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Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*

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Abstract Plantations of *Pinus* spp. constitute approximately 50% of the South African forestry industry. The first aim of this study was to develop a reliable inoculation technique to screen *Pinus* spp., for tolerance to infection by *F. circinatum*, which threatens pine forestry in South Africa. Inoculation of branches was compared with stem inoculations and we considered the number of branches or trees required to obtain statistically significant results. Furthermore, variation in the susceptibility of some *Pinus* families, clones and hybrids was considered. Results showed that branch inoculations were closely correlated with those from stem inoculations, and that it is important to consider branch and stem diameters when assessing susceptibility of trees. Subsequent trials using branch inoculations showed significant differences in *F. circinatum* tolerance amongst a range of pine species and hybrids of potential interest to forestry in South Africa. Significant differences in susceptibility were also found among clones of two *P. radiata* families. The most tolerant trees were *P. elliottii* × *caribaea* and *P. patula* × *oocarpa* hybrids, while the most susceptible species were *P. patula*, *P. greggii* and hybrids of these two. This is the first trial considering the susceptibility of *Pinus* hybrids, *Pinus* clones and some *P. patula* provenances, and the results indicate excellent potential for breeding for tolerance to pitch canker in South Africa.

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Application The accurate selection of disease tolerant planting stock for the South African forestry industry is crucially important for the continued sustainability of this important industry. The work described here provides valuable information on an artificial inoculation technique that will assist the industry in screening trees for tolerance to the pitch canker fungus, *F. circinatum*. It also provides some indication of the relative susceptibility of a number of *Pinus* spp., hybrids and families currently being evaluated in the country.

Keywords Forestry · Fungal disease · Inoculation · Resistance · Screening

Introduction

The South African forestry industry is one of the most important sources of employment and export capital to the country and it is based primarily on plantations of *Eucalyptus* (~40%) and *Pinus* (~50%) species (Anonymous 2003). Of the *Pinus* spp., *P. patula* Schlecth. is most widely and commonly planted. Other important species include *P. elliottii* Chapm. and to a lesser extent *P. taeda* L. *Pinus radiata* D. Don., makes up a very small portion of the pine resource, but is important in the Western Cape Province.

Disease and pest problems pose one of the greatest threats to the sustainability of commercial plantation forestry (Wingfield et al. 2002a; Wingfield 2004). One of the most important diseases threatening exotic plantation forestry is pitch canker, caused by *Fusarium circinatum* Nirenberg & O'Donnell (teleomorph: *Giberella circinata* Nirenberg & O'Donnell) (Correll et al. 1991; Dwinell et al. 1985; Gordon et al. 2001; Nirenberg and O'Donnell 1998; Wingfield et al. 1999, 2002a). Until 1989, *F. circinatum* was known only in the United States of America, where it first appeared in 1945 on *P. virginiana* Mill. in Florida (Hepting and Roth 1946). Originally this pathogen caused only occasional outbreaks of disease on *P. elliottii* in Florida (Dwinell and Phelps 1977) and in *P. taeda* seed orchards in North Carolina and Mississippi (Dwinell et al. 1977). However, in 1986 pitch canker appeared on *P. radiata* in California where it has subsequently caused a serious epidemic that threatens *P. radiata* in its native range (Gordon et al. 2001; McCain et al. 1987; Storer et al. 1997). The pathogen also occurs in Japan (Kobayashi and Muramoto 1989), Mexico (Rodriques 1989), South Africa (Viljoen et al. 1994), Chile (Wingfield et al. 2002b) and most recently Spain (Landeras et al. 2005).

Pinus radiata is extensively planted in countries such as Australia, Chile and New Zealand, where it forms the basis of substantial forest industries. The damage to *P. radiata* in its native range by *F. circinatum* has thus elevated this pathogen to being considered one of the most important threats to exotic plantation forestry in the Southern Hemisphere. Furthermore, this threat extends to South Africa where the local forestry industry relies heavily on *P. patula*, also known to be highly susceptible to *F. circinatum* (Hodge and Dvorak 2000; Viljoen et al. 1995; Wingfield et al. 1999).

Fusarium circinatum was first reported in South Africa in the early 1990's when the pathogen was discovered causing a seedling root and collar disease in a major forest production nursery (Viljoen et al. 1994). This fungus has subsequently spread to nurseries throughout the country where it has caused devastating losses. It has

also recently been found in several field plantings of trees up to 3-years-old, although it is not known whether these outbreaks are linked to nursery infections.

Pitch canker refers to a canker disease on the stems and branches of established trees, and thus, the manifestation of *F. circinatum* on seedlings in South Africa represents a different phase of the disease from that found on established trees elsewhere in the world (Wingfield et al. 1999, 2002a). Nonetheless, the fungus has caused debilitating losses in nurseries and newly established plantations. It is consequently considered the most important disease threat to the continued production of *P. patula* in South Africa. On established trees, pitch canker results in reduced timber quality because of stem deformation, reduced growth, seed contamination in seed orchards and in some cases tree death (Barnard and Blakeslee 1980; Barrows-Broaddus and Dwinell 1985; Dwinell et al. 1985; Schmidt and Underhill 1974).

If pitch canker were to appear on mature trees in South Africa, the only reasonable solution would be to plant disease tolerant trees. Techniques to identify planting stock with high levels of tolerance to infection by *F. circinatum* are thus desired. A number of studies have been conducted to identify differences in the susceptibility of *Pinus* spp. and provenances. Storer et al. (1998), reported variation in susceptibility of *P. radiata* to *F. circinatum*. Other researchers have also used inoculation trials to identify differences in susceptibility to the pathogen. For example, of particular interest to the South African situation is the work of the International Tree Conservation and Domestication programme (Camcore), a Co-operative that provides seed of promising *Pinus* spp. and provenances to commercial forestry companies for testing worldwide. Because of the importance of pitch canker, Camcore has actively engaged in testing key *Pinus* species for susceptibility (Hodge and Dvorak 2000). Through inoculation of seedlings, they have been able to show differences in susceptibility of *Pinus* spp. to infection by *F. circinatum*. In these studies, *P. radiata* and *P. patula* was shown to be amongst the most susceptible to infection.

The threat of pitch canker to the future of commercial pine forestry in South Africa is great. This is primarily because the most important species planted have high levels of susceptibility to the pathogen. Although the pitch canker fungus is common in nurseries, large trees have so far not been infected and natural selection cannot be used to identify disease tolerant trees. Previous studies have suggested that greenhouse inoculations could be used for this purpose (Gordon et al. 1998a, 1998b; Hodge and Dvorak 2000), although testing on larger trees would seem to be more natural and preferable. The aim of this study was to optimize an appropriate field-based screening technique that would allow for the rapid selection of disease tolerant individuals that might be used in subsequent tree breeding trials. A second objective was to compare important pine species, clones and hybrids for susceptibility to infection by the pitch canker fungus.

Materials and methods

Isolate for inoculations

An appropriate isolate of *F. circinatum* for inoculation trials was chosen from a randomly selected set of isolates collected from diseased seedlings in commercial nurseries in South Africa. All isolates were grown on 2% Malt Extract Agar (MEA)

(Biolab) for 7 days at approximately 25°C. Each isolate was inoculated onto ten, approximately 24-month-old, trees by removing a bark plug (5 mm diameter) and inserting an agar plug, of equal size, overgrown with the test fungus into the wound. The wounds and agar plugs were covered with Parafilm to prevent desiccation of the inoculum and the wounds. After 4 weeks, the bark was removed from around the inoculation site and lesion lengths were measured. The isolate giving the longest average lesion length was then chosen for subsequent inoculations

Comparison of branch and stem inoculations

To determine whether branch inoculations gave the same results as stem inoculations, as well as to evaluate different *P. radiata* clones for susceptibility to *F. circinatum* infection, inoculations were conducted on 3-year-old field-grown *P. radiata* trees. Trees were planted in 1997 at Karatara in the Western Cape Province. Twenty individuals from each of two *P. radiata* families (Family 1 and 2) were selected from open pollinated trees. Each of the twenty individuals per family was multiplied by vegetative propagation to produce thirty cuttings of each individual. A trial block was established using the forty different *P. radiata* genotypes and the thirty replicate clones of each genotype (1200 trees in total). The trees were planted using a randomised complete block design.

Four sets of inoculations were conducted on the test trees. The first two sets of inoculations were made on a single branch per tree. A third and fourth set of inoculations were conducted a year later on five branches per tree and later on the main stems of the trees. All inoculations were made using the selected virulent isolate of *F. circinatum* (FCC49). The fungus was grown on 2% MEA for 7 days at approximately 25°C.

Inoculations were performed by removing a 9 mm diameter bark plug from the branch or stem of each test tree, using a metal cork borer, to expose the cambium. An agar plug of equal size, overgrown with the fungus, was placed into each wound with the mycelium facing downwards and the wounds were sealed with masking tape to prevent desiccation. In all cases, lesion lengths were measured after 6 weeks. After measuring the results, all the inoculated branches were removed and destroyed. Subsequent inoculations were conducted on the same trees, after removal of the branches.

For the branch trials, the diameter of each branch at the point of inoculation was recorded to determine the effect of branch diameter on lesion development. After the inoculated branches had been removed from each tree, single inoculations were made into the stems of the same trees. Results of the main stem inoculations were also collected after 6 weeks and the circumference of the main stem at the point of inoculation was measured to determine possible effects of stem diameter on lesion length.

Screening of Pinus species and hybrids

Three trial sites were identified for screening different *Pinus* hybrids and species for susceptibility to *F. circinatum*. These trials represented existing selection trials of two forestry companies in South Africa, all located in the Mpumalanga Province of South Africa (Table 1). They were selected because the relative susceptibility to *F. circinatum* could be directly correlated with other data already collected for the

Table 1 Details of trials undertaken on different species and hybrid selections

Site	Elevation (m)	Trial design	Number of treatments	Composition
Graskop	1197	RCB with 4 replications & 6 tree line plots	59	*P. caribaea* var. *hondurensis* *P.elliottii* *P. elliottii* × *P. caribaea* *P.greggii* var *australis* *P. greggii* × *P. tecunumanii* *P. kesiya* *P. patula* var. *patula* *P. taeda* × *P. tecunumanii* *P. tecunumanii*
Sabie	980	RCB with 6 replications & 5 tree line plots	32	*P. elliottii* *P. elliottii* × *caribaea* *P. greggii* × *tecunumanii* *P. patula* × *greggii* *P. patula* × *oocarpa* *P. patula* × *radiata* *P. patula* × *tecunumanii*
Waterval boven	1800	RCB with 6 replications & 5 tree line plots	34	*P. greggii* var. *australis* *P. greggii* var. *greggii* *P. greggii* × *tecunumanii* *P. patula* var. *patula* *P. patula* × *greggii* *P. patula* × *oocarpa* *P. patula* × *radiata* *P. patula* × *tecunumanii* *P. taeda* × *tecunumanii*

trees. Each trial consisted of different numbers of treatments and a varying representation of species or hybrids. The Watervalboven and Sabie trials had some species and hybrids in common, while the Graskop trial consisted of species and hybrids that were unique to that site. Trees for the Watervalboven and Sabie trials were from cuttings taken from hedge plants representing hybrid families or seedlings representing pure species. Some seed orchard mixes of different families were also included among the treatments. For the Graskop trial material was obtained from seed collections.

Inoculations were conducted in December 2001 to ensure that trees were actively growing and environmental conditions were conducive to infection and growth of *F. circinatum*. The inoculation technique for these trees was based on prior experience gained from the *P. radiata* trials. It was thus known that inoculation of three branches per tree should yield reliable results. The technique described previously was followed and results were read 6 weeks after inoculation by measuring lesion lengths and diameters of each branch.

Statistical analyses

Data were analysed using SAS (SAS Institute Inc. 1999). All data were tested for adherence to the normal distribution. Data were analysed using General Linear Model (GLM) and Bonferoni t Confidence intervals were determined to distinguish between different treatments (representing either clones, hybrid families and pure species seedlings).

In addition to determining differences in tolerance, several other key questions around the methodology were addressed with the results from the *P. radiata* trials. These considered: (1) whether lesion length is associated with the diameter of branches or stems and whether an adjustment of lesion length is required for diameter; (2) whether lesions produced on branches would provide a similar reflection of tolerance to those produced on the main stems; (3) what number of branches would be required to provide a reliable assessment of susceptibility; (4) sensitivity of the inoculations; and (5) how many replicates of a clone would be necessary to obtain an accurate indication of susceptibility. Data from all trials (assessed in the Karatara, Watervalboven, Sabie and Graskop trials) were used to determine whether genotypes differ markedly in their susceptibility to infection by *F. circinatum*.

Where a most appropriate inoculation technique was being developed, stem and branch sizes were considered as co-variates. The four *P. radiata* trials were analysed as 14 separate data sets to check for conformity to the assumptions of the Analysis of Variance (ANOVA) and to effect transformations (guided by the Box–Cox method) where required. Where data were missing, Least Square Means were required to obtain accurate estimates of treatment means. These means were used to calculate correlations between branch and stem lesions. The Cronbach coefficient was calculated for each data set to determine the consistency of measurement across the replications. While these correlations were appropriate to indicate whether branches and stems provided equivalent information, regression modelling was employed to determine the number of branches that would be necessary to predict the stem value with a high degree of accuracy. Furthermore, a post-hoc power analysis was carried out on the data to determine how well the trials discriminated between different levels of tolerance and susceptibility. A prospective analysis of the discriminatory power of the experiments was also conducted.

Results

Comparison of branch and stem inoculations

After 6 weeks, lesions were visible on all the inoculated branches and on the main stems for all of the trials where branch inoculations were compared with stem inoculations on *P. radiata* at Karatara. The most disease tolerant trees had small lesions (10–30 mm), while the susceptible trees had much larger lesions (> 50 mm). In some cases, the inoculated branches were dead (Fig. 1), especially after the first set of inoculations (*P. radiata* single-branch trials), when the trees were younger and the branches smaller than those used in the subsequent inoculations. Statistically significant differences in lesion length were found between the various *P. radiata* clones from the two *P. radiata* families (Family 1 and 2) ($P < 0.0001$) (Figs. 2, 3).

Statistical analysis of the data showed that branch diameter was an important factor in assessing susceptibility of trees. This was clearly shown for a data set where un-adjusted lengths showed no correlation between branch and stem inoculations ($P > 0.05$, Pearson correlation coefficient of 0.2), while the same set of data, that had been adjusted with diameter as a covariate, showed a strong correlation between lesion length on branches and main stems ($P > 0.0001$, Pearson correlation coefficient of 0.81) (Table 2).

Fig. 1 Lesions produced on *P. radiata* 6 weeks after inoculation with *F. circinatum*. (**A**) Dead branch of susceptible tree, (**B**) lesions on branch

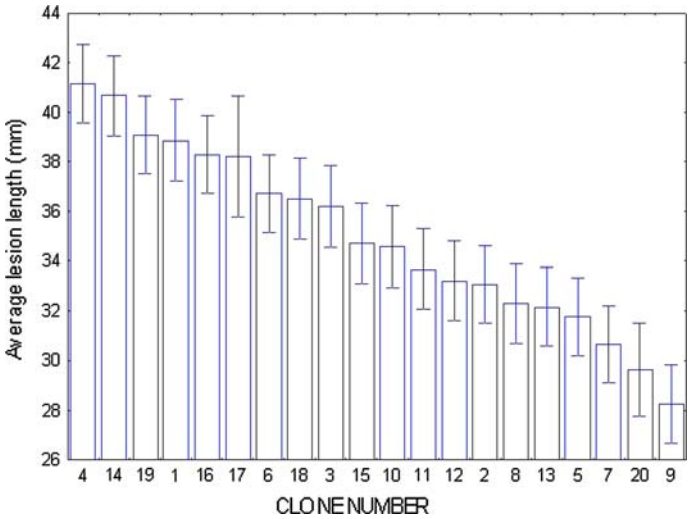

Fig. 2 Variation in susceptibility to *F. circinatum* infection for 20 clones in *P. radiata* family 1. Results based on inoculation of 5 branches for each of 30 cuttings per individual. (Pr < 0.0001; *R*-Square = 0.52; CV = 17.97)

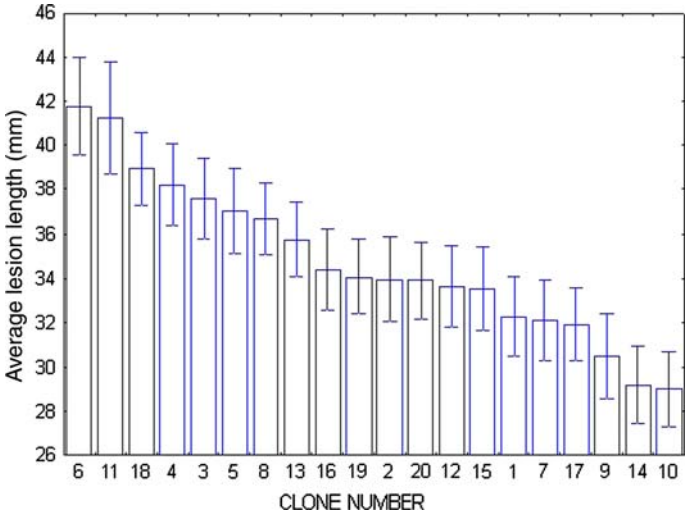

Fig. 3 Variation in susceptibility to *F. circinatum* infection between 20 clones in *P. radiata* family 2. Results based on inoculation of 5 branches for each of 30 cuttings per individual. (Pr < 0.0001; *R*-Square = 0.54; CV = 18.56)

A strong correlation was found between lesion lengths on different branches when five branches were inoculated on a single tree. Multiple regression analysis of these data showed that the inoculation of one branch gives an equally accurate indication of susceptibility as that based on an average of five branches per tree. (*R*-square values of more than 0.6 for all). Good correlations were also found between lesion length on five branches on single trees and lesions obtained from the inoculation on the main stems (Table 2). Correlations between the stem and single

Table 2 Pearson correlation[a] coefficients of results for clonal *Pinus radiata* inoculated with *F. circinatum*

Correlation	Family 1	Family 2
P. radiata single-branch 1& 2 trial	0.30	0.82
P. radiata five-branch trial	0.81–0.96	0.79–0.94
P. radiata single-branch 1 & main stem trial	0.81	0.59
P. radiata single-branch 2 & main stem trial	0.38	0.54
P. radiata five-branch & main stem trial	0.75–0.81	0.71–0.79

[a] $P > 0.0001$

branch inoculations were slightly lower than between the stem and 5-branch inoculations (Table 2).

''Power'' analysis (Statistical power analysis to determine sample size) showed that the experiments with 30 replicates are very efficient in exposing large differences in lesion size. Prospective power analysis showed that maintaining a $P = 0.05$ and 90% ''Power'' would be achieved with 20 replicates, or at most 25, assuming that the error remains the same. When calculating the power (i.e. 1-β) for the clonal experiments to determine how well they illustrate the differences between highest and lowest lesion measurements, the experiments were 99–100% efficient, at power $P = 0.05$ as well as $P = 0.01$ in determining firstly that the difference between the highest and lowest is real and measurable, and secondly that this difference could not have arisen by chance. Differences halfway down the range remained good at $P = 0.05$.

Calculation of the Cronbach coefficient for the lesion lengths in these experiments resulted in very high values (0.8 and greater). This confirms the high level of reliability of the technique used to inoculate and measure resultant lesions.

Screening of Pinus hybrids and families

Statistically significant differences ($P < 0.0001$) in susceptibility to infection by *F. circinatum* were obtained for all treatments and at all three sites. As was found in the *P. radiata* trials where inoculation technique was a primary aim, branch diameters were highly significant co-variants ($P < 0.0001$). Evaluations showed that *P. elliottii* × *caribaea* and *P. patula* × *oocarpa* hybrids in the trials were the most tolerant to infection. The most susceptible species were pure *P. patula* and *P. greggii*, while in general *P. greggii* hybrids were the most susceptible of all the hybrids.

Statistically significant differences were found between hybrids in the Watervalboven trial at a confidence level of 95% (R-square = 0.56) (CV = 26.76) (Fig. 4). All three branches gave statistically similar results when evaluated separately or in combination. The most susceptible selection in the trial was a *P. greggii* var. *greggii* selection, while the most tolerant hybrid was a *P. taeda* × *P. tecunumannii* family (Fig. 4). In general the *P. oocarpa* hybrids were more tolerant than the *P. greggii* hybrids.

As with the Watervalboven trial, results for the Sabie trial showed that all three branches gave the same results when evaluated separately or in combination. Statistically significant differences were found between hybrids at a 95% confidence

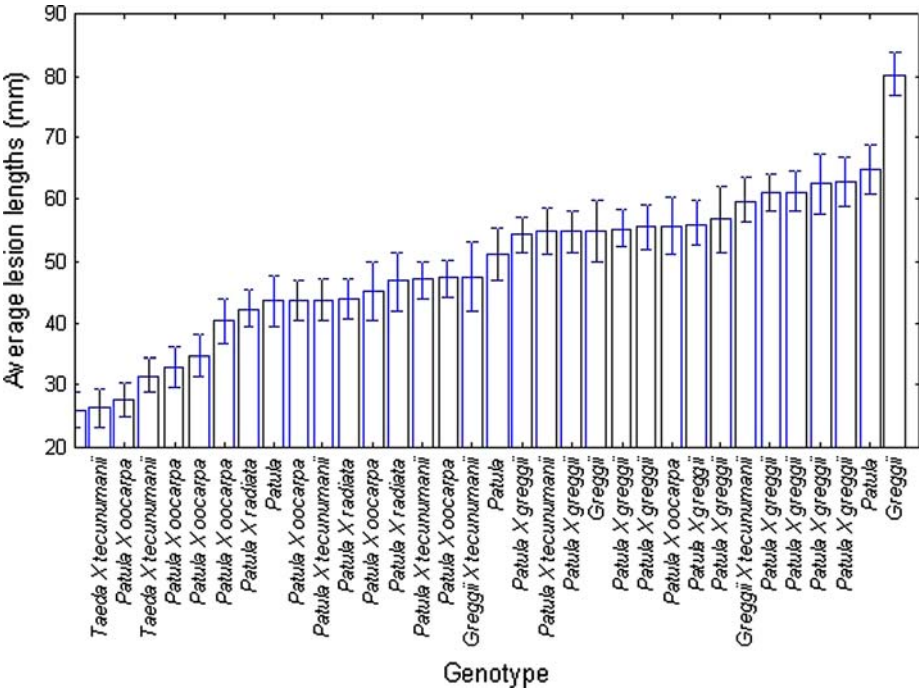

Fig. 4 Bar graph with error bars showing variation between *Pinus* hybrids and species for the Watervalboven trial. Lesion lengths were measured in millimetres

level (*R*- square = 0.4) (CV = 28.86). The most susceptible selection in the trial was a *P. patula* × *P. greggii* hybrid, while the most tolerant hybrid was a *P. patula* × *P. oocarpa* family, followed closely by a *P. elliottii* second generation seedlot (Fig. 5).

The Graskop trial showed good correlation between branches (*R*-square = 0.64, CV = 19.2) at a confidence level of 95%. As for the other trials, branch diameter played a significant role (Pr = 0.68) in results. Branches of the most susceptible trees were dead or dying and clear differences in lesion development were observed between hybrids. Results showed clearly that it is possible to distinguish between hybrids, in terms of their susceptibility to infection. The most susceptible selection planted in the trial was a *P. patula* selection, while the most tolerant hybrids were those of *P. elliottii* × *P. caribaea* family (Fig. 6).

Discussion

In this study, we have tested an inoculation method for trees in plantations and shown that it is possible to inoculate branches of trees in order to obtain a statistically reliable estimate of susceptibility to infection by *F. circinatum* under field conditions. This technique is simple and inoculations can be made relatively rapidly. It also has the important advantage of allowing evaluations of disease tolerance on older trees and under field conditions which reflect natural conditions more accurately than do nursery plants. Branch inoculation also allows survival of inoculated trees, which can be left to grow and be used later for breeding and seed production.

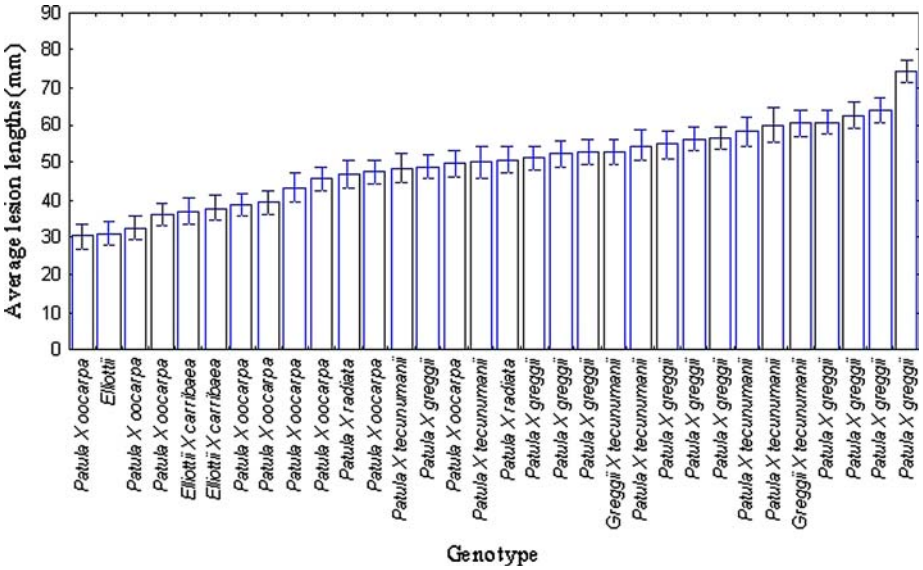

Fig. 5 Bar graph with error bars showing variation between *Pinus* hybrids and species for the Sabie trial. Lesion lengths were measured in millimetres

These trials have also shown that there is great potential for selecting disease tolerant planting material from current South African planting stock and thus to ensure that serious losses due to *F. circinatum* infection can be minimized in the future.

An important observation emerging from this study has been that branch diameter is an important factor in lesion development and that this must be considered when analysing data. Thus, where branch diameter was considered as covariate, much higher statistical significance was found, implying a truly accurate discrimination between clones, species or hybrids. Branch inoculations with *F. circinatum* have been used in several previous studies (Gordon et al. 1998a, b). In those studies, spore suspensions were applied to holes made in branches with small drills. None of these studies, however, calculated the possible impact of branch size on the results obtained. Hodge and Dvorak (2000), however, made a similar observation when they showed that an adjustment for height should be made for seedlings in nursery inoculations.

The initial studies with *P. radiata* clones, considering branch inoculations, showed that reliable results can be obtained where as little as one branch per tree is inoculated with *F. circinatum*. Despite these earlier results, the experiments with species and hybrids utilized three branches per tree because the material was expected to be far more variable than the clonal trial. This also made it possible to accommodate loss of branches due to wind or mechanical damage, during the course of the experiments and increased the precision of the experiments. In comparison with previous studies where results were measured only after periods ranging from 2 to 6 months (Dwinell and Barrows-Broaddus 1979; Hodge and Dvorak 2000), *F. circinatum* in our trials gave rise to lesions much more rapidly, with some branches dying within 6 weeks. This might be explained by the fact that the *F. circinatum* strain selected for inoculation in our trials was more virulent than those used by other researchers. Alternative explanations are that some of the material inoculated

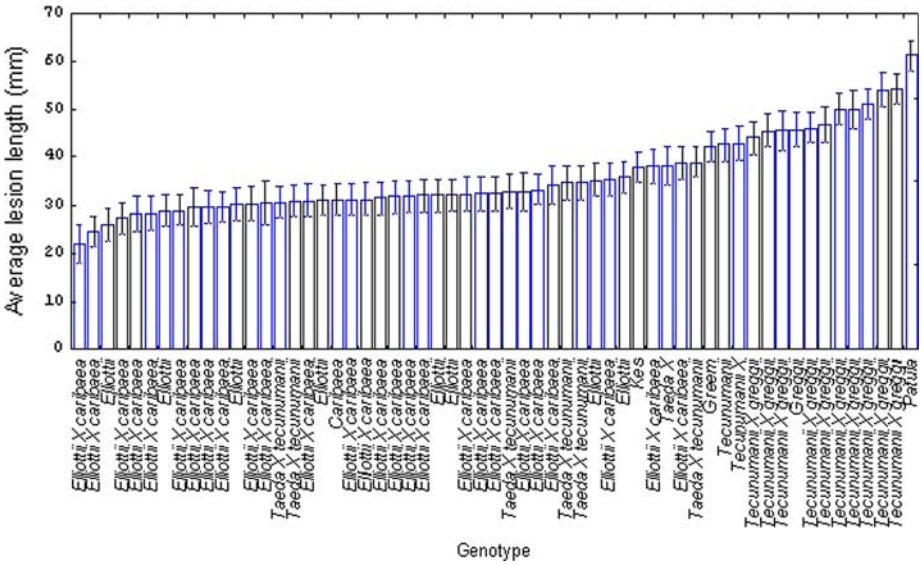

Fig. 6 Bar graph with error bars showing variation between *Pinus* hybrids and species for the Graskop trial. Lesion lengths were measured in millimetres

in South Africa is considerably more susceptible than those used in previous trials, or that environmental conditions in South Africa are more conducive to disease development.

Results of this study showed clear differences in susceptibility, both at a species and hybrid level as well as at a within species and at clonal level. There have been contrasting reports in the literature as to the variation in susceptibility within and between *Pinus* spp. Hodge and Dvorak (2000), for example, reported little or no tolerance to pitch canker when comparing collections from native populations, open pollinated families, full sib or control pollinated families of *P. radiata*. In contrast, several other researchers have reported considerable variation in susceptibility within *P. radiata* (Correll et al. 1991; Dwinell and Barrows-Broaddus 1979; Gordon et al. 1998a, b; Schultz et al. 1990) and *P. taeda* (Dwinell et al. 1977). These differences in results might be explained by the differences in inoculation techniques used and/or the differences in genetic sampling undertaken in the different studies.

The present study included a relatively small number of pure *P. patula* selections and *P. radiata* clones, mainly because trials available for pitch canker screening more strongly focussed on pine hybrids. Nonetheless, where pure *P. patula* and *P. radiata* were inoculated, statistically significant differences were observed in their susceptibility. Furthermore, there were several individual trees within families of these two pure species that showed high levels of tolerance to infection. Comparatively high levels of tolerance were also found among the limited number of *P. radiata* clones tested in this study. This suggests that it will be possible to grow these individuals and to use them as breeding stock to produce disease tolerant families.

It was clear from this study that hybrids between different *Pinus* spp. present great opportunities to develop planting stock tolerant to pitch canker. Within the hybrid selection trials, *P. patula* × *P. oocarpa* and *P. patula* × *radiata* hybrids, for example, showed variation in susceptibility, with some of these selections grouping

among the more tolerant material. This suggests that there are excellent opportunities not only to combine desirable wood properties, but also tolerance to pitch canker in future pine breeding. South African hybrid selections, including *P. oocarpa* and *P. caribaea*, were most tolerant to infection by *F. circinatum*. These results are consistent with those of Hodge and Dvorak (2000), who showed that *P. oocarpa* and *P. caribaea* were amongst the most tolerant in their trials. Our results also confirmed that pines within Sub-Section *Patula* (Hodge and Dvorak 2000) were the most susceptible to infection.

Pinus patula is one of the most important *Pinus* sp. utilised in commercial forestry in Africa, yet little is known regarding the genetic diversity of this tree on the continent. In contrast to the general situation in South African nurseries, suggesting that there is no tolerance to *F. circinatum* within *P. patula*, our results provide encouraging evidence to show variation in susceptibility between a limited number of *P. patula* selections commonly used in South Africa. This might have been expected as both *P. patula* and other *Pinus* spp., such as *P. gregii* var *gregii*, native to Mexico would have co-evolved with *F. circinatum*, which is apparently also native to that country (Britz et al. 2001). Excellent opportunities thus appear to exist to develop *P. patula* breeding stock that is resistant to *F. circinatum*, even from the genetic base of the species currently available in South Africa.

The average lesion lengths of the most tolerant selections in the three hybrid trials were 22 mm and greater. This clearly shows that even the more tolerant selections considered in this study have a high degree of susceptibility when a large number of individuals are considered. However, within these selections there were individual trees that had very small lesions. This indicates that the best opportunity for establishment of disease resistant planting stock will lie in selection of disease tolerant clones, based on the most resistant individuals in a family. In the longer term, these trees could then be used as breeding stock to produce seed from families with high levels of tolerance to the pith canker pathogen.

Selection for resistance to the pitch canker pathogen in South Africa will need to be an ongoing process. This is because *F. circinatum* is undergoing sexual reproduction in the country and new genotypes are emerging continuously (Britz et al. 2005; Viljoen et al. 1997). Thus, the pathogen is likely to adapt to selected, disease tolerant planting stock and durability of this trait might not be great. This is in contrast to the situation in California where no sexual reproduction has been found in the field and resistance would be more durable (Correll et al. 1992; Gordon et al. 1998a).

Current management of disease caused by *F. circinatum* in South Africa relies largely only on nursery hygiene practices and management. Despite concerted efforts, *F. circinatum* is continuing to cause large-scale problems in the country. Its recent appearance in Chile (Wingfield et al. 2002b) and Spain (Landeras et al. 2005) also highlights the fact that the pathogen is moving around the world. This, together with the low levels of resistance in commercial *P. radiata* populations from Chile and New Zealand in greenhouse inoculation trials (Hodge and Dvorak 2000), emphasizes the international importance of this pathogen. The only long-term solution to this disease must lie in the selection and breeding for disease tolerant trees. Results of this study provide encouraging evidence that this can be achieved and that rapid screening for disease tolerance will facilitate the process.

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