Effects of *Meloidogyne hapla* and *Macroposthonia ornata* on Cylindrocladium Black Rot of Peanut

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ABSTRACT

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Two peanut cultivars, four microsclerotial (ms) inoculum densities of *Cylindrocladium crotalariae* (0, 0.5, 5, and 50 ms per cubic centimeter of soil for Cylindrocladium black rot (CBR)-resistant NC 3033 or 0, 0.025, 0.25, and 2.5 ms per cubic centimeter of soil for CBR-susceptible Florigiant) and three inoculum densities of *Meloidogyne hapla* eggs or *Macroposthonia ornata* larvae and adult mixtures (0, 10^3, and 10^4 per 15-cm-diameter clay pot) were tested in all possible combinations in greenhouse factorial experiments. CBR severity on both NC 3033 and Florigiant plants increased in the presence of 10^3 and 10^4 *M. hapla* eggs. *M. ornata* increased disease severity at 10^4 nematodes per pot with 0.25 and 2.5 ms per cm^3 soil on Florigiant, but failed to affect the disease syndrome on NC 3033 in all combinations tested. Positions, but not the slopes of inoculum density-disease curves were changed by *M. hapla* on both peanut cultivars and by *M. ornata* on Florigiant only. ED_{50} values for *C. crotalariae* inocula decreased as nematode density increased with all combinations except those of *M. ornata* on NC 3033. Artificial injuries in the apical meristem region before inoculation with two ms led to more infections than when roots were either uninjured or injured further from the growth zone, suggesting that wounding may be an important factor in these interactions.

Since it was first described in 1966 by Bell and Sobers in Georgia (4), Cylindrocladium black rot (CBR), which is caused in peanut by *Cylindrocladium crotalariae* (Loos) Bell and Sobers (perfect stage *Calonectria crotalariae* (Loos) Bell and Sobers), has appeared in most peanut (*Arachis hypogea* L.)-growing areas of the southeastern United States. Although chemical control of CBR on peanut was not effective, resistance to *C. crotalariae* was reported (12,19). Intensive effort has been directed towards the development of commercially acceptable CBR-resistant peanut cultivars.

Sasser et al (16) reported nine plant-parasitic nematode species on peanut in North Carolina including *Meloidogyne hapla,*

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Macroposthonia ornata, Belonolaimus longicaudatus, Paratrichodorus minor, Helicotylenchus dihystera, Pratylenchus brachyurus, Hoplolaimus galeatus, Tylenchorhynchus claytoni, and *Xiphinema americanum.* These authors found negative correlations between populations of most of these nematodes and peanut yields. In a 1976 survey of 331 peanut fields in 17 counties of southwest Georgia, Motsinger et al (11) found *M. ornata* and *Meloidogyne* spp. in 97.0 and 9.7%, respectively, of fields sampled. *Meloidogyne* populations were 69% *M. hapla*, 25% *M. arenaria*, and 6% mixtures. Minton and Bell (10) observed severe discoloration and brown necrotic lesions on roots, pods, and pegs of plants of peanut cultivars Argentine and Starr due to *M. ornata* in a microplot experiment. Pod yield was reduced 50% by *M. ornata.*

Although the study of plant disease complexes has received much attention during the past two decades, only limited numbers of these investigations have included peanut or *C. crotalariae.* The purpose of this study was to determine: possible interactions between two of the major nematode pathogens of peanut crops in North Carolina (*M. hapla* Chitwood and *M. ornata* (Tayler) De Grisse) and *C. crotalariae* on CBR-resistant and CBR-susceptible peanut cultivars and the role of wounding by the nematodes in development of CBR on peanut.

MATERIALS AND METHODS

Nematodes. *M.hapla* and *M. ornata* populations were obtained from K. R. Barker, North Carolina State University, Raleigh. *M. hapla* inoculum was produced on tomato (*Lycopersicon esculentum* 'Rutgers') and eggs were extracted by the method described by Hussey and Barker (7). *M. ornata* inoculum was produced on corn (*Zea mays*) and extracted by centrifugal flotation (8). The isolate of *C. crotalariae* that was used originally was isolated from CBR-infected peanut roots in eastern North Carolina. Microsclerotial inoculum (ms) was produced on potato-dextrose agar (PDA), extracted, and standardized as described by Phipps et al (13). Peanut cultivars included were NC 3033 (CBR-resistant) and Florigiant (CBR-susceptible).

Inoculation tests. In tests involving simultaneous inoculations of *C. crotalariae* and nematodes, 0, 1,000, and 10,000 nematodes (eggs for *M. hapla* and mixtures of larvae and adults of *M. ornata*) and 0, 0.5, 5, and 50 ms per cubic centimeter of soil (for NC 3033) or 0, 0.025, 0.25, and 2.5 per cubic centimeter of soil (for Florigiant) were mixed in all possible combinations in a five-replication randomized complete block (RCB) arranged in a 3×4 factorial arrangement. Nematode and microsclerotial inocula were pipetted onto and manually mixed for 1 min with 1,400 cm^3 of steamed (84 C for 30 min) sand:sandy loam (3:2, v/v) in polyethylene bags. The mixture was placed in 15-cm-diameter clay pots and two peanut seeds were planted in each one.

In sequential inoculation tests, nematodes were added to the sand-soil mixture 2 wk prior to the addition of *C. crotalariae.* Approximately 450 cm^3 of soil with which *M. hapla* eggs or *M. ornata* were mixed, were placed in the bottom of the pot, and two 10-mm-diameter open-end glass tubes were inserted vertically opposite each other. Another 450 cm^3 of soil was added and two other tubes were inserted in such a way that they formed a plane perpendicular to that of the first two. The remaining 500 cm^3 of soil was added. Ms suspensions were pipetted 2 wk later into the tubes so that about 25% of the inoculum was introduced in each tube. Four tests were performed with NC 3033; two with *M. hapla* (one simultaneous and one sequential) and the remaining two with *M. ornata* (one simultaneous and one sequential). Three tests were performed on Florigiant; two with *M. ornata* (one simultaneous and one sequential) and the other one a simultaneous test with *M. hapla.* Disease progression was recorded for 8 wk on the basis of aboveground symptoms, which included wilting and death of main stem. Roots were washed free of soil, weighed, and rated for rot on a 0–5 scale in which 0 = no apparent rot and 5 = maximum rot (12). Shoot weights and final nematode populations also were determined. Final populations of *M. hapla* (juveniles) and *M. ornata* were obtained by mixing the contents of each pot with 4,000 ml of water. Subsamples (500 ml) were poured on two nested (420-μm over 38-μm) sieves. The material collected on the 38-μm sieve was centrifuged (5). *M. hapla* eggs were extracted as previously mentioned.

Wounding experiment. Two 1-wk-old Florigiant or NC 3033 seedlings were transplanted into a sterile sand:sandy loam (3:2, v/v) mixture over a 2- to 3-cm-thick vermiculite cushion in a lidded transparent plastic box with outlets for the shoot at one end and for drainage water at the other. The box was closed, wrapped in aluminum foil, and slanted over the lid side (inclination ~45 degrees) to encourage root growth against the lid. The box was carefully opened 7 days later to expose the growing roots and the following treatments were applied: two ms (collected on two nested sieves, 246 μm over 147 μm) were placed at the surface of intact lateral roots (about 0.5–1.0 mm in diameter) approximately 3 mm behind the root cap (hereafter referred to as tip inoculation); two ms in the same size range were incorporated in a 1-mm-diameter PDA disk and the agar disk was placed against the root tip 3 mm behind the root cap (hereafter referred to as tip nutrient inoculation); three fine punctures were made with thin needles 3 mm behind the root cap (hereafter referred to as tip wound + inoculation) and two ms were placed in the injured area. The same three treatments were applied to mature portions of main lateral roots selected at random (hereafter referred to as nontip treatments). Treatments were coded with colored pins inserted in close proximity to the treated spots. After completion of the inoculations, boxes were tightly closed and placed in the greenhouse at 25 ± 2 C. Two boxes were used per cultivar and five replications of each treatment were included per box. One week later, the boxes were reopened, the number of successful infections (rotted areas) was recorded, and each inoculation site was rated on a scale of 0–2 in which 0 = no apparent infection, 1 = visible infections <1 mm in length and 2 = visible infections >1 mm in length.

Statistical analyses. Analysis of variance (anova) were

TABLE 1. Effects of *Meloidogyne hapla* and *Macroposthonia ornata* on the severity of Cylindrocladium root rot at various inoculum density combinations on two peanut cultivars[y]

Treatment		Root rot per nematode level[z]					
		M. hapla (egg per pot)			*M. ornata* (larvae per pot)		
Cultivars	Fungal inoculum (ms per cm^3)	0	10^3	10^4	0	10^3	10^4
Florigiant	0.0	0.0 a	0.2 a	0.4 ab	0.0 a	0.6 ab	0.8 abc
	0.025	0.9 ab	0.5 ab	1.9 cd	0.9 bcd	1.5 cd	1.7 d
	0.25	2.3 cd	3.4 de	3.8 ef	2.9 e	3.2 e	4.5 f
	2.5	4.1 ef	4.4 ef	5.0 f	3.3 e	4.3 f	4.8 f
NC 3033	0.0	0.0 j	0.2 j	0.4 j	0.0 j	0.0 j	0.0 j
	0.5	0.4 j	0.6 j	1.5 k	0.5 j	0.6 j	0.3 j
	5.0	1.7 kl	2.4 l	3.6 m	2.2 k	2.6 k	2.8 kl
	50.0	3.4 m	3.7 m	5.0 n	3.8 lm	3.9 m	3.8 lm

[y] For a given nematode species on a given peanut cultivar, means followed by different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.

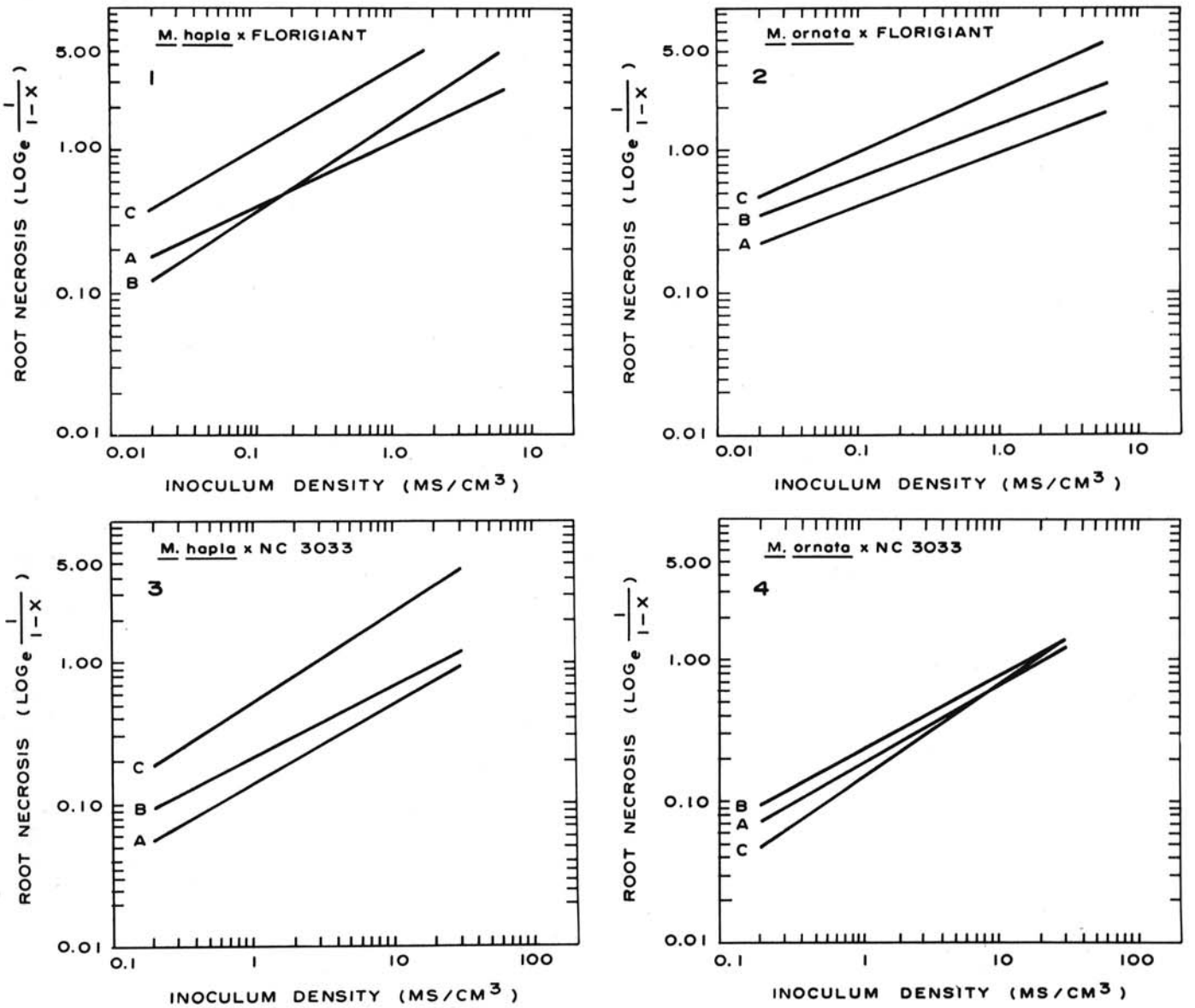
[z] Root rot was rated on a 0–5 scale in which 0 = no apparent rot and 5 = maximum rot.

performed according to appropriate models for 3 × 4 factorial arrangement in RCB design allowing significance tests for nematode and fungus main effects as well as for nematode × fungus two-factor interactions. Pertinent orthogonal contrasts were tested for significance and response curves were analyzed. Since simultaneous and sequential inoculations did not yield significantly different results, data from those treatments subsequently were combined to double the number of replications. A log-log transformation was performed with Gregory's multiple infection correction followed by a linear regression analysis (1). Slopes of inoculum density-disease curves were tested for homogeneity. Inoculum densities required for 50% disease (ED_{50}) were graphically determined for each nematode level on each peanut cultivar.

RESULTS

CBR-susceptible Florigiant. CBR severity increased as ms density increased from 0.025 to 2.5 ms per cubic centimeter of soil in the absence of nematodes (Table 1). Disease severity of 0.025 ms per cubic centimeter of soil was greater ($P = 0.05$) in the presence of 10^4 *M. hapla* eggs per pot than in the absence of nematodes. Similarly, disease severity was greater in the presence of 10^3 and 10^4 eggs per pot at 0.25 ms per cubic centimeter of soil than in the absence of nematodes. At 2.5 ms per cubic centimeter of soil, disease was so severe that the effect of *M. hapla* was not apparent. Disease severity at 10^3 and 10^4 eggs per pot with 0.25 ms per cubic centimeter of soil was similar ($P = 0.05$) to disease at 2.5 ms per cubic centimeter of soil in the absence of nematodes. Slope values of inoculum density-disease curves in the absence or presence of *M. hapla* did not differ ($P = 0.05$) but the position of the curve in the presence of 10^4 eggs per pot was different ($P = 0.05$) from that for treatment groups that received 10^3 eggs per pot and in the absence of nematodes (Figs. 1–4). Inoculum densities required for ED_{50} were 0.35, 0.29, and 0.05 ms per cubic centimeter of soil in fungus-only soil, 10^3, and 10^4 *M. hapla* eggs per pot, respectively (Table 2). Number of *M. hapla* eggs and larvae per pot 8 wk after inoculation of plants with *C. crotalariae* decreased ($P = 0.05$) as ms per cubic centimeter of soil was increased at all ms densities at both 10^3 and 10^4 eggs per pot (Table 3).

CBR severity also was increased in the presence of *M. ornata* (Table 1). However, no effect of the nematode was observed at 0.025 ms per cubic centimeter of soil. Disease was greater ($P = 0.05$) at 0.25 ms per cubic centimeter of soil with 10^4 *M. ornata* per pot than in the absence of nematodes. Disease also was greater at 2.5 ms



Figs. 1–4. Relation of peanut root necrosis severity and *Cylindrocladium crotalariae* inoculum density in the presence of **A,** 0, **B,** 10^3 or **C,** 10^4 eggs of *Meloidogyne hapla* or larvae and adults of *Macroposthonia ornata* per pot. Slopes of the curves do not differ ($P = 0.05$) within individual figures. Inoculum efficiency (position of curve) increased in treatment groups that received nematodes (Figs. 1C, 2B and C, and 3C) compared to those not infested with nematodes.

per cubic centimeter of soil with both 10^3 and 10^4 *M. ornata* per pot than in the absence of nematodes. Slope values of inoculum density-disease curves in the absence or presence of *M. ornata* did not differ ($P = 0.05$), but the position of the curve involving 10^4 *M. ornata* differed from that of the 10^3 *M. ornata* curve, which in turn was different from the curve generated when nematodes were not present (Fig. 2). The ED_{50} values were 0.42, 0.13, and 0.05 in soil not infested with nematodes and those containing 10^3 and 10^4 *M. ornata* per pot, respectively (Table 2). Number of *M. ornata* per pot 8 wk after inoculation with *C. crotalariae* was less ($P = 0.05$) for all ms densities at 10^3 nematodes per pot (Table 3). At 10^4 *M. ornata* per pot, number of nematodes recovered from pots decreased as ms per cubic centimeter of soil was increased. However, pots with 0.25 and 2.5 ms per cubic centimeter of soil had similar numbers of *M. ornata*.

With both nematodes, significant ($P = 0.05$) fungus × nematode two-factor interaction and significant nematode and fungus main effects ($P = 0.05$) occurred. The incubation period was shortened in the presence of both densities of both nematodes than with *C. crotalariae* alone (Fig. 5). The highest number of diseased plants occurred with sequential inoculation 10^4 *M. ornata* per pot at 2.5 ms per cubic centimeter of soil.

CBR-resistant NC 3033. Although *C. crotalariae* inoculum densities were greater by 20-fold at each level compared to Florigiant tests, disease enhancement in the presence of *M. hapla* was similar to that observed with Florigiant (Table 1). CBR severity increased as ms density increased from 0.5 to 50.0 ms per cubic centimeter of soil in the absence of nematodes. While the presence of 10^3 *M. hapla* eggs per pot did not increase ($P = 0.05$) CBR severity, disease severity increased at all levels of ms per cubic centimeter of soil at 10^4 *M. hapla* eggs per pot. CBR severity at 10^4 *M. hapla* per pot with 0.5 ms per cubic centimeter of soil was equal to CBR severity at 5.0 ms per cubic centimeter of soil without nematodes. Similarly, disease severity at 10^4 *M. hapla* per pot with 5.0 ms per cubic centimeter of soil was equal to 50.0 ms per cubic centimeter of soil with or without nematodes. Slope values of inoculum density-disease curves in the absence or presence of *M. hapla* did not differ ($P = 0.05$) but the position of the curve in the presence of 10^4 eggs per pot was different ($P = 0.05$) from that representing 10^3 eggs per pot and in the absence of nematodes (Fig. 3). The ED_{50} values were 17.5, 10.1, and 1.6 ms per cubic centimeter of soil in soil not infested with nematodes (fungus-only soil), 10^3, and 10^4 *M. hapla* eggs per pot, respectively (Table 2). Number of *M. hapla* eggs and larvae per pot 8 wk after inoculation of plants with *C. crotalariae* was less ($P = 0.05$) at 5.0 and 50.0 ms per cubic centimeter of soil than at 0.5 ms per cubic centimeter of soil or in nematode-only soil (Table 3).

No enhancement ($P = 0.05$) of CBR severity on NC 3033 was observed in the presence of 10^3 and 10^4 *M. ornata* per pot at any ms densities tested (Table 1). However, at 10^4 *M. ornata* per pot with 5.0 ms per cubic centimeter of soil, CBR severity was similar to that in soil infested with 50.0 ms per cubic centimeter of soil in the absence of nematodes. Slope values and position of inoculum

TABLE 2. *Cylindrocladium crotalariae* inoculum density[y] required for 50% disease (ED_{50}) on peanut cultivars NC 3033 and Florigiant in the presence of *Meloidogyne hapla* or *Macroposthonia ornata*

Nematode inoculum[z]	NC 3033 (*M. hapla*)	Florigiant (*M. hapla*)	Florigiant (*M. ornata*)
	ED_{50}	ED_{50}	ED_{50}
0	17.5	0.35	0.42
10^3	10.1	0.29	0.13
10^4	10.1	0.29	0.13
10^4	1.6	0.05	0.05

[y] Microsclerotia of *C. crotalariae* per gram of soil.
[z] Values for *M. hapla* are eggs per pot. Values for *M. ornata* are larvae and adult nematodes per pot.

TABLE 3. Effects of *Cylindrocladium crotalariae* on reproduction of *Meloidogyne hapla* and *Macroposthonia ornata* on two peanut cultivars

Treatments		Nematode numbers in thousands[x]					
		M. hapla[y] (eggs per pot)			*M. ornata*[z] (larvae per pot)		
Peanut cultivars	Fungal inoculum (ms per cm^3)	0	10^3	10^4	0	10^3	10^4
NC 3033	0.0	0	0.79 a	6.98 cd	0	8.08 a	48.1 c
	0.5	0	0.723 ab	7.24 c	0	8.03 a	32.8 d
	5.0	0	0.366 b	4.142 de	0	7.36 ab	18.3 e
	50.0	0	0.223 b	2.336 e	0	4.384 b	8.8 f
Florigiant	0.0	0	9.088 k	51.072 p	0	4.0 k	45.504 p
	0.025	0	6.432 l	38.46 q	0	1.8 l	29.696 q
	0.25	0	2.624 m	25.728 r	0	1.3 l	8.512 r
	2.5	0	0.992 n	4.294 s	0	1.4 l	3.6 r

[x] In each column for a given peanut cultivar, means followed by different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.
[y] *Meloidogyne hapla* inoculum densities: larvae only on NC 3033; larva + eggs on Florigiant.
[z] Inoculum densities of *Macroposthonia ornata*.

TABLE 4. Effect of root wounding and nutrient amendment on inoculation of two peanut cultivars with *Cylindrocladium crotalariae* after 2 wk[w]

Root parts inoculated	Inoculation treatment[x]	Percent successful infections		Lesion index[z]	
		Flori[y]	NC 3033	Flori[y]	NC 3033
Tip	None	10	0	0.1 a	0.0 a
	Nutrient	10	10	0.2 a	0.1 a
	Wounded	100	100	1.9 c	1.7 c
Nontip	None	10	0	0.2 a	0.0 a
	Nutrient	0	10	0.0 a	0.1 a
	Wounded	100	90	1.8 c	1.2 b

[w] Means followed by different letters are significantly different, $P = 0.05$, according to an LSD comparison.
[x] Nutrient consisted of a 1-mm-diameter PDA plug in which two microsclerotia were incorporated. Wounds were performed with thin pins.
[y] Flori = cultivar Florigiant.
[z] Based on a 0–2 scale in which 0 = no visible indication of infection; 1 = rotted area <1 mm in length; and 2 = rotted area >1 mm in length.

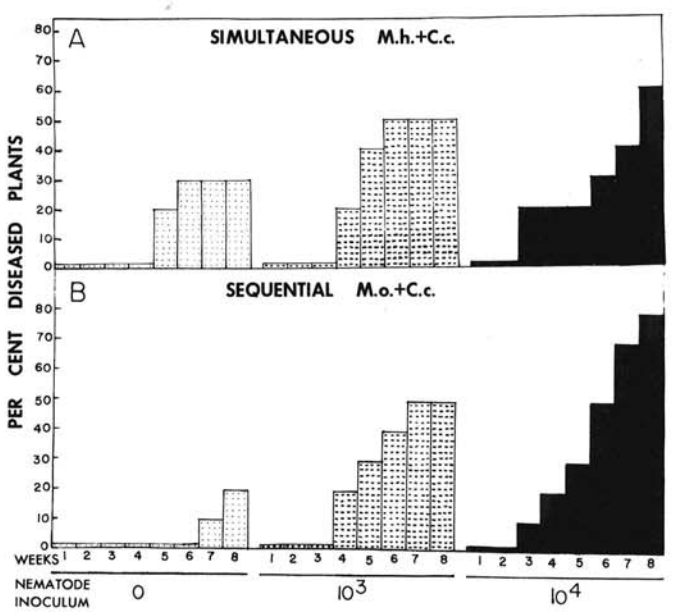


Fig. 5. Incidence of disease in Florigiant peanut plants (stem and foliage symptoms) grown in the presence of selected densities of *Meloidogyne hapla* (M.h.) eggs, *Macroposthonia ornata* (M.o.) larvae and adults, and *Cylindrocladium crotalariae* (C.c.) microsclerotia (ms) at 2.5 ms per cubic centimeter of soil. **A,** Simultaneous inoculation of plants with *M. hapla* and *C. crotalariae*. **B,** Sequential inoculation of plants with *M. ornata* and *C. crotalariae*.

density-disease curves were similar for all treatments (Fig. 4). The ED_{50} values were 11.0, 8.5, and 10.5 in fungus-only soil, 10^3 and 10^4 *M. ornata* per pot, respectively. Number of *M. ornata* per pot 8 wk after inoculation with *C. crotalariae* was less ($P = 0.05$) for all ms densities at 10^4 nematodes per pot (Table 3). This effect was less pronounced in the 10^3 *M. ornata* per pot treatments.

Wounding effect. Number of successful infections and lesion development was greater ($P = 0.05$) on roots wounded prior to inoculation with two ms than on nonwounded roots or in the presence of nutrient plugs (Table 4). The greatest percentage of successful infection and largest lesions resulted from the tip wound treatment (root apical meristem). The next most effective technique was the nontip wound, while nutrient amendment and placement of ms on nonwounded tissues were the least effective. Although a high percentage of nontip wound treatments caused lesions on both Florigiant and NC 3033, lesion development on older roots was less ($P = 0.05$) on NC 3033 than on Florigiant.

DISCUSSION

The factorial approach to pathogen interaction studies is an extremely useful technique that has not been adequately applied to most studies of this type. In this growing subdiscipline of plant pathology, it has become almost standard to say "fungus, nematode, both or neither" to refer to the experimental setup. Although this expression implies a valid 2 × 2 factorial arrangement, the usual number of inoculum combinations included in most tests is probably insufficient since countless population density conditions are likely to exist and eventually interact in the field. Factorial sets including three, four, or more densities of each organism should certainly give a better insight into the epidemiology of interaction problems.

Despite the independent pathogenic capabilities of *C. crotalariae* on peanut, greenhouse studies revealed an interaction (enhancement of CBR) between *M. hapla* and *C. crotalariae* on both CBR-susceptible Florigiant and CBR-resistant NC 3033. An interaction also was observed between *C. crotalariae* and *M. ornata* on CBR-susceptible Florigiant but not on the CBR-resistant NC 3033. The use of a different range of microsclerotial densities on the two cultivars was necessitated by the extreme degree of susceptibility of Florigiant to *C. crotalariae*. However, increased efficiency of *C. crotalariae* inoculum was demonstrated in the presence of *M. hapla* with both Florigiant and NC 3033. The ED_{50} values for Florigiant and NC 3033 were sevenfold and 12-fold greater, respectively, in soil not infested with nematodes compared to soil containing *C. crotalariae* and *M. hapla* (Table 2). A similar relationship was evident with *M. ornata* and Florigiant.

If we apply Vanderplank's principle of two-paths-to-susceptibility (17) to the present data, it appears that *M. hapla* increased both the amount of susceptible tissue (N), and the ease with which such tissue can be infected by *C. crotalariae* (a) on both Florigiant and NC 3033, whereas *M. ornata* apparently increased only a on Florigiant. Johnston (9) observed that fibrous roots of Florigiant are more susceptible to *C. crotalariae* than hypocotyls that may produce secondary periderm in response to infection. Root galls induced by *M. hapla* frequently are associated with extensive fibrous roots, providing more tissues to be infected by *C. crotalariae*. Baker (1) in his treatment of inoculum potential, stated that "the more roots, the greater proportion that encounter inoculum, and the more infection." Here, each extra fibrous root is an additional moving infection court that may encounter a microsclerotium that might not otherwise have taken part in the total infection. The probability for such an encounter increases with increasing ms density. In addition to providing extra tissues to be infected, *M. hapla* also may increase the nutrient supply both in the rhizophere and within the galls (2) for *C. crotalariae*. Johnston (9) reported that exogenous nutrient stimulates *C. crotalariae* infection. Not only did *M. hapla* enhance CBR on both cultivars, but also there was no apparent difference in the number of *M. hapla* induced galls between Florigiant and NC 3033.

Several mechanisms have been considered to explain the increased susceptibility of many nematode-infected plants to certain fungal pathogens (4,14). Wounding by the nematode (providing an entrance route for the fungus) was considered important in increasing susceptibility to various fungi for a long time (4). Powell (14), however, proposed that the increased capacity of certain *Meloidogyne* spp. to enhance Fusarium wilt on tobacco, when the nematode preceded the fungus by a few weeks, is an indication of more elaborate mechanisms. Most artificial wounding in these types of tests does not realistically mimic nematode injury. Passing a sharpened spatula through the root system in inoculation tubes has routinely been practiced (2). Such procedures may excise entire root segments, exposing their vascular systems.

The technique described here for mechanical injury, though not perfect, seems promising for the elucidation of many root pathogen phenomena. Mechanical injury behind the root cap using thin pins more closely mimics mechanical injuries associated with the penetration of juveniles of *Meloidogyne* spp. Although the actual size of the injuries was not controlled, the tip-wound treatment gave more successful infections when followed by inoculation with ms. The fact that wounded mature roots or root tips were more frequently infected by the fungus than nonwounded tips suggests that wounding due to the nematodes may have played an important role in the disease enhancement that was observed. However, the failure of *M. ornata* to affect CBR severity on NC 3033 seems to indicate that other mechanisms also were involved since *M. ornata* certainly does wound functional NC 3033 roots during its feeding. It is possible that the tissues wounded by *M. ornata* were more resistant to *C. crotalariae* invasion in NC 3033 than in Florigiant. Hirano (6), in his review of nematode-fungus studies in Japan, reports that injury from the penetration of one larva of *M. incognita* did not favor mycelium penetration of *Fusarium oxysporum* into tomato roots, but that injury resulting from larvae crowded in the meristematic region favored infection. Wounding by *M. hapla* larvae, whether initial (due to larval penetration) or final (due to the extrusion of egg masses by mature females) as well as increased quantity of fibrous roots, probably played a significant role in the interactions observed in this study (Fig. 6). Studies of the biochemical alterations within root-knot-induced giant cells or in the exudates of galled roots (18) have made a tremendous contribution to our understanding of disease complex problems. It seems that physical injuries and root proliferation interact with the biochemical and probably the physiological changes involved in these phenomena as suggested by the statement of Van Gundy, "....the rupturing of cortical cells by the nematode body at 28–35

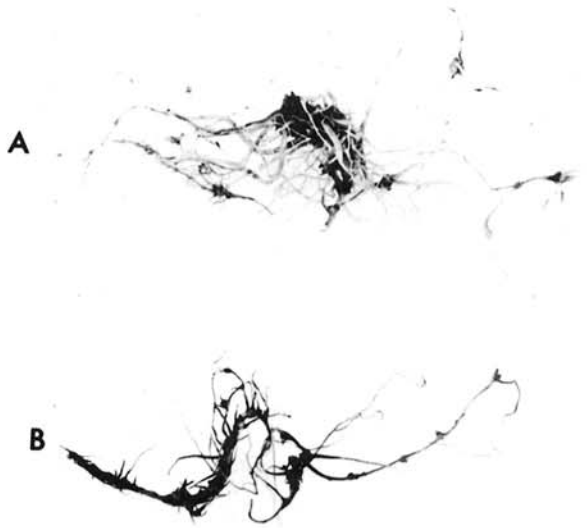


Fig. 6. Effects of inoculating cultivar NC 3033 plants with *Meloidogyne hapla* eggs and *Cylindrocladium crotalariae* on roots of cultivar NC 3033 peanut plants. **A,** *M. hapla* alone at 10^3 eggs per pot (note the extensive networks of fibrous roots around the galls). **B,** Galled roots in the presence of *C. crotalariae* at five ms per cm^3 (most fibrous roots around the galls have been destroyed).

days may provide a natural channel for xylem fluids to leak directly into the rhizosphere."

A decrease in nematode population as a result of concomitant inoculation with a fungus is quite common (14). Plant-parasitic nematodes are essentially obligate parasites. It was not surprising that the densities of both nematode species used in this experiment decreased as ms inoculum density was increased since the nutritive value of the roots apparently decreased in the presence of increasing *C. crotalariae* ms densities.

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