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Review

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

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Abstract: Damage or mortality from pathogens can reduce productivity of forest plantations, as well as significantly harm natural forest ecosystems. Genetic resistance within the host species is the first line of defense for tree species. Resistance breeding programs for the native fusiform rust and exotic (to North America) white pine blister rust diseases are two of the longest concerted efforts in forest trees, spanning more than 50 years. Advances in developing greater genetic resistance have been made in both pathosystems, but unique challenges and opportunities in each system translate to different approaches. Fusiform rust resistance programs have mainly emphasized complete resistance, while partial resistance plays a prominent role in white pine blister rust resistance programs. Advances in the development of molecular genetic tools now permit investigations in conifers and their associated rust pathogens. Good progress has been made in identifying resistant populations and understanding resistance in these pathosystems, and resistant stock is now being used extensively for reforestation and restoration. These programs represent great success stories brought to fruition by the

long-term efforts. However, continued support will be needed to enhance the level and fully realize the potential of durable genetic resistance in these invaluable North American conifer species.

Keywords: fusiform rust; blister rust; durable resistance; white pine; loblolly pine

1. Introduction

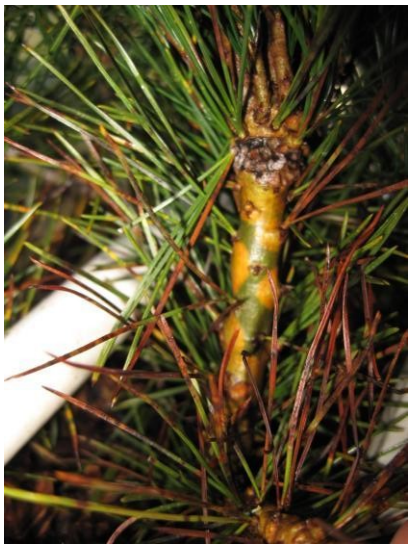
Rust diseases of forest trees have large economic and ecological impacts in North America. The two most notable affecting conifers are the native fusiform rust (FR) of the southern pines (Figure 1) and the non-native, invasive white pine blister rust (WPBR) of the white pines (also known as 5-needle pines) (Figures 2 and 3). Loblolly (*Pinus taeda*), slash (*P. elliottii*) and longleaf (*P. palustris*) pines are important components in native ecosystems as well as extremely valuable economically as the major species in large-scale, managed plantations in the southeastern United States. In these species, the fusiform rust pathogen (*Cronartium quercuum* f. sp. *fusiforme—Cqf*) can girdle stems and cause severe damage, wood defects and mortality especially in plantations of loblolly and slash pines. Together, these losses are estimated to exceed $140 million annually [1]. WPBR, caused by *Cronartium ribicola* J.C. Fisch. in Rabh., has resulted in high mortality in the economically important pines such as western white pine (*P. monticola*), sugar pine (*P. lambertiana*) and eastern white pine (*P. strobus*). In many areas in the West, the impact to western white pine and sugar pine is high enough that land managers are reluctant to replant with these species. In addition, high mortality in high elevation pines such as whitebark pine (*P. albicaulis*), southwestern white pine (*P. strobiformis*) and limber pine (*P. flexilis*) in native communities has resulted in ecosystem wide changes [2–4]. Due to a combination of white pine blister rust and mountain pine beetle outbreaks, whitebark pine has been proposed for listing under the Endangered Species Act in the U.S. [5], and similar concerns are present in Canada where it is protected under the federal Species at Risk Act (SARA). This concern has led to greatly increased efforts to find resistance to the WPBR fungus in whitebark pine as well as gene conservation efforts.

The identification and deployment of trees with natural genetic resistance to these rusts is key to restoring and maximizing the ecological role of many of the white pine species as well as the economic utility for white pine and southern pine species used in plantation forestry. Fortunately, genetic resistance to these rusts has been discovered in all pines species studied [6–15]. Resistance breeding programs, begun over 50 years ago, continue to produce trees with resistance in the U.S. and Canada [9,16,17]. During this period, knowledge has been gained and progress made in developing resistant material for reforestation and restoration, but further work is needed to increase the levels of resistance and to ensure its durability in the face of evolving pathogen virulence. The advent of new genomic tools opens up opportunities to gain and apply knowledge in the resistance programs to help ensure healthier forests in the future [18,19].

Figure 1. Fusiform rust, aecia on infected stem of slash pine right, and telia on infected leaf of northern red oak (*Quercus rubra*) on left. The pathogen is a macrocyclic, heteroecious rust fungus that alternates between the leaves of red oaks (*Quercus* section Lobatae) and stems of pines (*Pinus* section Diploxylon). Damage to oaks is minimal, but can be severe to pines.

Figure 2. White pine blister rust, caused by *Cronartium ribicola*, on bole of sugar pine (**right**) and *Ribes* leaf (**left**). The pathogen, *C. ribicola* is similar to *C. quercuum* f.sp. *fusiforme,* but alternates between the leaves of *Ribes* and needles (and eventually stems) of white pines (*Pinus* section Strobus).

Figure 3. Large, dying western white pine with hundreds on blister rust cankers on branches and main stem (**left**), and whitebark pine seedling with many stem infections, 13 months after artificial inoculation with *C. ribicola* (**right**).



A comparison of the underlying nature and challenges of increasing genetic resistance to the rusts in the southern pines and white pines may yield insights that can be used in operational tree improvement and research. The southern pine-FR pathosystem is native and presumably co-evolved. But silvicultural practices such as the large-scale establishment of even-aged pine plantations and intensive management including fertilization and fire suppression has led to increases in the abundance of some species of oaks, the native alternate hosts of *C. quercuum* f. sp. *fusiforme*. This is believed to have played a major role in the fusiform rust epidemics over the last few decades. In contrast, the white pine-WPBR pathosystem in North America involves a non-native pathogen that has now been present in North America for over 100 years and has killed millions of trees, dramatically altering forest ecosystems, in some locations and regions where native white pine species are present. The Eurasian white pine species that presumably co-evolved with the WPBR pathogen generally show much higher levels of genetic resistance than do North America white pine species [20,21]. However, in some cases with changing forest management and climate there is also high incidence of rust infection in some of the Asian white pines [22,23], and this may have parallels with the FR pathosystem or implications for the North American blister rust resistance programs. In this paper we will provide information on genetic resistance present in two pine groups and discuss field performance of resistant materials. We will also examine the new genomic tools that are available and the information they are providing, and provide some perspective as to what research and tree improvement efforts might provide in the next ten years. We will focus primarily on the WPBR resistance programs involving western white pine, and the FR resistance programs involving loblolly pine and slash pine where the most concerted efforts have occurred since the 1960s.

2. Resistance Testing

The basic steps involved in resistance breeding programs in forest trees include: (1) selection of candidate trees; (2) collecting seed from the candidate trees to use in short-term artificial inoculation

resistance testing; (3) characterizing the types of resistance; (4) selecting the top families and individuals within families (forward selection) and/or selecting the best parents for orchards based on their progeny performance (backward selection) for developing seed orchards and for breeding to increase resistance; (5) establishing field trials to directly assess resistance or to confirm the results of artificial inoculation trials and examine durability of resistance; and (6) operational use of resistant seedlings for reforestation or restoration. Short-term evaluation of seedling families for rust resistance is a key step in developing resistance. Although field progeny tests are often used without artificial inoculation trials for FR breeding southern pines [24–26], a combination of field and artificial inoculation tests are routinely used for WPBR. The use of small seedlings allows for the efficient testing of progeny of hundreds or even thousands of parent trees in a relatively short time period. In general, young seedlings are inoculated with rust basidiospores, the spore type produced on the telial host that are infectious on pines, under optimal conditions, and then they are assessed over time.

In this paper, for terminology, we use complete resistance to denote the resistance where generally no stem infection results from needle infections due, at least in part, to a hypersensitive-like response (HR) occurring in the needles (in the absence of virulent rust genotypes). In this case, the inheritance is conditioned by a single dominant major gene and also called major gene resistance (MGR). Much less is known about the inheritance or mode of action of the other types of resistances and terminology such as "slow rusting", "partial resistance" and "quantitative disease resistance" have been used to contrast it with the HR resistance. In this paper, for convenience, we use "partial resistance" to refer to the all non-HR types of resistances that are apparent after needle infection.

Beginning in the 1950s, efforts to breed southern pines (initial focus was on loblolly pine) with resistance to FR were undertaken. Barber [27] demonstrated wide variation in susceptibility from open-pollinated progeny of slash pine and it was observed that resistance was high in hybrids between shortleaf and slash and shortleaf and loblolly pines [28]; most selection for resistance has continued to focus on loblolly pine [24–26]. Surviving trees from high-hazard sites were clonally propagated by grafting, planted in seed orchards, and breeding for rust resistance had begun. As early as 1960 methods had been developed to mass inoculate seedlings with *Cqf* [29]. Following the establishment of the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC [30], a concentrated basidiospore spray (CBS) method was developed. This method allowed for uniform, mass-inoculations with specified target number of spores. In general, a method was developed and has been deployed for decades at the RSC where aecial spore collections are made from collection zones from throughout the range of FR in the southeast. These are used to produce bulk-inocula or less commonly, single-isolate inoculum. Cooperators submit seeds from open- or control-pollinated families and the focus is primarily to validate field progeny test results. The seedlings are grown for six to eight months prior to inoculations using the CBS method. Most of this work has focused on slash, longleaf and loblolly pine (and hybrids), and most evaluations have focused on complete/qualitative resistance.

For the white pine species, seedling testing is also done on young seedlings and is undertaken at several facilities in the United States and Canada [8,9,16,31]. Seedling progeny of field selections and advanced-generation selections have been tested. For the eight species of white pines in western North America, very young seedlings (usually two to four months old) are inoculated and screened for complete resistance, typically due to a hypersensitive response (HR) in the needles [10,12,32,33]. Older two-year old seedlings are usually used to evaluate a fuller suite of resistance related

traits [8,31]. Generally the inoculum used is a bulk basidiospore inoculum from the infected *Ribes* (alternate host of the pathogen) host from cultivated or wild plants. In some cases, sources of *C. ribicola* with high frequency of virulence (*vcr1* or *vcr2* genotypes) to HR in sugar pine or western white pine are used.

Assessment for HR is usually completed within six to twelve months after inoculation. In the tests for partial resistance, seedlings are evaluated for three to five years after inoculation for components of partial resistance such as presence and number of needle spots, shedding of infected needles (NS), presence or absence of stem symptoms (SS), number of stem symptoms (Figure 3), latency of stem infection, severity of infection, bark reactions (BR), and survival with stem infections (SSAL) [8,21,31]. The seedling testing regime has been in place for over five decades, with the more recent addition of assessing the number of stem infections and the severity of overall infection. Artificial inoculations at the USDA Forest Service's Dorena Genetic Resource Center (Dorena GRC) is very effective, generally producing needle infections on 95 to 100 percent of all seedlings in the test and stem symptoms on 100 percent of the seedlings in the most susceptible families. In addition, greater than 90 percent of the seedlings from almost all forest selections of western white pine and sugar pine show stem symptoms after artificial inoculation [8], giving an indication of the relatively low level and low frequency of genetic resistance in natural populations.

Table 1 presents a summary of data from nine (of 80) western white pine seedlots inoculated in two 2004 artificial inoculation trials, one with *Avcr2* geographic source of rust, and one with geographic source of rust with a high incidence of *vcr2* genotypes. Protocols for testing follow those used routinely at Dorena GRC for the last several decades [8,31]. The nine seedlots (eight families and one seed orchard bulk lot) represent a wide range of resistance in western white pine from high susceptibility, to complete resistance (HR), to differing levels of partial resistance.

Table 1. Resistance components [1] in two seedling artificial inoculation trials for western white pine for six partially resistant families (PR), one completely resistant family (CR), a F_2 orchard lot with partial resistance ("71") and a susceptible control family ("79"), one to five years after inoculation in 2004 with *Cronartium ribicola*. Geographic source of *Ribes* with known high incidence of virulent vcr2 rust used for inoculation of one trial (SY2003R1) and AVcr2 inoculum source used for second trial (SY2003R2). Note the strong contrast of the susceptible family ("79") with the other eight families in all components of resistance.

		vcr2 Inoculum								
Sow# [2]	**RT [3]**	**%SS2**	**%SS6**	**#SS2**	**%ESS**	**%BR_c**	**%BR_{all}**	**%SSAL6**	**%RSV4**	**%RSV6**
1	PR	50.0	73.3	2.0	68.1	13.3	53.3	41.7	73.3	56.7
4	PR	51.1	68.5	1.6	73.6	27.4	54.4	32.3	70.0	53.3
74	PR	59.3	92.6	2.8	64.8	25.9	88.9	39.4	83.0	41.9
20	PR	20.0	90.0	0.6	25.0	22.5	60.0	37.5	40.0	26.7
75	PR	11.6	84.7	0.4	14.3	52.3	76.4	76.2	88.8	76.8
73	PR	81.9	100	4.5	81.9	26.4	66.7	31.9	56.9	31.9
80	CR	93.0	96.3	6.2	96.7	0	14.1	3.3	48.5	7.0
71	PR	80.1	95.8	4.2	83.1	4.2	41.7	8.5	51.9	11.1
79	S	100	100	7.7	100	0	10.0	0	20.0	0

Table 1. *Cont.*

		Avcr2 Inoculum								
Sow# [2]	**RT**	**%SS2**	**%SS6**	**#SS2**	**%ESS**	**%BR_c**	**%BR_{all}**	**%SSAL6**	**%RSV4**	**%RSV6**
1	PR	53.3	80.0	1.8	68.5	33.3	73.3	61.1	86.7	70.0
4	PR	56.7	80.0	2.7	70.1	26.7	46.7	39.0	83.3	50.0
74	PR	55.2	72.2	3.3	81.1	24.1	58.1	37.8	66.7	53.3
20	PR	69.4	86.1	2.5	82.2	36.1	58.3	24.4	52.8	33.3
75	PR	20.0	85.9	0.1	20.8	51.1	85.9	78.6	90.5	74.3
73	PR	85.7	100	4.7	85.7	22.3	84.4	20.4	58.6	19.9
80	CR	8.1	24.3	0.4	33.3	14.8	14.8	66.7	96.7	83.3
71	PR	61.9	89.6	2.3	70.0	23.7	69.3	26.7	66.3	35.2
79	S	96.3	96.3	10.0	100	0	17.4	0	10.0	3.3

[1] %SS = percentage of seedlings with stem symptoms at approximately 1 year (SS2) and 5 years (SS6) post inoculation (p.i.); #SS2 = number of stem symptoms per tree at one year p.i.; %ESS = percentage of seedlings with stem symptoms one year p.i. *vs.* percentage in family 5 years p.i.; BR_c = percentage of seedlings with complete bark reaction; $\%Br_{all}$ = percentage of seedlings with complete or partial bark reactions; %SSAL6 = percentage of seedlings with stem symptoms that are alive 5 years p.i.; RSV = % of seedlings with infection (needle or stem) that are alive 3 years (RSV4), or 5 years (RSV6) p.i., respectively; [2] Sow # is the coded family identity within a particular trial. Families "20", "73", "75" are from forest collections (wild OP), "80" is from orchard collection (orchard pollen), families 1, 4, and 74 are from control crosses; [3] RT, resistance type.

The inclusion of a highly susceptible family in the trial is essential to determine the level of resistance that may be present in the other families. The inclusion of a very widely used operational seedlot provides the information on the level of resistance currently deployed. The other seedlots in Table 1 provide information on the potential level of resistance of the best current individual families. The "Bingham F_2" bulked seed orchard lot ("71" in Table 1) has been widely used in parts of the range of western white pine for decades as the source of genetically resistant seedlings for reforestation. At 15 months post inoculation (p.i.) the susceptible family ("79") generally had much higher percentage of seedlings with stem symptoms (%SS2) and higher number of stem symptoms per tree (#SS2) than the other seedlots (Table 1). In some cases, such as family "75", this difference was greater than 75% in both trials. By five years p.i. (%SS6) the difference between the susceptible and other seedlots had narrowed considerably, except for the CR family ("80") in the trial with the *Avcr2* source of rust. Seedlings in all partial resistance seedlots also showed much higher levels of complete bark reaction or partial bark reactions than the susceptible control (Table 1). All seedlots showed higher survival at both three (%RSV4) and five years p.i. (%RSV6) than the susceptible family (Table 1, Figure 4). In summary, relative to the susceptible control the partial resistant seedlots tend to show fewer stem symptoms per tree and a lower overall percentage of seedlings with stem symptoms, suggesting the fungus is being inhibited, but in most cases, not completely stopped. Once the stem is infected, most of the families with partial resistance show moderately high levels of bark reaction (partial or complete) where the fungus is being slowed or further inhibited (Figure 5). In addition, a percentage of trees with stem symptoms (normal cankers or bark reactions, SSAL6) survive through at least 5 years p.i., while all of those in the susceptible family are dead. Overall survival (stem infected or clean) of seedlings in the resistant families is generally substantially higher than the susceptible control at both three and five years p.i. It is also notable that the top families show moderate to substantially higher survival than the Bingham F_2 orchard seedlot (Table 1) used in reforestation, indicating the potential gain in resistance to come from continuing the tree improvement efforts.

Figure 4. Survival differences (five years after artificial inoculation) among susceptible western white pine family and several other seedlots in a trial at Dorena Genetic Resource Center (GRC). Family "75" on left adjacent to "79" (all dead and removed (see missing row in line with third metal tag from the left)) in 10 tree row plots (see Table 1 for rust resistance information on Families "75" and "79").

Another trait that has been observed (and could contribute to partial resistance) is "needle shed" a presumed form of defense response whereby the host sheds its infected needles [34]. The frequency of seedlings in a seedlot with visible needle lesions can decrease dramatically from the first assessment (approximately nine months p.i.) and the second assessment (around 15 months p.i.). In inoculation trials at Dorena GRC all seedlots generally have >98 percent of seedlings with needle spots at nine months p.i. For the seedlots in Table 1, 93 percent of the seedlings in the susceptible family still had needle spots present at second assessment *vs.* 51.5 percent for the *Cr2* family, and a mean of 49.0 percent for the seven partial resistance seedlots (averaged over both trials). The needle shed that occurs in the resistant families between the nine and 15 month p.i. assessments may be partially responsible for the reduced level of stem symptoms, or may be a consequence of the resistance reactions occurring during this time period. In any case, under the inoculation conditions and seedling culture present at Dorena GRC most seedling stems become infected and "needle shed" does not provide the level of protection that has sometimes been reported elsewhere [34]. The level of inoculum density, the inoculum source, the seedling culture and pre- and post-inoculation environment may play a role in the efficacy of some types of partial resistance, including needle shed. Field studies in British Columbia showed little efficacy of western white pine seedlots with the putative "needle shed" resistance [35].

Figure 5. Western white pine tree at Dorena GRC with multiple bark reactions (**left**), five years post-inoculation, in partially resistant family "75" (see Table 1 for details on family "75"); and western white pine tree (**right**) in field trial with >300 bark reactions (see [36] for trial details).

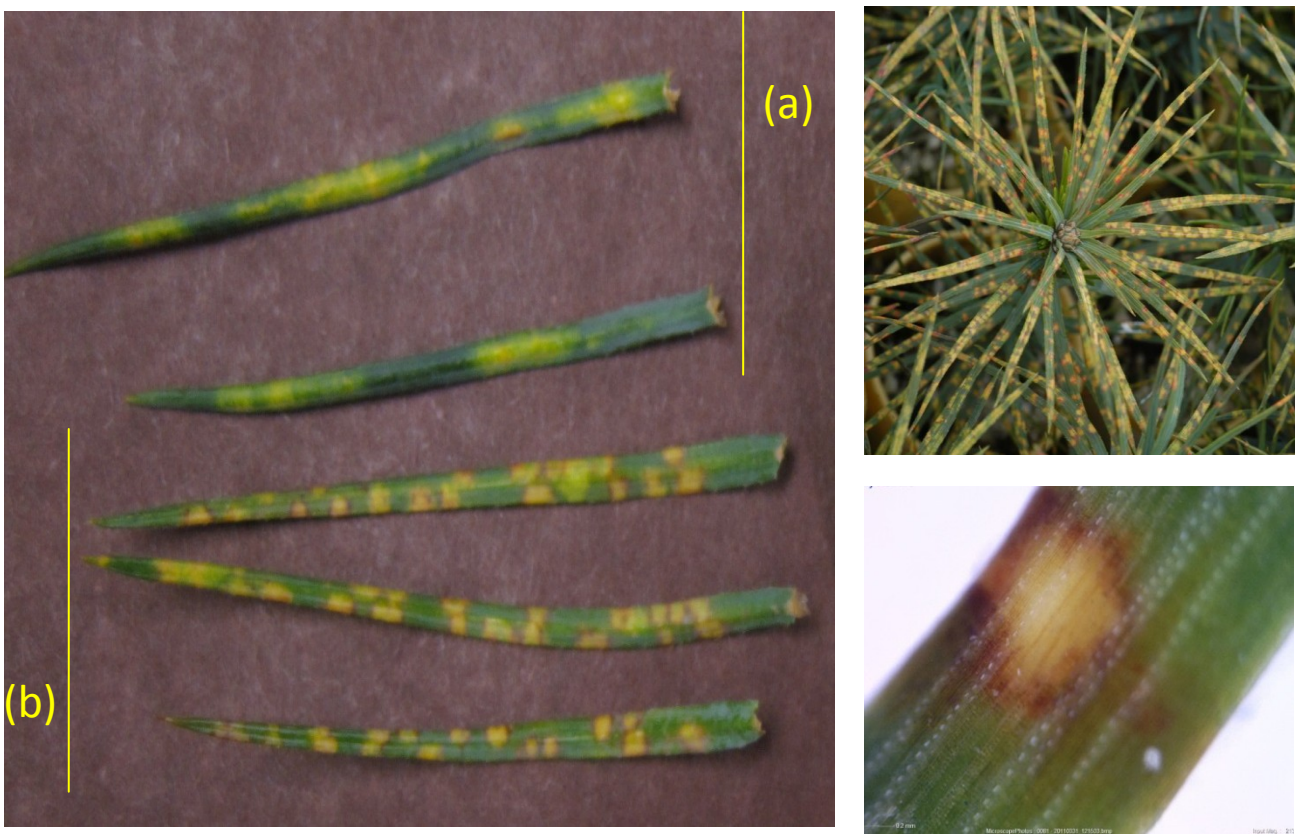
3. Mechanisms of Rust Resistance in Pines

Various types of resistance to pine stem rust are observed. This is expected in the naturally co-evolved FR pathosystems. But even in the WPBR pathosystems, resistance is present in natural populations of all species tested. Both complete resistance from R genes and several types of partial resistances have been documented in several of the white pines. All nine North American white pine species are generally much more susceptible than the white pine species of Europe and Asia, where the rust and white pines are thought to have co-evolved. Unfortunately, relatively little is known about the types of resistance in the Eurasian white pines [20,21].

3.1. Complete Resistance to WPBR in White Pines

Complete resistance from resistance (R) genes has been documented in four of the nine white pine species native to the US or Canada: sugar pine, western white pine, southwestern white pine, limber pine [10,12,32,33,37]. This resistance appears to restrict the fungus to the needles and has been described as a hypersensitive-like response [11,32,33,38] (Figure 6). Generally, seedlings with HR resistance do not get stem infections regardless of the number of needle infections, unless a virulent race of the rust is present. In sugar pine, western white pine, southwestern white pine and limber pine this resistance is conditioned by a single major dominant gene, *Cr1*, *Cr2*, *Cr3* and *Cr4*, respectively [10–12,33,37].

Figure 6. Western white pine showing: (a) susceptible needle infections; and (b) needle infections on a seedling with hypersensitive-like response.



Thousands of sugar pine and western white pine seedling families have been screened for resistance to WPBR, but only a very low frequency of complete resistance (HR) has been found (*Cr1* varying in frequency from ~0 to 0.08; *Cr2* from ~0 to 0.001), and the frequency of resistance varies geographically [11,39]. A slightly higher frequency of complete resistance (*Cr4* frequency varying from 0 to 0.139) has been found in limber pine in the portion of the range surveyed to date [12]. No complete resistance has been detected in whitebark pine families evaluated so far [21,39] or in eastern white pine although families, which exhibit HR-like mechanisms, which may contribute to complete resistance in this species, have been identified in eastern white pine [40,41]. Patton [42] originally selected a number of individual disease-free trees from high-hazard sites in the upper Midwest. These genotypes have served as a portion of a population used for breeding and screening for blister rust resistance in eastern white pine. Open-pollinated progeny from one of these selections, "P327", has consistently displayed resistance in artificial inoculations and field trials. Although not completely qualitative, needle infections rarely progress to stems in this family [43]. Close histological examination of the needle tissue following inoculations has revealed HR-like responses and concurrent proteomic analyses (see genomics section of this paper) have revealed up-regulation of defense-related proteins (NBS-LRR homologs) [41]. Interestingly, this same family also possesses an abundance of epistomatal wax, that due to occlusion of the open stomata, restricts entry by the pathogen, further reducing infection [44].

3.2. Partial Resistance to WPBR in White Pines

Partial or incomplete rust resistance responses have been documented in white pines [8,9,31,45–48]. Much less is known about the underlying mechanisms and their genetic control. Because of the quantitative nature of this resistance, it is presumed that the mode of inheritance is more complex than the HR resistance.

Some of the seedling families of western white pine and sugar pine parent trees selected in natural stands show higher survival than the susceptible controls in artificial inoculation trials at Dorena GRC. But the level of survival is generally relatively low with less than 15 percent of the seedlings surviving inoculations [8]. A few rare outstanding families perform better [47], as do some of the full-sib families made from seedlings selected in these trials (Table 1) [14]. Partial resistant families have a lower percentage of seedlings with stem symptoms within 15 months p.i. than the most susceptible families (Table 1) [14]. Western white pines are somewhat more resistant than sugar pines [8,49,50]. Breeding the top selections is showing promise to increase resistance (Table 1) [14,51]. Whitebark pine seedling families have higher levels and frequencies of partial resistance, in comparison to western white pine and sugar pine [52–54].

Families with partial resistance often show fewer stem infections, latent stem infection, bark reactions (complete or partial) and higher survival of seedlings with cankers and later mortality (Table 1). All of these responses are present among the seedlings in the top resistant families and different seedlings in the top families may show more than one [47] (Table 1). Little is known about the inheritance of the partial resistances including whether the genes for resistance occur in clusters or whether individual seedlings in a family show different phenotypes that might be expected with a quantitative inheritance. It may also be that similar phenotypes in different families arise from different underlying genetic mechanisms. Many of the sugar pine and western white pine within-family selections from rust screening or field trials are just beginning to reach reproductive maturity, but some of the early crosses show good promise [14,51]. The operational programs on the West Coast (CA, OR, WA, British Columbia) for western white pine and sugar pine are combining both complete and partial resistance in their seed orchard production populations.

3.3. Southern Pines and Fusiform Rust

Being a co-evolved pathosystem, a higher frequency of resistance alleles is found in the southern pine populations than in the white pines. However, their frequency is variable, depending on region or host ecotype [55]. For example, south Florida slash pine is much more resistant to FR where it occurs naturally, than slash pine is in its native range [55]. This may be due to both underlying genetic resistance and phenological differences that reduce infection frequencies [55]. This resistance is diminished greatly when south Florida slash pine is grown in the northern portion of the range slash pine [55]. Loblolly pine from the western portion of its range (Louisiana, Arkansas, *etc.*) is much more resistant to FR than eastern ecotypes [56]. Slash pine and loblolly pine had separate glacial refugia during the Quaternary; loblolly pine was found in Texas or Mexico (where *Cqf* continued to co-evolve with oaks in that region), and slash pine was found in south Florida or the Caribbean, where rust was rare or absent [55]. It is thought that high levels of disease in the eastern Gulf Coastal plain (>80%

incidence in many native stands) may be due to recent changes in the distribution of slash pine and loblolly pine in the post-Pleistocene era [55]. These species are encountering different pathogen populations, perhaps due to changes also in oak species distributions [55]. This, coupled with widespread increases in plantations of susceptible pines and increased oak abundance, due to fire suppression and land use changes have dramatically increased the presence of this disease in the region.

One key difference between FR and WPBR is *C. ribicola* infects pine hosts by way of stomata and thus, host-pathogen interactions occur first in needle, then in the twig or stem tissues—in FR, the pathogen does not need to infect needles and frequently directly penetrates stem tissues [57]. As a result, needle-level resistance such as HR or physical barriers (epistomatal waxes) are likely to be of less utility. Resistance in stem tissue is functionally the most likely mechanism for resistance in southern pines to *Cqf* [58].

Both complete and partial resistances have been observed in slash and loblolly pines [59]. Complete resistance in *Cqf* refers to the phenotype of the absence of a gall. It is the basis for resistance phenotyping in resistance programs and is under control of single, race-specific resistance genes. Partial resistance can display numerous phenotypes, including partial or reduced size galls and non-sporulating galls and these phenotypes are considered quantitative (under control of multiple genes) and non-race-specific. Although resistant slash pine families have been identified [60] most research on resistance to FR has been carried out in loblolly pine and has focused on complete resistance rather than partial resistance [15]. There are several reasons for this. First, the disease biology of FR is quite different from WPBR in that it is common for infected hosts to live for many years with infected main stems that can sporulate yearly for decades. With WPBR the host is frequently killed in less than five years following infection. This occurrence of varying degrees of partial resistance or tolerance in FR is difficult to evaluate. Second, the main objective of resistance programs for the southern pines is to reduce the impact of FR on the commercial pine forestry industry. As a result, infected trees, despite survival potential, may be unacceptable given the reduction in timber quality, reduction in growth potential and potential losses due to breakage.

Race-specific resistance to FR was first detected in loblolly pine in 1996 when Wilcox demonstrated the presence of *Fr1* [15]. Subsequently, eight additional R genes have been detected [61–63] and presumably interact with corresponding avirulence (*avr*) genes in *Cqf* [64]. These resistance genes have been mapped and molecular markers have been developed to assist in breeding efforts [65]. However, nothing is currently known about their function or relationships to other R genes in plants. Additional genes in slash pine have also been detected [66] and deployed in field studies [24–26], however, in general it is thought that complete resistance is less frequent and less stable in this species [67]. Partial resistance has been discovered in this species and may be useful in breeding programs [68]. In order to better understand the interaction and develop more precise, pathogen tailored resistance, more information about the *avr* genes is being sought. For example, *Avr1*, which triggers complete resistance through the interaction with *Fr1*, has been recently mapped in a heterozygous rust family [69].

3.4. Genetic Variability and Pathogen Evolution

The ability of pathogens to rapidly evolve to overcome resistance genes following deployment has long been observed in agricultural crops. This has resulted in a boom-and-bust cycle of breeding for resistance followed by breakdown as the pathogen acquires the ability to overcome the resistance mechanism. In pines, where breeding cycles are much longer than in agricultural crops and the host is long-lived, this problem is compounded. Complete resistance controlled by a single gene is particularly vulnerable to pathogen evolution since changes at a single pathogen gene should allow the pathogen to evade host recognition. Partial resistance should in theory be more stable than complete resistance as the selection pressure on the pathogen is lower. There are few long-term demonstrations of this durability, although current trial results are encouraging [49,70,71].

Races of *C. ribicola* that can overcome host resistance have been documented in sugar pine and western white pine [72]. Races of the pathogen that possess the *vcr1* allele can overcome the *Cr1* resistance gene in SP and the *vcr2* negates resistance provided by the *Cr2* gene in western white pine [72]. Recent genomic characterization in other pathosystems has shown that resistance genes can recognize pathogen effectors that facilitate infection and colonization of the host. Mutations in these effectors allow the pathogens to evade detection by corresponding R-genes [18]. A better understanding of these processes in WPBR will help in monitoring the appearance and spread of those *vcr* alleles.

In sugar pine and western white pine it appears that only one "effective" major gene for complete resistance is present in each species since wild type inoculum generally causes very high infection in all of the other thousands of families tested from throughout the range, and the virulent races (*vcr1* in sugar pine, *vcr2* in western white pine) cause stem infection of all known families with the complete resistance. Because of the nature of the resistance, presence of the corresponding *vcr* alleles in the pathogen population usually results in complete breakdown of the resistance. In areas where the *vcr* allele of the rust is present, the use of *Cr1* or *Cr2* alone provides little or no benefit above the susceptible control [36,39,49,71] (Figure 7). However, in some other areas, the *vcr* allele appears to be absent or in low frequency in the rust population and families with Cr1 or Cr2 alleles remain resistant [73]. The durability of the resistance provided by the *Cr1* and *Cr2* genes may be limited over the long-term, especially on sites of high rust hazard where *vcr* alleles that appear could rapidly spread. Deploying pine genotypes with both complete resistance and partial resistance may be an effective strategy for such sites. Monitoring of the *vcr* alleles in the rust populations would allow for better prediction and assessment of risk to the deployed resistant material.

In the FR pathosystem, it is likely that virulence to the R genes is present in varying frequencies in the pathogen population. Recent studies have revealed varied virulence to the nine known resistance genes to *Cqf* in loblolly pine, with some genes showing wide variation in responses to different inocula and others consistently providing resistance, regardless of inoculum source used [64,74].

To date, no studies have revealed any significant geographical structure or patterns regarding the specific avirulence alleles in *Cqf*. However, there appears to be genetic structure in the pathogen population in the southeast, with at least four distinct sub-populations being detected [75]. It may be that some differences in avirulence alleles are present in these sub-populations, but that has not been determined yet.

Figure 7. Western white pine families in a field trial in western Oregon, where blister rust genotypes with the vcr2 gene that overcomes the HR resistance are present (November 2012). The dead trees are from an open-pollinated orchard seedlot from a parent tree homozygous dominant for the complete (HR) resistance conditioned by the *Cr2* gene (*Cr2Cr2* × wind). At this site, families with only *Cr2* gene for resistance show 95 percent or greater stem infection, similar to the susceptible control family, while many of the families with partial resistance do significantly better (adjacent living trees shown in this photo).

4. Field Trials

4.1. Field Resistance to Fusiform Rust

Many field trials of loblolly and slash pine with various resistance phenotypes have been established in areas covering a range of rust hazards and likely covering much of the genetic variation in the rust. These trials generally show good gain in resistance from breeding, with a financial benefit:cost ratios of 2.2 to 20.4 [76] and gains are especially noted for loblolly pine [77–79]. Significant family × location interactions have been observed for resistant slash pine families (Figure 8), which may be due to increased virulence of local rust populations to this resistance or phenological phenomena in this host [60,79]. In general, resistance has greatly reduced incidence of FR, especially on high-hazard sites [77].

Figure 8. Two contrasting slash pine families, resistant (**left**) with 7% incidence and susceptible (**right**, showing branchy, deformed trees) with 87% incidence. Both are growing on a high hazard site for fusiform rust.

4.2. Field Resistance to WPBR in North America

By contrast, few field trials of western white pine and sugar pine have been established to closely monitor the efficacy of the different types of resistance. These trials are important since they reflect conditions that are different from the controlled inoculations. For example, field trials will reflect different climatic zones as well as diverse inoculum sources. They are essential to assess the durability of the resistance developed. Many of these trials comprising susceptible controls and resistant stock have been established in the PNW Region (OR and WA) since 1996. In addition, extensive field testing for rust resistance of sugar pine in northern California at the Happy Camp test site (where the *vcr1* race of rust is prevalent) is ongoing. These trials are now yielding valuable information on the relationship between the level of resistance in artificial inoculation trials *vs.* field trials as well as which type of resistances is most effective under field conditions [36,71]. A key role for well documented field sites will be to monitor the long term durability of resistance over time, and assess what components of partial resistance provide long-term survival.

In one set of field trials on three sites with high rust incidence the families with partial resistance (including "73" and "75" from Table 1 above) showed much higher levels of resistance (lower infection and higher survival) than the susceptible control [71]. In western white pine, visible stem infection of the partial resistant families is generally lower than that in artificial inoculation trials. Mortality lags behind stem infection and data is still being accumulated on the survival of these families. Families with partial resistance can perform well while susceptible control families and many families with complete resistance (HR) show very high levels of stem infection [36] (Figure 7). Two families with *Cr2* had high levels of stem infection at some sites, indicating that the frequency of *vcr2* can be high and vary from site to site [71].

White pine species vary in performance in resistance trials. Very few studies have evaluated field performance of resistance in eastern white pine and results have been inconsistent [80] with resistant

stock both performing better and worse than controls. Larger field trials are underway, but so far progress has been far less promising than with other species [81,82]. Sugar pine is generally more susceptible than western white pine [49,50], but some recent crosses of parents with partial resistance show excellent early promise [51]. Small field trials using resistant families of whitebark pine, limber pine and bristlecone pine (*P. aristata*) have recently been established and will serve to validate the results of short-term seedling screening and monitoring durability of resistance under different field environments and a changing climate.

5. Molecular Tools

An exciting development in the last two decades is the rapid advances taking place in the availability of genomic tools to investigate and help in the development of resistance to pine rust. In addition, the genomes of both the rust pathogens have recently been sequenced (Joint Genome Intstitute, [83,84]). Investigations underway will provide further insights into the underlying nature of resistance in the pines, the genetic diversity of the rusts, and the potential for increasing the efficiency of resistance programs using genomic selections or marker-assisted selection.

5.1. Comparative Mapping of Pinus Major R Genes Using SNP Markers

The sugar pine *Cr1* and the western white pine *Cr2* blister rust resistance genes, and loblolly pine fusiform rust *Fr1* gene have been genetically mapped [15,85–87]. This work provided a basis for using genomic approaches to map these R genes. This can be done by using a large number of single nucleotide polymorphism (SNP) markers. By comparing genes expressed by *Cr2*-resistant and *cr2*-susceptible populations of western white pine it is possible to identify transcripts that differ between these gene pools [88]. To construct fine and consensus *Cr2* maps, a full-sib *Cr2*-family was phenotyped and SNP markers in candidate plant defense-related genes and disease resistance genes were screened [89]. SNP markers from at least four functional genes belonging to the NBS-LRR and other disease resistance-related gene families, with tight linkage to *Cr2* in multiple elite western white pine seed families were found [90]. The resistance gene analog co-inherited with *Cr2* provides the best positional candidate for molecular characterization of *Cr2* in a future functional genomics study.

Comparing R genes in different pines could be useful. Sugar pine *Cr1* is localized on loblolly pine linkage group LG 11 [91,92]. Orthologs of *Cr1*- or *Cr2*-linked genes are also present in the genome of loblolly pine. Therefore, it is reasonable to believe that these *Cr2*-linked genes can be used for a comparative genomics study to address the question whether major resistance genes in different species are the same, which will help facilitate pyramiding these genes. As loblolly pine SNP maps are available [93] and an initial draft of its genome sequence is released [92], a further SNP mapping of nine loblolly pine R genes and comparison of those fine R genetic maps between subgenus *Strobus* and subgenus *Pinus* will help us understand more about the evolution of genetic resistance against *C. ribicola* and *C. quercuum f. sp. fusiforme*.

5.2. Molecular Mechanism of Major R Gene-Mediated Resistance

Plants have adapted various molecular resistance mechanisms to defend themselves from pathogen attack. Plant immunity is controlled by two layers of inducible responses: basal response triggered by conserved microbial features and specific response triggered by gene-for-gene recognition [94]. Pines with major R genes display a typical hypersensitive reaction (HR) on their needles after rust infection, presumably triggered by recognition of the *avr* product using an uncharacterized receptor encoded by the R gene [72,95]. Partial/quantitatively resistant or susceptible white pine seedlings show only basal responses through general defense pathways in response to pathogenicity factors in the absence of R proteins. Both *vcr1* and *vcr2* factors can overcome the corresponding R protein in sugar pine and western white pine with *Cr1*- or *Cr2*-genes, respectively, by manipulating plant physiological processes more suitable for rust to grow and spread in the seedlings.

Our knowledge about molecular mechanisms underlying *Pinus*-*Cronartium* interaction remains limited. No difference in regulated genes between compatible and incompatible interactions of loblolly pines infected by FR was found [86]. Approximately one hundred proteins were differentially regulated in *Cr2*-resistant and *cr2*-susceptible western white pine infected with *C. ribicola* [96]. The differentially expressed white pine proteins included intermediate factors functioning in the signal transduction pathways triggered by well-known plant R genes, heat shock proteins (HSPs), reactive oxygen species (ROS) scavenging enzymes. Comparison of the transcriptome of *Cr2*-resistant and *cr2*-susceptible genotypes revealed significant difference of global gene expression that affects the defense signaling pathways and metabolic pathways [88]. About one thousand differentially expressed genes, accounting for 2%–3% of the whole needle transcriptome, were identified during compatible (*cr2* to *avcr2*) and incompatible (*Cr2* to *avcr2*) WPBR interactions. Plant R candidates (NBS-LRR and RLK genes) were up-regulated specifically in resistant genotype following *C. ribicola* infection, suggesting a distinct role of these R candidate genes in the *Cr2*-mediated resistance. Biosynthesis and signaling pathways of multiple plant hormones (auxin, abscisic acid-ABA, and ethylene) were coordinately activated following rust infection, with indication that the auxin and ABA-mediated signaling pathways were involved in the *Cr2*-trigeered major gene resistance inside white pine needles. We also revealed a set of novel transcription factors in response to *C. ribicola* infection, some of them specifically responsive in the incompatible WPBR interaction. In addition, several families of pathogenesis-related (PR) proteins (PR1, PR2, PR3, PR5, PR6), ROS-related proteins (glutamine synthetases, thioredoxin-like proteins and peroxidases), and the ubiquitin–proteasome system (UPS) were also differentially expressed at transcriptional level between *Cr2*-resistant and *cr2*-susceptible seedlings following *C. ribicola* infection.

5.3. Genomic Selection of Partial Resistance

Genomic selection is a new plant breeding approach that uses statistical modeling to predict plants' performance with availability of DNA markers covering the whole genome and detailed phenotypic characteristics for multiple quantitative traits on a complete collection of germplasm in the breeding program. Genomics progress on the WPBR pathosystem with application of high throughput SNP genotyping technology makes it feasible to undertake the genome-wide association mapping of *Pinus*

partial resistance to *Cronartium*, which is even more important for whitebark pine, eastern white pine, and other *Pinus* species where no complete resistance has been discovered. Genome sequencing and RNA-seq analysis of the whole transcriptome have generated SNP data covering the whole genome for several *Pinus* species [97]. However, any association data between SNPs of candidate genes and partial resistance require verification using large populations (including both partial resistance and non-partial resistance seed families) with field phenotypic evaluation from different geographic areas. Phenotypic assessment of partial resistance to *C. ribicola* is difficult and takes several years after seedling inoculation [21]. Application of genomic selection for marker-assisted selection is still a challenging but feasible task in a WPBR breeding program.

As *Cr1* and *Cr2* can be overcome by virulent races, partial resistance (often also referred to as quantitative disease resistance (QDR)) to WPBR attracts special attention and some of it is quite impressive at least under moderate and high rust hazards [49,71]. Incorporation of multiple R genes and different types of partial resistances in an elite seed orchard appears to be the most effective approach for long-term WPBR durability [13,98]. However, currently we do not know how many genes or quantitative trait loci (QTL) underlie white pine partial resistance as well as the genetic effects contributed by each gene or QTL through their interaction with environmental factors.

Epicuticular waxes were observed as a partial resistance mechanism by reducing spore germination and subsequent infection through stomatal openings in eastern white pine [44]. Once rust germ tubes invaded needle tissues through stomata, histochemical analysis revealed that *C. ribicola* induced a build-up of physical barriers by collapsed cells adjacent to infected sites and deposition of cell wall bound phenolic compounds within the needle [40,99], suggesting this defense reaction may prevent systemic rust spread in partial resistant eastern white pine families. At the molecular level, a few studies revealed several *Pinus* gene families involved in defense response against *C. ribicola* infection. Plant R family of NBS-LRR proteins and multiple families of PR proteins, including chitinases (PR3), thaumatin like proteins (PR5), intracellular ribonuclease-like proteins (PR10), and anti-microbial peptides/proteins (AMPs), contributed in part to host resistance in the WPBR interactions [41,100].

The above investigations provided at least a small set of potential candidate genes for white pine partial resistance. Western blot analysis found that western white pine chitinase and AMP1 are protein biomarkers that could discern seed families with different defense mechanisms underlying stem partial resistance (such as slow canker growth and/or bark reaction) [101,102]. Further association study revealed SNPs and genotypes of western white pine genes for chitinase, PR10 proteins and AMPs were genetically associated with stem partial resistance phenotypes [103–105]. The proteins/enzymes encoded by these associated genes exhibited *in vitro* and *in vivo* antifungal activity [102,106], providing strong biochemical/functional links between gene polymorphisms/genotypes and resistance phenotypic traits.

In FR, both loblolly pine and *Cqf* genomes are near completion [83]. With a significant genomic resource for both host and pathogen, it is expected that new light will be shed on the underlying mechanisms of qualitative resistance in this pathosystem [95]. Already genomic selection models have been developed that work well for predicting fusiform rust resistance in loblolly pine [107]. Interestingly, due to the focus on qualitative resistance, certain models worked better than others with variation explained by different assumptions regarding equal contribution of markers to observed traits [107].

5.4. Tools to Monitor Pine Rusts

Understanding the genetic variability in pathogens is the cornerstone of monitoring for durability of resistance. In addition, patterns of migration and spread can inform us on current and past epidemiological processes [55]. This is important to identify sources of pathogen outbreaks and to provide prediction for risk of spread. One consistent observation is that genetic diversity is locally high in pine rusts. The genetic diversity is high within sites (usually around 90% of the total variation) [108–111] with the remainder of the diversity present among sites. This genetic diversity is even present at a very fine scale: 70% of the total genetic diversity found within a heavily sampled plantation was found to reside among aecia within cankers [75]. This data is consistent with a high rate of outcrossing in the rust. Spermatization, the process whereby insects carry the spermatia between cankers, creates highly variable genetic mosaics in pine rust cankers [75,111–113]. Extensive recombination was detected among haploid spermogonia sampled within populations [114]. This indicates a high potential for evolution and adaptation in these pathogens at a local level and therefore, a need for monitoring these rusts.

In addition, a high level of gene flow was estimated based on low measures of genetic differentiation among sampling sites from different geographic regions [108–110,114,115]. This is consistent with the nature of both aeciospores and urediniospores to be wind-borne and be involved in long-distance dissemination. Nevertheless, some regional structures are also apparent in both rusts. Eastern and western populations of *C. ribicola* are strongly differentiated, indicating their different introduction and colonization histories and a barrier to migration [116]. There is also regional differentiation among populations of FR in the southern US and the magnitude of this differentiation was closely associated with geographic distance between populations [75].

While these reports made use of a restricted number of polymorphic markers, new high-throughput single nucleotide polymorphism (SNP) genotyping and genome sequencing approaches will provide much greater information about the biology and epidemiology of rust fungi. The genome of the poplar rust was the first tree rust genome to be published [117]. It also alternates between conifers and deciduous hosts and shares very similar biology to the *Cronartium* rusts. Whole genome sequencing revealed unique features related to their biotrophic life-style [118]. A large repertoire of effector-like small secreted proteins is likely involved in facilitating host penetration and interactions. Nitrogen and sulfur assimilation pathways were found to be impaired, probably because of the reliance of the pathogen on host nutrient resources. Expanded families of amino acid and oligopeptide membrane transporters are probably involved in nutrition via acquisition of the host peptides by the rust. This information should form the basis for understanding what are the unique features of rusts and can be used to target processes that can be linked to adaptation.

A better understanding of what makes rust fungi so adaptable would help in our attempts to monitor and manage them. Secreted proteins are believed to be important in pathogenesis since they are likely involved in the pathogen interaction with the hosts. They have been found to modulate plant defense circuitry and enable parasitic colonization [119]. By comparing genomes of rusts that attack different hosts, the secretome (the ensemble of secreted proteins), was found to be enriched in genes under positive selection, indicating that different evolutionary constraints are acting on the rust secretome when compared to the rest of the genome [120]. Among these selected secreted proteins are likely

effectors of pathogenicity and the pattern observed is the hallmark of the coevolutionary arms race that occurs between hosts and pathogens, whereby effectors (such as avirulence alleles) are recognized by the host, generating resistance response, and rapidly overcome by the pathogen. This accelerated evolution of effectors compared to the rest of the genome is a key to ability of pathogens to overcome and adapt when resistance hosts are encountered. Our ability to detect and monitor these changes prior to their widespread dissemination is a key to monitor the durability of resistance.

6. Deployment of Resistant Stock

Deployment of southern pines resistant to fusiform rust has been a success. Seedlings from open-pollinated resistant parent trees or from control matings (full-sibs) have been deployed over millions of acres of forest plantations [59]. A variety of options are available to land managers who can utilize some unique resistant genotypes in very large plantings (narrow genetic base, but durable resistance) in relatively short rotations. It has been estimated that for every dollar spent on FR resistance research there is a return on investment (ROI) of $5–20 [76]. However, given the rapid genetic changes that occur in the pathogen population, combined with industry needs for new and improved phenotypic traits (fiber length, resin production, growth rate and form) continued screening for new resistance alleles is justified.

In the white pine species, seed from wind-pollinated seed orchards (western white pine, sugar pine, eastern white pine) or from parent trees in the field (whitebark pine and other high-elevation white pine species) will be used for reforestation or restoration. The genetic base will generally be large and the rotations longer than for the southern pines. For the high-elevation white pine species the long-term durability of resistance will be even more important since they will serve as the progenitors of future generations of natural regeneration since little or no breeding may be done. Recent data show that the most widely used F_2 seed orchard seedlot for reforestation in the Interior West (Bingham F_2 lot) provides greater relative resistance than the natural regeneration or first-generation orchard seedlot [121]. However, on the sites with highest rust hazard the infection of seedlings from this orchard seedlot has been very high making land managers reluctant to fully utilize this species. Additional breeding in western white pine and sugar pine will be needed to raise the resistance to levels more conducive to success for reforestation and restoration on sites of moderate to high rust hazard. Breeding is underway in the several programs and the top families show 40 percent or greater survival than susceptible families as well as notable increases above that of the Bingham F_2 orchard seedlot. Selections from these crosses can be used to establish new seed orchards with increased levels and diversity of resistance. New orchards with higher levels of resistance are urgently needed to fully realize the potential of western white pine and sugar pine in managed forests.

7. Discussion

Both of the pine rusts discussed in this paper can cause significant damage and mortality. The non-coevolved white pine blister rust pathosystem causes greater mortality than the co-evolved fusiform rust pathosystem. In the case of southern pines and fusiform rust, most of the infections occur when trees are relatively young and often damage the trees rather than kill them, greatly diminishing the economic value of some plantations. White pine blister rust can infect pines at any age, and can

cause extensive mortality (up to 100 percent) especially in seedlings and younger trees. Some trees can have hundreds or even thousands of cankers on the main stem and the branches. A major difference between the resistance programs for the southern pines and those for the white pines is the major focus on partial resistance traits in the white pine programs *vs.* the past and current emphasis on complete resistance in the southern pine programs. Several reasons for this difference exist: (1) the lack of complete resistance in some white pine species and in some portions of the range of species; (2) the detection and spread of the *vcr1* and *vcr2* virulent races in sugar pine and western white pine putting the long-term durability of complete resistance from *Cr1* and *Cr2* in doubt; (3) the presence of only one known effective R gene for complete resistance in both SP and WWP (*vs.* nine such genes in loblolly pine); and (4) the generally lower level of protection provided by partial resistance in the early generations of selection. Partial resistance is generally more difficult to evaluate (especially in the FR hosts) in seedling screening trials, but is thought to be under the control of multiple genes and to be more durable. Current field trials with western white pine in the Pacific Northwest show the promise of partial resistance, but also its limitation, notably that some trees in even the best current families become cankered and die. Further breeding will be needed to fully realize the potential benefits of partial resistance in western white pine and sugar pine.

A major question in all genetic resistance programs is whether the resistance will be durable. This question is even more germane for a forest tree species that will be expected to survive for decades or hundreds of years. For the time being, information on durability of resistance is primarily determined through monitoring trials or plantations with resistant plant stock or original parent trees that have been exposed to blister rust for decades. In western white pine, some seedlots show good survival after 30 or more years in areas of high infection. For whitebark pine, some parent trees survive decades after many others in the vicinity have perished, and their progeny show high levels of resistance in artificial inoculation trials. For loblolly or slash pine, there is currently limited information on durability in the field of R genes, but data from slash pine indicate that these genes may be overcome relatively quickly and durability of loblolly R genes (*Fr1 etc.*) is greater [60]. Future molecular dissection of resistance and virulence should help determine what types of resistances are likely to be durable.

Virulent genotypes of the blister rust are known to exist in several locations where white pines with HR resistance from *Cr1* or *Cr2* are planted, and it is likely that other cases will arise. However, a more worrisome scenario is the potential re-introduction of other more virulent races of blister rust from China, or the introduction of a second species of blister rust [23]. No information is currently available on the effectiveness of current partial resistances in North American white pines to either of these cases. A better understanding of the evolution of the pathogens and their patterns of pathogenic diversification are crucial to future efforts to ensure durability of host resistance.

8. Future Directions

Tree breeding is inherently a long-term endeavor, but the FR and WPBR resistance programs have made substantial progress in establishing large base populations, increasing the level of resistance for several species and producing seed for reforestation. Work on some of the high-elevation white pine species has only recently begun, but shows good promise. Interest in further examining rust resistance in longleaf pine has arisen. Protocols and advances made earlier with the commercial species for both

the southern pines and white pines will continue to facilitate the resistance evaluation in additional species. Partial resistance or the combination of complete resistance with partial resistance will be the key to developing durable resistance in the white pine species. For the southern pines, a better understanding of the underlying nature and durability of the genes for complete resistance is needed. A basic understanding of the frequency and geographic distribution of these genes in natural population may help formulate deployment strategies for forest plantations, however, the nature and extent of such plantations may call for different strategies.

Ideally, programs in both the WPBR and FR pathosystems would like to reduce the impact of rust to zero, but this is unrealistic, at least in the short-term and especially for the white pine species. The goals of the programs for the two pine-rust pathosystems currently vary. In the case of WPBR, complete resistance that reduces cankering to near zero levels on high hazard does not appear to be feasible, at least in the near future, with conventional breeding. The resistance programs in western white pine and sugar pine have begun breeding to increase resistance using the selections made within the artificial inoculation trials and this is expected to increase the level of partial resistance produced in future seed orchards. For species such as whitebark pine, where little or no breeding may be done, there may be high enough levels of partial resistance, at least in some populations, to provide effective restoration opportunities using seed collected from parent trees or developing seed orchards. In the southern pines, particularly in loblolly pine where nine R genes conferring complete resistance have been identified, there may be more prospects of achieving a very high percentage of canker-free trees in future plantations. However, more information is needed on the nature of the different resistance genes, the existence of virulent strains, and strategies for efficiently pyramiding or mixing these resistance mechanisms in production plantings to minimize evolution of virulent races of FR. The use of clonal plantings of highly resistant and highly productive clones (genotypes with at least several R genes) may be the most effective option in terms of maximizing rust projection in the short rotations of loblolly pine. However, caution in using such a limited number of genotypes will have to be weighed accordingly. For the long-term, further evaluation of partial resistance in the southern pines as a possible tool to incorporate into future breeding merits consideration.

The sequencing of the genomes of the two rust pathogens, along with that of the loblolly pine genome, and in the near future the sugar pine genome, will provide scientists with new information to help further understand the nature of the resistances and their potential durability in the face of evolving pathogens. In the case of the WPBR pathogen, these tools offer the potential to examine the worldwide genetic variation in the rust and the potential existence of new virulent or aggressive races that could still be inadvertently introduced into North America. These tools should also help facilitate understanding more about a second species of rust (in China) that appears to be virulent against at least some species of white pines [23].

For WPBR, a valuable option would be to test a subset of known resistant seedlots from several white pine species against geographic races of the rust from China or other Asian countries. With the added information gained in the last 10 years, there would be a chance to examine resistance in many of the North America white pines against potentially more virulent or aggressive races of rust or against another species of blister rust. This proactive measure would give assurance or provide extra incentive to minimize dangers of importation of new races of rust. However, additional funding would be needed to undertake this important work with international partners.

The potential impacts of climate change are unknown at this point, but the efficacy of some resistances could be affected by changing temperatures, expanded windows of infection, and the increased levels of rust spore densities that may occur in some cases. Seedling testing at Dorena GRC is generally done under milder conditions and warmer temperatures than are present in the field and this may help select for resistances that are effective under the warmer environments that may exists in the future in the native range of the white pine species. However, monitoring of field tests will be the definitive measure of assessing durability of resistance in the face of evolving pathogen and a changing climate.

The rust resistance programs for these pine species have made great progress to date and the resistant seedlings are being extensively planted. However, to maximize the economic and ecological benefits that these pines provide, it is essential that further research and breeding work continues. This work will help increase the levels of resistance as well as identify the types of resistance mechanisms that are durable. The genomic tools that are just becoming feasible to use in conifers will help accelerate progress in resistance development to improve forest health.

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Author Contributions

All authors contributed information from their personal work & expertises with these species and to the synthesis of ideas incorporated here.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Cubbage, F.W.; Pye, J.M.; Holmes, T.P.; Wagner, J.E. An Economic Evaluation of Fusiform Rust Research. *South. J. Appl. For.* **2000**, *24*, 77–85.
2. Fins, L.; Byler, J.W.; Ferguson, D.; Harvey, A.E.; Mahalovich, M.F.; McDonald, G.I.; Miller, D.; Schwandt, J.W.; Zack, A. Return of the Giants: Restoring Western White Pine to the Inland Northwest. *J. For.* **2002**, *100*, 20–26.
3. Harvey, A.E.; Byler, J.W.; McDonald, G.I.; Neuenschwander, L.F.; Tonn, J.R. *Death of an Ecosystem: Perspectives on Western White Pine Ecosystems of North America at the End of the Twentieth Century*. Gen. Tech. Rep. RMRS-GTR-208. US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2008; p. 10. Available online: http://www.fs.fed.us/rm/pubs/rmrs_gtr208.pdf (accessed on 13 December 2013).
4. Tomback, D.F.; Achuff, P. Blister Rust and Western Forest Biodiversity: Ecology, Values and Outlook for White Pines. *For. Pathol.* **2010**, *40*, 186–225.

5. US Fish and Wildlife Service. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition to list *Pinus albicaulis* as Endangered or Threatened with Critical Habitat. 2011. Available online: http://www.fws.gov/mountain-prairie/species/plants/whitebarkpine/ (accessed on 14 December 2013).
6. Kubisiak, T.L.; Amerson, H.V.; Nelson, C.D. Genetic interaction of the fusiform rust fungus with resistance gene *Fr1* in loblolly pine. *Phytopathology* **2005**, *95*, 376–380.
7. Bingham, R.T. *Blister Rust Resistant Western White Pine for the Inland Empire: The Story of the First 25 Years of the Research and Development Program*; General Technical Report INT-146; US Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station: Ogden, UT, USA, 1983; p. 45.
8. Kegley, A.J.; Sniezko, R.A. Variation in Blister Rust Resistance among 226 *Pinus monticola* and 217 *P. lambertiana* Seedling Families in the Pacific Northwest. In *Breeding and Genetic Resources of Five-Needle Pines: Growth, Adaptability and Pest Resistance*, Proceedings of IUFRO Working Party 2.02.15 Conference, Medford, OR, USA, 23–27 July 2001; Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., Eds.; Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; pp. 209–226. Available online: http://www.fs.fed.us/rm/pubs/rmrs_p032/rmrs_p032_209_226.pdf (accessed on 13 December 2013).
9. King, J.N.; David, A.; Noshad, D.; Smith, J. A Review of Genetic Approaches to the Management of Blister Rust in White Pines. *For. Pathol.* **2010**, *40*, 292–313.
10. Kinloch, B.B., Jr.; Dupper, G.E. Genetic Specificity in the White Pine-blister Rust Pathosystem. *Phytopathology* **2002**, *92*, 278–280.
11. Kinloch, B.B., Jr.; Sniezko, R.A.; Dupper, G.E. Origin and Distribution of *Cr2*, a Gene for Resistance to White Pine Blister Rust in Natural Populations of Western White Pine. *Phytopathology* **2003**, *93*, 691–694.
12. Schoettle, A.W.; Sniezko, R.A.; Kegley, A.; Burns, K.S. White Pine Blister Rust Resistance in Limber Pine: Evidence for a Major Gene. *Phytopathology* **2014**, *104*, 163–173.
13. Sniezko, R.A. Resistance Breeding against Nonnative Pathogens in Forest Trees—Current Successes in North America. *Can. J. Plant Pathol*. **2006**, *28* (Suppl.), 270–279.
14. Sniezko, R.A. Blister Rust Updates on Resistance and Field Trials: On the Road to Durable Resistance? A Tree Breeder's Perspective. In Proceedings of the 60th Annual Western International Forest Disease Work Conference, Tahoe City, CA, USA, 8–12 October 2012; Browning, J., Palacios, P.C., Eds.; WIFDWC: Tahoe City, CA, USA, 2013; pp. 81–90.
15. Wilcox, P.L.; Amerson, H.V.; Kuhlman, E.G.; Liu, B.H.; O'Malley, D.M.; Sederoff, R.R. Detection of a Major Gene for Resistance to Fusiform Rust Disease in Loblolly Pine by Genomic Mapping. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3859–3864.

16. McDonald, G.I.; Zambino, P.J.; Sniezko, R.A. Breeding Rust-resistant Five-needled Pines in the Western United States: Lessons from the Past and a Look to the Future. In *Breeding and Genetic Resources of Five-Needle Pines: Growth, Adaptability and Pest Resistance*, Proceedings of IUFRO Working Party 2.02.15 Conference, Medford, OR, USA, 23–27 July 2001; Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., Eds.; US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; pp. 28–50. Available online: http://www.fs.fed.us/rm/pubs/rmrs_p032.html (accessed on 13 December 2013).
17. Quesada, T.; Gopal, V.; Cumbie, W.P.; Eckert, A.J.; Wegrzyn, J.L.; Neale, D.B.; Goldfarb, B.; Huber, D.A.; Casella, G.; Davis, J.M. Association Mapping of Quantitative Disease Resistance in a Natural Population of Loblolly Pine (*Pinus taeda* L.). *Genetics* **2010**, *186*, 677–686.
18. Dangl, J.L.; Horvath, D.M.; Staskawicz, B.J. Pivoting the Plant Immune System from Dissection to Deployment. *Science* **2013**, *341*, 746–751.
19. Kovalchuk, A.; Keriö, S.; Oghenekaro, A.O.; Jaber, E.; Raffaello, T.; Asiegbu, F.O. Antimicrobial Defenses and Resistance in Forest Trees: Challenges and Perspectives in a Genomic Era. *Ann. Rev. Phytopathol.* **2013**, *51*, 221–244.
20. Hoff, R.J.; Bingham, R.T.; McDonald, G.I. Relative Blister Rust Resistance of White Pines. *Eur. J. For. Pathol.* **1980**, *10*, 307–316.
21. Sniezko, R.A.; Kegley, A.J.; Danchok, R. White Pine Blister Rust Resistance in North American, Asian, and European species—Results from Artificial Inoculation Trials in Oregon. *Ann. For. Res.* **2008**, *51*, 53–66. Available online: http://www.editurasilvica.ro/afr/51/1/sniezko.pdf (accessed on 14 December 2013).
22. La, Y.-J. Korean Successes in Controlling Blister Rust of Korean Pine. In Proceedings of Breeding and Genetic Resources of Five-Needle Pines Conference, 22–26 September 2008; Noshad, D., Noh, E., King, J., Sniezko, R., Eds.; Yangyang, Korea Forest Research Institute: Seoul, South Korea, 2009; pp. 1–9.
23. Zhang, X.Y.; Lu, Q.; Sniezko, R.A.; Song, R.Q.; Man, G. Blister Rusts in China: Hosts, Pathogens, and Management. *For. Pathol.* **2010**, *40*, 369–381.
24. Vergara, R.; White, T.L.; Huber, D.A.; Shiver, B.D.; Rockwood, D.L. Estimated Realized Gains for First-generation Slash Pine (*Pinus elliottii* var. *elliottii*) Tree Improvement in the Southeastern United States. *Can. J. For. Res.* **2004**, *34*, 2587–2600.
25. Vergara, R.; White, T.L.; Huber, D.A.; Schmidt, R.A. Realized Genetic Gains of Rust Resistant Selections of Slash Pine (*Pinus elliottii* var. *elliottii*) Planted in High Rust Hazard Sites. *Silvae Genetica* **2007**, *56*, 5.
26. McKeand, S.E.; Li, B.; Amerson, H.V. Genetic Variation in Fusiform Rust Resistance in Loblolly Pine across a Wide Geographic Range. *Silvae Genetica*. **1999**, *48*, 255–260.
27. Barber, J.C.; Dorman, K.W.; Bauer, E. *Slash Pine Progeny Tests Indicate Genetic Variation in Resistance to Rust*, Research Note 104; US Department of Agriculture, Forest Service, Southeastern Forest Experiment Station: Asheville, NC, USA, 1957; p. 2.
28. Henry, B.W.; Bercaw, T.E. Shortleaf-Loblolly Hybrid Pines Free of Fusiform Rust after 5 Years' Exposure. *J. For.* **1956**, *54*, 779.

29. Jewell, F.F. Inoculation of Slash Pine Seedlings with *Cronartium fusiforme*. *Phytopathology* **1960**, *50*, 48–51.
30. Cowling, E.; Young, C. Narrative History of the Resistance Screening Center: Its Origins, Leadership and Partial List of Public Benefits and Scientific Contributions. *Forests* **2013**, *4*, 666–692.
31. Sniezko, R.A.; Mahalovich, M.F.; Schoettle, A.W.; Vogler, D.R. Past and Current Investigations of the Genetic Resistance to Cronartium ribicola in High-elevation Five-needle Pines. In The Future of High-elevation, Five-needle White Pines in Western North America, Proceedings of the High Five Symposium, RMRS-P-63; Missoula, MT, USA, 28–30 June 2010; Keane, R.E., Tomback, D.F, Murray, M.P., Smith, C.M., Eds.; US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2011; pp. 246–264. Available online: http://www.fs.fed.us/rm/pubs/rmrs_p063/rmrs_p063_246_264.pdf (accessed on 14 December 2013).
32. Kinloch, B.B.; Littlefield, J.L. White Pine Blister Rust: Hypersensitive Resistance in Sugar Pine. *Can. J. Bot*. **1977**, *55*, 1148–1155.
33. Kinloch, B.B., Jr.; Sniezko, R.A.; Barns, G.D.; Greathouse, T.E. A Major Gene for Resistance to White Pine Blister Rust in Western White Pine from the Western Cascade Range. *Phytopathology* **1999**, *89*, 861–867.
34. McDonald, G.I.; Hoff, R.J. Resistance to *Cronartium ribicola* in *Pinus monticola*: Early Shedding of Infected Needles. USDA For. Serv. Res. Note INT-124; US Department of Agriculture, Forest Service: Ogden, UT, USA, 1970; p. 8.
35. Hunt, R.S.; Roke, G.; Jensen, G.D. Initiation of Stem Infection in Western White Pine by Blister Rust *Cronartium ribicola*. *Can. J. Plant Pathol.* **2007**, *29*, 18–24.
36. Kolpak, S.E.; Sniezko, R.A.; Kegley, A.J. Rust Infection and Survival of 49 *Pinus monticola* Families at a Field Site Six Years after Planting. *Ann. For. Res*. **2008**, *51*, 67–80.
37. Kinloch, B.B., Jr.; Parks, G.O.; Fowler, C.W. White Pine Blister Rust: Simply Inherited Resistance in Sugar Pine. *Science* **1970**, *167*, 193–195.
38. Sweeney, K.; Stone, J.; Cook, K.; Sniezko, R.; Kegley, A.; Schoettle, A. Are Needle Reactions in Resistance to *Cronartium ribicola* a Hypersensitivity Response? In *Gen. Tech. Rep. PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, OR, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 368–371. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_368.pdf (accessed on 14 December 2013).
39. Sniezko, R.A. US Department of Agriculture, Forest Service. Personal communication, 2013.
40. Jurgens, J.A.; Blanchette, R.A. Histology of White Pine Blister Rust in Needles of Resistant and Susceptible Eastern White Pine. *Plant Dis.* **2003**, *87*, 1026–1030.
41. Smith, J.A.; Blanchette, R.A.; Burnes, T.A.; Jacobs, J.J.; Higgins, L.; Witthuhn, B.A.; David, A.J.; Gillman, J.H. Proteomic Comparison of Needles from Blister Rust-resistant and Susceptible *Pinus strobus* Seedlings Reveals up Regulation of Putative Disease Resistance Proteins. *Mol. Plant-Microbe Interact* **2006**, *19*, 150–160.

42. Patton, R.F.; Riker, A.J. Lessons from Nursery and Field Testing of Eastern White Pine Selections and Progenies for Resistance to Blister Rust. In *Breeding Pest-Resistant Trees*; Gerhold, H.D., McDermott, R.E., Schreiner, E.J., Winieski, J.A., Eds.; Pergamon Press: London, UK, 1966; pp. 403–414.
43. Smith, J.A. Host-Pathogen Interactions in Rust Disease Pathosystems. Ph.D. Thesis, University of Minnesota, St. Paul, MN, USA, 2005; p. 108.
44. Smith, J.A.; Blanchette, R.A.; Burnes, T.A.; Gillman, J.H.; David, A.J. Epicuticular Wax and White Pine Blister Rust Resistance in Resistant and Susceptible Selections of Eastern White Pine (*Pinus strobus*). *Phytopathology* **2006**, *96*, 171–177.
45. Hoff, R.J.; McDonald, G.I. *Improving Rust-Resistant Strains of Inland Western White Pine*, Res. Pap. INT-245; US Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station: Ogden, UT, USA, 1980; p. 13.
46. Kinloch, B.B., Jr. Resistance to White Pine Blister Rust in Sugar Pine: Research at the Pacific Southwest Forest and Range Experiment Station. In Proceedings of the Servicewide Genetics Workshop: Breeding Insect and Disease Resistant Trees, Eugene, OR, USA, 19–23 July 1982; US Department of Agriculture, Forest Service: Berkeley, CA, USA, 1982; pp. 86–89.
47. Sniezko, R.A.; Kegley, A. Blister Rust Resistance Experiences in Oregon and Washington: Evolving Perspectives. In Proceedings of the 50th Western International Forest Disease Work Conference, Powell River, BC, Canada, 7–11 October 2002; Stone, J., Maffei, H., Eds.; US Department of Agriculture, Forest Service, Central Oregon Service Center: Bend, OR, USA, 2003; pp. 111–117. Available online: http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5280665.pdf (accessed on 15 January 2012).
48. Sniezko, R.A.; Kegley, A.J. Blister Rust Resistance of Five-needle Pines in Oregon and Washington. In Proceedings of the Second IUFRO Rusts of Forest Trees Working Party Conference, Yangling, China, 19–23 August 2002. Available online: http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5280720.pdf (accessed on 14 December 2013).
49. Kinloch, B.B., Jr.; Davis, D.A.; Burton, D. Resistance and Virulence Interactions between Two White Pine Species and Blister Rust in a 30-Year Field Trial. *Tree Genet Genomes* **2008**, *4*, 65–74.
50. Sniezko, R.A.; Bower, A.; Kegley, A. Variation in *Cronartium ribicola* Field Resistance among 13 *Pinus monticola* and 12 *P. lambertiana* Families: Early Results from Happy Camp. In *Breeding and Genetic Resources of Five-Needle Pines: Growth, Adaptability and Pest Resistance*, Proceedings of IUFRO Working Party 2.02.15 Conference, Medford, OR, USA, 23–27 July 2001; Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., Eds.; RMRS-P-32; US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; pp. 203–208. Available online: http//www.fs.fed.us/rm/pubs/rmrs_p032/rmrs_p032_203_208.pdf (accessed on 14 December 2013).

51. Kinloch, B.B., Jr.; Burton, D.; Davis, D.A.; Westfall, R.D.; Dunlap, J.; Vogler, D. Strong Partial Resistance to White Pine Blister Rust in Sugar Pine. In *Gen Tech Rep PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, OR, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 80–91. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_080.pdf (accessed on 15 December 2013).
52. Kegley, A.; Sniezko, R.A.; Danchok, R.; Savin, D.P. Blister Rust Resistance among 19 Families of Whitebark Pine, *Pinus albicaulis*, from Oregon and Washington: Early Results from an Artificial Inoculation Trial. In *Gen. Tech. Rep. PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, Oregon, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 311–315. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_311.pdf (accessed on 15 December 2013).
53. Sniezko, R.A.; Kegley, A.J.; Danchok, R.S.; Long, S. Variation in Resistance to White Pine Blister Rust among 43 Whitebark Pine Families from Oregon and Washington—Early Results and Implications for Conservation. In Proceedings of the Conference Whitebark Pine: A Pacific Coast Perspective, Ashland, OR, USA, 27–31 August 2006; Goheen, E.M., Sniezko, R.A., Eds.; US Department of Agriculture, Forest Service, Pacific Northwest Region: Portland, OR, USA, 2007; pp. 82–97. Available online: http://www.fs.fed.us/outernet/r6/nr/fid/wbpine/papers/2007-wbp-poster-kegley-a.pdf (accessed on 15 December 2013).
54. Sniezko, R.A.; Kegley, A.; Danchok, R. White Pine Blister Rust Resistance in *Pinus monticola* and *P. albicaulis* in the Pacific Northwest USA—A Tale of Two Species. In *Gen. Tech. Rep. PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, OR, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 262–266. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_262.pdf (accessed on 14 December 2013).
55. Schmidtling, R.C. Coevolution of Host/Pathogen/Alternate Host Systems in Fusiform Rust of Loblolly and Slash Pines. In *Rusts of Hard Pines*, Proceedings of IUFRO Workshop Party Conference, Athens, GA, USA, 16–21 September 1984; Barrows-Broaddus, J., Powers, H.R., Jr., Eds.; University of Georgia: Athens, GA, USA, 1985; pp. 13–20.
56. Powers, H.R., Jr.; Matthews, F.R. Comparison of Six Geographic Sources of Loblolly Pine for Fusiform Rust Resistance. *Phytopathology* **1980**, *70*, 141–143.
57. Miller, T.; Cowling, E.B. Infection and Colonization of Different Organs of Slash Pine Seedlings by *Cronartium fusiforme*. *Phytopathology* **1977**, *67*, 179–186.

58. Jewell, F.F.; Speirs, D.C. Histopathology of One and Two Year Old Resisted Infections by *Cronartium fusiforme* in Slash Pine. *Phytopathology* **1976**, *66*, 741–748.
59. Schmidt, R.A. Fusiform Rust of Southern Pines: A Major Success for Forest Disease Management. *Phytopathology* **2003**, *93*, 1048–1051.
60. Gramacho, K.P.; Schmidt, R.A.; Miller, T. Stability of Slash Pine Families with Major Gene and Partial Resistance to Single Gall and Mixed Gall Inocula of *Cronartium quercum* f. sp. *fusiforme* in Greenhouse Studies. *Forests* **2013**, *4*, 488–499.
61. Amerson, H.V.; Jordan, A.P.; Kuhlman, E.G.; O'Malley, D.M.; Sederoff, R.R. Genetic Basis of Fusiform Rust Disease Resistance in Loblolly Pine. In Proceedings of the 24th Southern Forest Tree Improvement Conference, Orlando, FL, USA, 9–12 June 1997; p. 403.
62. Echt, C.S.; Saha, S.; Krutovsky, K.V.; Wimalanathan, K.; Erpelding, J.E.; Liang, C.; Nelson, D. An Annotated Genetic Map of Loblolly Pine Based on Microsatellite and cDNA Markers. *BMC Genetics* **2011**, *12*, 17.
63. Amerson, H.V.; Nelson, C.D.; Kubisiak, T.L.; Kuhlman, E.G.; Garcia, S.A. North Carolina State University, Raleigh, NC, USA and USDA Forest Service, Southern Research Station, Saucier, MS, USA, *Forests* **2014**, submitted for publication.
64. Isik, F.; Amerson, H.V.; Whetten, R.S.; Garcia, S.A.; McKeand, S.E. Interactions of Fr Genes and Mixed-pathogen Inocula in the Loblolly Pine-fusiform Rust Pathosystem. *Tree Genet. Genomes* **2012**, *8*, 15–25.
65. Jordan, A.P. Fusiform Rust Disease Resistance and Genomic Mapping in Loblolly Pine. Master's Thesis, North Carolina State University, Raleigh, NC, USA, 1997; p. 105.
66. Goddard, R.E.; Schmidt, R.A. Early Identification of Fusiform Rust Resistant Slash Pine Families through Controlled Inoculation. In Proceedings of the 11th Southern Forest Tree Improvement Conference, Atlanta, GA, USA, 15–16 June 1971; pp. 31–36.
67. Schmidt, R.A.; Powers, H.R., Jr.; Snow, G.A. Application of Genetic Disease Resistance for the Control of Fusiform Rust in Intensively Managed Southern Pine. *Phytopathology* **1981**, *31*, 993–997.
68. Schmidt, R.A.; Gramacho, K.P.; Miller, T.; Young, C.H. Components of Partial Resistance in the Slash Pine-fusiform Rust Pathosystem. *Phytopathology* **2000**, *90*, 1005–1010.
69. Kubisiak, T.L.; Anderson, C.L.; Amerson, H.V.; Smith, J.A.; Davis, J.M.; Nelson, C.D. A Genomic Map Enriched for Markers Linked to *Avr1* in *Cronartium. quercuum* f.sp. *fusiforme*. *Fungal Genet. Biol.* **2011**, *48*, 266–274.
70. Sniezko, R.A.; Kinloch, B.B; Bower, A.D.; Danchok, R.S.; Linn, J.M.; Kegley, A.J. Field Resistance to *Cronartium ribicola* in Full-sib Families of *Pinus monticola* in Oregon. In *Breeding and Genetic Resources of Five-Needle Pines: Growth, Adaptability and Pest Resistance*, Proceedings of IUFRO Working Party 2.02.15 Conference, Medford, OR, USA, 23–27 July 2001; Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., Eds.; US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; pp. 243–249. Available online: http://www.fs.fed.us/rm/pubs/rmrs_p032/rmrs_p032_243_249.pdf (accessed on 14 December 2013).

71. Sniezko, R.A.; Danchok, R.; Hamlin, J.; Kegley, A.; Long, S.; Mayo, J. White Pine Blister Rust Resistance of 12 Western White Pine Families at Three Field Sites in the Pacific Northwest. In *Gen. Tech. Rep. PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, OR, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T, Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 356–367. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_356.pdf (accessed on 14 December 2013).
72. Kinloch, B.B., Jr.; Sniezko, R.A.; Dupper, G.E. Virulence Gene Distribution and Dynamics of the White Pine Blister Rust Pathogen in Western North America. *Phytopathology* **2004**, *94*, 751–758.
73. Hunt, R.S.; Jensen, G.D.; Ekramoddoullah, A.K.M. Confirmation of Dominant Genetic Resistance (*Cr2*) in U.S. White Pine Selections to White Pine Blister Rust Growing in British Columbia. In *Breeding and Genetic resoUrces of Five-Needle Pines: Growth, Adaptability and Pest Resistance*, Proceedings of IUFRO Working Party 2.02.15 Conference, Medford, OR, USA, 23–27 July 2001, Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., Eds.; RMRS-P-32; US Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; pp. 227–229.
74. Isik, F.; Amerson, H.V.; Whetten, R.W.; Garcia, S.A.; Li, B.; McKeand, S.E. Resistance Assessments of Elite Loblolly Pine Families to Fusiform Rust Inocula in Greenhouse Testing. *Can. J. For. Res.* **2008**, *38*, 2687–2696.
75. Kubisiak, T.L.; Roberds, J.H.; Spaine, P.C.; Doudrick, R.L. Microsatellite DNA Suggests Regional Structure in the Fusiform Rust Fungus *Cronartium quercuum* f. sp *fusiforme*. *Heredity* **2004**, *92*, 41–50.
76. Pye, J.M.; Wagner, J.E.; Holmes, T.P.; Cubbage, F.W. *Positive Returns from Investment in Fusiform Rust Research*; US Department of Agriculture, Forest Service, Southern Research Station: Asheville, NC, USA, 1997; p. 55.
77. Schmidt, R.A.; Holley, R.C.; Klapproth, M.C. Results from Operational Plantings of Fusiform Rust Resistant Slash Loblolly Pines in High Rust Incidence Areas in Florida and Georgia. In *Rusts of Hard Pines*, Proceedings of IUFRO Workshop Party Conference, Athens, GA, USA, 16–21 September 1984; Barrows-Broaddus, J., Powers, H.R., Jr., Eds.; University of Georgia: Athens, GA, USA, 1985; pp. 33–41.
78. Schmidt, R.A.; Holley, R.C.; Klapproth, M.C.; Miller, T. Temporal and Spatial Patterns of Fusiform Rust Epidemics in Young Plantations of Susceptible and Resistant Slash and Loblolly Pines. *Plant Dis.* **1986**, *70*, 661–666.
79. Schmidt, R.A.; Allen, J.E. Spatial Stability of Fusiform Rust Resistance in Slash Pine in the Coastal Plain of the Southeastern USA. In Proceedings of the First IUFRO Rusts of Forest Trees Working Party Conference, Saariselka, Finland, 2–7 August 1998; Jalkanen, O., Crane, R.P., Walla, J., Aalto, T., Eds.; Finnish Forest Research Institute: Rovaniemi, Finland, 1998; pp. 219–229.

80. Lu, P.; Derbowka, D. Breeding Eastern White Pine for Blister Rust Resistance: A Review of Progress in Ontario. *For. Chronicle* **2009**, *85*, 745–755.
81. Zsuffa, L. Poplar, White Pine, and Ornamental Tree Breeding at Ontario Forest Research Centre, Maple, in 1977 and 1978. In Proceedings 17th Canadian Tree Improvement. Association. Meeting, 27–30 August 1979, Gander, Newfoundland; Yeatman, Ed.; Canadian Forestry Service: Ottawa, Canada, 1979; pp. 133–138.
82. Sinclair, B. Screening White Pine for Blister Rust Resistance in Ontario: History and Status. In *White Pine Blister Rust Workshop*; Canadian Forestry Service: Ottawa, ON, Canada. 2003; pp. 24–27.
83. Joint Genome Institute. Available online: http://genome.jgi.doe.gov/Croqu1/Croqu1.home.html (accessed on 13 January 2014).
84. Hamelin, R.C. University of British Columbia. Personal communication, 2013.
85. Devey, M.E.; Mix, A.D.; Kinloch, B.B., Jr.; Neale, D.B. Random Amplified Polymorphic DNA Markers Tightly Linked to a Gene for Resistance to White Pine Blister Rust in Sugar Pine. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2066–2070.
86. Myburg, H.; Morse, A.M.; Amerson, H.V.; Kubisiak, T.L.; Huber, D.; Osborne, J.A.; Garcia, S.A.; Nelson, C.D.; Davis, J.M.; Covert, S.F.; *et al*. Differential Gene Expression in Loblolly Pine (*Pinus taeda* L.) Challenged with the Fusiform Rust Fungus, *Cronartium quercuum* f.sp. fusiforme. *Physiol. Mol. Plant Pathol.* **2006**, *68*, 79–91.
87. Liu, J.-J.; Ekramoddoullah, A.K.M.; Hunt, R.S.; Zamani, A. Identification and Characterization of RAPD Markers Tightly Linked to a Major Gene (*Cr2*) for Resistance to *Cronartium ribicola* (Fish.) in *Pinus monticola* (D. Don). *Phytopathology* **2006**, *96*, 395–399.
88. Liu, J.-J.; Sturrock, R.N.; Benton, R. Transcriptome Analysis of *Pinus monticola* Primary Needles by RNA-seq Provides Novel Insight into Host Resistance to *Cronartium ribicola*. *BMC Genomics* **2013**, *14*, 884.
89. Danchok, R.S.; Sniezko, R.A.; Long, S.; Kegley, A.; Savin, D.; Mayo, J.B.; Liu, J.-J.; Hill, J. Frequency of Hypersensitive-like Reaction and Stem Infections in a Large Full-sib Family of *Pinus monticola*. In *Gen Tech Rep PSW-GTR-240*, Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees, Eugene, OR, USA, 31 July–5 August 2011; Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., Eds.; US Department of Agriculture, Forest Service, Pacific Southwest Research Station: Albany, CA, USA, 2012; pp. 281–285. Available online: http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_281.pdf (accessed on 14 December 2013).
90. Liu, J.-J.; Williams, H.; Zamany, A.; Sturrock, R.N.; Sniezko, R.A.; Kegley, A. Discovery and Application of Single Nucleotide Polymorphisms (SNP) of Disease Resistance-related Genes for Selection of *Pinus monticola* with Genetic Resistance against *Cronartium ribicola*. *Can. J. Plant Pathol.* **2014**, in press.
91. Jermstad, K.D.; Eckert, A.J.; Wegrzyn, J.L.; Delfino-Mix, A.; Davis, D.A.; Burton, D.C.; Neale, D.B. Comparative Mapping in Pinus: Sugar Pine (*Pinus lambertiana* Dougl.) and loblolly pine (*Pinus taeda* L.). *Tree Genet Genom* **2011**, *7*, 457–468.

92. Conifer Genome Network. Available online: http://pinegenome.org/pinerefseq/ (accessed on 14 December 2013).
93. Eckert, A.J.; Pande, B.; Ersoz, E.S.; Wright, M.H.; Rashbrook, V.K.; Nicolet, C.M.; Neale, D.B. High-Throughput Genotyping and Mapping of Single Nucleotide Polymorphisms in Loblolly Pine (*Pinus taeda* L.). *Tree Genet Genom* **2009**, *5*, 225–234.
94. Jones, J.D.; Dangl, J.L. The Plant Immune System. *Nature* **2006**, *444*, 323–329.
95. Nelson, C.D.; Kubisiak, T.L.; Amerson, H.V. Unraveling and Managing Fusiform Rust Disease: A Model Approach for Coevolved Forest Tree Pathosystems. *For. Pathol.* **2010**, *40*, 67–72.
96. Zamany, A.; Liu, J.-J.; Ekramoddoullah, A.K. Comparative Proteomic Profiles of *Pinus monticola* Needles During Early Compatible and Incompatible Interactions with *Cronartium ribicola*. *Planta* **2012**, *236*, 1725–1746.
97. Lorenz, W.W.; Neale, D.B.; Jermstad, K.D.; Howe, G.T.; Rogers, D.L.; Bordeaux, J.M.; Ayyampalayam, S.; Dean, J.F.D. Conifer DBMagic: A Database Housing Multiple *de novo* Transcriptome Assemblies for 12 Diverse Conifer Species. *Tree Genet Genom* **2012**, *8*, 1477–1485.
98. Kinloch, B.B., Jr. White Pine Blister Rust in North America: Past and Prognosis. *Phytopathology* **2003**, *93*, 1044–1047.
99. Jacobs, J.J.; Burnes, T.A.; David, A.J.; Blanchette, R.A. Histopathology of Primary Needles and Mortality Associated with White Pine Blister Rust in Resistant and Susceptible *Pinus strobus*. *For. Pathol.***2009**, *39*, 361–376.
100. Richardson, B.A.; Ekramoddoulah, A.K.; Liu, J.-J.; Kim, M.-S.; Klopfenstein, N.B. Current and Future Molecular Approaches to Investigate the White Pine Blister Rust Pathosystem. *For. Pathol.* **2010**, *40*, 314–331.
101. Liu, J.-J.; Ekramoddoullah, A.K.M.; Zamani, A. A class IV Chitinase is Up-regulated upon Fungal Infection and Abiotic Stresses and Associated with Slow-canker-growth Resistance to *Cronartium ribicola* in Western White Pine (*Pinus monticola*, Dougl. ex D. Don). *Phytopathology* **2005**, *95*, 284–291.
102. Zamany, A.; Liu, J.-J.; Ekramoddoullah, A.K.; Sniezko, R. Antifungal Activity of a *Pinus monticola* Antimicrobial Peptide1 (PmAMP1) and its Accumulation in Western White Pine Infected with *Cronartium ribicola*. *Can. J. Microbiol.* **2011**, *57*, 667–679.
103. Liu, J.-J.; Sniezko, R.A.; Ekramoddoullah, A.K.M. Association of a Novel *Pinus monticola* Chitinase Gene (*PmCh4B*) with Quantitative Resistance to *Cronartium ribicola*. *Phytopathol.* **2011**, *101*, 904–911.
104. Liu, J.-J.; Hammet, C.; Sniezko, R.A. *Pinus monticola* Pathogenesis-related Gene *PmPR10-2* Alleles as Defense Candidates for Stem Quantitative Disease Resistance against White Pine Blister Rust (*Cronartium ribicola*). *Tree Genet Genom* **2013**, *9*, 397–408.
105. Liu, J.-J.; Zamany, A.; Sniezko, R.A. Anti-Microbial Peptide (AMP): Nucleotide Variation, Expression, and Association with Resistance in the White Pine-blister Rust Pathosystem. *Planta* **2013**, *237*, 43–54.

106. Verma, S.S.; Yajima, W.R.; Rahman, M.H.; Shah, S.; Liu, J.-J.; Ekramoddoullah, A.K.M.; Kav, N.N.V. A Cysteine-rich Antimicrobial Peptide from *Pinus monticola* (PmAMP1) Confers Resistance to Multiple Fungal Pathogens in Canola (*Brassica napus*). *Plant Mol. Biol.* **2012**, *79*, 61–74.
107. Resende, M.F.R., Jr.; Muñoz, P.; Resende, M.D.V.; Garrick, D.J.; Fernando, R.L.; Davis, J.M.; Jokela, E.J.; Martin, T.A.; Peter, G.F.; Kirst, M. Accuracy of Genomic Selection Methods in a Standard Data set of Loblolly Pine (*Pinus taeda* L.). *Genetics* **2012**, *190,* 1503–1510.
108. Et-touil, K.; Bernier, L.; Beaulieu, J.; Berube, J.A.; Hopkin, A.; Hamelin, R.C. Genetic Structure of *Cronartium ribicola* Populations in Eastern Canada. *Phytopathology* **1999**, *89*, 915–919.
109. Hamelin, R.C.; Beaulieu, J.; Plourde, A. Genetic Diversity in Populations of *Cronartium ribicola* in Plantations and Natural Stands of *Pinus strobus*. *Theor. Appl. Genet.* **1995**, *91*, 1214–1221.
110. Hamelin, R.C.; Doudrick, R.L.; Nance, W.L. Genetic Diversity in *Cronartium quercuum* f.sp. *fusiforme* on Loblolly Pines in Southern U.S. *Curr. Genet.* **1994**, *26*, 359–363.
111. Hamelin, R.C. Genetic Diversity between and within Cankers of the White Pine Blister Rust. *Phytopathology* **1996**, *86*, 875–879.
112. Hamelin, R.C.; Dusabenyagasani, M.; Et-touil, K. Fine-Level Genetic Structure of White Pine Blister Rust Populations. *Phytopathology* **1998**, *88*, 1187–1191.
113. Doudrick, R.L.; Nance, W.L.; Nelson, C.D.; Snow, G.A.; Hamelin, R.C. Detection of DNA Polymorphisms in a Single Urediniospore-derived Culture of *Cronartium quercuum* f.sp. fusiforme. *Phytopathology* **1993**, *83*, 388–392.
114. Hamelin, R.C.; Allaire, M.; Bergeron, M.J.; Nicole, M.C.; Lecours, N. Molecular Epidemiology of White Pine Blister Rust: Recombination and Spatial Distribution. *Phytopathology* **2005**, *95*, 793–799.
115. Kinloch, B.B.; Westfall, R.D.; White, E.E.; Gitzendanner, M.A.; Dupper, G.E.; Foord, B.M.; Hodgskiss, P.D. Genetics of *Cronartium ribicola*. IV. Population Structure in Western North America. *Can. J. Bot.* **1998**, *76*, 91–98.
116. Hamelin, R.C.; Hunt, R.S.; Geils, B.W.; Jensen, G.D.; Jacobi, V.; Lecours, N. Barrier to Gene flow between Eastern and Western Populations of *Cronartium ribicola* in North America. *Phytopathology* **2000**, *90*, 1073–1078.
117. Tuskan, G.; Difazio, S.; Jansson, S.; Bohlmann, J.; Grigoriev, I.; Hellsten, U.; Putnam, N.; Ralph, S.; Rombauts, S.; Salamov, A. The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **2006**, *313*, 1596–1604.
118. Duplessis, S.; Cuomo, C.A.; Lin, Y.-C.; Aerts, A.; Tisserant, E.; Veneault-Fourrey, C.; Joly, D.L.; Hacquard, S.; Amselem, J.; Cantarel, B.L.; *et al*. Obligate Biotrophy Features Unraveled by the Genomic Analysis of Rust Fungi. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9166–9171.
119. Hogenhout, S.A.; van Der Hoorn, R.A.L.; Terauchi, R.; Kamoun, S. Emerging Concepts in Effector Biology of Plant-associated Organisms. *Mol. Plant Microbe Interact* **2009**, *22*, 115–122.
120. Joly, D.L.; Feau, N.; Tanguay, P.; Hamelin, R.C. Comparative Analysis of Secreted Protein Evolution using Expressed Sequence Tags from Four Poplar Leaf Rusts (*Melampsora* spp.). *BMC Genomics* **2010**, *11*, 422.

121. Kearns, H.S.J.; Ferguson, B.A.; Schwandt, J.W. *Performance of Rust-resistant Western White Pine in Operational Plantations in Northern Idaho: 1995–2006*; Forest Health Protection Report 12-03; US Forest Service, Northern Region: Missolua, MT, USA, 2012; p. 27. Available online: http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5351277.pdf (accessed on 13 December 2013).