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Susceptibility of pines in South Africa to the pitch canker fungus *Fusarium subglutinans* f.sp. *pini*

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Fusarium subglutinans f.sp. *pini* (*F.s. pini*) is the causal agent of pitch canker of pines. The fungus has recently been found in South Africa on the diseased roots of *Pinus patula* seedlings, but has as yet not been detected on mature trees in commercial forests. Inoculation of 1-year-old *P. patula*, *P. elliottii* and *P. radiata* seedlings with isolates of *F.s. pini* resulted in canker development and shoot mortality. No significant differences in virulence were found among eight isolates of the pathogen on *P. elliottii* and *P. radiata*, but isolate MRC 6214 was significantly more virulent on *P. patula* seedlings than MRC 6209. Disease development was significantly more severe on *P. patula* and *P. radiata* than on *P. elliottii*. Pathogenicity tests on 4-year-old *P. patula* and *P. elliottii* trees yielded comparable results. Resinous cankers, similar to those described for pitch canker, developed on trees in the vicinity of inoculation points but development ceased before stems were girdled.

INTRODUCTION

Fusarium subglutinans f.sp. *pini* (*F.s. pini*) is best known as the causal agent of pitch canker of pines (Hepting & Roth, 1946; Dwinell *et al.*, 1985). The primary symptoms of the disease are resinous cankers on the main trunk and large branches, and shoot die-back in the upper crown (Blakeslee *et al.*, 1980; Dwinell *et al.*, 1985). In 1974, pitch canker reached epidemic proportions in plantations and seed orchards of *Pinus elliottii* (slash pine) (Dwinell & Phelps, 1977) and on *P. taeda* (loblolly pine) seed orchards in the southern United States (Dwinell *et al.*, 1977). The disease has more recently been reported from California (McCain *et al.*, 1987), Japan (Kobayashi & Muramoto, 1989) and Mexico (Santos & Tovar, 1991). Between 1990 and 1992, *F.s. pini* was responsible for a serious root disease of pine seedlings in South Africa (Viljoen *et al.*, 1994).

All pines grown in South Africa are exotic. Three of the most important commercial species planted are *P. patula* (45%), *P. elliottii* (27%), and *P. radiata* (Monterey pine) (9%) (Hinze, 1993). *P. patula* is native to Mexico, while *P. elliottii* and *P. radiata* occur naturally in the southern United States and California, respectively (Critchfield & Little, 1966). All three species therefore originate in areas of the world where pitch canker occurs, in many cases as a serious disease.

Pitch canker incidence is variable and depends on the inherent susceptibility of pine species, families or clones (Dwinell & Barrows-Broaddus, 1982). In the southern United States, *P. radiata* was found to be highly susceptible to infection, whereas *P. elliottii* was moderately susceptible to infection (Dwinell, 1978; Hepting, 1961). Variation in virulence also occurs among isolates of *F.s. pini*, but no relationship has been detected with geographic source or origin (Barrows-Broaddus & Dwinell, 1979).

The recent appearance of *F.s. pini* in South Africa is of considerable concern. The aim of the study was to determine the susceptibility of *P. patula*, *P. elliottii* and *P. radiata* in South Africa to *F.s. pini*. The relative virulence of different isolates of *F.s. pini* was also compared.

MATERIALS AND METHODS

Isolates

Isolates of *F.s. pini* and *F. oxysporum* recovered from roots of diseased *P. patula* seedlings were used for pathogenicity tests. These isolates are preserved in the culture collection of the Medical Research Council (MRC), PO Box 19070, Tygerberg, 7505, South Africa. Inoculum was prepared by transferring lyophilized cultures to carnation leaf agar (CLA) in Petri dishes and incubating them at 25°C for 14 days under cool-white and near-ultraviolet lights.

Inoculation of 1-year-old seedlings

One-year-old seedlings of the three pine species were inoculated with eight isolates of *F.s. pini* (MRC 6208, 6209, 6211, 6213, 6214, 6215, 6216 and 6217) and one isolate of *F. oxysporum* (MRC 6212). The seedlings were grown in 750-ml plastic bags in a nursery until inoculation. Twenty seedlings of each *Pinus* species were inoculated with each isolate of *F.s. pini* and *F. oxysporum*, making a total of 160 inoculated seedlings of each species. Twenty seedlings of each species were inoculated with sterile agar as controls.

At the time of inoculation, small vertical strips of bark (approximately 10 mm long and 1 mm wide) were cut from the stems of each pine seedling to expose the cambium. Mycelial strips cut from the cultures, similar in size to the wounds, were placed in contact with the cambium and covered with parafilm. The seedlings were kept in a growth room at 25°C under cool-white and dark-fluorescent lights set at a 12 h photoperiod. Lesion development was measured distal to the point of inoculation after 16 days. Random samples of five seedlings per treatment per species were selected for reisolation of the inoculated fungi.

Inoculation of trees under field conditions

In September 1992, 4-year-old *P. patula* and *P. elliottii* trees were selected for inoculation under field conditions. These trees were all cultivated from seed, and were planted in Graskop, Eastern Transvaal province. Inoculations were performed during spring, when temperatures normally fluctuate between 28 and 36°C. *F.s. pini* isolate MRC 6211 was used to compare the susceptibility of *P. patula* and *P. elliottii*. Isolate virulence was compared by inoculating 4-year-old *P. patula* with three isolates of *F.s. pini* (MRC 6208, 6214 and 6217) and two isolates of *F. oxysporum* (MRC 6210 and 6212).

Trees were inoculated by removing 11-mm diameter pieces of bark at chest height (approximately 140 cm) with a cork borer. Mycelial plugs from the culture were transferred aseptically, fitted into the cork borer wounds and covered with masking tape. Sterile agar was used for control inoculations. Twenty trees were inoculated per treatment and lesion development was assessed after 4 months by removing small pieces of the outer bark on the edges of cankers and measuring the length of each canker. Disease development was studied by dissecting cankers on randomly selected trees at the point of inoculation. Reisolation of the inoculated fungi was attempted at the interface of healthy and diseased tissue of five randomly selected trees for each treatment. Development of cankers on the remaining trees was followed over a subsequent 12-month period.

Statistical analyses of data

Analyses of variance were performed on the SAS/STAT system for personal computers (SAS Institute Inc., Cary, NC, USA). Data were compared using Tukey's studentized range (HSD) test.

RESULTS

Inoculation of 1-year-old seedlings

Symptoms developed on all three *Pinus* spp. inoculated with *F.s. pini*. Lesions were reddish brown, slightly sunken and sometimes produced resin (Fig. 1a). Resin production was more prevalent on *P. patula* than on *P. elliottii* or *P. radiata*. The wood beneath the bark was slightly discoloured, but seldom showed signs of colonization. Cankers eventually girdled the stems at the point of inoculation, resulting in wilting and die-back. No sporodochia developed on the stems, but hyphal growth and sporulation of the fungus could sometimes be seen externally, especially on *P. radiata* seedlings. No symptoms developed on seedlings inoculated with *F. oxysporum* or on the controls. The inoculated fungi could only be reisolated from seedlings inoculated with *F.s. pini*.

All the inoculated *P. patula* and *P. radiata* seedlings developed cankers, and 93% *P. elliottii* seedlings developed cankers. There was no significant ($P \leqslant 0{\cdot}05$) difference in susceptibility between *P. patula* and *P. radiata*. However, they were both significantly more susceptible to the fungus than *P. elliottii* (Table 1). Isolate MRC 6214 of *F.s. pini* was significantly ($P \leqslant 0{\cdot}05$) more virulent on *P. patula* seedlings than isolate MRC

Fig. 1 *Pinus patula* inoculated with *Fusarium subglutinans* f.sp. *pini* (*F.s. pini*). (a) Resinous lesion on 1-year-old seedlings. (b) Canker with resin pockets on the main stem of a 4-year-old *Pinus patula* tree. Bars = 10 mm.

Table 1 Susceptibility of 1-year-old *Pinus* seedlings to *Fusarium subglutinans* f.sp. *pini* (*F.s. pini*) and *F. oxysporum*

Fusarium species	Isolate	Lesion length (mm)[1]			
		P. patula	*P. elliottii*	*P. radiata*	Mean
F.s. pini	MRC 6208	23·25ab	8·35a	21·21a	17·60a
F.s. pini	MRC 6209	18·50b	9.85a	19·49a	15·95a
F.s. pini	MRC 6211	20·95ab	12·10a	19·17a	17·41a
F.s. pini	MRC 6213	22·45ab	13·15a	20·45a	18·68a
F.s. pini	MRC 6214	24·20a	11·45a	20·27a	18·64a
F.s. pini	MRC 6215	19·55ab	9·10a	21·04a	16·56a
F.s. pini	MRC 6216	19·45ab	8·90a	21·49a	16·61a
F.s. pini	MRC 6217	19·40ab	11·45a	21·17a	17·34a
F. oxysporum	MRC 6212	0·00c	0·00b	0·00b	
Control		0·00c	0·00b	0·00b	

[1] Mean lesion lengths (20 seedlings per isolate) followed by the same letter in each column do not differ significantly ($P \leqslant 0{\cdot}05$) according to Tukey's studentized range (HSD).

Table 2 Susceptibility of 4-year-old *Pinus patula* and *P. elliottii* trees to *Fusarium subglutinans* f.sp. *pini* (*F.s. pini*) under field conditions

	Lesion length (mm)[1]	
Pinus spp.	*F.s. pini*	control
P. patula	103·75a	11·00
P. elliottii	21·30b	11·00

[1] Mean lesion lengths (20 trees per treatment) followed by the same letter do not differ significantly ($P \leqslant 0{\cdot}05$) according to Tukey's studentized range (HSD).

6209 (Table 1). There were, however, no significant differences in virulence among the eight isolates of *F.s. pini* to *P. elliottii* and *P. radiata*.

Inoculation of trees under field conditions

Trees inoculated with *F.s. pini* exhibited stem cankers 4 months after inoculation and copious amounts of resin flowed from the points of inoculation (Fig. 1b). The bark was intact but slightly sunken. When bark was stripped from the cankered areas, dark brown discolouration and resin-soaking of the underlying wood could be seen (Fig. 1b). Large pockets of resin were present throughout the cankered area. The resin-soaking was most prominent in the outer wood but sometimes formed deeper wedges towards the pith. The cankers had not callused over, and had caused slight stem deformation. The fungus was easily reisolated from the canker margins. After 10 months, lesions showed signs of recovery, and a clear interface could be seen between live and dead tissue. At this stage the inoculated fungi could not be reisolated from cankered areas.

P. patula was significantly more susceptible to *F.s. pini* than *P. elliottii* (Table 2). The mean length of lesions on *P. patula* trees was almost five times longer than on *P. elliottii*. Cankers developed on all *P. patula* trees, but only 15% of *P. elliottii* trees developed lesions substantially larger than the initial wound. The three isolates of *F.s. pini* did not differ significantly ($P \leqslant 0{\cdot}05$) in virulence to *P. patula*. Trees inoculated with *F. oxysporum* or sterile agar developed no symptoms.

DISCUSSION

In this study we have shown that *P. patula* and *P. radiata* are highly susceptible to the pitch canker pathogen, whereas *P. elliottii* is only moderately susceptible. This is of particular concern because these three species constitute 80% of pines grown in South Africa and almost half of the total commercial forest investment (Hinze, 1993). It appears likely that the pathogen has been introduced into the country and outbreaks of pitch canker can probably be expected in the future. The disease has the potential to affect seriously one of the most valuable agronomic resources in southern Africa.

In the southern United States, 11 species of pine are naturally affected by pitch canker (Barrows-Broaddus, 1987). Of these, some are highly susceptible (*P. virginiana*, Virginia pine), some moderately susceptible (*P. elliottii*, *P. taeda*, *P. echinata*, shortleaf, *P. rigida*, pitch), and others highly resistant (*P. strobilus*, eastern white) to infection by *F.s. pini* (Dwinell & Barrows-Broaddus, 1982). In a subsequent study, *P. radiata* was also found to be highly susceptible (Dwinell, 1988). Our results are consistent with these reports, but also show that *P. patula* is highly susceptible.

All the *P. patula* and *P. radiata* seedlings or trees inoculated with *F.s. pini* developed lesions. However, extensive lesions were observed only on some *P. elliottii* seedlings and trees. Susceptibility of *P. elliottii*, therefore, appeared to be related to individual trees. Dwinell & Barrows-Broaddus (1982) reported that *P. elliottii* yielded highly susceptible, moderately susceptible and highly resistant families. Individual clones also vary greatly in their susceptibility (Phelps & Chelman, 1976; Barrows-Broaddus & Dwinell, 1984). Clonal variation may, therefore, provide sufficient genetic variation in resistance to select and breed pines as a preventative measure. Highly susceptible pine species also exhibit variation in susceptibility. This phenomenon has been noted for *P. virginiana* (Phelps & Chelman, 1976) and *P. radiata* (Schultz *et al.*, 1990). Breeding trials in South Africa should, in future, include the selection for resistance to *F.s. pini* in *P. patula* and *P. radiata*.

One isolate of *F.s. pini* in our study was found to be significantly more virulent to *P. patula* than another isolate of *F.s. pini*. The isolates did not, however, differ significantly in virulence to *P. elliottii* and *P. radiata*. This is consistent with previous studies where no variation in virulence among isolates was found within a population of *F.s. pini* (Kelley & Williams, 1982; Dwinell & Barrows-Broaddus, 1983; Correll *et al.*, 1991). Isolates of *F.s. pini* from pine in California were similar in virulence to those from the southern United States (Dwinell, 1988), while isolates from Japan were severely pathogenic to southern pine species (Muramoto *et al.*, 1993). It therefore appears that the worldwide population of *F.s. pini* belongs to a single pathotype.

The occurrence of *F.s. pini* in South Africa has been limited to *P. patula* seedlings in a forest nursery (Viljoen *et al.*, 1994). The seedlings grown in this nursery are subsequently planted out in the Transvaal and Natal areas. It is, therefore, possible that the fungus has already been introduced into these areas on asymptomatic infected seedlings but so far remains undetected. This form of introduction has previously been suggested by Blakeslee *et al.* (1981) and Kobayashi & Kawabe (1992).

Many pine-feeding insects are present in South Africa which could serve as vectors of the pitch canker fungus (Borthwick & van Rensburg, 1993). The deodar weevil (*Pissodes nemorensis*), which is well-known for its association with the pitch canker fungus in the United States (Blakeslee & Foltz, 1981), feeds on *P. patula*, but is especially severe on *P. radiata* in the western Cape (Borthwick & van Rensburg, 1993). An increase in incidence of pitch canker on slash pines in Florida was associated with large populations of deodar weevils, but also with drought and fertilization (Schmidt *et al.*, 1976). Thus, the development of disease in South Africa could be favoured both by the weather patterns, which include droughts and hail storms, and by management practice, which includes application of fertilizers.

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