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Genetic parameters for *Fusarium circinatum* tolerance within open-pollinated families of *Pinus patula* tested at screening facilities in South Africa and the USA

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The pine pitch canker fungus, *Fusarium circinatum,* has caused large-scale mortality of *Pinus patula* Schiede & Deppe ex Schltdl. & Cham. crops in South African nurseries. This disease is now managed with strict hygiene practices and mortality in commercial nurseries has been drastically reduced. During the last 10 years, however, the disease started to manifest in the field, impacting on post-planting survival. Tree breeders have identified selection and breeding of tolerant material as the likely long-term solution to this disease. This study demonstrates that, under greenhouse conditions with artificial inoculation of young seedlings, there is significant genetic variation in tolerance to *F. circinatum* among open-pollinated *P. patula* families. Tolerant families can be identified and can be utilised in breeding programmes and for seed production. The study provided strong evidence that these artificial inoculation experiments are highly repeatable within specific laboratories, with lower but still meaningful repeatability between different laboratories.

Keywords: artificial inoculation screening, genetic correlation, greenhouse inoculation, heritability, pitch canker fungus, repeatability

Introduction

Pinus patula Schiede & Deppe ex Schltdl. & Cham. is the most widely planted softwood species in South Africa with 338 923 ha, or 52.1% of the total planted softwood area (DAFF 2010). The wood of South African grown *P. patula* is utilised by various private and public concerns for wood, pulp and paper products. *Pinus patula* is grown in the summer rainfall regions of South Africa, including the provinces of Mpumalanga, KwaZulu-Natal and Eastern Cape (DAFF 2010). The production of these trees is, however, threatened significantly by disease caused by the pitch canker fungus (PCF), *Fusarium circinatum* Nirenberg & O'Donnell (Morris 2010; Mitchell et al. 2011).

Fusarium circinatum was first discovered in a South African nursery in 1991 (Viljoen et al. 1994) and it soon spread to all pine-growing nurseries in the country, causing large-scale mortality of nursery seedlings, especially *P. patula* (Mitchell et al. 2011). The disease is now managed in commercial nurseries with strict hygiene practices and mortality of seedlings has been drastically reduced (Morris 2010; Mitchell et al. 2011, 2012a). During the last 10 years, however, the pathogen started to cause mortality of recently planted seedlings in the field, impacting on post-planting survival of trees (Crous 2005). More recently, *F. circinatum* has also been found to cause pitch canker of mature trees in plantations and has been positively identified in 10-year-old *Pinus radiata* grown in the Western Cape province (Coutinho et al. 2007).

Artificial seedling inoculation trials of various pine species have indicated differential responses to *F. circinatum* and indicated that *P. patula* has intermediate tolerance (Hodge and Dvorak 2000). In another seedling inoculation study in which different provenances of *P. patula* and *P. tecunumanii* were evaluated, intermediate tolerance was confirmed, with differences in tolerance among provenances of *P. patula* (Hodge and Dvorak 2007). This intermediate tolerance has also been confirmed in a study in which pine species such as *P. elliottii*, *P. maximinoi*, *P. pseudostrobus* and *P. tecunumanii* were evaluated, and *P. patula* was included as a control (Mitchell et al. 2012b). Certain full-sib controlled-pollinated families of *P. patula* have also shown improved tolerance to *F. circinatum* in a greenhouse screening study (Mitchell et al. 2012c). In-field inoculations of two- to three-year-old trees of various *Pinus* species and hybrids also showed varying susceptibility to *F. circinatum*, between different pine species and between different families of species (Roux et al. 2007).

Artificial seedling inoculations offer the opportunity to identify breeding material with increased levels of disease tolerance. Five independent screening experiments were conducted at two different screening facilities during the period 2003 to 2007. A varying number of *P. patula* families were common among screening experiments, providing an opportunity to study repeatability between different screening events and facilities. In this study, a large number of open-pollinated families of *P. patula* were screened for their tolerance to *F. circinatum* in order to determine the range of tolerance among families. The five screening experiments were carried out in facilities at the

USDA Forest Service Resistance Screening Center (RSC) in Bent Creek, North Carolina, USA, and at the Forestry and Agricultural Biotechnology Institute (FABI) in Pretoria, South Africa. The objectives of the study were to determine the level of repeatability between screenings, the level of correlation among the different screening experiments and facilities, and if meaningful family variation in tolerance to *F. circinatum* was present in *P. patula*.

Materials and methods

RSC Bent Creek screening trials

Open-pollinated seed of 65 *P. patula* families was collected by Sappi Forestry Research and sent to RSC for each of the two screenings. A total of 14 of the screened families were common between the two RSC Bent Creek screening trials. The standard RSC control lot of *P. elliottii* susceptible to pitch canker (FA2) was also included. All seedlots were soaked in cold water for 24 h prior to sowing on 25 March 2003 (RSC-03) and 22 March 2004 (RSC-04) at the RSC. Seedlings were grown in Ray Leach containers (115 ml) for 21 weeks under standard RSC greenhouse conditions, with air temperature maintained at approximately 24 °C, and seasonal day/night length period. Four replications were included in the inoculation trials and families were represented by 20 plants per replication for a total of 80 trees.

Four isolates (S298, S396, S402 and S397) of *Fusarium circinatum*, which were originally collected in Georgia and Florida, were prepared according to procedures developed elsewhere (McRae et al. 1985). Spore-water suspensions of each of the four isolates were prepared at a concentration of 25 000 spores ml^{-1}. In the 2003 study, a mix of three of the four isolates (S298, S396 and S402) was also tested.

All seedlings were subjected to a protocol similar to that described previously (Oak et al. 1987). Seedlings were challenged with the pitch canker fungus and their resultant responses were used to gauge relative tolerance to infection. Inoculations were done when the seedlings were 21 weeks of age. The seedlings were wounded by severing the stem just below the apical meristem and the excised apical portion was removed. The seedlings were inoculated by atomising the aqueous spore suspension onto the fresh wounds. The atomised spore suspension was sprayed directly onto the wound surface from a distance of around 25 cm, passing three times over each tree.

Following inoculation the seedlings were returned to the greenhouse where they were maintained for 12 weeks during which pathogen colonisation was allowed to occur. Stem necrosis was assessed at 12 weeks after inoculation. Inoculated plants that were dead at the time of the assessment were excluded from the analysis. At the first assessment, the length of the stem from hypocotyl to wound, and the length of the stem necrosis caused by the pathogen were measured (this is referred to as height throughout the remainder of this report).

FABI Pretoria screening trials

Open-pollinated seed of 140, 18 and 73 *P. patula* families was collected by Sappi for the three different FABI Pretoria screening trials conducted in 2005, 2006 and 2007, respectively. A varying number of these families were common among the different FABI, as well as the RSC, screening trials. All seedlots were soaked in cold water for 24 h prior to sowing on 21 February 2005 (FABI-05), 12 February 2006 (FABI-06) and 7 August 2007 (FABI-07) at the Sappi Shaw Research Centre nursery, near Howick, South Africa. Seedlings were grown in Unigro 98 containers (90 ml) for 26 weeks in unregulated plastic-covered tunnels. The seedlings were then packed out into trial designs and delivered to the FABI screening facility and maintained under FABI greenhouse conditions, with air temperature maintained at approximately 25 °C, and seasonal day/night length period. Four replications were included in the inoculation trials and families were represented by 21 trees per replication for a total of 84 trees. The plants were allowed to acclimatise to the environment for a four-week period prior to inoculations being carried out. Treatments were challenged with three virulent strains of the pitch canker fungus (FCC 3577, FCC 3578 and FCC 3579) that are maintained in the FABI culture collection. These were used in equal concentration as a mix and the resultant responses were used to determine relative tolerance to infection.

The three *F. circinatum* cultures were grown on half-strength potato dextrose agar (PDA; Merck) for 10 d. The surface of these cultures was then washed from the Petri dishes into sterile glass containers using a 15% glycerol solution. Using a light microscope under 20× magnification, the number of conidia was counted using a haemacytometer. The spore concentration was then adjusted to 50 000 spores ml^{-1} with the sterile 15% glycerol solution. The inoculum was then kept on ice prior to the inoculations being carried out. During inoculation, each plant was topped by cutting the apical meristem tip off to expose a cut surface for the application of inoculum. The plants were inoculated by using a 2–20 µl micropipette and applying an amount of 10 µl per plant to the fresh cut surface on the stem (Porter 2010).

Following inoculation the plants were kept in the greenhouse where they were maintained for eight weeks during which pathogen colonisation was allowed to occur. Stem necrosis was then assessed after this inoculation period. Inoculated plants that were dead at the time of the assessment were excluded from the analysis. At assessment, the total length of each plant (in mm) and the length of the stem necrosis caused by the pathogen (in mm) were measured. The seedling height was measured from the root collar to the cut tip and the lesion length was measured from the inoculation point to where the tissue displayed no lesion expression. Stem-kill, the length of the stem necrosis divided by the total length of the plant, was calculated as a proportion for each plant.

Analysis

All statistical analyses were done using SAS 9.3 (SAS Institute, Cary, NC, USA). In all cases, the variables analysed were Dieback (DB) measured in millimetres, and Stemkill (SK) expressed as a percentage and calculated as Dieback/Seedling Height.

Single-experiment analyses

All five experiments were analysed individually to estimate single-experiment heritabilities. The experiments were analysed with SAS Proc Mixed using Seedling Height as a covariate, Rep and Block(Rep) as fixed effects, and

Family and Family*Block(Rep) as random effects. Single-experiment heritability (h^2) and an average family heritability (h_f^2) were calculated as follows:

$$h^2 = 3\sigma_f^2/(\sigma_f^2 + \sigma_{f\times b}^2 + \sigma_{error}^2) \text{ and}$$

$$h_f^2 = \sigma_f^2/(\sigma_f^2 + \sigma_{f\times b}^2/4 + \sigma_{error}^2/80)$$

where σ_f^2 = Family variance, $\sigma_{f\times b}^2$ = Family × Block(Rep) variance, and σ_{error}^2 = residual variance.

For the RSC experiments, the four replications were inoculated with different *F. circinatum* isolates, so the parameter $\sigma_{f\times b}^2$ = Family × Block(Rep) variance represents a composite of Family × Isolate interaction variance, plus the normal 'error' variance associated with Family × Block(Rep) interaction. For all experiments, a genetic correlation across replications ($r_{g\text{-}block}$) was estimated as

$$r_{g\text{-}block} = \sigma_f^2 / (\sigma_f^2 + \sigma_{f\times b}^2).$$

For the RSC experiments, this parameter can give some indication of the size of Family × Isolate interaction variance.

Multiple-experiment analyses by laboratory

The average genetic parameters across multiple experiments were calculated for each laboratory. SAS PROC Mixed was used with Seedling Height as a covariate, Experiment, Rep(Experiment) and Block(Rep*Experiment) as fixed effects, and Family, Family*Experiment and Family*Block(Rep*Experiment) as random effects. A RANDOM statement allowing for heterogeneous residual error variance was also used. Using these models, best linear unbiased predictions (BLUPs) of Family effects for DB and SK were calculated. Multiple-experiment heritability (h^2) and $r_{g\text{-}exp}$ were calculated as follows:

$$h^2 = 3\sigma_f^2 / (\sigma_f^2 + \sigma_{f\times e}^2 + \sigma_{f\times b}^2 + \sigma_{error}^2) \text{ and}$$

$$r_{g\text{-}exp} = \sigma_f^2 / (\sigma_f^2 + \sigma_{f\times e}^2)$$

where σ_f^2 = Family variance, $\sigma_{f\times e}^2$ = Family × Experiment variance, $\sigma_{f\times b}^2$ = Family × Block(Rep) variance, and σ_{error}^2 = residual variance.

In addition, a genetic coefficient of variation (GCV) was calculated as follows:

$$GCV = \sigma_f / \bar{u}$$

where $\bar{u}$ = the trait mean across all experiments.

For each family, the expected correlation between the predicted General Combining Ability (GCA) and the true GCA [Corr(g,$\hat{g}$)] was calculated as follows:

$$Corr(g,\hat{g}) = [(\sigma_f^2 - SEP) / \sigma_f^2]^{½}$$

where SEP = standard error of the prediction calculated by PROC Mixed.

Paired-experiment analyses

Pairs of experiments were analysed together using Proc Mixed with Seedling Height as a covariate, Experiment, Rep(Experiment) and Block(Rep*Experiment) as fixed effects, and Family, Family*Experiment and Family*Block(Rep*Experiment) as random effects. A SAS RANDOM statement was used to allow for heterogeneous family variances in the two experiments, and to directly estimate the genetic correlation between experiments ($r_{g\text{-}exp}$). Additional RANDOM statements were used to allow for different variances for Family × Block(Rep) and residual error in the two experiments. For the five experiments, there were 10 possible pairs of experiments, but two of the 10 pairs had only two or three common families, insufficient to provide for a good estimate of Family × Experiment interaction variance. All other pairs of experiments had between seven and 39 common families, with an average of 19 common families.

Multiple-experiment analyses

In order to calculate the genetic correlation between laboratories using data from all experiments simultaneously, all five experiments were analysed together using Proc Mixed with the same linear model as described above for the paired-experiment analyses. For this analysis, PROC Mixed RANDOM statements were used to specify a heterogeneous parameter model for the random effects associated with the two different laboratories, and the different experiments within each laboratory. Effectively, these statements allowed PROC Mixed to estimate a different family variance observed in the two laboratories, the genetic correlation between laboratories ($r_{g\text{-}lab}$), Family*Experiment and Family*Rep(Block) variance in each laboratory, and a different residual error variance in each experiment.

Results and discussion

Experiment means and genetic parameters

There were large differences in seedling size among the five experiments (Table 1), with heights ranging from 71.0 to 275.5 mm. The two tests done at the RSC had substantially larger seedlings, approximately two- to three-times as tall as the seedlings in the three FABI experiments, despite the fact that the seedlings in the RSC experiments were inoculated at 21 weeks of age versus 30 weeks of age in the FABI experiments. The growth differences between the two facilities may be due to differences in temperatures in the two greenhouses and different growing conditions in the two countries.

Dieback means were different in the RSC and FABI experiments, with higher values in the RSC experiments (from 80 to 132 mm) compared to the FABI experiments (from 14 to 31 mm). For the variable Stemkill, expressing stem necrosis as a percentage of seedling height, the means from the two laboratories were more similar, with Stemkill values of 42–51% at the RSC and 21–30% in the

Table 1: Summary statistics for five laboratory experiments to screen *P. patula* families for tolerance to *F. circinatum*

Experiment	No. of families	Height (mm)	Dieback (mm)	Stemkill (%)
RSC-03	65	272.5	130.9	50.9
RSC-04	65	209.5	81.3	42.3
FABI-05	140	78.9	21.5	29.6
FABI-06	18	115.7	30.6	27.8
FABI-07	73	71.0	14.1	20.9

Table 2: Genetic parameter estimates from single-experiment analyses of five laboratory experiments to screen *P. patula* families for tolerance to *F. circinatum*

Experiment	Mean	h^2	$r_{g\text{-blocks}}$	h_f^2	σ^2_{fam}	σ^2_{fxb}	σ^2_e	σ^2_{phen}
				Dieback (mm)				
RSC-03	130.9	0.27	0.64	0.79	815	458	7764	9037
RSC-04	81.3	0.41	0.71	0.85	386	157	2279	2822
FABI-05	21.5	0.26	0.72	0.81	39	15	398	452
FABI-06	30.6	0.30	0.63	0.80	10	6	81	96
FABI-07	14.1	0.32	0.61	0.80	15	9	115	139
				Stemkill (%)				
RSC-03	50.9	0.32	0.71	0.83	126	51	1023	1200
RSC-04	42.3	0.44	0.75	0.87	102	33	557	692
FABI-05	29.6	0.38	0.73	0.81	33	12	329	374
FABI-06	27.8	0.27	0.56	0.82	18	15	175	208
FABI-07	20.9	0.27	0.62	0.76	34	21	218	274

FABI experiments (Table 1). The rate of dieback may be related to the age of the seedlings, the degree of lignification in the stems, the different inoculum strains, and the conditions in the greenhouse, with higher temperatures favouring faster growth of the fungus.

Despite the large differences in mean growth, dieback, and stemkill, the single-experiment heritabilities were very similar across the five experiments. Average heritability for Dieback in the two RSC experiments was $h^2 = 0.34$ and in the three FABI experiments $h^2 = 0.29$ (Table 2). For Stemkill, average heritability was $h^2 = 0.38$ at the RSC and 0.30 at FABI (Table 2). For both Dieback and Stemkill, single-experiment family-heritability estimates were quite high, ranging from $h_f^2 = 0.76$ to 0.87, with an average $h_f^2 = 0.81$. This indicates that regardless of laboratory, tree size or rate of stem necrosis, an artificial screening experiment will produce similar family ranks.

Table 3: Means for isolate-replication in 2003 and 2004 RSC experiments to screen *P. patula* families for tolerance to *F. circinatum*. Stem necrosis measurements were taken at 20 weeks post-inoculation for the 2003 experiment, and 12 weeks post-inoculation for the 2004 experiment

Experiment	Isolate	Seedling height (mm)	Dieback (mm)	Stemkill (%)
RSC-03	S298	279.2	161.6	60.4
	S396	294.5	139.7	49.9
	S397	253.5	144.6	59.7
	S402	262.8	77.6	33.7
	Mean	272.5	130.9	50.9
RSC-04	S298	224.2	85.2	42.1
	S396	218.9	91.1	45.9
	S397	194.4	79.9	43.9
	S402	200.5	69.1	37.4
	Mean	209.5	81.3	42.3

Isolate × genotype interaction in RSC experiments

In the RSC experiments, four different *F. circinatum* isolates from the southern USA were used. One isolate was applied to each Block(Rep), thus confounding the effects of Block(Rep) and Isolate. In these experiments, *F*-tests indicated that there were statistically significant differences among Block(Rep)–Isolate for the two variables Dieback and Stemkill. The Block(Rep) treated with isolate S402 had the lowest amount of dieback and stemkill in both the 2003 and 2004 RSC experiments (Table 3).

In the same way that the effects of Block(Rep) and Isolate are confounded in the RSC experiments, the effects of Family × Block(Rep) interaction variance are confounded with Family × Isolate interaction variance. The parameter $r_{g\text{-block}}$ expresses the genetic correlation of family tolerance across different replications in the same experiment (Table 2). Alternately, one can interpret it as a measure of Family × Replication variance ($\sigma^2_{f\times b}$); if this variance is low, then family performance across replications will be highly correlated. In the two RSC studies, average $r_{g\text{-block}}$ was 0.68 for Dieback and 0.73 for Stemkill. In the three FABI experiments, average $r_{g\text{-block}}$ was 0.65 for Dieback and 0.64 for Stemkill (Table 2). The $r_{g\text{-block}}$ for the FABI experiments reflects only Family × Block(Rep) interaction, whereas the $r_{g\text{-block}}$ for the RSC experiments reflects Family × Block(Rep) interaction plus Family × Isolate interaction variance (if it exists). Although these data cannot provide a definitive result, the similarity of these parameters in the RSC and FABI experiments suggests that there is little Family × Isolate interaction variance for the four isolates used at the RSC. In other words, there may be differences in virulence among these four southern USA isolates, but families tolerant to one isolate are likely to be tolerant to others.

Genetic parameter estimates for laboratories

Multiple-experiment genetic parameter estimates for the two laboratories are presented in Table 4. The linear model specified homogeneous parameters across experiments (within laboratories) for the random effects Family, Family*Experiment, and Family*Rep(Block), and residual error variances among experiments. In general, the parameter estimates reflect the results from the single-experiment and paired-experiment analyses. Heritability estimates for Dieback and Stemkill are moderately high, ranging from 0.22 to 0.31. Genetic correlations between experiments in the same laboratory are very high, ranging from 0.78 to 1.00. Inspection of the single-experiment genetic parameters (Table 2) indicates that this assumption is probably more closely met for the variable Stemkill than for the variable Dieback, so perhaps the $r_{g\text{-exp}}$ values for

Table 4: Genetic parameter estimates for Dieback and Stemkill from multiple experiments to screen *P. patula* families for tolerance to *F. circinatum* at the RSC in the USA and at FABI in South Africa

Laboratory	Mean	h^2	GCV	$r_{g\text{-}exp}$	σ^2_{fam}	σ^2_{fxe}	σ^2_{fxb}	σ^2_e	σ^2_{phen}
				Dieback (mm)					
RSC	106.1	0.22	0.20	0.78	464	128	331	5 268	6191
FABI	22.1	0.30	0.25	1.00	31	0	13	266	310
				Stemkill (%)					
RSC	46.6	0.30	0.21	0.85	96	17	44	811	968
FABI	26.1	0.31	0.22	0.97	33	1	16	273	323

Stemkill (0.85 and 0.97 for the RSC and FABI, respectively) better reflect the true parameter values (Table 4).

The GCV values for Dieback and Stemkill seem substantial, ranging from 0.20 to 0.25 (Table 4). With intensive screening, it might be possible to find individuals up to 2 additive genetic standard deviations from the mean. This would be equivalent to 2*GCV, which equals a 40–50% reduction in Dieback or Stemkill.

Genetic correlations between experiments and laboratories

Results of the pairwise analyses are presented in Table 5. For experiments done in the same laboratory, the genetic correlations are very high. For the two RSC experiments, $r_{g\text{-}exp}$ = 0.93 for Stemkill and 0.95 for Dieback. For the two FABI experiments, $r_{g\text{-}exp}$ = 0.95 for Stemkill and 0.86 for Dieback. These genetic correlation values are very similar to those found in another South African study testing 17 common *P. patula* families in a nursery inoculation experiment (Mitchell et al. 2014). Genetic correlations between experiments done at the RSC and at FABI are lower, but are still relatively high, with average $r_{g\text{-}exp}$ = 0.70 for Stemkill and 0.69 for Dieback (Table 5). There were six pairs of RSC–FABI experiments where there were sufficient families (seven to 39) to estimate a genetic correlation. One of the pairs, RSC-03 and FABI-05T, with the lowest number (seven) of common families, had a much lower correlation than the other five. For Stemkill, the $r_{g\text{-}exp}$ for that pair was 0.38, whereas the other five experiments had an average $r_{g\text{-}exp}$ = 0.76. For Dieback, the $r_{g\text{-}exp}$ for that pair was 0.23, whereas the other five experiments had an average $r_{g\text{-}exp}$ = 0.78.

Note that these six estimates of $r_{g\text{-}exp}$ reflect the genetic correlation between family tolerance in an RSC experiment and a FABI experiment. The multiple-experiment analysis of all five tests was used to estimate the average between-laboratory genetic correlation (or in other words, the average $r_{g\text{-}exp}$ for an RSC-FABI pair of experiments). The resulting between-laboratory genetic correlation estimates were $r_{g\text{-}lab}$ = 0.63 ± 0.12 for Stemkill and $r_{g\text{-}lab}$ = 0.74 ± 0.12 for Dieback.

Finally, one can also examine the between-laboratory genetic correlation ($r_{g\text{-}lab}$) by comparing GCA predictions resulting from the two independent laboratories. Table 4 indicates a small amount of Genotype × Experiment interaction for Stemkill at the RSC ($r_{g\text{-}exp}$ = 0.85), and near-zero interaction at FABI ($r_{g\text{-}exp}$ = 0.97), so it is clear that repeat experiments in the same laboratory are evaluating essentially the same genetic trait. A duplicate trial at the RSC would help to average out the small amount of Genotype × Experiment interaction that exists.

Table 5: Genetic correlations (± SE) between pairs of experiments to screen *P. patula* families for tolerance to *F. circinatum*. Correlations for Stemkill (%) are above the diagonal, and those for Dieback (mm) are below the diagonal. The value in parentheses indicates the number of common families for each correlation. Values in bold are correlations between experiments done in the same laboratory, and values in italics are correlations between experiments done in different laboratories

	RSC-03	RSC-04	FABI-05	FABI-06	FABI-07
RSC-03		**0.93 ± 0.10** (14)	*0.38 ± 0.28* (14)	*0.83 ± 0.14* (14)	*0.66 ± 0.15* (29)
RSC-04	**0.95 ± 0.11** (14)		*0.96 ± 0.09* (17)	*0.85 ± 0.20* (7)	*0.51 ± 0.15* (39)
FABI-05	*0.23 ± 0.32* (14)	*0.90 ± 0.12* (17)			
FABI-06	*0.75 ± 0.18* (14)	*0.87 ± 0.18* (7)			**0.95 ± 0.10** (16)
FABI-07	*0.74 ± 0.14* (29)	*0.62 ± 0.14* (39)		**0.86 ± 0.13** (16)	

There were 11 families tested in two trials at the RSC that were also tested at FABI. In general, for this group of 11 families, there was good agreement between the BLUPs for Stemkill from the two independent laboratories (Figure 1), with an overall correlation of r = 0.78. There was a group of families that was clearly tolerant in both laboratories (i.e. negative predicted GCA values, indicating less Stemkill). There is a group of families that were average or below-average for tolerance in both laboratories (i.e. positive predicted GCA values, indicating higher Stemkill). There was one family that performed below-average at FABI (GCA = +3.8), but rather well at the RSC (GCA = −6.5). Considering all the data, it seems clear that families identified as tolerant in one laboratory will also be identified as tolerant in another laboratory.

A very important aspect of artificial inoculation studies under laboratory conditions is how these results relate to post-planting survival in plantations. Previous reported studies for *F. circinatum* have indicated that at the species and hybrid level, there was a good correlation between laboratory results and survival under post-planting plantation conditions (Mitchell et al. 2012b, 2012c). This study did not aim to provide evidence at the family level for this latter aspect.

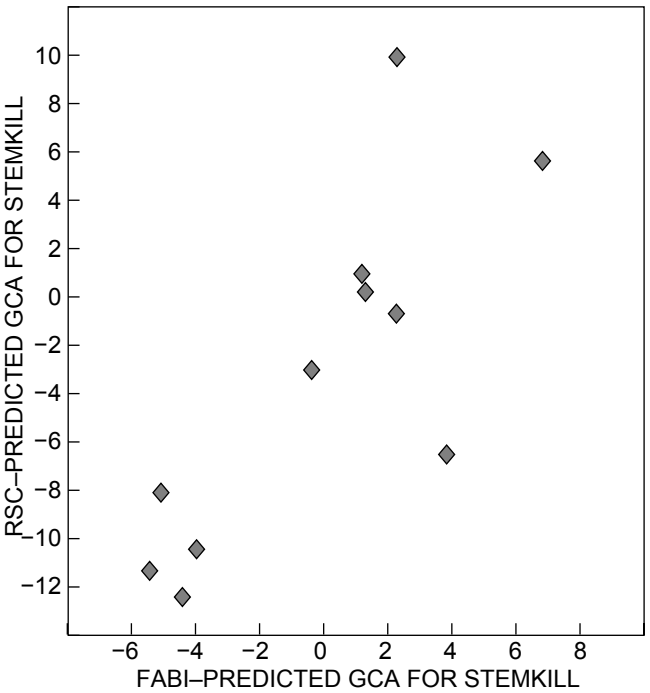

Figure 1: Comparison of predicted GCA values for Stemkill from *F. circinatum* tolerance screening in two laboratories (the RSC in the USA and FABI in South Africa). Data points are 11 families of *P. patula* tested in two tests at the RSC

Conclusions

The objectives of the study were to determine if meaningful family variation to tolerance to *F. circinatum* was present in *P. patula,* the level of repeatability between screenings, and the level of correlations among the different screenings and facilities. This study showed that artificial screening experiments can identify genetic variation among a large number of open-pollinated production *P. patula* families for *F. circinatum* tolerance. High repeatability was demonstrated, especially in the same laboratory, with individual tree heritability estimates of around 0.30 and family heritability of around 0.80 in these experiments, using four replications providing a total of 80 trees per family. High genetic correlation between experiments in the same lab ($r_{g\text{-}exp}$ around 0.86 to 0.95) was found. Genetic correlations between different laboratories were lower, but still fairly high considering that there were differences in greenhouse growing conditions, inoculation method, isolates and plant age at the time of inoculation.

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