University of Minnesota
College of Natural Resources
Department of Forest Resources

MINNESOTA TREE IMPROVEMENT COOPERATIVE

2002

ANNUAL REPORT

Prepared by:

Carolyn C. Pike
James C. Warren
Andrew David

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

SUPPORTING MEMBERS
Carlton County
Clearwater County
Hedstrom Lumber Company
Hubbard County
Minnesota Nursery and Landscape Association
Minnesota Association of Soil and Water Conservation Districts
Pine County

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 22nd year with a strong core of cooperators and many projects planned for the near future. In 2002 it was funded by 15 full members and 7 supporting members. Total dues received was $55,278. Dues were not paid by one member ($4,215), but may be recovered in 2003. Advancements were made in the genetics programs for all five species. Priorities for 2002 included completing pollination of white spruce, collecting data from red pine $GA_{4/7}$ trial, measuring 1993 white spruce comparison trial, collecting white spruce bud-break data, planting white spruce grafts into Co-op seed orchards, and orchard visits.

Regular Co-op expenses (supplies, travel) were $13,427 and $40,747 for personnel. A portion of Pike's and Warren's salary is paid by the white pine grant, reflecting the proportion of their time spent on those projects. Visits were made to 27 Co-op orchards by Pike, Warren and/or David. Dr. Andrew (Andy) David continues as Director, Carolyn (Carrie) Pike as Coordinator, and James (Jim) Warren as Research Fellow. The Advisory Committee met twice for business meetings, once each in January and March 2002. The annual 2001 workshop was held in January 2002 at the Cloquet Forestry Center. The 2002 workshop took place in November at the North Central Research and Outreach Center in Grand Rapids.

Co-op members now manage 39 seed orchards covering 151 acres. Cones were collected from white spruce and white pine orchards this year. A total of 110 controlled crosses were made at the white spruce breeding arboretum in Willow River, which should complete the second-generation population. Ten-year tree heights and diameters were measured at three out of four white spruce comparison trials. Cone and seed insects reared their ugly heads at two orchards, and warrant future monitoring/control.

Pike attended the state SAF meeting in St Cloud MN, the annual meeting of the Aspen/Larch genetics Co-op, and the Superior-woods tree improvement Co-op's annual workshop in Longlac, Ontario. Presentations were given for the NCROC Natural Resource seminar series, the MTIC annual workshop, and DNR silviculture workshop. A short introduction to tree improvement was given by David to Vermilion College students at Cloquet Forestry Center.

Outlook for 2003: Grafting at Itasca Greenhouses will begin this winter for Koochiching County's black spruce seed orchard. Grafting of white spruce for Blandin Paper Company will continue at Itasca greenhouses. At General Andrews Nursery in Willow River, white spruce grafting will continue for the MN DNR and other MTIC cooperators. Six reps of a new white spruce comparison trial will be planted in spring 2003, as well as three reps of the white spruce second generation material and a white pine trial. Five-year measurements will be taken on second-generation jack pine, white pine blister rust field trial, and Norway spruce provenance trial. Seeds from various red pine sources will be collected in preparation for a future red pine comparison trial.

2002 Annual Report

Minnesota Tree Improvement Cooperative

Table of Contents

INTRODUCTION

In its 22nd year of existence, membership in the Co-op's remains strong, and progress continues to be made. The Co-op works primarily with five major forest tree species native to the upper Midwest: black spruce, white spruce, jack pine, red pine and white pine. Now that the second-generation populations are nearly complete for two species, cooperators have expressed an interest in incorporating wood quality traits into future selections. Potential traits could be branch angle, specific gravity and possibly micro-fibril angle.

High priority activities for 2002 included completing controlled crosses of white spruce, collecting data from the red pine $GA_{4/7}$ trial, measuring the 1993 white spruce comparison trial, collecting bud-break data on white spruce, and regular orchard visits.

This report describes the Co-op's program and summarizes the activities and accomplishments from January 1 to December 31, 2002. It is organized into six sections: Administration, Finances, Seed Orchards, Species Reports, Applied Research Projects and Outlook.

ADMINISTRATION

Carrie Pike continues as the Coordinator of the Cooperative. This position consists of managing day-to-day activities, organizing and running business meetings, organizing the annual workshop, conducting field visits, assisting with field-work, and conducting statistical analysis on data from Co-op plantings. Dues payments are received and processed, and finances are managed as well.

Jim Warren's appointment was continued through 2002, and he worked 20% time for the Co-op. Jim's primary responsibility is to assist with field-related work, manage the white pine breeding arboretum at the Cloquet Forestry Center, and his computer expertise is invaluable in helping to manage the Co-op's tree record database in Microsoft Access. Pike and Warren are stationed at the Cloquet Forestry Center.

Andy David has entered his fourth year as Director of the Cooperative. In addition to teaching duties at the University of Minnesota in St Paul, Andy leads research projects, assists with MTIC long-term planning, and is Director of the Aspen/Larch Genetics Cooperative. Andy is stationed at the North Central Research and Outreach Center in Grand Rapids, Minnesota.

The Advisory Committee consists of representatives from each member of the Co-op. It met four times during 2002 for each of two business meetings and two workshops. Visits were made to 27 orchards by Pike, Warren and/or David. Orchards not visited in 2002 will be visited in 2003. Pike presented at three meetings. At the MTIC Fall workshop Pike presented the preliminary findings from ten-year measurements of the white spruce comparison trial. In

November Pike presented "Traditional breeding vs Genetic engineering: a comparison of the risks and benefits for forestry applications" at the NCROC Natural Resources Seminar Series in Grand Rapids, MN. In December 2002 Pike was invited to present "The Minnesota Tree Improvement Cooperative: Projects and Progress" at the MN DNR silviculture workshop given for the Division of Forestry held at the Cloquet Forestry Center.

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 39 seed orchards that are still used for seed collection. Seven orchards were established prior to 1981. Twenty-four orchards were established between 1981 and 1997; two were added in 1998, three in 1999, and three from 2000-2002. Five of the orchards contain improved first-generation material, four contain full-sib crosses, and the rest are first-generation seedling or clonal orchards. All but three of the Co-op's first generation seedling seed orchards have been rogued. A summary of the types and sizes of orchards managed by members of the Co-op is shown in Table 7. Table 8 lists all orchards by species and owner. These tables include only those orchards that are actively managed and utilized as seed sources.

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

	Black spruce	Jack pine	Norway spruce	Red pine	White pine	White spruce
1st Generation Clonal	10.0		0.6		13.3	25.6
1st Generation Seedling-Seed	8.2	40.8		42.2		4.1
2nd Generation Full-sib	3.0	6.4				Coming Soon!
Total acreage	21.2	47.2	0.6	42.2	13.3	29.7

Table 2. Seed orchards of Minnesota Tree Improvement Cooperative, 2002.

Species	Organization	Orchard	Established	Size(ac)	Live Trees
Black spruce	Blandin Paper Co.	Blackberry	22-May-78	2.5	596
Black spruce	Koochiching Co.	Big Falls	19-May-89	2.3	61
Black spruce	Koochiching Co.	Larsaybow	27-May-98	4	52
Black spruce	Minnesota DNR	Eaglehead	17-May-78	2.7	582
Black spruce	Minnesota DNR	Split Rock	27-May-92	2.4	262
Black spruce	Potlatch Corp.	Cloquet	01-May-78	3	580
Jack pine	Cass/Beltrami/Hubbard Co.	Deep Portage	08-Oct-82	3.4	492
Jack pine	Crow Wing Co./Minnesota DNR	County Line	01-May-99	2.6	1705
Jack pine	Crow Wing County	Crow Wing	04-Jun-85	2.1	320
Jack pine	Great Lakes Nursery Company	Pine Lake	24-May-88	1.7	270
Jack pine	IRRR Agency	Calumet	16-Sep-82	1.7	214
Jack pine	Minnesota DNR	Longprairie	18-May-84	4	495
Jack pine	Minnesota DNR	Nickerson	15-May-84	2.4	403
Jack pine	Potlatch Corp.	Gillogly Rd.	28-Jun-83	5.5	171
Jack pine	St. Louis County	Ellsburg Rd.	10-May-88	1.6	280
Jack pine	St. Louis County/IRRRB	Ellsburg Rd. East	12-May-99	3.9	2574
Jack pine	Wausau-Mosinee Paper Corp.	Barnes	27-May-88	4.1	549
Norway spruce	Blandin Paper Co.	College S.O.	23-May-01	0.55	152
Red pine	Cass/Beltrami/Hubbard Co.	Blind Lake	10-Sep-91	5.3	2249

Red pine	Minnesota DNR	Cotton	29-Jul-86	4.5	466
Red pine	Minnesota DNR	Eaglehead	25-Jun-81	3.6	390
Red pine	Plum Creek Timber Company	Ashwabay	17-Sep-85	5.5	401
Red pine	Plum Creek Timber Company	Petenwell	24-Apr-90	5.5	1576
Red pine	Potlatch Corp.	Gilogily Rd.	10-Jul-81	6.6	586
Red pine	St. Louis County	Ellsburg Rd.	09-May-88	5.5	536
Red pine	Wausau-Mosinee Paper Corp.	Mosinee	23-May-90	5.7	1174
White pine	Minnesota DNR	St. Francis	15-May-85	3	318
White pine	Rajala/Itasca County	Bass Lake	19-May-98	5.7	530
White pine	St. Louis County	Ellsburg Rd.	02-May-90	1.1	233
White pine	St. Louis County	Ellsburg Rd. East	21-Jun-99	2.5	245
White spruce	Blandin Paper Co.	Arbo	01-May-76	1.5	121
White spruce	Blandin Paper Co.	College	05-Sep-00	2.9	628
White spruce	Blandin Paper Co.	Latimer	15-May-67	4.1	224
White spruce	Itasca County	Fig. Eight Lake	02-Sep-87	1.1	187
White spruce	Lake County	Two Harbors	02-Sep-87	1	201
White spruce	Minnesota DNR	Cotton	01-May-77	12	206
White spruce	Minnesota DNR	Split Rock	02-May-02	2.3	100
White spruce	Potlatch Corp.	Cloquet	01-May-77	3.3	140
White spruce	St. Louis County	Ellsburg Rd.	11-May-88	1.5	212

Cone Collections

Cone collections were generally low this year in red and jack pine, but very high in white spruce and white pine. Details are provided in the species reports below.

Table 3. Cone collections from MTIC orchards in 2002.

Species	Orchard	Bushels
White spruce	Cotton	325.0
	Arbo	24.6
	Latimer	45.3
	Ellsburg Rd	12.0
	Two Harbors	5.5
White pine	St. Francis	100.0
	Ellsburg Rd	6.0
Jack pine	Long Prairie	60.0
Total:		578.4

SPECIES REPORTS

Black spruce

Status

Black spruce remains an important conifer for lowland plantings in Minnesota. Most planting is accomplished through aerial seeding. Most plantations are established on upland sites, where white spruce has more competitive growth rates than black spruce. Thus, progress in black spruce was advanced marginally in 2002.

Koochiching County's **Larsaybow** and **Big Falls** orchards were fertilized in the Spring of 2002. The remaining trees at **Big Falls** are growing well, and this orchard will continue to be used for cone collection. All new ramets will be planted in the **Larsaybow** orchard which has better soil moisture than the **Big Falls** site.

The MN DNR's **Eaglehead** orchard was topped for cone collections in 2001. Some trees in Blandin's **Blackberry** orchard were topped for collecting in 2001 as well, but most of the trees were left un-topped. Survival at the **Eaglehead** and **Blackberry** orchards is excellent.

A progeny test planted at **CFC** (in 1978) continues to thrive, and may be used as a source of scion material. Ten-year measurements are planned for Fall 2004 from an additional full-sib test at **CFC**.

Short and long-term planning

Grafting is expected to begin for Koochiching County's **Larsaybow** orchard in March 2003. Five Co-op orchards will continue to be maintained for seed collection. In general, compared to white spruce, black spruce has fewer insect and disease problems and wood quality is superior. Interest in improving black spruce has waned in recent years, but could see a resurgence if wood quality traits are valued over biomass.

White spruce

Status

In the past few years (1998, 2000, 2002), white spruce breeding has demanded a great investment of time in the early spring. In 1998, 90 crosses were made but seed set was very low. In 2000, a few additional crosses were made, but few cones were available for breeding. The seeds from 1998 and 2000 crosses are being grown at Potlatch tree nursery and will be planted in 2003. In 2002, the cone crop was excellent, and over 100 crosses were made. The seeds from the 2002 crosses are being cleaned this January 2003 by Kathy Haiby at NCROC in Grand Rapids (Thanks Kathy!!). If sown in Fall 2003, then seedlings should be out-planted in spring 2005.

Three white spruce orchards (**Ellsburg Rd**, **Fig.-8 Lake** and **Two Harbors**) have been genetically improved by adding new grafts from the top-25 ranked families. Ninety-one grafts were added to Lake County's **Two Harbors**, and 79 to Itasca County's **Fig.-8 Lake.** Fifty ramets were added to St. Louis County's **Ellsburg Rd** orchard in 2001, and filled all remaining open spaces. Blandin's **Latimer** and **Arbo** seed orchards were visited and are growing well. MN DNR's **Cotton** seed orchard had a bumper cone crop in 2002, and the trees have been topped to facilitate cone collections.

Two new improved first-generation orchards have recently been established: Blandin's **College** seed orchard was started in 2000, and the MN DNR's **Split Rock** was started in 2002. In addition, two new improved first-generation orchards are planned, one each by **Potlatch** and **Red Lake**. Potlatch is planning to replace the **Cloquet** orchard, the access to which was lost due to ownership changes. Planting will begin at Gilogly Rd in Spring 2003 using grafts made at General Andrews Nursery.

Short and long-term planning

Grafting will continue at Itasca Greenhouses for Blandin's **College** seed orchard. Grafting will also continue for one more year (hopefully the last) at General Andrews Nursery for the MN DNR and other cooperators. In Spring 2003, the first batch of second-generation material (crosses made in 1998 and 2000) will be planted at three sites (MN DNR, St Louis County, and Itasca County). The remaining 100 crosses that were produced in 2002 will be planted at three additional sites (MN DNR, Blandin, and Lake County). Six reps of a white spruce comparison trial will also be planted in Spring 2003, representing the best white spruce material from the first generation population.

The **Rabbit Lake** progeny test (planted 1986) was visited in Fall 2002. Mortality at this site was high immediately after planting. The remaining trees are growing well however, and 20-year measurements will be taken from portions of the trial in 2004. The MN DNR's **Ross Lake** progeny test will be evaluated in 2004. Portions of this trial might also be included in 20-year measurements.

Jack pine

Status

Jack pine remains the work-horse species of the Cooperative. The two remaining second-generation plantings (St. Louis County/IRRR Agency, Crow Wing County/MN DNR) continue to grow at breakneck speed. These sites will be five years old in 2003, and will be re-measured then.

The Co-op's first-generation orchards continue to flourish and produce seed. MN DNR's **Long Prairie** seed orchard was topped in Fall 2002. The Potlatch **Gillogly Rd.** is recouping from topping in 2001. No cones were collected in 2002 from **Gillogly Rd.**, MN DNR's **Nickerson**, **Crow Wing**, or **Ellsburg Rd.** seed orchards. IRRR Agency's **Calumet** orchard is thriving after topping in Fall 2001. Wausau-Mosinee's **Barnes** orchard was also topped in Fall 2001.

Cass-Beltrami-Hubbards's **Deep Portage**, DNR's **Bemidji**, DNR's **Link Lake**, and Potlatch's **Kallstrom** seed orchards are currently not being managed for cone collection. Great Lake Nursery and Lac du Flambeau (non-members) inherited **Pine Lake** and **Sand Lake** orchards respectively. **Sand Lake** orchard was visited in 2001 and remains in good condition. **Pine Lake** has not been visited in recent years and its status is not known. Red Lake also owns a seed orchard (**Red Lake**), which will likely be managed for future cone collections.

Short and long-term planning

Many years were spent creating the second-generation population that now resides at Crow Wing and St. Louis Counties. Five-year measurements will be taken from these populations in Fall 2003. In addition to measuring tree heights and diameters, wood quality traits may also be measured at this time. Branch angle, specific gravity, and gall rust incidence are potential traits to be measured.

Red pine

Status

Red pine, the single most planted tree in Minnesota, continues its run as the most vexing tree species for tree improvement specialists. The cone crop in 2002 was low, and the incidence of cone and/or seed insects is rising. There is simply not enough improved seed for Cooperators. Potlatch's **Gillogly Rd** orchard had a disappointing cone year and many shriveled (aborted) cones were found. Cone/seed insects may require control to prevent future losses. Plum Creek Timber Company's **Ashwabay** and **Petenwell** orchards are both in excellent condition. The cone crop at **Ashwabay** was non-existent in 2002, but the yet-to-be rogued **Petenwell** had its first sizeable crop. This orchard could be measured for roguing as early as 2003. The first-year cone crops at St Louis County's **Ellsburg Rd** and MN DNR's **Cotton** orchards was high, and will be ready for picking in 2003. MN DNR's **Eaglehead** orchard also did not produce a sizeable cone crop this year. Plans will be made to visit Cass-Beltrami-Hubbard's **Blind Lake** and Wausau-Mosinee's **Mosinee** orchards in 2003. These orchards are still several years from roguing.

The results of the selection and roguing plan for St Louis County's Ellsburg Rd orchard will be published in the journal Theoretical and Applied Genetics in 2003 entitled, "Comparison of selection methods for optimizing genetic gain and family diversity in red pine (*Pinus resinosa* Ait.) seedling seed orchard," by A.David, C.Pike and R. Stine.

Short and long-term planning

Orchards need to be monitored for cone and seed insects. Fertilization to induce flowering should be considered as part of a regular management regimen. The growth of **Blind Lake**, **Mosinee**, and **Petenwell** orchards needs to be monitored carefully to determine when roguing should occur.

White pine

Status

St Louis County's **Ellsburg Rd** produced its first abundant crop. **Ellsburg Rd East** is growing well. Rajala/Itasca County's **Bass Lake** seed orchard is growing well, fully enclosed and mowed routinely. Field grafting there should begin in either 2003 or 2004. MN DNR's **St Francis** orchard produced an abundant cone crop, which was picked in 2002.

The USFS Forest Service has done work in white pine for many years. A progeny test of plus-trees (parents of which reside in MTIC orchards) was planted in 1982 and 1983 by the Forest Service. Four replications were planted in 1982, and an additional four were planted in 1983. Of those planted in 1982, two plantings remain in good shape. Of those planted in 1983, three are in good to excellent condition. Pike, Warren and K. Haiby obtained twenty-year measurements at one of the sites (located outside Grand Marais Minnesota). The second site is located near in St Ignace Michigan and is expected to be measured in winter 2003.

Short and long-term planning

Orchards will continue to be managed for seed and cone production. Five-year measurements will be taken in fall 2003 from the blister rust field trials that were planted in 1999. Field grafting for Rajala/Itasca County's **Bass Lake** seed orchard should begin in either winter 2003 or 2004. In addition, three reps of a white pine regional provenance trial (work begun by Dr. Paul Anderson) will be planted in spring 2003.

APPLIED RESEARCH PROJECTS

Preliminary Results Of Observations Of Phenological Differences Between Two Sources Of White Spruce

Abstract

The timing of bud-break and shoot elongation was observed for each of two sources of white spruce in the spring of 2001 and 2002. Preliminary analysis indicates that bud-break and shoot elongation times were similar between sources, although Minnesota sources tended to be more advanced than Ontario sources at each date. This suggests that Ontario sources might be less prone to early frost damage than local sources, and would not widen the window of feeding for spruce budworm. Future studies on the feeding preferences of budworm and the seasonal growth phenology of improved and unimproved sources of white spruce would further this work.

Introduction

The purpose of this study was to investigate bud-break and shoot elongation 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 contain genotypes that originate from two geographic regions: northern Minnesota and southeastern Ontario. Studies have found that growth rates of sources from southeastern Ontario typically exceed most other sources (Nienstaedt & Teich, 1971; Stellrecht et al. 1974). The reason for this "superiority" of selections from Ontario is not known. Several possibilities exist: either Ontario trees have an extended growing season (breaking bud earlier or setting bud later) or these trees are physiologically more efficient, producing more wood than other sources over the same time period. Until these differences are elucidated, questions remain as to how improved trees outgrow other trees of the same species.

White spruce occurs across a wide geographic area, and exhibits a high level of genetic variability. Traits under strong genetic control (such as wood quality or bud break phenology) are less "plastic" than traits under weak genetic control, and are exhibited regardless of the environment in which the tree is grown. In the case of bud-break phenology, trees that break bud early may be more susceptible to early spring frosts. In addition, trees that break bud early could impact the overall feeding period of the spruce budworm (*Choristoneura fumiferana* (Clem)). Spruce budworm overwinters as a second-instar larva, and upon emergence in the spring feeds on the new buds of staminate flowers of white spruce or its preferred host balsam fir (Kucera and Orr, 1981). If no staminate flowers are present, larvae will feed on previous year's needles. Since older needles are less nutritious to the budworm than new growth, a tree that breaks bud after larvae have begun feeding may not be as desirable a target for the budworm. In other words, synchrony between budworm feeding and bud break in host trees is an important factor in the overall success of budworm population (See Mattson et al. 1991 for a list of references). This study attempts to lay the groundwork for understanding differences in growth characteristics between different sources of white spruce.

Methods

Data was collected from the Co-op's white spruce breeding arboretum located in General Andrews Nursery in Willow River, MN. This planting is a clone-bank of genotypes that are planted in various MTIC orchards. One ramet from each of 20 different clones was selected randomly for observation. Ten ramets originating from southeastern Ontario, and ten ramets originating from northern Minnesota were chosen at random. In 2001, the terminal leader of each subject tree plus one branch from each cardinal direction was selected at three different positions in the crown (lower, middle, upper) for a total of 13 branches per tree. Each selected branch was marked and observed throughout the spring. Results from 2001 indicated that variability among branches on a tree was small, so in 2002 only four branches per tree were used, one from each of four cardinal directions on the middle crown. Beginning on April 30, 2001, and again on May 17, 2002 each branch was observed and scored based on the stage of development of either the terminal or most distal bud. In 2001, observations were taken at eight dates at unequal intervals until May 23, 2001 (Table 1). In 2002, observations were taken at eleven dates until June 11, 2002. 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 – Elongation has begun, bud-cap is translucent and 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.

To make comparisons across years, changes in bud phenology were plotted against cumulative degree-days. Degree-days were estimated using maximum and minimum temperatures obtained from a NOAA weather station located in Moose Lake, MN, roughly eleven miles from the study site. For each day beginning in January of the calendar year, the maximum and minimum temperatures were obtained and averaged to calculate the mean daily temperature. A base temperature of 46.4 degrees, corresponding to the initiation of feeding by spruce budworm (Lysyk 1989), was subtracted from the mean daily temperature. When the difference was less than zero, no degree-days were accumulated for that date. If the difference exceeded zero, the difference became the number of degree-days for that date. Table 1 below provides an example of these calculations. Each stage of bud-break and elongation was scored and correlated with degree-days to observe trends over time and across two different years.

Table 1. Method used to calculate degree-days and cumulative degree-days.

Date (2002)	High temp.	Low temp.	Daily mean	Daily mean - base temp (base temp = 46.4)	No.deg days	No. cum. deg. days
April 7	42º	22º	32º	32 – 46.4 = -14.4	0	0
April 8	59	34	46.5	46.5 – 46.4 = 0.1	0.1	0.1
April 12	42	31	36.5	36.5 – 46.4 = -9.9	0	0.1
April 13	64	35	49.5	49.5 – 46.4 = 3.1	3.1	3.2

Statistical analysis

The average score for each tree was calculated from the 13 observations at each date in 2001, and from the four observations at each date in 2002. The mean score per tree was compared using a one-way ANOVA between Ontario and Minnesota sources at each date with factors “source” (MN vs Ontario), and “date” (each of 8 dates for 2001, 11 dates for 2002). Source and date were treated as fixed factors. Tukey’s HSD test was used to compare significance between sources at each date of observation. All analyses were performed in SAS (1999).

Results

The one-way ANOVA found significant differences at three different dates in 2002, (86, 265, 269 degree-days, Figure 1) which were verified by Tukey’s HSD. At these three dates, MN sources were significantly more advanced than Ontario. In 2001, differences were significant for one date only (159 degree days, Figure 2).

Discussion

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

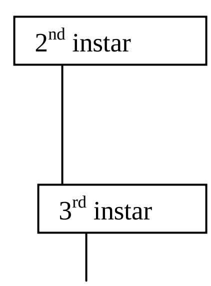
Due to the continuous nature of bud-break and shoot elongation, a categorical system was necessary to classify them efficiently in the field. Significant differences between mean scores of 1.5 and 1.9 would be imperceptible using such a system. Differences exceeding an entire category (ex. 2.0 vs 3.0 or 4.0) might indicate biological significance. The differences observed between sources were small, and the significance may have little biological importance.

In the field, individual trees were highly variable with regard to bud-break timing, and tree-tree variation played a stronger role in phenology than variation due to source alone. 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. An analysis on tree to tree variation is in progress, and will help to filter differences among trees from the same source.

This study was prompted by questions related to forest health and the use of improved material. These preliminary findings indicate that there are no dramatic differences between sources originating in southeastern Ontario and Minnesota. Therefore, improved material is not likely to be any more susceptible to forest pests than unimproved material. This work, and future studies, would also be beneficial to seed orchard managers. By refining the period of bud-break and shoot elongation, the efficiency of irrigation and fertilization could be improved by allowing a seed orchard manager to synchronize these practices 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 (Lysyk, 1989).

Date	Cum. Degree Days	Mean bud-break score Ont.	Mean bud-break score MN
April 30, 2001	75	0	0
May 3	119	2.3	2.3
May 7	142	3.2	3.1
May 9	159	3.6	3.8
May 11	180	3.9	4.0
May 14	215	4.7	4.5
May 18	273	5.8	5.9
May 23	316	6.2	6.3



Date	Cum. Degree Days	Mean bud-break score Ont.	Mean bud-break score MN
May 17, 2002	86	1.5	1.9
May 19	86	1.6	2.0
May 21	88	2.4	2.6
May 24	115	2.7	2.8
May 26	124	3.3	3.4
May 29	170	3.9	4.0
May 31	221	4.5	4.5
June 3	265	4.9	5.4
June 5	269	5.1	5.5
June 7	308	6.1	6.3
June 11	367	7.3	7.0

2nd instar

3rd instar

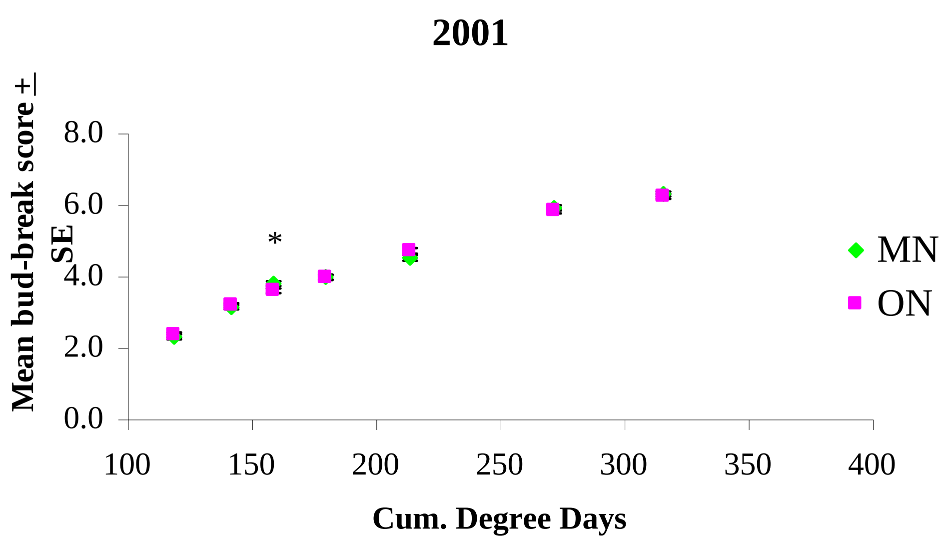


Figure 1. Mean bud-break score vs cumulative degree-days for 2001 Observations. MN sources were significantly more advanced at 159 degree-days only.

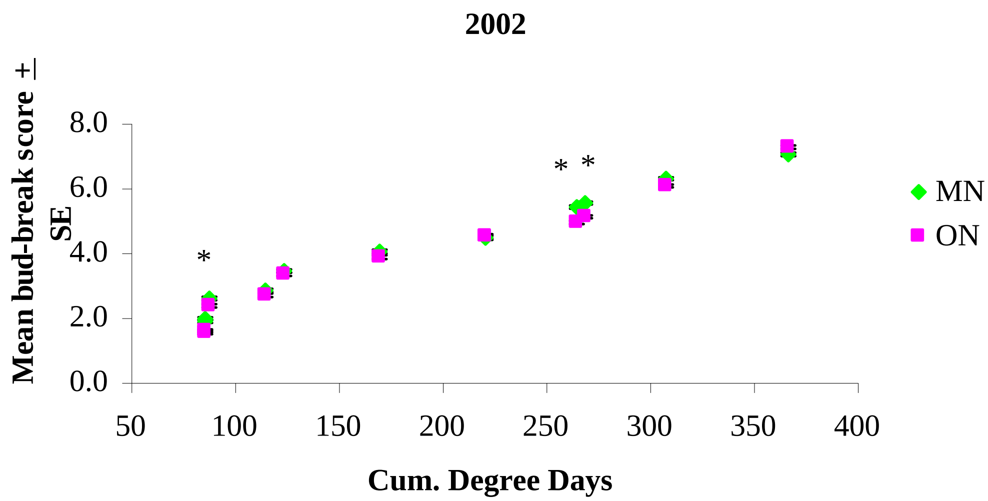


Figure 2. Mean bud-break score vs cumulative degree-days for 2002 observations. MN sources were significantly more advanced at 88, 265, and 269 degree-days.

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1993 White Spruce Comparison Trial: Results Of Ten-Year Measurements

Abstract

Ten-year heights and diameters of a white spruce comparison trial were taken from each of three sites. Differences among sites and sources were found to be highly significant. Norway spruce (planted at one of three sites) had significantly greater heights and diameters than all other sources. Trees originating from the MN DNR's Cotton seed orchard (containing grafts from southeastern Ontario) were significantly larger than woods run, or Ballbluff (a traditional collection site) sources. Tree volumes were 31% (Ontario sources) and 14% (improved Minnesota sources) greater, in terms of volume, than woods run material. Improved material is recommended for use in white spruce plantations in Minnesota, however, intensive management is imperative to increase survival.

Introduction

White spruce has been a component of the Minnesota Tree Improvement Cooperative since its inception in 1981. Even before the Co-op's creation, "plus-tree" selections from Minnesota forests were started in 1967 and progeny-tested at a site located in Grand Rapids. This test was later converted to a seed orchard ("Latimer" owned by Blandin Paper Company) and is now the main source of improved seed of local origin for the Co-op. Over time, the Co-op has included selections from other areas including southeastern Ontario, Michigan and Wisconsin. Provenance tests have indicated that material from southeastern Ontario is "superior" to local sources (Nienstaedt & Teich, 1971; Stellrecht et al. 1974) resulting in its dominant status in several orchards. As Co-op seed orchards have matured, seed was collected for testing purposes. The following report summarizes the results of one such test.

Methods

In 1992, seed was collected from four sites for this test. The sites included: Latimer seedling-seed orchard, a grafted seed orchard containing "plus-trees" selected from southeastern Ontario ("Cotton" seed orchard, in Cotton MN owned by MN DNR), a traditional seed collection site ("Ballbluff", located in Grand Rapids, MN), and two woods run seedlots, one each from Hibbing and Deer River, MN. Norway spruce seed was obtained from a stand in "Gunn Park" near Grand Rapids for planting at one of the sites.

All seed was germinated in April 1992 at Potlatch tree nursery in Cloquet, MN and grown in containers. Seedlings were planted in May 1993 at each of five locations: Grand Rapids MN (Blandin Paper Company), Orr MN (Potlatch Corp.), Gordon WI (Plum Creek Timber Company), Willow River MN (MN DNR). As of this printing, ten-year measurements have not yet been taken from Willow River. Each site consisted of a randomized complete block design with one 100 tree-block per source, per each of five reps. One block of red pine was included in each rep at Gordon WI, and one block of Norway spruce was included at Grand Rapids, MN for comparison.

In fall 1997, five-year heights were measured and reported in the 1998 MTIC Annual Report (Stine 1998). In Fall 2002, 10-year heights and diameters were measured. As many as three stems were measured per tree (for those with more than one stem at breast height), but only the largest diameter stem was included in the analysis. Heights were measured to the nearest centimeter with a height pole. Diameters were measured to the nearest millimeter with a tree caliper. Only one caliper measurement per tree was taken. The results of the 2002 measurements are reported below.

Statistics

Tree volumes were calculated using the standard equation for calculating volume of a cone:

$$\text{Tree Volume} = \pi * ⅓ \left(\left(½*\mathbf{DBH}\right)^2 \text{ Height}\right)$$

Tree heights, diameters, and volumes were analyzed with Analysis of variance (general linear models SAS, 1999) using the model:

$$\mu = L_i + R_j + S_k + L_i S_k + \varepsilon_{ijkl}$$

where "L_i" is the effect of Location, "R_j" is the effect of Rep, "S_k" is the effect of Source, and ε is the within-plot error. All effects were treated as fixed, and volumes were log-transformed to improve normality and improve heteroscaedasticity. Significant differences were analyzed at

α=0.05 and differences among sources were assessed using Tukey's HSD test. The analysis was also performed separately for each site using the reduced model:

$$\mu = R_j + S_k + \varepsilon_{ijkl}$$

where "R_j" is the effect of Rep and "$S_{k,}$" is the effect of Source, and ε is the within plot error. No rep*source interaction was tested. All statistics were performed on SAS (1999).

Results

Survival at Grand Rapids was the lowest among the three sites 44% (Table 1). At all three sites, between 12-16% of trees at all sites were smaller than breast height. Trees smaller than breast height were excluded from further analysis. The incidence of multiple-stemmed trees was highest at Gordon (9%) and less than 5% at Orr and Grand Rapids. Survival among sources was roughly similar (54%-65%) but Norway spruce had the lowest survival rate of all sources (32%).

For the complete model, all effects were significant (Table 2). Because the order of ranks and significance among sources for all sites was identical for tree height, diameter and volume, only volumes are reported here. Norway spruce was significantly largest, followed by Cotton, Latimer, Woods Run and Ballbluff material (Figure 1). The average volume for Cotton material (646 cm^3) was 31% larger than woods run (495 cm^3). Latimer material (564 cm^3) was 13% larger than woods run.

Due to the presence of the site*source interaction, the data was also analyzed at individual sites. Both factors (rep and source) were significant (ANOVA $p<0.05$). At Grand Rapids, Norway spruce was significantly larger than all white spruce sources, and no differences among other white spruce sources was detected (Figure 2). At Orr, Ballbluff was significantly less than all other sources and no other significant differences were found (Figure 3). At Gordon, Cotton and Latimer sources were similar and statistically larger than both Ballbluff and woods run sources (Figure 4).

Discussion

The sites differed greatly in terms of soils and forest type. The site in Gordon was planted adjacent to a red pine plantation on sandy, droughty, soils. Hardwoods, more than grass, were the main competing factor at this site. Blocks planted along the eastern edge of the planting suffered very high mortality due to competition by oak volunteers. This site also had the greatest incidence of multiple stemmed trees, presumably caused by an insect agent (tip weevil). The Orr site was more representative of a northern boreal forest with balsam fir, white birch, and black spruce growing adjacent to the planting. Besides grass, balsam fir, and spruce were common volunteers. The field at Grand Rapids was characterized mainly by heavy grass competition with nearby forests consisting mainly of aspen and birch. Factors related to competition were the most likely agents responsible for mortality at all three sites. No mortality-causing insect or disease agents were visible at any site.

While growth of Norway spruce was excellent, mortality was high (approximately 70%). The cause of the mortality is not known. An adjacent, younger stand of Norway spruce suffered winter burn in 2000 when cold temperatures were combined with little snow during the winter months. The absence of multiple stems on Norway spruce suggests that competition or climate, and not deer browse, influenced the mortality of this species.

Ballbluff performed poorly at all four sites, and is not recommended as a seed source for planting. Improved sources of both Minnesota and Ontario origin typically ranked better than woods run or Ballbluff, even though differences were not always significant at individual sites.

Genetic gains of white spruce for MTIC orchards have been estimated from the Co-op's open-pollinated progeny test to be 8-9% (height growth) and 15% (volume). After ten years, differences in volume among sources were highly evident. The mean volume for Cotton sources was 31% larger than woods run sources, and Latimer was 13% larger than woods run sources. Realized genetic gains (31% for Cotton) exceed estimates from progeny tests (15%). Advances in the genetic improvement of white spruce are clearly being made in plantations in the upper lake states.

Table 1. Characterization of mortality and stem form at three sites.

	Grand Rapids	Orr	Gordon, WI
% Missing trees	56%	15%	24%
% trees w/>1 stem	4%	1%	9%
% trees w/no dbh	16%	12%	14%

Table 2. ANOVA for log-transformed volumes. Type III Sum of Squares are shown.

Source	DF	SS	MS	F	P
Site	2	18.6	9.3	12.24	<.0001
Rep	4	16.5	4.1	5.44	0.0002
Source	4	77.6	19.4	25.60	<.0001
Site*Source	6	33.6	5.6	7.38	<.0001
Error	3377	2561.1	0.7		

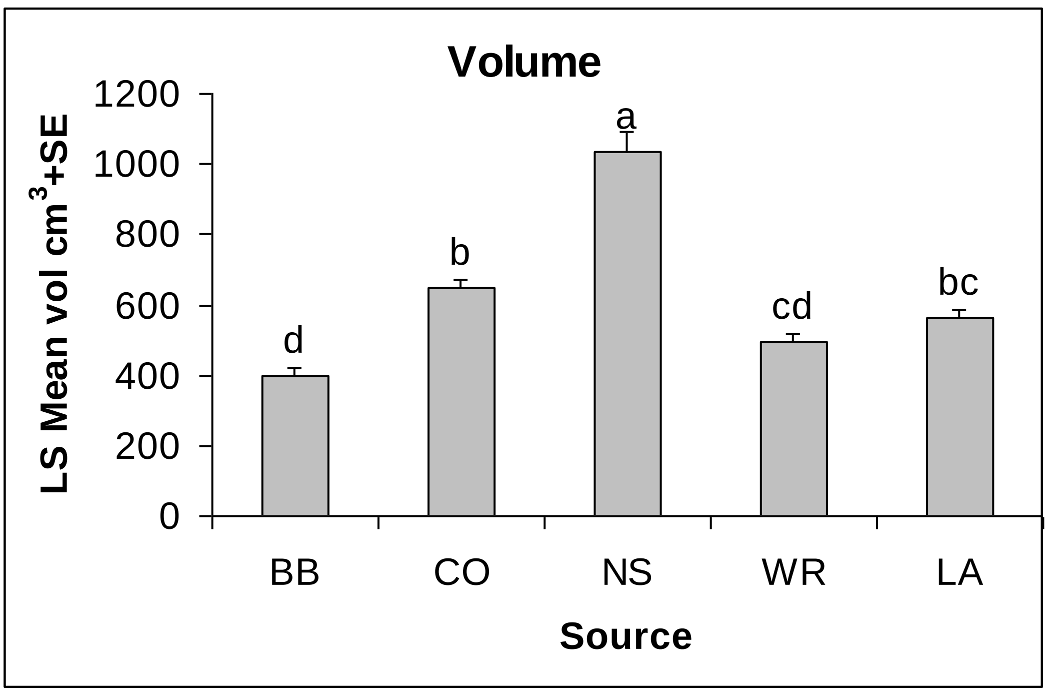


Figure 1. Mean least-squared mean volumes for all sources across sites. N=828, 751, 129, 840, and 846 for BB (Ballbluff), CO (Cotton orchard), NS (Norway spruce), WS (Woods Run), and LA (Latimer orchard) respectively. Letters indicate significant difference among sources ($\alpha = 0.05$, Tukey's HSD test).

Fig. 2. Grand Rapids

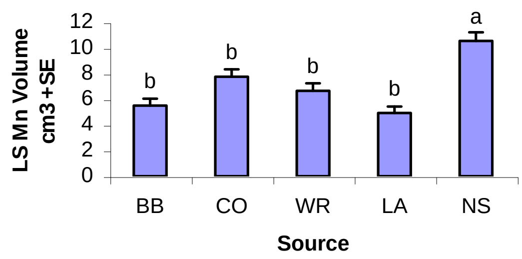


Fig. 3. Orr

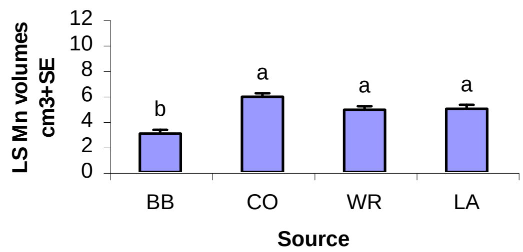
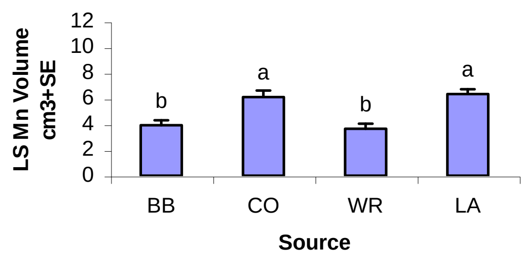


Fig. 4. Gordon, WI



Figures 2-4. Volumes (Least squared means) for each source at each location. Letters indicate significant differences among sources, using Tukey's HSD test with α=0.05.

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Red Pine Cone Induction For Sustainable Seedling Production

Abstract

Two red pine seed orchards were utilized in a study to test the effects of fertilization and systemically applied gibberellic acid ($GA_{4/7}$) on the production of male and female conelets. $GA_{4/7}$ was applied in two different concentrations, and across six different dates. Half of subject trees were fertilized in spring 2001 with ammonium nitrate at a rate of 400 pounds of N per acre. In spring 2002, male and female conelets were counted on subject trees. Fertilization had the greatest effect at increasing the number of female cones per branch tip at St Louis County, but was only marginally effective at Cotton. Fertilization negatively impacted the percent of male conelets but increased the number of female conelets per tip across sites. No significant differences between high and low concentrations were found at any date across sites. Future testing should focus on refining a sub-sampling procedure, and determining maximum threshold of tolerance to injections of $GA_{4/7}$ and fertilizer applications. Currently, fertilizing with ammonium nitrate remains the most cost-effective method for increasing cone production in red pine seedling-seed orchards.

Introduction

Red pine is an extremely important species in the Great Lakes region, and is the most commonly planted tree species in Minnesota. Many seed orchards are actively managed for seed production in the state however pines in general do not produce “good” cone crops on an annual basis. Typically, good cone crops are produced every three to seven years, with a bumper crop occurring every ten to twelve years (Burns and Honkala, 1990). Due to the periodicity of good cone crops in red pine, shortages of red pine seed are not uncommon.

The use of artificial growth hormones to induce premature conelet production in tree species has been well documented. Applications of $_{GA4/74/7}$ to stimulate early flowering are 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 aims to create a set of management guidelines for early cone 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. This would serve to improve the productivity of cooperative seed orchards both for commercial seed production and breeding programs.

Objectives

1. Identify the optimal time of year, and amount of stem injected $GA_{4/7}$ that will induce premature cone production in red pine.
2. Create a method for estimating cone crops from ocular or other sampling observations early in the growing season.
3. Determine how early red pine seed can be collected without affecting seed viability.

Methods

Two red pine seedling-seed orchards were utilized for this study: the St. Louis County orchard at Ellsburg Rd. (rogued in 2001) and MN DNR's Cotton orchard (rogued in 1991). Twenty-four different treatments composed of two different $GA_{4/7}$ concentrations (high and low), two different levels of fertilizer (fertilized and unfertilized), and six application dates (June 15-August 30) were tested. In addition, two controls did not receive a $GA_{4/7}$ injection; one was fertilized, the other not. This arrangement resulted in a total of 26 different treatments.

At each site, 15 trees were treated for each treatment, 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 15 trees that were not fertilized). This design resulted in 390 trees treated per site, for an experimental total of 780 trees.

The amount of $GA_{4/7}$ injected was determined by volume-tables based on the diameter of each tree. On each injection date trees were systemically injected with either a high (1.25 mg $GA_{4/7}/cm^2$) or low (0.75 mg $GA_{4/7}/cm^2$) concentration 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.

The fertilized trees were treated with ammonium nitrate (34 - 0 - 0) (slow-release granules) applied on June 19-20, 2001, at a rate of 400 lbs of N per acre. The average tree drip-line diameter at each site was used to determine the amount of fertilizer required per tree. At Cotton, a 12-foot drip-line resulted in 3.1 lbs of fertilizer per tree. At St. Louis Co, the average drip-line diameter of 8 feet required 1.35 lbs per tree. Fertilizer was applied by hand evenly across the drip line of each tree.

The treatment design and dates of application are shown in Table 1. Treatment effects were evaluated by counting male and female conelets in Spring 2002 and then calculating percent male cones, percent female cones and the number of female cones per branch tip.

Table 1. Treatment design. Each treatment was applied to three trees per rep. The actual date of injections is shown below.

Treatment No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
$GA_{4/7}$ Conc.	low	low	high	high	Low	low	high	high	low	low	high	high	low
Date	1	1	1	1	2	2	2	2	3	3	3	3	4
Fertilizer	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert

Treatment No.	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
$GA_{4/7}$ Conc.	low	high	high	low	Low	high	high	low	low	high	high	Control	Control
Date	4	4	4	5	5	5	5	6	6	6	6	0	0
Fertilizer	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert

Injections:

Date	Injection Date
1	17-Jun
2	30-Jun
3	13-Jul
4	30-Jul
5	16-Aug
6	31-Aug

Conelet counts

In Spring 2002, fifteen trees at each site were chosen at random for intensive sampling. Branches were accessed using a combination of "JLG lift" and orchard ladders (ranging from 12 to 16 feet in height). A different sub-sampling scheme was devised for counting male and female conelets. Codominant branches were selected over suppressed branches whenever possible. Three reps per site (234 per site; 468 trees total) were surveyed.

Three variables were measured, two which estimated the number of female conelets produced and one which estimates male conelet production. The "percent of females" was calculated by summing the total number of branch tips containing female cones and dividing by total number of branch tips. In addition, an estimate of the relative concentration of cones "females per tip" was calculated by summing the number of female conelets and dividing by the total number of branch tips containing female conelets. When no females were produced, i.e. the divisor for "females per tip"= 0, then a zero was substituted for the quotient. The average number of male conelets per branch tip was calculated by counting the total number of male clusters and dividing by the total number of branch tips.

Analysis of Variance was used to determine the significance of the 26 treatments. A preliminary model was used to test significance using the factors: site (two sites), rep (three reps), and treatments (26 total). The model was then expanded to compare effects of fertilization (fert vs unfertilized), date of injection (one control plus six dates), and rate of $GA_{4/7}$ injected (high vs low). Subsequent interactions (fertilization*date, rate*date, rate*fertilization, and rate*date*fertilization) were also tested across sites. Significant differences were further investigated using Tukey's HSD test. All statistical analyses were performed on SAS (1999).

Results

Across sites

Significant differences between the sites were found for all three variables. "Percent of females" was not significant for any other factor (Table 2) and was not included in further analysis. "Females per tip" and "percent males" were significant ($p < 0.05$), for effects of rep, treatment and interaction terms.

$GA_{4/7}$ injection rate was not significant for any variable (Table 3). Across sites, the number of females per tip was significantly greater at Date 4 vs Date 2 (Tukeys HSD $p<0.05$), but all other dates were statistically similar (Figure 1). The "percent males" was similar among all dates of injection. Fertilization increased "females per tip" significantly but also significantly decreased the "percent males."

Within sites

At Cotton fertilization did not significantly impact "females per tip" but did decrease the "percent males" (Table 3, Figures 2,3). At St Louis County, both variables were significant. Date of injection was only significant for "females per tip" at Cotton, but was not significant for any other variable at either site. Rate was not significant for any variable at either site.

Table 2. ANOVA significance level for site, rep and treatments (1-26). Non-significant effects are noted with "NS." "Sig" means that p-values were between 0.05 and 0.01. "Highly sig" means that p-value was less than 0.01.

Source	**Percent Females**	**Females per branch tip**	**Percent males**
Site	Sig.	Highly sig.	Highly sig.
Rep	NS	Highly sig.	Highly sig.
Treatment	NS	Highly sig.	Highly sig.
Site*Treatment	NS	Sig.	NS

Table 3. ANOVA p-values for rate, date, and fertilizer treatments. Non-significant effects are noted with "NS." No two- or three-way interactions were significant.

Source	**Females per branch tip**	**Percent males**
Rate	NS	NS
Date	0.0003	<0.0001
Rate*Date	NS	NS
Fert	0.0011	0.05

Table 4. Significance of p-values by site for "females per tip," "percent males," and "percent females" by site. No significance for any factor was found for "Percent females" at Cotton. Non-significant effects are noted with "NS." "Sig" means that p-values were between 0.05 and 0.01. "Highly sig" means that p-value was less than 0.01. No two- or three-way interactions were significant.

	Cotton		**St Louis**		
Source	**Females per tip**	**Percent males**	**Percent females**	**Females per tip**	**Percent males**
Rep	Highly sig.	Highly sig.	NS	Highly sig.	NS
Rate	NS	NS	NS	NS	NS
Fert	NS	Highly sig.	Highly sig.	Highly sig.	Highly sig.
Date	Highly sig.	NS	NS	NS	NS

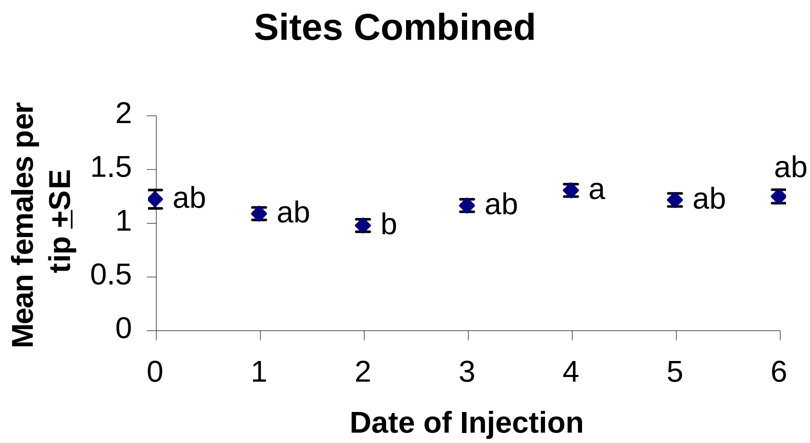

Figure 1. Mean number of "females per tip" at each date, across sites. Different letters indicate significant differences using Tukey's HSD at $p<0.05$. The control (no $GA_{4/7}$ injected) is shown at Date=0.

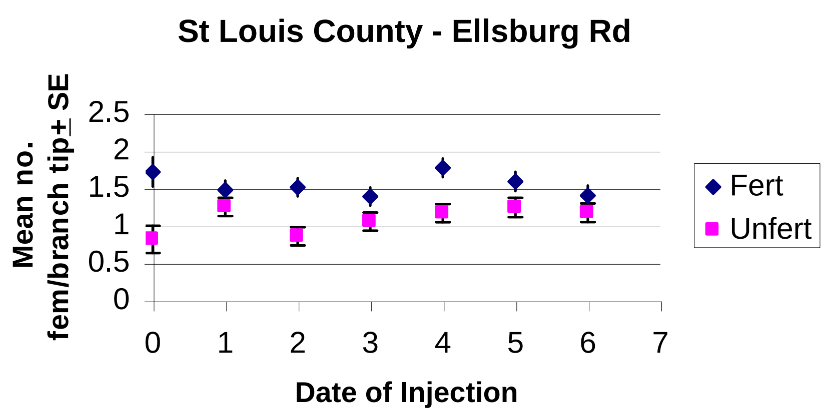

Figure 2. Mean number of "females per tip" at each date of injection for St Louis County's Ellsburg Rd site. The control (no $GA_{4/7}$ injected) is shown at Date=0.

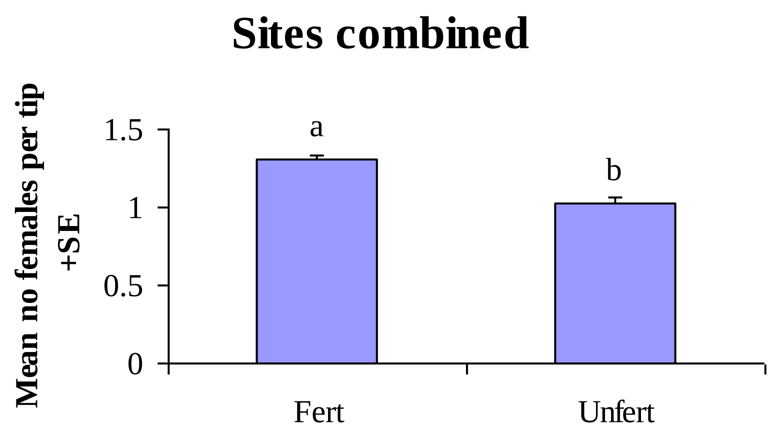

Figure 3. Mean number of “females per tip” at each fertilization treatment across sites. Means with different letters are significantly different using Tukey’s HSD at $p < 0.05$.

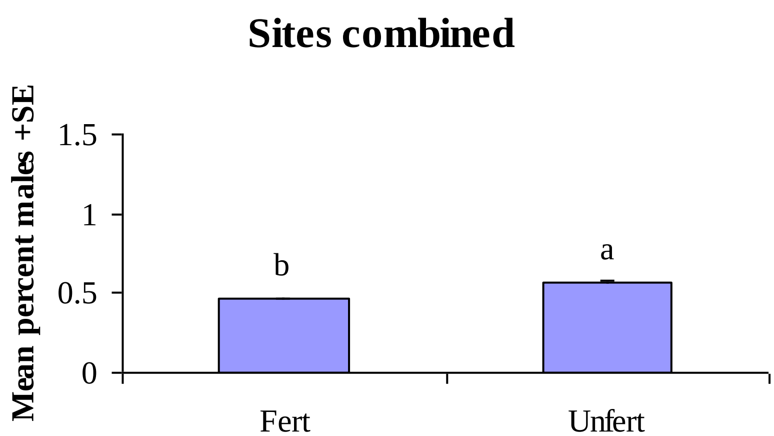

Figure 4. Mean number of male cones at each fertilization treatment across sites. Means with different letters are significantly different using Tukey’s HSD at $p < 0.05$.

Discussion

Fertilizer, more so than $GA_{4/7}$ rate or date of injection, had the most pronounced effect on the number of cones, both male and female. It effectively increased the number of female cones regardless of date of injection and decreased the number of male cones. Since the primary goal of the project was to increase the production of female cones, the effect on males is secondary. However, controls were not significantly different from any specific date, so the rates used did not have the expected impact. Fertilizer effects were more pronounced at St Louis County than at Cotton which is not surprising. Two characteristics of the sites may explain this discrepancy: trees at Cotton are older and larger, and the soil type has a greater clay component than at St Louis County. Because of the higher clay content, Cotton has a better capacity to retain nutrients and was better able to buffer the effects of the fertilizer. At the sandier St Louis site, the impact of fertilizer on cone production was more evident.

The concentration of $GA_{4/7}$ injected was not significant for any model which suggests that the rates used were not adequate to produce the expected result. A study to reveal "how much $GA_{4/7}$ is too much $GA_{4/7}$" might be useful to determine the threshold of $GA_{4/7}$ that can be tolerated by a tree. No newly dead trees or top-kill was observed during the cone counts.

Dates of injection did not have a large impact on the production of either male or female cones either. In fact, injecting at the early dates (dates 1-3) appeared to have a negative impact on the number of female cones produced. Injecting at date 4 resulted in the largest number of female cones per tip, however the mean differed significantly from only one other date (date 2) and not the control. The effect of injecting $GA_{4/7}$ may have been more pronounced if higher concentrations had been used.

It is possible that the sub-sampling procedure did not accurately reflect the number of conelets or was unable to capture differences in treatments. Further refinements of the sub-sampling scheme may improve accuracy of future tests. At this time, fertilizing with ammonium nitrate remains the most reliable, cost-effective management tool for increasing cone production in red pine seedling seed orchards.

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White Pine Blister Rust Update: Potential Resistance Mechanisms In Pinus Strobus To Infection By Cronartium Ribicola

Jason A. Smith[a], Todd A. Burnes[a], Joel A. Jurgens[a], Andrew J. David[b], and Robert A. Blanchette[a]

[a]Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN
55108-6030. [b]University of Minnesota, North Central Research and Outreach Center, Grand Rapids, MN 55744

Abstract
Although putative blister rust-resistant Pinus strobus were selected nearly 40 years ago, seedlings with increased resistance to blister rust are still unavailable. In the studies reported here, seedlings from open pollination and controlled crosses (factorial mating design) were subjected to artificial inoculation with Cronartium ribicola in an effort to facilitate selection of seed sources with resistance to blister rust. Disease incidence and severity data were collected and histology was used to study the infection process in needles of inoculated seedlings. Inoculation of seedlings from open pollination and controlled crosses indicate that seedlings with P. strobus selections P 30, P 312 or P 327 as a parent are more resistant than others tested. Specifically, seedlings with P 327 as a parent have a lower incidence of infection (approximately 10 – 20 % reduction in incidence compared to susceptible controls) and consistently longer survival following inoculations (393 days longer than susceptible controls). In addition, seedlings originating from P 327 had the lowest values for needle infection based on spot index (a rating used to evaluate disease severity). Histological studies indicate that different mechanisms of resistance to infection exist in the needles. Seedlings of P 327 respond to infection by producing a hypersensitive-like reaction where cells near the infection site die rapidly and inhibit the fungus from penetrating the tissue further. Seedlings from open-pollinated P 30 exhibit a concentration of phenolic compounds near the infection site that appears to restrict hyphal growth. Needles from infected and non-infected seedlings of P 327 (resistant) X H109 (susceptible) were also compared using scanning electron microscopy. Preliminary results suggest that non-infected seedlings have a greater proportion of stomata plugged with wax than infected seedlings. Grafts of the parent trees used in this study were made in 2002 and will be compared to seedlings and used to further elucidate the mechanisms of resistance. These results support previous findings by Patton done in the 1960's (University of Wisconsin) that selection P 327 shows promise for the development of blister-rust resistant P. strobus seedlings. In addition to selection P 327, selections P 30 and P 312 exhibit degrees of resistance that may result from different mechanisms. These results provide a basis for future selection and breeding programs aimed at developing blister rust-resistant P. strobus.

Introduction

Resistance to *Cronartium ribicola* has been found in several North American species in section *Strobus* of *Pinus*, including *P. monticola* and *P. lambertiana* (2,3). Major-gene resistance in these species has facilitated the development of blister-rust resistant seedlings for planting (3). The mechanisms of blister-rust resistance in these species are well characterized (2,3) and breeding efforts are underway to combine resistance traits and increase the durability of the resistance.

Despite the discovery of several rust-free *P. strobus* in high hazard areas nearly forty years ago (5), blister-rust resistant *P. strobus* seedlings are still unavailable for planting. Limited knowledge about the genetics of blister-rust resistance in *P. strobus* is further compounded by a lack of knowledge about what mechanisms are important for blister-rust resistance in this species. As a result, there has been little progress towards breeding blister-rust resistant *P. strobus.*

Grafts of putative resistant *P. strobus* selections are planted at the U.S.F.S Oconto River Seed Orchard in White Lake, Wisconsin (Fig. 1). These grafts are now being used for breeding and genetic improvement projects and seed from superior trees (and crosses) are being grown and tested for resistance to *C. ribicola*. In the studies reported here, seedlings from open pollination and controlled crosses (factorial mating) have been subjected to artificial inoculation with *C. ribicola* to further our understanding of the resistance behavior and possible mechanisms of resistance in these *P. strobus* selections.

Methods

Resistance screening

Seedling production - Seed from controlled crosses (factorial mating design) were collected and stratified for 12 weeks. After stratification, the seed were planted and grown in the greenhouse under supplemental lighting for 12 weeks.
Experimental design - Sixty 12-week-old seedlings per seed lot were randomly selected and placed in a randomized block design. For each experiment, 60 seedlings of the most susceptible selection (H 111) were included for comparison. Control seedlings were not inoculated.
Inoculations - Seedlings were inoculated by suspending telia-bearing leaves (from a monouredial isolate) over the seedlings for approximately 24 hours at 17 ^{O}C and 100% humidity. After 7 days, the seedlings were removed from the growth chamber and placed in the greenhouse and monitored for disease development. The inoculated seedlings were inspected visually for symptoms at 4 and 8 weeks post-inoculation. A rating scale called spot index (Table 1), was used to indicate the number of spots observed on each seedling. In addition, data were taken on the number of seedlings per seed lot with symptoms at each rating. For open pollinated seedlings, mortality rates from two experiments were determined and based on whether a plant was alive or dead on the date of data collection, which was taken every two weeks after inoculation. Mortality data were also taken weekly for seedlings (from open pollination) from replicated inoculations (2 experiments) used in histology studies.

Host-parasite Interactions

Needle histology - Secondary needles were collected from open-pollinated seedlings 7 weeks after inoculation and prepared for histological examination. 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 mm and stained with the periodic acid-Schiff technique and phoroglucinol-HCL.

Wax on needle surfaces - Primary needles from infected and non-infected inoculated seedlings of P 327 X H 109 were collected for SEM evaluation of wax occlusion of stomata. After coating with gold using a sputter-coater, the needles were evaluated under low vacuum, at room temperature using a Hitachi variable pressure S3500 scanning electron microscope. Images were collected at three pre-determined locations (using the x-axis). Stomata were considered occluded with wax when the wax covering the stomatal opening contained no gaps larger than 5 microns.

Results

Resistance screening -Inoculations of seedlings from controlled crosses indicates that seedlings with P 327 as a parent are most resistant. Seedlings with this selection as a parent have smaller, fewer spots than susceptible controls (**Fig. 2**) and survive longer following infection (**Fig. 5**). The lowest average spot index (at 4 weeks) (**Table 1**) for all inoculations was recorded for P 327 X H109 (spot index = 1.2) followed by P 312 X P327 (spot index = 1.5). Open pollinated P 327 had an average spot index of 1.9. In comparison, the susceptible seed source, H 111 had an average spot index of 3.0. Seedlings with P 327 as a parent also had lowest percentage of seedlings infected after 4 weeks. Only 66% of seedlings of P 327 X H109 and 74 % of P 312 X P327 were infected after 4 weeks, whereas 85% of seedlings of H 111 were infected. Survival of open-pollinated seedlings used in histology studies was greatest for selections P 327 and P 30 and worst for H 111. The average number of days required to reach 75% mortality was 212 for H111, whereas 605 days were required for P 327 (although in one study P 327 did not reach 75% mortality) and 597 days for P 30.

Host-parasite Interactions

Needle histology - Histological studies show that needle symptoms of seedlings from open pollinated P 327 are characterized visually by a small, bright yellowspot and microscopically exhibit a collapse of mesophyll cells and an absence of phenolic compounds (**Fig. 4**). The cells and transfusion tissue in close proximity to the infection were necrotic and vascular elements remained unaltered. Phenolic compounds were found deposited near the infection site in P 30 and P 312 and the hyphae were distorted and shrunken indicating inhibition of fungal development. Susceptible selections (H 111 and WI 352) had densely packed mycelium penetrating the vascular bundle and presumably not restricted by host responses.

Wax on needle surface - Of the 371 stomata observed on secondary needles of non-infected seedlings of P 327 X H109, 244 (66%) were occluded (**Fig. 3**) with wax (gaps were less than 5 microns). Of the 338 stomata observed on the secondary needles of infected seedlings of P 327 X H109, 67 (20%) were occluded with wax.

Discussion

The preliminary results from these studies indicate that there are different mechanisms responsible for resistance to *C. ribicola* in *P. strobus*. Inoculations have shown that selection P 327 exhibits smaller and fewer needle lesions. The fewer lesions may result from more stomatal wax that prevents entry of the fungus into the needle tissue. More experiments are underway to determine what role epistomatal wax plays in resistance in P 327.

The slower progression and smaller size of needle spots in P 327 may result from a hypersensitive-like response where the host cells die rapidly and prevent the fungus from penetrating the tissue. The accumulation of phenolic compounds and inhibition of fungal growth in cells of seedlings of P 30 and P 312 indicates that other mechanisms of resistance are present in these selections.

These studies have supported earlier findings by Patton et al. (5,7) and Hunt et al. (2) that selections P 327, P 312 and P 30 show promise for developing resistant *P. strobus*.

Further studies are underway to elucidate the mechanisms responsible for resistance to blister-rust in *P. strobus*. More seed sources are being tested and grafts will be used in inoculation studies during the fall of 2002.

Table 1 – Data from artificial inoculations of *P. strobus* selections (controlled crosses and open-pollination) 4 weeks after inoculation. Seedlings with P 327 as a parent are much less susceptible than other selections. The spot index values are as follows: 1 = 1-3 spots per seedling, 2 = 4-10 spots, 3 = 11-32 spots, 4 = 33-100 spots, 5 = 101-316 spots, 6 = 317-1000 spots and 7 = 1001-3162 spots.

Seed Source	Average Spot Index	Percentage of seedlings infected
P 327 X H109	1.2	66
P 312 X P 327	1.5	74
P 327 (open pollinated)	1.9	83
P 30 X P 343	2.7	84
H 111	3.0	85

Fig. 1 - (A) Blister-rust free *P. strobus* in a high hazard area of Minnesota and (B) grafts of putative blister-rust resistant selections of *Pinus strobus* planted at Oconto River Seed Orchard in White Lake, Wisconsin, USA.

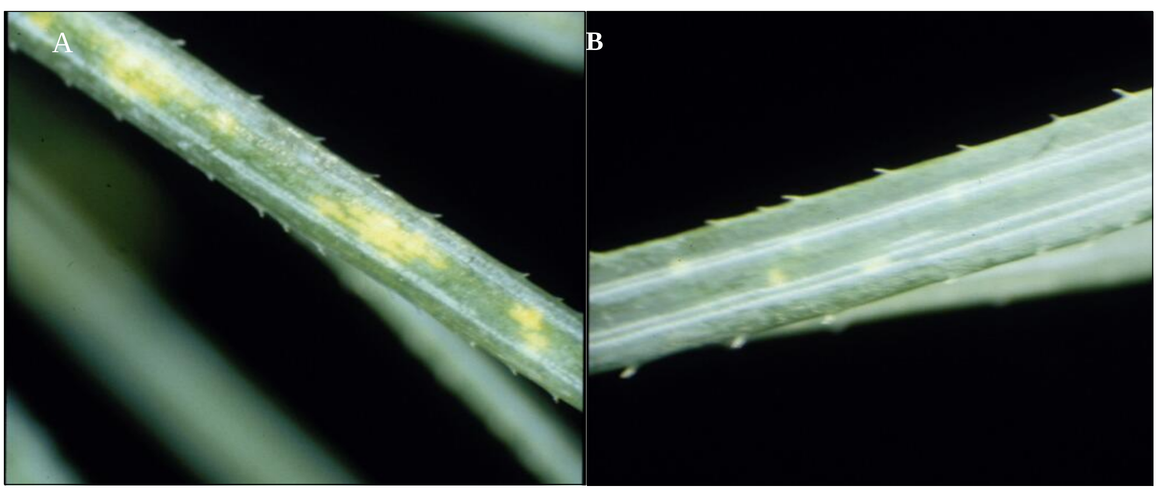

Fig. 2 – Needle lesions from inoculated seedlings of *P. strobus* selections H 111 (A) and P 327 X P 312 (B) at 4 weeks after inoculation. Note the reduced lesion frequency and size on P 327 X P312; whereas H 111 has larger, more numerous lesions.

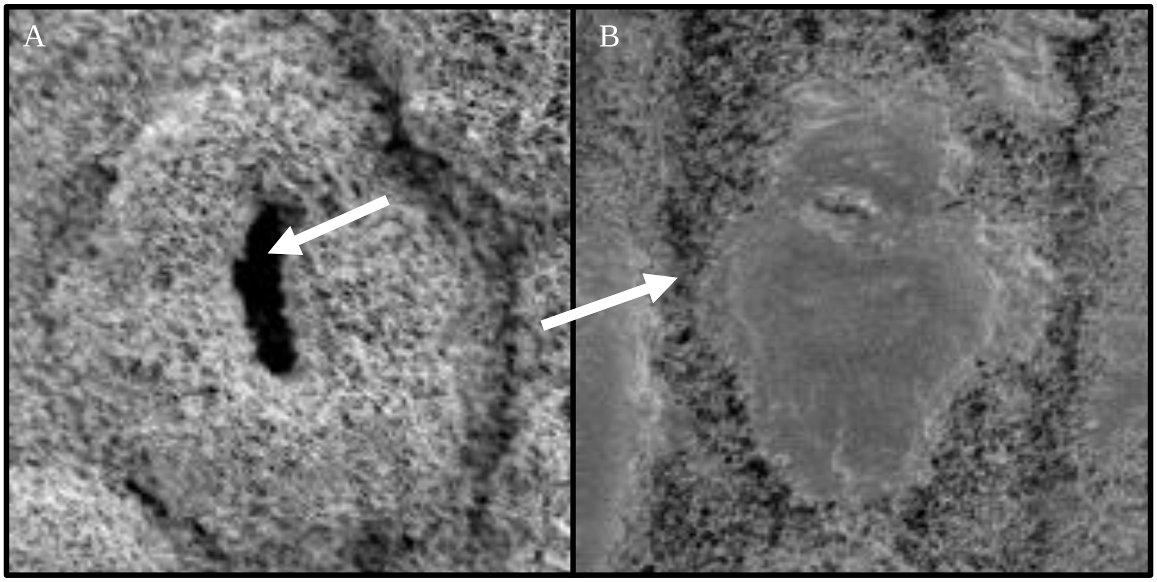

Fig. 3 – ESEM micrographs (8000x magnification) of stomata from infected (A) and non-infected (B) P 327 X H109 seedlings. Note uniform covering of epistomatal wax in B and large gap in wax at stomatal opening (white arrow) in A.

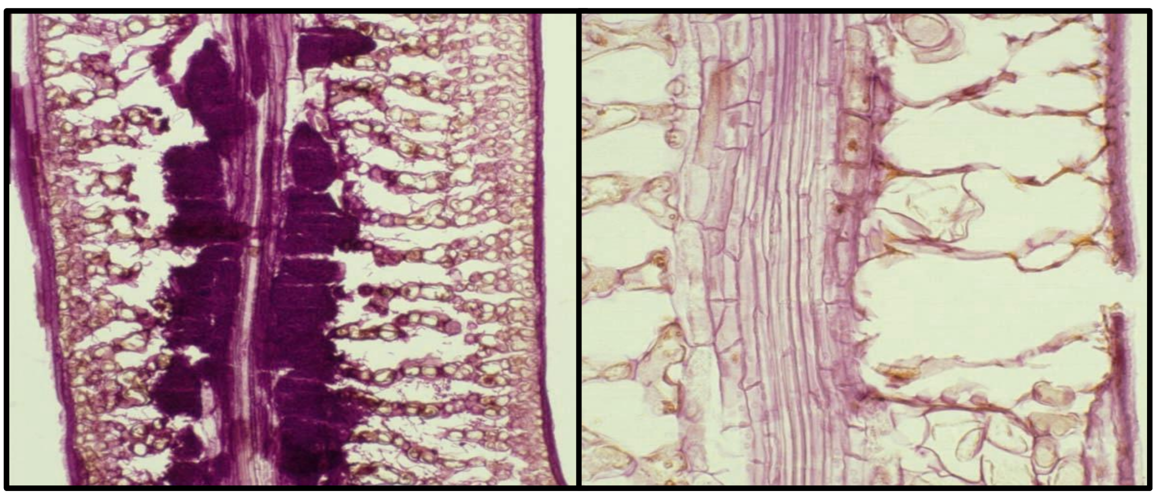

Fig. 4 –Micrographs of needles from susceptible (A – WI 352) and resistant (B – P 327) seedlings 7 weeks after inoculation. Note the collapsed mesophyll cells and lack of penetration of vascular bundles in B. In contrast, mesophyll cells remain intact in A and the mycelium has penetrated the vascular bundle.

Fig. 5 – Inoculated seedlings P 327 x P 312 and H 111 12 weeks after inoculation. Mortality is greater in H 111 (red arrows) than P 327 (black arrows).

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Identifying Suitable Sources Of White Spruce Seedlings For Underplanting In Shelterwood Situations

By: Andy David and Sarah Worrall, MS student

After harvesting white spruce there are two ways of regenerating the stand, one is to plant seedlings and the other is to utilize natural regeneration. The shelterwood silvicultural system relies upon natural regeneration where the new seedlings develop beneath a partially shaded microenvironment provided by residual trees. Although shelterwood systems have been successful in regenerating shade-tolerant conifers like spruce and balsam fir, if optimal regeneration conditions are not created fill planting is necessary to secure full occupancy of available growing space.

Because planting stock derived from tree improvement programs are evaluated based on their performance under open-grown conditions their performance in an underplanting context is untested. A question arises regarding the most suitable type of white spruce seedling for fill planting, is it better to use improved or 'woods run' seedlings?

Seedlings derived from different sources (i.e. first generation orchards, improved first generation orchards, second generation crosses and woods run, will be established in the nursery under shade cloth. Seedlings will be evaluated for height, diameter, volume, bud flush, and bud set under four light levels; 100%, 75%, 50%, and 25% of full sun light. Growth of individual trees will be evaluated using a regression analysis thereby eliminating the necessity of replication associated with a traditional ANOVA analysis. In this manner suitable sources of white spruce seedlings for underplanting in shelterwood situations will be identified.

OUTLOOK

The Minnesota Tree Improvement Cooperative is poised to advance into second-generation material for a second tree species in spring 2003. An abundant cone crop in spring 2002 permitted over 100 crosses to be made in white spruce, the seeds of which are expected to be sown in Fall 2003 and planted in Spring 2005. Five-year measurements of the second-generation jack pine population will be taken in 2003, which will include tree heights and diameters and perhaps wood quality traits. White pine blister rust research is advancing, and crosses will continue to be made to further our understanding of the mechanisms of resistance. One white pine orchard has reached maturity and was extremely productive in 2002.

Several new improved first-generation white spruce orchards are being established, which should provide Cooperators with ample amounts of improved seed. Cooperators have also begun to take a more active role in orchard management by fertilizing orchards and topping trees to facilitate cone collections.

Several additional plantings are planned for 2003, mainly a final comparison of first-generation white spruce, and a white pine regional/provenance trial. Seed from red pine orchards will be collected and stored for future testing. Selections in second-generation jack pine will be ongoing, and first-generation orchards will continued to be managed for cone production.

2003 COOPERATIVE WORK PLAN

White spruce:
- Plant ramets into MN DNR, Red Lake, and Potlatch orchards
- Plant white spruce comparison trial (six sites)
- Plant second generation material (three sites)

Red pine:
- Collect seed for comparison trial
- Fertilize orchards

White pine:
- Crosses at Oconto River Seed Orchard, and CFC breeding arboretum
- Plant three reps of regional provenance/progeny test (work begun by Dr. Paul Anderson)
- Maintain breeding arboretum
- Add additional ramets to breeding arboretum
- Visit USFS progeny tests
- Fifth year assessment and mortality survey of Zambino field trials (four sites)

Black spruce:
- Grafting winter 2002 for additions to Larsaybow seed orchard.

Jack pine:
- Fifth-year measurements of second-generation material (two sites)

Other:
- Fifth-year measurements of Blandin's Norway spruce trial (three sites)

APPENDIX

MINNESOTA TREE IMPROVEMENT COOPERATIVE

2002 ADVISORY COMMITTEE

MEMBERS	
Beltrami County	Greg Snyder, Bob Milne
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