Forest Ecology and Management xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Improving the resistance of eastern white pine to white pine blister rust disease☆

Carolyn C. Pike[a,*], Paul Berrang[b], Scott Rogers[b], Andy David[c], Carrie Sweeney[b], Julie Hendrickson[c]

[a] USDA Forest Service, Northeastern Area, 715 W. State Street, West Lafayette, IN 47907, United States
[b] USDA Forest Service, Eastern Region, Milwaukee, WI, United States
[c] University of Minnesota, Department of Forest Resources, St Paul, MN, United States

ARTICLE INFO

Keywords:
Eastern white pine
White pine blister rust
Seedling survival
Artificial inoculation
Pinus strobus
Cronartium ribicola
Artificial screening

ABSTRACT

Eastern white pine (EWP), *Pinus strobus* L., is an iconic forest tree in the north woods of eastern North America. White pine blister rust, caused by *Cronartium ribicola*, an invasive pathogen, entered North America in the early 20th century and infected all five-needled pines across the continent. Few genotypes of eastern white pine have demonstrated consistent, elevated resistance to the pathogen, so our objective was to identify additional genotypes with resistance. Since 1970, the USDA Forest Service has identified and grafted over 800 phenotypic plus tree selections from Michigan, Wisconsin and Minnesota. A protocol to artificially inoculate one-year old seedlings was used to screen 228 genotypes along with rust-resistant and susceptible standards across a four-year period, from 2010 to 2013. We identified 25 genotypes, based on survival assessed two years after inoculation, which exceeded the resistant standard. These genotypes will be grafted into new seed orchards to aid reforestation efforts aimed at restoring this keystone species to suitable habitats where regeneration is currently hindered by blister rust on privately-held land, state forests and national forests in the Lake States region.

1. Introduction

Eastern white pine, *Pinus strobus*, (EWP) is an iconic tree in North America, highly prized by European settlers for its use as ship masts and building material (Albion, 1926), and is recognized today as a keystone species in the eastern forest (Wendell and Smith, 1990). Large-scale exploitation of the species commenced in the late 1800s and early 1900s, and continued until wood resources were all but exhausted. Unfortunately, the white pine blister rust-causing pathogen *Cronartium ribicola* J.C. Fisch. entered North America on nursery stock shipped from Europe in the early 1900s, causing extensive mortality to regenerating white pine forests. The first known introduction on the east coast was noted in Geneva, NY in 1906 (Hummer, 2000), and later transport of infected nursery stock helped disperse blister rust across the region. In the span of a few decades, blister rust became a significant problem of all five-needled pines across North America (Maloy, 1997). In Minnesota, the combination of blister rust and herbivory, especially by white tailed deer (*Odocoileus virginianus*), resulted in decreased seedling survival, and a more simplified stand composition as the age-class distribution has skewed towards older, 100+ year trees (Vanderschaaf and Vongroven, 2013; White, 2012).

Blister rust has a complex life cycle that requires an alternate host, most commonly currant and gooseberry belonging to the *Ribes* genus, although other plants function as alternate hosts as well (Kaitera and Hiltunen, 2012). In the fall, basidiospores disperse from *Ribes* leaves to pine needles, a process that is enhanced when temperatures are cool and relative humidity is high (15.5 °C, 100% Relative Humidity). In North America, blister rust rapidly spread on native and cultivated *Ribes* spp., which were relatively common on the landscape. Many of the northern forests, where EWP is a dominant or co-dominant species and native *Ribes* are a common understory plant, are replete with lakes and topographical features that enhance humidity levels and provide convective surfaces that favor the fungus' propagation and spread. Cultivated *Ribes nigrum* is an epidemiologically important host in North America for blister rust (Van Arsdel et al., 2006), but has largely disappeared from the social and cultural ethos since it was connected with this pathogen. Extensive removal of native *Ribes* using mechanical, and later chemical methods (Martin and Spaulding, 1949) was attempted to

☆ This article is part of a special feature entitled "Resilience and Health of Eastern White Pines under Novel and Historical Factors", published in the Journal of Forest Ecology and Management 423, 2018.

* Corresponding author.
E-mail address: cpike@fs.fed.us (C.C. Pike).

https://doi.org/10.1016/j.foreco.2018.03.001
Received 30 October 2017; Received in revised form 27 February 2018; Accepted 1 March 2018
0378-1127/ Published by Elsevier B.V.

reduce the inoculum across a large swath of the eastern forests in the early 20th century. The impact of *Ribes* removal is not known, but one study suggested that survival of EWP in the decade after the arrival of blister rust was likely enhanced by the removal of *Ribes* in combination with other strategies (Stewart and Ritter, 1962; Van Arsdel et al., 2006).

After blister rust swept through North America, forests across the US and Canada were scoured in search of surviving trees to initiate a breeding program. These surviving trees were propagated by grafting into clone banks and seed orchards for federal, state and provincial governments in the United States and Canada. The Hartley plantation in Duluth, Minnesota was planted in 1914 with bare root nursery stock from unknown origin and continually monitored in a quest to locate trees with resistance. The first two infected pines were noted in 1927, and by the 1930s nearly 98% of the planting was infected, averaging over four infections per tree (Ritter, 1930). Two surviving trees were identified as having above-average resistance, bearing the namesake of Robert Patton (University of Wisconsin Madison): P-327 and P-312. Offspring from P-327 have been used in screening programs as a resistant standard demonstrating consistent, above-average levels of resistance in field and laboratory studies.

The largest known collection of seed sources selected for potential resistance to EWP was established in 1972–1974 near Tofte, Minnesota USA (47.57° N, 90.83° W). This site is located approximately six miles inland from Lake Superior in an area designated as being at high-hazard for blister rust (Van Arsdel, 1961; White et al., 2002). Over 40,000 open-pollinated tree seedlings were planted from over 800 mature mother trees from the forests in northern Minnesota (Ahlgren, 1979; Merrill et al., 1986). Each family was represented by five ten-tree row plots for a total of 50 progeny per mother tree at the site. Trees were planted at a roughly five foot (1.5 m) spacing, with a mixture of *Ribes* spp interplanted between rows to enhance exposure to the pathogen. By 1984, 97–99% of the seedlings were infected with rust. Differences in survival among families were significant, but rates of non-infection (number of trees that lacked any sign of the pathogen) were not significantly different among families (Merrill et al., 1986). In 1993, over 800 surviving trees selected as having no visible symptoms were field-tagged, and incorporated into a database. In 2015, fewer than 2000 live trees remained and no single family had more than five of its original 50 trees surviving.

Screening under controlled conditions is desirable to remove unwanted environmental variation that can mask gene expression for traits of interest. Artificial screening for resistance to blister rust entails cultivating and infecting *Ribes* leaves and infecting cultured white pine seedlings in an inoculation chamber or a nursery setting (Ahlgren, 1955; Riker et al., 1943; Riker and Patton, 1961). Needle lesions (spots), the first sign of infection, can be observed several months after inoculation. In side-by-side trials of EWP with western white pine (*Pinus monticola* Dougl. ex D. Donn.) and sugar pine (*P. lambertiana* Douglas), EWP averaged 36 spots per tree compared to 34 and 25 for western white pine and sugar pine, respectively (Sniezko et al., 2008). >90% of seedlings for all three species exhibited needle lesions. Infection severity is usually assessed by observing stem infections as a binary trait (infected vs not), or expressed as the percent of trees per family with early stem symptoms (after one year) or later (3 years or beyond) (Sniezko and Kegley, 2003). Screening for resistance in EWP has proven challenging, producing either too much infection from over-inoculating seedlings (Cliff and Isabel Ahlgren, personal communication), or inconsistent infection from cultural practices that failed to synchronize the plants' stomatal apertures with spore dispersal through inoculation (early efforts at USDA Forest Service). Programs that were successful in screening EWP found that no single genotype was fully immune, and that resistance occurred at a relatively low frequency in the population: in the aforementioned Tofte planting and at nursery experiments in Wisconsin fewer than 1% of one-year old seedlings expressed resistance (Merrill et al., 1986; Riker and Patton, 1961). In Ontario, scientists failed to identify alleles that conferred any meaningful resistance, leading them to establish a breeding program to hybridize EWP with Asian pine (Lu and Derbowka, 2009; Lu and Sinclair, 2006). Efforts to improve rust resistance in EWP in the US have failed to identify additional genotypes emerged with phenotypic resistance equivalent to P-327.

Resistance to blister rust in EWP is assumed to be largely polygenic (Smith et al., 2006a, 2006b; Zambino and McDonald 2003), which means that resistance results from certain combinations of alleles at many gene loci, complicating efforts to improve resistance through traditional breeding methods. Polygenic resistance exists in other five-needled pines including sugar pine (Kinloch et al., 2008) and in populations western white pine (Bingham et al., 1959). Acquisition of resistance in a breeding program, for instances where traits are polygenic, may entail inheritance of resistance genes from both parents. In wind-pollinated conifers, this may be achieved through controlled pollinations, or by sequestering seed orchards from susceptible pollen donors.

A number of mechanisms are associated with resistance in EWP, P-327, including a hypersensitive reaction (Jurgens et al., 2003), proteins that may confer disease resistance (Smith et al., 2006b), and occlusions of the stomata and/or biochemical characteristics of wax that may inhibit or repel fungal hyphae (Smith et al., 2006a). Progeny from EWP families selected as having putative resistance did not match the elevated resistance of selected western white pine families (Sniezko et al., 2008), leading to questions about the effectiveness of artificial screening. This new protocol developed for EWP has many similarities to western screening programs (Zambino, personal communication), and could provide a new opportunity to test the hypothesis that EWP families possess resistance that is heritable using artificial inoculations. The objective of the screening described here is to identify additional genotypes, also referred to as families, whose progeny (young seedlings) express resistance to white pine blister rust that exceeds P-327. Our long-term goal is to select a genetically diverse population of superior genotypes to plant in seed orchards as a seed source to reforest areas where blister rust remains an impediment to regeneration of EWP.

2. Materials and methods

Material for inoculation originated from a clone bank based at the USDA Forest Service's Oconto River Seed Orchard (ORSO), at 45°12′21.0″N 88°40′16.8″W in central Wisconsin, and from Tofte Minnesota (described earlier). The clone bank at ORSO includes a genetically diverse population of EWP of over 800 different genotypes from across the eastern US and Canada. Selections chosen for blister rust screening originated from the Lake States region only, and were selected if they were free of blister rust in a forest where blister rust infections were noted. Mother trees originated from two National Forests: the Chippewa National Forest (NF) in north central Minnesota (designated C-) and Superior NF in northeast Minnesota (S-). Selections were also acquired from the resistance program in Ontario (ON-), and from the state of Minnesota on land not affiliated with a national forest (MI-). Information on the locations of mother trees is maintained at the National Forest System but is not described further. Half-sibs for screening were derived from naturally open-pollinated collections at Tofte, open-pollinations that occurred in the wild of clones replicated at ORSO, or from mass-control pollination using a multi-genotype pollen mix (20–40 genotypes) of Minnesota-origin collected at ORSO. Each of four trials is described, herein referred to by the year that pine seedlings were germinated (2010, 2011, 2012, 2013). In the 2011 trial, twenty-two mother trees were each crossed with P-327, H-111, or were open-pollinated so that each family was represented by two full-sibs and one half-sib, resulting in 68 families. In 2010, 2012, and 2013, 26, 64, and 70 open-pollinated families were tested, respectively (Table 1) for each trial. Open-pollinated P-327 and H-111 were included as resistant and susceptible standards, respectively, in each trial.

3. Cultivation methods

Approximately 5000 EWP seedlings were germinated into individual Ray Leach Conetainer™ tubes, 10 cubic inches (164 cubic cm)