Root and Root Collar Disease of *Eucalyptus grandis* Caused by *Pythium splendens*

C. LINDE, M. J. WINGFIELD, and G. H. J. KEMP, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, South Africa

ABSTRACT

Linde, C., Wingfield, M. J., and Kemp, G. H. J. 1994. Root and root collar disease of *Eucalyptus grandis* caused by *Pythium splendens*. Plant Dis. 78:1006-1009.

A serious root and root collar disease of *Eucalyptus grandis* occurred in the Kwambonambi area of northern Natal during the past 4 yr. This disease typically occurs on young trees that rapidly wilt and die. *Pythium splendens* was consistently isolated from roots and root collars of dying trees. *P. splendens* inoculated on two different clones of *E. grandis* and on *Eucalyptus fastigata* was highly pathogenic. Isolates of *P. splendens* showed the same or an even higher degree of virulence than *Phytophthora cinnamomi,* a well-known *Eucalyptus* root pathogen, on the *E. grandis* clones tested. Virulence of *P. splendens* on *E. fastigata* was, however, less than that of *Phytophthora cinnamomi.*

The South African forestry industry is based mainly on plantations of exotic *Pinus* and *Eucalyptus* species in approximately equal proportions. *Phytophthora cinnamomi* Rands has occurred in scattered locations, causing minimal losses (30,33). This is in contrast with the widespread and destructive dieback of *Eucalyptus,* especially *Eucalyptus marginata* Sm., in southern and western Australia (15,17).

In high altitude areas of South Africa, *Phytophthora cinnamomi* is commonly known as a root pathogen of *Eucalyptus fastigata* Deane & Maiden and *Eucalyptus fraxinoides* Deane & Maiden (33). Their high susceptibility demanded the planting of other *Eucalyptus* spp. not susceptible to *Phytophthora cinnamomi.* The pathogen is also associated with root diseases of *Pinus* spp. in South Africa. However, only a few reports have been published on the latter host, and these were primarily from sites with poor drainage (4,30,33).

Serious but sporadic incidences of root diseases have been reported in pine and *Eucalyptus* nurseries in the Cape Province (4). These deaths were ascribed to factors such as poor drainage, as well as to *Phytophthora cinnamomi*-infested soil. Exclusion of these factors from nurseries resulted in a reduction in root diseases caused by *Phytophthora cinnamomi* (4).

In recent years, severe losses have occurred in 6- to 18-mo-old plantings of *Eucalyptus grandis* A.W. Hill ex Maiden at Kwambonambi in the Zululand Forestry Area (Natal Province). This disease is characterized by the reddening of the leaves (Fig. 1A) in December (summer). A good rainfall in November and December (average for the last 4 yr was 137 mm/mo), followed by a mid-summer drought in January (68 mm), are typical environmental factors associated with the disease. The trees rapidly wilt and die between January and March, following the girdling of the roots and root collars (Fig 1B).

In the past, *E. grandis,* which is the most important *Eucalyptus* sp. propagated in South Africa, has been virtually free of root diseases. This disease problem was, therefore, of particular concern. The objective of this study was to identify the causal agent of this important disease.

MATERIALS AND METHODS

Isolation and identification. Isolations were made from roots and root collars, as well as from soil in the root zone of dying trees. Samples were taken from 75 arbitrarily selected diseased trees in the Zululand area. Soils were baited with citrus leaf disks (6). Five leaf disks per soil sample were transferred to a selective medium for the isolation of Oomycetes in general (27) and to a hymexazol medium for the isolation of *Phytophthora* spp. (26). Roots and root collar segments were washed thoroughly in running tap water and plated onto both selective media. Transfers were made to cornmeal agar (CMA) for identification.

Oomycetous fungi consistently isolated from diseased trees and suspected as the causal agent were identified on the basis of morphological and cultural characteristics (29). Fifty mature asexual structures produced with the grass-blade culture technique (29) were mounted in lactophenol-cotton blue and measured. Twenty isolates of the suspected pathogen were crossed with two compatible strains of the heterothallic oomycetous fungus, *Pythium splendens* H. Braun, obtained from the Plant Protection Research Institute (PPRI), Pretoria, South Africa (PPRI 4722 (+) and PPRI 4723 (−)). All the isolates were cultured on CMA for 2 days prior to mating. Isolates of the suspected pathogen were transferred to opposite sides of the compatible mating types on CMA and incubated at 20 C. The presence of oogonia, antheridia, and oospores was determined 3 wk later by light microscopy. The presence of other fungal pathogens was investigated by incubating diseased plant material in moist chambers and making single-spore isolations from fruiting bodies on potato-dextrose agar (PDA).

Field trials. *1) Root inoculation.* An isolate of *P. splendens* isolated from diseased *E. grandis* roots (CBS 461.93), was inoculated in December 1993 in the roots of a seedling population of 20 *E. grandis* trees (3 yr old) planted in the Kwambonambi area. Inoculum of the *P. splendens* isolate was established on PDA. One root (5 cm diameter) of each tree was inoculated. The cambium of the lateral root was removed with a 10-cm-diameter corkborer, 15 cm from the tree collar. The wound was replaced with a disk of agar colonized with the test fungus and sealed with masking tape. Another 20 trees, which served as the controls, were inoculated with a sterile disk of agar and also sealed with masking tape. A completely random experimental design was utilized in the trial. Trees were examined for lesion and symptom development after 4 mo. Reisolations were made from inoculated and control plants on culture dishes containing pimaricin (27).

2) Stem inoculation. Two stem inoculation trials were conducted. In the first trial with the suspected pathogen, two clones of *E. grandis* were used. This was because clone 1 appeared to be more susceptible under field conditions than clone 2. Twenty trees (2 yr old) of each clone were inoculated at the Iona plantation near Mtunzini, Natal North Coast. Inoculum of a single isolate of the suspected pathogen was established on PDA in petri dishes. A 10-mm corkborer was used to make two wounds 180° from each other on each tree. Wounds were made 1.5 m above ground level. One wound was inoculated with a disk of agar colonized by the test fungus, and a disk of sterile PDA was inserted into the second wound, which served as the control. The wounds were sealed with masking tape,

Accepted for publication 2 May 1994.

and the trees were examined 10 wk later.

In the second stem inoculation trial, three different isolates of the suspected pathogen were compared. Ten trees each of three different *Eucalyptus* hosts were artificially inoculated. The trees for inoculation were as follows: a 2-yr-old clone of *E. grandis* near Kwambonambi, Natal North Coast; a 2-yr-old clone of *E. grandis* near Lydenburg, eastern Transvaal; and a 3-yr-old *E. fastigata* generated from seed near Lothair, southeastern Transvaal. The *E. grandis* clones were different from those used in the first stem inoculation trial.

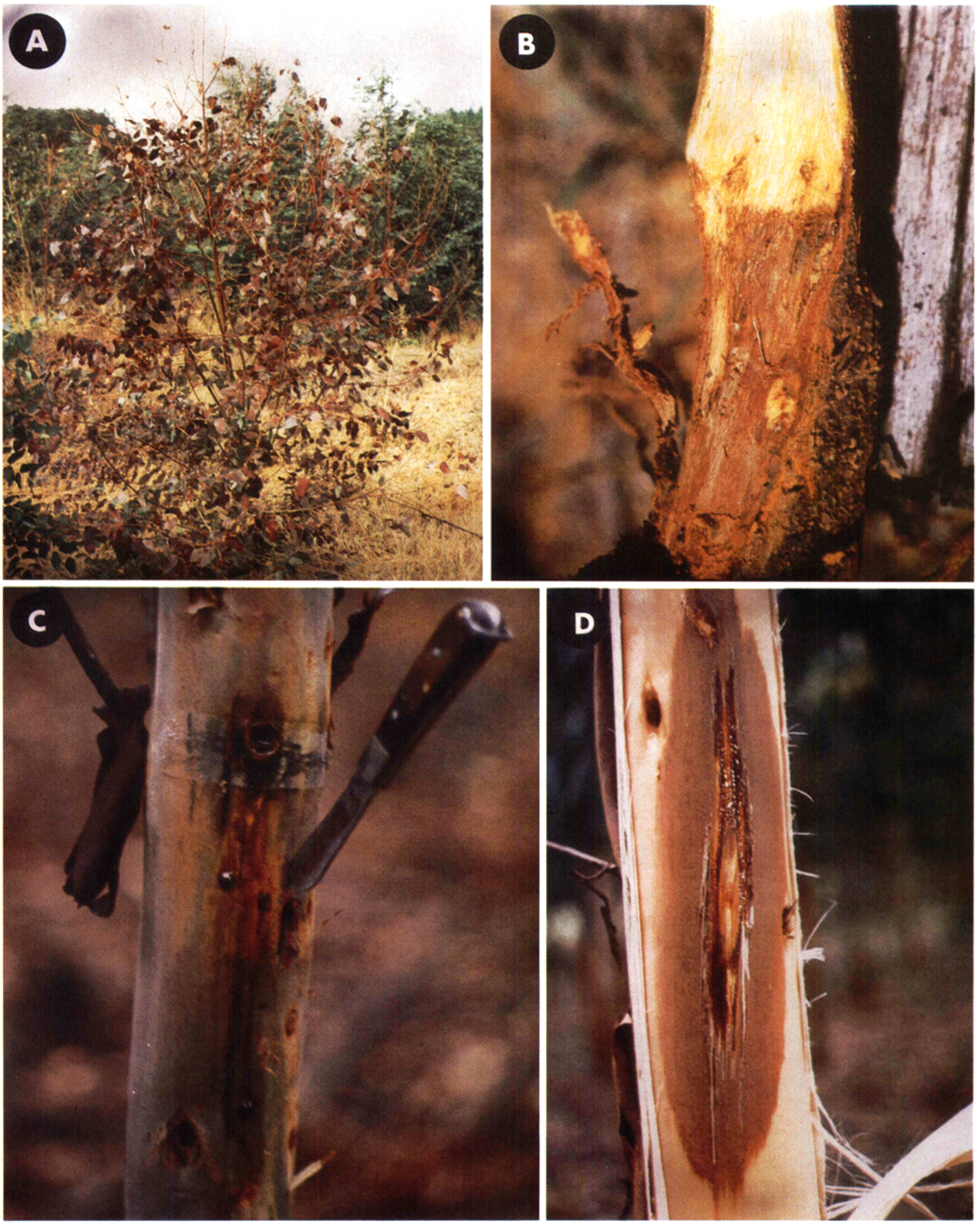

Fig. 1. (A) Red discoloration of leaves, the first observable symptom of *Eucalyptus grandis* infected with *Pythium splendens*. **(B)** Girdled root collar of *E. grandis* tree naturally infected with *P. splendens*. **(C)** Lesion on the outer bark of *Eucalyptus fastigata* 5 wk after inoculation with *P. splendens*. **(D)** Lesion on *E. fastigata* 5 wk after inoculation with *P. splendens* (outer bark removed).

The three different isolates of the suspected pathogen (CBS 460.93, CBS 461.93, and CBS 462.93) used in the pathogenicity studies were isolated from diseased roots of *E. grandis*. Two isolates of *P. cinnamomi* (CP 10 and CP 11) isolated from *E. fastigata* in eastern Transvaal were also inoculated for comparison. Inoculum preparation and inoculation procedures were the same as described for the first stem inoculation trial. However, lesions were examined after 5 wk rather than 10 wk as in the first trial. The experimental design followed for both trials was a complete randomized design.

Both field trials were conducted in summer when the host was thought to be most susceptible to fungal invasion (21,24). Lesion lengths in the secondary phloem were measured and used to indicate the virulence of each isolate inoculated (25). Reisolations were made from inoculated and control plants on culture dishes containing pimaricin (27).

Statistical analysis. The numerical data obtained throughout the study were statistically analyzed for variances and differences among isolates and hosts. Means were tested for significance according to Tukey's procedure (23).

RESULTS

Isolation and identification. A species of *Pythium* was isolated from diseased roots and root collars of 64 dying trees, as well as from soil in the root zone of 66 diseased *E. grandis* trees. Isolations were made on the selective medium not containing hymexazol. No other known plant pathogens were isolated from diseased plant material or soil. The fungus was identified as *P. splendens*. This identification was based primarily on characteristics such as the typical abundant globose hyphal swellings, 26.2–(31.5)–37.5 μm in diameter. Oogonia, aplerotic oospores, and diclinous antheridial cells were produced in all 20 isolates paired with the compatible isolate of *P. splendens* (PPRI 4722 (+)). Cultures representative of the species were deposited in the culture collection of the National Collection of Fungi, Pretoria.

Field trials. *1) Root inoculations.* Twelve of the inoculated *E. grandis* trees developed lesions that extended from the roots to the root collars, to form a root collar lesion similar to those observed under natural conditions. No lesions were observed in the other eight inoculated *E. grandis* or control trees. *P. splendens* was successfully reisolated from the 12 *E. grandis* trees that developed lesions, but could not be isolated from the eight trees without lesion development and the control trees.

2) Stem inoculations. Outer bark lesions (Fig. 1C) and secondary phloem lesions (Fig. 1D) with associated kino vein formation (25) occurred on *E. fastigata* and *E. grandis* clones inoculated with *P. splendens* and *Phytophthora cinnamomi*. Average lesion lengths which developed in clone 1 (538 mm) in the first stem inoculation trial were significantly ($P \leq 0.01$) longer than those of clone 2 (429 mm). Due to callus formation, no measurements were possible in control inoculations. The pathogens were reisolated from inoculated plants.

In the second stem inoculation trial, no significant differences in lesion lengths on clones of *E. grandis* in Natal were detected among isolates of *P. splendens* and *Phytophthora cinnamomi*. In contrast, isolate SI 2 of *P. splendens* was significantly ($P \leq 0.01$) more virulent to the *E. grandis* clone inoculated in Transvaal than was *Phytophthora cinnamomi* (Table 1). However, *Phytophthora cinnamomi* resulted in significantly ($P \leq 0.01$) longer lesions on *E. fastigata* than those associated with *P. splendens*.

Significant differences ($P \leq 0.01$) in virulence were detected among isolates of both *P. splendens* and *Phytophthora cinnamomi*. Although lesion lengths associated with *Phytophthora cinnamomi* (27.1 cm) as an average among hosts and isolates differed significantly ($P \leq 0.01$) from those associated with *P. splendens* (20.4 cm), lesion lengths associated with some isolates of *P. splendens* did not differ significantly from *Phytophthora cinnamomi*. Lesion lengths induced by isolates SI 2 and SI 3 of *P. splendens* as an average among hosts did not differ significantly from those associated with isolate CI 1 of *Phytophthora cinnamomi*. The lesion length induced by isolate SI 2 of *P. splendens* as an average among hosts was also equivalent to the more virulent isolate CI 2 of *Phytophthora cinnamomi*. Both it and *P. splendens* resulted in smaller lesions in the two *E. grandis* clones than in *E. fastigata*. No lesions developed in control inoculations (Table 1).

DISCUSSION

It was possible to show conclusively in this study that *P. splendens* is the primary cause of the root and root collar disease of established *E. grandis* trees in the Kwambonambi area of Northern Natal. *P. splendens* is known as a pathogen of young seedlings of *Eucalyptus* and *Pinus* species, although it is not frequently encountered (1,19,28). It also causes severe damping-off of both young *Pinus patula* and *E. grandis* seedlings and is thus an important nursery pathogen (28). Since the first report of *P. splendens* in South Africa, from *Carica papaya* L. (31), the fungus has been encountered only occasionally. In all cases, these reports have been from nonforestry hosts (2,3).

P. splendens has apparently never been associated with diseases of established *Eucalyptus* trees. It has, however, been suggested that *P. splendens,* as a component in a disease complex with *Phytophthora cinnamomi* and other *Pythium* spp., could cause a disease of *Eucalyptus* spp. in Australia (18). High populations of *Pythium* spp. have been detected in the soil of *Eucalyptus* plantations in the past (5,9). In these cases, they were considered simply soil inhabiting and nonpathogenic to established *Eucalyptus* trees under field conditions. This is in agreement with the situation in loblolly pine stands, peach orchards, and apple orchards, where *Pythium* spp. are considered minor components in tree decline (11,16,20). In contrast, the related fungus *Phytophthora cinnamomi* is a well-known component of the *Eucalyptus* dieback syndrome in Australia (10,17,32) and is also a serious pathogen of other woody plants (7,8,12–14).

Table 1. Lesion lengths (cm) on *Eucalyptus* hosts inoculated with different isolates of *Pythium splendens* and *Phytophthora cinnamomi*[w]

	Lesion lengths (cm)								
		P. splendens[x,y]				*P. cinnamomi*[x,y]			
Host	Control	SI 1	SI 2	SI 3	Mean[y]	CI 1	CI 2	Mean[y]	Overall mean[y]
NEGC	1.0 a	7.8 b	13.0 bc	6.4 b	9.1 a	10.8 bc	13.1 b	12.0 a	10.2 a
TEGC	1.0 a	23.4 cd	32.2 de	14.9 bc	23.5 b	8.0 bc	15.5 b	11.8 a	18.8 b
Eucalyptus fastigata	1.1 a	11.6 b	32.7 de	41.6 ef	28.6 c	50.4 g	64.5 f	57.5 d	40.2 c
Mean[z]	1.0 p	14.3 q	26.0 rst	21.0 rs	20.4 qr	23.1 rs	31.0 t	27.1 st	

[w] Abbreviations are as follows: SI = *P. splendens* isolates 1–3, CI = *Phytophthora cinnamomi* isolates 1 and 2, NEGC = Natal *Eucalyptus grandis* clone, and TEGC = Transvaal *E. grandis* clone.

[x] Each value is an average of 10 replicate trees per treatment. CV = 31.2%.

[y] Values in different columns followed by different letters differed significantly at $P \leq 0.01$ according to Tukey's procedures for comparison of means.

[z] Values in the row followed by different letters differed significantly at $P \leq 0.01$ according to Tukey's procedure for comparison of means.

Root collar lesions were successfully produced with artificial inoculation of *P. splendens* on the roots of *E. grandis* trees. However, these trees did not die as they ultimately do under natural conditions. It is believed that this was due to the fact that the inoculated trees were somewhat older than those observed dying in plantations. Moreover, under natural conditions, multiple infections probably led to tree death.

Although the disease under investigation in this study was of roots and root collars, we also inoculated stems to measure differences in virulence of *P. splendens* isolates and in clonal susceptibility. Stem inoculations could be justified by the fact that Shearer et al (22) showed that their results on *Eucalyptus* with *Phytophthora cinnamomi* corresponded with root inoculation. However, virulence is enhanced in stem inoculations (22,24).

As might be expected, different isolates of *P. splendens* differed in virulence. Tests to compare the virulence of *P. splendens* and *Phytophthora cinnamomi* showed that *P. splendens* is equally or even more virulent than *Phytophthora cinnamomi* on some of the *E. grandis* clones tested. The extensive lesions that developed on field-inoculated trees are indicative of a very high degree of virulence in *P. splendens*. *Phytophthora cinnamomi* was more virulent than *P. splendens* on *E. fastigata*. This is in agreement with the high susceptibility of *E. fastigata* to *Phytophthora cinnamomi* in South Africa (33).

Root pathogens such as *Pythium* spp. tend to have a wide host range, and no differences were expected in susceptibility of clones. In contrast, results of this study indicated significant differences in susceptibility among the *E. grandis* clones tested. This might be explained by the different environmental conditions at the inoculation sites, as well as genetic variability in *E. grandis*. These differences suggest that it might be possible to select for disease resistance in the field. However, the considerable variation in virulence among isolates of *P. splendens* could confound this opportunity.

The discovery of *P. splendens* as a pathogen of *Eucalyptus* in South Africa has resulted in considerable concern, because *E. grandis* is the most important species planted and the most severely affected. Future studies must, therefore, concentrate on developing strategies to control this important disease.

LITERATURE CITED

1. Campbell, W. A., and Hendrix, F. F., Jr. 1967. *Pythium* and *Phytophthora* populations in southern forest tree nurseries. (Abstr.) Phytopathology 57:457.
2. Darvas, J. M. 1979. Ecology of avocado root pathogens. South Afr. Avocado Grower's Assoc. Res. Rep. 3:31-32.
3. Doidge, E. M. 1950. The South African fungi and lichens to the end of 1945. Bothalia 51:1-1094.
4. Donald, D. G. M., and von Broembsen, S. L. 1977. The control of *Phytophthora cinnamomi* Rands in a South African forest nursery. South Afr. For. J. 100:50-55.
5. Gerrettson-Cornell, L., Dowden, H. G. M., Bridges, R. G., and Townsend, S. R. 1979. The incidence of *Phytophthora cinnamomi* and *Pythium* species in thinned Eucalypt plots. Plant Dis. Rep. 63:490-493.
6. Grimm, G. R., and Alexander, A. F. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. Phytopathology 63:540-541.
7. Kuhlman, E. G., and Hendrix, F. F. 1963. Phytophthora root rot of Fraser fir. Plant Dis. Rep. 47:552-553.
8. Lorio, P. L., Jr. 1966. *Phytophthora cinnamomi* and *Pythium* species associated with loblolly pine decline in Louisiana. Plant Dis. Rep. 50:596-597.
9. Marks, G. C., and Kassaby, F. Y. 1974. Pathogenicity of *Pythium* spp. and *Phytophthora drechsleri* to *Eucalyptus* spp. Aust. J. Bot. 22:661-668.
10. Marks, G. C., Kassaby, F. Y., and Reynolds, S. T. 1972. Die-back in mixed hardwood forests of eastern Victoria; a preliminary report. Aust. J. Bot. 20:141-145.
11. Mircetich, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.
12. Mircetich, S. M., Campbell, R. N., and Matheron, M. E. 1977. Phytophthora trunk canker of coast live oak and cork oak trees in California. Plant Dis. Rep. 61:66-70.
13. Mircetich, S. M., and Matheron, M. E. 1976. Phytophthora root and crown rot of cherry trees. Phytopathology 66:549-558.
14. Newhook, F. J. 1959. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers. N.Z. J. Agric. Res. 2:808-843.
15. Newhook, F. J., and Podger, F. D. 1972. The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. Annu. Rev. Phytopathol. 10:299-326.
16. Otrosina, W. J., and Marx, D. H. 1975. Populations of *Phytophthora cinnamomi* and *Pythium* spp. under shortleaf and loblolly pines in littleleaf disease sites. Phytopathology 65:1224-1229.
17. Podger, F. D., Doepel, R. D., and Zentmyer, G. A. 1965. The association of *Phytophthora cinnamomi* with a disease of *Eucalyptus marginata* forest in western Australia. Plant Dis. Rep. 49:943-947.
18. Pratt, B. H., and Heather, W. A. 1973. Recovery of potentially pathogenic *Phytophthora* and *Pythium* spp. from native vegetation in Australia. Aust. J. Biol. Sci. 26:575-582.
19. Robertson, G. I. 1973. Occurrence of *Pythium* spp. in New Zealand soils, sands, pumices, and peat, and roots of container grown plants. N.Z. J. Agric. Res. 16:357-365.
20. Sewell, G. W. F. 1981. Effects of *Pythium* species on the growth of apple and their possible causal role in apple replant disease. Ann. Appl. Biol. 97:31-42.
21. Shearer, B. L., Michaelsen, B. J., and Somerford, P. J. 1988. Effects of isolate and time of inoculation on invasion of secondary phloem of *Eucalyptus* spp. and *Banksia grandis* by *Phytophthora* spp. Plant Dis. 72:121-126.
22. Shearer, B. L., Michaelsen, B. J., and Warren, H. J. 1987. Comparative behavior of *Phytophthora* species in the secondary phloem of stems and excised roots of *Banksia grandis* and *Eucalyptus marginata*. Aust. J. Bot. 35:103-110.
23. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York.
24. Tippett, J. T., Hill, T. C., and Shearer, B. L. 1985. Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust. J. Bot. 33:409-418.
25. Tippett, J. T., Shea, S. R., Hill, T. C., and Shearer, B. L. 1983. Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. 31:197-210.
26. Tsao, P. H., and Guy, S. O. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. Phytopathology 67:796-801.
27. Tsao, P. H., and Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223:636-638.
28. Vaartaja, O. 1967. Damping-off pathogens in South Australian nurseries. Phytopathology 57:765-768.
29. Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. Studies in Mycology No. 21. Central bureau voor Schimmelcultures, Baarn, Netherlands.
30. Von Broembsen, S. 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the south-western Cape Province. Phytophylactica 16:221-225.
31. Wager, V. A. 1931. Diseases of plants in South Africa due to members of the Pythiaceae. Union South Afr. Dep. Agric. Bull. 105:1-43.
32. Weste, G. M., and Taylor P. 1971. The invasion of native forest by *Phytophthora cinnamomi*. I. Brisbane Ranges, Victoria. Aust. J. Bot. 19:281-294.
33. Wingfield, M. J., and Knox-Davies, P. S. 1980. Observations on diseases in pine and eucalyptus plantations in South Africa. Phytophylactica 12:57-60.