The tolerance of *Pinus patula x Pinus tecunumanii*, and other pine hybrids, to *Fusarium circinatum* in greenhouse trials

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Abstract

The field survival of *Pinus patula* seedlings in South Africa is frequently below acceptable standards. From numerous studies it has been determined that this is largely due to the pitch canker fungus, *Fusarium circinatum*. Other commercial pines, such as *P. elliottii* and *P. taeda*, show good tolerance to this pathogen and better survival, but have inferior wood properties and do not grow as well as *P. patula* on many sites in the summer rainfall regions of South Africa. There is, thus, an urgent need to improve the tolerance of *P. patula* to *F. circinatum*. Operational experience indicates that when *P. patula* is hybridized with tolerant species, such as *P. tecunumanii* and *P. oocarpa*, survival is greatly improved on the warmer sites of South Africa. Field studies on young trees suggest that this is due to the improved tolerance of these hybrids to *F. circinatum*. In order to test the tolerance of a number of pine hybrids, the pure species representing the hybrid parents, as well as individual families of *P. patula* x *P. tecunumanii*, a series of greenhouse screening trials were conducted during 2008 and 2009. The results indicated that species range in tolerance and hybrids, between *P. patula* and these species, are intermediate in tolerance to *F. circinatum*. Within *P. patula x P. tecunumanii*, large family variation exists when pollen from the high elevation source of *P. tecunumanii* is used. The results of these studies illustrate the importance of developing pine hybrid breeding programs to overcome the susceptibility of our pure species to pathogens such as *F. circinatum*.

Keywords; Forestry, disease tolerance, hybrids, greenhouse screening

Introduction

The successful establishment of South Africa's most important pine species, *Pinus patula* (DAFF 2010), is severely hampered by the pitch canker fungus, *Fusarium circinatum* (Mitchell et al. 2011). The greatest loss is seen in the field during the first six months post planting (Crous 2005). Currently, breeders are trying to improve the tolerance of *P. patula* to *F. circinatum* by screening for superior families using greenhouse inoculation studies. However, the level of tolerance within *P. patula* open-pollinated families does not seem to consistently translate into increased post-planting survival (A. Nel, Sappi forests, pers. com). In the immediate term, the problem can be overcome by planting *P. elliottii* and *P. taeda* on sites where *P. patula* is best suited. These, however, do not grow as well as *P. patula* on temperate sites, and have inferior wood properties for some applications (Kietzka 1988; Morris and Pallett 2000; Malan 2003).

As an alternative to planting pure species, there is a growing interest in South Africa to identify hybrids between *Pinus patula* and other pines to reduce the susceptibility of young *P. patula* seedlings to *Fusarium circinatum*. *Pinus patula x P. tecunumanii* is exhibiting good tolerance to *F. circinatum* (Roux et al. 2007) and growth (Nel et al. 2006; Kanzler et al. 2012), with numerous forestry companies deploying this hybrid on a commercial scale. Although *P. patula* x *P. tecunumanii* is exhibiting improved tolerance operationally, no selection for tolerance to *F. circinatum* has been carried out within the hybrid. Previous studies have shown that there is large provenance variation for tolerance to *F. circinatum* in both *P. patula* and *P. tecunumanii* (Hodge and Dvorak, 2006). This variation is closely related to the altitude where provenances naturally occur (Hodge and Dvorak, 2006). Provenances of *P. tecunumanii*, occurring below 1500 m (low elevation, *P. tecunumanii* (L)) in their home range are more tolerant to *F. circinatum* than those occurring above this altitude (high elevation, *P. tecunumanii* (H)) (Hodge and Dvorak 2000; 2006). Therefore, the variation in tolerance to *F. circinatum* among *P. patula* and *P. tecunumanii* suggests that *P. patula* x *P. tecunumanii* families are also likely to vary in tolerance. In addition to *P. tecunumanii*, *P. patula* has been successfully hybridized with a number of other species in South Africa including *P. oocarpa, P. elliottii, P. pringlei, P. greggii, P. taeda, P. herreare, P. maximinoi* and *P. caribaea.* A number of these, and other hybrids not necessarily with *P. patula*,

may in the future be planted in order to capture good characteristics which would include tolerance to *F. circinatum*.

The primary objective of this study was to examine the genetic control of *F. circinatum* tolerance among *P. patula x P. tecunumanii* hybrid families. A secondary objective was to assess the tolerance of a wide array of different pine hybrids in order to identify other potential hybrids that could be used to replace *P. patula* to reduce losses associated with *F. circinatum*. These objectives were investigated using artificial inoculation of seedlings and cuttings in a series of three greenhouse screening trials carried out in 2008 and 2009.

Materials and Methods

Pinus patula x Pinus tecunumanii

The first greenhouse trial (Trial 1: *P. pat x P. tec*) was conducted in November, 2008, and focused on the *P. patula x P. tecunumanii* hybrids. Included in the study were 75 *P. patula x P. tecunumanii* (L) families (*P. pat x P. tec*L) produced from crossing 13 *P. patula* parents and 12 *P. tecunumanii* (L) parents. There were also 24 *P. patula x P. tecunumanii* (H) families (*P. pat x P. tec*H) produced from crossing 10 *P. patula* parents and 7 *P. tecunumanii* (H) parents. All hybrid crosses were made using *P. patula* as the female parent, and *P. tecunumanii* as the male parent (see Appendix 1 for the specific crossing design). All hybrid families were screened for tolerance to *F. circinatum* using cuttings from juvenile seedling hedges. In addition, the trial also contained control-pollinated seedlings of 21 of the 75 *P. pat x P. tec*L and 6 of the 24 *P. pat x P. tec*H families. Finally, the trial also included open pollinated pure-species *P. patula* seedlings from the 13 *P. patula* mothers that were used as female parents of the hybrids, and bulk seedlings of a *P. elliottii* control (Table 1).

Other hybrids and species

In the second trial (Trial 2: Hybrids), cuttings from 14 hybrids (produced from 9 *Pinus* species), were screened in March 2009 (Table 1). Of the 14 hybrids, 6 were produced using *P. patula* as the female parent. Open-pollinated *P. patula* and *P. elliottii* seedlings, from commercial seed orchards, were included as controls. In order to compare the tolerance of the hybrids with the

pure species used to produce the hybrids, open-pollinated seedlings, representing 8 of the 9 parent species, were inoculated in a third trial (Trial 3: Species) in December 2009 (Table 1).

Plant growing conditions

The plants were raised in the Komatiland Forests Research nursery near the town of Sabie. Both plant types were grown in composted pine bark in plastic seedling containers with removable individual inserts of 0.09 dm3 capacity (Unigro98©), under covered greenhouse plastic and irrigated and fertilized where necessary. Cuttings were raised for approximately 9 months whilst seedlings were raised for approximately 7 months. At the end of the nursery phase, treatments were arranged in a randomized complete block design across, in most cases, 4 replications. Subject to plant availability each plot consisted of 12 - 22 plants. After packing the trials out, the plants were transported to the greenhouse screening facility at the University of Pretoria.

Inoculation procedure

Inoculation was carried out by removing the plant's apical bud with sharp secateurs and applying 500 spores of *F. circinatum* in a water solution to the wounded surface. The solution contained an equal mixture of 3 highly virulent South African isolates (CMW 3577, 3578, and 3579). Once inoculated, the trial was watered daily and assessed for lesion development 8 weeks after inoculation. Lesion development was read in mm from the tip of the seedling at the point of inoculation, to the point where the tissue showed no further visible necrosis. The seedling height, from the root collar to the wounded tip, was also measured. The proportion of lesion length, to the length of the seedling, was expressed as a percentage dieback. New shoot development, below the infected area, was measured in the *P. patula x P. tecunumanii* and species trial and is referred to as "resprout".

Statistical analysis – Trial 1: P. pat x P. tec

The statistical software package, SAS (SAS Institute, 2003) was used to analyse the data generated from the *P. patula x P. tecunumanii* trial (Trial 1). Pearson product moment correlations of family means for lesion length, percentage dieback, resprout and initial plant height were calculated, primarily to determine if there was a need to include height as a covariate

in subsequent models. Following this, a series of analyses of variance (ANOVAs) were done using SAS Proc GLM, and a series of variance component analyses were done using SAS Proc Mixed.

First, an analysis was done to examine if there were differences among the different species or hybrids and plant types (i.e., seedlings or cuttings). The data set included the open-pollinated seedlings of *P. patula* and *P. elliottii*, and both the seedlings and cuttings of *P. pat x P. tec*L and *P. pat x P. tec*H. The linear model included fixed effects for replicate (rep), species, and plant type, and random effects for family(species), type*family(species), rep*type*family(species), and initial height as a covariate. Duncan's multiple range tests were used to examine differences among species/hybrids - plant type combinations.

Next, a more focused analysis was done on the hybrid plants to examine if there was a difference in tolerance among the type of plant, i.e., seedlings and cuttings. The linear model for the combined data set (with both hybrids) included fixed effects for replicate, hybrid, plant type, and hybrid*type, and random effects for family(hybrid), type*family(hybrid), rep*type *family(hybrid), and initial height as a covariate. Separate ANOVAs were also done for the *P. pat x P. tec*L and *P. pat x P. tec*H data sets, with a reduced model eliminating effects for hybrid and hybrid*type. An ANOVA was also done on the open-pollinated *P. patula* data set to test for family differences in tolerance. The linear model included fixed effects for replicate, and random effects for family and rep*family, and initial height as a covariate.

Finally, variance component analyses for lesion length were done using SAS Proc Mixed to estimate genetic parameters for tolerance to *F. circinatum*. The analysis for the open-pollinated *P. patula* families used a model including fixed effects for replicate, and random effects for family and rep*family, and initial height as a covariate. Narrow-sense heritability (h^2) was estimated for lesion length for *P. patula* according to Dieters et al. 1995 as $h^2 = (3 \times \sigma^2_{female}) / \sigma^2_{phenotypic}$, where σ^2_{female} = variance due to *P. patula* female parent, and $\sigma^2_{phenotypic}$ = total phenotypic variance for lesion length. Separate variance component analyses were done for the *P. pat x P. tec*L and *P. pat x P. tec*H data sets using all plants of both types (cuttings and seedlings). The linear model included a fixed effect for replicate, and random effects for the *P.*

patula female parent, the *P. tecunumanii* male parent, the interaction of *P. patula x P. tecunumanii* parents, type*family, and type*rep*family, along with initial height as a covariate. For the hybrid data sets, the variance associated with *P. patula* female parent was taken to be the variance of general hybridizing ability (GHA) for *P. patula* ($\sigma^2_{GHA\text{-}pat}$), the variance associated with *P. tecunumanii* male parent was taken to be the GHA variance for *P. tecunumanii* ($\sigma^2_{GHA\text{-}tec}$), and the variance associated with the *P. patula x P. tecunumanii* interaction was taken to be the variance of specific hybridizing ability (SHA) ($\sigma^2_{SHA\text{-}pat\ x\ tec}$). Total phenotypic variance was estimated as the sum of all the variance components, and the percentage of variance accounted for each component was calculated. Total genetic variance among full-sib hybrid families was estimated as $\sigma^2_{G\text{-}FS} = \sigma^2_{GHA\text{-}pat} + \sigma^2_{GHA\text{-}tec} + \sigma^2_{SHA\text{-}pat\ x\ tec}$. Following Dieters et al. (1997), two separate estimates of heritability were calculated for the hybrid data sets as follows:

$$h^2_{pat} = (4 \times \sigma^2_{GHA\text{-}pat}) / \sigma^2_{phenotypic}$$

$$h^2_{tec} = (4 \times \sigma^2_{GHA\text{-}tec}) / \sigma^2_{phenotypic}$$

In addition, proportion of dominance (d^2) was calculated for the hybrid data sets as:

$$d^2_{pat\ x\ tec} = (4 \times \sigma^2_{SHA\text{-}pat\ x\ tec}) / \sigma^2_{phenotypic}$$

Statistical analysis – Trial 2: Hybrids, Trial 3: Species

The statistical software package GenStat 7.22 (2008) was used to analyse the data generated from the hybrid and species trials (Trials 2 and 3). Pearson product moment correlations of family means for lesion length, percentage dieback, resprout and initial plant height were calculated, primarily to determine if there was a need to include height as a covariate in subsequent models. Following this, an ANOVA was done on percentage dieback, lesion length, and length of the resprout with a model containing initial height as a covariate, and fixed effects for replicate, species/hybrid, and rep*species/hybrid. A Duncan's Multiple Range test was used to distinguish differences between species and hybrids.

Results

Trial 1: P. pat x P. tec

Plant height correlated significantly ($p<0.001$) with lesion length ($r = -0.41$), percent dieback ($r = -0.63$) and resprout ($r = -0.30$), and was used as a covariate in all analyses. Percent dieback and lesion length were highly correlated ($p<0.001$, $r = 0.92$) and lesion length was used to rank and compare treatments. There were clear differences for lesion length among species and hybrids. *P. elliottii* was the most tolerant (5.7 mm) followed by *P. pat x P. tec*L (7.5 mm), *P. pat x P. tec*H (17.1 mm) and *P. patula* (29.4 mm) (Table 2). There was no evidence for differences in tolerance among plant types (i.e., cuttings and seedlings) for lesion length (Tables 2, 3). When comparing percentage dieback, cuttings were significantly more tolerant than seedlings. Since lesion lengths for the cuttings and seedlings were similar, this seems attributable primarily to the fact that the cuttings were more than twice as tall as the seedlings. There was good evidence for significant differences in tolerance among hybrid families (Table 3). When each hybrid was analysed separately, families differed at the 10% level ($p\approx0.07$) and when the data was combined, differences among families were highly significant ($p=0.006$) (Table 3).

The range of family tolerance in *P. pat x P. tec*H was large where 23 out of the 24 families displayed lesions ranging from 5.5 to 25.4 mm in length, with the most susceptible family measuring 37 mm. Fifty percent of the *P. pat x P. tec*H families (centered around the median) displayed lesion lengths that ranged from 9 to 18 mm (Fig 1). The range in family tolerance of *P. pat x P. tec*L was smaller where 74 out of 75 families had lesion lengths ranging from 3.4 to 14.9 mm with the most susceptible family measuring on average 25.6 mm. Fifty percent of the *P. pat x P. tec*L families (centered around the median) displayed lesion lengths that ranged from 6 to 8 mm (Fig. 1). The range in tolerance of the 13 *P. patula* families was reflected by lesions from 17.3 to 40.3 mm in length where fifty percent (centered around the median) ranged from 25 to 32mm. Despite the large range, family differences in *P. patula* were not statistically significant ($p = 0.43$) (Table 3).

Family differences in *P. patula x P. tecunumanii* were primarily due to a result of the specific interaction between the *P. patula* and *P. tecunumanii* parents (i.e., $\sigma^2_{SHA\text{-}pat\ x\ tec}$), and to a lesser

extent the general ability of *P. patula* parents or *P. tecunumanii* parents to confer tolerance to their hybrid offspring (i.e., $\sigma^2_{GHA\text{-}pat}$ or $\sigma^2_{GHA\text{-}tec}$). This can be seen in Table 4 by comparing the *P. patula* and *P. tecunumanii* parental variance components to the *P. pat x P. tec* interaction variance component. In the case of *P. pat x P tec*H, SHA variance accounted for 9.6% of the phenotypic variance, while $\sigma^2_{GHA\text{-}pat}$ accounted for 6.4% of the phenotypic variance, and $\sigma^2_{GHA\text{-}tec}$ accounted for only 2.1% of the phenotypic variance (Table 4). The total genetic variance for hybrid families of *P. pat x P tecH* was 18.1% of the phenotypic variance. In the case of *P. pat x P tec*L, even though there was relatively little genetic variance among hybrid families (6.8% of the total phenotypic variance), SHA variance accounted for the bulk of that variance (4.2% of the phenotypic variance). The variation due to the *P. patula* and *P. tecunumanii* parents accounted for only 0.8% and 1.8% of the phenotypic variance, respectively (Table 4). Among the *P. patula* families, there was little genetic variation observed, corresponding to a narrow sense heritability of $h^2 = 0.06$ (i.e., 6% of the total phenotypic variance, Table 4). In contrast, there was more genetic control of tolerance in the *P. pat x P tec*H data set, with $h^2_{pat} = 0.25$ and $h^2_{tec} = 0.08$ (Table 4).

Trial 2: Hybrids, Trial 3: Species

Similar to the *P. patula x P. tecunumanii* data, lesion length correlated strongly with percent dieback both in the hybrid ($p<0.0001$, $r = 0.959$) and in the pure species trial ($p<0.0001$, $r = 0.946$), and was accordingly used to rank treatments. Seedling height had a significant negative effect on percent dieback in the hybrid ($p <0.001$, $r = -0.217$) and pure species trial ($p<0.0001$, $r = -0.263$) but had no effect on lesion length. Lesion length and resprout correlated negatively ($p<0.001$, $r = -0.450$) in the species trial indicating that the more tolerant species produced longer shoots after wounding and infection.

Pinus greggii var. *greggii* (*P. greg*N) ranked most susceptible (mean lesion length = 38 mm) in the species trial followed by *P. patula* (mean lesion length = 29.8 mm), *P. greggii* var. *australis* (*P. greg*S) (mean lesion length = 18.5 mm), *P. tecunumanii* (H) (mean lesion length = 9.2 mm), *P. elliottii* (mean lesion length = 8.8 mm), *P. caribaea* (mean lesion length = 8 mm), *P. tecunumanii* (L) (mean lesion length = 5.1 mm), *P. oocarpa* (mean lesion length = 4.2 mm) and *P. pringlei* (mean lesion length = 3.8 mm) (Table 5). All hybrids made with *P. patula* were

significantly more tolerant than *P. patula* (Fig. 2). In general, there was some correspondence between the tolerance of pure species, and the tolerance of *P. patula* hybrids. Hybrids made with *P. tecunumanii* (H), *P. greggii* (S), and *P. pringlei* were the most susceptible (Fig. 2) and more susceptible than *P. elliottii*. Hybrids made with *P. patula* and some of the most tolerant species (*P. oocarpa*, *P. tecunumanii* (L) and *P. elliottii*) were similar in tolerance to *P. elliottii*, and no different than hybrid combinations made between two tolerant species (Fig. 2). One surprising exception to this general pattern was that *P. patula* x *P. pringlei* was similar in tolerance to *P. pat* x *P. tec*H despite the fact that *P. pringlei* is highly tolerant as a pure species (Hodge and Dvorak 2000) which was also seen in Trial 3 (Table 5). This may be explained by the fact that there were an insufficient number of plants (28) of the hybrid, which were only represented across 2 replications, to obtain an accurate indication of the tolerance of this hybrid. This was also supported by a large standard error of the mean (3.44 mm).

Discussion

Standard quantitative genetics theory is based on assumptions of random mating populations of a pure species in genetic equilibrium. Hybrid populations are not in genetic equilibrium, and it is not clear that the concepts of additive genetic variance or heritability are appropriate for F_1 hybrids, or that hybrid heritabilities would predict genetic gain from forward selection of the best offspring of F_1 hybrids (Gordon 1999). However, there are no such difficulties when using hybrid progeny test data and genetic parameters to identify superior parents of hybrid families and to predict genetic gain from backward selection of the best hybrid parents and families. The parents of those families could then be re-crossed, and the resulting progeny mass propagated.

The data in this study suggest that substantial improvement in tolerance in *P. pat x P. tec*H can be made through identification of superior families. In this case family variation in tolerance is due mostly to the combination of specific parents (i.e., high $\sigma^2_{SHA\text{-}pat\ x\ tec}$, see Table 4), and this indicates that it will be necessary to screen all *P. pat x P. tec*H families. Relative to pure *P. patula*, there was improved tolerance of the *P. pat x P. tec*H hybrid to *F. circinatum*. Overall, however, *P. pat x P. tec*H was not as tolerant as *P. pat x P. tec*L. There was also much larger

variation seen among the 24 *P. pat x P. tec*H families tested, and some families were as susceptible as *P. patula* (Fig. 2).

With the very small amount of genetic variation observed among a large number of *P. pat x P. tec*L families tested, it is clear that it is not necessary to screen families of *P. pat x P. tec*L for *F. circinatum* tolerance. Although this hybrid is doing very well in field trials, it is restricted to warm temperate sites and is therefore likely to replace only a small portion of area planted to *P. patula*. It will, however, provide a good alternative to a number of other species such as *P. elliottii* and the popular *P. elliottii x P. caribaea* hybrid. Also, studies have shown that the tolerance of *P. tecunumanii* to frost is under genetic control (Mitchell et al. 2012) indicating that through selection the planting range of *P. pat x P. tec*L could be increased.

In the *P. patula x P. tecunumanii* trial, although the cuttings had slightly shorter lesion lengths, these differences were not statistically significant. There is, however, other evidence to suggest that cuttings are more tolerant, which concurs with general experience. In the nursery, cuttings appear less affected during outbreaks of *F. circinatum* (unpublished) and often survive better after planting (Mitchell et al. 2004). If this is so, it may be related to an increase in the maturation state of the plant (Zagory and Libby 1985; Mitchell et al. 2004). Elsewhere, studies on *P. radiata* have shown cuttings to be more tolerant than seedlings to *Endocronartium harknessii* (Power et al. 1994) and *P. taeda* cuttings show more tolerance to *Cronartium quercuum* than seedlings (Frampton et al. 2000). Further work in this field is needed.

Analysis of variance revealed no statistically significant variation in tolerance to *F. circinatum* amongst *P. patula* families (Table 3), and variance component analysis indicated low (although non-zero) heritability ($h^2 = 0.06$, Table 4). This lack of variation in tolerance could be explained by the fact that many of the *P. patula* seedlings died after inoculation due to their small size (average 68 mm), or from the limited number (13) families tested leading to an unbalanced representation across replications. Although the planting of *P. pat x P. tec*H may be extended to include some temperate sites, *P. patula* will remain an important species on high altitude sites due to its good growth and wood properties once successfully established. Therefore, if it is

possible, it would be very useful to identify *P. patula* families that are tolerant to *F. circinatum* although the genetic parameter estimates from this study suggest that this will be a challenge.

The tolerance of hybrids generated with tolerant species, and the improvement in tolerance when *P. patula* is hybridized with tolerant species, has been seen in field studies (Roux et al. 2007). The ranking of species in susceptibility to *F. circinatum* in our study is similar to that of Hodge and Dvorak (2000). Although, *P. greggii* var. *greggii* (*P. greg*N) was not used to make any of the hybrids tested, it was included in the species trial because it is known to be more susceptible than *P. patula* (Hodge and Dvorak 2000). It appears that, in general, tolerance to *F. circinatum* in pure species is closely related to the amount of tolerance that species is able to bring to a *P. patula* hybrid. Thus, one could expect a *P. patula x P. jaliscana* hybrid to exhibit good tolerance, based on the extreme tolerance of *P. jaliscana* as a pure species (Hodge and Dvorak 2000).

The success of other hybrids such as *P. elliottii x P. caribaea* (van der Sijde and Roelofsen 1986) and *P. patula x P. greggii* (Kietzka 2002) illustrates the potential that hybrid forestry offers as an alternative to pure species in South Africa. The *P. pat x P. tec*H hybrid is likely to become very important in the future due to its superior tolerance to frost compared to *P. pat x P. tec*L. *P. patula x P. tecunumanii* (H) has already been planted over a wide range of altitude classes including 1700 m above sea level with good success. Currently, it is the best hybrid alternative to *P. patula* on sites that experience light frost events and, by selecting provenances and families that are more frost tolerant (Dvorak et al. 2000; Mitchell et al. 2012), its planting range may be extended to include temperate sites. Although the immediate need is to identify hybrids between *P. patula* and species more tolerant to *F. circinatum*, there is a possibility that other hybrids may be deployed in the future in preference to *P. patula* on many sites. Currently, members of Camcore are testing a large number of hybrids over many sites in which *P. patula* is included as a control. More than 48 Camcore hybrid trials have been planted across Southern Africa and South America in the last 3 years testing over 15 different hybrids (J. Lopez pers. com 2011) with one local company testing approximately 40 pine hybrids. From these trials several hybrids are likely to show potential as an alternative to planting *P. patula*.

The findings presented here provide good evidence that the tolerance of *P. patula* to *F. circinatum* can be significantly improved by hybridizing it with *P. tecunumanii* and other tolerant species. Since *F. circinatum* is a major cause of seedling mortality after planting, the improved tolerance of *P. patula* hybrids may explain their better survival operationally. In the case of *P. patula x P. tecunumanii* the significant improvement in tolerance when the pollen is sourced from low elevation *P. tecunumanii* indicates that this hybrid need not be screened. However, there is large variation in the tolerance of hybrid families to *F. circinatum* when pollen is sourced from high elevation *P. tecunumanii* and care should be taken to screen these before large-scale commercial deployment. All other hybrids between *P. patula*, and species more tolerant to *P. patula*, display a significant improvement in tolerance to *F. circinatum*. Some of these hybrids are being tested in field trials and may prove to be valuable alternatives to *P. patula* in the future.

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References

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal*, vol. 205: 3 – 8.

Department of Agriculture, Forestry and Fisheries (DAFF) (2010) Report on commercial timber resources and primary roundwood processing in South Africa 2008/9. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Dieters MJ, Nikles DG, Toon PG, Pomroy P (1997) Genetic parameters for F1 hybrids of Pinus caribaea var. hondurensis with both Pinus oocarpa and Pinus tecunumanii. Canadian Journal of Forest Research, 27(7): 1024-1031.

Dieters MJ, White TL, Hodge GR (1995) Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). *Canadian Journal of Forest Research* 25: 1397-1408.

Dvorak WS, Hodge GR, Gutiérrez EA, Osorio LF, Malan FS, Stanger TK (2000) *Pinus tecunumanii*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative. College of Natural Resources, North Carolina State University. 188-209.

Frampton JF, Li B, Goldfarb B (2000) Early field growth of Loblolly pine rooted cuttings and seedlings. *Southern Journal of Applied Forestry*, Vol. 24: 98–105.

Gordon, IL (1999). Quantitative genetics of intraspecific hybrids. Heredity 83:757-764.

Hodge GR, Dvorak WS (2000). Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests*, 19: 241-258.

Hodge GR, Dvorak WS (2006). Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests*, 33:193 – 206.

Kanzler A, Payn K, Nel A (2012) Performance of two *Pinus patula* hybrids in Southern Africa. *Southern Forests* 74:19-25.

Kietzka JE (1988) *Pinus maximinoi*: A promising species in South Africa. *Southern African Forestry Journal* 145: 33 – 38

Kietzka JE (2002) “New” pine species and hybrids: Is there still potential? *Southern African Forestry Journal*. Vol 195: 89-92.

Malan FS (2003) The wood properties of South African timber resource for high-value solid wood products and its role in sustainable forestry. *Southern African Forestry Journal*, vol. 198:53-62.

Mitchell RG, Jones NB, Zwolinski J (2004) A review of on the effects of donor maturation on rooted conifer cuttings. *South African Forestry Journal*, vol. 204: 53-64.

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Mitchell RG, Wingfield MJ, Hodge GR, Dvorak W, Coutinho TA (2012) Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa. New Forests DOI: 10.1007/s11056-012-93.

Morris AR, Pallett R (2000) Site requirements and species matching: Pines. In: Owen D (ed) *South African Forestry Handbook, South African Institute of Forestry,* vol 1: pp 80-84.

Nel A, Kanzler A, Dvorak W (2006). Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. *In* Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. *Edited by* Fikret Isik. p 158-161.

Power AB, Dodd RS, Libby WJ (1994) Effects of hedging on maturation in Radiata pine: Western Gall Rust Susceptibility. *Silvae Genetica* 43: 1-7.

Roux J, Elsenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, Vol. 33: 109 – 123.

SAS Institute (2003) Cary, North Carolina, 27513, USA.

van der Sijde HA, Roelofsen JW (1986) The potential of pine hybrids in South Africa. *Southern African Forestry Journal*, March 1986: 5-14

Zagory D, Libby WJ (1985) Maturation-related resistance of *Pinus radiata* to Western Gall Rust. *The American Phytopathological Society*, 75: 1443-1447.

Table 1. The list of treatments in the three trials examining tolerance of *P. patula* and other species and hybrids to artificial inoculation with *Fusarium circinatum*.

Details	*Trial 1: P. patula x P. tecunumanii*	Trial 2: Various hybrids	Trial 3: Species trial
Date inoculated	11/2008	03/2009	12/2009
Date assessed	01/2009	06/2009	02/2010
Treatments	75 *P. patula x P. tecunumanii* (L)[1]	*P. patula x P. tecunumanii (L)*[1]	*P. patula*
	24 *P. patula x P. tecunumanii* (H)[2]	*P. patula x P. tecunumanii (H)*[2]	*P. elliottii*
	13 *P. patula*	*P. patula x P. oocarpa*	*P. greggii (N)*[4]
	P. elliottii (control)	*P. patula x P. elliottii*	*P. greggii (S)*[3]
		P. patula x P. pringlei	*P. tecunumanii (H)*[2]
		P. patula x P. greggii (S)[3]	*P. tecunumanii (L)*[1]
		P. elliottii x P. caribaea	*P. oocarpa*
		P. elliottii x P. taeda	*P. caribaea*
		P. elliottii x P. tecunumanii (H)[2]	*P. pringlei*
		P. tecunumanii (H)[2] *x P. caribaea*	
		P. tecunumanii (L)[1] *x P. caribaea*	
		P. tecunumanii (H)[2] *x P. oocarpa*	
		P. caribaea x P. oocarpa	
		P. caribaea x P. tecunumanii (L)[1]	
		P. elliottii (control)	
		P. patula (control)	

[1]*P. tecunumanii* (low elevation source), [2]*P. tecunumanii* (high elevation source)

[3]*P. greggii* var. *australis* (from southern Mexico), [4]*P. greggii* var. *greggii* (from northern Mexico)

Table 2. The mean values for the parameters assessed among *P. patula,* and *P. patula x P. tecunumanii* represented as cuttings and seedlings, after inoculation with *F. circinatum* in Trial 1.

Species/hybrid[1]	*Type*	*Freq.*	*Height (mm)*	*Lesion (mm)*	*Dieback (%)*	*Resprout (mm)*
P. elliottii	Seedlings	59	163.1	5.7 ^{A}	3.7 ^{A}	46.2 ^{A}
P. patula	Seedlings	461	68.7	29.4 ^{B}	42.6 ^{B}	30.6 ^{B}
*P. pat x P. tec*H	Cuttings	1275	155.9	16.1 ^{C}	12.1 ^{C}	28.3 ^{B}
*P. pat x P. tec*H	Seedlings	307	73.2	21.4 ^{C}	29.5 ^{D}	25.5 ^{C}
*P. pat x P. tec*L	Cuttings	4625	177.5	7.4 ^{A}	5.0 ^{A}	20.4 ^{E}
*P. pat x P. tec*L	Seedlings	1173	81.8	8.2 ^{A}	10.8 ^{E}	43.2 ^{E}

[1]High and low elevation *P. patula x P. tecunumanii* are indicated with H and L, respectively. For a given variable, means with different letters are statistically different ($p<0.05$).

Table 3. The results of an ANOVA on lesion length to determine family and type of plant (cutting *vs* seedling) differences for the two hybrids (*P. pat x P. tec*H, *P. pat x P. tec*L) and for *P. patula* to *F. circinatum* tolerance in Trial 1.

Source	DF	SS	MS	FValue	ProbF
a) *P. pat x P. tec*H[1]					
Rep	3	7578.2	2526.1	6.60	0.0004
Type (cutting/seedling)	1	22.2	22.2	0.06	0.8194
Family	23	57120.0	2483.5	4.04	0.0693
Type*Family	5	2903.3	580.7	1.43	0.2198
Rep*Type*Family	87	37839.0	434.9	2.26	0.0000
Height	1	24.6	24.6	0.13	0.7206
b) *P. pat x P. tec*L[2]					
Rep	3	4194.7	1398.2	5.92	0.0006
Type (cutting/seedling)	1	20.9	20.9	0.08	0.7835
Family	74	62258	841.3	1.78	0.0745
Type*Family	20	9178.2	458.9	1.89	0.0131
Rep*Type*Family	283	70760	250	4.46	0.0000
Height	1	881.9	881.9	15.75	0.0001
c) P. pat x P. tec combined[3]					
Rep	3	7687.9	2562.6	9.19	0.0000
Hybrid	1	41351	41351	65.83	0.0000
Type (cutting/seedling)	1	3.2	3.2	0.01	0.9161
Hybrid*Type	1	90.1	90.1	0.26	0.6131
Family (Hybrid)	97	119325	1230.1	2.51	0.0055
Type*Family(Hybrid)	25	11889	475.6	1.60	0.0345
Height	1	567	567	6.66	0.0099
Rep*Family(Hybrid*Type)	373	114237	306.3	3.60	0.0000
d) P. patula[4]					
Rep	3	4008.9	1336.3	1.85	0.1534
Family	12	10752	895.9	1.04	0.4356
Rep*Family	35	35255	1007.3	6.90	0.0000
Height	1	9213.7	9213.7	63.08	0.0000

1. ANOVA on all *P. patula x P. tecunumanii* treatments (from high elevation provenances only)
2. ANOVA on all *P. patula x P. tecunumanii* treatments (from low elevation provenances only)
3. ANOVA on all *P. patula x P. tecunumanii* treatments
4. ANOVA on all *P. patula* treatments

Table 4. Variance components[1] and genetic parameters for lesion length (assessing tolerance to *F. circinatum*) for hybrids of *P. patula* x *P. tecunumanii* High-elevation sources, *P. patula* x *P. tecunumanii* Low-elevation sources, and pure species *P. patula*.

	Data Set					
	*P. pat x P. tec*H		*P. pat x P. tec*L		*P. patula*	
Source	mm^2	%	mm^2	%	mm^2	%
P. patula parent	16.5	6.4	0.6	0.8	26.0	2.1
P. tecunumanii parent	5.3	2.1	1.4	1.8	-	-
P. patula x P. tecunumanii	25.0	9.7	3.3	4.2	-	-
P. pat * *P. tec* * type	0.2	0.1	3.2	4.1	-	-
Plot	18.4	7.2	13.1	16.9	99.1	39.6
Residual	192.3	74.6	56.0	72.1	146.1	58.3
Phenotypic	257.7	100.0	77.7	100	250.5	100
h^2	-	-	-	-	0.06	-
h^2_{pat}	0.25	-	0.03	-	-	-
h^2_{tec}	0.08	-	0.07	-	-	-
$d^2_{pat\ x\ tec}$	0.39	-	0.17	-	-	-

[1]Variance components for lesion length expressed in the units of measurement (mm^2) and percent of total phenotypic variation (%). Hybrids were tested both as two plant types, cuttings and seedlings. Estimated heritability (h^2) for *P. patula* was calculated as $(3 \times \sigma^2_{female}) / \sigma^2_{phenotypic}$ following Dieters et al. 1995.

Table 5. The mean lesion length, percent dieback and the ability to resprout of various pine species after inoculation with *F. circinatum*.

Species	Freq.	Height (mm)	Lesion (mm)	Dieback (%)	Resprout (mm)
P. greggii (N)	67	126.9	37.97 ^{A}	27.91 ^{A}	16.5 ^{A}
P. patula	57	118.3	29.65 ^{B}	23.38 $^{B/}$	18.4 ^{A}
P. greggii (S)	95	165.6	18.51 ^{C}	12.25 ^{C}	43.2 ^{B}
P. tecunumanii (H)	95	177.9	9.16 ^{D}	7.01 ^{DE}	59.2 ^{C}
P. elliottii	95	183.5	8.82 ^{D}	6.77 ^{D}	86.3 ^{D}
P. caribaea	87	149.7	7.97 ^{DE}	6.31 ^{DF}	81 ^{D}
P. tecunumanii (L)	89	150.8	5.07 ^{EF}	3.91 ^{FG}	96.9 ^{E}
P. oocarpa	95	158.1	4.21 ^{F}	3.28 ^{G}	117.1 ^{F}
P. pringlei	93	111.5	3.81 ^{F}	1.87 ^{H}	39.6 ^{B}

Note. Treatments are ranked from most to least tolerant based on lesion length. Treatments with different letters are significantly ($p<0.05$) different (Duncan grouping).

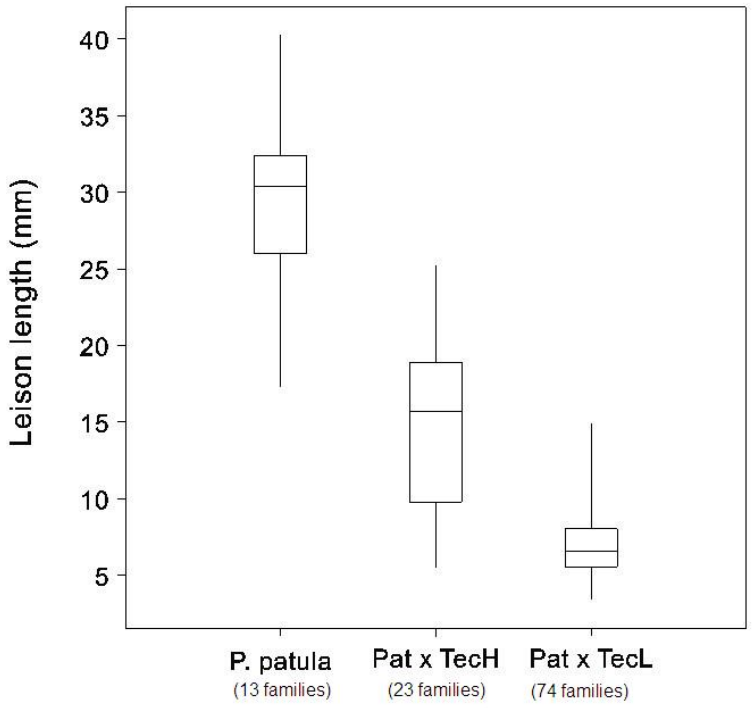

Figure 1. Box and Whisker plot showing family variation in *P. patula* and *P. patula x P. tecunumanii*. The blocks represent the center 50% (interquartile) values recorded. Horizontal lines within the blocks represent the median, vertical lines either ends of the box represent the range in susceptibility.

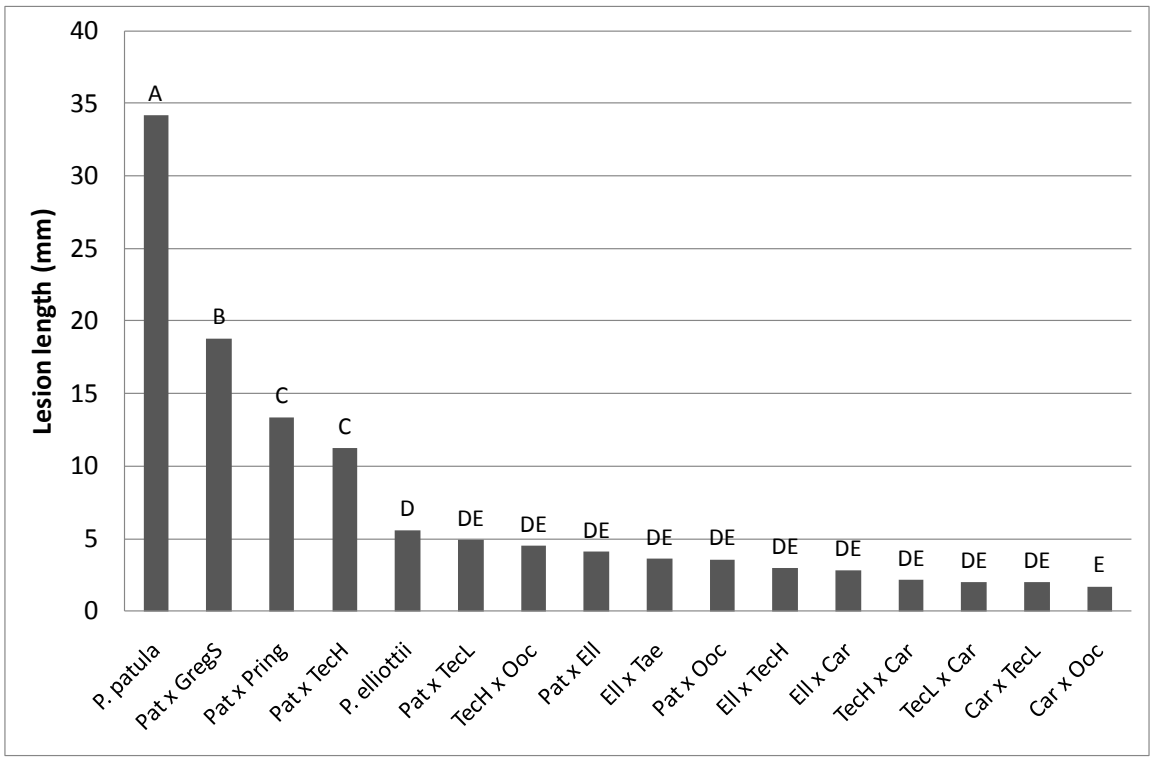

Figure 2. The variation in tolerance of a number of hybrids and *P. patula* and *P. elliottii* to *Fusarium circinatum* in Trial 2.

Appendix 1. The specific mating design used to produce a total of 99 *P. patula x P. tecunumanii* full-sib families with 13 *P. patula* female parents and 7 high elevation and 12 low elevation *P. tecunumanii* pollen parents.

	Female													
Male	Pat 1	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Pat 8	Pat 9	Pat 10	Pat 11	Pat 12	Pat 13	Total
TecH 1		*			*	**				*	**	*		6
TecH 2											*			1
TecH 3						**	*			*	**			4
TecH 4		*	*				**					*	*	5
TecH 5		**		*			*					*	*	5
TecH 6			*											1
TecH 7		*			*									2
TecL 1			**	*			**			*		*		5
TecL 2					*						*			2
TecL 3				*				*		*		*	*	5
TecL 4	*	*	*	**		*	*		*	*		*		9
TecL 5	*	*	**					*		**		*		6
TecL 6			**	*			**	*						4
TecL 7	*	**	*	*			**			*		**		7
TecL 8	*			*			**			*		*	*	6
TecL 9	*		**	*				*		*		*		6
TecL 10	**	*	**	**		*	*	*	**	*			*	10
TecL 11		*	*				**	*		*		**	*	7
TecL 12	**		**	*		*	**	*				*	*	8
Total	7	9	11	10	3	5	11	7	2	11	4	12	7	99

A single asterisk indicates that the full-sib family was represented by cuttings only, while double asterisk indicates that the family was represented by both cuttings and seedlings. TecL = P. tec (low elevation), TecH = P. tec (high elevation).