Discussion

The witches' broom disease of periwinkle in south Florida is associated with a polymorphic mycoplasmalike organism. The presence of virescence and phyllody along with the witches' broom, yellowing, and stunting symptoms are typical of a yellows group disease (3). Since branch proliferation is the predominant symptom, the disease has been termed witches' broom.

The relationship of periwinkle witches' broom to other plant diseases associated with mycoplasmalike organisms is not known, although the symptoms are distinct from those of aster yellows disease in periwinkle. Periwinkle witches' broom has been noted well outside of active lethal yellowing foci so it is highly doubtful that it is caused by the MLO associated with lethal yellowing disease of coconut palm (4, 5). Since no helical mycoplasmas were seen in infected tissue, it is unlikely that the pathogen is either *Spiroplasma citri*, cause of citrus stubborn disease, or the corn stunt spiroplasma. In any case, no helical mycoplasmas have been shown to produce virescence symptoms (1).

Periwinkle has been demonstrated to be a host for numerous plant mycoplasmas and MLO (1, 3), some of which can be differentiated on the basis of symptomatology. However, without side by side comparison it cannot be determined if periwinkle witches' broom is the same as the vinca virescence disease reported from *California* (2). Ultimately, the identification and separation of different mycoplasmal pathogens of plants will be possible when these agents are isolated in pure culture. Until such time, detailed taxonomic comparison of these pathogens must be based on symptomatology, host range, and vector relationships.

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CHEMICAL CONTROL OF MYROTHECIUM DISEASE OF GLOXINIA[1]

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Abstract. **The virulence of five isolates of** Myrothecium roridum **Tode ex Fr. was demonstrated on 'Improved Red Vevlet' gloxinia** (Sinningia speciosa **(Lodd.) Hiern.). Soil drenches of iprodione 50W at 1.0 g/liter gave effective, nonphytotoxic control of the leaf spot and crown rot phases of the disease. The leaf spot and crown rot were also controlled with soil drenches of captafol 4F at 7.1 ml/liter and 14.2 ml/liter, captan 50W at 4.8 g/liter and zinc ion maneb complex 80W at 3.6 g/liter. These treatments, however, were phytotoxic. Soil drenches of benomyl 50W at 0.6 g/liter and 1.2 g/liter, ethazole + thiophanate-methyl 40W at 0.7 g/liter and 1.3 g/liter, iprodione 50W at 0.5 g/liter, and RH 2161 2EC at 0.6 ml/liter and 1.2 ml/liter were not phytotoxic but were ineffective in controlling the disease.** In vitro **resistance of a gloxinia isolate of** M. roridum **to benomyl and ethazole + thiophanate-methyl fungicides was demonstrated.**

Myrothecium roridum Tode ex Fr. incites a collar rot and leaf spot of gloxinia (*Sinningia speciosa* (Lodd.) Hiern.) (3). During hot, damp weather the disease can be a serious threat to plant production. All ages of gloxinia are susceptible (3). The disease is most conspicuous when they are about 5 months old and beginning to flower. Because of the substantial investment of time and money required before plants reach this age, preventive measures against the disease are essential.

The present study was initiated to determine the virulence of 5 isolates of *M. roridum* from 5 sources on gloxinia, to assess disease control with chemicals, and to evaluate the resistance of a gloxinia isolate of *M. roridum* to fungicides containing benomyl or thiophanate-methyl.

Materials and Methods

Difco potato dextrose agar (PDA) cultures of each *M. roridum* isolate were grown under constant fluorescent light at 28°C for 7 days before storage as 5 mm discs under mineral oil in constant darkness at 10°C.

Experiment 1. Virulence of M. roridum isolates on gloxinia. The virulence of 5 isolates of *M. roridum* was determined on 'Improved Red Velvet' gloxinia. Inoculum was produced by growing each isolate on PDA under constant, cool-white fluorescent light at 28°C for 10 days. Conidia were harvested by adding deionized H_2O (DW) to the surface of cultures and rubbing with a rubber policeman. Conidial suspensions of each isolate were filtered through 2 layers of cheesecloth and adjusted to $1x10^6$ conidia/ml. These suspensions were used promptly to inoculate 4-week old seedlings.

Seedlings to be inoculated were removed from flats and large portions of their root systems were exposed. Groups of 12 seedlings were immersed in a conidial suspension or DW (control) for 5 minutes. The plants were then planted singly in 10 cm diam plastic pots containing a soil mix consisting of Florida peat, sand, vermiculite, and perlite (5:3:3:1 v/v ratio) supplemented with dolomite, hydrated lime, superphosphate, and a trace element mix. The soil mix (used for all subsequent plant experiments) was amended with 2.5 cc 14-14-14 Osmocote® time release fertilizer per pot and supplemented with water soluble fertilizer as needed. All seedlings were watered immediately after planting. Treatments were replicated 12 times in a randomized complete block design. All plants were rated for

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disease severity 4 and 12 weeks after inoculation. After the final disease rating, tissue isolations from plants representing all treatments were made on PDA.

Experiment 2. Phytotoxicity. Five-week old 'Improved Red Velvet' gloxinia seedlings were planted singly in 5 cm square plastic pots filled with potting mix amended with 0.8 cc 14-14-14 Osmocote/pot. Plants were watered in and immediately drenched with 15 ml/pot of suspensions of one of the following fungicides: benomyl 50W (Benlate 50W), captafol 4F (Difolatan 4F), captan 50W (Orthocide 50W), ethazole + thiophanate-methyl 40W (Banrot 40W), iprodione 50W (Chipco 26019 50W), RH 2161 2EC (Sisthane 2EC), and zinc ion maneb complex 80W (Manzate 200 80W). Treatments were replicated 8 times and randomized in a complete block design. Fresh weights of the above-ground portion of all plants were taken 3 weeks after treatment.

Experiment 3. Disease control. Five-week old 'Improved Red Velvet' gloxinia seedlings were planted in 10 cm plastic pots and grown for an additional 5 weeks. Eighty of these plants were then removed from their pots and large, uniform portions of their root systems manually removed before inoculation. Three groups of 25 of these plants were each root-dipped for 5 min. in individual conidial suspensions (2:5 x 10^6 conidia of isolate 4654/ml) prepared as for experiment 1. These inoculated plants and 5 control plants (dipped in DW for 5 min) were then planted singly in 15 cm plastic pots filled with soil mix amended with 10 cc 14-14-14 Osmocote/pot. Two hundred ml of the appropriate fungicide suspension were drenched over plant crowns after planting and again after 5 weeks. Treatments were replicated 5 times in a randomized complete block design. Eleven weeks after the initial treatment, plants were sacrificed for disease ratings, and tissue isolations were made from plants representing all treatments.

Experiment 4. In vitro response of three M. roridum isolates to two benzimidazole fungicides. PDA cultures of 3 isolates (4620, 4654, and 4708) were grown for 5 days at 28°C under constant, cool-white fluorescent light. Circular plugs (5 mm diam) were taken from the periphery of these cultures to individually "seed" PDA (in 10 cm Petri dishes) amended with 6 concentrations of either benomyl or ethazole + thiophanate-methyl. A control treatment was not amended. The Petri dishes were incubated at 28°C in complete darkness. The diameter of each colony was measured 3, 6 and 10 days after "seeding." Treatments were replicated 4 times in a completely randomized design.

Results

Experiment 1. Virulence of isolates of M. roridum on gloxinia. All isolates were pathogenic to 'Improved Red Velvet' gloxinia and no differences were found in virulence among the isolates (Table 1). Disease ratings with any of the isolates were statistically greater than that found for the naturally infected noninoculated water control treatment. The pathogen was reisolated from diseased plants representing all treatments.

Experiment 2. Phytotoxicity. Captafol 4F, captan 50W, and zinc ion maneb complex 80W were phytotoxic to 'Improved Red Velvet' gloxinia plants (Table 2). Plants treated with these fungicides were chlorotic and weighed statistically less than the H_2O control plants. Benomyl 50W at 1.2 g, ethazole + thiophanate methyl 40W, iprodione 50W and RH 2161 2EC were not phytotoxic. Fresh weight means of plants treated with these fungicides were statistically equal to that of the H_2O control plants. Similar results were obtained in a previous experiment.

Experiment 3. Disease control. Captafol 4F (7.1 and 14.2 ml/liter), captan 50W (4.8 g/liter), iprodione 50W (1.0 g/liter), and zinc ion maneb complex 80W (3.6 g/liter) were effective in controlling myrothecium disease (Table 2). Disease ratings for plants treated with these fungicides were statistically equal to that of the noninoculated control treatment plants and superior to that of the inoculated control treatment plants. Only the iprodione treatment was not phytotoxic. Captafol gave excellent disease control at both rates but was highly phytotoxic. It should be evaluated at lower rates to determine efficacy and phytotoxicity. Captain and zinc ion maneb complex were phytotoxic at all rates tested and gave disease control only at the higher rates. Benomyl 50W (1.2 g/liter), ethazole + thiophanate-methyl 40W (1.3 g/liter), and RH 2161 2EC (1.2 ml/liter) were

Table 1. Disease reaction of 'Improved Red Velvet' gloxinia to isolates of *Myrothecium roridum* three months after inoculation.

Isolate	Source	Disease rating[z]
Water control	—	1.5 a[y]
4654	Gloxinia (*Sinningia speciosa* (Lodd.) Hiern.)	3.4 b
4620	Kalanchoe (*K. blossfeldiana* Poelln.)	3.7 b
4308	Desert rose (*Adenium obesum* Roem. & Schul.)	4.0 b
4310	Geranium (*Pelargonium* x *hortorum* Bailey)	3.8 b
4582	Peperomia (*P. obtusifolia* A. Dietr.)	3.0 b

[z]Disease rating = mean of 12 replicates: 1 = no disease; 2 = slight leaf involvement and/or 1/4 stem girdled; 3 = moderate disease 1-2 large leaf spots and/or stem lesion girdling 1/2 stem; 4 = severe disease, several large leaf spots and 1/2-3/4 of stem girdled; 5 = extreme disease, numerous large leaf spots and stem almost entirely girdled; 6 = dead.
[y]Ratings followed by the same letter are not statistically different at the 5% level according to Duncan's multiple range test.

Table 2. Efficacy and phytotoxicity of fungicide drenches for the control of myrothecium leaf spot and collar rot of 'Improved Red Velvet' gloxinia.

Treatment	Rate (amt/liter)	Phytotoxicity rating[z]	Disease rating[y]
Water control—inoculated	—	—	4.0 ab
Water control—noninoculated	—	4.4 a	1.3 d
Benomyl 50W	1.2 g	4.0 ab	3.6 abc
Benomyl 50W	0.6 g	3.3 bcd	4.5 a
Captafol 4F	14.2 ml	1.5 fg	1.0 d
Captafol 4F	7.1 ml	1.2 g	1.0 d
Captan 50W	4.8 g	2.4 cde	1.9 cd
Captan 50W	2.4 g	2.7 cde	4.0 ab
Ethazole + thiophanate-methyl 40W	1.3 g	3.5 abc	3.9 ab
Ethazole + thiophanate-methyl 40W	0.7 g	3.5 abc	4.3 a
Iprodione 50W	1.0 g	3.7 abc	2.2 bcd
Iprodione 50W	0.5 g	3.6 abc	4.0 ab
RH 2161 2EC	1.2 ml	3.5 abc	3.2 abc
RH 2161 2EC	0.6 ml	3.5 abc	3.9 ab
Zinc ion maneb complex 80W	3.6 g	2.0 efg	1.8 cd
Zinc ion maneb complex 80W	1.8 g	2.3 def	3.7 abc

[z]Phytotoxicity rating = mean fresh weight (g) of 8 replicates/treatment (Experiment 2).
[y]Disease rating = mean of 5 replicates/treatment: 1 = no disease, 2 = slight disease, 1-2 small lesions on stem or leaf; 3 = moderate disease, 1-2 large leaf spots or stem lesion girdling 1/2 stem; 4 = severe disease, several large leaf spots and 1/2-3/4 of stem girdled; 5 = extreme disease, numerous large leaf spots and stem almost entirely girdled; 6 = dead. Inoculations were made with the 4654 isolate of *M. roridum* (Experiment 3). Means within a column followed by the same letter are not statistically different at the 10% level according to Duncan's multiple range test.

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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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).