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Pythium and *Phytophthora* species associated with eucalypts and pines in South Africa

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Abstract

Root and soil samples from *Eucalyptus* and *Pinus* plantations taken during routine surveys of South African forestry areas during 1990–1992, yielded various species of *Pythium* and *Phytophthora*. These include three species of *Phytophthora* and 20 species of *Pythium*. Six of these *Pythium* species have not previously been recorded in South Africa. The pathogenicity of the *Pythium* and *Phytophthora* spp. collected during the surveys was tested on established *Eucalyptus fastigata* and a clone of *E. grandis*.

Key words: *Eucalyptus* – *Pinus* – *Phytophthora* – *Pythium* – pathogenicity.

1 Introduction

South Africa is poorly endowed with natural forests and, therefore, plantations of suitable exotic species have been established for timber and paper production. The forestry industry is one of the largest and fastest growing sectors of the South African economy (ANONYMOUS 1992). Land utilized for forestry extends over an area of 1.5 million ha (VAN DER ZEL 1994) and includes mainly plantations of *Pinus* spp., *Eucalyptus* spp. and *Acacia* spp. *Pinus* and *Eucalyptus* spp. are extensively cultivated in more or less equal proportions and comprise 90 % of the total forestry operation (ANONYMOUS 1990). Because of the monoculture system used, diseases could result in reduced productivity (WESTE 1983; WINGFIELD et al. 1991). Little, however, is known of the root diseases associated with exotic forest-tree species in South Africa.

Various species of *Pythium* (*P.*) and *Phytophthora* (*Ph.*) have been associated with damping-off and root diseases of *Pinus* and *Eucalyptus* spp. under nursery conditions (VAARTAJA and SALISBURY 1961; VAARTAJA 1967; MARKS and KASSABY 1974). *Phytophthora cinnamomi* Rands and *Phytophthora cryptogea* Pethybr. and Laff. are the two most important oomycetous pathogens associated with *Eucalyptus* and *Pinus* spp. under field conditions (NEWHOOK 1959; PODGER and BATINI 1971; MARKS and KASSABY 1974; BUMBIERIS 1976; HEATHER et al. 1977; PODGER 1978; HAMM and HANSEN 1982). The importance of *Ph. cinnamomi* in forestry is exemplified by the disease of *Eucalyptus marginata* Sm. in western Australia (PODGER et al. 1965; WESTE 1974; SHEA et al. 1982), littleleaf disease of *Pinus echinata* Mill. and *Pinus taeda* L. in the south eastern USA (LORIO 1966), *Pinus radiata* D. Don. die-back in New Zealand (NEWHOOK 1959), chestnut decline in the USA and Europe (CRANDALL 1950; GRENTE 1961), oak decline in Iberia (BRASIER 1992), and red-oak disease in France (ROBIN et al. 1992).

In contrast to *Phytophthora* spp., *Pythium* spp. are generally not associated with root diseases of established *Eucalyptus* and *Pinus* spp. (MARKS and KASSABY 1974, 1976). This is despite the fact that *Pythium* spp. have frequently been associated with diseased *Eucalyptus* and *Pinus* spp. in the past (DAVISON and BUMBIERIS 1973; PRATT and HEATHER 1973;

MARKS and KASSABY 1974; OTROSINA and MARX 1975; GERRETTSON-CORNELL et al. 1979). However, PRATT and HEATHER (1973) suggested that *Pythium* and *Phytophthora* spp. can act either singly or in combination with *Ph. cinnamomi* to cause die-back of *Eucalyptus* trees. LORIO (1966) also suggested that *Pythium splendens* Braun, *Pythium vexans* de Bar. and *Pythium irregulare* Buis. in association with *Ph. cinnamomi,* may play an important role in *P. taeda* decline in Louisiana. However, the first evidence of a *Pythium* species, namely *P. splendens,* being pathogenic to *Eucalyptus* under field conditions, was presented in South Africa (LINDE et al. 1994b).

Phytophthora cinnamomi has previously been associated with a root disease of *Eucalyptus* and *Pinus* spp. under nursery conditions in South Africa (DONALD and VON BROEMBSEN 1977; DARVAS et al. 1978; VON BROEMBSEN 1981; 1984). *Pythium* spp., in particular *P. irregulare* and *P. ultimum* Trow, are well-known damping-off pathogens of *Pinus* spp. (DARVAS et al. 1978). *P. irregulare* has also been associated with the death of *Pinus patula* Schlecht. and Cham. during establishment (LINDE et al. 1994a). However, *Ph. cinnamomi* and the more recently recorded *P. splendens,* are the only oomycetous fungi associated with diseases of established *Pinus* and/or *Eucalyptus* spp. under field conditions. *Ph. cinnamomi* has been associated with root diseases of *Eucalyptus fastigata* Deane and Maid. and *Eucalyptus fraxinoides* Deane and Maid. in the south eastern Transvaal (WINGFIELD and KNOX-DAVIES 1980) and *Pinus clausa* Chapm. and *P. radiata* in northern Natal and the south eastern Cape Province (WINGFIELD and KNOX-DAVIES 1980; VON BROEMBSEN 1984). *P. splendens* has been associated with a root disease of 1–2-year-old *Eucalyptus grandis* Hill ex Maid. seedlings in northern Natal (LINDE et al. 1994b).

The objective of this study was to determine which species of *Pythium* and *Phytophthora* are present in South African eucalypt and pine plantations. Pathogenicity of these species on established *Eucalyptus* trees was also of interest.

2 Materials and methods

2.1 Isolation and identification

During routine surveys in the summer of 1990–1992, soil, as well as root samples, were collected from *Eucalyptus* and *Pinus* trees with root-disease symptoms. Soil and root samples were taken from four trees per site. One soil sample was sampled in the rhizosphere of each diseased tree. For soil samples, the top 5 cm of soil was removed and approximately 1 kg of soil sampled at a depth of 5–25 cm. Root samples consisted of diseased sections of adventitious roots with feeder roots. A total of 424 trees were examined for the presence of *Pythium* and *Phytophthora* species.

The six areas from which samples were collected (Fig. 1) included certain areas with specific root-disease problems: Natal Midland, area 1 (*E. smithii* Donn. ex Smith root disease); Zululand, area 2 (root disease of 1–2-year-old *E. grandis* trees); South eastern Transvaal, area 3 (*E. fastigata* die-back, as well as 1-year-old *E. dunnii* Maid., *E. macarthurii* Deane and Maid., and an *E. grandis* hybrid root disease); Eastern Transvaal Lowveld, area 4 (*E. smithii* root disease). The other areas (Fig. 1) had no specific root-disease problems in pines and eucalypts.

Each soil sample was divided into two parts and separately baited with *Citrus* leaf discs (GRIMM and ALEXANDER 1973). Distilled water was used to flood the soil to a depth of 1–2 cm. A total of 10 *Citrus* leaf discs (5 mm diameter) were floated on the water surface of each sample. After 2 days of incubation at room temperature, half of the leaf discs were transferred to a selective medium for the isolation of *Oomycetes* (TSAO and OCANA 1969), and the other half to a hymexazol medium for the isolation of *Phytophthora* spp. (TSAO and GUY 1977). Roots and root collar segments were thoroughly washed in running tap water and plated onto both selective media. Transfers were made to Difco Corn Meal Agar

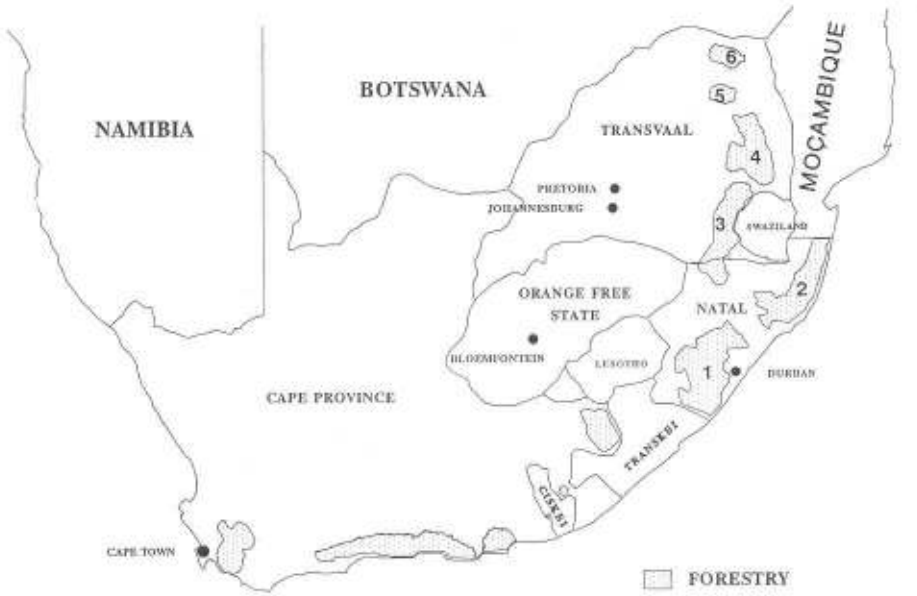

Fig. 1. Forestry areas of South Africa from which samples were collected: 1. Natal Midlands; 2. Northern Natal (Zululand); 3. South eastern Transvaal; 4. Eastern Transvaal (Lowveld); 5,6. North eastern Transvaal (Highveld)

(CMA) for identification. The recovery of each *Pythium* and *Phytophthora* sp. from soil and root samples was quantified by determining the number of trees per area examined from which at least one leaf disc (soil sample) or one root segment (root sample) was infected by a given species of *Pythium* or *Phytophthora*.

Asexual and sexual fruiting structures were stimulated to develop, using non-sterilized Petri's solution (TUCKER 1931). Chilling was used to stimulate zoospore formation and release (MENYONGA and TSAO 1966). Heterothallic species were stimulated to produce sexual structures by crossing with tester strains of known mating types. Isolates were identified based on morphological and cultural characteristics using the key described by STAMPS et al. (1990) for *Phytophthora* spp. and the key described by VAN DER PLAATS-NITERINK (1981) for *Pythium* spp.

The pathogenicity of the *Pythium* and *Phytophthora* isolates (Table 1) was tested on established *E. fastigata* and a clone of *E. grandis*. These host species were included due to their previously reported susceptibility to *Ph. cinnamomi* and *P. splendens*, respectively, in South Africa (WINGFIELD and KNOX-DAVIES 1980; LINDE et al. 1994b).

2.2 Field inoculation trials

Each of 20 species of *Pythium* and three species of *Phytophthora* collected, were artificially inoculated on established *Eucalyptus* trees. A single isolate of each species was used except in the case of *Ph. cinnamomi*, *P. splendens* and *P. irregulare*, where two isolates were included. Inoculum of these species for pathogenicity tests was produced by growing the fungi on CMA for 1 week at 25 °C in the dark.

A total of 10 trees each of the two different *Eucalyptus* hosts were inoculated with the fungi during the first week of February, 1992. The trial was repeated at the same time in 1993. Inoculation during summer was conducted because trees are known to be most

Table 1. Origin of *Phythium* (*P.*) and *Phytophthora* (*Ph*) isolates used in pathogenicity tests. Geographic origin of isolates. 1 = Natal Midlands; 2 = Northern Natal (Zululand); 3 = South eastern Transvaal; 4 = Eastern Transvaal (Lowveld); 5, 6 = North eastern Transvaal Highveld

Species	No.	Host	Origin
Ph. boehmeriae Saw.	1	*E. dunnii* roots	3
Ph. cinnamomi Rands	1	*E. fastigata* roots	3
Ph. cinnamomi	2	*E. fastigata* roots	3
Ph. parasitica Dast.	1	*Eucalyptus sp.* soil	3
P. acanthophoron Sid.	1	*P. elliotti* soil	2
P. angustatum Spar.	1	*P. elliotti* soil	2
P. aphanidermatum (Edson) Fitzp.	1	*E. grandis* soil	4
P. buismaniae van der Plaats-Nit	1	*P. patula* soil	4
P. hypogynum Middl.	1	*E. grandis* soil	2
P. intermedium de Bar.	1	*P. elliottii* soil	2
P. irregulare Buis.	1	*P. patula* roots	2
P. irregulare	2	*E. grandis* roots	6
P. myriotylum Drechs.	1	*E. grandis* soil	4
P. pyrilobum Vaart.	1	*P. patula* roots	1
P. rostratum Butler	1	*E. grandis* × *E. nitens* soil	5
P. salpingophorum Drechs.	1	*E. smithii*	4
P. spinosum Saw.	1	*E. smithii* roots	4
P. splendens Braun	1	*E. grandis* roots	2
P. splendens	2	*E. grandis* roots	2
P. tardicrescens Vanterp.	1	*E. grandis* soil	1
P. vexans de Bar.	1	*E. grandis soil*	2
P. violae Chest. & Hickm.	1	*E. smithii* soil	2
Pythium Group F	1	*P. patula* roots	4
Pythium Group G	1	*E. grandis* soil	2
Pythium sp. 1	1	*P. patula* soil	1
Pythium sp. 1	1	*P. patula* soil	1
Pythium sp. 2	1	*E. grandis* soil	2

susceptible to fungal invasion in summer (SHEARER et al. 1988). The trees to be inoculated included a 2-year-old clone of *E. grandis* near Kwambonambi, northern Natal (Natal *E. grandis* clone), and a 3-year-old *E. fastigata* near Lothair, south eastern Transvaal.

A 10-mm corkborer was used to make a wound on the stem of each tree at 1.3 m above the ground. Wounds were inoculated with 10-mm discs of CMA and colonized with the test fungi. From each host, 10 control trees were inoculated with a sterile CMA disc. Wounds were sealed with masking tape. Lesion development in the secondary phloem (inner bark; TIPPETT et al. 1983; SHEARER et al. 1987) and the outer bark were measured after 5 weeks to give an indication of the pathogenicity of each isolate.

2.3 Statistical analysis

Numerical data obtained in this study were statistically analysed for variances and differences among isolates and hosts. Means were tested for significance using Tukey's procedure for comparison of means (STEEL and TORRIE 1980). The aggressiveness of the *Pythium* and *Phytophthora* species to *E. fastigata* and *E. grandis* in the 1992 and 1993 trials was correlated using the Spearman's-rank correlation coefficient (r_s; STEEL and TORRIE 1980).

3 Results

3.1 Isolation and identification

A total of 20 *Pythium* spp. and three *Phytophthora* spp. were isolated and identified. Six of these species are reported for the first time in South Africa (Table 2). A number of isolates formed only sporangia or hyphal swellings, and did not produce oogonia, despite pairing with possible compatible mating strains. Those isolates with filamentous non-swollen sporangia were identified as Group F, and isolates with globose non-proliferating sporangia were identified as Group G (VAN DER PLAATS-NITERINK 1981). Isolates representing two species of *Pythium* could not be assigned to known taxa.

Table 2. ***Pythium* (*P.*) and *Phytophthora* (*Ph.*) spp. isolated from *Eucalyptus* and *Pinus* forestry areas in South Africa**

Species	Area[1]	Associated tree
Ph. boehmeriae Saw.	3	*E. dunnii* Maid.
	3	*E. macarthurii* Deane and Maid.
	3	*E. grandis*[2] Hill ex Maid
Ph. cinnamomi Rands	3	*E. dunnii*
	3	*E. fastigata* Deane and Maid.
	3	*E. macarthurii*
	3	*E. smithii* Donn ex Smith
Ph. parasitica Dast	3	*E. smithii*
P. acanthophoron Sid.	2	*P. elliottii* Chapm.
[3]*P. angustatum* Spar.	2	*P. elliottii*
P. aphanidermatum (Edson) Fitzp.	4	*E. grandis*[2]
[3]*P. buismaniae* van der Plaats-Nit.	4	*P. patula* Schlecht. and Cham.
P. hypogynum Middl.	2	*E. grandis*[2]
[3]*P. intermedium* de Bar.	2	*P. elliottii*
P. irregulare Buis.	1–6[4]	
P. myriotylum Drechs.	4	*E. grandis*[2]
P. Pyrilobum Vaart.	1	*P. patula*
[3]*P. rostratum* Butl.	2	*P. elliottii* Chapm.
	5	*E. grandis* × *E. nitens*
[3]*P. salpingophorum* Drechs.	4	*E. smithii*
P. spinosum Saw.	4	*E. smithii*
P. splendens Braun	2	*E. grandis*[2]
	2	*E. smithii*
P. tardicrescens Vanterp.	1	*E. grandis*[2]
P. vexans de Bar.	2	*E. grandis*[2]
	2	*E. grandis*[2]
[3]*P. violae* Chest. and Hick.	2	*E. smithii*
Pythium Group F	1–6[4]	
Pythium Group G	2	*E. grandis*[2]
Pythium sp. 1	1	*E. grandis*[2]
Pythium sp. 2	2	*E. grandis*[2]

[1] Areas represent those illustrated in Fig. 1
[2] *E. grandis* includes various clones and hybrids of *E. grandis*
[3] New reports for South Africa
[4] Species were isolated from every area stated in Fig. 1. The host list includes various *Eucalyptus* and *Pinus* spp. cultivated in South Africa

Most of the *Pythium* spp. were isolated from soil and not from roots (Table 3). *Pythium irregulare*, *P. splendens* and *Pythium* Group F were frequently isolated from roots of their respective hosts. *Ph. boehmeriae* and *Ph. cinnamomi* were also consistently isolated from soil as well as from roots (Table 3) in south eastern Transvaal.

Table 3. **Percentage isolation of *Pythium* (*P.*) and *Phytophthora* (*Ph.*) spp. from soil and roots of *Eucalyptus* and *Pinus* spp. in six different areas of South Africa**

Species[1]	No. trees[2]	No. of positive samples	
		Soil	Roots
Area 1	8		
P. irregulare		2	1
P. tardicrescens		3	0
Pythium Group F		7	6
P. pyrilobum		2	2
Pythium sp. 1		4	0
Area 2	212		
P. acanthophoron		12	2
P. angustatum		2	0
P. hypogynum		3	0
P. intermedium		45	0
P. irregulare		115	33
P. rostratum		63	0
P. splendens		67	54
P. vexans		78	9
P. violae		1	0
Pythium Group F		175	98
Pythium Group G		37	6
Pythium sp. 2		16	0
Area 3	92		
Ph. boehmeriae		12	13
Ph. cinnamomi		68	62
Ph. parasitica		3	0
P. irregulare		22	9
Pythium Group F		25	20
Area 4	96		
P. aphanidermatum		6	0
P. buismaniae		4	0
P. irregulare		12	1
P. myriotylum		17	0
P. salpingophorum		38	0
P. spinosum		43	9
Pythium Group F		73	52
Area 5	8		
P. rostratum		4	1
Pythium Group F		7	7
Area 6	8		
P. irregulare		2	1
Pythium Group F		7	5

[1] Areas are the same as those indicated in Fig. 1
[2] Each value represents the total number of samples examined from a specific area.

3.2 Field inoculation trials

Rapidly developing lesions associated with kino-vein formation in susceptible hosts were observed in both *Phytophthora* and *Pythium* inoculations. Results of pathogenicity tests on the *E. grandis* clone, indicate that the species of *Pythium* and *Phytophthora* fall into three groups based on their aggressiveness. The first group included *P. pyrilobum, Ph. cinnamomi* (1), *P. splendens* (2), *P. splendens* (1), *Ph. parasitica, Ph. cinnamomi* (2), *P. vexans, P. aphanidermatum* and *P. intermedium. Ph. boehmeriae, Pythium* Group G, *Pythium* sp. 2, *P. myriotylum, P. irregulare* (2), and *P. salpingophorum* also produced lesions which differed significantly ($p \leq 0.01$) from those of the controls and made up the second group of pathogenic fungi. The remaining species (*P. rostratum*, P. *spinosum, Pythium* Group F, *P. buismaniae, P. irregulare* (1), *P. acanthophoron, P. violae, P. hypogynum, P. angustatum* and *P. tardicrescens* did not differ significantly from the controls and comprise the third group. Apart from the 10-mm inoculation wound, no lesions developed in control inoculations (Table 4).

Species of *Pythium* and *Phytophthora* could also be divided into three groups, based on

Table 4. **Phloem lesion development on *E. fastigata* and a clone of *E. grandis*, 5 weeks after inoculation with species of *Phytophthora* (*Ph.*) and *Pythium* (*P.*) Each value represents an average of 10 trees**

Species	Lesion length (mm) *E. grandis* clone[1]	Species	Lesion length (mm) *E. fastigata*[2]
P. pyrilobum	149[j]	*Ph. cinnamomi* (1)	615[j]
Ph. cinnamomi (1)	137[hi]	*Ph. cinnamomi* (2)	518[i]
P. splendens (2)	137[hi]	*Ph. parasitica*	370[h]
P. splendens (1)	132[ghi]	*P. splendens* (2)	370[h]
Ph. parasitica	115[fghi]	*P. splendens* (1)	314[gh]
Ph. cinnamomi (2)	114[fghi]	*Ph. boehmeriae*	258[fgh]
P. vexans	109[efghi]	*P. irregulare* (1)	243[efg]
P. aphanidermatum	94[defghi]	*P. vexans*	224[defg]
P. intermedium	93[defghi]	*Pythium* sp. 2	189[cdefg]
Ph. boehmeriae	91[defgh]	*P. acanthophoron*	176[bcdef]
Pythium Group G	87[cdefgh]	*Pythium* sp. 1	175[bcdef]
Pythium sp. 2	79[cdefg]	*P. irregulare* (2)	171[bcdef]
P. myriotylum	76[cdefg]	*P. pyrilobum*	170[bcdef]
P. irregulare (2)	73[bcdef]	*Pythium* Group G	141[bcdef]
P. salpingophorum	67[bcdef]	*P. myriotylum*	138[bcdef]
P. rostratum	59[abcdef]	*P. salpingophorum*	134[abcdef]
P. spinosum	54[abcde]	*P. intermedium*	133[abcdef]
Pythium Group F	53[abcde]	P. angustatum	132[abcdef]
P. buismaniae	51[abcd]	*P. hypogynum*	119[abcde]
Pythium sp. 1	51[abcd]	*P. spinosum*	116[abcd]
P. irregulare (1)	47[abcd]	*P. tardicrescens*	105[abcd]
P. acanthophoron	46[abcd]	*P. aphanidermatum*	103[abcd]
P. violae	46[abcd]	*P. buismaniae*	86[abc]
P. hypogynum	39[abcd]	*P. rostratum*	69[abc]
P. angustatum	32[abc]	*Pythium* Group F	59[ab]
P. tardicrescens	20[ab]	*P. violae*	50[ab]
Control	10[a]	Control	10[a]

[a–j] Values in each column followed by different letters differ significantly at $p \leq 0.01$ according to Tukey's procedure for comparison of means

[1] CV = 32.9%

[2] CV = 27.9%

their aggressiveness to *E. fastigata*. The first group consisted of *Ph. cinnamomi* isolates that resulted in lesions significantly ($p \leq 0.01$) longer than those of any other species. *Ph. parasitica*, *P. splendens* (2), *P. splendens* (1) and *Ph. boehmeriae*, in that order of pathogenicity, also produced lesions which differed significantly ($p \leq 0.01$) from those of *Ph. cinnamomi*, but were still highly aggressive to *E. fastigata*. *P. irregulare* (1), *P. vexans* and *Pythium* sp. 2 resulted in lesions longer than those of *P. acanthophoron*, *Pythium* sp. 1, *P. irregulare* (2), *P. pyrilobum*, *Pythium* Group G and *P. myriotylum*, which differed significantly ($p \leq 0.01$) from the control and therefore comprises of an intermediate group of pathogens. Lesion lengths associated with the remaining species (*P. salpingophorum*, *P. intermedium*, *P. angustatum*, *P. hypogynum*, *P. spinosum*, *P. tardicrescens*, *P. aphanidermatum*, *P. buismaniae*, *P. rostratum*, *Pythium* Group F and *P. violae*) did not differ significantly from controls (Table 4).

3.3 Statistical analysis

Meaningful correlation ($p = 0.01$) between the pathogenicity of the species on *E. fastigata* ($r_s = 0.70$) and *E. grandis* ($r_s = 0.88$) in the two different inoculation trials were found. Therefore, only results of the first inoculation trial are presented (Table 4).

3.4 Lesion-length development in the outer bark

Lesion development in the outer bark of *E. fastigata* and a clone of *E. grandis* inoculated with *Pythium* and *Phytophthora* spp. was insignificant or absent in comparison to lesion development in the phloem. Only *Ph. cinnamomi* inoculated on *E. fastigata* was able to cause outer bark lesions (200 mm) significantly ($p \leq 0.01$) longer than those of the control inoculations where no lesions developed.

4 Discussion

This study has shown that many species of *Pythium* and *Phytophthora* are present in South African forest soils, though all species present may not have been identified with the isolation method used. Baiting with *Citrus* leaf discs favoured the isolation of fast-growing *Pythium* and *Phytophthora* spp., which masked the isolation of slow growing species. Furthermore, *Pythium* spp. like *P. irregulare* and *P. vexans*, were not inhibited by hymexazol and could possibly have prevented the isolation of slow growing *Phytophthora* spp. Therefore, to identify all the *Pythium* and *Phytophthora* spp. present in South African forest soils, various isolation techniques should be applied (TSAO 1990).

The results of this study suggest that *Pythium* spp. are more common in South African forest soils than *Phytophthora* spp. *Pythium* spp. have a cosmopolitan distribution and are common soil-inhabiting fungi (VAN DER PLAATS-NITERINK 1981). The high frequency of *Pythium* spp. from soil was, therefore, expected. Very little work has been done on *Pythium* spp. in South Africa, particularly in forestry (WAGER 1941; DARVAS et al. 1978; DENMAN and KNOX-DAVIES 1992). This probably explains why so many species were isolated for the first time in South Africa.

Phytophthora spp., including *Ph. cinnamomi*, *Ph. parasitica* and *Ph. boehmeriae*, are known as pathogens of *Pinus* and/or *Eucalyptus* spp. under field and nursery conditions (NEWHOOK 1959; OXENHAM and WINKS 1963; PODGER et al. 1965; LORIO 1966; PODGER and BATINI 1971; MARKS and KASSABY 1974; SHEARER et al. 1988). By contrast, *Pythium* spp. have only been associated with nursery diseases of *Pinus* and *Eucayptus* spp. (VAARTAJA and SALISBURY 1961; VAARTAJA 1965, 1967 CAMPBELL and HENDRIX 1967; MARKS and KASSABY 1974; DARVAS et al. 1978). The results of this study also support the view that, in

general, *Phytophthora* spp. are more aggressive than *Pythium* spp. on established trees. An exception is found in *P. splendens* which is more or equally aggressive than *Ph. cinnamomi* on *Eucalyptus* hosts (LINDE et al. 1994b).

Although *Pythium* and *Phytophthora* spp. are root pathogens, inoculation of these species took place on stems and not on roots. This more practical inoculation procedure was followed because it was previously shown that stem inoculations of *Ph. cinnamomi* correlate with root inoculation (SHEARER et al. 1987).

Inoculation studies on *E. fastigata* showed that *Ph. cinnamomi, Ph. parasitica, Ph. boehmeriae, P. splendens, P. irregulare* (1), *P. vexans,* and *Pythium* sp. 2 were the most pathogenic species. In comparison to *Ph cinnamomi,* the *Pythium* spp., except for *P. splendens,* appear to be insignificant. *P. irregulare* and *P. vexans* have previously been associated with pine and *Eucalyptus* decline (LORIO 1966; DAVISON and BUMBIERIS 1973; OTROSINA and MARX 1975). It was therefore not unexpected that these species would show some degree of aggressiveness. Lesions produced by pathogenic *Pythium* and *Phytophthora* spp. were associated with kino-vein formation which is typical of *Phytophthora* infection (TIPPETT et al. 1983).

Inoculation studies on *E. grandis* showed that it is a host which is more tolerant to *Pythium* and *Phytophthora* infection than *E. fastigata. Pythium pyrilobum* was the most aggressive species but the lesion it produced did not differ significantly from *Ph. cinnamomi, P. splendens, Ph. parasitica, P. vexans, P. aphanidermatum* and *P. intermedium.* This particular clone of *E. grandis* is therefore not a good host to inoculate when the aggressiveness of *Pythium* and *Phytophthora* species are compared.

Only *Ph. cinnamomi* was able to develop significant lesions in the outer bark of *E. fastigata.* No significant differences could be obtained in lesion lengths on the outer bark inoculated with the other *Phytophthora* or *Pythium* spp. This suggests that bark lesions are not a useful character on which to base comparisons of aggressiveness among species. Lesion development in the phloem, as described by TIPPETT et al. (1983), is a much more effective measure of aggressiveness for *Pythium* and *Phytophthora* spp.

Pythium and *Phytophthora* spp. tested under field conditions were considerably more pathogenic to *E. fastigata* than to *E. grandis.* These results are consistent with the reported virulence of *Ph. cinnamomi* to *E. fastigata* in South Africa (WINGFIELD and KNOX-DAVIES 1980). Although *E. fastigata* has been shown to be more susceptible to *Pythium* and *Phytophthora* infection than *E. grandis,* inoculation studies were also conducted on the latter species. This was because the cultivation of *E. fastigata* has been almost terminated due to its high susceptibility to *Ph. cinnamomi* (WINGFIELD and KNOX-DAVIES 1980). Furthermore, 74.2% of all the *Eucalyptus* planted in South Africa are *E. grandis* (SCHONAU et al. 1994) and, therefore, the most important *Eucalyptus* spp. in the South African forestry industry.

The *Pythium* and *Phytophthora* species used in this study, were isolated from commercial forests and not from nurseries. Extrapolation of the results to the nursery situation in South Africa is, therefore, not recommended. Nevertheless, those *Pythium* and *Phytophthora* spp. that are pathogenic, could play an important role in establishment deaths of *E. grandis* and *Pinus patula* under field conditions, as has been found with *Pinus patula* seedlings on previously cultivated lands (LINDE et al. 1994a). A very common *Pythium* sp. isolated from soil and roots of both *Eucalyptus* and *Pinus* spp., namely *Pythium* Group F, was not pathogenic to *E. grandis.* It is therefore possible that *Pythium* Group F is a common saprophyte that survives in dead roots which died of natural decline.

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Summary

A total of 20 *Pythium* and three *Phytophthora* spp. were isolated from root and soil samples from six areas in eucalypt and pine plantations in South Africa. Of these, six *Pythium* spp. were reported in South Africa for the first time. Most isolations of these fungi were from soil and not from roots. *Pythium* Group F was most consistently isolated from soil and roots of diseased trees and appears to be a common saprophyte in local soils. The species of *Pythium* and *Phytophthora* isolated in this study can be divided into groups based on their aggressiveness to *E. grandis* and *E. fastigata*. The species most aggressive to *E. fastigata* were *Ph. cinnamomi, Ph. parasitica, Ph. boehmeriae*, and *P. splendens*. On *E. grandis* trees, *Ph. cinnamomi, Ph. parasitica, P. aphanidermatum, P. intermedium, P. pyrilobum, P. splendens* and *P. vexans* were the most virulent species. *Ph. boehmeriae* and *Pythium* Group G were also pathogenic but less so than the above mentioned species. Of the most aggressive *Pythium* and *Phytophthora* spp., only *Ph. cinnamomi* is a common species which could be isolated from most of the forestry areas surveyed in South Africa.

Résumé

Les espèces de Pythium et de Phytophthora associées aux Eucalyptus et aux Pins en Afrique du Sud

En tout ont été 20 espèces de *Pythium* et trois de *Phytophthora* isolées d'échantillons de racines et de sol dans six plantations d'Eucalyptus et de Pins. Parmi elles, six espèces de *Pythium* sont des premières mentions pour l'Afrique du Sud. La plupart de ces champignons étaient issus d'isolements de sol et non pas de racines. Le groupe F de *Pythium* a été presque constamment isolé de sol et de racines d'arbres malades et apparaissait être un groupe saprophyte courant dans les sols locaux. Les *Pythium* et *Phytophthora* isolés dans cette étude peuvent être divisés en groupes sur la base de leur agressivité vis-à-vis de *Eucalyptus grandis* et *E. fastigata*. Les espèces les plus aggressives pour *E. fastigata* étaient: *Ph. cinnamomi, Ph. parasitica, Ph. boehmeriae* et *P. splendens*; pour *E. grandis*: *Ph. cinnamomi, Ph. parasitica, P. aphanidermatum, P. intermedium, P. pyrilobum, P. splendens* et *P. vexans*. Le groupe G de *Pythium* et *Ph. boehmeriae* étaient également pathogènes mais moins. Parmi les espèces les plus agressives de *Pythium* et de *Phytophthora*, seul *Ph. cinnamomi* est une espèce courante qui peut être isolée de la plupart des zones forestières étudiées.

Zusammenfassung

Mit Eucalyptus und Pinus assoziierte Pythium- und Phytophthora-Arten in Südafrika

Insgesamt wurden 20 *Pythium*- und drei *Phytophthora*-Arten aus Wurzel- und Bodenproben von sechs Gebieten mit Eucalyptus- und Pinusplantagen in Südafrika isoliert. Davon sind sechs *Pythium* spp. Erstnachweise für Südafrika. Die Pilze wurden hauptsächlich aus dem Boden und nicht aus den Wurzeln isoliert. Die *Pythium*-Gruppe F wurde sehr häufig aus Böden und Wurzeln erkrankter Bäume isoliert und scheint ein gewöhnlicher Saprophyt der lokalen Böden zu sein. Die in dieser Studie isolierten *Pythium*- und *Phytophthora*-Arten können aufgrund ihrer Aggressivität gegenüber *Eucalyptus grandis* und *E. fastigata* in Gruppen eingeteilt werden. Die aggressivsten Arten auf *E. fastigata* waren *Ph. cinnamomi, Ph. parasitica, Ph. boehmeriae* und *P. splendens*. Auf *E. grandis* waren dies *Ph. cinnamomi, Ph. parasitica, P. aphanidermatum, P. intermedium, P. pyrilobum, P. splendens* und *P. vexans*. *Ph. boehmeriae* und die *Pythium*-Gruppe G waren ebenfalls pathogen aber weniger aggressiv als die vorgenannten Arten. Von den aggressivsten *Pythium*- und *Phytophthora*-Arten kommt nur *Ph. cinnamomi* allgemein vor und konnte aus den meisten der in Südafrika untersuchten Forstgebiete isoliert werden.

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