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EVALUATION OF BIO-CON CRC-400 AS A PROTECTANT AGAINST DEVELOPMENT OF CROWN ROT OF PEPEROMIA AND ROOT ROT OF POTHOS[1,2]

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Abstract. **Bio-Con CRC-400, a bacterial and fungal soil inoculant, was applied at the recommended and twice the recommended rate and evaluated for protective action and growth stimulation of pothos and peperomia following inoculation with the pathogens** Pythium splendens **and** Phytophthora parasitica, **respectively. Bio-Con was applied at planting time to each of 2 soil mixes of peat and sand (1:1 and 3:1 v/v). After one week and an additional application of Bio-Con, the plants were inoculated with the respective pathogen. Within 7 days after inoculation, plants showed disease development. After 14 days in one of the 3 experiments, approximately 50% of the peperomia and 44% of the pothos were diseased in the inoculated controls and in the Bio-Con treatments. No symptoms of disease were detected in the noninoculated control treatments. No differences in the number of nodes, stem length, or fresh weight were detected between the Bio-Con and nontreated controls. No consistent differences in disease development or plant growth were noted between soil mixes.**

A substantial body of literature exists which examines the interactions among microbial antagonists, soil-borne plant pathogens, and the host crop (2, 3, 4, 5, 6, 8, 10, 11, 12). It is generally accepted that some microorganisms naturally occurring in a soil body serve to limit the amount of crop yield reduction resulting from infection by soil-borne root pathogens. Greenhouse studies have shown that under certain conditions the addition of isolates of microbial antagonists such as *Bacillus* sp., *B. subtilis,* and *Streptomyces* sp. have controlled soil-borne diseases such as *Rhizoctonia* or *Pythium* (4) and also increased growth of a variety of plant species (5). Numerous other examples of the biological control of plant pathogens are cited by Baker and Cook (2).

In December 1976, a new product, Bio-Con CRC-400, came to our attention through a local trade newsletter (News and Views—Apopka Growers Supply). One of the 8 claims of this product was the following: "Isolation or dilution of pathogens from the rhizosphere due to the extremely high concentrations of beneficial species of bacteria (direct competition for nutrients)." A review of the literature yielded no published accounts of this product's efficacy as a biological control agent.

In 1976, Smith and Strain (13) reported that pothos and peperomia each accounted for 3% of the $87 million dollar sales of Florida foliage. Golden Pothos, *Epipremnum aureum* (Linden and Andre) Bunt. (=*Scindapsus aureus* Engles), is commonly afflicted with root rot incited by the pathogen *Pythium splendens* Braun. Peperomia or pepperface, *Peperomia obtusifolia* (L.) A. Dietr. is often parasitized by *Phytophthora parasitica* Dast. (*P. nicotianae* B. de Haan var. *parasitica* (Dast.) Waterh.) with a resulting root, crown, and stem rot (Fig. 1 and 2). Tests were conducted to evaluate the efficacy of Bio-Con as a biological control agent for these two host-pathogen combinations and its effect on plant growth.

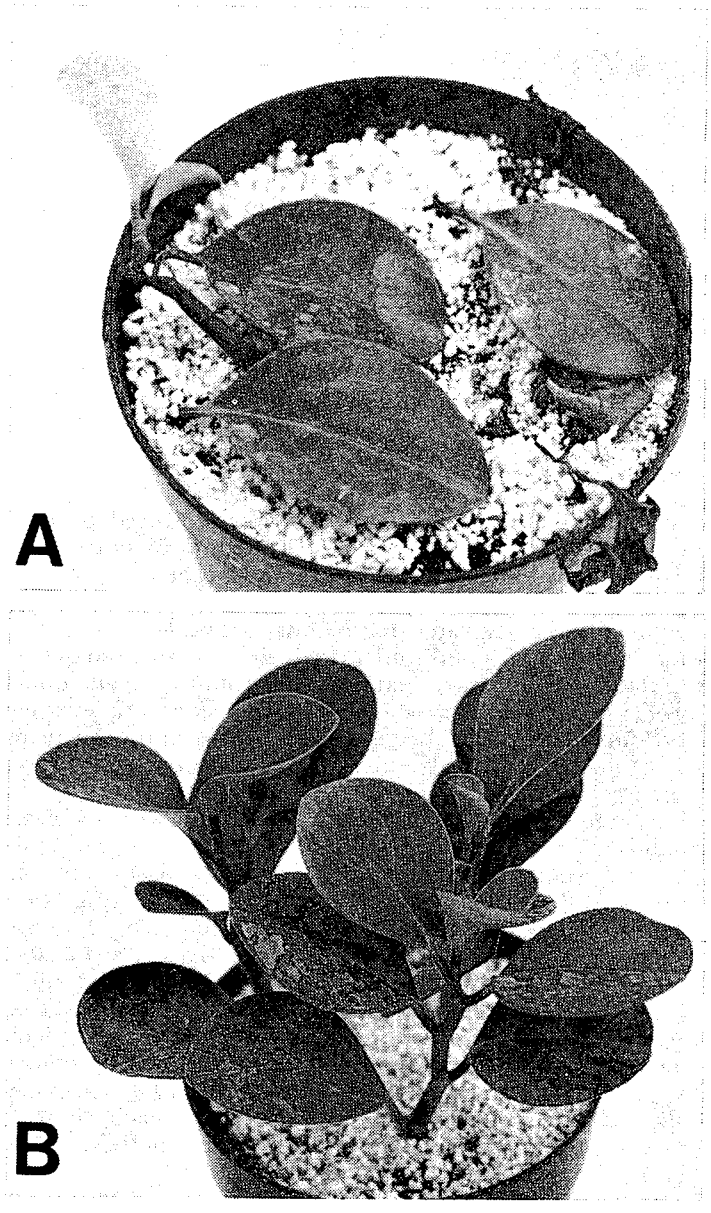

Fig. 1. Peperomia plants inoculated with *Phytophthora parasitica* (A) and plants treated as non-inoculated control (B) after 4 weeks. (Perlite used for photography purposes only).

Materials and Methods

The isolate of *Phytophthora parasitica* (R-117), supplied by J. F. Knauss (IFAS, ARC-Apopka), and the isolate of *P. splendens* were obtained from a diseased cutting of *Peperomia obtusifolia* and *Epipremnum aurenum,* respectively.

Inoculum of *P. splendens* was prepared from autoclaved (121 C for 20 min) millet seed (10 g of dry seed and 16 ml distilled water per petri plate) inoculated with a mycelial plug (5 mm diam) and incubated at room temp for approximately 7 days (9). Inoculum of *P. parasitica* consisted

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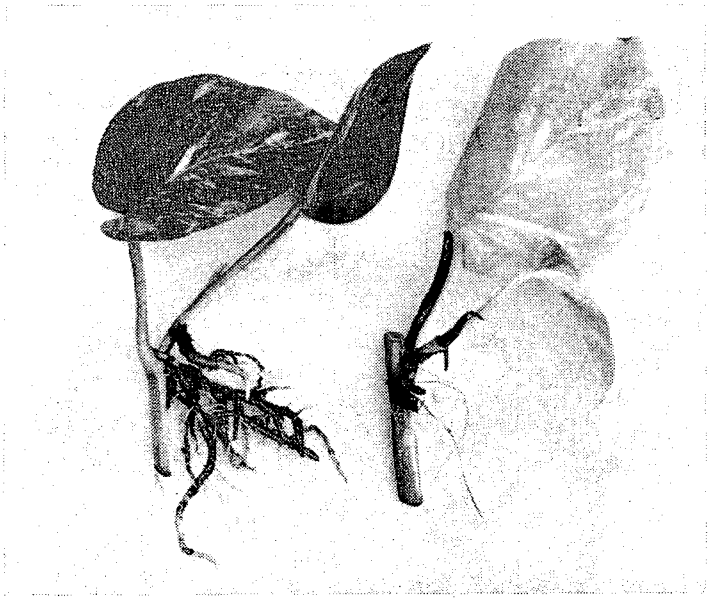

Fig. 2. Pothos plants inoculated with *Pythium splendens* after 4 weeks: Healthy (L) and Diseased (R).

primarily of chlamydospores which were induced by placing a mycelial plug into liquid V-8 medium in bottles and incubating for 4-5 weeks (14). The inoculum was prepared by rinsing the mycelial mat and comminuting in a specified volume of distilled water in a blender for 60 sec. Additional inocula of *P. splendens* and *P. parasitica* were prepared to simulate the disease situation that could prevail under nursery conditions. Unrooted pothos or peperomia cuttings were inoculated with the respective fungal pathogen using the inoculum outlined above. Single infected stems were then used as the inoculum.

In test 1, 3 rooted pothos and peperomia cuttings were established separately in a Canadian peat and sand mix (1:1 and 3:1 v/v) in 5 in (12.7 cm) diam plastic pots. In tests 2 and 3, unrooted pothos and peperomia cuttings were established only in the 3:1 (v/v) peat:sand mix. The soil media were each supplemented with 0.25 lb (0.11 kg) K_2SO_4, 1.5 lb (0.68 kg) $CaHPO_4$, 1.5 lb (0.68 kg) $CaCO_3$, and 4.5 lb (2.04 kg) dolomite for each 120 gal (454.2 liter) of mix. Potting media in all tests were steamed at 100 C for at least 6 hr. The pH of both mixes was 6.2. Plant material was provided by J. F. Knauss and was selected for uniformity in size and color. The selected single eye cuttings used in each replicate pot consisted of a 2nd, 3rd, and 4th node from each vine. Six to 10 replicates were used in each test.

In test 1, Bio-Con CRC-400 was prepared according to the manufacturer's directions and applied at the recommended dosage (.02 g/liter) and twice the recommended dosage at 100 ml per pot. The applications were made twice with a week interval between applications and were followed by the soil infestation with pathogens. Inoculation with *P. splendens* consisted of placing 1 g of infested millet seed into the center of each pot containing 3 rooted cuttings. Inoculation with *P. parasitica* consisted of applying 50 chlamydospores (with mycelial fragments) per sq. in. (6.5 cm^2) uniformly to the soil surface. The chlamydospore concentration was determined by averaging 6 to 8 sample counts from a standard hemacytometer.

In test 2, Bio-Con was used only at twice the recommended dosage and applied in the same manner as test 1. The same inoculation technique was used as described in test 1.

In test 3, the inoculation was done the same day as the single application of the Bio-Con at twice the recommended dosage. A previously infected stem of either pothos or peperomia together with a healthy stem were placed in the center of the pots containing unrooted healthy cuttings of either pothos or peperomia.

Truban 30W (5-ethoxy-3-(tricholoromethyl)-1,2,4-thiadiazole) was used for 2 of the treatments (tests 1 and 2) as a comparative control against the amount of inoculum used in the tests. One application of Truban (180 ppm a.i.) at 100 ml per pot was made one week before the inoculation.

During this study, both fungi were isolated from diseased tissues by plating surface-disinfested tissue (0.1% sodium hypochlorite for 4 min) on potato dextrose agar (PDA) or a selective medium (CMA-PVP) of Difco cornmeal agar containing 10 ppm pimaricin, 200 ppm vancomycin, and 100 ppm pentachloronitrobenzine (14).

Results and Discussions

Bio-Con CRC-400 applied at twice the recommended rate failed to significantly ($P \leq .05$) reduce disease incidence over the inoculated controls in either the 1:1 or 3:1 (v/v) peat:sand mixes for both host-pathogen combinations (Table 1). The results obtained with pothos in the 1 peat:1 sand (v/v) showed a failure by the Truban to control the pathogen. However, this did not occur in repeated tests. The same results, except for the Truban, were obtained using Bio-Con at the recommended rate. In test 2, using unrooted cuttings, similar results were obtained indicating Bio-Con was not protective. In test 3, Bio-Con did not protect healthy cuttings following introduction of the pathogens on infected stems (Table 2).

Treatment of rooted cuttings (test 1) with Bio-Con showed no growth stimulation over control plants in regard to stem length and number of nodes (Table 3). Fresh weight of both foliage species from test 2 in the Bio-Con control and the nontreated control were not significantly different (Table 4). Also, tissue N as determined by the micro-Kjeldahl method (7) was not affected by Bio-Con.

Baker (1) stated some of the problems involved with inoculating soils with a selected set of antagonists which will inhibit pathogens. Among these were "the microorgan-

Table 1. Effect of Bio-Con CRC-400[x] as a protectant of rooted cuttings of pothos and peperomia inoculated with *Pythium splendens* and *Phytophthora parasitica*, respectively, in two soil media after 4 weeks.

Treatment	Percent healthy plants[z] in soil media A and B[y]			
	Pothos		Peperomia	
	A	B	A	B
Control (noninoculated)	100a	100a	100a	100a
Bio-Con (noninoculated)	100a	100a	100a	100a
Bio-Con + Truban (inoculated)	89ab	94a	100a	100a
Bio-Con (inoculated)	83bc	44b	22b	33c
Control (inoculated)	61c	44b	28b	61b
Truban (inoculated)	61c	94a	100a	100a

[z]Percent healthy plants based on number of plants showing new leaf and root development divided by total plants (=18). Mean separated within columns by Standard Normal Deviate Test ($P \leq .05$).
[y]Soil medium A = 1 peat:1 sand (v/v); B = 3 peat:1 sand (v/v).
[x]Bio-Con applied two times at twice the recommended rate.

Table 2. Effect of Bio-Con CRC-400[y] as a protectant of pothos and peperomia unrooted cuttings inoculated with stems previously infected with *P. splendens* and *P. parasitica* after 4 weeks.

Treatment	Percent healthy cuttings[z] of Pothos	Peperomia
Control (noninoculated)	100	100
Control (inoculated)	61	78
Bio-Con (inoculated)	39	72

[z]Percent based on total cuttings (=18) in 6 pots; cutting determined healthy if development of new leaf and root growth present.
[y]Applied once at twice the recommended rate.

Table 3. Influence of Bio-Con CRC-400[x] on growth by rooted cuttings of Pothos and Peperomia after 4 weeks.

Treatment	Mean height[z] (cm) and number of nodes on plants in two soil media[y] Pothos A ht.	A nodes	Pothos B ht.	B nodes	Peperomia A ht.	A nodes	Peperomia B ht.	B nodes
Control	7.9	2.5	6.2	2.0	10.2	4.8	10.2	5.3
Bio-Con	7.6	2.1	4.6	2.0	8.5	4.9	9.9	4.7

[z]Mean height and nodes based on 6 pot replicates and 3 plants per pot.
[y]Soil medium A = 1 peat:1 sand v/v; soil medium B = 3 peat:1 sand v/v.
[x]Applied two times at twice the recommended rate.

Table 4. Influence of Bio-Con CRC-400[y] on growth by unrooted cuttings of pothos and peperomia after 6 weeks.

Treatment	Mean fresh weight[z] (g) change in tops of plants from initial to final weight Pothos initial (g)	Pothos final (g)	Pothos change %	Peperomia initial (g)	Peperomia final (g)	Peperomia change %
Control	6.6	14.7	123	18.0	43.8	143
Bio-Con	6.7	13.4	100	18.3	43.6	138

[z]Mean fresh weight based on 3 plants per pot and 10 pots.
[y]Applied 2 times at twice the recommended rate.

isms have a degree of specialization for different soils and pathogens, and that they must be mutually compatible among themselves." He further stated that if such microbial mixtures were ever found, their use would be restricted to horticultural situations which employed a biologically constant soil mix.

Moody & Gindrat (10) illustrated additional problems related to the actualization of biological control in practical situations. These include problems of stability and of colonization of the biological preparation by foreign microorganisms during manufacture and storage. Broadbent et al. (5), while emphasizing successful growth increases of forage, grain, vegetable, and ornamental crops resulting from seed and soil infestation with *Bacillus* spp. antagonistic to certain plant pathogens, also mention the high degree of variability encountered. For example, *Bacillus* spp. (Isolates DD32 and WW27) significantly reduced seed germination but significantly increased seedling weight while Isolates Tx1, A13, and AA43 had a nonsignificant influence on fresh weight. However, when mixed, these 5 isolates decreased shoot fresh weight significantly by 34.2% and germination by 21.8%.

The failure of the antagonists in Bio-Con CRC-400 either to reduce disease incidence or to increase growth of the pothos and peperomia control plants is indicative of either their lack of adaptation to the media employed in this investigation, the lack of ability to colonize the basal stem or root surface of these hosts, or the lack of biological activity as a competitor to the pathogenic organisms used in these tests. Whether disease control or growth enhancement is induced by this product over a wide range of hosts, pathogens, or environmental conditions remains to be ascertained by additional controlled experiments.

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