not phytotoxic at the higher rates tested but did not give disease control (Table 2). These fungicides need to be evaluated at higher rates to determine efficacy and the threshold rate of phytotoxicity. The pathogen was isolated from plants representing all but the captafol treatments. These data are similar to those in a previous experiment.

Experiment 4. In vitro response of three M. roridum isolates to two benzimidazole fungicides. Isolate 4654 of *M. roridum* was shown to be considerably less sensitive to benomyl and ethazole + thiophanate-methyl fungicides than isolates 4708 and 4620 (Table 3). Although all isolates grew to some extent on PDA amended with up to 200 ppm of either fungicide, only the 4654 isolate grew substantially at fungicide concentrations exceeding 100 ppm.

Discussion and Conclusions

Myrothecium collar rot and leaf spot is considered a major pathological problem in the culture of the florists' gloxinia (J. Sweet, personal communication). Two to 3 benomyl or ethazole + thiophanate-methyl soil/crown drench treatments are used routinely during a gloxinia production cycle for the control of this disease. Our experience, however, with the isolate of *M. roridum* (4654) used in disease control trials and subsequent *in vitro* tests with these fungicides, indicates caution should be exercised when using these materials.

In practice, *M. roridum* resistance to either benomyl or ethazole + thiophanate-methyl has not yet been recognized (J. Sweet, personal communication). Whether our resistant strain or others similar to it will emerge in the future as a pathologic threat to gloxinia production depends upon many factors. According to Dekker (1, 2), the emergence of fungicide resistance in the field is dependent upon, among other things, the type of disease involved and the fitness of resistant mutants.

Because of the soil-borne nature of myrothecium disease on gloxinia and the pot culture used in growing gloxinia plants, resistant strains of *M. roridum* would not be expected to spread easily from their point of origin. It is also doubtful, because *M. roridum* is soil-borne, that all sensitive strains would be eliminated by benzimidazole fungicide(s). Therefore, emergence of an insensitive strain could be counteracted by competition from remnant sensitive strains (1, 2). Decreased sensitivity may correspond to a decreased growth rate (*in vitro* growth of our insensitive isolate was considerably less than that of the other sensitive isolates) and virulence of the mutant strain (1, 2). A decreased ability of the resistant mutant to effectively compete with sensitive isolates due to these factors, would help explain the apparent lack of benzimidazole resistance problems in practice at this time.

Table 3. *In vitro* growth of three *Myrothecium roridum* isolates on PDA amended with benomyl and ethazole + thiophanate-methyl fungicides[z].

Concentration[y]	Isolate 4708		Isolate 4654		Isolate 4620	
	Benomyl	E + tpm	Benomyl	E + tpm	Benomyl	E + tpm
0	100 a[x]	100 a	100 a	100 a	100 a	100 a
1	99 ab	96 ab	98 abc	100 a	98 ab	99 ab
10	97 ab	100 a	98 abc	100 a	101 a	97 b
50	71 c	96 ab	100 a	102 a	98 ab	70 c
100	21 de	24 d	93 cde	95 bcd	21 d	17 e
200	19 e	7 f	91 de	79 f	5 g	12 f
500	5 f	0 g	89 e	16 g	0 h	0 h

[z]Ten days growth at 28°C without light.
[y]Concentration = ppm benomyl for benomyl fungicide, and ppm thiophanate-methyl for ethazole + thiophanate-methyl (E + tpm) fungicide.
[x]% of growth of unamended control, average of four replicates. Percentages for an isolate followed by the same letter are not statistically different at the 5% range according to Duncan's multiple range test. Data were analyzed for mean separation before conversion to %'s.

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Proc. Fla. State Hort. Soc. 93:183-186. 1980.

PATHOGENICITY OF CYLINDROCLADIUM FLORIDANUM ON SPATHIPHYLLUM SP. CV. CLEVELANDII[1]

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Abstract. **Beginning in late 1978 and continuing to the present, several Florida nurseries have incurred serious economic losses from a new root and foliar disease of** Spathiphyllum **sp. cv. Clevelandii incited by the fungal pathogen,** Cylindrocladium floridanum **Sobers & Seymour. Root disease severity is influenced by pathogen density, soil sterilization, and soluble salts. The root disease may eventually spread to the lower extensions of the petioles above the soil line. Foliar inoculation with the pathogen resulted in more lesions on the lower petioles than on the upper petioles and leaves.**

[1]Contribution No. 495, Bureau of Plant Pathology.

Spathiphyllum sp. cv. Clevelandii is a dark green aroid with porcelain white flowers. This cultivar, the origin of which is not certain (2) is often referred to by the common names of "Mauna Loa" and spathiphyllum. In December 1978, we associated the fungus *Cylindrocladium floridanum* Sobers & Seymour with a root rot of spathiphyllum from a south Florida nursery. Since that time, *C. floridanum* has been associated primarily with root and lower petiole rots and less frequently with upper petiole and leaf lesions of spathiphyllum in at least 15 Florida nurseries. To some of these nurseries, it has meant a serious economic loss. *C. floridanum* was described in 1967 by Sobers and Seymour (8) as a cause of a root and crown disease of peach seedlings (*Prunus* sp.) in Florida nurseries. This pathogen has been shown to cause a root disease of yellow-poplar seedlings in North Carolina nurseries (3) and to be of some economic importance as a root and foliar pathogen of *Rhododendron* spp. (6).

In this study, we conclusively demonstrate the pathogenicity of *C. floridanum* to spathiphyllum, describe symptomatology, and elaborate on soil treatments which affect disease. A preliminary report of the work has been published (7).

Materials and Methods

An isolate of *Cylindrocladium floridanum* (080-5789) obtained from roots of *Spathophyllum* sp. cv. Clevelandii was used throughout this study. This isolate was maintained on slants of potato dextrose agar (PDA) (4) and was transferred monthly. Seed-cultured spathiphyllums which were 4 months old were used for foliar pathogenicity studies, and tissue-cultured spathiphyllums were used for root pathogenicity studies. Inoculum for foliar pathogenicity studies was prepared by inoculating cornmeal agar (CMA) (Difco Laboratories, Detroit, MI) plates with stock cultures, allowing cultures to incubate at room temperature under intermittent lighting for 2-3 weeks. Three CMA cultures of the fungus were flooded each with 10 ml of sterile deionized water. A sterile glass rod was used to free conidia from the conidiophores, and the suspensions were pooled and made up to a volume of 100 ml. One drop of a wetting agent (Tween 80) was added to allow for greater wetting of the foliage. The conidia were counted using a hemacytometer. Inoculum for root pathogenicity studies was prepared by inoculating PDA plates from stock cultures, allowing the cultures to incubate for 3-4 weeks at room temperature (24 ± 2°C) under intermittent room lighting, scraping the cultures with a spatula, and grinding them in a mortar with a pestle in 5-10 ml of distilled water until the inoculum was uniform. The mixture was made up to a volume of 100 ml, and the number of fungal propagules (microsclerotia and conidia) was counted using a hemacytometer.

The seedlings for foliar pathogenicity tests were planted in a commercial soil mix (Metro Mix 500, W. R. Grace, Cambridge, MA) contained in 7.7 cm (3 inch) clay pots. Each plant was sprayed with 10 ml of a suspension containing 40,000 conidia per ml. Most plants were immediately placed and sealed in a plastic bag which contained a moistened paper towel. Some plants were not sealed. Controls were sprayed with water and Tween 80 and were treated in a similar manner to the inoculated plants. The bagged and nonbagged plants were placed in a greenhouse (27 ± 3°C, under a relative humidity of 70-100%), and the plants were removed from the bags at daily intervals up to 4 days. Lesions were counted on the 7th day, and some lesions were excised, surface-sterilized (2% commercial bleach for 3 min), and plated on PDA.

In the root pathogenicity study, there were 4 soils (autoclaved-leached; autoclaved-nonleached; nonautoclaved-leached; and nonautoclaved-nonleached). For each soil, there were 3 levels of inoculum (200, 20, 0 propagules per g of wet soil). There were 2 harvest times (14 and 20 days). Thus, there were 24 (4x3x2) treatments. It was deemed necessary to have all soils at the same approximate moisture level at the time of soil inoculation. The involved procedure that follows produced these 24 soils. Twenty-three hundred g of soil mix were placed in each of 8 sterilized, galvanized steel trays (50 cm x 36 cm x 8 cm) with perforated bottoms. Four of these 8 trays of soil were autoclaved at (121°C for 30 minutes), were covered with paper after cooling, and were not used for 48 hr. Two of the 4 autoclaved soils and 2 of the 4 nonautoclaved soils were leached with 13 liters of water sprayed over the surface. The soils of the 8 trays were transferred to separate plastic bags and were weighed. The weights of the bags were adjusted to the weight of the heaviest bag by adding water. Each of the 8 bags of soil was mixed and was subdivided into 3 portions of equal weight. Thus, 24 (8 x 3) soils of the same approximate moisture level were produced. Each of 3 subdivided portions received 200, 20, and 0 propagules per g of wet soil, respectively, which is equivalent to 800, 80, and 0 propagules per gram of dry soil. (Dry weights of soils were determined by weighing 25-100 g soil samples, incubating at 45°C for 48 hr, and reweighing the soils). Each of the soils was transferred to a plastic bag and was tumbled for 1 minute to distribute the inoculum. Ten 7.7 cm (3 in) clay pots were filled from each of the soils. A tissue-cultured spathiphyllum was planted in each pot. The pots (setting in clay saucers) were placed in a greenhouse (27 ± 5°C under relative humidity of 70-100%). The plants were harvested at 14 and 20 days. All roots were excised, surface sterilized, and plated on the selective medium of Griffin (5). After 5 days incubation in the dark at room temperature (25 ± 3°C), the number of infected and noninfected roots of each plant were enumerated, and percent infection was calculated. To determine pathogen recovery at the lower inoculum level, 1 g subsamples were taken and plated on the selective medium (5) at the time of planting and at the 2 harvest dates. The soil was removed 4 days after plating by gently washing the soil from the plates. The *Cylindrocladium* colonies (propagules) were counted, and propagules per g of dry soil were calculated.

Soluble salts were determined and were based upon the 1:2 dry weight extraction method (10).

Results

Symptomatology on infected plants. As a result of root disease, wilting of the newer foliage and premature yellowing and eventual collapse of the older foliage becomes evident in younger seedlings. By the time these symptoms are obvious, 30-40% of the roots may be decayed or missing (Fig. 1). The fungus *C. floridanum* was readily isolated from the infected roots. Eventually all of the roots may decay allowing the plants to be easily lifted from their soil substrate. In larger, mature plants which had much root necrosis there were also brown, irregularly-shaped lesions of various sizes on the petioles near the soil line (Fig. 2). These yielded *C. floridanum* upon culturing. Far less common under natural conditions than the root and lower petiole disease is the leaf spot disease. In artificial inoculations the leaf spots varied from brown circular (0.3-0.5 mm) spots (Fig. 3) to brown, irregularly shaped (1-3 mm) spots surrounded by yellow halos. These artificially produced spots resembled those which were produced under natural conditions and which had yielded *C. floridanum*.

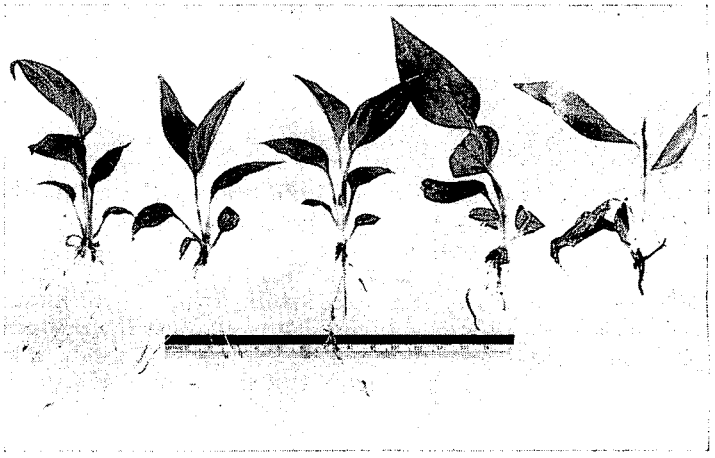

Fig. 1. Progressive root deterioration and discoloration of foliage of *Spathiphyllum* sp. cv. Clevelandii caused by *Cylindrocladium floridanum*.

Fig. 2. Petiole lesions caused by *Cylindrocladium floridanum* on a mature plant of *Spathiphyllum* sp. cv. Clevelandii.

Fig. 3. Leaf spots caused by *Cylindrocladium floridanum* on seedlings of *Spathiphyllum* sp. cv. Clevelandii.

Koch's postulates and factors affecting disease severity. In a foliar inoculation test (Table 1) of *C. floridanum* on spathiphyllum, the fungus was recovered consistently and in pure culture from representative leaf and petiole lesions. Accordingly, *C. floridanum* is now established as a cause of petiole and leaf lesions of spathiphyllum. Regardless of the time spent in the plastic bag (humidity chamber), there were more petiole lesions than leaf lesions (Table 1). The total average number of lesions per plant increased with time.

In a preliminary experiment where 10 tissue-cultured spathiphyllums were planted in an autoclaved soil mix which was infested with *C. floridanum*, the plants developed symptoms similar to those previously described, and *C. floridanum* was recovered in pure culture from all infected plants. These isolations along with those completed from the root inoculation test presented in Table 2 establish *C. floridanum* as the cause of the root disease of spathiphyllum. The effects of autoclaving the soil mix, leaching of the soil mix, pathogen density, and time of harvest were considered in the experiment presented in Table 2. The percentage data were transformed into arcsin values (9), and an analysis of variance was performed on these data. Autoclaving of the soil mix significantly increased the percentage of roots infected with *C. floridanum* at the 5% level. Leaching of the soil mix from 6300 ppm to 4200 ppm significantly decreased the percentage of roots infected at the 5% level. Increasing the inoculum from 80 to 800 propagules per g of dry soil mix significantly increased the percent root infection at the 1% level. There were no significant differences between the 2 harvest dates. At the lower inoculum level on the first harvest in the nonautoclaved leached soil mix there were no infected roots. At the second harvest date for the same soil, 18% of the roots plated were infected with the pathogen. When soils from these 2 harvest dates were assayed for pathogen populations (Table 3), no propagules were detected in the soils from the 14 day harvest, corresponding to 0% infection and 8 propagules per g of dry soil were detected in the 20 day harvest, corresponding to 18% infection. Immediately following mixing of soil and

Table 1. Foliar inoculation of seedlings of *Spathiphyllum* sp. cv. Clevelandii with conidia of *Cylindrocladium floridanum*.

Inoculum	Days in humidity chamber	Average number[z] of lesions per plant		
		Petioles	Leaves	Total
4 x 10^5 conidia	0	1.1	0.5	1.6
−(V)	0	0.0	0.0	0.0
4 x 10^5 conidia	1	3.2	0.0	3.2
4 x 10^5 conidia	2	4.5	0.1	4.6
4 x 10^5 conidia	3	4.5	1.5	6.0
4 x 10^5 conidia	4	6.8	1.1	7.8
−(V)	4	0.0	0.0	0.0

[z]Each value represents the mean of 20 replications.

inoculum (Day 0), no propagules were detected in the 2 nonautoclaved soil mixes whereas 33 and 38 propagules per g of dry soil were detected in the autoclaved soil mixes. When the soil was assayed at the 2 harvest times, leaching correlated with decreased recovery of propagules compared to nonleached soils.

Table 2. Percent infection of roots of *Spathiphyllum* sp. cv. Clevelandii with *Cylindrocladium floridanum* as a function of soil treatment, pathogen density, and time.

Soil treatment		Percent infection[z] at day 14 & day 20		
		Propagules per gram of dry soil		
		800	80	0
Autoclaved	Leached	66 & 87	7 & 28	0 & 0
Autoclaved	Nonleached	97 & 100	30 & 59	0 & 0
Nonautoclaved	Leached	48 & 34	0 & 18	0 & 0
Nonautoclaved	Nonleached	71 & 66	42 & 21	0 & 0

[z]Each value represents the mean of 10 replications.

Table 3. Recovery of *Cylindrocladium floridanum* from soils at the time of planting *Spathiphyllum* sp. cv. Clevelandii and at 14 and 20 days after planting.

Soil treatment		Propagules[z] per gram of dry soil			
		Added	Recovered		
		Day 0	Day 0	Day 14	Day 20
Autoclaved	Leached	80	38	14	81
Autoclaved	Nonleached	80	33	117	169
Nonautoclaved	Leached	80	0	0	8
Nonautoclaved	Nonleached	80	0	143	81

[z]Each value represents the mean of 5 replications.

Discussion

The root, petiole, and leaf spot disease of *Spathiphyllum* sp. cv. Clevelandii affecting several Florida nurseries is caused by *Cylindrocladium floridanum*. Koch's postulates were fulfilled. This is the first report of a *Cylindrocladium* species on an aroid (family Araceae).

The root disease is usually established before symptoms become apparent. To the grower, this elusiveness most often results in infested soils and infected plants being moved to new places within the nursery before the problem is noticed or taken seriously and makes a clean-up and control program substantially more difficult.

The root disease was more serious where the soil mix had been autoclaved prior to infestation and planting. The competitive advantage of a soil-borne pathogen in a soil which has been steamed or fumigated is well known (1). Since propagules of the pathogen were not detected in the soil subsamples that were taken from recently infested nonautoclaved soils but were detected in soil subsamples that were taken from recently infested autoclaved soils, this suggests that *C. floridanum* is a poor competitor. We are not recommending that growers who are having *Cylindrocladium* problems on spathiphyllum forsake soil treatments. We would encourage the use of treated soils in control programs for this or for any soil-borne pathogen. We would advise growers not to allow these treated soils to become infested with *C. floridanum*. Treated soils should be stored in protected, raised, and cleaned areas.

The root disease was less serious where the soil mix had been leached to lower the soluble salts. In soils which had been leached, pathogen populations remained low at the 2 harvest dates compared to nonleached soils. This effect of leaching may reflect a predisposition of the plant to greater infection at the higher salts levels and/or a stimulatory effect of higher salts levels upon the pathogen. A soluble salts level of 6300 ppm is not considered high by the standards of the 1:2 dry weight extraction procedure for a soil high in organic matter (9). We would suggest that soluble salts be maintained at a moderate level (3000-5000 ppm) and moisture be maintained at adequate levels, where *C. floridanum* is causing disease on spathiphyllum.

Under natural conditions, the foliar disease usually appears at or near the soil line, but leaf spotting may sometimes occur. In inoculation tests, there were more petiole spots than leaf spots. This may reflect a differential susceptibility of the 2 tissues to the pathogen and (or) run-off and accumulation of inoculum on the lower petioles. The number of lesions increased with time in the humidity chambers. This would suggest an increasing effectiveness of the primary inoculum under wet and humid conditions.

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