

Fusiform Rust Fr Genes by Bulked Inocula Interactions in Loblolly Pine

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Challenging progeny of loblolly pine (*Pinus taeda* L.) from known mother trees with single genotype lines of the fusiform rust fungus (*Cronartium quercuum* sp. *fusiforme*, hereafter called *Cqf*) has shown that single-gene interactions can predict disease expression with high accuracy in controlled inoculations (Wilcox et al. 1996; Jordan 1997). A host tree carrying a *R* (resistance) allele at a specific *Fr* gene performs as a resistant (non-diseased) host when the challenging pathogen has an avirulence (*Avr*) allele at the corresponding pathogen gene; however, if the challenging pathogen carries a virulence (*avr*) allele at the corresponding gene the interaction results in disease even when the *R* allele is present. Hosts having only *r* (non-resistance alleles) typically develop disease when challenged with either an *Avr* or *avr* pathogen isolate. Considerable progress has been made in understanding the *Fr* gene resistance system by using controlled inoculations with single-genotype fungal isolates, but the response of *Fr* genes to mixtures of pathogen samples has not been investigated thoroughly. Understanding these interactions is crucial to tree improvement programs' abilities to increase genetic gains through deployment of pine genotypes that are resistant to fusiform rust disease under field conditions, where many different pathogen genotypes may be present. In this study, we investigated the interactions of maternally inherited *R* and *r* pine genotypes in progeny challenged with different bulked *Cqf* inocula to evaluate their responses and to draw conclusions on deployment of *R* pine genotypes.

MATERIALS AND METHODS

The responses of loblolly pine seedlings that carry *r* or *R* alleles (inherited from the maternal parent) to 10 different bulked *Cqf* inocula were analyzed in six open-pollinated families that were known to carry a resistance (*R*) allele for one of the eight *Fr* genes (*Fr1-8*) known at the inception of the study. These families were identified as segregating for fusiform rust resistance in previous works by Amerson and co-workers (Wilcox et al, 1996; Jordan 1997; Amerson et al 1997; Li 2003; Amerson unpublished data). We used 10 bulked *Cqf* inocula from 10 different geographic regions in the southern United States for inoculations. Each of the inocula was a 30 gall mix, derived by blending ten gall collections from three different sites within a collection area (Isik et al. 2008). Pine seedlings were challenged with the basidiospores (50,000 bsp./ml except for one inoculum at 20,000bsp.ml) following standard RSC protocols (Knighten et al. 1988). DNA markers (RAPD) were used to assess whether a seedling had inherited a recessive *r* allele (susceptible form of gene) or dominant *R* allele (resistant form of gene) from the maternal parent. Diseased and disease-free plants with *R* and *r* genotypes were counted for each inoculation-by-family combination. A generalized linear mixed model with logit link function

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was fit to disease phenotype (1=diseased, 0=no disease) to test the significance of main effects and the interaction term.

RESULTS AND DISCUSSION

We detected significant differences among bulked pathogen inocula for causing disease ($P < 0.001$). Overall, half-sib family response to bulked inocula was also significant. Family F3 with R3 allele was the most resistant (with 0.22 probability of developing disease), while family F2 with R2 had the highest level of disease (0.62).

Seedlings which inherited only recessive *r* alleles from the maternal parents did not interact because they developed high levels of disease incidence across all 10 inocula. Among seedlings with *R* alleles, R3 displayed consistent disease resistance across all inocula (the disease incidence ranged from 0.0 to 0.14), which suggests that the corresponding *avr3* allele is very rare in the pathogen populations sampled by the ten bulk pathogen inocula tested. Other *R* alleles (e.g., Fr4, Figure 1) had more interactive disease-phenotype (disease vs. no disease) responses across inocula, indicating that the corresponding *avr* alleles are more frequent in some of the bulk inocula than in others.

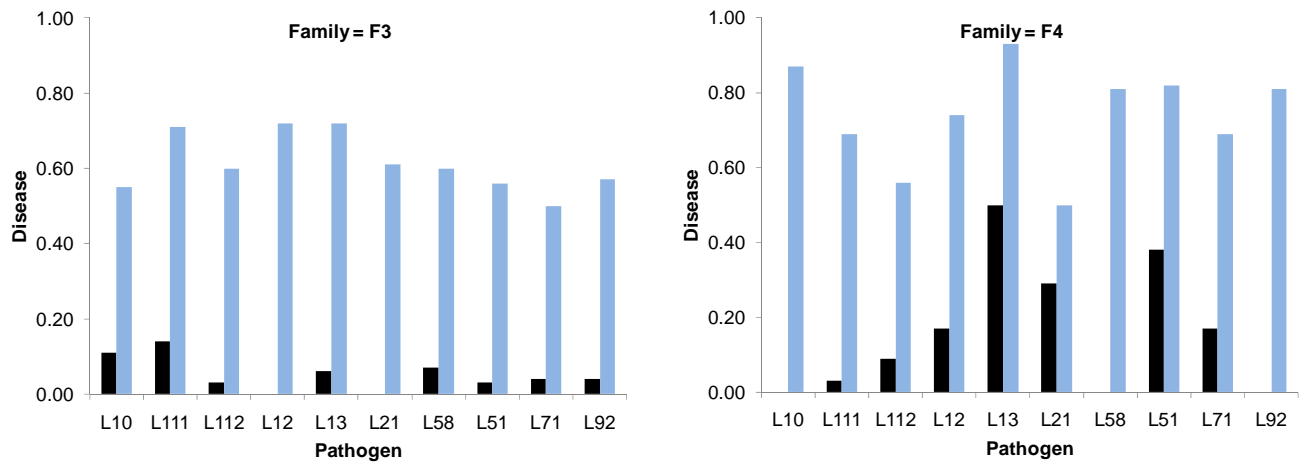


Figure 1. Response of *R* and *r* pine genotypes in family F3 and in F4 to 10 different pathogen inocula. The black bars are the genotypes with the resistance *R* allele and the blue bars are genotypes which inherited the recessive *r* allele from the maternal parent. *R* genotype by inocula was not significant in family F3, but it was highly significant in family F4.

The results suggest that stable resistance to fusiform rust disease can be achieved in the short term by planting mixtures of pine families carrying different resistance (*R*) alleles. A long-term breeding strategy of combining or “stacking” multiple *R* alleles in individual pine families or clones, enhanced if possible with quantitative or horizontal resistance, may produce more stable disease resistance and such a strategy should be pursued for loblolly pine.

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