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Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa

Jolanda Roux [*], Michael J. Wingfield

Tree Pathology Cooperative programme, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, South Africa

Abstract

Various disease symptoms occur on *Acacia mearnsii* in South Africa, of which black butt, on older trees, is the most common. Other less commonly reported symptoms include gummosis, cracks, discoloured lesions and die-back. These diseases are of unknown aetiology. During a 2-year period, a survey of diseases on *A. mearnsii* was conducted in two major commercial wattle-growing areas of South Africa. Samples were collected from all symptomatic tissue on randomly selected trees in each area. A wide range of fungi were isolated, including species of *Phytophthora*, *Seiridium*, *Sphaeropsis*, *Fusarium*, *Diplodia*, *Ceratocystis* and *Botryosphaeria*. Of these, *Phytophthora* spp. were isolated only from basal lesions and soil, whereas the *Diplodia* and *Fusarium* spp. were the most frequently isolated from diseased tissue on aboveground parts of trees. *Phytophthora parasitica* and *Ceratocystis albofundus*, which are well-known pathogens of *A. mearnsii*, were excluded from the pathogenicity tests. All other fungi isolated, and particularly those belonging to genera that are known plant pathogens, were used in pathogenicity tests to determine their possible role in diseases. For each isolate, 20 saplings were inoculated in the field, and the resultant lesion lengths were measured. Only the *Phytophthora* spp., *Botryosphaeria* sp. and *Sphaeropsis* sp. produced noticeable lesions. From the surveys and pathogenicity tests, it is clear that many fungi are associated with diseases of *A. mearnsii*, and that these deserve further study. © 1997 Elsevier Science B.V.

Keywords: *Acacia mearnsii*; Disease survey; Pathogenicity

1. Introduction

During the past century, various disease symptoms have been recorded on black wattle (*Acacia mearnsii* de Wild.) planted in many parts of the world. Some of these diseases have proved to be serious, while reports of others have been based on single events. The cause of many of the disease symptoms has never been determined, and those such as black butt, remain enigmatic.

Black butt is the best known disease of black wattle in South Africa and is characterised by black discoloration of the bark at the base of trees (Sherry, 1971; Zeijlemaker, 1971; Wingfield and Kemp, 1993). This is followed by subsequent cracking of the bark, and exudation of gum. The cause of the disease has been attributed to *Phytophthora nicotianiae* var. *parasitica* (Dastur.) Waterhouse (Zeijlemaker, 1971; Zeijlemaker and Margot, 1971). This

* Corresponding author. Tel.: 0/27/51/4012124. Fax: 0/27/51/4482004. E-mail: rouxjo@wwg3.uovs.ac.za.

association was based on the fact that the fungus was regularly isolated from black basal cankers, as well as on the results of pathogenicity tests. Over time, the black basal discoloration on the stems spreads higher up the trees towards their tips. In severe cases, the entire main stem of the trees can be discoloured. Isolations from these advanced lesions have, however, never yielded *P. parasitica*, and the cause of these advanced lesions remains unknown.

Ceratocystis wilt was first recorded in 1989 from the Natal Midlands (Morris et al., 1993). Although little is known of the disease, the causative agent has been identified as a new species of *Ceratocystis*; *Ceratocystis albofundus* De Beer, Wingfield and Morris (De Beer, 1994; Wingfield et al., 1996). Symptoms of the disease include wilt and die-back of the trees, discoloured lesions on the stems and branches, blister lesions, and discoloration of the wood (Morris et al., 1993; Wingfield and Kemp, 1993; De Beer, 1994). It is not yet known how this fungus is spread, or what its distribution is in the wattle-growing areas of South Africa.

Various other disease symptoms have been described on *A. mearnsii*, but their aetiology has not been characterised (Zeijlemaker, 1968; Sherry, 1971). These include isolated cracks in the stems, from which gum may be exuded, and mottle-like lesions. Zeijlemaker (1971) suggested that the mottled lesions, characterised by black spots or patches on the bark, are caused by *P. parasitica*. The authors were, however, never able to isolate this fungus from symptomatic tissue above the base of the trees. Other disease symptoms have also been described on *A. mearnsii* and these have been comprehensively noted elsewhere (Roux et al., 1995). Many trees are currently also dying of unknown cause, and this is of concern to the wattle industry. These trees show symptoms of wilting, wood discoloration and bark discoloration; a comprehensive study of these diseases is urgently required.

The last comprehensive study of wattle diseases in South Africa was conducted in the 1960s (Roux et al., 1995). The identification of the new wilt pathogen, *C. albofundus*, in 1989, suggested that new diseases may have appeared during the intervening period. The aim of this study was, therefore, to conduct comprehensive surveys of *A. mearnsii* in two major commercial wattle growing areas of South

Africa. Pathogenicity tests were conducted using selected fungi, isolated during these surveys, to determine their role in wattle diseases.

2. Materials and methods

2.1. Surveys

Disease surveys were conducted in two commercial wattle-growing areas and extended over a 22-month period. These areas included the Bloemendal Experimental Farm at Pietermaritzburg (KwaZulu/Natal Midlands) and various farms surrounding the town of Piet Retief (South Eastern Mpumalanga Province). A small number of trees were also sampled from bush wattle stands in Alexandria (Eastern Cape Province). During the study period, samples of diseased trees were also received by the diagnostic service of the Tree Pathology Cooperative Programme (University of the Orange Free State) from other areas, and the results of these collections were included in this study.

During the survey period, a total of 328 diseased trees were sampled. Of these, 196 trees were from the South Eastern Mpumalanga province, 132 from KwaZulu/Natal and 10 from Alexandria. Symptoms from which isolations were made included black butt, other basal cankers, mottled bark, cracks and red-to-black discoloured lesions on the stems (Fig. 1), pith and wood discoloration (Figs. 2 and 3), die-back (Fig. 4) and wounds exuding gum. Isolations were also made from insect wounds, hail-damaged tissue and blister-like lesions. In addition to diseased plant tissue, soil samples were collected from the base of every tree sampled, and these were screened for *Pythium* and *Phytophthora* spp.

Plant material was surface-sterilised with 98% ethanol for 30 s, and the outer bark was removed with a sterile scalpel. Samples, approximately 2 mm², cut from the leading edge of lesions, were then plated onto Potato Dextrose Agar (PDA) [200 g peeled potato, 2% Oxoid Agar, 2% Merck D(+)-Glucose]. Plates were incubated at 25°C (alternating 12 h light with 12 h darkness) until fungal growth appeared. Each colony was transferred to a separate Petri dish of PDA by either excising a drop of spores, a fruiting body, or a small piece of mycelium.

Fig. 1. Disease symptoms occurring on *A. mearnsii* in South Africa: black, sunken discoloration on the stem.
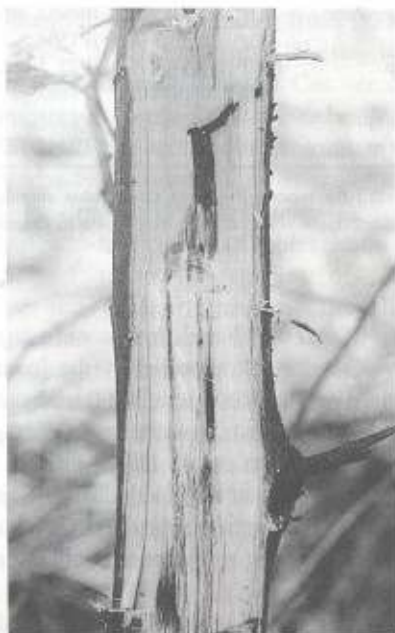

Fig. 2. Disease symptoms occurring on *A. mearnsii* in South Africa: discoloration of the wood.

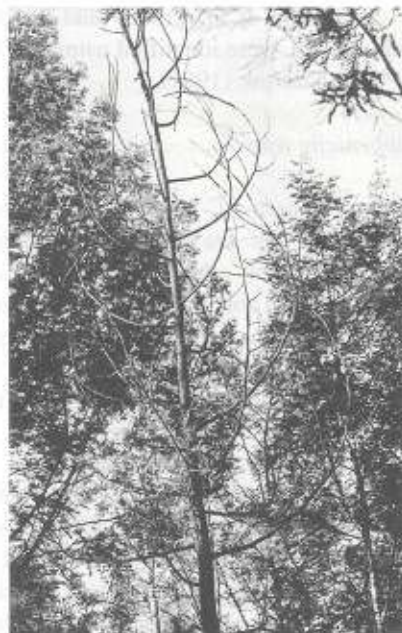Fig. 3. Disease symptoms occurring on *A. mearnsii* in South Africa: pith discoloration.

Fig. 4. Disease symptoms occurring on *A. mearnsii* in South Africa: die-back of a sapling, typical of Ceratocystis wilt.

In addition to PDA, the plant material was also plated onto PARP and PARPH media (Ribeiro, 1978). The latter media are selective for the isolation of Oomycetous fungi such as *Phytophthora* and *Pythium* spp. (Tsao and Ocana, 1969; Tsao and Guy, 1977). The symptomatic plant material was also placed in moist chambers and incubated to induce the production of fungal fruiting structures. Spores from the fruiting structures that developed were, thereafter, transferred to PDA. Soil samples were baited using the citrus leaf disc method (Grimm and Alexander, 1973), and leaf discs were plated onto the two selective media (PARP and PARPH).

2.2. Identification of fungi

All ascomycetous fungi isolated were identified from PDA plates using standard mycological keys. For identification, *Phytophthora* and *Pythium* isolates were plated onto CMA (1.7% Difco corn meal agar). Blocks of agar were cut and placed in non-sterilised Petri's solution to induce sporangium and oospore formation (Ribeiro, 1978). A piece of grass, boiled in water for 10 min, was placed on each agar block (Van der Plaats-Niterink, 1981) and incubated in the dark. *Phytophthora* spp. were identified using the keys of Stamps et al. (1990) and Ho (1981), while *Pythium* spp. were identified using the keys of Van der Plaats-Niterink (1981).

2.3. Pathogenicity tests

Only those genera of fungi isolated most frequently, or those that are known as pathogens of either *Acacia* spp. or other plants, were used in pathogenicity tests (Table 1). Although *C. albofundus* and *P. parasitica* were isolated during these surveys, they were not included in these tests because their pathogenicity to *A. mearnsii* is well known (Morris et al., 1993; De Beer, 1994).

Two isolates were selected from each of the fungal taxa to be tested for pathogenicity and these were cultured on PDA (3.9% Merck Potato Dextrose Agar) at 25°C for 14 days in the dark. For the pathogenicity tests, each isolate under consideration was inoculated into 20 *A. mearnsii* trees under field conditions. The inoculation studies were conducted on the Bloemendal Experimental Farm near Pieter-

Table 1
List of fungi used in field pathogenicity tests

Genus[a]	Isolate number	
	March, 1995	September, 1995
Botryosphaeria dothidea	–	JP2721
Cylindrocladium candelabrum	JNP93, JP2522	JNP93, JP2579
Diplodia sp. A	JNP237, JNP335	JNP237, JNP335
Diplodia sp. B	JNP871, JP1902	JNP871, JP2699, JP2706
Diplodia-like	–	JP2669
Fusarium sp 1.	JP1713, JP1442	JP1713, JP1442
Fusarium sp. 2	JP1921, JP2080	JP1921, JP2080
Fusarium sp.3	JP2458	JP2458, JP2739
Fusarium sp.4	JP1881	JP1881
F. acuminatum	JP2231	JP2231
F. graminearum	–	JP1402, JP2447
F. oxysporum	JP1497	JP1497
F. solani	–	JNP947, JP1242
F. subglutinans	–	JNP972
Gliocladium roseum	JP2334, JP2603	JP2334, JP2603
Glomerella sp.	–	JNP2668, JNP2670
Pestalotiopsis guepinii	–	JP1019, JP2533
Pestalotiopsis sp.	JP1916, JP2223	JP1916, JP2223
Phoma sp.	JP2502, JP2518	JP2500, JP2502, JP2518, JP2527
Phytophthora boehmeriae	JP5, JP7	JP5, JP7
P. meadii	JP1967	JP1967
Pythium irregulare	JP2575	JP1452, JP1453, JP1504
Seiridium sp.	JP22, JA42, JNP306, JP1032	JP22, JA42, JNP305, JP1032
Sphaeropsis sp.	–	JP1204, JP1771
Verticillium sp.	–	JP2747, JP2750

[a] Fungi selected for inoculation were those most abundantly isolated or those thought to potentially contribute to diseases of *A. mearnsii*.

maritzburg (KwaZulu/Natal Province), using plants of improved seed stock supplied by the Institute for Commercial Forestry Research (ICFR). These inoculations were done in two separate trials during 1995. The first inoculation was done during March (Autumn) and this was repeated during September (Spring). The September inoculations included a number of isolates that were not available during the earlier inoculations.

For each tree, a piece of bark was removed with a 9-mm cork borer. A piece of agar, taken from the actively growing margin of the test fungus, was

placed into each wound and covered with masking tape, to prevent desiccation of the wound and fungus. The results were read after 6 weeks by measuring the length of the lesions produced on the outer bark. Samples were taken from the trees for re-isolation of the test fungi.

2.4. Statistical analyses

The data obtained from the pathogenicity tests were statistically analysed for variances and differences among isolates, using a two-factorial analysis of variance (Schefler, 1980).

3. Results

3.1. Survey and identification

A wide range of fungal genera and species were isolated from diseased *A. mearnsii* trees (Tables 2 and 3). These included *C. albofundus*, *Cylindrocladium candelabrum* Viegas, *Fusarium* spp., *Pestalotiopsis* spp., *Phoma* spp. and *P. parasitica* that have previously been recorded from *A. mearnsii*. Various genera of fungi that are not known from *A. mearnsii* before were also isolated. These included *Botryosphaeria dothidea* (Moug.) Ces. *et* de Not., *Diplodia* spp., *P. boehmeriae* Sawada, *P. meadii* McRae, *Sphaeropsis* sp. and a *Seiridium* sp. The most abundantly isolated fungi were species of *Diplodia*, *Fusarium* and *Pestalotiopsis*.

The *Diplodia*-like cultures (Tables 2 and 3) were all black and resembled species of *Diplodia*, *Sphaeropsis* and/or *Botryosphaeria*. They, however, did not sporulate in culture, making their identification impossible (For reference purposes they will be referred to as *Diplodia*-like, since most of those that did sporulate were *Diplodia* sp. A). Some of the isolates were induced to sporulate by plating them onto water agar plates (1.6% Biolab agar) containing a sterile piece of *Eucalyptus* leaf. All of those that sporulated were of a *Diplodia* sp. (referred to as *Diplodia* sp. A), with the exception of 16 isolates that were species of *Sphaeropsis*, *Lasiodiplodia*, *Glomerella* or *B. dothidea* (Tables 2 and 3). *Diplodia* sp. A appeared to be identical to the fungus

Table 2
List of fungi isolated from diseased *A. mearnsii*

Fungi	Areas and number of isolates		
	Kwazulu/Natal	Mpumalanga	Eastern cape
Alternaria sp.	–	–	1
Aplosporella sp.	1	–	–
Bartalinia sp.	2	7	–
Botryosphearia dothidea	3	1	–
Camarosporium sp.	1	1	–
Cephalosporium sp.	–	2	–
Ceratocystis albofundus	1	3	–
Chaetomium sp.	–	2	–
Cladosporium sp.	1	67	–
Coleophoma sp.	–	1	–
Curvularia sp.	–	7	–
Cylindrocarpon sp.	–	–	1
Cylindrocladium candelabrum	6	37	–
Cytospora sp.	2	1	1
Diheterospora sp.	1	–	–
Diplodia sp. A	31	7	–
Diplodia sp. B	55	71	–
Diplodia-like (non-sporulating)	51	72	–
Drechslera sp	–	1	–
Epicoccum sp.	12	15	2
Fusarium spp.	23	89	–
F. acuminatum	–	2	–
F. graminearum	–	2	–
F. oxysporum	5	9	–
F. proliferatum	–	1	–
F. solani	2	8	–
F. subglutinans	1	–	–
Gliocladium roseum Bainier	7	46	–
Gliomastix sp.	1	–	–
Glomerella sp.	4	–	–
Harknesia sp.	1	–	–
Helminthosporium sp.	–	1	–
Lasiodiplodia theobromae (Pat.) Griff. and Maubl.	2	–	–
Leptosphaerulina sp.	1	–	–
Libertella sp.	–	1	–
Microsphaeropsis spp.	2	7	–
Nigrospora oryzae	4	1	–
Pestalotiopsis sp.	110	14	–
Pestaloptiopsis sp. 2	4	117	–
Pestalotiopsis sp. 3	78	85	–
Phacidium sp.	1	–	–
Phoma sp.	1	15	2
Phomopsis sp.	3	3	–
Phytophthora	–	8	–

Table 2 (continued)

Fungi	Areas and number of isolates		
	Kwazulu/Natal	Mpumalanga	Eastern cape
boehmeriae			
P. meadii	–	2	–
P. parasitica	5	–	–
Pithomyces sp.	2	–	–
Pleurocytospora sp.	–	2	–
Pythium spp.	5	–	–
Rhinocladiella sp.	8	10	–
Seiridium sp.	15	17	1
Sphaeropsis sp.	3	9	–
Trichoderma sp.	3	22	–
Tryblidopycnis pinastri Höhn	2	–	–
Verticillium sp.	6	–	–

known as *Sphaeropsis sapinea* f. sp. *cupressi* Solel, Madar, Kimchi and Golan (synonym: *Diplodia pinea*), the cause of a canker disease of *Cupressus sempervirens* var. *stricta* and *horizontalis* in Israel (Solel et al., 1987). The remaining *Diplodia*-like cultures that did not sporulate, all showed the same colony morphology as those identified as *Diplodia* f. sp. *cupressi* and are thought to represent that taxon.

Many of the samples analysed showed blue discoloration of the wood and pith (Figs. 2 and 3). The most abundantly isolated fungi from this symptom were a *Sphaeropsis* sp. and *B. dothidea* (Tables 2 and 3). These fungi also grew and sporulated abundantly on the symptomatic tissue when it was placed in a moist environment.

Diplodia sp. B was commonly isolated and had white colonies, with conidia of an average length of 10 μm compared to those of *Diplodia* sp. A, which were 25–30 μm long and had black colonies. The former was the second most abundantly isolated fungus, but could not be identified to the species level (Tables 2 and 3).

Ceratocystis albofundus was isolated only four times during the survey period (Tables 2 and 3). The first, third and fourth of these isolations were made from two different farms in the Piet Retief area while the second was from the Pietermaritzburg area. Isolations of this fungus originated from a range of symptoms, including black butt symptoms on older trees and younger trees exuding gum from wounds.

Various *Fusarium* spp. were isolated with a relatively high frequency (Tables 2 and 3). These include *F. acuminatum* Ell. and Ev. sensu Gordon, *F. oxysporum* Schlecht, *F. proliferatum* (Matshushima) Nirenberg, *F. solani* (Mart.) Saac., *F. subglutinans* (Wollenw. and Reinking) Nelson, Toussoun and Marasas and *F. graminearum* Schwabe. These species were especially abundant in insect galleries found in trees from the Piet Retief area.

P. parasitica was isolated from black basal cankers (black butt) on 4-year-old trees from Bloemendal, KwaZulu/Natal Province, as well as from mottled lesions within a 1-metre zone aboveground on 18-month-old seedlings in the Stanger area, KwaZulu/Natal. The 18-month-old seedlings did not have the basal discoloration typical of black butt. They did, however, exude gum from cracks at the bases of trees and from more advanced mottled lesions with cracks, higher on the stems. *P. parasitica* was not isolated from the Piet Retief area. Apart from *P. parasitica*, two other species of *Phytophthora* were isolated from basal cankers. These include *P. boehmeriae* and *P. meadii*.

With the exception of the *Phytophthora* spp., no correlation could be made between the type of symptom, and the fungus isolated. Isolations from soil samples mainly yielded *Pythium* spp. These included *P. irregulare* Buisman, *P. ultimum* Trow var. *ultimum*, *P. acanthophorum* Sideris, *P. vexans* de Barry and *Pythium* Group F. None of these species was isolated from the diseased plant material; their role in diseases seems doubtful.

3.2. Pathogenicity tests

Most of the fungi tested for pathogenicity had not resulted in any lesion development after 6 weeks, in either the March or September inoculation trials. These included *Gliocladium roseum* Bainier, *Phoma* spp. and *Pestalotiopsis* spp. *Phytophthora* and *B. dothidea* isolates produced the largest lesions after 6 weeks (Tables 4 and 5). Isolates of the *Fusarium* spp., *Seiridium* sp. and *Sphaeropsis* sp. also gave rise to lesions, but these were relatively small. In the March inoculations, the largest lesions were produced by *P. boehmeriae* and *P. meadii*. One of the unidentified *Fusarium* spp. also produced large lesions in these inoculations. For the March inocula-

Table 3

Symptoms from which isolations were made and the fungi associated with them

Basal canker	Wood discoloration	Stem/branch canker	Hail/insect wound
Aplosporella sp.			
Bartalinia sp.		*Bartalinia* sp.	
Botryosphaeria dothidea	*Botryosphaeria dothidea*	*Botryosphaeria dothidea*	*Botryosphaeria dothidea*
		Camarosporium sp.	
		Ceratocystis albofundus	
		Chaetomium sp.	
Cladosporium sp.		*Cladosporium* sp.	*Cladosporium* sp.
			Coleophoma sp.
		Curvularia sp.	*Curvularia* sp.
Cylindrocarpon sp.			
Cylindrocladium candelabrum		*Cylindrocladium candelabrum*	
Cytospora sp.	*Cytospora* sp.	*Cytospora* sp.	
Diplodia sp. A		*Diplodia* sp. A	
Diplodia sp. B		*Diplodia* sp. B	*Diplodia* sp. B
			Drechslera sp.
Epicoccum sp.	*Epicoccum* sp.	*Epicoccum* sp.	*Epicoccum* sp.
Fusarium sp.	*Fusarium* sp.	*Fusarium* sp.	*Fusarium* sp.
			F. graminearum
			F. solani
Gliocladium roseum	*G. roseum*	*G. roseum*	*G. roseum*
Gliomastix sp.			
	Glomerella sp.		
Harknesia sp.			
		Helminthosporium sp.	
Lasiodiplodia sp.			
Leptosphaerulina sp.			
	Libertella sp.		
Micropshaeropsis sp.	*Microsphaeropsis* sp.	*Microsphaeropsis* sp.	
Nigrospora oryzae		*Nigrospora oryzae*	*N. oryzae*
Pestalotiopsis sp.	*Pestalotiopsis* sp.	*Pestalotiopsis* sp.	*Pestalotiopsis* sp.
		Phacidium sp.	
		Phoma sp.	*Phoma* sp.
Phomopsis sp.	*Phomopsis* sp.		
Phytophthora boehmeriae			
Phytophthora meadii			
Phytophthora parasitica			
		Pleurocytospora sp.	
Pithomyces sp.			
Pythium irregulare			
Rhinocladiella sp.	*Rhinocladiella* sp.	*Rhinocladiella* sp.	
Seiridium sp.	*Seiridium* sp.	*Seiridium* sp.	
Sphaeropsis sp.	*Sphaeropsis* sp.	*Sphaeropsis* sp.	*Sphaeropsis* sp.
Trichoderma sp.	*Trichoderma* sp.	*Trichoderma* sp.	
		Verticillium sp.	

tions, other fungi that produced lesions were *C. candelabrum*, *Seiridium* sp. and *Fusarium* spp. (Table 4). Results for the latter fungi are, however, inconclusive since only one of the isolates of each species produced lesions, while the other isolate appeared to be non-pathogenic.

In the September inoculations, the largest lesions were produced by *B. dothidea* (Table 5). This isolate was not included in the March inoculations, due to the fact that it was only collected later in this study. The second largest lesions were produced by *Sphaeropsis* sp. Lesions were also produced by both

Table 4

Lesion lengths produced on 18-month-old *A. mearnsii* by different fungi in March 1995 inoculations

Fungus[a]	Isolate number	Lesion length (mm)[b,c]
Seiridium sp.	JA42	15.85a
Cylindrocladium candelabrum	JNP93	16.00a
Fusarium sp. 3	JP2458	21.60a
Phytophthora boehmeriae	JP5	31.10b
P. meadii	JP1967	32.95b
P. boehmeriae	JP7	45.35c
Control		15.00a

[a] Other fungi inoculated (Table 1) did not result in any noticeable lesion development and have thus been omitted from this table.
[b] Each value represents an average of 20 trees per isolate; CV = 31.80%.
[c] Values in each row followed by a different letter differ significantly at *P* = 0.01.

Table 5

Lesion lengths on 36-month-old *A. mearnsii* produced during September 1995 inoculations

Fungus[a]	Isolate number	Lesion length (mm)[b,c]
Glomerella sp.	JNP2668	16.30a
Fusarium sp. 3	JP2458	16.35a
Pestalotiopsis sp.	JP2739	16.35a
Glomerella sp.	JNP2670	16.80a
Cylindrocladium candelabrum	JNP93	17.05a
Phytophthora meadii	JP1504	17.30a
Cylindrocladium candelabrum	JP2579	17.30a
Seiridium sp.	JP1032	17.50a
Phytophthora meadii	JP1967	17.95a,b
Glomerella sp.	JNP2668	18.95a,b,c
Seiridium sp.	JNP305	21.20a,b,c,d
Sphaeropsis sp.	JP1204	21.25a,b,c,d
Pestalotiopsis guepinii.	JP1019	21.90a,b,c,d
Phytophthora boehmeriae	JP5	24.25b,c,d
P. boehmeriae	JP7	25.00c,d
Sphaeropsis sp.	JP1771	26.65d
Botryosphaeria dothidea	JNP2721	50.30e
Control		15.00a

[a] Other fungi inoculated did not result in any noticeable lesion development and have been omitted from this table.
[b] Each value is an average of 20 measurements per isolate; CV = 29.30%.
[c] Values followed by different letters differ significantly at *P* = 0.01.

isolates of *P. boehmeriae*, isolates of *Pestalotiopsis* and *Seiridium* sp. Small lesions were formed after inoculation with *Glomerella* sp., *C. candelabrum*, *Seiridium* sp., *Pythium irregulare* and various *Fusarium* spp. (Tables 4 and 5). Results for the *Seiridium* spp. were inconclusive, since some of the isolates produced lesions, while the others did not.

Significant differences (*P* = 0.01) in virulence were detected between isolates of *B. dothidea*, *Sphaeropsis* and the other fungi tested for the September inoculations (Table 5). For the March inoculations, significant differences (*P* = 0.01) in virulence were noted between the *Phytophthora* isolates (*P. meadii* and *P. boehmeriae*) and the other fungi tested (Table 4). The only notable differences in virulence between the March (autumn) and September (Spring) inoculations were with the *Phytophthora* isolates, which produced smaller lesions in the spring inoculations.

4. Discussion

The results of this survey of diseased *A. mearnsii* have emphasised the fact that there are many disease symptoms on this species that are of unknown origin. Many fungi have been isolated from these symptoms. Some of them, such as *C. albofundus* and *P. parasitica*, are known pathogens of *A. mearnsii* in South Africa. Most of the fungi isolated during this study, however, have not previously been associated with diseases of these trees. Many of these fungi are common saprophytes, as was confirmed in the pathogenicity tests. Others, such as *B. dothidea* and the *Sphaeropsis* sp. were shown to be virulent and potentially important pathogens.

The isolation of *C. albofundus* from Piet Retief represents a first report of this fungus from the Mpumalanga Province. It has been isolated now throughout the wattle growing areas in South Africa, namely Mpumalanga, KwaZulu/Natal and the Eastern and Western Cape (Roux and Wingfield, unpublished; Morris et al., 1993). The wide distribution of this fungus, and the fact that it was originally isolated from a *Protea* sp. (Gorter, 1977; Morris et al., 1993), lead us to believe that it is endemic in South Africa.

The low frequency of isolation of *C. albofundus* from the KwaZulu/Natal and Piet Retief areas during a 20-month period, raises many questions regarding its importance. This is an extremely virulent fungus and has been thought to threaten wattle growing in South Africa (Morris et al., 1993; De Beer, 1994). Typical symptoms of the associated disease, Ceratocystis wilt, are abundant in the field. These symptoms include wilting and die-back of trees, red-to-black discoloration of the bark, and wood discoloration of trees (Morris et al., 1993; De Beer, 1994). Although a very large number of isolations were made from these symptoms during different periods of the year, the fungus was isolated only 4 times. This might indicate that it is not as abundant and serious as previously thought. Alternatively, the fungus is known to be difficult to isolate and it might not have been detected, despite its presence on trees.

It was surprising in this study to find more than one species of *Phytophthora* associated with wattle disease. *P. parasitica* has previously been shown to cause black butt on *A. mearnsii* in South Africa (Sherry, 1971; Zeijlemaker, 1971). These authors suggested that *P. parasitica* might be the cause of two symptoms, namely black butt and mottled diseases, and that these two symptoms are associated with different climatic conditions. In the present study, the fungus was recovered from typical black butt symptoms on older trees and from mottled symptoms at the base of younger trees. The younger trees did not have the typical black butt symptoms. This indicates that the mottle lesions may represent the initial stages of black butt. We were unable to isolate *P. parasitica* from mottle, or any other lesions, occurring higher up on trees. We, therefore, believe that the mottle symptoms represent the initial stage of infection by a variety of different pathogens.

P. boehmeriae and *P. meadii* are known pathogens of various plants, including *Eucalyptus* spp., *Pinus* spp. and *Hevea brasiliensis* [Muell.] Arg. (Ribeiro, 1978). This fungus has also been reported as the cause of diseases on high altitude *Eucalyptus* spp. such as those planted in the Piet Retief area (Linde et al., 1994). *P. boehmeriae* was isolated only from the Mpumalanga province, while *P. parasitica* was only isolated from KwaZulu/Natal consistent with the findings of Sherry (1971) and Zeijlemaker (1971). The isolation of two different species from different climatic areas could indicate that the species have different distributions. All three species of *Phytophthora* produced significant lesions in the pathogenicity tests, indicating that more than one *Phytophthora* sp. may be responsible for the diseases of *A. mearnsii* in South Africa.

The cause of the advanced lesions forming on trees suffering from black butt remains unknown. Because no specific organism was isolated from any specific symptom, we suspect that *P. parasitica*, or other *Phytophthora* spp., infect the base of trees, causing the initial symptoms and weakening of the trees. This would then provide entry sites for secondary or opportunistic pathogens to infect and cause advanced lesions, which may eventually cover the whole stem.

The association of species of *Botryosphaeria* and *Sphaeropsis* with diseases of *A. mearnsii*, and their relatively high levels of virulence, indicates that they play a role in diseases. Species of *Botryosphaeria* and *Sphaeropsis* are serious pathogens of plantation trees, such as *Eucalyptus* and *Pinus* spp. in South Africa (Swart et al., 1985; Wingfield and Kemp, 1993; Smith et al., 1994; Smith, 1995). Typical symptoms of both these genera include blue-to-black discoloration of the pith and wood (Swart et al., 1985; Shearer et al., 1987; Smith et al., 1994), similar to those found during this study. These fungi tend to be opportunistic and are frequently associated with adverse environmental conditions such as drought, hail, wind and frost damage (Ramos et al., 1991; Smith, 1995). During the past 3 years, *A. mearnsii* trees in South Africa have been subjected to severe drought, as well as insect, hail and frost damage. This has placed the trees under stress and has also probably promoted infection by opportunistic pathogens.

The results of this study indicate that there are many species of fungi on diseased black wattle that have not previously been recorded. Preliminary pathogenicity tests indicate that they include a number of potentially important pathogens. The fact that no fungus was consistently isolated from any specific symptom (Table 3) suggests that many of the pathogens are of an opportunistic nature, and that adverse environmental factors have promoted infection. Further (and more detailed) study is required to understand the role of these fungi in diseases.

Acknowledgements

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