Fungi Associated with Damping-off of Slash Pine Seedlings in Georgia

J. W. HUANG, Graduate Student, Department of Plant Pathology, University of Georgia, and E. G. KUHLMAN, USDA Forest Service, Southeastern Forest Experiment Station, Athens, GA 30602

ABSTRACT

Huang, J. W., and Kuhlman, E. G. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Dis. 74:27-30.

A survey of fungi occurring in pine seeds, diseased pine seedlings, and pine bark mulch at southern pine nurseries identified 41 taxa in 23 genera. Pathogenicities of 35 representative isolates from 12 species or taxa of fungi were tested on slash pine seedlings. Isolates of *Fusarium moniliforme* var. *moniliforme, F. oxysporum, F. fusarioides, F. solani, Alternaria alternata, Rhizoctonia solani* AG-4, binucleate *Rhizoctonia*-like fungus CAG-3, *Pythium aphanidermatum, Penicillium expansum*, and *Cladosporium cladosporioides* caused preemergence damping-off. Isolates of *R. solani*, a binucleate *Rhizoctonia*-like fungus, and *P. aphanidermatum* also caused significant amounts of postemergence damping-off. *F. moniliforme* var. *subglutinans*, *F. m.* var. *moniliforme*, and *F. m.* var. *intermedium* initiated infections from seedborne inoculum to the cotyledons. *F. m.* var. *subglutinans* was the most virulent of these three varieties of *F. moniliforme*. In growth chambers at 20 and 30 C, *F. m.* var. *moniliforme*, and *F. m.* var. *intermedium* needed higher temperatures to cause infections. This is the first report of *F. m.* var. *intermedium* causing a plant disease.

Additional keywords: *Fusarium proliferatum*, loblolly pine

Forest tree nurseries in the South grew 418 million seedlings of loblolly pine (*Pinus taeda* L.) and slash pine (*P. elliottii* Engelm. var. *elliottii*) in 1980 (28). Each year in Georgia, 40 million extra seeds are planted to offset losses due to nonviable seeds and damping-off of seedlings. Many reports indicate that fungi play an important role in these two problems (9,20). However, little information is available on fungus species that cause damping-off of slash pine.

In general, damping-off fungi can be either seedborne or soilborne (5). *Fusarium* spp. have been recovered from the interior of southern pine seeds (2,4). Some seedborne *Fusarium* spp. have been recognized as important causal agents of damping-off of pine seedlings (5). Tint (26) indicated that certain species of *Fusarium* can reduce emergence of conifer seedlings. The role of the different taxa of the section Liseola have been inadequately evaluated. Whether pine bark used to mulch nursery beds can carry damping-off pathogens has not been studied.

Our study identified fungi isolated from pine seeds, diseased pine seedlings, and pine bark mulch, and tested their pathogenicity to slash pine seedlings.

This research was supported in part by a grant from the Georgia Forestry Commission. The first author was supported by a fellowship from the National Science Council of the Republic of China.

Accepted for publication 7 July 1989.

MATERIALS AND METHODS

Isolation and identification. From April to July 1987, seedlings with symptoms of damping-off, stunting, or chlorosis were collected from Clarke, Crawford, and Tattnall counties, Georgia. Pine bark mulch was also collected. Specimens were stored in an ice chest to prevent heating. Seeds of slash and loblolly pines were obtained from seed orchards and clone banks in Baldwin, Greene, and Houston counties, Georgia.

Seeds, diseased seedlings, and pine bark mulch were surface-sterilized with 1% sodium hypochlorite for 3 min, then placed on 2% water agar (WA), potato-dextrose agar with 300 μg/ml streptomycin sulfate (PDA+), and Nash-PCNB medium (21) to isolate fungi. A total of 2,200 seeds; 600 segments (approximately 0.6–0.8 cm) of cotyledon, stem, and root of diseased seedlings; and 600 pieces (approximately 1.2 × 0.5 cm) of pine bark mulch were plated out. Hyphal tips or single spores were taken from individual colonies on 2% WA, PDA+, and Nash-PCNB medium and grown on Czapek's agar, hemp seed medium, malt extract agar, potato-dextrose agar (PDA), V-8 medium (27), or 2% WA for identification (1,3,6,22,23). Anastomosis groups of *Rhizoctonia* were determined by pairing with tester isolates (BN-07 CAG-3 and Rhs-81 AG-4, provided by D. R. Summer) (17).

Pathogenicity tests. *Soil inoculation.* Soil collected from Clarke County was treated with methyl bromide (250 lb per acre) and aged in a greenhouse for 45 days before use. Inoculum was prepared in the different substrates and added to the soil at a rate of 150 g infested substrate per 1500 g soil. Each fungus was grown for 10 days in autoclaved plant material: raw potato chips for *Rhizoctonia*, eggplant chips for *Pythium*, celery stem segments for *Fusarium*, and oatmeal with 15% water content for *Alternaria, Cladosporium*, and *Penicillium*. Before the seeds were sown, the inoculum density of the fungi in the soil was determined with various selective media: Nash-PCNB medium for *Fusarium* (21), Ko and Hora's medium (18) with a multiple soil-pellet sampler method (13) for *Rhizoctonia*, Burr and Stanghellini's medium for *Pythium* (7), and PDA+ for *Alternaria, Cladosporium*, and *Penicillium*. In no case did the inoculum level exceed the following: 5×10^4 propagules per gram of soil for *Alternaria, Cladosporium, Penicillium*, or *Fusarium*; 60 propagules per gram of soil for *Pythium*; or 8 cfu per gram of soil for *Rhizoctonia*.

Viable seeds of the slash pine family Emanuel-5 were surface-sterilized in 1% sodium hypochlorite (with 0.001%

Table 1. Taxonomic designations and sources of representative fungi tested for their pathogenicity to slash pine seedlings

Taxonomic designation	Host/substrate	Origin[a]	Representative isolate numbers
Fusarium moniliforme	Pine bark mulch	3	BB-02
var. *moniliforme* Sheld.	Slash pine seed	1	CS-02, CS-03, FMM-01, FMM-04
(= *F. moniliforme* Sheld.)	Slash pine seedling	1	WH-05
F. m. var. *subglutinans* Wollenw. & Reink.	Slash pine seed	1	FMS-03
(= *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas)	Loblolly pine seed	1	FMS-05[b]
F. m. var. *intermedium* Neish & Leggett	Loblolly pine seedling	1	CS-01
(= *F. proliferatum* (Matsushima) Nirenberg)	Slash pine seed	1	FMI-05, FMI-06
F. oxysporum Schlecht.	Slash pine seedling	2	MN-01, MN-11
		3	VW-05
	Loblolly pine seedling	1	WH-01, WH-03
		2	MN-06
		3	VP-02
F. solani (Mart.) Sacc.	Slash pine seedling	2	MN-02, MN-03
		3	VW-04
F. fusarioides (Frag. & Cif.) Booth	Loblolly pine seedling	3	VS-01
Pythium aphanidermatum (Edson) Fitz.	Pine bark mulch	3	BB-09, BB-10
	Slash pine seedling	3	VS-01, VW-02, VW-06
Rhizoctonia solani Kühn AG-4	Pine bark mulch	3	BB-08
Rhizoctonia-like binucleate fungus CAG-3	Loblolly pine seedling	1	WH-10, WH-11
Alternaria alternata (Fr.) Keissler	Slash pine seedling	1	WH-09
		2	MN-09
Penicillium expansum Link	Slash pine seedling	1	WH-12
Cladosporium cladosporioides (Fres.) de Vries	Slash pine seedling	1	WH-13

[a]Numbers indicate the following Georgia counties: 1 = Clarke; 2 = Crawford; 3 = Tattnall.
[b]Provided by Jane Barrows-Broaddus.

Tween 80) for 15 min, rinsed in 2% H_2O_2 for 5 min, and washed twice in distilled water (J. Barrows-Broaddus, *unpublished*). They were then germinated in a petri dish with wet sterilized paper towels at 25 C. After 7 days, 10 seeds with hypocotyls approximately 3 mm in length were sown in a plastic flat (15 × 12 × 6.5 cm) of soil infested with each fungus. Control seedlings were planted in soil with noninfested substrate; most of these emerged 7 days after planting. The flats were maintained in the greenhouse at 25–30 C for 5 wk. The percentages of seedlings with preemergence and postemergence damping-off were recorded. The causal agents were confirmed by placing surface-sterilized, symptomatic segments of seedlings on 2% WA.

Table 2. Other fungi occurring in the seeds, diseased seedlings, and pine bark mulch of southern pines

Fungus	Seed		Seedling		Pine bark mulch
	Loblolly	Slash	Loblolly	Slash	
Alternaria sp.	+[a]	···	+	···	···
Aspergillus niger Tiegh.	+	+	···	···	···
A. terreus Thom.	···	+	···	···	···
Candida spp.	···	···	···	···	+
Cephalosporium spp.	+	···	···	+	···
Ceratocyystis spp.	+	···	···	···	+
Chaetomium spp.	+	···	···	···	···
Cylindrocarpon spp.	···	···	···	+	···
Diplodia spp.	···	+	···	···	···
Dothiorella spp.	···	+	···	+	···
Fusarium equiseti (Corda) Sacc.	+	···	+	···	+
F. semitectum Berk & Rav.	+	+	+	+	+
F. ventricosum Appel & Wollenw.	···	···	···	+	···
Monilia spp.	···	+	···	···	···
Mucor spp.	···	+	···	···	+
Nigrospora spp.	···	+	···	···	···
Penicillium aculeatum Raper & Fennell	+	···	+	···	+
	+	···	+	···	+
P. boytryosum Batista & Maia	···	···	···	+	···
P. nigricans Bainier & Thom	+	···	···	···	···
Penicillium spp.	···	···	+	···	+
Pestalotia flavidula Tassi	···	+	···	···	···
Pestalotia spp.	+	···	···	+	+
Phoma eupyrena Sacc.	···	···	+	···	+
Rhizopus spp.	+	+	···	···	···
Sphaeropsis spp.	+	+	···	···	+
Stilbum spp.	···	···	···	···	+
Trichoderma lignorum (Tode) Harz.	···	+	···	···	+
T. viride Pers. ex Fries	···	···	···	···	+
Trichoderma spp.	···	···	+	···	+

[a]+ = Fungus was isolated from the host/substrate in this study.

Seed inoculation. Seeds of slash pine family Emanuel-5 were sterilized using the 1% sodium hypochlorite–2% H_2O_2 method. Two isolates each of three varieties of *F. moniliforme* (Table 1) were cultured on PDA slants at 25 C for 2 wk under cool-white fluorescent lights (12-hr photoperiod). Germinating seeds were soaked in a conidial suspension (10^6 spores per ml) of one of the fungi or in distilled water only. After air-drying for 3 hr, 10 seeds treated with each isolate were planted in fumigated soil in a plastic flat. The seedlings were then transferred to a growth chamber and grown under cool-white fluorescent lights (12-hr photoperiod) at 20 and 30 C for 5 wk. Each week dead seedlings (combined preemergence and postemergence damping-off) were recorded and the presence of the pathogen was confirmed.

Data analysis. Each treatment was replicated four times, and all experiments were repeated at least once. Statistical

analyses were run on the SAS/STAT System for Personal Computers (SAS Institute Inc., Cary, NC). We used Tukey's Studentized range (HSD) test for comparisons of pathogenicity among the fungi, and PROC GLM for interaction between the fungi and growth chambers (20 and 30 C).

RESULTS AND DISCUSSION

In this study, 41 taxa in 23 genera of fungi were isolated and identified from pine seeds, diseased seedlings, and pine bark mulch (Tables 1 and 2). We determined pathogenicities on slash pine seedlings for 35 isolates from 12 taxa with high recovery frequencies (Table 1). In previous records, *Pythium, Rhizoctonia,* and *Fusarium* were major pathogens in damping-off of conifer seedlings in southern forest nurseries (12,16). Our data on slash pine seedlings confirm those observations (Table 3 and Fig. 1). Soil inoculations with isolates of *F. m.* var. *moniliforme* (= *F. moniliforme*), *F. oxysporum, F. fusarioides, F. solani, Alternaria alternata, R. solani* AG-4, binucleate *Rhizoctonia*-like fungus CAG-3, *P. aphanidermatum, Penicillium expansum,* and *C. cladosporioides* caused preemergence damping-off. Isolates of *R. solani* AG-4, binucleate *Rhizoctonia*-like fungus CAG-3, and *P. aphanidermatum* also caused significant amounts of postemergence damping-off without cotyledonary infections (Table 3). With seed inoculations, *F. m.* var. *subglutinans* (= *F. subglutinans*), *F. m.* var. *moniliforme*, and *F. m.* var. *intermedium* (= *F. proliferatum*) caused both preemergence and postemergence damping-off with 80% incidence of cotyledonary infections (Fig. 1).

Various species of *Pythium* have been shown to occur frequently in forest nurseries (29). *P. irregulare* and *P. aphanidermatum* have been reported to be highly pathogenic to seedlings of shortleaf and loblolly pine but much less so to slash pine (11). In our study, isolates of *P. aphanidermatum* from slash pine seedlings with damping-off and from pine bark mulch were virulent pathogens of slash pine seedlings (Table 3).

Rhizoctonia spp. have been described as nonspecialized and highly virulent pathogens (24,25). A binucleate *Rhizoctonia*-like fungus CAG-3 caused blight of longleaf pine seedlings in nurseries in Florida (8). *R. solani* AG-4 from pine bark mulch and a binucleate *Rhizoctonia*-like fungus CAG-3 from a loblolly pine seedling were highly pathogenic to slash pine seedlings (17). Our source of *R. solani* AG-4 was a storage pile of pine bark near nursery beds of the Reidsville nursery in Tattnall County, Georgia. We also isolated *R. solani* AG-4 from pine bark mulch from a nursery bed with damped-off slash pine seedlings. Apparently, *R. solani* may be disseminated when nursery operators

Table 3. Preemergence and postemergence damping-off of slash pine seedlings grown in soil infested with pure cultures of fungi isolated from seeds and seedlings of southern pine and pine bark mulch

Name	Isolate	Preemergence damping-off (%)	Postemergence damping-off (%)
Fusarium moniliforme var. *moniliforme*	BB-02	30 cdefghi[z]	0 e
	CS-02	45 abcde	0 e
	CS-03	50 abc	0 e
	WH-05	23 efghij	5 de
F. m. var. *intermedium*	CS-01	15 ghij	0 e
F. oxysporum	MN-01	63 a	0 e
	MN-06	13 hij	5 de
	MN-11	58 ab	0 e
	VW-05	13 hij	0 e
	VP-02	20 fghij	0 e
	WH-01	50 abc	0 e
	WH-03	25 defghij	3 de
F. solani	MN-02	43 abcdef	0 e
	MN-03	35 bcdefgh	0 e
	VW-04	18 ghij	5 de
F. fusarioides	VS-01	35 bcdefgh	0 e
Pythium aphanidermatum	BB-09	65 a	35 b
	BB-10	65 a	10 de
	VW-01	65 a	18 cd
	VW-02	48 abcd	43 ab
	VW-06	65 a	33 bc
	VWB-01	55 ab	38 ab
Rhizoctonia solani AG-4	BB-08	63 a	37 ab
Binucleate *Rhizoctonia*-like fungus CAG-3	WH-10	48 abcd	52 a
	WH-11	25 defghij	53 a
Alternaria alternata	MN-09	50 abc	18 cd
	WH-09	38 cde	3 de
Penicillium expansum	WH-12	35 bcdefg	0 e
Cladosporium cladosporiodes	WH-13	45 abcde	0 e
Control None	...	10 ij	0 e
Control Celery stem	...	8 ij	0 e
Control Oatmeal	...	5 j	0 e
Control Potato chip	...	10 ij	3 de
Control Eggplant chip	...	5 j	0 e

[z]Within columns, data followed by the same letter do not differ significantly at $P = 0.05$ according to Tukey's Studentized range (HSD) test. Data were recorded for 35 days after planting.

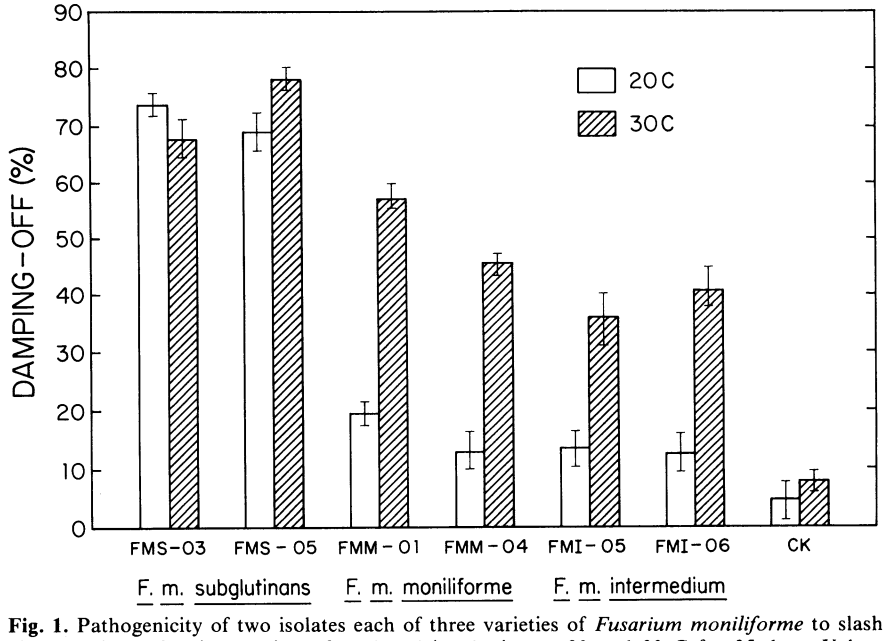

Fig. 1. Pathogenicity of two isolates each of three varieties of *Fusarium moniliforme* to slash pine seedlings after inoculation of seed and incubation at 20 and 30 C for 35 days. Values are the means of four replications and indicate significant ($F = 7.05$, $P = 0.01$) interactions between isolates of the fungi and growth chambers (temperatures). Bars represent ± one standard deviation.

mulch seedbeds with pine bark infected with *R. solani* after seedbed soil is fumigated with methyl bromide.

Fusarium spp. were a major group of fungi isolated from healthy and diseased seedlings (5). Hodges (14) proved that a combination of *F. oxysporum* and *Sclerotium bataticola* Taub could cause black root rot of pine at high temperatures. In our experiment, *F. oxysporum, F. solani*, and *F. fusarioides* caused preemergence damping-off of slash pine but did not cause postemergence damping-off (Table 3). Isolates of *F. oxysporum* varied in virulence from nonpathogenic to highly pathogenic.

In recent years, varieties of *F. moniliforme* (anamorphs of *Gibberella fujikuroi*) were described (19). We frequently isolated *F. m.* var. *subglutinans*, *F. m.* var. *moniliforme*, and *F. m.* var. *intermedium* from pine seeds and seedlings. Isolates of these three varieties could move from the unshed seedcoat inoculated with a spore suspension to colonize cotyledons and cause seedling death (Fig. 1). Our isolates of *F. m.* var. *subglutinans* had the highest virulence to slash pine of these three varieties of *F. moniliforme*. Figure 1 shows that *F. m.* var *subglutinans* caused damping-off at 20 and 30 C, while *F. m.* var. *moniliforme* and *F. m.* var. *intermedium* incited damping-off only at 30 C. Apparently *F. m.* var. *moniliforme* and *F. m.* var. *intermedium* need a higher temperature to initiate cotyledonary infection of slash pine. Although *F. m.* var. *intermedium* has been reported to be toxigenic to animals (22), its pathogenicity on plants is first recorded in this study.

Hodges (15) found that species of *Aspergillus* and *Penicillium* make up almost 50% of the fungi isolated from southern forest tree nursery soils, and that *Penicillium restrictum* Gilman and Abbott (= *Penicillium expansum*) is very abundant in Georgia. In East Africa, the saprophytic fungi *Aspergillus, Mucor, Rhizopus, Trichoderma*, and *Trichothecium* on the seedcoats of *Pinus patula* invade tissues of germinating seed and kill the seedlings under favorable conditions (10). Farag et al. (9) indicated that *C. cladosporioides* can cause significant preemergence losses in *Cupressus sempervirens*. Likewise, we found that *A. alternata, C. cladosporioides*, and *Penicillium expansum* can cause significant preemergence damping-off of slash pine.

In our study, we also recovered *Diplodia, Dothiorella, Phoma, Sphaeropsis, Pestalotia*, and *Ceratocystis* from pine bark and pine seeds (Table 2). Because these fungi are not associated with damping-off of pine seedlings, we did not study their pathogenicity.

LITERATURE CITED

1. Ainsworth, G. C., Sparrow, F. K., and Sussman, A. S., eds. 1973. The Fungi: An Advanced Treatise. Vol. 4A. A Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecti. 621 pp.; Vol. 4B. A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi. 504 pp. Academic Press, New York.
2. Anderson, R. L., Belcher, E., and Miller, T. 1984. Occurrence of seed fungi inside slash pine seeds produced in seed orchards in the United States. Seed Sci. Technol. 12:797-799.
3. Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams & Wilkins Co., Baltimore. 364 pp.
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. 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.
6. Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.
7. Burr, T. J., and Stanghellini, M. E. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soil. Phytopathology 63:1499-1501.
8. English, J. T., Ploetz, R. C., and Barnard, E. L. 1986. Seedling blight of longleaf pine caused by a binucleate *Rhizoctonia solani*-like fungus. Plant Dis. 70:148-150.
9. Farag, S. A., Shehata, M. R. A., and Omran, T. A. 1977. Studies on damping-off of *Cupressus sempervirens* seedlings in Egypt. I. Seed borne fungi associated with damping-off cypress seedlings. Alexandria J. Agric. Res. 25:319-322.
10. Gibson, I. A. S. 1957. Saprophytic fungi as destroyers of germinating pine seed. East Afr. Agric. J. 22:203-206.
11. Hendrix, F. F., Jr., and Campbell, W. A. 1968. Pythiaceous fungi isolated from southern forest nursery soils and their pathogenicity to pine seedlings. For. Sci. 14:292-294.
12. Hendrix, F. F., Jr., and Campbell, W. A. 1985. Tree Diseases: Recognition—Impact—Management. University of Georgia Press, Athens, GA. 81 pp.
13. Henis, Y., Ghaffar, A., Baker, R., and Gillespie, S. L. 1978. A new pellet soil-sampler and its use for the study of population dynamics of *Rhizoctonia solani* in soil. Phytopathology 68:371-376.
14. Hodges, C. S., Jr. 1959. Studies on black root rot of pine seedlings. Ph.D. dissertation. University of Georgia, Athens. 120 pp.
15. Hodges, C. S., Jr. 1962. Fungi isolated from southern forest tree nursery soils. Mycologia 54:221-229.
16. Hodges, C. S., Jr., and Ruehle, J. L. 1969. Nursery diseases of southern pines. U.S. Dept. Agric. For. Serv., For. Pest Leafl. 32:1-8.
17. Huang, J. W., and Kuhlman, E. G. 1989. Recovery and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia*-like fungi in forest nurseries. Plant Dis. 73: 968-972.
18. Ko, W. H., and Hora, F. K. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61:707-710.
19. Kuhlman, E. G. 1982. Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section Liseola. Mycologia 75:759-768.
20. Munjal, R. L. 1976. Effect of seed mycoflora on pre-emergence and post-emergence seedling rots of some important conifers in the Himachal Pradesh. Indian J. Mycol. Plant Pathol. 6:27-31.
21. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
22. Nelson, P. E., Toussoun, T. A., and Marsas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park. 193 pp.
23. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
24. Saksena, H. K., and Vaartaja, O. 1960. Descriptions of new species of *Rhizoctonia*. Can. J. Bot. 38:931-943.
25. Saksena, H. K., and Vaartaja, O. 1961. Taxonomy, morphology, and pathogenicity of *Rhizoctonia* species from forest nurseries. Can. J. Bot. 39:627-647.
26. Tint, H. 1945. Studies in the *Fusarium* damping-off of conifers. I. The comparative virulence of certain *Fusaria*. Phytopathology 35:421-439.
27. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Purdue University Press, Lafayette, IN. 239 pp.
28. U.S. Department of Agriculture Forest Service. 1980. Forest planting, seeding and silvical treatments in the United States 1980 report, FS-368. 15 pp.
29. Vaartaja, O., and Bumbieris, M. 1964. Abundance of *Pythium* species in nursery soils in South Australia. Aust. J. Biol. Sci. 17:436-445.