

Article

Genetic Control of Pitch Canker Response in Southern Pine and Southern Pine Hybrids

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Abstract: *Fusarium circinatum* causes pine pitch canker (PPC) disease and associated symptoms such as resinous lesions, mechanical weakness, and crown dieback that may lead to mortality in *Pinus* and *Pseudotsuga* spp. There are no ameliorative techniques available for the disease, and the genetic resistance among populations to support commercial plantation deployment has not been well characterized. In this study, we characterize the genetic control of PPC disease tolerance (and/or resistance) and predict the tolerance of families in existing breeding populations: open-pollinated (OP) half-sib and control-pollinated full-sib (FS) slash pine (*Pinus elliottii* var. *elliottii* Engelm.), OP loblolly pine (*Pinus taeda* L.), and advanced-generation OP hybrid slash × *P. caribaea* (*Pinus elliottii* var. *elliottii* Engelm. × *Pinus caribaea* var. *bahamensis*, *caribaea*, and *hondurensis*) using *F. circinatum* isolates obtained from three locations in Georgia and FL, USA. We describe a new experimental design that improves the accuracy of breeding value predictions, provides more precise genetic parameter estimates, and facilitates comparisons within and among taxa as well as comparisons among isolates. We found strong evidence for genetic control of the ratio of stem damage by *F. circinatum*, especially in slash pine and slash × *P. caribaea* hybrids. Loblolly and slash × *P. caribaea* hybrids exhibited less damage than slash pine. We observed a spectrum of virulence among *F. circinatum* isolate sources, which were not equally virulent in different pine taxa.

Keywords: pine pitch canker; forest tree disease; disease screening; tree breeding; tree improvement



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1. Introduction

Background and Review of Literature

Fusarium circinatum is a damaging necrotrophic fungal pathogen that causes pine pitch canker disease (PPC). The disease is characterized by heavy resinous lesions, crown dieback, mechanical weakness in stems, loss of vigor, and mortality [1]. Native to Central America, *F. circinatum* is naturalized to the Southeast region of the United States and many other regions of the world where North American *Pinus* species are commercially grown [2,3]. North American *Pinus* species are planted and managed for fast growth across their native ranges and are major species of the planted forest estates in South America, South Africa, Southeast Asia, Australia, and New Zealand. Pitch canker is especially devastating to the stands of *P. radiata* in Chile. Pine hybrids with resistance to PPC are now replacing the severely impacted *P. patula* forests of South Africa and elsewhere [2,4,5]. Although pitch canker is not presently found in New Zealand or Australia, its introduction is an issue of high concern based on its impacts in Europe, South America, and South Africa [2].

PPC infection is typically caused by the introduction of *F. circinatum* conidia into wounds and may be vectored by water, wind, or insects [6]. *F. circinatum* does not require an alternate host in its life cycle and can spread from tree to tree [3]. Symptoms of the disease are more frequently observed in trees with mechanical damage typically from high wind events and high nutrient availability from a silvicultural regime [7,8]. An overabundance of nitrogen is the most well-described factor that increases the risk of infection in production stands [9]. Forest landowners across the lower US Southeast and Gulf Coast regions have observed an increase in outbreak frequency and severity of PPC in commercial stands of loblolly pine (*Pinus taeda* L.) and slash pine (*P. elliottii* var. *elliottii* Engelm.). In nurseries, *F. circinatum* can severely damage a crop of seedlings without proper seed coat treatments, soil sanitation, and prophylactic fungicide treatments (e.g., Proline™) [10].

As there is no cost-effective chemical treatment for pitch canker remediation in commercial stands; genetic tolerance is the main approach to mitigate PPC damage. In the US Southeast, endemic fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) has traditionally been the most damaging pathogen in plantation forestry. Consequently, advances in genetic resistance captured in breeding programs have provided a robust response to rust infection risk, largely due to its prevalence and the proven utility of field-based phenotypes for resistance [11–15]. While screening for PPC tolerance has occurred in collaborative projects with academia, industry, and the United State Forest Service (USFS), there are no domestic loblolly or slash pine breeding programs producing seed from populations with verified pitch canker tolerance, principally because field data have not been useful in screening for pitch canker resistance.

Various studies have demonstrated differences in tolerance among *Pinus* species in the Australes, Oocarpa, and Patula sections as well as variation among species and families within species [16,17]. These and other inoculation studies show that varieties of *P. caribaea* are less susceptible to damage than *P. elliottii* var. *elliottii* or *P. taeda*. Among the species of the Patula and Oocarpa groups, there is a wide spectrum of tolerance. While inter-taxa variation in susceptibility to PPC response precipitating stem damage is reasonably well characterized, there is considerably less information concerning intra-species genetic variation, especially in *Pinus* spp. relevant to the US Southeast. Studies in the 1970s and 1980s reported significant genetic variation within slash pine populations, with heritability estimates for tolerance (variously defined as narrow-sense heritability and “repeatability”) near 25% [18]. Estimates in loblolly pine are limited to two studies by Quesada et al. and Kayihan et al. estimating clonal repeatability and narrow-sense heritability around 30%, respectively [12,19].

The UF | IFAS Cooperative Forest Genetics Research Program (CFGRP) has executed controlled screenings of slash pine with some success in identifying tolerant and susceptible families [18,20]. However, there are few descriptions of genetic control, including an affirmative delineation between resistance and tolerance as a response mechanism. These screenings, along with those from other *Pinus* spp. suggest that there is genetic variation in PPC tolerance and that some populations have a spectrum of inherent genetic tolerance [17]. The lack of information concerning genetic tolerance to *F. circinatum* limits our ability to guide deployment and improving tolerance has not been an explicit target for breeding populations of loblolly or slash pine. Although some groups have examined the merit of using genomic tools for characterizing pitch canker tolerance in loblolly pine, these analyses have not been utilized to improve tolerance in breeding programs [19,21].

In a preliminary study, we used the existing USFS Resistance Screening Center (RSC) methodology to assess the response to diseases affecting *Pinus* sp. The standard RSC design uses six experimental units per family or seed lot, each composed of twenty trees in a tray screened in two “runs” that represent independent screenings that are typically undertaken on sequential days. For this assay, the measured phenotype is the proportion of stems in the tray with 33% or more lesion coverage, which is used to infer the percent of “damaged” seedlings.

For taxa comparisons and genetic parameter estimation, the existing RSC method is inadequate. Inference on the experimental unit is information-poor: a count of trees meeting a threshold does not stratify relative stem damage (total damage and moderate damage count the same) and so does not account for intra-family variation. The traditional method also requires 120 seedlings per family and provides only 4 total degrees of freedom to estimate the significance of differences in stem damage (USFS RSC testing manual, unpublished). Internal studies found that the families with the highest and lowest damage scores, while demonstrably different on a visual basis, are not statistically different because of poor experiment power (Figure A1).

The screening we describe here was motivated not only by the need to construct more precise estimates of genetic merit in breeding populations but also in response to the increase in PPC outbreaks in the region and the recent discovery that the virulence of reference isolates used by the USFS RSC differ significantly and that some had become avirulent [22].

Consequently, we created and tested a new experiment design with greater power to differentiate among treatments and additional information to improve the accuracy of genetic parameter estimates. We screened slash pine families from the UF | IFAS CFGRP along with loblolly pine and slash $\times$ *P. caribaea* hybrids from industry breeding programs to provide comparisons among North American pine taxa. The goals of this screening include obtaining breeding value predictions to guide operational deployment and genetic parameter estimates to guide the development of pitch canker resistance. We used an experiment designed to improve the precision of breeding value estimates for pitch canker tolerance, measured as the percentage of seedling stems with lesions following inoculation. The design also provided genetic parameter estimates for pitch canker tolerance and for virulence comparisons among newly identified isolates [23]. In doing so, we provide a case study that provides supporting evidence for changes in the screening protocols currently used by the USFS RSC.

2. Materials and Methods

2.1. Genetic Resources

Families from slash pine (*P. elliottii* var. *elliottii* Engelm.), loblolly pine (*P. taeda* L.), and hybrid *P. elliottii* var. *elliottii* $\times$ *P. caribaea* (including *bahamensis*, *caribaea*, and *hondurensis* varieties) populations were screened for stem damage caused by *F. circinatum*.

Sixty slash pine families were tested, a sample of commercially relevant parents in orchards managed by members of the UF | IFAS CFGRP and the Texas A&M Western Gulf Cooperative Tree Improvement Programs (WGFTIP). There were fifty-three CFGRP and seven WGFTIP families. Of these, forty-seven were open-pollinated (OP), and all thirteen full sibs (FS) were CFGRP families. Six CFGRP OP families were collected from different orchards and one genetic entry represented an OP seed orchard mix. These genetic entries were used for internal gain predictions. While the FS and OP families from CFGRP populations are connected using a pedigree that tracks relatedness among seed lots, the OP WGFTIP families were treated as unrelated half-sib families.

The twelve loblolly pine families represent a small sample of commercially relevant OP material suitable for deployment in the US Southeast. Twenty hybrid slash $\times$ *P. caribaea* families represented OP seed lots collected from F2 selections that are commercially deployed in southeastern Queensland, Australia. This hybrid has also been established in South Africa, South Florida, and various tropical and subtropical regions worldwide. While the primary purpose of including slash $\times$ *P. caribaea* hybrid and loblolly families in the screen was to compare differences in symptom development among taxa, the use of pedigreed families also allowed for the comparison of genetic parameters from the different populations.

These isolates were collected in 2019 from Suwannee County, FL, Volusia County, FL, and Wilcox County, GA. The isolates were chosen because their pathogenicity varied on two susceptible slash pine families [22]. Further information concerning these isolates,

including draft genomes, can be found in Fulton et al. [23]. The isolates have become standard for RSC pitch canker screening.

2.2. Experiment Design

A total of 103 seed lots were tested, along with known “tolerant” loblolly and “susceptible” slash pine families the USFS RSC uses as experiment controls. In all, 7200 trees were arranged into 1800 row plots of 4 seedlings each. These plots were randomized in an unbalanced nested incomplete block design. A total of 360 metal trays were used as incomplete blocks with trays accommodating 20 seedlings arranged as 5-row plots of 4 seedlings each. The dimensions of the screening trays were the determinate factor in the size of incomplete blocks. Twenty incomplete blocks were assigned to 1 of 18 replications and treatments were further nested within the 3-pitch canker isolates so that 6 replications of each pine treatment were inoculated with each of the 3-pitch canker isolates. The scheme for this design is found in Figure 1.

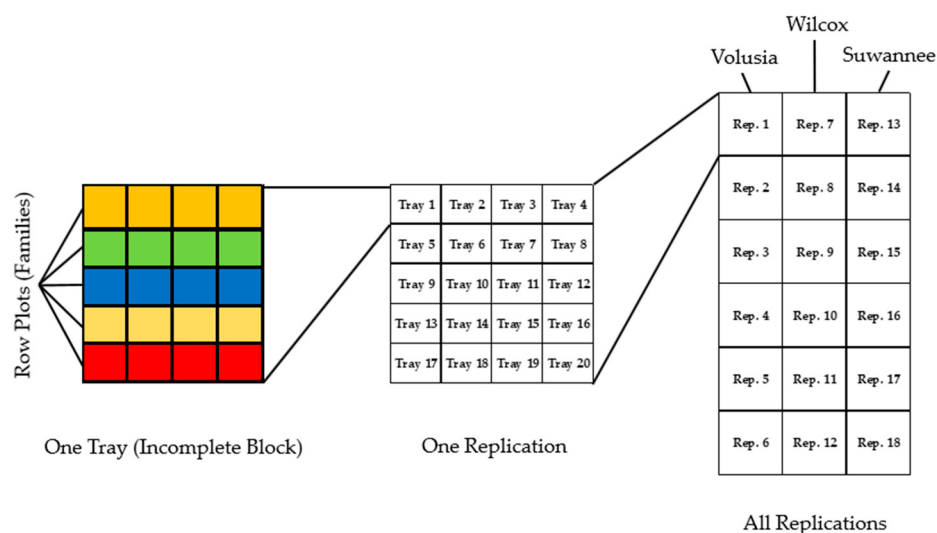


Figure 1. A graphic representation of the experiment design. Each tray is designed as an incomplete block with five four-tree row plots. Each replication had 20 such trays (i.e., 20 incomplete blocks). Each of these replications was assigned to one of three isolate treatments.

Seeds from slash pine and slash $\times$ *P. caribaea* hybrid pine populations were directly planted with no prior stratification as recommended in the RSC protocol. Loblolly pine seed lots were stratified for 45 days prior to planting. Seedlings were grown in a 3:2:1 peat:vermiculite:perlite mixture and fertilized two times prior to inoculation (3 weeks and 5 weeks post-transplant) and then once (3 weeks) post-inoculation followed thereafter every 6 weeks. Fertilizer was a soluble 15-30-15 (N:P:K) dispensed at 1:100 using Dosatron injector into water stream. After 10 weeks of growth, a total of 7172 out of 7200 seedlings originally assigned in the experiment design were of sufficient size to be included in the screening. The remaining 28 cells (7-row plots) were filled with filler trees which were inoculated and then excluded from the analysis. Testing procedures followed the standard RSC protocol.

The fungal isolates were revived from the confetti pieces on PCNB agar. Spores produced from the confetti pieces were used to infect “storage” trees to rejuvenate the fungus using live trees before inoculating the test trees. The fungus was then cultured out of the infected storage trees with the use of several media including PCNB plates, APDA plates, Carnation agar plates, and water agar plates to produce spores for test inoculations. Spores were scraped from plates to produce independent aqueous spore suspensions for each isolate and were standardized to 100K spores/mL using a hemocytometer. Each seedling was cut and then hand sprayed once with a prescribed isolate from each of the

3 fusarium isolates. Seedlings were watered and monitored for 24 weeks. Total stem height and distance from the top of the seedling to the margin of the lesion were measured to estimate a damage ratio as the percentage of the stem that was damaged (ranging from 0 to 100%).

2.3. Linear Model and Variance Component Estimation

A linear mixed model was constructed to evaluate the screening data sets including and excluding seedlings with no lesion development that may have been escapes, i.e., where the inoculation process failed. Rather than a generalized linear mixed model assuming a binomial or truncated distribution, a more parsimonious and traditional linear mixed model was used [24]. The model was parameterized as shown in Equation (1):

$$y = \mu = Xb + Zu + e \quad (1)$$

where y is the response or the proportion of total stem length that was damaged by PPC and defined as the ratio of the lesion length to the total height. μ is the mean, X is the design matrix for fixed effects with solutions in a vector denoted b , Z is the design matrix for random effects with solutions in a vector denoted u , and e is the error.

Fixed effects account for three taxa, three different *F. circinatum* isolates, the interaction of taxon and isolate, and a term for replication nested within the isolate. Random effects describe row plots nested within replicates and the double Kronecker product describes the implicit model including a pedigree-based numerator relationship matrix (A-matrix) nested within isolate that is disconnected along a block diagonal where block diagonal elements represent levels of pine taxa. This double-Kronecker product allows for taxon-specific variance component estimates with an internal covariance matrix that incorporates the A-matrix within each isolate. This model could account for numerous variance assumptions, including homogeneity or heterogeneity of variance component estimates for each pedigree within each of the isolates used for the screening.

Incomplete blocks were designed for the purpose of randomization and assignment of plots; however, they were excluded from the model because they are redundant and accounted for with the row plots. The incomplete block effect would be retained in the model if row plots were treated as experimental units instead of individual trees.

The model was assessed with multiple covariance parameterizations for the term describing the A matrix nested within *F. circinatum* isolate and convergence was assessed using loglikelihood solutions. A model with a common correlation and heterogeneous variance estimates was found to minimize likelihood estimates. Diagonal elements of the covariance matrix (additive variance at each isolate) were assumed heterogeneous and off-diagonal elements were fixed as equal and heterogeneous providing solutions for intra-isolate, inter-taxon pairwise, or group genetic correlations for predicted stem damage. The common correlation term was selected for parsimony based on relative log-likelihood. In all, this model requires estimates of nine additive variance components (one for each of three isolates in the three taxa) and three common correlations/covariances (one for each taxon). For a visual expression of the covariance model, see Figure 2.

This model was evaluated with ASReml-R 4.1 [25]. Data arrangement, data summaries, and post-processing of variance components, statistics, and best linear unbiased predictions (BLUPs) were executed in R [26]. Breeding values of clones were calculated as BLUPs and extracted from respective model results.

Estimates of within-taxon narrow-sense heritability were calculated as shown in Equation (2):

$$h_{ns}^2 = \frac{\text{mean}(\sigma_{Volusia}^2, \sigma_{Suwannee}^2, \sigma_{Wilcox}^2) * \rho_b}{\text{mean}(\sigma_{Volusia}^2, \sigma_{Suwannee}^2, \sigma_{Wilcox}^2) + \sigma_p^2 + \sigma_e^2} \quad (2)$$

where $\sigma_{Volusia}^2$ is the A-inverse numerated additive variance component (same for two other isolates), ρ_b is the within-taxon group type-B genetic correlation, σ_p^2 is the plot(block(rep))

variance component, and σ_e^2 is the residual error. Estimating the heritability with the inclusion of the intra-isolate genetic correlation provides more conservative estimates.

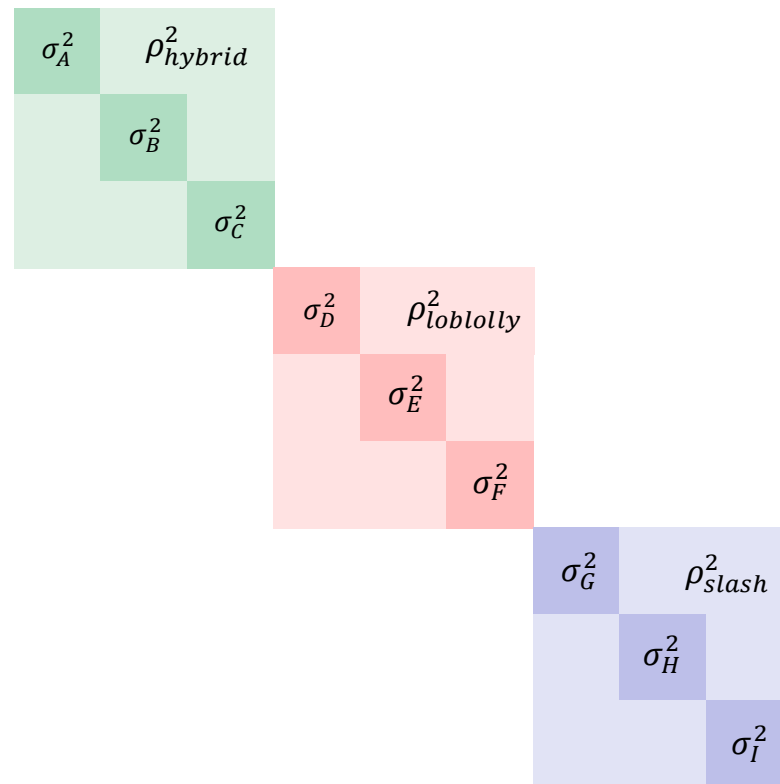


Figure 2. A visualization of the covariance matrix specification to account for independent taxa, individual pedigree-based additive variances, and genetic correlations within taxa and among isolates. σ_A^2 corresponds to the additive variance for slash $\times$ *P. caribaea* hybrids with the Suwanee isolate, σ_B^2 corresponds to the additive variance for slash $\times$ *P. caribaea* hybrids with the Volusia isolate, and so on. Genetic correlations within a taxon are denoted ρ_{hybrid}^2 and so on.

3. Results

3.1. Summary Statistics

A sizeable proportion of seedlings (1182) had no visible damage and were either resistant seedlings or treatment escapes. A further 198 were excluded for mechanical damage or other reasons unrelated to screening: 7 slash $\times$ *P. caribaea* hybrid entries, 2 loblolly entries, and 189 slash pine entries. To evaluate the impact of escapes on genetic parameter estimates and breeding value predictions, data were analyzed including and excluding records with damage scores of 0%. For the reduced data set that excluded potential escapes, 511 out of 1874 slash $\times$ *P. caribaea* hybrid seedlings (27.3%, 20.4 seedlings/family), 222 out of 902 loblolly seedlings (24.6%, 17.3 seedlings/family), and 449 out of 4199 slash seedlings (10.7%, 6.7 seedlings/family) were removed.

Table 1 summarizes the average damage ratios stratified by taxon and inoculum. The data did not follow a normal distribution both because of the high number of end-range outcomes of 0% or 100% damage and because the response variable is bounded. Figures 3 and 4 show the distribution of damage ratios stratified by taxon, family within taxon, and isolate.

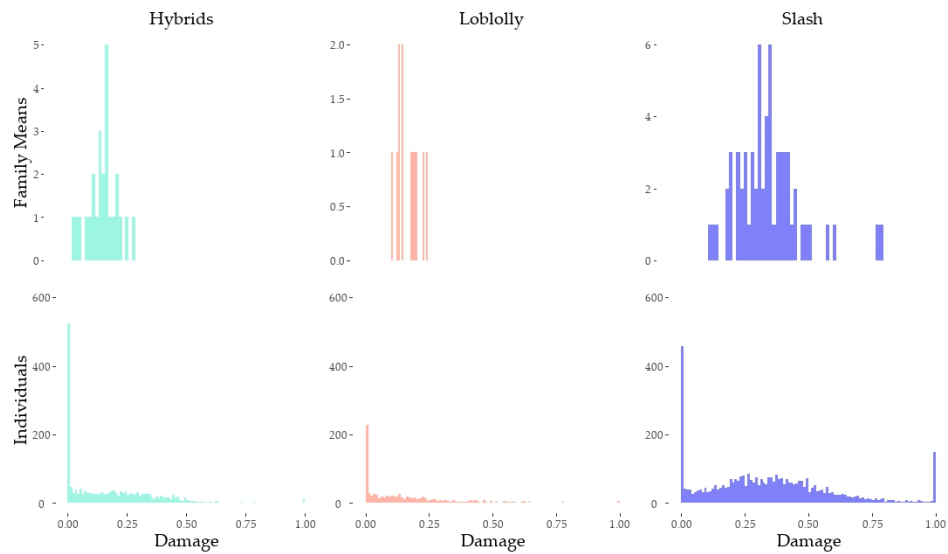


Figure 3. Histograms of individual and raw family mean damage stratified by taxon. These his-to-grams include all data including records identified as potential escapes.

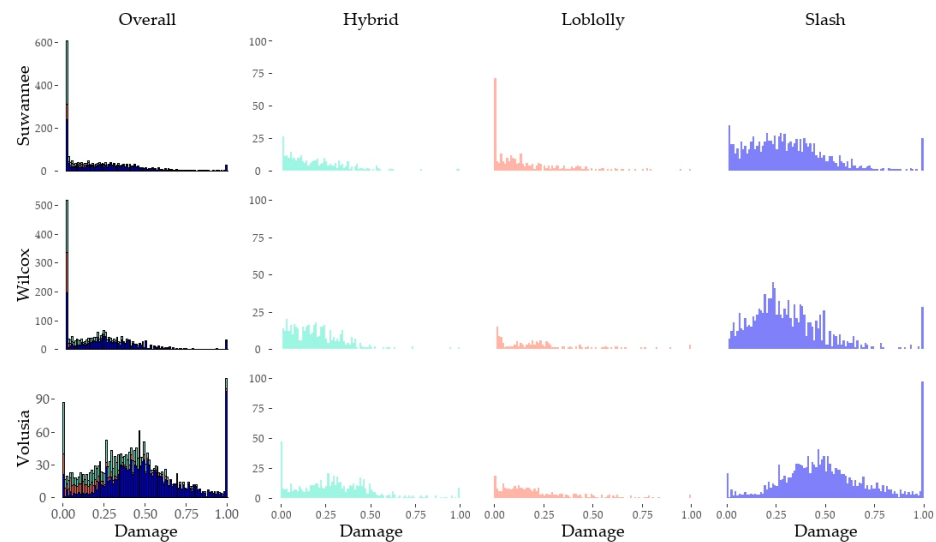


Figure 4. Histograms of percent stem damage on individual trees stratified by isolate. The complete set of records (including all taxa) are seen in the first column. Records are stratified by taxa in the second (hybrid slash $\times$ *P. caribaea*), third (*P. taeda*), and fourth (*P. elliottii* var. *elliottii*) columns. These histograms include all data including records identified as potential escapes.

Table 1. Average damage ratios stratified by taxon and isolate for full and reduced data sets.

Category	Full Data Set			Reduced Data Set		
	Mean Damage	s.e.	n	Mean Damage	s.e.	n
Hybrid	0.184	0.19	1874	0.253	0.19	1363
Loblolly	0.177	0.2	902	0.234	0.2	680
Slash	0.349	0.25	4199	0.391	0.24	3749
Suwannee	0.205	0.21	2282	0.276	0.21	1693
Volusia	0.421	0.25	2362	0.437	0.24	2276
Wilcox	0.217	0.21	2331	0.278	0.19	1823
Sum			6975			5792

3.2. Model Effects and Genetic Parameters

3.2.1. Fixed Effects

Each fixed effect was significant when the models were fit with data that included and excluded potential escapes. From Figures 3 and 4, it is evident that slash is the most susceptible pine species, and the Volusia isolate is the most virulent. The significance of the interaction between taxa and isolate seems to be largely attributable to changes in the loblolly linear predictions. Changes in the ranking of slash $\times$ *P. caribaea* hybrid families across taxa are negligible, and interactions of the slash families are small relative to loblolly. Although the Volusia isolate is clearly more virulent than the other two isolates, it is shown to be the least virulent isolate for a select set of loblolly families included in the screening. Solutions for the fixed effects of each model are found in Table 2:

Table 2. Solutions for fixed effects from the model including all data and data with potential escapes excluded. Solutions indicate the predicted addition or subtraction of the predicted damage ratio. P-scores were functionally equivalent for fixed terms in each model, with every term including the replication–isolate nested interaction, a blocking effect, significant at $p < 0.001$.

Fixed Effect	Fixed Term Level	Full Data Set		Reduced Data Set	
		Probability	Solution	Probability	Solution
Mean	Mean	<0.001	0.098	<0.001	0.180
	Hybrid		0.000		0.000
Taxon	Loblolly	<0.001	0.068	<0.001	0.037
	Slash		0.193		0.156
	Suwanee		0.000		0.000
	Wilcox		0.009		−0.003
Isolate	Volusia	<0.001	0.229	<0.001	0.160
	Hybrid*Suwanee		0.000		0.000
	Hybrid*Wilcox		0.000		0.000
	Hybrid*Volusia		0.000		0.000
Isolate * Taxa	Loblolly*Suwanee	<0.001	0.000	<0.001	0.000
	Loblolly*Wilcox		−0.081		−0.004
	Loblolly*Volusia		−0.151		−0.126
	Slash*Suwanee		0.000		0.000
	Slash*Wilcox		−0.024		−0.011
	Slash*Volusia		0.048		0.065

3.2.2. Random Effects and Genetic Parameters

Random effects for respective models are listed in Table A1 with heritabilities and linear combinations of the random effects reported in Table 3. Heritability was calculated as defined in Equation (2). ASReml internally computes a common correlation as a linear combination of covariances and variances with the “corh” covariance specification option [25]. These internal estimates also include Taylor-series derived estimates of standard errors. Estimates are assumed homogeneous for each pairwise relationship of isolates within a taxon. These genetic correlation estimates correspond to both the tolerance among isolates for each taxon and a pseudo-type-B genetic correlation that describes a genotype-by-environment (G $\times$ E) interaction.

Table 3. Estimates of narrow-sense individual tree heritability with standard errors and genetic correlations. Heritability was estimated following Equation (2). Genetic correlation is estimated directly in ASReml as a linear combination of the intra-taxon additive variances at each of the isolates and common intra-taxon covariance.

	Taxon	Narrow Sense Her- bitability	s.e.	Genetic Correlation	s.e.
Full Data Set	Hybrid	0.35	0.08	0.78	0.15
	Loblolly	0.16	0.31	0.52	0.25
	Slash	0.41	0.06	0.62	0.08
Reduced Data Set	Hybrid	0.36	0.13	0.58	0.18
	Loblolly	0.19	0.42	0.56	0.24
	Slash	0.52	0.07	0.71	0.07

Narrow-sense heritabilities are strong for the slash $\times$ *P. caribaea* hybrid taxon and pure slash but low and statistically insignificant for loblolly. Each of these taxa represents samples from a deeply pedigreed population not selected for PPC tolerance, possibly contributing to narrow-sense heritability estimates higher than those typically observed in traits such as volume accumulation, stem straightness, or fusiform rust resistance.

As an additional verification, correlations among BLUP means specific to each isolate were calculated for each taxon considering the full and reduced data sets with isolates representing independent and different testing environments associated with PPC isolates Table 4. In the model where potential escapes were excluded, BLUPs were also estimated for individuals with no data to preserve the integrity of the model comparisons. These correlations were generally quite strong, indicating that information gleaned from predictions is not highly leveraged by the presence or absence of possible escapes in the experiment.

Table 4. Correlations of BLUPs estimated for all selections in the pedigree and for parents (uniquely named clones in breeding programs or suitable for commercial deployment). Correlations are calculated for all records then stratified by taxon.

Taxon	Parents/Selections	Parents and Progeny
All	0.97	0.98
Hybrid	0.93	0.97
Loblolly	0.92	0.97
Slash	0.97	0.97

Figure 5 shows an interaction plots of the within-taxon genetic correlation among isolates. These predictions consider only the genetic effects estimated for a given parent tested with a different isolate, not the whole prediction including fixed effects for taxon, isolate, and the taxa-isolate interaction.

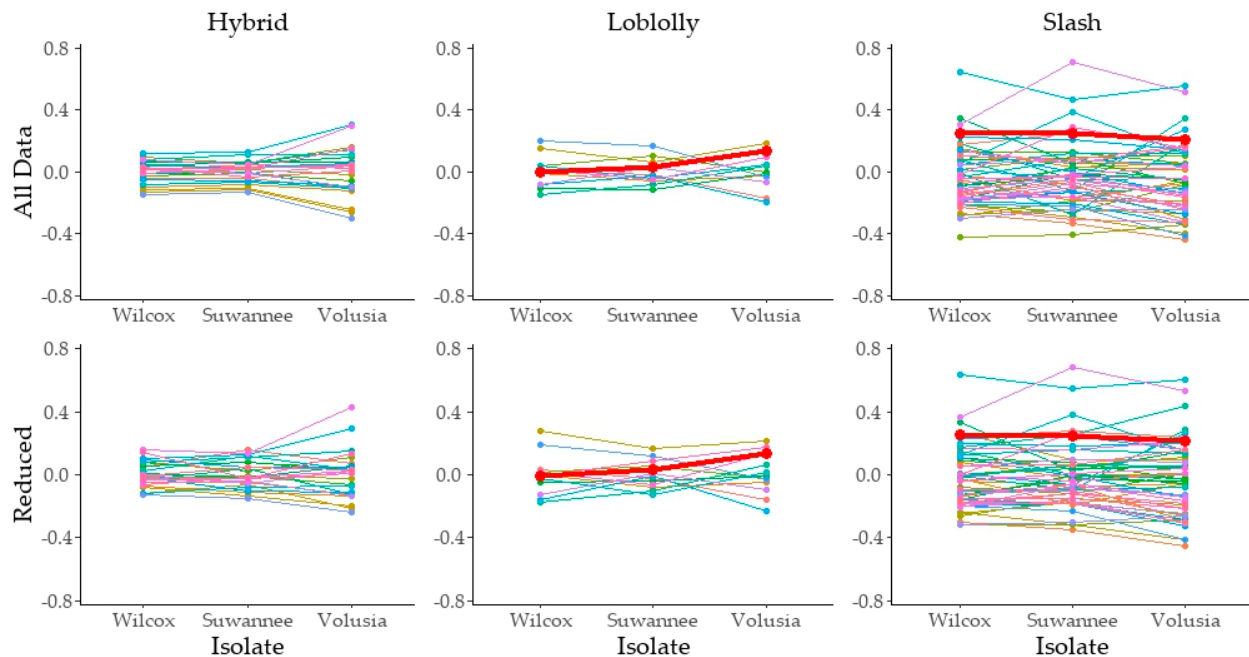


Figure 5. Interaction plot of BLUPs for parent and ancestors (i.e., excluding progenies) stratified by taxon for both the full and reduced (i.e., potential escapes excluded) data sets. Thick red bars indicate the internal RSC check lots: MVLB for loblolly and U17 for slash.

4. Discussion

4.1. PPC Tolerance across Taxa and Isolates

We found that slash pine is significantly less tolerant than loblolly or the slash $\times$ *P. caribaea* hybrids to the three *F. circinatum* isolates used for this screening. The mean damage ratio in slash pine seedlings was significantly greater, approximately 2-fold in data sets including and excluding potential escapes (Tables 1 and 2). The significant fixed effects for taxa from each model support Hodge and Dvorak's findings that pure *P. elliotii* var. *elliotii* is more susceptible to PPC than *P. caribaea* varieties, as the hybrid of the two was significantly more tolerant [16,17]. The *P. taeda* families were more tolerant than both slash $\times$ *P. caribaea* hybrids and pure slash pine. Although these differences are statistically significant, the relatively small number of loblolly and slash $\times$ *P. caribaea* hybrid pine families preclude broad inter-taxa conclusions until more families are screened.

Seedlings from all three taxa were assigned randomly to trays, and there was no prescribed pattern of row plots of one taxon being near or far from another. The analysis to affirm data integrity revealed no differences among locations in trays; escape rates were functionally uniform among each of the rows (plots) and columns (seedling position in row plot). While we cannot rule out that some unknown factor in execution lead to this difference in potential escapes between the taxa, it is also possible that the data support the hypothesis that loblolly and slash $\times$ *P. caribaea* hybrids are not only more tolerant when infected but also more resistant to infection by *F. circinatum* than slash pine. Future experiments are required to test this hypothesis.

The significance of the taxa–isolate interaction limits inferences of main effects for taxa and isolate. There is some level of theoretical confounding between the fixed-effect interaction and the random effect corresponding to genetic correlation within a taxon among isolates. However, these terms are distinct in an important way: the fixed effect accounts for broad species/taxon level interactions, and the random term accounts for differences in how individual families might perform. In future analyses, it might be more appropriate to screen more families in a single taxon and to define a heterogeneous covariance structure (or a factor-analytic approximation) to account for differences between isolates.

The largest differences in taxa performance when different isolates were used are associated with the performances of loblolly pine families, where there was relatively little PPC damage and there were few families tested. Results indicate that including loblolly in the analysis to account for all records and analysis excluding escapes provides estimates for taxa and isolates that have useful interpretations. The interaction of loblolly with isolate warrants further investigation as the two isolates sourced from the extreme end range of natural loblolly distribution were demonstrably less pathogenic (Table 2, Figure 6).

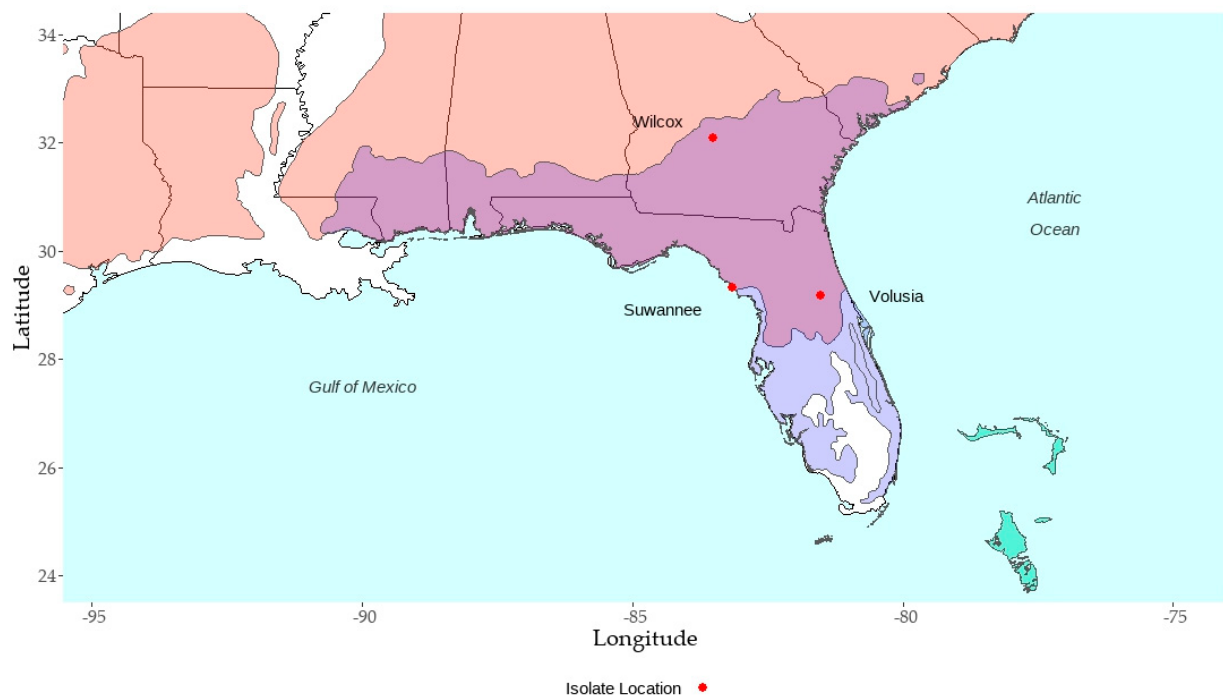


Figure 6. Map of isolate collection locations overlaid on a map of the natural range of *P. caribaea* (light green), *P. taeda* (salmon), light purple zone overlapping with slash pine), and *P. elliotii* var. *elliotii* (overlapping light purple zone and lilac). Note that Wilcox, the northern-most isolate was demonstrated to be the most pathogenic for loblolly pine.

The Volusia isolate was most virulent for slash, least virulent for loblolly, and had the most heterogeneous response for slash $\times$ *P. caribaea* hybrids (Table 2, Figure 4). The Volusia isolate was the most southerly and farthest along the proposed path of *Fusarium circinatum* invasion from Central America [2,3,27]. The significant taxa–isolate interaction, along with observed variation in isolate performance and the stem damage ratio, motivates further study of the genetic architecture of response and virulence, including a study of longitudinal and latitudinal gradations in tolerance and virulence for tree hosts and *F. circinatum* isolates, respectively. This suggests that a gradation in both fungal pathogenicity and genetic tolerance (or resistance) may exist and should be explored with additional screening of both slash and loblolly families. Pathogenicity would be a more supported hypothesis given the very recent introduction of *F. circinatum* to the eastern end of the slash and loblolly pine ranges, following Gordon’s assertion about *F. circinatum* originating principally from Mexico [27]. Given that slash $\times$ *P. caribaea* hybrids exhibited the most heterogeneous response to the Volusia isolate, it could mean that this isolate had evolved to recognize *P. caribaea* less effectively than isolates taken from sources closer to its proposed Central American origin. These hypotheses support conclusions drawn by Schmidtling et al. concerning refugia of *P. taeda* and would further explain the putative co-evolution of *P. taeda* and *P. caribaea* varieties and pitch canker [28].

4.2. Genetic Parameters

Genetic correlations among isolates within taxa may be interpreted as a measure of the genotype-by-environment (G×E) interaction. In tree breeding, developing distinct populations for different environments is suggested if genetic correlation estimates are less than 0.66 [29]. In this study, for both data sets and among all three taxa, estimates are relatively modest (0.51–0.78) (Table 3). The strength of correlations varied among taxa and data sets, indicating that, like the case of the fixed taxa by isolate interaction term, pedigrees in different taxa relate in different ways to the virulence of a given isolate.

Estimates of narrow-sense heritability of PPC tolerance in slash pine have not been reported frequently or recently. Here, we show that heritability is strong in slash pine and hybrid *P. caribaea* × slash pine (Table 3). Genetic parameter estimates differ little in the analysis using both the full data set and the data set with potential escapes excluded. Heritabilities were shown to be marginally higher for the data set including potential escapes. This could be simply associated with a better normality approximation of the data or could be associated with true escapes being excluded.

In loblolly pine, estimates for narrow-sense heritability are moderate to low and are within the expected ranges for other traits ($h^2_{ns} \sim 0.15$). However, the small number of tested families, generally high PPC tolerance, high standard errors, and observed interaction with isolates make population inferences inappropriate for the loblolly pine population. This experiment demonstrates considerably lower estimates than those reported by Kayihan et al. and Quesada et al. [12,19]. Assuming that there is no factor uniquely affecting experimental integrity for experimental units in loblolly, it seems that the relatively low spectrum of susceptibility limits inference on genetic differences in this study. There is evidence for a spectrum of tolerance among loblolly families, but to obtain estimates appropriate for inference, many more families would need to be screened as in Kayihan et al. and Quesada et al. While these studies demonstrate that loblolly has a spectrum of genetic tolerance, the families screened here were so tolerant that there is little evidence that the differences are meaningful or practical to describe. The principal inference from these data concerning loblolly pine is that it is highly tolerant to PPC [16,17]. Further inferences concerning genetic control and G×E would require further specific examination.

4.3. Informing Deployment Decisions in Slash Pine

The PPC response is nearly impossible to test using traditional multi-year progeny tests for many reasons. Conditions leading to PPC infection in progeny trials offset the assessment of other objectives including stem health, environmental homogeneity, and expression of other traits under genetic control such as stem form and growth. The environmental density of spore loads of *F. circinatum* is functionally unpredictable with currently available tools and would not reach each tree in a test uniformly. Perhaps most importantly, PPC is often observed in stands 15 years and older, by which time the integrity of progeny trial design is compromised. The correlation between results from controlled inoculations of seedlings in the greenhouse with field resistance/tolerance is unknown for PPC disease. Significant interactions between host and isolate add complexity because it is impractical to produce estimates for all possible *F. circinatum* isolates. However, this limited study suggests that rank changes in the performance of families may not be practically meaningful with different isolates (Figure 5).

We report the only published effort in which rigorous screening techniques have been used to guide deployment decisions with respect to PPC in the US Southeast. For commercial deployment in the US Southeast, G×E has largely been ignored for most traits beyond broad adaptation for winter minimum temperatures [30]. In deployment, the typical commercial solution for G×E is to use the mean predicted response across the environment multiplied by intra-environment genetic correlations centered on the predicted mean for breeding value estimates, assuming a single meta-environment with isolates of similar virulence. Interactions do impact predictions; however, BLUP regression across all environments prevents families with a heterogeneous predicted performance

from being deployed in risky areas and favors families with homogeneously favorable predicted performance. For commercial use in slash pine, breeding values for families that are derived from the full data set and commensurate gain calculations will be used to match tested families to sites based on historical and predicted risk for PPC infection. In slash pine, this information was integrated into the CFGRP GRADE™ tool. BLUP estimates and corresponding genetic gains cannot be described as direct predictions but are instead measures of relative risk. Gain estimates should be used to characterize and identify the most appropriate families for deployment based on perceived acceptable risk thresholds for PPC, as would be the case for other disease traits.

As in the case of other commercially relevant traits, there are many difficulties in interpreting the importance and magnitude of interactions between genotypes and environments. On one side, rigorous statistical analysis demonstrates that there are meaningful differences between predicted performances when the environment is changed by exposing a different member of a pedigree to different environments. On the other, these different predictions cannot account for how environments vary over the life of a test and indeed a commercial stand.

5. Conclusions

These data demonstrate strong evidence for genetic variation and potential for capture of genetic gain in pitch canker tolerance that may be deployed from commercially relevant southern yellow pine populations. They also confirm that meaningful differences exist in the virulence of isolates and agree with previous studies indicating tolerance to PPC varies among and within *Pinus* taxa. We further find that the new screening procedure offers a much more powerful design to discriminate amongst pine families for PPC tolerance and to also quantify the virulence of PPC isolates.

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Conflicts of Interest: The funders had no role in the design of the study or in the collection, analyses, interpretation of data and writing of the manuscript. Commercially relevant results from the loblolly pine population are limited to use by ArborGen; results for hybrid slash $\times$ *P. caribaea* are limited to use by HQPlantations; results from this analysis relevant to slash pine are open for equal use by all CFGRP members.

Appendix A

Table A1. Variance components for random effects of respective models, along with standard errors (*italicized*), genetic correlations in bold, and their Z ratios. Each effect is statistically significant.

Effect	Full Data Set			Reduced Data Set		
	Component	s.e.	Z Ratio	Component	s.e.	Z Ratio
Rep*Block*Plot	0.003	<i>0.00</i>	6.76	0.004	<i>0.00</i>	8.02
Hybrid Correlation	0.784	<i>0.15</i>	5.35	0.581	<i>0.18</i>	3.21
Hybrid(Suwanee)	0.006	<i>0.00</i>	2.86	0.015	<i>0.00</i>	4.49
Hybrid(Volusia)	0.028	<i>0.00</i>	7.85	0.031	<i>0.00</i>	8.29
Hybrid(Wilcox)	0.007	<i>0.00</i>	3.07	0.010	<i>0.00</i>	3.49
Loblolly Correlation	0.517	<i>0.25</i>	2.04	0.555	<i>0.24</i>	2.30
Loblolly(Suwanee)	0.024	<i>0.00</i>	5.72	0.031	<i>0.00</i>	6.28
Loblolly(Volusia)	0.026	<i>0.00</i>	5.97	0.032	<i>0.00</i>	6.91
Loblolly(Wilcox)	0.027	<i>0.00</i>	6.03	0.043	<i>0.01</i>	6.29
Slash Correlation	0.623	<i>0.08</i>	7.95	0.707	<i>0.07</i>	9.83
Slash(Suwanee)	0.034	<i>0.00</i>	10.95	0.032	<i>0.00</i>	9.45
Slash(Volusia)	0.033	<i>0.00</i>	10.96	0.035	<i>0.00</i>	10.40
Slash(Wilcox)	0.027	<i>0.00</i>	9.61	0.026	<i>0.00</i>	8.29
Error	0.014	<i>0.00</i>	8.30	0.007	<i>0.00</i>	3.54

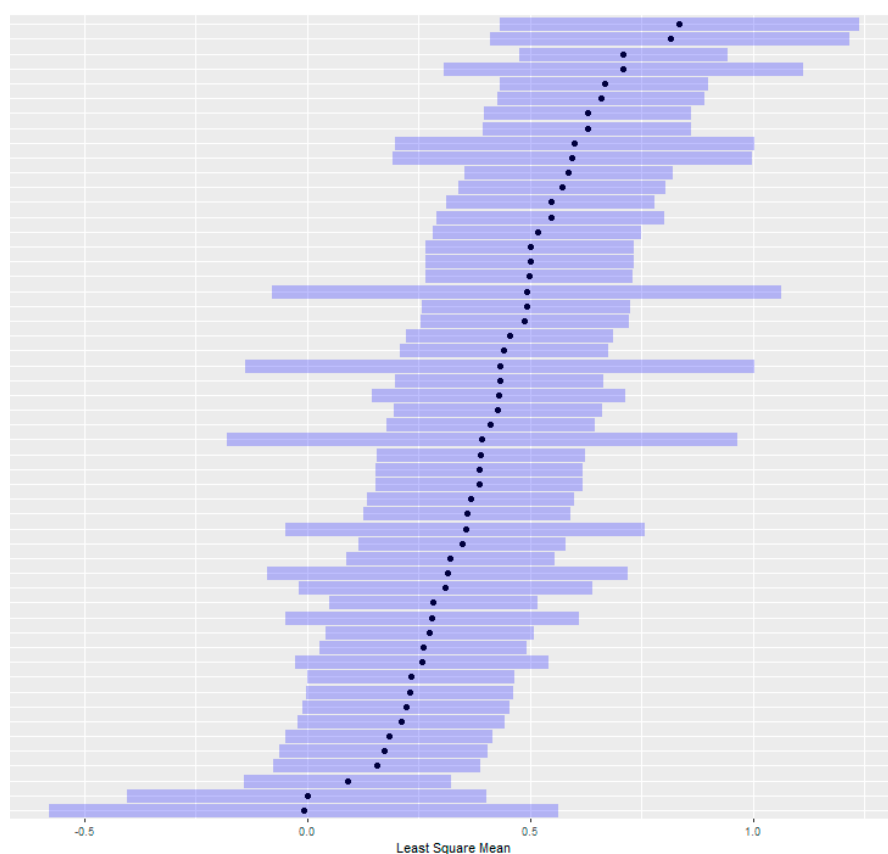


Figure A1. Least square means of full-sib CFGRP slash pine families screened using traditional USFS RSC protocols with confidence intervals. Note that the most tolerant (lower least square mean) families have overlapping confidence intervals with the least tolerant, indicating a lack of statistical differences.

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