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Reproduction of *Meloidogyne* spp. on Resistant Peanut Genotypes from Three Breeding Programs

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Abstract: Three described species of root-knot nematode parasitize peanut (*Arachis hypogaea*): *Meloidogyne arenaria* race 1 (Ma), *M. hapla* (Mh), and *M. javanica* (Mj). Peanut cultivars with broad resistance to *Meloidogyne* spp. will be useful regardless of the species present in the field. The objective of this study was to determine whether peanut genotypes with resistance to *M. arenaria* originating from three different breeding programs were also resistant to *M. hapla* and *M. javanica.* The experiment used a factorial arrangement (completely randomized) with peanut genotype and nematode population as the factors. The five peanut genotypes were 'COAN' and AT 0812 (highly resistant to Ma), C209-6-13 (moderately resistant to Ma), and 'Southern Runner' and 'Georgia Green' (susceptible to Ma). The four nematode populations were two isolates of Ma (Gibbs and Gop) and one isolate each of Mh and Mj. On COAN or AT 0812, both Ma and Mj produced <10% of the eggs produced on Georgia Green. On the peanut genotype C209-6-13, Ma and Mj produced about 50% of the eggs produced on Georgia Green. None of the resistant genotypes exhibited a high level of resistance to Mh. The lack of resistance to Mh in any cultivars or advanced germplasm is a concern because the identity of a *Meloidogyne* sp. in a particular peanut field is generally not known. Breeding efforts should focus on moving genes for resistance to *M. hapla* into advanced peanut germplasm, and combining genes for resistance to the major *Meloidogyne* spp. in a single cultivar.

Key words: Arachis hypogaea, Meloidogyne arenaria, M. hapla, M. javanica, peanut, reproduction, resistance, root-knot nematode.

Three described species of root-knot nematode parasitize peanut (*Arachis hypogaea*). *Meloidogyne arenaria* race 1 is found throughout the peanut-producing region of the United States and is the dominant species in Texas and the Southeast (AL, FL, GA, and SC), where it reduces peanut yield by 3% to 15% annually (Dickson, 1998; Koenning et al., 1999; Minton and Baujard, 1990). *Meloidogyne hapla* is the dominant species parasitizing peanut in more northerly states (OK, NC, and VA), though this species is not as damaging to peanut as *M. arenaria* (Koenning and Barker, 1992; Minton, 1984). *Meloidogyne javanica* is a common parasite of peanut in Egypt (Tomaszewski et al., 1994); however, most populations of this nematode are not able to reproduce on peanut (Hartman and Sasser, 1985). In the United States, three populations of *M. javanica,* one each from Georgia, Texas, and Florida, are pathogens of peanut (Lima et al., 2002; Minton et al., 1969; Tomaszewski et al., 1994). Peanut yield suppression caused by *M. javanica* was found to be similar to that caused by *M. arenaria* (Abdel-Momen and Starr, 1997).

Two advanced germplasm lines of *A. hypogaea* have been developed with genes introgressed from wild species. The first, TxAG-6, was developed from a complex hybrid of *Arachis batizocoi* × (*A. cardenasii* × *A. diogoi*) (Nelson et al., 1989; Starr et al., 1995). The germplasm TxAG-7 was derived from a backcross of *A. hypogaea* ('Florunner') × TxAG-6 (Simpson et al., 1993). TxAG-7 is resistant to *M. arenaria, M. javanica,* and an undescribed *Meloidogyne* sp. from Texas. However, in additional backcross generations with Florunner, resistance to *Meloidogyne* spp. segregated independently, suggesting that resistance to individual species is conditioned by different genes (Abdel-Momen et al., 1998). The cultivar COAN contains a single dominant gene for resistance to *M. arenaria* originating from *A. cardenasii* and introgressed through TxAG-6 (Burow et al., 1996; Choi et al., 1999; Simpson and Starr, 2001). The resistance to *M. arenaria* is linked to RFLP markers on linkage group 1 (Choi et al., 1999). COAN is also resistant to *M. javanica* (Simpson and Starr, 2001); however, it is unknown whether the same gene confers resistance to both *Meloidogyne* spp.

The second interspecific germplasm, GP-NC WS 5 and GP-NC WS 6, was developed from a hybrid of *A. hypogaea* (PI 261942) × *A. cardenasii* (Stalker et al., 2002). The resistance in these germplasm lines is conferred by two dominant genes: *Mae* conditions resistance to egg production and *Mag* conditions resistance to gall formation (Garcia et al., 1996; Stalker et al., 2002). The two genes are linked to the Z3 RAPD marker on linkage group 1 (Garcia et al., 1996). It is not known whether *Mae* is the same gene that suppresses egg production in COAN, or whether the resistance in GP-NC WS 5 and GP-NC WS 6 is effective against other *Meloidogyne* species besides *M. arenaria.* The genotype AT 0812 came from a cross of GP-NC WS 5 and AT 108, and contains the Z3 RAPD marker (Anderson, unpubl.). In a third breeding program, several advanced breeding lines with moderate levels of resistance to *Tomato spotted wilt virus* (TSWV) and *M. arenaria* were identified (Holbrook et al., 2003; Timper et al., 2000). The breeding lines came from a cross between *A. hypogaea* 'MARC-1' and an interspecific germplasm (PI 261942 × *A. cardenasii*) and is designated the C209-6 family of genotypes. The interspecific germplasm was obtained from H. T. Stalker while it was still segregating for nematode resistance; therefore, it is related to but not identical to GP-NC WS 5. COAN, GP-NC WS 5, and AT 0812 express a high level of resistance

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to *M. arenaria,* whereas the C209-6 genotypes express a moderate level of resistance to the nematode (Anderson et al., 2002; Holbrook et al., 2003; Simpson and Starr, 2001; Stalker et al., 2002). All three genotypes are runner-type peanut.

Peanut cultivars with broad resistance to *Meloidogyne* spp. will be useful across the peanut-producing region of the United States and will reduce the need for species identification of root-knot nematode within a field. The objective of this study was to determine whether peanut genotypes with resistance to *M. arenaria* originating from three different breeding programs were also resistant to *M. hapla* and *M. javanica.*

Materials and Methods

Nematode inoculum. Four isolates of *Meloidogyne* were tested: two isolates of *M. arenaria,* and one isolate each of *M. hapla* and *M. javanica.* The two isolates of *M. arenaria,* both from Tift County, Georgia, were selected because they had shown differential reproduction on peanut (Noe, 1992). The Gop isolate (= GA-8) (Noe, 1992) produced twice as many eggs on 'Florunner' peanut as did the Gibbs isolate (= GA-7). The Gop isolate of *M. arenaria* was provided by J. P. Noe. *Meloidogyne javanica* and *M. hapla* were isolated from peanut in Texas and were provided by J. L. Starr. The nematodes were cultured alternately on tomato (*Lycopersicon esculentum* cv. Rutgers) and peanut cv. Georgia Green to reduce potential contamination from *M. incognita* (a parasite of tomato but not peanut). Eggs were collected from roots of tomato using 0.05% NaOCl (Hussey and Barker, 1973).

Reproduction on peanut. Two experiments were conducted. The first experiment compared reproduction of *M. arenaria* Gibbs and Gop isolates, and *M. javanica.* The second experiment compared reproduction of *M. arenaria* Gibbs and *M. hapla.* Both experiments used a two-way factorial arrangement of treatments with peanut genotype and nematode isolate as the factors in a completely randomized design. The five peanut genotypes were COAN and AT 0812 (highly resistant to Ma), C209-6-13 (moderately resistant to Ma), and 'Southern Runner' and 'Georgia Green' (susceptible to Ma). The soil used in the experiments was a loamy sand (82% sand, 9% silt, 7% clay, 1% organic matter; pH 5.3) that had been steam heated at 100 °C for 6 hours to kill potential plant pathogens. Peanut genotypes were planted two seeds per pot (10-cm-square pots, 700 cm^3 of soil) and thinned to one plant per pot after germination. Nematode eggs were distributed between two holes (3-cm deep) at the base of the plant 2 to 3 weeks after planting and covered with soil. Each pot was infested with 8,000 nematode eggs. The treatments were completely randomized on a single bench in a greenhouse where soil temperatures varied between 20 °C and 35 °C. Both experiments were performed twice, with six to eight replicates per treatment in each trial of the experiment.

Nematode eggs were extracted from the peanut roots 58 to 63 days after inoculation by the following method. The entire root system of a single plant was cut into ca. 5-cm pieces, weighed, placed in a 1-liter flask, and agitated for 4 minutes in a 1% NaOCl solution (Hussey and Barker, 1973). Eggs were collected and rinsed with tap water on nested 150- and 25-µm-pore sieves, and counted using a dissecting microscope. A three-way analysis of variance was used to determine whether there were interactions between peanut genotype, nematode population, and experimental trial. Differences ($P \leq 0.05$) among peanut genotypes within a nematode population were determined by Fisher's LSD test.

Results

Reproduction on peanut—M. arenaria *and* M. javanica. Results from the two trials of Experiment 1 are presented separately (Fig. 1) because reproduction of the different nematode isolates on the peanut genotypes were not consistent between the trials (trial × nematode × genotype interaction, $P < 0.0001$). In both trials, nematode reproduction was greatest on Georgia Green

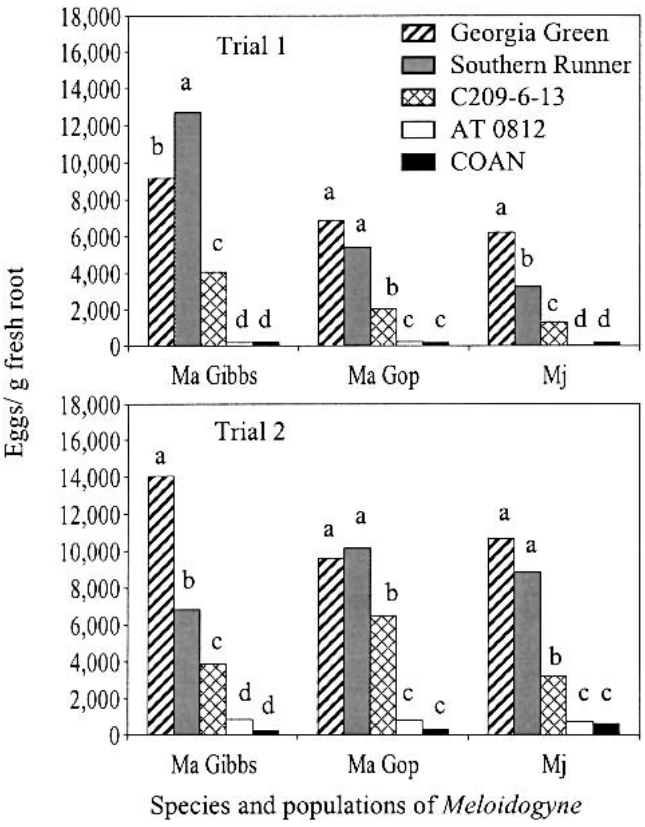

Fig. 1. Reproduction of *Meloidogyne arenaria* (Ma) Gibbs isolate, *M. arenaria* Gop isolate, and *M. javanica* (Mj) on resistant and susceptible peanut (*Arachis hypogaea*) genotypes. Georgia Green and Southern Runner were the susceptible controls. Bars within a nematode isolate with the same letter are not different ($P > 0.05$).

and Southern Runner, intermediate on genotype C209-6-13, and least on COAN and AT 0812 when the nematode isolates were pooled ($P < 0.0001$). In Trial 1, there were differences ($P < 0.0001$) among the nematode populations, with *M. arenaria* Gibbs producing the greatest number of eggs and *M. javanica* producing the least number of eggs; however, in Trial 2, there were no differences among the nematode populations. Also in Trial 1 but not in Trial 2, *M. arenaria* Gibbs produced more eggs than *M. arenaria* Gop on the susceptible peanut cultivars and C209-6-13 ($P < 0.05$). Although there was an interaction ($P < 0.0004$) between peanut genotype and nematode population in both trials, the relative reproduction on the resistant genotypes was consistent among nematode populations and between trials (Fig. 1). However, differences between the two susceptible genotypes, Georgia Green and Southern Runner, were not consistent among nematode isolates or between trials. For example, *M. arenaria* Gibbs produced more eggs on Southern Runner than on Georgia Green in Trial 1, but the reverse occurred in Trial 2 (Fig. 1).

Reproduction on peanut—M. arenaria *and* M. hapla. In Experiment 2, the results from the two trials were similar and, therefore, were combined (Fig. 2). There was an interaction between nematode species and peanut genotype ($P = 0.004$), which was due to differential reproduction of *M. arenaria* and *M. hapla* on the resistant peanuts. The resistant genotypes COAN, AT 0812, and C209-6-13 supported the same amount of *M. hapla* reproduction as the susceptible genotypes (Fig. 2). Reproduction of *M. hapla* was lower ($P \leq 0.05$) on COAN than on Georgia Green or on AT 0812, but it was not different from Southern Runner or C209-6-13. The relative reproduction of *M. arenaria* on the peanut genotypes was similar to that observed in Experiment 1, except reproduction on Southern Runner was similar to C209-6-13. On the susceptible genotypes, *M. hapla* produced more eggs than did *M. arenaria* Gibbs ($P \leq 0.05$).

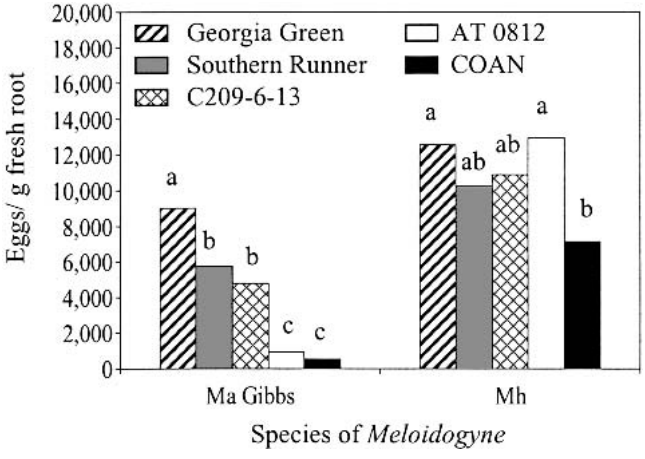

FIG. 2. Reproduction of *Meloidogyne arenaria* (Ma) Gibbs isolate and *M. hapla* (Mh) on resistant and susceptible peanut (*Arachis hypogaea*) genotypes. Georgia Green and Southern Runner were the susceptible controls. Bars within a nematode isolate with the same letter are not different ($P > 0.05$).

DISCUSSION

Although resistance is a relative concept, nematode reproduction <10% of the susceptible genotype is considered a high level of resistance (Hussey and Janssen, 2002). *Meloidogyne arenaria* and *M. javanica* were similar in their ability to reproduce on COAN or AT 0812, producing <10% of the eggs produced on Georgia Green. On the peanut genotype C209-6-13, both *M. arenaria* and *M. javanica* produced about 50% of the eggs produced on Georgia Green. None of the resistant genotypes exhibited a high level of resistance to *M. hapla*. Even though reproduction of this nematode was less on COAN than on Georgia Green, it was not different from Southern Runner, the other susceptible control. In several instances, Southern Runner appeared to have some resistance to *M. arenaria* (Trial 2 of Experiment 1 and Experiment 2) or *M. javanica* (Trial 1 of Experiment 1). Perhaps this cultivar is segregating for a gene conferring moderate resistance to these nematode species.

The similarity of the resistance in COAN and AT 0812, both in terms of the level and breadth of resistance, suggests that the same resistance gene(s) is expressed in both genotypes. The resistance in both COAN and AT 0812 was introgressed from the wild peanut *A. cardenasii,* which contains several dominant genes, all independently conferring resistance (Starr and Simpson, 1991). Although the Z3 RAPD resistance marker, which is found in *A. cardenasii,* GP-NC WS 5, and AT 0812, is also found in COAN (H. T. Stalker, pers. comm.), we cannot rule out the possibility that different, closely linked genes or minor effect genes are involved.

We did not observe any consistent differences in reproduction between *M. arenaria* and *M. javanica* on susceptible or resistant peanut. In the first trial of Experiment 1, *M. javanica* produced fewer ($P < 0.05$) eggs than *M. arenaria* Gibbs on Southern Runner and C209-6-13, but produced similar egg numbers as *M. arenaria* Gop. However, in the second trial, *M. javanica* and *M. arenaria* Gibbs produced similar numbers of eggs on all genotypes. These results differ from those of Abdel-Momen and Starr (1997), who found that *M. arenaria* produced greater numbers of eggs than *M. javanica* on Florunner peanut. In our study, reproduction of *M. hapla* was greater than *M. arenaria* on the susceptible peanuts, whereas other studies have found that reproduction of *M. arenaria* was greater than (Koenning and Barker, 1992) or similar to (Hirunsalee et al., 1995) *M. hapla* on peanut. The most likely explanation for these disparate results is differences in aggressiveness of the nematode isolates used in the different studies. Isolates

of *M. arenaria* race 1 are known to vary in their reproductive rate on peanut. For example, the Gop isolate (= GA-8) produced twice as many eggs as the Gibbs isolate (= GA-7) on Florunner (Noe, 1992). We were not able to confirm the differential reproduction of the Gop and Gibbs isolates on either Georgia Green or Southern Runner. Perhaps the Gop isolate has become less aggressive over time or differences in the relative viability of the egg inoculum for the Gop and Gibbs isolates affected the results. The latter point highlights the need for determining percentage egg hatch of inoculum, particularly in studies comparing reproduction of different nematode isolates or species.

The results of this study indicate that the source of resistance in COAN, AT 0812, and C209-6-13 will be effective in suppressing populations of *M. arenaria* and *M. javanica,* but not *M. hapla.* The latter nematode is considered less damaging to peanut than *M. arenaria;* nevertheless, it still causes yield loss (Koenning and Barker, 1992). The lack of resistance to *M. hapla* in any cultivars or advanced germplasm is a concern because the identity of a *Meloidogyne* sp. in a particular peanut field is generally not known and is assumed to be the dominant species in the region: *M. arenaria* in southern latitudes and *M. hapla* in northern latitudes of peanut production. However, there are populations of *M. hapla* in all peanut-producing states in the United States except Florida (Dickson, 1998; Norton et al., 1984). In Texas, *M. hapla* has been found in mixed populations with *M. arenaria* and an undescribed *Meloidogyne* sp. in five peanut fields in Collinsworth County and in one potato field in the south of the state (Frio County) (Starr, pers. comm.). Extensive surveys are needed to determine the frequency and distribution of *M. hapla* in Alabama, Georgia, and South Carolina. Furthermore, breeding efforts should focus on moving genes for resistance to *M. hapla* into advanced peanut germplasm. Several wild *Arachis* species, including *A. cardenasii,* and the complex hybrid TxAG-6 contain genes for resistance to *M. hapla* (Nelson et al., 1989). Combining genes for resistance to multiple species of *Meloidogyne* in a single peanut cultivar will allow that cultivar to effectively manage root-knot nematodes over a wide range of environments, and may reduce selection of virulent species in fields with mixed populations.

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