Enhancement of Cylindrocladium crotalariae Root Rot by Meloidogyne arenaria (Race 2) on a Peanut Cultivar Resistant to Both Pathogens[1]

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Abstract: Two populations of *Meloidogyne arenaria* (race 2, incompatible on peanut) enhanced development of Cylindrocladium black rot (CBR) on CBR-resistant peanut cv. NC 3033 in greenhouse factorial experiments. Nematode populations 256 and 486 (0, 10^3, 10^4 eggs per 15-cm pot) were tested in all combinations with *Cylindrocladium crotalariae* (0, 0.5, 5, 50 microsclerotia per cm^3 of soil). Root-rot index increased in the presence of either population. Positions but not slope values of inoculum density-disease curves were changed by both populations, indicating increased efficiency of microsclerotia when peanuts were grown in the presence of these nematodes. Although little or no reproduction occurred with either nematode population on NC 3033, larvae of 256 and 486 penetrated roots. *Meloidogyne arenaria* 486 did not induce root galls and was not successful in establishing feeding sites. *Meloidogyne arenaria* 256 produced a few very small eliptical galls and had a range of success in establishing a feeding site, varying from no giant cell development to large giant cell with production of a few eggs. *Key words:* root rot, Cylindrocladium black rot, *Arachis hypogea,* groundnut.

Most investigations on nematode disease complexes have dealt with fungus pathogens, *Meloidogyne* species being the prevalent nematode component (1). Certain *Meloidogyne* species may promote normally saprophytic organisms to pathogenic status (14) and may allow known fungal pathogens to cause much more damage in the presence than in the absence of these nematodes (9,13). Madamba et al. (8) demonstrated that various *Meloidogyne* species penetrate nonhost plants. Physiological changes and root wounding are thought to be important in the interaction involving *M. hapla* Chitwood or *Macroposthonia*

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ornata (Raski) DeGrisse and *Cylindrocladium crotalariae* (Loos) Bell and Sobers on peanut, *Arachis hypogea* L. (4).

Cylindrocladium black rot (CBR) has appeared in most peanut growing areas of the southeastern United States and has been reported in Japan (10). Although race 1 of the peanut root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) is a serious pathogen on peanut, race 2 does not reproduce on this crop even though it is found in North Carolina peanut growing areas (17). This report describes enhancement of CBR by incompatible populations of *M. arenaria* race 2 on CBR-resistant peanut cultivar NC 3033 (11). This appears to be the first report of an incompatible host-nematode relationship being involved in fungal disease enhancement.

MATERIALS AND METHODS

Inoculum preparation: M. arenaria (race 2) International Meloidogyne Project populations 256 (from Colombia, South America) and 486 (from North Carolina) and *M. hapla* were increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Nematode eggs were extracted with sodium hypochlorite (6). Freshly hatched nematode larvae were collected from heavily galled tomato roots on Baermann funnels in a mist chamber (19). The isolate of *C. crotalariae* was from peanut in eastern North Carolina. Microsclerotia (ms) grown for 3–4 wk on potato dextrose agar cultures were extracted and standardized as described by Phipps et al. (12).

Nematode populations with fungus: Three levels of *M. arenaria* (race 2) (0, 10^3, 10^4 eggs per pot) and four *C. crotalariae* inoculum densities (0, 0.5, 5, 50 ms per cm^3) were tested in all possible combinations for each population in a five-replication-randomized complete block, using a 3 × 4 factorial design. Eggs of nematode population 256 were simultaneously mixed with ms in 1,400 cm^3 of steamed sand:sandy loam (3:2). Soil mix was placed in 15-cm-d clay pots into which two 3-d-old peanut seedlings were transplanted. In a sequential inoculation experiment, eggs of nematode population 486 were introduced into the soil 2 wk before the introduction of ms. Eggs were mixed in 450 cm^3 soil mix and placed in the bottom of the pot with two 10-mm-d open-end glass tubes placed vertically and opposite each other. Another 450 cm^3 soil mix were added, two other tubes were inserted vertically in a plane perpendicular to that formed by the first two, and the remaining 500 cm^3 soil mix was added. Suspensions of ms equal in volume were pipetted 2 wk later into each of the tubes.

All experiments were conducted with CBR-resistant peanut cv. NC 3033 at 25 ± 2C for 8 wk in the greenhouse. CBR resistance in NC 3033 is inoculum dependent (11). Progression of foliar symptoms (4), root-rot index (15), and final nematode populations (6) were determined. For purposes of data transformation, root-rot index was expressed as percent (X) and each disease value was transformed using Gregory's multiple infection correction factor of $\log_e \frac{1}{1-X}$. The transformed data (a measure of successful fungus infections) and inoculum density were plotted on a log-log scale and linear regression was used to find the best fitting lines (2). Slopes of inoculum density-disease curves were tested for homogeneity. Effective dosages of ms required for 50% disease (ED_{50}) were graphically determined for each nematode level (4).

Penetration study: Individual peanut seedlings were transplanted into 5-cm-d clay pots containing sandy loam to which 200 larvae of *M. arenaria* 256 or 486 or *M. hapla* had been added and mixed. Three replications of each nematode population were examined at 24, 48, 72, 96, and 120 h after transplanting. Roots were washed free of soil, cut into 5-mm lengths and stained overnight in acid fuchsin lactophenol (16). Larvae inside roots were counted after destaining in clear lactophenol for 72 h.

Histology: Plants were grown for 8 wk in sandy loam soil infested with 5,000 eggs of *M. arenaria* 256 or 486 or *M. hapla* per pot. Roots were examined histologically as described by Johnston and Beute (7) to determine the extent of female development and host response for each population.

RESULTS

Nematode populations with fungus: In experiments with *M. arenaria* (race 2) pop-

Table 1. Effects of *Meloidogyne arenaria* (race 2) International Meloidogyne Project populations 486 and 256 on the root-rot index of Cylindrocladium black rot on peanut cv. NC 3033.*

Fungal inoculum (microsclerotia per cm³)	Root-rot index†					
	M. arenaria 486 (Eggs per pot)			*M. arenaria* 256 (Eggs per pot)		
	0	10^3	10^4	0	10^3	10^4
0.0	0.0 a	0.2 a	0.9 ab	0.0 a	0.5 a	1.0 ab
0.5	0.5 a	1.2 ab	1.9 bc	0.9 ab	1.6 bc	1.9 bc
5.0	2.0 bc	3.0 cde	3.8 de	2.7 cd	3.4 de	3.9 ef
50.0	3.2 de	4.1 ef	4.8 f	3.6 de	4.3 ef	4.6 f

*For a given nematode population, means followed by different letters are significantly different at the 5% level according to Duncan's multiple-range test.

†Root-rot index based on a 0–5 scale where 0 = no apparent rot and 5 = maximum rot.

ulations 256 or 486, root-rot severity increased as ms density and nematode level increased. The pattern of response to ms densities was similar at each nematode level (Table 1). Root-rot severity was greater with ms in combination with either nematode than with ms alone. Significant differences ($P = 0.05$) were noted in root-rot severity for the main factors of fungus inoculum density and nematode level, but the fungus × nematode two-factor interaction term was not statistically significant. Slope values of inoculum density-disease curves did not differ ($P = 0.05$) for either nematode population (Figs. 1, 2). Position of curve with population 256 was statistically different

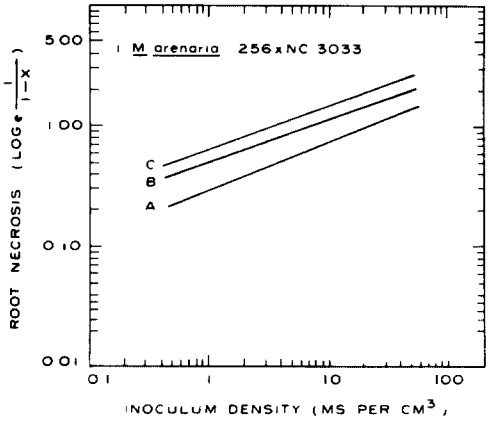

Fig. 1. Relation of peanut cv. NC 3033 root-necrosis severity and *Cylindrocladium crotalariae* microsclerotia density in the presence of various densities of *Meloidogyne arenaria* (race 2) International Meloidogyne Project population 256. (A) 0 egg per pot. (B) 1 × 10^3 eggs per pot. (C) 1 × 10^4 eggs per pot. Slopes of curves do not differ ($P = 0.05$). Fungus inoculum efficiency (position of curve) was increased ($P = 0.05$) in the presence of nematodes.

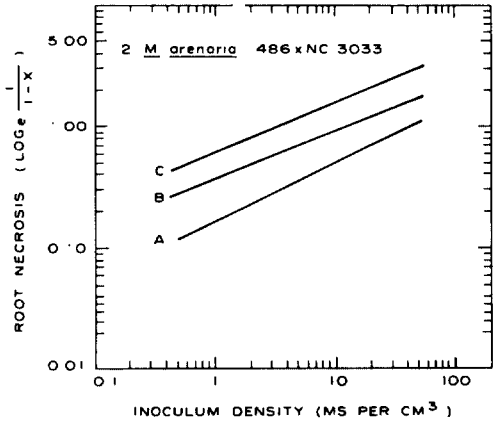

Fig. 2. Relation of peanut cv. NC 3033 root-necrosis severity and *Cylindrocladium crotalariae* microsclerotia density in the presence of various densities of *Meloidogyne arenaria* (race 2) International Meloidogyne Project population 486. (A) 0 egg per pot. (B) 1 × 10^3 eggs per pot. (C) 1 × 10^4 eggs per pot. Slopes of curves do not differ ($P = 0.05$). Fungus inoculum efficiency (position of curve) was increased ($P = 0.05$) in the presence of nematodes.

from other treatments for the comparison of 0 with 10^4 eggs per pot. All comparisons of curve position for population 486 levels were significantly different. ED_{50} values indicate that the presence of either *M. arenaria* population increased the inoculum efficiency of *C. crotalariae* ms (Table 2). There was a 15-fold increase in ms efficiency (ED_{50}) for population 486 in the presence of 10^4 eggs per pot. An almost eightfold increase in inoculum efficiency of ms occurred when population 256 was added.

No foliar disease symptoms occurred in any nematode-alone treatment. Plants with foliar symptoms of CBR appeared earlier and more frequently in the presence of

Table 2. The number of *Cylindrocladium crotalariae* microsclerotia per cm³ of soil required for 50% disease (ED_{50}) on peanut cv. NC 3033 in the presence of Meloidogyne arenaria (race 2) International Meloidogyne Project populations 256 or 486.

Nematode inoculum eggs per pot	ED_{50}	
	M. arenaria 256	*M. arenaria* 486
0	7.7	16.0
10^3	2.0	4.0
10^4	1.0	1.1

either nematode population than when nematodes were absent (Fig. 3). A few males with two gonads were observed for both populations of *M. arenaria.*

Penetration study: Larvae of *M. arenaria* 356 or 486 and *M. hapla* each penetrated NC 3033 roots at a constant rate over time. Percentage of larval penetration for the two *M. arenaria* populations was slightly less than that of *M. hapla* larvae (Fig. 4).

Histology: Meloidogyne arenaria 486 did not induce root galls and was not successful in establishing feeding sites in NC 3033 peanut roots. Most sections indicated migration in the cortex or stele of roots; occasionally a small nematode (Fig. 5A) and small or dead giant cells (Fig. 5B, C) were observed. Giant cells were frequently delineated and restricted by periderms. *Meloidogyne arenaria* 256 induced a few very small eliptical root galls and varied in

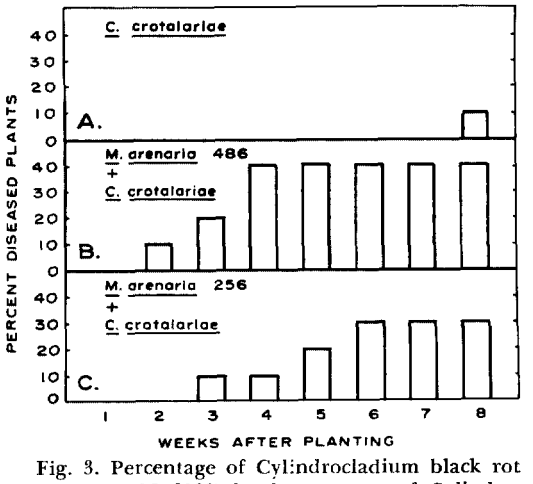

Fig. 3. Percentage of Cylindrocladium black rot on peanut (NC 3033) in the presence of *Cylindrocladium crotalariae* (50 microsclerotia per cm³ soil) alone (A) or in combination with *Meloidogyne arenaria* (race 2) (10^4 eggs per pot) International Meloidogyne Project populations 486 (B) or 256 (C).

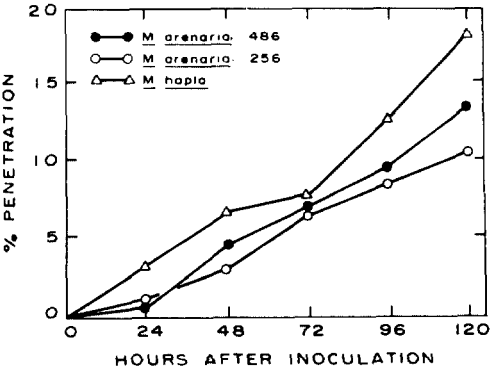

Fig. 4. Percentage of peanut cv. NC 3033 roots penetrated by larvae of *Meloidogyne hapla* (compatible) and *M. arenaria* (incompatible, race 2) International Meloidogyne Project populations 256 and 486.

successful establishment of feeding sites. Disease response in roots ranged from evidence of migration with no development to small females and small or dead giant cells to a successful feeding site with large giant cells (Fig. 5D), large females, and a few eggs.

DISCUSSION

Root-knot nematodes have been reported to predispose plants to attack by many other pathogenic (13) or normally saprophytic organism (14). Previous studies were conducted with compatible nematode-host combinations; i.e., nematodes were able to penetrate, feed, and reproduce. In this study two populations of *M. arenaria* (race 2), incapable of reproduction on peanut, were found to enhance CBR on CBR-resistant NC 3033.

When either population of *M. arenaria* was combined with *C. crotalariae,* root-rot severity was increased in an additive manner. This additive effect was indicated by statistical significance for fungus and nematode main effects but nonsignificant fungus × nematode interaction. Additive enhancement was also indicated because slopes of curves from log-log transformed data were not different, but positions of curves were different in at least some comparisons. The effect of *M. arenaria* (race 2) on CBR root-rot severity can be interpreted as increasing the inoculum efficiency of ms of *C. crotalariae.*

No apparent difference in disease re-

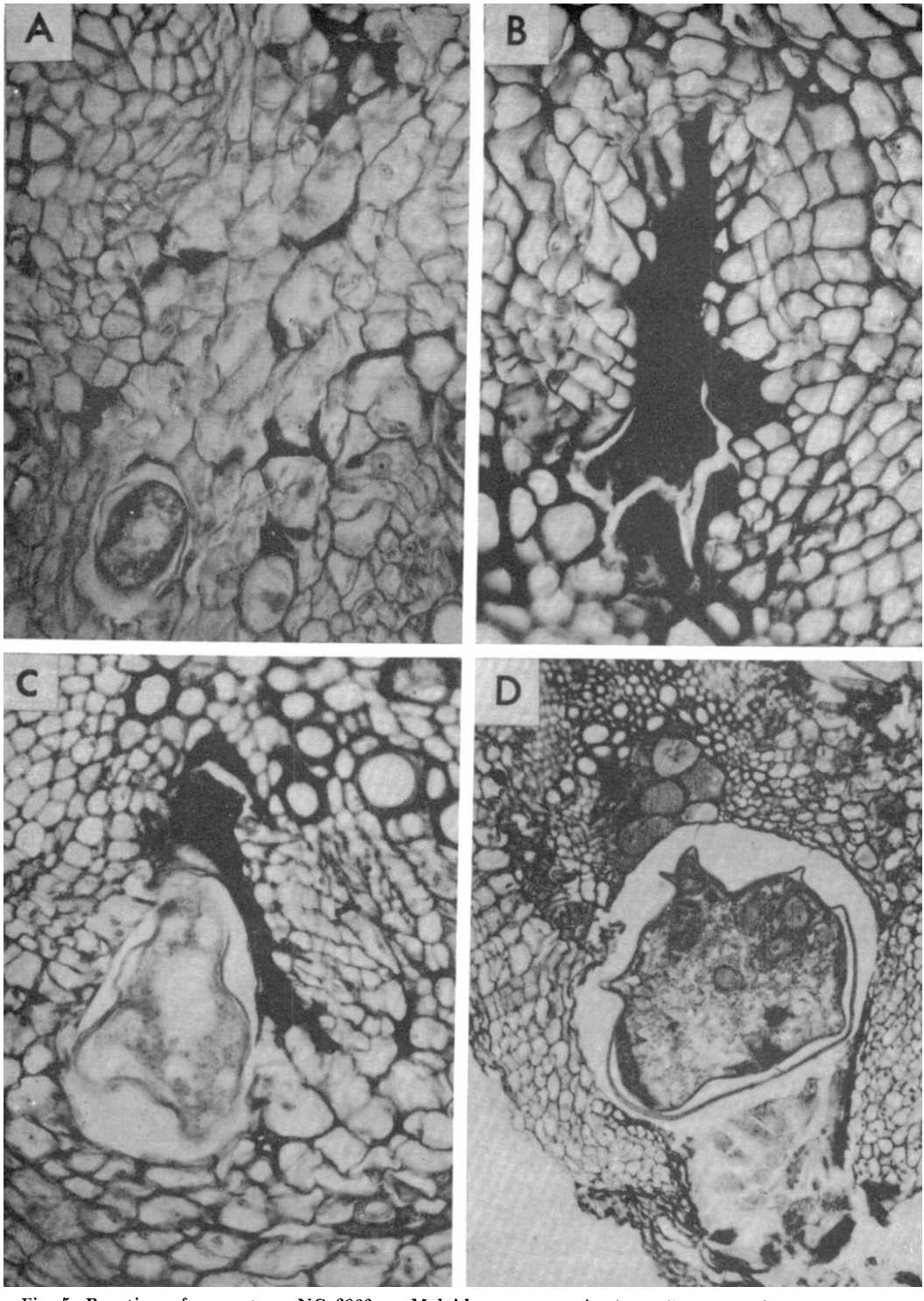

Fig. 5. Reaction of peanut cv. NC 3033 to *Meloidogyne arenaria* (race 2) International Meloidogyne Project populations 486 or 256. A) unsuccessful feeding sites and small individual of 486 (X340); B) degenerated giant cells induced by 256 (X285); C) degenerated giant cells and underdeveloped female of 256 (X200); D) successful feeding site of a female of 256 (X87).

sponse was observed when eggs of population 486 were incorporated into the soil prior to ms, or when eggs of population 256 and ms were mixed with soil initially or simultaneously. This type of response has been reported for similar studies with *M. hapla* and *Macroposthonia ornata* (4). The lower ED_{50} values for population 256 indicate that 25 C was more favorable for disease development when the experiment was conducted with population 256.

The percentage of plants with foliar CBR symptoms for either population of *M. arenaria* (incompatible) did not increase after 4–6 wk, but in a study with *M. hapla* and *M. ornata* (both compatible relationships), new aboveground symptoms continued to occur throughout the 8-wk experiments (4). Apparently, the effect of *M. arenaria* (race 2) populations on CBR occurred primarily in the first weeks of the experiment when larvae were wounding and inducing other changes in the roots during penetration.

Although *M. arenaria* (race 2) on peanut is not a compatible relationship, larvae of populations 256 and 486 were able to penetrate roots at a rate similar to that of *M. hapla* (compatible), which is in agreement with Madamba et al. (8). Some larvae probably failed to establish sufficiently successful feeding sites for female development, but others developed into males and exited from roots (18). Histological examinations of roots after 8 wk also indicated movement of *M. arenaria* (race 2) larvae within roots with no success (486) or varying success (256) in establishing feeding sites. CBR was enhanced on CBR-susceptible 'Florigiant' by *M. hapla* and *Macroposthonia ornata* and on CBR-resistant NC 3033 by *M. hapla* (4). *M. hapla* (endoparasite) and *M. ornata* (ectoparasite) are compatible with both cultivars. Wounding (initial penetration and that of second generation larvae) with giant cell formation or wounding from external feeding occurred during those experiments. Although greenhouse experiments with *M. ornata* and *C. crotalariae* on NC 3033 did not indicate an interaction, a microplot field test did provide evidence of a subtle enhancement of CBR (5). It has been shown that more successful infections by *C. crotalariae* occur on mechanically wounded peanut roots than on nonwounded roots (4). Root injury associated with penetration of larvae, and exit of resulting males, may be a major component of the interaction reported here between *M. arenaria* (race 2) and *C. crotalariae*. Nevertheless, physiological changes associated with giant cell development cannot be discounted (3).

The factorial approach to pathogen interaction studies with more than two levels of each organism is an extremely useful technique which has not been adequately applied to most studies of this type. Countless population density conditions exist and eventually interact in the field. Use of several densities of each organism should provide clearer insight into the epidemiology of other interaction problems.

When high initial nematode populations are carried over from previous crops or weeds, enhancement of other soil-borne diseases may occur when nematodes are incompatible, as reported herein, as well as compatible with the current host plant (4).

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