Volume 2: Spring 2015

REVIEW

Breeding Efforts with Eastern White Pine (*Pinus strobus* L.)for Resistance to Blister Rust (*Cronartium ribicola*)

Tami Rahkola

Abstract

Eastern white pine, *Pinus strobus* L., is native to North America, and is susceptible to attack by the blister rust, *Cronartium ribicola* J.C. Fischer in Rabenhorst, a fungus native to Asia. The pines in Eurasia have some resistance to this fungus through constant exposure, and the ensuing molecular arms race. The pines in North America descended separately from their Eurasian relatives, and do not possess the same resistance. In the early 20th century, some North American pines were found growing amongst infected neighbors. These trees and their clones have since been used for both molecular investigations, and intraspecific breeding programs in the search for natural resistance. When intraspecific programs proved less than successful in other countries, they turned to interspecific efforts. These programs have had success with crossing eastern white pine to the Himalayan blue pine, to increase resistance, while also maintaining the growth characteristics of eastern white pine.

*Biology Department, University of Minnesota Duluth,

Introduction

Eastern white pine, *Pinus strobus* L., is native to North America and it is attacked by the blister rust, *Cronartium ribicola*. This rust is a fungus that is native to Asia and has devastating effects on white pines. Native white pine once covered vast regions of the United States, and it has long been valued around the world for the quality of its wood, wide climactic adaptability, and its ecological value (Blada and Popescu 2004; Radu 2008). Eastern white pine (*P. strobus*) was imported to Europe in the 1700's to be cultivated for its easily adaptable and robust growth characteristics (Blada and Popescu 2004). When re-imported to the U.S. in the 1900's, the damaging rust fungus came with it (David et al. 2012). As every white pine native to North America is susceptible to this fungus (United 2014), it has spread throughout the U.S. and Canada.

Though every North American white pine is susceptible to infection, many native white pines in Europe and Asia have a natural resistance (Blada and Popescu 2004). The blister rust fungus originated in Asia, which kept it within close proximity to the native Eurasian white pines (Van Arsdel and Geils 2011). When two species are in a **symbiotic** relationship, and one is harmed by that relationship, the harmed species must develop a way to hinder the relationship in order to survive. This is commonly known as a **selection pressure** within **natural selection**. In response to a host's developed resistance, the pathogen must then overcome that resistance in order to survive. The interaction of the two species, while in contact, is defined in the Geographic Mosaic of Coevolution (**GMC**) (Thompson 1999). The interaction between the Eurasian pines and blister rust fits into this mosaic: the two have survived alongside each other for millennia (Van Arsdel and Geils 2011). The pines in North America also fit into this mosaic in that they have descended outside of constant interaction, termed a "cold spot." The definition of which is detailed later in this review. Therefore, they do not possess the same resistance

Symbiotic: An interdependent relationship - may not be beneficial to one of the parties involved

Selection pressure: An environmental pressure which can result in the adaptation of a new trait and thus drive natural selection

Natural selection: The individuals best suited to their environment are more likely to reproduce and pass on their traits to offspring

Corresponding Author:
Tami Rahkola
rahko032@d.umn.edu

GMC
Geographic mosaic of coevolution – occurs when local variation in species interactions produces a spatially variable pattern of reciprocal adaptation in these species (Craig, in press)

Intraspecific
Occuring within a species

Interspecific:
Occurring between different species

as the Eurasian pines.

The initial search for naturally occurring resistance in white pines began nearly 100 years ago. As early as the 1930's, healthy *P. strobus* trees were found living amongst infected *P. strobus,* and plants from the *Ribes* genus, without showing any signs of infection (King et al. 2010; Patton and Riker 1953). *Ribes* plants are the alternate host for the *C. ribicola* fungus. Researchers since have been using these apparently healthytrees and their clones in **intraspecific** breeding programs. Through isolating the best trees from each study, a database of the most resistant pure *P. strobus* has been gathered (David et al. 2012). More recently, molecular techniques have also been used in the effort to find resistance genes. With a few other related North American white pines possessing a gene which offers some weak resistance (King et al. 2010; Sniezko et al. 2014), it was thought that *P. strobus* would also have those resistant genes. However, none have yet been found in *P. strobus*.

Intraspecific breeding programs in Canada and Romania were discontinued due to lack of intraspecific success, and financial constraints; however, **interspecific** programs were continued (Blada and Popescu 2004; Lu and Derbowka 2009). Interspecific efforts bred *P. strobus* to both related North American white pines, and related Eurasian species, to find crosses that would produce non-sterile, resistant progeny. Maintaining the growth characteristics of *P. strobus* was also a concern, due to its economic value. Additional concerns include making the offspring acceptably cold-hardy, and reducing the cost of breeding programs. The cross of *P. strobus* to the Eurasian species, *Pinus wallichiana* A.B. Jacks, has been showing success in the interspecific programs, by producing hybrid progeny that maintain both the resistance of *P. wallichiana,* and the growth of *P. strobus*.

This review will first briefly discuss the survival of *C. ribicola*, to facilitate the need for resistant trees. Then a short review is given on some of the genetic research done, which is followed by a discussion on two different breeding programs in use for obtaining natural resistance in *P. strobus*. The intraspecific breeding programs have provided material for genetic research, along with the best rates of natural resistance that can be found in pure *P. strobus*. The interspecific breeding programs have shown success with the hybridization between *P. strobus* and the Eurasian species *P. wallichiana*, to produce fertile, rust-resistant offspring. Bringing together the intraspecific and interspecific breeding programs may hold the key to breeding blister rust resistant progeny into this integral species.

Research

Survival of Cronartium ribicola

When a host species and a parasitic organism are in a harmful symbiotic relationship, the two develop mechanisms by which to resist each other. As mentioned prior, this can be clarified through the GMC. The GMC is well documented in Thompson (1999): When two organisms are actively involved in a relational process of natural selection, the interaction is termed a "hot spot." When each species has gained adequate strategies to overcome each other's resistance, the interaction is less intense and becomes less hot, where the two can coexist while continuing to adapt to each other's presence. If a related species is not involved in this molecular arms race (term from Ravensdale et al. 2011), the region it resides in is termed a "cold spot" (Thompson 1999). If the pathogen is then introduced to that cold spot, the resident related species does not possess the adapted resistance to the pathogen.

The molecular arms race and GMC have been well documented in other species

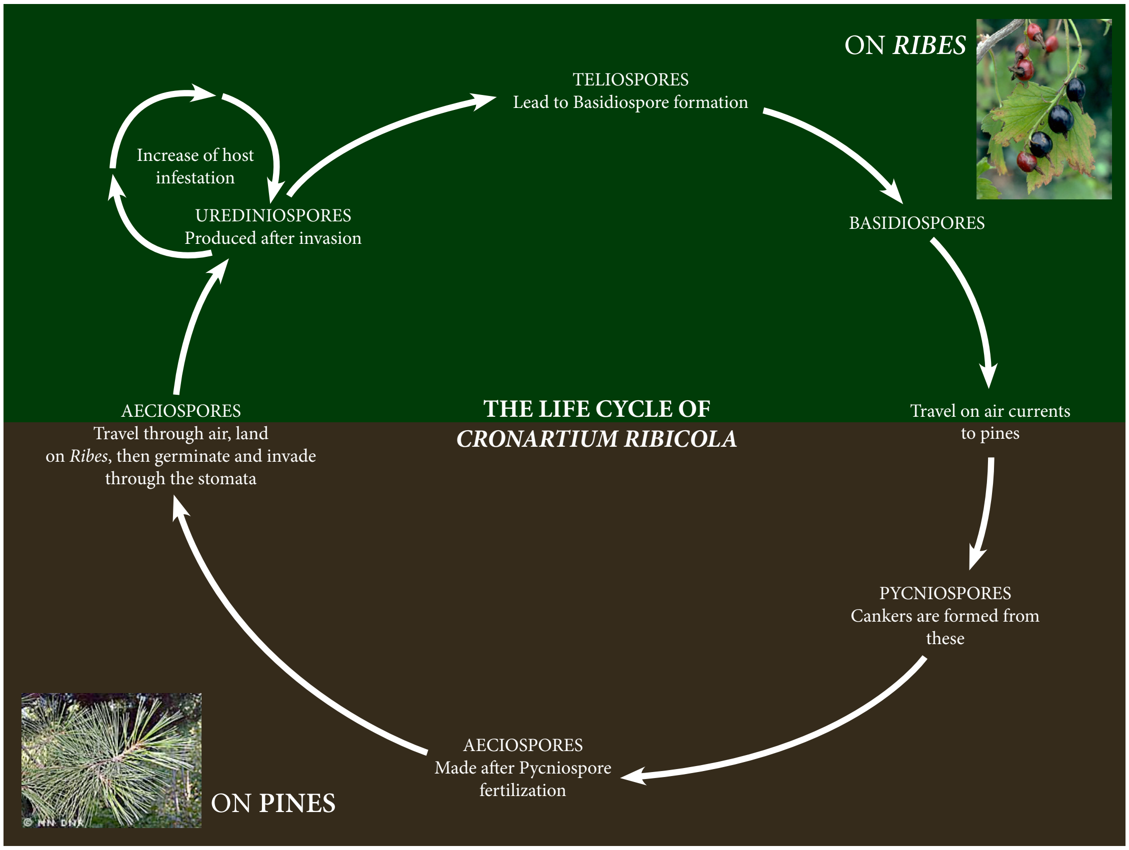

Figure 1. The life cycle of *Cronartium ribicola*. This complex cycle takes place on two separate hosts: Members of the *Ribes* genus (such as gooseberries and currants), and pines in the *Quinquefoliae* section of the *Pinus genus*. Figure adapted from Schwandt et al. (2013). Pictures from Minnesota Department of Natural Resources.

(Ravensdale et al. 2011; Thompson 1999), and it can be inferred to be occurring in the blister rust – white pine pathosystem. The ancient history of *C. ribicola* and pines is found within that of rust fungi and the ancestors of **angiosperms** and **gymnosperms** (Van Arsdel and Geils 2011). The fungi adapted their life cycle to alternate between the angiosperms and the gymnosperms. Those gymnosperm species were the early ancestors of today's *Pinus* and *Strobus* (pine species) subgenera. As the earth and climate changed, the gymnosperms that ended up in cooler latitudes, away from the rust, lost their resistance to it. Those that remained in contact with the rust in warmer regions continued to adapt in their natural resistance (Van Arsdel and Geils 2011).

Angiosperms: Seed bearing plants

Gymnosperms: Cone bearing plants

Constant exposure to *C. ribicola,* by pines, is necessary in order to retain resistance. This is due to the fact that the *C. ribicola* life cycle requires a live host and two different species (Schwandt et al. 2013). This bi-host life cycle has provided the fungus with the need to continually change, in order to combat the resistance effects of its two hosts (King et al. 2010). The alternate host of the *C. ribicola* life cycle includes the *Ribes* plant family, such as gooseberries and currants. These plants host three of the five phases. The other two phases are completed on white pine in the *Quinquefoliae* subsection of the *Pinus* genus (Dalton et al. 2010), see Figure 1. Aeciospores that have been produced during the life cycle on pine will land on a *Ribes* plant, germinate, and then invade the leaf through the stomata (Kearns et al. 2008). Urediniospores are then produced, which

GIS
Geographic information system, computerized managment of multiple layers of geographic and/ or spatial data

increase host infestation (Kearns et al. 2008; Schwandt et al. 2013). Teliospores are then gathered on the undersides of *Ribes* leaves, which lead to basidiospore formation (Kearns et al. 2008). Basidiospores are caught up on air currents to land on and infect white pine.

When a basidiospore lands on a pine needle, it invades through the stomata (Schwandt et al. 2013). A canker subsequently forms where pycniospores are; these are carried by insects to be fertilized (Schwandt et al. 2013). The aeciospores which cause the "rust" colored canker are made after fertilization. It is the aeciospores which infect the *Ribes* plant to reproduce and continue the life cycle. However, when the fungus is inside the tree, it works its way down the branch toward the main trunk at approximately two inches per year (Schwandt et al. 2013). When the fungus reaches the trunk, it cuts off the nutrient supply within the tree, and the tree dies (Albers and Albers 1998; Kearns et al. 2008; Schwandt et al. 2013). The younger the leaf or needle is when a spore lands on it, the more susceptible it is to invasion by the fungus (Dalton et al. 2010; Schwandt et al. 2013).

The risk of blister rust infection is also directly related to the topography, elevation, and climate of an area (Van Arsdel et al. 2006; Van Arsdel and Geils 2011). The climate in Minnesota is especially advantageous to blister rust, as it has the cool, moist summers necessary for blister rust to thrive (Van Arsdel et al. 2006; Van Arsdel and Geils 2011; White et al. 2002). Trees in northeastern Minnesota are especially susceptible, having been classified as a Zone 4 by Van Arsdel in the 1960's (Van Arsdel and Geils 2011). This is the highest-risk zone, because it provides the conditions necessary for intense infection (Van Arsdel et al. 2006; Van Arsdel and Geils 2011). This assessment was supported in 2002 by more modern technology, such as **GIS**, and high-definition maps (White et al. 2002). The trees in a zone category of 4 are especially susceptible to invasion, so the healthy trees found amongst the diseased trees and *Ribes* plants have been thought to have some type of resistance.

Resistance in Pinus strobus

Three primary methods have been used in the search for blister rust resistance in white pines. Two of the methods relate to breeding programs, and are described later in this review. The other method includes using molecular approaches to investigate the hypersensitive reaction, and possible genetic resistance, of the potentially resistant trees. A hypersensitive reaction is not an inherited trait, but is a reaction gained through exposure to a parasitic organism (Fernandes 1990). An elementary explanation of such would be: when a person is exposed to the chicken pox virus, their immune system will adapt to, and eventually fight it off, but that response will not be passed on to their biological children.

Hypersensitive responses in *P. strobus* are evidenced by non-inherited ontogenic resistance, which means that as a tree ages, it is better able to withstand infection. These reactions include a physical barring of the invading fungal hyphae. This can include polyphenolic compound buildup (Figure 2, part B) which restricts the ability of the fungus to invade. Also, an increase in cell size and number around the invasion, along with mesophyll cell collapse and death around the invading hyphae, are forms of ontogenic resistance (Dalton et al. 2010; David et al. 2012; Jurgens et al. 2003). However, the reactions were not found to be consistent among different family crosses and intercrosses used in the Jacobs et al. (2009) study, which suggested more than one mechanism as being responsible.

The few families that possess some partial resistance are thought to do so through a polygenic reaction with some environmen-

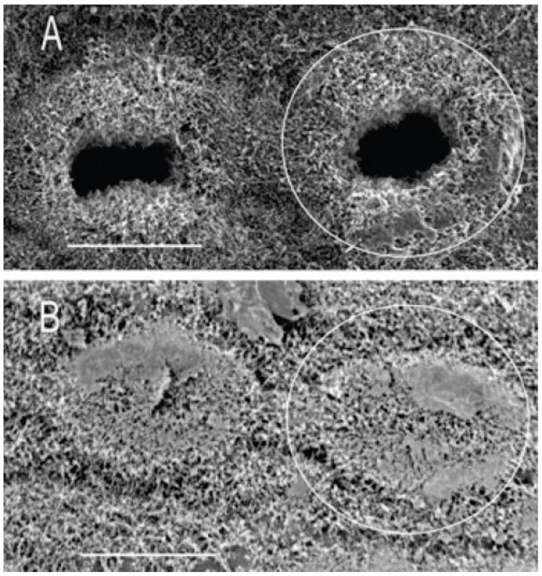

Figure 2. Environmental scanning electron microscopy image: The stomata of two different *Pinus strobus* individuals. **A** shows the stomata of clone H111, a blister-rust susceptible *P. strobus*. **B** shows the stomata of clone P327, a blister-rust resistant *P. strobus*. The stomata in **B** are wax-occluded, which aid in the physical barring of fungal hyphae. Image from USDA General Technical Report PSW-GTR-240 (David et al. 2012). Used with permission.

tal influence (indicated in Lu et al. 2005; Sniezko et al. 2014). The underlying factors of hypersensitive reactions are as yet undetermined (Sniezko et al. 2014), and the molecular search for resistant genes in *P. strobus* has thus far been unsuccessful. This may be due to lack of genetic diversity within the population. The intense logging in the 1900's reduced *P. strobus* distribution (Mehes et al. 2007; Steen-Adams et al. 2007), which potentially reduced the genetic variation necessary to withstand the fungus. When a tree population is reduced significantly, the interaction between **stands** becomes disjointed and can lead to reduced gene flow (Mehes et al. 2007; Rantam et al. 2014), which results in increased inbreeding and less genetic variation. One study found a 26% reduction in allelic diversity from a stand that had been harvested, compared to a nearby stand that had been left intact (Rajora et al. 2000). This suggests that heritable, resistant genes may need to be introduced to *P. strobus* through other means, which can be accomplished with careful breeding programs.

Stand
Tree stands - a group of trees in one area

Intraspecific breeding programs

Intraspecific breeding programs are those which breed individuals from the same species together, in an effort to increase desired characteristics. Intraspecific breeding for *P. strobus* resistance to blister rust began in the Great Lake States in Wisconsin in the 1930's when A.J. Riker and colleagues found potentially resistant trees, and tested their offspring (David et al. 2012; Patton and Riker 1958). Similarly, Patton and colleagues at the University of Wisconsin searched the Great Lake States for resistant trees. They found P327 (P for Patton (Jacobs et al. 2009)) outside of Duluth, MN (David et al. 2012), which has proven to be one of the most resistant *P. strobus* found. Clones of it are still being used for resistance investigations, such as in Jacobs et al. (2009), Jurgens et al. (2003) and Smith et al. (2006). The initial resistance research programs led to further program development in the Great Lake States.

An intraspecific breeding program for *P. strobus* began in Minnesota in the 1960's, when seeds from ostensibly resistant trees were collected from regions in northern Minnesota. The Quetico-Superior Wilderness Research Foundation, the USDA Forest Service, and the University of Minnesota, collaborated in an effort to establish a program whose aim was to produce trees with higher natural resistance levels (David et al. 2012). The seeds were planted in Tofte, MN in 1972 and 1974, and monitored for 37 years. While the final survival rate in the program was 2.7%, the end result still produced the most genetically diverse collection of pure *P. strobus* with high resistance, and the best 52 individuals

cpDNA
Chloroplast DNA, contains photosynthetic genes

mtDNA: Mitochondrial DNA - inherited from the female parent only

Fluorescence in-situ hybridization:
Used to find specific DNA sequences on a chromosome by a fluorescent-molecule tag, which binds to the desired sequence

rDNA
Ribosomal DNA - translates mRNA into proteins

ISSR
Inter-simple sequence repeat marker - short, repetitive segments of DNA. Used in DNA fingerprinting, to distinguish identity and / or kinship

RAPD
Random amplified polymorphic DNA - used to amplify short segments of random DNA, commonly used to define the relatedness of plants

within this population were selected for future studies (David et al. 2012).

Intraspecific resistance breeding programs have also been conducted in other countries, though the focus here will be on the Canadian and Romanian programs. A review of other European studies is provided in Blada and Popescu (2004). Canadian efforts to find resistance began in 1908 when a nursery using 400 *P. strobus* trees imported from Germany was reduced to a mere 35 trees after 39 years (King et al. 2010; Lu and Derbowka 2009). These remaining individuals were picked for future studies, being presumed to have resistance to the fungus. However, intraspecific experiments using these showed almost every result to be 100% mortality (Lu and Derbowka 2009). Resistance programs in Romania were begun in 1974 for the same reasons as in the U.S. and Canada (Blada and Popescu 2004). However, the intraspecific programs were discontinued (Blada 2004), for the same reasons as they were in Canada. The poor survival rates of intraspecific crosses, coupled with high breeding program cost, turned the focus in these other countries to interspecific hybridizations.

Interspecific breeding programs

Interspecific programs are those which breed together individuals from different, though related, species in an effort to bring together desired characteristics from each. Another important benefit of interspecific breeding programs, when using a species such as *P. strobus*, is that genetic diversity can be increased. As with any species, when a breeding population gets small, the ways in which the present alleles can combine is reduced. This can reduce the ability of the species to overcome pathogens. When introducing the genome of a related species, the genetic diversity is increased, and the offspring have more allele recombination options. This gives the offspring more of a chance to inherit any resistance alleles present in a parent. Therefore, using a related species is beneficial in increasing the overall diversity of a population, as well as potentially introducing resistance.

Interspecific programs in Canada and Romania were begun in 1946 and 1977 respectively (Blada and Popescu 2004; Lu and Derbowka 2009), and they initially crossed a variety of species with *P. strobus* to find compatible matches. The desired matches would produce viable, fertile offspring that would be resistant to blister rust, while also retaining the desirable growth characteristics of *P. strobus* (Blada and Popescu 2004; Lu and Derbowka 2009).

Pinus wallichiana has proven to be one of the best crosses in these programs. *P. wallichiana* possesses its own cold-hardiness, high genetic diversity, climactic adaptability, and leading forest value (Lee et al. 1998; Siddiqui et al. 2013 a, b). Its native region in the Himalayas also encompasses a wide range of elevations and ecotypes (Khan 2004). As a Eurasian species, it possesses some resistance to blister rust (Blada 2004; Lu and Derbowka 2009). Even though it is a Eurasian species, it is closely related to *P. strobus*: Using closely related species is a crucial step in finding compatible crosses.

Traditionally, the relatedness of trees (phylogenies) was classified based on morphological characteristics, such as seed type and cone scales (Gernandt et al. 2005). This relatedness within the 100+ member *Pinaceae* family has recently been investigated through molecular techniques, to discern the genetic relation between them. The techniques have included the use of **cpDNA**, (Gernandt et al. 2005; Tsutsui et al. 2009), **mtDNA** (Tsutsui et al. 2009), **Fluorescence in-situ hybridization** of different **rDNA** sites (Cai et al. 2006), **ISSR** and **RAPD** markers (Kovacevic et al. 2013; Mehes et al. 2007), and

LEA
Late embryonic abundant genes which are turned on late in development

an intron within a **LEA**-like gene (Syring et al. 2007). Though all of the results show that the genome of *P. strobus* is incredibly large, and the exact relatedness with other species is unknown, each study maintains that *P. strobus* and *P. wallichiana* are in the same sub-section, *Quinquefoliae,* and are closely related.

Using these related species, *P. strobus* and *P. wallichiana*, has shown success in cross-breeding for retaining the desired resistance and growth characteristics. Canadian efforts additionally desire to increase *P. strobus* parentage and cold hardiness in the offspring (Lu and Derbowka 2009; Lu and Sinclair 2006). The efforts to increase cold hardiness in the Canadian progeny began, due to the first generation of hybrids being susceptible to cold (Lu and Sinclair 2006), though this was not an issue in Romania (Blada and Tanasie 2013). The second backcrossed generation has shown 87.5% *P. strobus* parentage, with the end goal to produce no less than 93.75% *P. strobus* parentage (Lu and Derbowka 2009). Additionally, as the *P. strobus* percentage increased in subsequent backcrossing, the ability to withstand the cold appeared to increase as well (Lu and Sinclair 2006; Lu et al. 2007).

The gender of each parent species was found to play a role in how much variability there was in passing on the desired traits of resistance and growth (Blada 2004; Blada and Popescu 2004; Lu and Derbowka 2012; Lu and Sinclair 2006). Studies using both *P. strobus* females crossed to *P. wallichiana* males, and *P. strobus* males crossed to *P. wallichiana* females, produced great variability in the offspring survival rates (Blada and Popescu 2004; Lu and Sinclair 2006), as much as 50-100% (Lu et al. 2005). Romanian studies using female *P. strobus* and male *P. wallichiana* showed that the resistance passed on from the males was not significant between the individuals used (Blada and Popescu 2004), meaning that each male passed on the same level of resistance as the others. The families that showed increased resistance in Canada, had heterozygous mother trees to contribute resistance, which did so around 50% of the time (Lu et al. 2005). The offspring of a double hybrid cross [(*P. strobus* x *P. wallichiana*) x (*P. strobus* x *P. wallichiana*)], that was then bred back to *P. strobus,* has shown as much as 71% resistance (Lu et al. 2005). While this demonstrates that the *P. wallichiana* resistance is heritable, it could take up to six crosses and backcrosses to regain lost resistance (Lu and Derbowka 2009) if careful selections are not made.

In addition to survival, height is one of the important growth characteristics recorded in both Romanian and Canadian studies (Blada and Tanasie 2013; Blada et al. 2013; Lu and Sinclair 2006), as the growth characteristics of *P. strobus* make it valuable for its lumber. What each male *P. wallichiana* was able to pass on in terms of height was found to be significant in Romanian studies (Blada 2004). The growth characteristics of pure *P. strobus*, *P. wallichiana*, and their hybrid progeny were recorded in Romanian (Blada and Tanasie 2013; Blada et al. 2013), and in Canadian studies (Lu et al. 2005). The results showed that the hybrid progeny obtained nearly the same, or better, heights as the *P. strobus* parent. These growth rates were noted in both young trees and mature trees. A young tree can also be accurately assessed for resistance, which can be seen in seedlings as young as 1 year. (Lu et al. 2005; Lu and Derbowka 2009; Lu and Derbowka 2012). This is beneficial, as the process of finding resistant trees is a costly, time consuming process.

Conclusion

For over a century, blister rust has been spreading through forests, killing white pine needed for economic and ecological purposes. The complex life cycle of *C. ribicola* will continue to contribute to its ability to adapt within its molecular arms race. Increasing the genetic diversity of native *P. strobus* is essential for it to have a chance to compete for survival. Thus far, attempts to find a genetic basis for blister rust resistance in *P. strobus* have been unsuccessful. The search for resistance through intraspecific breeding programs has produced trees with the highest possible rates of genetic diversity and resistance, within pedigreed *P. strobus*.

The successful use of *P. strobus* and *P. wallichiana* in interspecific hybridization programs have been accomplished in Canada and Europe for over 60 and 40 years, respectively (Blada and Popescu 2004; Lu and Derbowka 2009). These programs have shown that the hybridization between *P. strobus* and *P. wallichiana* can produce offspring with increased resistance, while maintaining the desired growth characteristics of *P. strobus*, which is important in a tree valued for its ecological and economic value. The offspring are also able to be backcrossed to the parent species for increased percentage of that parentage. This is essential, as the ultimate goal of any interspecific program would be to have the end product pollenated by existing native *P. strobus*, which would increase the genetic diversity of natural stands (Lu and Derbowka 2009). Even as molecular techniques advance to isolate possible genetic resistance, obtaining genetically diverse populations is essential for future resistance. Combining the interspecific and intraspecific programs, by using the best families from each, could provide a genetically diverse, two-pronged approach to combat this pervasive disease

.

Acknowledgements

I would like to thank Dr. Timothy Craig, Dr. Shannon Stevenson and Dr. Elizabethada Wright for their support and editing contributions to this review, and to the DJUB. I also especially thank Dr. Timothy Craig for his advisement throughout the writing of this review. I am grateful to Alicia Boltz for her assistance in navigating InDesign, along with her editing contributions to Figure 1. I extend a thank you to the peer reviewers who helped to improve this article for publication. I would finally like to thank Dawn Voges and the DNR at Lake Vermilion – Soudan Underground Mine State Park for the internship opportunity which introduced me to the problem between blister rust and white pines.

Author Biography

Tami Rahkola is a junior at the University of Minnesota Duluth. She is pursuing a B.S. in Biology, with an emphasis on organismal biology, and a minor in Environmental Science. After graduation, she plans to pursue a career that relates to natural resource management. In her free time, she enjoys reading and writing, along with hiking, camping, fishing and horseback riding with her son.

References

Albers M and Albers J. 1998. White Pine: How to prune for blister rust. Minnesota Department of Natural resources. Field Tip Number 10.

Blada I. 2004. Genetic variation in growth and blister-rust resistance in a *Pinus strobus* x *P. wallichiana* hybrid population. Silvae Genetica. 53(1): 33-41

Blada I and Popescu F. 2004. Genetic research and development of five-needle pines (*Pinus* subgenus *Strobus*) in Europe: An overview. USDA Forest Service Proceedings RMRS-P-32. 51-60

Blada I and Tanasie S. 2013. Growth, straightness and survival at age 32 in a *Pinus strobus* x *P. wallichiana* F_1 hybrid population (Experiment 2). Annals of Forest Research. 56(1): 15-30

Blada I, Tanasie S, Dinu C, Bratu I. 2013. Growth, straightness and survival at age 32 in a *Pinus strobus* x *P. wallichiana* F_1 hybrid population (Experiment 1). Annals of Forest Research. 56(2): 269-282

Cai Q, Zhang DM, Liu ZL, Wang XR. 2006. Chromosomal localization of 5S and 18S rDNA in five species of subgenus *Strobus* and their implications for genome evolution of *Pinus*. Annals of Botany. 97(5): 715-722

Craig, TP. In Press. The geographic mosaic of coevolution. Encyclopedia of Evolutionary Biology. R.M. Kilman, editor. Elsevier, Amsterdam

Dalton DT, Postman JD, Hummer KE. 2010. Comparative infectivity of *Cronartium ribicola* aeciospores and urediniospores in genotypes of *Ribes nigrum*. Plant Disease. 94(4): 461-464

David A, Berrang P, Pike C. 2012. White pine blister rust resistance research in Minnesota and Wisconsin. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest areas. General Technical Report PSW-GTR-240. U.S. Dept. of Ag. 46-53

Fernandes GW. 1990. Hypersensitivity: A neglected plant resistance mechanism against insect herbivores. Environmental Entomology. 19(5): 1173-1182

Gernandt DS, Lopez GG, Garcia SO, Liston A. 2005. Phylogeny and Classification of *Pinus*. International Association for Plant Taxonomy. 54(1): 29-42

Jacobs JJ, Burnes TA, David AJ, Blanchette RA. 2009. Histopathology of primary needles and mortality associated with white pine blister rust in resistant and susceptible *Pinus strobus*. Forest Pathology. 39(6): 361-376

Jurgens JA, Blanchette RA, Zambino PJ, David A. 2003. Histology of white pine blister rust in needles of resistant and susceptible eastern white pine. The American Phytopathological Society. 87(9): 1026-1030

Kearns HSJ, Jacobi WR, Burns KS, Geils BW. 2008. Distribution of *Ribes*, an alternate host of white pine blister rust, in Colorado and Wyoming. Journal of the Torrey Botanical Society. 135(3): 423-437

Khan SR. 2004. Genetic variation in Blue Pine and applications for tree improvement in Pakistan, Europe and North America. Fort Collins, CO: USDA Forest Service Proceedings RMRS-P-32.

King JN, David A, Noshad D, Smith J. 2010. A review of genetic approaches to the management of blister rust in white pines. Forest Pathology. 40(3-4): 292-313

Kovacevic D, Nikolic B, Drinic SM, Bojovic S, Dodos T, Rajcevic N, Marin PD. 2013. Genetic Relationships among some *Pinus*, *Picea* and *Abies* species revealed by RAPD markers. Genetika. 45(2): 493-502

Lee SW, Choi WY, Norbu L, Pradhan R. 1998. Genetic diversity and structure of blue pine (*Pinus wallichiana* Jackson) in Bhutan. Forest Ecology and Management. 105(1-3): 45-53

Lu P, Sinclair RW, Boult TJ, Blake SG. 2005. Seedling survival of *Pinus strobus* and its interspecific hybrids after artificial inoculation of *Cronartium ribicola*. Forest Ecology and Management. 214(1-3): 344-357

Lu P and Sinclair RW. 2006. Survival,

growth and wood specific gravity of interspecific hybrids of *Pinus strobus* and *P. wallichiana* grown in Ontario. Forest Ecology and Management. 234(1-3): 97-106

Lu P, Colombo SJ, Sinclair RW. 2007. Cold hardiness of interspecific hybrids between *Pinus strobus* and *P. wallichiana* measured by post-freezing needle electrolyte leakage. Tree Physiology. 27(2): 243-250

Lu P and Derbowka D. 2009. Breeding eastern white pine for blister rust resistance: A review of progress in Ontario. The Forestry Chronicle. 85(5): 745-755

Lu P and Derbowka D. 2012. Effects of seedling age on blister rust resistance assessments in eastern white pine and its hybrid backcrosses. Canadian Journal of Forest Research. 42(1): 67-74

Mehes MS, Nkongolo KK, Michael P. 2007. Genetic analysis of *Pinus strobus* and *Pinus monticola* populations from Canada using ISSR and RAPD markers: Development of genome-specific SCAR markers. Plant Systematics and Evolution. 267(1-4): 47-63

Patton RF and Riker AJ. 1958. Blister rust resistance in eastern white pine. Proc. 5th Northeast. Forest Tree Improvement Conference. 46-51

Radu S. 2008. *Pinus strobus*: Past and future in Europe. A page of silvicultural history and international scientific cooperation. Annals of Forest Research 51: 133-140

Rajora OP, Rahman MH, Buchert GP, Dancik BP. 2000. Microsatellite DNA analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. Molecular Ecology. 9(3): 339-348

Rantam W, Rajora OP, Finkeldey R, Aravanopoulos F, Bouvet J-M, Vaillancourt RE, Kanashiro M, Fady B, Tomita M, Vinson C. 2014. Genetic effects of forest management practices: Global synthesis and perspectives. Forest Ecology and Management 333(SI): 52-65

Ravensdale M, Nemri A, Thrall PH, Ellis JG, Dodds PN. 2011. Co-evolutionary interactions between host resistance and pathogen effector genes in flax rust disease. Molecular plant pathology. 12(1): 93-102

Schwandt J, Kearns H, Byler J. 2013. White pine blister rust general ecology and management. Insect and disease management series. Forest Health Protection and State Forestry Organizations. USDA Forest Service. 14.2

Siddiqui MF, Shaukat SS, Ahmed M, Khan N, Khan IA. 2013a. Vegtation-environment relationship of conifer dominating forests of moist temperate belt of Himalayan and Hindukush regions of Pakistan. Pakistan Journal of Botony. 45(2): 577-592

Siddiqui MF, Shaukat SS, Ahmed M, Khan N, Khan IA. 2013b. Age and growth rates of dominant conifers from moist temperate areas of Himalayan and Hindukush regions of Pakistan. Pakistan Journal of Botany. 45(4): 1135-1147

Smith JA, Blanchette RA, Burnes TA, Jacobs JJ, Higgins L, Witthuhn BA, David AJ, Gillman JH. 2006. Proteomic comparison of needles from blister rust-resistant and susceptible *Pinus strobus* seedlings reveals upregulation of putative disease resistance proteins. The American Phytopathological Society. 19(2): 150-160

Sniezko RA, Smith J, Liu JJ, Hamelin RC. 2014. Genetic resistance to fusiform rust in southern pines and white pine blister rust in white pines – a contrasting tale of two rust pathosystems – current status and future prospects. Forests. 5(9): 2050-2083

Steen-Adams MM, Langston N, Mladenoff DJ. 2007. White pine in the northern forests: An ecological and management history of white pine on the Bad River Reservation of Wisconsin. Environmental History. 12(3): 614-648

Syring J, Farrell K, Businsky R, Cronn R, Liston A. 2007. Widespread genealogical non-monophyly in species of *Pinus* subgenus *strobus*. Systematic Biology. 56(2): 163-181

Thompson JN. 1999. Specific hypotheses on the geographic mosaic of coevolution. The American Naturalist. 153(S5): S1-S14

Tsutsui K, Suwa A, Sawada K, Kato T, Ohsawa TA, Watano Y. 2009. Incongruence among mitochondrial, chloroplast and nuclear gene trees in *Pinus* subgenus *strobus* (*Pinaceae*). Journal of Plant Research. 122(5): 509-521

United States Department of Agriculture Forest Service. 2014 April 29 [cited 2015 Jan 18]. White pine blister rust. Available from http://www.fs.fed.us/research/invasive-species/plant-pathogens/white-pine-blister-rust.php

Van Arsdel EP, Geils BW, Zambino PJ. 2006. Epidemiology for hazard rating of white pine blister rust. In: Guyon J. comp. Proceedings of the 53rd Western International Forest Disease Work Conference. Jackson, WY. Ogden, UT: U.S. Dept. of Agriculture, Forest Service, Intermountain Region. 49-61

Van Arsdel EP and Geils BW. 2011. Blister rust in North America: What we have not learned in the past 100 years. In: Fairweather, M., Proceedings of the 58th annual Western International Forest Disease Work Conference. Flagstaff, AZ: U.S. Department of Agriculture, Forest Service, Forest Health Protection. 61-69

White MA, Brown TN, Host GE. 2002. Landscape analysis of risk factors for white pine blister rust in the mixed forest province of Minnesota,U.S.A. Canadian Journal of Forest Research. 32(9): 1639-1650