UNIVERSITY OF MINNESOTA
College of Natural Resources
Department of Forest Resources

MINNESOTA TREE IMPROVEMENT COOPERATIVE

2001

ANNUAL REPORT

Prepared by:

Carolyn C. Pike and Andrew David

December 18, 2001

MEMBERS
Beltrami County
Blandin Paper Company
Cass County
Crow Wing County
Iron Range Resources and
Rehabilitation Board
Itasca County
Itasca Greenhouse Inc.
Koochiching County
Lake County
Minnesota DNR
Division of Forestry
Potlatch Corporation
Rajala Companies
Red Lake Nation
St. Louis County
The Timber Company
Wausau-Mosinee Paper Corp
USDA Forest Service
University of Minnesota
Department of Forest Resources
NCFES Forest Pathology and Genetics

SUPPORTING MEMBERS
Carlton County
Clearwater County
Hedstrom Lumber Company
Hubbard County
Minnesota Nursery and Landscape
Association

TECHNICAL ADVISORS
USDA Forest Service State and Private
Forestry
USDA Forest Service North Central Forest
Experiment Station
University of Minnesota
College of Natural Resources

EXECUTIVE SUMMARY

The Minnesota Tree Improvement Cooperative has entered its twenty-first year serving forestry organizations in the Upper Midwest. It was funded by seventeen full members and six supporting members. Total dues received was $54,614. Dues were not paid by two members in 2001 ($6,445) but are expected to be recovered in 2002. One member is not expected to renew in 2002 (USDA NCFES), but one new member has been added (Band of Red Lake Chippewa). Advancements were made in genetics programs for all species. Priorities for 2001 included several applied research projects, third-year measurements of jack pine, and regular orchard visits.

Regular Co-op expenses were $21,150 for general operating expenses and $37,129 for personnel. The white pine grant, appropriated by the Minnesota Legislature in 1997, is now administered directly through the University. It will be renewed biannually and continues to reflect the portion of Pike and Warren's salaries dedicated to white pine work. Cooperative staff made about 82 on-site visits with cooperators, resulting in 478 hours (Pike) and 407 hours (Warren) of work on specific projects. Dr. Andrew David continues as the Director and Carrie Pike as the Coordinator of the Co-op. Jim Warren's part-time appointment was continued and he currently works 20% time for the Co-op. The Advisory Committee held two regular meetings, and a workshop is planned for January 2002.

Cooperative members now manage 39 seed orchards covering about 151 acres and containing approximately 22,000 trees. Cones were collected only from jack pine orchards this year. No crosses in white spruce were completed due to flower asynchrony. Tree heights were measured at the 2nd generation jack pine plantings. In two red pine seed orchards, a total of 790 trees were injected with $GA_{4/7}$ in a study which will be analyzed Spring 2002. Four orchards plus the white spruce breeding arboretum were fertilized with Ammonium Nitrate to improve flower production for next year.

Pike attended a 2-1/2 week International Short Course in Forest Genetics at North Carolina State University in June and presented at the Annual Meeting of the Superior Woods Tree Improvement Council and at a white pine workshop in Grand Rapids. Pike and Warren attended the state SAF meeting in Ely, MN in February, where a poster on the red pine roguing scheme at St. Louis County was presented.

Outlook for 2002: Grafting will begin for Koochiching County's black spruce seed orchard. Grafting will also be done for Blandin's new improved first generation seed orchard, which was established in 2001. Grafting is completed for the MN DNR's new improved first generation seed orchard, and planting should begin in 2002. Three established first-generation orchards will receive additional ramets in Spring 2002. Breeding in white spruce will continue, and breeding in white pine will hopefully begin in Spring 2002. Flowers will be counted at the red pine $GA_{4/7}$ trials at St. Louis and the DNR. A white spruce comparison trial will be measured in Fall of 2002.

2001 Annual Report

Minnesota Tree Improvement Cooperative

Table of Contents

INTRODUCTION

During the Minnesota Tree Improvement Cooperative's twenty-one year existence, members have made great strides in improving the genetic quality of seedlings planted in Minnesota and

Wisconsin. The Co-op has worked primarily with five major forest tree species native to the Upper Midwest: black spruce, white spruce, jack pine, red pine, and white pine. Tests to identify the realized genetic gains for most of these species have begun, since orchards are now mature enough to produce seed. In recent years, second generation material has been produced for jack pine, and crosses in white spruce are ongoing. In 2001, the Co-op was funded by fourteen Full Members and three Supporting Members (see Appendix). Two full members and two supporting members did not pay dues in 2001, but are expected to pay in 2002.

High-priority activities during the year included several projects and regular orchard visits, and measurements. Projects included a white spruce bud-break study, experimental $GA_{4/7}$ injections at two red pine orchards, and height measurements of second-generation jack pine trials.

This report describes the Co-op's program and summarizes the activities and accomplishments from January 1 to December 31, 2001. It is organized into five sections: administration, finances, seed orchards, species reports, and outlook.

ADMINISTRATION

Carrie Pike has continued as Coordinator of day-to-day activities of the Co-op. Pike conducts field visits, writes and sends monthly and annual reports, conducts breeding, and oversees field work at orchards. Dr. Andy David, Director, oversees Co-op activities and is primarily involved in long-term planning. This includes, but is certainly not limited to, developing breeding plans, managing research projects, and providing academic and technical assistance.

Jim Warren, who was hired in 1999 as a part-time plot technician, continues to work part-time for Co-op and white pine projects. Currently, he works 20% time for the Co-op assisting with field plantings and tree breeding and has been the primary technician for several new Co-op projects. He continues to assist in managing the Co-op's tree database. For the white pine project most of his work takes place at the white pine breeding arboretum at the Cloquet Forestry Center. Tasks include managing the irrigation system, monumenting ramets, conducting tree breeding, monitoring flowering, fertilizing, and watching for pest problems. He also assists with the four blister rust field trials planted in 1999.

The Advisory Committee consists of representatives from each member of the Co-op. It met twice during 2001 for business meetings. The annual workshop was postponed until January 2002 to accommodate our speaker's busy schedule. The workshop will focus on enhancing forest productivity through silvicultural practices, and will be presented by Dr. H. Lee Allen of the Forest Productivity Cooperative in Raleigh, North Carolina.

About 82 on-site visits were made by Pike, Warren, and David this year to sites managed by cooperators for the purpose of visits and various projects. A total of 478 hours (Pike) and 407 hours (Warren) were dedicated to field work and visits to cooperators. The number of site visits remained very high this year due to white spruce breeding, and establishing the red pine project. Other visits included collecting bud-break data for white spruce, and measuring second-generation jack pine sites. Pike and Warren attended the Minnesota SAF meeting held in Ely Minnesota in February 2001. At that meeting, Pike presented a poster summarizing the red pine roguing scheme used at St Louis County. Pike also attended the annual meeting of the Superior-Woods Tree Improvement Council in Thunder Bay, Ontario in October 2001 where she presented the Co-op's program. She also attended the "International Short-course in Forest Genetics," held in Raleigh NC from May to June 2001.

SEED ORCHARDS

Seed orchards are the primary means by which genetically improved material is produced for use in commercial-scale planting programs. Since 1967, members of the Co-op have established 41 seed orchards, many of which are still used for seed collection. Most of those orchards (25) were established between 1981 and 1997, three were added in 1998, five in 1999, one in 2000, and two in 2001. Five of the orchards contain improved first-generation material, four contain full-sib crosses, and the rest are first generation seedling or clonal orchards. Nearly all the first generation orchards are rogued. A summary of the types and sizes of orchards managed by members of the Co-op is shown in Table 5. Table 6 lists all orchards by species and owner.

Table 1. Acres of seed orchard by species and orchard type.

	Black spruce	White spruce	Jack pine	Red pine	White pine
1st generation, seedling-seed	**11.2**	4.1	43.0	42.2	
1st generation, clonal		22.2			11.8
Improved first-generation, clonal	3.0	2.9			
2nd generation, full sib	1.3		9.5		
Total acreage	15.5	29.2	52.5	42.2	11.8

Table 2. Seed orchards of Minnesota Tree Improvement Cooperative, 2001.

Species	Organization	Orchard	Established	Size (ac)	Live Trees
Black spruce	Blandin Paper Company.	Blackberry	05/22/78	2.5	596
	Koochiching Co.	Big Falls	05/19/89	2.3	61
	Koochiching Co.	Larsaybow	05/27/98	1.1	52
	Minnesota DNR	Eaglehead	05/17/98	2.7	582 [a]
	Minnesota DNR	Split Rock	05/27/92	2.4	261 [a]
	Potlatch Corp.	Cloquet	05/05/78	3.0	580 [a]
Jack pine	Cass/Beltrami/Hubbard Co.'s	Deep Portage	10/08/82	3.4	492 [a]
	Crow Wing Co.	Crow Wing	06/04/85	2.1	320
	IRRRB	IRRRB	09/16/82	1.7	245
	Minnesota DNR	Bemidji	05/30/84	2.5	406 [a]
	Minnesota DNR	Nickerson	05/15/84	2.4	407
	Minnesota DNR	Staples	05/18/84	4.0	501
	Wausau-Mosinee Paper Corp.	Barnes	05/27/88	4.1	548
	Potlatch Corp.	Cloquet	06/28/83	5.5	171 [a]
	Potlatch Corp.	Kallstrom	05/01/74	2.8	282 [a]
	St. Louis Co.	Ellsburg Rd.	05/10/88	1.6	280
	St. Louis Co./ IRRRB	North Side	05/12/99	3.8	2574
	Potlatch Corp.	Haltberg Rd.	05/11/99	2.6	1800 [b]
	Crow Wing Co./MN DNR	County Line	05/07/99	2.6	1705
Red pine	Cass/Beltrami/Hubbard Co.'s	Blind Lake	09/10/91	5.3	2249 [a]
	The Timber Company	Petenwell	04/24/90	5.5	1576
	The Timber Company	Ashwabay	09/17/85	5.5	405
	Minnesota DNR	Cotton	07/29/81	4.5	466 [a]
	Minnesota DNR	Eaglehead	06/25/81	3.6	388 [a]
	Wausau-Mosinee Paper Corp.	Mosinee	05/23/90	5.7	1174 [a]
	Potlatch Corp.	Cloquet	07/10/81	6.6	586
	St. Louis Co.	Ellsburg Rd.	05/09/88	5.5	539
White Pine	Minnesota DNR	St. Francis	05/15/85	3.0	318
	Rajala/Itasca Co.	Bass Lake	05/19/98	5.7	530
	St. Louis Co.	Ellsburg Rd.	05/02/90	1.1	233
	St. Louis Co.	Ellsburg East	05/20/99	2.3	245
White spruce	Blandin Paper Company	Latimer	05/15/67	4.1	224
	Blandin Paper Company	Arbo	05/01/76	1.5	121
	Blandin Paper Company	College	9/05/00	2.9	538
	Itasca Co.	Fig.-8-Lake	09/02/87	1.1	110
	Lake Co.	Two Harbors	09/02/87	1.0	121
	Potlatch Corp.	Cloquet	05/01/77	3.3	140
	St. Louis Co.	Ellsburg Rd.	05/11/88	1.5	169
	Minnesota DNR	Cotton	05/01/77	12.0	206 [a]

[a] Indicates that orchard has not been surveyed in the previous five years.
[b] Haltberg Rd orchard was damaged by hailstorm and will be surveyed in the future.

Cone Collections

Cone collections were low this year, but after the 2000 bumper crop, it was neither surprising nor disappointing. Jack pine was the only species that produced an appreciable number of cones, and five cooperators collected from their respective orchards.

SPECIES REPORTS

Black spruce

Status

Several members of the Co-op have interest in black spruce and continue to manage their orchards for seed production. Three seedling-seed orchards, established in 1978, are managed for cone production. Cones are picked periodically, not on an annual basis. No black spruce cones were collected this year from any orchard, however collections were made in 2000 from two orchards.

Koochiching County is the only member establishing an improved first-generation clonal orchard. Trees at **Larsaybow** were visited and fertilized this spring, and survival is good. The **Big Falls** orchard will be used for seed as it becomes available, but management will be limited to cone collections and periodic mowing. Blandin's **Blackberry** seedling-seed orchard will continue to be managed and cones picked periodically. The grass at Potlatch's **Cloquet** seedling-seed orchard continues to be mowed but seed hasn't been picked for some time.

In other parts of Minnesota, white spruce is preferred over black spruce, due to faster growth. However, in parts of Northern Minnesota, where lowlands represent a significant proportion of the landscape, interest in this species remains strong.

Short and long-term planning

Black spruce rootstock is being prepared at Itasca Greenhouse, Inc. and grafting for new ramets at Larsaybow could begin as early as winter 2002. The survival of black spruce grafts has been historically low for the Co-op, however, grafting success in Eastern Canada is reportedly greater than 90%. Contacts in Canada will hopefully provide some insight into our problems.

A seedlot of improved black spruce will be included in the upcoming white spruce comparison trial. The seedlings for this trial are now being prepared by Potlatch nursery and will be planted in Spring 2003. The seedling-seed orchards should continue to be managed for seed production.

White spruce

Status

The theoretical genetic gains in white spruce generally exceed those of all other Co-op tree species. Several factors contribute to this: it has a broad geographic range across North America, and exhibits a high degree of genetic diversity. It is also an important timber species, and has been well studied. As a result, the Co-op has benefited from studies that have identified Southeastern Ontario as a superior source of white spruce material. The vast majority of clones in Co-op seed orchards are derived from this region. A second set of white spruce material in the Co-op consists of Minnesota sources selected from wild stands based on growth. The Minnesota material is represented in Blandin's **Latimer** (formerly Zigmund) orchard. This site was originally planted as an open-pollinated progeny test in 1967, and later converted to a seed orchard.

Since 1988, white spruce seed orchards in the Co-op have produced more than 1,300 pounds of seed, enough to meet the basic needs of most organizations. Because of the availability of seed, members have expressed an interest in maximizing genetic gains in orchards. Grafting efforts over the past few years produced clones for two improved-first generation orchards. Three other orchards will receive these clones to fill in empty spaces, or to replace clones that performed poorly in progeny tests.

Results from progeny tests form the basis of the Co-op's genetic selections. Five replications of an open-pollinated white spruce progeny test were planted in 1986 to test as many families as possible, emphasizing those already planted in Co-op seed orchards. The test was measured and analyzed after five, eight, and ten growing seasons. In Summer 1998, the Lake County progeny test (**Finland)** was thinned to half its size. Blandin/Itasca County's progeny test at **Nine-Mile** was thinned in the Summer of 1999. **Nickerson** (MN DNR) was thinned in the Summer of 2000. In the Fall of 2000, 15-year measurements (tree heights and diameters) were taken at all three sites. St. Louis County's **Rabbit Lake** and MN DNR's **Ross Lake** were not thinned or measured in 2000 due to high mortality at both sites.

Family ranks generated from the ten-year measurements were used to rogue three of the Co-op's orchards. Gains in height growth from roguing generally fall in the range of eight to nine percent above the orchard mean. Results from the 2000 measurements are included in this report, and are being used to generate lists of the top 25 clones to replace clones in seed orchards, and to determine mating pairs for breeding.

Blandin's **Latimer** orchard is being used for Mass Controlled Pollinations (MCP), which theoretically should produce the highest genetic gains of any other white spruce seed. This work is being done by Quintin Legler, contracted through Itasca Greenhouse. Lake County's **Two Harbors** and Itasca County's **Figure-8-Lake** orchards will receive replacement ramets that are being grown at the DNR's General Andrews Nursery. These ramets should be available for planting in Spring 2002. St Louis County's **Ellsburg Rd** orchard received new ramets last spring, and additional replacements will be made in 2002.

The Co-op currently has two seed source comparison trials, both of white spruce. The first, planted in 1993, was measured in 1997. The findings were reported in the 1998 Annual Report. Ten-year measurements will be made in 2002. The second comparison trial was established in 1995. This trial compared a patented "Elite" white spruce from Forgene, Inc., containerized seedlings from seed orchard material, and bare-root seedlings from woods-run material. It was measured Fall 1999 after five growing seasons. The findings were reported in the 2000 annual report. A third trial, planned for planting in Spring 2003, will contain several improved sources including Blandin's "high gain" seed produced from MCP. Other sources to be tested include rogued **Cotton** material, rogued **Latimer** material, unrogued Potlatch **Cloquet** material, and a woods run source. In addition, a seedlot of improved black spruce (rogued **Blackberry** material) will be planted for comparison.

Short and long-term planning

Controlled crosses will be made if flowers are present in the breeding arboretum. In Spring 2001, all trees needed for breeding were fertilized with ammonium nitrate and injected with $GA_{4/7}$.

The last remaining grafts will be ready to outplant in Spring 2002 to the MN DNR's new improved first-generation seed orchard, and to the three existing County orchards. Some additional grafting might be necessary for Blandin's new **College** orchard, which had the unfortunate luck of being half-planted prior to the completion of the 15-year progeny test measurements. Some of the planted grafts will be removed and replaced with new grafts once completed.

Status of white spruce 2nd generation

A breeding arboretum established at General Andrews Nursery has been used to breed the second generation population. An assortative breeding scheme (single pair mating) was determined from family performances in the progeny test. In this breeding plan, the top-ranked clone is crossed with the second ranked clone; third is crossed with fourth ranking, etc. While this scheme has limitations in terms of identifying general combining ability (the results of one clone mating with several others), this scheme maximizes the number of unrelated families for the next generation. Controlled crosses were made using this design in Spring 1998 and 2000. In 2001, crosses failed due to the asynchronous production of male and female flowers. Flowers were abundant on most trees during 1998, allowing the completion of about half the required crosses. A total of 52 families were produced from breeding efforts in 1998 and 2000, and seed from 46 of those families was sent to Potlatch nursery for germination in Fall 2001. These seedlings will be planted in Spring 2003. In the meantime, the remaining crosses will be completed and planted at a later date. Six families created in 1998 will be kept in cold storage and germinated with the remaining crosses in order to make valid statistical comparisons between the two groups.

15-year results of white spruce progeny test

Methods

In 1986, the Minnesota Tree Improvement Co-op planted an extensive progeny test of white spruce to assess growth properties of families in seed orchards. Open-pollinated seeds were collected from clonal seed orchards, seedling-seed orchards, and open-pollinated progeny tests located in Wisconsin and Minnesota. Families included in the test originated from selections made in Southeastern Ontario, Northern Minnesota, Wisconsin and Upper Michigan. A total of 17,256 seedlings representing 292 parent trees were planted on five sites. Forty-seven of the original 292 families were planted at all five locations. The experiment is a block design with five replications of four-tree row plots. One border row is planted around the entire planting.

Tree heights were measured at all five tests after five, seven, and ten years of growth. Between 1998 and 2000 three sites were thinned: Nickerson (MN DNR), Nine-mile (Blandin/ Itasca County) and Finland (Lake County). Thinning was done by systematically reducing the row plots from four to two trees. Two of the sites, Ross Lake (MN DNR) and Rabbit Lake (St Louis County) were not thinned or re-measured due to high mortality. In 2000, after 15 growing seasons in the field, tree heights and diameters were measured at the three thinned sites. Tree heights were measured with a hypsometer to the nearest cm, and diameters at breast height (2.5 m) were measured to the nearest millimeter using a metric tree caliper. Tree diameters were converted to meters to estimate volume using the equation for a cone:

$$Volume(meters)^3 = \frac{\Pi * (0.5 * (DBH))^2 * Height}{3}$$

Volumes were then transformed using a cube-root function to improve normality and heteroscaedasticity. An Analysis of Variance was run on the 47 families that were common to all three sites to test significance of blocking and site effects, and to evaluate genotype x environment interaction using the model:

$$\mu = S_i + R_j(S_i) + F_k + S_iF_k + R_j(S_i)F_k + \varepsilon_{ijkl}$$

where "S_i" is the effect of Site, "R_j" is the effect of Rep, "F_k" is the effect of Family, and ε is the within plot error. Variance components were generated (Proc Varcomp SAS, 1999) using the same 47 common families for tree heights, diameters, and transformed volumes. Least Squared Means of transformed volumes were estimated for all 292 families, and families were ranked in descending order. Individual tree heritabilities were calculated from the above variance components using the equation:

$$h^2 i = \frac{4 * \text{Var(Family)}}{\text{Var(Family)} + \text{Var(Rep)} + \text{Var(Site)} + \text{Var(Family)} * (\text{Rep(Site)})}$$

where Var(Family)= variance due to differences among families
Var(Rep) = variance due to differences among reps within sites
Var(Site)=variance due to differences among sites
Var(Family*Rep(Site)) = variance due to the interaction of family with replications in sites.

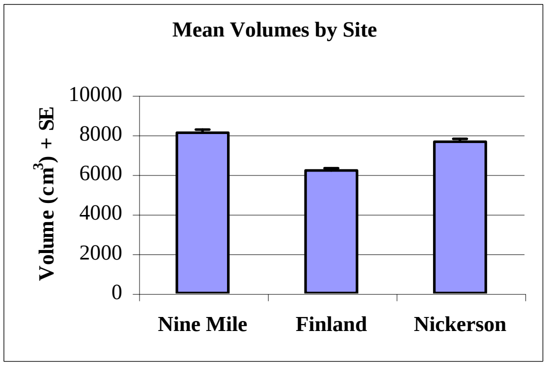
Results

Transformed volumes and diameters differed significantly among sites (F=3.77, p=0.05, Table 1). Tree heights did not differ significantly among sites (Volume: F=0.77, p=0.49). The largest diameter occurred in Nine-Mile (15.5 cm) and the largest height was found in Nickerson (8.7 m). No significant g x e interaction was present for any variable. Figure 1 shows the mean volumes, heights, and diameters for each site: Significant differences for tree volumes (Table 1) were found for reps (F=4.02, p<0.001) and families (F=2.06, p=0.002). Individual tree heritabilities for transformed volumes, heights and diameters were .20, .19, and .20 respectively.

Table 1. ANOVA table for transformed volumes. A mixed model is used with fixed effects for Family and Rep. Site and its interactions are random.

Source	Df	SS	MS	F	P
Planting	2	0.094	0.047	3.77 [a]	0.054
Rep(Planting)	12	0.15	0.013	4.02 [b]	<0.0001
Family	46	0.295	0.006	2.06 [c]	0.002
Planting*Family	92	0.288	0.003	1.00 [b]	0.484
Rep(Planting)*Family	524	1.669	0.003	1.50	<0.0001
Error	524	1.12	0.002		
Total	1203	3.646			

Error Terms used for F calculations:
[a] = Rep(Site)
[b] = Family*Rep(Site)
[c] = Site*Family



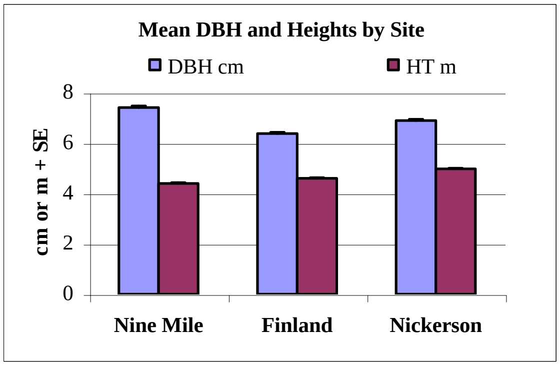


Figure 1. Mean volumes, diameters and heights for each of the three test sites. Number of living trees = 1584, 1688, and 1665 for Nine-Mile, Finland, and Nickerson respectively.

Table 2. Ranks (based on LS means of transformed volumes) and family origin for the top-performing family in the progeny test.

FAMILY	15-year Ranks	Family Origin
619.1	1	Lake Tomahawk progeny test - Origin unknown
563.1	2	Lake Tomahawk progeny test - Origin unknown
358.1	3	Latimer progeny test (Minnesota sources)
613.1	4	Forest, Wisconsin
576.1	5	Lake Tomahawk progeny test - Origin unknown
454.1	6	Douglas, Ontario
610.1	7	Cook, Minnesota
468.1	8	Douglas, Ontario
614.1	9	Ashland, Wisconsin
422.1	10	Beachburg, Ontario
418.1	11	Douglas, Ontario
508.1	12	Bradley, Maine
392.1	13	Denbeign, Ontario
426.1	14	Douglas, Ontario
566.1	15	Lake Tomahawk progeny test - Origin unknown
400.1	16	Beachburg, Ontario
137.1	17	Latimer progeny test (Minnesota sources)
484.1	18	Beachburg, Ontario
616.1	19	Lake Tomahawk progeny test - Origin unknown
568.1	20	Menominee, Wisconsin
419.1	21	Douglas, Ontario
494.1	22	Beachburg, Ontario
487.1	23	Beachburg, Ontario
285.1	24	Latimer progeny test (Minnesota sources)
403.1	25	Beachburg, Ontario

Discussion

The three thinned sites have excellent survival and growth. All three sites should be grown to full rotation and measured every five years. Additional data related to stem form and wood quality would also be beneficial to utilize in future selections.

The lack of a significant family by site interaction indicates that family performance is not strongly influenced by site. However, 15-year data is far from the typical rotation age of white spruce, and measurements should continue to be taken to verify this. A previous analysis on eight-year heights found the genotype by interaction effect to be barely non-significant (Klevorn 1995). Block effects were significant for all three variables, and thus blocking was useful in reducing the variation within sites. This makes our ability to judge differences among families more accurate.

The significant differences observed among families is proof that even among "plus" tree selections, growth characteristics differ widely. Ontario sources performed consistently well, with 14 families in the top 25 ranks. The origin of families collected from the Lake Tomahawk progeny test is not known at this time, and may include some Ontario sources. Only four of the Minnesota sources were ranked in this group, but again, some of the unknown families at Lake Tomahawk could also include Minnesota sources. Exact origins of the trees tested at Latimer are also not available at this time, but may someday be excavated from the basement of a local tree improvement guru.

The heritabilities calculated from the 15-year data are larger than those estimated on 8-year data from the same three sites (0.13 by Klevorn, 1995). The 1995 study used tree heights only on unthinned plantings. Nienstadt and Reimenschneider (1985) reported heritabilities of 0.158 at age nine, increasing to 0.247 at age 15 for tree heights of a white spruce progeny test in Wisconsin. The implication of this apparent increase in heritability with age is not known. Future measurements would determine if heritabilities continue to increase with age.

Data from the 15-year measurements was used to make selections for improved first-generation clonal orchards. These new grafts are being planted in two new orchards, and used to fill-in space at three additional existing orchards. An assortative-mating breeding scheme was designed from the ranks of the 292 families, from which a second-generation population will be produced.

Literature Cited

Klevorn, Rick E., 1995. Genotype-environment interaction for height growth in white spruce. MS Thesis. University of Minnesota. 50pp

Nienstaedt, Hans and Riemenschneider, Don E. 1985. Changes in heritability estimates with age and site in white spruce, *Picea glauca* (Moench) Voss. Silvae Genetica. 34(1): 34-41.

Jack pine

Status

Jack pine is one of the most reliable species that the Co-op works with in terms of its potential for genetic gains, seed production capabilities, and shear hardiness. As a seed orchard tree, it's fast growth results in early seed production and quicker turn-around for each generation. Members of the Co-op manage 12 first-generation seedling-seed orchards that are a variety of ages, all of which have begun to produce seed. Cone collections in any given year may not be large, but the supply for seed is generally met within the Co-op. Four second-generation full-sib populations were planted in 1999, and measured in 2001. The results are shown below.

In 2001, trees at Wausau-Mosinee's **Barnes** seed orchard were top-pruned, and several bushels of cones were collected. **Crow Wing** county's seed orchard has produced good cone crops even after extensive top-pruning in 1996. The trees now have a form very conducive to picking. **Crow Wing** collected a total of 16.5 bushels in 2001. At St Louis County's **Ellsburg Rd**. jack pine orchard, 14 bushels of cones were collected this year. Top-pruning at this orchard has also resulted in trees that are more easily managed. Potlatch's **Gillogly** Rd. orchard was topped last fall, resulting in a collection of 34 bushels, and we look forward to their future cone crops. The MN DNR collected 17 bushels from their **Long Prairie** orchard.

Short and long-term planning

Continued maintenance of second-generation populations will be the main focus of the jack pine program for a while. With the second-generation population in the ground, breeding efforts have temporarily shifted to other species. Orchards should continue to be managed for seed production. Nearly all of the jack pine seed orchards have now been topped.

Three first-generation seedling-seed orchards in Wisconsin may become the source of material for the second-generation orchards for Wisconsin cooperators. Pike visited two of the three orchards this spring, and a time-line for this project needs to be determined. Breeding could begin soon after the white spruce breeding is completed.

Status of second generation population

Introduction

The Co-op's jack pine seed orchards were established using open pollinated (OP) seed from "plus" trees selected from forest stands in Minnesota and Wisconsin. Twelve orchards were planted across Northern Minnesota and Wisconsin from 1976 – 1988. The number of families represented in each orchard varied from 80 to 214, with a total of 420 different families planted. The orchards were planted in a block design with single tree plots. Tree heights were measured and analyzed in 1990 from seven orchards that were planted between 1982 and 1985. Using this data, trees were ranked in each orchard. The top performing trees in each orchard that were exclusive to that orchard, were mated by an assortative (single-pair) mating scheme between 1993 - 1995.

These crosses resulted in 143 new families that were sown at IRRRB greenhouse in Spring 1998, and planted at four different locations in Northern Minnesota in Spring 1999. One site St. Louis in partnership with IRRRB received all 143 families. The three other sites, owned by Crow Wing County (in partnership with the MN DNR), Potlatch Corp., and Cass County (in partnership with Beltrami and Hubbard Counties) were planted with 100 families each. Since planting, one site was lost due to deer browse and subsequent hail-damage (Potlatch), and another site was destroyed by vole feeding during Winter 2000 at Cass County. The Crow Wing/MN DNR and St Louis/IRRRB sites remain in excellent condition.

Methods / Results

Tree heights were measured using a height pole in Fall 2001(after three growing seasons in the field) at the Crow Wing Co. and St. Louis Co. sites. The results of those measurements are shown below:

Site	No. Live Trees	% live trees	Mean Ht (cm)	St. dev.	Min Ht	Max Ht
Crow Wing / MN DNR	1705	66%	116.1	20.7	25	186
St. Louis / IRRRB	2362	63%	83.1	17.6	23	130

Mortality was slightly higher at St. Louis/IRRRB, where some low spots periodically fill with water. At Crow Wing/MN DNR, most mortality was due to pocket gophers. Regular discing and poison baiting is used to control this pest. Tree heights were generally larger at the Crow Wing/MN DNR site.

Future Plans

Tree heights will be measured again after five years of growth, and probably every two to three years after that. Once trees exceed breast height, diameter measurements could be used to estimate tree volumes. Measurements of stem form and branch angle may also be taken to characterize the families. Plans for future controlled crosses in these sites will be discussed once flower production commences. There are many possibilities for advancing the jack pine program at this point. Members will be consulted and their needs incorporated into our future breeding plans. In the meantime, sites must be maintained and monitored for flower production and growth in upcoming years.

Red pine

Status

The Co-op's red pine orchards are still young, and are not producing seed on a regular basis. An experiment was begun in 2001 to test the efficiency of $GA_{4/7}$ to enhance and/or initiate flower production either alone, or in combination with fertilization. The Co-op manages eight orchards, ranging from 6 to 17 years of age. All but three have been rogued. Of the un-rogued orchards, The Timber Company's **Petenwell** in south central Wisconsin will be ready for roguing in the next five years, followed by Cass County's **Blind Lake** and Wausau-Mosinee's **Mosinee** orchard. Potlatch's **Gillogly Rd.** orchard was fertilized this spring with two different concentrations of ammonium nitrate. Although no formal measurements will be taken to evaluate the efficacy of the different levels of fertilization, the orchard will be visited in the Spring 2002 to see if there are detectable differences in flower production.

Short and long-term planning

The Co-op's main focus with red pine is to increase seed production. The Co-op's members are in need of a steady supply of seed for this species. In Spring 2002 the $GA_{4/7}$ experiment will be evaluated for effects on flower production. Results from measurements taken at St. Louis County's **Ellsburg Rd.** red pine orchard indicate that reasonable genetic gains can be made in this species (see section below). In 2002, seed needs to be collected for a red pine comparison trial to determine if these theoretical genetic gains can be realized. Unrogued orchards need to be monitored annually for growth and mortality in preparation for future measurements.

Red Pine Flower Induction for Sustainable Seedling Production

Due to widespread red pine seedcrop failures in Minnesota during the past eight years there is now a shortage of locally adapted red pine seed available for sowing. As a result, seed that is not locally adapted is imported for use in Minnesota. Seedlings not adapted to local climatic conditions are more prone to frost injury, and suffer slower growth rates. Cooperators have established seed orchards of red pine based on locally adapted trees, but these seed orchards are approximately 3-5 years away from reaching (natural) sexual maturity.

Gibberrelic Acid ($GA_{4/7}$) is a safe and effective treatment for stimulating flower production. Applications of $GA_{4/7}$ to stimulate early flowering is becoming more common in spruces and other conifers (Smith, 1998, Eysteinsson and Greenwood, 1993). However, due to the limited natural range of red pine (Little, 1971) and the fact that it is operationally planted only in the upper Great Lakes region, little is known about the conditions that stimulate flower production.

This project will result in management guidelines for early flower stimulation in red pine seed orchards through the utilization of a common flower-inducing chemical, $GA_{4/7}$, in combination with seed orchard management techniques. The long-term goal of this project is to establish a procedure for inducing and/or enhancing flower production in red pine. This would serve to improve the productivity of Co-op seed orchards both for commercial seed production and breeding programs.

Project Goals and Objectives

- Determine best amount of systemically-injected $GA_{4/7}$ to stimulate flowering in red pine.
- Determine optimal time to apply $GA_{4/7}$ to stimulate flowering in red pine.
- Determine the impact of fertilization alone, and in combination with $GA_{4/7}$.

Methods

Two seedling-seed orchards were utilized for this study: the St. Louis County red pine at Ellsburg Rd. (rogued in 2001) and MN DNR's Cotton seedling-seed orchards. There were 4 treatments: 1) $GA_{4/7}$, 2) fertilization 3) $GA_{4/7}$ with fertilization and 4) control. Within each $GA_{4/7}$ treatment there were 2 application rates (high and low), on each of 6 dates starting on June 15th and thereafter every 15 days until August 30th. There were 15 trees for each application level on each date for a total of 180 trees for the $GA_{4/7}$ treatment, 180 trees for the $GA_{4/7}$ with fertilization treatment, and 30 trees for the control treatment (15 trees that were fertilized and not injected; and 15 trees with no $GA_{4/7}$ or fertilization). This design resulted in 390 trees treated per site, for an experimental total of 780 trees.

Ammonium Nitrate (34 - 0 - 0) was applied to the fertilizer treatments on June 20 at 400 lbs per acre. The average tree dripline diameter at each site was used to determine the amount of fertilizer required per tree. At Cotton, a 12 foot dripline resulted in 3.1 lbs of fertilizer per tree. At St. Louis Co, the average dripline diameter of 8 feet required 1.35 lbs per tree.

The amount of $GA_{4/7}$ injected was determined by volume tables based on the diameter of each tree (Smith, 1998). On each injection date, 60 trees were systemically injected with either a high (1.25 mg $GA_{4/7}/cm^2$) or low (0.75 mg $GA_{4/7}/cm^2$) rate of $GA_{4/7}$ dissolved in 95% Ethanol. Two holes were drilled at breast height on opposite sides of each tree receiving an injection. The required amount of $GA_{4/7}$ was injected into the hole with a 200 – 1000 µl Fisherbrand Finnpipette pipetter.

Most of the experimental setup and treatment implementation was done by Jim Warren and Carrie Pike. An account of the number of working hours (wh) required for each aspect of the experiment are as follows: field setup (locate and tag treatments) 22.5 wh, fertilizer treatments 36 wh, and $GA_{4/7}$ injections 54 working hours.

Treatment effects will be evaluated by counting both male and female flowers in Spring 2002. The fertilizer treatment will be reapplied in Spring 2002. If flower production is bountiful, we will count and collect mature cones to develop a method for estimating cone crop and collect bulk seed to determine the earliest collection date for viable seed. In 2003, any residual effects of $GA_{4/7}$ will be observed, female cones counted, and cone crop data analyzed to determine the best time and amount of $GA_{4/7}$ to apply.

White pine

Status

The white pine grant will continue to fund white pine research in Minnesota, but it is now being directly administered through the Ag. Exp. Station at the University. This eliminates much of the paper work originally required by the grant, and means that the University has more control over how the funds are spent. Pike and Warren work part time on a variety of field tasks related to seed orchard establishment and tree breeding for white pine.

The Co-op's white pine seed orchards are growing well. The DNR's **St. Francis** seed orchard, planted in 1985, appears as though it will have a bumper cone crop for picking in 2002. This represents the first substantial cone crop of white pine in the Co-op. A visit was made there in Fall 2001 to inventory the orchard.

Rajala/Itasca County's **Bass Lake** orchard has received all the available ramets from IRRRB. Seventy-five unplanted spaces in the orchard were filled with rootstock for future field grafting. These locations are flagged with orange tape so as not to be confused with grafts. The fence built around the orchard in 1999 has held up well, and trees that were previously damaged by deer browse have mostly recovered. Grass competition in the orchard is heavy, despite repeated mowings. Rubber mats placed around the bases of trees have prevented grass from shading out some of the smaller ramets. Overall growth of orchard trees is excellent.

Flower production at the Cloquet Forestry Center **breeding arboretum** has been minimal, with mostly male flowers being produced. This has limited our capacity to make crosses, so none have been made there to date. The site will be fertilized in Spring 2002, and trees may be injected with $GA_{4/7}$ to promote flowering.

St Louis County manages two orchards, **Ellsburg Rd** and **Ellsburg Rd East**. **Ellsburg Rd** was field grafted in 1990 using scion material from the Forest Service with putative resistance to blister rust. Additional clones were added to **Ellsburg Rd East** in 1999.

White pine topping trial

This trial was established at the Cloquet Forestry Center in Spring 2000 to observe the effects of $GA_{4/7}$ injections and topping on flower production and stem form. In Spring 2001, only a few male flowers were produced, which was not enough to validate any statistical analysis. The trees will be visited again in 2002 observe stem form effects of the topped trees.

Blister rust field trials

In 1999, five field trials were planted in four locations to test the field resistance of white pine families included in the screening research. Mortality surveys were conducted at four of these sites and survival was generally excellent. The site at Itasca County sustained damage from deer browse and heavy vegetative competition. Hand weeding around each tree needs to be done in order to conduct a survey. A visit to this planting is planned for Spring 2002 to evaluate the condition of the seedlings.

Tofte

In the Spring of 2001, Pike, David, Warren, and Bob Stine visited the white pine progeny test planted by Cliff Ahlgren in 1960's. In Fall of 2000, funding by the Wilderness Research Foundation in Ely was used to map the locations of the remaining "plus" trees at the site using a Global Positioning Unit (GPS). Jim Warren was the lead technician on the project. The planting originally contained some 50,000 seedlings, and it has been historically difficult to locate specific families from a map. It is now possible to locate a specific tree of interest using GPS coordinates.

Short and long-term planning

Future work with white pine includes making controlled crosses of promising families at the Cloquet Forestry Center's breeding arboretum. Trees in the arboretum will also be fertilized in Spring 2002. Regular orchard maintenance such as irrigation and mowing will be done as needed. Some pesticide application may be necessary in Spring 2002 to reduce the population of Pine bark adelgid observed in some trees. Some top-pruning will be necessary as well to maintain tree heights within range of an orchard ladder for breeding.

SELECTION AND CHARACTERIZATION OF RESISTANCE IN EASTERN WHITE PINE TO WHITE PINE BLISTER RUST

Robert A. Blanchette, Joel Jurgens, Jason Smith and Todd Burnes
Department of Plant Pathology
University of Minnesota
St. Paul, Minnesota 55108-6030

I. Understanding Mechanisms of Resistance in White Pine

Introduction

Since its introduction to North America nearly a century ago, white pine blister rust, caused by *Cronartium ribicola* J. C. Fisch has devastated eastern white pine (*Pinus strobus*) populations throughout their range. Although some native western white pine species have been found to posses "major gene" resistant against blister rust, which is characterized by a hypersensitive reaction, eastern white pine appears to exhibit a more qualitative resistance. When compared to susceptible families, some selected eastern white pines have lower rates of stem infection, slower tissue colonization, and reduced or delayed mortality. This report elucidates both susceptible and resistant mechanisms in secondary needles of five open pollinated, half-sib eastern white pine families.

Materials and methods

The seed used from this study were obtained from the USFS Oconto River Seed Orchard, White Lake WI and are from five open pollinated, half-sib families. The original source of WI 352 and H 111 were collections made by foresters in Langlade County Wisconsin in 1966 and Hiawatha county Wisconsin in 1965. There collections were based on phenotypic selections of white pine trees that were relatively canker free in areas that had a high occurrence of blister rust. Seedlings from families with "P" as the precursor were selections made by Prof. Robert Patton, University of Wisconsin in the 1950's, again basing his collections on blister rust free trees in areas of high rust occurrence. Selection P 30 was collected in Dunn County WI and P 312 and P 327 were collected in St. Louis County MN.

Seed from the five families were stratified and sown in a greenhouse for 5 months and placed in an incubation chamber the day before inoculation. Leaves of *Ribes nigrum* bearing telia of *C. ribicola* strain W14.1B were suspended above the seedlings then were removed and the seedlings were incubated for an additional 96 hours to insure infection. Secondary needles were collected from seedlings seven weeks after infection and prepared for histological examinations. Needle tissues were fixed in formalin-acetic acid-ethyl alcohol (FAA), dehydrated in a tertiary-butyl alcohol series and embedded in paraffin. Serial sections were cut at 12-14 μ and stained with the periodic acid-Schiff technique and phloroglucinol.

Results

Susceptible Reactions

Susceptibility and resistance of families was determined based on mortality rates after inoculation (Figures 1 and 2). The first susceptible histological characteristic is most common in both families H 111 and WI 352 and is distinguished by large areas of densely packed mycelium located around the vascular bundle (Figure 3). Hyphae grow through intercellular spaces with haustoria present in mesophyll and transfusion cells as well as vascular elements.

Shortly after haustoria development in mesophyll cells, the cytoplasm appeared granulated and the nucleus was disrupted. Mesophyll cells were also found to collapse at this stage of the infection process. Transfusion cells appear to maintain cellular organelles after haustorial development and often were completely filled with hyphae. Infected vascular tissue did not become ramified by hyphae. When needles exhibiting this type of reaction are stained with phloroglucinol, there is no indication of polyphenolic compound accumulation. The hyphae continue to radiate through intercellular spaces from the original point of infection toward the needle tip and at a greater rate toward the base of the needle. Similar reactions have been observed in needles from all families involved in this study.

Another characteristic identified in the susceptible families WI 352 and H 111 is a smaller region of densely packed hyphae, again located around the vascular bundle (Figure 4). The large areas of disruption seen in the first example are not apparent. However, the fungus has moved at a much faster rate toward the main stem by means of individual hyphae. These individual strands grow rapidly through intercellular spaces in the needle infecting both mesophyll and transfusion cells. Hyphae and haustoria are also present in vascular cells, but they do not advance toward the needle base at the same rate as intercellular hyphae. This susceptible reaction also lacks polyphenolic production in response to infection.

Resistant Reactions

Resistance characteristics have also been identified in histological studies from three different families. The resistance observed is supported by both microscopic observations and mortality data (Figures 1 and 2). Families P 30, P 312 and P 327 show a greater capacity for survival when compared to families H 111 and WI 352 which do not display any of the following “resistant” characteristics.

Resistant Reaction One

A resistant characteristic common to family P 30 is shown in Figures 3 and 4. These micrographs show the same infection in a needle stained using two different reagents. In Fgure 5 a confined area of infection is indicated by the magenta color located around the vascular bundle after staining with periodic acid–Schiff’s reagent. The infection was localized and apparently unable to expand due to polyphenolic compounds located around this zone. These phenolic compounds stained with phloroglucinol have been linked to resistance in blister rust and many other disease systems and can be seen as a reddish, pink color in Figure 6. A limited number of hyphae are located in the intercellular spaces around the confined mycelial mass, but appear shrunken in appearance and are not forming haustoria in mesophyll, transfusion or vascular cells.

Resistant Reaction Two

A different reaction has been identified in needles from family P 312 and can be observed in Figures 7 and 8. These needles react to infection by producing abundant, abnormally large cells and copious amounts of polyphenolics substances. The macroscopic appearance of this reaction is characterized by a bright yellow spot that is slightly swollen. After phloroglucinol staining the surrounding cells are stained, indicating phenolic compounds (Figure 8). This reaction appears to stop the proliferation of the fungus allowing the needle and seedling to survive.

Resistant Reaction Three

A third resistant characteristic observed involves the collapse of mesophyll cells and an absence of phenolic compound accumulation (Figures 9 and 10). The macroscopic appearance of this infection is a bright yellow spot and can be seen in needles from family P 327. In these needles it appears that the host reacts to infection by killing cells around the infection site reducing the available cells for the obligate parasite to colonize. This creates both a physical barrier that inhibits the fungus from future colonization and reduces the food source required for survival.

Susceptible Reactions in Family WI 352 & H 111

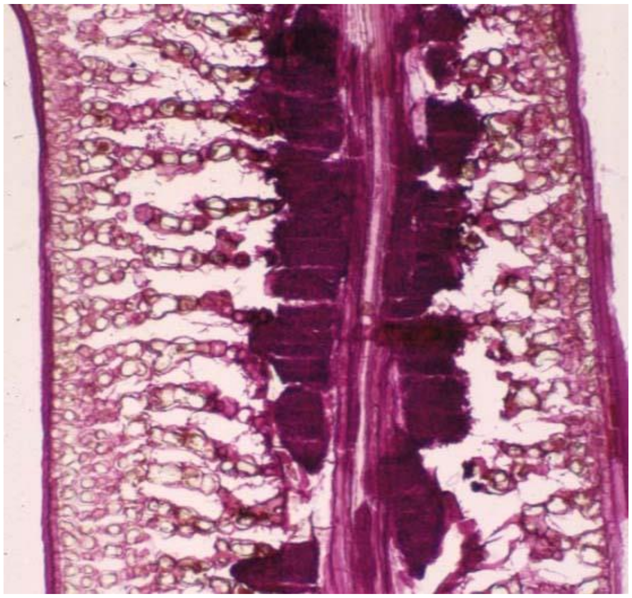
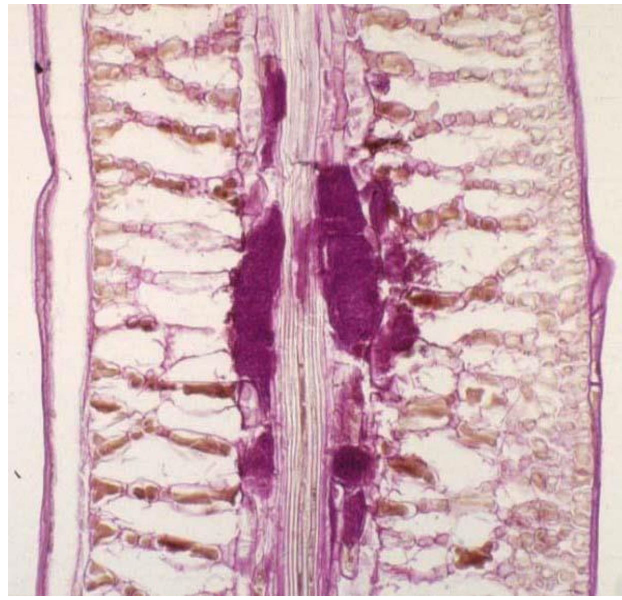


Fig. 3. Large areas of densely packed mycelium located around the vascular bundle. Section stained with Schiff's reagent.



ıyFig. 4. Individual hyphae radiating out from a :erelatively small mycelial mass.

Resistant Reaction in Family P 30

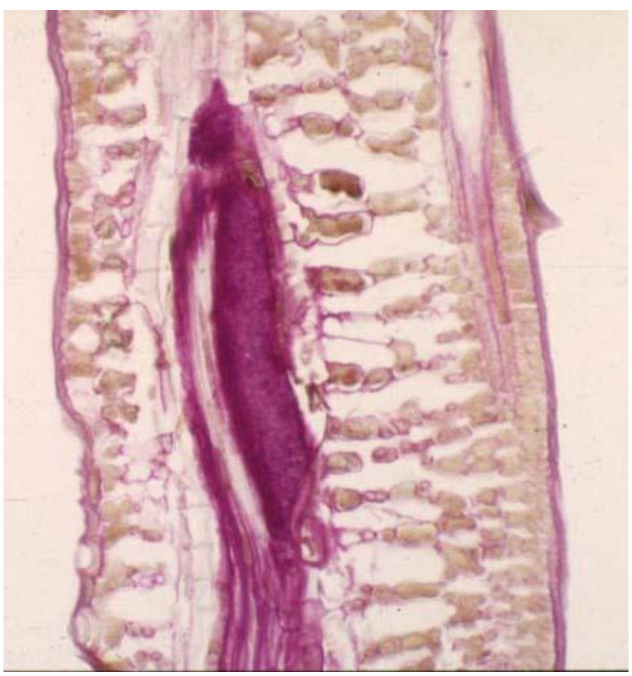


Fig. 5. Confined area of infection is indicated by the magenta color located around the vascular bundle after staining with periodic acid–Schiff's reagent

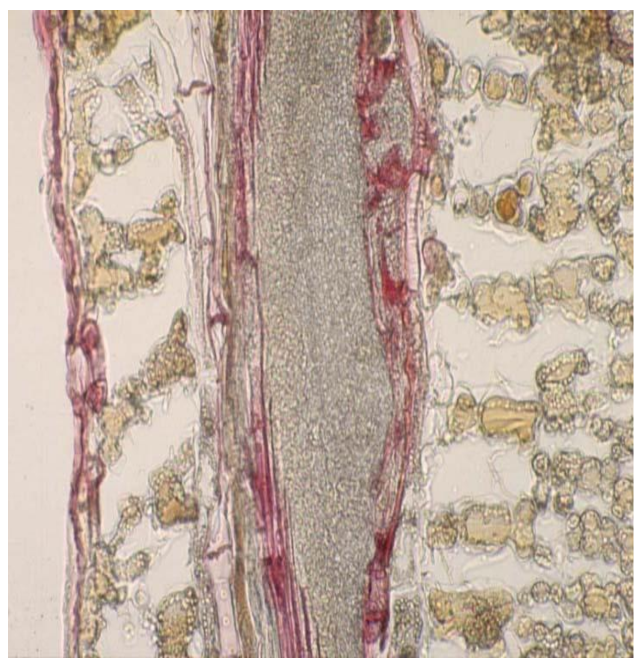


Fig. 6. The infection was localized and unable to expand due to polyphenolic compounds located around the mycelial mass.

Resistant Reaction in Family P 312

Fig. 7. In response to infection, needles produce abundant, abnormal cells.

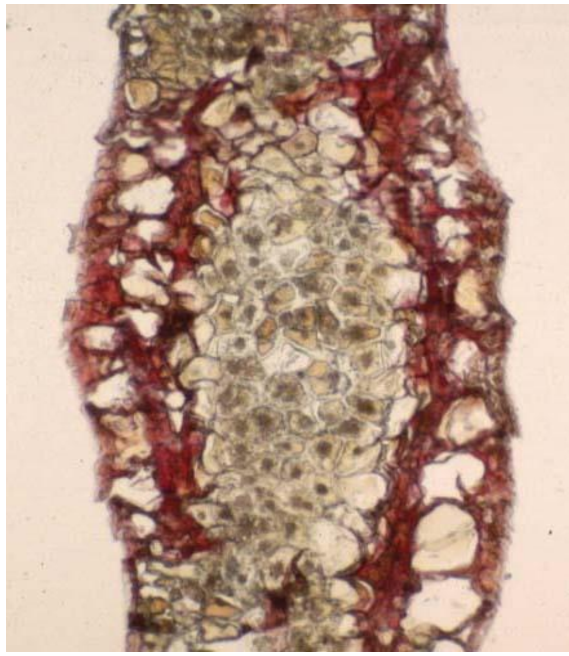


Fig. 8. Prolific polyphenolic appears to inhibit the fungus production.

Resistant Reaction in Family 327

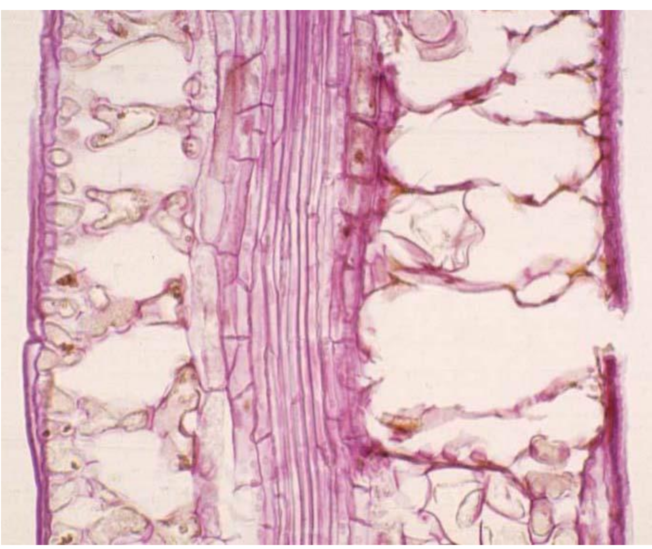


Fig. 9. Collapsed mesophyll cells create a physical barrier and reduce the food source available for the obligate parasite.

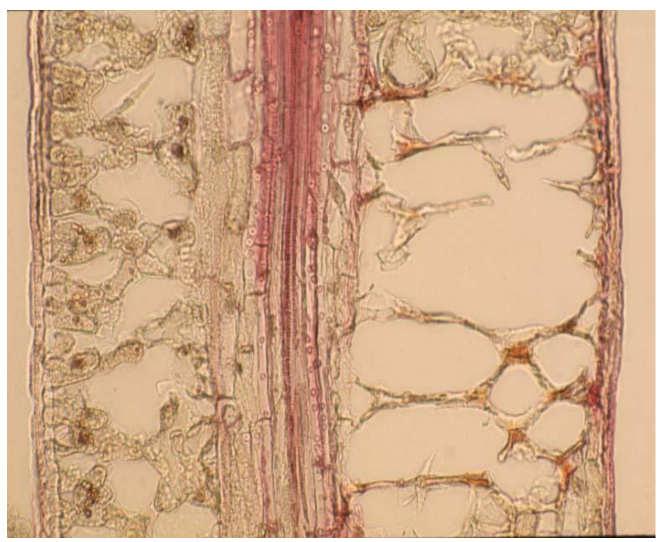


Fig. 10. Needle reaction to infection does not include polyphenolic production.

Future Investigations

Experiments are continuing to elucidate additional details of host resistance mechanisms. Recently, families of known lineage have been inoculated and collected for histological investigation. These new families will be examined for evidence of similar mechanisms of resistance that will help correlate resistance and susceptibility and make selection of resistant families more precise.

II. Greenhouse screening experiments for resistance

Screening experiments previously carried out at the US Forest Service Experiment station in Rhinelander, Wisconsin are now being done at the University of Minnesota. One set of inoculations completed with four eastern white pine seedlots was done and the ranking of seedlots compared to mortality data previously obtained by Dr. Paul Zambino at the US Forest Service (Table 1). Results indicated that our screening techniques and methods used were working appropriately. Seedlings from seedlots P312xP327 and ON 469 had the least amount of mortality after 12 months as compared to seedlots P343xH109 and H111. Seedlots P312, P327 and ON469 have demonstrated resistance in the field and seed lot H111 was selected from the field for silvicultural traits but not for blister rust resistance. Preliminary data from these and other greenhouse studies suggest that traits for resistance may be observed comparing mortality data among eastern white pine seedlots. Further inoculations will help determine correlation between mortality and level of resistance.

Additional blister rust screening was done on 36 eastern white pine seedlots from Oconto River seed orchard in Wisconsin. The results of inoculations were inconclusive since infection rates were lower than previous studies. This was likely due to viability of frozen spores that were used and minor fluctuations in environmental conditions. These same 36 eastern white pine seedlots will be grown and inoculated again within the next 8 months (Table 2). Early symptom development on needles and mortality data will be taken. This data will allow us to compare specific crosses of known field resistance with other resistant and susceptible white pine families. It will provide more precision in our resistance evaluations than those based only on open pollinated seedlots.

During this past spring and summer we have been propagating *Ribes nigra* to obtain sufficient numbers for inoculating the plants with urediospores to obtain telia and eventually basidiospores for use to inoculate white pine seedlings. Propagation is done by cutting terminal sections of older plants and placing them into wet vermiculite in a moist environment for 3 to 4 weeks or until adequate roots develop. *Ribes nigra* plants are than transplanted into 4-inch pots and are ready to inoculate in 3 to 4 weeks.

Inoculation is done by using a urediospore suspension and spraying this onto the underside of *Ribes nigra* leaves. It takes approximately 50 to 60 *Ribes nigra* plants for each inoculation of 10 seed lots. After inoculation plants are placed in a growth chamber at 20 C for 4 weeks or until urediospores are produced. The plants are than placed into a growth chamber with 20 C day and 15 C night temperatures and a 12-hour photoperiod. It will take an additional 4-5 weeks for the production of telia on the leaves and at this time leaves can be used to inoculate pine seedlings or can be stored for future inoculations. A separate inoculation is done with 100 *Ribes nigra* plants just for the purpose of collecting urediospores for future inoculations.

Table 1. Percent mortality of eastern white pine seedlings 12 months after inoculation with blister rust

Eastern White Pine Crosses	US Forest Service Inoculation 1	US Forest Service Inoculation 2	University of Minnesota Inoculation
H111	80	78	78
P343xH109	70	73	62
P312xP327	63	62	36
ON 469	63	40	15

Table 2. Eastern white pine seed lots included in experiments that are underway

Crosses Female x Male		
P312xP327	P030xP327	P343xP343
H111	P312xP312	P312xP343
P327xP30	H109xP327	P018xP343
P327xP18	P030	P030xP343
P312xP30	P343	P312
P312xP18	P030xP312	P343xP030
P18xP312	ON 469xON 469	P018xP030
P30xP30	H109	P327xP343
H109xP312	P327	P327xP312
P343xP018	P018	P343xP327
H111xP327	P343xH109	P327xH109
		ON 469

III. Identifying resistant white pine

Introduction and Background

White pine blister rust, caused by the basidiomycete *Cronartium ribicola* J.C. Fisch. Ex Rabenh., is one of the most damaging forest diseases yet observed in North America. There is great interest in restoring eastern white pine (*Pinus strobus*) in its native range (14). However, efforts to re-establish white pine have been largely unsuccessful due to this pathogen (14).

As white pine blister rust spread throughout the range of eastern white pine this century, it was observed that a few individual trees remained unaffected (8). The lack of disease on these trees has led to breeding projects aimed at developing seedlings that are putatively resistant to the disease (8,13). Unfortunately, many years have passed since resistant individuals were first located and disease-resistant planting stock is not yet available.

There are several problems commonly encountered when screening eastern white pine for resistance to blister rust. First, there is a lack of understanding about how the pathogen attacks the host and what traits in the pine may confer resistance (15). Although much is known about the epidemiology of the disease, little is known about host-pathogen interactions or mechanisms of resistance (1,6,15). Second, screening seedlings poses several problems (7,15,19). Inoculation procedures have been described and used for decades, but unfortunately infection is often low and not uniform (15,19). Although natural inoculation may be feasible when testing progeny (19), having mixed genotypes of the pathogen does not allow for detailed host-pathogen interaction studies. In addition, variability among seedlings makes it difficult to establish patterns of disease morphology such as lesion types (15,19) and further complicates studies aimed at understanding the interaction between the host and pathogen. Due to this variation, it is not known whether putatively resistant *P. strobus* germplasm react in a consistent manner to infection by *C. ribicola*.

Although tissue culture has been used to develop assays for screening for resistant phenotypes (3), use of vegetative propagation to study blister rust resistance has been very limited. Low seed production has limited large-scale development of resistant seedlings of sugar pine (20). However, protocols have been described to propagate white pines from fascicles (20). This approach could be useful for studying resistance mechanisms in pine.

Most studies aimed at understanding resistance to blister rust have focused on western species of pine (not eastern white pine) and have been conducted by geneticists (not pathologists). Their work has provided us with detailed inheritance data and it suggests that a major gene for resistance is present in some of the native species of white pine (12,13). These studies have also facilitated in identifying lesion morphologies or resistance classes. One of the most studied is the hypersensitive response (HR) associated with major gene resistance (3,10,11,12). In addition to HR, other morphologies include reduced needle-lesion frequency (15,16), early needle drop/senescence (16), and bark corking (2) and other degrees of resistance such as failure of the pathogen to move from the needle lesion to the main stem.

From the classical genetics approach, great strides have been made in understanding the resistance to the disease in primarily sugar pine (8,13). However, few studies have attempted to describe the host mechanisms of resistance. The importance of the stomata as the mode of penetration by *C. ribicola* was determined early on (9,15,16). From this starting point, the infection process has been described in some detail (6,16,17,18). The mode of entry of the fungus has been well-documented (16). Germinating basidiospores produce germ tubes that enter the outer stomatal chamber and penetrate through apertures between the guard cells (16). Upon entry a substomatal vesicle is produced that produces an infection hypha that develops into mycelium that penetrates the mesophyll cells (16).

Only a few studies have been dedicated to determining mechanisms involved in resistance. Boyer (2) conducted studies on changes in phenols in infected eastern white pine as well as auxins in relation to stem resistance. Unfortunately, this early work has not carried on. More recently, Ekramoddoullah has characterized protein changes in infected tissue (5) and generated monoclonal antibodies to basidiospore proteins that could be used to better characterize the host-pathogen interaction early in the infection process (4).

Several studies in the past have noted an apparent relationship between epistomatal wax and lack of penetration by developing hyphae of *C. ribicola* (7,15, 16). These explanations have been based on observations that the needles from unsuccessful inoculations had stomata that were occluded with wax (including the gap between the guard cells) (15). Previous studies have suggested that because the wax deposits occlude the stomatal antechambers, the developing hyphae are prevented from entering and subsequent development of the substomatal vesicles is aborted (15). It has been suggested that that the mechanisms involved in the reduced needle-lesion frequency resistance are likely to take place prior to penetration of the hyphal peg between the guard cells at the stomata (15). Thus, these wax deposits may be involved in the "reduced needle-lesion frequency" resistance (15). This has not been substantiated, however. No studies have looked at wax biochemistry in relation to resistance or the effect of epistomatal wax on basidiospore germination.

One limitation to studying the relation between wax occlusion and the infection process has been the process of tissue fixation during preparation for electron microscopy. Traditional protocols use solvents that destroy needle wax (15). Thus, it has been virtually impossible to look at germinating spores and the needle surface (as it would appear *in vivo*) at the same time. Only recently, with the advent of environmental scanning electron microscopy has this become possible.

Although it remains implied that "resistant" individuals of eastern white pine occur, it has not been substantiated whether the lack of disease is the result of specific resistance to the disease or a non-specific host response. One of our goals is to determine if blister rust-resistant eastern white pine exist by characterizing the mechanisms by which the host avoids damage by the pathogen.

Objectives and Research Approach

The three main objectives of the study are: (i) to determine if needles from resistant *Pinus* genotypes exhibit consistent lesion morphology when infected with a strain of *Cronartium ribicola* (ii) to determine the role of epistomatal wax in resistance to blister rust needle infections and (iii) to compare hypersensitive responses among several species and determine if HR genotypes express systemic acquired resistance when challenged with *Cronartium ribicola*.

It is our intention to further investigate the "reduced needle-lesion frequency" and "hypersensitive response (HR)" mechanisms. Little is currently known about the events in host tissue leading up to and following the appearance visual symptoms associated with these reactions. A better understanding of these mechanisms will be beneficial to breeding programs and attempts to use biotechnology to enhance resistance in white pine.

Here we describe preliminary studies using a protocol developed to inoculate hydroponically grown one-year shoots as well as fascicles of the same approximate age. This work will facilitate future studies that require a homogenous host population and eliminates the variability of seedling populations that are unsuitable for these studies. In addition, environmental scanning electron microscopy (ESEM) studies were initiated to compare the needle surface morphology of a resistant and susceptible phenotype of *P. strobus*. Initial results suggest that there are major differences in the epidermal wax between these phenotypes. This tool (ESEM) will allow us to look at the infection process without destroying the wax layer as in previous studies. In addition to these studies, hundreds of *Ribes nigrum* cuttings have been rooted and inoculated with *Cronartium ribicola*. Secondary infections have occurred and are being maintained in order to provide fresh inoculum. Telia have recently been produced and the leaves are being collected and stored for future pine inoculations. A collection of five-needled pine species, hybrids and cultivars has been obtained and is being maintained in containers and will serve as stock plants for material to be used in future studies.

Project Summary to Date

Hydroponic Inoculations

In April 2001, cuttings of the current year's growth were taken from parent trees WI342 (resistant) and H109 (susceptible) at the Oconto River Seed Orchard and shipped next day air back to the laboratory in St. Paul. Upon arrival the cuttings were refrigerated and processed the next day.

Shoots and fascicles were processed and placed in a non-circulating hydroponic solution (Fig. 1). The shoots and fascicles were cultured in a growth chamber set at 22 ^{o}C. After one week, a subset of the shoots and fascicles were set aside and cultured indefinitely to determine how long they would remain viable. The rest were inoculated using frozen telia-containing *Ribes nigrum* leaves. The germination rate of the basidiospores was about 70%. After an incubation period, the cuttings and fascicles were taken out of the incubation chambers and maintained in the growth chamber. After 6 weeks 100 % of the H109 shoots and 65% of the fascicles were alive and 90% of the WI342 shoots and 50% of the fascicles were alive. In addition, 40% of the H109 shoots had lesions typical of those caused by *C. ribicola*. After 8 weeks 60% of the H109 shoots and 30% of the fascicles were alive and 25% of the WI342 shoots were alive and 10% of the fascicles were alive. Further studies are being done to optimize these methods (fascicles will be cultured on other media and the inoculation procedure is being modified).

***Ribes nigrum* inoculations**

Cuttings from *Ribes nigrum* were taken from greenhouse grown stock plants and propagated under mist and after roots developed (approximately 2 weeks) the cuttings were transplanted to 8 cm pots and grown under artificial lights in the greenhouse for 6 weeks. After 6 weeks the plants were inoculated and placed in a growth chamber set at 18 ^{o}C. After 2 weeks in the growth chamber uredia developed on the lower surface of the leaves. One growth chamber is used to encourage secondary infections by uredia. Another growth chamber is set at 15 ^{o}C at night and 20 ^{o}C during the day to encourage telial production. All plants are being fertilized every two weeks with a soluble fertilizer.

As of the end of August, telial production has occurred on plants that were inoculated in early July. The leaves are being collected and stored for use in pine inoculations during the coming months. More *Ribes* are being propagated and inoculated to provide inoculum throughout the coming year.

Environmental Scanning Electron Microscopy Studies

Needles from H109 and WI342 were evaluated using the environmental scanning electron microscopy (ESEM) mode of the Hitachi S3500N scanning electron microscope. This new tool provides a way to look at needle surfaces exactly as they are *in vivo*. The needle surface does not become altered from the fixation process.

Early results indicate that when needles of the same age are compared, the resistant (WI342) needles have stomata that are completely occluded with wax, whereas the susceptible (H109) needles have a large gap in the wax over the stomatal opening and cracks in the wax were observed between the guard cells (Fig. 2). These results confirm earlier studies conducted by Patton and Spear and will be the focus of further studies.

In addition to these studies, some other germplasm were studied using ESEM including variants of *P. strobus*. One variant is of particular interest. *P. strobus* 'Bennett Clumpleaf' is a horticultural variety selected due to the strange trait of fascicles of needles remaining attached so five needles appear as one large needle. The ESEM studies revealed that these needles are arranged so that the stomata are located on the inside of the bundle, not exposed to the outside (Fig.3). The needles normally remain in this arrangement for at least one-year. This trait may be a novel mode of resistance for two reasons. First, since the *C. ribicola* enters through the stomata, this variant is avoiding the pathogen for at least one year. Second, it has long been observed that needles are most susceptible for that first year. Avoiding infection for one year may be significant and this variant will also be used in further studies to look at how anatomy and development may aid in resistance.

***Pinus* germplasm collection development**

During the late winter and Spring of 2001 seed, seedlings and grafted plants of *Pinus strobus* cultivars and other five-needled pines were purchased from nurseries and seed dealers. After several months of stratification the seed was planted and the plants were containerized and placed on drip irrigation in an outside nursery. Throughout the season the plants have been fertilized and repotted when needed. These plants will be used in host-resistance comparison studies. After serving as stock plants for this project, they will be planted and evaluated for horticultural potential in Minnesota.

Conclusion

In this summary we have outlined our work that is aimed at better understanding resistance mechanisms in eastern white pine to infection by *Cronartium ribicola*. It is our hope that these studies will aid in the development of resistant eastern white pine. The ability to inoculate successfully shoots or fascicles of pine would eliminate the problems of using a seedling population to study host-pathogen interactions. Identifying the traits that confer resistance will have wide reaching implications for breeding programs and will hopefully be a major step in process of developing blister rust resistant eastern white pine.

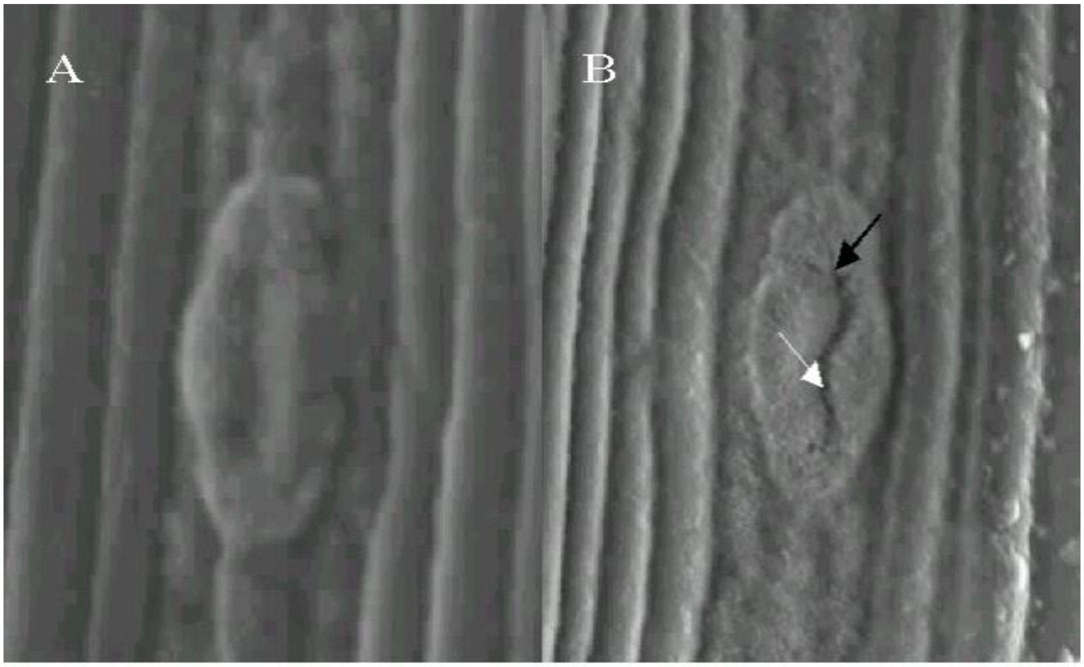


Fig. 2 – ESEM micrographs (8000x magnification) of stomata from resistant (A – WI342) and susceptible (B – H109) *Pinus strobus* germplasm. Note uniform covering of epistomatal wax in A and large gap in wax at stomatal opening (white arrow) and between the guard cells (black arrow) in B.

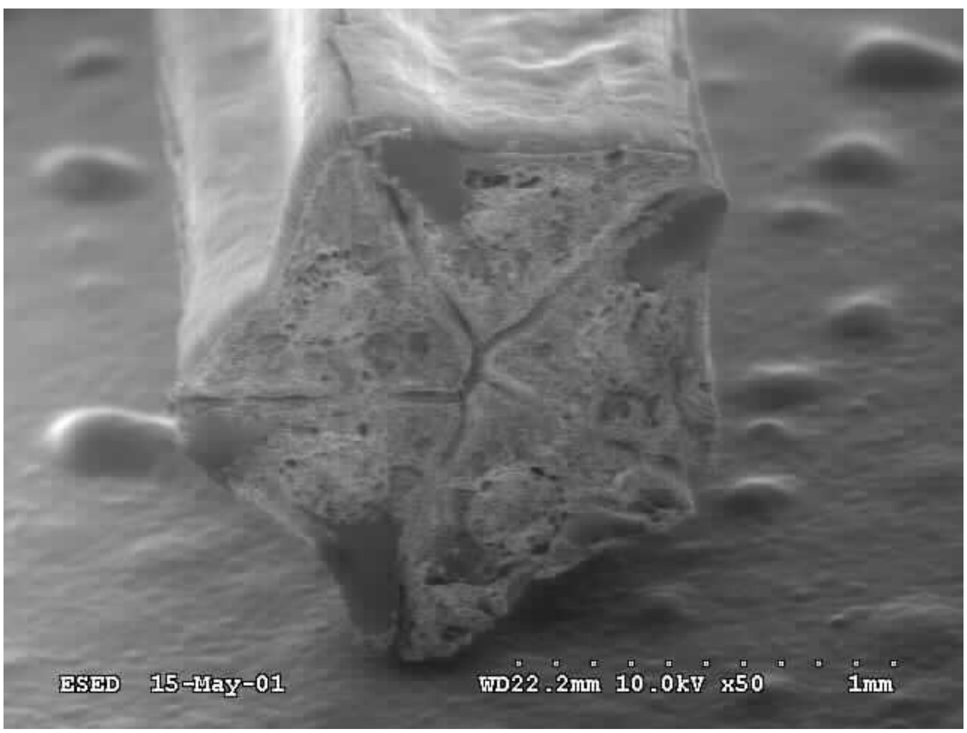


Fig. 3- *P. strobus* 'Bennett Clumpleaf' fascicle cross-section. Note the absence of stomata on the outside of the bundle.

Literature Cited

1. Boyer, M.G., Isaac, P.K. 1964. Some observations on white pine blister rust as compared by light and electron microscopy. *Canadian Journal of Botany* 42: 1305-1309.

2. Boyer, M.G. 1964. Studies on white pine phenols in relation to blister rust. Canadian Journal of Botany: 42-979-987.

3. Diner, A.M., Mott, R.L. 1982. A rapid axenic assay for hypersensitive resistance of Pinus lambertiana to Cronartium ribicola. Phytopathology 72: 864-865.

4. Ekramoddoullah, A.K.M., Taylor, D.W. 1996. Production and characterization of monoclonal antibodies to the white pine blister rust fungus, *Cronartium* ribicola. Canadian Journal of Plant Pathology 18: 14-18.

5. Ekramoddoullah, A.K.M., Hunt, R.S. 1993. Changes in protein profile of susceptible and resistant sugar pine foliage infected with the white pine blister rust fungus Cronartium ribicola. Canadian Journal of Plant Pathology 15: 259-264.

6. Hansen, E.M., Patton, R.F. 1975. Types of germination and differentiation of vesicles by basidiospores of *Cronartium ribicola*. *Phytopathology* 65: 1061-1071.

7. Hansen, E.M., Patton, R.F. 1977. Factors important in artificial inoculation of Pinus strobus with Cronartium ribicola. Phytopathology 67: 1108-1112.

8. Heimburger C. 1962. Breeding for disease resistance in forest trees. *Forestry Chronicle* 38: 356-362.

9. Hirt, R.B. 1938. Relation of stomata to infection of *Pinus strobus* by *Cronartium* ribicola. Phytopathology 28: 180-190.

10. Hoff, R.J., McDonald, G.I. 1972. Resistance of *Pinus armandii* to *Cronartium* ribicola. Canadian Journal of Forest Research 2: 303-307.

11. Hoff, R.J., McDonald, G.I. 1975. Hypersensitive reaction in *Pinus armandii* caused by Cronartium ribicola. Canadian Journal of Forest Research 5: 146-148.

12. Kinloch, B.B., Littlefield, J.L. 1977. White pine blister rust: hypersensitive resistance in sugar pine. *Canadian Journal of Botany* 55: 1148-1155.

13. Kinloch, B.B. 1972. Mechanisms and inheritance of rust resistance in conifers. In: Biology of rust resistance in forest trees. Misc. Publ. 1221. Washington, DC: U.S. Department of Agriculture, Forest Service: 119-129.

14. Ostry, M.E. 2000. Restoration of white pine in Minnesota, Wisconsin and Michigan. *HortTechnology* 10(3): 542-543.

15. Patton R.F., Spear, R.N. 1980. Stomatal influences on white pine blister rust infection. Phytopathologia Mediterranea 19: 1-7.

16. Patton, R.F., Johnson, D.W. 1970. Mode of penetration of needles of eastern white pine by Cronartium ribicola. Phytopathology 60: 977-982.

17. Robb, J., Harvey, A.E., Shaw, M. 1975. Ultrastructure of tissue cultures of *Pinus monticola* infected by *Cronartium ribicola*. II. Penetration and post-penetration. *Physiological Plant Pathology* 4: 9-18.

18. Robb, J., Harvey, A.E., Shaw, M. 1975. Ultrastructure of tissue cultures of *Pinus* monticola infected by Cronartium ribicola. I. Prepenetration host changes. Physiological Plant Pathology 4: 1-8.

19. Patton, R.F. 1972. Inoculation methods and problems in testing eastern white pine for resistance to *Cronartium ribicola*. In: Biology of rust resistance in forest trees. Misc. Publ. 1221. Washington, DC: U.S. Department of Agriculture, Forest Service: 373-385.

20. Stiff, C.M., Wenny D.L., Dumroese, R.K., Roberts, L.W., and Stiff, C.T. 1989.Establishment of western white pine shoots *in vitro* using needle fascicles. *Canadian Journal of Forest Research* 19: 1330-1333.

FLOWER INDUCTION ON YOUNG GRAFTED WHITE PINE MATERIAL

This study was conducted by Paula Pijut of the USDA Forest Service and investigated the effects of $GA_{4/7}$ injections on flower production in white pine. Two sites were used: the white pine breeding arboretum at the Cloquet Forestry Center, and the St. Louis County white pine orchard. At St. Louis, ten trees from each of six genotypes were chosen in 2000. At the CFC breeding arboretum, only three trees per genotype are available, so the study required thirty different genotypes to cover all the different treatment levels. Trees were injected once every two weeks, beginning on March 15th, 2000 and ending October 25th for a total of four injections per tree. Within the $GA_{4/7}$ treatment, there were two application rates, high (52 mg/0.2 ml ETOH) and low (25 mg/0.2 ml ETOH). All trees receiving the $GA_{4/7}$ treatment were fertilized with ammonium nitrate (34-0-0) on May 30th, and again on June 27th, 2000 for a total application rate of 50 kg N per hectare. One control ramet per clone was fertilized, and the second was not fertilized. For statistical analysis, injection times were divided into four major periods: early, mid, late, and very late (Table 1). Flowering data was collected June 12, 2001.

Table 1. $GA_{4/7}$ injection dates and period designations for analysis.

Dates of injection	**Injection time period**
March 15, 29, April 12, 26	EARLY
May 17, 31, June 14, 28	MID
July 12, 26, Aug 9, 23	LATE
September 13, 27, October 11, 25	VERY LATE

Results

The analysis for this data is not yet completed. A complete analysis and discussion will be submitted in a future monthly report. These are some preliminary observations:

- At both sites, only male strobili were induced.
- Treatment effects were more pronounced at St. Louis than at Cloquet.
- There was no consistent pattern for strobili production among genotypes with respect to the dosage and time of injection.
- Results from the Cloquet site suggest no differences between early and mid injection times and between high and low doses.
- Early times were generally better for enhancing male strobili than were later times. Further analyses might indicate if these differences were significant.
- There was no clear effect of high and low dosage rates.
- Fertilization did not produce a significant effect for all genotypes.

CHARACTERIZATION OF BUD-BREAK TIMING IN TWO SOURCES OF WHITE SPRUCE

Objectives / Methods

The purpose of this study was to investigate bud-break timing among different genotypes of white spruce (*Picea glauca* Moench) utilized in seed orchards of the Minnesota Tree Improvement Cooperative. The Co-op's seed orchards are planted with genotypes that originate from two geographic regions: Northern Minnesota and Southeastern Ontario. This study was undertaken to address concerns about the potential susceptibility of improved white spruce to damage from early-spring frosts and feeding from the spruce budworm (*Choristoneura fumiferana* (Clem)). In this study, a method for scoring bud-break in white spruce was assessed, and bud-break timing was correlated with degree-days for future studies. Comparisons between the two sources were made to characterize relative differences in bud-break timing and shoot elongation. No evidence exists to implicate genetically improved sources of white spruce as being more susceptible to budworm feeding damage than un-improved sources.

Data for this study was collected from the Co-op's white spruce breeding arboretum located in General Andrews Nursery in Willow River, MN. This planting contains a clone-bank of genotypes that are represented in various MTIC orchards. One ramet from each of 20 different clones was selected for observation. Ten genotypes from Southeastern Ontario, and ten genotypes from Northern Minnesota were selected at random, without regard to any phenotypic characteristics. The Minnesota sources represent selections made from Blandin's Latimer open-pollinated progeny test in Grand Rapids, MN. On each tree, thirteen branches were flagged with string. The leader, plus one branch from each cardinal direction were selected at three different positions in the crown (lower, middle, upper). Beginning on April 30, 2001, each branch was observed and scored based on the stage of development of either the terminal or most distal bud. Observations were taken for a total of eight days at unequal intervals until May 23, 2001 (Table 1). The scale used to score the stages of bud-break consisted of nine levels, described below, which are based on those developed by Alfaro et al., 2000:

1 – Bud appears dormant
2 – Bud is visibly swollen, scales starting to peel back
3 – New scales make up less than half of the total length of the bud
4 – New scales make up more than half of the total length of the bud
5 – Bud cap is either translucent or cracked, needles are visible beneath it
6 – Bud cap has broken apart from cap, lowermost needles have emerged
7 – New growth has a brush-like appearance, needles appear to originate from one point
8 – New growth has a feather-like appearance, needle bases begin to separate
9 – Needles are widely separated from expanding shoot.

Using a baseline of 8°C (46.4° F) (Lysk 1989), cumulative degree-days were estimated for the nursery using maximum and minimum temperatures obtained from a weather station in Moose Lake, MN.

Statistical analysis

The average score for each tree was calculated from the 13 observations at each date. Scores were grouped and compared between Ontario and Minnesota sources. The data was analyzed using a Repeated Measures ANOVA with date as the repeated measure, and each genotype as the subject (general linear models SAS 1996).

Results / Discussion

There were no significant differences between sources across dates (Repeated Measures F=.08 p>.05). When considering each date separately, significant differences between the two sources existed for one of the eight dates (F=4.6, p=.03). The average scores for each source were plotted to see if any large differences in bud-break timing were evident (Figure 1).

The phenology of improved sources of white spruce is not well understood. The timing of bud-break is just one of many factors that influence tree growth patterns. Theoretically, trees that break bud earlier, or set buds later may benefit from an extended growth period. However, Trees that break bud early may be more susceptible to damage from late frosts, or have an increased or decreased susceptibility to an important native insect, the spruce budworm. The implications of tree improvement efforts on tree health need to be considered. Budworm feeding habits have been correlated to degree-days in several studies (Bean and Wilson 1964; Lysyk 1989). While no budworm was present in the Willow River nursery, the dates that bud-break occurred coincided with published reports of budworm feeding activity (Lysyk 1989).

In this study the timing of bud-break stages did not differ significantly between Ontario and Minnesota material. More information could be gathered from increased replication of sites, ramets, and clones. This preliminary study looked at only clonal material. Observations in several sites across different climatic zones might elucidate genotypic and environmental effects more clearly. In addition, Co-op progeny tests could be analyzed to correlate scores between parents and offspring to quantify the amount of heritable genetic variation. Observations taken throughout the growing season might help to identify timing of growth spurts, and identify sources with potential susceptibility to late frosts, and insect feeding. In addition, by correlating periods of rapid shoot elongation with degree days, managers may improve the efficiency of irrigation and fertilization by better synchronizing management activities with tree growth.

Table 1. Date, cumulative degree-days, and bud-break scores for Minnesota and Ontario sources. Lines at right indicate the budworm instar whose emergence correlates with that degree-day (Lyskyk 1989).

Dates	Cum. Degree Days	Mean Score (Minnesota)	Mean Score (Ontario)
April 30	75	1.0	1.0
May 3	119	2.3	2.3
May 7	142	3.1	3.2
May 9	159	3.8	3.6
May 11	180	4.0	3.9
May 14	215	4.5	4.7
May 18	273	5.9	5.8
May 23	316	6.3	6.2

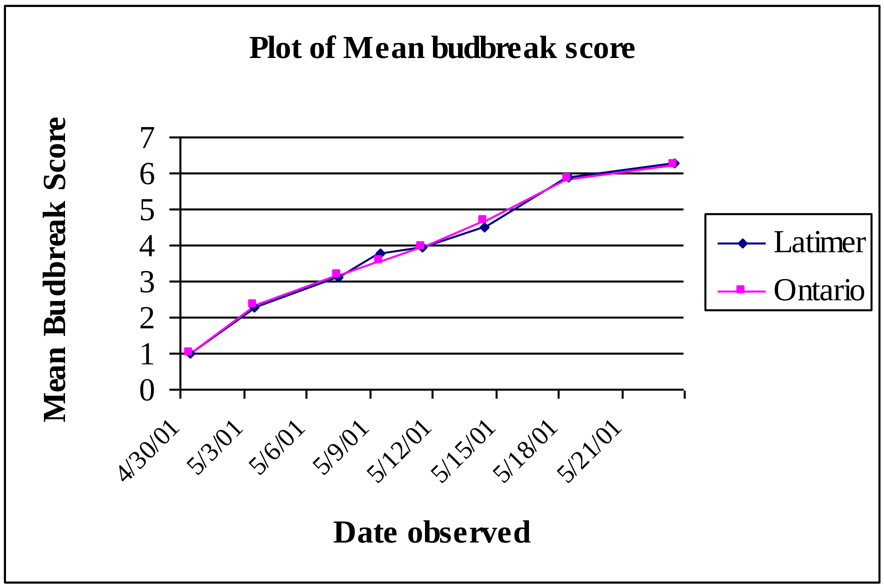


Figure 1. Mean budbreak score for each source at each observation date. No significant differences for sources were found across dates.

Literature cited

Alfaro, R. I.; Lewis, K. G.; King, John N.; El-Kassaby, Y. A.; Brown, G., and Smith, L. D. 2000. Budburst phenology of Sitka spruce and its relationship to white pine weevil attack. Forest Ecology and Management. 127(1-3): 19-29.

Bean, J. L. and L.F. Wilson. 1964. Comparing various methods of predicting development of the spruce budworm, Choristoneura fumiferana, in northern Minnesota. J. Econ. Entomol. 57(6): 925-928.

Lysyk, T.J. 1989. Stochastic model of eastern spruce budworm (Lepidoptera: Tortricidae) phenology on white spruce and balsam fir. J. Econ. Entomol. 82(4): 1161-1168.

Mattson, W.J.; Haack, R.J.; Lawrence, R.K.; Slocum, S.S. 1991. Considering the nutritional ecology of the spruce budworm in its management. For. Ecol. Mgmt. 39: 183-210.

COMPARISON OF SELECTION METHODS FOR OPTIMIZING GENETIC GAIN IN A RED PINE (*PINUS RESINOSA* AIT.) SEEDLING SEED ORCHARD

Introduction

Red pine has been an important tree species in the Minnesota Tree Improvement Cooperative since its inception in 1981. To date, eight red pine seedling-seed orchards have been established, and five have been rogued. All five orchards were rogued using family selection based on tree height measurements only. The average genetic gain at 15% selection intensity for these orchards was 4.6 %. Other selection methods that incorporate information on individuals as well as families may increase the genetic gains possible in seed orchards. In addition, selection methods that incorporate volume calculations may further increase genetic gains when applied to this species. We hypothesized that a combined index selection based on tree volumes would maximize genetic gains within a seed orchard when compared to family selection.

Methods

The red pine seedling seed orchard managed by St. Louis County is located near Central Lakes, Minnesota at approximately $47^0$18' North latitude $92^0$29' West longitude. This orchard consisted of 108 families from "plus" trees selected in Minnesota and Wisconsin, arrayed in single-tree plots in 24 replications for a total of 2382 planted seedlings (not all families were fully replicated). Seedlings were planted in May 1988 as 1-0 containerized stock at 10 x 10 ft spacing, and were 12 growing seasons from planting at the time of measurement. In the Fall of 1999, individual tree heights were measured to the nearest 0.1 ft using a range pole and diameters were measured at breast height (four feet or 1.5 m above ground) to the nearest 0.5 inches using metal calipers. Volume was calculated using a formula for total cubic foot stem volume (Gevorkiantz and Olsen 1955; Ek, 1985) converted to cubic meters:

$$v = \left[\left((dbh)^2 \times (ht) \times (0.005454)\right) \times \left(0.42 + \left(0.006(30 - ht)\right)\right)\right] \times (0.02832)$$

where dbh is diameter in inches, and ht is height to the top of the live crown measured in feet. Eight families with low representation in the orchard (number of individuals per family less than five) were removed from analysis, leaving 100 families.

Estimated genetic gain was calculated for nine different selection intensities (10%, 12%, 15%, 17%, 20%, 23%, 25%, 27% and 30%), but are only reported for four (15%, 20%, 25%, and 30%). Four selection methods were also tested, following formulas from Falconer (1989): (1) individual selection, (2) family selection, (3) family plus within family selection (FWFS) and (4) combined index selection. Individual tree volumes were adjusted for differences among replications (Cotterill 1987; Cotterill and Dean 1990). Determination of retained individuals for each selection intensity was accomplished using adjusted individual volumes to rank individuals. For individual selection, the top 15% trees were kept based on their calculated tree volumes. In family selection, family means were estimated, and the top families are retained in the orchard. For FWFS, the top seven individuals are retained from each of the top families. Family means and heritabilities were used to create an index value (*index*) for combined selection:

$$\text{index} = \left(\text{vol}_i * \left(h_i^2\right)\right) + \left(\text{vol}_f * \left(h_f^2\right)\right)$$

where vol_i is the volume of the individual (adjusted for replication effects), vol_f is the mean volume of the family (adjusted for replication effects), and h^2_i and h^2_f are the individual and family heritabilities respectively.

Results

Overall plantation survival was high at 93.3%. The average height was 5.6 m (18.4 ft); average diameter was 10.4 cm (4.1 in.); and the plantation mean volume was 0.957 m^3 (33.8 ft^3). Analysis of variance for individual tree volumes indicated significant differences were present among replications (p = 0.0355) and families (p =0.0002) (Table 1). Individual (h_i^2) and family (h_f^2) heritabilities were calculated at 0.18 and 0.335 respectively.

Table 1. ANOVA for volume in the St. Louis County red pine seedling seed orchard.

Source	Df	Type III SS	MS	F	Prob>F
Rep	23	8.836	.384	6.42	.0002
Family	99	13.213	0.1333	2.23	.0355
Rep x family	2072	131.472	0.0635	1.06	.4823
Error	16	0.958	0.0598		
Total	2209				

Estimated genetic gain for volume ranged from 8.0% (Combined index selection at 15% selection intensity) to 4.5% (FWFS at 30% selection intensity). The combined index selection method always generated the largest estimated genetic gain, followed by individual selection, and either family selection or FWFS (Table 2). The highest genetic gain, 8.9%, was obtained at the 10% level for combined index selection. The lowest was 4.5%, obtained by family selection at 30% intensity (Table 2).

Table 2. Theoretical genetic gains for each of four selection methods, at four different selection intensities.

	----Selection Intensity----			
Selection Method	15%	20%	25%	30%
Combined Index	8.0	7.1	6.5	6.1
Individual	7.7	6.8	6.2	5.8
FWFS	7.6	6.6	5.6	4.5
Family	7.4	6.7	6.1	5.5

Discussion

The high survival rate at this orchard allowed for a well balanced statistical design (average family size equals 22.1 individuals), to estimate genetic parameters. Narrow sense heritability for individual stem volume (h^2_i= 0.18) compared favorably with other pine species such as *P. tecunumanii* (0.15), *P. oocarpa* (0.29), and *P. elliottii* (0.12), (Hodge and Dvorak 1999; Moura et. al 1998; Dieters et al 1995, respectively) indicating that volume is a trait under low to moderate genetic control in this population of red pine.

Despite the inherently low level of natural genetic variation in red pine, all four selection methods tested resulted in a range of theoretical genetic gains from 4.5 to 8.9%, indicating that improvements in volume are possible. Combined index selection would result in an 8.9% increase in stem volume when applied at a 10% selection intensity, and 8.0% at a 15% selection intensity. This is considerably greater than estimates obtained using family selection at a 15% intensity on tree heights, where gains in other Co-op orchards were as low as 1.5%. The gains calculated for St. Louis County using combined index selection are similar to the stem volume increase of 9.0% reported for a 290 family, ten year old, red pine seedling seed orchard in Wisconsin (Guries and Ager 1980). Combined index selection also retains a greater number of families over family selection, thereby retaining more genetic diversity within the seed orchard.

Within each selection intensity, the four selection methods provided similar theoretical genetic gains, never varying by more than 1.6% in the four selection intensities shown (Table 2). For each selection intensity, genetic gains estimated by family selection were lowest. This effect is due to the retention of all the trees within a family (both good and bad performers) with this selection method. This method also retains the fewest number of families, thus increasing the chances of inbreeding within the orchard. FWFS selection excludes the poorer performing trees within a family, but fixes the number of trees included at seven, which excludes some of the better performing individuals in the best families. Individual selection consistently ranked second for estimated genetic gain across all selection intensities. This selection method includes the best performing individuals, but does not account for heritability and family effects. The genetic gains predicted by combined index selection were consistently higher than all other selection methods for all selection intensities. This method weighs both family and individual performance according to their respective heritabilities. The advantage of including heritabilities in the calculation is that the amount of "additive genetic variation," (the variation that is most easily inherited) is considered. This allows for an increase in genetic gain over individual selection and also increases the number of families selected relative to family selection.

It should be noted that the measurements and selection simulations are based on 13 year- old trees that have not reached rotation age. Family differences may assert themselves later in the rotation, changing the individuals or families that would be retained. Conversely, family differences may become less distinct over time. Height and volume calculations made on red pine seedling seed orchards in Wisconsin indicated that families and stands were a significant source of variation at age 6 but that by age 10 stands had become a nonsignificant source of variation (Lester 1976; Guries and Ager 1990). Selection after 12 growing seasons in the field may be early, but the orchard must be rogued to retain full crowns, maximizing seed production. Earlier measurements may have detected a trend regarding family performance, and indicated whether family differences could be expected to increase or decrease in this population of red pine.

Literature Cited

Cotterill, P.P. 1987. Short note: On estimating heritability according to practical applications. Silvae Genet. 36:46-48.

Cotterill, P.P. and Dean, C.A. 1990. Successful tree breeding with index selection. CSIRO Publications, Victoria, Australia. 80 p.

Dieters, M.J., White, T.L. and Hodge, G.R. 1995. Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). Can. J. For. Res. 25(8):1397-1408.

Ek, A.R. 1985. A formula for the total cubic foot stem volume of small trees in the lake states.

North. J. App. For. 2:3.

Grevorkianz, S.R. and Olsen, L.P. 1955. USDA Forest Service Techical Bulletin No. 1104. Lake States Forest Experiment Station. 51p.

Guries, R. and Ager, A. 1980. Red pine seedling seed orchards: 10 year results. Forestry Research Notes, Department of Forestry, University of Wisconsin. No. 242, 4 p.

Hodge, G.R. and Dvorak, W.S. 1999. Genetic parameters and provenance variation of *Pinus tecumumanii* in 78 international trials. For. Gen. 6(3):157-180.

Lester, D.T. 1976. Height growth of red pine families in seedling seed orchards 6 years after planting. Forestry Research Notes, Department of Forestry, University of Wisconsin. No. 196.

Moura, V.P.G., Dvorak, W.S. and Hodge, G.R. 1998. Provenance and family variation of *Pinus oocarpa* grown in the Brazilian cerrado. For. Ecol. Mgt. 109(1-3):315-322.

OUTLOOK

The Minnesota Tree Improvement Cooperative has ended its 21st year with a flurry of activity. Co-op orchards remain productive in most species, and we have begun to investigate different aspects of seed production with several applied research projects. Continued management of seed orchards is recommended to maximize seed production. Fertilization, mowing, and pest control should continue on a regular basis. In coming years, the application of the flower-inducing hormone $GA_{4/7}$ may enhance seed production in orchards. A $GA_{4/7}$ project in red pine was initiated in 2001 to establish a protocol for its application to this species.

The first set of measurements of the second-generation population of jack pine were taken in 2001. Controlled crosses of white spruce will continue into 2002 until at least 100 crosses are complete. Half of the white spruce crosses are being grown in the Potlatch Nursery. Crosses may also be made in white pine in 2002 (flowers permitting), and in Wisconsin jack pine orchards in future years. The Co-op's original goals of improving genetic gain and maintaining a broad genetic base for a variety of important tree species, remain the same for the second-generation material as well as the first.

Most of the Co-op's breeding programs have focused on improving height growth. Other factors including volume and wood quality will be included in future selections to further improve the quality of orchard seed. White pine researchers have improved our understanding of the mechanisms of blister rust resistance. A protocol for screening seedlings for resistance to blister rust has been tested and refined. Seeds for a large comparison trial of white spruce are also at the Potlatch Nursery, and set to be planted in 2003. Additional comparison trials testing different sources of red pine may begin in 2002. Given all that has happened with the nation's economy in 2001, and the fact that we have lost several members, there remains a solid core of members supporting tree improvement in Minnesota.

2002 COOPERATIVE WORK PLAN

White spruce:

- Outplant new ramets to MN DNR, Blandin, Lake, Itasca and St. Louis County orchards
- Plan and prepare sites for 2nd generation
- Continue breeding at General Andrews Nursery
- Measure 1993 comparison trials

Red pine:

- Count flowers on GA trials
- Increase level of seed orchard management
- Collect seed for comparison trial

White pine:

- Make controlled crosses at breeding arboretum at Cloquet Forestry Center
- Management of new orchards: Rajala/Itasca, St Louis
- Continue research related to blister rust resistance

Black spruce:

- Grafting winter 2002 for additions to Larsaybow seed orchard

Jack pine:

- Control weeds at 2nd generation sites
- Manage 1st generation orchards and collect cones as needed

Other:

- January 2002 workshop
- Organize Fall 2002 workshop
- Database upkeep
- Cooperator visits

APPENDIX

MINNESOTA TREE IMPROVEMENT COOPERATIVE

2001 ADVISORY COMMITTEE

MEMBERS	
Beltrami County	Greg Snyder
Blandin Paper Company	Jim Marshall
Cass County	Mike Wadman
Crow Wing County	Tom Cowell
The Timber Company	Joel Aanensen
Iron Range Resources and Rehabilitation Board	Dan Jordan
Itasca County	Mike Gibbons
Itasca Greenhouse Inc.	Bill Sayward
Koochiching County	Tim Probst
Lake County	Bill Nixon
Minnesota DNR - Forestry	Rick Klevorn
Potlatch Corporation	Mike Fasteland
Rajala Companies	Beth Jacqmain
St. Louis County	George Kirk, Mark Pannkuk
Univ. of Minnesota Dept. of Forest Resources	Alan Ek
USDA Forest Service NCFES Forest Pathology and Genetics	Charles Michler, Paula Pijut
Wausau-Mosinee Paper Corp.	Pat Scheller
SUPPORTING MEMBERS	
Carlton County	Milo Rasmussen
Clearwater County	Bruce Cox
Hedstrom Lumber Co.	Howard Hedstrom
Hubbard Co.	Allen Lysdahl
Minnesota Nursery and Landscape Association	Bob Fitch