Strong Partial Resistance to White Pine Blister Rust in Sugar Pine

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Abstract

Quantitative resistance to white pine blister rust in 128 controlled- and open-pollinated sugar pine families was evaluated in a "disease garden", where alternate host *Ribes* bushes were interplanted among test progenies. Overall infection was severe (88%), but with great variation among and within families: a 30-fold range in numbers of infections per tree, and a 10- fold range in trees with completely inactive, or aborted infections. Inheritance of these partial resistances was strong, with significant amounts of both additive and non-additive genetic variance evident. They will be most useful in combination with major gene resistance (MGR) by reducing the probability of infections virulent to MGR.

Key words: *Cronartium ribicola*, *Pinus lambertiana*, partial resistance

Introduction

Sugar pine (*Pinus lambertiana* Dougl.), one of the most susceptible of white pines to white pine blister rust (*Cronartium ribicola*), has a major gene (*Cr1*) that confers virtual immunity to the disease, but is vulnerable to specific virulence from a complementary gene (*vcr1*) in the pathogen. Partial resistance (PR; also known as slow rusting, SR) is a suite of traits that reduces the amount and rate of infection through lowered receptivity to infection and incompatibility reactions in the host when infections do occur. Partial resistance has been shown to be effective and durable to blister rust in white pines (King et al. 2010, Kinloch et al. 2008). Combining the two types of resistance in synthetic lines could be mutually reinforcing by sanitizing all inoculum lacking *vcr1* with *Cr1* and by reducing the probability of infection by *vcr1* with PR.

Methods

To assess the degree and inheritance of PR, 128 sugar pine progenies with different pedigrees were exposed to the pathogen in a plantation at Happy Camp (HC) in northern California. Happy Camp has been used since 1958 as the principal site for breeding, testing, and evaluating sugar pine and other white pines by the Genetic Resources Unit of the Pacific Southwest Region, USDA Forest Service, with the objective of developing durable resistance to blister rust. Here, resistant parent selections were grafted in a clone bank and interbred when reproductively mature, and different cohorts of test progenies established every year. Seedlings are naturally challenged by blister rust inoculum (enhanced by interplanting alternate host *Ribes* sp. among pines in the test plots). Surviving seedlings are allowed to remain in place indefinitely, and many reach reproductive maturity. Although many parent trees were known to carry *Cr1*, this gene was effectively neutralized by the overwhelming prevalence of *vcr1* inoculum on the site, allowing other kinds of resistance to express.

Test progenies consisted of four main groups: 1) a full-sib and half-sib matrix of 75 families controlled-pollinated (CP) among select parents; 2) 14 families of the same select parents that were wind or open-pollinated (OP) by the ambient pollen cloud on the site (_xW(HC)); 3) 33 families from

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cone bearing parents of uncertain genotype that had survived the chronic epidemic at HC, also pollinated from pollen on site (?xW(HC); and 4) four multi-tree seed lots, each mixed and bulked from four different natural stands.

The full- and half-sib group was made from parents a) known from previous experience to transmit relatively high resistance (SR) or susceptibility (fast rusting, FR) to their offspring; b) F1s of some of the selects; and c) F1s of other trees on the site of unknown or uncertain resistance genotypes that had survived many years of the chronic epidemic. Controlled crosses were made among the 16 parents in this group in all available combinations, emphasizing known SR x SR, SR x FR, and FR x FR genotypes (see tables 1 and 2).A mixed general linear model (GLM) was used to account for repeated measurement of families. The SAS (v. 9.2) MIXED procedure was used to perform paired t-tests with the Satterthwaite adjusted degrees of freedom and the Bonferroni's level of significance adjustment.

All parents were SR phenotypes, by virtue of identified characteristics or survival under chronic high-hazard exposure to the disease. This included two that were known to produce highly susceptible (FR) offspring (i.e., were FR genotypes, though SR phenotypes). Because crowns damaged by rust rarely bear cones, adequate numbers of FR phenotypes are difficult to find.

We hoped to make enough crosses for a balanced partial diallel, but this goal was frustrated because of sugar pine's notoriously erratic reproductive behavior. Many crosses were not successful, resulting in a severely unbalanced mating design. The shortage of susceptible control genotypes was particularly conspicuous. Nevertheless, we were able to extract two factorials of unrelated seed and pollen parents from the matrix of crosses to make estimates of additive and non-additive variances.

We expected that F1 parents would generally transmit the greatest amount of SR to offspring, followed by SR parent (P1) selects. We also expected on-site pollen to have a major effect. Since this pollen derived overwhelmingly from both select SR and other parents surviving the chronic epidemic, we presumed it would carry strong resistance, especially if additive genetic variance is predominant. In effect, it would simulate pollen in a seed orchard of inter-pollinating selected parents, and allow estimates of realized heritability. Thus, we expected the open-pollinated progenies from the select SR parents would perform well; less than resistant selects, but probably somewhat better than the other 33 open-pollinated seed parents. We expected the FR crosses to depress expression of SR and we assumed the bulked seed lots would represent average susceptibility in the wild population.

Table 1—Mean number of infections per tree on sugar pine families

A) Controlled- and wind-pollinated families:

Parents:	Pollen																x W (HC)
Seed	K71	K86	K44	K23	K33	K72	K14	K36	K71 x K73	K86 x K44	K71 x K44	K70 x K43	K87 x K19	K36 x K73	K16 x K17	K36 x K17	
K71		2.2	1.5			1.6	3.3			1.0			2.1	2.8	2.5		2.0
K86	1.2		1.3	2.4	1.1	1.7		3.2	1.7		1.1		1.0	2.0	1.2		2.2
K44																	2.0
K23	2.7				2.1					1.0			2.6				3.3
K33	2.2	1.7							2.6	1.1		1.4					4.9
K72																	2.8
K14	3.6	3.3	4.1	5.9		6.1		11.8		3.1			5.4	10.2		10.2	7.2*
K36		2.7	3.9							2.8							6.3
K71 x K73	1.6		3.4			3.2		5.4									3.3
K70 x K43		1.4		1.4			2.7	3.6		0.4				2.8			2.2
K87 x K19			1.2					5.4	4.3	1.0							3.2
K36 x K73	3.8	3.8	3.3					8.3					4.2				6.1
K16 x K17		2.1		3.3	1.6	3.0		2.8	2.3	0.7	2.2	1.5	1.5	4.2			3.0
K36 x K17		2.8	2.8						5.5	1.7							4.5
Parent mean[1]	**2.3**	**2.0**	**2.7**	**2.7**	**1.7**	**3.1**	**5.8**	**5.0**	**3.3**	**1.4**	**1.6**	**1.9**	**2.7**	**4.5**	**2.2**	**4.6**	**3.5**

Half-sib mean: 3.0

Full-sib range: 0.4 - 11.8

Half-sib parent mean/W(HC) mean $r_p = 0.82$

x W (wild stand)

B) Group means:

Legend:		No. families in type	Seed	Pollen	Mean	Range		Rank Obs.	Rank Exp.
	SR select parent	12			**1.8**	1.1	2.7	**2**	5
	F_1 of SR select parent	9			**1.9**	1.0	3.4	**3**	2
	F_1 survivor	20			**2.3**	1.0	3.8	**5**	4
	FR select parent	9			**4.0**	2.7	6.1	**8**	8
		0			-	-	-	**-**	1
		8			**2.2**	0.4	5.5	**4**	3
		3			**3.7**	2.8	5.4	**7**	7
		5			**2.8**	1.5	4.2	**6**	6
		8			**6.1**	2.7	11.1	**9**	9
		1			**11.8**	-	-	**10**	10
		75			**4.06**			r_p =	**0.90**
		33	**?**	**x W (HC)**	**5.00**	2.1	10.5		
		4	**Bulked mix**	**x W (wild)**	**4.79**	4.2	5.6		

[1] includes both seed and pollen entries.

Table 2—Percent of sugar pine families with no active infections from blister rust

A) Controlled- and wind-pollinated families:

Parents:	Pollen																
Seed	K71	K86	K44	K23	K33	K72	K14	K36	K71 x K73	K86 x K44	K71 x K44	K70 x K43	K87 x K19	K36 x K73	K16 x K17	K36 x K17	x W (HC)
K71		41.4	54.7			35.7	17.8			73.0			44.4	30.7	28.0		23.6
K86	55.3		37.5	36.2	51.4	34.1		16.7	37.8		67.1		32.8	40.0	43.5		21.4
K44																	31.0
K23	27.0				15.8					52.1			20.6				7.7
K33	24.1	38.1							25.4	44.1		29.0					16.3
K72																	20.5
K14	26.8	16.2	9.6	8.2		6.9		0.0		23.5			12.9	0.7		1.4	6.56*
K36		20.6	13.5							41.5							14.3
K71 x K73	50.7		4.4			9.6		13.6									20.0
K70 x K43		36.6		34.0			10.5	22.0		73.2				23.5			22.4
K87 x K19			42.4					2.9	16.0	58.7							17.0
K36 x K73	20.3	12.9	17.8					6.1					8.7				7.0
K16 x K17		45.2		15.2	21.3	25.0		14.1	23.4	64.4	46.6	26.9	29.6	10.8			18.2
K36 x K17		20.3	18.1						20.0	30.8							5.1
Parent Mean[1]	**37.8**	**36.0**	**24.7**	**26.1**	**31.1**	**22.2**	**11.2**	**15.1**	**22.3**	**51.2**	**56.9**	**32.0**	**26.9**	**17.1**	**30.3**	**18.1**	**16.5**

Half-sib mean: 28.70

Full-sib range: 0 - 73

C-P parent mean:xW(HC) mean: r_p= 0.60

* x W (wild)

B) Group means:

Legend:		No. families in type	Seed	Pollen	Mean	Range	Rank	
							Obs.	Exp.
	SR select parent	12			**37.6**	16 - 55	**4**	5
	F_1 of SR select parent	9			**40.5**	4 - 73	**3**	2
	F_1 survivor	20			**28.9**	13 - 45	**5**	4
	FR select parent	9			**15.1**	8 - 27	**8**	8
		0			**-**	-	**-**	1
		8			**41.6**	16 - 46	**2**	3
		3			**26.2**	14 - 42	**6**	7
		5			**19.9**	9 - 30	**7**	6
		8			**8.8**	1 - 22	**9**	9
		1			**0.0**	-	**10**	10
		75			**24.3**		$r_{p=}$	**0.95**
		33	**?**	**x W (HC)**	**11.90**	2 - 25		
		4 bulk lots	**Bulked mix**	**x W (wild)**	**7.7**	3 - 11		

[1] includes both row and column entries

Progenies were established in 2006 with an average of 57 trees each in single-tree plots in 75 blocks. In all, 7,545 trees in 128 controlled- or open-pollinated families were planted and evaluated. Disease was assessed in 2010 and 2011, after overall infection was nearly complete on susceptible controls. Separate infections were counted on each seedling in 2010, usually in their incipient stages (fig. 1A), and determined to be either typical (normal) for the disease or reactive. Reactions were of two main kinds. The first, bark reactions (BRs), caused infections to abort or 'cork out' (Struckmeyer and Riker 1951), leaving a necrotic scar beneath and surrounding the infection site (fig. 1B). BRs ranged in size from "micro-" necrotic lesions surrounding the base of the infection court of the short shoot to lesions many cm long. The other reaction was a blighting (BL) of the infected shoot, from the point of infection distal to the end of the shoot (fig. 1C). Both reactions often occur on the same tree, and reactions of both types often expressed only partially: death and collapse of most diseased tissue, but with some activity remaining on the margins of the lesion (see Kinloch and Davis 1996 for further details). Trees with typical infections, or those with incomplete reactions, were classed as normal/susceptible; trees with BRs, BLs, or both were classed as reactive, but only if disease symptoms were in complete remission and the tree had no active infections (NAI).

Results

The epidemic started early, with 3 successive wave years from 2007 to 2009. By spring 2011, 88 percent of all trees were infected and 9 percent were dead from rust. Individual families ranged from 44 to 100 percent infected and 0 to 51 percent dead.

The most useful indexes of resistance were low receptivity to infection (LRI), measured by numbers of separate infections on individual trees, and the percent of trees in individual families with no active infections (percent NAI). Mean number of infections for all families (CP and OP) was 3.7 and ranged from 0.39 to 11.80. Within families, there was great variability among trees, many having no infections and ranging as high as 92. For percent NAI, the mean of all families was 22.1, and ranged from 0.0 to 81.1. Correlation between the two indexes was -0.71.

Tables 1A and 2A summarize data for numbers of infections and percent NAI, respectively, for the matrix of individual full-sib families of select parents; half-sib means of select parents; and select xW(HC) means. Part B of tables 1 and 2 compare families grouped according to parental generation (P1, F1) or parental genotype (SR, FR) with each other, and with the 33 families that were open-pollinated from the ambient pollen cloud at HC (?xW(HC)), and the four seed lots bulked from wild stands.

Figure 1—Reactions of sugar pine seedlings to infection by blister rust. A) Typical incipient infections, susceptible phenotype; note pink discolorations, swelling at base of short shoots; four separate infections are visible, but will soon coalesce. B) Micro bark reactions on slow rusting (SR) phenotype. About 10 are visible. C) Blight reactions on SR phenotype resulting from several separate infections.

For both indexes the general ranking of the four main group means, from most resistant to most susceptible, was: the CP matrix of full/half-sib select parents > OP select parents > OP non-select parent survivors > OP bulk seed lots from wild stands. None of OP family group means were significantly different from one another (figs. 2 and 3).

The greatest variation was among individual full-sib families, which ranged (resistant to susceptible) from 0.39 to 11.8 infections/tree and from 73.0 to 0.0 percent NAI. Half-sib family means from P1 and F1 selects ranged from 1.4 to 5.8 infections/tree, and from 56.9 to 11.2 percent NAI. The 14 families from open-pollination of the P1 and F1 parents ranged from 2.0 to 7.2 infections/tree, and 31.0 to 5.1 percent NAI. Correlations between these OP families and their CP counterparts were 0.82 for infections/tree and 0.60 for percent NAI.

The nine full-sib family groups generally ranked as expected, with correlations between observed and expected values of 0.90 and 0.95 for numbers of infections and percent NAI, respectively (tables 1B and 2B). Half-sib means varied greatly, with strong resistance from families with parents K86 and/or K44 in their pedigrees, and the greatest from the F1 (K86xK44). But this performance was not always consistent; for example, K44, as pollen parent, performed relatively poorly with another F1 select (K71xK73; table 2). None of the groups was statistically different from others, with the sole exception of the single FR x FR family (K14xK36), which was significantly more susceptible than all other groups (figs. 4 and 5). The negative influence of the FR parents was pervasive: groups with FR, taken as a whole (coded red, figs. 4 and 5), were significantly different from groups lacking FR (coded black, figs. 4 and 5).

Discussion

For a species so generally susceptible to an introduced disease, sugar pines show remarkable variability in resistance to blister rust: a 30-fold range in mean numbers of infections among full-sib families, and a ten-fold range in percent of trees with no active infections (tables 1 and 2). The modest correlation (-0.71) between the two indexes indicates that they can be selected for separately or simultaneously for development of stable resistance.

Although the expression and underlying causes are different, both mechanisms are highly effective, either in reducing the probability of infection occurring (LRI) or the probability of an infection becoming lethal (percent NAI). Both are strongly inherited; several of the best select parents in this test have shown consistent performance in several previous trials (Kinloch and Davis 1996, Kinloch et al. 2008). Still, modes of inheritance remain unclear. From the two factorials, we estimated additive effects to consist of 78 and 58 percent of the total genetic variance in LRI and percent NAI, respectively, but both estimates have large standard errors of the estimated means. In a related pathosystem, Isik et al. (2003) estimated that epistatic effects were nearly equal to additive effects for resistance to fusiform rust in loblolly pine, and we have observed that certain sugar pine parents (e.g., K71, K36) sometimes exhibit specific rather than general combining abilities to blister rust resistance (Kinloch and Davis 1996, Kinloch et al. 2008). Although most quantitative genetic traits are additive, partial resistance to disease can be controlled by specific interactions with pathogens (Krenz et al. 2008) or even gene-for-gene interactions (Antonovics et al. 2011).

The lack of a stronger effect of on-site pollen (_xW(HC)) on progeny performance was contrary to expectations, implying that pollen from the ambient cloud at HC does not carry particularly strong or abundant genes for SR that combine additively. Neither of the two open-pollinated groups, select xW(HC) or ? xW(HC), were significantly different from controls represented by the four bulk seed lots from natural stands or from each other (figs. 2 and 3). Seed parents of the ? xW(HC) group, on the other hand, showed wide variation in resistance (tables 1B and 2B). Taken together, the data suggests that both additive and non-additive effects are important in controlling resistance.

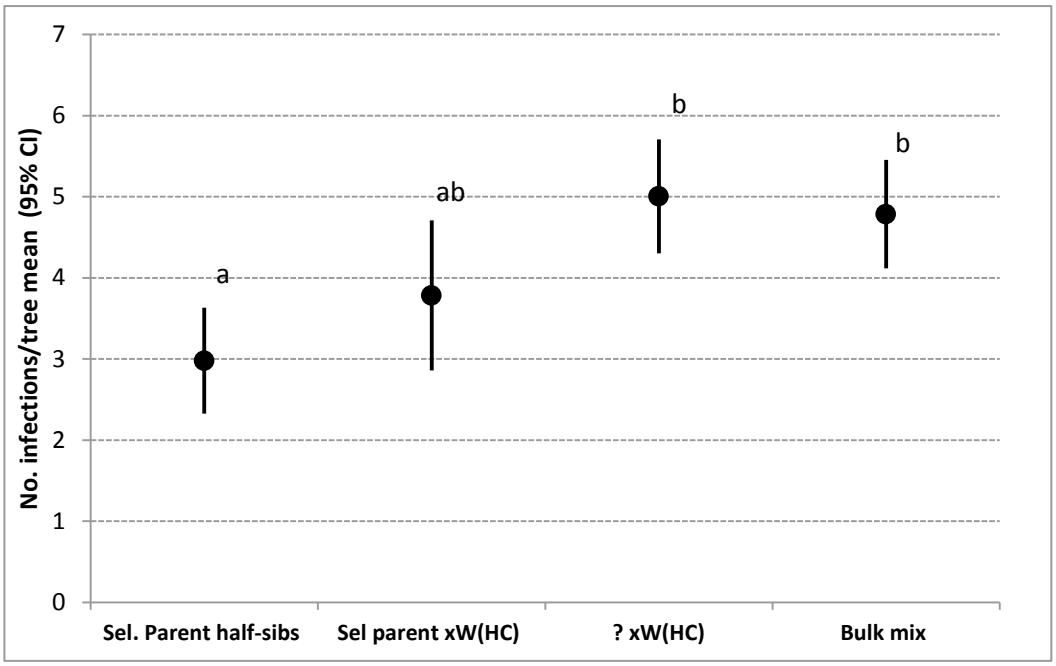

Figure 2—Means and 95 percent confidence intervals for numbers of infections/family in four main open-pollinated groups of sugar pine progenies. Groups with the same letter are not statistically different; the six pairwise comparisons were tested using a Bonferoni's adjusted-a=.05/60.0082to attain an experimentwise error rate a=0.05.

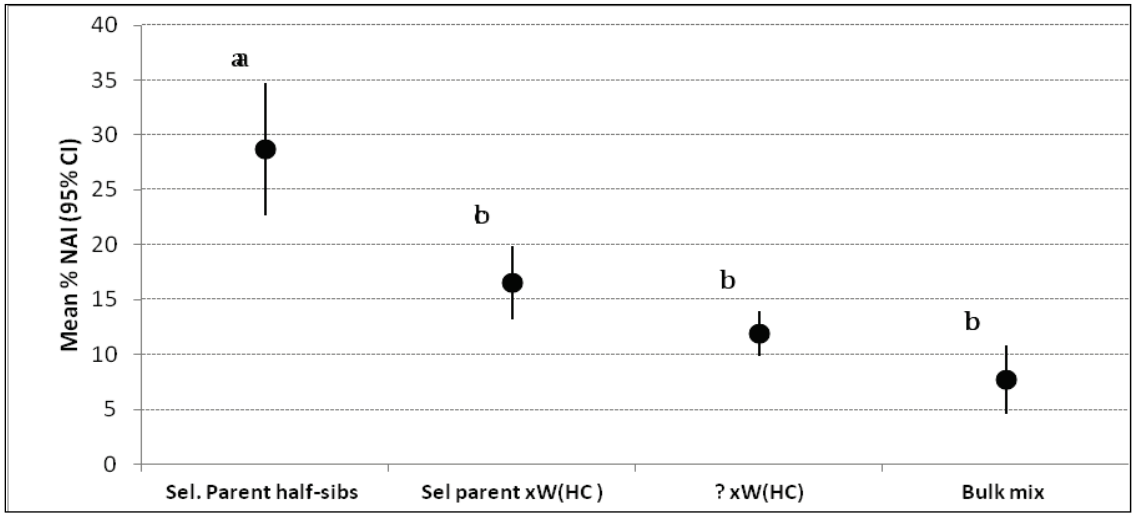

Figure 3—Means and 95 percent confidence intervals for percentage of trees with no active infections/family (percent NAI) in four main open-pollinated groups of sugar pine progenies. Groups with the same letter are not statistically different; the six pairwise comparisons were tested using a Bonferoni's adjusted-a=.05/6=0.0082 to attain an experimentwise error rate a=0.05.

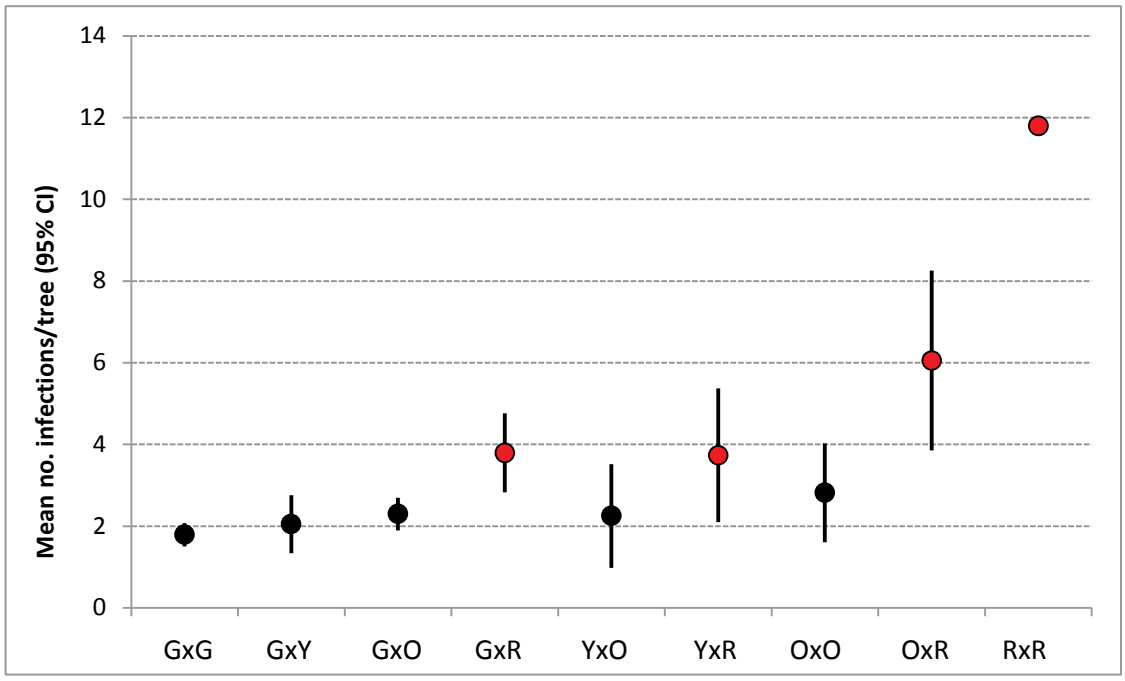

Figure 4—Means and 95 percent confidence intervals for numbers of infections/family in nine full-sib groups of sugar pine progenies. (cf., table 1 or 2 for group color codes. The mean of the groups in red is significantly different than the mean of groups in black).

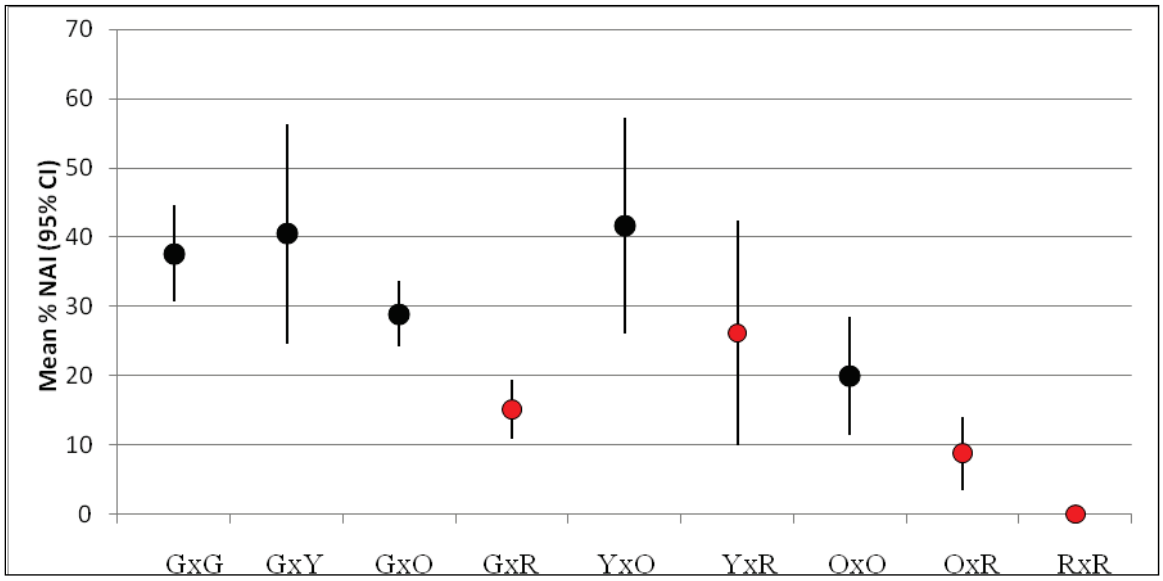

Figure 5—Means and 95 percent confidence intervals for percentage of trees with no active infections/family (percent NAI) in nine full-sib groups of sugar pine progenies. (cf., table 1 or 2 for group color codes. The mean of the groups in red is significantly different than the mean of groups in black).

Regardless of the type of inheritance of PR, there is increasing consensus that major resistance genes are more effective when deployed in a background containing quantitative resistance traits (Brun et al. 2010, Wulff et al. 2011). Quantitative resistance genes can work additively with R genes, prolonging their durability, probably through reduction in pathogen effective population

size and especially the number of virulent mutants that could be selected by R-genes (McDonald, 2010). Accordingly, the highest value of PR in breeding sugar pine is to protect Cr1 from vcr1 in synthetic populations with combined pedigrees. Cr1 will protect against all ambient inoculum except that containing vcr1. Concentrated PR genes will sanitize a great proportion of incoming inoculum, reducing the probability of vcr1, which is rare in natural populations, from becoming epidemic (Kinloch and Davis 1996, Kinloch et al. 2008). The prospect of developing more durable resistance in sugar pine is good.

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