pathogenic and that isolates from four different conifer species were able to infect subalpine fir.

Why did this epidemic occur on fir in Norway? First of all, it might be a new *Neonectria* species occurring in the country, or a genetic change (mutation) may have taken place within *N. ditissima*. *N. ditissima* has not been described as a pathogen in conifers, but is well known from broadleaf trees, including maple (*Acer* spp.), birch (*Betula* spp.), horse chestnut (*Aesculus hippocastanum*), willow (*Salix* spp.), ash (*Fraxinus*), beech (*Fagus*), *Sorbus*, and especially apples (*Malus* spp.) (Butin, 1995; Ellis and Ellis, 1997).

Secondly, weather conditions during the 2007 growing season were ideal for fungal infections: wet and cool. In Akershus County in southeastern Norway, there was also a serious outbreak of larch canker (*Lachnellula wilkommii*) on western larch (*Larix occidentalis*) in 2008 (Talgø et al., unpublished data). This disease is normally a problem only in wetter regions such as western Norway, and thus the outbreak in 2008 may have been a consequence of the weather conditions in 2007.

After the two described findings of *Neonectria* sp. on subalpine fir, we have received only one report about dieback of subalpine fir Christmas trees resembling *Neonectria* sp., and thus far the situation does not seem alarming for Christmas tree production.

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Copper-based fungicide field trials against CSNN: Results from five countries

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Introduction

Current-season needle necrosis (CSNN) has been a serious foliage disorder on true fir Christmas trees and bough material in Europe and North America for more than 25 years. Approximately 2 weeks after budbreak, needles get chlorotic spots that later turn necrotic, and severely affected needles are often shed (Figure 1).



Figure 1. Current-season needle necrosis (CSNN) on Nordmann fir (*Abies nordmanniana*). Left: Red spots, bands, or needle tips are characteristic symptoms of CSNN. Right: Severe needle shedding can be a consequence of CSNN. Photos: V. Talgø and I.M. Thomsen

Symptoms have been observed on noble fir (*Abies procera*), Nordmann fir (*A. nordmanniana*), and grand fir (*A. grandis*) on both continents. CSNN was reported from the U.S., Denmark, and Ireland as a physiological disorder with unknown etiology, but most likely associated with calcium deficiency and/or influenced by climatic factors (Chastagner et al., 1990). However, in Norway, Germany, and Austria, the symptoms were associated with the fungus *Kabatina abietis* (Butin and Pehl, 1993). In 2007, a fungus that morphologically resembled *K. abietis* was isolated from needle samples from Nordmann fir from Norway, Denmark, Germany, the U.S., and Austria (Talgø et al., 2008). Recent research in Norway showed that *K. abietis*, included in the reference culture that Butin and Pehl (1993) had delivered to Centraalbureau voor Schimmelcultures (CBS) in the Netherlands, was identical to *Hormonema dematioides*, the imperfect stage of *Sydowia polyspora* (Talgø, 2009). Inoculation experiments confirmed that the fungus was pathogenic. In the future, *Sydowia polyspora* should be used rather than *Kabatina abietis*.

Objectives

In order to test the theory that the primary cause of CSNN is a fungus and therefore may be prevented by fungicides, fungicide trials were carried out in Austria, Denmark, Germany, Norway, and the U.S. (Figure 2). For more than a decade, copper oxychloride has been used in Norway against various needle diseases, and it was believed to prevent CSNN as well (Terje Pundsnes, Norsk Pyntegrønt, pers. com.). In 2008, copper oxide became the copper-based fungicide approved for this purpose in Norway. As copper is a wide-spectrum fungicide, it was reasonable to expect it would be effective against *S. polyspora*, and thus most of the trials in the different countries in 2008 were carried out with a copper compound.



Figure 2. Fungicide trial with Nordmann fir (*Abies nordmanniana*) in Norway, 2008. The plants were imported from a Danish nursery in spring 2008, and at that time the 2007 needles had obvious CSNN symptoms. *Sydowia polyspora* had been found on needle samples from the same nursery prior to import. Photo: V. Talgø

Methods and materials

The experimental design varied (see Table 1), but in every case both treated and untreated Nordmann fir had CSNN symptoms on the previous year's needles (2007). Treatments were carried out three times during flushing, using copper-based fungicides, mainly copper oxide (Nordox 75WG). The trees were scored for CSNN symptoms on the 2008 needles at least once during late summer or autumn, and the percentage of trees with severe, intermediate, or insignificant damage is given below (Table 2).

Table 1. Overview of field trials in Nordmann fir (*Abies nordmanniana*) in the five participating countries. In the fourth column, the number of untreated trees is given in parentheses. In Norway V, three different rates and a backpack sprayer (not motorized) were used. In Norway I–IV only the highest rates given by the manufacturer were used, and fungicide was applied with mist sprayers on tractors.

	Product ^a	Treatment dates	Number of trees	Spray method
Norway I	Nordox 75WG	9/6, 25/6, 3/7 (late)	10 (10)	Overhead mist spray
Norway II	Nordox 75WG	21/5, 30/5, 9/6 (early)	10 (10)	2x overhead mist spray, 1x single tree
Norway III	Nordox 75WG	26/5, 6/6, 12/6 (mid)	10 (10)	2x overhead mist spray, 1x single tree
Norway IV	Nordox 75WG	26/5, 6/6, 12/6 (mid)	10 (10)	2x overhead mist spray, 1x single tree
Norway V	Nordox 75WG, Kopperkalk Bayer Delan WDG	16/5, 30/5, 9/6 (early)	80 (16)	Single tree ^b
Denmark	Nordox 75WG	15/5, 22/5, 27/5	20 + 20 (40)	Single tree ^b + overhead mist spray
Germany	Cuprozin WP	20/5, 28/5, 6/6, 25/6	20 (25)	Single tree
USA	Nordox 75WG	15/5, 29/5, 11/6	20 + 21 (41)	Single tree
Austria A	Cuprozin WP	20/5, 1/6, 10/6	20 (20)	Single tree
Austria B1	Cuprozin WP	20/5, 1/6, 10/6	20 + 20 (20)	Single tree
Austria B2	Cueva			

^a Active ingredients are: copper oxide in Nordox 75WG, copper oxychloride in Kopperkalk Bayer, copper hydroxide in Cuprozin WP, copper octanoate in Cueva, and dithianon in Delan WDG.

^b All single tree sprays were sprayed with backpack equipment.

Results

Results from the fungicide treatments are given in Table 2.

Table 2. Incidence (%) of trees with no (or slight), medium, or severe CSNN after treatments with copper-based fungicides during flushing of Nordmann fir (*Abies nordmanniana*). Significant differences ($P \leq 0.05$) between treated and untreated trees were found in only a few cases (numbers marked in **bold**). Results from Norway V are not shown.

Country	Treated plants (%)			Untreated plants (%)		
	None/ slight	Medium CSNN	Severe CSNN	None/ slight	Medium CSNN	Severe CSNN
Norway I	100	0	0	100	0	0
Norway II	100	0	0	100	0	0
Norway III	70	20	10	50	40	10
Norway IV	60	30	10	30	0	70
Denmark	45	32	23	25	45	30
Germany	25	45	30	30	45	25
USA	15	51	34	15	48	37
Austria A	30	45	35	30	35	25
Austria B1	12	58	30	0	65	35
Austria B2	35	55	10			

The **Norwegian** trials did not yield consistent results. Norway I and II had no CSNN symptoms on any of the trees, treated or not. In Norway III and IV, there were clearly more symptoms on untreated trees than on treated trees. In Norway V, there were generally more symptomatic needles on untreated compared to treated trees, but none of the fungicides reduced CSNN significantly.

In the **Danish** trial, 45% of the treated Nordmann fir had no CSNN symptoms versus 25% of the untreated plants. However, the number of plants with severe symptoms was almost the same in both treated and untreated trees. Both Nordmann fir plants in beds with overhead spraying and in single tree treatments had CSNN symptoms (Figure 3). In Table 2, data from the two trials were pooled.



Figure 3. Selected pairs of Nordmann fir (*Abies nordmanniana*) at Gl. Kirstineberg pot-in-pot nursery. All plants had CSNN symptoms and needle loss on 2007-year shoots. Trees with yellow labels (left) were removed before overhead spraying and returned to the plot immediately after spraying. Note that in the top photo the untreated tree has symptoms on 2008 needles, as would be expected. However, in the lower photo the situation is reversed. The tree with a blue label, i.e. the fungicide-treated tree, has severe symptoms on the 2008-year shoots, and the untreated tree of this pair has no symptoms. Apart from the selected trees, many other plants in the rows of fungicide-treated trees had CSNN symptoms, indicating that spraying with copper did not work. Photos: I.M. Thomsen

In the **German** trial, there were fewer trees without CSNN among the sprayed trees. On the contrary, a higher number of the treated trees had symptoms on more than 50% of the shoots compared with the untreated trees. In other words, symptoms were more severe on sprayed trees, although the difference was not significant. Ortiva/Amistar (azoxystrobin) and Systhane 20 EW (myclobutanil) were also tried, but these fungicides had no effect. The total amount of trees with CSNN was less in 2008 than the year before, but the percentage of trees with severe damage was the same.

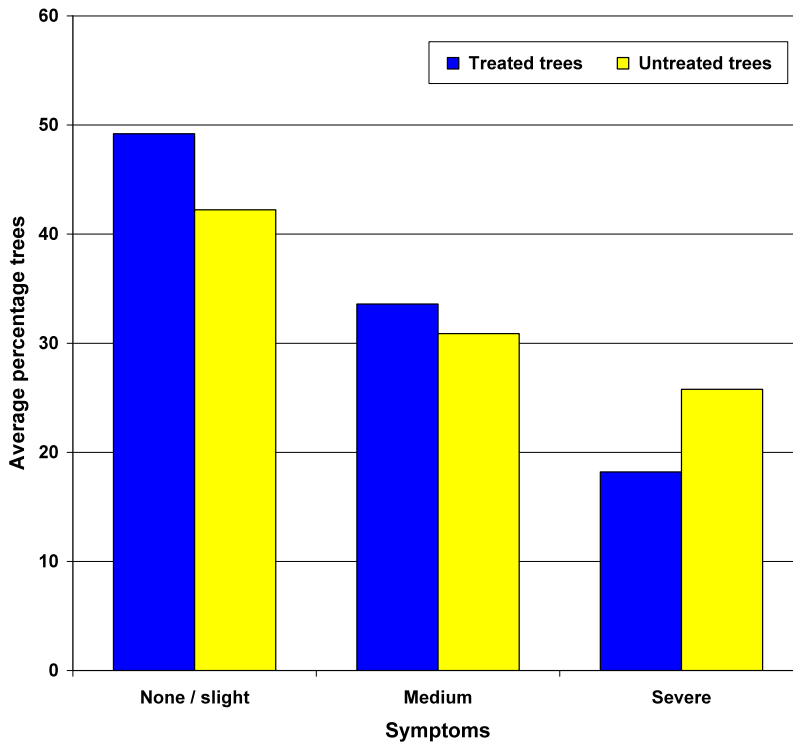


Figure 4. Mean percentage of experimental trees in the three symptom categories (none/slight, medium, and severe), based on whether they were treated with fungicides (blue) or not (yellow). The averages were calculated from field trials in Denmark, Norway (except Norway V), Germany, the U.S., and Austria.

In the **U.S.** trials, the treated and untreated plots had the same number of trees with severe symptoms and no symptoms. In one trial, treated trees had the most needle damage, but the reverse was true for the other experiment. In Table 2, data from the two trials were pooled. Sprays were also carried out in *A. grandis* and *A. procera*, but the copper treatments had no effect. All of the noble fir had extensive CSNN symptoms on new shoots at the time of assessments.

In the **Austrian** trials, more of the sprayed trees had severe symptoms in one case (B2), whereas the other experiment had more treated trees without CSNN and fewer trees with severe symptoms. On one-half of both treated and untreated trees, there was no change in disease severity from 2007 to 2008. For the other half there was mostly an improvement on the treated trees and more CSNN on the untreated trees. In the experiment with Cueva, none of the trees had symptoms in 2008, treated or not.

Overall, copper-based fungicides had only slight or no effect against CSNN on Nordmann fir. Severe symptoms of CSNN were slightly less pronounced on treated trees (Figure 4), but the difference was not significant. In several cases, treated Nordmann fir was just as damaged as untreated.

Discussion

Isolations from symptomatic needles and inoculation tests in Norway clearly indicated that *S. polyspora* is involved in the development of CSNN (Talgø, 2009). However, none of the fungicides used in the field trials was effective. Laboratory tests confirmed that copper-based fungicides were not effective against *S. polyspora*, but other fungicides prevented spore germination and mycelia growth (V. Talgø, unpublished data). The lack of results may be related to a failure of copper rather than an indication that the disease may not be controlled by

fungicides. There is still much work to be done to further field test fungicides that showed an effect in the laboratory.

The necessary number of treatments and the optimal timing needs to be determined. It should be calculated whether fungicide spraying is economically feasible compared to potential losses. The gain could increase substantially if the fungicide was also effective against fire weed rust (*Pucciniastrum epilobii*) and other fungi that infect needles during flushing if conditions are right.

In Denmark, getting approval for use of fungicides on Christmas trees is likely to be difficult, as most of the potential candidates are either restricted or under re-evaluation with limited chances of being cleared for continued use, and none is approved for Christmas tree production at present. The situation is less restrictive in other countries.

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WEEDS

Test of mixtures of herbicides: Accurate mixed with diflufenican for weed control in Christmas trees

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Introduction

In Denmark, as well as in most other EU (European Union) countries, there is increased concern for the environmental effect caused by chemical weed control. All of the traditional soil herbicides—atrazine, cyanazine, diuron, hexazinone, simazine, and terbuthylazine—have been banned in all of the EU during the past 10 years.

During the past 10 years, a lot of new herbicides have therefore been tested in Denmark for use in Christmas trees (Figure 1). Most of the newly tested herbicides are sulfonylureas, and some of them have now been registered via an off-label registration for use in Nordmann fir (*Abies nordmanniana*). The weed control period for most sulfonylureas is too short, so experiments are being carried out to improve the control period by adding the soil herbicide diflufenican to a sulfonylurea.

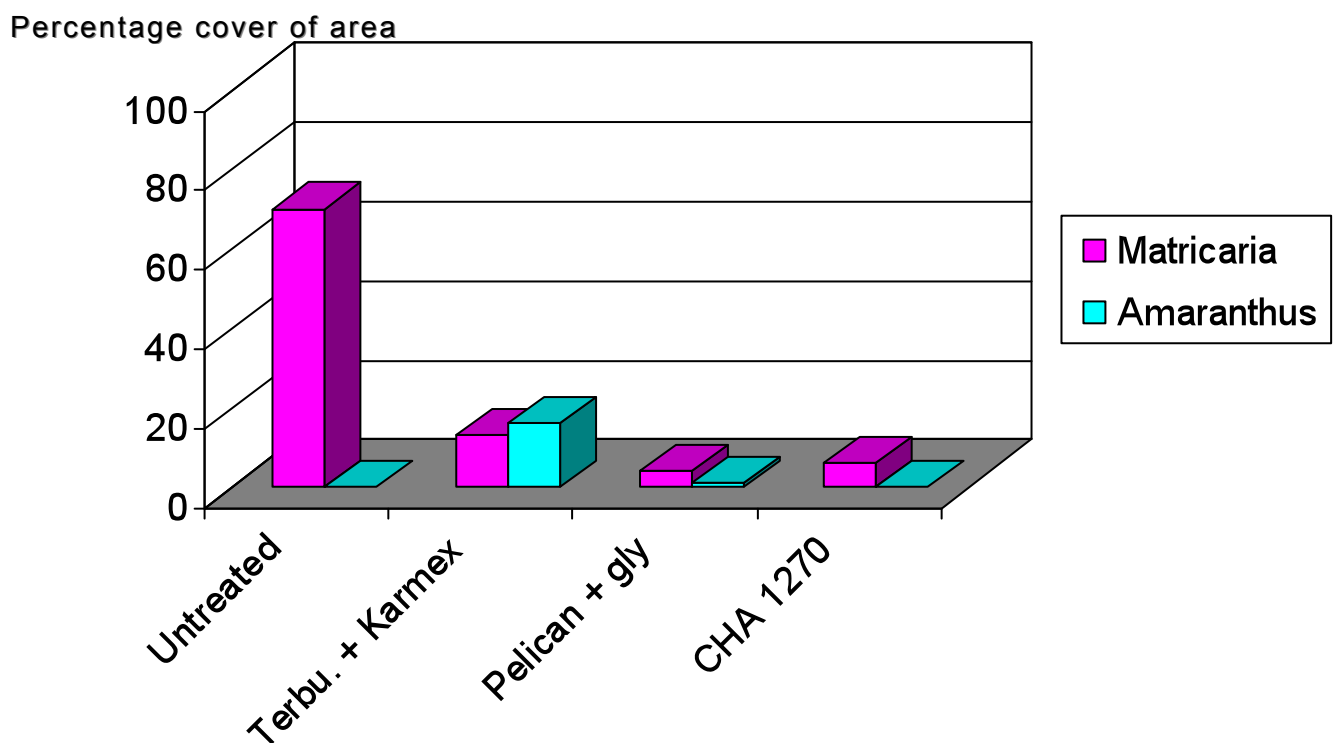


Figure 1. Vegetation control of *Matricaria maritima* and *Amaranthus retroflexus*, 2007.

Accurate

Accurate contains 20% metsulfuron methyl. It contains the same active ingredients as the trade product Ally, but Accurate is a different formulation that seems to be gentler on the trees and gives less risk of needle discoloration compared to Ally. Accurate is not yet registered in any of the European countries, but is expected to be registered in late 2009.

Diflufenican

Various trade products contain 500 g/l diflufenican. It is a very active soil herbicide. When mixed with metsulfuron methyl or glyphosate, it gives good and long-lasting weed control (Figure 2). Diflufenican is registered in several European countries.



Figure 2. Pelican + glyphosate 0.4 + 2.2 l/ha. Applied April 29, 2007.

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Transitioning weed suppression from Roundup Original to Roundup Powermax with backpack and mistblower sprayers

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Introduction

North Carolina Fraser fir Christmas tree growers have learned to use very low rates of glyphosate herbicide to suppress weeds and beneficial ground covers such as Dutch white clover. Monsanto sponsored two studies to evaluate use of Roundup Powermax in place of Roundup Original, on which weed suppression research was based. In 2008, Powermax was evaluated next to Roundup Original using conventional backpack sprayer practices. In 2009, the work compared midsummer application with mistblower sprayers to backpack sprayers.

Materials and methods

Both studies were conducted with a generic formula of Roundup Original and the new Roundup Powermax formulation provided by Monsanto. In 2008, three studies were established to compare the two materials. Two row plots were established in which the lower branches of the interior 30 trees were intentionally sprayed with herbicide mix. Backpack sprayer treatments were made at three locations in May, June, and July using 8 ounces of Original and 5.5 ounces of Powermax per acre. Damage to lower branches was evaluated 3 weeks later, along with subjective evaluation of weed suppression. Damage was recorded as number of shoots injured and the maximum length of damage observed per tree.

Building on the results of the 2008 backpack sprayer work, the 2009 studies incorporated both mistblower and backpack sprayer treatments. 2009 treatments were made to larger blocks (from $\frac{1}{4}$ to $\frac{1}{2}$ acre) on five farms to adequately evaluate mistblower application. Treatments were made in June and July to evaluate injury potential in the tender window during June and July. Rates of 4, 3, and 2 ounces of Original and 2.7, 2.1, and 1.4 ounces of Powermax were applied per acre with tractor-powered mistblower sprayers. Additional applications of PowerMax were applied only in July at rates of 2.7 and 4.1 ounces per acre. The highest rates were repeated using backpack sprayers as a comparison. Only lower branches were sprayed with backpack sprayers. TK nozzles were used intentionally to ensure the likelihood of injury using backpack sprayers. The whole tree, including the leader, was sprayed using mistblowers. Tree injury was evaluated at 3 weeks after treatment and again after trees were sheared. Number of shoots injured, length of damage in inches, and severity of injury was recorded. Further injury evaluations will be conducted after foliage maturity in 2010.

Results

In the 2008 backpack sprayer study, no damage was observed in May. Foliage injury was observed at only one of the three locations in June and may have been aggravated by operator error. Backpack sprayer injury occurred on branch tips close to where sprayer nozzles passed. Minor injury to branch foliage observed in early July was similar for both Roundup Original and Powermax. The pattern of injury at each site was different. Treatments induced the same level of injury at one site, more with Powermax at the second site, and more with Original at the third

site, effectively cancelling out differences. The percentages of trees injured are shown in Figure 1.

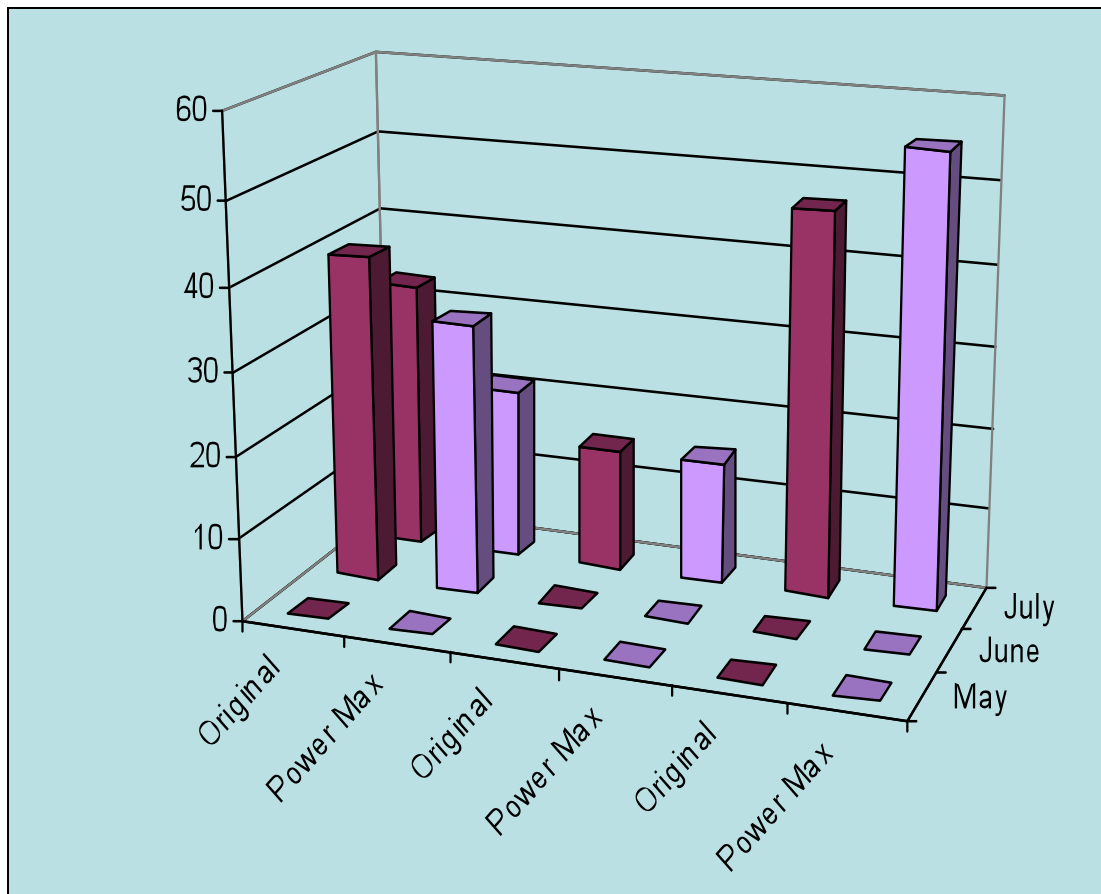


Figure 1. 2008 Roundup Original and Powermax study—percentage of trees injured using backpack sprayers.

The results of the 2009 mistblower/backpack sprayer study repeated the pattern of no foliage injury from May treatments despite onset of foliage flushing. Tree injury was observed in both June and July, but final results after both treatments are reported. All June and July treatments resulted in some damage. Backpack sprayer injury occurred only on branch tips close to sprayer nozzles. Mistblower damage occurred to terminals and some top lateral branches. The number of lateral branches injured by treatment is shown in Figure 2. A significant difference was observed between sprayer type, with many more branches injured by the backpack sprayer. It also appeared that more branches were injured using Powermax than when using Roundup Original with the backpack sprayer.

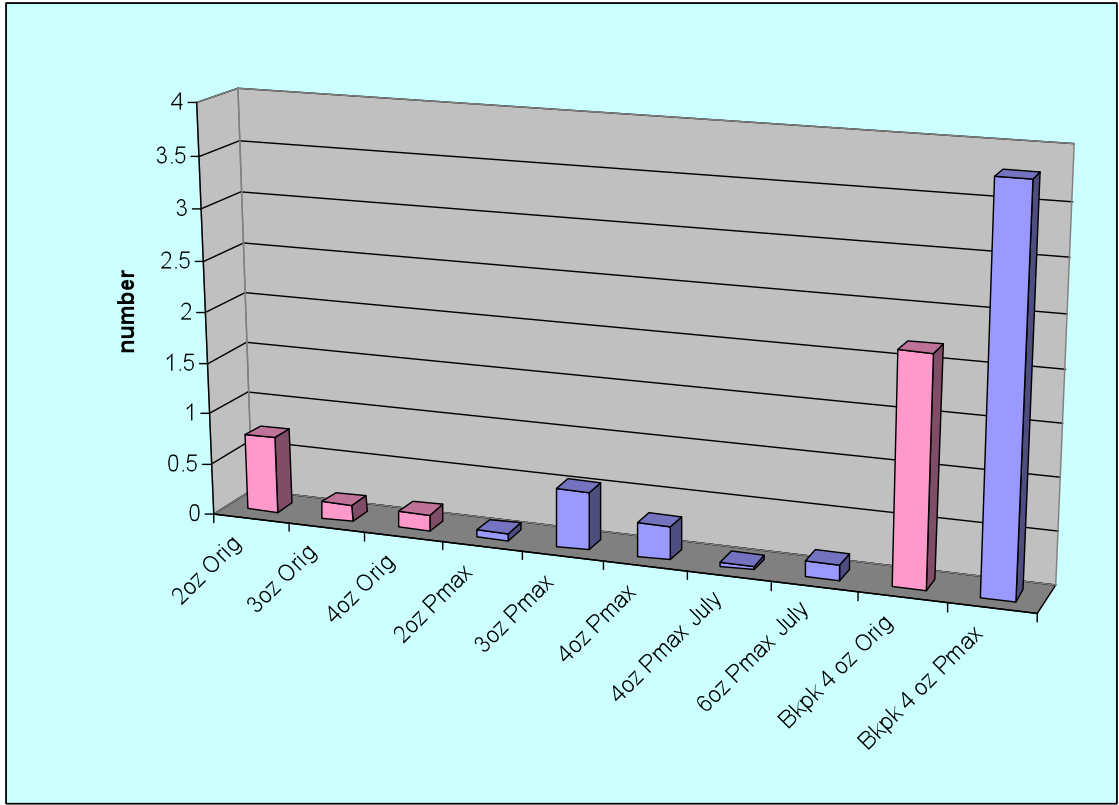


Figure 2. Average number of branches injured by mistblowers and backpacks.

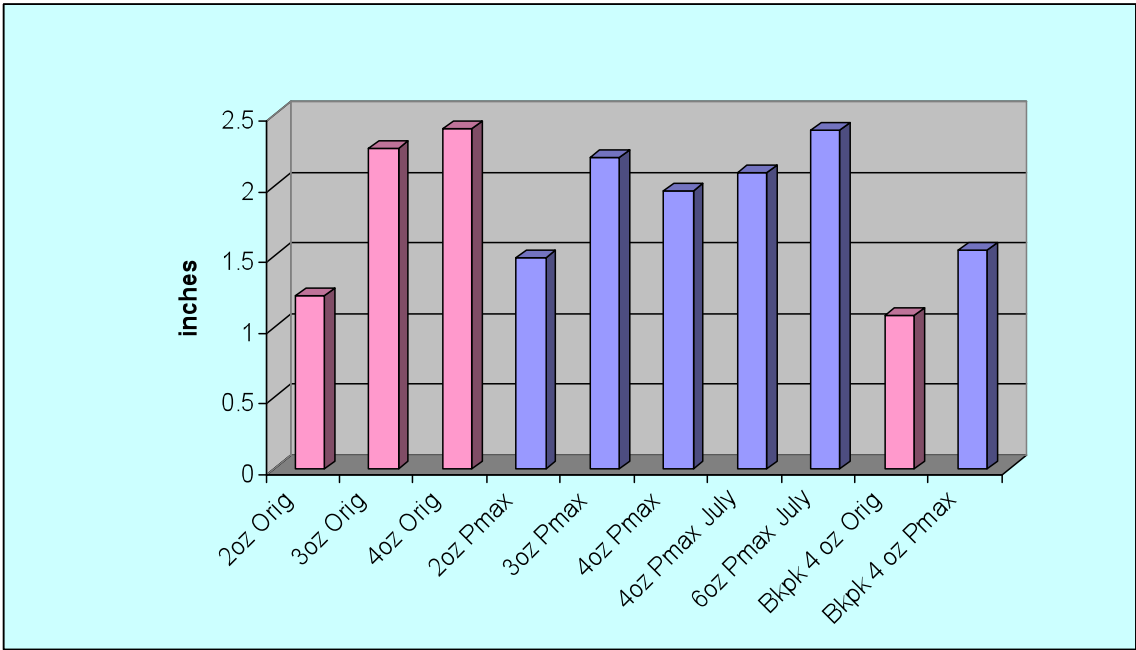


Figure 3. Length of branch and terminal injury (inches) by mistblowers and backpacks.

When the length of injury is examined in Figure 3, no difference was observed between equal rates of Original and PowerMax. However, the lowest rate of both materials did yield shorter average length of injury compared to the middle and higher rates of herbicide. The length of injury to terminals damaged by mistblower application was longer than that observed from backpack application, as might be expected from the length of different shoots. While both terminals and side shoots were actively growing at the time of treatment, most terminals were 1 to 2 feet tall and side shoots were 4 to 8 inches.

Figure 4 shows the percentage of terminals injured before and after shearing. Looking at terminal injury prior to shearing in July, the number of trees injured increased with rate for both Roundup Original and PowerMax. However, equal rates of the two products were not different. The greatest number of terminals injured was from the 4.1-ounce-per-acre rate of PowerMax applied in July. Both low and high rates were significantly different from the intermediate treatments of PowerMax prior to shearing. After shearing, most of the differences in injury disappeared. Only the 3- and 4-ounce rates of Original still showed injury on a slightly higher percentage of terminals. Much of the injury to terminals was located primarily on the tips of terminals and therefore was removed when the terminal was cut to length.

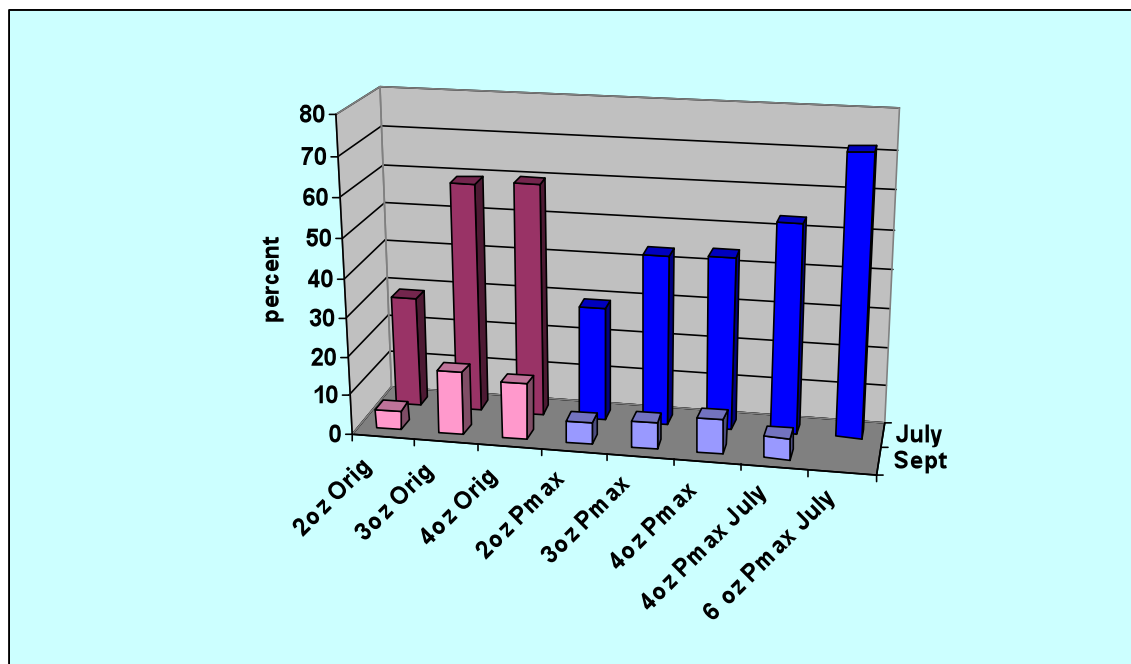


Figure 4. Percentage of terminals injured by mistblower treatments before and after shearing.

When the length of injury is examined on farms where trees were sheared (three farms instead of five), a rate effect is more visible after shearing than before for the June and July treatments (Figure 5). The low rate showed the shortest length of injury remaining. However, the July-only treatments of PowerMax at 2.7 and 4.1 ounces actually show a further decrease in length of injury after shearing, presumably because the injury was limited to the tips. There was no difference between formulations of Roundup. There was no reduction in the length of damage from shearing side branches.

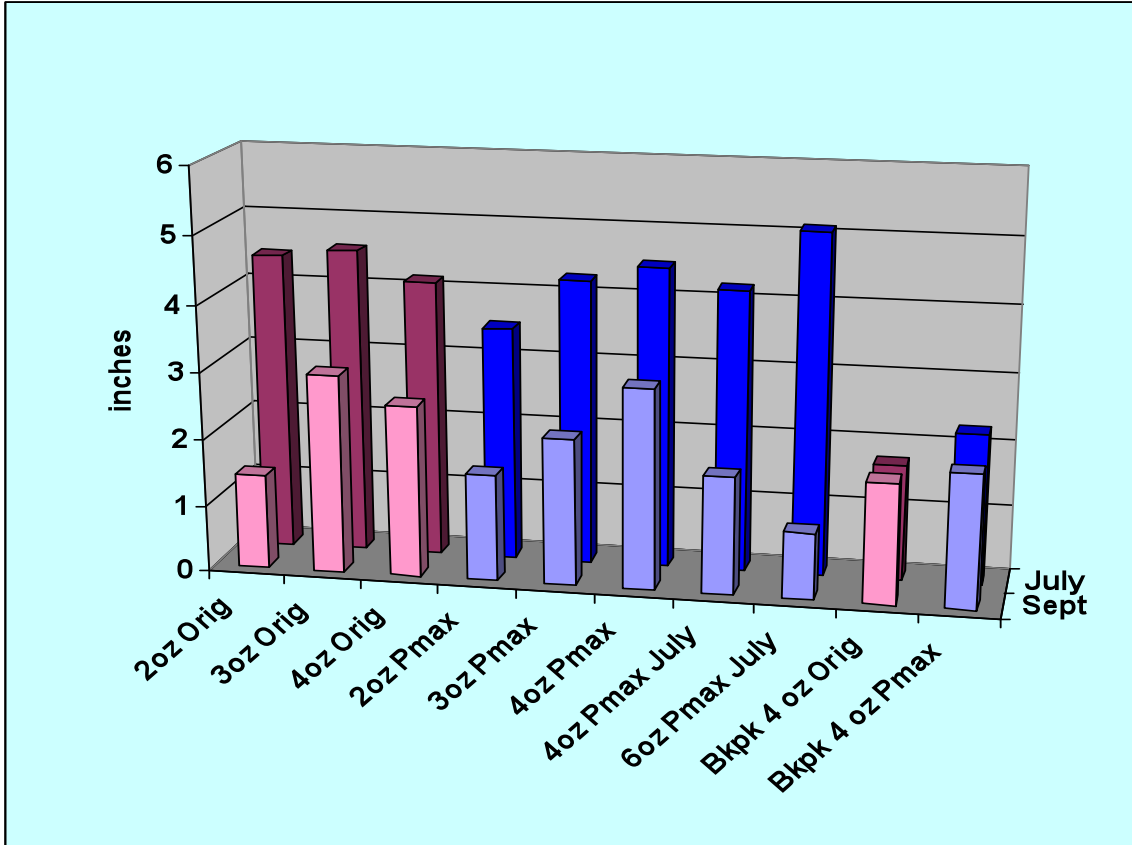


Figure 5. Length of injury before shearing (July) and after shearing (September) from applications of Roundup herbicide (various formulations and rates). Data is average of observations on three farms.

Discussion

In 2008, the differences between Roundup Original and PowerMax applied by backpack sprayer suggested a similar risk of injury. However, in 2009, more branches appeared to be injured by PowerMax using the backpack sprayer. This pattern did not hold true for mistblower applications, where no difference between materials was observed. With 2 years of data available, it would seem that PowerMax could still be substituted for Original with only slightly more risk.

While significantly less damage was observed at the lowest applied rate, there is some question whether this rate is useful. The effectiveness of the herbicide was noticeably less at the reduced rate. At this level, adjustments to timing or application technique may be more effective than reductions in rate.

Levels of injury in these studies show a potential for risk in June and July, with seasonal variations regardless of the type of sprayer. The mistblower study results suggest that much of the damage will be sheared off, leaving a majority of trees unblemished. This holds true where terminals are cut, but not where trees are left with natural tops. It seems unwise to recommend mistblower application during June or early July. Growers can use backpack sprayers with a safer nozzle during the tender window to minimize risk. This still is the safest choice. However, growers have pushed treatment limits to the point where this work was needed and will, in all likelihood, keep doing so.

INSECT AND VERTEBRATE PESTS

Elongate hemlock scale, balsam twig aphid, and balsam woolly adelgid in North Carolina Fraser fir

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The elongate hemlock scale (*Fiorinia externa*) (EHS) has emerged as a serious pest of Fraser fir (*Abies fraseri*) in the mountains of North Carolina, Virginia, and Tennessee over the past decade. This pest has been present in the region for at least 15 years, during which time the insect has expanded its range, resulting in increased pesticide applications for Christmas tree farmers. North Carolina Cooperative Extension specialists, county extension agents, and technicians have closely monitored the expansion of EHS in affected areas and educated growers on how to properly identify EHS. North Carolina Cooperative Extension research and demonstrations have targeted both chemical choice and application timing to determine the best control methods available to Christmas tree farmers in the region.

Several years of studies have determined that maximum EHS control is achieved when chemical applications of Dimethoate + Asana (esfenvalerate) are made in July, August, and early September. Mid-April through early May also provide good EHS control, and this timeframe also coincides with the typical treatment window for balsam twig aphid (*Mindarus abietinus*) (BTA) on Fraser fir. Recent NCCE research has also determined that late-summer and early-fall treatments can provide BTA control the following spring as well. This provides the opportunity to combine multiple pest treatments into one application through scouting and proper pesticide choice and timing of application. This approach exemplifies the Integrated Pest Management strategies applied in Fraser fir production in North Carolina.

Elongate hemlock scale

History of elongate hemlock scale in North Carolina

The elongate hemlock scale has been a problem pest of conifers in the eastern United States for many years. It was first observed in Queens, New York in 1908 (Sasscer, 1912) and has spread throughout the northeastern United States, west to Ohio, and as far south as North Carolina and Tennessee (Lambdin et al., 2005). The scale was first observed in western North Carolina at the Biltmore Estate in Asheville in 1993 (Sidebottom, personal communication), but was not documented causing damage to Fraser fir on Christmas tree farms until approximately 10 years later, when the scale began appearing on a small number of farms well north of Asheville. From 2003 through 2009, the scale continued to expand its range throughout the mountains of North Carolina, Virginia, and Tennessee, and it is now considered a serious pest of Fraser fir.

The increased activity of EHS has coincided with several weather patterns, including a period of drought and abnormally warm fall, winter, and early spring temperatures. These conditions possibly contributed to its rapid spread, due to the longer season of scale activity and more favorable conditions for survival. In addition, for many years North Carolina Christmas tree growers relied on Lindane and Di-Syston 15 G (disulfoton) for chemical insect control of the balsam woolly adelgid (*Adelges piceae*) (BWA) and BTA. Lindane has been demonstrated to control EHS with a single application, and granular Di-Syston has little effect on scale natural

enemies (Sidebottom, personal communication). Therefore, this common practice may have inadvertently kept scale numbers in check. Lindane was no longer manufactured by 1998, and supplies kept by growers dwindled by 2001, about the same time scale numbers rapidly increased.

Life cycle of elongate hemlock scale

This species overwinters as fertilized females or eggs which are deposited beneath a waxy cover (Lambdin et al., 2005). Females may continue to lay eggs through early summer. One female may produce a total of 20 eggs in a lifetime. In 3 to 4 weeks, eggs hatch into first instar nymphs called crawlers, which migrate to new needles on the same plant or are dispersed to other hosts through wind and bird movements. In the southern Appalachian mountains, EHS crawlers can emerge throughout the year, but peak release appears to occur in spring and early summer. Feeding and molting occur in place. Females have three developmental life stages after the egg, and males have five. During this time the scales continue to add to their protective covering, which is also covered by the waxy cuticle of the needle on which they are feeding. When mature, males emerge as tiny winged insects, mate with the female, and die. Mated females start to lay second-generation eggs 6 to 8 weeks after mating. Individuals that develop from these eggs mature and overwinter. Adult females may live up to 1 year (Lambdin et al., 2005).

Damage created by elongate hemlock scale

Low levels of EHS populations often go unnoticed due to few visible symptoms, unless scouting for other pests reveals the scale on randomly selected trees. These low levels of scales can be present for 1 to 3 years before the easily identified white waxy secretions are seen on the foliage of Fraser fir. Some farms have no visible signs of EHS one year, and the following year may have many trees with easily recognizable EHS.

Most growers find EHS on trees in midsummer while shearing and tagging trees. This is when the males are actively creating the white waxy secretions that are the most commonly recognized indications of EHS presence. This white coloration can make trees unmarketable if it is on a very large area of the foliage. This discoloration can be reduced or eradicated when chemical applications are made during the summer and early fall to control EHS. In some instances, scale feeding will result in needles with mottled yellow spots.

Control of elongate hemlock scale

The EHS is a difficult pest to control due to the protection provided to the insect by its scale covering as well as the covering of the cuticle of the needle on which the scale is feeding. The extended period in which the crawlers emerge also makes control more difficult without multiple chemical applications during the growing season.

Products such as Dimethoate and Asana are now commonly used to control Fraser fir pests, and both of these products provide control of EHS, especially when combined (Sidebottom, 2009). The addition of Prev-Am, which is a citric acid based adjuvant, can also help to increase control provided by either product. Control of EHS can be achieved either in the spring treatment window for BTA, which is in late April or early May, or with a summer application made between July and mid-September. Heavily infested fields may require applications during both treatment windows in order to achieve maximum control.

Balsam twig aphid and balsam woolly adelgid

Balsam twig aphid control with fall chemical applications

Traditional control of BWA and BTA on Fraser fir has been applied in spring prior to budbreak, which can be difficult due to weather and the busy spring workloads for growers. Spring treatments with materials such as Asana or Talstar (bifenthrin) have also created problems with secondary pests, such as hemlock rust mites (HRM) and spruce spider mites (SSM).

Many growers began to make chemical applications for BWA in the fall to reduce issues with mite flare-ups and to take advantage of cooler conditions for spraying and more time to schedule around the weather. After scouting the following spring revealed an absence of BTA, research was conducted to evaluate the effectiveness of fall treatments on spring BTA populations. Several years of observations have determined that Talstar in particular, when applied in the fall, will control BTA the following spring.

Balsam twig aphid life cycle

The BTA has an unusual life cycle, different from any of the other pests of Fraser fir. There are three distinct adult forms that are produced one after another (Nettleton and Hain, 1982). Each has a role in the life cycle, and each must be produced in succession for the life cycle to be complete. The first form, the stem mother, increases aphid numbers. The second form is winged and allows the aphid to spread to other areas. The final form produces the overwintering egg.

The aphid both “oversummers” and overwinters as a small, black, tear-drop shaped egg with white waxy rods covering it. Eggs can be found anywhere on the tree, but are most common on the shoots produced that year. These eggs begin to hatch in the spring from early March to late April. Unlike some insects that all emerge at the same time, it takes several weeks for all the BTA eggs to hatch. This is a survival mechanism for the aphid. If the spring is warm, the earliest hatching eggs will survive and quickly mature to the stem mother, who will begin to reproduce. However, if freezes occur after the eggs start to hatch and the young aphids die, more eggs will hatch later, ensuring the survival of the species.

The small green aphids that hatch from the eggs feed on the previous year’s needles. Each aphid molts three times, becoming a little larger with each successive molt. This form of the adult aphid is female. These stem mothers produce live aphids without male fertilization or eggs being laid. Their young are clones, genetically identical to the mother (Nettleton and Hain, 1982). This allows a quick buildup of twig aphids. Usually this occurs just prior to or just as the buds start to open. The adult stem mother and her offspring are easy to find, often feeding on the buds as they start to break.

The young aphids that the stem mother produces also go through three molts. At maturity some of them will be like the stem mother, reproducing greatly. However, at some point the stem mother’s offspring will be different, having wings at maturity. These are also all female and also lay live young.

Though this stage has wings, they are not as useful to the aphid as the wings of a fly or bee. They are only strong enough to lift the aphid away from the tree for the wind to blow it to another location. If it turns out to be another fir tree, the aphid will continue to feed. If not, it will die. These winged aphids are found in May and June in western North Carolina.

Balsam woolly adelgid life cycle

Sometimes incorrectly called the balsam woolly aphid, the BWA is actually very different from an aphid. Aphids continually walk around on a plant, probing plant cells with their feeding tubes. Adelgids are sedentary pests, much like a scale insect. The crawler is the only stage in the BWA life cycle that can move from place to place. All other stages feed from the same location from a feeding tube sunk into the bark. The feeding tube cannot be moved.

The BWA overwinters as an immature nymph (Balch, 1952). These are small, black, and tent-shaped with a row of short, white, waxy filaments running down the middle and around the edge. The nymphs start to mature to the adult in March or April. As nymphs mature, they get plumper and produce a woolly covering of wax to protect themselves from predators. The purple-black adult is completely hidden from view by this covering.

Honey-colored, oblong eggs are laid in a clutch behind the female. In the laboratory, females have been reported to produce as many as 200 eggs, but the number produced in nature is far fewer and may depend on the vigor of the host tree. Eggs hatch within a month to produce the next stage in the life cycle, the crawler.

The crawler is similar in appearance to the egg, only with eyes and legs. The crawler has no mouthparts. It must search out a suitable site to feed within several days or die. Once the crawler finds a suitable site, it never moves again. It molts in place to the nymph stage and sinks its feeding tube into the bark. The feeding tube is as long as the crawler. Feeding throughout the rest of the insect's life will occur in specialized cells just below the bark. The insect will eventually molt to the adult and lay eggs in that same spot. Although the nymphs and adults do not crawl about, legs are still apparent when the insect is examined under the microscope.

During the growing season, a nymph will molt to an adult in about 1 month. Two or three generations are produced each year (Arthur and Hain, 1984). More generations are produced at lower elevations. The different generations are not synchronous, and during the growing season all stages of the BWA—egg, crawler, nymph, and adult—can often be found. This complicates control, as the eggs are not affected by pesticide applications.

Chemical choice and timing for effective fall control of balsam twig aphid

Research was conducted over a 3-year period to evaluate all commonly used pesticides for control of BWA in Fraser fir and their effectiveness at controlling spring BTA populations with fall treatments. The chemicals evaluated were Asana, Astro (permethrin), Mavrik (fluvalinate), Thiodan (endosulfan), Talstar, and horticultural oil. Labeled rates of each product were applied with a high-pressure sprayer in August, September, and October, and follow-up sampling and scouting was done the following April and May to evaluate BTA control and to look for the presence of hemlock rust mites and spruce spider mites. Talstar consistently gave the best control, while Asana, Astro, and Mavrik also provided good control. The importance of Asana plus Dimethoate providing spring BTA control is due to its use for treatment of EHS during the late summer and early fall, which would be able to eliminate one pesticide application the following spring. Continued evaluation of the effectiveness of applications of Asana made for EHS and its effects on spring BTA populations will continue in 2010.

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Host resistance screening for balsam woolly adelgid: Early results from 13 fir species

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Abstract

Nearly all Fraser fir Christmas trees produced in North Carolina need to be treated one or more times during their 5- to 10-year rotation to prevent or lessen damage caused by the exotic balsam woolly adelgid (BWA; *Adelges piceae* Ratz.). These pesticide applications result in an annual cost to the industry estimated at \$US 1.53 million, not including direct losses due to BWA damage or increased miticide control costs associated with BWA treatments.

A BWA resistance screening trial was established in a greenhouse at the Upper Mountain Research Station in Ashe County, NC. The study included 13 fir species (4-year-old seedlings), some representing the range of known susceptibility and some of unknown susceptibility. After one season (summer/fall), one-half of the study was dismantled, and seedlings were brought back to the lab for assessment of first instar settlement. The following spring, the remainder of the study was dismantled, and the seedlings were brought back for assessment of BWA development and egg production. Early results are reported.

Introduction

Since its introduction onto Mount Mitchell in 1955, the balsam woolly adelgid (*Adelges piceae*) has caused 90–95% mortality of mature Fraser fir (*Abies fraseri*) in native stands. The initial wave of mortality has passed, and regeneration has taken place in the native range of Fraser fir. Cycles of mortality and regeneration are expected, with populations decreasing over time. Fraser fir is an important component of the Christmas tree industry, and BWA is a major pest in plantations, destructive and expensive to control.

BWA is a piercing-sucking insect specific to the genus *Abies*, and all fir trees are susceptible to this insect at some level. The basic biology of BWA is well known, but little is known about host resistance and the mechanisms of resistance.

Objectives

Our long-term objective is to develop BWA-resistant Fraser fir trees for native stand restoration and the Christmas tree industry.

Our short-term objective is to screen for resistance across multiple fir species (of equal age, grown under the same conditions, with insects from the same source) and to observe the reactions of both host and insect on the various species.



Figure 1. Young *Abies* seedlings in the greenhouse.

Methods

Thirteen (13) fir species, spanning the range of BWA susceptibility, were grown in a greenhouse for 4 years (Figure 1), then transported to an open greenhouse in Ashe County, NC. Species, listed by susceptibility (most to least), are: *A. fraseri* (3 seed sources), *A. balsamea*, *A. balsamea* var. *phanerolepis*, *A. lasiocarpa* var. *arizonica*, *A. koreana*, *A. procera*, *A. concolor*, *A. alba*, *A. firma*, and *A. veitchii*. Species representing unknown susceptibilities include: *A. bommuelleriana*, *A. equi-trojani*, and *A. pindrow*.

The experimental design:

- 4 blocks
- 13 species
- 5 treatment trees
- 2 time repetitions (August/September)
- 600 treatment trees
- 120 controls (treated with an insecticide)
- 720 total study trees

Trees were placed in six rows, with one treatment tree per species within each row (randomly assigned) and one control per species (randomly assigned) throughout the plot. In August and September, the trees were exposed to BWA by suspending three logs (2 m in length) of BWA-infested Fraser fir over each treatment plot, allowing crawlers to drop onto the trees under the logs (Figures 2 and 3), mimicking natural dispersal.



Figure 2. Logs suspended over one plot.



Figure 3. Greenhouse after the initial (August) infestation of trees with BWA.

After one season (summer/fall), one-half of the trees were cut, placed individually into plastic bags, and stored at 27°F pending assessment. The remaining trees were collected the following spring. Assessed variables included branch and bole length, life stages, egg production, and gout development.

Results

The results reported here represent the trees (N = 60) from Block 1, Rep 1 (August infestation) for both groups (Group 1: collected after 3–4 months; Group 2: collected after 7–8 months, following winter dormancy). These are very preliminary results.

BWA crawlers appear to settle preferentially on the buds of young fir trees (Table 1). In Group 1, in trees of almost every species, there were at least a few instars that were showing signs of molting to another phase of development (e.g., first to second or third instar), but none to the adult stage. Individual trees reacted to the insect in the form of swelling (gouting) at the feeding site, although over half of the species did not exhibit this reaction.

Table 1. Mean heights, BWA instar settlement sites, total settled instars, signs of development, signs of tree response to BWA (after one season, before winter dormancy).

Species	Height (cm)	Branch				Total BWA	Moulted Instars?	Gouting?
		Under Scales	Leaf base on branch	Buds	Other			
		----- (% of total BWA) -----						
<i>A. alba</i>	19	12	6	72	6	141	y	n
<i>A. balsamea</i>	30	7	15	76	1	188	y	y
<i>A. bals</i> var. <i>phan</i>	55.7	---	10	85	---	20	y	n
<i>A. bornmuelleriana</i>	17.75	13	20	58	3	144	y	n
<i>A. concolor</i>	31.9	17	1	48	11	148	y	y
<i>A. equitrojani</i>	24.5	6	1	57	7	138	y	n
<i>A. firma</i>	30.5	13	10	63	2	168	y	n
<i>A. fraseri</i> -Mt Mitchell	33.8	13	23	59	2	696	y	n
<i>A. fraseri</i> -Richland Bals	37	16	5	70	3	398	y	y
<i>A. fraseri</i> -Roan Mtn	40.4	12	8	65	4	373	y	y
<i>A. koreana</i>	37.4	10	1	62	3	217	y	n
<i>A. lasio</i> var. <i>arizonica</i>	19	---	7	90	---	29	y	y
<i>A. pindrow</i>	19.9	10	4	66	1	112	n	n
<i>A. procera</i>	29.5	23	7	60	1	162	y	y
<i>A. veitchii</i>	23.5	5	5	81	5	199	y	y

In trees from Group 2 (following winter dormancy), BWA adult development and egg production occurred primarily at the bases of buds, under old bud scales, and at the base of needles. Settled instars remained on virtually every branch, but adult development was often scattered, suggesting a high mortality rate in the first instar phase. Egg production per female varied widely within some species, e.g., 2–71 for *A. fraseri*, with the most extreme range in *A. equi-trojani*, with females producing 2–167 eggs. Females on *A. lasiocarpa* var. *arizonica* were somewhat more consistent in fecundity. However, within a given species, one tree may have few egg-producing adults, while another tree within the same species may have relatively high populations of egg-laying adults. For example, *A. balsamea* var. *phanerolepis* had one tree (393.5-cm branch length) with 4 adults and a total of 20 eggs, while another (382-cm branch length) had 131 adults with 2,064 eggs. The reasons for this range of development within a given species are not yet known; we need to assess more trees before any firm conclusions can be drawn. It may be as simple as placement within the treatment plot; one tree may have been in a more protected area.

No development to egg production took place on *A. alba* or *A. veitchii*, and very little on *A. koreana* and *A. bornmuelleriana* (Figure 4).

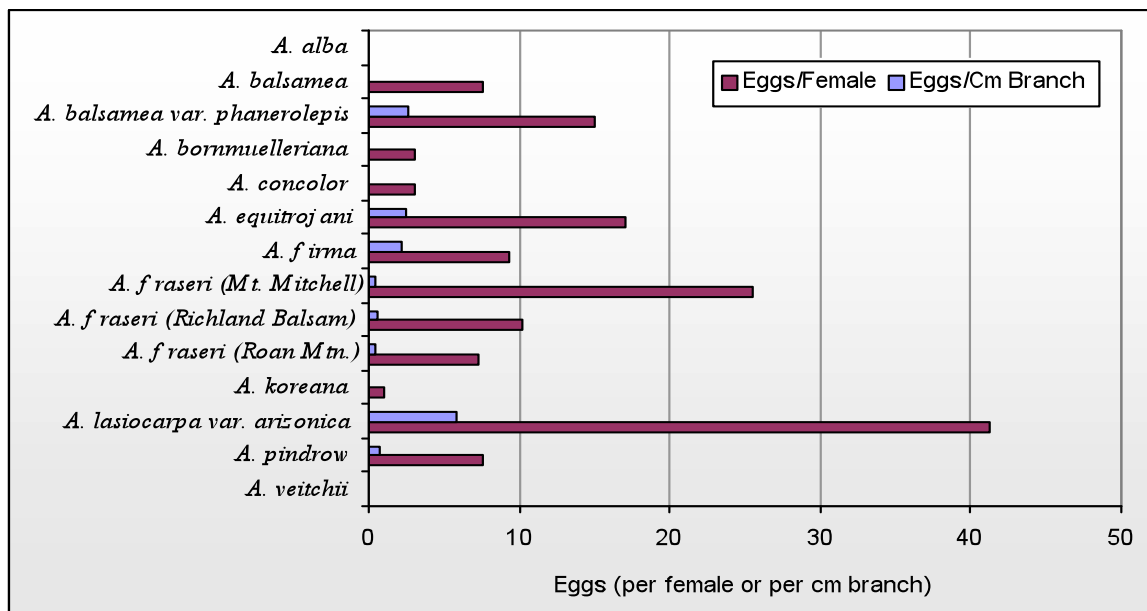


Figure 4. BWA development on branches—fecundity (eggs/female); eggs per cm branch.

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Predicting and timing of control for Douglas-fir needle midge in Michigan

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Abstract

In other parts of the country where Douglas-fir needle midge (*Contarinia pseudotsugae* Condr.) occurs natively, it can be a very destructive pest, and infestation of new needles can be as high as 100%. Needle loss is an especially serious problem because the value and salability of Christmas trees is determined largely by tree appearance. In plantations with severe infestations, we have seen intolerable needle loss, which has made the trees unsalable. Heavily infested trees may take several years to recover. Damaged needles are frequently bent and swollen. Initially, the damaged area is pale in color, but as the season progresses, it will darken and eventually turn brown. When trees are heavily infested, they will have no needles left by harvest time, making the trees unable to be sold or used for the greens market.

In Michigan, the first Douglas-fir needle midges were found in 2003 in Missaukee and Osceola counties. Since then, the pest has slowly spread to other locations in surrounding areas, but it may be more widespread than we realize. Michigan's market niche is its ability to provide customers with more than eight different species of Christmas trees. According to the Michigan Agricultural Statistics Rotational Survey, Douglas-fir is tied for second with Fraser fir with 7,600 acres grown in the state, accounting for more than \$US 5,000,000 in annual sales, plus the sales of greens for wreaths and roping. If we are unable to determine effective timing and control materials for Douglas-fir needle midge, this pest can potentially limit our ability to grow high-quality Douglas-fir to meet the market demand.

Materials and methods

Douglas-fir needle midge overwinters as larvae in the soil under infested trees. In early spring, larvae pupate, and adult midges emerge to mate and lay eggs in the needles of the expanding buds. Douglas-fir needle midge is a native invader that has expanded its range because of artificial transport into a new habitat. Like other invaders, it probably arrived without a complement of specialized natural enemies, e.g., parasitoids.

Since this is a new pest to Michigan, we need to determine when emergence occurs. To monitor the emergence of adult midges, we placed traps under previously infested trees in a 10-acre field of Douglas-fir at the end of April. We used several trap types (black plastic, white plastic, and cardboard boxes) to determine whether one was more effective than the others. Traps were monitored daily to determine first emergence, peak emergence, and when emergence ends. Weather data were collected to help time this emergence with growing degree days.

In 2008, once emergence was detected, we then evaluated the effectiveness of three different spray schedules.

Four treatment spray trials were set up in the plantation. The treatments were as follows:

1. Control (no pesticide application)
2. Spray at midge emergence
3. Spray at midge emergence and 5 days later
4. Spray at midge emergence, 5, and 10 days later
5. Spray at midge emergence, 5, 10, and 15 days later

(Note: The insecticide used was Lorsban 4E)

Results and discussion

The highest trap catches for the entire study were in the cardboard boxes on the north side of the tree (Figure 1). However, the first midges were detected in the black plastic traps placed on the south side of the tree.

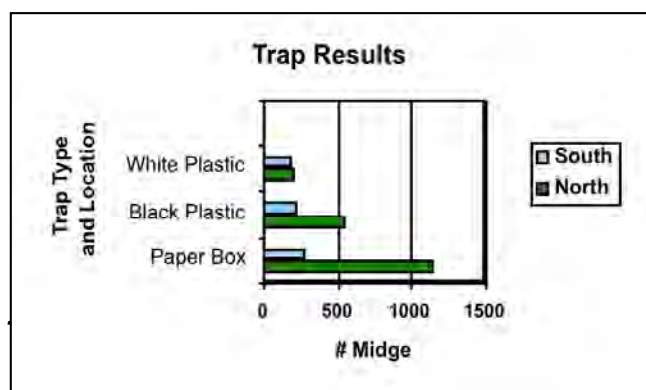


Figure 1. Trapping results by trap type and location under the tree.

We monitored for adult emergence in 2008 and 2009. Table 1 and Figure 2 show the trapping data for both years.

Table 1. Trapping results, 2008 and 2009.

	2008	2009
First midge adults	20 May (218 GDD50)	21 May (215 GDD50)
Peak emergence	3 June (330 GDD50)	1 June (273 GDD50)
Emergence ended	11 June (470 GDD50)	15 June (376 GDD50)

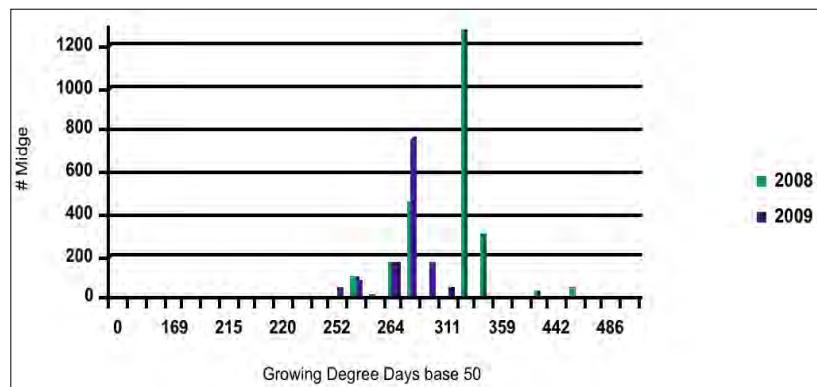


Figure 2. Trapping results, as related to growing degree days, 2008 and 2009.

We evaluated trees for damage levels in the different spray trials in September 2008. The damage criteria were none, slight, moderate, heavy, and very heavy. In both the heavy and very heavy categories, the trees would be considered unsalable due to needle loss. All of the insecticide treatments had significant control of Douglas-fir needle midge over the control block (no insecticides). However, there was no added benefit to spraying more than at emergence or emergence plus 5 days (Figure 3).

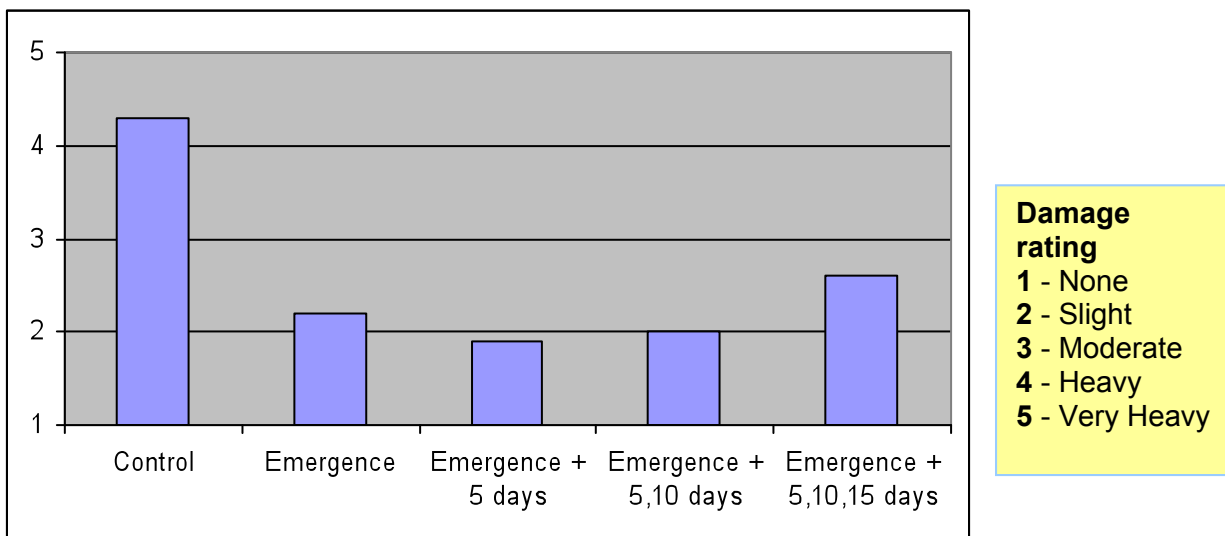


Figure 3. Comparison of Douglas-fir needle midge control with various spray timings.

In 2009, sticky traps were placed in the field. We placed red, blue, white, and yellow traps throughout the plantation. We found adults on the yellow sticky traps at the same times that we collected them from the other traps in the field. The other colors of traps were not as effective.

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Identifying alternatives to commercial deer repellents

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Introduction

Deer browsing continues to limit Christmas tree production in areas of western North Carolina. Research since 2006 has reduced the cost of deer-repellent treatments by evaluating bulk ingredients, spreader stickers, and alternative application techniques, including repellent fencing and mechanization.

Objectives

To evaluate commercial deer repellents and low-cost bulk alternatives and to evaluate alternative application techniques.

Materials and methods

Over the winter of 2008–2009, separate studies were conducted to evaluate bulk repellents, repellent spreader-stickers, and alternative methods of application. Blocks with uniform edges were selected at cooperating farms. Alternative repellent studies were installed at four farms. Treatment blocks were about 10 rows wide by the depth of the block, usually 15 to 24 trees. Three replications were installed at three farms and two replications at the fourth. Treatments included high, medium, and low rates of five alternative bulk repellents and two similar commercial products. Materials included egg powder, putrescent (inedible) egg powder, casein powder, spray-dried red blood cells, and anti-coagulated slaughterhouse blood. These materials were tested against egg- and blood-based commercial products: DeerStopper and Plantskydd. Treatments were applied using backpack sprayers once in early winter during November or

early December and again in late January or early February. Sites were monitored during the winter. Final evaluations were conducted in the spring prior to budbreak.

Studies were conducted at two farms to evaluate rates of Clearspray spreader-sticker and to compare brands of spreader-stickers used with deer repellent. Treatment blocks were about 8 rows wide by the depth of the block, usually 15 to 24 trees. Three replications were installed at one farm and two replications at the other. Deerstopper was used as the basic deer repellent across all spreader-sticker plots. Rates of Clearspray included 1.5, 3, 6, and 12 oz per gallon of water. Brands of spreader-sticker included labelled rates of Bond, Clearspray, Plyac, and Croplife. Treatments were applied using backpack sprayers once in early winter during November or early December and again in late January or early February. Sites were monitored during the winter. Final evaluations were conducted in the spring prior to budbreak.

Mechanization studies were conducted at two farms in 2008. A mistblower sprayer was used at one site (Figure 1). Mistblower treatments included broadcast spray across the block, perimeter spray, and 6-row band sprays with the cannon pointed down. An ATV with an electric sprayer was used at the other site. Treatments included spraying two rows from a rear machine bar and wicking two rows with a repellent-soaked paint roller (Figure 2). Both sites included check plots with no repellent and backpack sprayer checks with traditionally applied repellent. Plantskydd was used as the deer repellent in all treatments. Fifteen row blocks were used for the mistblower without replication due to space restrictions. Eight row blocks were replicated three times for the ATV. A repellent fence was included at the ATV site to protect an especially steep block. Treatments were applied once in early winter (November or early December) and again in late January or early February. Sites were monitored during the winter. Final evaluations were done in the spring prior to budbreak.



Figure 1. Mistblower sprayer.



Figure 2. ATV wick applicator.

Results

The bulk deer repellents performed as well or better than their commercial counterparts. Figure 3 shows the total number of deer bites counted (percent of trees browsed multiplied by the average bites per tree) per treatment weighted for different block sizes across the four 2008 studies. The two bulk egg products worked as well as Deerstopper. The spray-dried red blood cells worked as well as Plantskydd. Any blood product failed to provide full control at the McInnis farm under severe browsing pressure (green bars), although the egg products worked

well. Relying more on taste than odor, the hydrolyzed casein incurred more bites than either the blood or egg products but still provided a vast reduction in the number of trees browsed as compared to the check plots.

These studies failed to substantially answer the more complex question of identifying the optimum rate for each of these bulk materials. High, medium, and low rates of bulk and commercial repellents were applied at four of the five studies with medium and high rates applied at the fifth. Only at the Tucker farm did the amount of browsing increase slightly across repellents as the rate of repellent decreased. At the other farms, repellent rates ranked inconsistently.

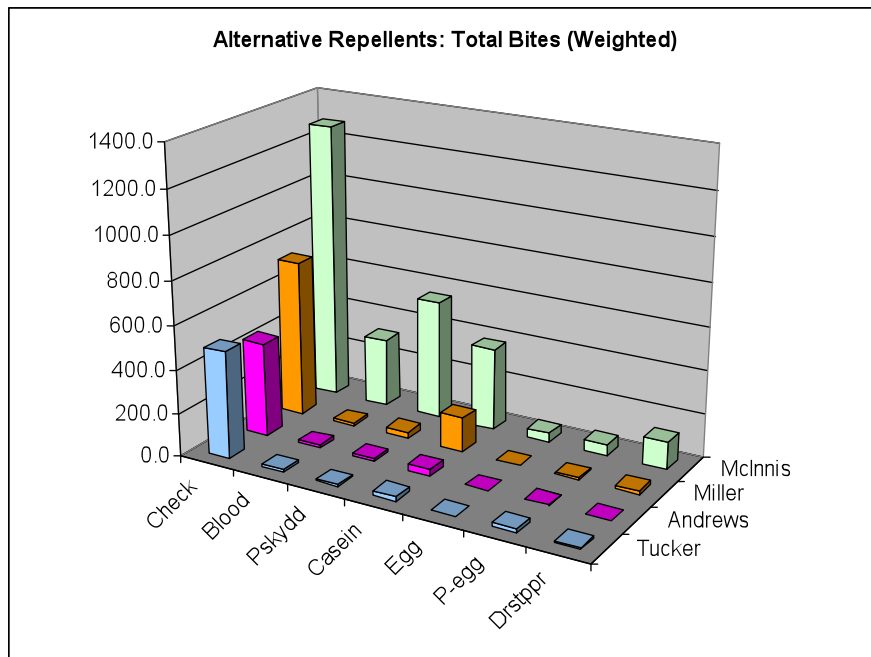


Figure 3. Total deer bites counted at four 2008 alternative deer-repellent studies.

Another question we sought to answer in 2008 was the importance of spreader-stickers. No trend was visible among the rates of Clearspray applied despite extensive browsing in the check plots. The one plot treated with Deerstopper without any Clearspray did about as well as those treated with different rates of sticker. Results of the alternative sticker studies at two farms also reflected fairly uniform repellent control. Check plots at both sites were heavily browsed. At the Tom Miller site, all spreader-sticker treatments worked across three replications with almost no bites in treated areas. At the Lovern site, Bond, Clearspray, and Croplife may have performed slightly better than Plyac. Unfortunately, treatments of Deerstopper alone were not included in either of these studies. Further work is needed to definitively identify the need for and choice of spreader-stickers.

The results of the alternative sprayer studies can be seen in Figure 4. The ATV and Mistblower sprayer treatments reduced browse damage compared to the checks but incurred slightly more browse damage than the conventional backpack sprayer treatments. The differences among unreplicated mistblower treatments may reflect differences in deer traffic across a long narrow block more than repellent activity. The six-row treatment applied more repellent per acre and should have provided slightly more protection, but was located along the most-travelled deer trail.

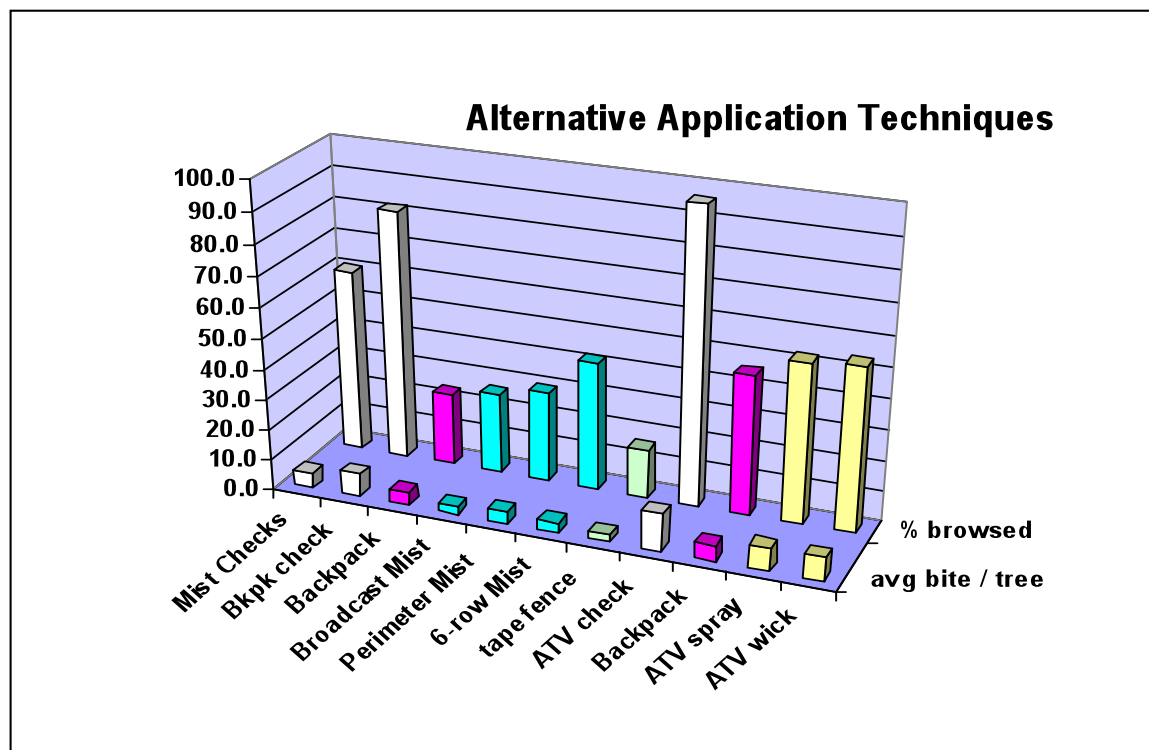


Figure 4. Combined results of two 2008 alternative sprayer studies.

Discussion

The alternative bulk repellent studies presented here show that several bulk materials can be used in place of expensive commercial products. The inedible egg powder and spray-dried red blood cells are both processed for pet and livestock feeds. Even with shipping and handling, these materials cost less than 10% of their commercial counterparts. The costs of different commercial and bulk materials are represented in Figure 5.

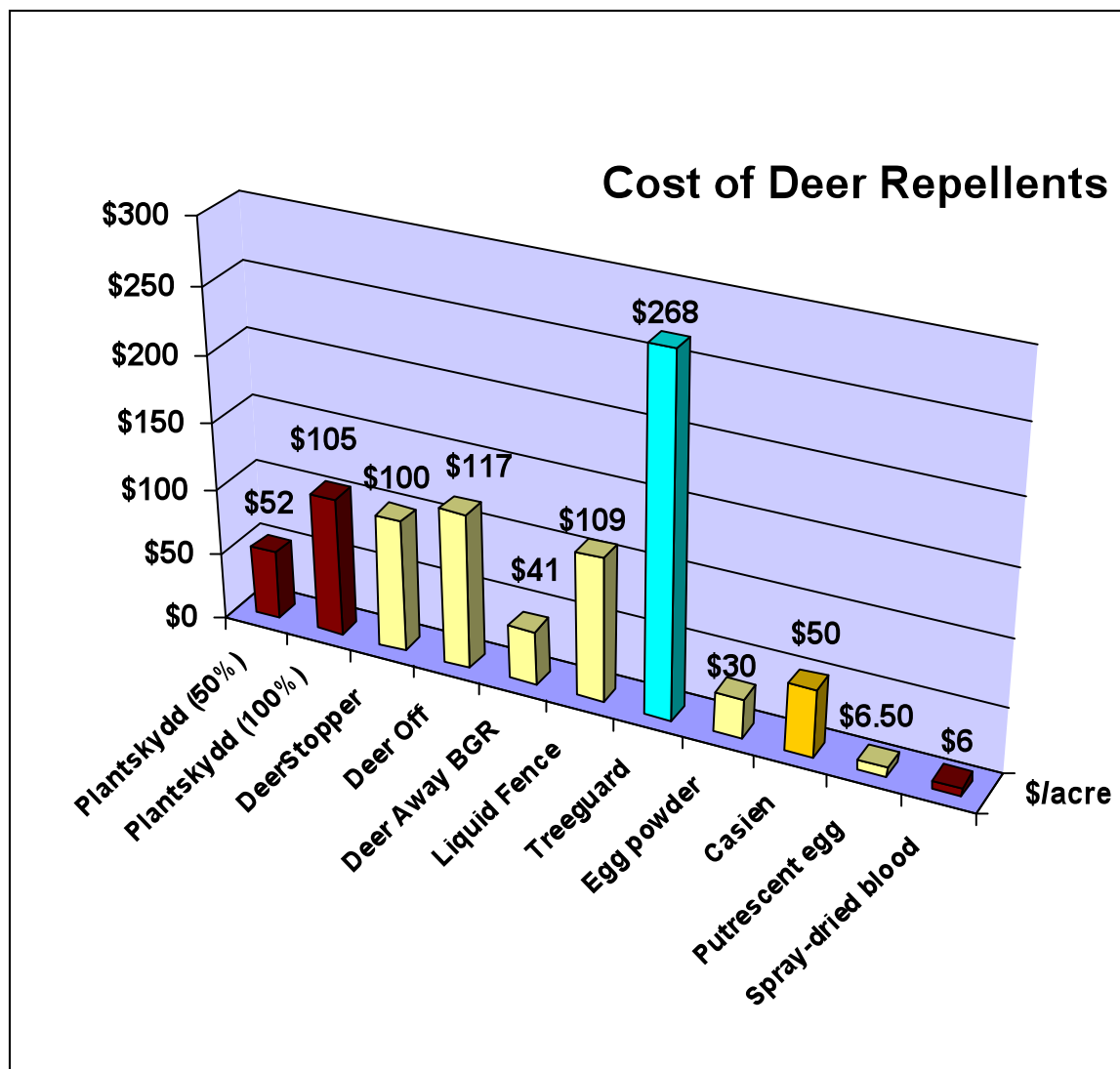


Figure 5. Cost of commercial and alternative bulk deer repellents per application.

While additional research will be needed to satisfactorily identify optimum rates of bulk repellents, we do have more than 1 year's evidence that these materials work. With the low cost of both blood and egg materials, a grower could use the high rate (9–10 pounds per acre) and still save most of the cost of commercial equivalents. While the lighter rates may work in most places, the higher rates represent less risk of deer becoming accustomed to the product.

Another question we sought to answer in 2008 was the importance of spreader-stickers. Currently, we use 6 ounces of Clearspray per gallon of repellent mix to help glue the repellent to the foliage. If 10 gallons of repellent are applied per acre, about half a gallon of Clearspray would be used at a cost of \$13, or about equivalent to the cost of the bulk repellents. Using less or a cheaper sticker could make repellent application more affordable and represents the greatest additional cost savings once bulk repellents are selected.

Both forms of mechanized application worked but also represent increased risk of deer browsing. The mistblower applications dilute the rate of repellent applied to the foliage of trees. The ATV sprayer applications put out a similar rate to backpack sprayers but did not provide as uniform coverage. We observed more deer browse damage on lower branches in ATV plots than is typically seen with backpack applications. Mechanized sprayers may have a role where deer browsing pressure is light or where manual labor is unavailable.

Perhaps the most important and oft-repeated result of the 2008 deer-repellent research is the consistent damage in our check plots. Except for the one site deer did not touch, every check plot incurred greater damage than our repellent treatments. Not only were the percentage of trees browsed approaching 100% at many check plots, but many trees had few terminal branches or buds left. With few remaining buds, these trees will take several years to recover if they survive at all. The case for protecting Fraser fir Christmas trees from deer browsing by any means couldn't be demonstrated more clearly.

CULTIVATION TECHNIQUES

Test of various methods of application of NAA for leader length retardation on Nordmann fir

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Introduction

In Denmark, as well as in most other European and North American countries, there is increased concern for controlling the leader growth on various species of Christmas trees. Many growth retardants have been tested for that purpose during the past 20 years. Today most growers prefer to use naphthyl acetic acid (NAA).

In Denmark, a NAA product called TopStar was registered by the Danish Environmental Agency in 2008 for reducing leader growth on Christmas trees of Nordmann fir (*Abies nordmanniana*).

Experiment

In an experiment in Denmark in 2008, three different methods of application were tested: (1) spraying of the leader with a small compression sprayer, (2) roller application on the leader with the "Easy Roller," and (3) over-top spraying of whole trees.

The experiment was designed as a randomized block experiment with four blocks. All treatments were made with a concentration of 1½% of trade formulation, which has been recommended for leader application in Denmark for many years.

Results

All three methods gave the same and a highly significant growth reduction of the leaders compared to the untreated control (Figure 1). None of the treatments affected the length of the side shoots.

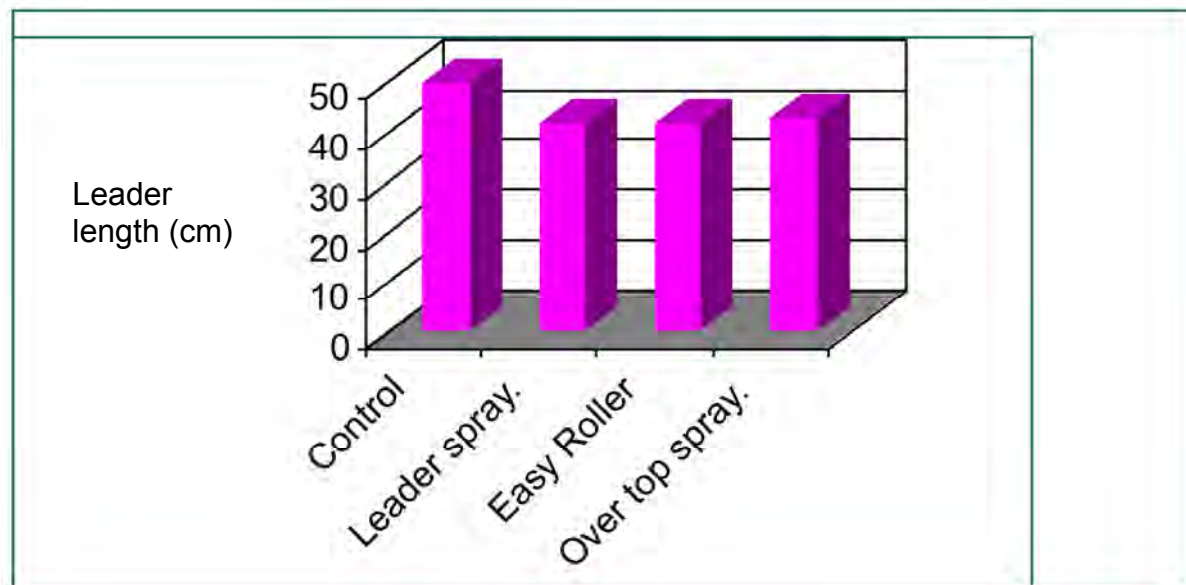


Figure 1. Growth regulation of leaders on Nordmann fir, mid-June (1½% TopStar).

All three application techniques might produce discoloration of the needles on side shoots, while discoloration of the leaders was of minor importance. Both the leaders and side shoots might have some bends and curves if the application is not carried out at the appropriate stage of shoot development (Figure 2). To minimize discoloration and bending, application should take place when the leaders have achieved a length of 11–15 cm.

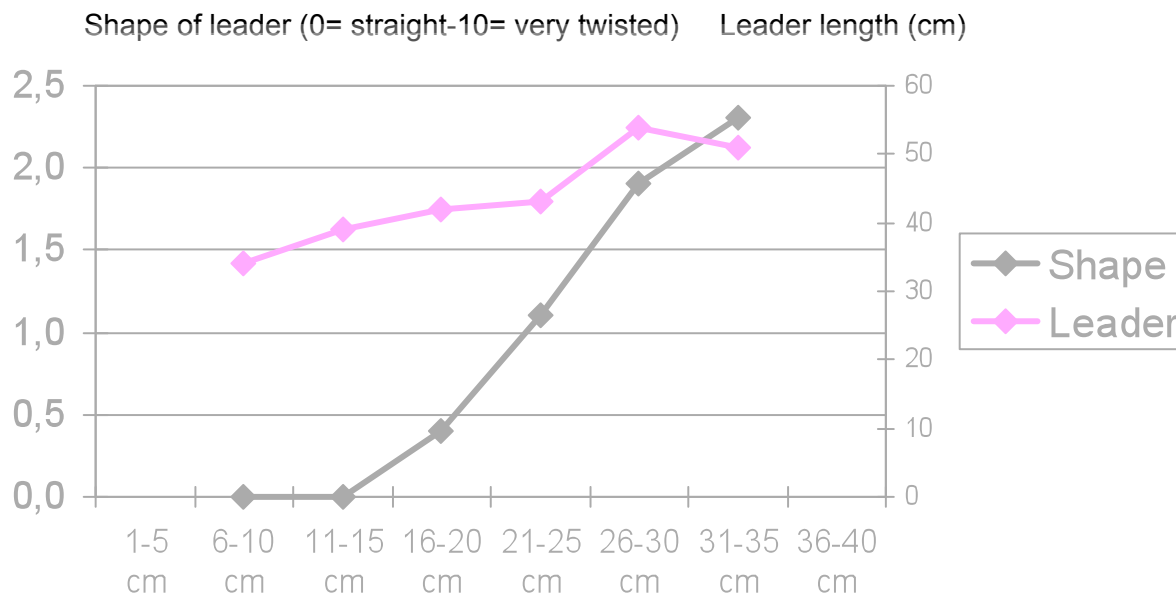


Figure 2. Leader spraying on Nordmann fir, mid-June. Importance of leader length at application.

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The influence of needle location and sampling technique on nutrient concentration

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Crop nutrient assessment using tissue sampling relies on two standard protocols: sampling when nutrient concentration is relatively stable and selecting plant tissue related to plant nutrient sufficiency. These protocols for Christmas trees vary by growing region as shown by needle sampling instructions.

All needle sampling instructions share the directive to sample current-season growth from the upper part of the tree. In some production areas, growers are told to sample the north side, south side, or just the top of trees. Michigan growers are told to collect 2–3 oz of needles from current-year foliage located in whorls on the upper one-third of the crown on the north side of the tree (Rothstein, 1996). Danish growers are directed to take needles from the upper whorl pointing southward (Pederson et al., 2006). In Pennsylvania, growers are told to clip 20 to 30 sections (2 to 4 inches long) of current-season growth from the top third of the trees (Bates, 2002). Instructions in North Carolina are similar (Rideout, 2002): collect at least 40 inches of shoots by clipping two shoots of new growth per tree from the upper one-third of the tree.

We were curious whether needle position influences nutrient concentration.

Nutrient concentration by whorl

To examine needle nutrient concentration difference by whorl, needles were collected separately from the top three whorls of noble fir (*Abies procera*) Christmas trees for 3 years from five locations. Nutrient concentration for whorl position is given in Table 1. For statistical analysis, sites were used as a replicate. Nutrient concentration differences in a column are noted by differing letters after the concentration. Columns without letters following the concentration did not have different nutrient concentration by whorl position, $p = 0.05$.

Table 1. Noble fir needle nutrient concentration by whorl.

Whorl	Nutrient										
	N	P	K	S	Ca	Mg	B	Mn	Zn	Fe	Cu
	----- g/kg -----					----- mg/kg -----					
Top	13.1	1.5	9.9a	1.3a	3.2b	1.1	39a	418	39	152	4.1a
Second	13.8	1.5	7.9b	0.9b	3.7b	0.9	31ab	368	33	115	3.7b
Third	13.6	1.5	7.0b	0.8b	4.6a	0.8	27b	353	33	113	3.7b

The small differences in needle nutrient concentration and lack of difference for the nutrient of primary concern, N, did not provide a reason to direct needle collection from a specific whorl.

Top versus bottom of tree

A second comparison of needle nutrient concentration was made by collecting needles in September from the top and bottom of Douglas-fir (*Pseudotsuga menziesii*), noble, grand (*Abies Grandis*), Nordmann (*Abies Nordmanniana*), and Turkish fir (*Abies bornmuelleriana*) Christmas trees.

Table 2 shows nutrient concentration from samples taken from the upper half and lower half of five Christmas tree species. Needle nutrient concentration in bold typeface is different from top to bottom.

Table 2. Comparison of needle nutrient concentration for needles collected from the top half or bottom half of five Christmas tree species.

Tree	Position	Nutrient									
		N	P	K	S	Ca	Mg	Cu	B	Mn	Zn
		g/kg					mg/kg				
Noble fir	Top	10.9	1.6	6.7	0.7	3.7	1.2	2.31	37.7	69	20.9
	Bottom	10.4	1.7	5.2	0.6	4.6	1.2	2.09	42.5	68	18.8
Grand fir	Top	14.6	2.0	8.3	0.9	9.0	1.8	3.53	71.6	112	24.9
	Bottom	15.6	1.8	6.6	0.8	11.3	2.0	2.72	71.8	106	18.0
Douglas-fir	Top	14.9	1.3	8.8	0.7	3.1	1.2	3.66	13.5	116	19.2
	Bottom	15.3	1.3	8.1	0.8	3.4	1.3	3.46	12.6	110	17.7
Nordmann fir	Top	15.3	1.4	9.6	0.6	2.7	1.0	3.44	22.9	67	24.0
	Bottom	14.5	1.4	8.1	0.5	4.2	1.3	2.03	17.3	85	28.4
Turkish fir	Top	14.6	1.5	10.3	0.7	4.0	1.3	3.97	15.1	79	28.5
	Bottom	14.0	1.3	7.6	0.6	5.1	1.6	3.10	11.9	79	32.9

Our standard recommendation has been to sample the upper one-third of the tree. This recommendation is being kept since needle nutrient concentration differences between the top and bottom positions were measured in four of the five species sampled.

Needles compared to small branches with needles attached

A third comparison of needle nutrient concentration was made by collecting needles and 15-cm long branches in January from Douglas-fir, noble, grand, Nordmann, and Turkish fir Christmas trees. A total of 11 samples were collected from 4 locations. In addition, Fraser fir (*Abies fraseri*) needles and small branches were collected from the upper one-third of the same 10 trees each month by clipping a single 10-cm branch section from 1 location.

The relationship of branch-with-needles-attached and needles alone on N and K concentrations for Douglas-fir, noble, grand, Nordmann, and Turkish fir Christmas trees is shown in Figure 1. The N needle-branch line slope of 0.75 is low enough to influence N sufficiency interpretation and subsequent recommendations for N addition. Using current needle N standards, N would likely be recommended even when no increase in color or economic return would be gained.

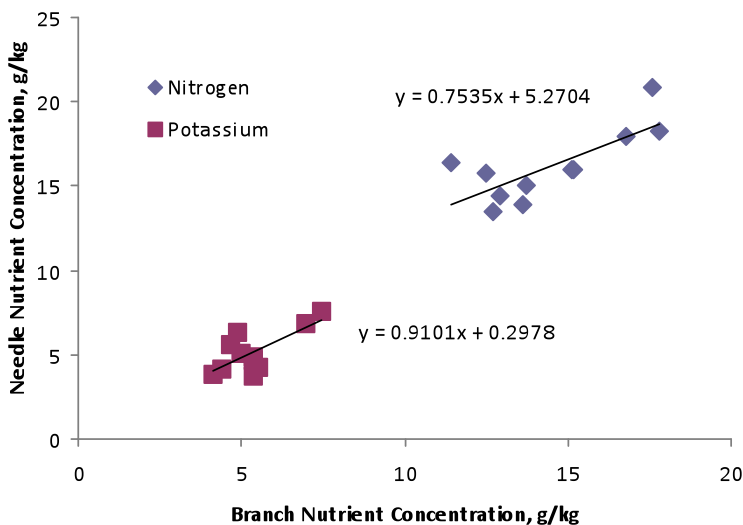


Figure 1. The relationship of N and K concentration for five Christmas tree species from two needle sampling techniques: branches with needles attached and needles only.

The temporal relationship of Fraser fir N concentration of branches with needles attached and needles is shown in Figure 2. The differences are of sufficient magnitude to influence interpretation of N sufficiency. The branch-with-needles-attached and needle P concentration for Fraser fir is shown in Figure 3. This relationship is similar to the branch-with-needle and needle P relationship for Douglas-fir, noble, grand, Nordmann, and Turkish fir Christmas trees and for the relationship of other nutrients in Fraser fir.

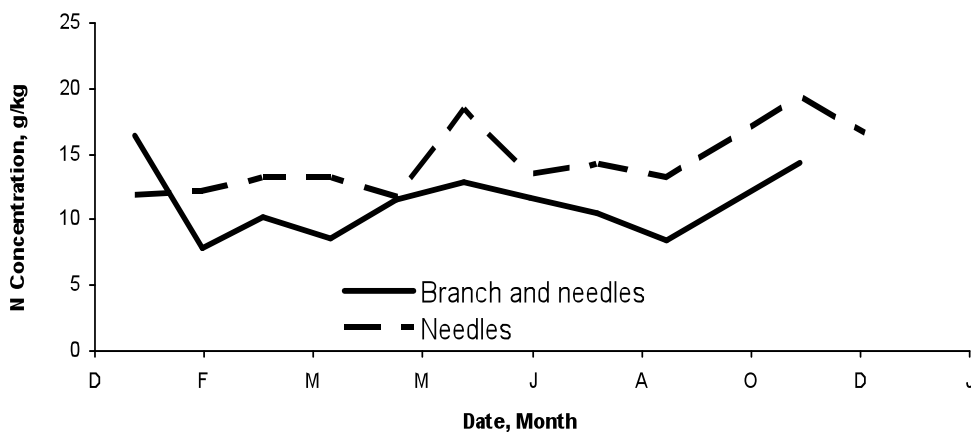


Figure 2. The comparison of temporal N concentration in Fraser fir using two needle sampling techniques: branches with needles attached and needles alone.

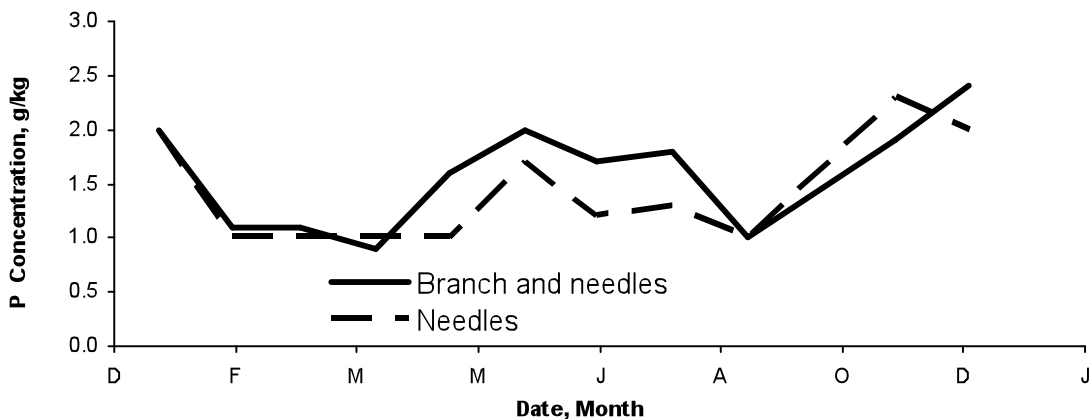


Figure 3. The comparison of temporal P concentration in Fraser fir using two needle sampling techniques: branches with needles attached and needles alone.

Currently, branches with needles are sampled in North Carolina and Pennsylvania. Samples are dried and needles are removed from branches before analyzing for nutrients. This practice removes complications of branch analysis while capturing ease of sampling.

Some Danish advisors and laboratories are sampling and analyzing branches with needles attached. This practice may provide similar results in a single-species growing area, especially if the N concentration is high, approaching 20 g/kg.

Conclusion

Nutrient concentration differs little with whorl position, differs between top and bottom of trees for many nutrients of most species, and differs between branches with needles attached and needles alone for N and Mn. For Pacific Northwest growers, we recommend collecting needles from the upper one-third of trees by pinching or removing individual needles, since laboratories are not accustomed to drying and removing needles from short branches.

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Growth, quality, and economic value of Fraser fir sheared with varying leader lengths in North Carolina, USA

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In the early years of Christmas tree production, Fraser fir (*Abies fraseri* [Pursh] Poir.) was harvested mostly as wild trees from isolated mountain peaks in western North Carolina, southwest Virginia, and eastern Tennessee. Later, trees were grown in plantations well below the elevation of natural stands, and shearing became a standard cultural practice. As the years passed, shearing intensity increased and tree density became heavier in response to consumer preferences. Stands normally are harvested over 3 years, with the fastest growing trees harvested first. Using this harvest system, large Christmas trees historically have been produced by high-grading plantations, i. e., the best trees were removed first (thinning from above), usually at a height of 6 to 8 feet (1.8 to 2.4 m), and the lowest quality, slower growing trees were retained longer to eventually yield taller trees.

Shearing studies were initiated in 1998 to examine the effect of shearing date on growth and quality of Fraser fir (Hinesley and Derby, 2004a, b). Those studies, as well as others, showed that the ideal time to shear is July and August, soon after cessation of shoot elongation (Brown and Heiligman, 2002; Powell, 1982). Perhaps most significant, shearing in July and August using a 12-inch (30-cm) leader reduced growth by an average of 38% compared to non-sheared trees; if done later, say October, the reduction was $\geq 50\%$. This suggests that the industry should consider “lightening up” shearing intensity, i.e., leave longer leaders.

During the past 15 years, I have suggested that Fraser fir growers dedicate a portion of their production to trees with a more open density. The value of a tree is determined first by its height; second by its density. If trees can be “stretched” during shearing (i.e., use longer leaders), they reach commercial height quicker and/or grow to a taller size in a given number of years, which reduces costs and increases profit. The objective of this study was to determine

the effect of leader length on growth, quality, and economic value of Fraser fir Christmas trees in North Carolina.

Experimental design

The experiment began in 2001 when trees were 3 to 4 feet (0.9 to 1.2 m) tall. Trees were selected for uniformity from a 30 row x 23 column area. Each of the 30 rows contained 9 shearing treatments (Table 1) and two replications (single-tree plots) of each treatment.

Table 1. Leader lengths used 8 years in Fraser fir Christmas trees.

Tmt #	Ht when shearing began ^z (ft)	Leader lengths, by year							
		2001	2	3	4	5	6	7	2008
		inches ^z							
1	3	14	12	10	10	10	10	10	10
2	3	12	12	12	12	12	12	12	12
3	3	14	14	14	14	14	14	14	14
4	3	16	16	16	16	16	16	16	14
5	5	--	12	12	12	12	12	12	12
6	5	--	14	14	14	14	14	14	14
7	5	--	16	16	16	16	16	16	14
8	3	18	16	14	12	12	12	12	12
9	5	--	18	18	18	18	18	16	14

^z 1 m = 3.28 ft; 1 inch = 2.5 cm.

Measurements

Height and USDA grade have been recorded for each tree annually since 2005. Basal stem diameter (5 to 7 cm above groundline) was measured on all residual trees in 2008. Fresh weight was recorded for each tree harvested in 2008. The value of individual trees was based on its commercial height, USDA grade, and the price schedule of the grower.

Harvesting

Harvesting began in 2005. Approximately equal numbers of trees were removed annually from each treatment (Table 2) to maintain adequate growing space for residual trees. About one-third of the trees were still present after the 2008 harvest. The tallest trees were 14 to 15 feet (4.3 to 4.5 m) in 2008. In the analysis, the stand was assumed to have been clear-cut in 2008.

Table 2. Height, USDA grade, stem diameter, and value of Fraser Fir sheared with various leader lengths^z.

Shearing tmt	no. trees	Height ^y (ft)	USDA grade ^x	Stem diam. ^w (in.)	Wholesale value (\$)
1	60	8.9	1.05	4.92	59
2	60	9.4	1.22	5.00	74
3	60	10.6	1.32	5.47	106
4	60	11.3	1.42	5.51	129
5	60	9.9	1.25	5.08	81
6	60	10.8	1.30	5.47	116
7	60	11.6	1.35	5.63	138
8	60	10.6	1.25	5.51	112
9	60	12.2	1.58	5.67	133

^z Harvesting began in 2005 and ended in 2008.

^y 3.28 ft = 1 m; 1 inch = 2.5 cm.

^x Value of 1.05 = 95% USDA premium or grade 1, and 5% grade 2;
Value of 1.58 = 42% USDA premium or grade 1, and 58% grade 2.

^w Computed for residual trees in July 2008; n = 24 to 34.

Results, conclusions, and application of results

- To effectively use a shearing system with longer leaders, soil nutrient levels and pH must be optimum based on soil testing and proper fertilization. Otherwise, budset and branch production will be too sparse to yield dense trees.
- There is huge variation among trees sheared alike, emphasizing the importance of genetics in tree performance. Response to a particular shearing method might also vary among genotypes (i.e., shearing x genotype interaction). Research is underway at another location to examine that hypothesis.
- Short leaders (10 and 12 inches, 25 to 30 cm) result in many more “horns” in the top whorl compared to longer leaders. This is likely an issue with dominance and sink strength. Earlier research with Fraser fir (Hinesley and Snelling, 1995) showed that removing lateral buds from the leader reduces its growth and tends to shift growth into branches of the upper whorl.
- Longer leaders yielded taller trees—a plus (Tables 2 and 3). On the other hand, average tree grade tended to decrease—a negative. Looking at the extremes (Table 2), 95% of the trees harvested from Treatment 1 were USDA grade 1 or premium, compared to 42% for Treatment 9. The value of a particular shearing regime therefore depends on the trade-off between larger trees of lower average grade versus smaller trees of higher average grade. The average value of trees consistently grown with 10- to 12-inch

(25- to 30-cm) leaders (Treatments 1, 2, and 5) was \$71, compared to \$133 for those grown with 16 to 18-inch (40- to 45-cm) leaders (Treatments 4, 7 and 9; Table 2).

Table 3. Height and fresh weight of Fraser fir Christmas trees harvested in 2008.

Shearing Tmt	No. trees	Avg height (ft)	Fresh weight (lbs)
1	12	9.6	107
2	5	10.1	94
3	13	11.9	139
4	19	12.6	147
5	10	10.6	117
6	11	11.9	132
7	14	12.7	151
8	11	12.1	146
9	12	13.4	150

^z 3.28 ft = 1 m; 2.2 lb = 1 kg.

- In the beginning, we hypothesized that using longer leaders would yield taller trees with less weight and smaller trunk diameters. In agreement with earlier research (Hinesley and Derby, 2004a, b), results confirmed that tight shearing (i.e., short leaders) greatly reduced the potential growth of Fraser fir. Trees sheared with long leaders (Treatments 4, 7, 9) were about 39% heavier than those with 10- and 12-inch leaders (Treatments 1, 2, and 5) (Table 3). They were also 3 to 3.5 feet (1 m) taller, but had a lower average grade (Table 2).
- Longer leaders increase the risk from factors such as late frost. Freeze damage can cause uneven density or a tiered appearance, which reduces quality and value.
- Minimum leader length should be 14 inches (35 cm) for trees ≥ 10 ft tall (3 m). With shorter leaders, it is harder to avoid “shoulders” in the upper crown.
- Growing larger trees intermixed with a smaller crop requires relatively narrow taper to prevent crowding and thinning of crowns at the base. Narrow crowns have some advantages: (1) less weight per tree, (2) smaller diameter bales (more trees per load), and (3) ability to display trees in smaller areas of a room, especially in corners. To the extent possible, when deciding which trees to remove or leave, retain the highest quality trees as long as possible.
- For trees selected to be grown taller during the rotation, delay onset of shearing until they are 5 ft (1.4 m) tall. It is a mistake to initiate shearing of Fraser fir when trees are too small. If a tree is targeted to be grown to 10 ft (3.0 m) or more, there is no

justification to begin shearing when it is less than 3 ft (0.9 m) tall, assuming no defects or injuries requiring corrective pruning. For example, in trees that were sheared with 16-inch (40-cm) leaders, delaying shearing from 3 ft (Treatment 3) to 5 ft (Treatment 7) increased harvest weight by almost 10% (Table 3).

- The best way to realize an advantage from longer leaders is to identify early in the rotation the best trees (perhaps 5% to 10% of the crop), and shear those to produce taller trees during the rotation. Good genotypes naturally grow fast and are dense, often requiring less shearing than regular trees. By identifying the best trees early and shearing them with a different system, one can capture a greater portion of their growth potential. This approach, combined with better genetic selection of fast-growing, high-quality stock, can help maximize profit and efficiency. Ultimately, the greatest potential gains will arise by applying optimum shearing systems to clonal material.

Literature

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Bud removal for tree shaping: Hormonal and growth pattern effects

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In a conifer tree, such as Nordmann fir, *Abies nordmanniana* Spach, the leader bud and its immediate surroundings play a decisive role in growth allocation between stem and branches and thus in the tree's crown shape and ornamental value. The hormonal relationship between tree-top buds in young trees was recently studied in their natural state and after surgical removal in early July of either the leader bud (decapitation) or the subapical whorl branch buds (destipitation).

The two bud types showed consistent cytokinin profile differences but parallel seasonal dynamics in cytokinins and auxin (IAA). After bud excision, a succession of zeatin-group cytokinins increased dramatically in the stem. Supernormal levels were maintained through autumn and persisted in spring in the destipitated trees, but had returned to normal in the decapitated trees. The treatments also affected the remaining tree-top buds; after a short wound

reaction, these buds experienced a large surplus of cytokinins. The following spring, this high level persisted in the leader bud of destipitated trees, but not in whorl buds of decapitated trees. Conspicuous growth pattern changes followed from destipitation, few from decapitation. The former included increased leader length and width, higher bud set, and larger new buds. Growth reactions suggest that resource allocation to main branch buds inhibits leader growth in normal trees, a kind of “lateral control.” Data suggest that subapical leader tissues beneath the apical bud group are a primary source of cytokinin regulation.

Introduction

Growth allocation is an important quality feature in trees. In Danish Christmas tree production, about 10% of the production cost goes into correcting tree crown shape; nevertheless, an estimated 20% of trees are rejected because of poor shape. In the United States, 37–53% of potential tree growth is spent in correcting tree shape by shearing (Hinesley and Derby, 2004).

Growth allocation is also a challenging biological question. Among the multitude of growth points in the tree crown, how are resources distributed in such a manner that the inherent architecture of the species in question is expressed and optimized for individual survival?

In the conifer tree architecture, almost everything depends on the way in which the leader bud interacts with the whorl of main branch buds that surrounds it in the extreme tree top. These whorl buds are the immediate offspring of the leader meristem. Clearly they are founders of the branch system that comprises a tier: if they receive many resources, the branches grow long and the tree gets broad. If they receive much compared to the leader bud, the tiers of subsequent years will tend to be closely set, and the crown will thus turn dense. In contrast, strong leader growth that leaves less to the branches results in a slender and open crown with widely spaced tiers (Figure 1). Presumably, the relationship between the leader bud and the subapical whorl buds may change over the years to accommodate passage through the tree’s life phases.

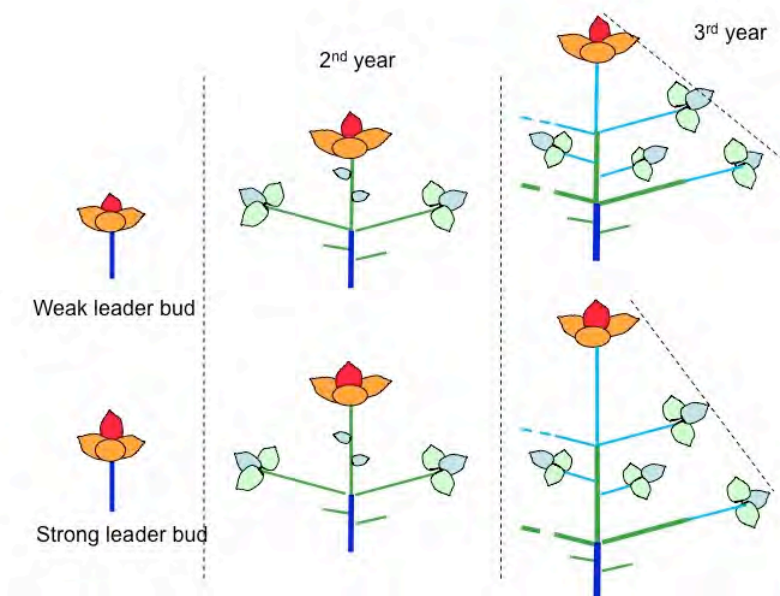


Figure 1. Crown shape consequences of growth allocation between leader bud and whorl buds. Top: a weak leader meristem, favoring whorl bud development. Bottom: a strong leader meristem, leaving proportionally less resources to its whorl buds, resulting in a steeper outline and a more open crown. Crown shape differences are subtle at first, but tend to increase over time.

If there is a natural trade-off between slender crown shape and density, and the tree may change its allocation pattern over time, how can the ideal of a slender and regularly dense tree

be obtained for Christmas tree production? An understanding of the principles of growth allocation, and ultimately tools to regulate it, could solve our problem. Thus, we need to understand how buds acquire their identity and influence each other within the tree. What makes a leader bud a leader and not a branch whorl bud?

We have previously learned that a leader bud stripped of the neighboring whorl buds will produce a new leader that is about 10% longer and 20% wider than normal, and sets as much as 300% more interwhorl buds (Rasmussen et al., 2003 a, b). Now we wanted to understand more about the hormonal processes that had such dramatic morphological effects.

The objective of this study, more fully detailed in Rasmussen et al. (in press), was to investigate the relationship between the leader bud and the subapical buds. We wanted to learn if there were natural hormonal differences between bud types and whether we could influence one bud type by interfering with the other.

Methods and materials

Four-year-old trees in pot culture and ambient growth conditions were used. In early July, when the buds had just differentiated on the expanding shoots, we decapitated trees by removal of the young leader bud. Other trees we destipitated by removing the developing whorl buds. We monitored treated trees hormonally by subsequent destructive random sampling, 1 hour, 1 week, and several months after treatment, the last sample being taken just before budbreak the following spring. In autumn, growth performance in the tree top was recorded. A set of similar control trees was monitored by regular seasonal samplings to obtain a background pattern. Plant samples were analyzed using high-pressure liquid chromatography and electrospray tandem mass spectrometry (Rasmussen et al., 2009); results are presented in picomol per g fresh tissue weight.

Results and discussion

Leader and whorl buds at time of excision

Overall cytokinin concentration was about two times higher in the leader bud tissues than in the whorl buds (Table 1). The great majority of cytokinins consisted of zeatin, its riboside and ribotide forms, primarily zeatin riboside, a compound with high biological activity and mobility. In spite of that difference in cytokinin levels, the developing whorl buds contained about two times as much cytokinin glucosides. These are considered to be temporarily or permanently inactivated forms. The cytokinin differences between bud types were maintained throughout the season of development (Rasmussen et al., 2009). Studies of cytokinin-metabolism mutants in other plant species have shown a relationship between shoot apical meristem size and cytokinin content (Matsumoto-Kitano et al., 2009), and recent research also suggests a role for cytokinin in determining bud identity (Kyojuka 2007).

Table 1. Nordmann fir leader bud versus whorl buds at time of surgery (9 July). Data analyzed by means of HPLC and electrospray tandem MS.

*1 Means \pm s.e. of mean

*2 Mann-Whitney U-test, 0-hypothesis being no difference between bud types

*3 pmol/g FW

*4 nmol/g FW

*1	Leader bud	Whorl bud	P \leq *2
Total cytokinins*3	536.90 \pm 45.93	205.29 \pm 35.82	0.005
Cytokinin glucosides*3	4.65 \pm 1.16	10.82 \pm 2.59	0.05
ABA*4	6.41 \pm 0.45	6.44 \pm 0.83	n.s.
IAA*3	1.12 \pm 1.12	12.43 \pm 6.55	n.s.

Auxin concentrations were rapidly decreasing (Figure 2) and thus very variable from tree to tree. Leader and whorl bud auxin profiles were very similar and resembled that of other bud types analyzed. This pattern would thus seem to be “normal behavior” of buds over time and not carry any information on bud identity. After the initial decrease, auxin concentration stayed close to or under detection level during summer while needle differentiation was taking place within the buds. One may speculate that this minimum is required to prevent tissue expansion and premature bud burst. After a rise in August, a level of about 50 pmol was maintained through winter. In spring, there seemed to be a further rise in auxin when approaching bud burst.

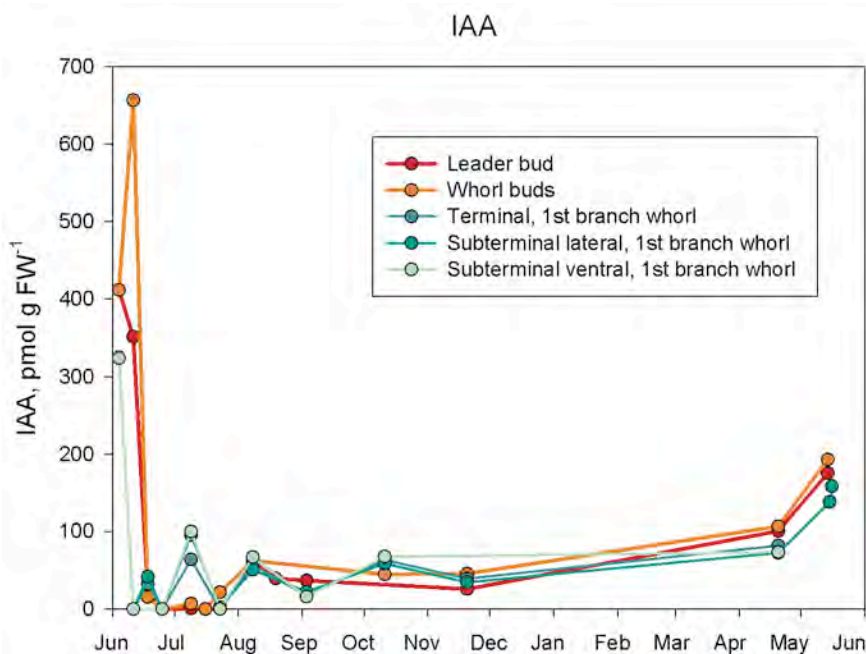


Figure 2. Annual auxin pattern in Nordmann fir buds. Leader bud, whorl buds and buds of the uppermost whorl branch.

Reactions to bud excision

Immediate or delayed reactions on auxin levels could not be detected either in buds remaining after bud excision or subtending stem tissues (data not shown). In contrast, the stem tissues in question reacted within 1 hour with a strong increase in zeatin ribotide, and within 1 week a strong increase in the de-phosphorylated form, zeatin riboside, was added (Figure 3). High levels of zeatin riboside were subsequently found both in autumn and early spring in the destipitated trees, whereas the content eventually returned to control levels in the decapitated trees. With some delay, the buds that remained after bud excision reacted similarly to the subtending stem tissues. That is, the leader bud remaining after destipitation experienced supernormal levels until budbreak the following spring, whereas the whorl buds remaining after decapitation eventually stabilized at control levels (Rasmussen et al., in press)

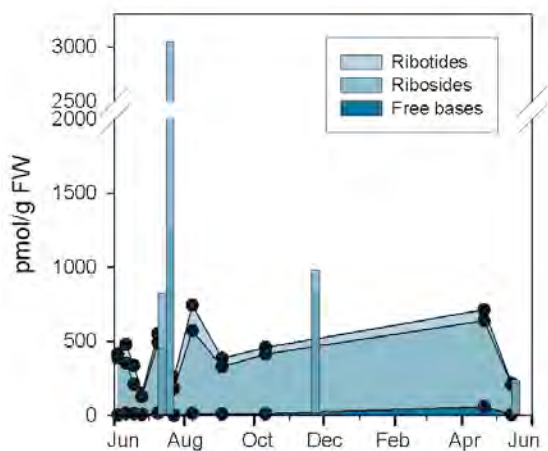
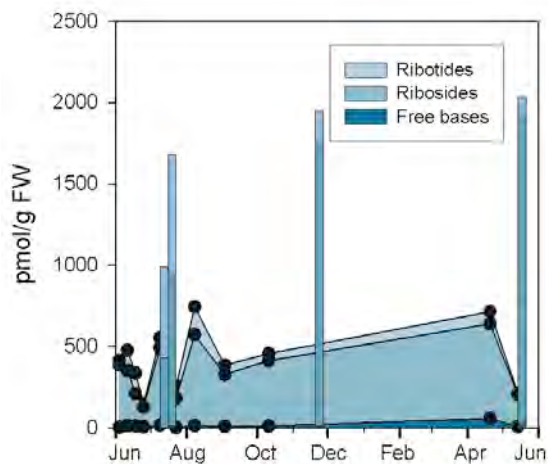


Figure 3. Cytokinin reactions in Nordmann fir in stem tissues under buds that were excised. Seasonal profile of control trees shown as graphs, treated trees as bars. Cytokinins are subdivided into free bases (trans-zeatin, dehydrozeatin, isopentenyladenine), ribose conjugates of the three (ribosides), and phosphorylated forms of ribosides (ribotides).

Growth pattern reactions were consistent with the above: strong effects were observed after destipitation and only minor ones after decapitation (Figure 4). The growth stimulation of the leader length, width, bud size, and bud numbers are all consistent with elevated cytokinin, which might otherwise have been obtained by external application (e.g., Little, 1984).

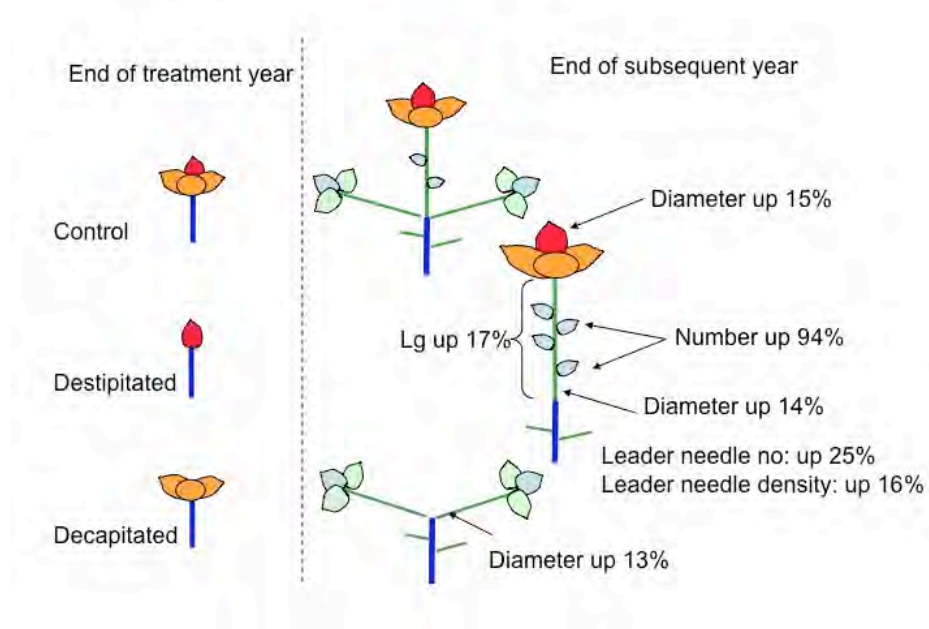


Figure 4. Morphological and morphometric results of destipitation and decapitation in Nordmann fir. All differences statistically significant at $P < 0.05$ or less, except needle density of leader ($P = 0.056$).

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IUFRO BUSINESS MEETING

Group 2.09.02 Christmas Trees

Sept. 15, 2009—Forestry Club Cabin, Oregon State University, Corvallis, Oregon, USA

Held in conjunction with the 9th International Christmas Tree Research and Extension Conference

Coordinator: John Frampton, North Carolina State University

Meeting for 2011—Typically alternates between USA and Europe. Options include:

- First choice—Austria with Karl Schuster and a strong Association there
- Second option—Norway (hope to wait until 2015)

The Czech Republic is an option when their Christmas tree industry develops. Timing of meeting will depend on when it fits best for the host. August–September seems to work for most attendees.

Newsletter and Web—Pascal Nzokou volunteered to continue to produce the newsletter and Bert Cregg will take over in future issues. Should come out in timely manner, perhaps every 6 months to 1 year.

Website is hosted by IUFRO personnel. Having a representative from each country or state is helpful.

Officers—Chal Landgren, coordinator; Ulrik Nielsen, first deputy; Pascal Nzokou, second deputy; Karl Schuster, third deputy; Bert Cregg, newsletter.

Funding—For future meeting it might be nice to consider some type of scholarship or stipend to encourage students to attend. There were Guatemalan students interested in this meeting, but no extra funds were available.

Ideas for collaboration

- Species hybridization using germplasm or species important to each area/region.
 - Interest group—Bert Cregg (convener/lead), John Frampton, Rick Fletcher, Pascal Nzokou, Jan Stejskal, Jaraoslav Koblíha, AnneMargaret Braham
- Marketing real trees and limiting artificial trees, US Check-Off program, market development in all regions
 - Claus Christensen (lead), Jill L. O'Donnell, Jeff Owen, Mike Bondi, Steinar Haugse
- Climate change impacts on Christmas tree production
 - Iben Thomsen (lead), Pascal Nzokou, Jan Stejskal, Lars Pederson, Claus Christensen, Eric Hinesley
- Coordinated species collections and selections similar to former IUFRO collections
 - Interest group—Rick Bates (lead), John Frampton, Rick Fletcher, Dennis Fullbright, Chal Landgren, Ulrik Nielsen, AnneMargaret Braham, Mark Vodak

- Develop broad teams of expertise. Integrate findings so there is broader applications across the regions (e.g., similar data is collected in ways to have broad use across regions). No team formed
- Help in integrating molecular work across institutions and projects. No team formed

Provided by AnnMargaret Braham, North Carolina State University, and Chal Landgren, Oregon State University