First Report of *Fusarium subglutinans* f. sp. *pini* on Pine Seedlings in South Africa

A. VILJOEN and M. J. WINGFIELD, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, South Africa, and W. F. O. MARASAS, Programme on Mycotoxins and Experimental Carcinogenesis, Medical Research Council, P.O. Box 19070, Tygerberg, 7505, South Africa

ABSTRACT

Viljoen, A., Wingfield, M. J., and Marasas, W. F. O. 1994. First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. Plant Dis. 78:309-312.

A study of fungi responsible for root rot of *Pinus patula* seedlings in a forest nursery yielded *Fusarium subglutinans* f. sp. *pini* and *F. oxysporum*. *F. s. pini* was isolated most frequently from diseased seedlings and proved to be the most virulent fungus in pathogenicity tests conducted on germinated seed and 2-mo-old seedlings. Inoculation of 3-yr-old trees with *F. s. pini* yielded symptoms reminiscent of pitch canker. This is the first report of *F. s. pini* causing root disease of pine seedlings and is also the first record of the fungus in the Southern Hemisphere.

Fusarium species are well-known pathogens in forestry nurseries in many parts of the world (7,27). Diseases associated with this group of fungi include seed decay, damping-off, root rot, and stem cankers (1,5,16,19, 21–23,25). *Fusarium* spp. are particularly damaging to conifer seedlings such as Douglas-fir and pine (7,16,17). Many species of *Fusarium* are associated with root diseases of conifer seedlings. *Fusarium oxysporum* Schlechtend.:Fr. has been identified as a major pathogen in bare-root (5,6) and container-grown seedlings (17). Other *Fusarium* spp. known to cause disease of conifer seedlings include *F. acuminatum* Ellis & Everh., *F. avenaceum* (Fr.:Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. lateritium* Nees:Fr., *F. moniliforme* J. Sheld., *F. sambucinum* Fuckel, *F. solani* (Mart.) Sacc., and *F. sporotrichioides* Sherb. (7,17).

F. subglutinans (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas, previously isolated from slash pine (*Pinus elliottii* Engel. var. *elliottii*) and loblolly pine (*P. taeda* L.) seed (16) and container-grown conifer seedlings (17), was found to initiate infections from seedborne inoculum to the cotyledons of slash pine seedlings (16). The well-known causal agent of pitch canker of *Pinus* spp. in the United States (12,13,15) was designated as a specific forma specialis, *F. subglutinans* f. sp. *pini*, by Correll et al (10). This fungus has also been isolated from strobili, seed cones (2,4,11,18), and the stems and root collars of first-year slash pine seedlings (1). It has not, however, been regarded as a serious root pathogen of pine seedlings.

During 1990, a major forest nursery in the Eastern Transvaal province of South Africa reported devastating losses of containerized *P. patula* Schlechtend. & Cham. seedlings (Fig. 1A). Disease onset was approximately 3 mo after planting, and losses were due to root rot rather than to pre- or postemergence damping-off. The primary aim of this study was to determine the cause of the disease affecting *P. patula* seedlings.

MATERIALS AND METHODS

Isolation and identification of pathogens. One hundred symptomatic *P. patula* and 50 asymptomatic *P. patula* and *P. elliottii* seedlings were initially collected in the Ngodwana nursery in the Eastern Transvaal during June 1990. Thereafter, 20 symptomatic *P. patula* seedlings each were examined on three further occasions (November 1990, June 1991, and August 1991).

Seedlings were removed from their cells in ultraviolet-stabilized white plastic containers together with the composted bark medium in which they were cultivated, kept in open plastic bags, and examined within 48 hr of collection. Bark medium was first carefully removed from the roots by hand and by washing. The roots were then surface-disinfested with 100% ethyl alcohol for 1 min, rinsed three times in sterile distilled water, and damp-dried on absorbent paper toweling. Pieces (3–5 mm) of tissue were cut from the margins of diseased areas on the main and lateral roots. The infected root tissue was aseptically transferred to malt extract agar (20 g of Biolab malt extract, 20 g of Biolab agar, 1,000 ml of H_2O) modified with 0.1% novobiocin to obtain maximum fungal development. Two isolations were made from each plant, one from the main and one from the lateral roots. Four pieces of tissue were placed in every petri dish, which were then incubated at 25 C under cool-white fluorescent illumination. To evaluate the possible presence of pythiaceous fungi, similar isolations from diseased roots were prepared on PARP medium (26). Material on PARP medium was incubated in the dark at 25 C and observed for mycelial development over a 1-wk period.

In addition to isolation on agar media, pieces of root tissue or whole surface-disinfested seedlings were placed on moist paper towels in plastic petri dishes. This material was incubated at 22–25 C under cool-white fluorescent lights for 72 hr. Developing mycelium was transferred to new petri dishes for identification.

Single-conidium transfers were made from developing *Fusarium* mycelia to 2% carnation leaf agar (CLA) (20 g of Biolab agar, 1,000 ml of H_2O, one or two 5-mm irradiated carnation leaves per petri dish) and potato-dextrose agar (PDA) (39 g of Difco PDA powder, 1,000 ml of H_2O) for identification purposes (20). Single-conidium isolates were maintained on CLA slants at 4 C. Some representative strains were lyophilized for further study.

Pathogenicity of isolates. Twelve isolates representing the two *Fusarium* species obtained from diseased *P. patula* seedlings were selected for pathogenicity tests. These isolates have all been deposited in the culture collection of the Medical Research Council (P.O. Box 19070, Tygerberg, 7505, South Africa).

Pathogenicity of *Fusarium* spp. was evaluated in three different tests. In the first two, *P. patula* seed (clone 35, S/N 23517) was used. Seeds were first disinfested in sodium hypochlorite (3.5% m/v) for 60 sec, then washed three times in sterile distilled water and dried on sterile filter paper in a laminar flow cabinet. The seeds were divided into two groups. One group was placed on sterile moist filter paper in petri dishes until germination before planting, and the other was planted directly to obtain 2-mo-old seedlings for inoculation. In the third pathogenicity test, 3-yr-old *P. patula* trees were inoculated under field conditions.

Inocula of the 12 *Fusarium* isolates included in seedling pathogenicity tests were prepared through cultivation on

Accepted for publication 22 September 1993.

sterile oat kernels as follows: Single-conidium isolates were grown on CLA at 25 C under cool-white and near-ultraviolet fluorescent lights. After 14 days, *Fusarium*-covered agar disks (11 mm in diameter) were cut from the CLA with a cork borer. Three disks of each isolate were then transferred to 25 g of oat kernels that had been water-soaked and autoclaved (20 min, 100 kPa, 121 C) on two consecutive days in 250-ml Erlenmeyer flasks. The flasks were incubated under cool-white fluorescent lights at 25 C for 14 days and shaken by hand every second day to obtain even distribution and growth.

Composted bark medium typical of that used under commercial conditions was used as growth medium for plants. The bark medium was first water-soaked and autoclaved on two consecutive days, then was placed in 1,300-ml plastic planting pots (200 × 150 × 60 mm). Twenty germinated *P. patula* seeds were placed, evenly spaced, on the surface of the bark medium. Two oat kernels colonized by the test fungus were placed alongside each pine seed, about 10 mm apart. The seeds and inoculum were then covered with a thin layer of sterile pine bark. For controls, seeds were planted alongside sterile oat kernels. Three pots were used, for a total of 60 seeds per treatment. The pots were arranged in a completely randomized block design in a growth room at 24 C under 12 hr day/night diurnal illumination and were watered regularly. Pre- and postemergence damping-off was recorded after 4 wk.

The 2-mo-old seedlings were inoculated by means of spore suspensions. Isolates of *Fusarium* spp. to be tested were grown for 14 days on PDA in the dark. They were then flooded with 10 ml of sterile distilled water. Micro- and macroconidia were dislodged from these cultures by rotating the petri dish before decanting the liquid into 1-L Erlenmeyer flasks. The suspensions were then diluted to 10^5 conidia per milliliter.

The seedlings, planted in sterile pine bark medium with 20 seeds per pot, were thinned to 15 plants per pot. A 200-ml spore suspension was flooded onto the seedlings in each pot, with three pots per treatment. Treatment with the spore suspensions was repeated once each week for three consecutive weeks. Controls received sterile distilled water. The pots were arranged in a completely randomized block design in a growth room at 24 C and watered regularly. The percentage of dead and dying seedlings as a result of root rot was recorded 2 and 4 mo after the first treatment.

One isolate of *F. s. pini* was selected for inoculation of stems of established 3-yr-old *P. patula* trees under field conditions. The fungus was grown on CLA for 14 days under near-ultraviolet fluorescent and cool-white illumination. Agar disks (11 mm in diameter) were cut from the colony with a cork borer. Bark was removed from stems of 20 *P. patula* trees at breast height using a sterile 11-mm cork borer. The agar disks with the fungus were then placed into these wounds and covered with masking tape. Sterile agar was used for control inoculations. After 10 wk, the trees were observed for symptom development and the length and width of lesions were measured.

The inoculated *Fusarium* isolates were reisolated from diseased tissue.

Data analysis. Analyses on pooled data were run on the SAS/STAT System for Personal Computers (SAS Institute, Cary, NC). Comparisons of pathogenicity among isolates and species were done by Tukey's studentized range (HSD) test.

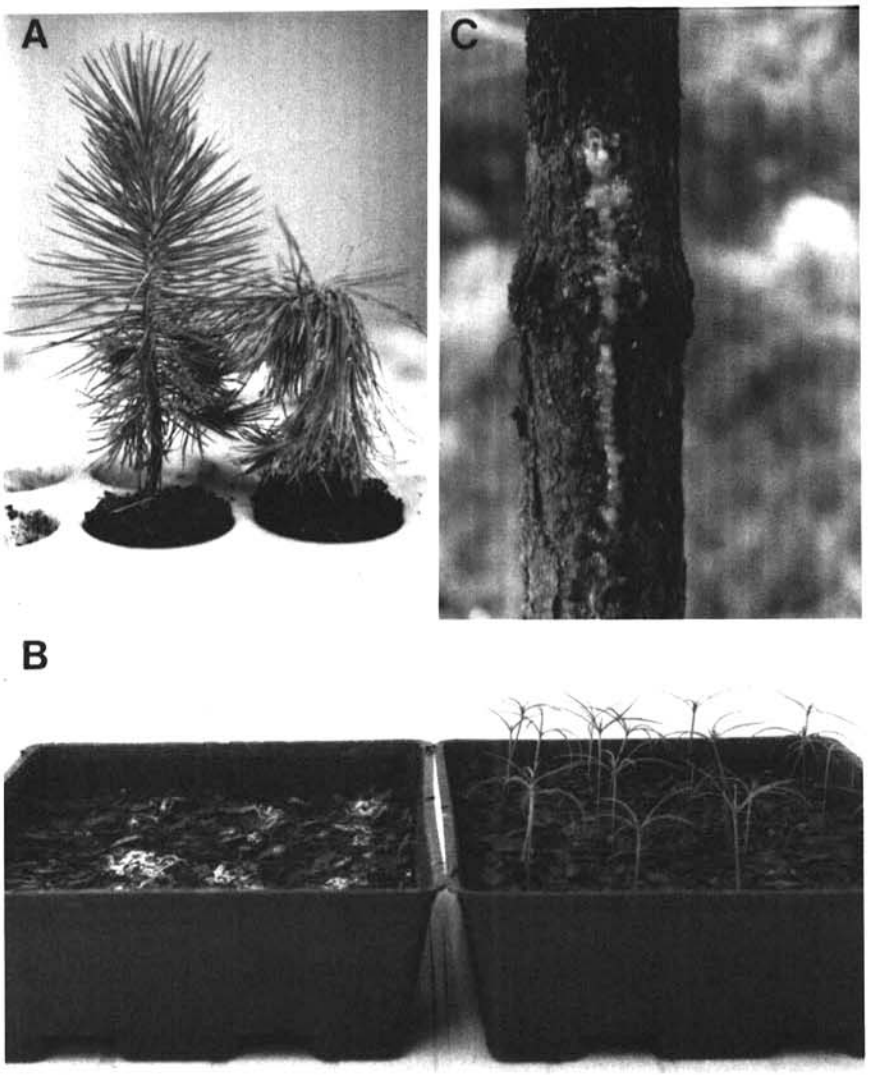

Fig. 1. Infection of *Pinus patula* with *Fusarium subglutinans* f. sp. *pini*: **(A)** Diseased seedling with typical symptoms (right) compared with healthy plant under field conditions. **(B)** Pots with (left) seed inoculated with *F. s. pini* (mycelial growth is visible on the surface of the bark medium) compared 4 wk after inoculation with (right) uninoculated controls. **(C)** Cankered stem of a 3-yr-old tree showing pitch flowing from the bark 10 wk after inoculation with *F. s. pini*.

RESULTS

Isolation and identification of pathogens. Two *Fusarium* species, *F. s. pini* and *F. oxysporum*, were consistently isolated from roots of diseased *P. patula* seedlings at each of the four sampling dates (June 1990, November 1990, June 1991, and August 1991). Isolation frequencies of the two species were 90, 89, 96, and 94% and 10, 11, 4, and 6%, respectively. The two species were distinguished by the formation of microconidia on polyphialides and by the absence of chlamydospores in *F. s. pini* (20). Isolates of *F. s. pini* were compared further with authentic pitch canker isolates M-935, M-1290, and M-3834 (obtained from P. E. Nelson, Pennsylvania State University, University Park) and found to be similar in cultural characteristics and morphology.

Of the two species, *F. s. pini* was isolated most often, and in comparison, *F. oxysporum* was relatively uncommon. Almost all root pieces plated on malt extract agar yielded *Fusarium* isolates. *F. s. pini* also developed in injured and wounded areas throughout the root

systems that had been incubated on moist paper in petri dishes. No *Fusarium* species were isolated from healthy seedlings. The only other fungi isolated were well-known saprophytes such as species of *Penicillium, Aspergillus*, and *Trichoderma*. No pythiaceous fungi were isolated despite the use of an appropriate selective medium.

Pathogenicity of isolates. Isolates of *F. s. pini* and *F. oxysporum* killed newly germinated *P. patula* seed inoculated with colonized oats kernels. Symptoms associated with both *Fusarium* spp. included pre- and postemergence damping-off. Where preemergence damping-off occurred, the seed coats and coleoptiles of germinated seeds were heavily colonized. In the case of postemergence damping-off, collars of seedlings were girdled. In seedlings from seed inoculated with *F. s. pini*, the stems that had collapsed as well as the surrounding bark medium later became heavily colonized (Fig. 1B). None of the control seedlings died.

There was a significant ($P = 0.05$) difference between the two *Fusarium* species in ability to cause damping-off (Table 1). *F. s. pini* isolates resulted in death of all the inoculated newly germinated seedlings within 4 wk (Table 1). In contrast, *F. oxysporum* isolates caused significantly ($P = 0.05$) less damping-off, and some isolates were significantly less aggressive than others (Table 1).

Mortality of established seedlings was lower than that of newly germinated seeds when inoculated with both *F. s. pini* and *F. oxysporum* isolates (Fig. 2). Some seedlings died in the first 2 mo after inoculation (Fig. 2); during the subsequent 2 mo, this was increased. Although the number of seedlings killed by *F. oxysporum* increased during the latter period, infection by *F. s. pini* was greater (Fig. 2). Very few control seedlings died, and there was no change in the death rate of these seedlings after 2 mo.

Symptoms developing on seedlings infected by *F. s. pini* and *F. oxysporum* in the growth room experiments were similar and resembled the root rot symptoms found in the nursery. The tops of the young seedlings wilted before the onset of needle discoloration. The seedlings very seldom collapsed, and roots were underdeveloped, with discolored lesions and necrosis of the cortex. The *Fusarium* species used as inoculum could be reisolated from both the diseased roots and the inoculated soil.

As in the case of the germinated seeds, *F. s. pini* was significantly more aggressive to 2-mo-old *P. patula* seedlings than was *F. oxysporum*. At 2 and 4 mo after inoculation, mean mortality was 18 and 51%, respectively, for *F. s. pini* and 6 and 16%, respectively, for *F. oxysporum* (significantly different at $P = 0.05$).

Stems of all 20 3-yr-old *P. patula* trees inoculated with an isolate of *F. s. pini* showed lesion development. Inoculations resulted in copious amounts of pitch flowing from the bark (Fig. 1C). When the bark was removed, dark brown lesions spreading in length and width were observed (average: 75 cm long and 46 cm wide). The pitch-soaked lesions mainly affected the cortex but also extended into the pith in the vicinity of the inoculation point. These symptoms resemble those described for pitch canker in the United States (15). No lesions developed on the control inoculations, where inoculum points were callused over.

F. s. pini and *F. oxysporum* could be recovered from diseased newly germinated seedlings, the roots of dying established seedlings, and the bark medium in the root area. *F. s. pini* was also reisolated from the necrotic lesions on the stems of inoculated *P. patula* trees.

DISCUSSION

Isolations from diseased *P. patula* seedlings and pathogenicity tests in this study showed that *F. s. pini* is the primary pathogen responsible for the nursery disease under investigation. *F. oxysporum* is also associated with this disease but appears to be less aggressive. This is the first report of *F. s. pini* causing root disease of seedlings and is also the first record of this pine pathogen in the Southern Hemisphere.

Newly germinated *P. patula* seeds are

Table 1. Damping-off of *Pinus patula* seedlings 4 wk after inoculation of germinated seeds with isolates of *Fusarium subglutinans* f. sp. *pini* and *F. oxysporum*

Species Isolate[y]	Damping-off (mean percentage)		
	Preemergence	Postemergence	Total
F. s. pini			
MRC 6208	63.33	36.67	100.00 a[z]
MRC 6209	76.67	23.33	100.00 a
MRC 6211	38.33	61.67	100.00 a
MRC 6213	80.00	20.00	100.00 a
MRC 6214	36.67	63.33	100.00 a
MRC 6215	66.67	33.33	100.00 a
MRC 6216	63.33	36.67	100.00 a
MRC 6217	45.00	55.00	100.00 a
F. oxysporum			
MRC 6207	30.00	23.33	53.33 c
MRC 6210	50.00	21.67	71.67 b
MRC 6212	33.33	35.00	68.33 bc
MRC 6804	36.67	45.00	81.67 b
Control	3.33	3.33	6.66 d

[y] Culture collection of the Medical Research Council, Tygerberg, South Africa.
[z] Mean percentages (20 seeds per pot, replicated three times) followed by the same letter do not differ significantly ($P = 0.05$) according to Tukey's studentized range (HSD).

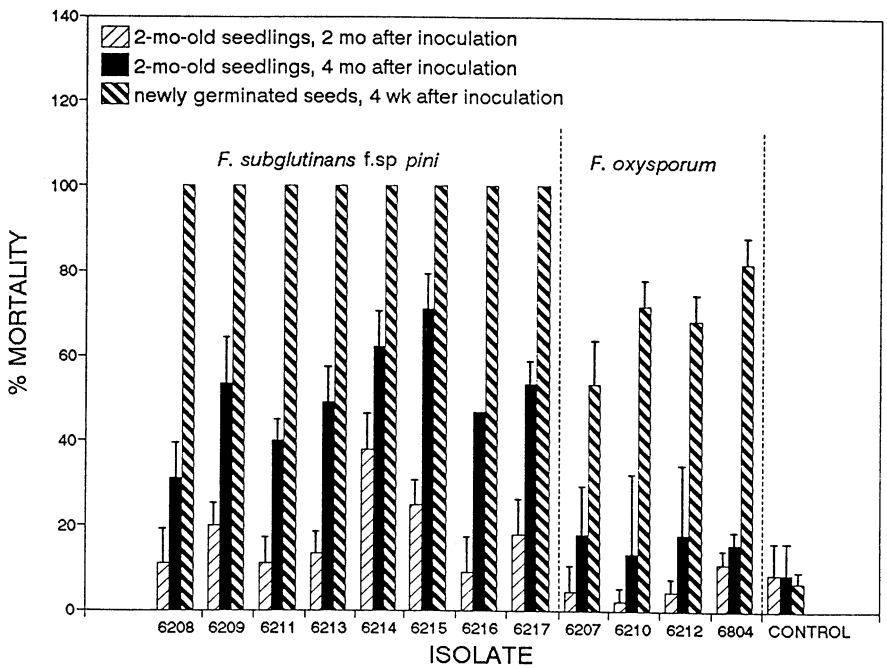

Fig. 2. Effect of *Fusarium subglutinans* f. sp. *pini* and *F. oxysporum* on newly germinated *Pinus patula* seedlings 4 wk after inoculation and on 2-mo-old *P. patula* seedlings 2 and 4 mo after inoculation. Bars are means of three replicates; vertical lines represent standard error.

extremely susceptible to *F. s. pini*, and *F. oxysporum* also caused death of these germinated seeds. This confirms previous findings where *F. s. pini* (2,4) and *F. oxysporum* (7), commonly associated with pine seed, caused damping-off in pathogenicity tests (5,16).

Seedlings in the nursery under investigation tend to die after they have become well established. In contrast, results of this study indicate that contact of seedlings with *F. s. pini* during the early stages of seedling development usually results in death of all plants. We therefore assume that *F. s. pini* is not present or in contact with seedlings in nature at the time of planting. It is unlikely that the composted bark medium or the seeds themselves are the primary source of inoculum.

The ability of *F. oxysporum* to kill established pine seedlings may depend on isolate virulence differences within the species (8). Although all isolates of *F. s. pini* caused complete damping-off of *P. patula* seedlings, they differed in aggressiveness on established seedlings 2 and 4 mo after inoculation (Fig. 2). Bloomberg and Lock (8) suggested that a high inoculum dose might reduce pathogenic differences among strains of *F. oxysporum*. This situation might also be true for *F. s. pini* on *P. patula* seedlings.

Losses of pine seedlings in nurseries around the world, attributed to *F. oxysporum* in the past, might be caused by *F. s. pini*, since *F. s. pini* resembles *F. oxysporum* in culture on PDA and can be confused with this species (20). This is further supported by the fact that *F. s. pini* is known to be a seedborne pathogen (2,4) and has previously been isolated from seedlings (16,17).

F. subglutinans, like *F. oxysporum*, is able to cause diseases of numerous hosts (9). Correll et al (10) assigned strains of *F. subglutinans* pathogenic to pines to a specific forma specialis. They proposed that the "pitch canker" pathogen be designated *F. subglutinans* f. sp. *pini*. Pine pitch canker isolates, however, have also resulted in decay of gladiolus corms (3). Although the designation "forma specialis pini" is therefore perhaps not technically correct, we believe that isolates of *F. subglutinans* pathogenic to pine, including the isolates pathogenic to gladiolus, represent a distinct group within the species. Until this complication of the nomenclature has been sufficiently elucidated, we choose to follow the lead of Correll et al (10) and refer to *F. s. pini*. South African isolates of *F. subglutinans*, obtained from diseased roots of *P. patula* seedlings, were able to cause symptoms similar to those of pitch canker (11,15) on the stems of 3-yr-old *P. patula* trees. Accordingly, we believe that the local isolates represent *F. s. pini*.

Pitch canker causes severe damage to various pine species throughout the United States (10,12). Our study has indicated that *P. patula*, an exotic tree species that is widely cultivated throughout South Africa, is highly susceptible to infection by South African isolates of *F. s. pini*. Factors that might contribute or predispose trees to infections, such as wounds caused by hail and insects as well as drought (24), are often experienced locally. This fungus, therefore, has the potential to become one of the most serious pathogens of *P. patula* and possibly other *Pinus* spp. in South Africa.

Results of this study established that *F. s. pini* has the ability to cause devastating losses in pine nurseries. Inoculum in the nursery could originate from sources such as wind and irrigation (7) or from reused seedling containers (17). Various insects have also been found in the growing medium (*unpublished*), and these could play a role in inoculum spread. Insects, acting both as vectors and as wounding agents, have been demonstrated to contribute to disease spread in pines (11,14,24). The source of inoculum and conditions facilitating its buildup in the affected nursery deserve urgent attention. Effective programs to monitor the distribution of the fungus, comprehensive pathogenicity tests, and control strategies should be implemented to prevent serious losses to pine seedlings and trees in South Africa.

ACKNOWLEDGMENTS

We thank W. J. Swart and M. D. Fair for assisting with the statistical analysis and P. E. Nelson for providing isolates for comparative studies. We also acknowledge the financial support of the South African Forestry Industry, SAPPI Ltd., and the Foundation for Research Development.

LITERATURE CITED

1. Barnard, E. L., and Blakeslee, G. M. 1980. Pitch canker of slash pine seedlings: A new disease in forest tree nurseries. Plant Dis. 64:695-696.
2. Barrows-Broaddus, J. B. 1987. Pitch canker. Pages 42-49 in: Cone and Seed Diseases on North American Conifers. J. R. Sutherland, T. Miller, and R. S. Quinard, eds. North Am. For. Comm. Publ. 1.
3. Barrows-Broaddus, J., and Dwinell, L. D. 1980. Decay and colonization of gladiolus corms by the pine pitch canker fungus. Phytopathology 70:847-850.
4. Barrows-Broaddus, J., and Dwinell, L. D. 1985. Branch dieback and cone and seed infection caused by *Fusarium moniliforme* var. *subglutinans* in a loblolly pine seed orchard in South Carolina. Phytopathology 75:1104-1108.
5. Bloomberg, W. J. 1971. Diseases of Douglas-fir seedlings caused by *Fusarium oxysporum*. Phytopathology 61:467-470.
6. Bloomberg, W. J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. Phytopathology 66:1090-1092.
7. Bloomberg, W. J. 1981. Disease caused by *Fusarium* in forest nurseries. Pages 178-187 in: *Fusarium*: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park.
8. Bloomberg, W. J., and Lock, W. 1972. Strain differences in *Fusarium oxysporum* causing diseases of Douglas-fir seedlings. Phytopathology 62:481-485.
9. Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, England.
10. Correll, J. C., Gordon, T. R., McCain, A. H., Fox, J. W., Koehler, C. S., Wood, D. L., and Schultz, M. E. 1991. Pitch canker disease in California: Pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). Plant Dis. 75:676-682.
11. Dwinell, L. D., Barrows-Broaddus, J. B., and Kuhlman, E. G. 1985. Pitch canker: A disease complex of southern pines. Plant Dis. 69:270-276.
12. Dwinell, L. D., Kuhlman, E. G., and Blakeslee, G. M. 1981. Pitch canker of southern pines. Pages 188-194 in: *Fusarium*: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park.
13. Dwinell, L. D., and Phelps, W. R. 1977. Pitch canker of slash pine in Florida. J. For. 75:488-489.
14. Fox, J. W., Wood, D. L., and Koehler, C. S. 1991. Engraver beetles (Scolytidae: *Ips* species) as vectors of the pitch canker fungus, *Fusarium subglutinans*. Can. Entomol. 123:1355-1367.
15. Hepting, G. H., and Roth, E. R. 1946. Pitch canker, a new disease of some southern pines. J. For. 44:742-744.
16. Huang, J. W., and Kuhlman, E. G. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Dis. 74:27-30.
17. James, R. L., Dumroese, R. K., and Wenny, D. L. 1991. *Fusarium* diseases of conifer seedlings. Pages 181-190 in: Proc. 1st Meet. IUFRO Work. Party S2.07-09 (Diseases and insects in forest nurseries). J. R. Sutherland and S. G. Glover, eds. Victoria, BC, Canada.
18. Miller, T. 1984. Diseases of female strobili in southern pine seed orchards. Pages 15-20 in: Proc. Cone Seed Insect Work. Party Conf. H. O. Yates, ed. Ashville, NC.
19. Morgan, P. 1983. Fusarium stem rot of Douglas-fir seedlings. Plant Dis. 67:441-442.
20. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
21. Pawuk, W. H. 1978. Damping-off of container-grown longleaf pine seedlings by seedborne fusaria. Plant Dis. Rep. 62:82-84.
22. Peterson, G. W., and Smith, R. S. 1975. Forest Nursery Diseases in the United States. U.S. Dep. Agric. For. Serv. Handb. 470.
23. Rathbun, A. E. 1922. Root rot of pine seedlings. Phytopathology 12:213-220.
24. Schmidt, R. A., Wilkinson, R. C., Moses, C. S., and Broerman, F. S. 1976. Drought and weevils associated with severe incidence of pitch canker in Volusia County, Florida. Univ. Fla. Inst. Food Agric. Sci. Prog. Rep. 76-2.
25. Sutherland, J. R., and Van Eeden, E. 1980. Diseases and insect pests in British Columbia forest nurseries. B.C. Minist. For./Can. For. Serv. Joint Rep. 12.
26. 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.
27. Viljoen, A., Wingfield, M. J., and Crous, P. W. 1992. Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: A review. S. Afr. For. J. 161:45-51.