Selection of *Pinus* spp. in South Africa for tolerance to infection by the pitch canker fungus

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Abstract   The increasing threats from pests and diseases demand that the South African forest industry explores options to deploy alternative pine species in plantation development. This is especially true for species, such as *Pinus patula* Schiede and Deppe ex Schltdl. and Cham., which are highly susceptible to the pitch canker fungus *Fusarium circinatum*. Losses due to *F. circinatum* have been confined mostly to nurseries and at field establishment resulting in a significant cost to the industry. Although, the fungus has not as yet resulted in stem and branch infections on established *P. patula* in South Africa, it has caused pitch canker on other, more susceptible species such as *P. radiata* D. Don., and *P. greggii* Engelm. ex Parl. As alternatives to *P. patula*, on the warmer and cooler sites in South Africa, families of *P. elliottii* Engelm var. *elliottii*, *P. tecunumanii* (Schw.) Eguiluz and Perry, *P. maximinoi* H. E. Moore and *P. pseudostrobus* Lindl. were screened for tolerance to infection by *F. circinatum* in greenhouse studies. Seedlings were wounded and inoculated with spores of *F. circinatum*. Lesion development following inoculation was used to differentiate the levels of tolerance between families. The results showed that *P. maximinoi*, *P. pseudostrobus*, and the low elevation variety of *P. tecunumanii* are highly tolerant to infection with very little family variation. The narrow sense heritability estimates for these species were less than 0.06. In contrast, *P. elliottii* showed good tolerance with some family variation and a heritability of 0.22, while the high elevation source of *P. tecunumanii* showed a high degree of family variation and a heritability of 0.59. These results provide the industry with valuable information on pine species tolerant to *F. circinatum* that could be used as alternatives to *P. patula* in South Africa.

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Introduction

Pitch canker is a serious disease of pines caused by the fungus *Fusarium circinatum* (anamorph) that has a sexual state (teleomorph) known as *Gibberella circinata* (Nierenberg and O'Donnell 1998; Britz et al. 2005). Although, the exact origin of pitch canker is unknown, studies indicate that the pathogen may be endemic to Mexico (Guerra-Santos 1999), southeast USA (Wikler and Gordon 2000) or as far south as Central America (Dvorak et al. 2009). The first discovery of the disease in South Africa was 1991 (Viljoen et al. 1994) suggesting that *F. circinatum* is an introduced pathogen into this country (Wikler and Gordon 2000). From vegetative compatibility group, and allelic frequency studies, the South African population has the third highest degree of genetic diversity after Mexico and the south-eastern USA (Viljoen et al. 1997; Wikler and Gordon 2000; Britz et al. 2005). Accepting that the South African population is in all probability young, several possibilities for its diversity have been suggested. Many genotypes could have been initially introduced (Viljoen et al. 1997), subsequent introductions have occurred (Britz et al. 2005) or sexual reproduction is occurring (Viljoen et al. 1997).

Pitch canker can result in branch die-back, stem cankers and in large-scale mortality (Dwinell and Phelps 1977; Bethune and Hepting 1963; Storer et al. 1999). Consequently, *F. circinatum* is considered one of the most serious pathogens threatening plantations of non-native *Pinus* spp. worldwide (Wingfield et al. 2008). The susceptibility of *Pinus* spp. to *F. circinatum*, however, varies considerably. Thus, some species, such as *Pinus radiata* and the northern provenances of *P. greggii* are highly susceptible whilst other species including *P. jaliscana* and *P. oocarpa* display high levels of tolerance to infection (Hodge and Dvorak 2000). This indicates that the threat of *F. circinatum* can be reduced by planting more tolerant species in high risk areas.

In South Africa, *P. patula* is the most important softwood planted (Department of Agriculture, Forestry and Fisheries 2008) and was the first pine species to be associated with the pitch canker fungus (Viljoen et al. 1994). The disease has now spread to most pine growing nurseries in the country where it is managed with varying degrees of success. The most significant effect of *F. circinatum* on *P. patula*, however, can be seen on young seedlings that become infected and die after establishment (Crous 2005; Mitchell et al. 2011). More recently, pitch canker was discovered on mature *P. radiata* and *P. greggii* trees in the Southern and Eastern Cape provinces of South Africa (Coutinho et al. 2007; Roux 2007). Given the speed at which the nursery disease has progressed since it was first identified, and the current situation with growing areas of mature trees being infected, it seems reasonable to expect that pitch canker may spread to other areas and species, which could include mature *P. patula* trees.

In the long term, tree breeders in South Africa hope that the tolerance of *P. patula* to *F. circinatum* could be improved by including tolerance as a selection criterion in their breeding programmes (Mitchell et al. 2011). In the shorter term the tolerance of *P. patula* can be improved through hybridization with more tolerant species. The simplest immediate solution, however, is to deploy alternative species that are known to be tolerant to infection by *F. circinatum*, at least in high-risk sites (Mitchell et al. 2011).

South Africa is fortunate to have a wide range of site types that are suited to a number of *Pinus* species (Morris and Pallett 2000). Although, *P. patula* is planted across many of these sites, *P. elliottii* and *P. taeda* have been planted in increasing numbers in the last decade as substitutes due to their tolerance to *F. circinatum* (Hodge and Dvorak 2000). In South Africa, *P. taeda* is considered highly tolerant to *F. circinatum* (Hodge and Dvorak 2000; Roux et al. 2007). However, due to its specific site requirements (well drained soils that are a minimum of 750 mm deep, in areas that receive a minimum of 950 mm of rain per year, and on sites below 1,400 m (Schönau and Grey 1987; Morris and Pallett 2000; Zwolinski and Hinze 2000), few areas are suitable for this species. This, together with its tolerance to *F. circinatum*, makes it less important to screen the local population of *P. taeda*.

Pinus elliottii is less tolerant to *F. circinatum* than *P. taeda* (Hodge and Dvorak 2000) and is South Africa's second most important pine crop (Department of Agriculture, Forestry and Fisheries 2008). Elsewhere, large variation in tolerance to *F. circinatum* has been found between *P. elliottii* families, which have enabled breeders to improve the overall tolerance of the species (Rockwood et al. 1988; Blakeslee and Rockwood 1999). This suggests that it would be important to screen the South African *P. elliottii* selections for tolerance to *F. circinatum*.

There are a number of other *Pinus* species that show potentially high levels of tolerance to *F. circinatum* in research trials and in commercial plantings. On warmer sites, *P. tecunumanii* and *P. maximinoi* are the most promising (Kietza 1988; Dvorak et al. 2000, 2002; Gapare et al. 2001). Fewer species are available on the temperate and cooler sites that perform as well as *P. patula*. Similar performance can be achieved from the Mexican pine, *P. greggii*, on cold and dry regions in South Africa (Dvorak et al. 1996) but the susceptibility of the species to *F. circinatum* (Hodge and Dvorak 2000) does not make it a suitable alternative to reduce the risk of pitch canker. *Pinus pseudostrobus*, however, has also shown potential in these regions (Coetzee 1985) and is tolerant to *F. circinatum* (Hodge and Dvorak 2000). It is already being deployed commercially by one company. Although, *P. tecunumanii*, *P. maximinoi* and *P. pseudostrobus* are known for their higher levels of tolerance to *F. circinatum* than *P. patula*, little is known about the degree of within family variation to infection by the pathogen.

In this study, families of *P. maximinoi*, *P. pseudostrobus*, *P. tecunumanii* and *P. elliottii*, were screened for their tolerance to *F. circinatum*. This was achieved using greenhouse inoculations. The objectives of the study were to determine whether meaningful family variation, in tolerance to *F. circinatum*, exists in these species which would indicate that their general tolerance could be further improved though breeding and selection.

Materials and methods

Plant material

A number of Camcore trials were planted in the early 1980's testing the performance of unimproved *P. tecunumanii* and *P. maximinoi* families from various provenances (localities) in Mexico and Central America. Camcore is an international tree conservation and domestication programme at North Carolina State University, USA. The organization is actively involved in the collection of wild populations of pine species for conservation and domestication in areas outside of their natural distribution. The trials were situated on Komatiland Forests' property, South Africa. The seed of these two species, collected for

this study, was harvested from trees that had been selected for superior growth and stem form based on their breeding values in the Camcore trials. In the original field design, families were grouped by provenance and, therefore, the selections were likely to be pollinated by surrounding trees of the same provenance. However, there would also be some natural cross pollination between trees of different provenances. This is relevant because the seed collected would not necessarily represent a pure provenance.

Pinus tecunumanii

The *P. tecunumanii* seed was harvested, in 2004 and 2005, from 73 selected trees, representing 12 provenances in the Camcore trials (Table 1). Of the 73 trees, 49 were sampled from 4 provenances that occur below 1,500 m above sea level (low elevation or LE) in Honduras. The other 24 selections represented 8 provenances above this altitude (high elevation or HE). With the exception of one high elevation provenance (Las Trancas), which is found in Honduras, all others originate in Mexico and Guatemala.

The *P. tecunumanii* seed was sown in September 2006 in preparation for inoculating the seedlings in April 2007 (Table 2). When the seedlings were 6-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 88 seedlings or 22 seedlings per plot. Seedlings of *P. patula* and *P. elliottii*, from a commercial seed orchard, were included as controls in the trial.

Pinus maximinoi

The *P. maximinoi* seed was harvested, in 2005 and 2006, from 105 selected trees representing 13 provenances across Mexico, Guatemala and Honduras (Table 1). In this collection, most provenances were represented by 4–13 trees. The seed was sown in July 2007 in preparation for the March 2008 inoculation. When the seedlings were 7-months-old,

Table 1 *Pinus tecunumanii* and *P. maximinoi* provenances screened for tolerance to *Fusarium circinatum*

Ecotype	Country	Provenance	Families	Country	Provenance	Families
Pinus tecunumanii				*Pinus maximinoi*		
High	Guatemala	KM 47	1	Guatemala	Coban	13
High	Guatemala	San Jerónimo	5	Guatemala	San Jerónimo Guatemala	19
High	Guatemala	San Lorenzo	1	Guatemala	San Juan Sacateqequez	18
High	Honduras	Las Trancas	3	Guatemala	San Lorenzo	1
High	Mexico	Chempil	8	Honduras	Dulce Nombre de Copan	8
High	Mexico	Jitotol	3	Honduras	El Portillo	16
High	Mexico	Montebelo	6	Honduras	Marcala	4
High	Mexico	Napite	1	Honduras	Tatumbla	6
Low	Honduras	Jocón	3	Mexico	Altamirano	4
Low	Honduras	San Esteban	9	Mexico	Coapilla	1
Low	Honduras	San Francisco	15	Mexico	La Cañada	1
Low	Honduras	Villa Santa	22	Mexico	Monte Cristo	1
				Mexico	San Jerónimo Chiapas	13
			77			105

Table 2 Details of plant material, trial layout and dates for inoculation of seedlings inoculated with *F. circinatum*

	P. tecunumanii	*P. maximinoi*	*P. elliottii*	*P. pseudostrobus*
Date sown	09/2006	07/2007	03/2009	05/2009
Date inoculated	03/04/07	19/03/08	01/12/09	01/12/09
Date assessed	30/05/07	21/05/08	04/02/10	04/02/10
Families tested	49 LE** and 24 HE*	104	49	33
Mean plot size	22	14	11	20
Replications	4	4	4	4
Mean plants per treatment	88	56	44	80
Range of plants per treatment	58–96	12–64	20–64	51–95
Controls	*P. elliottii*	*P. taeda*	*P. elliottii*	*P. elliottii*
	P. patula	*P. elliottii*	*P. patula*	*P. patula*
		P. patula	*P. taeda*	*P. taeda*
		*P. tecH**		
		*P. tecL***		

* *P. tecunumanii* (high elevation)

** *P. tecunumanii* (low elevation)

they were packed out in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 56 seedlings. Seedlings of *P. tecunumanii* HE, *P. tecunumanii* LE, *P. taeda*, *P. elliottii* and *P. patula* were included in the trial as controls (Table 2).

Pinus elliottii

Seed, from a total of 49 open pollinated 2nd generation *P. elliottii* families, was provided by Komatiland Forests from their breeding programme for this trial. Seed from the highly susceptible *P. elliottii* clone FA2 (Hodge and Dvorak 2000, 2007) was provided by Camcore and included in the study. All seed was sown in March 2009 in preparation for screening the seedlings in December 2010. When the seedlings were 8-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 44 seedlings. Commercial open pollinated seedlings of *P. patula*, *P. elliottii* and *P. taeda* were included as controls (Table 2).

Pinus pseudostrobus

Seed of 33 selected *P. pseudostrobus* trees, in a commercial stand, was supplied by Komatiland Forests for this trial. The seed was sown in May 2009 in preparation for screening the seedlings in December 2010, together with the *P. elliottii* trial. When the seedlings were 7-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 80 seedlings. As with the *P. elliottii* trial, open pollinated seed of *P. elliottii*, *P. taeda* and *P. patula* was included as controls (Table 2).

The seedlings in all the trials were raised in the Komatiland Forest's Research nursery at Sabie, South Africa (S25°03.22′, E30°46.859′). The local climate can be described as

warm temperate with a mean annual temperature of 15°C and approximately 1,300 mm of rain predominantly during the summer months. Seedlings were raised in composted pine bark in the Unigro tray, consisting of loose inserts, under plastic covering. Each individual insert is square in shape with a top end width of 37 mm, length of 100 mm, and volume of 90 ml. The side walls have pronounced internal ridges to prevent root spiraling. Granular fertilizer [2:3:2 (N:P:K)] was applied as needed. No fungicides were applied to the seedlings during their establishment. Due to the different seed collection dates the trials were raised at different times in the nursery.

Inoculation procedures

The seedlings, for each trial, were transported to a greenhouse at the University of Pretoria, specifically erected for the purpose of screening pine families for tolerance to *F. circinatum*, on different occasions (Table 2). The greenhouse had a wet-wall cooling system and a separate heating system to maintain air temperatures of approximately 25°C. The plants were left to acclimatise for 1–4 weeks before the seedlings were inoculated.

The inoculum was prepared on the same day that the seedlings were inoculated, using a equal mixture of conidia from three highly virulent South African isolates (CMW 3577, 3578, and 3579) of *F. circinatum* that are maintained by the Tree Protection Co-operative Programme (TPCP) in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. The isolates were cultured on half strength Potato Dextrose Agar (PDA; 2 g potato extract, 10 g dextrose, and 7.5 g agar/l distilled water) under sterile conditions for 7 days at 25°C prior to inoculation. Cultures were flooded with sterile water containing 15% glycerol and spores were dislodged using a glass "hockey stick". The spore concentration was determined using a haemocytometer, viewed under a light microscope at 20× magnification, and adjusted to 50,000 spores/ml. The tubes of inoculum were maintained on crushed ice prior to inoculation.

In the greenhouse, the apical bud of each seedling was removed using sharp secateurs, and 10 µl inoculum (500 spores per infection site) was immediately applied to the wounded tip using a micro-pipette. Once inoculated, plants in the trial were watered daily and assessed for lesion development 8-weeks after inoculation. The lengths of lesions were measured from the tips of the seedlings, at the point of inoculation, to the point where the tissue showed no visible necrosis. The seedling height, from the root collar to the wounded tip, was also measured. For each inoculated seedling the proportion of lesion length to the length of the seedling at the time of inoculation was expressed as a percentage of die-back. In many seedlings, new shoots (re-sprouts) had formed below the lesions and the length of these was measured. All measurements were recorded in millimetres (mm).

Statistical analyses

The statistical package SAS version 9.1.3 (SAS Institute 2008) was used to carry out the data analysis. An analysis of variance (ANOVA) was conducted on the data (seedling height, percentage die-back, lesion length, and length of the re-sprout) for each of the four inoculation trials. In order to correct for the influence that the seedling size could have had on the variables, initial height was analysed as a covariate (Hodge and Dvorak 2000). A Pearson correlation coefficient analysis was carried out to determine the relationship that these variables had on each other. As the trials were analysed on separate occasions, different tests were used to determine treatment differences. A *t* test was used to compare differences between provenance and species means in the *P. tecunumanii* data-set and a

Student–Newman–Keuls (SNK) test was used to test for differences between family means. A Duncan Multiple Range test was used to determine treatment differences in the *P. maximinoi*, *P. elliottii* and *P. pseudostrobus* datasets. Narrow sense heritability was estimated following the method described by Dieters et al. ([1995]) for open-pollinated families $\left(h^2 = \left(3 \times \sigma^2_{\text{female}} \right) / \sigma^2_{\text{phenotypic}} \right)$.

Results

After inoculation, lesions were observed in *P. patula* control seedlings within 7 days. Shortly thereafter, the *P. tecunumanii* (HE) treatments developed lesions followed by lesions in *P. elliottii*, families of *P. tecunumanii* (LE), *P. maximinoi* and *P. pseudostrobus*. Sprouts were seen developing below the lesion on those species that took longer to develop lesions. Previous studies have shown that lesion length and the percentage dieback can be influenced by the height of the seedling (Hodge and Dvorak [2000]) or the diameter of an infected branch in field studies (Roux et al. [2007]). In these cases taller seedlings, or thicker branches, reduced lesion development. It has also been found that lesion length and percentage dieback correlate well (Hodge and Dvorak [2000]). However, it has not been reported that the ability of the seedling to produce sprouts, below the lesion, may be related to the tolerance of the seedling.

Pinus tecunumanii

The effect of seedling height influenced percent die-back and the length of re-sprout significantly ($P < 0.0001$), except for lesion length at the family level. The Pearson Correlation Coefficients between means at the provenance and family level indicate highly significant relationships for all comparisons. For provenance, the relationship between lesion length and percentage dieback was the strongest ($r = 0.99$) and lesion length was used to rank treatments. The correlation between lesion length and the degree of re-sprout was strongly negative at the provenance ($r = -0.78$) and family ($r = -0.76$) levels, indicating that treatments with longer lesions produced shorter sprouts.

In this part of the study *P. tecunumanii* (LE) displayed the greatest level of tolerance to infection with a mean lesion length of 2.78 mm (Table 3). This did not differ significantly from the lesion lengths obtained for *P. elliottii* (3.11 mm). In contrast, *P. tecunumanii* (HE) had an average lesion length of 9.61 mm. This was significantly greater than lesions on *P. tecunumanii* (LE) and the *P. elliottii* control, but not as long as those on *P. patula* (22 mm) (Table 3). When assessing provenance variation, there were no differences in lesion lengths between the four low elevation provenances, while there were large

Table 3 Mean values of the variables measured for the species tested as controls, against *P. tecunumanii*

Species	Height (mm)	Variables measured		
		Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula	136.67[C]	22.00[C]	16.89[C]	2.49[C]
P. tec (high)	124.24[B]	9.61[B]	8.20[B]	9.11[B]
P. elliottii	148.34[A]	3.11[A]	2.41[A]	10.66[B]
P. tec (low)	147.68[A]	2.78[A]	2.37[A]	21.78[A]

Species that share the same letter (Duncan grouping) are not significantly different

Table 4 Mean values of the variables measured for the species tested as controls, and *P. tecunumanii* provenances

Provenance	Ecotype	Country	Families	Variables measured		
				Lesion	Dieback	Re-sprout
P. patula	Control		Mix	22.01[A]	16.89	2.48
San Jerónimo	HE	Guatemala	5	11.48[B]	10.65	7.56
Montebello	HE	Mexico	6	10.88[B]	9.19	9.31
Chempil	HE	Mexico	8	10.19[B]	8.48	8.95
Jitotol	HE	Mexico	3	8.1[C]	6.28	10.83
Las Trancas	HE	Honduras	3	5.72[D]	4.76	8.99
San Esteban	LE	Honduras	9	3.1[D]	2.68	21.42
San Francisco	LE	Honduras	15	2.79[D]	2.41	20.87
P. elliottii	Control		Mix	3.09[D]	2.39	10.66
Jocón	LE	Honduras	3	2.73[D]	2.25	22.79
Villa Santa	LE	Honduras	22	2.61[D]	16.89	22.41

Provenances that share the same letter (Duncan grouping) are not significantly different

differences among the high elevation provenances (Table 4). The high elevation provenance from Honduras, Las Trancas, was the most tolerant of this variety and similar in tolerance to the other *P. tecunumanii* (LE) provenances from Honduras (Table 4). There were also no significant differences in lesion lengths between the 49 low elevation families screened (SNK grouping not shown) and all low elevation families were similar to the *P. elliottii* control (Fig. 1). On the other hand, large family variation in lesion lengths occurred in the high elevation source (Fig. 1).

Pinus maximinoi

The effect of seedling height, at the time of inoculation, influenced percentage die-back moderately ($r = -0.22$) and had a weak, but significant, effect on the length of the re-sprout ($r = -0.12$). Seedling height had no effect on lesion length. The relationship between lesion length and percent die-back was extremely strong ($r = 0.92$) and lesion length was used to rank treatments. The overall mean lesion length of *P. maximinoi* (3.19 mm) was no different to that on *P. tecunumanii* (LE) (2.69 mm) (Table 5). Both of these species also showed the greatest ability to re-sprout. *P. maximinoi* (3.19 mm) was significantly more tolerant than *P. taeda* (4.95 mm), *P. elliottii* (5.91 mm), as well as *P. tecunumanii* (HE) (8.63 mm). The *P. patula* seed orchard control was more susceptible than all other treatments with an average lesion length of 21.04 mm (Table 5).

In this part of the study there was little variation between provenances (Table 6), or families (Fig. 2), of *P. maximinoi* in lesion length with only those at the extreme range of infection differing significantly (Duncan grouping not shown). In this comparison, provenances represented by fewer than 4 families, were excluded. The lack of family variation corresponded to a very low narrow-sense heritability estimate ($h^2 = 0.014$) for lesion length.

Pinus elliottii

The effect of seedling height influenced percent die-back caused by *F. circinatum* ($P < 0.001$). Similar to the other studies, lesion length and percent dieback were strongly
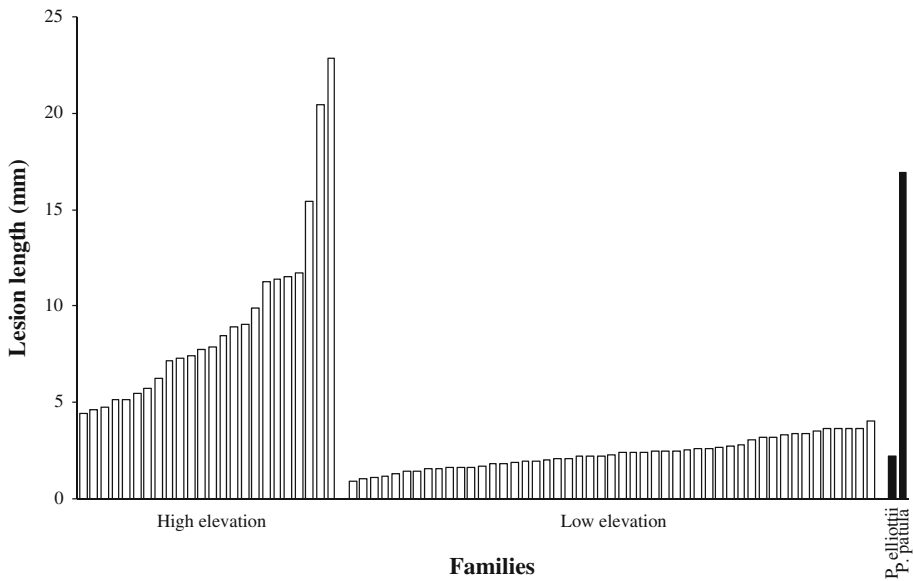
Fig. 1 Mean lesion *length* (corrected for *height*) for all *P. tecunumanii* families screened for tolerance to *F. circinatum* compared to the controls (*P. elliottii* and *P. patula*). Within ecotype, treatments are ranked from most to least tolerant. *Narrow-sense* heritability (Dieters et al. 1995) was calculated at 0.59 for the high elevation ecotype and 0.01 for the low elevation ecotype

Table 5 Mean values of the variables measured for the species tested as controls, against *P. maximinoi*

Species	Height (mm)	Variables measured		
		Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula	139.92[A]	21.04[A]	16.39[A]	18.99[BC]
P. tecHE	107.37[C]	8.63[B]	8.93[B]	22.00[BA]
P. elliottii	109.66[C]	5.91[C]	6.02[C]	17.27[C]
P. taeda	123.33[B]	4.95[C]	4.22[D]	15.87[C]
P. maximinoi	95.68[D]	3.19[D]	3.84[DE]	25.44[A]
P. tecLE	136.47[A]	2.96[D]	2.19[E]	24.11[A]

Species that share the same letter (Duncan grouping) are not significantly different

related ($r = 0.94$) and lesion length was again used to rank treatments. Lesion length had a weak, but significant, relationship with the ability for the families to re-sprout ($r = -0.28$) indicating that families with shorter lesions produced longer shoots. Overall, *P. elliottii* produced the longest shoots followed by *P. patula* then *P. taeda*.

Pinus taeda ranked the most tolerant to *F. circinatum* with a mean lesion length of 4.2 mm (Table 7). Statistically, the lesion lengths were no different to the overall mean lesion length of *P. elliottii* (5.73 mm). However, *P. taeda* was more tolerant than *P. elliottii* when comparing percentage die-back. *P. patula* was the most susceptible with a mean lesion length of 23.7 mm (Table 7). Family variation, among *P. elliottii*, was greater than in *P. maximinoi* and *P. tecunumanii* (LE) (Fig. 3). Most families ranked more susceptible than *P. taeda*. However, there were also a number of families that ranked more tolerant

Table 6 Mean values of the variables measured for the species tested as controls, and *P. maximinoi* provenances (represented by at least 4 families)

| Provenance	Country of origin	Families	Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula (control)		Mix	21.04[A]	16.39[A]	18.99[FE]
P. tecunumanii—high (control)		Mix	8.63[B]	8.93[B]	22.00[DEC]
P. elliottii (control)		Mix	5.91[C]	6.02[C]	17.27[F]
P. taeda (control)		Mix	4.95[DC]	4.22[DFE]	15.87[F]
Altamirano	Mexico	4	3.64[FE]	4.00[DFE]	21.64[DE]
San Juan Sacateqequez	Guatemala	18	3.32[FE]	4.07[DFE]	25.96[BAC]
San Jerónimo Chiapas	Mexico	13	3.28[FE]	3.28[FE]	25.16[BDAC]
El Portillo	Honduras	16	3.21[F]	3.21[F]	24.80[BDAC]
Dulce Nombre de Copan	Honduras	8	3.20[F]	3.20[F]	24.78[BDAC]
San Jerónimo Baja Verapaz	Guatemala	19	3.17[F]	3.91[DFE]	25.02[BDAC]
Coban	Mexico	13	3.06[F]	3.87[DFE]	26.73[BA]
Tatumbla	Honduras	6	2.98[F]	3.92[DFE]	24.98[BDAC]
Marcala	Honduras	4	2.91[FG]	3.35[GFE]	27.39[BA]
P. tecunumanii—low (control)		Mix	2.70[FG]	2.19[G]	24.11[BDAC]

Provenances that share the same letter (Duncan grouping) are not significantly different
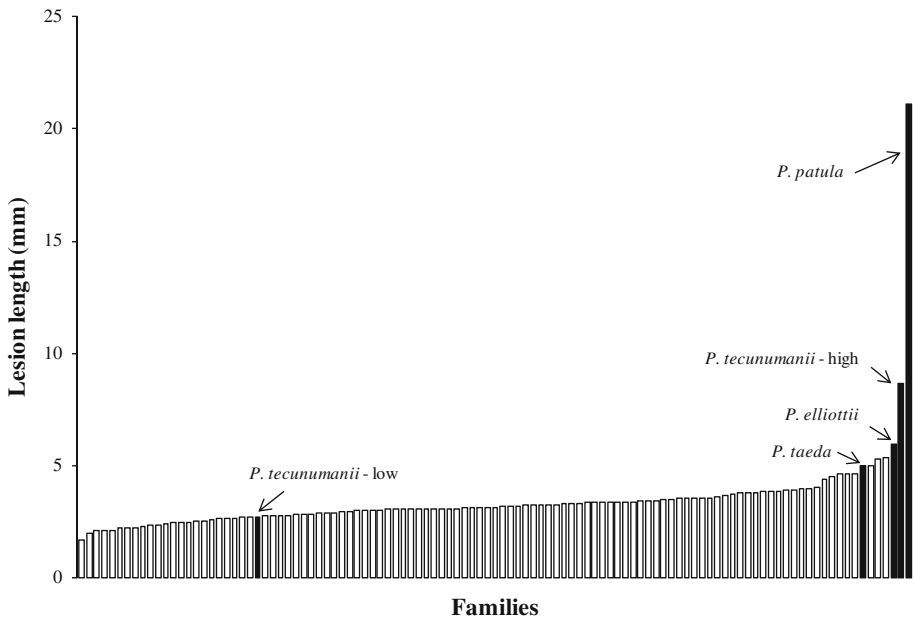
Fig. 2 Mean lesion *length* (corrected for *height*) for all *P. maximinoi* families screened compared to the controls. Treatments are ranked from most to least tolerant. *Narrow-sense* heritability (Dieters et al. 1995) was calculated at 0.014

Table 7 Comparison of the responses to inoculation with *F. circinatum* on *P. elliottii* with those on *P. patula* and *P. taeda* based on lesion length, die-back and the ability of plants to re-sprout

Species inoculated	Height (mm)	Disease characteristics		
		Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula (control)	148.34	23.7[A]	9.46[A]	10.40[B]
P. elliottii	124.24	5.3[B]	2.44[B]	24.19[A]
P. taeda (control)	136.67	4.2[B]	1.53[C]	9.97[B]
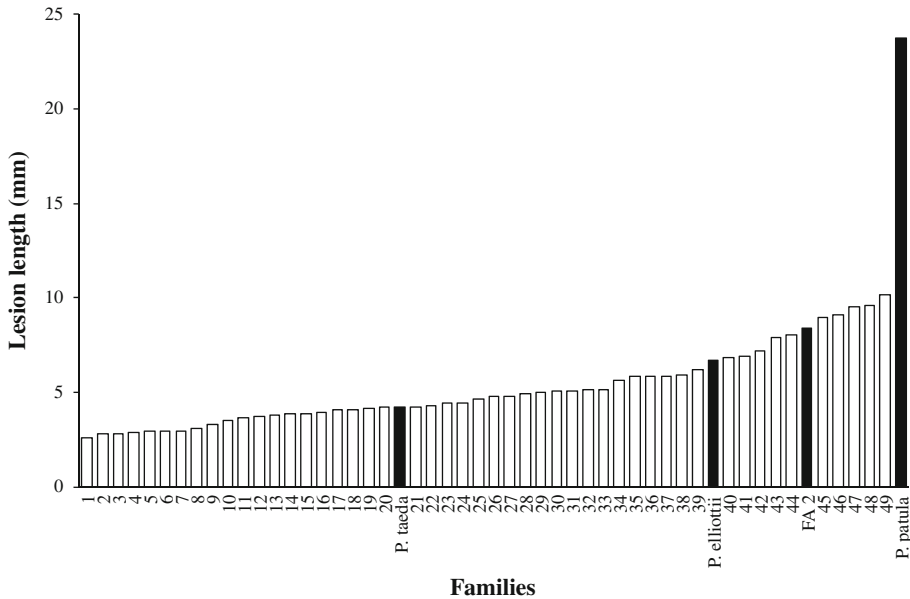
Fig. 3 Mean lesion *length* (corrected for *height*) for all *P. elliottii* families inoculated with *F. circinatum* compared to the *P. patula* and *P. taeda* controls and the susceptible *P. elliottii* control (FA2). Treatments are ranked from most to least tolerant. *Narrow-sense* heritability (Dieters et al. 1995) was calculated as 0.22

than *P. taeda*. The *P. elliottii* family FA2, previously used as a susceptible control by Hodge and Dvorak (2000, 2007), ranked as the 6th most susceptible in this study (Fig. 3). Narrow sense heritability was estimated at 0.22.

Pinus pseudostrobus

As with all the other trials, seedling height at the time of inoculation with *F. circinatum* influenced the percentage dieback. In this case, lesion development was also influenced by seedling height. Neither lesion length, nor the percentage die-back, influenced the ability of plants to re-sprout. Similar to all the other trials, lesion length and percentage dieback were strongly correlated ($r = 0.99$) and lesion length was used to rank the families.

Compared to the controls, *P. pseudostrobus* was the most tolerant to *F. circinatum* with a mean lesion length of 3.7 mm followed by *P. taeda* (7.1 mm), *P. elliottii* (10.8 mm) and *P. patula* (24.6 mm) (Table 8). Family variation was low with 32 of the 33

Table 8 Comparison of the responses of inoculation with *F. circinatum* on *P. pseudostrobus* with those on *P. patula, P. elliottii* and *P. taeda* based on lesion length, die-back and the ability of plants to re-sprout

Variety	Height (mm)	Variables measured		
		Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula	136.67	24.6[A]	21.7[A]	10.6[A]
P. elliottii	124.24	10.8[B]	10.2[B]	42.6[B]
P. taeda	148.34	7.1[C]	5.8[C]	17.3[C]
P. pseudostrobus	147.68	3.7[D]	2.6[D]	21.3[C]

Species that share the same letter (Duncan grouping) are not significantly different

P. pseudostrobus families ranked more tolerant than *P. taeda* (Fig. 4). Heritability was estimated at 0.06.

Discussion

The results of this study clearly show that the risk that *F. circinatum* poses to young and mature stands of *P. patula* in South Africa could be largely overcome by planting alternative species. *Pinus tecunumanii* (HE), suitable to warm-temperate and sub-temperate sites, showed the greatest variation in tolerance, where many families ranked more susceptible than *P. patula*. This indicates that screening families of this variety is strongly recommended before selecting those for deployment. This is particularly important if
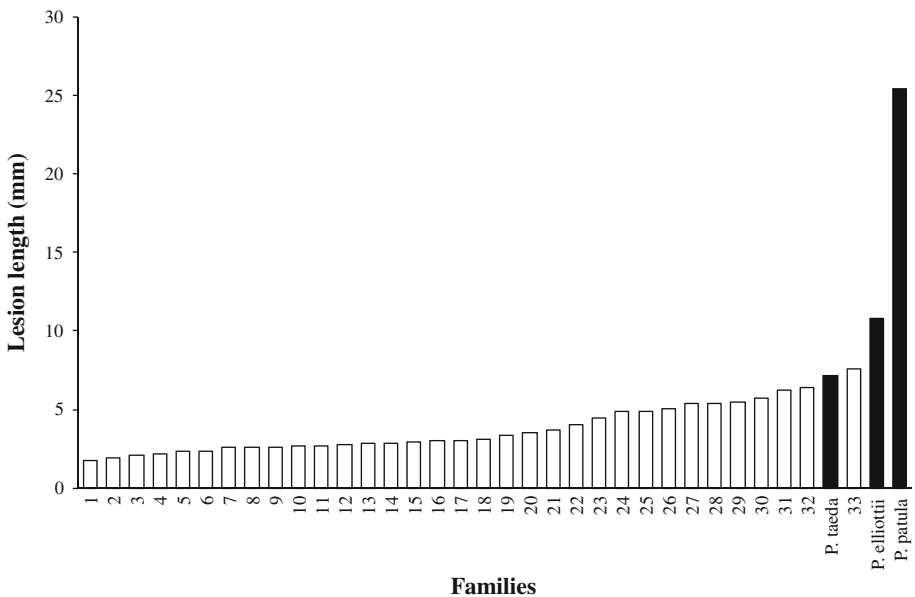
Fig. 4 Mean lesion *length* (corrected for *height*) for all *P. pseudostrobus* families screened compared to the controls. Treatments are ranked from most to least tolerant. *Narrow-sense* heritability (Dieters et al. 1995) was calculated at 0.06

families of *P. tecunumanii* (HE) are used as hybrid partners with *P. patula*. *Pinus pseudostrobus*, a species that could be significantly improved in growth through breeding, showed considerable tolerance to *F. circinatum* in this study. This species could, therefore, become an important alternative on the temperate sites of South Africa in the future. *P. maximinoi*, and *P. tecunumanii* (LE), showed excellent tolerance to *F. circinatum* with little family variation indicating that these could be planted on the warmer sites, without concern that they may become infected with *F. circinatum*. *Pinus elliottii*, a highly adaptable species suitable to a wide range of sites, showed sufficient variation in tolerance to suggest that screening families and selecting those with high levels of tolerance to infection by *F. circinatum* should be considered when deploying this species.

Pinus tecunumanii

The differences seen at the ecotype level where *P. tecunumanii* (LE) was significantly more tolerant to *F. circinatum* than *P. tecunumanii* (HE) has been previously reported (Hodge and Dvorak 2000). In this study the mean lesion length of *P. tecunumanii* (HE) was longer than *P. elliottii* which is opposite to that reported by Hodge and Dvorak (2000). Although, the tolerance of *P. tecunumanii* (LE) was similar to the mean of *P. elliottii*, where large family variation could be seen (Fig. 3), neither provenance nor family differences existed. This illustrates the high level of tolerance of this source. This was further demonstrated by the strong tendency to re-sprout after inoculation. The variation in tolerance of various *P. tecunumanii* (HE) provenances to *F. circinatum* has also been reported elsewhere (Hodge and Dvorak 2007).

The very large variation in tolerance of the 24 *P. tecunumanii* (HE) families proved to be strongly heritable ($h^2 = 0.59$). A similar heritability estimate ($h^2 = 0.58$) was calculated for 14 *P. tecunumanii* (HE) families collected from a seed orchard in Colombia (Isaza 2008). Although, a few *P. tecunumanii* (HE) families in this study were more susceptible than the *P. patula* control (Fig. 1), the overall mean for this variety indicated much higher levels of tolerance than *P. patula*. Furthermore, the high level of heritability indicates that the tolerance of *P. tecunumanii* (HE) could be improved upon relatively easily. It also suggests that families of *P. tecunumanii* (HE) should be screened for susceptibility before deployment, while those from the low elevation ecotype need not be screened. The high degree of provenance variation in the high elevation ecotype, compared to the lack of variation in the low elevation ecotype, has also been reported for traits other than susceptibility to infection by *F. circinatum*. For example, Malan (2006) found marked differences in mean inter-node length, branch diameter, and air-dried wood density between different high elevation provenances whilst no significant variation could be found for these traits amongst low elevation provenances.

In recent years tree breeders have learnt that *P. tecunumanii* hybridizes easily with *P. patula* and that the hybrid can outperform *P. patula* in field trials (Nel et al. 2006). Field inoculation studies on 3-year-old trees have shown that the tolerance of this hybrid to *F. circinatum* is superior to that of *P. patula* (Roux et al. 2007). The higher level of tolerance to *F. circinatum* is clearly seen by improved survival of the hybrid over *P. patula* in areas of little or no frost (Mitchell, unpublished). However, the results of this study suggest that not all of the hybrids made with families of the high elevation variety will be more tolerant to *F. circinatum*. Therefore, due to the susceptibility to frost, particularly in the low elevation ecotype (Dvorak et al. 2000), as well as susceptibility to *F. circinatum* in the high elevation ecotype (Hodge et al. 2000), care should be taken when planting this hybrid on a large scale without prior selection.

Pinus maximinoi

Kietza ([1988](#)) recommended planting *P. maximinoi* in South Africa in order to broaden the species base planted "should one of our major pine species (*P. patula*, *P. elliottii*, and *P. taeda*) become subject to major pest or disease problems". Although, the tolerance of *P. maximinoi* to *F. circinatum* was probably unknown at the time, it was later established that it is more tolerant than *P. patula* (Hodge and Dvorak [2000](#)).

The main constraints to planting *P. maximinoi* are its susceptibility to frost during establishment (Dvorak et al. [2000](#)) and the production of heavy branch whorls that reduce sawn board timber quality (Malan [2006](#)). However, saw millers are now able to remove the knot clusters to produce long lengths of clear timber (Malan [2006](#)). Although, *P. maximinoi* is sensitive to frost, which can result in establishment failure, it often survives better than *P. patula*. In a number of Camcore trials planted during 2008, the survival of *P. maximinoi* was consistently better than *P. patula* with an average survival across all trials of 96% compared with 80% for *P. patula* (data not shown). This indicates that many of the warmer and wetter sites currently planted to *P. patula*, could be replaced with *P. maximinoi*. These would include areas between 15 and 17°C mean annual temperature with >1,000 mm mean annual rainfall. This constitutes approximately 11% of the summer rainfall regions of South Africa where *P. patula* would be planted (Mitchell unpublished).

Pinus elliottii

Due to the greater tolerance to *F. circinatum*, *P. elliottii* survives well after planting, making it a popular species among foresters. However, the general tolerance of this species may not be sufficient to eliminate the risk of a pitch canker outbreak in South Africa. In the southern USA, mortality of infected *P. elliottii* stands has reached levels of 25% with as much as 98% of all trees showing infection (Blakeslee and Oak [1980](#)). Fortunately, large genetic variation in tolerance to *F. circinatum* exists in the species, which has allowed breeders to identify and select tolerant individuals (Rockwood et al. [1988](#)). Blakeslee and Rockwood ([1999](#)) reported narrow-sense heritability estimates around 0.25 in both greenhouse and field studies, similar to the $h^2 = 0.22$ estimated in this study, so genetic improvement of resistance within *P. elliottii* is certainly possible. This was supported by the fact that the susceptible family (FA2), used in the studies by Hodge and Dvorak ([2000](#), [2007](#)), ranked as the 6th most susceptible treatment in the present trial. Using family FA2 as a benchmark, several families were equally susceptible in this sample, highlighting the importance of screening *P. elliottii* in South Africa. Although, the *P. elliottii* commercial seed orchard seed lot was more tolerant than family FA2, a number of families ranked more tolerant than the commercial *P. elliottii* seedlot and also *P. taeda* (Fig. [3](#)).

Due to the adaptability of *P. elliottii* to a wide range of sites in South Africa, the species will continue to be planted on a large scale until alternative species and hybrids are deployed in greater numbers. The species has been planted in all areas where *P. patula* has been planted including sub-tropical sites not suited to *P. patula*. However, the growth of the species is inferior to that of *P. patula* on most sites, particularly those in temperate regions (Darrow and Coetzee [1983](#); Morris and Pallet 2000). Relative to alternative species, such as *P. tecunumanii* and *P. maximinoi*, the easy accessibility of seed makes *P. elliottii* an attractive alternative to *P. patula*. However, it should be cautioned that the slower growth of *P. elliottii* compared to *P. patula* (Morris and Pallett [2000](#)) could cost more than the cost that *F. circinatum* might cause to *P. patula* (Mitchell et al. [2011](#)).

Pinus pseudostrobus

Currently, few other species, suited to the cold regions of South Africa, grow as well as *P. patula* (Darrow and Coetzee 1983; Coetzee 1985; Morris and Pallett 2000). The limited choice is particularly challenging for breeders as the majority of South Africa's afforested regions experience mild to severe frost in the winter months. Of all the Mexican and Central American pines suited to colder regions, *P. pseudostrobus* shows the greatest tolerance to *F. circinatum* (Hodge and Dvorak 2000).

Hodge and Dvorak (2000) found that *P. pseudostrobus* was moderately resistant to infection by *F. circinatum,* where it was more tolerant than *P. elliottii*, somewhat less tolerant than the high elevation variety of *P. tecunumanii* and significantly less tolerant than *P. taeda*. The *P. pseudostrobus* seed used in the present study came from selections in a commercial stand with good growth such as described by Coetzee (1985). Little is known regarding the origin of these selections, however, in this study most families ranked more tolerant than *P. taeda*. One possible explanation is related to seedling size at the time of inoculation. In the Hodge and Dvorak (2000) study, the *P. pseudostrobus* seedlings were much smaller than most of the other species and the covariate adjustment for height may have not been sufficient to correct for this factor and to properly compare the tolerance of *P. pseudostrobus*. In contrast, in the present study, the *P. pseudostrobus* seedlings were of very similar height to the *P. taeda, P. elliottii* and *P. patula* controls.

Conclusion

In this study, the subtropical species, *P. tecunumanii* (LE) and *P. maximinoi*, were highly tolerant to infection by *F. circinatum* with little meaningful family variation. This suggests that they could be deployed with little concern of infection by the pathogen. Due to their susceptibility to frost, these species will be limited to the warmest areas where *P. patula* is currently planted. The sub-tropical to warm temperate species, *P. elliottii*, was less tolerant than *P. tecunumanii* (LE) and *P. maximinoi*, and displayed sufficient family variation with strong heritability. This result suggests that *P. elliottii* families should be screened and selected for tolerance to *F. circinatum* prior to decisions being made for large scale deployment. This species is highly adaptable and probably the most suitable alternative to *P. patula* for planting on warm temperate sites. The warm to sub-temperate species *P. tecunumanii* (HE) was less tolerant to infection *F. circinatum* than *P. elliottii* and displayed very large family variation with extremely high heritability. This indicates that it should be screened for tolerance to *F. circinatum* prior to deployment. Disease screening will be especially important where *P. tecunumanii* is considered for hybridization with *P. patula*. In contrast, the cold temperate species, *P. pseudostrobus*, showed extreme tolerance to *F. circinatum* with little to no family variation. Through further breeding and selection for growth and other good characteristics, this species may become an alternative to *P. patula* on cold sites.

Overall results of this study have shown that there is good opportunity to develop alternative species to *P. patula* in areas where the pitch canker fungus limits the successful production of this species. The results are generally consistent with those from prior studies, field observations and prior studies where established trees have been used in inoculations (Roux et al. 2007). It is, however, important to recognise that this study was based on seedling evaluations, which clearly provide an indication of resistance to *F. circinatum*, but may not fully encompass the field situation. It is well-known that trees

respond differently to infection by pathogens at different stages of development and field experiments extending the limits of this study need to be conducted.

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