Reducing the Impact of Blister Rust on White Pine in Minnesota

Final Report

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EXECUTIVE SUMMARY

The 1997 Minnesota State Legislature provided $300,000 for research to reduce the impact of blister rust on eastern white pine in Minnesota. The research focused on several areas. One area was development of methods to better predict rust hazard on both a regional and site scale. Existing work on selection and testing of trees for increased growth rates and blister rust resistance was accelerated. To support the genetics work, research on flower induction, rapid screening for rust resistance, and histological examination of infected material was initiated.

A new rust hazard map for northeastern Minnesota was created using inventory information, climate, topography, distance to water, and several other factors. It shows that even within areas previously classified as Ahigh hazard,@ there are places where the rust hazard is quite lower. New GIS maps are available for land managers to help make decisions about the level of blister rust to anticipate when growing white pine.

Selection of fast growing and disease resistant trees, and subsequent establishment of seed orchards was accelerated as part of the research project. In addition, a breeding arboretum containing promising clones was expanded using selected material from an earlier progeny test.

To support shortened generation cycles (breeding-testing-selecting), research on flower induction was initiated. A foliar spray application of gibberellic acid during the period of rapid shoot elongation induces both male and female inflorescences, but not consistently across all genotypes. The study continues in order to increase the quantity and consistency of both male and female flowering. Stem injection trials were initiated in May 1999 as an alternative to foliar spray. The critical time of year for stem injections, hormone concentrations, effects of tree fertilization, and effects on pollen viability will all be determined as the study continues.

A rapid and reliable method was created for early screening of blister rust susceptibility. Five-month-old seedlings are inoculated with blister rust, and resistant families can be identified within 1 1/2 years. This is an improvement over less reliable methods that require up to 5 years for results. Field plantings of the families tested using the accelerated method were planted to measure how well early screening results correspond to field results.

Histological examinations of the early stages of seedling colonization of both resistant and susceptible white pine seedlings were conducted. In a very short time, remarkable progress was made in understanding what actually occurs within a needle when blister rust infection occurs. This work is useful in helping to understand potential defense mechanisms, and supports research on the ability to induce greater resistant responses in trees by biological or chemical additives to the soil or surfaces of the tree.

Future research is building on information developed during the first two-year period. Refinements to the flower induction and early screening techniques are being developed. Preparations are being made for breeding work in the arboretum. Internal biological and chemical methods of blocking infection are also being explored.

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INTRODUCTION

Starting in the mid 1980s, interest in regenerating and managing eastern white pine (*Pinus strobus* L.) in Minnesota has grown dramatically. The interest was spurred by the desire among many interests to increase the number and distribution of white pine across the landscape. That desire was coupled with the realization that, despite numerous and serious pests, growing white pine was possible under the right conditions. Prior to that time, there was a general consensus that growing white pine was rarely successful or too expensive.

Several items led to the change in thinking about white pine. First, scientists began to better understand the historic role of white pine in Minnesota ecosystems, and its value to wildlife and ecological processes. Along with its aesthetic value, this understanding led to a consistent call for more white pine in Minnesota=s northern forests. At the same time, natural resource managers recognized that white pine populations were increasing on their own, with little or no human assistance. Some small research projects determined the conditions under which this natural regeneration was occurring, allowing managers to purposefully grow white pine in favorable conditions.

The concern with growing white pine in Minnesota, particularly the northeastern portion of the state, is three major pests; white tailed deer (*Odocoileus virginianus*), white pine weevil (*Pissodes strobi* (Peck)), and white pine blister rust (*Cronartium ribicola*). Perhaps nowhere else in the natural range of eastern white pine are these three pests as prevalent in combination as they are in northeastern Minnesota. Deer browse young trees from the time they are seedlings until they grow out of reach. The weevil attacks trees during the sapling to pole stage, killing the leader and creating crooks in the main stem. Blister rust can infect trees of all ages, but is particularly deadly for younger trees.

To find effective and efficient ways of overcoming these and other challenge, the Minnesota Department of Natural Resources commissioned a group of scientists in 1996 to develop a set of strategies for increasing the number and distribution of white pine in Minnesota[1]. Among the recommendations made was one urging research in the areas of deer predation, regeneration systems, genetic improvement, and blister rust management.

Responding to the recommendations in the report, the 1997 Minnesota State Legislature funded $1.5 million for white pine programs, including $300,000 for research to reduce the impact of blister rust on eastern white pine in Minnesota. The research focused on several areas. One area was development of methods to better predict rust hazard on both a regional and site scale. For the longer term, existing work on selection and testing of trees for increased growth rates and blister rust resistance was accelerated. To support genetic improvement efforts, research on flower induction, rapid screening for rust resistance, and histological examination of infected material was initiated. Each project is described below.

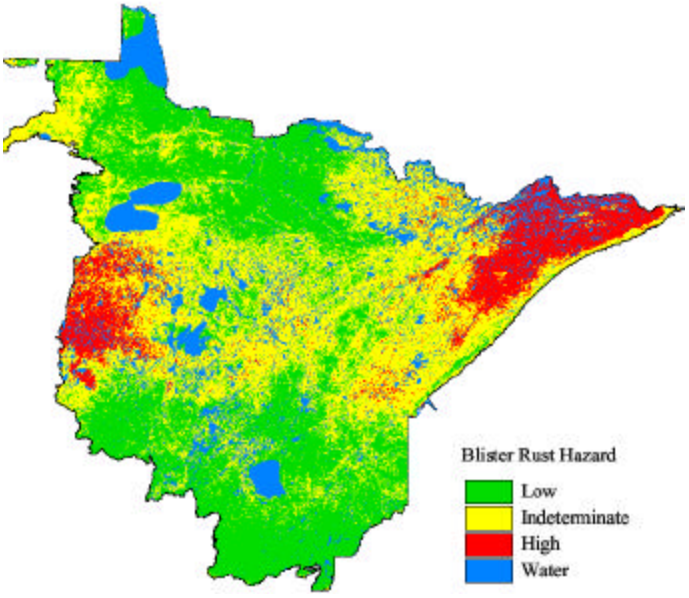
[1]State of Minnesota, Department of Natural Resources. 1996. Minnesota=s white pine, now and for the future. A report by the white pine regeneration strategies work group. Dec. 19, 1996. 66 pp.

RUST HAZARD RATING

Regional Scale

For nearly 40 years, foresters have used the rust hazard map developed by Van Arsdel to identify regions in the state more or less susceptible to blister rust infection. This map has been useful, but was based on broad climate and topographic patterns. It did not identify areas within the broad hazard zones were infection risk was either higher or lower than the broad zone.

Tony Brown, Mark White, and Dr. George Host, from the Natural Resources Research Institute, created a new hazard zone map for northeastern Minnesota using modern GIS techniques. The map is shown below, and the entire report is available at http://www.nrri.umn.edu/rustmap.



Site Level

Several publications have been written to describe site selection and cultural techniques to minimize the incidence of blister rust. Following a review of the literature, Scott McDougal provided the following summary of available information.

Landscape Position

Where to plant is one of the most important decisions made when attempting to reduce losses from white pine blister rust. Landscape position affects the likelihood of blister rust infection by subtly altering environmental conditions. The blister rust fungus requires cool moist conditions to cause infection. Subsequently, warm and dry sites provide less favorable conditions for infection than cool wet sites. Locations favoring the fungus include frost pockets, the bases of slopes, north or western facing slopes, and small gaps in an otherwise continuous canopy. Locations that do not favor infection are hill tops, slope shoulders, south to east facing slopes, and large canopy openings, such as clear-cuts or open fields. Blister rust may still occur on these sites, but the incidence of infection will be lower.

There are several strategies for reducing the incidence of blister rust when growing white pine in Minnesota. They can be used singly, or to be more effective, in combination with one another.

Plant only on sites unfavorable to blister rust - Reduces infection rates

Pros: - Maximizes success on a given parcel of land
Cons: - Such sites may not be available
- Reduces total area available for planting

Blister rust will always be more common on trees in landscape positions that favor the formation and persistence of dew. Landscape position is less critical in southern Minnesota where higher temperatures discourage rust, and sites favorable to the fungus are smaller. In the south, rust occurs only on the coldest and wettest sites. Landscape position becomes increasingly important when moving northward because temperatures are cooler and the size of sites favorable to the fungus become larger. Therefore, the further north the site, the more important it is to seek landscape locations that are unfavorable to blister rust.

Plant white pine at high density - This allows for high infection rates yet still provides sufficient trees as the stand ages.

Pros: - promotes rapid height growth
- encourages early natural pruning
- provides natural correction for trees attacked by white pine weevil
Cons: - more expensive
- may be inefficient use of planting stock
- may require additional thinning

Plant white pine at high density in the central and northern parts of the state when planting open fields, or when planting in landscape locations favorable to blister rust infection. Spacing as low as 6 x 6 feet for open-grown plantations increases the chance of obtaining an adequate stocking of crop trees. Use lower densities when planting beneath an overstory. In the south and on sites that do not favor blister rust, wider spacing is more appropriate and will increase the time until plantations need thinning.

Plant underneath an existing overstory - This often prevents the formation of dew on needles, which is necessary for blister rust infection.

Pros: - requires lower planting densities
- reduces competition from other vegetation
- overstory can intercept spores
- helps to reduce susceptibility to white pine weevil
Cons: - requires an overstory
- overstory may require thinning prior to planting
- overstory will probably require subsequent thinning and/or removal
- reduces growth rates

This strategy should be considered in the central part of Minnesota, particularly when planting in landscape positions that favor blister rust, and is strongly recommended in all locations in northern Minnesota. In the south, underplanting is counter-productive and therefore not recommended. Candidate stands for underplanting should be on favorable landscape positions, feature mature or overmature trees, and contain little vegetation in the lower canopy or on the ground. Mature oak, aspen, paper birch, red and jack pine, and

existing white pine stands all make good candidates. Avoid late-successional species stands like sugar maple and basswood or multi-storied stands. The overstory should be thinned to about 50-70 percent crown cover, but gaps within the overstory should not be larger than **2** the diameter of the height of the overstory trees.

Pathological pruning - Removes those branches most susceptible to infection.

Pros: - provides an opportunity to remove existing infections
- provides opportunities for corrective pruning
- promotes better wood quality
- promotes height growth

Cons: - expensive
- requires several entries into the stand
- potentially harmful if done improperly

Pathologists strongly recommend pruning on all sites in the north and on planting sites which favor blister rust in central Minnesota. Begin pruning after trees have reached 2 feet in height, and repeat every two or three years until the trees are branch-free to a height of 9 feet. Never remove more than 1/2 the living crown at any given time, and always leave 1/2 to 2/3 of the height of the tree with live branches. For timber production, prune only those trees that will eventually become crop trees. During pruning, also remove infected branches within the crown if they can be reached. Pathological pruning for blister rust requires pruning to a minimum height of 9 feet. Additional silvicultural pruning for timber production should continue to a height of 17 feet.

Ribes eradication - Removes inoculum source

Pros: - Protects trees from short distance spore spread

Cons: - expensive
- plants may grow back if roots not completely removed
- may require several attempts to achieve significant reductions
- is a questionable practice from an ecological point of view

In the early part of the century, eradication was the primary blister rust control practice. Beginning in the 1950's, the large-scale use of eradication decreased dramatically because of questions concerning its effectiveness. Since then however, new information about spore movement and survival has shown that eradication can be effective as a short distance control measure. Spores generally travel farther distances and survive longer in the north than they do in the south, and therefore eradication within a plantation is most effective in the south. Similarly, a Ribes-free zone surrounding plantings must necessarily be wider in the north than in the south to achieve similar protection. Despite location, all sites will benefit from eradication, but the benefit is the greatest in the south.

A successful white pine regeneration program would probably include a combination of these strategies. Location within the state, planting objectives, and available resources will help determine which combination of strategies to use. Table 1 table summarizes the control strategies.

Table 1. Blister rust control strategies.

Control Measure	**Location in Minnesota**		
	South	Central	North
Landscape Location	Avoid canopy gaps and frost pockets	Avoid canopy gaps, frost pockets, and the bottoms of valleys	Favor hill and ridge tops, south and east facing slopes, and large openings
Planting Density	Use normal spacing	Plant high density in open plantings and on landscape positions favorable to the fungus	Plant high density on all sites, except when underplanting
Underplanting	Not recommended	Can be used, not essential	Highly recommended, unless planting in large openings
Pruning	Not necessary, but can be used for additional protection	Prune if Ribes are present or for extra protection	Recommended on all sites
Ribes Eradication	Eradicate within plantation and for x feet around plantation	Eradicate within plantings and for a distance of x feet around plantings	Consider eradication only for additional protection

Evaluating Planting Sites

To demonstrate how the information presented above might be used to help select appropriate planting sites for white pine, McDougal prepared a preliminary scoring table (Table 2) to help determine the probable incidence of blister rust on specific types of sites. Although the values derived from the table need to be tested, the system offers a method for helping to make site specific decisions about planting white pine.

The table assigns values to various landscape and cover class features. A potential planting site would be evaluated by assigning the appropriate value based upon the geography and cover class characteristics found at the site. The values are then summed to arrive at a score for that particular site. The score gives an indication of the relative incidence of blister rust expected at that location - the higher the score, the more likely blister rust will be a problem. The scores represent relative incidence for each part of the state. For instance a location with a value of 8 in the north will have more rust than a site with a value of 8 in the south.

Table 2. Relative probability of blister rust infection based on site characteristics and location.

Site Characteristics	Southern Minnesota	Central Minnesota	Northern Minnesota
Ribes			
Yes	6	4	2
No	0	0	1
Position			
Base of slope	8	9	10
On slope	2	3	4
Shoulder	-2	0	0
Flat	0	0	0
Aspect			
N-E	2	2	3
S-E	0	0	0
N-W	2	3	4
S-W	0	1	1
% Slope			
0-3	0	0	2
3-20	4	4	4
20-50	2	2	2
50-100	0	0	0
>100	-2	0	0
Cover Class			
Complete Overstory	0	0	0
Small Opening	6	8	10
Large Opening/Open Field	0	1	2
Brush	4	5	6

adapted from VanArsdel, 1961

0-8 = low incidence of blister rust
9-15 = moderate incidence of blister rust
>15 = high incidence of blister rust

Example 1

Suppose you were considering a planting site in northern Minnesota that is located on a 25% slope with a Northeast aspect. The site is an old field with Ribes present. Using the above chart, select the values in the Northern Minnesota column and work down the chart assigning appropriate values.

Since there is Ribes present, select value 2	R=2
Landscape position is Aon the slope@, select value 4	P=4
Aspect is northeast, select value 3	A=3
Slope is 25%, select value 2	S=2
Cover class is Aopen field@, select value 2	C=2
Sum the values to determine score	Score = 13

A score of 13 means you can expect a moderate amount of blister rust for northern Minnesota. Depending upon your objectives you would probably want to consider additional blister rust control measures such as pathological pruning or planting at higher densities.

Example 2

You are considering a planting site in southern Minnesota that is an open field on flat ground with no Ribes present.

No Ribes, select value 0	R=0
Landscape position is flat, select 0	P=0
Since it is flat there is no aspect, select 0	A=0
Since it is flat slope is 0, select 0	S=0
Cover class is Aopen field@, select 0	C=0
Sum the values to determine score	Score = 0

A score of 0 indicates that you can expect very little if any blister rust on this site. Additional control measures are probably not needed on this site.

This chart is intended solely as a guide to assist in planting site evaluation and should not be used as a substitute for a thorough on the ground evaluation. Each planting site is unique and all the variation that exists in nature can not possibly be accounted for in a simple evaluation tool. Most white pine plantings, particularly in northern Minnesota, will require additional blister rust control measures. Furthermore competition, deer browse, and white pine weevil are other factors that can affect the growth and survival of white pine and need to be addressed if they are a problem.

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GENETIC IMPROVEMENT

Reducing the impact of blister rust on white pine through genetics can follow two paths. One path is to develop trees that are faster growing. This helps limit rust infection by getting the crown of the tree above the critical 9 foot level (below which most infection takes place) more rapidly. In situations where white pine trees are planted under an overstory canopy, genetically improved, faster growing trees can compensate for some of the loss typically associated with growing trees in the shade. They also have the added advantage of growing out of the reach of deer more quickly than unimproved trees.

The second path is to develop trees which are genetically resistant to blister rust. Past work on eastern white pine has shown that resistance is controlled by multiple genes, meaning it is more difficult to identify and then fix in a gene pool. However, once in place, such resistance is likely to be more stable across generations and against multiple strains of the fungus.

Research in both areas was supported by the grant, and are described below.

Increased Growth

Approximately 55 high performing trees (excellent growth rates, good form, no blister rust) were selected across northern Minnesota for inclusion in the program to genetically improve growth rates. Scions were collected from each tree and grafted onto rootstock. Following two or three years in controlled conditions, the first 231 grafts were outplanted in a seed orchard in the spring of 1998. Additional grafts were outplanted in 1999.

As early as possible, cones will be collected from these trees so that growth performance of the individual clones can be measured. When available, flower induction technology will be used to increase cone and seed production. In a relatively short time, this orchard should be producing significant quantities of faster growing white pine for commercial purposes.

Blister Rust Resistance

Various researchers have worked over many years to develop genetic resistance to blister rust in eastern white pine. A major roadblock was the time needed to complete the evaluation, often 20 years or more. Careers and funding were rarely long enough to complete one generation of testing, let alone the four or five generations of selection, breeding, and testing thought necessary to fully develop a rust resistant population.

With that constraint in mind, a significant portion of the funding from this grant was used to support research that would help shorten the time needed to create new generations of trees, and to evaluate the performance of those trees. Traditional field plantings and tests have been maintained, but in ways that should allow much more rapid advancement. It is hoped that the time needed to create and test one generation of trees will move from 20-25 years to about five years.

Even with the emphasis on reducing generation time spans, it is important to keep track of test material planted by earlier researchers. One such set of material is a series of seven plantings made between 1982 and 1987 by Cliff Ahlgren. Grant funds were used to hire a person (Jim Warren) to cut brush and weeds from all the plantings, re-establish plot markers, and conduct mortality and blister rust surveys. Because mortality was so high and in some cases it was impossible to accurately identify trees, an analysis of the blister rust data from these plantings did no reveal any trends related to genetics.

These plantings have major limitations because of the narrow genetic base represented, high levels of relatedness, and inadequate statistical design. They almost all suffer from severe weevil damage. However, now that they are in useable condition, they provide an interesting set of material for potential use in the future if they are properly maintained.

Breeding Arboretum

A key component of the research effort is a breeding arboretum established at the Cloquet Forestry Center. It contains three ramets of nearly 200 clones, selected from a planting near Tofte, Minnesota. The Tofte planting was established in the mid-1970s for the purpose of testing resistance to blister rust. After more than 20 years of growth and several evaluations, fewer than two percent of the original trees were growing vigorously and did not have blister rust. The 200 clones in the breeding arboretum came from among those trees and represent the bulk of the base population that will be used in future breeding and testing.

The breeding arboretum is fenced to protect the trees from deer and other animals. Funds from the grant were used to enlarge the area to accommodate additional plant material. A shallow well was installed to feed the existing drip irrigation system, which is enlarged as trees are planted. Tree care, monumenting, and vegetation management were also funded by the grant.

FLOWER INDUCTION

Flower induction research was conducted by Dr. Paula Pijut of the USDA Forest Service North Central Research Station. Fifty-three grafted eastern white pine clones (with three ramets per clonal plot), at the Cloquet Forestry Center-Breeding Arboretum, were treated with foliar spray applications of gibberellic acid ($GA_{4/7}$) (one ramet), ProCone **J**, (one ramet), with one ramet as a control. Trees were sprayed (500 mg/L) weekly during the period of rapid shoot elongation (mid-May through July; for a total of 11 applications) for 1998. Inflorescence data were collected June 15, 1999.

Both male and female inflorescences were produced, but not on all genotypes tested. Twenty-five genotypes out of 53 produced male inflorescences (pollen-cone clusters) in 1999. The number of clusters per tree and the average number of pollen cones per cluster varied across genotypes (Table 3). Not taking into account genotype selection, the total number of male inflorescence clusters and the mean number ($\pm$ SE) of pollen cones per cluster produced on eastern white pine treated with $GA_{4/7}$, ProCone **J**, or untreated controls were: 643, 7.1 $\pm$ 0.3; 574, 7.9 $\pm$ 0.3; and 79, 8.3 $\pm$ 0.8, respectively.

Nineteen genotypes out of 53 produced female inflorescences. The total number of female inflorescences per tree varied across genotypes, but the mean number ($\pm$ SE) of female inflorescences per shoot was not significantly different (Table 4). The total number of female inflorescences and the mean number ($\pm$ SE) of female inflorescences per shoot produced on eastern white pine treated with $GA_{4/7}$, ProCone **J**, or untreated controls were: 87, 1.1 $\pm$ 0.1; 130, 1.3 $\pm$ 0.1; and 33, 0.6 $\pm$ 0.1, respectively.

Ten genotypes produced both male and female inflorescences. Control trees that flowered may be a result of spray drift.

It appears that a foliar spray application of $GA_{4/7}$ or ProCone **J** at 500mg/L (for a total of 11 weekly applications) during the period of rapid shoot elongation will induce both male and female inflorescences. The study continues in order to increase both male and female flowering across all genotypes tested. Stem

injection trials of $GA_{4/7}$, ProCone **J**, or ethanol (controls) were initiated in May 1999. The critical time of year for stem injections, concentration of $GA_{4/7}$ or ProCone **J**, fertilization of trees, and effects on pollen viability will be determined as the study continues.

Table 1. Number of male inflorescence (pollen-cone) clusters and mean number (± SE) of pollen cones per cluster on eastern white pine trees sprayed with $GA_{4/7}$, ProCone **J**, or control trees[a].

	GA4/7		ProCone **J**		Control	
Genotype Number	No. of Clusters	Cones per Cluster	No. of Clusters	Cones per Cluster	No. of Clusters	Cones per Cluster
310	1	2	2	7.5 ± 4.5	0	0
2210	0	0	2	5.5 ± 1.5	0	0
240	39	3.7 ± 0.5	47	25.1 ± 1.9	1	1
1040	20	6.2 ± 0.9	11	3.9 ± 1.0	0	0
2950	325	7.3 ± 0.4	48	12.4 ± 1.8	0	0
2940	59	5.7 ± 0.4	37	5.5 ± 0.6	0	0
2490	96	11.6 ± 0.9	41	7.1 ± 0.8	0	0
10300	5	8.8 ± 2.8	83	6.7 ± 0.6	0	0
7170	1	1	0	0	0	0
5211	0	0	6	5.5 ± 1.6	0	0
2560	73	6.6 ± 0.6	109	6.9 ± 0.5	65	14.3 ± 1.0
3881	5	10.2 ± 3.3	0	0	0	0
1640	0	0	15	6.7 ± 0.9	0	0
2840	0	0	24	6.3 ± 1.0	1	5
3551	7	4.1 ± 0.6	15	3.5 ± 0.4	1	10
10280	1	2	0	0	3	5.3 ± 1.2
7160	3	6.3 ± 1.8	20	6.1 ± 1.5	0	0
5270	5	6.6 ± 2.3	0	0	0	0
10	0	0	45	7.8 ± 0.8	0	0
4531	0	0	7	3.1 ± 0.6	0	0
580	0	0	9	3.1 ± 0.7	0	0
40	0	0	0	0	2	2.5 ± 0.5
10750	2	10.5 ± 6.5	53	6.2 ± 0.6	0	0
6200	1	2	0	0	0	0
6550	0	0	0	0	6	9.0 ± 3.4

[a]Trees sprayed with 500 mg/L of $GA_{4/7}$ or ProCone **J** weekly from mid-May through July, 1998. Control trees were left unsprayed. Inflorescence data collected June 15, 1999.

Table 2. Number of female inflorescences per tree and mean number (± SE) of inflorescences per shoot on eastern white pine trees sprayed with $GA_{4/7}$, ProCone **J**, or control trees[a].

	GA4/7		ProCone **J**		Control	
Genotype Number	No. per Tree	No. per Shoot	No. per Tree	No. per Shoot	No. per Tree	No. per Shoot
3100	3	1.3 ± 0.3	0	0	0	0
4500	0	0	1	1	0	0
1040	3	1.3 ± 0.3	0	0	3	1
500	2	1	3	1.7 ± 0.3	0	0
10300	4	1.8 ± 0.3	2	1	0	0
2690	17	1.7 ± 0.2	21	2.6 ± 0.3	0	0
2840	28	1.6 ± 0.1	48	1.4 ± 0.1	8	1.4 ± 0.3
2841	0	0	4	1	0	0
3120	6	1.2 ± 0.2	9	1.3 ± 0.2	0	0
6900	3	1.3 ± 0.3	4	1.8 ± 0.5	3	1.7 ± 0.3
3551	3	1	7	1.6 ± 0.2	6	1.3 ± 0.2
10280	2	2	0	0	0	0
5270	4	1	5	1.2 ± 0.2	0	0
10	0	0	4	1.5 ± 0.3	0	0
2470	0	0	0	0	2	1.5 ± 0.5
2170	10	1.4 ± 0.2	10	2.3 ± 0.2	4	1.5 ± 0.3
10750	0	0	8	1.9 ± 0.2	0	0
6200	1	5	0	0	0	0
6500	1	3	0	0	0	0

[a]Trees sprayed with 500 mg/L of $GA_{4/7}$ or ProCone **J** weekly from mid-May through July, 1998. Control trees were left unsprayed. Inflorescence data collected June 15, 1999.

EARLY SCREENING

A major focus of the grant was development of a mechanism that would allow reliable screening of white pine seedlings for blister rust resistance at a very young age. Dr. Paul Zambino of the USDA Forest Service North Central Forest Experiment Station led the research in this area. There were four main components to the research.

Accelerated Artificial Screening for Resistance

Four experiments were conducted to test the ability to detect differences in resistance among families in greenhouse-grown plants of families of eastern white pine inoculated with the white pine blister rust fungus and to examine the effect of seedling maturity on resistance. Two experiments used Amature@ seedlings bearing the early, simple Aprimary@ needles, plus two flushes of the more mature Asecondary@ needles in fascicles. In these two experiments, seedlings were inoculated at 16 months and 13 months, respectively, with a second exposure to rust approximately two months after the first inoculation. Two experiments with younger seedlings used 5-month-old seedlings cut to a crown of only primary needles, each inoculated on a single occasion. Standard preparation of 5-month seedlings for accelerated screening for these and subsequent experiments has been to cut seedlings to a 2.0 cm crown, removing secondary needles within the remaining crown. An alternate treatment in which the lowest 0.5 cm of primary needles and cotyledons was removed, and a crown of only 20 primary needles allowed to remain was included in only the first experiment

that utilized 5-month-old seedlings. For each inoculation, seedlings were incubated at 100 percent RH, 18 C beneath leaves of *Ribes nigrum* bearing telia of strain WI4.1B until a density of 6,000 basidiospores/cm^2 was present across all six blocked replications of the experiment. Leaves were then removed, and plants incubated an additional 96 hours to allow basidiospores to infect needles before being moved to the greenhouse.

Seed for experiments was obtained from Richard Meier from clonal nurseries at Oconto River Seed Orchard (ORSO), Nicolet National Forest, USDA. Resistant clones (clones P18, P30, P312, P327, P343, selections by R. Patton at the Univ. Wisconsin; and clones ON538, ON469, selections by C. Heimburger, Ontario) and clones not known to carry resistance, but maintained at ORSO for superior silvicultural traits (H111, MI12, O122, SE22, U13, WI342, WI352) were sources of open-pollinated (half-sib) and controlled cross (full-sib and self) families. Thirteen open-pollinated families derived from these parents were common to all experiments, but additional open-pollinated families and controlled crosses were included in most experiments.

Numbers and sizes of needle spots, extent of stem infection, and mortality were determined every two weeks for each plant throughout each experiment. Data collection is complete for one experiment using mature seedlings 16 months old at time of inoculation, and both experiments using 5 month-old seedlings. Additional mortality data is being collected from mature seedlings inoculated at 11 months.

Because stems of all plants of even the most resistant families became infected in most experiments, it is apparent that the resistant families used in this study lack mechanisms of resistance that eliminate infections in the needles. Thus, resistance in eastern white pine does not correspond to the single-dominant resistance traits (MGR major gene resistance) found in western white pine (*Pinus monticola*) and sugar pine (*Pinus lambertiana*).

Examples of mortality curves for selected susceptible (H111, WI352) and resistant (P312, P327) families across three experiments are shown in Figures 1-3. The susceptible families had more rapid onset of mortality and reached 50 percent mortality more rapidly than resistant families in all three experiments. Onset and progress of mortality was more rapid in seedlings inoculated at 5 months vs. 16 months, but there were also differences in rate of mortality between the two experiments inoculated at 5 months. Distinct mortality classes could not be discerned within open pollinated or controlled cross families, indicating that resistance may potentially be quantitative and multigenic in nature. This hypothesis is being further tested by examining patterns of resistance in a diallel cross among six resistant and two susceptible parents. Data are currently being taken from an experiment that inoculated eighteen controlled crosses of the diallel and two standard open-pollinated seedlots used as susceptible controls at 5 months.

If resistance is polygenic, then characteristics of the mortality curve can be used to differentiate families or even mixed seedlots with potentially useful levels of resistance from susceptible seedlots. Two potential measures of assessing differences in quantitative resistance (time until 50 percent mortality, and area under mortality curve at two times the time until 50 percent mortality) are presented in Figures 4 and 5, respectively. With either measure, seedlots derived from parents previously selected for resistance fared better than unselected half-sib families. However, additional refinement of selection criteria may be needed. For example, differences were not as distinct in the second experiment testing 5-month-old seedlings, in which onset of mortality was the most rapid, as in other experiments.

Three approaches are under investigation for sharpening differences among families: 1) Choice of different selection criteria derived from the mortality curve; 2) Use of selfs, controlled crosses, or crosses using known mixed pollen sources; 3) Use of biological (plant-growth-promoting rhizobacteria) or chemical (elicitor) materials to induce systemic acquired resistance. Regarding the second approach, in those cases

where comparisons can be made in the experiments reported, resistance appeared to be greater in seedlings derived from controlled selfs or controlled crosses among resistant trees than for half-sib families from the same parents (Cross P327 x P312 in Figure 2; selfs and crosses in Figures 4 and 5). Random mating among a small set of resistant parent clones may also be useful for obtaining a high level of resistance among progeny. This is demonstrated in Figure 3 by the high levels of resistance of a bulk seedlot from a small, isolated nursery at ORSO that contains only the 5 resistant Patton clones P18, P30, P312, P327, P343 (1995 ORSO Aselect@ bulk seed).

Figure 1.

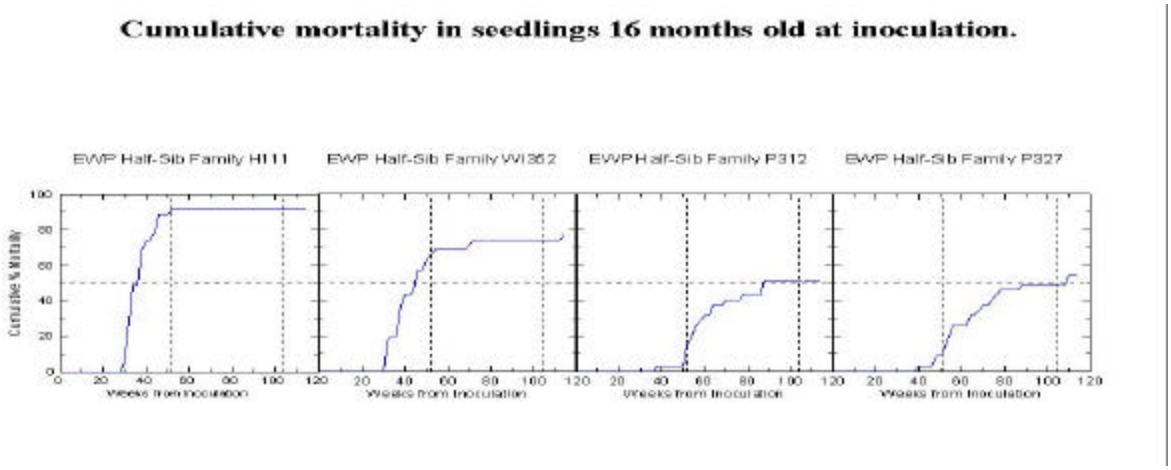


Figure 2.

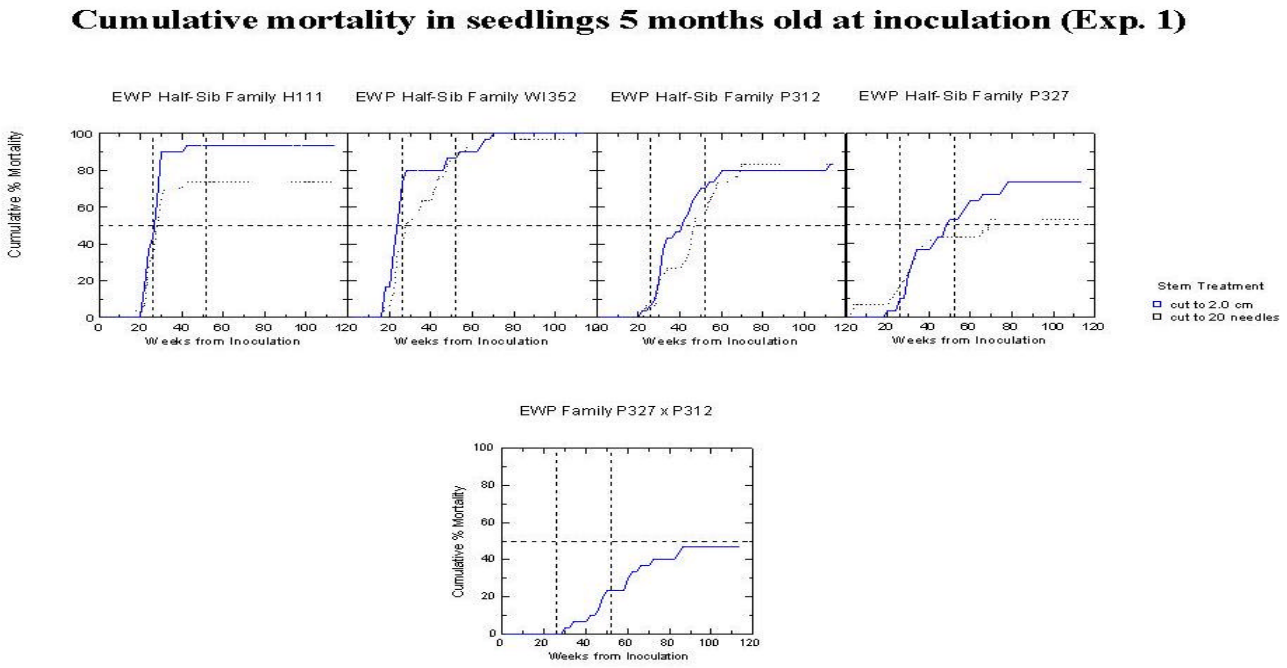


Figure 3.

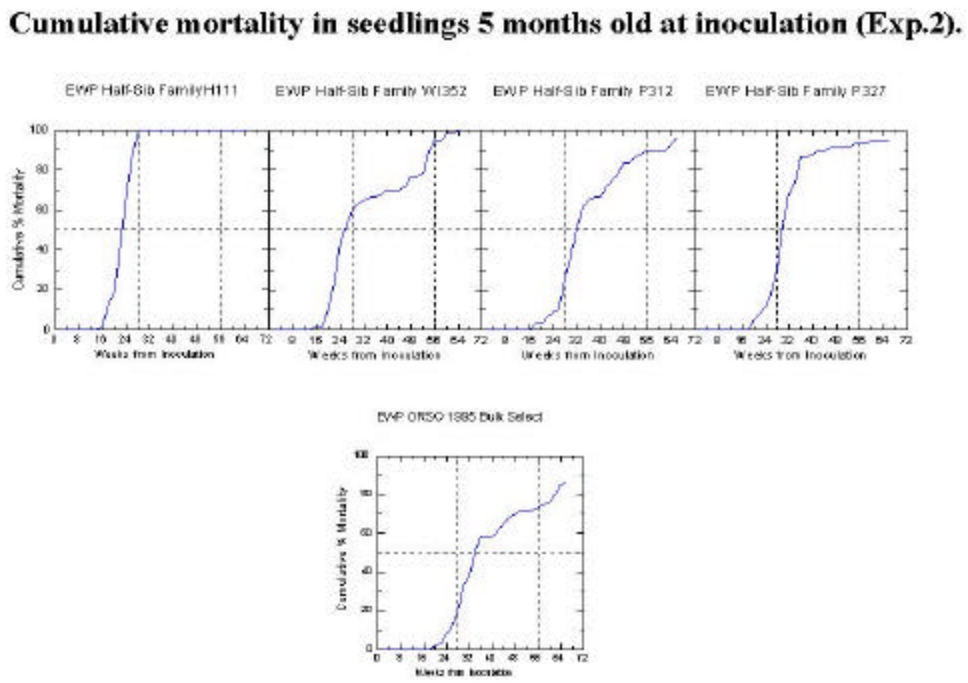


Figure 4.

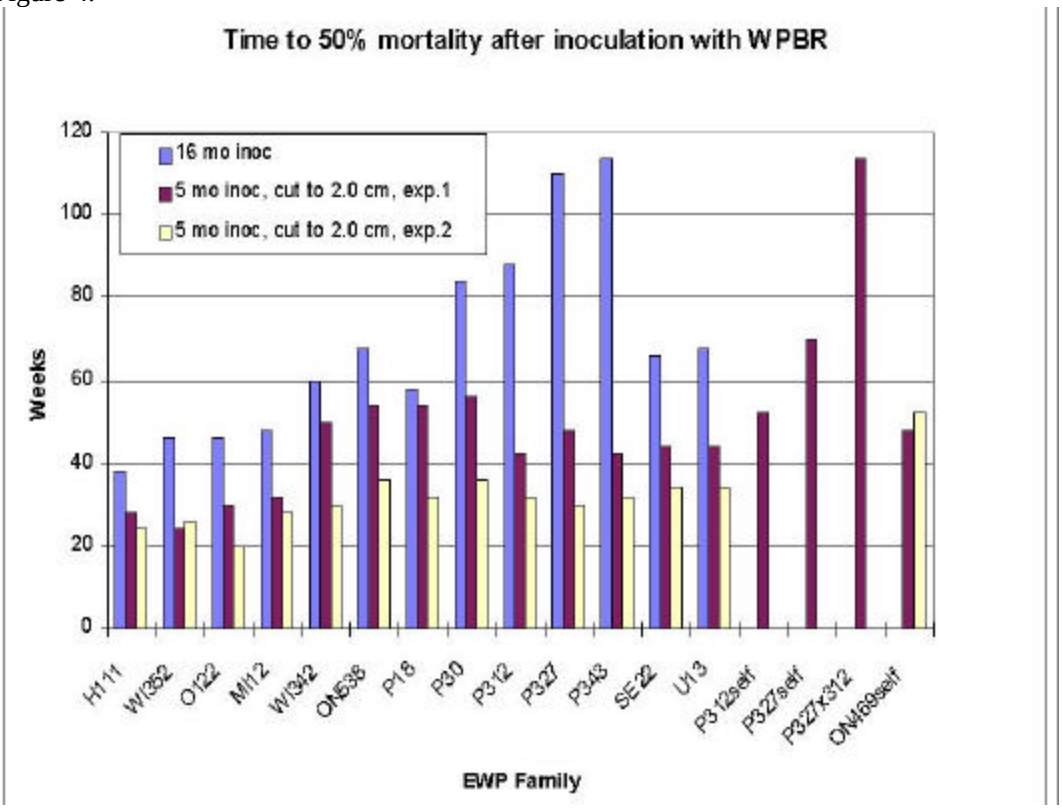
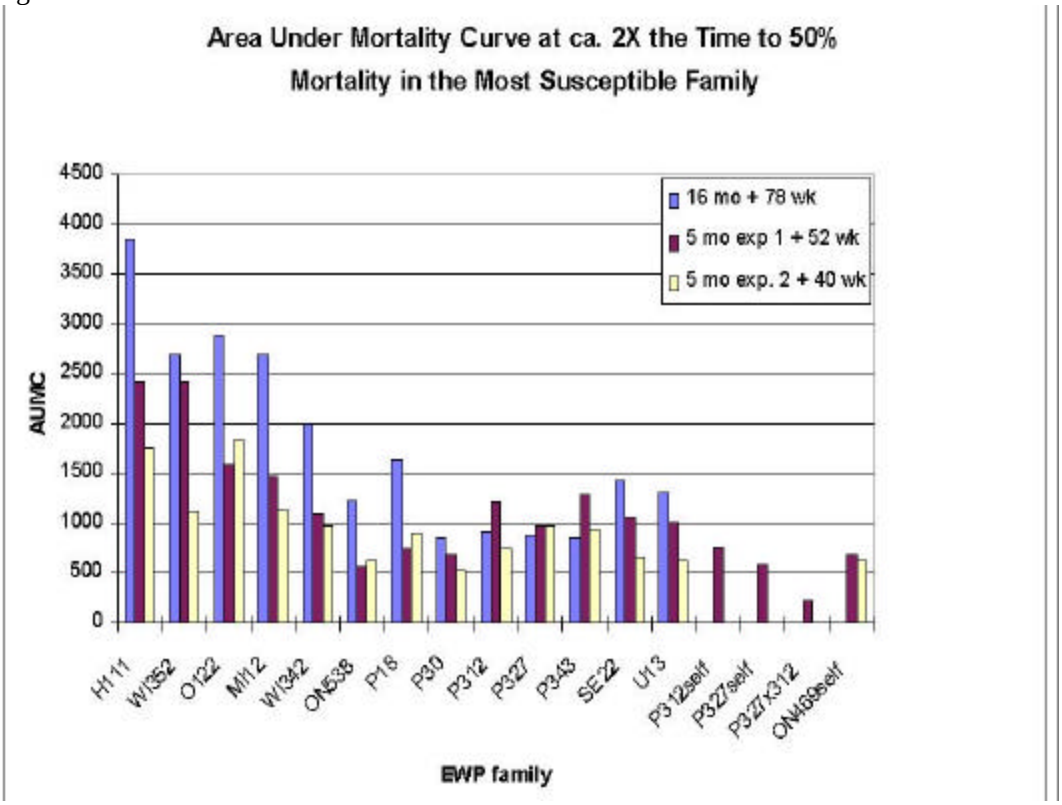


Figure 5.



Correspondence Between Seedling Assays and Field Resistance

To determine if levels of resistance detected in the greenhouse are appropriate to provide resistance under different field conditions, field experiments were planted in 1999 at three locations in Minnesota (St. Louis Co., Itasca Co., and Superior National Forest) and one location in Wisconsin (ORSO, Nicolet National Forest). Each experiment contained 18 families, in a complete block design with 54 blocked replications (3 replications of a 18x18 Latin square). An additional experiment at ORSO contained two ramets of each of 324 individuals previously tested by accelerated screening of cut plants. The 324 individuals comprise 18 individuals for each of 18 families included in the original screening experiment. Infection and rate of canker growth on branches of these experiments will be monitored for 5 years and correlated to family and individual estimates of resistance obtained from early screening. Coordination of field experiments is being provided by Dr. Robert Stine and Carrie Pike of Univ. of Minnesota, Cloquet Forestry Center.

Histological Mechanisms of Resistance:
Cooperators Dr. Robert Blanchette and graduate student Joel Jurgens at the University of Minnesota, Dept. of Plant Pathology, are examining the histology of early stages of seedling colonization of resistant and susceptible families in eastern white pine versus western white pine, in which several resistance mechanisms are known. Cooperative involvement in these studies has included obtaining resistant and susceptible families of eastern white pine (5 families from ORSO) and western white pine (15 families from cooperator Dr. Richard Sniezko, USDA-FS, Doreena Tree Improvement Center); inoculating 8 experiments thus far, at 5, 10, 15, and 20 weeks after emergence; and taking symptom and mortality data.

The eastern and western white pine material inoculated at 20 weeks will be analyzed as an Aaccelerated screening@ experiment to test which non-needle-based form(s) of resistance in western white pine (slow canker growth, bark reaction) have mortality curves similar to those of resistant eastern white pine. Additional inoculations are being planned to provide additional material for staining.
Two experiments have been conducted to test if external factors can affect disease reaction, histology, and the success of Aaccelerated screening@. In these experiments, two resistant (ON469 open pollinated and P312 x P327) and two susceptible (H111 open pollinated and families of eastern white pine were exposed to four treatments: control, seed treatment with plant-growth-promoting rhizobacteria (commercial strains SE34-*Bacillus pumilus* and 99-166-*Serratia marcescens* from Gustafson, Inc., supplied by cooperator Dr. Scott Enebak, Auburn Univ.), or a chemical elicitor (Actigard, Novartis, Inc.), before inoculation at 10 weeks of age. Data are being collected from these experiments.

Genetic Diversity of the Rust Fungus:

DNA markers in blister rust do not directly identifying differences among strains in their virulence to pines or Ribes. However, DNA markers can be used to investigate rust population structure and dynamics. This information is in turn useful in assessing how virulent rust strains might become established and spread within the Lakes region.

Progress has been made in four areas: 1) A method was developed for extracting high quality DNA from all spore stages of blister rust; 2) 60 RAPD primers were identified that produce reproducible markers in selected rust isolates; 3) 24 pure-genotype rust strains representing a cross section of hosts and geographic regions were supplied to cooperator Dr. Richard Hamelin, Univ. Lavalle, Ste. Fay, Quebec, who showed a high degree of genetic differentiation between the isolates from eastern white pine in Minnesota and eastward, versus limber pine and other hosts in western South Dakota and westward (Gst = 38%, based on 45 markers generated using 5 RAPD primers). This confirms that the rust was introduced into the east and west coast regions in separate introductions from Europe and that there has been low gene flow between the two regions to date. 4) Western Minnesota is the most likely area where the western rust population could become incorporated into the eastern rust population, and vice versa. Thus, additional strains from eastern white pine in Wisconsin and Minnesota, limber pine in South Dakota and Wyoming, and western white pine and whitebark pine in Idaho, Washington, and Oregon are being studied to test if strain mixing can be detected across these regions. These strains were sent to Dr. Hamelin, who is now testing their diversity using a combination of RAPD markers developed at both labs, as well as 2 codominant markers developed at his lab. Preliminary results from these strains confirms that eastern and western populations differ significantly, and also indicate diversity is much higher in the east than in the west.

In related work, there has been a renewed interest by horticulturists in producing currants commercially. Widespread culture of susceptible Ribes species could provide a means for strains to spread between the eastern and western populations and increase local disease pressure. Use of resistant cultivars could minimize this risk, if resistance is effective against all strains of the rust. Tests were conducted to determine whether the two main sources of resistance of commercial currant cultivars (the Cr gene in black currants, and the unnamed resistance of the Aimmune@ red currant cultivar AViking@) were effective against the predominant rust strains. Cultivars carrying the Cr gene were tested and found to be resistant to 23 strains from diverse regions and hosts in North America, including strains virulent on sugar pine and western white pine carrying MGR resistance. However, two separate sources of the red currant cultivar AViking@ that were tested were readily infected by all 23 strains, and developed uredinia and telia comparable to those of other susceptible red currants. A third source of this cultivar has been located and will be tested.

HISTOLOGICAL STUDIES

As noted in Zambino=s work, the material he is producing has provided an opportunity for histological examinations of the early stages of seedling colonization of both resistant and susceptible white pine seedlings. In a very short time, remarkable progress has been made in understanding what actually occurs within the needle when blister rust infection occurs. The work was conducted by Dr. Bob Blanchette and Joel Jurgens, Plant Pathology Department, University of Minnesota.

White pine blister rust infects needles and progressively moves into the branch and main stem of 5-needled pines. The fungus attacks the phloem and cambial region causing a diffuse canker that gradually kills the tree. Seed and grafting material from white pines found to be free of the disease have been selected over the past several decades and outplanted. Seedlings from these open pollinated trees have been grown in the greenhouse and tested for resistance to the disease by Dr. Paul Zambino, USDA Forest Service, using newly developed techniques. These inoculation studies showed that some of the selected white pine resists infection better than others. To gain insight into the trees defense reactions occurring in these trees, histological and micromorphological investigations were initiated. Characterization of resistance mechanisms in different seed sources of eastern white pine and knowledge of how these defense systems restrict invasion by the fungus will provide a better understanding of basic mechanisms responsible for resistance. This information will be of great value to rapidly screen new seed sources and help develop trees with the most significant capacity for resistance.

Research investigations involve microscopic examination of inoculated seedlings that represent tolerant and susceptible reactions to white pine blister rust. Inoculated needles and stems of white pine are sampled, fixed to prevent cellular changes from occurring, and embedded so the delicate tissue can be sectioned. Sections, a few microns in thickness, are made and a wide array of different histological stains are used to observe the spatial relationship of the invading rust fungus and to identify the chemical and morphological reactions occurring in the host. Histological reagents, such as phloroglucinol, are used to identify phenolic compounds produced by the tree to stop the infection and Schiff=s reagent or dyes such as analine blue and orseillin BB are used to observe fungal hyphae and cellular changes in the host. Observations using scanning and transmission electron microscopy are also used to elucidate the ultrastructural aspects of the host-pathogen interaction.

White pine blister rust infection starts with infection from a germinating basidiospore though the needle stomata. Once inside the needle, the fungal hyphae grow intercellularly down the needle into the branch and then move into the main stem of the tree. A small chlorotic area, usually first apparent on the underside of the needle, represents a successful infection. Eastern white pine seed sources tested show a large amount of variation with respect to the extent of needle lesions. Seedlings from susceptible families have very diffuse needle lesions with a mottled yellow and green coloration. These Asusceptible@ lesions usually increase to cover several cm of the needle within a few weeks after infection. Micrographs of sections made from needles of susceptible families show little to no restriction of hyphal growth within the needle tissue. The hyphae grow intercellularly throughout the mesophyll cells producing large numbers of intracellular branched haustoria. The haustoria are absorbing structures originating from hyphae that penetrate the host cell walls and obtain nutrients from the living cells. The colonization of the needle and subsequent host response causes significant symptom development resulting in lesions on the needles.

Families that show resistance to the invasion by the rust fungus have much smaller zones of infection and more brilliant yellow coloration. These spots rarely grow more than a few centimeters down the needle regardless of the amount of time after infection. Sections from these reaction zones show areas where hyphae are localized and further growth is restricted. The defense compounds produced by the tree around the infected area appear to be produced in relatively small concentrations but serve to compartmentalize the

infection. Since the amount of reaction compounds produced by the adjacent cells is not extensive, there is a large amount of variation in the success of individual reaction zones to stop the invading fungus. Another factor that appears to affect colonization of host cells by the fungus is the extent of cell death at the infection site. The rust fungus causing white pine blister rust is an obligate parasite and needs live host cells to stay alive. Rapid death of cells adjacent to the infected area can effectively restrict the fungus since colonization cannot continue. In some eastern white pine families that showed varying amounts of resistance to blister rust after inoculation, cell death helped to localize the infection.

None of the eastern white pines tested to date are immune to white pine blister rust but many seed sources show some tolerance to the disease. In these trees, the infection is slow to move within the needles and reaction zones stop the fungus from gaining entry into the main stem of the seedling. Current investigations are being done to further characterize the specific components produced in these reaction zones so the most effective chemical barriers can be identified in seed sources showing the highest levels of tolerance to the disease. See Figures 6 through 20 for photos and micrographs of various infections.

Investigations have also focused on the mechanisms of resistance found in western white pine families that have previously been identified with varying degrees of resistance to blister rust. A wide range of macroscopic observations have shown differences in the intensity of the needle reaction, reduced size of needle lesions, slow canker formation, etc. Microscopic observations of all these reactions are being done to categorize specific differences that occur and compare them to reactions observed within eastern white pine selections.

These microscopic observations demonstrate that in some selections of eastern white pine there is an identifiable degree of resistance to blister rust. The mechanism of resistance appears similar to the defense reactions found in some resistant selections of western white pine. Additional screening of white pine families and continued characterization of the resistant mechanisms will lead to the identification of superior sources of resistant trees with strong defense reactions. These trees will be valuable additions to breeding programs and future establishment of resistant eastern white pine in the United States.

Another area of research interest is the ability to induce greater resistant responses in trees to disease by biological or chemical additives to the soil or surfaces of the tree. The ability of specific strains of rhizobacteria to colonize plant roots and induce a general response that prevents subsequent infection by pathogens is a new field of study showing great promise. Chemicals that elicit plant defense responses are also being used as preventative spray treatments to protect against diseases. These novel ways of inducing greater host responses are being tested on susceptible eastern white pine to ascertain if they will be successful in controlling white pine blister rust. Monitoring of pine host responses in these studies is being carried out with histological and ultrastructural procedures.

FIG. 6. Susceptible white pine seedling inoculated in the greenhouse showing characteristic needle lesions associated with white pine blister rust infection.

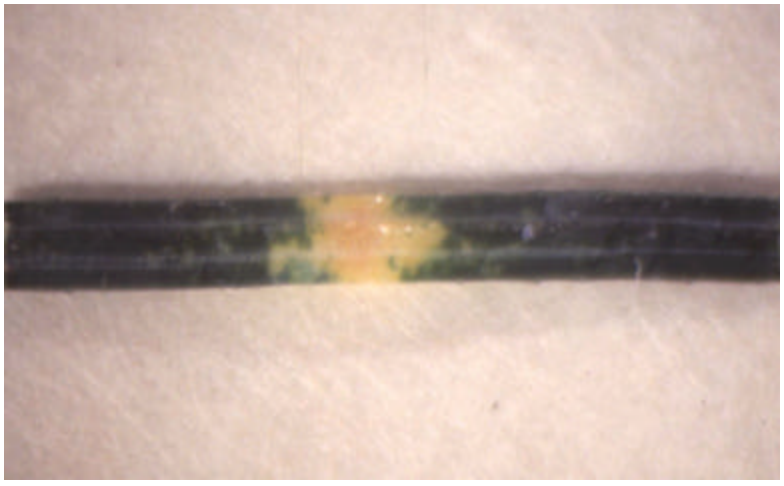


FIG. 7. Resistant reaction at site of infection on an eastern white pine needle. Lesion (yellow area) is restricted to a localized zone around the infection site. Photo taken 6 weeks after infection.

FIG. 8. Susceptible reaction on needle of eastern white pine showing widespread infection. Yellow mottled areas occur in the needle tissue around the unrestricted infection. Photo taken 6 weeks after infection.

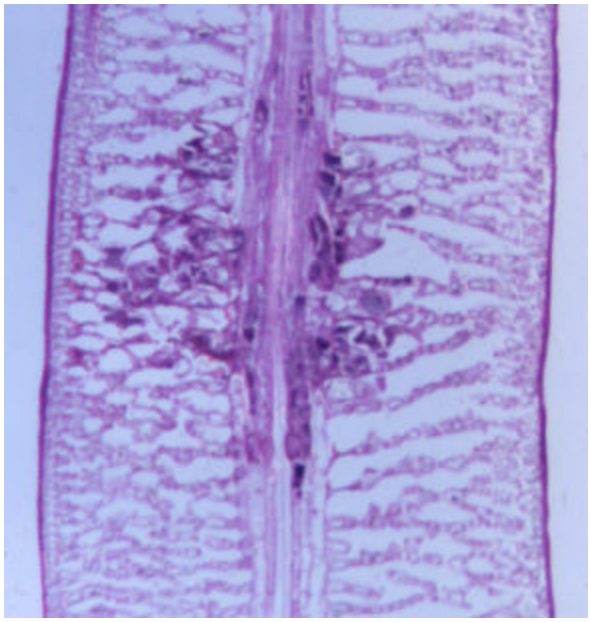


FIG. 9. Section of a needle showing a resistant reaction zone 4 weeks after inoculation. The host response is concentrated around the immediate vicinity of the infection site. Hyphae of the rust fungus are confined to a small area. Dark regions are host defense compounds. Section stained with periodic acid – Schiff's reagent.

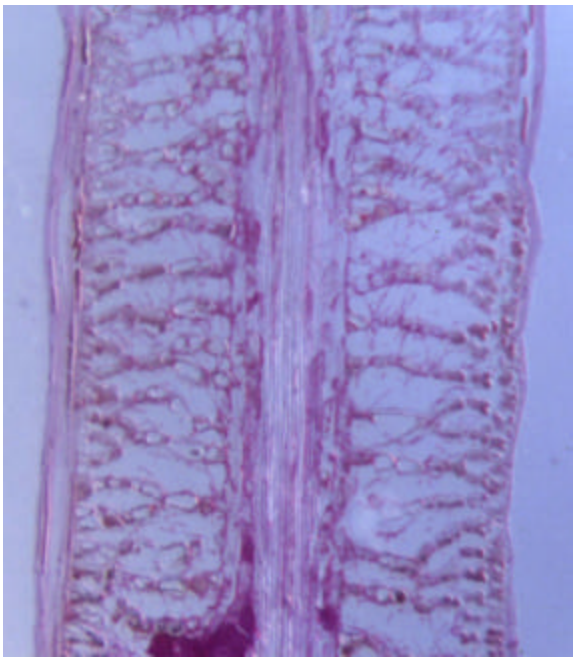


FIG. 10. Section of a needle showing a susceptible reaction 4 weeks after infection. Intercellular hyphae growing without obstruction can be seen throughout the needle. A diffuse host response is evident. Section stained with periodic acid – Schiff's reagent.

FIG. 11. Micrograph showing a section of a resistant needle stained with phloroglucinol, which is used to identify phenolic compounds. The bright red area indicates a positive reaction for these defense compounds. Sample taken 5 weeks after infection.

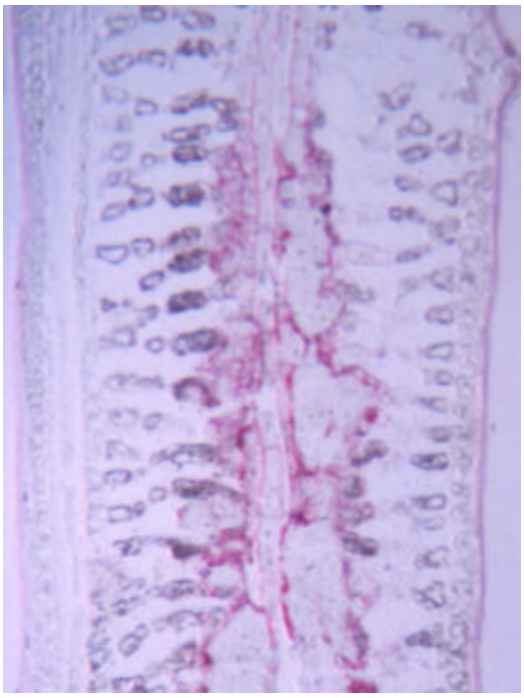


FIG. 12. Susceptible needle stained with phloroglucinol showing an extensive area of infection and a reduced phenolic reaction. Seedlings with this type of reaction have a diminished capacity to produce these defense compounds. Sample taken at 5 weeks after inoculation.

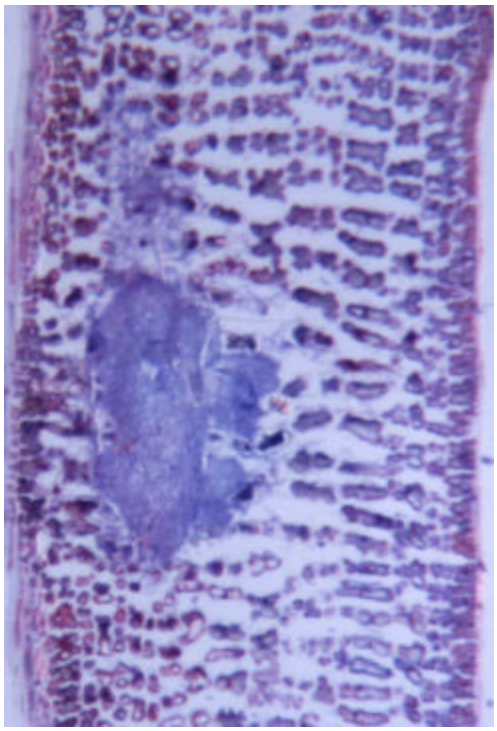


FIG. 13. Resistant needle stained with orseillin BB and aniline blue 5 weeks after inoculation. This stain is use to differentiate fungal hyphae and host tissue. Infected area is indicated by the dark blue region.

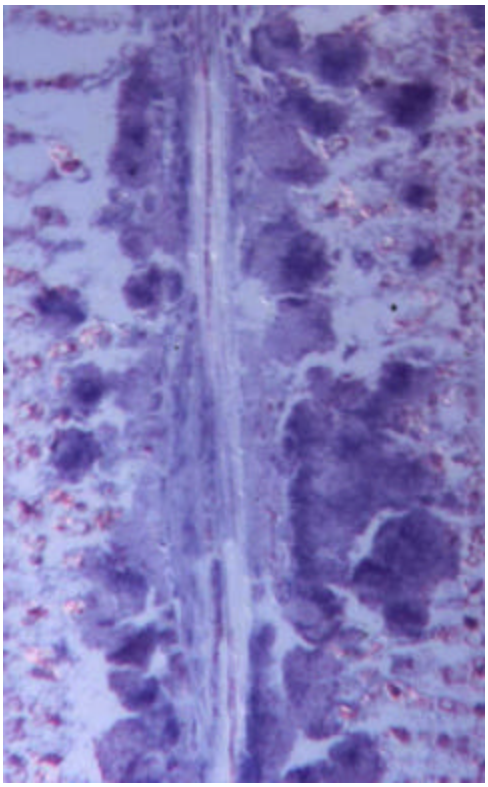


FIG. 14. Section of needle from a susceptible seedling showing large area of disrupted cellular morphology. Section stained with orseillin BB and aniline blue. Sample taken 5 weeks after inoculation.

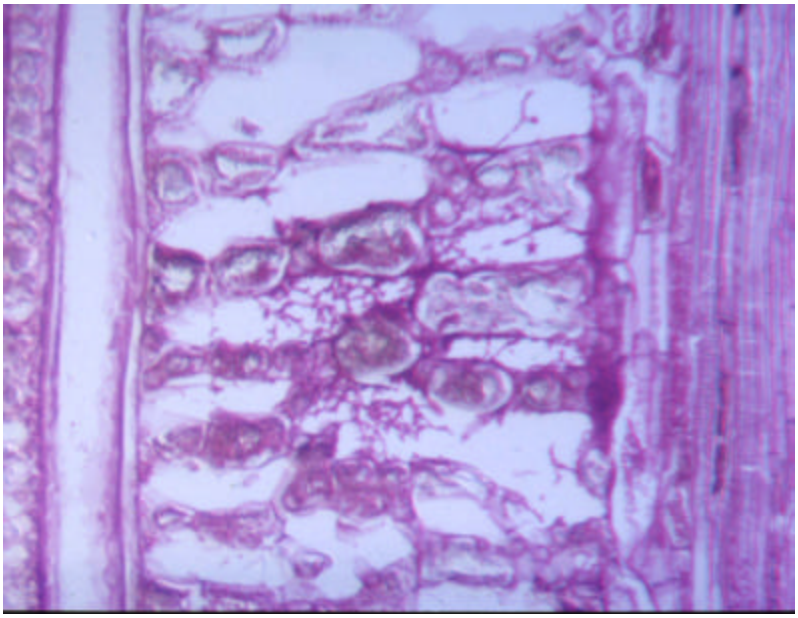


FIG. 15 Section of a needle exhibiting a resistant reaction and a localized zone where hyphae are present. The trees reaction has produced defense compounds around the infection and relatively few cells have been affected. Sample taken 5 weeks after inoculation. Section stained with periodic acid – Schiff's reagent

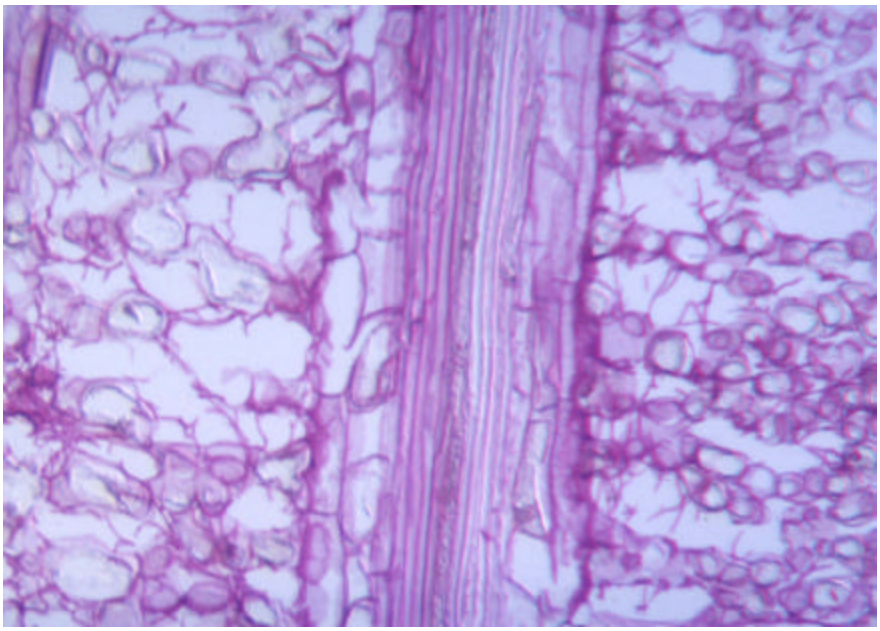


FIG. 16. Section of a needle from a susceptible seedling showing response to infection. Hyphae are numerous and widely dispersed throughout a large portion of the needle. Stained with periodic acid – Schiff's reagent. Sample taken 5 weeks after inoculation.

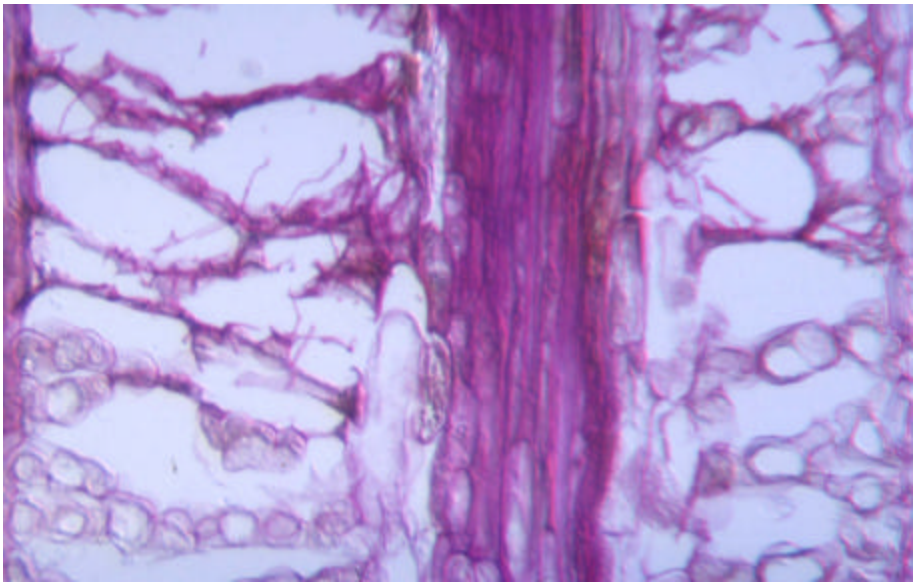


FIG. 17. Section of a needle showing a resistant reaction to infection. The mesophyll cells, typically associated with nutrient absorption by the fungal haustoria, have collapsed restricting the fungus. Colonization past this zone of affected cells did not occur.

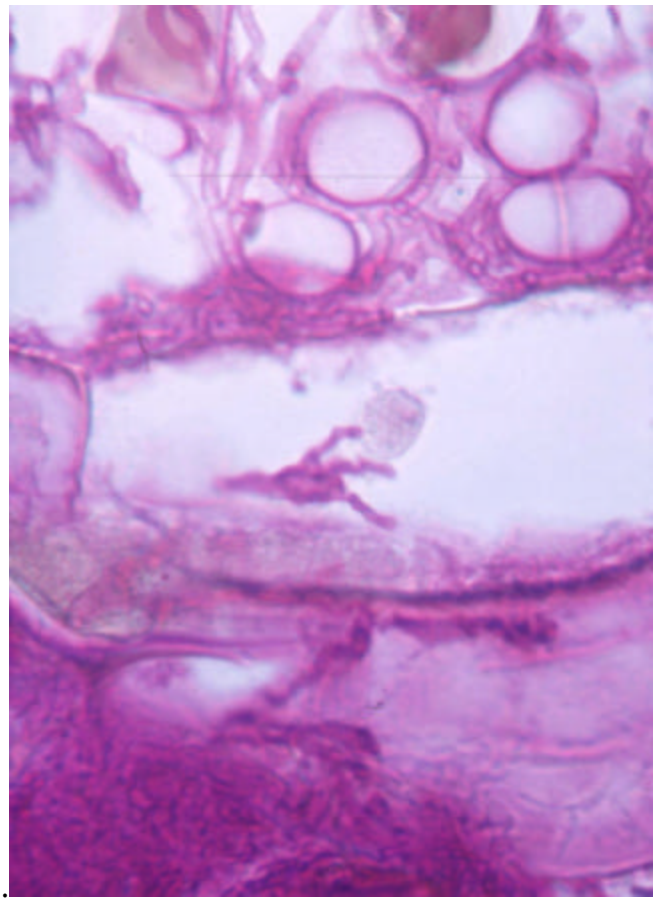


FIG. 18. A branched haustorium, the rusts nutrient absorbing structure, within a live cell.

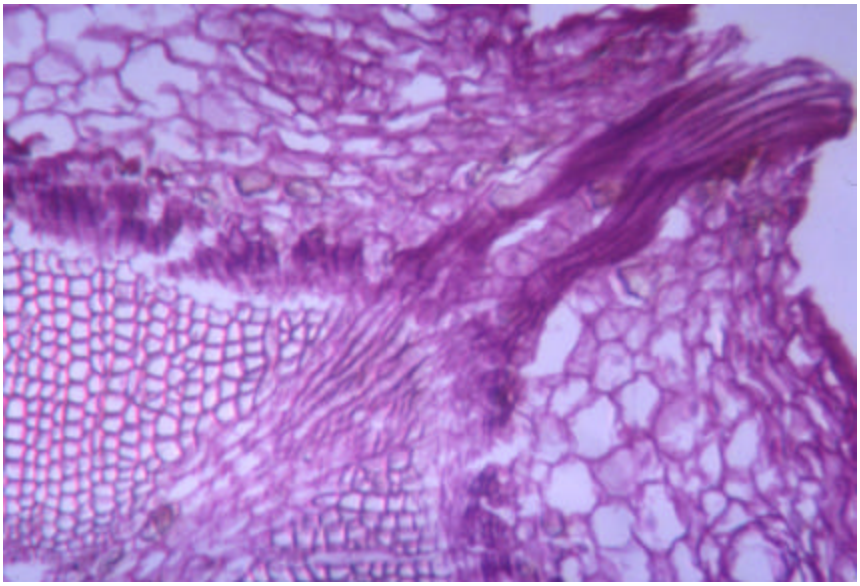


FIG. 19. Cross section of a resistant stem showing the node of an infected needle with no evidence of infection in the stem cells 5 weeks after inoculation. Section stained with periodic acid – Schiff's reagent.

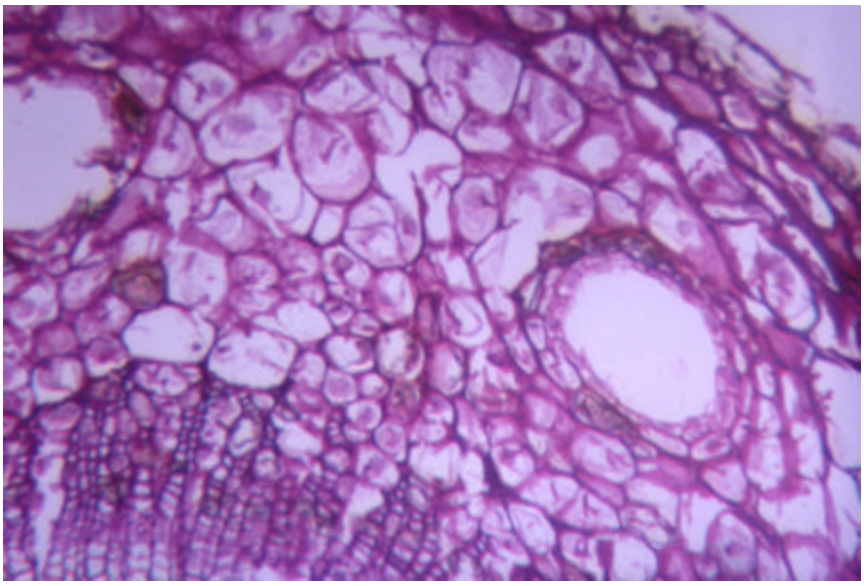


FIG. 20. Cross section of a susceptible stem showing extensive hyphal and haustoria development 5 weeks after inoculation. At this stage of infection the main stem appears swollen and discolored. Section stained with periodic acid – Schiff's reagent.

FUTURE RESEARCH

Work during the next two-year period will concentrate on four general areas; primarily continuing projects already started. Abstracts of the research projects are provided below.

Flower Induction on Young Grafted Material

Timing is critical for optimizing induction of female and male flower formation. Environmental factors such as light intensity, temperature, soil moisture, and nutrition also may affect the response of eastern white pine to these treatments. Recent reports in other *Pinus* species indicate that treatments during shoot elongation tend to promote pollen cones and treatments after shoot elongation tend to promote seed cones. Therefore one method may not be adequate to promote the production of both male and female flowers simultaneously. Flowering response can also be influenced by application technique. Stem injections of $GA_{4/7}$ or ProCone **J** into xylem-conducting tissues of developing shoots, branches, and main stems of various conifer species have been shown to promote flowering.

The study will utilize two different grafted white pine populations, one at the Cloquet Forestry Center, Cloquet, MN, and the second located at the St. Louis County Seed Orchard, near Cotton, MN. Both of these populations were selected for putative rust resistance. Data to be collected on a yearly basis includes tree height, number of male and/or female flowers produced per treated and control shoots, and phytoxic effects (precocious needle senescence, etc.) if any.

Results of this study will advance the knowledge of flower phenology in eastern white pine. This will give breeders and seed orchard managers a method of bringing trees into flower sooner and more reliably. This decrease in the amount of time required to reach sexual maturity will aid white pine breeding efforts for genetic resistance to blister rust by decreasing the time between generations. Knowing how to optimize flower induction in white pine using chemical and/or cultural treatments will be beneficial to operational seed

orchards in the Lakes States area by providing synchronous flowering of both pollen and seed cones across entire seed orchards.

Early Screening for Blister Rust Susceptibility

During the first two years of research, a rapid and reliable method was created for early screening of blister rust susceptibility in eastern white pine. This novel method inoculates 5-month-old seedlings cut to 2 cm of primary needle growth with blister rust and identifies resistant families within 1 1/2 years from planting. This is an improvement over less reliable methods that require up to 5 years for results.

During the next two years, experiments designed to verify the efficacy of the early screening method will be concluded and data collection and statistical analysis can begin. As inoculum level, environment, and seedling maturity can each influence infection and colonization, resistance detected by seedling screening may by higher or lower than is needed for field-planted trees. Experimental plots planted in summer of 1999 will determine what levels of resistance in 5 month-inoculated seedlings correspond to useful levels of resistance in the field but will need maintenance and monitoring in the next biennium.

Clones (ramets) derived from cuttings from the original 5-month white pine seedlings (ortets) used in two early screening experiments have produced fascicled needles and can now be inoculated as "mature" seedlings. If there is a high correlation between resistance of ortets and ramets, then early screening can not only be used to discriminate between families, but between resistant and susceptible individuals of the same family. This discrimination may be useful for developing DNA markers linked to resistance and for selecting highly resistant individuals from locally adapted families for planting in nurseries. If readily infected, clones might also be used to test specificity of resistance to specific strains or resistance at different field locations, preventing unexpected breakdowns in field resistance previously noted for some mechanisms of resistance.

In the past year, eastern and western white pine seedlings inoculated at different ages and showing macroscopic symptoms of infection were used in cooperative studies to examine the histology of eastern white pine resistance and determine if resistance appeared qualitatively different from western white pine mechanisms that have been overcome by virulent strains. Additional experiments are being conducted to confirm suspected relationships between cellular appearance, certain macroscopic symptoms of early colonization, and resistance. A portion of the plants in these latest studies have also been treated with non-specific biotic (1) and chemical elicitors of resistance to determine if resistance can be induced in pine, and whether histologic indicators of non-specific and rust-specific resistance are similar.

The early screening method, in combination with the field testing that validates the method, will give breeders a tool to evaluate seedlings for levels of resistance to blister rust at a very early age. This will enable breeders to rogue out susceptible seedlings from planting programs and evaluate parents based on progeny performance. Comparing resistance mechanisms in eastern and western white pine will identify the potential for eastern white pine to be susceptible to the virulent blister rust races that impact western white pine.

Histological Characterization of Rust Resistance Mechanisms

Much of this work utilizes white pine seedling material generated by the early screening research. Histological methods are used to examine the response of white pine tissue that is infected by blister rust to elucidate the mechanistic nature of susceptibility and resistance. If blister rust resistance is heritable within families (i.e. resistant progeny in one seedlot share a resistance mechanism) then breeding programs should be established to cross individual eastern white pine with similar mechanisms of resistance. Results have demonstrated it will be possible to characterize the tolerance to blister rust observed in some white pine families.

Examinations in the first biennium demonstrated distinct differences in needle reaction to infection. Needles from trees in more resistant families have an intense reaction to the fungus and tend to impede growth of the fungus through the needle. Needles from trees in more susceptible families have less intense reactions and allow extensive growth of fungal hyphae throughout the needle.

The histological and ultrastructural investigations will provide the mechanistic basis of susceptibility and resistance for the early blister rust research. Differences in resistance mechanisms among white pine families may indicate that parents crossed in breeding strategies must be of similar resistance mechanisms to maintain resistance to blister rust in the progeny. In addition to the comparison of eastern and western white pine, mechanisms of susceptibility and resistance will help determine the susceptibility of eastern white pine to blister rust. Evaluation of eastern white pine treated with rhizobacteria or Actigard and challenged with blister rust will indicate whether elevated resistance levels are due to a heightening of natural resistant mechanisms or a new resistance mechanism.

Field Testing and Establishment of Seed Orchards

The three projects described above are providing critically important tools and information for understanding genetic resistance to blister rust in eastern white pine. An equally important facet is the production of genetically improved seed with elevated levels of blister rust resistance. The objectives in this section are designed to accrue potential breeding material and bring new and existing seed orchards into flower as early as possible. Once these orchards are flowering and the resistance mechanisms for each individual are understood, a breeding plan that maximizes resistance to blister will be initiated. Several different field projects are underway, supported in part by forestry organizations and agencies in Minnesota interested in white pine. Some grant monies will be used to assist and administer these field-based projects.

In the long run these actions are structured to provide suitable amounts of germplasm for breeders to work with once blister rust screening and evaluation methods have been developed. In the short-term these sites will also provide material for flower induction studies.