Relation of *Meloidogyne hapla* and *Macroposthonia ornata* Populations to Cylindrocladium Black Rot in Peanuts

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ABSTRACT

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Five field experiments were established in *Cylindrocladium crotalariae*-infested peanut fields from 1976 to 1978. Populations of *Macroposthonia ornata, Meloidogyne hapla*, and *Cylindrocladium crotalariae* microsclerotia were determined in each field three times during each growing season. Correlations between populations of these organisms and the severity of Cylindrocladium black rot (CBR) were analyzed using multiple regression models. In all tests, CBR-resistant cultivar NC 3033 sustained less disease than CBR-susceptible Florigiant and final populations of microsclerotia were higher on Florigiant than on NC 3033. Correlations and the partial correlations of *M. hapla* and *C. crotalariae* populations with CBR severity were generally significantly positive on both cultivars. Correlations between *M. ornata* and the disease were either less pronounced than those with *M. hapla* or not significant. Final populations of *M. ornata* were consistently greater on NC 3033 than on Florigiant. In microplot tests, *C. crotalariae* combined with *M. ornata* caused more disease on Florigiant but not on NC 3033 than did *C. crotalariae* in the absence of *M. ornata*. Reproduction factor (ie, final population/initial population) for *M. ornata*, however, was higher on NC 3033 than on Florigiant.

Additional key words: *Arachis hypogaea*, disease interaction, ring nematode, root knot nematode

Most nematode-fungus interaction studies have been conducted in greenhouse environments, but both microplot and field studies have been reported (2,12). Wallace (16) emphasized the multifactorial nature of disease causation and the need for multiple regression analyses including several independent variables (determinants of disease) for determining disease causality in complex environments.

Few fungus-nematode interaction studies have included peanut (*Arachis hypogaea* L.) or *Cylindrocladium crotalariae* (Loos) Bell and Sobers. In a greenhouse study a disease enhancement interaction was observed between *Meloidogyne hapla* Chitwood and *C. crotalariae* on Cylindrocladium black rot (CBR)-resistant NC 3033 and CBR-susceptible Florigiant (5). An interaction was also observed between *Macroposthonia ornata* (Taylor) De Grisse and *C. crotalariae* but only on Florigiant.

Of the nematodes associated with peanuts (6,7,8,13,15), *M. hapla* and *M. ornata* are the most frequent (8) and most damaging (13,14) in most southeastern states (USA). The present field and microplot study was initiated to investigate the effect of *M. ornata* and *M. hapla* on the capacity of *C. crotalariae* to cause CBR of peanut under field conditions.

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MATERIALS AND METHODS

Field test. Five peanut fields in four counties of North Carolina were selected for tests from 1976 to 1978. Fields were selected on the basis of high CBR incidence on peanut during previous years. In 1976, tests were established in Northampton and Martin counties. In 1977, one test was established in Halifax County, and in 1978 tests were established in Bladen and Martin counties.

Soil in a randomized complete block design (three replications in 1976, five in 1977 and 1978) was treated by incorporating aldicarb (10% granules), 3.36 kg a.i./ha, in 8- to 10-cm in 30-cm bands or by injecting dibromochloropropane (DBCP) 12.5 kg a.i./ha, about 15 cm deep in chisel plow rows. Treatments were applied before planting CBR-resistant NC 2033 and CBR-susceptible Florigiant peanuts in four 12-m rows per plot. Data were taken only from the two center rows. Controls were not treated.

Populations of *C. crotalariae* microsclerotia (ms) were determined by the elutriation method described by Phipps et al (11), before planting (May), midseason (late July or early August), and at the end of the growing season (late September or early October). Populations of *M. ornata* and *M. hapla* were also determined from the same samples by isolating nematodes with the centrifugal flotation method (3). Before peanuts were planted, *M. hapla* eggs were extracted by the method of Byrd et al (4). Identification

of these two nematode species involved mensurations for *M. ornata* and differential hosts and perineal patterns for *M. hapla*.

At midseason and at the end of the growing season, diseased plants were counted and transformed to percent of germinated seeds. Two independent estimators, using a 0–5 index, visually assessed root rot on a per-plot basis. Only diseased plants were counted in 1976.

Multiple regression models were used to correlate nontransformed nematode and/or ms populations to percent diseased plants and root rot index. The 1976 data from Martin and Northampton counties were combined, and those from Bladen, Halifax, and Martin counties in 1977 and 1978 were combined. The data from one of the two fields in Halifax County were analyzed separately because *M. hapla* occurred only in that field.

Microplot tests. Microplots (76-cm in diameter) were set in methyl bromide-treated Norfolk sandy loam at the Central Crops Research Station at Clayton, NC, in 1978. In the laboratory, 10,000 chlamydospores of *Glomus macrocarpus* (from J. P. Ross) and about 5 g of Rhizobium inoculum were mixed in polyethylene bags with 1 kg of sterile sand and transferred to microplots not inoculated or inoculated with five *M. ornata* nematodes, five *C. crotalariae* ms, or five *M. ornata* nematodes plus five *C. crotalariae* ms, each inoculation amount per cubic centimeter computed on the basis of 60 cm depth. All treatments were replicated five times with four plants of NC 3033 or Florigiant per microplot in randomized complete block design.

The experiment lasted from 24 May to 5 October. From 22 August to 4 October, diseased plants (aboveground symptoms) were counted every other week. At the end of the experiment, total number of diseased plants, root rot index, and final populations of both *C. crotalariae* and *M. ornata* were determined as described for field tests.

Table 1. Effect of cultivar and nematicides on Cylindrocladium black rot incidence and final populations of *Cylindrocladium crotalariae* and *Macroposthonia ornata* in Northampton and Martin counties in 1976[x]

Treatments		Percent diseased[z] plants	Final populations	
Cultivars	Nematicides[y]		*C. crotalariae* (ms/g of soil)	*M. ornata* (in thousands)
Florigiant	Control	27.1 b	30.0 mn	5.740 b
	DBCP	42.1 a	61.2 l	3.852 bc
	Aldicarb	19.1 c	43.2 m	3.513 c
NC 3033	Control	13.5 c	21.2 n	9.886 a
	DBCP	14.1 c	21.4 n	4.833 bc
	Aldicarb	13.4 c	15.4 n	2.786 c

[x] Means followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.
[y] DBCP = dibromochloropropane, 12.5 kg a.i./ha; aldicarb, 3.36 kg a.i./ha.
[z] Number of plants with aboveground symptoms expressed as a percent of germinated seeds.

Table 2. Effect of cultivar and nematicide treatment on Cylindrocladium black rot incidence and severity and final populations of *Cylindrocladium crotalariae, Macroposthonia ornata*, and *Meloidogyne hapla* in 1977 and 1978 (Halifax, Bladen, and Martin counties, 1978)[w]

Treatments[w]		Percent diseased[x] plants	Root rot[y] index	Final populations		
				C. crotalariae	Nematodes per 473 cm^3 of soil	
Peanut cultivar	Nematicides			(ms/g of soil)	*M. hapla*[z]	*M. ornata*
Florigiant	Control	31.8 a	2.9 a	64.1 b	3,932 a	2,450 b
	DBCP	23.3 b	2.5 a	42.1 b	592 cd	1,140 b
	Aldicarb	26.4 ab	2.9 a	102.1 a	2,288 b	2,143 b
NC 3033	Control	3.6 c	0.6 b	6.9 c	912 c	3,859 a
	DBCP	3.4 c	0.6 b	3.6 c	264 d	1,139 b
	Aldicarb	3.7 c	0.9 b	6.6 c	680 cd	4,736 a

[y] In a column, means followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.
[w] Each figure is the mean of 15 observations (five per field). DBCP = dibromochloropropane, 12.5 kg a.i./ha; aldicarb, at 3.36 kg a.i./ha.
[x] Number of plants showing aboveground symptoms expressed as a percent of germinated seeds.
[y] Based on a scale of 0–5; where, 0 = no apparent rot and 5 = total decay.
[z] Populations of larvae in field in Halifax County only.

Table 3. Correlations[a] of Cylindrocladium black rot incidence and severity with final populations of *Cylindrocladium crotalariae* (ms) and *Macroposthonia ornata* (mo) on two peanut cultivars in 1977 and 1978

	Percent diseased plants		Root rot index	
	Florigiant	NC 3033	Florigiant	NC 3033
Mo	0.111	0.199	0.246	0.342*
ms	0.229	0.100	0.267	0.262
mo + ms	0.230	0.231	0.533**	0.307
Root rot index	0.627**	0.049	...	...

[a] All correlation coefficients are based on 45 observations (15 per field). *, $P = 0.05$; **, $P = 0.01$.

RESULTS

Field tests. In all five field experiments, disease was more severe and *C. crotalariae* final populations (ms) were higher on Florigiant than on NC 3033, and *M. ornata* final populations were often higher on NC 3033 than on Florigiant (Tables 1 and 2).

In 1976 (Martin and Northampton counties), more disease occurred in DBCP-treated Florigiant plots than in control plots. Final populations of *M. ornata* were lower in NC 3033 plots treated with aldicarb or DBCP and in Florigiant plots treated with aldicarb than in untreated plots. Ms density was highly correlated with percent diseased plants on Florigiant ($r = 0.910$, $P = 0.01$) but less on NC 3033 ($r = 0.414$, $P = 0.05$). Inclusion of both ms and *M. ornata* in the model produced no increase in correlation coefficient over that obtained with ms alone.

In 1977 and 1978 (Bladen, Halifax, and Martin counties), final populations of *M. ornata* were positively correlated ($P = 0.05$) with root rot on NC 3033 (Table 3). Ms density was not correlated with root rot on either cultivar. Inclusion of both *M. ornata* and ms in the model produced higher correlation coefficient than ms alone with root rot. Percent diseased plants were positively correlated with root rot only on Florigiant (Table 3).

M. hapla was present only in one field in Halifax County in 1977 (Table 4) and was highly correlated with percent diseased plants and root rot on NC 3033 ($P = 0.01$) but less so on Florigiant ($P = 0.05$). Ms density was highly correlated ($P = 0.01$) with percent diseased plants and root rot on Florigiant but less so ($P = 0.05$) on NC 3033. No significant correlation was obtained between *M. ornata* populations and any of the disease assessment variables. Correlation coefficients were higher with *M. hapla* alone than with ms alone on NC 3033, whereas they were higher with ms alone than with *M. hapla* alone on Florigiant. Including *M. ornata* in the model with ms and/or

M. hapla did not increase the correlation coefficients over those obtained with ms and/or *M. hapla* alone. The inclusion of *M. hapla* and ms together in the model produced higher correlation coefficients than those obtained with either variable alone in some cases (eg, percent disease plants and root rot on Florigiant or root rot on NC 3033).

Microplot tests. Root rot was more severe on Florigiant than on NC 3033 (Table 5, Fig. 1). *M. ornata* did not significantly alter root rot severity caused by *C. crotalariae* on either cultivar (Table 5). Final populations of *C. crotalariae* were higher on Florigiant than on NC 3033, whereas populations of *M. ornata* and the corresponding reproduction factors (final/initial population) were the highest on NC 3033 in the absence of *C. crotalariae*. *M. ornata* increased the incidence of disease on Florigiant but not on NC 3033, compared with the *C. crotalariae*-alone treatment (Fig. 1).

DISCUSSION

Florigiant was generally more severely diseased than NC 3033 in these tests, as expected, given the high level of resistance of the latter (9,17). The higher final ms populations on Florigiant than on NC 3033 were also expected (10).

The significant positive correlations between final populations of *C. crotalariae*, *M. hapla*, and/or *M. ornata* and CBR in field tests indicates that these nematodes as well as *C. crotalariae* can affect CBR development in the field. Increased values of correlation coefficients when *M. hapla* or *M. ornata* were included simultaneously with *C. crotalariae* in the model suggest interactions between these nematodes and *C. crotalariae* in the field. Correlation of *M.*

Table 4. Correlations[a] between Cylindrocladium black rot severity and final populations of *Cylindrocladium crotalariae* (ms), *Macroposthonia ornata* (Mo), and *Meloidogyne hapla* (Mh) in 1977

	Percent diseased plants		Root rot index	
	Florigiant	NC 3033	Florigiant	NC 3033
Mo	0.028	0.323	0.096	0.218
Mh	0.533*	0.779**	0.468	0.839**
ms	0.675**	0.577*	0.690**	0.507
Mo + Mh	0.593	0.798**	0.558	0.841**
Mo + ms	0.678*	0.584	0.703*	0.507
Mh + ms	0.679*	0.846**	0.690*	0.871**
Mh + Mo + ms	0.690	0.848**	0.705*	0.872**

[a] r values are based on 15 observations. *, $P = 0.05$; **, $P = 0.01$.

Table 5. Effects of *Cylindrocladium crotalariae* and *Macroposthonia ornata* on Cylindrocladium black rot severity on NC 3033 and Florigiant in microplots

Soil inoculation (no./cm^3)			Final populations[y]		
C. crotalariae microsclerotia	*M. ornata* nematodes	Root rot index	*C. crotalariae*	*M. ornata*	Reproduction factor[z]
Florigiant					
0	0	0.1 c	0	0	0
	5	0.3 c	0	8.575 b	3.43
5	0	4.3 a	64.3 a	0	0
	5	4.9 a	48.9 b	6.578 b	2.63
NC 3033					
0	0	0.0 c	0	0	0
	5	0.1 c	0	19.225 a	7.69
5	0	2.3 b	40.1 c	0	0
	5	2.9 b	35.3 c	7.737 6	3.09

[y] Means of four of five replications. In a column, numbers followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.
[z] Reproduction factor for *M. ornata* = final population/initial population.

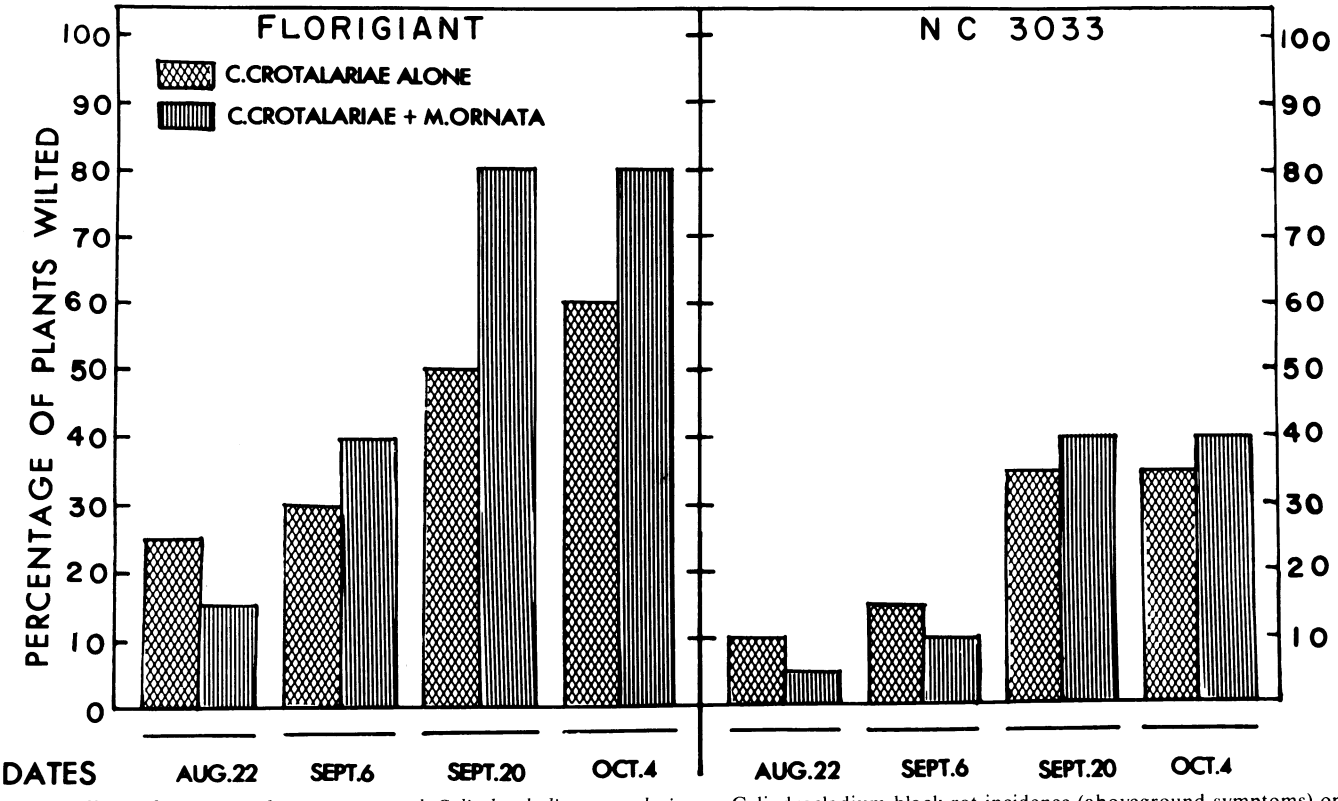

Fig. 1. Effects of *Macroposthonia ornata* and *Cylindrocladium crotalariae* on Cylindrocladium black rot incidence (aboveground symptoms) on peanut cultivars.

hapla with CBR symptoms was even higher than that of the primary causal agent, *C. crotalariae*, on NC 3033. However, the consistently higher correlation of *M. hapla* with CBR on NC 3033 than on Florigiant indicates that this nematode may contribute to the CBR syndrome more on CBR-resistant cultivar NC 3033 than on Florigiant. The fact that Florigiant is so highly susceptible to *C. crotalariae* (9) may have obscured the effect of *M. hapla* on that cultivar, especially under the high density (42–102 ms/cm^3) in these tests.

Correlations in field plots involving *M. ornata* generally were either not significant or less conspicuous than those involving *M. hapla* or *C. crotalariae*. Higher correlation between *M. ornata* and CBR on NC 3033 than on Florigiant (Table 3) are apparently in disagreement with the greenhouse findings that *M. ornata* had no significant effect on NC 3033 or on CBR on that cultivar (5). Such discrepancies could be due to higher *M. ornata* populations associated with NC 3033 than with Florigiant in field tests.

In contrast to the situation with *M. ornata, M. hapla* was found only in one field and at relatively low population densities during this study. Inferences that can be made regarding *M. hapla* are therefore based on one year's data from one field. Correlations obtained between *M. hapla* and CBR symptoms were strikingly higher than those obtained between *M. ornata* and CBR symptoms. On this basis it seems reasonable to assume that *M. hapla* is more effective in predisposing both peanut cultivars to CBR than is *M. ornata*.

The low correlations of *M. ornata* final populations with CBR symptoms on both peanut cultivars may result from the fact that high populations of this nematode are required to affect CBR severity on Florigiant (5). Basically, plant growth responses are related to initial nematode populations (1,15). It is logical to assume that initial nematode populations are important where nematodes are suspected to be predisposing agents in disease complexes since the argument is that nematodes have negligible motility and a relatively low reproductive rate. In the present study, however, initial nematode populations were generally small, occasionally under a detectable level. Midseason populations (from mid-July to mid-August) generally gave no significant correlations with CBR. Thus, correlations obtained between nematodes and CBR in this study were obtained from final nematode populations.

Although adequate nematode control must be included in peanut production, use of nematicides to modify nematode densities in these tests did not affect the incidence of diseased plants or severity of root rot in the treated plots compared with plots receiving no nematicides.

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