Pathogenicity of Pythium myriotylum and Other Species of Pythium to Caladium Derived From Shoot-Tip Culture

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ABSTRACT

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Isolates of *Pythium* obtained from diseased caladium roots were identified as *P. irregulare, P. myriotylum,* and *P. splendens*. Pathogenicity studies with these species of *Pythium* at 24 C and 32 C showed *P. myriotylum* and *P. splendens* to be infective. The shoot, corm, root, and total plant weights after 40 days showed that only *P. myriotylum* reduced plant growth significantly ($P < 0.05$) at 32 C. No significant differences ($P < 0.05$) were noted for any of the species at 24 C. Greenhouse inoculations with *P. myriotylum* significantly slowed ($P < 0.05$) the germination of corm pieces and resulted in significant losses ($P < 0.01$) in total plant growth after 28 weeks.

Additional key words: Caladium hortulanum, root infection.

Caladium (*Caladium hortulanum* Birdsey) is an important ornamental plant species grown for its attractive variegated foliage. Commercial cultivars of caladium are propagated exclusively by vegetative means, and proof of pathogenicity by phytopathogens has been hindered because pathogen-free material has not been available. Heat treatment or chemical surface sterilization is not effective in ridding vegetative material of such phytopathogens as dasheen mosaic virus (DMV) which has been reported uniformly infecting the cultivar Candidum (5, 6). Recently, Hartman (5) obtained pathogen-free plants of this cultivar through the aseptic culture of shoot-tips and, by using this material, demonstrated that DMV can reduce caladium yields 30-50% (4, 7).

The purpose of this study was to identify isolates of *Pythium* obtained from diseased caladium roots and determine the relative pathogenicity of these isolates using pathogen-free caladiums derived from shoot-tip cultures.

MATERIALS AND METHODS

Isolates of *Pythium* obtained from diseased roots of field-grown caladiums were designated 112, 120, 121, 1934, 3513, and 3605. These isolates were maintained on test tube slants of potato-dextrose agar (PDA) and hemp agar [HA (oil from 30 g of hemp seed plus 20 g of Difco agar in 1 liter of water)] at room temperature (approximately 25 C). Sexual reproductive structures of the Pythium isolates were formed in cultures on HA after 7 to 10 days. Sporangia were formed in HA or were induced in fresh mycelium formed from active cultures on lima bean agar (LBA) blocks placed in sterile tap water in petri plates. Zoospores were induced by replacing the sterile tap water with fresh supplies of distilled water. Measurements of 25 oogonia, oospores, and/or sporangia were recorded for each isolate. Identifications were based upon comparing recorded data with published descriptions (1, 10, 14, 15).

Inoculum for studies in environmental growth chambers was prepared by infesting sterilized millet seed (10 g per petri plate) with each isolate and incubating at room temperature for 7-10 days. One petri plate of infested millet seed was mixed uniformly with approximately 500 cc of a steam-sterilized sand-peat (1:1, v/v) potting medium. Pathogen-free Candidum plants, derived from shoot-tip culture according to the method described by Hartman (5), were grown for 6 weeks in 5-cm diameter clay pots containing the potting medium. These plants were transplanted with root ball intact into 10-cm diameter clay pots containing approximately 300 cc of the infested potting medium. The pots were then set in 10-cm diameter saucers in environmental growth chambers operating at 94-97% relative humidity and with a light intensity of 10,760 lux derived from a combination of fluorescent and incandescent lights operating at a day length of 12 hours. Temperatures were set at either 24 ± 1 or 32 ± 1 C, and the plants were watered as needed to maintain continuous saturation. Fifty milliliters of a fertilizer solution, prepared by adding 2 ml of Ortho 12-6-6 and 2 ml of a 0.5% manganese solution to a liter of water, were applied biweekly to each plant.

Inoculum for studies in the greenhouse was prepared by uniformly mixing 10 g of 7- to 10-day-old infested

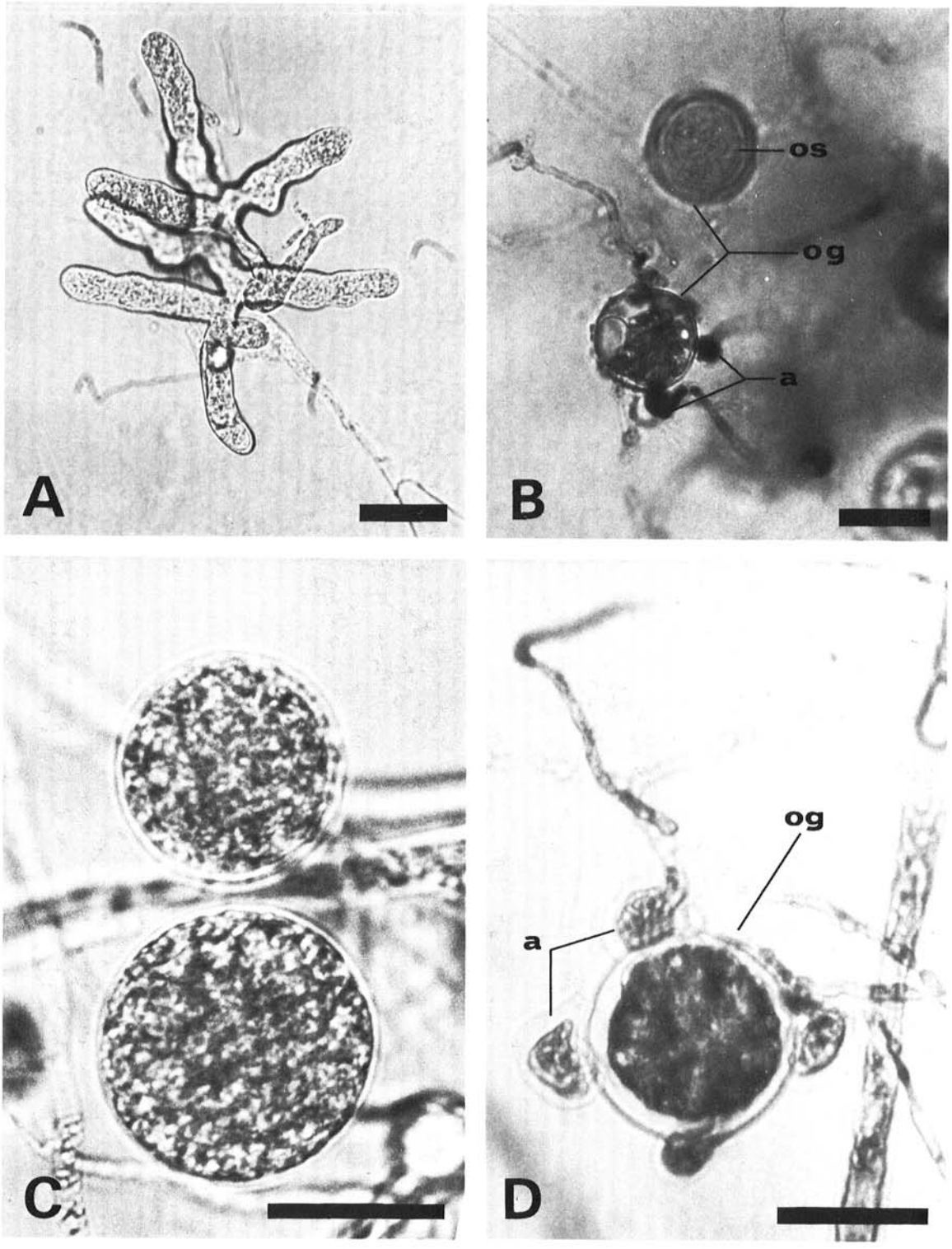

Fig. 1-(A-D). Reproductive bodies in culture of isolates of *Pythium* spp. from caladium roots: **A)** sporangium complex of *P. myriotylum;* **B)** oogonium (og), antheridia (a), and oospore (os), representative of each isolate of 112, 121, and 1934 of *P. myriotylum;* **C)** dense sporangia of *P. splendens;* **D)** oogonium (og), and antheridia (a) of isolate 3605 of *P. splendens*. Scale bar is 20 μm.

millet seed with approximately 1,400 cc of steam-sterilized muck soil in 15-cm clay pots. The muck was obtained from a commercial caladium field in Highlands County, Florida. Pathogen-free cultivar Candidum corms were cut into pieces (approximately 0.9 g), each of which contained a single bud. Four corm pieces were planted in each 15-cm diameter pot of *Pythium*-infested muck. The pots were placed in saucers and watered individually by means of polyethylene tubes (1.14 mm in diameter). With this watering system, equal volumes of water were applied to all pots which were watered as needed to maintain continuous saturation. The greenhouse temperatures varied from 21-25 C at night to 32-37 C during the day. One hundred ml of the liquid fertilizer (described above) were added biweekly to each pot. Control plants were treated identically to inoculated plants in both environmental growth chamber and greenhouse tests except that sterilized millet seed was used in place of infested seed.

Data were obtained by removing plants from the pots and washing the potting mix from the roots. The intact plants were air dried at room temperature for 24 hours and then subdivided into shoot, corm, and roots prior to weighing. Experiments in the growth chamber were conducted at least twice with five plants per treatment. The greenhouse experiment was conducted once using five or 10 plants per treatment depending on the harvest period.

RESULTS

Identification of Pythium spp. isolates.—Isolates 121 and 1934 were identified as *P. myriotylum* Drechs. based on their formation of smooth oogonia (mean diameters 27.0 and 27.5 μm, respectively) with aplerotic oospores (mean diameters were 22.6 and 22.4 μm, respectively). Two to six crook-necked clavate, diclinous antheridia were present for each oogonium (Fig. 1-B). The middle of the antheridial cell was arched distinctly. Isolate 112 formed numerous oogonia with aborted oospores. Oogonia with nonaborted oospores, as well as antheridia, were very similar in size and description to isolates 121 and 1934 and, therefore, isolate 112 was likewise designated as *P. myriotylum*. Lobate sporangia were formed in water by all three isolates on hyphae derived from cultures on LBA blocks or boiled grass blades (Fig. 1-A). Isolate 112 formed many more lobate sporangia than the other two isolates. Mycelia of all three isolates grew with a mean radial extension of 2.1 mm per hour on LBA at 40 C.

Isolate 3605 was identified as *P. splendens* Braun based on its production of abundant smooth oogonia (mean diameter 29.8 μm) with aplerotic, thick-walled oospores (mean diameter 23.2 μm). One to four clavate, monoclinous, and diclinous antheridia were present on each oogonium (Fig. 1-D). The antheridial cell averaged 9.9×12.4 μm. Numerous dense, large sporangia (mean diameter 32.6 μm) were formed readily (Fig. 1-C). The formation of oospores by this isolate was further confirmed by oospore production in each of 20 single hyphal-tip cultures. Although heterothallism occurs predominantly in *P. splendens*, this isolate must be considered homothallic (11).

Isolate 120 produced abundant echinulate oogonia (mean diameter 16.6 μm) with aplerotic thin-walled oospores (mean diameter 15.0 μm). The size of the oogonia varied considerably from 9.9 to 19.8 μm. The sparsely formed spines on the terminal and intercalary oogonia were $1.0 - 2.0 \times 8.9$ μm and rounded at the apex. One to two clavate, monoclinous, and diclinous antheridia were observed for each oogonium. The antheridial cell averaged approximately 8.2×11.5 μm. Barrel-shaped intercalary sporangia and terminal spherical sporangia were formed abundantly and varied considerably in size. This isolate was identified as *P. irregulare* Buis.

Isolate 3513 produced abundant echinulated oogonia (mean diameter 13.7 μm). The spines on the predominantly terminal oogonia were 1.1×2.4 μm and were rounded at the apex. One clavate, diclinous antheridium was observed for each oogonium. Sporangia and zoospores were not formed by the general procedures outlined or by culturing the isolate on boiled grass blades (14). Because of insufficient data for speciation, the identity of this isolate remains as *Pythium* sp.

Pathogenicity by Pythium spp.—Caladium root infection, as evidenced by reddish brown lesions and root rot, occurred from inoculations with isolate 3605 of *P. splendens* and isolates 112, 121, and 1934 of *P. myriotylum* at 24 and 32 C (Fig. 2-B). Isolations of the fungi from infected roots on a selective agar medium (13) and subsequent identification confirmed the presence of these isolates. No symptoms of root infection by isolate 120 of *P. irregulare* or isolate 3513 of *Pythium* sp. were evident at either temperature. When shoot, corm, root, and total plant weights of inoculated plants were compared with the noninoculated control, only isolates 112, 121, and 1934 of *P. myriotylum* caused significant losses ($P < 0.05$) at 32 C (Table 1, Fig. 2-A). No significant losses ($P < 0.05$) were detected at 24 C for any of the isolates. A comparison of the pathogenicity of the isolates with each other on the basis of total plant weight at 24 C showed that *P. irregulare, P. myriotylum,* and *P. splendens* were not significantly different ($P < 0.05$) from each other. However, at 32 C, isolates 112 and 1934 of *P. myriotylum* differed significantly ($P < 0.05$) from isolates 120 of *P. irregulare,* 3605 of *P. splendens,* and 3513 of *Pythium* sp.

Exposure of caladium corm pieces in the greenhouse to soil infested with isolate 112 of *P. myriotylum* resulted in significant reductions ($P < 0.05$) in the rate of corm germination at 3 and 4 weeks after planting (Table 2). Shoot, corm, and total plant weights of the inoculated plants were significantly reduced ($P < 0.01$) as early as 8 weeks after planting (Table 3). Significant differences were detected at each harvest time. At the conclusion (28 weeks) of the experiment, highly significant losses ($P < 0.01$) in plant weights were noted. A 41% difference occurred in the total plant weights between the inoculated and noninoculated treatments. Oospores of *P. myriotylum* were detected in infected roots, but not in healthy roots (Fig. 2-C).

DISCUSSION

Although high inoculum levels, favorable moisture conditions, and generally favorable temperatures were employed in these studies, only *P. myriotylum* and *P.*

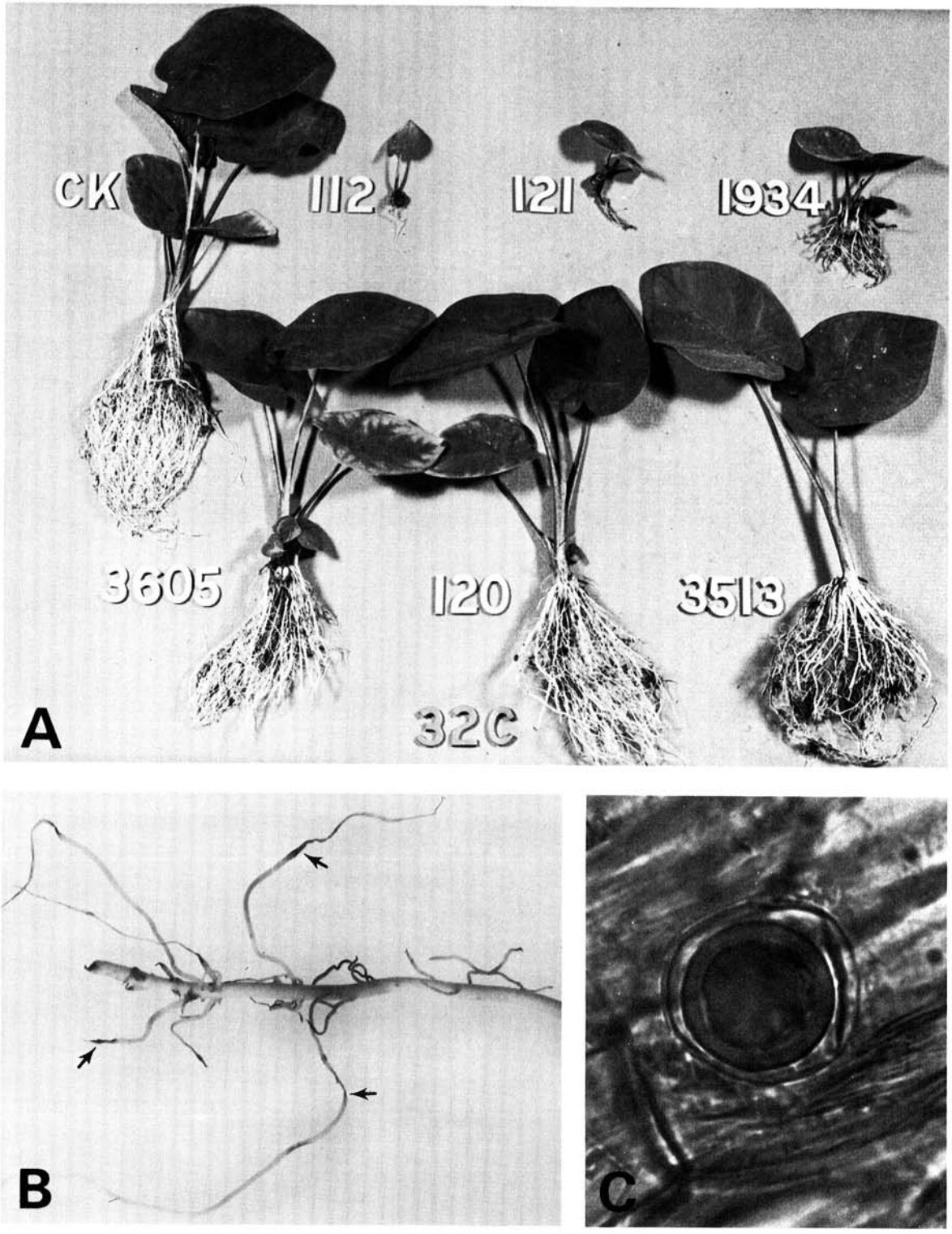

Fig. 2-(A-C). Pathogenicity of isolates of *Pythium* spp. to caladium: **A)** effect of isolates 112, 121, and 1934 of *P. myriotylum* in relation to the other isolates of *Pythium* inoculated to Candidum caladium at 32 C after 40 days in the environmental growth chamber; **B)** caladium roots infected by *P. myriotylum;* **C)** oospore of *P. myriotylum* imbedded in infected root.

TABLE 1. Pathogenicity of *Pythium* isolates to caladium cultivar Candidum at two temperatures after 40 days in environmental growth chambers

Pythium sp. isolates[b]	Mean weights[a] (g) of plant parts							
	Shoot		Corm		Root		Total	
	24 C	32 C	24 C	32 C	24 C	32 C	24 C	32 C
120	2.13 A*	3.52 A*	1.02 AB*	2.13 A*	0.31 AB*	0.68 A*	3.46 AB*	6.33 A*
3605	1.07 AB*	2.71 A*	0.76 B*	1.15 BC*	0.20 AB*	0.24 BC*	2.03 BC*	4.10 AB*
3513	2.41 A*	3.38 A*	1.91 A*	1.89 AB*	0.42 A*	0.59 AB*	4.74 A*	5.86 A*
112	0.12 B*	0.69 B	0.26 B*	0.72 C	0.01 B*	0.03 C	0.39 BC*	1.44 C
121	0.32 B*	0.96 B	0.43 B*	0.64 C	0.01 B*	0.03 C	0.76 BC*	1.63 BC
1934	0.40 B*	0.34 B	0.71 B*	0.41 C	0.02 B*	0.02 C	1.13 BC*	0.77 C
Control (non-inoculated)	1.68*	3.28*	0.86*	2.00*	0.23*	0.55*	2.77*	5.83*

[a]Means in columns followed by the same letter are not significantly different ($P < 0.05$) using Duncan's new multiple range test. Asterisk (*) denotes means that were rated not significantly lower ($P < 0.05$) than the control by Dunett's test (12). The standard error of the mean (Sx) for the shoot, corm, root, and total plant was 0.484, 0.291, 0.115, and 0.820, respectively. Data are from one of two experiments involving five plants per treatment.

[b]Identity of isolates is as follows: 120 = *P. irregulare;* 3605 = *P. splendens;* 112, 121, and 1934 = *P. myriotylum;* and 3513 = *Pythium* sp.

TABLE 2. Germination of caladium cultivar Candidum corm pieces 3-8 weeks after inoculation with *Pythium myriotylum* in the greenhouse

Time after planting (weeks)	Germination of corms[a]	
	Noninoculated (%)	Inoculated (%)
3	54	29
4	84	67
5†	86	83
6†	87	85
7†	89	88
8†	93	89

[a]Data are based on 160 corm pieces in each treatment. Dagger (†) denotes period after planting in which per cent germination was not significantly different ($P < 0.05$) for the inoculated and noninoculated corms.

splendens infected the caladium roots. However, only *P. myriotylum* significantly reduced caladium plant development at 32 C in the growth chambers. This favorable high temperature for development of *P. myriotylum* is in agreement with pathogenicity studies with this fungus on other hosts (1, 2, 3, 8, 9). This appears to be the first report of the pathogenicity of *P. myriotylum* to caladium. Although *P. splendens* and *P. irregulare* are regarded as pathogens on other hosts (10, 14), the isolates used in this study resulted in only slight infectivity by *P. splendens*. Thus, the role of these isolates may be secondary.

The aggressiveness of *P. myriotylum* was well demonstrated in the greenhouse test where this fungus slowed the germination of corm pieces and subsequently caused poor plant development at all harvest periods resulting in yield losses of 41% compared to the noninoculated controls. Temperatures (25-35 C) in the greenhouse were generally favorable for pathogenicity by this fungus. In the greenhouse, as well as in the growth chamber studies, very few inoculated plants were killed by *P. myriotylum*.

TABLE 3. Comparison of weights of plant parts of Candidum caladium harvested at different times after inoculation with *Pythium myriotylum* (isolate 112) in the greenhouse

Plant part	Treatment	Mean weights[a] (g) at harvest					
		8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	28 weeks
Shoot	Control	2.65	6.49	13.50	18.39	25.78	20.93
	112	1.44**[b]	2.91**	8.07*	10.19[NS]	14.51*	12.54**
Corm	Control	0.82	1.21	2.84	3.68	5.39	9.63
	112	0.52**	0.82[NS]	1.49**	1.76*	3.92[NS]	5.65**
Root	Control	0.12	0.33	0.51	0.51	0.55	0.91
	112	0.12[NS]	0.12*	0.17**	0.23**	0.26**	0.24**
Total plant	Control	3.59	8.03	16.85	22.58	31.72	31.47
	112	2.08**	3.85**	9.73**	12.18*	18.69*	18.43**

[a]Means are based on 10 plants per treatment at the 8 and 28 week harvests; the remaining means are based on five plants per harvest.

[b]Asterisks indicate: * = significantly different from the respective control at LSD ($P = 0.05$). ** = significantly different from the respective control at LSD ($P = 0.01$). NS = not significantly different from the respective control. The magnitudes of the variances at the different harvest times were significantly different ($P < 0.05$) using Bartlett's test (11).

In Florida, caladiums are grown commercially over a 7- to 8-month period generally starting in March. During this time, the presence of *P. myriotylum* in combination with high soil temperatures and favorable moisture levels could cause significant losses to the growers.

Species of *Pythium* have been associated frequently with phytopathogens such as *Rhizoctonia solani* Kuehn, *Fusarium* spp., and *Sclerotium rolfsii* Sacc., but their individual role as pathogens has not been clearly defined. The use of pathogen-free Candidum caladiums has aided greatly in the evaluation of the relative importance of the species of *Pythium* used in this study. With this pathogen-free material, additional studies can be conducted on the influence of specific inoculum densities and the interaction of *P. myriotylum* with dasheen mosaic virus and different fungi.

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