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Infection, Reproduction Potential, and Root Galling by Root-knot Nematode Species and Concomitant Populations on Peanut and Tobacco[1]

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Abstract: Single populations of *Meloidogyne arenaria* races 1 (MA1) and 2 (MA2) and *M. hapla* (MH), and mixed populations of MA1 + MA2 and MA1 + MH with four inoculum levels of eggs were tested on peanut cv. 'Florigiant' and *M. incognita*-resistant tobacco cv. 'McNair 373' in a greenhouse experiment. Root infection, female development, and reproduction of MA2 on peanut and MA1 on resistant tobacco were limited at 2 and 6 weeks. MA1, MH, and MA1 + MH on peanut had similar root infection (total parasitic forms per root unit) at both 2 and 6 weeks, and similar female development and reproduction potentials at 6 weeks. MA2 tended to depress root infection, female development, and reproduction of MA1 on peanut. MH had little effect on MA1 on this crop. On tobacco, MA2 population had greater incidence of root infection than did MH at 2 weeks. The two nematode species had similar development in roots at 6 weeks. All of these processes were restricted when either MA2 or MH was present together with MA1. As initial inoculum level of parasitically fit populations increased, relative infection ratio on both peanut and tobacco, and reproduction factor on peanut decreased. Populations that had high infection incidence and reproduction rates induced greater root galling than did other populations. Root galling was suppressed in the presence of antagonistic response between nematode populations.

Key words: Arachis hypogaea, infection, interaction, *Meloidogyne arenaria, M. hapla*, nematode, *Nicotiana tabacum*, peanut, reproduction potential, root-knot nematode, tobacco.

Plant-parasitic nematodes generally occur in polyspecific communities because many species have overlapping host ranges (18). Concomitant populations of these parasites can interact with each other to affect reproductive capacity, and these interactions can alter the etiology of associated plant disease (5). Sedentary endoparasitic nematodes are highly specialized organisms that develop complex relationships with host plants, including altered host physiology (12). Interactions between two sedentary endoparasites are generally mutually suppressive because of the competition for available feeding sites (17). However, stimulatory interactions or neutral situations also occur (5). Evidence of interactions has been found between *Heterodera* and *Meloidogyne* spp., *Rotylenchulus reniformis* and *Meloidogyne* spp., and among species of *Meloidogyne* (5,11). Several factors have been demonstrated to affect the interaction of concomitant species, resulting in greater community prominence for favored taxa. These factors include temperature, density-dependence, fecundity, and time-dependence (5,19).

Tobacco (*Nicotiana tabacum* L.) and peanut (*Arachis hypogaea* L.), two major crops in the southeast United States, are damaged by more than one species of root-knot nematode. *Meloidogyne arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid & White) Chitwood are the most damaging species on tobacco, whereas *M. hapla* Chitwood usually causes slight damage (2). For peanut, *M. arenaria* race 1 and *M. hapla* are the major nematode pathogens (15). Because concomitant infestations of *M. arenaria* races 1 and 2 and *M. hapla* are common in peanut and tobacco fields in North Carolina (20), the interactions among these nematode populations on both crops are potentially important. To date, interaction

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studies of *Meloidogyne* species have been limited (5,9,10,13). This research was initiated to determine the effects of interactions between races 1 and 2 of *M. arenaria* and between *M. arenaria* race 1 and *M. hapla,* on root infection, nematode development, reproduction potential, and root damage on peanut and *M. incognita*-resistant tobacco.

Materials and Methods

Plant culture: Meloidogyne arenaria race 1-susceptible peanut 'Florigiant' and *M. incognita* (MI) races 1 and 3-resistant tobacco 'McNair 373' were tested in a greenhouse experiment for 2 and 6 weeks. Peanut seedlings were obtained by germinating seeds in vermiculite for 1 week. Tobacco seedlings were derived from germinating seeds in a 1:1:1 mixture of steam-sterilized sand:field soil (loamy sand: 80% sand, 15% silt, and 5% clay):metromix 220 (Grace Sierra Horticultural Products Co., Milpitas, CA) for 3 weeks and transferred singly into a mixture of sterilized sand: field soil (1:1) in 48-cell plastic bedding-plant containers in which the seedlings were kept for an additional 3 weeks. Both peanut and tobacco seedlings were then transferred singly into 1:1 mixture of sterilized sand and field soil in 10-cm-d and 15-cm-d clay pots for 2-week and 6-week harvests, respectively. Each 10-cm-d pot was placed in an empty 15-cm-d clay pot to prevent contamination among neighboring plants. Plants were watered twice daily and fertilized weekly with 20-20-20 N-P-K.

Nematode inoculation: Populations of *M. arenaria* race 1 (MA1) and race 2 (MA2) and *M. hapla* (MH) were increased separately on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in a greenhouse. Egg inocula were collected by the NaOCl(0.5%)-extraction method (8). The four concentrations of egg inocula used for single populations of MA1, MA2, and MH, and for combined (1:1) populations of MA1 plus MA2, and MA1 plus MH, were: 2,000, 4,000, 8,000, and 16,000 eggs per plant (pot). All nematode populations and selected combinations were inoculated to both peanut and tobacco. The combinations (per 10^3 eggs) included: 1 + 1, 2 + 2, 4 + 4, and 8 + 8 for MA1 + MA2 and MA1 + MH. Inoculation was done 5 days after transplanting the seedlings by adding a 30-ml egg suspension to the soil around the base of each plant and covering with sterilized sand:soil mixture for 1-cm depth. Pots were arranged by crop and harvest date on greenhouse benches. Within crop and date, five nematode populations × four inoculum levels were arranged in a randomized complete block design with five replicates. Plants were kept in a greenhouse with approximately 22–32 C minimum-maximum temperatures during the experiment.

Nematode and root assays: For the 2-week harvest, plants were removed from pots and root systems were washed and weighed. A representative 1-g root sample from each root system was stained with NaOCl-acid fuchsin-glycerin (4) to determine the number of nematodes infecting roots. For the 6-week harvest, washed roots were weighed and rated for gall development (0–100% of root system galled per root system). The number of nematodes per gram of root was determined and percentage infection was calculated as previously described. Other 1-g samples were used to determine the number of root galls and egg masses, and to extract and count eggs (3). Reproduction factor (RF) was calculated from final egg number per root system ÷ initial inoculum rate.

Data analyses: Nematode-count data were transformed by $\log_{10}(X + 1)$ to standardize variances before analyses. All nematode and crop response data were subjected to analysis of variance, and Waller-Duncan mean comparisons were performed. Root-infection and reproduction-factor data at 6 weeks were regressed against initial inoculum levels. Because of some missing values for assessing interactions in tobacco, only nematode–population treatment means are presented. Correlations were calculated to determine relationships of reproduction and root-

galling data to nematode population density in roots.

Results and Discussion

Infection of roots by different nematode populations varied with initial inoculum (Pi) levels, except for the least-parasitically-fit populations, for both peanut (MA2) and tobacco (MA1) (Table 1). At 2 weeks after inoculation, almost all nematodes found in roots were swollen juveniles, and at 6 weeks more females were observed than juveniles, except those of MA2 on peanut and MA1 on MI-resistant tobacco. No males were found in roots at the 6-week harvest for either crop (Table 1).

Regardless of Pi level, MA1, MH, and MA1 + MH had more nematodes in peanut roots than did MA2 and MA1 + MA2 at both 2 and 6 weeks (Table 1). Infection of MA2 was very limited at 2 weeks, and was much lower than those of other nematode populations at 6 weeks. The number of MA1 in roots was slightly less than that of MH at 2 weeks. The two single populations (MA1 and MH) had similar numbers in peanut roots at 6 weeks. The combined population of MA1 + MH, with half Pi density of each, had similar infection capacities compared to the single population with full density of Pi at both 2 and 6 weeks. The concomitant populations of MA1 and MA2 generally had lower nematode total numbers in roots than did MA1 alone at both 2 and 6 weeks (Table 1).

At 6 weeks, nematodes per gram of peanut roots of MA1, MH, and MA1 + MH decreased over those for 2 weeks due to the growth of root systems, whereas those of MA2 increased (Table 1). However, the total number of all populations per root system increased. The majority of nematodes in peanut roots at 6 weeks was females, except those of MA2 in which only a few females had reached maturity. At 6 weeks, numbers of juveniles in roots of MA2 and MA1 + MA2 were significantly higher than those of MA1, MH, and MA1 + MH.

As Pi level increased, the number of nematodes in peanut roots increased but the percentage infection decreased (data not included). Relative infection levels

Table 1. Numbers of nematodes in roots of peanut 'Florigiant' and tobacco 'McNair 373' at 2 and 6 weeks after inoculation of different *Meloidogyne* spp. populations in greenhouse.

Treatment	Nematodes/g root				Total nematodes/root	
	2 weeks		6 weeks			
	Vermiform juveniles	Swollen juveniles	Swollen juveniles	Females	2 weeks	6 weeks
			Peanut			
MA1	2 a	134 a	10 b	73 a	883 a	2,414 ab
MA2	0 c	3 c	23 a	3 c	17 c	631 c
MH	2 ab	198 a	2 d	85 a	1,510 a	2,208 ab
MA1 + MA2	1 bc	55 b	24 a	38 b	399 b	1,507 b
MA1 + MH	1 a-c	168 a	5 c	88 a	1,290 a	2,493 a
Pi	NS	**	**	**	**	**
			Tobacco			
MA1	1 ab	3 d	7 ab	7 c	36 d	559 b
MA2	2 a	141 a	8 a	70 a	1,291 a	3,216 a
MH	1 ab	54 b	2 c	72 a	551 b	2,874 a
MA1 + MA2	0 b	44 b	5 bc	50 b	309 b	1,530 b
MA1 + MH	0 b	13 c	5 ab	26 b	95 c	1,185 b
Pi	NS	**	**	**	**	**

All data are means of five replicates of Pi treatments (see Materials and Methods). Statistical analyses of nematode count data were based on $\log_{10}(X + 1)$ transformed data.

MA1 = *Meloidogyne arenaria* race 1; MA2 = *M. arenaria* race 2; MH = *M. hapla*.

Means within column followed by the same letter are not different according to Waller-Duncan k-ratio *t* test (k-ratio = 100).

*,** indicate significant difference at $P = 0.05$ and 0.01, respectively; NS indicates no significant difference.

tended to decline as Pi level increased. This density dependence was more prominent at 6 weeks than at 2 weeks, especially in MH and MA1 + MH, for which infection significantly decreased as Pi level increased.

MA1 infection was limited in MI-resistant tobacco roots. Numbers of MA2 in tobacco roots were greater than those of other populations at 2 weeks (Table 1). At 6 weeks, root populations of MA2 and MH were similar. The combined population of MA1 + MA2 had greater levels in roots than did MA1 + MH. At 6 weeks, the majority of nematodes in MI-resistant tobacco roots were females, except those of MA1, in which approximately half of juveniles reached maturity as females (Table 1). An increase in Pi level resulted in greater population densities of nematodes in tobacco roots, but infection efficiency tended to decrease as Pi level increased. The reduction, however, was not significant until 6 weeks. The initial density effect of MA2 and MH populations was significant.

MA2 population produced no visible egg masses, and only limited numbers of eggs were found per gram of peanut roots (Table 2). Although MA1 produced more egg masses than MH, egg numbers per gram of root were similar. MA1 plus MA2 in a mixed population produced fewer egg masses and eggs per g root than MA1 alone. Egg masses in MA1 and MA1 + MH were similar, but both were greater than those of MH. This mixed population produced similar egg numbers to those produced by either MA1 or MH population alone. RF values of MA1 and MH were not significantly different, and the mixture of the two nematodes had a greater RF than either single population at the lowest Pi (Table 2). In contrast, the RF of MA1 was restricted by the presence of MA2.

Reproduction of MA1 on MI-resistant tobacco was very restricted compared to MA2. In contrast, MA2 and MH produced similar numbers of egg masses and eggs (Table 2). RF of MA2 was greater than that of MH, however. Production of egg masses and eggs by MA2 and by MH were suppressed by MA1 (in the mixed populations). An increase of Pi resulted in greater reproduction of most nematode populations (data not included). However, Pi level had almost no effect on RF, except in MH, in which RF was slightly reduced as Pi increased.

MA2 nematode induced few galls on

TABLE 2. Numbers of egg masses, eggs, and root galls per gram of root, and root-gall indices on peanut 'Florigiant' and tobacco 'McNair 373' at 6 weeks after inoculation of different *Meloidogyne* spp. in greenhouse.

Treatment	Egg masses/ g root	Eggs/ g root	RF (Pf/Pi)	Root-gall indices (0–100)	Galls/ g root
			Peanut		
MA1	39 a	17,738 a	76 ab	19 a	79 a
MA2	0 d	64 c	0.3 d	0 d	3 c
MH	23 b	14,363 a	63 b	11 c	88 a
MA1 + MA2	14 c	5,143 b	24 c	14 bc	40 b
MA1 + MH	32 ab	23,915 a	105 a	15 b	91 a
Pi	NS	**	**	**	**
			Tobacco		
MA1	2 d	208 c	1 d	4 b	15 c
MA2	66 a	20,540 a	95 a	11 a	92 a
MH	38 ab	11,215 a	64 b	7 b	72 a
MA1 + MA2	27 bc	6,597 a	26 c	7 b	44 b
MA1 + MH	19 c	3,367 b	17 cd	4 b	40 b
Pi	NS	**	**	**	**

All data are means of five replicates of pooled Pi treatments (see Materials and Methods). Statistical analyses of nematode-count data were based on $\log_{10}(X + 1)$ transformed data. Root-gall indices based on visual estimate of percentage of root galled.

MA1 = *Meloidogyne arenaria* race 1; MA2 = *M. arenaria* race 2; MH = *M. hapla.*

Means within column followed by the same letter are not different according to Waller-Duncan k-ratio *t* test (k-ratio = 100). *,** indicate significant difference at $P = 0.05$ and 0.01, respectively; NS indicates no significant difference.

peanut roots. MA1 caused a higher root-gall index than did MH and the mixed populations (Table 2). However, numbers of galls induced by MA1, MH, and MA1 + MH were similar and greater than that by the MA1 + MA2 mixed population. Gall development by MA1 was suppressed by MA2.

Tobacco roots inoculated with the varied nematodes had limited gall indices at 6 weeks (Table 2). Nevertheless, numbers of galls per g root caused by MA2 and MH were greater than those caused by MA1 and the two mixed populations. Induction of root galls by MA2 and MH nematodes was suppressed by the presence of MA1 nematode (in the mixed populations).

Numbers of females in parasitically fit populations (MA1 and MH on peanut, and MA2 and MH on resistant tobacco) in roots were usually more closely correlated with nematode reproduction and root galling than were numbers of juveniles in roots (data not included). For example, the respective correlation coefficients for numbers of females of MA1 in peanut roots at 6 weeks vs. root-gall indices, galls per g root, egg masses per g root, and eggs per g root were 0.52*, 0.73**, 0.59**, and 0.74**. Juvenile numbers of MA1 in peanut roots were better correlated with root galls and egg numbers at 2 weeks than at 6 weeks. Similar correlations were observed for MH. No significant correlations were detected for any of these parameter combinations for MA2 in peanut roots. Respective correlation coefficients for numbers of MA2 females in tobacco roots at 6 weeks vs root-gall index, gall per g root, egg masses per g root, and eggs per g root were 0.67**, 0.84**, 0.90**, and 0.87**. In contrast, the numbers of MA1 females in tobacco roots were significantly correlated only with galls per g root (0.47*).

Successful host–parasite relationships of sedentary endoparasitic nematodes depend on their ability to locate and invade host roots, reach suitable feeding sites, induce a compatible metabolic sink for a permanent feeding site, develop fully into the reproductive stage, and produce progeny. The numbers of nematodes observed in roots at different sampling times may not reflect more than the rate of initial penetration, as indicated in other studies (1,6, 7,14,21,22). For example, Arens et al. (1) found that during 2–8 days after inoculation, numbers of *M. javanica, M. arenaria,* and *M. incognita* in susceptible roots of tobacco were different, but at 10 days no significant differences were found. Because most nematodes in roots at 2 weeks were juveniles in this study, these numbers could reflect only the successful infections (suitable feeding site had been established). The total population density of parasitically fit nematodes in roots at 6 weeks reflected their potential for reproduction (i.e., the majority were females). Fewer than half of the nematodes in roots of the less parasitically fit populations developed into females. Failure of juveniles to develop further has been observed in several resistant or unsuitable host plants, including resistant peanut lines (14,16).

As root growth increased, total nematodes per root system at 6 weeks increased over those at 2 weeks, indicating that after 2 weeks, root infection was still occurring. However, at 6 weeks, root-nematode density (number per g-root) of high-density populations on peanut (MA1, MH, and MA1 + MH) and MI-resistant tobacco (MA2) were restricted. The dilution of nematode per gram-root at 6 weeks could, in part, be due to the fast growth of root systems without extended infection. Also, juveniles in high-density populations may have egressed the roots when overcrowding occurred (7,21).

Among nematode populations, number of egg masses may not accurately represent the number of eggs because egg mass size varied with nematode species, or populations, and host crops. For example, MA1 produced more egg masses on peanut than did MH, but the two populations produced similar amounts of eggs, suggesting that MH had more eggs per egg mass than did MA1. Between two root-galling parameters (root-gall indices and gall numbers per gram-root) gall number correlated better with female population densities in roots than did gall indices.

Root-gall index of MH nematode may have been underestimated because of the smaller size of galls compared to those of other root-knot nematodes. For example, MH had a smaller root-gall index on peanut than did MA1, but they had similar gall numbers.

The status of host suitability (based on nematode development and reproduction) to one nematode may change when that population interacts with other(s), depending on type, or character, of the nematode interaction. Information on host suitability, aggressiveness of mixed nematode populations, and interactions among different populations will aid in the selection of appropriate crops and rotations to be used in multispecies-infested soil. Inappropriate crop selection might result in an increase of an alternate nematode population. Multiple benefits occur if a crop is selected that expresses a suppressive interaction among nematode populations.

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