Pathogenicity of *Pythium aphanidermatum* to Chrysanthemum in Combined Inoculations with *Belonolaimus longicaudatus* or *Meloidogyne incognita*[1]

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Abstract: Rooted cuttings of 'Iceberg' chrysanthemum in steamed soil were inoculated with the nematodes *Belonolaimus longicaudatus*, and *Meloidogyne incognita*, alone and combined with *Pythium aphanidermatum*, a fungus pathogen of chrysanthemum. *B. longicaudatus* alone severely restricted the root system; with *P. aphanidermatum* also present, plant weight and height were further reduced and onset of symptoms was earlier. *M. incognita* + fungus interaction was similar but less intense. The fungus suppressed egg production of *M. incognita* but not the reproduction of *B. longicaudatus*. However, all three pathogens combined significantly suppressed reproduction of both nematodes and caused greatest inhibition of plant growth. *Key Words: Meloidogyne incognita, Belonolaimus longicaudatus, Pythium aphanidermatum*, Chrysanthemum, Pathogenicity, Fungus-nematode interactions.

The literature on interaction of plant-parasitic nematodes and plant-pathogenic fungi has been reviewed by Powell (10) and Pitcher (9). Only a few studies have involved a chrysanthemum host and most of these are on the *Meloidogyne-Fusarium* complex. Littrell and Heald (5) proved that *Meloidogyne hapla* Chitwood increased the pathogenicity of *Fusarium oxysporum* Schlecht on *Chrysanthemum morifolium* Ramat. (Hemsl.) (var. 'Yellow Delaware'). Johnson and Littrell (4) demonstrated a similar effect by *M. javanica* in the same fungus-host system.

Several *Meloidogyne* spp. (1, 4, 5, 7, 8) and the fungus *Pythium aphanidermatum* (Edson) Fitzp. (2, 12) singly parasitize *C. morifolium* causing severe growth reduction, especially in the sandy soils of the Southern Coastal Plain. No report showed the effects of *Belonolaimus longicaudatus*, Rau, alone or combined with *Pythium* on chrysanthemum. Therefore, this study was designed (i) to study the effect of *P. aphanidermatum, B. longicaudatus,* and *M. incognita* (Kofoid & White) Chitwood singly and together on *C. morifolium* and (ii) to study the effect of combination of pathogens on reproduction of each nematode species.

Materials and Methods

This study involved the interaction of *M. incognita, B. longicaudatus,* and *P. aphanidermatum* on *C. morifolium* (var. 'Iceberg').

The cuttings were planted in 15-cm clay pots containing a 2:1:4 mixture of steamed builder's sand, peat moss, and Tifton sandy loam. A nutrient solution (700 g of a commercial fertilizer mixture, VHPF®[3], 123 g of KNO_3, and 227 g of $MgSO_4$ in 84 liters of tap water) was added at the rate of 100 ml per pot each week for 2 weeks, and then 100 ml per pot semiweekly until the experiment was terminated 40 days after inoculation. Tap water was applied as needed.

Treatments, each replicated eight times, were as follows: (i) non-inoculated control; (ii) *B. longicaudatus;* (iii) *M.incognita;* (iv)

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[3] Miller Chemical Company, Baltimore 15, Maryland. VHPF® contains 6% nitrogen, 25% available phosphoric acid, 15% potash and minor elements. Mention of trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may be suitable.

P. aphanidermatum; (v) *B. longicaudatus* + *P. aphanidermatum*; (vi) *M. incognita* + *P. aphanidermatum*; and (vii) *B. longicaudatus* + *M. incognita* + *P. aphanidermatum*.

B. longicaudatus were hand-picked from soil samples from turf grasses and increased for stock cultures in the greenhouse on millet (*Pennisetum typhoideum*). Larvae of *M. incognita* obtained from tomato roots and *B. longicaudatus* were surface-disinfested in 0.001% 8-quinolinol sulfate for 30 min and then rinsed with tap water.

Nematode inoculum levels corresponded to a moderate infestation with approximately 750 (larvae and adults) or 2,000 (larvae) per pot of *B. longicaudatus* or *M. incognita*, respectively. The fungus was originally isolated from infected chrysanthemum then cultured in 500-ml potato broth cultures. The mycelia from 2-day-old (20 C) 500-ml potato broth cultures were blended in 1 liter of water, yielding a suspension approximately 1 mg (dry wt)/ml which was used to inoculate plant roots.

Plants were inoculated as follows: (i) pots were filled with soil, and a 5 × 5 cm cylindrical depression made in the surface; (ii) nematodes suspended in 10 ml of water were distributed at the periphery of the depression; (iii) plants were set into the depressions and the soil was washed gently in around the roots; and (iv) fungal inoculum was syringed into the root zone, 10 ml at each of three injection sites. The potted plants were placed in a greenhouse where the soil temperature ranged from 32–38 C during the day and 22–28 C at night. The terminal bud was removed from each plant to promote branching and 4 hr of incandescent light was provided during the night to prevent flowering.

Relative growth rates were determined by measuring the lengths of axillary branches. Symptoms were rated 1–5 (1 = no symptoms and 5 = plants dead) and recorded four times during the experiment. At harvest, roots were carefully removed from the soil, washed, blotted, and weighed. Fresh plant top weight was also recorded.

Final populations of *M. incognita* were estimated in terms of the total number of eggs produced per root system. Two, 2-g samples of chopped root fragments (about 1 cm long) with 40 ml of 10 percent NaOCl solution (Loewenberg, Sullivan, and Schuster [6]) were placed in separate 150-ml beakers and stirred for 5 min, then enough water was added to make 100 ml of suspension. Duplicate 1-ml aliquots of the suspension were placed in dishes and eggs were counted.

B. longicaudatus was extracted by carefully removing the soil from roots in 6 liters of tap water. The soil-water suspension was stirred vigorously and allowed to settle for 15 sec before a 1-liter sample was removed from the container. This suspension was sieved through a 20- and 325-mesh sieve; the sievings from the 20-mesh screen were discarded while those from the 325-mesh sieve were processed according to Jenkin's centrifugal sugar flotation method (3). Samples were then placed in calibrated counting dishes and nematodes were counted. The counts were multiplied by six to give a population estimate for each pot.

Results and Discussion

Significant nematode-fungus interactions were observed in nematode-fungus combinations. Plants infected with *M. incognita* or *B. longicaudatus* showed suppressed terminal growth beginning the second through the sixth week after inoculation (Fig. 1), decreased total plant weight (Table 1), and severely restricted root systems even though root weight was not reduced (Fig. 2-C). *M. incognita* produced no reduction in plant weight. Galls were very small, and root systems were comparable in weight with those of control plants (Fig. 2, A & E). *B. longicaudatus* with *P. aphanidermatum* reduced

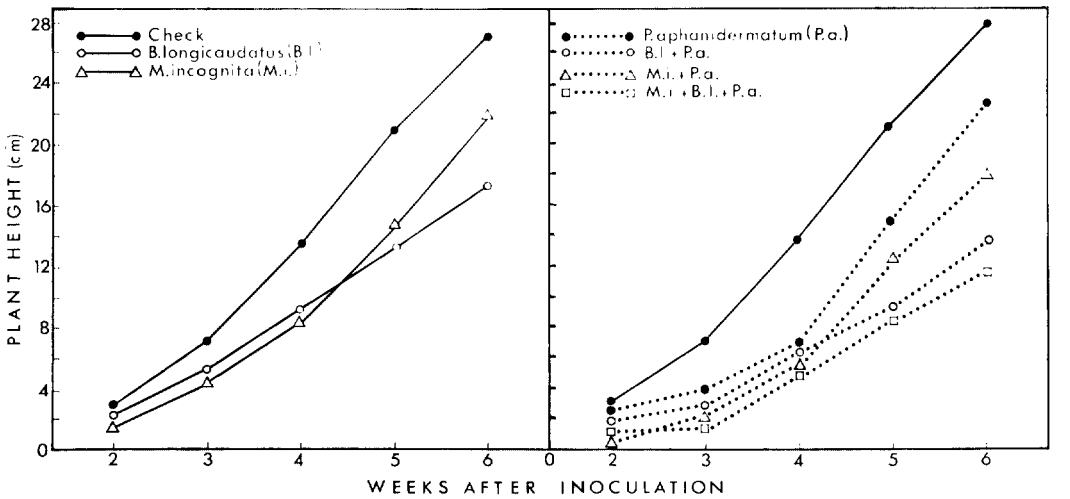

FIG. 1. Plant height measurements of 'Iceberg' chrysanthemum 2–6 weeks after single and combined inoculations with *Pythium aphanidermatum*, *Belonolaimus longicaudatus*, and *Meloidogyne incognita*.

plant height (Fig. 1), weight (Table 1), and root system size (Fig. 2, D). Root systems attacked by *B. longicaudatus* + *P. aphanidermatum* were more severely stunted and malformed than those infected by each pathogen alone (Fig. 2, B, C and D). Secondary and tertiary rootlets generally were absent, and the remaining roots were blunt and malformed. Disease symptoms were more severe and appeared earlier in combined inoculations (Table 1). The combined effects of *M. incognita* + *P. aphanidermatum* caused a greater reduction in plant height than either pathogen alone (Fig. 1). The root system was less extensive (Fig. 2, E & F), and weight was reduced significantly (Table 1). The most severe reduction in plant growth was caused by a combination of both species of nematodes with *P. aphanidermatum* (Fig. 1, Fig. 2 G). Cumulative reduction in total plant weight resulting from single inoculations with each pathogen was 66 percent, while actual reduction from combined inoculations was 74 percent. This clearly indicates a synergistic effect (Table 1).

Disease symptoms induced by *P. aphanidermatum* in this experiment were slight when compared with damage observed under field conditions. Symptoms normally ob-

TABLE 1. Disease index and percent reduction in fresh weight of 'Iceberg' chrysanthemum after inoculation singly with *Pythium aphanidermatum* and in combination with *Meloidogyne incognita* and *Belonolaimus longicaudatus*.

Treatment	Disease index[a]				% Reduction[b]	
	19[c]	26	33	40	Root wt.	Total wt.
P. aphanidermatum	1.0	1.1	2.0	2.0	45	35
M. incognita	1.0	1.0	1.0	1.0	14	12
B. longicaudatus	1.0	1.0	1.0	1.0	10	19
P. aphanidermatum + *M. incognita*	1.1	1.3	2.0	2.6	40	36
P. aphanidermatum + *B. longicaudatus*	2.3	3.0	4.0	4.2	62	65
P. aphanidermatum + *M. incognita* + *B. longicaudatus*	2.0	2.8	3.8	4.1	72	74
LSD .01 =					22	17

[a] Disease index: 1–5 where 1 = no symptoms and 5 = plants dead, mean of 8 replications.
[b] Percent reduction based on non-inoculated controls.
[c] Days after inoculation.

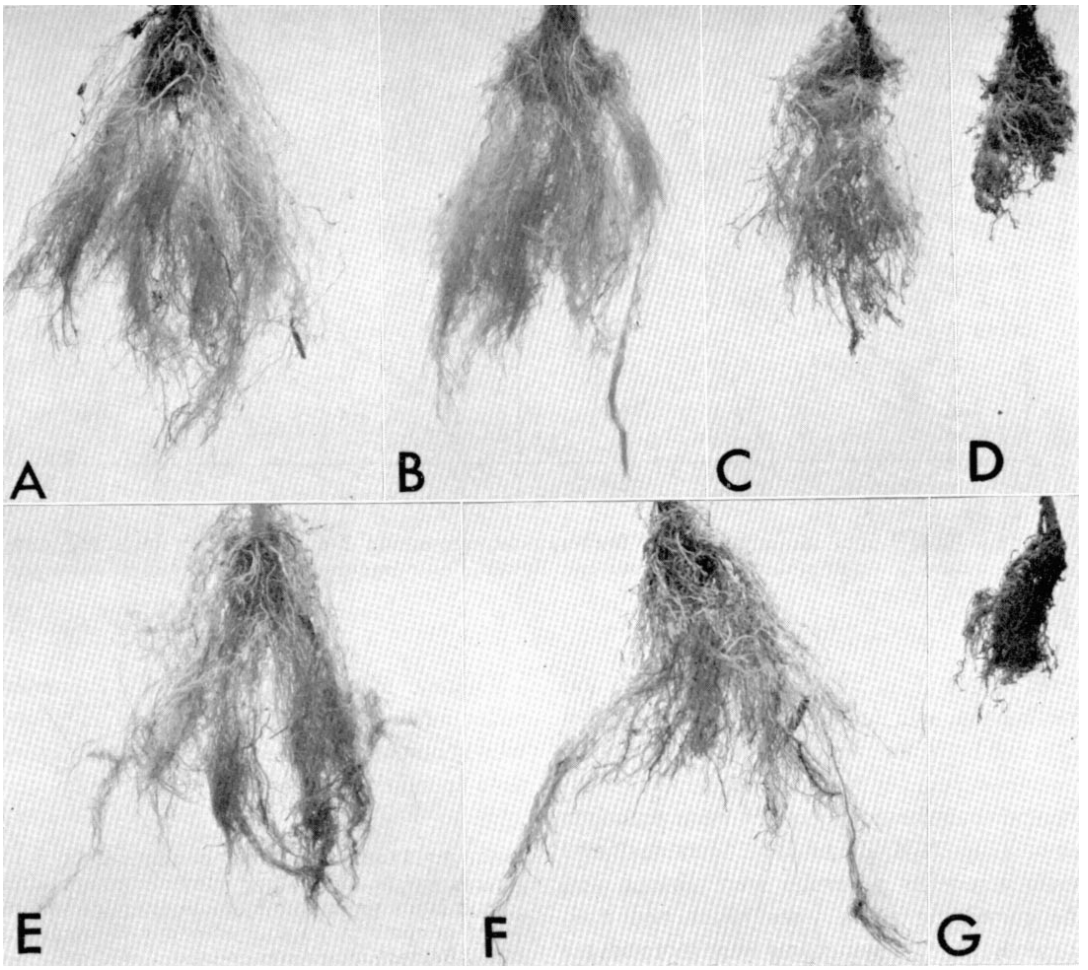

FIG. 2. Root systems of 'Iceberg' chrysanthemum 6 weeks after single and combined inoculations. **A.** non-inoculated; **B.** *Pythium aphanidermatum*; **C.** *Belonolaimus longicaudatus*; **D.** *Pythium aphanidermatum* + *Belonolaimus longicaudatus*; **E.** *Meloidogyne incognita*; **F.** *Meloidogyne incognita* + *Pythium aphanidermatum*; **G.** *Meloidogyne incognita* + *Belonolaimus longicaudatus* + *Pythium aphanidermatum*).

served are severe root and stem necrosis, with a blackening of the stem at the crown line often causes death of plants (2). This indicates that parasitic nematodes in field-grown plants may be interacting with *Pythium* spp. on roots to cause severe disease symptoms. Seldom in nature does one find only one potential pathogen present, but rather an array of microorganisms.

In addition to nematode-fungus interaction effects on disease severity, combined inoculations had varying effects on nematode reproduction. In the presence of *P. aphanidermatum M. incognita* produced fewer eggs (Table 2). Powell and Nusbaum (11) studied combined root-knot nematode and black shank fungus infections in tobacco histologically, and showed that fungus hyphae invaded giant cells and other diseased tissue in the infection court causing degeneration

TABLE 2. Reproduction of *Meloidogyne incognita* and *Belonolaimus longicaudatus* in mono-cultures and in combined inoculations with *Pythium aphanidermatum* on 'Iceberg' chrysanthemum.

Treatment	Number/plant[a]
	M. incognita[b]
M. incognita	37.2
M. incognita + *P. aphanidermatum*	3.9
M. incognita + *P. aphanidermatum* + *B. longicaudatus*	0.9
	B. longicaudatus[c]
B. longicaudatus	21.5
B. longicaudatus + *P. aphanidermatum*	17.1
B. longicaudatus + *P. aphanidermatum* + *M. incognita*	6.0

[a] *M. incognita* eggs × 1,000, *B. longicaudatus* × 100 nematodes.
[b] LSD .01 = 7.7.
[c] LSD .01 = 7.7.

of these tissues. If this relationship existed in our combined inoculations of *M. incognita* and *Pythium,* development of nematodes would be prevented, thus reducing their reproductive capability. Reproduction of *B. longicaudatus* apparently was not hindered by fungus infection of roots. Only in combined nematode inoculations with the fungus was reproduction of *B. longicaudatus* hindered (Table 2). This may be partially explained by the very limited root system remaining to support the nematode population.

Other studies were planned to gain an insight into mechanisms involved in these nematode-fungus interactions on chrysanthemum.

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