Control of Fungi Associated with Cankers of Greenhouse Roses

L. E. SWEETS, Assistant Professor, Department of Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames 50011, F. L. PFLEGER, Associate Professor, F. C. MORGAN, Assistant Extension Specialist, Department of Plant Pathology, University of Minnesota, St. Paul 55108, and J. R. MIZICKO, Plant Pathologist, Niagara Seed Operation of Moran Seed, Inc., El Macero, CA 95618

ABSTRACT

Sweets, L. E., Pfleger, F. L., Morgan, F. C., and Mizicko, J. R. 1982. Control of fungi associated with cankers of greenhouse roses. Plant Disease 66:491-494.

Of six fungi isolated from rose canes, *Botryodiplodia theobromae, Botrytis cinerea, Coniothyrium fuckelii,* and *Trichothecium roseum* proved to be pathogenic on the rose cultivars Belinda and Golden Fantasy. Inoculations were made by pinning colonized wheat seed to recently pruned rose canes, wrapping the inoculated canes with a wet paper towel, and covering them with a plastic bag. *Alternaria alternata* and *Pestalotia palmarum* were not pathogenic on either cultivar when inoculated using the same procedure. Benomyl, applied as a weekly spray beginning 3 wk after inoculation, reduced canker development caused by *Botyrodiplodia theobromae, Botrytis cinerea, C. fuckelii,* and *T. roseum* on Golden Fantasy, whereas on Belinda benomyl was only effective against *T. roseum*. Chlorothalonil, applied as a weekly spray beginning 3 wk after inoculation, reduced canker development caused by *Botrytis cinerea* and *T. roseum* on Golden Fantasy but only reduced canker development caused by *T. roseum* on Belinda. This is the first report of either *Botryodiplodia theobromae* or *T. roseum* on roses.

During 1976 and 1977, isolations were made from roses with cane cankers and cane dieback obtained from commercial greenhouses. Fungi isolated from these canes included *Alternaria alternata* (Fr.) Keissler, *Botryodiplodia theobromae* Pat., *Botrytis cinerea* Pers. ex Fr., *Coniothyrium fuckelii* Sacc., *Pestalotia palmarum* (Cooke), and *Trichothecium roseum* (Pers.) Link ex. S. F. Gray.

Reports of *Alternaria* spp. on roses are limited primarily to bud blights or leaf spots. An *Alternaria* sp. isolated from chrysanthemum and from King aster has been shown to be pathogenic to buds of Tropicana and Red American Beauty roses, and symptoms similar to those produced experimentally have been observed in field-grown roses (2). *A. circinans, A. brassicae, A. brassicae* var. *microspora,* and *Alternaria* sp. have been reported to cause leaf spots of cultivated roses (6). A *Botryodiplodia* sp. has been isolated from dry rose canes in Brazil (4). The pathogenicity of the isolate was demonstrated by wounding and inoculating canes of the rose cultivars Super Star and Happiness. *Botrytis cinerea* causes bud and twig blight of roses (1,7). *C. fuckelii* causes stem canker or common canker of roses and is commonly associated with this plant (6,8,9). Several *Pestalotia* spp. have been reported to be associated with leaf, stem, and bud necrosis on roses (3,6).

The purpose of this study was to determine the pathogenicity of the six

Paper 11,506, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 55108.

Accepted for publication 30 August 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Table 1. Mean canker length on rose canes inoculated with one of six fungi

Fungus	Canker length (mm)[a,b]	
	Belinda	Golden Fantasy
Control	1.1	0
Alternaria alternata	2.0 NS	2.1*
Botryodiplodia theobromae	44.7*	54.5*
Botrytis cinerea	22.8*	54.0*
Coniothyrium fuckelii	21.9*	108.7*
Pestalotia palmarum	3.3*	4.4*
Trichothecium roseum	28.3*	54.9*

[a] Mean canker length based on measurements from nine canes 3 mo after inoculation.

[b] Values represent results of *t*-test comparison between each fungus and the control for each cultivar. NS = not significant, * = significant at the 5% level.

fungi isolated from rose canes and to test the effectiveness of certain fungicides against those pathogenic fungi.

MATERIALS AND METHODS

Isolates of *A. alternata, Botryodiplodia theobromae, Botrytis cinerea, C. fuckelii, P. palmarum,* and *T. roseum* used in these experiments were obtained from rose canes collected from commercial greenhouses in the St. Paul, MN, area during the winter of 1976–1977. Isolates were maintained in pure culture on Difco potato-dextrose agar (PDA) plates or in tubes containing soil.

Pathogenicity tests. Erlenmeyer flasks containing 100 g of wheat seed and 50 ml of distilled water were autoclaved twice at 24-hr intervals for 30 min. Mycelial plugs 5 mm in diameter were taken from PDA plates of each of the six fungi and transferred to the flasks. Flasks were incubated at 22–25 C for 2 wk.

The rose cultivars Belinda and Golden Fantasy were used for pathogenicity tests. Five young canes per plant on seven plants of each cultivar were cut back with disinfected pruning shears to an internode, leaving a stub about 50 mm long. One kernel of infested grain was impaled on a T-pin, and the pin was inserted into the cut end of the cane until the kernel was in contact with the cut surface. One fungus was used to inoculate the five pruned canes on one plant of each cultivar. A similar procedure was used for the remaining five fungi. A pin with no inoculum was inserted into the cut ends of the pruned canes on the control plant for each cultivar. A wet paper towel was wrapped around the cane just below the cut surface. The cane, inoculum, and paper towel were then covered with a plastic bag. The 14 plants (one control and six inoculated plants of each cultivar) were returned to the greenhouse bench and kept at 24 C. The pathogenicity test was repeated once.

During the first test, one of the five plastic bags on each of the 14 plants was removed 3 wk after inoculation, and the canes were examined for canker development (Fig. 1). The remaining four plastic bags on each plant were removed 3 mo after inoculation. Observations and measurements of canker size were made on each cane. Isolations were made from all inoculated canes to confirm the presence of the fungus originally used to inoculate the canes. In the second test, all five plastic bags on each of the 14 plants were removed 3 mo after inoculation, and canker length on each cane was measured.

In vitro fungicide test. The four commercial fungicides used were mancozeb (zinc ion and manganese ethylene bisdithiocarbamate; Manzate 200, 80W); benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; Benlate 50 W]; cupric hydroxide (Kocide 86 W); and chlorothalonil (tetrachloroisophthalonitrile; Bravo 75 W). Each fungicide was suspended in 1-L volumes of sterile PDA at concentrations of 0, 100, 300, and 500 μg/ml. Mycelial plugs 5 mm in diameter were aseptically transferred from the periphery of PDA plates containing actively growing colonies of the six fungi to PDA plates amended with the four chemicals. Linear mycelial growth of the six fungi was measured and recorded every second day. The experiment had five replicates.

In vivo fungicide test. The rose cultivars Belinda and Golden Fantasy and the fungal isolates *Botryodiplodia theobromae, Botrytis cinerea, C. fuckelii,* and *T. roseum* were used in the in vivo fungicide test. Twelve plants of each cultivar were inoculated by the previously described inoculation procedure. Each fungal isolate was used to inoculate five canes on three plants of each cultivar. After inoculation, plants were returned to a greenhouse bench. After 3 wk, all of the plastic bags were removed, observations and measurements of canker size on each cane were made, and fungicide sprays were applied.

One plant per cultivar per isolate (total of eight plants) was sprayed with benomyl at the rate of 600 mg/L of water. Similarly, one plant per cultivar per isolate (total of eight plants) was sprayed with chlorothalonil at the rate of 900

Table 2. Effect of five doses of four fungicides on linear mycelial growth on each of six fungi on potato-dextrose agar

Fungicide / Fungus	Mycelial growth (mm)[y] at dose (μg/ml) 0	10	100	300	500
Benomyl					
Alternaria alternata	30.0 a[z]	32.3 a	24.7 ab	21.7 ab	17.0 b
Botryodiplodia theobromae	63.0 a	3.0 b	0 b	0 b	0 b
Botrytis cinerea	49.7 a	50.7 a	40.3 a	18.0 b	10.0 b
Coniothyrium fuckelii	16.0 a	0 a	0 a	0 a	0 a
Pestalotia palmarum	48.7 a	0 b	0 b	0 b	0 b
Trichothecium roseum	54.3 a	0 b	0 b	0 b	0 b
Chlorothalonil					
A. alternata	30.0 a	28.3 a	27.0 a	23.7 a	23.7 a
Botryodiplodia theobromae	63.0 a	25.3 b	15.7 b	24.7 b	0 c
Botrytis cinerea	49.7 a	18.3 b	18.3 b	6.0 b	12.0 b
C. fuckelii	16.0 a	0 a	0 a	0 a	0 a
P. palmarum	48.7 a	33.0 ab	27.3 b	18.0 b	21.7 b
T. roseum	54.7 a	50.3 a	42.7 ab	36.7 b	34.0 b
Cupric hydroxide					
A. alternata	30.0 a	34.0 a	30.0 a	19.0 ab	7.7 b
Botryodiplodia theobromae	63.0 a	66.3 a	53.0 a	51.7 a	35.7 b
Botrytis cinerea	49.7 a	42.3 a	17.7 b	11.3 b	10.3 b
C. fuckelii	16.0 a	16.0 a	15.0 a	12.7 a	9.0 a
P. palmarum	48.7 a	49.3 a	31.3 b	10.0 c	8.3 c
T. roseum	54.7 a	49.3 a	40.0 a	1.7 b	16.3 b
Mancozeb					
A. alternata	30.0 a	30.7 a	16.0 ab	8.0 b	11.7 b
Botryodiploida theobromae	63.0 a	31.0 b	6.3 c	0 c	3.3 c
Botrytis cinerea	49.7 a	53.3 a	55.0 a	56.7 a	52.3 a
C. fuckelii	16.0 a	5.3 a	3.7 a	0 a	0 a
P. palmarum	48.7 a	50.3 a	48.0 a	16.7 b	22.0 b
T. roseum	54.7 a	51.0 a	33.3 b	23.7 b	23.0 b

[y] Means of five observations collected on each of three dates.
[z] Numbers followed by the same letter in a row are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

Table 3. Effect of benomyl and chlorothalonil on canker development caused by four rose-cane pathogens on the cultivars Belinda and Golden Fantasy

	Canker length (mm)[y]					
	Control		Benomyl		Chlorothalonil	
Fungus	Belinda	Golden Fantasy	Belinda	Golden Fantasy	Belinda	Golden Fantasy
Botryodiplodia theobromae	35.8 b[z]	56.3 a	26.6 bc	24.6 c	34.4 b	52.8 a
Botrytis cinerea	27.9 c	64.4 a	29.5 c	43.7 b	49.3 b	48.7 b
Coniothyrium fuckelii	13.7 bc	44.8 a	11.9 c	15.4 bc	18.8 b	44.7 a
Trichothecium roseum	40.4 b	59.3 a	20.3 cd	29.9 bc	12.5 d	26.7 c

[y] Based on measurements at 1, 3, and 6 wk. Values for *Botryodiplodia theobromae* are means of 12 measurements; those for other fungi are means of 15 measurements.
[z] For each fungus, means followed by the same letters in a row are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

mg/L of water. Both fungicides were applied weekly for 8 wk. The remaining set of eight inoculated plants was not treated with any fungicide. Canker development and dieback on all plants were measured weekly. After three fungicide applications, one cane from each plant was removed and pieces cultured on PDA to determine if the pathogen originally used for inoculation could still be recovered from the plants.

RESULTS

Pathogenicity test. The mean length of cankers on nine canes of Belinda or Golden Fantasy roses 3 mo after inoculation with each of the six rose cane fungi is given in Table 1. On both cultivars, only the initial 2 mm of the canes inoculated with *A. alternata* was affected, and attempts to reisolate the fungus from the affected areas were unsuccessful. *P. palmarum* did not develop beyond 3–4 mm into the cane tip in either cultivar. However, the fungus was reisolated from three of nine of the Belinda inoculated canes and two of nine of the Golden Fantasy canes. *Botryodiplodia theobromae, Botrytis cinerea, C. fuckelii,* and *T. roseum* all grew into the canes from the original point of inoculation, and these four fungi were reisolated from all inoculated canes on both cultivars.

In vitro fungicide test. Benomyl-amended PDA suppressed mycelial growth of *Botryodiplodia theobromae, P. palmarum,* and *T. roseum* at doses of 10 μg/ml; *A. alternata* and *Botrytis cinerea* were inhibited at doses of 500 and 300 μg/ml, respectively. *C. fuckelii* was determined statistically to be unaffected at any dose of benomyl (Table 2).

Chlorothalonil-amended PDA inhibited mycelial growth of *B. cinerea* and *Botryodiplodia theobromae* at doses of 10 μg/ml, whereas *P. palmarum* and *T. roseum* mycelial growth was suppressed at doses of 100 and 300 μg/ml, respectively. At the tested doses of chlorothalonil, the mycelial growth rates

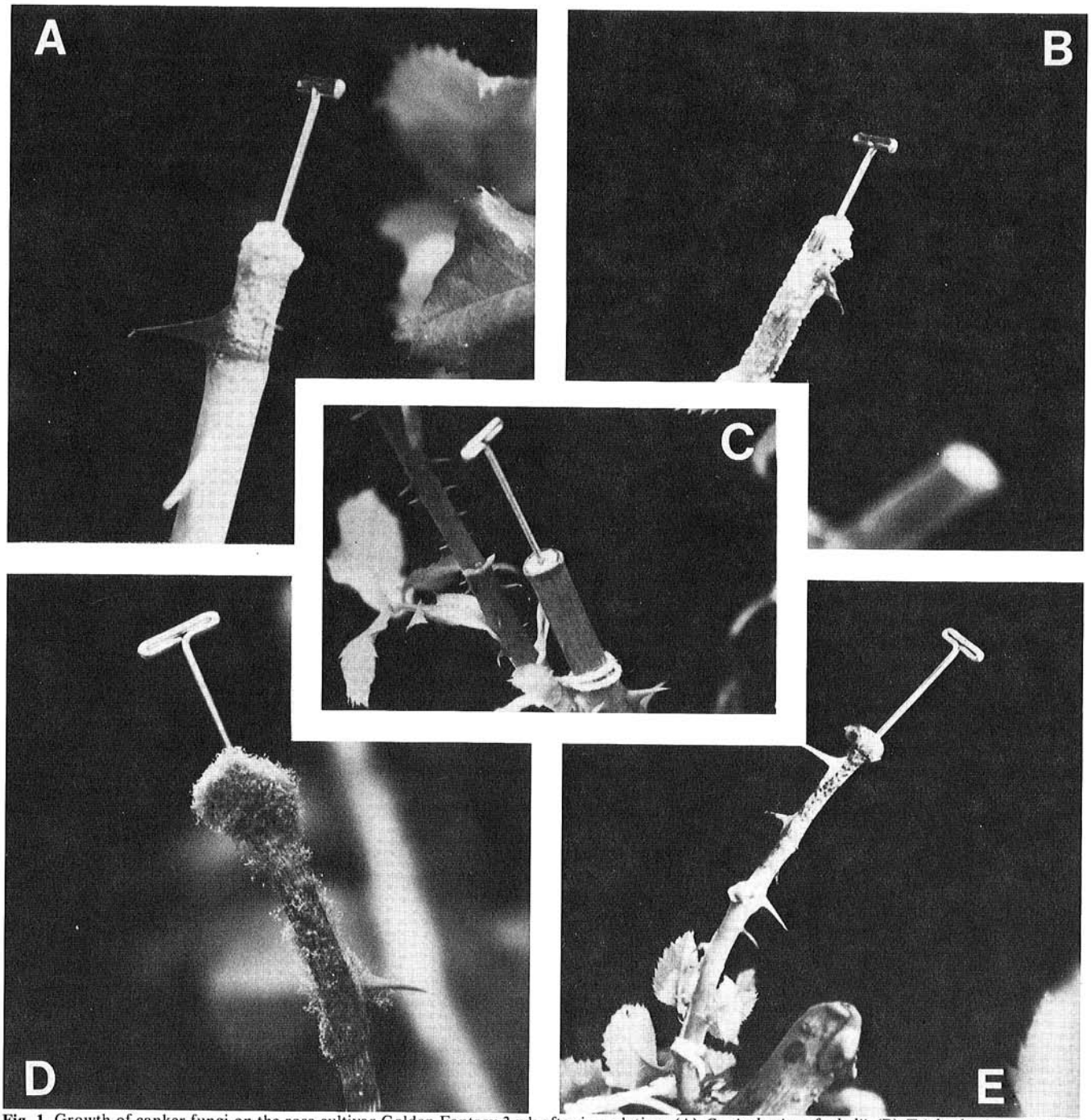

Fig. 1. Growth of canker fungi on the rose cultivar Golden Fantasy 3 wk after inoculation: **(A)** *Coniothyrium fuckelii,* **(B)** *Trichothecium roseum,* **(C)** control, **(D)** *Botrytis cinerea*, and **(E)** *Botryodiplodia theobromae.*

Table 4. Effect of three weekly applications of benomyl or chlorothalonil on recovery of test fungi from inoculated canes

	Recovery of test fungus per treatment (%)					
	Control		Benomyl		Chlorothalonil	
Fungus	Belinda	Golden Fantasy	Belinda	Golden Fantasy	Belinda	Golden Fantasy
Botryodiplodia theobromae	100	100	100	100	94	100
Botrytis cinerea	100	81	62	6	69	17
Coniothyrium fuckelii	100	100	100	100	100	67
Trichothecium roseum	75	100	62	50	58	58

of *A. alternata* and *C. fuckelii* were not significantly different from the control (Table 2).

PDA amended with Kocide inhibited *Botrytis cinerea* and *P. palmarum* mycelial growth at doses of 100 μg/ml. Mycelial growth of *A. alternata, Botryodiplodia theobromae,* and *T. roseum* was restricted at doses of 500, 500, and 300 μg/ml, respectively. Mycelial growth of *C. fuckelii* was not suppressed by Kocide at any dose (Table 2).

PDA amended with Manzate 200 suppressed mycelial growth of *Botryodiplodia theobromae* at 10 μg/ml, whereas it inhibited *A. alternata, P. palmarum,* and *T. roseum* at doses of 300, 300, and 100 μg/ml, respectively. Mycelial growth of *Botrytis cinerea* and *C. fuckelii* was determined statistically to be unaffected by any dose of Manzate 200 (Table 2).

In vivo fungicide test. Developing cankers on all plants were measured weekly just before spraying. Mean length of cankers based on measurements at 1, 3, and 6 wk is given in Table 3. For all four fungi, the mean canker length on Golden Fantasy plants sprayed with benomyl was not statistically different than the unsprayed controls for *Botryodiplodia theobromae, Botrytis cinerea,* or *C. fuckelii.* However, the mean canker length on Belinda inoculated with *T. roseum* and sprayed with benomyl was significantly less than on the unsprayed control. Chlorothalonil applied to Golden Fantasy resulted in less canker growth caused by *Botrytis cinerea* and *T. roseum* than on the control but was not effective in controlling *Botryodiplodia theobromae* or *C. fuckelii.* When chlorothalonil was applied to Belinda, canker size caused by *T. roseum* was less than the control, cankers caused by *Botryodiplodia theobromae* and *C. fuckelii* were not statistically different from the control, whereas canker size caused by *Botrytis cinerea* was greater than that of the control (Table 3).

Botryodiplodia theobromae and *C. fuckelii* were isolated from 100% of the canes originally inoculated with either of these fungi except from chlorothalonil-treated Belinda and Golden Fantasy plants. *Botrytis cinerea* was isolated from 100% of the unsprayed *Botrytis*-inoculated Belinda canes and from a majority of unsprayed, *Botrytis*-inoculated Golden Fantasy canes and sprayed, *Botrytis*-inoculated Belinda canes. However, *Botrytis cinerea* was isolated from only 6% of benomyl-sprayed, *Botrytis*-inoculated Golden Fantasy canes and 17% of chlorothalonil-sprayed, *Botrytis*-inoculated Golden Fantasy canes. *T. roseum* was isolated from 100 and 75% of the unsprayed, *Trichothecium*-inoculated Golden Fantasy and Belinda canes, respectively. Isolations from Belinda and Golden Fantasy canes inoculated with *T. roseum* and sprayed with chlorothalonil or benomyl resulted in 50–60% recovery (Table 4).

DISCUSSION

Attempts to inoculate fresh pruning cuts with *A. alternata* and *P. palmarum* were unsuccessful. However, these fungi can colonize dried rose tissue already infected by other fungi.

Under the high-humidity conditions of the pathogenicity test, *Botryodiplodia theobromae* invaded inoculated canes of both cultivars. The pathogen was not limited by nodes and moved into the main stems on plants of both cultivars. *Botrytis cinerea* usually causes bud and shoot blights of roses, and *C. fuckelii,* the cause of common canker on roses, is usually considered a weak wound parasite (1,7–9). *T. roseum* is usually found as a saprophyte on decaying plant tissue or as a secondary parasite on apple fruit and has been reported from soil (5). However, with the inoculation procedure used in the pathogenicity test, it was found that *Botrytis cinerea, C. fuckelii,* and *T. roseum* invaded inoculated canes of both rose cultivars, but with these fungi there was a difference in susceptibility of the cultivars. On Belinda, these three fungi invaded the stubs but were usually limited by the first node and seldom reached the main stem of the plant; on Golden Fantasy, they caused extensive cane dieback and occasionally plant death. None of the fungicides applied to inoculated canes of Belinda or Golden Fantasy caused a complete halt in canker development. This represents the first report of either *Botryodiplodia theobromae* or *T. roseum* on roses.

ACKNOWLEDGMENTS

We thank E. L. Stewart, Mycologist, Department of Plant Pathology, University of Minnesota, for assistance in identification of *Alternaria alternata* (Fr.) Keissler and *Pestalotia palmarum* (Cooke).

LITERATURE CITED

1. Deacon, G. E. 1933. Some effects of *Botrytis cinerea* on roses. Trans. Br. Mycol. Soc. 17:331-333.
2. Engelhard, A. W. 1980. Alternaria pathogenic on blooms of roses, chrysanthemums, and King asters. (Abstr.) Phytopathology 60:1539.
3. Guba, E. F. 1961. Monograph of *Monochaetia* and *Pestalotia.* Harvard University Press, Cambridge, MA. 342 pp.
4. Pitta, G. P. B., and Teranishi, J. 1973. *Phomopsis* e' *Botryodiplodia* associados à seca de roseiras. O Biológico 39:21-23.
5. Rifai, M. A., and Cooke, R. C. 1966. Studies on some didymosporous genera of nematode-trapping hyphomycetes. Trans. Br. Mycol. Soc. 49:147-168.
6. U.S. Department of Agriculture. 1960. Index of Plant Diseases in the United States. Handb. 165. 531 pp.
7. Waterman, A. M. 1928. Rose diseases: Their causes and control. U.S. Dep. Agric. Farm. Bull. 1547. 19 pp.
8. Waterman, A. M. 1930. Diseases of rose caused by species of *Coniothyrium* in the United States. J. Agric. Res. 40:805-827.
9. Westcott, C. 1934. Brand canker of rose, caused by *Coniothyrium wernsdorffiae* Laubert. N.Y. Agric. Exp. Stn. Ithaca Mem. 153. 39 pp.