White Pine Blister Rust Resistance Research in Minnesota and Wisconsin

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Abstract

The exotic fungus *Cronartium ribicola* causes the disease white pine blister rust on five-needled pines throughout North America. Although the effects of this disease are perhaps better known on pines in the western portion of the continent, the disease has also impacted regeneration and growth of eastern white pine (*Pinus strobus* L.), especially in the upper Great Lakes region of Minnesota, Wisconsin, Michigan, and Ontario. This paper summarizes some of the early white pine blister rust research in Minnesota and Wisconsin, particularly the Moose Fence site near Tofte, Minnesota, the consistently high resistance level of genotype P327, and how the Minnesota Tree Improvement Cooperative (MTIC) housed at the University of Minnesota and the USDA Forest Service Oconto River Seed Orchard (ORSO) are working together to advance the state of blister rust resistance in eastern white pine.

Introduction

At the turn of the 20th century, the exotic fungus *Cronartium ribicola* was brought to North America on seedling stock imported from Europe. Although there were multiple introductions across the continent, the results were similar; five-needled pines could become infected and die wherever the fungus entered an ecosystem that contained these pines and *Ribes* species that could act as an alternate host (Van Arsdel and Geils 2011). Forest pathologists came to recognize the life cycle of *Cronartium ribicola* or white pine blister rust in its new environment and combating the disease took on a two-pronged approach. One option was to physically remove individual *Ribes* plants. This method was cumbersome as it required a large, committed workforce to survey vast acreages on a repeated basis. The second option was to search for genetic resistance to the new disease in the native population.

One of the first plant pathologists to search for and find eastern white pine trees with putative resistance to white pine blister rust was A.J. Riker at the University of Wisconsin. By the mid-1930s, Riker recognized that, while most young white pines were susceptible to the new disease, there were rare individuals (roughly 1 in 400) that remained healthy. In the late 1930s, he identified 163 young white pine trees in Wisconsin that exhibited no external signs of infection despite coexisting with *Ribes* and the blister rust fungus for at least 15 to 20 years (Riker and Kouba 1940). In the early 1940s, Riker and his team artificially inoculated the progeny of these trees in a nursery setting and found differences between the offspring of selected and unselected trees (Riker et al. 1943). Although not complete resistance, this research was among the earliest evidence of natural occurring genetic resistance in eastern white pine and it offered hope that a genetic basis for full resistance was possible. These results also spawned the development of an active rust research group in the Lake States that included Cliff and Isabel Ahlgren at the Quetico-Superior Wilderness Research Foundation, Bob Patton at the University of Wisconsin, and Gene Van Arsdel also of the University of Wisconsin and U.S. Department of Agriculture, Forest Service (USDA FS), as well as many more

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applied state and federal employees. The methodology established by Riker also set a precedent for the rust screening efforts by the USDA FS in Wisconsin and in the western states.

However, the efforts of these researchers notwithstanding, a high level of heritable resistance remained elusive in eastern white pine. Unlike the western five-needled pines where outdoor nursery screening techniques identified a stably inherited source of major gene resistance (MGR), time and time again eastern white pine researchers found increased levels of resistance, but not complete resistance. Due to the apparent lack of MGR in eastern white pine, researchers began to see the need for a large-scale, long-term experiment that would expose the most promising genotypes to a high level of fungal inoculum. Such an experiment, if allowed to continue long enough, would create a population of eastern white pine with a higher than average level of genetic resistance.

Moose Fence Planting Near Tofte, Minnesota

In the 1960s, Cliff and Isabelle Ahlgren proposed a large-scale, long-term experiment and began collecting cones from putative rust resistant white pine along the north shore of Lake Superior and in the Boundary Waters Canoe Area Wilderness. By the late 1960s, the Quetico-Superior Wilderness Research Foundation, the USDA FS, and the University of Minnesota had entered into an agreement to establish an eastern white pine blister rust disease garden trial in a high blister rust risk area. The goals of the project were to provide a source of eastern white pine with higher than average rust resistance that could be used as a source of seed for reforestation purposes, and could serve as a source of advanced generation breeding material. This idea came to fruition when a total of 43,176 open pollinated 3-year-old eastern white pine seedlings from 873 families were planted in two stages (1972 and 1974) at a site near Tofte, Minnesota. The 873 mother trees came from 22 sites in two general areas in northern Minnesota: the Boundary Waters Canoe Area Wilderness and the north shore of Lake Superior, with a 23rd site in northern Wisconsin (Merrill et al. 1984). The 8.9 ha "Moose Fence" site was fenced to keep out moose (*Alces alces*) (hence the name) and interplanted with various *Ribes* species, the obligate alternate host, to increase the spore load and the chance for disease incidence.

In the fall of 1984, after these seedlings had spent 11 or 13 years in the field (14 or 16 years from seed), each tree in the 10 tree row plots was scored for survival and evidence of previous infection by white pine blister rust. The results of this initial screening indicated that overall survival ranged from 38 percent (1972 planting) to 41 percent (1974 planting). The frequency of seedlings with no evidence of previous infection was very low at 1.0 percent and 0.5 percent (1972 and 1974 respectively). Furthermore, in the 1972 cohort, the two best families had 14 percent non-infected seedlings while the two best families in the 1974 cohort had a 6 percent non-infected rate. This information suggested two things: the frequency of uninfected individuals occurred at a very low frequency in the natural population, and the data do not support the existence of a major gene for resistance (MGR) among the maternal parents (Merrill et al. 1984).

Subsequent to the 1984 assessment, surviving trees were evaluated for vigor and rust and 888 non-infected trees with high vigor were selected and permanently tagged with aluminum tags on nylon rope tied at the base of a lower branch. These 888 non-infected trees represented 2.1 percent of the total number of seedlings planted and were two to four times higher than the estimate of non-infected seedlings from the 1984 assessment. This difference is likely the result of stringent initial scoring in 1984 which used the terminology, "no evidence of previous infection" before a seedling was considered non-infected. This would have resulted in seedlings with marks on stems or branches (potential hail or insect damage) being left out of the non-infected category. Additionally, when the trees were tagged, their larger size relative to the 1984 scoring undoubtedly made visualization of the entire bole and all stems more difficult, potentially resulting in missed infections.

After the 1993 growing season, at approximately 20 years of age, the tagged trees at the Moose Fence site were scored for survival, vigor, and evidence of rust. Rust incidence was scored on a 1 to 4 scale, with 4 representing a tree free of rust, 3 a tree with inactive canker(s), 2 a tree with active canker(s), and 1 indicating a tree dead from rust. Vigor was scored on a 1 to 4 scale with 4 being the most vigorous and 1 representing dead. Only 802 of the 888 tagged trees could be found because, at roughly 20 years of age, a combination of factors were rendering the monumentation in the planting difficult to use. The serpentine layout of the 10 tree family plots, small size of the aluminum stakes, considerable mortality, and non-linear rows due to 1.5 m by 1.5 m spacing on shallow, rocky soils, all contributed to making navigation in the plantation extremely difficult. Of the 86 missing trees, 67 were never found, while 19 trees were found in future assessments. These 67 lost trees represent trees that either died or lost their tags due to breakage or self-pruning of their lower branches where the aluminum tags were attached. Results of this 1993 scoring indicated that 747 (93.1 percent) of the 802 assessed trees were uninfected while 4.6 percent had inactive cankers and 2.2 percent had active cankers. There were no dead trees among the 802 assessed (table 1), although there may have been dead trees among the 67 that could not be found.

Table 1—Number of select eastern white pine trees by rust score category (4 = uninfected, 3 = inactive canker(s), 2 = active canker(s), 1 = dead) and average rust score at the 20, 30, and 37 year assessments[a]

		Rust	Score				Rust score	
Year	**Age**	**4**	**3**	**2**	**1**	**Not found**	**Average**	***N* =**
<1993	11-19 years	888						888
1993	20 years	747	37	18	0	86	3.9	802
2003	30 years	516	191	95	0	0	3.5	802
2010	37 years	442	55	255	47	3	3.1	802
2003	30 years	552	194	97	0	0	3.5	843
2010	37 years	465	59	266	48	5	3.1	843

[a] 888 trees were initially selected and tagged sometime between 1984 and 1993 of which only 802 could be found in 1993. The 843 trees in 2003 and 2010 represent the 802 tagged trees plus the inclusion of 32 uninfected trees likely among the 86 lost trees in 1993 and 9 trees found from the original 888.

Prior to the 30 year assessment in 2003, an additional 32 non-infected trees (rust score = 4) were tagged permanently with numbers from 900 to 931. These trees were most likely from the group of 67 trees that had lost their original tags prior to the 1993 assessment. The remaining 35 trees from the group of 67 were unaccounted for and were either infected and not tagged or died of unknown causes, most likely blister rust. The 2003 assessment recorded rust scores on the same 1 to 4 scale for 843 trees (table 1). The percentage of uninfected trees dropped approximately one-third between age 20 and age 30. Despite this drop in the average health of the population, it should be noted that the majority dropped just one classification, while approximately 70 trees dropped two classifications. None of the tagged individuals died between the 20 and 30 year assessments.

Despite the lack of mortality among tagged trees, by age 30 it was obvious that blister rust was actively working in the planting. Large, standing dead trees could be seen throughout the plantation while other trees had fantastically contorted trunks due to multiple cankers. The frequency of tagged trees in the plantation appeared to be increasing over time as non-tagged trees died at a faster rate. Surprisingly, some trees that appeared to be growing vigorously were infected, but had not been tagged. Presumably these trees were infected before the tags were put on the original 888 uninfected trees. Therefore, these vigorous but infected trees had existed with the disease longer than any of the selected tagged trees and, borrowing the parlance from western North American breeding programs, we began to think of these trees as potential “slow rusters”.

In 2010, a complete census of the plantation was performed, placing the remaining live trees into one of three categories: 1) previously tagged, 2) not previously tagged, with cankers but appearing healthy, and 3) not previously tagged, with cankers and appearing unhealthy and/or likely to die within the next year. The census results indicated that there were a total of 1,274 trees alive after 37 years, a survival rate of 2.9 percent. The 1,274 surviving trees were categorized as follows: 794 were previously tagged, 346 had cankers but appeared otherwise healthy, and 134 were cankered, appeared unhealthy, and/or were likely to die within the next year (table 2).

Table 2—Thirty-seven year census results for the Moose Fence eastern white pine blister rust trial near Tofte, Minnesota

Category	Description	Number alive
1	Previously selected	794
2	Possible slow-rusting genotypes	346
3	Severely cankered or likely to die within 1 year	134
Total		1,274

After the 2010 census, all the category 1 trees and the best of the category 2 trees were evaluated for vigor (very good, good, fair, poor), rust score (1 to 4 scale as previous), the number and type of cankers (round or linear), presence or absence of callus at the canker edge, and the percentage of the stem alive at the most infected point. This process identified 114 trees (84 category 1 trees and 30 category 2 trees) that are considered potential slow rusters and will be followed over time. Of these 114 potential slow rusters, 52 were selected based on percent bole alive (> 80 percent), with preference given to linear vs. round cankers, two or fewer cankers, rust score = 3, and the subjective "this looks like a good slow ruster". All selections had callus formation around the canker edges. Scions from these 52 trees were collected and grafted in 2011 and were outplanted in spring 2012. These 52 trees represent 43 category 1 trees and 9 category 2 trees.

The trial at Tofte has provided some very valuable long-term research results. It is obvious from the rust score numbers in table 1 that a wave of mortality is working through the tagged trees. Because most of these trees are dying due to cankers that are located within 3.0 m of the ground (personal observation), we know that these trees were at least 3.0 m tall before they were infected. Since it is taking at least 10 to 20 years for them to die from blister rust, there is a period of time where the tree is still functional while the pathogen is a) moving through the branch towards the bole or b) latent in the branch and/or bole. As the remaining trees have few lower branches to become infected and the incidence of blister rust infection is partially ontologically controlled (Patton 1961), we expect that the rate of new infections will decrease in the future. The health of currently infected trees, whether the infection is latent or not, is expected to decrease over time. The census indicated that after 37 years, 2.9 percent of the trees were still alive, although that is expected to drop to 2.6 percent by the 40th year in the field as the category 3 trees succumb to the disease. In retrospect, this 2.6 percent survival rate is remarkable considering there is no evidence for MGR in the population, the trial was established in a high blister rust risk area, and *Ribes* spp. were interplanted among the seedlings.

By comparing survival rates between selected trees and the population as a whole it is clear that early selection for uninfected trees with high vigor during the second decade was successful in this population. The initial census of the trial (Merrill et al. 1984) indicated total survival was approximately 40 percent at 12 years. After 37 years, total survival was down to 2.9 percent with the selected trees representing 62.3 percent of the survivors. Furthermore, the fact that the selected trees had an 89.4 percent survival rate (794 of 888 trees) at age 37 and that 52.7 percent of the selected trees (468 of 888) were still considered uninfected roughly 20 years after selection, is additional evidence that early selection was effective in this population.

After close to 40 years of constant selection under real world conditions, it is clear that the Moose Fence planting has tremendous practical value. The remaining trees represent the largest genetically diverse source of potentially resistant eastern white pine anywhere in North America and most likely the world. As such, Moose Fence is a unique site that provides locally adapted seed and genotypes with increased genetic resistance to white pine blister rust that can be used for reforestation purposes and advanced generation breeding.

The Genotype P327

The Moose Fence planting at Tofte is not the only source of genetic material being used in Minnesota or Wisconsin. Dr. Patton at the University of Wisconsin oversaw a large white pine blister rust selection and screening program. He and his team scoured woods and plantings throughout Minnesota, Wisconsin, and Michigan for potential rust resistant trees and tested their scion and open pollinated progeny at several locations throughout Wisconsin. The most famous of these Patton selections is P327 which was identified in a planting of unknown origin outside of Duluth, Minnesota. Because P327 is consistently one of the last families to reach 25 percent, 50 percent, and 75 percent mortality levels in greenhouse screening trials, it is often used as a positive control.

Jurgens et al. (2003) used histological techniques to identify a potential mechanism of resistance among a portion of open pollinated P327 seedlings. Using various stains he was able to differentiate the fungal mycelium from mesophyll cells as the hyphae moved into and through the needle of infected white pine seedlings. Figure 1 compares infected needles from progeny of P327 and a susceptible control H109. What is clear from the figure is that mesophyll cells in P327 collapse just ahead of the fungal hyphae slowing down the spread of the fungus which requires live cell tissue to survive. Conversely, needles of the very susceptible H109 are packed with mycelium allowing the hyphae to progress through the needles to twigs and branches.

More recent research (Smith et al. 2006) has shed some light on stomatal status in P327. Environmental scanning electron microscopy (eSEM) images demonstrate that needles of P327 have occluded stomates (fig. 2). These occlusions were shown to be similar to the waxes that envelope the needles and are hypothesized to act as a physical barrier that prevents fungal hyphae from entering the needles. In theory, the more susceptible clones like H111 lack occluded stomates, allowing blister rust hyphae an opportunity to enter the needles following spore germination on the needle surface.

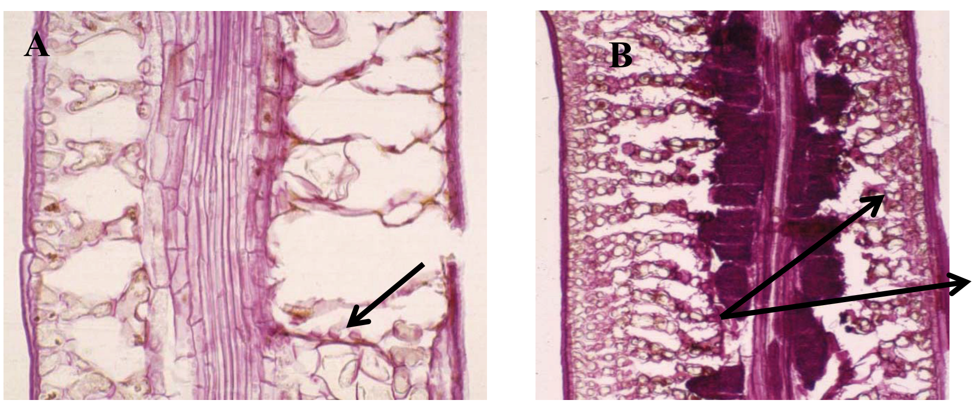

Figure 1—Needle sections stained with periodic acid-Schiff's reagent reveal (A) lysed mesophyll cells ahead of blister rust hyphae in an open pollinated seedling of P327, and (B) densely packed mycelium surrounding the vascular bundle in an open pollinated seedling of the more susceptible H109.

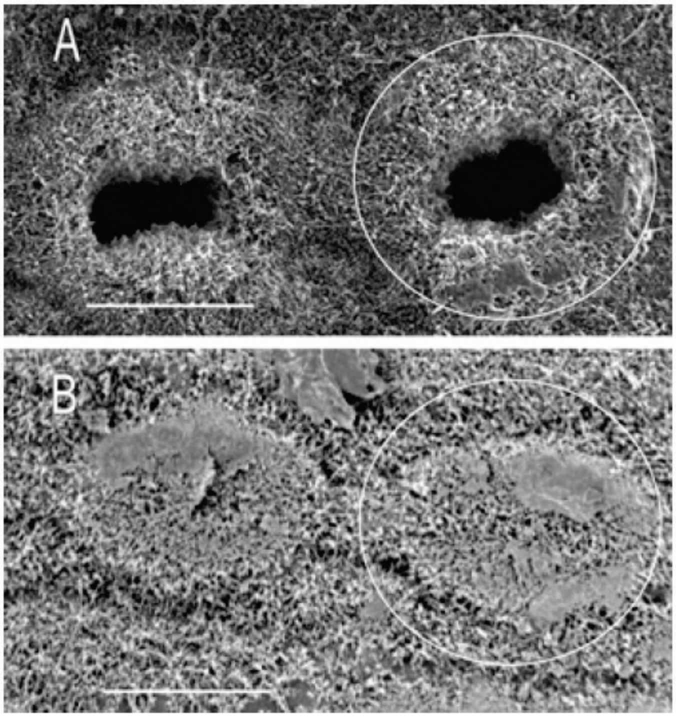

Figure 2—Environmental scanning electron microscopy image of secondary needles showing (A) open stomates in susceptible H111, and (B) wax occluded stomates in more resistant P327.

USDA Forest Service Oconto River Seed Orchard

The USDA FS's Oconto River Seed Orchard (ORSO) near Langlade, Wisconsin serves as the source of seed for white pine regeneration efforts in Region 9. The bulk of this seed comes from an interim seed orchard comprised of 30 Heimberger and five Patton selections. Future plans call for the establishment of at least one new clonal seed orchard of local origin where the increased resistance to white pine blister rust is well documented. At ORSO there is also a grafted clone bank (roughly 1,200 selections) from northern Minnesota, northern Wisconsin, and the upper peninsula of Michigan. Clonal material of non-infected and slow rusting Tofte genotypes has been kept separate to facilitate tracking known origins.

One of ORSO's mandates is to screen for blister rust resistant white pine. This is accomplished using an indoor system that was refined (starting in 2004) using suggestions from Paul Zambino (research pathologist; formerly at the USDA FS Rocky Mountain Forest Research Station). A typical example would use open pollinated seedlings with a mixture of primary and secondary needles from mother trees in the orchards or clone bank and expose them to blister rust spores at a spore load of approximately 10,000 spores/cm^2. The seedlings are sown in the greenhouse in February or March and inoculated in November of the first year. In May of the second year they are scored for percent of seedlings with foliar symptoms and the severity of symptoms before being placed outside. In mid-summer of the second year, the seedlings are scored for the percent of seedlings with stem cankers and their severity. Ideal families would have many seedlings with foliar symptoms, indicating exposure and initial infection, but a decided lack of stem symptoms indicating some level of resistance. The seedlings are followed for an additional 2 to 3 years while they are evaluated for evidence of resistance mechanisms, although at this time there is no formal, definitive end to the evaluation period. Initial work has shown that the 10,000 spores/cm^2 appears to provide sufficient inoculum to produce foliar symptoms across all families without killing all seedlings. The 10,000 spores/cm^2 level also demonstrates a definite dosage effect associated with susceptible and resistant families. In the most recent results ORSO has identified four to five additional genotypes out of 34 tested that show promise for elevated levels of blister rust resistance.

University of Minnesota's Tree Improvement Cooperative

The Minnesota Tree Improvement Cooperative (MTIC) is based on the industry-governmental organization-university model and is headquartered at the University's Cloquet Forestry Center. It is comprised of 14 full members and six supporting members who focus their efforts on five conifer species native to Minnesota, including white pine. The MTIC's white pine program has been active since the mid-1980s. Among the cooperators with full membership, there are five 1st generation clonal seed orchards totaling 3.4 ha. These seed orchards are comprised of genotypes selected by earlier researchers for increased blister rust resistance in nursery and early field trials. Sources include: USFS ORSO, University of Wisconsin, and the Quetico-Superior Wilderness Research Foundation. The MTIC has been collecting open pollinated cones from individuals in these seed orchards and at Tofte in anticipation of establishing a progeny trial in 2014.

The MTIC also manages two other long-term white pine plantings. One site, the Moose Fence trial mentioned earlier, is jointly managed with the USDA FS, Superior National Forest, Tofte Ranger District while the second planting is a white pine breeding arboretum located at the Cloquet Forestry Center in Cloquet, Minnesota. As of August 2011, the white pine breeding arboretum has three ramets each of 214 Tofte genotypes and 94 non-Tofte genotypes; primarily selections from Minnesota and Wisconsin. In the spring of 2012, grafts of the 52 potential slow rusting genotypes from Tofte were planted at Grimsbo Field, increasing the total to over 350 individual genotypes in the breeding program. Current plans are to breed these selections both for testing increased resistance using the ORSO method and to establish more traditional field based progeny tests.

In summary, MTIC and ORSO have programs that have benefited from previous white pine blister rust research. As a result of this early work, both organizations have been able to establish seed orchards and provide their stakeholders with eastern white pine seed for reforestation needs. Early selections such as P327, which consistently produces progeny that outlive other families in blister rust screening trials, have formed the bulk of this early research. However, recognition is growing that, to support white pine reforestation efforts across Minnesota and Wisconsin, there is a need for locally adapted germplasm of known origin and more genetic diversity than existed in the early selections. The value of the Moose Fence site at Tofte is that over the past 40 years there has been active selection for increased blister rust resistance in a population that is genetically diverse and adapted to growing conditions that are found in northern Minnesota and northern Wisconsin. As a result, both the MTIC and ORSO have been working cooperatively to critically evaluate individual trees within Moose Fence and determine the genetic value of both uninfected and slow rusting phenotypes.

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