Effect of *Meloidogyne incognita, M. hapla,* and *M. javanica* on the Severity of Fusarium Wilt of Chrysanthemum[1]

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Abstract: Rooted cuttings of *Chrysanthemum morifolium* 'Yellow Delaware' (Fusarium-susceptible) and 'White Iceberg' (Fusarium-resistant) were greenhouse-grown in: (i) non-infested soil; (ii) soil infested with *Fusarium oxysporum* alone; (iii) soil infested with *Meloidogyne incognita, M. javanica* or *M. hapla*; and (iv) each nematode separately plus the fungus. All nematode species infected roots of both cultivars and caused characteristic root-knot symptoms but did not appreciably affect growth measured by plant weight. Nematodes did not break Fusarium wilt resistance of 'White Iceberg'; however, wilt symptoms appeared earlier and were more severe among 'Yellow Delaware' plants inoculated with *Meloidogyne javanica* and *F. oxysporum* than with similar combinations of the fungus and *M. incognita* or *M. hapla* or with the fungus alone.

The extensive literature on interactions of plant-parasitic nematodes and plant-pathogenic fungi has been reviewed by Powell (13) and Pitcher (11). The root-knot nematode-Fusarium complex is considered one of the most important interactions. Very little research has been published on nematode-fungus interactions in chrysanthemums. Littrell and Heald (6) demonstrated that *M. hapla,* combined with *Fusarium oxysporum* (Schlecht), increased wilt symptoms on *Chrysanthemum morifolium* 'Yellow Delaware.'

Several *Meloidogyne* spp. parasitize *C. morifolium* (2, 6, 8, 10). Therefore, we decided (i) to study the effect of *M. incognita, M. hapla,* and *M. javanica* on the severity of Fusarium wilt in a susceptible cultivar and (ii) to determine whether these species of root-knot nematode will break resistance to *F. oxysporum.*

MATERIALS AND METHODS

This study involved the interaction of three species of root-knot nematodes, *M. incognita* (Kofoid & White) Chitwood, *M. hapla* Chitwood and *M. javanica* (Treub) Chitwood; an isolate of *F. oxysporum* Schlecht. from 'Yellow Delaware' (5); and two cultivars of *C. morifolium,* 'Yellow Delaware' (Fusarium-susceptible) and 'White Iceberg' (Fusarium-resistant).

The plants were grown in steam-treated vermiculite in wooden flats for 14 days and then transferred to 15.2-cm clay pots containing a 3:1 (v/v) mixture of methyl bromide-treated (454 g/1.4 m^3) Tifton sandy loam and builder's sand sieved through a 0.64 cm screen. A nutrient solution (700 g of a commercial fertilizer mixture, VHPF®, 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 100 ml per pot biweekly until the experiment was terminated 125 days after inoculation. Tap water was applied as needed.

The treatments were as follows: (i) non-inoculated control; (ii) *F. oxysporum* alone; (iii) *M. incognita* alone; (iv) *M. hapla* alone; (v) *M. javanica* alone; (vi) *M. incognita* plus *F. oxysporum*; (vii) *M. hapla* plus *F. oxysporum,* and (viii) *M. javanica*

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plus *F. oxysporum*. Each treatment was replicated eight times.

Nematode inoculum level was approximately 1800 larvae per kg soil (corresponding to a heavy infestation) supplied via 10 well-developed egg masses per pot hand picked from 50-day-old populations on heavily-galled tomato roots.

Inoculum levels were approximately 3800, 3600, and 2840 eggs per pot of *M. incognita, M. hapla,* and *M. javanica,* respectively. The number of eggs per egg mass were estimated by the sodium hypochlorite method described by Loewenberg, Sullivan, and Schuster (7). Plants were inoculated as follows: (i) pots were filled with soil, and a 5 × 5 cm depression made at the surface; (ii) egg masses in 50 ml of water were distributed at the periphery of the depression; (iii) roots of plants lifted from the flats of vermiculite were dipped into undiluted fungus inoculum obtained by growing the fungus for 4 days at 28 C in Hoagland's No. 2 nutrient solution with 2% glucose; and (iv) the plants were then set into the depressions and the soil washed gently in around the roots. The potted plants were placed in a greenhouse where the temperature was maintained at 18 to 28 C. The terminal bud was removed from each plant to promote branching. Artificial light was provided for 4 hr during the dark period and discontinued after 6 weeks to allow flowering.

Relative growth rates were determined by measuring growth of axillary branches. Fusarium wilt symptoms were expressed as a wilt index (Table 2) and recorded six times during the experiment. At the conclusion of the experiment, roots were carefully freed from soil, washed, blotted and weighed. Root gall indices were rated as described in Table 1.

RESULTS AND DISCUSSION

All nematode species infected 'Yellow Delaware' and 'White Iceberg' and produced characteristic root-knot symptoms. *M. incognita* galled roots of both cultivars more severely than the two other nematodes (Table 1). A higher percentage of galling developed on 'Yellow Delaware' plants inoculated with the fungus plus nematodes of each species than when inoculated with each nematode alone. This may have been due to the more limited root system of plants inoculated with the fungus. Also, *F. oxysporum* may have predisposed plants to nematode attack.

The galling of 'White Iceberg' plants was not significantly different whether the nematodes were acting alone or in combination with the fungus. Apparently the galling reaction was independent of the presence of the fungus around resistant roots.

Inoculations with nematodes and *Fusarium* combined did not break wilt resistance in 'White Iceberg.' Wilt symptoms appeared earlier and were more severe on 'Yellow Delaware' inoculated with *M. javanica* and *F. oxysporum* than with the fungus alone or in combination with the other nematodes (Table 2). On 'Yellow Delaware' *M. hapla* did not increase wilt severity over that caused

TABLE 1. Mean root-gall indices of two chrysanthemum cultivars inoculated with *Meloidogyne incognita, M. hapla,* and *M. javanica* singly and combined with *Fusarium oxysporum*.

	Mean root-gall indices[a]					
Cultivar	M.i.[b]	M.h.	M.j.	M.i. + F	M.h. + F	M.j. + F
Yellow Delaware	3.7	2.8	2.7	4.7	4.6	4.5
White Iceberg	5.0	2.1	2.3	4.7	2.2	2.2

LSD .05 for two treatment means on the same cultivar 0.5

[a] Root-gall index, mean of 8 replications: 1 = no galls, 2 = 1–25%, 3 = 25–50%, 4 = 50–75%, and 5 = 75–100% of roots galled.

[b] M.i., M.h., M.j. and F = *M. incognita, M. hapla, M. javanica,* and *F. oxysporum,* respectively.

TABLE 2. Effect of *Fusarium oxysporum* singly and combined with *Meloidogyne incognita, M. hapla,* and *M. javanica* on severity of Fusarium wilt of 'Yellow Delaware' chrysanthemum.

Treatment[a]	Weeks after inoculation					
	4	5	6	9	11	18
Control	1.0[b]	1.0	1.0	1.0	1.0	1.0
F. oxysporum	1.0	2.5	3.0	4.5	4.6	4.7
M.i. + F	1.0	2.5	2.8	4.6	4.8	5.0
M.h. + F	1.0	2.5	2.7	4.1	4.2	4.4
M.j. + F	2.0	3.3	3.7	4.8	4.8	5.0

[a] F., M.i., M.h., and M.j. = *F. oxysporum, M. incognita, M. hapla,* and *M. javanica,* respectively.

[b] Relative wilt index, mean of 8 replications: 1 = no symptoms, 2 = light yellowing, 3 = moderate yellowing, 4 = severe browning, and 5 = dead; 'White Iceberg' plants in all treatments received a wilt index of 1.0.

by *F. oxysporum* alone. Results of this test were not in agreement with those of Littrell and Heald (6), who demonstrated that *M. hapla* increased severity of wilt. They used a different population of *M. hapla* and also reported a temperature range of 21–31 C, slightly higher than in this study. These may be important variables.

Plant growth responses are presented graphically in Figure 1. 'White Iceberg' plants were generally less stunted by nematodes alone and in combinations with the fungus than 'Yellow Delaware' plants. In most cases early stunting was either absent or slight, and this trend continued throughout the experiment. With *M. incognita* alone on 'White Iceberg,' a slight stimulation of plant height 3 weeks after inoculation was followed by a decline after 8 weeks as compared to control plants. Early stunting of 'White Iceberg' plants inoculated with *M. hapla* and *M. javanica* alone was followed by more severe stunting 6 weeks after inoculation. In most cases, marked early stunting occurred in 'Yellow Delaware' plants inocu-

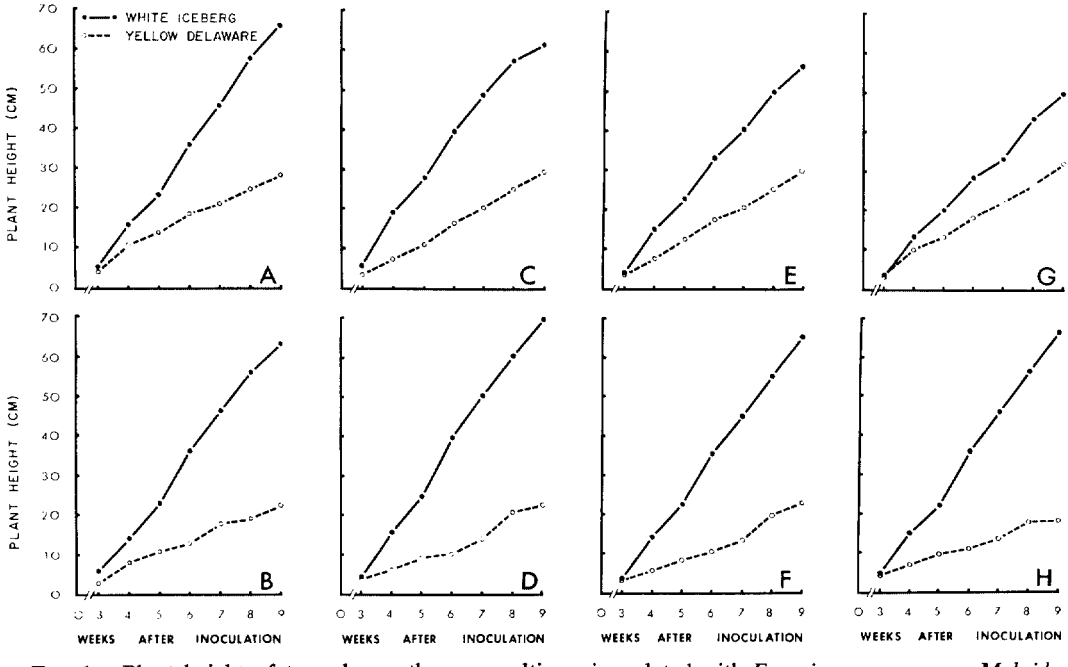

FIG. 1. Plant height of two chrysanthemum cultivars inoculated with *Fusarium oxysporum, Meloidogyne incognita, M. hapla,* and *M. javanica*: (A) Control—no inoculum, (B) *F. oxysporum,* (C) *M. incognita,* (D) *M. incognita* + *F. oxysporum,* (E) *M. hapla,* (F) *M. hapla* + *F. oxysporum,* (G) *M. javanica,* and (H) *M. javanica* + *F. oxysporum.*

lated with *F. oxysporum* alone and *M. incognita, M. hapla,* or *M. javanica* plus *F. oxysporum.*

In most nematode-Fusarium disease complexes reported in the literature, nematodes increased wilting in both wilt-resistant and susceptible plants (1, 3, 4, 9, 12). Porter and Powell (12) reported that wilting of tobacco plants was more severe when nematodes were applied 2 or 4 weeks prior to the fungus. They suggested that some change in host physiology was necessary before the fungus could become established. Apparently nematode-fungus interactions depend on the host and nematode species involved. We found that only *M. javanica* increased the appearance and severity of wilt. Bowman and Bloom (1) demonstrated that *M. incognita* broke resistance to Fusarium wilt in tomato when the nematodes were applied 2 weeks prior to fungal inoculation. In our study neither *M. incognita, M. hapla,* nor *M. javanica* broke resistance to Fusarium wilt in 'White Iceberg.' Further investigations are planned to study the effects on wilt resistant and susceptible cultivars of chrysanthemum when root-knot nematodes are applied prior to fungal inoculation.

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