Sixth International Christmas Tree Research & Extension Conference

September 14 - 19, 2003
Kanuga Conference Center
Hendersonville, NC

Proceedings

Hosted by
North Carolina State University

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John Frampton, Organizer and Editor

Forward

During September 2003, North Carolina State University hosted the 6th International Christmas Tree Research and Extension Conference. This conference was the latest in the following sequence:

Date	Host Organization	Location	Country
October 1987	Washington State University	Puyallup, Washington	USA
August 1989	Oregon State University	Corvallis, Oregon	USA
October 1992	Silver Fall State Park	Silver Falls, Oregon	USA
September 1997	Cowichan Lake Research Station	Mesachie Lake, British Columbia	Canada
July/August 2000	Danish Forest and Landscape Research Institute	Vissenbjerg	Denmark

The conference started September 14th with indoor presentations and posters at the Kanuga Conference Center, Hendersonville, N.C., and ended September 18th in Boone following a 1½ day field trip. The conference provided a forum for the exchange of scientific research results concerning various aspects of Christmas tree production and marketing. The 74 attendees represented 12 states as well as Canada (5), Denmark (9), Norway (2) and South Africa (1). Most attendees were professionals who support the Christmas tree industry.

The wooded seclusion of the Kanuga Conference Center provided an ideal setting for the conference. The 35 talks and 27 posters were presented in the Balthis-Rodwell Building, but a favorite location for socializing and informal exchanges of ideas during breaks was the lounge's rocking chair porch with its view of Kanuga Lake. Attendees lodged in the rustic comfort of cottages with living rooms and were spoiled by good food and unlimited servings at the dining hall.

On the evening of September 16th, Gary Chastagner led a roundtable discussion on issues facing Christmas tree research and extension. Among the topics of discussion was the erosion of funding within the Cooperative Extension Service and from state appropriations. This predicament has been worsened by the recent decline in the use of real Christmas trees. A need to better understand Christmas tree markets and anticipate customer preferences was recognized. A related topic discussed was the need in the United States to develop a national research team to help identify and coordinate Christmas tree research on issues that have country-wide impact such as developing market strategies, improving convenience and addressing environmental concerns. Such a committee could help provide the National Christmas Tree Association with guidance on allocating resources from its recently established research endowment fund. Many in the group expressed a feeling of isolation with respect to Christmas tree colleagues and information. The group agreed that holding this conference biennially and that alternating hosts between North America and Europe would be beneficial.

One hundred percent of attendees returning evaluation forms agreed that they gained valuable knowledge at the conference. And, perhaps equally important to the information learned at this meeting were new contacts made. Each of us left the conference with new friends and an expanded network of experts to call on, not to mention a head full of new ideas and approaches.

The 7th International Christmas Tree Research and Extension Conference will be hosted by Michigan State University in 2005. I, for one, am looking forward to it.

John Frampton

Conference Organizer
and Proceedings Editor

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Table of Contents

Oral Presentations 1

Christmas Tree Research and Extension – an Overview for North Carolina 2

Propagation & Genetics 3

Breaking Dormancy in Tree Seeds, with Special Reference to Fir (*Abies*) Species..... 4

Clonal Propagation of Christmas Trees through Somatic Embryogenesis, Why and How?.. 5

Cloning *Abies* Research and Commercial Possibilities. 12

In vitro Plant Regeneration and *Agrobacterium tumefaciens*-mediated Transformation in Christmas Trees 13

Noble Fir Current Season Needle Necrosis in Denmark – Status for Genetic Variation and Effect of Site 17

Genetic Improvement of Virginia Pine for Christmas Tree Production..................... 18

Genetic Variation in Fraser Fir 19

Diseases 20

Christmas Tree Diseases in Norway 21

Managing Rhabdocline Needlecast on Douglas-fir in Pennsylvania.......................... 22

Heterobasidion annosum associated with Mortality of Christmas Trees in the Pacific Northwest.. 26

Phytophthora Root Rot of Fir in Michigan 27

Phytophthora Root Rot Mortality in Fraser Fir: Genetic Variation in an Infested Progeny Test 32

Susceptibility of Conifer Shoots to Infection by *Phytophthora ramorum*.................. 33

Arthropods & Weeds..34

The Di-Syston Project: Development of a Hand Operated Metered Closed System Applicator For Christmas Tree Producers in Western North Carolina................ 35

Arthropod Pest Management in Christmas Trees in the Pacific Northwest 41

Pest Management Control in Christmas Trees Grown in the Southern Appalachians 42

Achieving Results Through On Farm Research, A Case Study: Impact of Round-Up Original Applications on Fraser fir Foliage during Early Shoot Elongation...... 51

Responses of Christmas Tree Species and Selected Weeds to Herbicides................. 56

Nutrition...57

Growing Christmas Tree with Reduced Environmental Impact from Fertilization: Alternative Fertilizers and Split Treatments.. 58

Growing Christmas Tree with Reduced Environmental Impact from Fertilization: Alternative Methods for Fertilizer Application 60

Manganese Deficiency in Colorado Blue Spruce 61

Production 62

Changes in Soil Physical Conditions Over Multiple Christmas Tree Cropping Cycles 63

Triazine and Mycorrhizae Observations over Multiple Christmas Tree Cropping Cycles 72

Soil and Needle Nutrient Changes over Christmas Tree Cropping Cycles 78

Evaluating the Potential of *Abies* Species in Eastern North Carolina 89

Shearing Fraser Fir Christmas Trees... 90

Scotch Pine Shearing Regimes - Tree Grade Relationships...................................... 92

Bud Manipulation as a Tool for Regulation of Crown Structure in Young Trees...... 93

Terminal Bud Selection in Fraser Fir Christmas Trees 96

The Dynamics of Labor in North Carolina’s Christmas Tree Industry 97

Precocious Cone Production in Fraser Fir 98

Post-Harvest 107

Identification of Canaan Fir Trees with Superior Needle Retention 108

The Keepability of Douglas-fir Progeny from Texada Island, B.C. 109

Late Season Moisture Levels of Christmas Trees on Retail and Wholesale Lots in Washington, Oregon and California 111

Poster Presentations 113

Pests 113

Protecting Christmas Trees from Root Feeding Insects 114

Bark Responses of Fraser Fir to Balsam Woolly Adelgid Infestations 116

Susceptibility of Various True Firs to Insect Pests in the Pacific Northwest 118

Deer Management in Fraser Fir Christmas Trees 120

Evaluating Grub Control Products 123

Diseases & Disorders 125

Management of Phytophthora Root Rot in Christmas Tree Plantations of Fraser fir with Fungicides 126

Screening *Abies* Species for Resistance to *Phytophthora cinnamomi* 128

Identifying Possible Chemical and Cultural Management Strategies for *Phytophthora cinnamomi* in California's Sierra Foothill-grown Christmas Trees: An On-farm Pilot Study 130

The Dynamic Status of Douglas-fir Needlecasts in New York State Christmas Tree Plantations 132

Effect of Annosus Root Rot on the Keepability of Noble Fir Christmas Trees 133

Genetic Variation in *Phytophthora cinnamomi* Isolated from Fraser Fir 135

Sensitivity of *Phytophthora ramorum* to Fungicides 137

Cavity Formation below the Bud Crown in Nordmann fir 139

Current Season Needle Necrosis (CSNN) in Nordmann Fir 141

Environment..143

Macroinvertebrate Surveys to Monitor Water Quality below Christmas Tree Farms 144

Nutrition...146

Soil and Plant Nutritional Status of Fraser Fir Christmas Tree Fields – A Survey .. 147

Nitrogen Management in Christmas Tree Production 148

Accumulation of Biomass and Nutrients in Nordmann Fir Christmas Trees in Denmark 150

Post-Harvest 152

Effect of Harvest Date on Needle Retention by Various True Firs 153

Propagation & Genetics 154

Virginia Christmas Tree Growers - Virginia Dept. of Forestry Christmas Tree Species Test Plantings.. 155

Clonal Propagation of Fraser Fir through Somatic Embryogenesis 156

True Firs: New Conifers for Michigan Landscapes.. 157

Improving the Rooting Ability of Fraser Fir Cuttings 159

Transferring Technology in Fraser Fir Production: Determining Commercial Viability of Asexual Propagation of Fraser Fir with On-Farm Demonstrations 161

Genetic Conservation and Evolutionary History of Naturally Fragmented Fraser Fir (*Abies fraseri*) Populations 162

High Efficiency *in vitro* Plant Regeneration and the Accumulation of Peroxidase in Virginia pine (*Pinus virginiana* Mill.).. 164

Photographs..166

List of Participants..171

Oral Presentations

Christmas Tree Research and Extension – an Overview for North Carolina

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The research and extension activities focusing on the Christmas tree industry at NC State University reflect the importance of this estimated $100+ million (wholesale) industry to North Carolina. The research and extension programs encompass areas such as soils and nutrition, tree production and culture, genetics and tree improvement, disease management, and insect management. Approximately 33 persons are involved in an incredible array of projects. A striking hallmark of the Christmas tree research and extension programs in North Carolina is the multidisciplinary approach to the work coupled with strong teamwork between State agencies, the University, and the Christmas tree associations. This overview is an attempt to provide a sense of breadth and depth of activities involved in the research and extension work on behalf of the Christmas tree industry in North Carolina.

Propagation & Genetics

Breaking Dormancy in Tree Seeds, with Special Reference to Fir (*Abies*) Species

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As with other gymnospermous trees, seeds of fir (*Abies*) species generally display a wide range of dormancy. Dormancy level, or degree, may vary from one crop year to the next among the same parent trees; among parent trees in the same stand in any one crop year; among cones on the same parent tree, and from seed to seed in the same cone. Standard stratification/prechilling treatments usually fail to accommodate such wide variances in seed dormancy. A 3- or 4-week stratification treatment often fails to satisfy the requirements for breaking deep dormancy, so some seeds are lost for seedling production. If the stratification duration is doubled, or tripled, non-dormant and less-dormant fir seeds tend to chit in the refrigerator, again usually being lost for seedling production. The stratification-redry (strat-redry) procedure, first described in the 1980s, is reviewed. This method overcomes a wide range of dormancy levels by controlling seed moisture content during an extended chilling period. Subsequently, most, if not all, of the viable seeds germinate synchronously. A previously-unpublished, new, simplified method for applying the strat-redry procedure, that relies only on changes in seedlot weight, is described.

Clonal Propagation of Christmas Trees through Somatic Embryogenesis, Why and How?

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BACKGROUND

Christmas tree production is a growing business in Norway. According to Statistisk Sentralbyrå the current gross value is 250 million Norwegian kroner, ca 30 milllon US $. Most of the production is for the Norwegian market, where trees imported from Denmark has a large market share. The major producers in Norway are located in Rogaland County nearby the "oil capitol" Stavanger. The ambition of the larger producers is to compete in the European market where approximately 60 million trees are sold annually. This market is gradually expanding because the Christmas tree tradition is spreading to new countries in the region and due to a general increase in purchasing power. It will be particularly challenging for Norwegian Christmas tree growers to compete in the European market because the oil industry has pushed labor and capital costs to higher levels than in other European countries.
If Norwegian Christmas tree production is to be competitive the most labor demanding operations in the production process has to be kept at a minimum or they have to be mechanized.

The two most common Christmas tree species in the European market are Norway spruce (*Picea abies*) and Noble fir (*Abies nordmanniana*). By using plant material that is well adapted to the climatic conditions at the site and skilled trimming, typically 30-50 percent merchantable trees can be obtained. Trimming is defined as all activities to shape the tree. Assuming that: a) the cumulative time to trim a tree is 10-15 minutes; b) labor cost is more than 20 US $ per hour; c) only half of the trees can be sold, makes it clear that trimming is a major component of the overall production cost. This cost could be substantially reduced by use of plant material that yield high quality trees only. Skogforsk (Norwegian Forest Research Institute) has deployed a large number of genetic field tests of Norway spruce. These are well designed and some have been assessed for Christmas tree quality with promising results (Nyeggen and Skage 2000, Nyeggen and Skage 2001). During the last years there has been a shift in buyer preference from spruce to fir in the Norwegian Christmas tree market. The potential of firs, and Sub alpine fir in particular, as a Christmas tree in the Norwegian and the European market has however been recognized before. Oppland Forest Society established different types of genetic tests with Norway spruce and Sub Alpine fir already in 1989. The tests comprised provenances, open pollinated progenies, progenies from controlled crosses and rooted cuttings (clones) from various families. We will present some of the results from the tests in Sub alpine fir hoping to provide an answer the why in the title of this paper.

Even before the test results had been fully analyzed it became evident that some clones and some full sib families consisted mostly of high quality trees. To make use of such high quality clones an efficient propagation method is needed. Oppland Forest Society therefore established a collaboration with Skogforsk to develop a method for clonal propagation of Norway spruce and Sub Alpine fir through somatic embryogenesis. In the second section we will present our results as we give an overview of somatic embryogenesis in conifers.

CHRISTMAS TREE FIELD TEST

Materials and methods

The provenance, full sib family and clonal tests were established in 1989 at Biri Plant Nursery. Fourteen provenances were tested, each represented with 3 to 71 trees. The full sib families were from crossings between six fathers with a variable number of totally 39 mothers. Each family was represented with 3 to 56 trees. The 322 clones were produced mostly from two full sib families with 2 to 18 ramets within each clone. The provenance test was planted at two sites, Oladalen and Fluberg. Oladalen is warmer but spring frosts are more frequent. The full sib family and the clonal tests were planted at this site. All provenances were not present at both sites and separate analysis has therefore been conducted for each site. In all tests the height and number of branches in each whorl was recorded in 1989. Three trained observers evaluated each tree for Christmas tree quality by assigning a value, 1, 2 or 3 to the tree depending on whether the tree could be sold as it was without trimming (1), could be sold after trimming (2), could not be sold in any case (3). Similarly, the degree of attack by the Sibirian fir aphid (*Adelges sibirica*) was scored in three classes, little to none, moderate and severe. Variance components and Best Linear Unbiased Predictors (BLUP) for the traits height and branch number were computed using Proc Mixed in SAS™. The variables Christmas tree quality and aphid attack (coded as 1,2,3) were treated as if they were continuous and analyzed in the same way as height and branch number. To assess the possible gains that can be obtained through selection of the five percent best in each group (provenance, open pollination, controlled pollination and clones) the groups were ranked according to the BLUP values for quality. The five percent best were selected and the analysis run again to estimate the environmental variance component in this selected group. This variance was thereafter used to compute the share of trees in class 1, 2 and 3 in the selected population assuming that an individual tree’s probability of ending in one or the other class due to environmental factors can be described with a normal distribution.

Results

Quality in provenances

In the provenance test the overall percentages of trees in the three quality classes at the best site were 22 in class 1, 38 in class 2 and 40 in class 3. At the other, more frost prone site the corresponding numbers were 9, 32 and 59 percent. Thus if a Christmas tree plantation of Sub alpine fir is established from a broad selection of provenances at suitable sites in Eastern Norway one cannot expect the total yield of merchantable trees to be higher than 60 percent even if they are trimmed. If the best provenance was selected the proportion of trees in class 1 increased to 38 percent and the class 3 decreased to 21 percent, at the best site. At the frosty site, Oladalen, only 24 percent of the trees were in class 1 and 36 percent were in class 3. It should be noted that the best provenance at Fluberg was among the worst at Oladalen, which underlines the importance of matching the phenolgical characteristics of the plant material to the climatic conditons of the site. The limitations by selection at the provenance level only are equally clear, since there is still a large proportion of trees that cannot be sold and trees that needs expensive trimming even in the best provenances.

Quality in full sib families

The overall quality of the progeny from the 49 controlled pollinations was rather low, with 16 percent of the trees in class 1, 30 percent in class 2 and 54 percent in class 3. The quality in the two best full sib families was much higher however, 57 percent in class 1, 36 percent in class 2 and 7 percent in class 3.

Quality in clones

A majority of the 322 clones were from two full sib families that displayed below average quality. Overall only 10 percent of the trees were in class 1, 31 percent in class 2 and 59 percent in class 3. Selection of the five percent best clones produced a dramatic change in these figures: 67 percent in class 1, 32 percent in class 2 and 1 percent in class 3. Consequently, the non-merchantable class was practically eliminated. These predicted values obviously do not include the probability of total plantation failure due to snow break, insects etc. since there were no such events during the test.

Correlation between traits

In the provenance test the number of provenances was too low to permit assessment of correlations between traits. In the full sib families however some correlations were evident. There was a negative correlation (-0.45) between branch number and the quality class, indicating that trees with many branches in the whorl had higher quality. Height and branch number was also correlated (r=0.65), whereas height and quality was not correlated at all (r=0.05). In spite of this, the shortest family had the highest quality and none of the taller families had high quality. The average height of the three best families was 114 cm compared to 132 cm for the whole population. This may suggest that it will be difficult to avoid loss of height growth if one select for superior quality in full sib families. A larger test is needed to clarify this issue however. In the clones there was also no correlation between height and quality. As in the full sib families none of the best clones were among the tallest. There were however, several clones with average height that had superior quality. Moreover, the height of the five percent best clones was similar to the overall population average, 142 cm.
A weak correlation (r=0.19) between height and the number of branches in the whorl was present among the clones. Overall the correlations between traits were lower among the clones than among the full sib families.

Conclusions

The provenance trial demonstrates that the plant material must be tested at representative sites. Poorly adapted provenances caused poor quality and gave a high fraction of trees that could not be sold. The full sib family test shows that the share of merchantable trees can be increased substantially and the trimming cost can be reduced trough selection of the best full sib families. As expected however, selection of clones is the most efficient in all these regards. With clones it also appears less likely that intensive selection for quality will cause reductions in height growth. Provided that there exist an efficient protocol for cloning the use of clones circumvents the problems with irregular seed supply.

SOMATIC EMBRYOGENESIS FOR CHRISTMAS TREE PRODUCTION

The first reports on how to regenerate plants from somatic embryos of Norway spruce appeared almost 20 years ago (Chalupa 1985, Hakman and von Arnold 1985). Since then research in many laboratories have answered enough questions to enable regeneration of plants from many clones in several other conifers as well. CellFor, a commercial laboratory in Canada, has proved this point through annual deployment of several million plants from somatic embryos of *Pinus taeda.* However there are other conifers, Sub alpine fir and Fraser fir are but two examples, where all questions have not been answered yet. This was recently pointed out in a critical review of the method as such (Bornman 2002). Awareness of problems is obviously important since it is the first step in their solution. Another recent review (Stasolla and Yeung 2003), more optimistic on the behalf of this technology, emphasized that solutions have been found and will be found. We share this optimism. Here we give an overview of the different steps in somatic embryogenesis and present some of our results that are important from a production point of view.

Initiation

The first step is to initiate the cultures from some explant material. Generally, the most responsive explant is immature zygotic embryos, where the response can be between 70 to 100 percent. In *Picea* species it is also possible to initiate cultures from mature embryos. Several studies in *Picea* have shown that there is a strong genetic control over initiation (see Kvaalen et al. 2001 and references therein). Consequently; those embryos that forms embryogenic tissue represents a selected population from a genetic point of view. A correlated selection response in other traits such Christmas tree quality is therefore a possibility that should not be overlooked (Ekeberg et al. 1993, Kvaalen et al. 2001). To avoid this potential problem the initiation response should be as close to 100 percent as possible. Better initiation protocols are therefore needed. We have developed a medium for Sub alpine fir based on analysis of female gametophytes. With this medium we obtained higher initiation frequencies in most families than with the commonly used Shenk and Hildebrandt medium. We were also able to rescue cultures that started to decay on the S&H medium (Kvaalen et al., unpublished). In Norway spruce ca 70 percent of mature zygotic embryos cultured on the AL medium or a similar medium, produced embryogenic tissue compared to ca 40 percent on the LP medium (von Arnold and Erikson 1981) that is often used in spruce.

With further development of the culture system close to 100 percent initiation could be possible.

Proliferation

This is the step where the tissue is maintained or bulked up before maturation treatment. On solid medium the growth rates are ca 7 percent per day which means that the tissue mass is more than doubled every second week. For bulking up tissue liquid medium cultures are preferable because the growth rates are much higher (20 percent per day) and the maturation step can be done more efficiently. Liquid medium culture is not without problems however. In Nordmann fir the embryos lost their suspensor cells and the growth declined after some months in culture (Nørgaard 1992). The cultures may also loose their ability to form mature embryos after some months in liquid medium. These problems may be connected. Currently we are testing some additives that appear to reduce or completely solve these three problems in both Norway spruce and Sub alpine fir, although further studies are needed to confirm the results.

Maturation

To switch the embryos from proliferation it is necessary to remove auxin and cytokinin from the medium and include abscisic acid (von Arnold and Hakman 1988). Inclusion of polyethylene glycol in the medium (PEG) Attree et al. (1991) or other osmotic agents can further enhance maturation. In Norway spruce we have found that a stepwise increase in PEG concentration from 5 to 10 percent promote embryo maturation in most clones (Latkowaska et al. 1998). Often however, many clones in a species do not respond to treatments with ABA and PEG. One answer to this is emerging. Find (2002) found that Nordmann fir clones could be stimulated to produce embryos when treated with an auxin antagonist. These results fit well with what we have found in Norway spruce, where 22 of 22 clones produced embryos after they were given an optimal auxin level for two subcultures before the maturation treatment (Kvaalen unpublished). Taken together these results suggest that most clones in spruce and fir are capable of producing mature embryos if they are given the right conditions both during the proliferation and maturation steps.

Desiccation

This step was introduced in conifers by (Roberts et al. 1990). It represented a major step forward as the subsequent germination frequency increased from 20 to 80-90 percent. Mature seeds of many conifers must also be desiccated before they reach full germination potential. With some of our Norway spruce clones we find that this step can be omitted if the maturation medium contains at least 10 percent PEG during the last part of maturation. By further increasing the PEG concentration and extending the maturation period it may be possible to omit the desiccation step all together. Completely dried embryos can be sent around the world just like seeds.

Germination

If the quality of the somatic embryos is good the germination frequency is similar to what we find in seeds, 85-95 percent. In Norway spruce we have found some important differences between seeds and somatic embryos. Whereas seeds show little response to light quality and germinate both in light and darkness, somatic embryos are sensitive to light quality (Kvaalen and Appelgren 1999). Blue light has a strong inhibitory effect on root elongation but stimulates shoot elongation. Red light on the other hand stimulates root elongation but the onset of shoot elongation is delayed. This can be used in practice by subjecting the embryos to red light for the first three weeks until the root is at least 2-4 cm long. During this first period we also use activated charcoal in the germination medium to soak up residual ABA (Latkowska et al. 1998). Thereafter the plants are grown under blue light for 4-6 weeks more until the shoot (epicotyle) is 2-4 cm long. Then the plants can be transplanted to peat plugs.

Transfer to soil and *ex vitro*

This step usually poses no problem from a biological point of view if the plants have sufficiently large shoot to support further growth by photosynthesis alone. Such plants are easy to acclimatize to ex vitro conditions. Once the plants have reached this far, the mortality is low although there is some variation between clones.

CONCLUSIONS

Several conifer species can be mass propagated through somatic embryogenesis. Commercial laboratories like Cellfor has scaled up the production and can supply superior planting stock. In all species where the biological qestions related to initiation, proliferation, maturation and germination have been answered large scale production should thus be possible.

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Cloning *Abies* Research and Commercial Possibilities.

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Nordmann fir (*Abies nordmanniana*) is economically the most important forest tree in Denmark, and it is grown on a large scale exclusively for the production of Christmas trees. The production is cost- and labour intensive, and the single plant represents a high value for the grower. Plants of Nordmann fir are produced from seeds, and due to genetic variability about 20 % of the produced trees are discarded prior to harvesting and only 20 % are rated as high quality even in the best plantations. The total production of Christmas trees in Europe and North America averages about 170 mio. trees per year.

For this reason, the Christmas tree industry has great commercial interest in the development of methods for clonal propagation of conifers. Clonal propagation offers the opportunity for fast propagation of improved genetic ressources developed by traditional tree improvement programs. Furthermore, clonal propagation provides a stable supply of uniform and high quality plants. However, traditional methods such as propagation by cuttings are hampered by poor rooting and by the branch like appearance of the produced trees.

Woody Plant Biotech Aps (WPB) is a Danish based company specialised in clonal propagation of forest trees. WPB is presently about to commercialise newly developed and patented methods for clonal propagation of conifers. The methods are based on somatic embryogenesis and have shown to be very efficient for nordmann fir from which the first 15,000 thousand plants are ready for larger scale field trials and clone selection.

Using cloned *Abies* seedlings will generate huge productivity gains by the producers of Christmas trees, ornamental greens and timber. Accordingly, nurseries and producers that utilize this technique will prosper.

In vitro Plant Regeneration and *Agrobacterium tumefaciens*-mediated Transformation in Christmas Trees

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ABSTRACT

The effects of different plant growth regulators on in vitro adventitious shoot formation in Virginia pine (*Pinus virginiana* Mill.) were quantitatively evaluated in this investigation. In TE basal medium supplemented with 2.0 mg/l indole-3-acetic acid (IAA) and 0.5 mg/l N^6-benzyladenine (BA), organogenic callus was formed after 3 to 6 weeks of culture. Organogenic calli were transferred to TE basal medium supplemented with 0.1 mg/l indole-3-butyric acid (IBA) and 2.0 mg/l N^6-benzyladenine (BA) for 6 to 9 weeks, where they differentiated into numerous small shoot primordia. In TE basal medium supplemented with 0.1 mg/l indole-3-butyric acid (IBA) and 1.0 mg/l N^6-benzyladenine (BA), growth and elongation of adventitious shoots were promoted. After elongated shoots were transferred to TE medium containing 0.01 mg/l α–naphthalene acetic acid (NAA) for 6 weeks, adventitious roots were formed at the base. *Agrobacterium tumefaciens* strain GV3850 harboring the plasmid pBIN-mGFP5-ER was used to transform mature zygotic embryos of Virginia pine, Fraser fir (*Abies fraseri*), and Nordmann fir (*Abies nordmanniana*). Five hundred to one thousand embryos were used in transformation of each species. The frequency of transient *gfp*-expressing embryos was 21%, 26%, and 13% for *Pinus virginiana*, *Abies nordmanniana,* and *Abies fraseri,* respectively. These results provide useful information for the establishment of an efficient *Agrobacterium tumefaciens* -mediated transformation system for stable integration of economically important genes into Christmas tree species.

INTRODUCTION

Christmas tree improvement programs use open-pollinated seeds from selected trees, and this warrants identification and use of the best available material by growers. Once good performance such as branching habit, good stem, and tip moth resistance are identified in the tested families, good quality seed from existing seed orchards will provide growers with genetically improved high quality trees. Clonal propagation of provides another alternative for the Christmas tree industry. In vitro regeneration of Virginia pine provides opportunities to genetically transform this species via particle bombardment or *Agrobacterium tumefaciens*-mediated transformation and to produce genetically modified trees with insect, disease, drought resistance, and salt tolerance (Chang et al. 1991, Jang and Taiter 1991). Although plant regeneration by organogenesis on cotyledon explants in Virginia pine has been achieved (Chang et al. 1991), it is has been difficult to use this procedure for genetic transformation. Protocols for genetic transformation in Virginia pine need to be improved including clonal tissue culture procedures. The present study was undertaken to establish a novel protocol for the induction of adventitious shoots and roots in Virginia pine and for regeneration of plant from shoots, as well as preliminary work on gene transfer.

MATERIAL AND METHODS

Mature seeds collected in 1994 from open-pollinated cones of Virginia pine (*Pinus virginiana)*, Nordmann fir (*Abies nordmanniana*), and Fraser fir (*Abies fraseri*) were kindly provided by John Frampton of the Christmas Tree Program, Raleigh, North Carolina USA and by Clyde Leggins, Morganton Ace Hardware Co., Morganton, North Carolina, respectively. Seeds were rinsed thoroughly under running tap water for 30 min. The seeds were disinfected in 50% (w/v) Clorox for 45 min, rinsed five times in sterile, distilled water and soaked overnight in sterile water. Mature zygotic embryos were aseptically excised from disinfected seed and placed horizontally on TE (Tang et al. 2001) callus induction medium.

The binary expression vector pBIN*m-gfp5-ER* was used (Haseloff and Amos 1995). The pBIN*m-gfp5-ER* contains the *m-gfp5-ER* gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter, the nopaline synthase polyadenylation region, and the selectable marker gene, *nptII*, which confers resistance to kanamycin. The binary vector pBIN*m-gfp5-ER* was introduced into three *Agrobacterium tumefaciens* strains GV3850 by electroporation. Transformation experiments were done 3 days after mature zygotic embryos of pine were transferred to callus induction medium for selecting embryos not contaminated by natural bacteria. *Agrobacteriua*, grown for either 1 or 2 days to an optical density (OD_{600} nm = 0.8-1.0) of bacteria in 3 ml of YEP broth, were centrifuged and re-suspended in plant medium. To obtain large quantities of transformed tissues for further analysis, selected organogenic cultures were again introduced on a callus proliferation medium. After 4–6 weeks, the cultures were actively producing 5–10 mg of tissue each week, and they were then used to prepare DNA for Southern blot analysis.

RESULTS

Adventitious shoots were induced by culture of organogenic calli (Fig. 1) on TE medium containing 0.1 mg/l indole-3-butyric acid and various concentrations of BA ranging from 0.5 – 8 mg/l (0.5, 1, 2, 4, and 8 mg/l). The highest rates of adventitious shoot induction from organogenic calli derived from 1994 seeds and 2002 seeds (data not shown) were 61.5% and 65.5%, respectively.

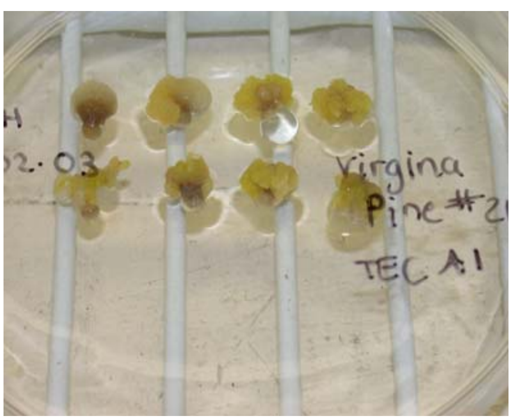

Figure 1. Callus induced from Virginia pine zygotic embryos.

Figure 2. Virginia pine shoots.

The maximum induction frequency of adventitious shoot formation was obtained on TE medium containing 0.1 mg/l IBA and 2 mg/l BA. Induction of adventitious shoot formation from cultured organogenic calli of Virginia pine is typical of other members of the genus *Pinus,* such as loblolly pine, in that a combination of IBA and BA is effective in adventitious shoot induction (Tang et al. 2001), and is different from the genus *Abies* (Thorpe and Murashige 1970). Adventitious shoot induction frequencies in Virginia pine were similar to those previously reported for loblolly pine (62.1%) (Tang et al. 2001). In TE basal medium supplemented with 0.1 mg/l indole-3-butyric acid (IBA) and 1.0 mg/l N^6-benzyladenine (BA), growth and elongation of adventitious shoots were promoted (Fig. 2). Adventitious shoots grew from 0.3-0.5 cm to 1.5 to 3 cm in 6 weeks. After elongated shoots were transferred to TE medium containing 0.01 mg/l α–naphthalene acetic acid (NAA) for 6 weeks, adventitious root was formed at the base (Fig. 3). With this technique, we have produced 5,000 pieces of calli and 2,000 shoots.

Figure 3. Virginia pine shoots with roots.

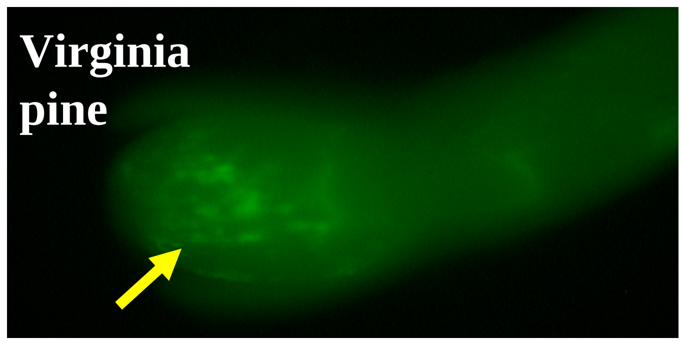

Figure 4. Virginia pine cotyledon tissues transformed with green fluorescent protein gene (*gfp*).

Our primary experimental results demonstrated that in vitro and in vivo monitoring of *gfp* expression permitted a rapid and easy discrimination of transgenic calli, and drastically reduced the quantity of tissue to be handled and the time required for the recovery of transformed plants. Integration of the *m-gfp5-ER* was confirmed by visual observation in Virginia pine (Fig. 4), Nordmann fir, and Fraser fir. The frequencies of transient expression of *gfp* for Fraser fir, Nordmann fir, and Virginia pine were 9.7%, 11.6%, and 17.2%, respectively. The frequencies of *gfp* expressing calli for Fraser fir, Nordmann fir, and Virginia pine were 1.0%, 2.5%, and 3.5%, respectively. This research will be useful in the production of transgenic Christmas trees.

CONCLUSIONS

Plant regeneration via shoot and root organogenesis can be affected by culture media, the nature of the plant materials, and relative concentrations of auxin and cytokinin. Since mature zygotic embryos can be used as explants at any specific season for plantlet regeneration, this method may be used to study the effects of certain components in the culture medium on organogenesis in tissue-culture shoot organogenesis protocols with high repeatability. Adventitious shoots appear on most of these organogenic calli. This system may offer a key to genetic transformation of Virginia pine, based on in vitro organogenesis. Availability of clonal techniques is of particular importance for Virginia pine, since improved seed are not readily available. The present work demonstrates that induction of somatic organogenesis via callus production in Virginia pine and regeneration of plantlets has been accomplished. The *Agrobacterium tumefaciens*-mediated transformation method has had a major impact on tree improvement research. Although the benefits of *Agrobacterium*-mediated transformation method could be valuable in genetic transformation of conifers, efforts to apply these methods to other tree species have not always been successful. Tree transformation can now be performed routinely in laboratories that have expertise in plant tissue culture and transformation, allowing *in planta* analysis of multiple DNA constructs. In this work, we have obtained transient expression of *gfp* from three Christmas tree species, and this should foster application of similar transformation methods to other plants and/or tree species.

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Noble Fir Current Season Needle Necrosis in Denmark – Status for Genetic Variation and Effect of Site

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On several occasions a foliage disorder "red needles" occur on noble fir grown for Christmas trees and greenery production in Denmark. Its common to several species and countries and known as "Current season needle necrosis" – CSNN – and is a poorly understood physiological disorder occurring during shoot elongation (Chastagner and Riley 2000, Chastagner 1997).

In the late 1980's several noble fir provenance and progeny trials were established in Denmark: a) Madsens provenance trial - comprising 29 Danish sources (4 sites), b) Christmas Tree Growers Association's noble fir progeny and provenance trial – comprising 4 Danish, 5 US provenances and 72 open pollinated single tree progenies (12 sites), and c) a test related to a grafted clonal seed orchard - comprising 64 open pollinated single tree progenies and 4 Danish provenances (2 sites). All sites are mostly located in eastern Denmark and one locality in southern Sweden.

Within series site differences are very pronounced. In the series (b) comprising 12 sites damage varied between 1 % and 26 % in the same year and for the same genetic material (Danish and US provenances). Damages were in general most severe on former farmland and on sites with extensive weed control. Less damage was seen on sites established under forest conditions. Four sites in the provenance trial (a) showed similar differences, 1 % to 16 %, and the only farm site again showing the highest level of damages.

Substantial differences were seen (b) between the Danish and US provenances – the later having much higher levels of damages, 40 % in average, compared to the Danish sources having in average 8 % of the trees damaged. However also between Danish provenances significant differences were seen (a) ranging from 11 % to 23 % with an average of 14 %. The two most damaged sources originated from the same stand.

Based on the data from the open pollinated progenies (b) the heritability for CSNN was estimated between 0.10-0.50, and estimates were very dependent on the damage level. Rather large differences were seen between families, and breeding for improved resistance to CSNN is an interesting possibility. Based on estimated breeding values for the selected mother trees (b) no correlation was seen between CSNN and several growth and morphological traits.

Preliminary investigations on the nutrient content in the needles of Danish vs. US provenances show significant differences for magnesium (Mg) and calcium (Ca), and will be further discussed.

Genetic Improvement of Virginia Pine for Christmas Tree Production

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Virginia pine is grown as a Christmas tree species throughout the coastal plain and piedmont regions of the South. In 1998, the N.C. State University Christmas Tree Genetics Program established a progeny test series to evaluate first and second generation open-pollinated families (n=120) of Virginia pine that had previously been selected for growth and stem form in forestry tree improvement programs. The four sites, two in the Piedmont and two in Coastal Plain of North Carolina, were managed under a Christmas tree cultural regime including shearing. Height, quality and other characteristics were measured annually and, after the fourth growing season (rotation age), three Christmas tree growers independently assessed retail value. Height, quality and retail value at age four were all under strong genetic control (family mean h^2 =0.87, 0.71 and 0.77, respectively). Use of the best five families evaluated would increase Christmas tree retail values by $2.40/tree (18.7%) and $3.94/tree (34.8%) relative to the checklot ($12.86) and overall ($11.33) means, respectively.

Family and individual-tree Best Linear Unbiased Predictors (BLUPs) for Christmas tree retail value were employed to guide field selection. In 2002, a total of 58 elite trees were chosen from the 14,893 surviving trees and grafted onto greenhouse rootstock. During 2003, a seed orchard and clone bank containing the best 12 unrelated clones, and all selected clones, respectively, were established in Morganton, N.C. It is anticipated that commercial seed production and advanced-generation breeding will commence in two to four years.

Recent results from rooted cutting research are being applied to clonally multiply the best selection on a pilot scale. For this selection, over 100 grafts as well as the original ortet were severely cut back to produce juvenile appearing shoots which displayed vigorous growth and long primary needles. Cuttings from these shoots are currently being rooted. Hopefully, after one additional propagation cycle, a sufficient number of juvenile behaving stock plants can be licensed to a commercial nursery capable of producing and marketing clonal planting stock to Christmas tree growers. If this effort proves successful, in the future, additional selections may be clonally multiplied for commercial use.

Genetic Variation in Fraser Fir

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The natural range of Fraser fir is limited to high elevations in the mountains of western North Carolina, eastern Tennessee and southwestern Virginia. It occurs in six disjunct populations: Great Smoky Mountains National Park, the Balsam Mountains, the Black Mountains, Roan Mountain, Grandfather Mountain and Mount Rogers. In a provenance/progeny study established in 1983, trees from southern seed sources (Richland Balsam and Clingman's Dome) and lower elevation classes (5000 and 5500 ft.) performed best for Christmas tree production. Further, traits important for Christmas tree production appeared to be under strong genetic control ($h^2_{OP\ Family}$= 0.73 for height, 0.72 for crown density and 0.87 for wholesale value). In 1994, during a bumper cone year for Fraser fir, a large-scale seed collection effort was conducted across the species' entire range. In 2000, seedlings grown from the seeds of this collection were established in a larger, more comprehensive provenance/progeny test series. Early results from this test series will be discussed.

Diseases

Christmas Tree Diseases in Norway

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Traditionally Norway spruce (*Picea abies*) has been the dominant Christmas tree in Norway, but different species of fir (*Abies* spp.) have taken increased market shares over the last two decades. Approximately 1 million Christmas trees are planted yearly, and 70 % is fir. The aim is to plant 2 million trees per year by 2005, to meet the competition with imports. Nordmann fir (*A. nordmanniana*) is the most commonly planted fir, but also subalpine (*A. lasiocarpa*), noble (*A. procera*), Siberian (*A. sibirica*), and other fir-species are used. During a resent survey in Christmas tree plantations in Norway it became evident that a number of diseases are able to reduce the quality or completely destroy the trees. Rhizosphaera needle cast (*Rhizosphaera kalkhoffii*) is often observed damaging species of both spruce and fir. In nordmann fir we have reason to believe that infections with *Thysanophora penicillioides* also result in severe needle cast. In 2002, symptoms of interior needle blight syndrome were identified for the first time in Norway on subalpine fir. The stomata of the brown needles were covered with black pseudothecia of *Phaeocryptopus nudus*. Damages caused by three different rust fungi have been observed in several Christmas tree plantations; *Pucciniastrum epilobii* on nordmann fir, and *Chrysomyxa abietis* and *Thekopsora areolata* on Norway spruce. Shoot blight caused by grey mold (*Botrytis cinerea*) is sometimes seen in dense plantations in humid, coastal areas. In 2002, *Cytospora* sp. was identified from canker on subalpine fir and *Nectria* sp. from canker on white fir (*A. concolor*). In 2003, *Kabatina* sp. has been found to cause needle necrosis on noble fir, and *Schlerophoma* sp. has been identified on damaged new shoots on Norway spruce. *Pestalotiopsis* sp. is common on brown needles from different species of spruce and fir. *Phomopsis juniperovora* is frequently found on buds that have failed to open in spring, and on damaged needles and shoots from spruce and fir. However, the fungus is mainly a problem on small plants where the fungus has girdled and killed thousands of small trees, both in nurseries and plantations. In nurseries *Gremmeniella abietina*, *Sirococcus* sp., grey mold, and damping off agents like *Pythium* sp., *Fusarium* sp., *Cylindrocarpon* sp., and *Rhizoctonia* sp. have caused damage. Different nematodes are causing problems both in nurseries and plantations. In a number of plantations of nordmann fir and Norway spruce a phytoplasma is suspected to sporadically cause generations of short needles. It results in strange looking trees with reduced marketability.

Managing Rhabdocline Needlecast on Douglas-fir in Pennsylvania

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INTRODUCTION

Douglas fir (*Pseudotsuga menziesii* subsp. *glauca*) has a rapid growth rate, is very adaptable, and possesses excellent post harvest characteristics. These factors have made Douglas-fir the most widely planted Christmas tree species in Pennsylvania. However, intermountain seed sources of Douglas-fir are susceptible to Rhabdocline needlecast (*Rhabdocline pseudotsugae*). There is also significant variation in the susceptibility of intermountain seed sources to Rhabdocline. Generally, trees derived from the Lincoln National Forest tend to be moderately-highly susceptible to Rhabdocline. Unfortunately, the Lincoln NF provenance represents the majority of Douglas-fir acreage planted in Pennsylvania.

Recommendations for reducing potential Rhabdocline needlecast infection include: 1) avoiding planting near ponds, low lying areas that remain wet and on northern exposures, 2) providing adequate spacing between trees to improve air circulation, and 3) maintaining good weed control. Even if these recommendations are followed, chemical control is often required to prevent or eradicate Rhabdocline. Applications of chlorothalonil (Bravo) usually provide effective control, however several problems still exist. In Pennsylvania, three and sometimes four applications of chlorothalonil are necessary to achieve reliable control. New infections on sites that most would consider “low Rhabdocline risk” are becoming more commonplace. Poor weather conditions during the May-early June infection period make it difficult to obtain suitable spray coverage on many farms. Even when the recommended spray program is followed, needlecast control with chlorothalonil is sometimes variable.

Recently the strobilurin azoxystrobin, marketed under the trade names Heritage, Quadris, and Abound received registration for a wide range of diseases on numerous crops. Azoxystrobin fungicides have received a great deal of attention due to the fact that they are systemic and possess curative activity to varying degrees in many crops. An additional advantage is that these fungicides have very low toxicity, minimal environmental impact and no carry-over. Quadris has a Christmas tree label for needlecast diseases, however many questions exist concerning how best to use this relatively new fungicide in a chlorothalonil-based Rhabdocline control program.

This study was undertaken to determine the efficacy of Quadris for Rhabdocline control on Douglas fir and to provide information on application rate and timing when used in combination with Bravo.

MATERIALS AND METHODS

Two farms in Schuylkill County, PA and one farm in Clinton County, PA were chosen as test sites. Represented in the study were two tree sizes (8-10 ft, 5-8 ft), high and low farm management levels and moderate-high Rhabdocline infection pressure. All trees were from the Lincoln NF provenance. Quadris, plus a non-ionic surfactant, was applied at a high (16 oz/acre) or low (8 oz/acre) rate as the first or second spray within a standard Bravo-based control program (Table 1). Treatments containing a Quadris spray were also compared to a control and the standard Bravo treatment. Control trees were not sprayed with a fungicide. Spraying began in early May 2002, when approximately 10% of the trees in the experimental blocks had broken bud. Sprays were applied using a Solo backpack mist blower and spray volume was equivalent to approximately 100 gallons/acre. A single tree was considered a replicate and each treatment was replicated 6 to 8 times, depending on the site. The study used a completely randomized design at all sites. During February-March 2003, trees were examined for the presence of Rhabdocline needlecast and rated on 0-50 scale. Assessment of infection was made by first rating whole-tree infection levels using a needlecast severity rating system, where 1 = no obvious infection, 2 = light-moderate infection of foliage, bottom ¼ of crown, 3 = moderate-severe infection bottom ¼; light-moderate infection second ¼, 4 = severe infection bottom half, light-moderate third ¼, and 5 = severe infection, bottom ¾ of the crown. A disease incidence rating for each tree was then taken by randomly removing, at knee height, a 3-4 inch stem section from the N, S, E and W sides of the tree. Ten needles were selected from each section and if the needle had Rhabdocline lesions it was counted. The average of the four stem sections yielded a 0-10 average incidence rating. An overall disease index was determined by multiplying the needlecast severity rating by the disease incidence rating. Air temperature, rainfall and relative humidity were also measured at each site in an attempt to determine probable infection periods.

Table 1. Chlorothalonil (Bravo) and azoxystrobin (Quadris) treatment combinations for Spring 2002 Rhabdocline study.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	---	---	---	---	---	---
Standard	B[1]	B	---	B	---	B
#1	Q(H)[2]	---	B	---	---	B
#2	Q(H)	---	---	B	---	B
#3	Q(L)[3]	---	B	---	---	B
#4	Q(L)	B	---	B	---	B
#5	B	Q(H)	---	B	---	B
#6	B	Q(L)	---	B	---	B
Spray Date	5/1	5/8	5/15	5/21	---	6/7

[1] B=Bravo; rate: 1 quart/acre

[2] Q(H)=Quadris High; rate: 16 ounces/acre
[3] Q(L)=Quadris Low; rate: 8 ounces/acre

RESULTS AND DISCUSSION

Each test site yielded similar needlecast severity ratings and the data is presented as an average over the three sites (Figure 1). Control trees at each site had a high level of infection, indicating that infection-producing conditions existed during the test period. Climate data from each site revealed likely infection periods on May 9-10 and May 13-14. The May 9-10 infection period occurred one day after the second Bravo spray in the Standard and #4 treatments, and one day after the first Quadris spray for treatments #5 and #6. Bravo, applied the day before this infection period, offered a high degree of control in the Standard and #4 treatments. When the second Bravo spray was replaced by a high and low Quadris spray, treatments #5 and #6 respectively, infection levels increased. This indicates that even a high rate of Quadris was not as effective in controlling Rhabdocline as Bravo. Results also suggested that Quadris, used as the initial spray, does not offer adequate protection against Rhabdocline infection. Quadris was applied 8 days prior to the May 9 infection period in treatments #1, #2, and #3 and infection rates were only slightly lower than the untreated controls. Quadris is approximately twice as expensive as Bravo per treated acre. If Quadris is to become a cost effective alternative to Bravo, control would need to extend beyond 7-10 days. At this time, azoxystrobin does not appear to offer cost-effective control of Rhabdocline on Douglas fir when compared to the industry standard chlorothalonil.

Rhabdocline needlecast is the most important disease limiting production of Douglas-fir in Pennsylvania. As long as Lincoln NF or other susceptible provenances are grown, Rhabdocline will continue to be a problem, particularly during cool, moist springs. However, resistant individual trees from the Lincoln source are common on Pennsylvania farms. If these repositories of genetic resistance also possess frost tolerance, good growth rate, color and form, they may serve as future seed sources or stock for asexual propagation.

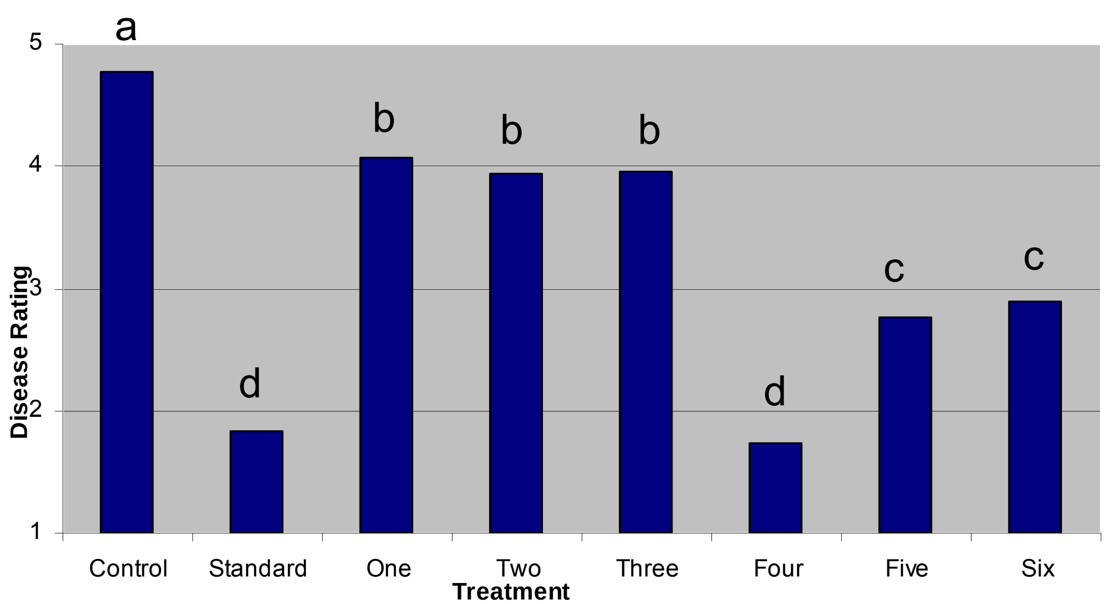

Figure 1. Needlecast severity ratings averaged over the three test sites. 1 = no obvious infection, 2 = light-moderate infection of foliage, bottom ¼ of crown, 3 = moderate-severe infection bottom ¼; light-moderate infection second ¼, 4 = severe infection bottom half, light-moderate third ¼, and 5 = severe infection, bottom ¾ of the crown. Bars with the same letter are not significantly different.

ACKNOWLEDGEMENTS

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Heterobasidion annosum associated with Mortality of Christmas Trees in the Pacific Northwest

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Historically, Annosus root rot, caused by *Heterobasidion annosum* has seldom been a problem in Pacific Northwest (PNW) Christmas tree plantations. During the past four years, the prevalence of this disease has increased significantly in 2nd and 3rd rotation noble and Fraser fir plantings. During 2001, 21 field plots were established in grower plantations to obtain a better understanding of the losses associated with this disease. Trees in these plantations were originally planted between 1997 and 2000. A total of 32,496 trees in these plots were examined in 2001 and 2002 for above-ground symptoms such as branch flagging, wilting, and death associated with this disease. Data were also collected on the number of missing trees and number of replanted seedlings prior to the end of 2000. In 2001, the percentage of symptomatic trees ranged from 0.2 to 11.2%. If missing and replanted trees were included, the maximum percentage reached 29.2% in 2001. During 2002, losses increased in most fields. Maximum losses in 2002 reached 27.2% (42.2% if missing/replanted trees are included) in one Fraser fir and 16.8% (21.9% if missing/replanted trees are included) in one noble fir plantation. Annosus root rot was detected on noble, Fraser, grand, and Nordmann fir, as well as Douglas-fir. Noble and Fraser fir appear to be very susceptible to this disease.

Annosus root rot spreads via airborne spores that are able to infect freshly cut stumps and via root-to-root contact when roots of a healthy tree come in contact with an infected root or stump. Preliminary spore trapping data during 2002 indicates that *H. annosum* spores are present throughout the harvest season in PNW plantations with Annosus root rot infected trees. Stump removal prior to replanting or treatment of freshly cut stumps to protect them from infection by airborne spores is commonly recommended in forest situations to control this disease. These management practices have not been commonly used in PNW plantations. Of the 21 fields being monitored, limited disease has occurred where stumps had been removed prior to replanting. Additional data will be collected from these plots during the next couple of years in an effort to determine the extent of losses caused by this disease throughout a complete rotation.

Phytophthora Root Rot of Fir in Michigan

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P. cactorum, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. cryptogea*, *P. gonapodyides*, and *P. megasperma*, have been associated with diseased roots and stems in Christmas tree and Phytophthora root rot of true firs is well documented and more than seven species including forest tree nurseries in the Pacific Northwest and/or North Carolina (Shew and Benson, 1981; McCain and Scharpf, 1986; Chastagner et al,1994)

Phytophthora root rot of Fraser fir is of special concern to Michigan Christmas tree growers as this fir species represents a premium tree to the industry. Root rot of Fraser fir has not be a serious problem to most growers as long as the trees are planted in non-conducive soils that are light and well drained. However, even with the best of intensions, growers occasionally extend Fraser fir production to marginally conducive soils that may retard water drainage, especially during spring runoff. In these fields, Phytophthora root rot can cause devastating losses in young Fraser fir nursery stock as well as in older trees on plantations.

A *Phytophthora* survey of nurseries growing fir seedlings in Michigan in 1986 indicated that two species, *P. cactorum* and *P. citricola*, were present on *Abies balsamea*, *A. concolor*, *A. fraseri*, and *A. procera* in the lower peninsula (Adams and Bielenin,1988). Species infecting fir in plantations was not reported.

In order to manage plant diseses, it is imperative to develop an understanding of all species involved in a patho-system such as root rot as each species occupies it own niche and has its own life cycle. If proper management strategies are to be employed including cultural controls, use of fungicides and initiation of breeding programs for selection of root rot resistance the exact species, and in some cases the exact strains of the pathogen need to be well known. This information will also help develop a fundamental understanding of the disease through time as routine isolation and characterization of the pathogen may turn up more or less aggressive strains, additional species or new pathogens that cause similar symptoms.

Therefore, in 2001 we initiated a study in Michigan to determine the *Phytophthora* species associated with diseased Fraser fir in nursery beds and plantations where seedlings and transplanted trees, respectively, showed symptoms of Phytophthora root rot. Our short term objective was to determine the *Phytophthora* species associated with Fraser fir cropping systems in order to approach our long term objective of managing Phytophthora root rot in nursery beds, and determining the response of other firs to the *Phytophthora* species prevalent in Michigan.

MATERIALS AND METHODS

Phytophthora isolates were recovered from the rhizosphere of diseased or dying Fraser fir trees and seedlings from nurseries and plantations around the state. Occasionally, soil away from the root zone was sampled. Rhizosphere soil was sampled by baiting soil samples with rhododendron leaves after the method of Themann and Werres (1999). Single hyphae of resulting cultures were selected and placed on V8 agar to ensure purity.

To determine species identity, culture morphology was examined along with specific reproductive or resting structures such as sporangia, chamydospores and oospores. DNA was extracted and the internal transcribed spacer (ITS) region was amplified with ITS1-ITS4 and sequenced with ITS5-ITS4 primers or digested with restriction enzymes for random fragment-length polymorphisms (RFLP). Sequences were aligned with DNA Star software and Blast searches were conducted to compare the sequences and identify the *Phytophthora* isolates recovered (Cooke and Duncan, 1997).

Pathogenicity assays were conducted by potting Fraser fir in the greenhouse and incorporating into the soil sterile oat seeds infested with various strains of the pathogen (Shew and Benson,1981).

RESULTS AND DISCUSSION

Over 60 *Phytophthora* isolates were collected from eight plantations and nurseries. More than twenty of these isolates have been characterized to species (Table 1 and Fig. 1) and many have been tested for pathogenicity on Fraser fir (Table 2).

Table 1. *Phytophthora* species found associated with Michigan Christmas tree culture.

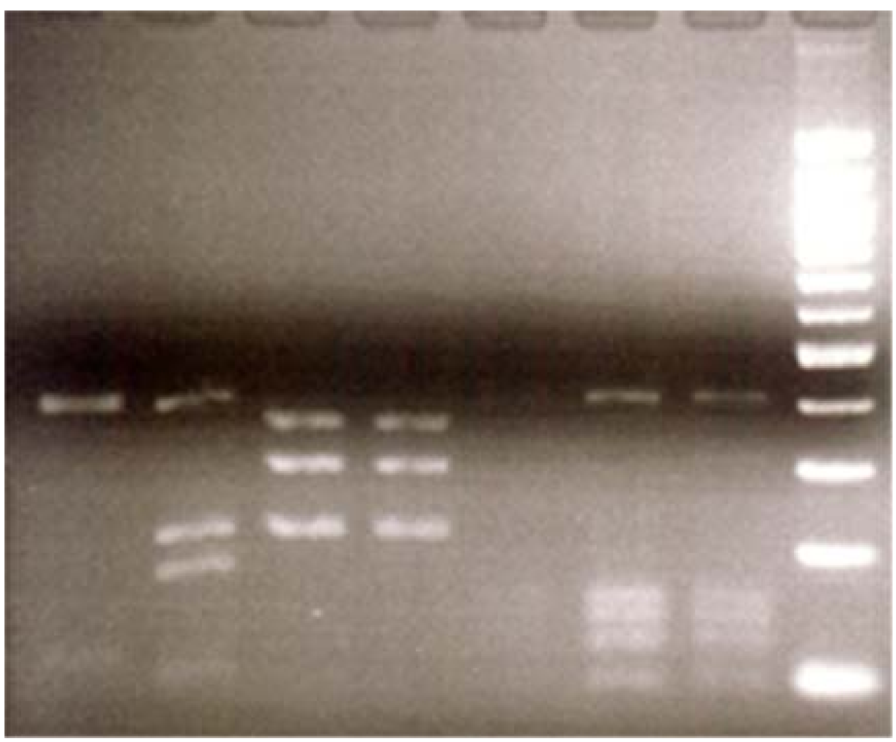
Location	Species	Number of farms
Field	*P. citricola*	4
	P. cactorum	3
	P. megasperma	2
	P. cryptogea	1
	P. medicaginis	1
	P. europa	1
Transplant bed	*P. citricola*	3
	P. cactorum	2
	P. nicotianae	1
Seedling bed	*P. citricola*	1
	P. cactorum	1
Pond/river water used for irrigation	*P. gonapodyides*	2

Similar to the findings of Adams and Bielenin (1988), the most common species in Michigan remains *P. citricola* and *P. cactorum*. Other species were found associated with the roots of dying Fraser fir, but other than *P. cryptogea*, the role these species play in pathogenicity if any, is not well understood (Table 2). Also, and as might be expected, some of the same species associated with symptomatic or dying trees in nursery beds were also associated with dying Fraser fir in plantations.

Table 2. *Phytophthora* species and strains tested for pathogenicity on Fraser fir.

Species	Number of strains tested	Number dead out of 11
P. citricola	1	11/11
P. cactorum	3	5/11; 9/11; 11/11
P. cryptogea.	2	11/11; 11/11
P. megasperma	2	0/11; 1/11
Rye seed control	1	1/11

Figure 1. RFLPs generated from an 800 base pair PCR-generated ITS region of *Phytophthora* isolates cut with *Msp*1. Lane 1, *P. nicotianae*; lane 2, *P. cactorum*; lanes 3 & 4, *P.citricola*; lane 5 is empty; lanes 6 & 7, *P.megasperma*; and lane 8, DNA size standard markers.



The findings in this study were applied to a severe root rot epidemic occurring at a Michigan Fraser fir plantation. Both *P. crpytogea* and *P. megasperma* were commonly found in the rhizosphere of symptomatic and dying Fraser fir in this field. However, it was obvious that the root rot was, at first, directly associated with the nursery stock from an out-of-state nursery since diseased trees immediately stopped at a row in the middle of a landing planted with Fraser fir obtained from an in-state nursery. In this landing containing 14 rows of Fraser fir, the first eight had disease and the last six were without disease or symptoms of root rot. Since *P. cyptogea* was prevalent on the dying trees in the field, and *P. cyptogea* had not been found in Michigan nurseries, it was not surprising when the grower explained he had run out of nursery stock from the out-of-state nursery and switched to the in-state nursery material in the ninth row of the landing. The *P. megasperma* associated with the trees was not very aggressive in greenhouse pathogenicity assays and its role in disease development is unknown (Table 2).

Finally, these data were also useful when surveying the field in question. Not only were Fraser fir brought in from different nurseries, but the Fraser fir stock was separated by a landing (16 rows) of Canaan fir. The Canaan fir however did not develop root rot under these field conditions even though the drainage pattern of the field representing the original disease focus passed directly through the Canaan fir planting. Another landing of Fraser fir planted to the distal side of the Canaan fir also developed Phytophthora root rot associated with *P. cryptogea*, but two years later. This disease pattern strongly supports the supposition that the oomycete was carried by the drainage water through the Canaan fir planting initiating root rot on the Fraser fir distal to the original focus of the epidemic. Yet, the passage of *P. crytogea* through this landing did not lead to root rot developing on the Canaan fir suggesting some level of field tolerance or other inhibitory agents of disease were present in the Canaan fir.

It is hoped that a better understanding of this disease and the development of management schemes and disease resistance are outcomes of these studies.

ACKNOWLEDGEMENTS

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Phytophthora Root Rot Mortality in Fraser Fir: Genetic Variation in an Infested Progeny Test

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Fraser fir (*Abies fraseri* (Pursh.) Poir.), which is native to a small number of isolated ridgetops in North Carolina, Tennessee, and Virginia, is widely grown in the Southern Appalachians for the fresh-cut Christmas tree market. Phytophthora root rot presents a serious economic limitation to these Christmas tree growers, with the majority of root rot damage caused by *Phytophthora cinnamomi* Rands. In 2000, a Fraser fir progeny test in Avery County, North Carolina, was unintentionally infested by *P. cinnamomi*. Using data from the 94 open-pollinated families at the test site, we analyzed seedling mortality with three objectives: 1) to analyze the genetic variation within the six major Fraser fir subpopulations for potential Phytophthora root rot resistance, 2) to estimate genetic parameters for these traits; and 3) to explore using a Geographic Information System (GIS) neighborhood analysis as a tool for separating potential genetic resistance to Phytophthora from uneven exposure to the pathogen.

Family within source was the only variance component significantly different from zero. When the populations were analyzed separately, family variance was also significant in some sources. The results from these analyses, and from future analyses of additional mortality at the site, were compared to results from a greenhouse inoculation test in progress during the summer of 2003. In this test, Fraser fir from 120 families and all six seed sources are being inoculated with a single *P. cinnamomi* isolate.

Our GIS analysis generated information on the proportion of dead trees near each individual in the plot. When we used this data as a covariate term in our genetic analysis of variance, the results did not result in different variance estimates and genetic parameters than without the use of the GIS-generated neighborhood mortality data. This may indicate that the single-tree non-contiguous plot design for the field study worked to reduce differences in Phytophthora exposure among Fraser fir families.

Susceptibility of Conifer Shoots to Infection by *Phytophthora ramorum*

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Phytophthora ramorum is the pathogen that causes sudden oak death (SOD), which was first detected on tanoak (*Lithocarpus densiflorus*) in Marin County, California in 1995. Two conifers, Douglas-fir and coast redwood, are among the naturally infected hosts that have been reported for this pathogen in California. The pathogen has also recently been isolated from grand fir Christmas trees in California.

There are a large number of different types of conifers that are grown as Christmas trees and in conifer nurseries in the Pacific Northwest (PNW). In an effort to better understand the potential impact this pathogen might have on the Christmas tree and conifer nursery industries, a series of inoculation studies were conducted to determine the potential susceptibility of foliage and shoots from 25 conifers and nine sources of intermountain Douglas-fir to *P. ramorum*.

A series of initial experiments were conducted with coastal Douglas-fir to determine the optimal growth stage for infection. Detached shoots were either sprayed with water or a suspension containing a mixture of zoospores (41,000 spores/ml) from three Oregon isolates of *P. ramorum*. The shoots were then sandwiched between two pieces of moistened filter paper in petri plates and incubated under lighted conditions at 19C for 7 to 14 days. The susceptibility of 25 conifers to *P. ramorum* was determined by collecting samples of 2002 shoots from trees grown at WSU Puyallup just after bud break in 2003. In addition, samples were also collected from 9 different seed sources of intermountain Douglas-fir. Detached shoots from each species of tree were inoculated and incubated as indicated above.

Symptoms associated with *P. ramorum* infections included needle blight, a shoot blight resulting from needle infections, and stem lesions resulting from the growth of the pathogen from infected needles/shoots into the 2002 wood. Growth stage had a significant effect on susceptibility. Douglas-fir shoots that were inoculated just after bud break were much more susceptible than those that were inoculated 2 to 8 weeks later. Twenty of the 25 conifers tested, including many of the important species that are used as Christmas trees throughout the world, were susceptible to *P. ramorum*. Some true firs were highly susceptible. The susceptibility of the different intermountain Douglas-fir seed sources was highly variable.

The results of these studies indicate that many different types of conifers have the potential to be susceptible to *P. ramorum* under certain conditions. At this point, it is unclear how likely it would be for disease to develop on these species under field conditions. At the field sites in California where conifers have been infected, the trees are growing in close proximity to other highly susceptible hosts that have the potential to produce high levels of inoculum.

Arthropods & Weeds

The Di-Syston Project: Development of a Hand Operated Metered Closed System Applicator For Christmas Tree Producers in Western North Carolina

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ABSTRACT

The purpose of this project is to develop a closed system applicator to apply 8 gram dose of Di-Syston 15 G (disulfoton) an organophosphate to Fraser fir (*Abies fraseri*) Christmas trees in western North Carolina. Approximately 49% of the acreage is treated yearly for these pests. Fraser fir production in western North Carolina has a wholesale value of $72 – 100 million, representing 15% of the national Christmas tree market(). Di-Syston 15 G is used to control balsam twig aphid (*Mindarus abietinus*) and to give season-long control of the spruce spider mite (*Oligonycus ununguis*). Other pesticides for these pests are foliar sprays which can not be applied with adequate coverage for control during the treatment window for the balsam twig aphid. North Carolina has a special local needs 24(c) label for the use of Di-Syston 15 G at 20 – 30 pounds per acre, approximately 1/10th the federal rate. Incorporation is not required on the 24 (c) label because it is impossible on steep slopes with a perennial crop surrounded by ground covers.

Currently Di-Syston 15 G is most commonly applied with a bucket and spoon or other hand-held, homemade device. . This application technique has been perceived by the US EPA and others as high risk to the applicator. During the Food Quality Protection Act review process for disulfoton, Western North Carolina Christmas Tree Growers wrote letters to the US EPA expressing their concerns about the possible loss of the product. In response the US EPA decided that a new hand held metered closed system applicator had to be developed. Efforts have been concentrated on developing an effective metered system that is cost effective durable, and acceptable to the growers. From November 1999 to December of 2002, Extension took a lead role in facilitating and developing a solution that all parties felt acceptable including the North Carolina Department of Agriculture & Consumer Science, Bayer Corporation, the Christmas tree growers, and the makers of the SELECT-A-FEED, the applicator which was modified to make the present model. Grower meetings, US EPA tour, workshops, and other educational opportunities were employed to reach a positive outcome. In April of 2003, a field test was conducted to monitor the exposure of workers to disulfoton and to demonstrate the durability of the applicator.Concern for applicator safety has been a major issue in maintaining the North Carolina 24 (c) label. The prototype for a new applicator has been developed by modifying an applicator for Marathon insecticide called the Select-A-Feed. Modifications include: 1) replacing the cone with a cap to attach the 2.5 gallon container directly, 2) making the S-curved slide trigger mechanism so chemical dust doesn't escape, 3) using a water jet cut blade instead of stamping to increase the tightness of the blade.

USE OF DI-SYSTON

Di-Syston 15 G is used on 49-65% of the Fraser fir acreage in western North Carolina to control the balsam twig aphid (*Mindarus abietinus*) and spruce spider mite (*Oligonycus ununguis*). These pests result in both cosmetic damage and growth loss (North Carolina Ag Statistics). Left unchecked, either pest can reduce tree quality and therefore market value, or delay harvest for one to two years. Estimated losses from poor control are from $600 to $6,000 per acre per year (Jeff Owen, personal communication).

Other materials control these pests including Provado (imidacloprid), Endeavor (pymrtrozine), Asana (esfenvalerate), Dimethoate for twig aphid control, and Savey (hexythiazox), Dimethoate, and Sanmite (pyridaben) for spider mite control. However, it is difficult to get adequate coverage using these foliar applied liquids in the narrow treatment window for twig aphids (two to four weeks). A high-pressure sprayer provides excellent coverage but is slow and labor intensive and results in high worker exposure to the pesticide. The relatively quick application with an air-blast mist blower results in poor coverage and control and has enormous risks from drift. Di-Syston 15 G provides quick, effective application in mountainous terrain. Mist blowers are also too expensive for the majority of Christmas tree growers that produce less than 10 acres of Christmas trees (Sidebottom 1996).

For the past ten years, Di-Syston 15 G has been a part of the Fraser fir Integrated Pest Management program. Use of this material provides excellent control, particularly of spider mites. Additional miticide applications have been required when other materials were used. Though extremely toxic to mammals, Di-Syston 15 G use has resulted in little impact on local ecosystems. David Lenat with the Division of Water Quality Biological Assessment Unit of the North Carolina Department of Health and Natural Resources observed that "Christmas tree farming has little negative effect on the fauna of adjacent stream with the use of integrated pest management and adequate stream buffer zones(Lenat,1998)." Natural predators including *Harmonia* lady beetles, hover fly larvae, and predatory mites are commonly found a week after Di-Syston application.

In addition, work is being conducted at North Carolina State University to determine economic thresholds and scouting protocols to reduce the need for balsam twig aphid treatment (Sidebottom 1994). Unfortunately, observations from 1998 – 2001 have indicated that most Fraser fir to be marketed that fall do require a pesticide treatment for the balsam twig aphid(Sidebottom, 1999)

There is a 24 (c) Special Local Needs Permit for granular Di-Syston in North Carolina at the rate of 20 to 30 pounds per acre. This label does not require incorporation into the soil as the federal label does. Many growers apply this material with a spoon and a bucket or some other homemade, handheld device. Some growers apply granular Di-Syston with a Soda-Flo fertilizer spreader attached to a motorcycle. Usually there is a mixer/loader who keeps either the motorcycles or buckets filled with material, and several applicators at each farm for quick and easy application.

Three quarters of the state's growers are small enough that they can apply the material themselves in one day or less. Larger growers that must hire workers only apply Di-Syston for part of the day for 2 to 5 days during a 2 to 3 week period. Therefore Di-Syston applications are made in 1 - 7 days, which conforms to EPA's definition of short-term dermal exposure. During application, workers commonly wear the Tyvek disposable suits, chemical resistant gloves, and a dust/mist-filtering respirator (MSHA/NIOSH approval number prefix TC-21C) as required on the Di-Syston 15 G label.

EPA TOUR AND RECOMMENDATIONS

The EPA is currently reviewing disulfoton under the Food Quality Protection Act of 1996. Five scientists from EPA including Christina Scheltema, the product manager for disulfoton, Mike Goodis, Harry Craven, Mike Hennessey, and Sayed Tadayon, toured western North Carolina June 25-27, 2000, to learn about the pests controlled, alternative pesticides and economic impact of this material. They were given an informational packet containing 25 documents on Di-Syston 15 G use and toured 7 farms in 5 counties. Their major concerns with granular Di-Syston use were:

1) Too great an exposure to the material by workers. Though the application period is short, and applicators wear protective equipment, the MOE (margin of exposure) for the bucket and spoon applications of granular Di-Syston is still considered too great.
2) Potential for exposure of wildlife to the unincorporated material. Small mammals were of particular interest.

It was suggested that a closed system applicator for Di-Syston 15 G to reduce worker exposure be developed.

To be workable, the new applicator must apply a metered amount to each Christmas tree in the field equivalent to a teaspoon or 8-8.4 grams of formulation. Other design criteria are as follows:

1. It must be light-weight – holding no more than 18 pounds of chemical – so that it can be carried up and down steep slopes.
2. The applicator needed to handle ½ to ¾ of an acre.
3. It must deliver the material at the ground at the drip line of the tree.
4. It must be a closed system.
5. It must be economical for growers to use.

Currently, Di-Syston 15 G is sold in 10 and 50 pound paper bags with plastic linings. Bayer has indicated that it will place the material into 2.5 gallon jugs.

DEVELOPMENT OF THE APPLICATOR

A prototype having many of these features has been designed by Leo Bennet of Cedar Creek Nursery and Carlton Hinnant of Hinnant's Nursery and Landscaping in Johnston County, North Carolina. They were asked to modify their Select-A-Feed and Select-A-Feed Jr. (Figure 1) which apply fertilizers and Marathon insecticides respectively to containerized nursery materials. Since Marathon uses a similar carrier as Di-Syston 15 G the modifications were expected to be minor.

The prototype was displayed at a meeting in Winston-Salem, NC, on January 5, 2001, to North Carolina Christmas tree growers and representatives of the North Carolina Cooperative Extension Service, North Carolina Department of Agriculture & Consumer Sciences, Bayer Corporation, and Sotera Systems. A videotape of this meeting was sent to Christina Scheltema with EPA.

One important change to the Select-A-Feed was a tighter fit for the slide trigger mechanism so that dust from the product would not escape. The trigger mechanism is an S-curve system. When the trigger is pulled, it opens the top and closes the bottom. When it is released, it closes the top and opens the bottom to release it to a tube. The tighter fit for the prototype was accomplished by using delron. This plastic is very durable, and allows the metal portion of the trigger mechanism to slide smoothly back and forth. To achieve the tolerances required, the slot through which the trigger mechanism slides is cut with a water jet.

Tooling costs for the new applicator were estimated by Leo Bennet and Carlton Hinnant to be $30,000. Grant monies were sought so that these costs would not be passed on to the growers, as well as to reduce the risks to the developers. A $14,000 Pesticide Environmental Trust Fund grant was obtained from the North Carolina Department of Agriculture & Consumer Sciences in February 2001 to for this purpose. The North Carolina Christmas Tree Association donated $5,000 and various county associations donated $2,800. The EPA Strategic Ag Initiative funded the remainder 21,800.

The last valve used in the prototype is one designed by Sotera Systems, Inc. for a closed system application of liquids. This valve becomes jammed with the Di-Syston 15 G granules. Wes Wall with New Perspectives developed a valve that would allow for the material to be dumped, however the cost of this particular valve far exceeded what was acceptable. Finally a metal ball valve was used to turn on and off the flow. This was utilized after the decision that a dump valve was not practical. The ball valve can be turned to allow flow of product. This particular valve was economical and practical.

The final product uses the actual jug as the reservoir, a ball valve to allow the flow into the metering device, opposing paddles to meter out the amount of product, and a bolt located in the cap portion of the applicator to cut out the seal. The last design met with grower approval, and was then incorporated into field trials.

SUMMARY

The final product was field tested in April of 2003, and the analysis of the worker exposure data will be completed in January of 2004. Bayer has granted Avery County up to $65,000 to pay for the analysis, and they have also agreed not to pass on the costs of the new packaging for the logistics test in spring of 2004. At this point, the new applicator exists, the concern of worker exposure has been adequately answered, Bayer has agreed to package it in 2.5 gallon jugs, the EPA has agreed to allow the application of this product if it is done via a closed system applicator, the Select-A-Feed company has agreed to make the applicators, and the growers have agreed to steward the product and its application system. The growers now have a product and practical applicator.

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Arthropod Pest Management in Christmas Trees in the Pacific Northwest

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Several arthropod pest species attack commercial christmas trees in the pacific northwest united states. We have been evaluating various control agents for several pests that infest christmas trees. In particular, we have focused on root aphids, adelgids and eriophyid mites. The insecticide, aphistar has been found to be a particularly effective control for root aphids. Aphistar is applied to foliage and when applied at a rate of 8 oz. Product /acre, controls these root pests for up to 6 weeks on noble fir. A new insecticide, acetamidprid was evaluated for control of adelgids. Acetamidprid applied to foliage at rates of 0.05 lb ai/100 gallons water and 0.1 ai/100 gallons water provided excellent control of adelgids on douglas fir. Thiodan and mesa were evaluated for control of an eriophyid mite, nelepella longoctonema on noble fir. Thiodan was the most effective product, but mesa also provided control of this species. Although we have focused on pesticides for control of christmas tree pests so far, our goal is to develop integrated pest management for pests attacking christmas trees that will integrate selective pesticides and biological controlevaluating grub control products

Pest Management Control in Christmas Trees Grown in the Southern Appalachians

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ABSTRACT

In 2001, a pest management survey was conducted to determine growers' pest control practices. The 54-question survey was mailed to 1,013 growers whose names were taken at random from county extension mailing lists from 12 counties in western North Carolina. Several growers from Tennessee and Virginia were also on these lists and were included in the survey. There were 336 growers that filled out some portion of the survey representing 14,746 acres of Christmas trees. Growers were asked questions about their pesticide use in 2000, scouting practices, farm size, equipment for pest control, as well as damage from pests. Information was compared to a prior survey conducted in 1995 by Steve Toth at North Carolina State University. The average grower managed 44.4 acres, up from 22.7 acres in 1994. Many growers were using lower than labeled rates of post-emergent herbicides such as Roundup (glyphosate) to control ground covers. The use of Simazine, a broad spectrum, pre-emergent herbicide had declined from 72% of the acreage to 39%. It was estimated that growers use an average of 1.72 pounds active ingredient per acre. A lack of calibration of herbicide applications with backpack sprayers was identified as a problem that kept growers from achieving their ground cover management goals. There were also changes with insecticide/miticide use. Use of dimethoate which did not have a clear Christmas tree label in 1994 increased to 21% of the acreage. Growers use dimethoate to control the rosette bud mite (*Trisetacus fraseri*), the hemlock rust mite (*Nalepella tsugifoliae*) which has been an increasing problem, and to control spider mites (*Oligonychus ununguis*). Di-Syston 15 G (disulfoton) was the primary insecticide used, though the use had declined from 65% of the acreage in 1994 to 50% in 2000. Di-Syston is used to control the balsam twig aphid (*Mindarus abietinus*) and spider mites. Lindane was still the predominate material to control the balsam woolly adelgid (*Adelges piceae*), even though it was no longer being manufactured in 2000. Only 53% of growers own a high-pressure sprayer which is necessary to control the woolly adelgid and rosette bud mite.

THE NEED FOR A NEW PESTICIDE USE SURVEY

In 1995, Dr. Steve Toth, pesticide impact specialist at North Carolina State University, conducted a pesticide use survey of pesticide use in 1994 by the Christmas tree industry in western North Carolina. This information was the basis of the Crop Profile written by Jill Sidebottom and was important in understanding what products are important to Christmas tree production.

However, another survey was needed because of many changes that have occurred since 1994. Many pesticides are undergoing review under the Food Quality Protection Act of 1996, particularly Di-Syston 15 G, Dimethoate and Goal. Pesticide rates were not asked for in the

1995 survey, and many growers use materials at lower than labeled rates – particularly when chemical mowing (see below). It was not known how growers were applying pesticides or what type of pesticide application equipment they had available to them. Also, there were several new pests of Fraser fir including the hemlock rust mite, and it was important to document changes in pest pressure.

THE SURVEY METHOD

Mailing lists of Christmas tree growers in twelve western North Carolina counties were obtained from county extension centers. The counties chosen included Alleghany, Ashe, Avery, Buncombe, Haywood, Jackson, Macon, Madison, Mitchell, Swain, Transylvania, and Yancey. These counties encompass the entire Fraser fir producing region. There were 1,786 growers included in these county mailing lists. Approximately 57 % of growers were selected randomly to receive surveys resulting in 1,013 surveys being mailed.

The survey design was similar to the one used by Steve Toth. The surveys were mailed in March of 2001. A reminder card was mailed to all growers 10 days later. A second copy of the survey was mailed to those who hadn't responded about 3 weeks after the initial mailing. To do this, the number corresponding to each grower was written on the stamped return envelope that was initially sent. However, surveys were anonymous. Once the survey was received, and the name of the grower was marked off the list, the survey itself could no longer be identified as any one grower's.

There were 57 questions in the survey in five sections – grower production, ground cover management, insect/mite control, disease control and a section of miscellaneous questions. These questions were for the pest management practices in 2000. The survey was returned by 47.6% or 336 Christmas tree growers. They represent 14,746 acres of production. Only a small portion of the survey results is reported here.

To determine information on pesticide use, growers were asked to list each pesticide used in 2000 under the appropriate section. Growers were asked to write down the number of acres on which each material was used and the number of applications made in 2000. The use rate was also asked based on either the amount of formulation mixed with 100 gallons of water and the gallons of spray applied per acre, or the number of ounces of formulation mixed into a backpack sprayer and the number of backpack sprayers applied per acre. This information was compared to each grower's total acreage.

GROWER PRODUCTION

The average farm size was 44.4 acres and the average tree harvest reported was 7,033 trees in 2000. More than ½ of all growers, 51.3%, managed less than 10 acres of Christmas trees. However, they only made up 4.7% of the total survey acreage. There were 10.8% of growers that managed 100 acres or more. They account for 73.2% of the survey acreage. This was similar to results from the 1997 North Carolina Department of Agriculture Survey that estimated that 50% of the Christmas tree acreage in western North Carolina is in the hands of less than 4% of the growers.

Only 23.8% of growers surveyed considered themselves full-time growers and are fully self supporting from their Christmas tree business. About 1/3 of growers, 33.0%, had a business partner on at least some of their trees. These business arrangements can affect pest management. The part-time grower has to work around another full-time job making it difficult to be in the field to scout or apply a pesticide in a timely manner. Growers who have a business partner have to accommodate that partner's opinions on pest control. Delays may also occur as the partners consult with each other on the best way to manage a pest problem. There were 5.4% of growers surveyed that manage other people's Christmas tree farms.

Only 53.5% of surveyed growers reported owning a high-pressure sprayer, the type of application equipment required to make applications for the balsam woolly adelgid. Other growers would have to hire a commercial pesticide applicator or borrow another growers. Even fewer growers, 17.1%, own an air-blast mist blower which can be used for the quick application of materials against the hemlock rust mites and balsam twig aphid. Many of these growers use dimethoate. The US Environmental Protection Agency had indicated that they may require the use of an enclosed cab for worker safety when applying with a mistblower. Most growers owning an air-blast mist blower do have an enclosed cab on their tractor – 15.5%. However, not all are properly outfitted for pesticide application.

GROUND COVER MANAGEMENT

Christmas tree growers in western North Carolina are taught that they manage not one but two crops – the Christmas trees themselves and the ground covers growing around them. Ground covers reduce problems with erosion. The soil is kept cooler, allowing Fraser fir roots to grower closer to the surface of the soil where fertilizers are applied. The 10 most commonly reported problem weeds from the survey in order are: poke weed (38.4% of responses), ragweed (34.5% of responses), briars (28.2% of responses), morning glory (20.8% of responses), poison ivy (18.4% of responses), foxtail (13.7% of responses), thistles (12.9% of responses), bindweed (11.4% of responses), grasses (11.4% of responses), and pigweed (10.2% of responses). Flowering ground covers may provide a habitat for many natural predators that feed on other pests. If ground covers are growing, there is less of an area for problem weeds to occur as many of these only grow on disturbed soil.

Growers were asked to mark responses that best describe their method of ground cover management (Table 1). There was an average of 1.5 responses to this question reflecting that growers use different ground cover management strategies in different situations. For instance, many growers use pre-emergent herbicides in the row under the trees while the trees are small, and then stop this practice as the trees get older and can shade out weeds for themselves.

Growers commonly use herbicide suppression or "chemical mowing" to stunt weed growth. This is where lower than labeled rates of post-emergent herbicides such as Roundup are applied to stunt weeds. The timing of application and herbicide rates are important as there is a fine line between applying too much herbicide and killing more of the ground cover than desired, or applying too little and not getting a good stunt. Also, Christmas trees can be injured by these practices.

Table 1. Responses to 2001 survey question #10: "What best describes your method of ground cover management? Check all that apply for all ages of trees."

Ground Cover Management Practice	Percentage of 324 Respondents*
Bare ground management	3.7%
Mechanical mowing or weed-eater only – no herbicides used	9.9%
Sow ground cover**	12.3%
Broadcast herbicide suppression of all groundcovers	25.0%
Herbicides banded in row to kill weeds under tree, herbicide suppression of middles	39.5%
Herbicides banded in row to kill weeds under trees, mow middles	54.6%

* The sum of the percentages in this column will exceed 100% as growers were instructed to check all answers that applied.
** Sown ground covers listed by respondents include: clover, creeping red fescue, rye, wheat, barley, flowering plants and oats.

Herbicide use has changed considerably from 1994 to 2000 (Table 2). In particular, the use of Simazine has declined considerably as well as the use of Goal, Stinger and Vantage. Grower use of Simazine continues to decline as growers shift to leaving less bare ground. The Goal/Stinger/Vantage mixes were used to chemical mow before Roundup was used so widely.

Table 2. Responses to the 2001 survey question #14: "Which of the following herbicides were applied to your Christmas tree fields in 2000?" as compared to responses to a similar question in the 1995 Pesticide Use Survey.

□HERBICIDE□1995 Pesticide Use Survey HERBICIDE	1995 Pesticide Use Survey	2001 Pest Management Survey
Roundup (glyphosate)	93.7%	94.9%
Simazine (simazine)	72.2%	38.8%
Goal (oxyflurofen)	43.8%	20.7%
Vantage (sethoxydim)	23.9%	14.7%
Stinger (chlorpyralid)	22.8%	11.7%
Garlon (triclopyr)	---	7.8%
Crossbow (premix of 2,4-D plus triclopyr)	0.9%	3.1%

Atrazine (atrazine)	0.1%	2.7%

* The sum of the percentages in this column exceed 100% as growers were instructed to check all answers that applied.

Growers were asked how they applied these herbicides. Of the 299 people responding to this question, 96.7% made herbicide applications with backpack sprayers, 8.4% made applications with an air-blast mistblower, and 7.4% made applications with some sort of vehicle mounted sprayer such as a tractor or 4-wheeler. The North Carolina Cooperative Extension Service currently does not recommend herbicide applications with an air-blast sprayer. Most of these are with Roundup.

The rates of Roundup varied greatly (Table 3). About half of the growers are using Roundup at rates considered appropriate for chemical mowing. Roundup is being used at appropriate rates for killing problem weeds by almost one-third of the growers (Table 3). The average rate for Roundup use was calculated at 0.64 pounds active ingredient per acre which is less than the range of killing rates. However, more than one-quarter of growers are using Roundup at a rate too high for chemical mowing and too low for a killing effect (Table 3). These results match observations made by County Extension Agents in North Carolina. Growers may be intending to use Roundup for chemical mowing, but are using it at too strong a rate and killing more weeds than they intend. It is important to note that these rates are effective at killing weeds if Roundup is mixed with another material such as Garlon. These rates may also be effective at killing weeds in the spring when plant growth is tender.

Table 3. Roundup rates and their effectiveness in ground coverage management as determined from the 2001 Pest Management Survey.

Range Of Roundup Rates in Ounces Formulation per Acre	Effectiveness of Use Rate	Percentage of 212 Respondents Using that Rate
3 ounces per acre	Too low to be effective even for chemical mowing	0.4%
4 to 12 ounces per acre	Chemical mowing rates	37.7%
14 to 21 ounces per acre	Too high for chemical mowing, too low for killing*	28.3%
24 to 48 ounces per acre	Recommended killing rate	27.8%
>48 ounces per acre	Excessive rate except for hardest to kill woody vines	5.7%

* These rates may be effective at killing weeds when mixed with another material such as Garlon.

The typical ground cover management practice for western North Carolina Fraser fir Christmas tree growers is to apply Simazine in the tree row in the spring, especially in young trees, then use chemical suppression of Roundup two or three times through the growing season at rates of 4 to 12 ounces of formulation per acre. Fall treatments of killing rates of Roundup or Garlon are used to control problem weeds as needed.

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INSECT/MITE CONTROL

Growers were asked which for which pests did they apply an insecticide and/or miticide (Table 4). Growers continue to treat most for the three major pests of Fraser fir, the balsam twig aphid, spruce spider mite and balsam woolly adelgid. Treatments for two eriophyid mites, the hemlock rust mite and rosette bud mite, continue to increase.

Table 4. Responses to the 2001 survey questions #24: “For which insects and mites did you use an insecticide or miticide to control in 2000?” as compared to responses to a similar question in the 1995 Pesticide Use Survey.

PEST	1995 Pesticide Use Survey	2001 Pest Management Survey
Balsam twig aphid	89.1%	71.7%
Spruce spider mite	37.4%	54.5%
Balsam woolly adelgid	37.8%	48.5%
Hemlock rust mite	6.5%	20.2%
Rosette bud mite	5.4%	11.9%
White grubs	2.4%	3.3%
Cinara aphids	---	3.0%
Spruce gall adelgid	0.7%	2.4%
Root aphids	---	2.1%
Bagworms	---	1.2%
Pine bark adelgid	1.7%	0.9%
Scale insects	---	0.3%
Termites	---	0.3%
Pine sawflies	0.3%	---

* The sum of the percentages in this column will exceed 100% as growers were instructed to check all answers that applied.

Insecticide/miticide has also changed a great deal from 1994 until 2000. As in 1994, Di-Syston 15 G and Lindane are the first and second most widely used materials, though the percentage of trees treated with Di-Syston has declined (Table 5). Use of Lindane is slightly higher, which is surprising because Lindane is no longer being manufactured. By 2001, however, growers had begun to run out of their supplies of Lindane. The use of Dimethoate is up considerably (Table 5). In 1994, Dimethoate was not considered labeled for use on Fraser fir but this changed in 1998 when Clean Crop came out with a Dimethoate label that included Christmas trees as a labeled site of application. Growers use Dimethoate to control the RBM and HRM. The mix of Dimethoate + Savey controls both BTA and SSM. As with Lindane, any use of Morestan in 2000 is by growers who are using up old material.

Table 5. Responses to the 2001 survey questions #25: "Which of the following insecticides/miticides were applied to your Christmas tree fields to control insects and/or mites in 2000?" as compared to responses to a similar question in the 1995 survey.

INSECTICIDE	1995 Pesticide Use Survey	2001 Pest Management Survey
Di-Syston 15 G (disulfoton)	64.58%	49.6%
Lindane (lindane)	21.67%	23.8%
Dimethoate (dimethoate)	2.34%	21.2%
Asana XL (esfenvalerate)	11.82%	16.6%
Lorsban (chlorpyrifos)	5.75%	8.4%
Savey (hexythiazox)	---	5.4%
Morestan (oxythioquinox)	14.33%	3.0%
Thiodan (endosulfan)	---	2.2%

* The sum of the percentages in this column exceed 100% as growers were instructed to check all answers that applied.

Growers were asked how they applied insecticide and miticides in 2000 (Table 6). Responses reflect growers' various methods for controlling several pests. Spring applied Di-Syston 15 G, which is a granular, controls twig aphids and spider mites and is the most common method of controlling these pests (Table 6). However, if growers need to control the balsam woolly adelgid too with a high pressure sprayer, they will may treat during the spring window and control twig aphids too. Hemlock rust mites are often controlled with a mistblower.

The typical insect/mite control for growers in western North Carolina is to treat for grubs before planting if needed. Growers treat the year of sale and year before sale for the balsam twig aphid. The balsam woolly adelgid is controlle once or twice in a rotation of 5 to 10 years. Growers will treat for the mite pests such as spider mite, rust mites and rosette bud mites as needed.

SUMMARY

This information has been used to update the Crop Profile and to develop a Pest Management Strategic Plan for Christmas Trees in western North Carolina, eastern Tennessee and southwestern Virginia. Comparisons with future surveys will help document change as growers continue to adopt IPM practices.

Table 6. Responses to the 2001 survey question #26: "How did you apply insecticides/miticides for the following pests in 2000? If a single application was made to control several pests, mark the same method of application under each pest. If you didn't control a particular pest, leave it unmarked."

Method	Balsam Twig Aphid		Spruce Spider Mite		Hemlock Rust Mite		Balsam Woolly Adelgid	
	Number of Respondents	Percentage of 311 Respondents	Number of Respondents	Percentage of 253 respondents	Number of Respondents	Percentage of 83 Respondents	Number of Respondents	Percentage of 176 Respondents
Di-Syston 15 G	209	67.2%	160	63.2%	27	32.5%	20	11.4%
High pressure sprayer	50	16.1%	43	17.0%	25	30.1%	110	62.5%
Mistblower	24	7.7%	22	8.7%	15	18.1%	15	8.5%
Backpack sprayer	14	4.5%	13	5.1%	7	8.4%	8	4.5%
Backpack mistblower	1	0.3%	1	0.4%	1	1.2%	3	1.7%
Vehicle mounted pressure sprayer	13	4.2%	14	5.5%	8	9.6%	20	11.4%

* The sum of the percentages in this column exceed 100% as growers were instructed to check all answers that applied.

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Achieving Results Through On Farm Research, A Case Study: Impact of Round-Up Original Applications on Fraser fir Foliage during Early Shoot Elongation

Doug Hundley
Director, Avery Co. IPM Program

My name is Doug Hundley. I am an IPM technician in the Cooperative Extension Office in Avery County North Carolina. I have had the pleasure of managing an IPM Program based in the County and focused on Fraser fir production. I have over 85 tree grower members in the program and have spent more than ten years working one on one with these tree farmers. I have and continue to teach them IPM scouting skills and data based treatment decision making in the management of both insect and weed pests.

Recognizing the basic IPM principle, our goal has always been to learn to manage both of these types of pests not necessarily eliminate them. Our efforts have always been to do this as economically and environmentally friendly as possible.

I have always been warned that because of the nature of my program, which is teaching the tree growers to scout rather then scouting for them, that I might work myself out of a job. To some extent, I'm glad to say, they have been right. However, because of the long term working relationships I have with these growers, I've come to enjoy looking at pest management questions with these growers. We've initiated large field research projects over the last few years. We call this work "On Farm, Grower Participatory Research". Nationally I understand it's called On Farm Research.

What I would like to do here is to share my experience with On Farm Grower Research, for short, by telling you about one project I have been involved with for three years. This is a study that that we hope will be useful to Fraser fir tree growers in their groundcover management efforts.

First, let me say some things about OFR in general terms. Research can be done at the university with a Phototron where there is a high degree of control and very specific changes can be managed. Alternatively, research can be done at the systems level looking at whole farm systems where there is low control of variables. Each of these approaches has their value for advancing certain types of knowledge.

On Farm Research or "OFR" is a segment of this spectrum that has been not been utilized nearly as much as it could be. There are certain projects that are very suitable to OFR.

Where does OFR fit in? In many cases farmers have been the innovators in influencing new ideas or new methods, then university personnel came to understand the mechanisms by which those processes and methods succeed.

The strength of OFR is to recapture this source of innovation and include the grower, in the actual process of research. Why not? The growers possess the common sense and problem solving capabilities needed. They are often the origin of many practical and pertinent questions and some times have the answers too. They have great experience and large numbers of trees. The tree growers are a monumental resource.

On Farm Research requires a slightly different outlook and different model. OFR gets away from the expert based model and moves towards partnership. The OFR model changes the role of Extension personnel from being the bringer of expert knowledge to being the facilitator to coordinate research initiatives that address key questions growers may have. OFR should be responsive to farmer's questions. Then bring in the experimental design and the use of statistics to answer their questions in a way that farmers can understand and everyone can have confidence in.

Now a little background; It may be hard to tell this prior to the upcoming tours later this week, but you will soon see that we grow Fraser fir on mountain sides, highly erodible mountain sides. It is a given here, that groundcovers are necessary for the long term production of Fraser fir in Western NC.

For over 15 years NC State Extension has been involved in the development of a technique for managing groundcover while leaving the groundcover alive to assist with top soil stabilization and erosion control.

After much work in the 1980's, NC Extension began recommending the use of Stinger, Vantage, and Goal for a new "sod suppression" type weed control in Fraser fir Christmas tree production in the early 1990's. This was important as a sustainable and environmental alternative to broadcast pre-emergent herbicides encouraged in the 1970's. A mix of these three herbicides at low rates could be safely sprayed over tree growth during any most any week of the summer. It would also achieve weed suppression for up to 6 weeks. Among growers it became known as "chemical mowing".

It is effective during most years but very expensive at ($40-50 per acre in chemical cost alone). Labor is cheap because the speed of application is fast with no need to keep the spray off the trees. However, many growers were still uncomfortable with the price.

Shortly after being introduced to the idea of chemical mowing, the growers began using low rates of Round-up (6-12oz/A) to achieve the same suppression. They often found it to be more effective.

Extension was following and duplicating their efforts and reminding them to minimize contact with tree foliage from mid April to mid August. This is the time period of most active Fraser fir growth.. The growers continued to increase their use of these suppression rates of Round-up and developed a "sloppy directed approach". This meant they did lightly hit some lower limbs on the trees, and might get a little damage, but usually damage they could live with. As the growers practiced this they began to notice weeks of the summer when little or no damage occurred while they hit the tree foliage.

As a member of the Extension Service and a tree grower I can say that we had all began wondering what the real damage thresholds were with Round-up on Fraser fir during the growing season. But without research education and little to no calibration practiced with the backpack sprayers, the growers remained satisfied with the "sloppy directed" approach. The chemical cost with Round-up was low and the labor costs of application were a little high, but worth it. Still the question of unknown damage thresholds remained.

I was finally approached by a grower with this question in the fall of 2000. He asked me "How am I getting away with hitting my trees in July with Round-up? Keep in mind he didn't really know what rate per acre he was using. I offered to help him figure out how or why he was "getting away with it". He took me up on my offer.

I had just attended an OFR Class sponsored by SARE, and taught by Keith Baldwin and Scott Marlowe. So I was ready to apply the OFR model to this intriguing question. In addition to Keith and Scott, I also asked Dr. Joe Neal, our NC STATE Weed Science Specialist to help us and he obliged. This grower with the question, Bill McNeely and I recruited an Avery County "On Farm Research" Group to look at the question. The group and I began the 2001 Round-up Tolerance Study that winter.

I encouraged the growers to participate in every aspect of the project; the design, the implementation, and the evaluation of the study. The project has been a pleasure for the growers and myself. I trust that I can speak for them. The growers have had the opportunity to learn as much as they wanted to about the process of field research. I hope they will continue to pursue it on their own if they wish.

Our first year of work involved 5 different farms and 19 plots of trees. We were testing rates of Round-up Original ranging from 4-12oz. per acre. We were making applications during three different windows of time or Fraser fir growth stages, through the summer weed season. We also made multiple applications on the same trees. Most importantly, we were applying the Round-up non-directed. We intentionally hit at least the bottom third of all trees. This would allow us to get complete weed contact. This change made it different from the sloppy directed applications.

The results of the first year were very encouraging. The higher rates caused unacceptable damage during a certain time/new growth window. The lower and middle rates caused little or no damage through out the season. Weed suppression was very good with the lower and middle rates. They were successfully suppressing the groundcover without damaging it severely.

The results of this first year of study encouraged us to not only continue but expand the project. The growers had a few new ideas that would turn out to be very significant.

We have been testing the effects of delivering the herbicide with lower amounts of water. We have tested different nozzles.

I believe, most importantly, we tested tools and techniques to improve the calibration and accuracy of backpack applications by both individuals and groups of worker in the field. We have tested a new pressure or flow regulator sold by Solo. We have studied the effects of multi-year applications So you can see why we needed so many farms, growers, and trees.

In 2003, we have been fine tuning rates, dates, and nozzle configurations to find the safest and most effective way to manage the ground cover during June, the most tender time for Fraser foliage. We are evaluating the best choices based on the data and the grower's opinions.

Most importantly I would like to say that Round-up Classic, at these rates, is very selective. It tends to leave unharmed, desirable groundcovers like strawberry, dutch white clover, violets, nimblewill grass and other low growing, desirable, perennial groundcovers . At the same time these rates stunt the undesirable weeds such as tall and competitive annuals, perennial grasses and broadleaves. We hope this shift will result in a living, weed controlling, groundcover.

It is a delicate practice and requires an accuracy and calibration of backpack applications that growers don't normally do. This year we exported this new Round-Up Weed Suppression practice to other Fraser fir counties by involving them in the 2003 Study. . As new growers begin to try the practice each year, there is normally an immediate positive reaction. Having another 8-12 growers from neighboring counties contributing their results to ours has helped us fine tune the practice and increased our confidence in the Practice. At the same time we now have these County Agents in Mitchell, Watauga, Ashe and Allegany leading the way with this Practice within their own counties. Most these gentlemen are here today, they are Jeff Vance, Jerry Moody, Jim Hamilton, Colby Lambert, David Isner and Bryan Davis.

The growers in the study are reducing their use of Princep and Goal dramatically. This is accounting for significant reductions in herbicide expenses. This non-directed Round-up suppression practice obviously has significant economic value as well as environmental.

This year we have had the opportunity to watch several fields of trees demonstrate the results of 2-3 years of continuous practice. We are seeing large acreages of dutch white clover and other desirable plants that are controlling summer annual weeds and grasses.

Many fields this year required only two applications. The full understanding of the effects of this practice on the groundcover composition is yet to be fully understood or appreciated. However we are finding it very interesting and encouraging. This is truly a long term ground cover systems process.

The best evidence of the success of OFR and this Study may be in the numbers. Our 2001 OFGR group consisted of 5 growers and they invested 2,500 trees in the study. The next year our 2002 group expanded to 10 growers and over 500,000 trees were treated in the study with in Avery Co. In 2003 the Study expanded into adjoining counties.

It is notable I think that while this has been a large and complicated study out of necessity, it has naturally disseminated the information as it has developed. This may turn out to be typical of OFR. I don't know.

If the growers are involved in the research and the work answers questions most important to them, and the work is done on their own farms, there may be a quick response and a quick dissemination of information. Maybe this is simply another illustration of the influence growers have over other growers. Maybe OFR is a new opportunity for Extension to continue to use this well known aspect of human nature.

In summary I would simply like to say that the growers I have been working with will tell you, "Seeing it work on their farm, in their own trees, is convincing". I personally would add that the more they play a part in the research the more they will "trust what they are seeing".

Responses of Christmas Tree Species and Selected Weeds to Herbicides

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Research studies were conducted in 2003 to evaluate potential and standard herbicides for Christmas tree production. In the first study, herbicides were evaluated for use on 10 plug-grown conifer species. Treatments were applied on April 16, and included flumioxazin (BroadStar, 100 lb/A), flumioxazin (SureGuard, 0.17 lb ai/A), metolachlor (Pennant Magnum, 1 lb ai/A), isoxaben (Gallery, 0.5 lb ai/A), trifluralin (Treflan, 0.5 lb ai/A), oxadiazon (Ronstar G, 100 lb/A), metolachlor plus isoxaben, and lactofen (Cobra, 0.125 lb ai/A). Crop injury was generally low from treatments. Weed control was good with flumioxazin and metolachlor plus isoxaben, but was not acceptable with trifluralin, metolachlor alone, or granular herbicides.
In separate studies, herbicides were evaluated for preemergence and postemergence control of knawel (*Scleranthus annuus*) in seedling blue spruce. Treatments were applied May 22, and included flumioxazin (SureGuard, 0.25 lb ai/A) alone and in mixture with metolachlor (Pennant Magnum, 1.5 lb ai/A) and oryzalin (Surflan, 2 lb ai/A), flumioxazin (0.38 lb ai/A), simazine (Princep, 1.5 lb ai/A) and imazaquin (Image, 0.375 lb ai/A). Simazine and imazaquin were applied alone and in mixture with metolachlor. At four weeks after treatment, no visible injury was observed. Postemergence knawel control was at least 72% with all herbicide treatments. Control was high at 95% with flumioxazin plus metolachlor, but did not differ from flumioxazin (0.38 lb ai/A) or imazaquin plus metolachlor. Preemergence knawel control exceeded 92% with all herbicide treatments.

A third study evaluated herbicides for hoary alyssum (*Berteroa incana*) control in a Fraser fir plantation. Treatments were applied on April 24, and included simazine (Princep, 1.5 lb ai/A) plus oxyfluorfen (Goal 2XL, 0.5 lb ai/A), flumioxazin (SureGuard, 0.4 lb ai/A) plus glyphosate (Roundup Original, 1 lb ai/A), glyphosate, triclopyr (Garlon, 1.5 lb ai/A), lactofen (Cobra, 0.25 lb ai/A), lactofen plus clopyralid (Stinger, 0.08 lb ai/A), and halosulfuron (Manage, 0.047 lb ai/A). All treatments except flumioxazin plus glyphosate were applied in mixture with pendimethalin (Pendulum, 3 lb ai/A). At six weeks after application, tree injury was less than 5% from all treatments. Hoary alyssum control was high with triclopyr at 100%, but did not differ from control with simazine plus oxyfluorfen, flumioxazin plus glyphosate, or glyphosate alone.

Nutrition

Growing Christmas Tree with Reduced Environmental Impact from Fertilization: Alternative Fertilizers and Split Treatments

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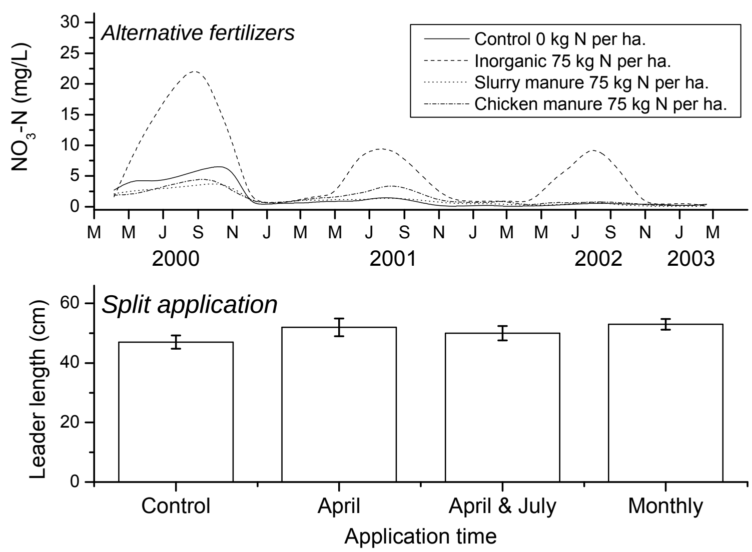
In two experiments (I and II), methods for reducing the environmental impact of fertilization was tested by measuring N leaching and Christmas tree quality on 3-year-old (2000) Nordmann fir (*Abies nordmanniana*) during four growing seasons.

I) Effects from the application of 75 kg N*ha^{-1} as slurry manure, chicken manure and traditional inorganic fertilizer were compared to untreated trees in experiment I on a former forest site.
II) Application of 75 kg N*ha^{-1} as inorganic fertilizer during the growing season as split treatments (i) only April (standard), (ii) April and July and (iii) monthly from April to September were compared to untreated trees in experiment II on a former farm site.

Input and output N and other nutrients were determined on a monthly basis. Growth and tree quality as well as needle chemistry were recorded on a yearly basis outside the growing season.

In experiment I the use of organic fertilizer resulted in a 50 percent reduction of the N concentration (1.4 mg N*l^{-1}) in the leachate compared with the inorganic fertilizer treatment. Furthermore, mean N concentration in the leachate in the treatment with organic fertilizers did not differ from the untreated control. There was a general tendency for decreasing soil water concentrations during the study period. In experiment II, split application resulted in only minor differences in N leaching between treatments (April and July>monthly>April>control). The ranking of N concentration in the needles in experiment I (inorganic>chicken manure>slurry manure>control) and in experiment II (monthly>April and July>April>control) reflected partly the soil water concentration with highest concentration.



Only few differences in Christmas tree quality were observed in experiment I due to a low soil nutrient status, stand age, and competition from weed. In experiment II the split treatments resulted in significant differences between treatments with monthly application as the fastest growing, most vigorous and best colored, but also with the highest number of damaged trees.

The results indicate that leaching of N may be reduced by use of organic fertilizer without compromising a high Christmas tree quality. In contrast split treatment on a monthly basis resulted in too many damages.

Growing Christmas Tree with Reduced Environmental Impact from Fertilization: Alternative Methods for Fertilizer Application

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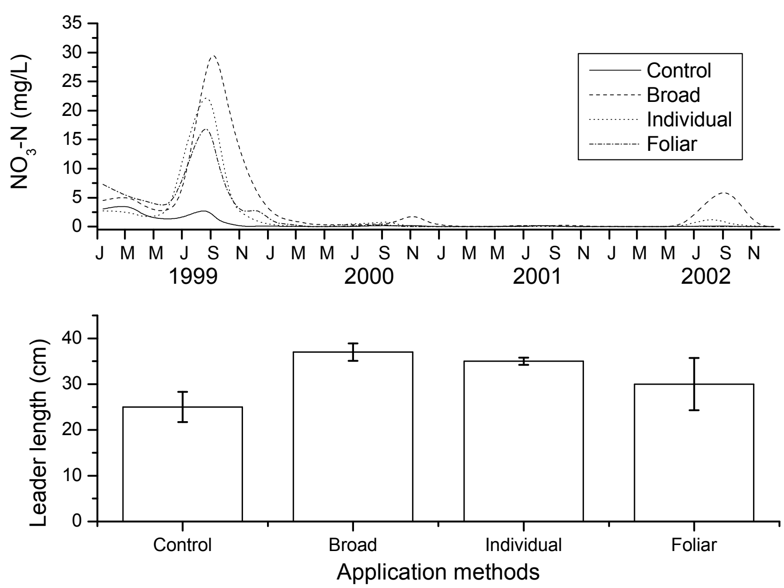
Effects of i) foliar fertilization, (ii) individual plant fertilization, (iii) traditional broad fertilization, and (iv) control without fertilization were investigated for nitrate leaching and Christmas tree quality in a Danish fertilization experiment on three former farm sites. The investigation was carried out from 1999 to 2003 in three 4-year-old stands (1999) of Nordmann fir (*Abies nordmanniana*). Input and output N and other nutrients were determined on a monthly basis. Christmas tree quality included responses on growth (height and leader length), vigor (number of buds, (internodial) branches and needle length), color and damages/health as well as needle chemistry and was measured on a yearly basis outside the growth period.

During the first year elevated nitrate leaching was observed but over time the leaching was reduced concurrently with age although modified by precipitation. Given a 50 kg input of nitrogen per ha, nitrate leaching was largest with broad fertilization (mean concentration in soil water 5.4 mg $N*l^{-1}$) followed by the treatment of individual plants (2.3 mg $N*l^{-1}$), foliar fertilization (2.2 mg $N*l^{-1}$) and the control (0.5 mg $N*l^{-1}$). The concentration of N in the needles showed the same distribution among treatments as the leaching figures, which indicates a clear correlation with accessibility of N. Both broad fertilization and individual fertilization resulted in a significantly faster growth, more vigorous trees with a significantly improved color relative to the untreated trees and foliar fertilized trees.

The results indicate that the use of alternative fertilization techniques such as foliar fertilization and individual plant fertilization may reduce the N leaching by almost 50 percent. However, only the individually fertilized trees are qualitatively as good as the broadly fertilized trees.



Manganese Deficiency in Colorado Blue Spruce

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Manganese (Mn) deficiency frequently occurs in row crops grown on New Jersey coastal plain soils. Heavy applications of limestone and a high soil pH are often associated with Mn deficiency. The tendency for Mn deficiency to occur in woody perennials is less well-known.

A case of suspected Mn deficiency was recently observed on blue spruce Christmas trees growing on a farm in Burlington County, New Jersey. The soil series is an Adelphia sandy loam with a soil pH of 6.2. The soil test (Mehlich-3 extraction method) for Mn was only 3.2 ppm and the calculated availability index was only 20 (an index value below 25 predicts Mn deficiency in soybean). The needles on the trees exhibited symptoms of chlorosis that are commonly associated with Mn deficiency in other plants. Symptoms of Mn deficiency can be, however, confused with other nutrient deficiencies or other causes of plant stress.

An investigation of the suspected Mn deficiency was started in June 2002, just as the trees were initiating new growth. Untreated trees were compared to trees treated either once or twice with a foliar spray of 2 lbs/acre per application of Mn as manganese sulfate (32% Mn). The first foliar treatment was applied when the trees' new growth was 2 inches long. For the two-spray treatment, the second application was made 2 weeks following the first spray. The experimental trees were evaluated for color in late July using a visual color range from 0 (worst) to 9 (best).

Visual observation clearly showed that the Mn treatments did improve tree color. The average color rating was 5.2 for the untreated trees, 6.0 for the trees receiving a single Mn treatment, and 7.3 for the trees that were sprayed twice. The trees that were sprayed twice had significantly better color than untreated trees, or trees that were sprayed only once.

The study was continued in 2003, with a third foliar Mn spray applied two weeks after the second. Early indications indicate that trees receiving a third application had slightly better color than those receiving two applications.

To date, preliminary findings suggest guidelines for treating Mn deficiency on blue spruce include: testing for determining Mn deficiency; using manganese sulfate fertilizer and applying at a rate of two pounds of Mn per acre per application; applying at least two Mn foliar sprays in June spaced two weeks apart, starting when the new growth is at least two inches in length; and, since the problem of Mn deficiency likely reoccurs annually, applying foliar Mn sprays every spring.

Production

Changes in Soil Physical Conditions Over Multiple Christmas Tree Cropping Cycles

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ABSTRACT

This report summarizes the soil physical properties portion of a long-term productivity study of Christmas tree fields in Western Oregon and Washington. The study was conducted to determine whether hypothesized changes in soil conditions are occurring over multiple rotations of Christmas trees. Aggregate stability was found to decline slightly from early to later rotations. Soil resistance as measured by an electronic readout penetrometer was found to decline for some pairs, but there was no statistically significant trend. About 10% of all sites had either aggregate stability or resistance measurements that would significantly impact site productivity, and would benefit by remedial treatments.

INTRODUCTION

Christmas tree production is important to Oregon's economy, consistently ranking as one of the top ten agricultural crops for income production. Oregon has held this position now for over 2 decades. Plantation production began during the 1960's, with considerable growth in acreage and sophistication of practices during the 1980's and 1990's. The same fields are commonly used for multiple rotations of Christmas trees, often without even varying the species grown. In order for Oregon and the Pacific Northwest to maintain leadership in tree production, growers and researchers need to understand potential changes in soil productivity, particularly physical properties brought on by multiple cropping cycles.

In the last 20 years, growers have increasingly commented that succeeding rotations are of lower quality than expected, given improvements in tree genetics, fertilization, and other practices. Specifically, compared to Christmas trees in first rotations, late rotations were described as having a lower grade, growing slowly, and growing at an uneven rate across the fields. These observations have produced concern that field productivity is declining with multiple rotations of Christmas trees, which will ultimately result in increased costs, lower returns to growers and loss of competitive advantage with other Christmas tree producing regions.

A number of speculations have been advanced regarding soil changes contributing to an alleged decline in field productivity. Soil chemical changes, loss of mycorrhizae, pesticide build-up and compaction have all been suggested. A leading speculation is that repeated travel with rubber-tired equipment, particularly during spring and fall dates with moist soils, is compacting soil near the trees. Compaction results in reduced soil aeration, root density, and rooting volume, all which have been connected to reduced productivity (Dexter, 1987, Johnson and Beschta, 1980). Forest research with conifer species commonly grown in the Pacific Northwest for Christmas trees has generally shown decreased growth and tree health when soils are compacted by equipment or other means (Froelich, 1979; Zou, 2000), although this relationship is not without skeptics (Miller and Anderson, 2002).

METHODS

Experimental Design

Measurements were conducted on selected soil properties in “paired” fields which were proximate to one other, and which were as similar as possible with respect to species, soil type, slope, aspect, and usage prior to being planted to Christmas trees. The main difference in the “paired” fields was that one was continuously cropped with Christmas trees for at least three rotations (late rotation fields), while the other field was in its first rotation (early rotation fields). Pairs of fields were selected at 18 locations in western Oregon and 4 locations in southwest Washington. Twenty-two pairs, a total of 44 fields, were included in the study.

With the exception of rotation age, conditions between pairs of fields were as similar as possible. Between locations, soil, climatic and management conditions were very dissimilar. Conditions at each of the locations were in the range typical for Christmas tree fields in western Oregon and Washington (all of these fields were judged suitable for sustained Christmas tree production). Management practices such as field preparation, tillage, sub-soiling, liming, pesticide use, and fertilizing varied markedly between locations. Consequently, large variations in the parameters among locations are to be expected. Field histories were collected to account for variation due to differences in cultural practices within the paired fields.

The overall purpose of the study was to look for trends and causes for the overall range of sites being examined. Consequently, the data set has been viewed and analyzed in its aggregate, as intended in the original experimental design. Making comparisons between individual pairs must be done with caution. Without replicated observations at each location, it is very difficult to judge whether differences between pairs is the result of natural variation, or the result of prolonged cropping with Christmas trees. Analysis of data within pairs is valid, and has provided some good observations for the Christmas tree producers who manage the sites associated with the pairs.

Soil Density

Our hypothesis was that repeated travel with rubber tired equipment would compact soil near the trees and restrict the volume of soil occupied by roots. Soil density was measured with an electronic recording penetrometer, which records resistance to the penetration of a slender metal probe into the soil in kiloPascals (kPa). The recording penetrometer was used to measure soil resistance at 14 to 31 locations at depths from 25 to 600 mm in 25mm increments.

Penetrometer data were collected psuedo-randomly from three different positions along the tree rows, referred to as the drip line, the middle, and the tire track. Data locations were determined by following a “Z” shape in each field, with a penetrometer measurement taken after walking a certain number of paces totaling 20-40 feet apart. More paces were walked between readings in large fields than in smaller fields so that the overall shape was roughly proportional within each field. Sampling areas varied in size from one to five acres.

Occasionally rocks were hit by the penetrometer and no measurements could be taken at depths below the rock. In these cases, regardless of the previous measures of soil resistance, the penetrometer would return a reading of over 4000 kPa. Because rocks were hit over almost the entire range of penetrometer readings, points where rocks were encountered contained a varying amount of information. These points were omitted from the analysis of soil resistance because the data were incomplete and the final reading of each point was misleading. Omitting these censored points reduced the number of penetrometer readings to a range of 6 to 31 points per site.

Soil density is generally equated with soil compaction. The more dense the soil, the greater the resistance to the probe, and the greater the force required to press the probe into the soil. The penetrometer continuously records the force exerted as the probe is pressed into the soil up to the 600 mm (24 inch) depth of the probe. These densities were subsequently plotted.

Soil Aggregate Stability and Particle Size

At each site, a soil sample was taken for physical and chemical characterization. Particle size analysis and aggregate stability were determined using the OSU soil physics laboratory. Aggregate stability and particle size analysis were determined on the soil samples obtained from 0 to 3 inch depth. Each soil sample was the composite of 15 to 20 cores obtained with a sampling tube having a 1.25-inch diameter opening. Laboratory tests for aggregate stability used standard procedures with repeated crushing of wet soil aggregates.

RESULTS AND DISCUSSION

Soil Compaction

Penetrometer measurements taken in the tire track, in the row center and at the dripline were not different, indicating uniform soil density in the area sampled. The proximity of measurements and movement of force can explain this uniformity of measurements from equipment traffic. The “tire track” measurement was between the “drip line” and “center” measuring points. Force from a rubber-tired vehicle is transferred downward and laterally. The lateral transfer of force will compact soil adjacent to the track. Since the “drip line” and “center” measurement were made close to the “tire track”, the values are quite similar. Also, more than one third of the fields, because of the use of helicopters, have had a history of limited equipment traffic.

Average measurements for early and late rotations were compared. Statistical analysis showed no difference in resistance between early and late rotations in the surface foot of soil.

Resistance generally increased from the surface to a depth of 15 to 20 inches and then decreased, as shown in Figure 1. Increased resistance can also be an indication of the increase of subsurface clay content. Figure 1 also illustrates that tillage on a given location reduced resistance measurements. At a depth of 5 inches, soil that had been tilled had a lower resistance than soil that had not been tilled, regardless of rotation.

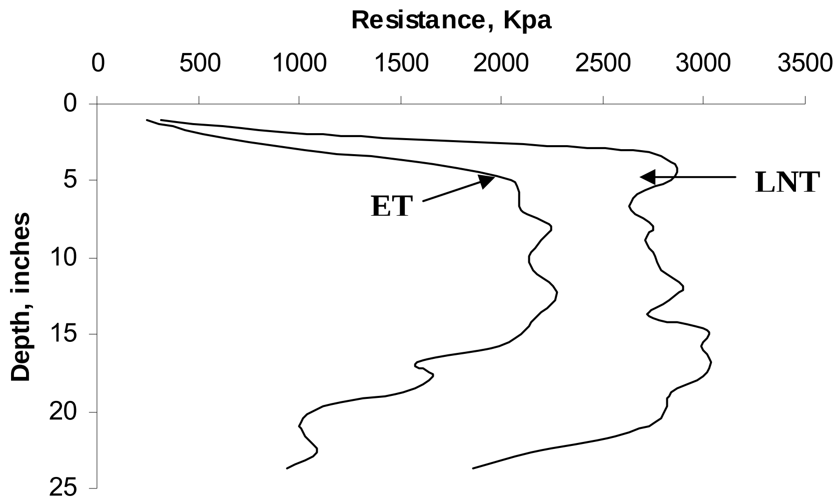

Figure 1. Selected average soil resistance with depth from an early and late rotation pair. LNT indicates a late rotation with no tillage before planting, and ET indicates a first rotation with tillage.

In some tilled and non-tilled late rotation plantations, soil between 4 and 20 inches had resistance measurements high enough to impede root growth. The late not tilled field resistance data (LTN) shown in Figure 1 is an example of elevated resistance and probably soil compaction in the surface of fields. Resistance measurements above 2000 Kpa are generally associated with impeded root growth. Figures 2 and 3 below illustrate one pair where a significant difference occurred between early and late rotations.

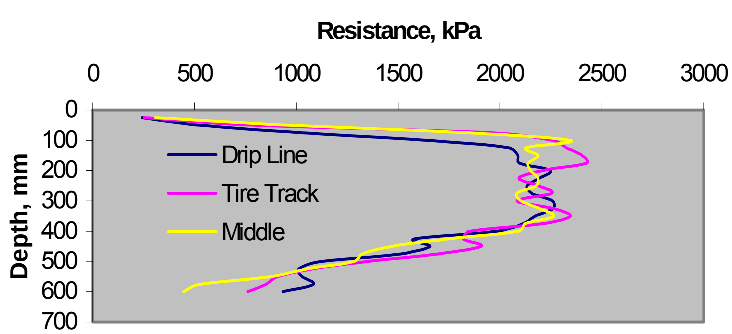

Figure 2. Resistance profile for Pair 11, Site 21.

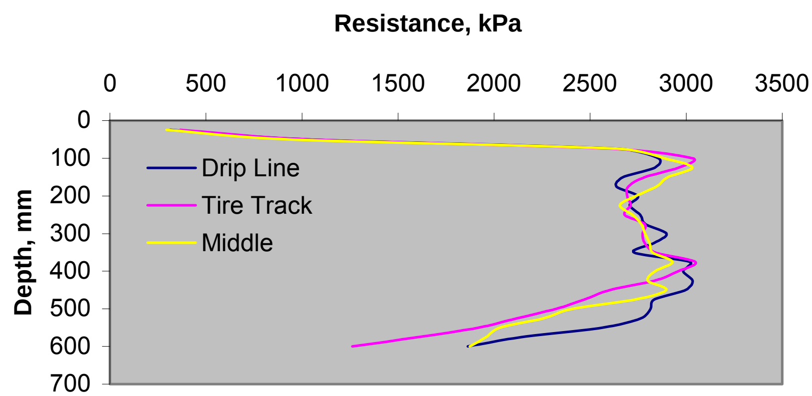

Figure 3. Resistance profile for Pair 11, Site 22.

No significant relationship existed overall between rotation number and soil resistance, but overall many sites had limited soil volumes with resistance readings low enough to avoid impeding root growth (Table 1). For the sake of this analysis, we used a kPa of 2500 as the point at where root growth will be severely impeded. Research seems to indicate roots are restricted at about 2000kPa, and stops for many tree species when resistance is greater than 3000 kPa.

Variance in management practices, particularly tillage among the late rotations may have masked any difference, as some late rotation fields are tilled before replanting, and different growers have used various methods and timings. The cause of increased resistance was not determined by this study. Resistance measurements indicated compacted conditions on some sites that could limit effective rooting depth, suggesting an expected benefit from appropriate deep ripping or other tillage.

Table 1. Soil depth with relatively unimpeded root growth.

Soil Depth to Reach 2500 Kpa	# of Sites	% of Sites
<6 inches	4	9
<12 inches	14	32
<18 inches	22	50
>24 inches	22	50

Aggregate Stability
An aggregate is a group of soil particles (Klute, et al, 1986). Stable aggregates maintain their cohesion when wetted. Unstable aggregates disintegrate. When unstable aggregates at the soil surface disaggregate, the soil particles fill soil pore spaces and restrict the movement of soil air and water. Tillage, repeated driving or walking, and lack of cover crop, are factors detrimental to aggregate stability. Aggregate stability measurements generally varied little from field to field (Figure 4). Aggregate stability was above 90% in 36 out of a total of 44 fields. Twenty of these fields were first rotation fields and 16 were late rotation fields.

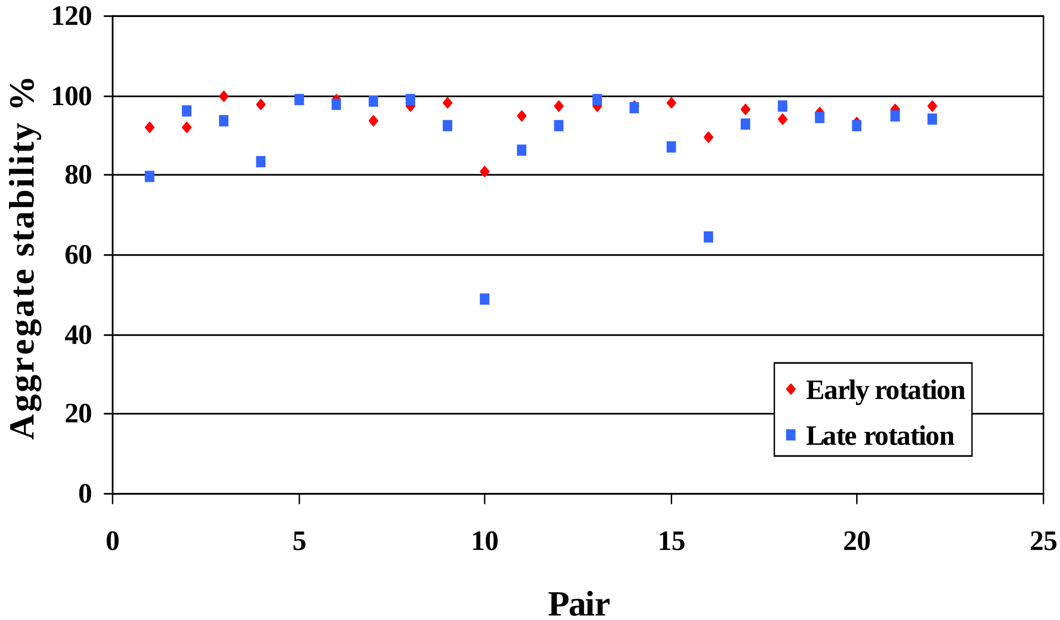

Figure 4. Aggregate stability (%) plotted against pair for early and late rotations.

No relationship between aggregate stability and textural class was found. The textural class of approximately three-fourths of the fields was silt loam or silty clay loam, (see Table 2.)

Table 2. Number of fields in each USDA textural class.

USDA Textural Class	***Number of fields***
Loam	6
Silt Loam	22
Clay Loam	6
Silty Clay Loam	10

Analysis of aggregate stability by individual pairs provided some interesting comparisons (Figure 5). Aggregate stability was almost always lower in late rotation fields than first rotation fields, but there were some minor exceptions like pair 18. The average difference between early and late fields, 5.5% was statistically significant. This slight decrease in aggregate stability in older rotations is unlikely to explain tree decline as the values are well above those expected to impact growth.

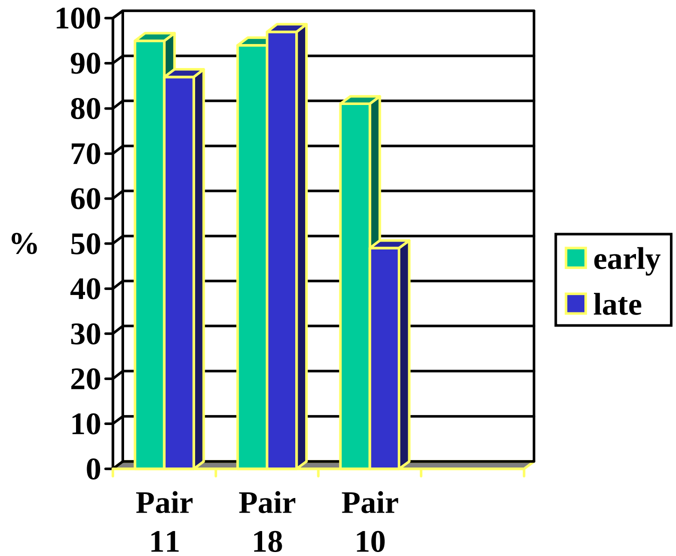

Figure 5. Aggregate stability differences for selected pairs.

At two locations, lower aggregate stability in late rotation fields compared to first rotation fields was particularly notable. Aggregate stability in the first rotation field at the pair 10 location was 81% compared to 49% in the late rotation field (Figure 5); at another second location, aggregate stability was 90% and 65% in first and late rotation fields, respectively. Cultural practices imposed on these fields over multiple rotations are potentially the cause of these differences in aggregate stability, although without replicated observations at these locations it is not possible to unequivocally draw this conclusion. Never the less, these observations serve as signals that cropping of multiple rotations of Christmas trees, under the right combination of soil conditions and soil management practices, can potentially lead to a loss of aggregate stability, which can in turn lead to soil erosion and reduced tree growth. Erosion is of particular concern if the soil clay content is greater than 35% and aggregate stability is less than 50%.

Cynthia Cooper originally analyzed the aggregate stability data. The presence of two outliers prompted John Hart to question if those outliers disproportionately affected the results of that analysis. The aggregate stability percentages were further analyzed with the Wilcoxon signed rank test for paired data so that the effects of the outliers would be minimalized. The aggregate stability data are displayed in Figure 4 and the two outliers are visible well below the other data points. Both outliers are late rotation sites. Incidentally, the two lowest aggregate stability readings from the early rotation sites are the sites matched with the two outliers. Note that for most of the pairs, early rotation has higher aggregate stability – sometimes quite a bit higher.

An analysis of the 22 paired plots between rotation types with the Wilcoxon signed rank test results in a p-value of 0.0124 (exact permutation test). Therefore, the results of the nonparametric test show evidence of a location shift in the median aggregate stability between rotation types, indicating declining aggregate stability with later rotations, even when the outliers are removed from the data set.

REPORT SUMMARY

Decreases in aggregate stability and specific site problems with high soil resistance measurements indicate a potential to negatively impact soil physical properties with the practices being used in the Pacific Northwest to produce Christmas trees. Although the data collected does not suggest that the sky is falling, there is sufficient evidence of decline to be a warning to growers. The parameters measured are valuable for individual growers, and will help guide remedial treatments where necessary. The experimental techniques will also be valuable in educating Christmas tree farm managers regarding detecting and treating soil physical property problems.

ACKNOWLEDGEMENTS

Many individuals contributed to the design and execution of this research project. The authors wish to acknowledge the contributions of Aria Beekman of the Linn Soil and Water Conservation District, Jim Boyle, Efren Cazares, Cynthia Cooper, Maria Dragila, and Manuela Huso, all of Oregon State University. They also wish to thank the participating growers in northwest Oregon and southwest Washington for their cooperation; this work could not have been done without them.

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Triazine and Mycorrhizae Observations over Multiple Christmas Tree Cropping Cycles

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ABSTRACT

The "buildup" of soil active triazine herbicides from repeated applications of Atrazine and/or Hexazinone is a concern of many Christmas tree growers. Could this be causing productivity decline in later rotations? Might herbicides and/or changes in soil chemistry or physical properties have an impact on mycorrhizae colonization?

In hopes of answering these questions a series of paired study plots were established. The design and rationale of the paired plots have been described elsewhere (Hart, 2003). With regard to Atrazine and Hexazinone, 9 pairs were used and tested, for Desethyl Atrazine 6 pairs and for mycorrhizae, 2 pairs. Pairs for triazine testing were selected from among pairs with the longest and most intensive use of triazine herbicides. Testing was performed on the 3-12 inch soil horizon, the primary rooting area for Douglas and noble fir Christmas trees.

The mycorrhizae evaluations required destructive sampling of 10 trees on each plot. On each sample tree, 200-300 root tips were removed for observation.

Results for the triazine evaluations provided the triazine levels for both the primary products (Atrazine or Hexazinone) or breakdown products (Desethyl Atrazine) lower than expected from application histories and known half-lives of these materials in the soil.   These levels are unlikely to cause productivity decline for conifers.

The mycorrhizae evaluations were cursory due to the small sample size. Limited observations suggest that on 2 pairs, mrchorizal colonization was not different between early and late rotations. Also the levels of colonization on those pairs was considered 'adequate", compared to levels expected in forest situations.

INTRODUCTION

Triazine herbicides such as hexazinone and Atrazine have been used as residual grass control products on Christmas trees since the 1950's. Growers are familiar with symptoms of successful control of target weeds, and occasionally damage to non-target trees. Growers are less familiar with degradation of these products in the soil, breakdown products, and their impacts. Some growers are concerned that the long-term use of triazine products may be creating an accumulation in the soil profile resulting in poor tree performance.

Likewise, many growers are realizing the important role that mycorrhizae play in water and nutrient uptake. Questions arise as to the impact of herbicides, and other pesticides on these fungi and if there may be declines in mycorrhizal populations with advancing rotations.

Little systematic evaluations of these factors in Christmas trees have been attempted. In the spring of 2001 we initiated a field study of these issues.

METHODS

For the triazine soil analysis, we elected to evaluate soil samples within the primary rooting zone of the trees (3-12" soil depth).

Plot pairs were selected based on site histories of Atrazine and hexazinone applications, particularly in the later rotation plots. Triazine application histories were provided by growers and are summarized in table 1 below

Table 1. Pair triazine application histories.

Pair #	First Rotation	Later Rotation
1	1998-2000 Atrazine- est. 5 lbs/ac.	1972-1991 At. + Vel, 1992- Atrazine 95-01 Atra. est. 5 lbs./acat unknown rate-
2	91-95 at. @ 4lbs/ac,95-00- accord	78-85 vel/+atra. @2 lbs/ac each,86-99 accord + atra @4lbs/ac.
3	No herbicides	1985-99 rotate between Atra. or Vel. in alternate years @ 4 lbs./ac
4	1999- 4 lbs Atrazine	1965- 1985 4 lbs Atra. Every yr./1985- 2001 4 lbs of Atraz every 3rd yr.
8	99 Atra 4 lbs, 2000-Atra=5 lbs	95+ 4 lbs,96-97=Atr@ 5lbs, 98= Atra @ 4lbs, 99 Vel + Atra @ 2 qts+ 2lbs 00= atra@ 5lbs, 02 Atra@4 lbs
12	2001-02= 2 lbs Hexazinone, No Atraz	1957-1980 Atra. 5 lbs, 80's- 2+2 Atra and Hexazinone, 90's- Hexazinone @2/ac
15	99 Atra 4 lbs, 2000-Atra=5 lbs	95+ 4 lbs,96-97=Atr@ 5lbs, 98= Atra @ 4lbs, 99 Vel + Atra @ 2 qts+ 2lbs
17	97= 1.5 qts vel.,98=3 qts vel par, 99 & 00= 4lbs atraz.	98= 2 qts vel, 99- 01= 4lbs atra.
20	2002- Hexazinone 3 pts./ac.	1973-1984 Atra. Max. rate, 1985-1989 Vel. 2 qt./ac.

We selected two pairs (1 and 7) on which we conducted a bioassay. Here we compared the sprouting of a grass (annual rye), on the soil pairs with a garden soil with no Triazine application history.

For the mycorrhizae evaluation, we selected 2 pairs with a long history of herbicide use. At each site, 10 trees were dug out and root tips (200-300) were cut from around the root zone. These were refrigerated and sent to Efren Cazares, OSU Forest Science mycologist, for processing and visual examination. No evaluation of species was performed, color was noted. Evaluation was made without reference to plot history.

RESULTS AND DISCUSSION

Bioassay

Results of the bioassay, showed that grass germination on the first rotation and late rotation soils were similar and comparable to the garden soil. (grass growth on the garden soil was more vigorous, yet this is likely a result of differing nutrient availability and soil pH, rather than triazine level)

Triazine Levels
As a reference point, we needed to determine damage thresholds on other crops to compare with our data. On loam soils the visible injury threshold in soil for selected crops appears in table 2.

Table 2. Soil atrazine levels for selected crop species.

Crop	Visible injury (ppm)	Dead or Dying (ppm)
Alfalfa	.11-.33	.25-3.3
Grasses	.19-.43	.4-1.41
Oats	.13-.53	.63-4.17
Tomato	.15-.36	.47-6.82
Wheat	.17-.31	.35-1.26

(after Frank,R. 1983)

We are not aware of studies listing sensitivity thresholds of noble or Douglas-fir. We suspect that sensitivities are far higher than the listed crops in table 2 since Atrazine commonly is used to kill listed crops, without visibly impacting conifers.

Comparing early and late rotation pairs provides a starting point for analysis. With the exception of pair 8, soil Atrazine levels are lower in the 3-12 soil depth in early rotation fields as compared to late rotation sites.

Looking at the Atrazine soil levels in Figure 1 as compared to thresholds listed in table 2, we note that late rotation fields (except for pair 1) are below the mean values for visible injury levels for most crops. So despite years of repeated Atrazine applications, soil Atrazine levels remain low in all but one pair.

Pair 1 levels in the late rotation is likely a result of recent Atrazine applications as opposed to the total number of applications over time. Many of the late rotation fields have received up to 30 years of Atrazine exposure, yet soil Atrazine levels at depth remain below threshold levels of sensitive crop species.

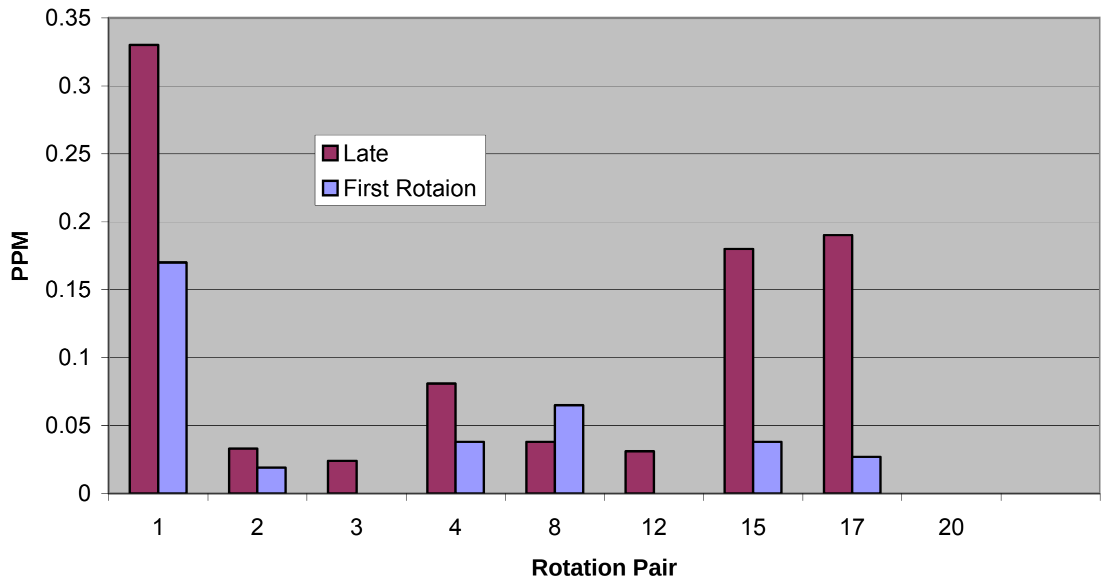

Figure 1. Paired soil Atrazine levels.

Hexazinone (Figure 2) applications provide a slightly different picture than Atrazine. Based on the plot history data, many pairs have a long history of hexazinone use, while only pairs 12,15 and 20 show residual hexazinone. This indicates hexazinone is not likely to accumulate at the 3-12" soil level overtime. Further, based on plot history reports hexazinone levels in Figure 2 likely represent recent applications. We were not able to detect hexazinone in soils two years following use. (note on fig. 2 - 0 values indicate levels below detection limits)

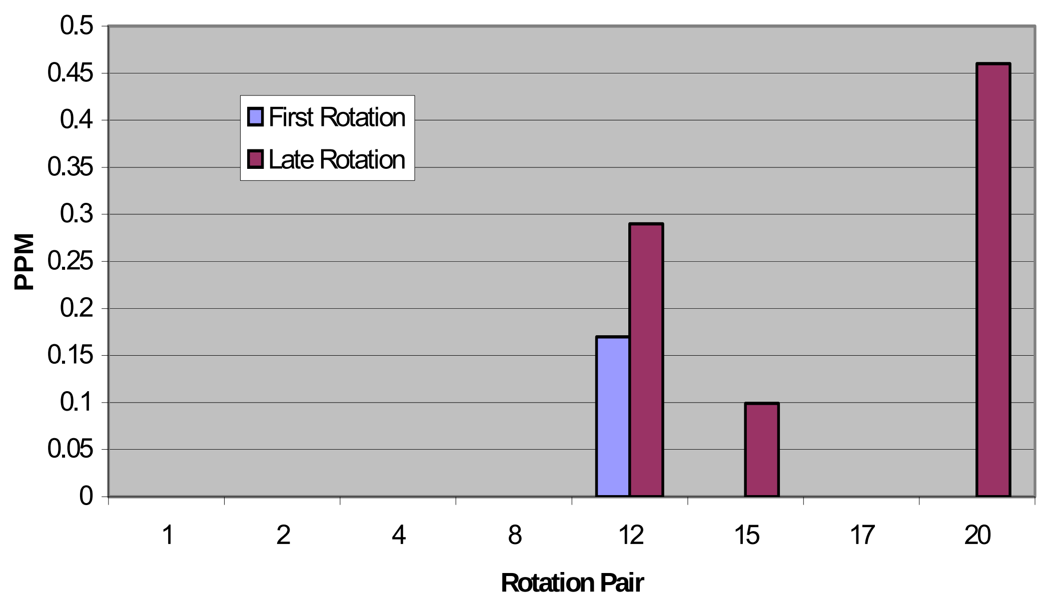

Figure 2. Paired soil Velpar levels.

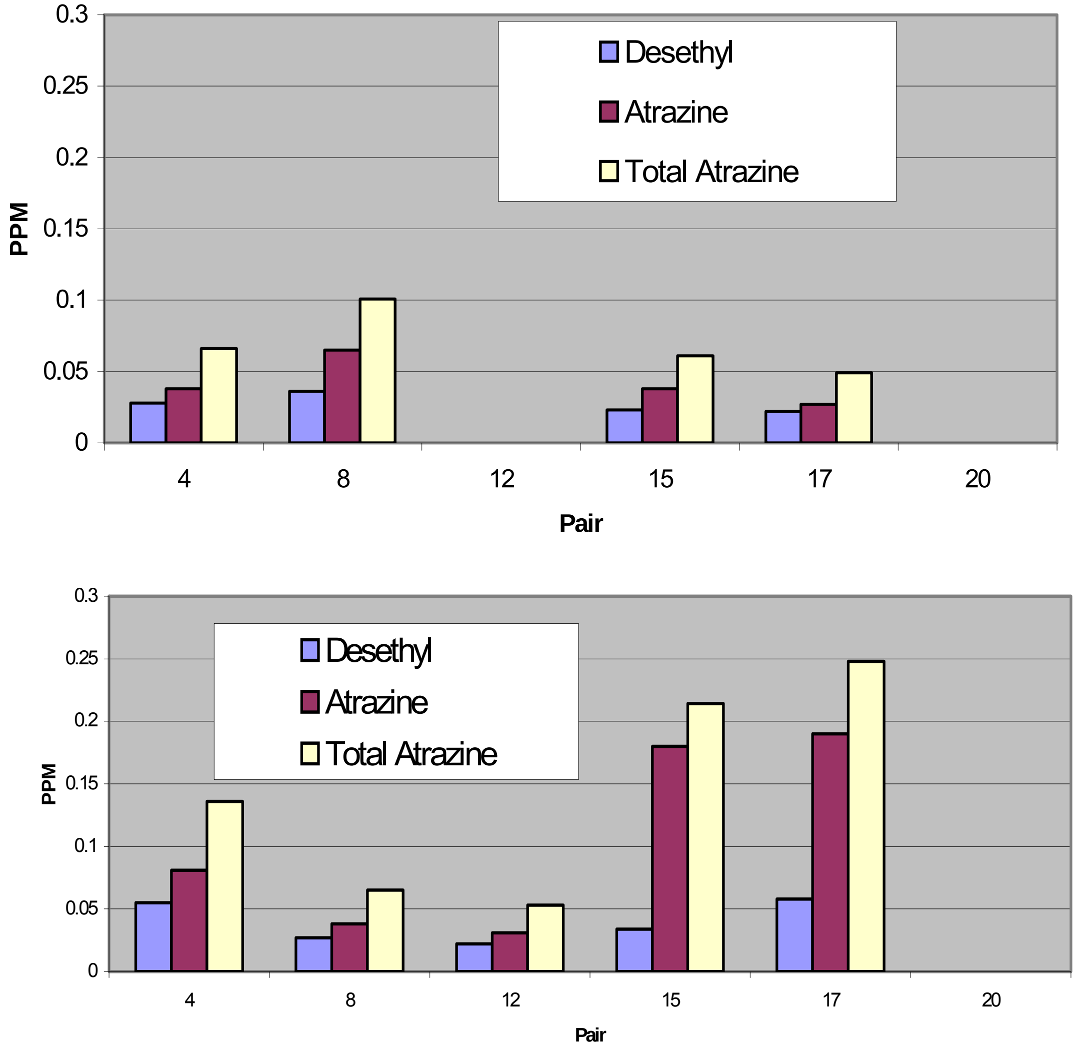

Figure 3. Early (upper) and late (lower) rotation total Atrazine.

Atrazine degrades into compounds that are not herbicidal, yet some of the first breakdown products, such as desethyl Atrazine have herbicidal properties. Figures 3a and 3b looks at the total amount of Atrazine in the soil profile (3-12"). By looking at the sum (light colored bars in fig 3a and 3b above) of Atrazine plus desethyl Atrazine, we may arrive at a more comprehensive view of the history of Atrazine in soil.

Late rotation fields have received more total Atrazine, reflected in the heights of the bars in Figure 3b. Comparing total Atrazine levels on these pairs, values are below the mean injury thresholds for many of the sensitive crops listed in Table 2.

Mycorrhizae Evaluation

Evaluations of mycorrhizae are summarized in Table 3 below. These evaluations were performed using a dissecting microscope and counting mycorrhizal and non-mycorrhizal root tips. Though limited in the extent, mycorrhizal colonization of root tips is similar in the sample fields. (Cazares, Personal Communications, 2002)

Table 3. Mycorrhizae colonization.

Field Name	% Mycorrhizal Root Tips Identified
Rainier—1st	79
Rainier—4th	77
Blodgett—1st	63
Blodgett—3rd	77

SUMMARY

Atrazine (both the primary compound and desethyl Atrazine) and hexazinone applications do not appear to be accumulating in the 3-12" rooting depth. Limited bioassay results confirm this conclusion, as do triazine residual tests of soils.

The mycorrhizae evaluations were limited but are useful as an observational analysis. Given these limitations, the colonization levels we found on 2 plot pairs appear adequate.

LITERATURE CITED

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Soil and Needle Nutrient Changes over Christmas Tree Cropping Cycles

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ABSTRACT

Christmas tree growers lament that second and third rotation crops do not grow as quickly, mature evenly, or yield as well compared to first rotation trees. They speculated that the reduction in growth was caused by continual use of herbicides, inadequate nutrients, or mycorrhizal decline.

To begin unraveling the mystery of reduced productivity, measurements were conducted of selected soil properties in "paired" fields which were close to one another and which were as similar as possible with respect to species, soil type, slope, aspect, and usage prior to being planted with Christmas trees. The main difference in the "paired" fields was that one had to have been continuously cropped with Christmas trees for at least three rotations (late rotation fields), and the other field had to be in its first rotation (first rotation fields). Needles from trees in 32 of the 44 fields were sampled and analyzed.

We measured extractable soil, P, K, Ca, Mg, total N, total C, soil pH, and tissue nutrient concentration. Soil for nutrient analysis and pH was obtained incrementally from 0 to 3 inch, 3 to 12 inch, and 12 to 18 inch depths.

The average extractable Ca was low at all sampled depths for both rotation ages. Extractable Ca was significantly lower, 5.4 compared to 4 meq/100 g soil, for samples obtained between 3 and 12 inches in late rotation fields compared to first rotation fields. The same magnitude of reduction, approximately 25%, was measured for extractable K in the 3 to 12-inch soil depth. The average extractable K for first rotation sites was 177 ppm and the average for older rotation sites was 139 ppm. No additional differences were measured.

In contrast to measurement in the soil, the average tissue concentration of Ca and K did not differ with rotation age. Tissue Mn was the only element to differ with rotation age. Tissue Mn was higher in later rotations than first crop needles.

Even though soil Ca was low, tissue Ca was adequate. Tissue K concentration was low in some fields. Nutrients do not seem to limit tree growth. For the parameters measured, a few slight differences were found, with no evidence that a single property was responsible for productivity decline. The low Ca soil test values and tissue Mn concentration that increases with stand age indicate that growers need to monitor and adjust soil pH.

INTRODUCTION

Christmas tree production is important to Oregon's economy, with consistent ranking as one of the top ten agricultural crops for income production. Oregon leads the U.S. in Christmas tree production, by harvesting 8.8 million trees in 2000 (U.S. Department of Commerce, 2000). Oregon has held this position now for over two decades. The same fields are commonly used for multiple rotations of Christmas trees. Depending on market demand, species may change from one rotation to the next. In order for Oregon and the Pacific Northwest to maintain leadership in tree production, growers and researchers need to understand potential changes in field conditions brought on by multiple cropping cycles.

In the last 20 years, growers have increasingly commented that succeeding rotations are of lower quality than first rotations. Specifically, compared to Christmas trees in first rotations, late rotations were described as having a lower grade, growing slowly, and growing at an uneven rate. These observations have produced concern that productivity is declining with multiple rotations of Christmas trees, which will ultimately result in increased costs and lowered returns to growers.

A number of speculations have been advanced regarding soil changes contributing to a decline in field productivity. The list of possible agents causing the decline in productivity includes: 1) compaction, 2) an accumulation of the active ingredients in atrazine and hexazinone, two commonly used herbicides, 3) cultural practices including repeated applications of pesticides adversely changing mycorrhizal abundance or ecology, and nutrient depletion, resulting from tree harvest and soil acidification.

Little data has been collected for a systematic evaluation of these factors or to measure differences in soil or tree parameters between rotation ages. In the spring of 2001, we initiated a systematic evaluation of the situation.

METHODS

Measurements were conducted of selected soil properties in "paired" fields which were proximate to one other and which were as similar as possible with respect to species, soil type, slope, aspect, and usage prior to being planted to Christmas trees. The main difference in the "paired" fields was that one had to have been continuously cropped to Christmas trees for at least three rotations (late rotation fields), and the other field had to be in its first rotation (first rotation fields). Pairs of fields were selected at 18 locations in western Oregon and 4 locations in southwest Washington. Twenty-two pairs, a total of 44 fields, were included in the study.

With the exception of rotation age, conditions between pairs of fields were as similar as possible. Yet, between locations, soil, climatic and management conditions were very dissimilar. Conditions at each of the locations were in the range typical for Christmas tree fields in western Oregon and Washington. Management practices such as field preparation, tillage, sub-soiling, liming, pesticide use, and fertilizing varied markedly between locations. Consequently, large variations in the parameters among locations are to be expected. Field histories have been collected to account for variation due to differences in cultural practices.

Soil samples were collected from the surface to 3 inches, from 3 to 12 inches, and from 12 to 18 inches. Fifteen to 20 samples were collected at random locations in each field. The Central Analytical Laboratory at Oregon State University performed soil and tissue analyses.

During the late summer and early fall of 2002, we obtained needle samples from 32 Christmas tree plantations from which soil nutrient and other measurements were gathered in 2001. Needles could not be obtained from all sites as some had been recently replanted and young trees reflect conditions in the nursery for two seasons after transplanting to the field.

The data was analyzed in its aggregate with paired t-tests p = 0.10.

RESULTS AND DISCUSSION

Soil Nutrient Measurements

Soil pH Average soil pH was similar for first and late rotation fields, decreasing 0.2 unit with depth (Table 1). The differences were non-significant. The average soil pH for all depths sampled was between 5.0 to 5.6, the range recommended for Douglas-fir and Noble-fir Christmas tree production in western Oregon (Bondi et al., 1994). Even though the range for surface soil pH in older rotations is larger than the surface soil range for early rotations, the soil pH from only three older rotation sites was above 5.6.

Table 1. Mean and range of soil pH for first and late Christmas tree rotations from three soil depths.

	Rotation			
Sampling Depth	First		Late	
inches	Average	Range	Average	Range
0 to 3	5.4	4.5 to 6.3	5.4	4.8 to 7.0
3 to 12	5.6	4.8 to 6.2	5.4	4.8 to 6.2
12 to 18	5.6	5.0 to 6.1	5.6	5.1 to 6.0

Without liming, surface soil in any western Oregon or Washington cropping system acidifies through time. Nitrogen fertilization, practiced by many Christmas tree growers, decreases soil pH. Top-dressing ammonicial nitrogen sources such as urea decreased surface soil pH 0.1 unit /100lb N/a in western Oregon grass seed production (Gingrich et al., 2003).

Liming explains the lack of soil pH decline with multiple rotations when averaged over 22 locations. Some locations are known to have been limed, others not. The average soil pH for older rotation fields not receiving lime is 5.2 for the surface 3 inches and 5.4 for the 3 to 12-inch sampling depth.

Soil acidification does not appear to be the primary cause of a decline in Christmas tree growth or productivity. Even though soil pH did not differ between rotations and average soil pH was within the range for Christmas tree production, the lower soil pH in late rotation fields not limed, the lower soil pH in the 3 to 12-inch sampling depth, and soil pH relationship with other nutrients should be considered before dismissing soil pH as one portion of the cause for decline in productivity. Other nutrients that should be examined are soil and tissue calcium, soil and tissue magnesium and tissue manganese. For ease of arrangement of the material in this paper, tissue data is presented in the "Needle Nutrient Concentration" section.

Calcium and Magnesium Average calcium soil test values from all sampling depths tended to be lower in late rotation fields than in first rotation fields (Table 2). The difference was significant ($p = 0.10$) only for the 3 to 12-inch sampling depth, the area in which most Christmas tree roots are located. Many average soil test calcium values were lower than 5 meq/100g soil, the amount recommended for adequate growth of Christmas trees (Bondi et al., 1994).

Table 2. Mean and range of calcium for first and late Christmas tree rotations from three soil depths.

Sampling Depth inches	Rotation: First		Rotation: Late	
	Average	Range	Average	Range
	meq/100 g soil			
0 to 3	4.7	0.2 to 10.8	4.1	0.4 to 10.7
3 to 12	5.4	0.1 to 13.1	4.0	0.2 to 11.9
12 to 18	5.2	0.1 to 11.8	4.8	0.2 to 11.7

As found in many other woody perennial crops, less than optimum soil test values do not necessarily produce below normal tissue nutrient concentration. This fact was true for calcium in the needles sampled. No needle calcium concentration was below the suggested Oregon State University threshold of 0.25% (Table 6).

Although changes in soil Ca are often accompanied by changes in soil pH, no relationship was evident from the data in Tables 2 and 6. However, plotting soil test calcium vs soil pH showed that low soil pH was weakly (R^2 0.36) associated with low soil test calcium (Figure 1). Soil test calcium tends to decline with multiple crops of Christmas trees. Even so, needle calcium concentrations remain sufficient for adequate tree growth.

Magnesium (Mg) levels are normally lower than Ca, but in many other respects, the two nutrients behave similarly in the soil. Magnesium soil test values were similar for both rotations and no significant differences were measured (Table 3). All average soil test magnesium values were above the 0.4-meq/100 g soil threshold used for Christmas tree production in Oregon (Bondi et al., 1994).

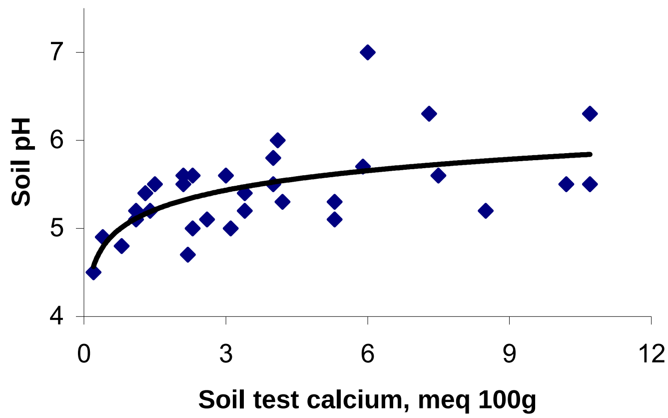


Figure 1. The relationship of soil pH and soil test calcium from the surface three inches from all fields sampled.

Table 3. Mean and range of magnesium for first and late Christmas tree rotations from three soil depths.

Sampling Depth inches	Rotation: First Average	Rotation: First Range	Rotation: Late Average	Rotation: Late Range
	------------------------------ meq/100 g soil ------------------------------			
0 to 3	1.18	0.1 to 3.4	1.24	0.1 to 4.1
3 to 12	1.29	0.1 to 4.9	1.22	0.1 to 4.6
12 to 18	1.52	0.1 to 5.2	1.65	0.1 to 6.2

Potassium Like calcium, potassium (K) concentrations tended to be lower in late rotation fields than for first rotation fields (Table 4). Soil test potassium in the 3 to 12-inch depth of late rotation fields was significantly lower ($p = 0.10$) than soil test potassium for the same depth in early rotation fields. Unlike soil test calcium, all average soil test potassium values were above the threshold used for Christmas tree production in Oregon, 75 ppm (Bondi et al., 1994). Soil test potassium values were highest in the 0 to 3 inch sampling depth, likely a result of repeated top-dress applications with potassium-containing fertilizer.

In 11 of the 44 fields, potassium soil test values were below 75 ppm, a deficient level, in the 3 to 12-inch depth. This sampling depth is important since it is the location of most Christmas tree roots and assumed to be critical in the uptake of nutrients. Fields with deficient soil test potassium values were equally divided between first and later rotation fields.

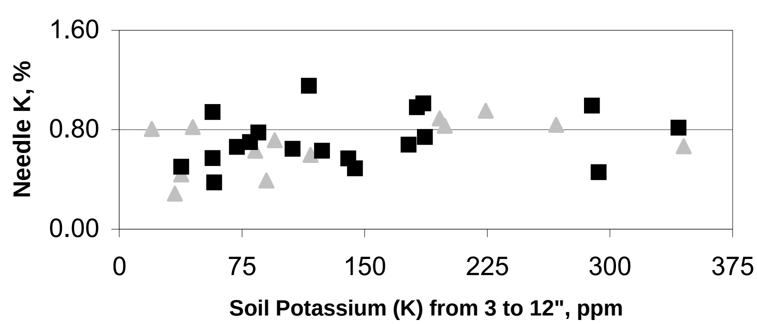
Table 4. Mean and range of potassium for first and late Christmas tree rotations from three soil depths.

Sampling Depth inches	Rotation: First Average	First Range	Late Average	Late Range
	ppm			
0 to 3	235	35 to 573	196	74 to 428
3 to 12	177	34 to 463	139	38 to 342
12 to 18	154	20 to 408	130	37 to 436

Each crop of trees removes an estimated 80 lb K/acre from the field, or 240 pounds in three rotations. Harvest removal of K could easily account for a portion of the soil K decline observed.

The relationship of soil test potassium and needle potassium concentration is a puzzle since the supply in soil is adequate for most fields, but the average needle potassium concentration is below the 0.8% OSU critical concentration (Table 6). Needle potassium concentration is independent of soil test (Figure 2). When soil test potassium is greater than 75 ppm, approximately twice the number of samples were measured with a needle potassium concentration below 0.8% than when soil test less than 75 ppm. The critical potassium needle concentration of 0.8% is supported by McEvoy. Conversely, Turner reported that increasing the needle concentration with potassium fertilizer from 0.34 to 0.44% did not increase growth or color of Douglas-fir Christmas trees.

Figure 2. The relationship of potassium soil test from the 3 to 12-inch sampling depth and needle potassium concentration. The light colored triangles represent early rotation fields and black squares late rotation fields.



Use of lower potassium needle concentration and the Cate-Nelson technique did not improve the relationship between needle and soil potassium. A factor other than K supply is likely the reason for our puzzle. Possibly, dry late summer weather and lack of transpiration that would move potassium to the needles caused the depressed tissue potassium concentration. The low needle potassium concentration is not viewed as the primary reason for decline in productivity. Even so, we hope to pursue the reason for the low potassium needle concentration.

Phosphorus Average phosphorus soil test values were identical in first and late rotation fields at all soil depths (Table 5). The average P soil test value in the 0 to 3-inch sampling depth was 28 ppm, which is almost twice the 15 ppm the threshold used for Christmas tree production in Oregon (Bondi et al., 1994). Elevated soil test phosphorus at the soil surface is normal and expected when phosphorus containing fertilizer is top-dressed without tillage.

Table 5. Mean and range of phosphorus for first and late Christmas tree rotations from three soil depths.

	Rotation			
Sampling Depth	First		Late	
inches	Average	Range	Average	Range
	ppm			
0 to 3	28	10 to 76	28	6 to 103
3 to 12	21	9 to 68	21	5 to 81
12 to 18	14	6 to 34	14	4 to 43

Although average phosphorus soil test values for the top foot of soil were adequate for tree production, phosphorus soil test values from the 3 to 12-inch depth of nineteen fields were below the adequate level for Christmas tree production, less than 15 ppm. Nine of these fields were first rotation fields and 10 were second rotation fields. Average tissue P concentration was below the Oregon State University critical level for both rotations (Table 6). Low phosphorus soil test and needle concentration appear industry wide and similar for rotation age. Inadequate phosphorus may be limiting production on some sites; it is not likely the cause of productivity decline in late rotations.

Nitrogen and Carbon Most nitrogen and carbon in the top 18 inches of soil are components of organic substances, microbial biomass and cellulose compounds collectively termed soil organic matter. No differences between rotation age at any depth were measured for either element, and total amounts of both elements decreased with soil depth.

Needle Nutrient Concentration

The average needle concentration of most nutrients from first rotation trees was above the OSU critical concentration and not significantly different from late rotation trees (Table 6). The average concentration of phosphorus was only slightly below the OSU critical level, the same for both rotations, and not a concern for both first and later rotation trees. The average potassium concentration for both rotation ages was substantially lower than the OSU critical concentration and probably caused by very dry late summer conditions. The lowest needle concentrations of nitrogen, phosphorus, and potassium likely limited the growth of trees in both rotations.

Table 6. High, low, standard, and average Christmas tree needle nutrient concentration.

Nutrient	OSU Critical Concentration	Average Needle Concentration		Range	
		First Rotation	Late Rotation	First Rotation	Late Rotation
	- - - - - - - - - - - -	- - - - - - - - - - - -	% - - - - - - - - - - - -	- - - - - - - - - - - -	- - - - - - - - - - - -
N	1.60	1.66	1.59	1.32 to 2.03	1.30 to 2.00
P	0.15	0.14	0.14	0.10 to 0.21	0.11 to 0.20
K	0.80	0.68	0.72	0.28 to 0.95	0.37 to 1.15
S	0.06	0.11	0.11	0.08 to 0.14	0.08 to 0.14
Ca	0.25	0.46	0.42	0.25 to 0.80	0.27 to 0.62
Mg	0.07	0.12	0.12	0.07 to 0.17	0.06 to 0.17

The average nitrogen concentration for later rotation trees was not significantly lower than nitrogen concentration from first rotation trees, but slightly lower than the OSU critical concentration for growth and color development of Douglas-fir. For sufficient needle color, we recommend 1.6% N in needles of Douglas-fir Christmas trees and 1.4% N in needles of Noble-fir Christmas trees a year before harvest.

Needle calcium concentrations for all fields sampled were above the OSU critical level for calcium. Although not all fields were sampled, we expected, based on soil test calcium, some low needle calcium concentrations would be found. Even though all needle calcium concentrations were sufficient, growers should be cautious about decreasing soil calcium.

The average needle concentration for all micronutrients was above the OSU critical level (Table 7). The concentration of boron from some fields was below the OSU critical level. The average manganese concentration was significantly higher in late rotations than first rotations.

Table 7. High, low, standard, and average Christmas tree micronutrient needle nutrient concentration.

Nutrient	OSU Critical Concentration	Average Needle Concentration		Range	
		First Rotation	Late Rotation	First Rotation	Late Rotation
	- - - - - - - - - - - -	- - - - - - - - - - - -	ppm	- - - - - - - - - - - -	- - - - - - - - - - - -
B	15	18	19	9 to 34	10 to 52
Cu	3	6	5	3 to 10	3 to 9
Mn	25	325	416	83 to 570	140 to 1010
Zn	10	26	27	19 to 33	13 to 36

Manganese was the only element measured to have a significantly higher average concentration in late rotations than first rotations ($p = 0.068$). The increase in needle manganese concentration follows decrease in soil pH (Figure 3). Even though we did not measure a significant decrease in soil pH with rotation age, the increase in needle manganese is an indicator that growers should measure soil pH between rotations.

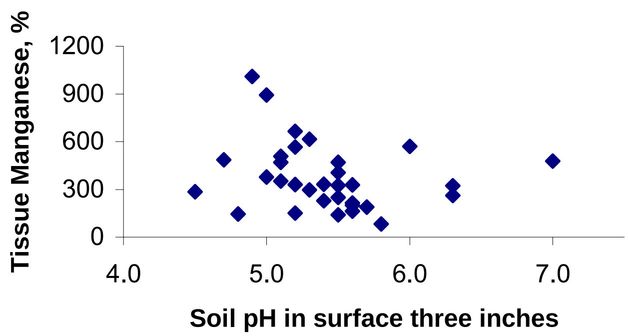


Figure 3. The relationship of surface soil pH and needle manganese concentration for all fields.

SUMMARY

The increase in tissue manganese and decrease in soil calcium with rotation age are industry trends, but not likely the single factor responsible for decline in Christmas tree productivity. Some early and late rotation fields produced trees with nitrogen, phosphorus, potassium, and boron needle concentration low enough to limit tree growth. The low nutrient concentrations, other than phosphorus, were not an industry-wide problem, but one problem that contributes to depressed productivity. Growers should monitor tissue annually from the third year that the trees are in the field to the year before harvest.

No single factor measured was clearly responsible for decline in Christmas tree productivity. Similar to the soil chemical data and needle nutrient concentration, hexazinone and atrazine accumulation, mychorrizal decline, and soil physical properties do not appear to be a single factor causing decline in productivity with advancing rotation age. However, combinations of chemical and physical restraints to root development in combination with production moving to sites that might not be "prime" or optimum for Christmas tree production provide fuel for thought and speculation.

Let's begin with genetics. Tree selections are planted on sites for which they might not be best suited. The profitable production of trees, especially Noble-fir may result in planting selections for Christmas trees in warm, dry, low elevations sites not well suited for the species. Noble-fir planted in these conditions may be stressed by lack of moisture in late summer and early fall.

Lack of moisture in late summer and early fall is logically a problem for all Christmas tree species. Consider that aggregate stability decreased with rotation age. Aggregate stability decline contributes to a decrease in water infiltration. Decreased infiltration of late spring rain could contribute to a lack of moisture in mid-summer or even early summer in some years.

Logic that moisture stress is a primary cause of productivity decline is reinforced by the soil resistance measurements that showed root growth was likely impaired at several sites, especially late rotation fields that were not ripped or tilled between rotations.

Uneven or "patchy" growth reported by growers is commonly associated with a soil related problem rather than herbicide or insect injury or many plant diseases.

Low soil pH also restricts roots growth. The difference between plants that grow and those not growing can change within 0.1 pH unit and from plant to plant within a row. Logically, when the soil pH from a field is measured by obtaining subsamples from 20 locations within a field, some locations will have soil pH below the average for the aggregate sample. When a soil pH from a field is reported as 5.0 for the top 18 inches of soil, some locations in the field likely have a lower soil pH. The lower soil pH in these locations could singly or in conjunction with another factor, slow tree growth and be called a decline in productivity by growers.

When all aspects of the data and Christmas tree production are considered, management is the likely cause of productivity decline. We proposed to test this hypothesis by planting the same genetic stock on large plots of an old rotation field with lime and ripping/tillage treatments. Our funding was denied. We currently are discussing options of working with growers that are tilling and liming older fields. In addition, another treatment, surface mulch is being considered.

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Evaluating the Potential of *Abies* Species in Eastern North Carolina

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Christmas tree farms in the Piedmont and Coastal Plain of North Carolina are almost entirely choose and cut operations done on a part-time basis. Most growers cultivate a variety of tree species, however, fir (*Abies*) species have historically not been grown in the Piedmont and Coastal Plain because survival is poor. Factors thought to be associated with poor survival include heat and phytophthora root rot. Although the species grown in the Piedmont and Coastal Plain make excellent Christmas trees, many potential tree buyers prefer fir. The addition of choose and cut fir species would enhance market and income potential for eastern North Carolina growers.

Recent research on one Piedmont and one Coastal Plain site indicated that momi fir (*A. firma* Sieb. & Zucc.), Nordmann fir (*A. nordmanniana* (Steven) Spach.), and Turkish fir (*A. bornmuelleriana* Mattf.) are apparently heat and phytophthora tolerant with about half of the seedlings surviving four growing seasons. On the Piedmont site, seedlings from these same species survived equally well with grafted Fraser fir (*A. fraseri* (Pursh) Poir.) scions.

The surviving seedlings are being evaluated for continued survival, quality, and growth and additional seedlings of these three species have been planted on these two sites in 2003 with a goal of better understanding how to improve these characteristics.. An additional Coastal Plain site in Pitt County was planted in 2003 to evaluate 12 species for Christmas tree production with and without drip irrigation. These species include momi fir, Nordmann fir, Turkish fir, concolor fir (*A. concolor* (Gord. and Glend.) Hilder.), and Guatemalan fir (*A. guatemalensis* var. *jaslicans* Mart.). First growing season survival is excellent for momi, Nordmann, and Turkish fir, but moderate to poor for concolor and Guatemalan fir.

Shearing Fraser Fir Christmas Trees

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ABSTRACT

Fraser fir [*Abies fraseri* (Pursh) Poir.] Christmas trees were sheared to fixed leader lengths once annually (two experiments) over a 4-year period on schedules ranging from July to March. Early shearing (July) reduced potential growth (dry weight) of the upper crown (top three internodes, including branches) by 38%, compared with non-sheared controls; when done later (October or March), the reduction was about 50%. In the upper crown, trees sheared in July were 16% to 33% heavier (dry weight basis) than those sheared in August, or later. Early shearing (July) resulted in the smallest loss of potential growth, and yielded the largest and heaviest branches, with significantly more foliage and lateral shoots. In the upper crown, foliage constituted about 30% of the biomass for all shearing dates; in 3-yr-old branches, it was about 50%.

Sheared trees averaged two to three leaders, regardless of date. Trees sheared in July produced the longest leaders as well as 35% to 66% more internodal branches on the residual leader, compared to later shearing. Internodal branches on the leader were almost twice as long on sheared trees, compared to non-sheared controls. The ratio of abaxial to adaxial buds on the lower part of the leader was about 1:1, with little variation among shearing dates. Distance from the base of the leader to the first abaxial branch also showed no clear pattern with shearing date. In comparisons of bud types (>whisker= and >bubble=) for next year's leader, some differences, although statistically significant, were of small absolute value, and were considered negligible in commercial shearing practice.

Compared to later dates, shearing in July and August yielded significantly more 2nd-order lateral branches on 3-year-old branches, followed by greater elongation of those laterals. Branch density on 3-year-old branches as well as leaders was greatest for early shearing. Length, mass, and projected area of individual needles were smallest on non-sheared trees. Needles were larger on sheared trees, and reached maximum size for October and March. Shearing in July and August yielded the highest quality and retail value; the least favorable time was late fall (October).

Among shearing treatments, early shearing (July) resulted in the smallest reduction of potential growth, and yielded the largest and heaviest branches, with significantly more foliage and lateral shoots, all of which would be expected to optimize crown density. The least favorable time to shear was late fall (October). Results are summarized in two publications.

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Scotch Pine Shearing Regimes - Tree Grade Relationships

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In the past 20 years or so Scotch pine has become an increasingly attractive host for a variety of insect and disease pests. Managing these pests has become a major challenge to growers who must annually apply one or more pesticides to maintain tree quality. Most of these problems become more serious and difficult to control in the last two years of the rotation. Even with regular and timely pesticide applications most growers experience significant loss that results in reducing harvest yields to only 60 to 70 percent of those trees initially planted. If rotation length could be reduced by one year significant increases in yield would occur, and reductions in pesticide applications would result. Modification of shearing practices might permit this to occur provided tree height and quality were not adversely affected. Five different terminal-length shearing prescriptions were established. These were (1) grower normal, which served as a control, (2) 10-inch terminal, (3) 12-inch terminal, (4) 14-14-12-12-10-inch terminal, and (5) 16-14-14-12-10-inch terminal. In late June of 2000 the trees were sheared according to the first year prescriptions for each treatment. This procedure was repeated in late June of 2001, 2002, and 2003. In September of each year total tree height and USDA grade was determined for each tree in each treatment. For all regulated shearing treatments the maximum total tree height difference is approximately 12 inches. While this difference might be viewed as sizeable, it was expected because of differences among treatments. It is not reason for significant concern if trees are sold in the customary 5-1/2 to 7 foot size class, especially when increases in average grade are considered. For trees in the 12-inch and 14-inch treatments height differences were even less, averaging about 0.7 foot. Careful attention to regulating length of the terminal shoot during annual shearing can and does influence tree height and grade. It has been demonstrated that annual shearing following a prescribed shearing regime will result in the production of trees of an acceptable height and with an average higher grade than conventionally followed shearing practices. Furthermore it is possible to reduce the length of a typical 7 to 9-year rotation and still produce trees of a size readily acceptable to the market place.

Bud Manipulation as a Tool for Regulation of Crown Structure in Young Trees

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BACKGROUND

In the eyes of most people, the regular conical shape is what defines a desirable Christmas tree. Poor quality in Christmas trees is often due to an irregular crown structure. The European Christmas tree market does not encourage visible shape adjustment by pruning or shearing. While required to be symmestic and harmonious in shape, the trees must also show the natural tiers. *Abies nordmanniana* has become a dominating species in the Danish production, not only on account of its superior needle retension, but also because the naturally regular crown structure only requires minor growth adjustments to satisfy the taste of the consumer.

Understanding the rules and means by which the tree allocates its biomass production is a key to any successful, unobtrusive shape regulation. The grower is confronted with two main problems that are inherent to the growth pattern of *A. nordmanniana*: a) excessive leader growth in the later years of culture, which tends to create a too open upper crown, and b) poor height increase and excessive expansion in breadth in newly planted young trees. Excessive leader growth may be controlled either by mechanical or by chemical means. Methods have been developed in recent years for controlled wounding, either at the base of the tree (global growth inhibition which also affects branch growth) or on the leader itself (local g.i., Pedersen & Theilby, 2000). The instruments used are rotating branch cutting gear or modified secateurs. Chemical growth inhibition is only applied locally on the leader and involves spraying or brushing with phytohormones or removal of young needles on the leader (presumably removing phytohormone production sites). Combined with a fairly reliable growth forecast system as we are now developing, regulation of eccessive leader growth may be administered in proportion to the expected growth. Some producers achieve a high success rate in terms of first class trees, but this requires considerable experience and skill.

The problem of poor leader growth in newly planted trees has recieved much less attention. Not only does it prolong the culture time but it disadvantages the trees in relation to weeds. As we have developed empirical models of allometric relationships in *A. nordmanniana,* it has furthermore become clear that the slower tree becomes broader than a fast-growing tree of equal height. The young tree with an undesirably small height-breadth relationship apparently cannot adjust this with later growth.

STUDY AND RESULTS

When in the third year the trees acquire the first set of lateral branches, the branch needles vastly outnumber those placed on the leader, and so the role of the leader as the main photosynthetic structure is over. We assumed that buds compete with each other for resources and that from year three there exists a strategic choice within the young tree between gaining height by allocation to the leader bud and adding to the photosynthetic apparatus by allocation to the lateral buds and branches. Experiments aimed at directing more growth into the leader of young trees by pruning of lateral buds and branches proved highly effective (Rasmussen et al., 2003a). Manipulations took place in April on 3-year-old trees and effects were noted at the end of the first and the second growth seasons. Treatments increased the leader growth in the same year by up to 30% in length and 45% in width. The width of the stem at tree base was not affected by removal of whorl buds but removal of other lateral buds increased stem width. Removal of whole branches, however, reduced stem width. Some treatments enhanced height growth also in the second year. We measured the root growth both in terms of total root length and number of root tips in selected treatments and found that removal of buds seemed to delay root development, but in late summer the root data were no different from the control plants. After removal of whole branches, however, root development not only lagged behind but was significantly smaller by the end of the season.

CONCLUSION AND PERSPECTIVES

Bud removal is thus potentially useful for stimulating leader growth without any apparent negative side effects, but branch removal generated a significant loss of biomass.

Bud set was vastly enhanced by the treatments, both with respect to bud sizes and numbers (Rasmussen et al., 2003c). These results suggests that fundamental mechanisms of growth allocation were affected by the treatments. Ongoing research into the phytohormonal characteristics of buds in various positions in the crown (Hansen-Moeller et al., 2003; Rasmussen et al., 2003b) and the effects of their removal may establish new tools for the shaping of the trees.

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Terminal Bud Selection in Fraser Fir Christmas Trees

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Numerous pruning and shearing studies have been conducted on Fraser Fir Christmas tree farms in North Carolina. Terminal bud selection and timing have been key variables. Five terminal bud selection studies were conducted during the fall 2000 and summer and fall of 2001 on Fraser fir Christmas trees in Avery County, North Carolina. Treatments included selection of a large bud, a prolapsed bud, and a small bud, and as a check, a 12 inch cut with random bud selection. Bud types represented a strong tree-to-tree bias which determined the single tree, 30+ replication design. Bud selection categories were distinct as opposed to being selected in relation to other buds on the same terminal. Strong treatment differences were observed in the fall, but bud selection appeared to be insignificant in the summer. Selecting a small bud in the fall yielded terminal shoots that were shorter and contained fewer buds than the other treatments. Many terminal shoots resulting from small buds were bypassed by stronger buds lower on the stem. The tallest terminals with the most buds in the fall studies grew from large and prolapsed buds with the 12-inch cut producing terminals that were intermediate between the two extremes.

The Dynamics of Labor in North Carolina's Christmas Tree Industry

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There are over 1,600 Christmas tree growers in North Carolina who grow 20 percent of the Christmas trees in the United States. A mail survey and personal interviews with industry participants were carried out in 2001-2002 in western North Carolina to document labor trends. Social exchange theory served as the conceptual platform for examining the social costs/benefits of working in this industry. Preliminary results indicate that around 80% of the industry's workforce is Hispanic. Many growers indicated that they began hiring Hispanic workers in the early 1990s due to a lack of 'reliable' local labor. Advantages of hiring Hispanic workers include 'good work ethic', reliability, and availability. Disadvantages include legal status issues and the language barrier. Based on interviews with Hispanic workers, availability of steady work was the major advantage of working in the industry while legal status and the language barrier are shared concerns. Further analysis of the perspectives of each group should offer suggestions for developing appropriate programs for industry participants.

Precocious Cone Production in Fraser Fir

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ABSTRACT

Fraser fir trees often produce large amounts of cones in Michigan Christmas tree plantations. In heavy cone years growers must devote significant labor resources to picking cone buds. Ironically, *Abies* species are considered poor cone producers. Most of our knowledge of cone production in *Abies* and related conifers comes from studies directed at improving cone production in seed orchards. In this article we review the basic biology of flowering in *Abies*. We also review some of the internal and environmental factors that control flowering in confers. We discuss how knowledge of flowering biology and environmental control may be used to reduce flowering in Fraser fir Christmas tree plantations and our current research on reducing early coning in Fraser fir.

INTRODUCTION

Heavy cone production is a frequent problem in Fraser fir (*Abies fraseri*) Christmas tree plantations in Michigan. Unlike other members of the Pinaceae, cones of true firs (genus *Abies*) do not persist entirely. Instead, the cone scales shed in the fall and only the cone stalks remain. The remnant cone stalks are unsightly and can reduce the value of the Christmas trees or render them unsalable. The number of cones on a given tree can vary from none or a few to several hundred (Fig. 1). Besides reducing the aesthetic value of a tree, the rapidly growing cone buds demand large amounts of the tree's energy reserves. If cones are allowed to develop then current season needle growth may be significantly reduced (Powell, 1974; Powell, 1977). In Christmas tree plantations growers typically pick within a few weeks of cone bud-break. When the cone buds are less than 3 cm (1.25") long they can be easily pinched off. However, cone picking must be done by hand and can require significant amounts of labor (Fig. 2).

As part of an on-going program to optimize production of Fraser fir for Christmas trees in Michigan, we are investigating methods to eliminate precocious cone production. In this paper, we review literature related to the biology of cone production in firs and factors influencing cone production. We then suggest how these factors may be modified to reduce the incidence of coning in Fraser fir.

BIOLOGY OF CONE PRODUCTION

The anatomy and morphological development of numerous *Abies* species (including *A. amabilis, A. grandis, A. lasiocarpa*) has been described in detail by Owens and his co-workers (Owens, 1984; Owens and Morris 1998; Owens and Molder 1977; Owens and Singh, 1982). Although the specific timing of certain events varies somewhat by species, the general phenology is similar. The development of cones in firs occurs in a two-year cycle (Table 1). In year one, vegetative and reproductive buds develop on the current-year's growing shoots. Initially the buds are undifferentiated and may develop into vegetative (shoot) buds or reproductive (cone or pollen) buds. At about the time the shoots cease elongation; hormonal signals in the tree cause some of the developing buds to differentiate into reproductive buds. These buds continue to develop over the first year but cannot be readily distinguished from vegetative buds. In the second year, cone buds grow and develop rapidly before the vegetative flush. Cones continue to mature and are pollinated in the summer and the seed are shed in the fall.

Factors affecting cone production

Flower development in conifers is a complex process and year to year variability in cone production is common for most species (Bonnet-Masimbert and Webber, 1995; McDonald 1992). Flowering in *Abies* appears to be even more inconsistent than other conifers. While environmental and endogenous control of conifer flowering is not completely understood, there are several factors that are known to influence flowering. For the most part, our knowledge of these factors comes from studies directed at improving flowering of conifers in seed orchard production. Results of these studies indicate hormonal relations, endogenous patterns, temperature, water availability, nutrition, and tree size or age may influence conifer cone production.

Hormones

Gibberellins are the hormones most consistently associated with flower production in conifers (Daoust et al. 1995; Eysteinsson and Greenwood, 1995, Smith and Greenwood, 1995) Application of gibberellins increases flower cone production in a number of conifer species. GA_3 induces flowering in Cuppresaceae and Taxodiace whereas mixtures of less polar GAs, GA_4 and GA_7 are most effective in Pinaceae (Owens and Blake 1985) Addition of $GA_{4/7}$ increased the number of cone flowers of Douglas-fir from 9.2 per tree to 59 compared to root-pruned only treatments (Ross et al. 1985). Ross et al. (1985) also reported that application of $GA_{4/7}$ increased the percent of trees with flowers. Although the mechanism of the effect of GA on flowering is not clear, application appears to cause a shift towards reproductive development during bud differentiation (Eysteinsson and Greenwood 1995).

Endogenous patterns

Most temperate confers do not produce heavy cone crops every year. Intervals between heavy cone crops vary from 2 to 7 years for temperate members of the Pinaceae (Owens, 1995). McDonald (1992) examined cone crop loads of several conifers in forest stands in California. *Abies concolor* produced the most infrequent cone crops compared with

Douglas-fir, ponderosa pine, and sugar pine; producing cone crops in only 6 of 23 years studied. Powell (1977) observed a trend of biennial cone production in *Abies balsamea*. Powell proposed that the biennial pattern was the result of altered source-sink relations. In heavy cone years, developing cones provide strong sinks for photosynthates. Developing shoots (including the next year's buds) receive less photosynthate, produce shorter shoots with short needles and the developing buds remain vegetative. In the subsequent year few cones develop resulting in greater allocation of photosynthate to developing shoots and buds and increased differentiation to reproductive buds. In Fraser fir Christmas tree plantations we have observed a related trend. When cone buds are not picked, the needles and shoots that develop in the current year are much shorter than on those on which the cones are picked.

Temperature and water stress

Both temperature and water stress affect cone development (Owens and Blake 1985). However, it is often difficult to separate the two effects since warm years are frequently drier as well. Moreover, treatments designed to increase tree temperature also increase transpiration resulting in water stress. Owens et al. (2001) increased flower production in an *Abies amabilis* seed orchard by erecting small clear plastic tents over the trees during the late-spring and summer. Air temperatures inside the tents increased up to 8^{o} above ambient. Placing the tents over the trees increased the average number of cones per tree from 3-8 to 22-24 compared to related treatments without tents. Water stress and root pruning have been used to enhance flowering in conifers (Ross 1991). Observations by Daoust et al. (1995) suggest that drought stress and elevated temperatures during the period of bud differentiation increased seed cone production in *Picea glauca*.

Nutrition

Flowering generally increases with increased plant nutrition (Owens 1995). Arnold et al. (1992) developed DRIS indices for populations of high cone yielding and low cone yielding Fraser fir trees. DRIS analysis indicated phosphorus most consistently limited cone development. Nitrogen was the second limiting element of cone production based on DRIS. Flower production in Douglas-fir increased with nitrogen fertilization up to 800 lbs/acre (Ebell and McMullin 1970). The form of nitrogen fertilizer is also important. Nitrate fertilizers may increase flower production up to ten-fold compared to ammonium sources (Ebell, 1972; Ebell and McMullan 1970).

Tree age/ size

The age at which conifers become reproductive varies widely among species. However, most conifers do not produce significant cone crops until age 15-45 years (Owens 1995). Among North American firs, Fraser fir and balsam fir are considered the earliest to flower. In a test plantation near East Lansing, Michigan we observed cones on trees three years after planting as 2-3 seedlings (i.e., 8 years from seed). We also observed extremely early (<8 years) flowering in Korean fir and Korean x Balsam hybrids in our exotic fir test plots. Cone production also increases with tree size. Seki (1994) found that cone production in *Abies mariesii* increased as an exponential function of trunk diameter.

RESEARCH APPROACHES TO REDUCING FLOWERING

Based on the developmental patterns of cones, we are investigating two approaches to eliminating cone production. First, we are evaluating the use of flower thinning agents that are commonly used in the tree fruit industry. These are caustic chemicals that cause fruit tree flowers to abort. Wilthin and ammonium thiosulfate are two products are presently on the market for flower thinning fruit trees. In the spring of 2001 we initiated trials to evaluate the effectiveness of Wilthin to thin Fraser fir cones. Results from an on-farm trial Ingham County, Michigan indicated that Wilthin at a high rate (8%) stopped the development of over 60% of the cones on the trees that were treated (Fig. 3). A second on-farm trial Oceana County, Michigan yielded similar results in 2001. In 2002 and 2003 the experiments were repeated at the on-farm site in Ingham County. In the subsequent trials Wilthin and ammonium thiosufate did not stop cone development and we observed significant needle phytoxicity.

A second approach to eliminating cone production is to disrupt the internal chemical signals that cause some of the undifferentiated buds on the current year's shoot to become next year's cone buds. From research on promoting flowering in seed orchards, we know that a hormone, gibberillic acid (GA), increases cone production in many conifers, including true firs. Several plant growth retardants used in the floriculture trade are GA inhibitors (Grossman, 1990; Rademacher 1991). These compounds retard growth of greenhouse crops by inhibiting GA synthesis or GA translocation. In the spring of 2003, we treated 50 trees each with one of five PGR's. The trees were treated three times on a bi-weekly basis beginning when current year's short growth was nearly complete. The trees will be scored in the spring of 2004 for cone production.

SUGGESTIONS FOR GROWERS

As indicated in the foregoing discussion, cone flowering in *Abies* is a complex process controlled by a variety of potentially interacting factors. Some standard practices in Christmas tree culture may contribute to increased flowering. For example, growers typically maintain a high level of fertility, which may promote flowering (Arnold et al. 1992). At present, it seems unlikely that a single approach will completely eliminate flowering. However, growers may consider modifying cultural practices to reduce flowering.

- Use ammonium sources of nitrogen rather than nitrate
- Irrigate trees to reduce moisture stress when buds are differentiating (current year's shoots are 50% to 100% elongated).
- Overhead irrigation, if available, may be used for cooling on warm days when buds are differentiating.
- Flower thinning agents tested to date are not consistently effective and caused phytoxicity to needles.
- Pruning reduces the number of cones per tree by reducing shoot length but does not affect cones per length of shoot (Copes 1973).

Table 1. Bud development in true firs (*Abies*).

<table>
<tr><td rowspan="3">Bud development</td><td colspan="3" rowspan="3">Dormant Vegetative buds</td><td colspan="4" rowspan="3">Bud-scale initiation</td><td colspan="2" rowspan="3">Bud differentiation</td><td colspan="4">Mesosporophyll initiation and development</td><td colspan="2">Dormant Pollen-cone bud</td></tr>
<tr><td colspan="4">Bract initiation</td><td colspan="2">Dormant seed-cone bud</td></tr>
<tr><td colspan="4">Leaf initiation</td><td colspan="2">Dormant vegetative bud</td></tr>
<tr><td rowspan="2">Shoot development</td><td colspan="3" rowspan="2">Dormant</td><td colspan="2">Bud Enlargement</td><td colspan="4">Shoot elongation</td><td colspan="4" rowspan="2">Shoot maturation</td><td colspan="2" rowspan="2"></td></tr>
<tr><td colspan="6">Flushing</td></tr>
<tr><td>Cone development</td><td colspan="2">Dormant seed-cone bud</td><td colspan="3">Cone-bud development</td><td>Pollination</td><td colspan="2">Rapid seed-cone growth</td><td>Fertilization</td><td colspan="2">Cone and seed maturation</td><td>Seed shed</td><td colspan="3"></td></tr>
<tr><td></td><td>Jan</td><td colspan="2">Feb</td><td>Mar</td><td colspan="2">April</td><td>May</td><td colspan="2">June</td><td>July</td><td>Aug</td><td>Sept</td><td>Oct</td><td>Nov</td><td>Dec</td></tr>
</table>

Adapted from Owens (1984)

Table 2. Reported ages for first cone crop of North American fir species.

Species	Age of first cone crop (years)
A. amabilis	20-30
A. balsamea	15
A. concolor	40
A. grandis	20
A. lasiocarpa	20
A. bifolia	50
A. magnifica	35-45
A. magnifica var. *shastensis*	30-40
A. procera	20
A. fraseri	15

Compiled from Silvics of North America

Table 3. Plant growth retardants (PGR's) applied in current cone reduction research.

Trade name	Active ingredient	Rates
B-Nine®	daminozide	5000 ppm
Bonzi®	paclobutrazol	60 ppm
Sumagic®	uniconazole	15 ppm
Cycocel®	chlormequat	1500 ppm
A-rest®	ancymidol	100 ppm

Figure 1. Christmas tree-sized Fraser fir trees may produce upwards of 1000 cones.

Figure 2. Removing fir cone buds by hand is labor intensive and can add significantly to a grower's costs.

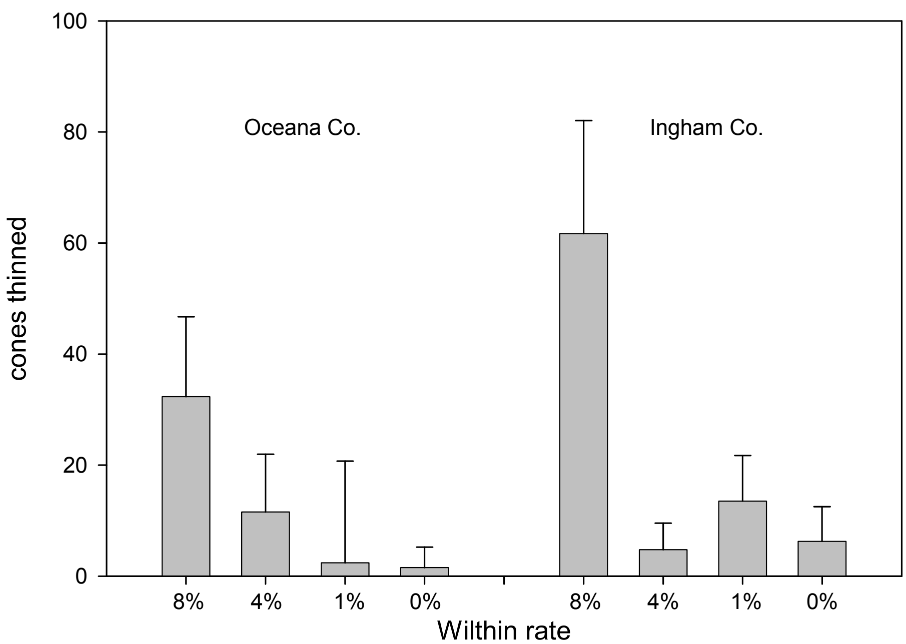

Figure 3. Effectiveness of various rates of Wilthin in inhibiting development of Fraser fir cone development at two locations in Michigan.

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Post-Harvest

Identification of Canaan Fir Trees with Superior Needle Retention

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There is considerable interest in growing various types of true firs for use as Christmas trees. In addition to high quality foliage characteristics, some species, such as noble and Fraser fir also have excellent moisture and needle retention characteristics that make them excellent Christmas trees. Needle retention is an important trait when selecting for high quality Christmas trees and extensive testing at WSU Puyallup has shown that many true firs that are being evaluated for use as Christmas trees have a high level of tree-to-tree variation with respect to needle retention when trees are allowed to dry. Recent studies with Nordmann fir indicate that detached branches can be used to determine the potential needle retention characteristics of whole trees. Studies in Denmark also indicate that needle retention in Nordmann fir is under strong genetic control.

At WSU Puyallup, a series of studies are underway to identify various species of true firs with excellent needle retention. There are a number of growers who are interested in growing Canaan fir, particularly in areas where Fraser fir cannot be grown because of Phytophthora root rot. Previous studies at WSU Puyallup have shown that about 25% of Canaan fir trees will exhibit moderate to severe loss of green needles within 1 week if they are allowed to dry. In an effort to identify potential sources of Canaan fir that consistently do not shed needles, branches were harvested from 94 trees grown near Wooster, OH. Although commonly called "Canaan" fir, the sources of trees used in this study are sometimes also referred to as "West Virginia seed sources" of balsam fir because they come from four areas in West Virginia, of which only one is the Canaan Valley. Four branches were harvested from each tree during November (1999 and 2000) and late October (2001) and then shipped to Puyallup for testing. Branches were displayed dry and the extent of needle loss was rated over a 10-day period.

Results from these tests indicate that needle loss varied by tree and year. Branches from three of the trees did not loose any needles during these tests. Nineteen trees had less than 1% needle loss and branches from 72 of the trees had unacceptable levels of needle loss. Tests are also currently underway to determine if clonally propagated material from some of these trees will have similar needle retention characteristics when grown in the Pacific Northwest. If so, testing of progeny from these trees should also be conducted to determine if it would be beneficial to use these trees to establish a clonal seed orchard to provide a source of Canaan fir with superior needle retention

The Keepability of Douglas-fir Progeny from Texada Island, B.C.

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A recently completed Pacific Northwest (PNW) Douglas fir progeny trial indicated that trees from several open pollinated families of Douglas-fir from Texada Island, B.C. have the potential to make high quality Christmas trees. This trial included 48 progeny (46 from B.C. plus 2 Oregon standards), which were planted in replicated plots at 8 sites in western Oregon and Washington in 1997. The first evaluation step involved rating the progeny at all sites for traits such as bud beak timing, height, grade and pest susceptibility. These measurements were completed in September, 2002. Progeny were ranked on the basis of tree value derived from both grade and height rankings. In addition to these observable tree quality characteristics, there can be significant differences in the postharvest keepability of cut trees that can influence the choice of specific sources of conifers that are suitable for the production of Christmas trees with superior postharvest quality. During fall 2002, an additional experiment was conducted to compare the keepability of trees from the 6 top performing Texada Island sources with two sources of Douglas-fir that are commonly grown as Christmas trees in the PNW.

On November 15, 2002, ten trees from each of the different sources of Douglas-fir were harvested from one of the 1997 progeny test plots near Rochester, WA. Each trees was tagged, baled and transported to WSU Puyallup were they were stored in an unheated barn until they were set up and displayed indoors on November 19th. At the time of setup, moisture levels of the trees were measured with a pressure chamber and pairs of trees with similar moisture levels from each source were assigned to each of five blocks. Individual trees were unbaled and shaken to remove loose needles. On November 19th, one of the pair of trees from each source was displayed with it's freshly cut base continually in water while the other tree was displayed dry. The temperature of the display room was maintained at 20C and the relative humidity was maintained between 40 and 50%. During the 34 days that trees were displayed, changes in moisture levels, needle loss, and tree quality were assessed as indicated below:

Results of this test confirm the benefits of displaying trees in water. Trees displayed in water had higher moisture levels and higher quality ratings than trees that were displayed dry. In general, trees displayed in water had quality ratings of 'good' or higher for 3 weeks while trees displayed dry only had such ratings for about 1 week. This test also indicates that there were significant differences in the keepability of trees from some of the sources tested. Although the overall quality of the different Texada Island sources was highly variable, source 135 consistently had the highest quality ratings irrespective of display condition. Additional testing is needed to confirm these test results and determine what effect production site has on the keepability of Douglas-fir trees. Finally, five family sources were grafted into a production seed orchard with numerical emphasis given to those that appear to have superior keepability.

Late Season Moisture Levels of Christmas Trees on Retail and Wholesale Lots in Washington, Oregon and California

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Moisture status is one of the most important factors affecting the quality and safety of cut Christmas trees. Last year, a survey was conducted to determine the moisture status of Christmas trees on retail and wholesale lots in Washington, Oregon, and California. Trees on 63 lots were sampled between December 15 – 22, 2002. Retail lots included 35 traditional lots, 14 large retail stores with nurseries (e.g. Home Depot, Lowe's, Wal-Mart, and Fred Meyer), 9 retail stores without nurseries (e.g. grocery stores and pharmacy/variety stores), two choose-and-cut growers who sell precut trees, and one retail nursery/garden center. Most of the samples were collected from displayed trees. When possible, samples were collected from different types of lots that were in close proximity to each other so that the moisture status of trees from these lots could be compared with each other. Baled trees at two wholesale distribution facilities in Los Angeles were also sampled.

Of the lots sampled, 55 were in California ranging from Yreka in the north to Palm Desert in the south, 6 were in western Oregon and 5 were in western Washington. Samples were collected from about 1,500 trees on these lots and the percent moisture content of each sample was determined. Noble fir (568), Douglas-fir (493), grand fir (212), and Fraser fir (122) accounted for 93% of the sampled trees. In addition, samples were also collected from a limited number of Canaan fir, white fir, Shasta fir, balsam fir, Nordmann fir, and Scots pine. Most of the trees came from Oregon and Washington, although a few were from Idaho/Montana, California, North Carolina, or Canada.

With the exception of the trees at the retail stores that do not typically have nurseries, the moisture contents of the trees sampled during this survey were very similar to those of freshly-harvested trees. Based on moisture content, there was no difference in the trees from traditional lots and those from large retail stores that typically include a nursery. The moisture contents of the trees at the two choose-and-cut farms and the retail nursery were also very similar to the moisture contents of the trees at the traditional lots and the retail stores with nurseries.

The results of this survey indicate that the moisture content and quality of trees available to consumers last year at a variety of retail lots in Washington, Oregon, and California was generally very good. However, at a number of the large retail stores without nurseries, many of the trees were very dry compared to the other types of lots. For example, in Palm Desert, the moisture content of Douglas-fir trees from a traditional retail lot, where the retailer protected his trees in storage and sprayed the trees with water at night ranged from 93 to 160% while the moisture content of the trees a the grocery store across the street ranged from 30 to 81%. In addition, the store was giving trees away and a number of customers were loading trees onto their cars that had already dried to the point where they were a potential fire hazard. These results and observations indicate that the industry needs to continue to educate retailers about the importance of proper care and handling of trees on retail lots, particularly those who are not used to caring for plant material.

Poster Presentations

Pests

Protecting Christmas Trees from Root Feeding Insects

Richard S. Cowles
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Three groups of root-feeding insect pests are challenging to Christmas tree growers in the northeastern United States, white grubs (larvae of scarab beetles), root weevils, and conifer root aphids. Different strategies may be used for managing these pests, however, protection of the root systems with imidacloprid, a systemic insecticide, while being grown in seedling and liner beds, and a coating of the root system with bifenthrin, a long-residual pyrethroid, at the time of planting are methods that have managed these pests and improved plant health. Detailed information on the economics of oriental beetle larval injury to fraser fir became available when the State Forest Nursery run by the Connecticut Department of Environmental Protection ran out of imidacloprid product in 1999 and left two 300-foot long liner beds untreated. Untreated plants were chlorotic or died in 2000, but their color improved in 2001. Trees were dug during the spring of 2002, from which data on the grade of the resulting plant material was recorded. The net loss from white grub feeding from a single season of leaving the trees unprotected was calculated to be $33,168 per treated acre. Representatives of these trees were then planted in experimental plots to determine their growth performance over time. In 2002, the average growth of the terminal leader was 2.0 cm for previously injured trees, and 23 cm for trees protected in the liner bed from white grub feeding.

Protecting Christmas Trees from Root-Feeding Insects

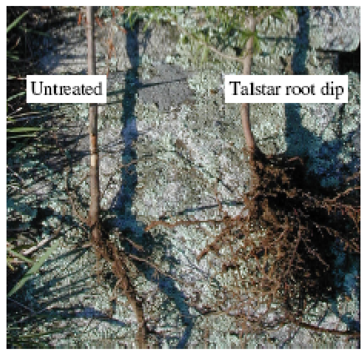
Richard S. Cowles, Conn. Agric. Expt. Station, Valley Laboratory, Windsor, CT 06095

Summary

Three groups of root-feeding insect pests are challenging to Christmas tree growers in the northeastern United States, white grubs (larvae of scarab beetles), root weevils, and conifer root aphids. Different strategies may be used for managing these pests, however, protection of the root systems with imidacloprid, a systemic insecticide, while being grown in seedling and liner beds, and a coating of the root system with bifenthrin, a long-residual pyrethroid, at the time of planting are methods that control these pests and have improved plant health. Detailed information on the economics of oriental beetle larval injury to Fraser fir became available when the State Forest Nursery run by the Connecticut Department of Environmental Protection ran out of imidacloprid product in 1999 and left two 300-foot long liner beds untreated. Untreated plants were chlorotic or died in 2000, but their color improved in 2001. Trees were dug during the spring of 2002, from which data on the grade of the resulting plant material was recorded. The net loss from white grub feeding from a single season of leaving the trees unprotected was calculated to be $33,168 per treated acre. Representatives of these trees were then planted in experimental plots to determine their growth performance over time. In 2002, the average growth of the terminal leader was 3.8 cm for previously injured trees, and 25.4 cm for trees protected in the liner bed from white grub feeding. In 2003, there was no difference in the growth rate of these two groups of trees, so one year of white grub injury resulted in three years of lost growth potential. The newly registered Flagship (thiamethoxam) is labeled for protection of Christmas trees and nursery beds from white grubs.

Oriental beetle adult

Four species of injurious white grubs

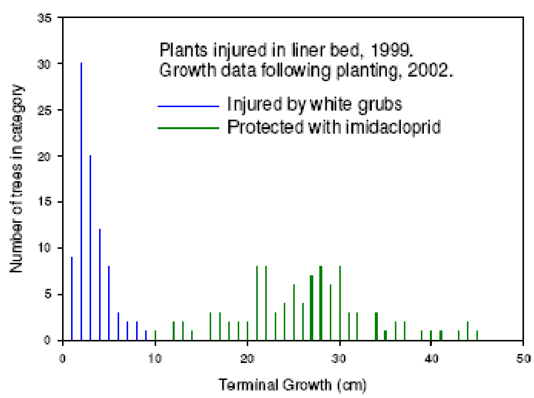


Girdling and root thinning on the nursery seedling on the left was prevented for the plant on the right by dipping the roots in a 640 ppm suspension of bifenthrin at the time of planting. Bifenthrin has unusual long-residual properties when protected from sunlight and is virtually insoluble in water, implying that it remains in close association with the root tissues after planting.

Economic effect of imidacloprid treatment

Status	Number in grade: good	Number in grade: poor	$/bed	$/Acre
Treated	1,904	24	$1,922	$46,128
Untreated	255	380	540	12,960

Not protecting trees in their first year in the liner bed from oriental beetle grub feeding resulted in a loss of $33,168 per acre.



Bark Responses of Fraser Fir to Balsam Woolly Adelgid Infestations

Jennifer Emerson[1], John Frampton[2] and Fred Hain[3]
[1]Graduate research assistant, Dept. of Forestry, [2]Associate Professor, Dept. of Forestry and [3]Professor, Dept. of Entomology, N.C. State University

The bark traits of thickness, color, roughness, hardness, and concentration of juvabione, an insect juvenile hormone analogue, were studied in Fraser fir, *Abies fraseri* (Pursh) Poirit, as a response to balsam woolly adelgid, *Adelges piceae* (Ratzeburg), infestation. Geographic variation and height positional effects of these responses were quantified. Seasonal variation in juvabione concentration was also investigated. An understanding of these responses and selection of adelgid-resistant genotypes would be of great value both to the Christmas tree industry and to preserving natural stands of Fraser fir.

NC STATE UNIVERSITY

Bark Responses of Fraser Fir to Balsam Woolly Adelgid Infestations

Jennifer Emerson[1], John Frampton[2], and Fred Hain[3]

[1]Graduate research assistant, Dept. of Forestry, [2]Associate Professor, Dept. of Forestry and [3]Professor, Dept. of Entomology, North Carolina State University

Objectives:

1. To determine if there is any seasonal variation in juvabione concentration of Fraser fir (*Abies fraseri* [Pursh] Poir.) bark
2. To determine how the bark traits of thickness, hardness, roughness, color, and juvabione concentration vary with height in infested Fraser fir trees
3. To determine seed source and family variation for these bark traits

Balsam Woolly Adelgid

The balsam woolly adelgid, *Adelges piceae* (Ratzeburg), is native to central Europe and was first seen in the United States in Maine in 1908 (Kotinsky 1916). European fir species generally are not seriously affected by the balsam woolly adelgid (BWA), but for many North American fir species, a BWA infestation results in crown dieback or tree death. In 1957, Fraser fir trees in the southern Appalachians were first found to be infested with BWA (Speers 1958). Fraser fir has been shown to be one of the most susceptible of the North American fir species (Mitchell 1966) and the adelgids have spread to all natural Fraser fir stands, causing severe mortality. In some fir species, a thickened outer bark is produced as a result of BWA infestation, and this seems to help in recovering from the infestation. Bark thickness, as well as color, roughness and hardness were investigated here.

Juvabione

The wood of some *Abies* species has been found to contain juvabione, an insect juvenile hormone analogue. When juvabione has been topically applied to BWA, it seems to inhibit egg production in the adelgid (Fowler 1999). One possible mechanism of resistance in fir trees is the production of juvabione, which may affect the growth and development of the BWA feeding on it. This study will look at juvabione concentrations in the bark of Fraser fir to determine if this may be a resistance mechanism against the balsam woolly adelgid.

A stand at Clingman's Dome killed by the balsam woolly adelgid. Photo by Ronald F. Billings

Methods

All the samples for this study were collected from Purchase Knob, located in Haywood County, NC at an elevation of 1350m, from the remaining trees in and around a Fraser fir progeny test established in 1983. The genetic test consisted of open-pollinated progeny from five provenances. To determine seasonal variation in juavabione concentration, cork borer samples 9mm in diameter were collected from five groups of 25 trees each monthly from May through October of 2002. The samples in each group were pooled together for the analysis of juvabione concentration by gas chromatography. The outer 2 mm of bark was separated from the rest of the sample, as this is the only part of the bark that the adelgid's stylet comes into contact with, and these two sections were analyzed separately.

Bark samples approximately 25cm^2 in size were collected in August, 2002 from each living tree remaining in the progeny test. Tree height, BWA infestation level, degree of apical dominance loss, and the presence or absence of branch gouting was measured in the field. In the lab, the number of new woolly masses and the number of second instars were counted. The thickness, color, roughness, hardness, and an estimate of the degree of bark reaction to the infestation were also recorded for each sample, as well as the juvabione concentration of the outer 2mm of bark.

Infested Fraser fir bole with bark sample removed

Bark samples approximately 25cm^2 in size were collected from five different heights of six infested trees. Wood samples were also collected from just beneath each bark sample. The same bark traits as above were measured in the lab. The juvabione concentration in the wood was also analyzed in addition to the outer 2mm of bark.

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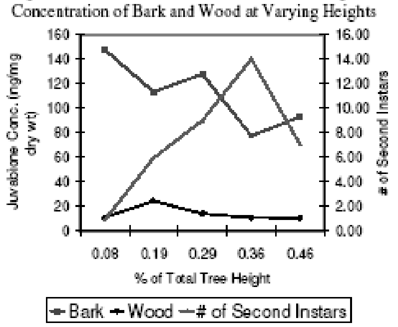
Speers, C.F. 1958. J. of Forestry 56:515-516.

Preliminary Results

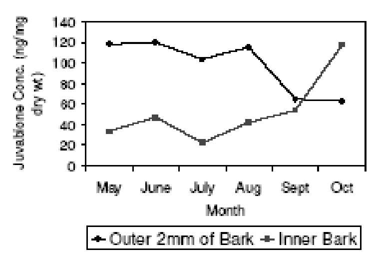
Figure 1. Number of Second Instars and Average Juvabione Concentration of Bark and Wood at Varying Heights



All samples have been collected, the physical bark traits measured, and are in the process of being analyzed for juvabione concentration. Although previous work has been done on juvabione concentrations in the wood of *Abies* species, this is some of the first information we have on bark juvabione content. If the trends shown in these graphs prove to be statistically significant, it could help in determining if this is a resistance mechanism against the balsam woolly adelgid.

In the infested trees tested, juvabione concentrations in the outer 2mm of bark were greater than those in the wood at all tree heights. The sample height with the lowest average bark juvabione concentration also had the greatest average number of adelgid second instars present (Figure 1). Juvabione concentrations in the outer 2mm of bark appear to decrease from May to October, while concentrations in the inner portion of the bark increase late in the season (Figure 2).

Figure 2. Seasonal Variation in Juvabione Concentration



Susceptibility of Various True Firs to Insect Pests in the Pacific Northwest

Gary Chastagner, Art Antonelli, Kathy Riley, John Stark, and Paul Kaufmann
Washington State University, Research and Extension Center, Puyallup, Washington 98371

There are a number of different true firs (*Abies* spp.) that are grown as Christmas trees. In 1996, a replicated planting containing balsam, Canaan, grand, Fraser, Korean, noble, Nordmann, Turkish, and white firs was established at WSU Puyallup. Four to 8 trees of each species were planted in each of four different blocks and no insecticides have been applied to this planting. During the past four years, data have been collected regarding the susceptibility of these trees to spruce spider mites (*Oligonychus ununguis*) and balsam twig aphids (*Mindarus abietinus*).

There were significant differences between species with regard to the numbers of overwintering spider mite eggs on shoots, the location of eggs on the shoots, and the extent of visual damage associated with this pest. Overall, eggs were more prevalent on the lower surfaces of shoots. With some species such as white fir, there were also a high number of eggs associated with the shoot terminal buds. Based on number of eggs and greatest extent of visual damage, the Fraser, Canaan, and balsam firs have been the most susceptible trees in this test. No visible damage and very few if any eggs have been detected on the Turkish, grand, Shasta, Nordmann and noble firs. Given the variation in susceptibility of the hosts in this trial to spider mites, growers might want to focus on scouting for eggs on the lower surface of the shoots at the point of needle attachment as well as the terminal bud on highly susceptible hosts.

In addition to spider mites, balsam twig aphid also caused severe damage to some of the grand fir trees in this plot. No twig aphid damage has been observed on any of the other species.

Susceptibility of Various True Firs to Insect Pests in the Pacific Northwest

Gary Chastagner, Art Antonelli, Kathy Riley, John Stark, and Paul Kaufmann,
Washington State University, Research and Extension Center, Puyallup, WA 98371

INTRODUCTION

Production of true fir (*Abies* spp.) Christmas trees has increased significantly during the past 20 to 30 years in the Pacific Northwest (PNW). Noble fir now accounts for about 45% of the 13.2 million trees harvested each year. Other common species include grand and Fraser fir. In addition, there is limited production of a number of other true firs, such as Nordmann, Turkish, white, Shasta, Canaan, Korean, and balsam fir.

Pest management is an important aspect of the production of high quality Christmas trees. Three of the most common pests on true firs in the PNW are spruce spider mite (*Oligonychus ununguis*), balsam twig aphid (*Mindarus abietinus*), and balsam woolly adelgid (*Adelges piceae*).

Spider mite feeding causes a "stippling" of the foliage. Initially, it is confined to the base of the needles, but can extend over the entire needle when mite populations are high (Fig. 1). Monitoring for this pest is based on examination of the stems and needles for the presence of reddish orange eggs (Fig. 2) and the dark green to black mites (Fig. 3) with orange legs. Webbing also becomes evident as time goes on.

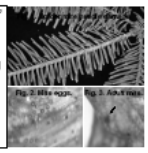
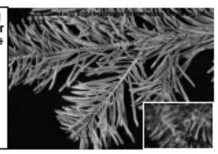
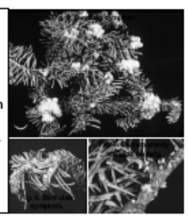


Fig. 2. Mite eggs. Fig. 3. Adult mite.

Balsam twig aphid feeding can result in a distortion or twisting of needles and the new growth (Fig. 4). The aphids often are covered with a waxy covering and the honeydew they produce often attracts yellowjackets and supports the growth of unsightly sooty mold.



Balsam woolly adelgids can cause significant damage to some types of true firs. Needle yellowing and loss are the first symptoms of this pest. Later, a swelling of the branch nodes (Fig. 5) and terminal buds becomes evident and the branch tips may curve downward like a "bird claw" (Fig. 6). On highly susceptible host such as Fraser fir, feeding by this pest can kill the trees. These adelgids are hard to find but the presence of white woolly masses (Fig. 7) confirms their identity.



Although the susceptibility of the true firs that are commonly grown as Christmas trees in the PNW to these pests is well known, there is limited information concerning the susceptibility of their effects on the less commonly planted species. During the past 4 years, data have been obtained relating to the susceptibility of 10 true firs to these pests.

METHODS

A combination of laboratory and field assessments were used to monitor the susceptibility of 10 true firs to spider mites, balsam twig aphids, and balsam woolly adelgids in a replicated planting at WSU Puyallup. This planting was established in 1996 and consists of 20 rows of trees on 6-foot centers. Four to 8 trees of each species were planted 3 feet apart in each of four blocks (Figure 8).

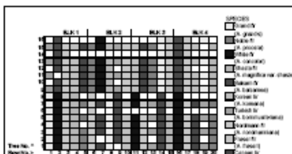


Fig. 8. Tree plot design at Puyallup, WA.

Detached shoot assessments: Two 2-year-old branch samples were collected from the lower third of each tree during October 1999 and January 2002. These samples were examined with the aid of a dissecting microscope to rate the severity of spruce spider mite, balsam twig aphid, and balsam woolly adelgid damage and look for the presence of spider mite eggs and adelgid crawlers.

Detached shoot assessments (contd):

Spider mite - Damage was rated on a scale of 0 to 3, where 0 = none, 1 = slight (random areas of chlorosis), 2 = moderate (random areas of chlorosis with some uniform discoloration at base of needles), and 3 = severe (random areas of discoloration, but with very pronounced discoloration at base of needles). The numbers of spider mite eggs on 1.5 inches of shoot and the terminal bud (January 2002 rating only) was also rated on a scale of 0 to 3, where 0 = none, 1 = 1-20, 2 = 11-25, and 3 = >25.

Balsam twig aphid – Damage on current season growth was rated on a scale of 0 to 3, where 0 = none, 1 = slight twisting of some needles, 2 = moderate needle twisting, and 3 = severe needle twisting.

Balsam woolly adelgid - The nodes on each branch sample were examined for balsam woolly adelgid damage. Damage was rated on a scale of 0 to 3, where 0 = none, 1 = slight swelling, but no downward curling of the shoot, 2 = moderate, but pronounced nodal swellings, and 3 = severe nodal swelling with downward curling of the shoot. In 1999, the number of crawlers on a 1.5 inch long portion of the current season and one-year-old shoots on each sample was also determined.

Whole tree field assessments:

During the winter of 2002/03 the extent of spider mite and balsam twig aphid damage evident on the trees in the field was assessed by rating the average damage on branches in the middle portion of each tree using the rating scales described above. In addition, the effect of the damage on the marketability of the whole tree was also rated on a scale of 0 to 3, where 0=no damage, marketable, 1=slight damage, still marketable, 2=moderate damage, not marketable, and 3=severe damage, not marketable.

RESULTS

There was considerable variation in the extent of visual damage caused by pests on the *Abies* spp. included in this test. For example, balsam and grand fir were severely damaged by spider mites and twig aphids, respectively, while much less or no damage from either of these pests was seen on Korean, noble, Nordmann, and white fir (Fig. 9)

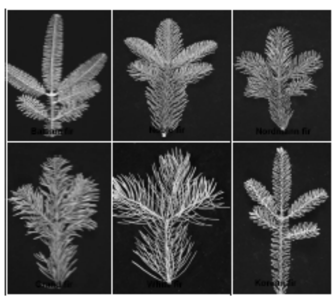


Figure 9. Extent of visual spider mite and balsam twig aphid damage on 6 of the tested *Abies* species.

SPIDER MITE - There were significant differences between species with regard to the numbers of overwintering spider mite eggs on shoots, the location of eggs on the shoots, and the extent of visual damage associated with this pest. On the samples collected in 1999, the extent of damage was higher on the one-year-old shoots than the current season shoots, but there was no difference in the numbers of eggs on the different age classes (Table 1). Eggs were much more prevalent on the lower surfaces of the shoots that were sampled in 2002 (Table 2). With some species such as white fir, there was also a high number of eggs associated with the shoot terminal buds (Table 3).

Table 1. Differences in spider mite damage and egg ratings based on age of shoot.

Shoot age	Damage rating	Egg rating
One-year-old	1.20 a	0.43 a
Current season	0.83 b	0.39 a

Overall ratings for 10 species. Numbers in columns followed by the same letter are not significantly different, P=0.05

Table 2. Differences in the number of spider mite eggs on the upper and lower surfaces of shoots.

Location	Egg rating
Lower surface	0.79 a
Upper surface	0.04 b

Overall ratings for one-year-old and current season growth for 10 species. Numbers followed by the same letter are not significantly different, P=0.05.

Table 3. Egg ratings for shoot terminal buds in 2002.

Species	Egg rating[1]
White	1.8 a
Fraser	1.3 b
Balsam	0.3 c
Canaan	0.1 c
Grand	0.0 c
Korean	0.0 c
Nordmann	0.0 c
Shasta	0.0 c
Turkish	0.0 c
Noble	0.0 c

[1] The numbers of eggs on the terminal bud was rated on a scale of 0 to 3, where 0 = none, 1 = 1-10, 2 = 11-25, and 3 = >25.

Based on the number of eggs that was evident on the detached shoots and the extent of visual damage in the field assessments, the Fraser, Canaan, and balsam firs were the most susceptible trees to spider mite damage in this test (Tables 4 and 5). The marketability of these species, and to a lesser extent the Korean fir was adversely affected by spider mite damage (Table 5).

Table 4. Shoot damage and egg ratings on 1999 and 2001 current season growth.

	Damage rating		Egg rating	
Species	1999	2001	1999	2001
Fraser	0.7 a	2.6 a	1.4 a	1.8 a
Canaan	0.4 ab	2.6 a	0.8 ab	2.0 a
Balsam	0.1 b	2.0 b	0.3 bc	1.9 a
Korean	0.2 ab	1.4 c	0.2 bc	0.8 b
White	0.0 b	0.9 d	0.0 c	1.1 b
Turkish	0.0 b	0.4 e	0.1 bc	0.2 c
Grand	0.0 b	0.1 ef	0.0 c	0.0 c
Noble	0.0 b	0.1 f	0.1 c	0.0 c
Nordmann	0.0 b	0.0 f	0.1 bc	0.0 c
Shasta	-	0.0 f	-	0.0 c

Numbers in columns followed by the same letter are not significantly different, P=0.05, DMRT.

Table 5. Visual field assessment of spruce spider mite damage on *Abies* species grown at WSU Puyallup.

	Damage rating		
	Shoots[1]		
Species	2002	2001	Tree[2]
Fraser	2.1 b[3]	2.9 a	2.8 a
Balsam	2.5 a	2.9 a	2.4 b
Canaan	1.8 c	2.4 b	1.7 c
Korean	0.7 d	1.1 c	0.7 d
White	0.1 e	1.2 c	0.3 e
Grand	0.0 e	0.0 d	0.0 e
Nordmann	0.0 e	0.0 d	0.0 e
Shasta	0.0 e	0.0 d	0.0 e
Turkish	0.0 e	0.0 d	0.0 e
Noble	0.0 e	0.0 d	0.0 e

[1] Rated on a scale of 0-3, 0=slight, 2=moderate and 3=severe.
[2] Rated on a scale of 0-3, 0=none, 1=slight, still marketable, 2=moderate, not marketable, and 3=severe, not marketable.
[3] Numbers in columns followed by the same letter are not significantly different, P=0.05, DMRT.

BALSAM TWIG APHID - Balsam twig aphid caused severe damage to some of the grand fir trees in this plot. In 2001, the overall damage rating on the detached shoots was 0.9. The field assessment rating for the grand fir was 1.8 and the overall tree quality of marketability was 1.3. No twig aphid damage was observed on any of the other species.

BALSAM WOOLLY ADELGIDS – Although balsam woolly adelgid crawlers were found on all of the species included in this test, damage was primarily limited to a few of the Fraser fir (Table 6).

Table 6. Number of balsam woolly adelgid crawlers and damage ratings.

	Number	Damage rating	
Species	1999	1999	2001
Fraser	14.6 a	0.20 a	0.30 a
Canaan	8.7 b	0.00 b	0.00 b
Balsam	6.5 b	0.00 b	0.00 b
Grand	6.4 b	0.00 b	0.00 b
Korean	3.7 c	0.00 b	0.00 b
Shasta	3.1 c	0.00 b	0.00 b
Noble	3.1 c	0.00 b	0.00 b
White	2.3 c	0.02 b	0.00 b
Turkish	1.6 c	0.00 b	0.00 b
Nordmann	1.3 c	0.02 b	0.00 b

Numbers in columns followed by the same letter are not significantly different, P=0.05, DMRT.

CONCLUSIONS

Given the variation in susceptibility of the hosts in this trial to spider mites, growers might want to focus on scouting for eggs on the lower surface of the shoots at the point of needle attachment as well as the terminal bud on highly susceptible hosts. Based on the twig aphid damage ratings, we can comfortably caution growers on the susceptibility of Grand fir, but further testing of the other fir species utilizing methods of artificial infestations away from Grand fir is needed. We cannot preclude the possibility that this pest demonstrated a strong preference for Grand fir in our setting. Likewise, among the species tested, it appears that Fraser fir is more susceptible to balsam woolly adelgid, which limits the potential use of this species unless growers employ management programs to control this pest. Overall, these experiments also demonstrate some real potential for several foreign *Abies* spp. to be utilized for avoidance of some of our serious *Abies* spp. pests.

ACKNOWLEDGEMENTS

Seedlings to establish this planted were provided by Weyerhauser, Lawyer Nursery, and Clifty View Nursery. The Pacific Northwest Christmas Tree Association provided portions of the funding to support this project. The technical assistance of Jackie Kimball is gratefully acknowledged.

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September 2003

Deer Management in Fraser Fir Christmas Trees

Jeff Owen and David Isner,
Extension Christmas Tree Specialist and Alleghany County Agent, NCCES

Damage from deer to Fraser fir Christmas trees grown in the mountains of North Carolina has escalated over recent years. In a 2001 NC Christmas tree pest management survey, about 70 percent of the growers in Ashe, Alleghany, and Watauga Counties reported problems with deer as opposed to 30 percent or less of growers in other fir growing counties. Smaller problem areas exist in Mitchell and Yancey Counties. Regionally, deer browsing has increased the number of cull trees per acre, extended crop rotations by a year or two, and increased production costs. Current deer control strategies that include the use of hunting and repellents yield mixed and often disappointing results.

In the fall of 2002, six Fraser fir growers cooperated in two different demonstrations in fields of 1 and 2 year old Christmas trees. Care was taken to insure that all plots on a farm had the same exposure to field edges and access to deer. Single large treatment blocks were used on each farm to minimize potential effects from one treatment to another. Replication was provided by multiple farm sites. Baseline height, width, and damage data was collected in November, 2002. Similar data was collected prior to bud break in April of 2003.

One demonstration compared four deer repellents including Plantskydd, Deer-Away Big Game Repellent, Deer Off, and Deer Stopper to check plots with no treatment. These putrescent repellents have consistently rated better than other repellents in research studies conducted across the nation in various crops. Initial results indicate that all four repellents reduced the amount of deer damage compared to the control. However, the need for mid-winter re-application reduces their reliability of all the repellents tested. In a hard winter like 2003, re-treatment is nearly impossible at the same window when deer feeding is likely to be greatest. Deer Off effectively deterred deer, but clogged sprayers and is not recommended.

The other 2003 demonstration compared one repellent to one commercially available fencing system. Plantskydd, the most widely used putrescent repellent among NC Fraser fir growers, was used next to a temporary, solar-powered electric, double-slant fence. The fence deters deer with a depth perception optical illusion and a high voltage shock. Check, repellent alone, fence alone, and fence plus repellent treatments were established. Initial results indicated that the fencing, the repellent, and the combined fencing plus repellent reduced deer feeding as compared to the check plots. Deer tracks could be found inside the fence plots at one site but grazing was visibly reduced. There was slightly less damage in the integrated repellent plus fencing treatment than in either the fencing or repellent alone.

Both deer repellents and fencing hold promise as deer management tools. Fencing has a high up-front cost but has an effective 10-year life. Repellents cost less to apply initially but growers incur repeat annual costs. Both alternatives are only effective if maintained throughout the season. The extent and duration of deer damage currently function as an economic treatment threshold. Only under the most severe deer pressure are growers resorting to the up-front investment in fencing. As the economics are better understood, fencing will become a more viable alternative where deer are a problem.

2003 research in deer management has expanded on the work done in 2002. Six repellents including Deer Away BGR, Deerstopper, Plantskydd, Tree Guard, Liquid Fence, and Gamestop are being tested at 3 farms. A replicated study of Plantskydd was installed that examines two rates, two timings, and the addition of two different stickers. The 2002 fencing plus repellent studies were expanded to include the Plotmaster repellent rope fence. Plots of deer repellant applied by tractor-mounted mist blowers have been established and will be monitored. Repellents have also been applied to test plots in nursery beds of native rhododendrons and azaleas to deter deer and rabbit feeding. Results from these studies will be analyzed after data collection in the spring of 2004.

Funded by: NC State University 2002 IPM Grant and NCCTA & NCSU 2003 Christmas Tree Research Grant.

NC STATE UNIVERSITY

Deer Management in Fraser Fir

2002 NC State IPM Grant

Jeff Owen, David Isner, Colby Lambert, Jim Hamilton, Bryan Davis

Demonstration Objectives:

1. Select and integrate deer management tools
2. Collaborative implementation & monitoring
3. Identify cost effective alternatives

Integrated Deer Fencing & Repellents

Treatments

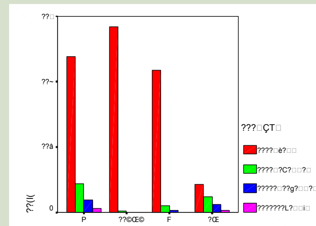
- ♦ Plantskydd (P)
- ♦ Temporary double-slant electric fence (F)
- ♦ Fence & Plantskydd (F +P)
- ♦ Check (CK)

Results (3 sites) :

- ♦ Growth, Year 1 (height & width)
  Not Significant in first year (ANOVA)
- ♦ Damage Categories, Year 1
  Top, lateral, & side damage frequencies
  F, F + P had low damage frequencies
  P, CK had higher damage frequencies (CHI SQ)
- ♦ Spring Damage Frequencies

P	22%	(69 / 307)
F + P	1%	(2 / 286)
F	6%	(13 / 231)
CK	48%	(39 / 82)

A relationship exists between treatments and the frequency of damage (CHI SQ)

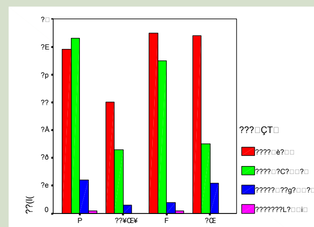


Integrated Fencing: Spring Damage Rating

3 farm summary: Moderate pressure

Results (Tucker Farm site) :

- ♦ Widespread light damage
- ♦ Fewer moderate & severely damaged trees inside the fence



Integrated Fencing: Spring Damage Rating

Tucker Farm: Severe Deer Pressure

Repellents

Treatments (Re-application cycle)

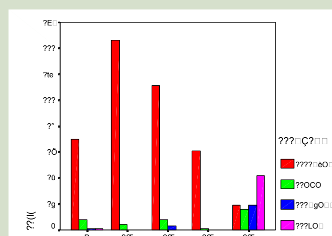
- ♦ Plantskydd (3 month cycle) (P)
- ♦ Deer Off (2 month cycle) (DF)
- ♦ DeerStopper (1 month cycle) (DS)
- ♦ Deer-Away BGR (2 month cycle) (DA)
- ♦ Check (CK)

Results (Zack Farm) :

- ♦ Growth, Year 1 (height & width)
  CK tree width differed from all repellents (ANOVA)
- ♦ Spring Damage Frequencies

P	12%	(10 / 80)
DA	3%	(4 / 146)
DF	9%	(11 / 122)
DS	2%	(1 / 62)
CK	80%	(77 / 96)

A relationship exists between treatments and the frequency of damage (CHI SQ)

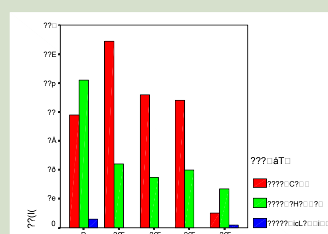


Repellents: Spring Damage Rating

Zack Farm: Severe Deer Pressure

Results (Tucker Farm) :

Higher frequency of damage (see chart)



Repellents: Spring Damage Rating

Tucker Farm: Severe Deer Pressure

2003 Work:

NCCTA Research Grant:

- ♦ Continue 2002 demonstrations
- ♦ Mistblower application of repellents
- ♦ Rope fencing using repellents
- ♦ Expanded region

Evaluating Grub Control Products

Jeff Vance[1] and Jill Sidebottom[2]
[1]County Extension Director, Mitchell County, NCCES, and [2]Extension IPM Conifer Specialist

Grubs are the immature stage of Scarab Beetles. They damage Fraser fir by eating the roots off the plant causing above ground symptoms of yellow stunted growth and if damage is severe the plant will die. Control of grubs has traditionally been accomplished through ground cover management and the use of Triumph (Isazofos). This material is no longer being manufactured, so there was a need for on-farm trials to look at new materials that may be used as a preplacement for this product.

The trial was set up to look at the materials Flagship (Thiamethoxan), Dylox (Trichlorifon) and Marathon (Imidacloprid). In the trial Dylox (Trichlorifon) was applied alone and with a soil wetting agent in the spring. Flagship (Thiamethoxan) and Marathon (Imidacloprid) were applied in the summer. There were three applications equaling five rows that were fifty-eight feet long. The application was made as an in-row broadcast application that was 3 ft. X 58 ft.. On June 1, 2002 the wetting agent was applied. On June 2, 2002 the Dylox (Trichlorifon) was applied. Evaluations of these treatments were completed on June 13 and July 3, 2002. On June 24, 2002, the Flagship (Thiamethoxan) and Marathon (Imidacloprid) were applied to one of the five rows in the prior treatments. This application was evaluated in September 2002. Evaluations were made by digging up three trees in each replication, prior to application and recording the number of grubs found. Following the applications three more trees were dug up and the number of grubs found were recorded.

Results indicate that Dylox (Trichlorifon) reduced the grub populations considerably. Following the Dylox (Trichlorifon) application with another application of Flagship (Thiamethoxan) or Marathon (Imidacloprid) did not significantly reduce the number of grubs found. The materials Flagship (Thiamethoxan) and Marathon (Imidacloprid) applied alone varied little from no treatment. Work is continuing with these materials being applied earlier in the season. Due to unavoidable circumstances the materials Flagship (Thiamethoxan) and Marathon (Imidacloprid) used in this trial were applied later in the season than desired.

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Evaluation of Grub Control Products

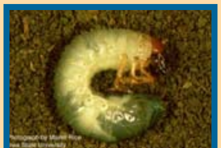
Jeffery Vance, Mitchell County Extension Director
Jill R. Sidebottom, Mountain Conifer IPM Specialist
North Carolina Cooperative Extension Service

INTRODUCTION:

White grubs are often a problem in Fraser fir Christmas trees in western North Carolina when seedlings are planted in old pastures. Growers are encouraged to leave a living ground cover around trees and to maintain those using chemical mowing to reduce grub damage, but that isn't always adequate for control. For growers needing to use a pesticide, the best control used to be obtained with Triumph (Isazofos). This material is no longer being manufactured, so there was a need for on-farm trials to look at new materials that may be used as a replacement for this product. The trial was set up to look at the following materials:

- ♦Dylox (Trichlorfon) is an older organophosphate, labeled by Bayer for use in the landscape only.
- ♦Flagship (Thiamethoxan) is not currently labeled, but has a vegetable label as Actara.
- ♦Marathon (Imidacloprid) is currently labeled for grub control, though few growers use it because of its expense. Previous trials have not proven successful with control.

Many growers currently will use either Diazinon, Sevin (Carbaryl) or Lorsban (Chlorpyrifos) to control grubs, with poor results.



White grubs are larvae of scarab beetles.

Yellowing and wilting seedlings are the first signs of grub feeding.

When the plant is pulled up, grub damage is confirmed by the loss of roots and chewing marks on the main roots. Grubs may or may not be found.

STUDY DESIGN:

The site for this study was a Fraser fir field with a prior history of grub problems. Trees were between 2-3 feet in height and were in a field with substantial ground cover of native perennials. Dylox was applied first as a knock-down product. It was either applied alone or with the use the day before of a soil wetting agent in an attempt to allow deeper penetration of the product. These treatments occurred on June 1 and 2, 2002, and were compared to untreated trees. There were three replications and blocks were 5 rows wide and 58 feet long. On June 24, these blocks were subdivided. The inner three rows received either Flagship, Marathon, or no additional treatment. Grub counts were taken after all treatments and prior to any treatments by digging up three trees and shifting through the soil in a one-foot square around them to find grubs.

Rates were:

♦*Soil wetting agent*	8 ounces/1,000 square feet
♦*Dylox 80 SP*	3 3/4 ounces/1,000 square feet (8.1 pounds active ingredient per acre)
♦*Flagship 25WP*	8 ounces per acre (0.125 pounds active ingredient per acre)
♦*Marathon 0.5 G*	1.8 pounds/1,000 square feet (0.4 pounds active ingredient per acre)

TREATMENTS	**Average grubs found in nine plants**
Grub counts made June 1	
Pretreatment grub counts	5.0
Grub counts made June 13	
Untreated check	0.9
Dylox alone	0.9
Dylox + wetting agent	3.0
Grub counts made September 3	
Untreated check	3.3
Dylox alone	2.0
Dylox + Flagship	1.3
Dylox + Marathon	1.3
Flagship	4.7
Marathon	3.3

RESULTS & DISCUSSION:

The application of Dylox has shown to reduce the grub populations considerably. Following the Dylox application with another application of Flagship or Marathon did not significantly reduce the number of grubs found. The materials Flagship and Marathon applied alone varied little from the control. Work is continuing with these materials being applied earlier in the season or with multiple applications each season.

Another question being investigated is which species of grubs are present. Previous research conducted at NC State University determined that the May and June beetle grubs, *Phyllophaga anxia*, *P. fusca* and *Polyphylla comes* were the predominate species damaging Fraser fir (Kard & Hain, 1990). However, growers have also had damage from the Asiatic garden beetle grub (*Maldera castanea*), the masked chafer grub (*Cyclocephala* sp). Also the black vine weevil grub (*Otiorhynchus sulcatus*) has damaged seedlings. Observations are continuing to determine if other species are also important.

Special thanks to Craig Akins (area nursery specialized agent and Tommy Beutell (cooperating grower).

Diseases & Disorders

Management of Phytophthora Root Rot in Christmas Tree Plantations of Fraser fir with Fungicides

D. M. Benson and J. R. Sidebottom
Dept of Plant Pathology, and Dept of Forestry, respectively, North Carolina State University, Raleigh.

Phytophthora root rot caused primarily by *Phytophthora cinnamomi* is an important disease of Fraser fir grown for Christmas trees in western North Carolina. Growers use disease free plants and select well-drained planting sites to avoid the disease, as host resistance in Fraser fir is not available. In sites where the disease occurs the use of fungicides to prevent infection may offer promise to manage the disease through the remainder of the rotation. To test this idea, field plots on a six tree by six tree grid were established in field plantings of Fraser fir where Phytophthora root rot was present in four sites in western North Carolina in 2001. Aliette and two formulations of mefenoxam, Subdue Maxx and Subdue 1 GR were applied either three or two times annually, respectively, over a 2-yr period with the first application in May each year at new shoot growth. Incidence of Phytophthora root rot was recorded initially and subsequently each spring and fall. Disease incidence was 8% or less in all plots initially across the four sites. Both Aliette and Subdue prevented an increase in disease over years at two of three sites compared to trees in the untreated control. At the Avery 1 site, disease increased 30% in the untreated control compared to only 1 and 2% increase in Aliette and Subdue plots, respectively, after 2 yr. At the Avery 2 site, only the Subdue 1 GR had less disease (5% increase) than the other treatments. At the Watagua site, only a 4% increase in disease incidence was observed over 2 yr, so no differences in treatments were found. At the Ashe site, no disease developed in any plots after 2 yr. In separate nursery plots, potted fir transplants were treated with Aliette, Subdue, Biophos, Vital, Stature-DM, and Zerotol and then inoculated with rice grains colonized by *P. cinnamomi*. Biophos, Vital, and Stature-DM are new fungicides that will be labeled in the near future. At the end of the growing season top weight and root rot were assessed. Trees treated with all fungicides tested except Subdue Maxx and Zerotol had significantly less root rot than the untreated control. Our results suggest that several fungicides are effective in stopping the progress of Phytophthora root rot in field or nursery plots of Fraser fir.

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Management of Phytophthora Root Rot in Christmas Tree Plantations of Fraser fir with Fungicides

D. Michael Benson and Jill R. Sidebottom,
Dept. of Plant Pathology, and Dept. of Forestry, respectively, North Carolina State University, Raleigh, NC 27695

Introduction

Phytophthora root rot caused by several *Phytophthora* spp. is a serious disease problem of Fraser fir *(Abies fraseri)* used for Christmas tree production in western North Carolina (Fig. 1). A recent survey by Benson and Grand (2000) recorded an average 9% loss of Fraser fir in the state due to this disease, however, individual fields had up to 70% loss. In the past growers have relied on use of disease-free transplants and site selection to avoid major disease losses. Transplant beds are often treated with preventative fungicides to assist in production of disease-free transplants. Although fungicides have not been used in field plantings, they may offer value in high hazard sites with poor drainage or to finish a crop through the last 1 to 2 years of a rotation.

Objectives

• To evaluate the effect of several fungicides to prevent further development of Phytophthora root rot in field plantings of Fraser fir used for Christmas tree production.

•To evaluate several new fungicides to prevent development of Phytophthora root rot in Fraser fir transplants

Materials and Methods

Field plots

•Four permanent plots of Fraser fir with root rot caused by *P. cinnamomi* were established in Ashe, Avery (2), and Watauga Counties in western NC in 2001 (Fig. 2).

• Plots were laid out with enough trees per treatment such that each treatment was a 6 x 6 grid with three replications in a randomized complete block design.

• Treatments were Aliette 80W (fosetyl-Al) at 5 lb/100 gal sprayed to run-off; Subdue Maxx 2E (mefenoxam) at 1.25 gal/ 50 gal/ acre, in a directed spray uniformly over the plot using a flood nozzle and backpack sprayer; Subdue 1GR (mefenoxam) at 250 lb/acre applied uniformly over the plot by a broadcast spreader (Fig. 2).

• Aliette was applied May, July, and Sept. each year, while Subdue was applied May and Sept.

• Plots were assessed twice each year for symptoms and mortality due to Phytophthora root rot over the next 2 yr.

Transplant trial

• Three-year-old Fraser fir seedlings were collected from a nursery bed on 23 Apr, 2002, and transplanted to pine bark : peat moss (6:1 v/v) in 3-gal pots the following day.

• Plants were allowed to break bud and grow out for 3 wk, and then fungicides (Fig. 7) were applied on 16 May as a spray to run-off or a drench of 500 ml/pot.

•Five days later, rice grains colonized by *P. cinnamomi* were placed in holes around the pot to a depth of 4 cm and covered (9 grains/pot).

•There were 10 replications of each treatment including untreated, and non-infested controls arranged in a randomized complete block design.

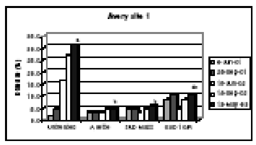
•Aliette and Subdue Maxx were re-applied on a 60-day schedule for a total of three applications. The other fungicides were re-applied on a 30-day schedule for a total of five applications.

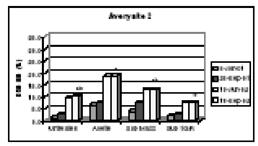
•Since plants had not developed foliar symptoms of root rot by mid-July, a second inoculation with *P. cinnamomi* as described above was done on 31 July, 2002.

• Foliar ratings were made on 5 Sep and 9 Oct (Fig. 4) and top weights and root rot ratings (1= healthy, 3= severe root necrosis, 5= dead plant) were taken on 8 Oct (Fig. 5).

Fig. 1. Development of Phytophthora root rot in a field planting of Fraser fir. In addition to symptomatic trees, the large gaps show where many trees were lost to disease in previous years.

Fig. 2. Application of granular fungicide by Jill (foreground) and directed spray by Jerry (background) to prevent further development of Phytophthora root rot in a field planting of Fraser fir.





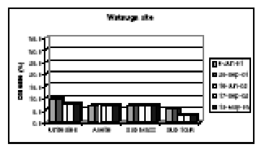


Fig. 3. Disease incidence in Fraser fir due to Phytophthora root rot for three field sites treated with Aliette or Subdue over 2 yrs.

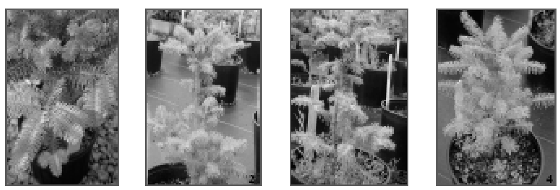
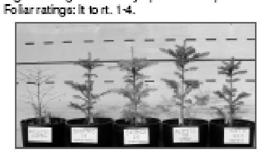
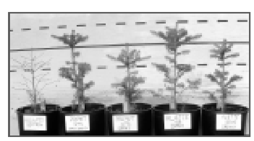


Fig. 4. Progression of symptom development in Fraser fir infected with *P. cinnamomi*. Foliar ratings: lt to rt. 1-4.





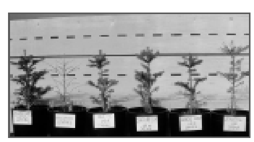
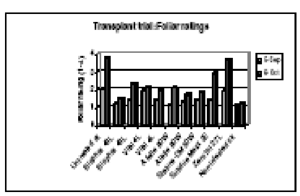
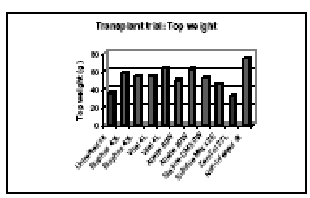


Fig. 6. Foliar symptoms of Phytophthora root rot on Fraser fir in the transplant trial. Biophos (lf. photo), Vital (=Prophyt, rt. top photo), and Stature-DM (rt. top photo) were as effective as Aliette sprays or drenches. ZeroTol (rt. bottom photo) did not prevent root rot. Note dead inoculated control (lf. in each photo) for comparison.





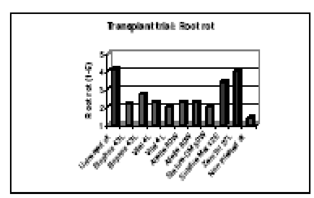


Fig. 7. Foliar ratings, top weight, and root rot rating for Fraser fir transplants treated with various fungicides to prevent Phytophthora root rot caused by *P. cinnamomi*. Biophos (128 and 256 fl oz.) and Vital (32 and 64 fl oz.) low rate sprayed to run-off and drenched; while high rate and Aliette (80 oz) sprayed to run-off only. Aliette (12.8 oz); Stature-DM (6.4 oz); Subdue Maxx (0.5 fl oz) and ZeroTol (256 fl oz.) all drenched. Treatments with red bars are not different than the untreated check.

Results

Field plots

• Initially, disease incidence was 8% or less in all plots.

• Both Aliette and Subdue prevented an increase in disease over years at one of two sites compared to trees in the untreated control.

• At the Avery 1 site, disease increased 30% in the untreated control compared to only a 1 and 2% increase in Aliette and Subdue plots, respectively, after 2 yr (Fig. 3).

•At the Avery 2 site, only the Subdue granular prevented an increase in disease compared to the increase in the Aliette treated plots.

•At the Watauga site no increase in disease was found over years and thus no comparison of fungicide effectiveness could be made.

•At the Ashe site no disease was apparent in the plot, even though it adjoined a highly infested area (Fig. 1).

Transplant trial

• Symptoms developed between 4 to 8 wk after the second inoculation.

• Aliette was as effective as a spray (80 oz) or a drench (12.8 oz) (Fig. 6-7).

• Biophos, Vital, and Stature-DM were comparable to Aliette and better than Subdue Maxx for preventing Phytophthora root rot, although applications were on 30-day vs 60-day schedule (Fig. 6-7).

•ZeroTol drenches did not prevent root rot development (Fig. 6-7).

Fig. 5. Diseased fir roots (left) compared to healthy (right)

Discussion & Conclusions

• Applications of fungicides to Fraser fir in field plantings has the potential to prevent further development of Phytophthora root rot.

  • Application was quickest with Subdue materials, as Aliette spray applications required more time per tree

  •Wholesale tree prices coupled with years to harvest and cost of materials and labor to apply fungicides will be important factors for use of a fungicide management strategy for Fraser fir

• Several new fungicides such as Biophos, Vital, and Stature-DM have the potential to prevent Phytophthora root rot caused by *P. cinnamomi*.

Literature Cited

Benson, D. M. and Grand, L. F. 2000. Incidence of Phytophthora root rot of Fraser fir in North Carolina and sensitivity of isolates of *Phytophthora cinnamomi* to metalaxyl. Plant Dis. 84: 661-664.

Acknowledgements

The authors gratefully acknowledge the assistance of Jerry Moody, Avery County, Colby Lambert, Ashe County, David Tudor, Watauga County, and Bryan Davis, BMP Technician, for the field plot work and Kala Parker and Billy Daughtry for the transplant work.
Financial support by the North Carolina Christmas Tree Association and the N. C. Agricultural Research Service.

Screening *Abies* Species for Resistance to *Phytophthora cinnamomi*

John Frampton[1], Mike Benson[2], Emily Hudson[3], Jianfeng Li[4] and Anne Margaret Braham[4]
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The Fraser fir [*Abies fraseri* (Poir.)] Christmas tree industry in the Southern Appalachians is significantly impacted by losses due to root rot caused by *Phytophthora cinnamomi* Rands. Since Fraser fir exhibits little or no resistance to this pathogen, identification of resistance in other *Abies* species will be useful. Thirty-two species (50 taxa) of *Abies* were grown for two and three years in a greenhouse. Up to 20 seedlings/taxa from each age were inoculated in each of two reps at two times (June and July 2003). Seedling mortality was recorded biweekly after inoculations. Preliminary results (8 weeks from inoculation) confirm the extreme susceptibly of Fraser fir. Momi (*A. firma* S. & Z.) and pindrow (*A. pindrow* Royle) fir appear to be relatively resistant while other species exhibit varying degrees of resistance/susceptibility.

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Screening *Abies* Species for Resistance to *Phytophthora cinnamomi*

John Frampton[1], Mike Benson[2], Emily Hudson[1], Jianfeng Li[1], & Anne Margaret Braham[1]
[1]Department of Forestry, [2]Department of Plant Pathology, North Carolina State University, Raleigh, N.C. 27695 USA

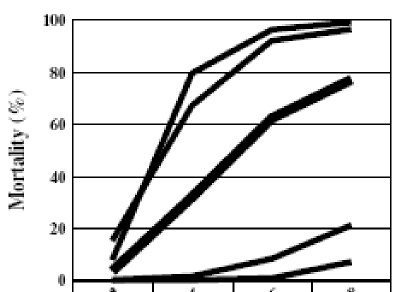
Objective: To evaluate the relative resistance of *Abies* species to root rot caused by *P. cinnamomi*.

Background: The Fraser fir [*Abies fraseri* (Poir.)] Christmas tree industry in the Southern Appalachians is significantly impacted by losses due to root rot caused by *Phytophthora cinnamomi* Rands. In North Carolina, this disease results in an estimated $1.3 million in direct losses and an additional $4.5 million in lost potential revenue annually. Since Fraser fir exhibits little or no resistance to this pathogen, identification of resistance in other *Abies* species may prove useful. Species possessing significant resistance could be used 1) as an alternative crop species to Fraser fir, 2) as rootstock for growing Fraser fir on high hazard sites and 3) in a long-term hybridization and backcrossing breeding program.

A. firma shows significant resistance to *P. cinnamomi* inoculation

Methods: Thirty two species (50 taxa) of *Abies* were grown for two and three years in a greenhouse. Up to 20 seedlings/taxa from each age were inoculated in each of two reps at two times (June and July 2003). Inoculations were performed by inserting two rice grains colonized with *P. cinnamomi* approximately 1 cm deep into the medium. Inoculated seedlings were placed in an outdoor lath house and irrigated twice daily to promote disease development. Seedling mortality will be assessed biweekly through 16 weeks after inoculations.

Preliminary Results: Mortality developed rapidly in most species (see figure). Of the 50 taxa under evaluation, 36 had 75% or more mortality eight weeks after inoculation. Results so far confirm the extreme susceptibly of Fraser fir. Momi (*A. firma* S. & Z.) and pindrow (*A. pindrow* Royle) fir appear to be relatively resistant while other species exhibit varying degrees of resistance/susceptibility (see table).



	2	4	6	8
A. firma	0%	0%	1%	7%
A. fraseri	8	79	96	99
A. pindrow	0	1	8	21
A. veitchii	15	67	92	96
Overall Mean	3	32	62	77

Weeks from Inoculation

Figure: Mortality curves for two resistant species, two susceptible species, and the overall mean of the of the 50 taxa at eight weeks from inoculation.

Table: Shows the % mortality at week 8 for each of the 50 taxa; % is the mean of June and July data

Species	Common Name	Mortality (%)
A. alba	European Silver fir	70
A. amabilis	Pacific Silver fir	96.9
A. balsamea	New England var. of Balsam fir	100
A. balsamea var. phanerolepis	Nova Scotia var. of Balsam fir	99.4
A. balsamea var. phanerolepis	WV Canaan var. of Balsam fir	87.5
A. balsamea	Balsam fir 'Cooks Blue Improved'	99.4
A. borisii-regis	King Boris fir	41.8
A. bornmuelleriana	Turkish fir	34.4
A. bracteata	Bristlecone fir	88.7
A. cephalonica	Grecian fir	71
A. chensiensis	Shensi fir	61.2
A. cilicica	Cilicica fir	36.8
A. concolor	White fir	60
A. delavayi	Delavay fir	90
A. equi-trojani	Trojan Horse fir	55.1
A. ernestii	Ernest fir	77.4
A. fabri	Fabri's fir	67.5
A. fargesii	Farges fir	80
A. firma	Momi fir	6.9
A. fraseri NC 81-18'	Fraser fir	99.6
A. fraseri NC 81-19'	Fraser fir	100
A. fraseri NC 81-31'	Fraser fir	97.4
A. georgei	var. of Delavay fir (George fir)	93.1
A. grandis	Grand fir	96.8
A. guatemalensis	Guatemalan fir	73.2
A. holophylla	Manchurian fir	72.5
A. koreana	Korean fir	96.9
A. lasiocarpa var. arizonica	Subalpine fir	98.7
A. magnifica var. CA Red	California Red fir	99.3
A. magnifica var. shastensis	Shasta Red fir	98.7
A. nephrolepis	Manchurian fir	97.4
A. nordmanniana	Ambrolauri var. of Nordmann fir	68.2
A. nordmanniana	Borshomi var. of Nordmann fir	67.5
A. nordmanniana	Kogot var. of Nordmann fir	64
A. nordmanniana	Nordmann fir - Romanian fam. 10B	63.8
A. nordmanniana	Nordmann fir - Romanian fam. 11B	62.5
A. nordmanniana	Nordmann fir - Romanian fam. 14B	21.7
A. nordmanniana	Nordmann fir - Romanian fam. 1A	31.3
A. nordmanniana	Nordmann fir - Romanian fam. 3B	47.5
A. nordmanniana	Nordmann fir - Romanian fam. 4C	43.8
A. nordmanniana	Nordmann fir - Romanian fam. 8B	71.3
A. nordmanniana	Nordmann fir - Romanian fam. 9M	38.8
A. nordmanniana	Turkish var. of Nordmann fir	54.7
A. numidica	Algerian fir	77.5
A. pindrow	West Himalayan fir	21.9
A. procera var. glauca	Noble fir	97.4
A. procera var. OR	Noble fir	83.8
A. sachalinensis	Sachalin fir (Sakhalin)	94.4
A. siberica	Siberian fir	61.3
A. veitchii	Veitch fir	96.3

Identifying Possible Chemical and Cultural Management Strategies for *Phytophthora cinnamomi* in California's Sierra Foothill-grown Christmas Trees: An On-farm Pilot Study

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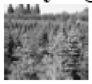
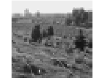
Root rot caused by *Phytophthora cinnamomi* has been identified as a serious problem in Christmas trees grown in the Sierra foothill region of California; yet, growers have few management options for this disease. This preliminary study, replicated five times in a complete randomized block design in a naturally infested field, begins to investigate the effects of two chemical treatments, mefenoxam (Subdue Maxx™, Syngenta), currently registered in California, applied as a root dip at planting or during the season as a soil drench, and fenamidone (Bayer), a new material not yet registered in California, applied during the season as a soil drench. We also included a cultural treatment, planting trees on six-inch raised beds, and four species of Christmas trees grown in our region: Douglas Fir, Noble Fir, Nordmann Fir, and White Fir, in order to try to identify species which may be more tolerant of the disease. The trial was planted in February 2003 and this poster presentation will include results to date.

Identifying chemical and cultural management strategies for *Phytophthora cinnamomi* in California's Sierra foothill Christmas trees: an on-farm pilot study.





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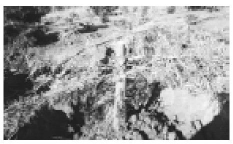
Introduction

Agriculture in the Sierra foothill region of California is characterized by small family farming operations that rely mainly on direct marketing for sales. Christmas trees present a lucrative crop for these growers as sales from "choose and cut" operations have increased in recent years. However, root rot caused by *Phytophthora cinnamomi*, (*P.c.*), has been identified as a serious problem for Christmas tree growers in this region. This pilot study begins to investigate potential tools to help manage *P.c.* in California's Sierra foothill Christmas tree farms.



A White fir exhibiting the typical signs of *Phytophthora* infection: "bleeding" on the trunk at and just above the soil line. When cut into, the canker under the bark appears red-brown in color.

Goal: To identify promising management strategies for root rot, *Phytophthora cinnamomi*, in naturally infested Christmas tree farm soils.

Objectives:

1. To identify Christmas tree species which may confer some resistance to *P.c.* infection.
2. To evaluate the effect of mefenoxam, (Subdue Maxx™, Syngenta), applied either as a root dip prior to planting or as a soil drench during the season, on *P.c.* infection.
3. To evaluate the effect of fenamidone, (Bayer- not yet registered in California), applied as a soil drench during the season, on *P.c.* infection.
4. To evaluate the effect of planting Christmas trees on raised beds on *P.c.* infection.

Methods

Christmas trees were planted on February 21, 2003 in two adjacent blocks with a history of *P.c.* located on the same Christmas tree farm in Camino, CA. Four species were planted. Table 1. shows the species, size and root health of the trees planted.

Table 1: Christmas tree species planted in 2003 *Phytophthora* trial.

Species planted	Obtained from*	Size (plug-bed)	Notes
Douglas Fir (*Pseudotsuga menziesii*)	U.S.F.S nursery, Placerville, CA. (local)	P0-2	Large root system-strong trees
White Fir (*Abies concolor*)	CDF nursery, Magalia, CA.	P2-0	Weaker root system-smaller trees
Nordmann Fir (*Abies nordmanniana*)	Lawyer nursery, Olympia, WA.	P1-2	Large root system-strong trees
Noble Fir (*Abies procera*)	Weyerhaeuser Co., Tacoma, WA.	P1-1	Weaker root system-smaller trees

* All trees were donated from growers and from the local U.S.F.S. nursery. These contributions are gratefully acknowledged.

The following treatments were applied:

- Control-untreated.
- Subdue Maxx™ at planting at a rate equivalent to10x the labeled 2-0 transplant rate: 0.5 pt. Subdue/gal. One gallon of solution was mixed and tree roots were soaked in the solution for 30 minutes prior to planting.
- Subdue Maxx™ during the season using the labeled rate for 2-0 transplants of .05 pt. Subdue/gal. A volume of 2 pints solution per tree was hand-applied at the base of each tree on three dates: March 31, June 4 and July 18.
- Fenamidone during the season at a rate equivalent to 14 oz./100 gal. A volume of 2 pints of solution per tree was hand applied at the base of each tree on three dates: March 31, June 4 and July 18.
- Raised beds. Prior to planting, a mound approximately six inches in height was created and the tree was planted into the middle of the mound.

Five replicates of the Doug Fir and White Fir, and three replicates of the Nordmann and Noble Fir were blocked and treatments were randomly applied in two sections of the field.

Trees were inspected for visual symptoms of *P.c.* infection (browning of entire seedling) on June 3, June 11, July 15, and August 27. In order to confirm *P.c.* infection as the cause of seedling death, trees with visual symptoms were dug up on June 11 and July 15 , examined for signs of J rooting or other obvious damage, and taken to the State Dept. of Forestry lab in Davis, Ca. for culturing. Tissue from roots were plated on to specialized media (PARP) for *Phytophthora* fungi. Cultures were maintained at room temperature in the dark for one to three weeks until potential fungal growth appeared.

Soil samples were collected from each irrigation line where trees were planted in the study. A composite of three samples approx. 6 inches in depth was collected, mixed and pear baited for *P. cinnamomi*. Samples were placed in labeled plastic bags containing a firm pear and sterile water. After three to five days the pears were removed and tissue from along the margins of any areas of discoloration was plated on specialized media and incubated in the dark for approximately one week.

At left : This White Fir (foreground with arrow) is planted on a mound but exhibiting symptoms of *P.c.* infection, while the Doug Fir (background), also planted on a mound, appears healthy. _At right :_ Tom Smith samples seedlings to take to the lab for culturing.

Table 2. Percent of Christmas trees (by species) exhibiting visual signs of *Phytophthora* infection in treated and untreated plots across both experimental blocks.

Species	Untreated Control	Subdue™ dip	Subdue™ drench	Fenamidone drench	Planted on mound
Doug Fir	0% (n=5)	0% (n=5)	0% (n=5)	0% (n=5)	0% (n=5)
White Fir	100% (n=5)	80% (n=5)	40% (n=5)	60% (n=5)	60% (n=5)
Nordmann Fir	0% (n=3)	0% (n=3)	0% (n=3)	0% (n=3)	0% (n=3)
Noble Fir	67% (n=3)	33% (n=3)	67% (n=3)	0% (n=3)	33% (n=3)

Table 3. Results of lab culturing of seedling tissue to confirm *P.c.* infection from treated and untreated plots. n=number of trees in each treatment with visual symptoms of *P.c.* (+) indicates number of trees positively cultured for *P.c.*; (-) indicates number of trees that did not culture *P.c.*; (*lost) indicates number of cultures lost due to contamination; (! NS) indicates number of trees that show visual signs of infections but have not yet been sampled.

Species	Untreated Control	Subdue™ dip	Subdue™ drench	Fenamidone drench	Planted on mound
White Fir	n=5 1 (+) 1 (-) 3 (*lost)	n=4 1 (+) 1 (*lost) 2 (!NS)	n=2 1 (+) 1 (!NS)	n=3 1(+) 1 (-) 1 (!NS)	n=3 1 (+) 1 (-) 1 (!NS)
Noble Fir	n=2 2 (+)	n=1 1 (!NS)	n=2 1 (-) 1 (!NS)	-	n=1 1 (!NS)

Results to date (Sept. 2003): Of the twelve soil samples taken, seven cultured positively for *P.c.* using the pear bait assay. Table 2 shows the percent of each species in each treatment that showed visual signs of *P.c.* Only White Fir and Noble Fir seedlings died, although it should be noted that these seedlings had the weakest root structure at planting. None of the trees sampled exhibited J-rooting, although lack of fibrous roots was noted in some. Table 3 shows the results of culturing to confirm death due to *P.c.* Culturing for *P.c.* was difficult and slow: all seedlings sampled June 11 were lost due to contamination of lab cultures. Those seedlings exhibiting symptoms on August 27 have not yet been cultured. Therefore, it is difficult to evaluate the effects of the treatments at this time. Doug Fir and Nordmann Fir appear to offer some "resistance" to *P.c.* and this should be further investigated.

Acknowledgements. Thanks to Rapetti Farms, Kid's Inc., Indian Rock Tree Farm and Pat Trimble, U.S.F.S. Nursery, for supplying seedling trees for this study. Thanks to Syngenta and Bayer Corporation for supplying chemical materials. Thanks to Randy Rapetti, Mike McGee and Mark Visman for their cooperation, and to Gary Chastagner and Mike Benson for helpful suggestions.

The Dynamic Status of Douglas-fir Needlecasts in New York State Christmas Tree Plantations

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Douglas-fir (*Pseudotsuga menziesii*) is a popular choice with Christmas tree growers in many northeastern states because it tolerates a wide range of soils and weather conditions and yields a tree with good form, needle retention, and color. Most growers are able to get one rotation of a crop in and out of a new site with few pest problems, but as second and third generation trees are grown, problems with Rhabdocline and Swiss needlecasts seem to be inevitable. Management of these diseases with three annual applications of chlorothalonil (as Bravo® or Daconil®) in spring has traditionally been successful albeit costly. Anecdotal reports from growers in recent years (since 2000) suggest that this fungicide is not as effective as it once had been and in 2003, we conducted the first stage of a planned multi-year survey to more clearly document the nature of these diseases as they occur in the state. Microscopic examination of sections of mature apothecia on needles with Rhabdocline needlecast from trees throughout the state indicated that the predominant pathogen in most of the state, was *R. pseudotsugae* rather than the expected *R. weirii*, with *R. pseudotsugae* found at nearly 90% of collection sites. Furthermore, it became readily apparent during the survey, that Swiss needlecast (caused by *Phaeocryptopus gauemanii*) was a much more significant disease in both managed and unmanaged plantations than reports from growers previously suggested. Despite the ready availability of educational resources, we found that many growers were still unable to distinguish between the two diseases. This latter observation causes us to question the reliability of results of unsupervised grower surveys.

Effect of Annosus Root Rot on the Keepability of Noble Fir Christmas Trees

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Washington State University, Puyallup, WA. 98371

Annosus root rot, caused by *Heterobasidion annosum* has become an increasing problem in 2nd and 3rd rotation Pacific Northwest noble fir Christmas tree plantations during the past 4 years. Newly planted seedlings become infected when their roots come in contact with infected roots or stumps from the previous crop. Once infected, the pathogen spreads up the roots towards the base of the tree where it continues to spread and eventually kills the tree. Over 20% of the trees have been killed within five years of planting in some plantations. In some instances, infected trees are harvested before this disease kills them. On these trees, there is typically a staining of the woody portion of the stem that is evident upon harvest.

One way to minimize the losses caused by this disease is to harvest infected trees prior to their death. In fields with high levels of disease, growers will begin harvesting trees once they reach a marketable size. The staining that is evident in the wood relates to the area of the stem that has been colonized by the pathogen. Since this tissue may not be able to translocate water as efficiently as healthy stem tissue, a study was done to determine if there was a relationship between the extent of staining present at harvest and the postharvest keepability of trees. Twenty-two noble fir trees with varying levels of staining were obtained from a group harvested on December 5, 2002. The trees were baled and transported to Puyallup and stored outdoors. On January 13, 2003, the base of each tree was recut and trees were then displayed with their bases in water in a room maintained at 20C. The percentage of the stem surface area that was stained was determined for each tree and ranged from 0 to 56.7%.

In addition to killing trees, the results of our testing indicate that the presence of staining associated with Annosus root rot has a significantly adverse affect on the postharvest keepability of noble fir Christmas trees. Within 4 to 7 days of being displayed in water, the trees with the highest degree of staining had noticeably lower moisture contents than the other trees. After 21 days, the percent moisture contents (%MC) of the trees without any staining ranged from 127.4 to 157.2%. Trees with 1.9 to 19.8% staining had %MC that ranged from 122.0 to 153.1%. The %MC of the trees with 31.2 to 35.8% staining ranged from 94.2 to 116.9%. The trees with the highest degree of staining (36.2 to 56.7%) had %MC that ranged from 19.7 to 96.8% after 21 days. Additional studies are needed to confirm these results and determine if this disease also adversely affects the postharvest keepability of Fraser fir Christmas trees, which are also highly susceptible to this pathogen.

Effect of Annosus Root Rot on the Keepability of Noble Fir Christmas Trees

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INTRODUCTION

Annosus root rot, caused by *Heterobasidion annosum* has become an increasing problem in 2nd and 3rd rotation Pacific Northwest (PNW) noble fir Christmas tree plantations. Almost 30% of noble fir trees have been killed within five years of planting in some plantations. Newly-planted seedlings become infected when their roots come in contact with infected roots or stumps from the previous crop. Once infected, the pathogen spreads up the roots towards the base of the tree until it eventually kills it (Figure 1).

Figure 1. Noble fir Christmas trees that have been killed by Annosus root rot.

Once this disease becomes established in a plantation, trees that are adjacent to the infected ones are also at risk of being killed as the pathogen spreads from infected to healthy trees via root-to-root contacts. Infected trees in these "root rot" areas commonly do not show any above-ground evidence of infection until just prior to death. If these trees are harvested before they are killed, the only evidence of infection is a stain on the cut woody portion of the stem that is evident upon harvest (Figure 2).

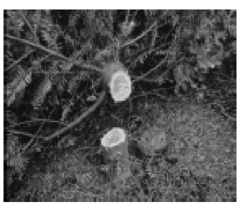


Figure 2. Extensive staining of the stem of an Annosus root rot-infected noble fir Christmas tree just prior to it's death.

In an effort to minimize losses in plantations where this disease is killing trees, some growers are harvesting healthy appearing trees in root rot areas within plantations as soon as they reach a marketable size. If the disease has already spread to these healthy-appearing trees, there will generally be varying levels of staining evident on the cut stems.

The staining that is evident in the wood relates to the proportion of the stem that has been colonized by the pathogen. Since this colonized tissue may not translocate water as efficiently as healthy stem tissue, a study was done to determine if there was a relationship between the extent of staining present at harvest and the postharvest keepability of trees.

METHODS

Twenty-two noble fir trees with varying levels of Annosus root rot staining were harvested from a grower's field on December 6, 2002 for use in this experiment (Figure 3). Following harvest, the trees were baled and transported to Puyallup where they were stored outdoors. On January 13, 2003, the base of each tree was recut and the percentage of the stem surface area that was stained was determined. The diameter and height of each tree was also measured. Trees were then displayed with their stems in water in a room maintained at 20C and 32 to 52% relative humidity for 21 days. During this time, changes in the moisture content of each tree were monitored. Tree quality was also rated on a scale of 1 to 6, where 1 = poor/not acceptable, 2 = fair, 3 = good, 4 = very good, and 6 = excellent.

Figure 3. Noble fir trees with various levels of staining being harvested and baled for test.

RESULTS

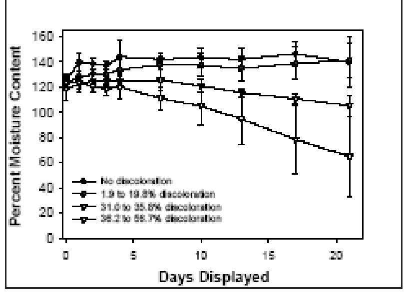
The height and stem diameter of the trees used in this test ranged from 1.4 to 2.4 meters and 4.8 to 10.7 cm, respectively (Table 1). The percentage of the stem surface that was stained ranged from 0 to 56.7%.

Table 1. Tree height, stem diameter and percentage of stem surface area stained.

Tree No.	Height (m)	Diameter (cm)	% stained
8	1.6	6.0	0.0
9	1.6	5.0	0.0
10	1.4	6.5	0.0
13	1.7	8.3	0.0
14	1.5	5.5	0.0
20	1.7	6.3	0.0
12	1.6	7.0	1.9
11	1.8	7.0	9.5
16	1.6	6.0	10.1
5	1.5	9.2	18.3
2	1.7	7.0	19.8
21	1.7	6.2	31.0
15	1.6	6.7	31.2
3	1.4	4.8	34.2
1	1.7	7.3	35.8
18	1.6	6.7	35.8
22	1.4	5.2	36.2
6	1.9	8.5	37.2
4	1.5	6.0	40.0
7	1.9	7.2	42.2
17	1.5	6.0	43.1
19	2.4	10.7	56.7

Within a few days of being displayed, the trees with the highest degree of staining had noticeably lower moisture contents than the other trees (Figure 4). After 21 days, the percent moisture content of the 6 trees without any staining averaged 140.1% while the moisture content of the 6 trees with with the highest degree of staining (36.2 to 56.7% staining) averaged 66%.

Figure 4. Changes in the moisture content of displayed noble fir Christmas trees with various percentage of stem staining.



The extent of staining also affected tree quality ratings. Initial tree quality ratings ranged from 4 to 6. As trees dried, tree quality ratings decreased. After 21 days, the average tree quality ratings of the trees without staining averaged 3.7, while the trees with the highest degree of staining averaged 1.7.

Regression analysis indicated that there was a highly significant negative correlation between the percentage of stem surface staining and moisture content and tree quality (Figure 5).

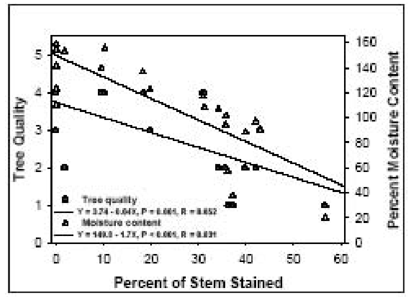


Figure 5. Correlation between stem staining and tree quality and moisture content after 21 days of display in water.

CONCLUSIONS

Noble fir are generally considered to have the best postharvest quality characteristics of any Christmas tree species grown in the PNW. Previous work has shown that when this species is displayed in water, it can maintain high levels of moisture and tree quality for up to 6 weeks. The results of these tests indicate that the presence of staining associated with Annosus root rot has a significant, adverse affect on the postharvest keepability of noble fir Christmas trees that are displayed in water.

The extent of staining on infected trees typically decreases the further above the ground the stem is cut. Although growers typically cut trees off as close to the ground as they can to maximize the height of the tree, it may be possible for growers to minimize the adverse affects of this disease on postharvest quality by simply removing an additional section of the lower portion of the stem on any tree that has a stained stem at harvest.

Additional studies are needed to determine if this disease also adversely affects the postharvest keepability of Fraser fir Christmas trees, which are also highly susceptible to this pathogen in the PNW.

ACKNOWLEDGEMENTS

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Genetic Variation in *Phytophthora cinnamomi* Isolated from Fraser Fir

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Root rot disease caused by *Phytophthora cinnamomi* Rands limits or prevents Fraser fir Christmas tree production on many sites in the Southern Appalachians. To understand genetic variation in this pathogen, genomic DNA was extracted from 34 single zoospore cultures of *P. cinnamomi* and 1 culture of *P. dreschsleri* Tucker isolated from Fraser fir Christmas trees from 5 different North Carolina counties. DNA fingerprints of these isolates were developed by amplified fragment length polymorphism (AFLP) technique using five primer pair combinations (*EcoR* I-AC with *Mse* I-AG, -CG, -GG-, -CT and -TC). Isolates were determined to all be the A2 mating type and showed two distinct fingerprinting patterns. Variation within each pattern was minimal. These results suggest that two distinct introductions of *P. cinnamomi* were brought into the area and that no or little genetic change has subsequently occurred. Presumably, present geographic distribution patterns reflect that of the original introductions and/or recent spread.

NC STATE UNIVERSITY

Genetic Variation of *Phytophthora cinnamomi* Isolated from Fraser Fir (*Abies fraseri)*

Jianfeng Li[1], John Frampton[1] and D.Michael Benson[2]

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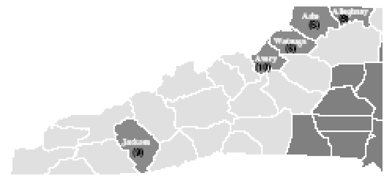
Fraser fir has a small natural range limited to the mountains of western North Carolina, eastern Tennessee and southwestern Virginia. It is the main species for the North Carolina Christmas tree industry. *Phytophthora cinnamomi* , the cause of root rot, is a major limitation to Fraser fir production.



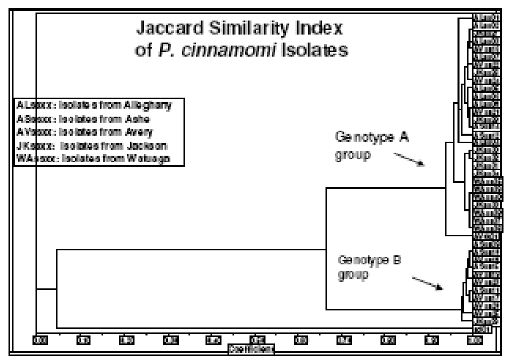
Number of *P. cinnamomi* isolates and counties

Objective: To investigate the genetic relationships among isolates of *P. cinnamomi* collected from Fraser fir of western North Carolina.

Materials and Methods

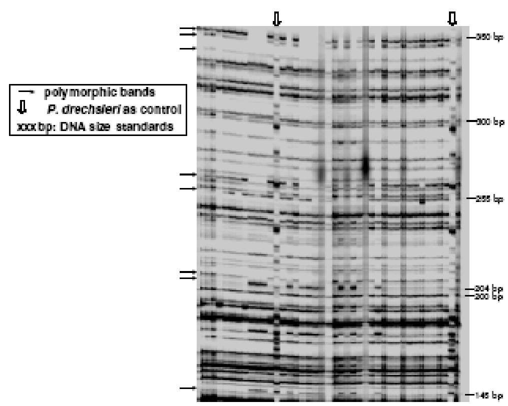
- 39 isolates of *P. cinnamomi* and 1 isolate of *P. drechsleri* from 5 western North Carolina counties were used in the study.
- Single genotype cultures were obtained by single zoospore technique, agar disks of each isolate were paired with A1 and A2 tester strains for oospore production in the mating type tests.
- CMA disks were incubated in V-8 broth for 1 week at 24° C for mycelia growth.
- DNeasy® Plant Mini Kit (Qiagen) was used for DNA extraction, and DNA was digested by *EcoR* I / *Mse* I, ligased by T4 DNA.
- Non-selective reaction was set for 15 sec at 94°C, 30 sec at 60°C, 1 min + 1 sec/cycle at 72°C for 28 cycles, then 2 min at 72°C, with E+A /M +C primer combination.
- Five screened *EcoR* I / *Mse* I primer combinations were used in the selective reaction following the protocol of Myburg *et al* *.
- AFLP images were analyzed with Keygene®, and the data matrix was analyzed with NTSYSpc, TreeView and other phylogenetics software.

Primer Combination	Polymorphic Bands Scored		
	Total	Between *P.cinnamomi* and *P.drechsleri*	Among *P.cinnamomi*
E+AC/M+AG	45	32	13
E+AC/M+CG	57	38	19
E+AC/M+GG	60	49	11
E+AC/M+CA	32	19	13
E+AC/M+TC	55	41	14
Total	249	179	70

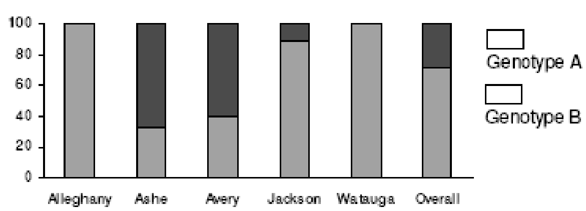


Results and Discussion

- Of the 39 *P. cinnamomi* isolates tested for mating type, 37 isolates were A2 and two failed to form oospores with either tester.
- The AFLP pattern of the isolate of *P. drechsleri* was distinctively different from those of isolates of *P. cinnamomi*.
- Isolates of *P. cinnamomi* from five different counties were divided into two cluster groups, genotype A and genotype B. All isolates from Alleghany and Watauga counties were genotype A, while isolates from Ashe, Avery and Jackson counties were either genotype A or B. Variations in each group were not apparent.
- Although genotype A occurred most frequently on Fraser fir, a second genotype (genotype B) of *P. cinnamomi* has become established in some plantations. The low genetic diversity among isolates suggests that asexual reproduction is the dominant means of dissemination of this pathogen.



**Section of AFLP Gel Image
Primer E+AC/M+CA**



Distribution of genotypes of *P. cinnamomi* isolates among five counties

Authors of this poster wish to thank owners of the farms from which the fungal isolates in this study were collected.

* Myburg *et al.* 2001. High-throughput AFLP analysis using infrared dye-labeled primers and automated DNA sequencer. BioTechniques 30: 348-357.

Sensitivity of *Phytophthora ramorum* to Fungicides

Gary Chastagner[1] Everett Hansen[2] Kathy Riley[1] Wendy Sutton[2]
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The recent detection of *Phytophthora ramorum* on ornamental plants at several commercial nurseries in Oregon and Washington has generated a lot of concern within the Pacific Northwest (PNW) nursery and Christmas tree industries. *Phytophthora ramorum* is the pathogen that on oaks, causes sudden oak death (SOD), which was first detected on tanoak trees in Marin County, California in 1995. On other hosts, it has been referred to as Ramorum leaf blight or dieback. By the end of 2002, it had spread to 12 counties in California and a small area in southwestern Oregon. Earlier this spring, *P. ramorum* was detected for the first time on plants in commercial ornamental nurseries in Oregon, Washington, and British Columbia.

In 2002, a federal quarantine was put in place that prohibits the movement of hosts of this pathogen from areas where it occurs unless they are certified to be free of this pathogen. Although many hosts have been shown to be susceptible to *P. ramorum* under controlled conditions, the officially regulated hosts are limited to those that have been infected under natural conditions. Douglas-fir is one of 22 currently regulated hosts of this pathogen. Recently, Ramorum shoot dieback was confirmed on grand fir Christmas trees at a site in California that is adjacent to a mixed wooded area containing highly susceptible bay laurel trees. Grand fir is currently listed as a provisional host, but will be added to the official regulated host list pending the completion of some additional inoculation studies.

Although no *P. ramorum* has been detected in any Christmas tree fields or conifer nurseries in the PNW, these industries are at risk of potential disruptions in the shipment of trees and nursery stock if efforts to eradicate this pathogen are unsuccessful and the pathogen spreads into other areas in western Oregon and Washington. In an effort to identify fungicides that are effective in protecting conifers from this pathogen, the sensitivity of *P. ramorum* to 20 fungicides that are commonly used to manage Phytophthora diseases in agricultural and nursery crops was determined under laboratory conditions. The effect of each fungicide on mycelial growth and germination of zoospores was assessed by comparing the extent of growth or the percentage of germinated spores on fungicide-amended media to growth and germination on unamended media.

These studies indicate that a number of the tested fungicides were very effective in inhibiting *P. ramorum* mycelial growth and zoospore germination. This included systemic materials like dimethomorph as well as contacts like maneb. Mycelial growth was much more sensitive to some fungicides than were spores (e.g. mefenozam), while spore germination was more affected than mycelial growth with others (e.g. chlorothalonil). Additional studies are in progress to determine the effectiveness of these materials in protection Douglas-fir seedlings from infection by *P. ramorum*.

Sensitivity of Phytophthora ramorum to Fungicides

Gary A Chastagner[1], Everett Hansen[2], Kathy Riley[1], and Wendy Sutton[2]

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The Pacific Northwest (PNW) leads the United States in the production of Christmas trees and has extensive production of conifer nursery stock.

The recent identification of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) as a host of *Phytophthora ramorum* has the potential to disrupt the shipment of Christmas trees and conifer nursery stock grown in the PNW. *Phytophthora ramorum* is the pathogen that, on some oaks, causes sudden oak death (SOD) and was first detected on tanoak (*Lithocarpus densiflorus*) in Marin County, California in 1995. By the end of 2002, SOD had spread to 12 counties in California and a small area in southwestern Oregon. In the spring of 2003, *P. ramorum* was detected for the first time on plants in commercial ornamental nurseries in Oregon, Washington, and British Columbia.

Shoot dieback on Douglas-fir in California caused by *Phytophthora ramorum*.

Unlike most Phytophthora diseases, infections by *P. ramorum* appear to be limited to the above-ground portions of plants, i.e. the leaves, shoots and stems or trunks. With some hosts, such as coast live oak and tanoak, SOD has killed thousands of large trees in California. For other hosts, most infections appear to be limited to the foliage or small shoots and these diseases are sometimes referred to as Ramorum leaf blight or dieback. Although there are many hosts that have been shown to be susceptible to *P. ramorum* under controlled conditions, the officially regulated hosts are limited to those that have been infected under natural conditions.

APHIS has established a federal quarantine that prohibits the movement of known *P. ramorum* hosts out of areas where this pathogen occurs unless they have been certified to be disease free. Over 90% of the Christmas trees produced in the PNW are shipped to markets throughout the U. S. and a number of foreign countries during a limited period each fall. Most of the Douglas-fir seedlings produced in the PNW are utilized in the PNW, but they are commonly shipped from one area to another.

FUNGICIDE STUDIES

There are a number of systemic and contact fungicides that are commonly used in disease management programs to control Phytophthora diseases on various agricultural and nursery crops. Limited information is currently available regarding the sensitivity of *P. ramorum* to these fungicides. Some work is currently in progress in California to determine the effectiveness of applications of systemic fungicides to the trunks of trees to prevent the development of stem cankers. Additional research is needed to determine the effectiveness of systemic and contact types of fungicides to control foliar infection, such as those that occur on Douglas-fir.

In late 2002, a project was initiated to identify fungicides that potentially could be used to control *P. ramorum* on Christmas trees and conifer nursery stock. This project has two objectives:

1. Determine the sensitivity of *P. ramorum* to fungicides that are commonly used to manage Phytophthora diseases in agricultural and nursery crops.

2. Determine the effectiveness of these fungicides in protecting Douglas-fir needles and shoots from *P. ramorum* infection.

METHODS

Three Oregon isolates of *P. ramorum* that were originally obtained from tanoak were screened for their sensitivity to 20 fungicides under laboratory conditions (Table 1). A series of studies were conducted to determine the effect each fungicide had on the growth of mycelia and germination of zoospores.

Mycelial Growth: Mycelial plugs from each isolate of *P. ramorum* were placed on the surface of cornmeal agar media that had been amended with various concentrations of each fungicide. Inoculated plates were incubated at 17C and the extent of mycelial growth was measured after 10 to 12 days.

Spore Germination: Tests were also conducted to determine the effect of different concentrations of each fungicide on the germination of zoospores. Zoospores from each isolate were placed on fungicide-amended cornmeal agar media and incubated at 17C for 16 to 17 hours.

The effect of each fungicide on mycelial growth and spore germination was accessed by comparing the extent of growth or the percentage of germinated spores on the fungicide-amended media to growth and germination on unamended media.

Table 1. List of fungicides tested.

Trade Name	Common Name	Source
Champ Formula 2 F	copper hydroxide	NuFarm Americas Inc
Chipco Signature 80WDG	fosetyl-al	Aventis
Curzate 60WG	cymoxanil	DuPont
Daconil Ultrex 82.5% WDG	chlorothalonil	Syngenta
Dithane 75DF	mancozeb	Dow AgroSciences
DPX-JE874-389 50DF	famoxadone (famoxate)	DuPont
Gavel 75DF	mancozeb 66.7%, zoxamide 8.3%	Rohm and Haas
Heritage 50W	azoxystrobin	Syngenta
IKF-916 400SC	cyazofamid	ISK Biosciences
Insignia 20WG	pyraclostrobin	BASF Corp
Maneb 75DF	maneb	Cerexagri
Omega 500F	fluazinam	Syngenta
Phostrol (53.6%)	phosphorous acid	NuFarm Americas Inc
Phyton 27	copper sulfate pentahydrate	Source Technology Biologicals
Polyram 80DF	metiram	BASF Corp
Reason 500SC	fenamidone	Bayer Crop Science
Stature DM 50WP	dimethomorph	SePro Corporation
Subdue Maxx (21.3%)	mefenoxam	Novartis
Tanos 50DF	cymoxanil, famoxadone 25%	DuPont
Terrazole 35WP	etridiazole	Uniroyal Chemical Co.

RESULTS

A number of the tested fungicides were very effective in inhibiting *P. ramorum* mycelial growth and zoospore germination. This included systemic materials like Stature as well as contacts like Maneb (Figures 1-4 and Table 2). Mycelial growth was much more sensitive to some fungicides than were spores (i.e. Subdue Maxx), while spore germination was more affected than mycelial growth with others (i.e. Daconil Ultrex).

Figure 1. Effect of Maneb on mycelial growth

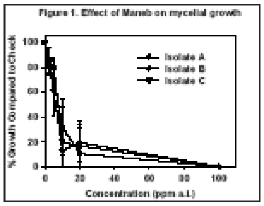

Figure 2. Effect of Stature on mycelial growth

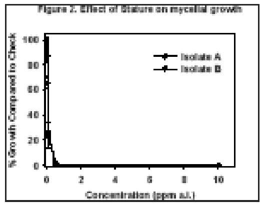

Figure 3. Effect of Maneb on zoospore germination

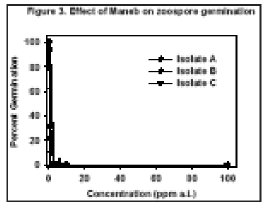

Figure 4. Effect of Stature on zoospore germination

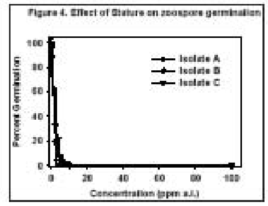

Table 2. Relative sensitivity of *Phytophthora ramorum* mycelial growth and zoospore germination to fungicides.

Fungicide	Sensitivity Rating[1]	
	Growth	Germination
Champ Formula 2 F	3	3
Chipco Signature 80WDG[2]	4	4
Curzate 60WG[2]	2	3
Daconil Ultrex 82.5%WDG	5	1
Dithane 75DF	2	1
DPX-JE874-389 50DF[2]	5	4
Gavel 75DF	1	1
Heritage 50W[2]	4	1-3
IKF-916 400SC[2]	5	2
Insignia 20WG[2]	5	2-4
Maneb 75DF	2	1
Omega 500F	3(?)	1
Phostrol (53.6%)[2]	3	5
Phyton 27	2	2
Polyram 80DF	2	1
Reason 500SC[2]	5	2-3
Stature DM 50WP[2]	1	1
Subdue Maxx (21.3%)[2]	1	5
Tanos 50DF[2]	2	3
Terrazole 35WP[2]	2	4

[1]Rated on a 1 to 5 scale: 1 = >90% reduction at <10 ppm a.i.; 2 = >90% reduction at 10 to 100 ppm a.i.; 3 = >90% reduction at 100 to 500 ppm a.i.; 4 = >90% reduction at 500 to 1,000 ppm a.i.; and 5 = <90% reduction at 1,000 ppm a.i..

[2]systemic activity

CONCLUSIONS

Based on the results of these laboratory studies, it appears that *P. ramorum* is highly sensitive to a number of common fungicides, some of which are already registered for other diseases on many of the hosts that are susceptible to this pathogen. In addition, several fungicides such as Chipco Signature and Phostrol, which are generally not effective in the types of laboratory tests we conducted, can provide excellent control of various Phytophthora diseases under field and nursery conditions. Therefore, it is likely that these materials will also be effective in controlling *P. ramorum*.

The next step in this project is to conduct a series of greenhouse trials to determine the effectiveness of these fungicides in protecting Douglas-fir seedlings from infection by *P. ramorum*. Depending on the traditional method of application for each fungicide, materials will be applied as foliar sprays to the needles and stems or as soil drenches. Seedlings will then be inoculated with *P. ramorum* and the effectiveness of each treatment in protecting the seedlings will be assessed based on symptom development, traditional isolation onto laboratory media and PCR testing to detect the pathogen.

ACKNOWLEDGEMENTS

This project is being supported by the Oregon Department of Agriculture and the Washington State Commission on Pesticide Registration. The assistance of Weyerhaeuser and the B. C. Ministry of Forestry, who provided seedlings for this project and the various chemical companies who provided products is greatly appreciated.

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August 2003

Cavity Formation below the Bud Crown in Nordmann fir

Iben Margrete Thomsen
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Formation of cavities below the bud crown in conifers was first described by Lewis & Dowding (1924). The cavity was said to appear due to the breaking down of the old medullae during the autumn. Subsequently, other authors have also described the bud crown cavities in conifers but there have been few attempts to explain the cause and effect of this cavity.

Studies of buds of balsam, Fraser, Nordmann, Turkish, grand, and noble fir showed that balsam and Fraser fir had cavities below almost every bud by mid-winter. Grand fir and noble fir rarely had cavities, even when freeze treatment was used to induce cavity formation. Nordmann and Turkish fir usually did not have cavities, but freeze treatment (-20ºC for 8 hours) made cavities appear in most trees. Cavities were detected by cutting shoots open longitudinally. They would often enlarge, if the shoots were subjected to drying after freezing. However, if the shoots were immersed in water, the cavities would close up, even though exposed by cutting.

In Denmark, dead buds and abortive shoot elongation in Nordmann fir have been associated with the presence of cavities and discoloration below the bud crown. The symptoms are quite variable:

- dead buds that never break
- shoots die immediately after bud break, when they are less than 1 cm (2,5 inches) long
- shoots wilt either at the tip or the base when they reach 5-8 cm (12-20 inches)
- shoots being deformed or pale in color.

When damaged shoots or dead buds are cut longitudinally, an orange-brown discoloration becomes visible, often associated with a cavity.

To determine the time of cavity formation, several Danish sites with damaged trees were selected. Shoots were collected from trees and examined during winter and early spring. The incidence of cavities rose during the study period, and finally almost 80% of trees had at least one shoot with a bud crown cavity. Some trees had very few cavities (2-5% of shoots examined), while other trees had many (25-50% of shoots). No trees had cavities before the first frost episode in late December.

At this point, it is unclear whether the formation of cavities is the cause of the observed damages in Nordmann fir, or whether they are induced by the other factors such as frost and drought that have also been speculated to be the cause of the bud break problems. Solving this riddle will require more research.

Cavity formation below the bud crown in Nordmann fir

Iben M. Thomsen
Danish Forest and Landscape Research Institute

Cavities below buds

Formation of cavities below the bud crown in conifers was first described in 1924. The cavity was said to appear due to the breaking down of the old medullae during the autumn or winter.

Cavities can be detected by cutting shoots open longitudinally. They often enlarge, if the shoots are subjected to drying. If the shoots are then immersed in water, the cavities mostly close up, even though exposed by cutting.

Abies species

Bud studies in Balsam, Fraser, Nordmann, Turkish, Grand, and Noble fir have shown:

- Balsam and Fraser fir had cavities below almost every bud by mid-winter.
- Grand fir and Noble fir rarely had cavities, even when freeze treatment was used to induce cavity formation.
- Nordmann and Turkish fir usually did not have cavities, but freeze treatment (-20ºC for 8 hours) made cavities appear in most trees.

Time of cavity formation

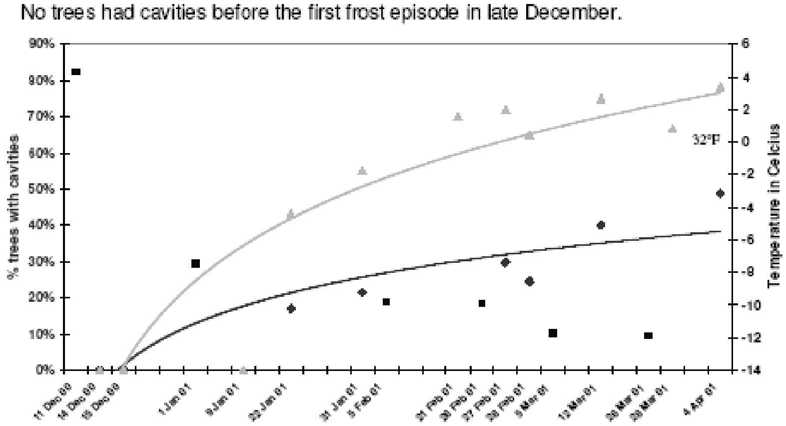
To determine the time of cavity formation, several Danish sites with damaged Nordmann firs were selected. Shoots were collected from trees and examined during winter and early spring.

The incidence of cavities rose during the study period, and finally almost 80% of trees had at least one shoot with a bud crown cavity.

In average 30% of the shoots had cavities under buds.

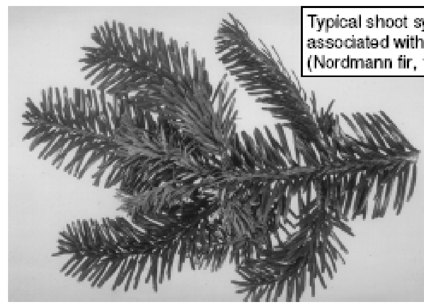
Some Nordmann firs had very few cavities (2-5% of shoots examined) while other trees had many (25-50% of shoots).

No trees had cavities before the first frost episode in late December.



Average percentage of shoots (red dots) and trees (green dots) with cavities below the buds in relation to the lowest mean temperature (blue squares) in Celcius (0 °C = freezing point) recorded till then. There is an increase in amount of cavities from January to March. No cavities were recorded before the temperature dropped below freezing point.

The presence of cavities may not be the cause of the observed damages in Nordmann fir, but could be induced by the same factors causing the bud break problems. Likewise, it has not been proven that frost and drought are involved in the formation of cavities. Solving the riddle will require more research.

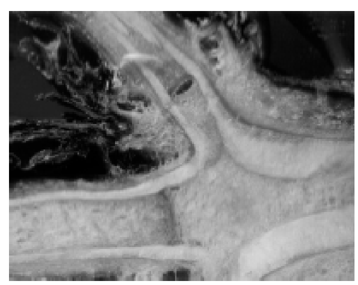


Typical shoot symptom associated with cavities. (Nordmann fir, 1982)

Damage related to cavities

In Denmark, dead buds and abortive shoot elongation in Nordmann fir have been associated with the presence of cavities and discoloration below the bud crown. The symptoms are quite variable:

- Dead buds that never break.
- Shoots die immediately after bud break, when they are less than 1 cm (2,5 inches) long.
- Shoots wilt either at the tip or the base when they reach 5-8 cm (12-20 inches).
- Shoots being deformed or pale in color.



When damaged shoots or dead buds are cut longitudinally, an orange-brown discoloration becomes visible, often associated with a cavity. (Nordmann fir, 1983).

Cavities can be seen below the buds as early as January. (Nordmann fir, 2001)

Current Season Needle Necrosis (CSNN) in Nordmann Fir

Iben Margrete Thomsen[1] & Bent Christensen[2]
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Current season needle necrosis (CSNN) is a common foliage disorder of noble fir and grand fir in the Pacific Northwest (Chastagner, 1997). The initial symptoms of CSNN are reddish brown or tan colored bands and tips on the newly developed needles. No pathogens have been proved to be involved, and the causes seem to be related to nutrient translocation and weather conditions. Application of calcium chloride during shoot elongation has been shown to reduce symptoms, and shading of trees also lessens the incidence of CSNN. The severity of CSNN differs from one year to the next. There seems to be a genetic factor, as some trees are obviously much more prone to CSNN.

In Denmark, Nordmann fir (*Abies nordmanniana*) is the most common Christmas tree species. Noble fir is mainly used for greenery production. Symptoms similar to CSNN have been observed in both species for more than 20 years. The economic consequences are high in the years following a severe attack. Christmas trees are not sheared in Denmark and must therefore have at least 3 years of undamaged needles to be marketable.

The needle symptoms observed in Nordmann fir have not yet been proved to be CSNN. However, during a severe outbreak experienced in 2002 several factors pointed in this direction. The damage appeared in early July after 6-8 days of hot weather. Prior to this, flushing had happened during fairly cool and moist weather. The distinctive red bands and needle tips were very similar to damage also seen on Grand and Noble fir. Shaded trees and shoots had little damage. Several trees had severe shedding by the end of July, but others shed needles more slowly during August or later.

It is proposed to initiate a project in order to: 1) verify the diagnosis of CSNN on Nordmann fir, 2) investigate the specific conditions which prompts the disorder and 3) test various prevention methods. The greatest benefit of this CSNN research project would be the development of procedures to induce the symptoms on susceptible host material under standard conditions. Hopefully, tests could then be conducted on progeny of known seed sources, as the trait is expected to show high heredity in Nordmann fir. Depending on the financial support, the project may include noble fir.

Current Season Needle Necrosis (CSNN) in Nordmann fir

Iben M. Thomsen[1] & Bent K. Christensen[2]
1) Danish Forest and Landscape Research Institute
2) Danish Christmas Tree Growers' Association

Skov & Landskab

Current Season Needle Necrosis

CSNN is a common foliage disorder of noble fir and grand fir in the Pacific Northwest.

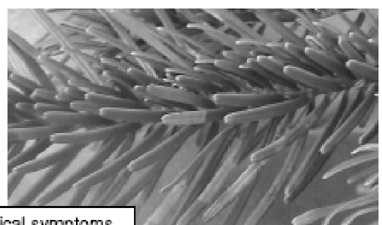
The initial symptoms of CSNN are reddish brown or tan colored bands and tips on the newly developed needles.

Causes seem to be related to nutrient translocation and weather conditions.

Application of calcium chloride during shoot elongation has been shown to reduce symptoms in US.

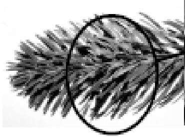
Shading of trees also lessens the incidence of Current Season Needle Necrosis.

There seems to be a genetic factor, as some trees are obviously much more prone to CSNN.



Typical symptoms on Nordmann fir in July. (2002)

Fungal attack in dead needle tissue is common, but no pathogens have been proved to be involved.
Late August, 2002



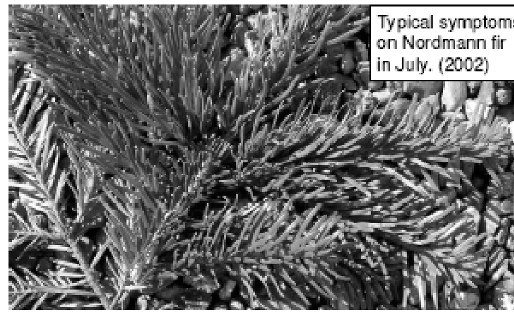
Proposed project

We are currently applying for funds regarding a project to:

1) Verify the diagnosis of CSNN on Nordmann fir
2) Investigate the specific conditions causing CSNN by using controlled climate conditions and susceptible plants.
3) Test various prevention methods.

The greatest benefit of this CSNN research project would be the development of procedures to induce the symptoms on susceptible host material under standard conditions. Hopefully, tests could then be conducted on progeny of known seed sources, as the trait is expected to show high heredity in Nordmann fir.

Depending on the financial support and interest from e.g. the US, the project may include noble fir.



Typical symptoms on Nordmann fir in July. (2002)

Nordmann fir

In Denmark, Nordmann fir (*Abies nordmanniana*) is the most common Christmas tree species. Symptoms similar to CSNN have been observed in both Nordmann and noble fir for more than 20 years.

The economic consequences in Nordmann fir are high in the years following a severe attack. Christmas trees are not sheared in Denmark and must therefore have at least 3 years of undamaged needles to be marketable.

The needle symptoms observed in Nordmann fir have not yet been proved to be CSNN. However, during a severe outbreak experienced in 2002 several factors pointed in this direction.

- Flushing happened during fairly cool and moist weather in May (temperatures around 65°F).
- In the beginning of June there were 6-8 warm, dry and sunny days (temperatures above 75°F).
- The damage on Nordmann fir became obvious in early July (mostly) or late June.
- The distinctive red bands and needle tips were very similar to damage also seen on Grand and Noble fir.
- Shaded trees and shoots had little damage.
- Many damaged trees also had symptoms on older needle sets, indicating genetic predisposition.

Many trees had severe shedding by the end of July, but others shed needles more slowly during August or later.

Environment

Macroinvertebrate Surveys to Monitor Water Quality below Christmas Tree Farms

Jill R. Sidebottom
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In the southern Appalachians, Christmas trees are produced in areas where tourism and retirement homes bring people to the area that have little prior contact with farming. Growers are often criticized for using too many pesticides, and citizens are concerned that these pesticides are adversely affecting the quality of streams in the area. In 1999, a benthic macroinvertebrate (MI) survey was conducted below five Christmas tree farms. The percentage of total MI's that were mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), and riffle beetles and larvae were determined for each site and compared to a nearby reference creek in an undisturbed location. The presence of the first three insect orders commonly referred to as EPT's are indicators of good water quality. Streams were sampled from 3 to 7 times through the year. Representative mayflies, stoneflies and caddisflies were identified to species from three of the sample sites. At four of the sites, the percentage of stoneflies was statistically lower below Christmas trees and the percentage of riffle beetles higher when compared to the reference creek. This difference may be due to the loss of riparian buffers and erosion from farm roads in Christmas tree fields. At one farm, stoneflies disappeared entirely when the grower did some grading upstream. The site where there were no statistical differences in the percentage of EPT's between the Christmas tree farm and reference creek was at a farm which had had continuous Christmas tree production since 1956, but had excellent stream buffers. There were 19 species of EPT's found below Christmas tree farms that had a water quality index of 1.0 or less. These species are highly intolerant of pollution. These observations suggest that water quality remains good in streams below Christmas tree farms.

NC STATE UNIVERSITY

Macroinvertebrate Surveys to Monitor Water Quality Below Christmas Tree Farms

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Special thanks to: *Christy Bredenkamp, Tommy Beutell, Christy Esposito-Edwards. Steve Fraley, Doug Hundley, Jerry Moody, Homer Sides, Mike Stroot, Denver Taylor, David Tucker, Jeff Vance, & Jerry Washington. This work was funded by an NCSU water quality grant.*

NATURE OF THE WORK: In the southern Appalachians, Christmas trees are produced in areas where tourism and retirement homes bring people that have little prior contact with farming to the area. Growers are often criticized for using too many pesticides, and citizens are concerned that these pesticides are adversely affecting the quality of streams in the area. In 1998 and 1999, a benthic (dwelling on the bottoms of streams) macroinvertebrate (MI) survey was conducted below five Christmas tree farms to determine if Christmas tree production was having an adverse effect on water quality. The percentage of total MI's that were mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), and riffle beetles were determined for each site and compared to a nearby reference creek in an undisturbed location on the same sample date.

Streams were sampled from 3 to 7 times through the year. On each date, three samples were taken with a kick-net from riffles and compiled to make a single sample. A random 1/6-portion of the sample was taken and all the macroinvertebrates were counted and identified to order. At least 100 macroinvertebrates were counted in each sample. If there were not 100 in the first portion, a second 1/6 portion was taken and so on until at least 100 were counted. These counts were converted to a percentage of the total. These percentages, and not the actual counts, are reported. Representative mayflies, stoneflies and caddisflies (commonly referred to as EPT's) were also identified to species from three of the sample sites by Steve Fraley with the Tennessee Valley Authority.

Sample site in Alleghany County.

MI's such as caddisflies may indicate clean water.

Abundant riffle beetles may indicate problems with sediment.

WHY MACROINVERTEBRATES?: Benthic macroinvertebrates are recognized as reliable, low cost indicators of stream health and water quality. Sampling is relatively easy and inexpensive. MI's range in their pollution tolerances and sensitive species will respond quickly to stress. MI's are also abundant and serve as primary food source for fish.
Insect photos courtesy Dr. J. Rutherford, Wilfrid Laurier University

RESULTS & DISCUSSION: At four of the five sites, the percentage of stoneflies was statistically lower below Christmas trees and the percentage of riffle beetles higher when compared to the reference creek. These differences may be due to the loss of riparian buffers as well as erosion from farm roads in Christmas tree fields. At one farm, stoneflies disappeared entirely when the grower did some grading upstream. The percentage of EPT's did not change in response to spring pesticide applications of herbicides and Di-Syston 15 G. Streams without cover were also warmer and had greater extremes in daily high and low temperatures.

The site where there were no statistical differences in the percentage of EPT's between the Christmas tree farm and reference creek was at a farm in Jackson County having continuous Christmas tree production since 1956. This site had particularly good stream buffers.

The North Carolina Biotic Index is a listing produced by the North Carolina Division of Environmental Management (DEHNR) for tolerances of North Carolina benthic macroinvertebrate species. The listings range from 0 (most sensitive) to 10 (most tolerant). There were 19 species of EPT's found below Christmas tree farms that had a water quality index of 1.0 or less. These species are highly intolerant of pollution. These observations suggest that water quality remains good in streams below Christmas tree farms, though growers can further protect streams by allowing riparian buffers to grow up around creeks.

These results are similar to findings from a 1998 study conducted by the North Carolina Department of Environment, Health and Natural Resources which concluded that when growers used IPM practices, effects of tree production on streams were minimal.

Bank erosion contributes to sediment in a local stream. Also important contributors to erosion are farm roads and sites being cleared of timber to plant Christmas trees.

TABLE 1. Percentage of groups of macroinvertebrates found in a reference creek (A) and below a Christmas tree farm (B) in Jackson County. This farm has had continuous Christmas tree production since 1956 and there are no statistical differences in the percentage of groups of MI's.

MI's	12/5/98		3/18/99		5/20/99	
	A	B	A	B	A	B
Mayflies	44	77	22	56	41	63
Stoneflies	18	18	16	10	11	9
Caddisflies	7	8	10	14	7	5
Total EPT's	69	70	48	80	59	77
Riffle Beetles	5	21	22	9	12	13

TABLE 2. Percentage of groups of macroinvertebrates found in a reference creek (A) and below the Christmas tree farm (B) in Alleghany County pictured above. When percentages of macroinvertebrates were analyzed over time, the percentage of stoneflies were significantly lower and the percentage of riffle beetles were significantly higher below Christmas trees as compared to the reference creek.

MI's	1/7/99		2/2/99		3/10/99		4/15/99		5/25/99		7/13/99		8/19/99	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mayflies	65	59	47	56	55	67	51	48	55	30	29	44	41	24
Stoneflies	17	9	21	3	14	3	27	1	5	2	17	4	16	4
Caddis-flies	8	4	9	8	5	5	7	2	6	5	11	9	8	8
Total EPT's	90	72	77	67	74	75	85	51	66	37	57	57	65	36
Riffle beetles	3	22	4	31	8	21	4	38	3	57	10	19	11	46

Nutrition

Soil and Plant Nutritional Status of Fraser Fir Christmas Tree Fields – A Survey

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A comprehensive nutritional survey was conducted on 230 sites throughout the Fraser fir Christmas tree production area in the mountains of western North Carolina. Soil was sampled at depths of 0-5, 5-10, 10-20, and 30-50 cm. Samples were analyzed for P, K, Ca, Mg, Mn, Zn, Cu, pH, exchangeable acidity, CEC and humic matter. Texture was determined by particle size analysis. Needle samples from each site were analyzed for N, P, K, Ca, Mg, S, Mn, Zn, Cu, Fe, and B. Site information collected included condition of the trees, tree age, rotation number, aspect, slope, elevation, and soil series. Soil test (Mehlich 3) P was high at the soil surface, but decreased drastically with increased depth. Levels averaged over 200 ppm in the 0-5 cm sample, but averaged less than 50 ppm in the 10-20 cm samples. The majority of the P in the soil is most likely above the active root system of the trees. Average tissue P levels was 0.19%, which is at the lower limit of the established sufficiency range. Average tissue N and K levels were within their sufficiency ranges, while Ca was below its sufficiency range. The relationship of soil nutrient level to tree tissue nutrient level was poor as determined by regression analysis. Like P, soil Ca levels decreased with depth. Soil pH also decreased with depth, most likely due to surface application of lime. The low tissue P and Ca levels indicate a need to develop P and lime (or gypsum) incorporation methods that work on steep slopes.

Nitrogen Management in Christmas Tree Production

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The production of Christmas trees is an agricultural activity with the potential to contribute significant amounts of reactive nitrogen to the environment - one that has received relatively little attention in sustainable agriculture research. In Michigan alone there are *ca.* 60,000 acres of land devoted to Christmas tree production, with annual sales valued at $40 million at the producer level (Michigan Agricultural Statistics Service 2000). Several lines of evidence suggest that Christmas tree production in Michigan may be contributing substantially to N pollution of regional ecosystems. First, conventional N management strategies add very large amounts of nitrogen to Christmas tree plantations. Current recommendations range as high as 170 kg N ha^{-1} y^{-1} (Koelling 2001), which exceeds the national average of 145 kg N ha^{-1} y^{-1} applied to corn - the agronomic crop with the highest nitrogen demand (Fixen and West 2002). Depending on growth stage, these additions of N range from 2 to 3 times the apparent demand of the crop, indicating great potential for N loss to the environment. Secondly, conventional operational process is for all of this fertilizer to be added in a single application, early in the spring when potential for leaching losses is high and cold soil limits root uptake. Finally, Christmas trees are often planted on sandy soils in Michigan with low exchange capacity, further increasing the potential for leaching losses of N. In the spring of 2002 we initiated a study of the response of Fraser fir plantations to varying rates of N addition. We added nitrogen to 4-year old trees at rates corresponding to 0, 50, 100, and 200 percent of the amount recommended for trees of that age. These treatments were applied at two sites: one at the MSU Forestry Department's Tree Research Center (TRC), where the trees had never before been fertilized and the other at a commercial farm (CF) in Missaukee County where the trees have been fertilized continuously since planting. At each site we installed tension lysimeters below the rooting zone of three randomly selected trees in each plot to monitor leachate N concentrations. We are also measuring tree response to fertilization in terms of growth and foliar N concentrations.

Nitrogen Management in Michigan Christmas Tree Plantations

David Rothstein and Audria Holcomb
Forestry Department Michigan State University
Jill O'Donnell
MSU Extension

INTRODUCTION

There is increasing concern in the scientific community over the effects of anthropogenic inputs of reactive nitrogen (N) to the global ecosystem (Ayers et al. 1994; Smil 2001; Galloway and Cowling 2002). Negative effects of N enrichment include: nitrate (NO3-) and nitrite (NO2-) contamination of drinking water, eutrophication of aquatic ecosystems, losses of biodiversity in terrestrial ecosystems, acidification of soils and groundwater, and global climate change induced by emissions of nitrous oxide (N2O). Nearly ¾ of global anthropogenic release of reactive N compounds is associated with fertilizer inputs in food and fiber production (Galloway and Cowling 2002).

The production of horticultural crops such as Christmas trees is an agricultural activity with the potential to contribute significant amounts of reactive nitrogen to the environment - one that has received relatively little attention in sustainable agriculture research. In Michigan alone there are ca. 60,000 acres of land devoted to Christmas tree production, with annual sales valued at $40 million at the producer level (Michigan Agricultural Statistics Service 2000). Several lines of evidence suggest that Christmas tree production in Michigan may be contributing substantially to N pollution of regional ecosystems. First, conventional N management strategies add very large amounts of nitrogen to Christmas tree plantations. Current recommendations range as high as 170 kg N ha-1 y-1 (Koelling 2002), which exceeds the national average of 145 kg N ha-1 y-1 applied to corn - the agronomic crop with the highest nitrogen demand (Fixen and West 2002). Because Christmas trees in Michigan are often planted on sandy soils with low exchange capacity, the potential for leaching losses is likely to be particularly high.

Table 1. Current N fertilizer recommendations for Fraser fir in Michigan (from Koelling 2002).

Years After Planting	N Fertilization Rate (g/tree)
2	14
3	21
4	28
5	42
6+	56
Harvest Year	140-170

METHODS

In the spring of 2002, we initiated a study of the response of Fraser fir plantations to varying rates of N addition. We added nitrogen to 4-year old trees at 0, 14, 28, and 42 g N per tree (0, 47, 95 and 126 kg N/ha). These rates correspond to 0, 50, 100, and 150 percent of the amount recommended for four-year-old trees in Michigan (Table 1; Koelling 2002). Nitrogen was added to these trees in a manner designed to mimic conventional farm operations. Specifically, N was added as ammonium sulfate ((NH4)2SO4) in a single surface application around the drip line of each tree. These trees were planted in 1998 on a Capac Loam soil at the MSU Forestry Department's Tree Research Center (TRC) in East Lansing, MI. Prior to this experiment the trees had never before been fertilized. Fertilizer was applied May 21st in 2002 and on May 3 in 2003 to 16 plots, of 25 trees each, laid out in a randomized block design (n=4).

Each spring we measured the height, leader length, lateral buds and basal diameter of all trees. Throughout the 2003 growing season we collected soil cores (20 cm depth) from the dripline of 2 randomly-selected trees per plot. These were extracted in 2M KCl to determine mineral N (NH4+ and NO3-) availability. In October of 2002 we collected foliage to determine N concentrations using a Carlo-Erba NA1500 Elemental Analyzer. We used allometric biomass equations developed for Fraser fir in North Carolina (Hinesley and Wright 1989) to estimated growth in stemwood, branches and needles based on changes in diameter. We used these estimates, together with measures of N concentration, to estimate the amount of N taken up by each tree (aboveground only) over the 2002 growing season.
On May 1, 2002 we installed tension lysimeters below the rooting zone (50-60 cm depth) of three randomly selected trees in each plot to monitor leachate N concentrations. Lysimeters were sampled ca. weekly between April and June of both 2002 and 2003. In both years, lysimeters had dried out by mid June. Michigan experienced a severe drought in the late summer and autumn of 2002 so that we were unable to collect any soil leachate throughout that autumn. Lysimeter samples were analyzed for NH4+ and NO3- using an Alpkem Flow Solution IV Autoanalyzer. We converted lysimeter NO3- concentrations to leaching losses using water flux estimated on a daily time step using climate data from a local climate station. We used a modified bucket model (Reed 2003) to estimate water yield from potential evapotranspiration (modified Penman-Monteith method), rainfall, and soil waterholding capacity. On days with a net water yield, we estimated leaching losses as the product of water yield (L/m2) and lysimeter N concentration (mg/L). We only report leaching data for 2003 because, due to the drought, we estimated no net water yield after fertilizer was applied on May 21st.

RESULTS

We found that springtime applications of fertilizer produced marked increases in available soil N that were sustained throughout the growing season (Fig. 1). Notice also that extractable N levels in soil remained elevated in the 28 and 42 g/tree treatments in early spring of 2003 – a full year after the 2002 fertilization. Control plot trees, which have never been fertilized, had foliar N levels below the 1.5% deficiency threshold defined by Fisher (1996) for Fraser fir in Michigan (Fig. 2). Fertilization resulted in a significant increase in foliar N relative to the control, however, there was no apparent response to additions above the lowest rate of 14 g/tree. Fertilization had no effect on rates of 2003 height extension (Fig. 3) or basal diameter growth between 2002 and 2003 (Fig. 4). In addition, we could find no evidence that fertilized trees took up any more N than control trees (Fig. 5). Our estimates of aboveground N uptake correspond to 38, 16 and 12 % of fertilizer inputs in the 14,28 and 42 g/tree treatments respectively. Taken together these data suggest that there is no benefit to adding N at levels above 14 g/tree (47 kg/ha).

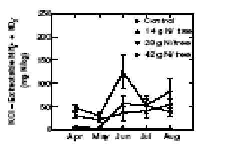

Figure 1. Seasonal patterns of available soil N in 2003. Soil inorganic N concentrations differed significantly among treatments (P<0.0001) and among sample dates (P<0.0001). There was a significant treatment by date interaction (P=0.014).

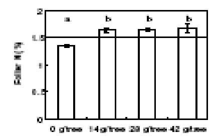

Figure 2. Autumn 2002 foliar N concentrations by fertilization treatment. There was a significant effect of fertilization level on foliar N (P = 0.004), however there was no response to N beyond 14 g/tree. The dashed line at 1.5% represents the N deficiency threshold for Fraser fir in Michigan as determined by Fisher (1996).

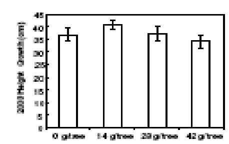

Figure 3. 2003 height extension by fertilization treatment. There was no significant effect of fertilization level on height growth (P = 0.406).

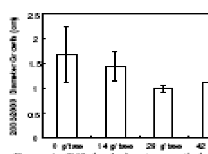

Figure 4. 2002 basal diameter growth by fertilization treatment. There was no significant effect of fertilization level on diameter growth (P = 0.439).

In contrast, we clearly see that the environmental costs of fertilization increase markedly with increasing levels of N addition. Nitrate concentrations of leachate water increased consistently with each increment of added N (Fig. 6). Leachate NO3- concentrations were well above the EPA-mandated threshold for safe drinking water of 10 mg/L as N. In fact, we frequently recorded lysimeter NO3- concentrations in excess of 200 mg N/L in the highest addition rate treatment (data not shown). This pattern of lysimeter NO3- concentration resulted in clear increases in leaching losses of N with increasing additions of fertilizer (Fig. 7). Leaching losses for 2003 will likely increase when we water flux commences again in the autumn. Clearly, at even the lowest application rate, N is being added at rates that exceed crop demand, resulting in substantial hydrologic losses.

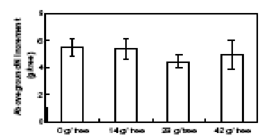

Figure 5. Nitrogen accumulation in aboveground tissues from spring 2002 to spring 2003 by fertilization treatment. There was no effect of fertilization level on on N increment (P = 0.789). Aboveground N increment represented 38, 16 and 12 % of fertilizer inputs in the 14, 28, and 42 g/tree treatments respectively.

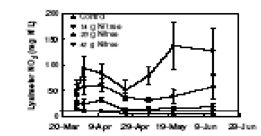

Figure 6. 2003 lysimeter NO_3^- concentrations by fertilization treatment. Lysimeter NO_3^- concentrations differed significantly among treatments (P < 0.001), but not among sampling dates (P = 0.254). The dashed line at 10 mg NO_3^--N/L corresponds to the EPA's safe drinking water threshold.

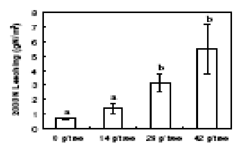

Figure 7. Spring 2003 N leaching by fertilization treatment. Higher levels of N fertilization significantly increased the amount of N leached (P = 0.028).

CONCLUSIONS

- Some N fertilization is necessary – unfertilized trees appear N deficient.
- We can find no benefit to N additions above 47 kg/ha.
- N additions above 47 kg/ha result in N escaping the rooting zone, potentially contributing to groundwater contamination.

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- Michigan Christmas Tree Association

Accumulation of Biomass and Nutrients in Nordmann Fir Christmas Trees in Denmark

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Accumulation of biomass and nutrients was measured in 7-years-old trees in 1997 (age after planting) in a Christmas tree stand (Abies nordmanniana). Four fertilizer treatments and one unfertilized reference treatment were examined. The treatments consisted of inorganic NPK 23-3-7 in four doses corresponding to an N addition of: 35 kg ha^{-1} yr^{-1}, 70 kg ha^{-1} yr^{-1}, 138 kg ha^{-1} yr^{-1} and 276 kg ha^{-1} yr^{-1}.

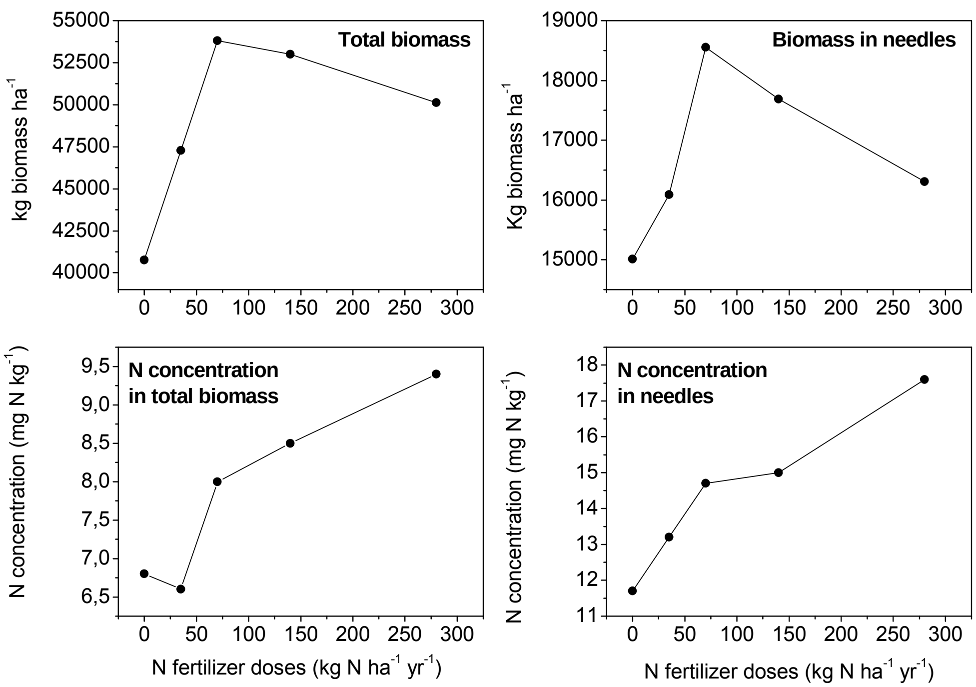

Figure 1. The total biomass and needle biomass accumulated in standing trees and the N concentration in total biomass and needles of standing trees.

The accumulation of biomass increased with increasing doses of fertilizer up to 70 kg N ha^{-1} yr^{-1} (figure 1). Doses higher than 70 kg N ha^{-1} yr^{-1} did not increase the biomass accumulation further. Instead a decreasing biomass amounts compared to 70 kg N ha^{-1} were observed, especially in the needles. The N concentration in both total biomass and needles rose with increasing fertilizer doses, revealing luxury consumption.

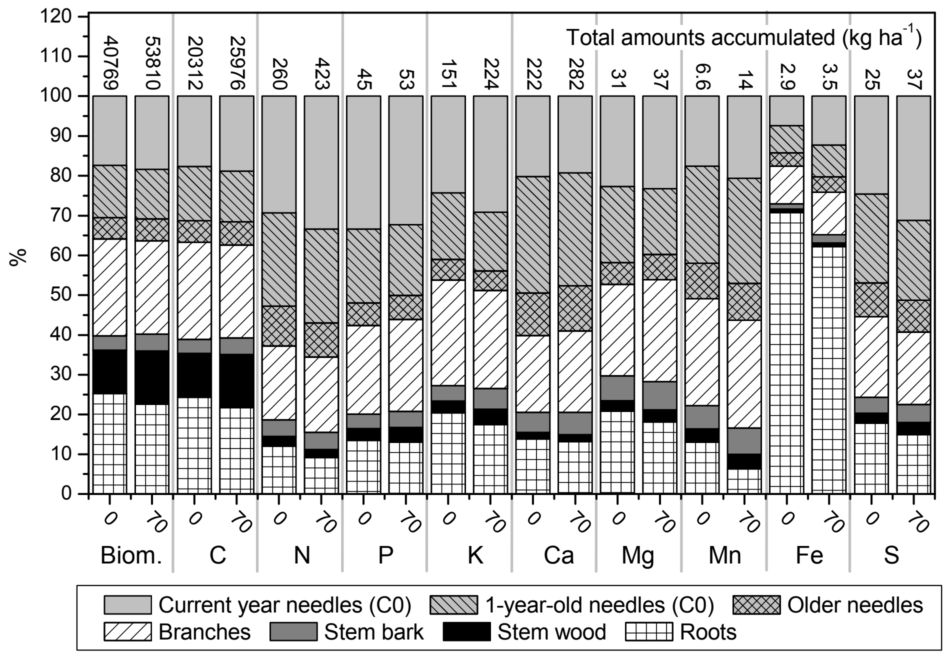

Figure 2. Relative distributions of biomass and nutrients into various plant parts are depicted for the unfertilized reference treatment (0) and the treatment with 70 kg N ha^{-1} yr^{-1}. The numbers above the columns show amounts of accumulated biomass (Biom.) and nutrients. The figure represents averages for the repeated treatment plots.

Biomass accumulation increased with approx. 30 % in plots fertilized with 70 kg N ha^{-1} yr^{-1} compared to unfertilized plots. The accumulation of nutrients was also influenced by increasing doses of fertilizer, but at different magnitudes depending on the nutrient e.g. N and K were more affected than P. Even though accumulation of biomass and nutrients was clearly affected by fertilization, the treatments had a notably small effect on the relative distribution of biomass and nutrients in the various plant parts (figure 2).

Post-Harvest

Effect of Harvest Date on Needle Retention by Various True Firs

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The shedding of green needles as trees dry is a serious problem with some conifers grown as Christmas trees. Research has shown that there are a number of factors that determine the extent of needle loss. One factor that is often assumed to predispose trees to needle loss is early harvest. However, it is likely that in most situations it is the extent of cold that trees have been exposed to prior to harvest that influences needle retention and not the actual harvest date. Work at North Carolina State University has shown that this is the primary reason for sporadic needle loss problems with Fraser fir when there is an unusually warm fall prior to harvest.

Although there is considerable interest in growing various true firs (*Abies* spp.) as Christmas trees, some of these have potentially serious needle loss problems. In 1996, a replicated planting of 10 true firs (balsam fir, Canaan fir, Fraser fir, grand fir, Korean fir, noble fir, Nordmann fir, Shasta fir, Turkish fir, and white fir) was established at WSU Puyallup. Four to 8 trees of each species were planted in each of four blocks. In 2002 and early 2003, branches were harvested from each tree to assess needle retention. Branches were displayed in a postharvest room maintained at 18C for 10 days. Needle loss was evaluated after 3, 7 and 10 days by gently rubbing the needles on each branch and rating the extent of 2002 and 2001 needles that were lost. Moisture contents were also determined for a small subset of branches in order to determine the rate of moisture loss.

Noble fir was the only species that did not have any needle loss during this test. On samples that were harvested on October 22nd, the percentage of Korean and Shasta fir that exhibited needle loss was 9.1 and 13.0%, respectively. Fifty to 67.9% of the balsam, Fraser, and grand fir trees and over 80% of the Canaan, Nordmann, Turkish, and white fir trees had needle loss problems at this time. The actual needle loss varied from less than 1% to the loss of all the needles on the branch.

Exposure to cold temperatures prior to harvest is known to reduce needle loss in some species. There was an unusually cold period during late October and early November in 2002. Nighttime temperature dropped to about –6C for six days starting on October 30th. Even though delaying harvest until early December or mid-January 2003 significantly reduced needle loss by some species, it did not eliminate it. For example, even when branches were harvested in January, 19 to 75% of the Nordmann, Turkish, and white fir trees shed needles. It appears that a better understanding of factors affecting needle loss by these species is needed if they are going to be used in the production of high quality Christmas trees.

Propagation & Genetics

Virginia Christmas Tree Growers - Virginia Dept. of Forestry Christmas Tree Species Test Plantings

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Beginning in the Fall of 1997 the VCTGA and the VA. Dept. of Forestry formed a partnership to test plant tree species new to Virginia Choose and Cut Christmas Tree growers. Twenty seven species of Fir and twelve other tree species were planted by the Spring of 1999. The test site is located in the Coastal Plain of Virginia in New Kent County. Elevation is 59 feet at the New Kent planting. To date four Fir species, one Arizona Cypress, and the Leyland Cypress have survived and are recommended for small test plantings. The Canaan Fir { West Virginia Seed Source } and the Leyland Cypress have performed best on the New Kent site and are also growing well on a second site in the Coastal Plain of Virginia in James City Co.

Clonal Propagation of Fraser Fir through Somatic Embryogenesis

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There is a demand for an efficient clonal propagation technique for the production of Christmas trees, and for fir-species in particular, due to a large genotypic variation of the desired traits and a low natural production of seeds. Multiplication by cuttings can only provide limited quantities and not always optimal quality of the clonal material. An alternative method to produce clonal material is through somatic embryogenesis. This technique is based on seeds that are clonally propagated into somatic embryos capable of forming unlimited numbers of trees of the same genotype. Some of the advantages with plant production through somatic embryogenesis are

- The plantlets have a non-disrupted connection between root and shoot, as opposed to plantlets produced from cuttings.
- The multiplication rate is very high. In Norway spruce, the growth rate in bioreactors has been shown to exceed 30% per day (F.W.). This implies the possibility to produce 1 million plantlets from one single tree in a year.
- The production of plantlets can be automated, providing a cost effective alternative.
- The interesting genotypes can be kept during field testing; the embryogenic cultures can be preserved under liquid nitrogen for an unlimited time period.

Somatic embryogenesis methods has been successfully established for many commercially important tree species, e.g. Norway spruce, Loblolly pine. The Fir-species are however regarded as recalcitrant for SE, and only a few reports on successful regeneration of plants are available to date. In Norway, the use of somatic embryogenesis for Christmas tree production has however been implemented by a national nursery, in collaboration with the Norwegian Forest Research Institute (see Kvaalen et al. at this meeting). At the Forest Biology Unit at IPST at GT, Atlanta, GA, we were recently able to initiate SE from mature seeds of Fraser fir. Embryogenic cultures are being established, and we will further work on the methods for maturation and propagation of plants.

True Firs: New Conifers for Michigan Landscapes

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[2]*Michigan State University, Department of Forestry*
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Conifers are among the most widely grown woody ornamentals in Michigan. Unfortunately, many of the conifers that are currently planted in Michigan and the Upper Midwest such as Colorado blue spruce and Austrian pine have been over-planted and are subject to several serious pests. The goal of this project is to identify new, exotic true firs (Members of genus *Abies*) that are adapted to Michigan climate and soils. True firs have considerable aesthetic and ornamental appeal. Many true firs are high-value specialty trees that are valued by collectors and can be marketed to high-end landscape outlets. Identifying the adaptability of these species will expand the market for growers and retailers and increase the range of conifer choices for Michigan landscapers and homeowners. Over the past ten years, Dr. Mel Koelling and MSUE State Christmas Tree Extension agent Jill O'Donnell have developed an accession of over 30 exotic fir species and hybrids from throughout the world. Presently the trees, ages 3-7 years, are growing in a plantation at the Kellogg Experimental Forest. The trees were established at a relatively tight spacing to evaluate their potential as new Christmas tree species for Michigan. While this spacing has allowed evaluation for Christmas trees, the trees need to be thinned to maintain the planting past the rotation length for Christmas trees of 7-9 years. Working cooperatively with the Kellogg Forest staff we have a initiated a project to transplant trees from 20 species to three MSU Horticultural stations (Horticulture Research and Teaching Center near East Lansing, Northwest Horticulture Experiment Station near Traverse City and Clarksville Horticulture Research Station) to evaluate their adaptability to varying sites and to expand the evaluation to include use as landscape ornamentals.

Once the transplanting is completed we will conduct a long-term study to evaluate the selected species and hybrids based on ornamental characteristics and:

- Drought tolerance
- Cold tolerance
- pH tolerance
- Pest resistance.

True Firs: New Conifers for Michigan Landscapes

Bert Cregg, Mel Koelling, Jill O'Donnell, Katrina Schneller, Grant Jones

Department of Horticulture, Department of Forestry, Michigan State University Extension

Michigan State University, East Lansing, MI 48824

Synopsis of Research:
Twenty true fir (*Abies spp.*) species and hybrids (Table 1) from a species test at the Kellogg Experimental Forest were transplanted to three Horticultural experiment stations and evaluated for stress tolerance, pest resistance, and ornamental characteristics (Figures 1-5).

Figure 1: Fir plots at Kellogg Experimental Forest

Figure 2: Digging fir trees in the fall of 2002

Table 1: Fir trees used in the trials.

Noble fir (NOB)	*Abies procera*
Nordman fir	*Abies nordmanniana*
Korean fir (KOR)	*Abies koreana*
Corkbark fir (COR)	*Abies bifolia*
Sub-alpine fir (SUB)	*Abies lasiocarpa*
Turkish fir	*Abies nordmanniana* Ssp. *equi-trojani*
Needle fir (NEE)	*Abies holophylla*
Veitch fir (VEI)	*Abies veitchii*
Nikko fir (NIK)	*Abies homolepis*
Ernest fir (ERN)	*Abies chensiensis*
Siberian white fir	*Abies nephrolepis*
Korean x Balsam (KXB)	*Abies koreana x balsamea*
Fraser x Nikko (FXN)	*Abies fraseri x homolepis*
Korean x Veitch (KXV)	*Abies koreana x veitchii*
Red fir	*Abies magnifica*
Siberian silver fir	*Abies siberica v. argenta*
Sakhalin fir	*Abies sachalinensis*
Greek fir	*Abies cephalonica*
Momi fir	*Abies firma*
Mayr Sakhalin fir	*Abies sachalinensis* var. *mayriana*

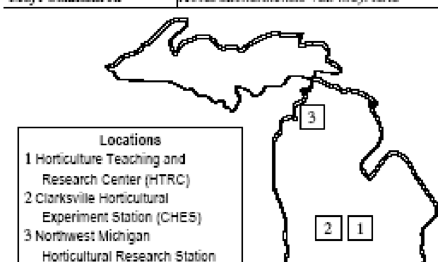


Figure 3: Trial locations for ornamental evaluation of true firs

Figure 4: Planting Firs at the Clarksville Horticultural Experiment Station in the Fall of 2002

Figure 5: Horticulture Research and Teaching Center in the Spring of 2003

Specific Objectives:
The project is divided into two phases: 1) an extensive evaluation of the species based on survival and growth at the three test locations and 2) a more intensive evaluation of physiological characteristics of the trees transplanted to the Horticulture Teaching and Research Center near MSU.

The overall goal of the proposed project is to identify species and hybrids of true firs (*Abies spp.*) that are suitable for Michigan landscapes. The specific objective is to evaluate the selected species and hybrids based on:

- Ornamental characteristics
- Drought tolerance
- Cold tolerance
- pH tolerance
- Pest resistance

Specific Methods and Procedures
Evaluation of the tolerance of the species to environmental stresses will be based on a combination of growth responses and physiological responses (Figures 6-7). For Example, initial measurements on shoot gas exchange indicate significant inter-specific variations in water use efficiency (Figure 8), suggesting opportunities to select for improved drought tolerance.

Figure 6 (above): Recording shoot photosynthesis using a Li-Cor 6400.

Figure 7 (Right): Measuring volumetric soil moisture using a portable TDR system.

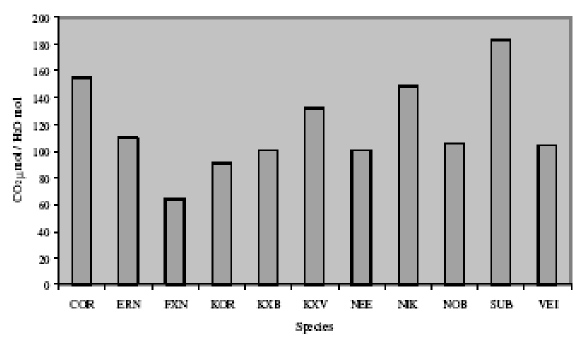


Figure 8: Preliminary Water Use Efficiency levels compiled from all three location, July 2003

Potential Impact on Michigan Plant Agriculture/Industries:
Conifers are among the most widely grown woody ornamentals in Michigan. Unfortunately, many of the conifers that are currently planted in Michigan such as Colorado blue spruce and Austrian pine have been over-planted and are subject to several serious pests. The proposed research will identify new, exotic true firs that are adapted to Michigan climate and soils.

Improving the Rooting Ability of Fraser Fir Cuttings

John Frampton[1] and Chris Rosier[2]
[1]Associate Professor, Dept. of Forestry, North Carolina State University, Raleigh, N.C., and Technical Forester, Smurfit-Stone Container Corporation, Fernandina, FL, USA

Two studies were conducted to better understand factors influencing the rooting ability of Fraser fir cuttings. In the first study, season (fall, winter and summer), auxin type [indolebutryic acid (IBA) and napthaleneactic acid (NAA] and auxin concentration (0, 1, 2, 4, 8, 16 or 32 mM) were evaluated. Cuttings from 3-0 or 4-0 seedlings rooted best (90-100%) when collected in summer (June) and treated with either 4-16 mM (~800-3200 ppm) IBA or 2-8mM (~375-150 ppm) NAA. In the second study, age (3, 5 or 7 years from planting) and severity of stumping were evaluated. The 3- and 5-year-old trees were stumped to the bottom whorl or left intact while the 7-year-old trees were stumped to the bottom 1, 3 and 5 whorls or left intact. Rooting ability decreased with tree age and increased with the severity of stumping.

Improving the Rooting Ability of Fraser Fir Cuttings

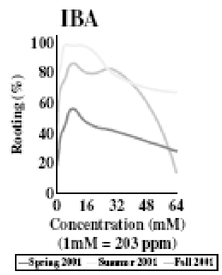
John Frampton and Chris Rosier

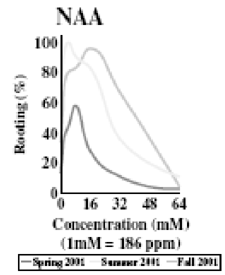
Christmas Tree Genetics Program, Department of Forestry, North Carolina State University, Raleigh, N.C., 27695-8002

Objective: To understand how various factors influence the rooting ability of Fraser fir cuttings in order to develop an economically feasible clonal propagation system.

Season and Auxin Study

- ❖ What is the best season for collecting and rooting cuttings: Fall (August), Winter (February) or Summer (June)?
- ❖ What is the best auxin (rooting hormone) to treat cuttings with: IBA (indolebutyric acid) or NAA (napthaleneacetic acid)?
- ❖ What is the best auxin concentration to treat cuttings with: 0, 1, 2, 4, 8, 16, 32 or 64 mM?

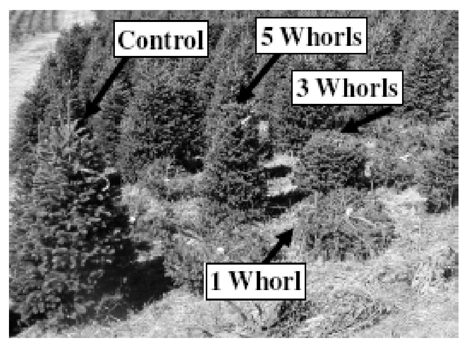




Conclusions

Fraser fir cuttings from 3-0 or 4-0 seedlings rooted best (90-100%) when:

- ❖ Collected in summer (June)
- ❖ Treated with either:
  - 4 – 16 mM (~800-3200 ppm) IBA or
  - 2 –8 mM (~375-1500 ppm) NAA



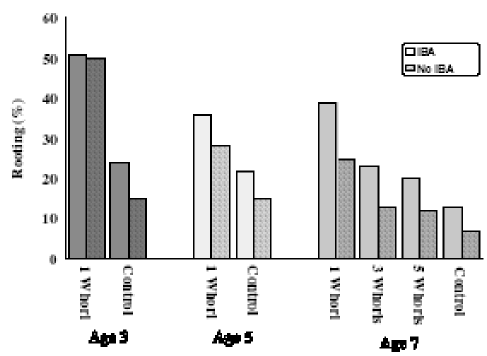
Age and Stumping Study

- ❖ Which age roots best: 3, 5 or 7 years in the field?
- ❖ How does stumping effect rooting ability:
  - stumped to bottom whorl or control (Ages 3 and 5)
  - stumped to bottom 1, 3 or 5 whorls or control (Age 7)?

Conclusions

Rooting ability in Fraser fir:

- ❖ Is higher for younger trees
- ❖ Increases with the severity of stumping



Thanks to Joe Shoupe and the NC DFR Linville River Nursery for donating seedlings. Also, thanks to the following growers for donating trees: Waightstill Avery, Wayne Ayers, Sam Cartner, Tom & Rock Hall and Jack Wiseman

Transferring Technology in Fraser Fir Production: Determining Commercial Viability of Asexual Propagation of Fraser Fir with On-Farm Demonstrations

Jerry T. Moody and John Frampton
North Carolina Cooperative Extension Service, Newland, NC, and North Carolina State University, Raleigh, NC, USA

Fraser fir (*Abies fraseri* (Pursh) Poir.) is indigenous to particular areas of the Southern Appalachians and is commercially important as Christmas Trees and Greenery. In Avery County approximately 4500 acres are in production and is estimated that 1,000,000 trees are harvested annually. One production problem is that not all trees mature similarly. It takes 2-3 years to clear an acre of trees. Fraser fir is grown from seed either from natural areas such as Roan Mountain or private orchards. This results in high level of genetic variability within given fields. Clonal reproduction would allow the cultivation of superior and similar plants based on the growers specific needs. Asexual reproduction has been shown to be possible. This project was designed to transfer the research information into actual commercial demonstrations. Three sites were selected to demonstrate commercial viability. The first site was a commercial propagation facility, the second was a cold frame greenhouse, and the third was commercial outdoor seedling production area. The three sites propagated Fraser Fir from trees that were cutoff allowing the regeneration of multiple terminals. Cuttings were taken in July and propagated over the next three months and will be followed into the field over the rotation.

Genetic Conservation and Evolutionary History of Naturally Fragmented Fraser Fir (*Abies fraseri*) Populations

Kevin M. Potter[1] and John Frampton[2]
[1]Graduate Research Assistant and [2]Associate Professor, Department of Forestry, Box 8002, North Carolina State University, Raleigh, NC 27695-8002

The fragmentation of plant populations may have detrimental consequences for the exchange of genetic material between and among those populations. This, in turn, could increase the risk of extinction in isolated patches as a result of disease, pests, environmental changes, and genetic drift. The isolated populations of Fraser fir (*Abies fraseri* (Pursh.) Poir.), a popular Christmas tree species that occurs naturally on a handful of Southern Appalachian ridgetops, provide an opportunity to investigate the genetic consequences of such forest fragmentation.

The first objective of this project is to better understand the relationship between the size and isolation of Fraser fir populations and their genetic diversity. We hypothesize that smaller, more isolated Fraser fir populations are less genetically diverse than those that are larger and closer to other populations. The second objective is to use genetic molecular genetic analyses (amplified fragment length polymorphisms [AFLPs], microsatellites, and sequencing data) to examine the recent evolutionary history of the eastern fir taxa: Fraser fir, balsam fir (*Abies balsamea* (L.) Mill.), and intermediate fir (*Abies balsamea* var. *phanerolepis* Fern.). We hypothesize that Fraser fir populations are fragments of a once-contiguous eastern North American fir species during the Pleistocene Epoch. The current north-to-south clinal variation in several traits of the eastern firs, including cone bract exsertion, may have occurred as the range of fir shifted from south to north at the end of the Pleistocene. This variation may have been accentuated with the fragmentation of the Fraser fir and intermediate fir populations.

The results of this research may assist efforts to conserve the genetic diversity of Fraser fir by determining which populations are the most genetically unique, and therefore in most need of protection. Fraser fir also offers a rare opportunity to investigate the impact of long-term fragmentation on the genetic variability of a forest tree species.

NC STATE UNIVERSITY

Genetic Conservation and Evolutionary History of Fraser Fir (*Abies fraseri*)

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Fraser fir cones

Fraser fir, Mt. Mitchell, N.C.

Introduction

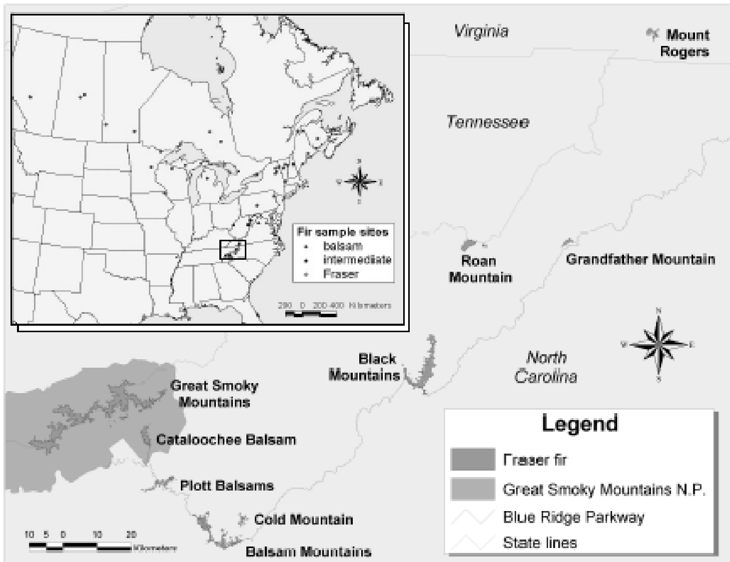
Fraser fir (*Abies fraseri* (Pursh.) Poir.), is a popular Christmas tree species that occurs naturally atop only a handful of Southern Appalachian ridges. With a range of 18,500 hectares, it faces several threats to its survival in its natural stands:

❑ Logging and slash fires may have reduced the extent of Southern Appalachian Fraser fir-red spruce (*Picea rubens* Sarg.) forest to less than half its historical area (Pyle 1984).

❑ Within the last 50 years, an exotic pest, the balsam woolly adelgid (*Adelges piceae*), has inflicted severe mortality in old-growth Fraser fir stands (Dull *et al.* 1988).

❑ A model predicts the likely extinction of spruce-fir forest with a global mean increase of 3° C (Delcourt and Delcourt 1998).

Fraser fir populations



Fraser fir samples have been collected from eight of the nine major Fraser fir population centers. Balsam fir samples (inset map) have been collected from throughout that species' range, including intermediate fir samples in Virginia and West Virginia.

Methods

❑ <u>**Define existing Fraser fir populations and compile information about each**</u>, including size and distance to other populations.

❑ <u>**Mathematically model gene flow and diversity among these populations**</u>, to predict which are most likely to disappear under varying scenarios.

❑ <u>**Use molecular genetic analyses**</u> to establish the genetic diversity (and extent of inbreeding) of each Fraser fir population, and to establish phylogenic relationships among eastern North American *Abies* taxa.

- ➢ Amplified fragment length polymorphisms (AFLPs)
- ➢ Simple sequence repeats (SSRs)
- ➢ DNA sequencing

Evolutionary History

Balsam fir, Mount Greylock, Mass.

Fraser fir is closely related to balsam fir (*A. balsamea* [L.] Miller), which has a wide northern distribution. The origin of this relationship is not clear, however.

Was Fraser fir a separate species before the end of the last glaciation, 10,000 years ago? Or did it diverge as the climate warmed and fir shifted north?

The species are identified by cone morphology: Fraser fir has exserted bracts (see above), while balsam fir bracts are typically not exserted. However, a variety of balsam fir (known as intermediate, bracted or Canaan fir) has slightly exserted bracts (see below right).

This variety (*A. balsamea* var. *phanerolepis*) occurs:

❑ At isolated high-elevation sites in Virginia and West Virginia.

❑ Along the coast of Maine and the Maritime provinces of Canada.

❑ On the highest Northern Appalachian peaks.

What does the existence and geography of intermediate fir tell us about the evolutionary history of Fraser fir and balsam fir?

Objectives

This ongoing research project has three main objectives:

❑ <u>**Objective 1:**</u> To better understand the relationship between the genetic diversity of Fraser fir populations and their size and isolation.

Hypothesis: Smaller, more isolated populations have less genetic diversity and are more likely to disappear.

❑ <u>**Objective 2:**</u> To examine the recent evolutionary history of the eastern North American fir taxa: Fraser fir, intermediate fir, and balsam fir.

Hypothesis: Fraser fir did not exist as a separate species during the Pleistocene Epoch, but separated from balsam fir since the end of the glaciation.

❑ <u>**Objective 3:**</u> To create a gene conservation plan for Fraser fir. We intend to use the results of our analyses to outline a strategy for preserving the genetic diversity of Fraser fir.

Bracted balsam fir, Mount Mansfield, Vermont

Expected Results

Richland Balsam, N.C.

This research aims to assist efforts to conserve the genetic diversity of Fraser fir by determining which populations are the most genetically unique.

Preserving genetic diversity from all Fraser fir sources would ensure that material is available for future Christmas tree breeding improvements.

It would also create a source of material for the reintroduction of Fraser fir into natural stands, if such action should become necessary.

Research on the origin of Fraser fir should offer insight into how forest tree species migrate and adapt to changing climatic conditions.

Finally, this project will make conclusions about the impact of long-term fragmentation on the genetic viability of a forest tree species.

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High Efficiency *in vitro* Plant Regeneration and the Accumulation of Peroxidase in Virginia pine (*Pinus virginiana* Mill.)

Wei Tang*, Latoya C. Harris, Ronald J. Newton
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Plant tissue necrosis and subsequent cell death are usually observed during in vitro regeneration in conifers, especially in plant regeneration via somatic organogenesis in pine species. Cell death is correlated with the elevated levels of peroxides. Virginia pine (*Pinus virginiana* Mill.) is a source of pulpwood in the southeastern United States on poor quality sites and has been the staple for the Christmas tree industry in the south since its inception. In this investigation, the effects of antibiotics on in vitro regeneration of Virginia pine were evaluated. Antioxidants polyvinylpolypyrrolidone (PVPP) and dithiothreitol (DTT) were found to improve callus formation, shoot growth, and shoot rooting by inhibiting tissue necrosis during the initiation of cultures and subculture of shoots. These treatments enabled the recovery of high frequency regeneration plants through somatic organogenesis. Compared to the control, the frequencies of callus formation, shoot growth, and shoot rooting increased 15%, 26%, and 19%, respectively, by addition of 0.5%PVPP and 0.2% DTT. Higher peroxidase activity of tissue cultures during transfer and subculture from callus proliferation, to shoot differentiation, and to rooting media was observed. The addition of these antioxidants significantly reduced and inhibited browning. Clonal propagation of Virginia pine provides an alternative for the Christmas tree industry. In vitro regeneration of Virginia pine also provides opportunities to genetically transform this species via particle bombardment or *Agrobacterium tumefaciens*-mediated transformation and to produce genetically modified trees with insect, disease, drought resistance, and salt tolerance.

High efficiency in vitro plant regeneration and the accumulation of peroxidase in Virginia pine (*Pinus virginiana* Mill.)

Wei Tang*, Latoya C. Harris, Ronald J. Newton

Department of Biology, Howell Science Complex, East Carolina University, Greenville, NC 27858-4353

1. Abstract Plant tissue necrosis and subsequent cell death are usually observed during in vitro regeneration in conifers, especially in plant regeneration via somatic organogenesis in pine species. Cell death is correlated with the elevated levels of peroxides. In this investigation, the effects of antioxidants on in vitro regeneration of Virginia pine were evaluated. Antioxidants polyvinylpolypyrrolidone (PVPP) and dithiothreitol (DTT) were found to improve callus formation, shoot growth, and shoot rooting by inhibiting tissue necrosis during the initiation of cultures and subculture of shoots. These treatments enabled the recovery of high frequency of regenerated plants through somatic organogenesis. Compared to the control, the frequencies of callus formation, shoot growth, and shoot rooting increased 15%, 26%, and 19%, respectively, by addition of 0.5%PVPP and 0.2% DTT. Higher peroxidase activity of tissue cultures during transfer and subculture from callus proliferation, to shoot differentiation, and to rooting media was observed. The addition of these antioxidants significantly reduced and inhibited browning. Clonal propagation of Virginia pine provides an alternative for the Christmas tree industry. In vitro regeneration of Virginia pine also provides opportunities to genetically transform this species via particle bombardment or *Agrobacterium tumefaciens*-mediated transformation and to produce genetically modified trees with insect, disease, drought resistance, and salt tolerance. Virginia pine (*Pinus virginiana* Mill.) is a source of pulpwood in the southeastern United States on poor quality sites and has been the staple for the Christmas tree industry in the south since its inception.

2. Materials and methods Mature seeds collected in 1994 from open-pollinated cones of Virginia pine, were kindly provided by John Frampton of Christmas Tree Program, Raleigh, North Carolina, USA. Seeds were rinsed thoroughly under running tap water for 30 min. The seeds were disinfected by immersion in 70% v/v ethanol for 30 s, then in 50% (w/v) Clorox for 45 min, and rinsed five times in sterile water. Mature zygotic embryos were aseptically removed from the seeds and placed horizontally on a solidified callus induction medium. The basal media used for organogenic callus induction consisted of TE medium (Tang et al. 1998) containing 0.5 mg/l N6-benzyladenine (BA), 2 mg/l indole-3-acetic acid (IAA), 500 mg/l glutamine, 500 mg/l casein enzymatic hydrolysate (CH), 30 g/l sucrose, and 0.35% (w/v) Phytogel (Sigma), pH 5.8. Shoot differentiation medium consisted of TE containing 0.1 mg/l IBA and 2 mg/l BA. Rooting consisted of TE containing 0.01 mg/l NAA. The influence of antioxidants on callus induction, shoot differentiation, and rooting was assessed by adding 0.5%PVPP and 0.2% DTT to TE culture media. Peroxidase enzyme activity was determined at room temperature with a spectrophotometer (470 nm) following the formation of tetraguaiacol in 3 ml reaction mixture containing 1 ml of 0.1 M phosphate buffer, 1 ml of 15 mM 2-methoxyphenol, 1 ml of H_2O_2, and 0.5 µl enzyme extract. One unit of peroxidase activity (U) represented the amount of enzyme catalyzing the oxidant of 1 mol of guaiacol in 1 min.

Table 1 Influence of 0.5%PVPP and 0.2% DTT on peroxidase activity (U/g FW) extracted from Virginia pine tissues cultured on TE culture medium at different culture stages. The enzyme activity was determined at 0d, 1d, 2d, 3d, 4d, and 5d after subculture. Each treatment was replicated three times, and each replicate consisted of 0.5 grams tissue. Data represent the mean of three replicates.

Culture stages		Culture time					
		0d	1d	2d	3d	4d	5d
Callus induction	0.5% 0.2%	21.3	31.8	32.2	34.3	23.8	21.5
	0	21.2	36.2	43.1	54.2	55.2	55.1
Shoot differentiation	0.5% 0.2%	18.9	30.1	31.3	32.8	21.2	20.4
	0	18.8	34.7	41.7	53.1	54.7	53.8
Shoot rooting	0.5% 0.2%	19.2	31.1	32.1	34.9	23.5	21.3
	0	19.3	35.4	47.3	56.8	57.1	56.2

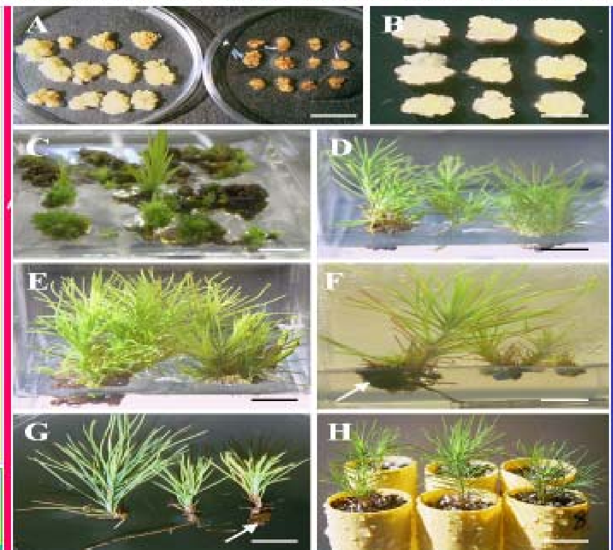

Fig. 1. Different stages of shoot organogenesis and plantlet regeneration in Virginia pine. A Organogenic calli formed from mature zygotic embryos with 0.5%PVPP and 0.2% DTT (left) and without 0.5%PVPP and 0.2% DTT (right); B Proliferation of organogenic calli; C Differentiation of organogenic calli; D Elongation of adventitious shoots; E Clusters of adventitious shoots; F Rooting of elongated shoot; G Roots were formed on medium with 0.5%PVPP and 0.2% DTT (left and middle) and browning callus was formed on medium without 0.5%PVPP and 0.2% DTT (right); H Regenerated plantlets established in soil. (A-C, Bars = 0.5 cm; B-D, Bars = 1 cm).

3. Results Tissues cultured on medium without 0.5% PVPP and 0.2% DTT had increasing peroxidase activity. Peroxidase activity of tissues cultured on medium with 0.5%PVPP and 0.2% DTT decreased 4 day after subculture (Table 1). After 9 weeks, organogenic calli (Figure 1A and B) were induced from mature zygotic embryos cultured on TE medium containing 0.5 mg/l BA and 2 mg/l IAA. Culture of over 4000 isolated mature zygotic embryos from 1994 seeds showed a maximum induction frequency of 44% on TE medium containing 0.5% PVPP and 0.2% DTT; induction frequency of callus on TE medium without 0.5% PVPP and 0.2% DTT was 39% (Table 2). Adventitious shoots (Figure 1C, D, and E) were induced by culture of organogenic calli on TE medium containing 0.1 mg/l IBA and 2 mg/l BA. The highest rates of adventitious shoot induction from organogenic calli derived from 1994 seeds was 78% on TE medium with 0.5% PVPP and 0.2% DTT and 62% on TE medium without 0.5% PVPP and 0.2% DTT, respectively (Table 2). Induction of adventitious shoot formation from cultured organogenic calli of Virginia pine is typical of other members of the genus *Pinus*, such as loblolly pine, in that a combination of IBA and BA is effective in adventitious shoot induction (Tang and Ouyang 1999). Adventitious shoot induction frequency increased by 26% on TE medium with 0.5% PVPP and 0.2% DTT, compared to the control. After elongated shoots were transferred to TE medium containing 0.01 mg/l α−naphthaleneacetic acid (NAA) for 9 weeks, adventitious roots (Figure 1F and G) formed at the base. Seventy-six shoots produced 1-3 roots after 9 weeks of culture. On TE medium with 0.5% PVPP and 0.2% DTT, 32 of 150 shoots produced roots (Table 2). Regenerated plantlets were established in soil (Figure 1H).

Table 2 Influence of antioxidants (0.5% PVPP and 0.2% DTT) on the percentages (%) of callus induction, adventitious shoot formation and shoot rooting in Virginia pine. Each treatment was replicated three times, and each replicate consisted of 30-90 zygotic embryos, 30-90 pieces of callus tissues (each callus is 0.5 cm × 0.5 cm in size) or shoots. Values represent the mean of three replicates.

Culture stages		Culture time of Virginia pine embryos, calli, and shoots					
		4wks	5wks	6wks	7wks	8wks	9wks
Callus induction	0.5% 0.2%	21.7	32.2	41.2	42.1	43.7	44.3
	0	17.3	21.8	34.2	36.3	37.8	38.5
Shoot differentiation	0.5% 0.2%	38.9	50.1	71.3	72.2	73.2	77.5
	0	28.2	34.3	58.7	50.1	59.7	61.5
Shoot rooting	0.5% 0.2%	11.2	13.1	18.1	19.9	21.1	21.4
	0	10.3	11.4	16.3	16.9	17.1	18.0

4. Conclusions Plant regeneration via shoot and root organogenesis can be affected by culture media, the nature of the plant materials, and relative concentrations of auxin and cytokinin. In this investigation, we use antioxidants to improve in vitro regeneration efficiency in Virginia pine. Since mature zygotic embryos can be used as explants at any specific season for plantlet regeneration, this method may be used to study the effects of certain components in the culture medium on organogenesis in the tissue-culture shoot organogenesis protocol with high repeatability. Adventitious shoots appeared on most of the organogenic calli. This system may offer a key to genetic transformation of Virginia pine, based on in vitro organogenesis. Further studies in the development of tissue-culture shoot organogenesis protocols in Virginia pine should be to improve the efficiency of shoot differentiation and to increase the frequency of root formation. Availability of clonal techniques is of particular importance for Virginia pine, since improved seed are not readily available. The present work demonstrates that induction of somatic organogenesis in Virginia pine and regeneration of plantlets has been accomplished. Further work is underway to improve the efficiency of shoot induction and rooting and to transform this species by *Agrobacterium*-mediated gene transfer using the improved organogenic procedure that we describe here.

Photographs


Kanuga


Kanuga


Kanuga


of Round-Up Original
ns of Fraser Fir Foliage
arly Shoot Elongation







NORTH CAROLINA FOREST SERVICE
Young Offenders Forest Conservation Program

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