Journal of Nematology 35(1):115–118. 2003.

Mechanism of Resistance to *Meloidogyne arenaria* in the Peanut Cultivar COAN[1]

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Abstract: Resistance to *Meloidogyne arenaria* in the peanut cultivar COAN is inherited as a single, dominant gene. The mechanism of resistance to *M. arenaria* in COAN was evaluated in three experiments. In the first experiment the number of second-stage juveniles (J2) of *M. arenaria* penetrating roots of the susceptible cultivar Florunner was higher than the number of J2 penetrating roots of the resistant peanut cultivar COAN ($P < 0.05$). In a second experiment it was determined that the root size and number of potential infection courts (root tips) were similar for the two peanut cultivars. The number of nematodes emigrating from roots of COAN after penetration was greater than emigrated from roots of Florunner ($P < 0.05$). Necrotic host tissue was rarely observed in roots of COAN infected with *M. arenaria,* suggesting that resistance to *M. arenaria* does not involve a necrotic, hypersensitive response. Most of the J2 observed in roots of COAN were restricted to the cortical tissue, with only 1 of 90 J2 observed being associated with the vascular cylinder, whereas in Florunner >70% of the J2 were associated with vascular tissues. Resistance in COAN may be due to constitutive factors in the roots.

Key words: Arachis hypogaea, emigration, host resistance, hypersensitive reaction, *Meloidogyne arenaria,* peanut, penetration, root-knot nematode.

The root-knot nematode *Meloidogyne arenaria* Neal (Chitwood) is one of the most important pests of peanut in the United States (Ingram and Rodríguez-Kábana, 1980; Motsinger et al., 1976; Wheeler and Starr, 1987). Until recently, there was no peanut cultivar with resistance against this highly damaging nematode, which can cause yield reduction at population densities as low as 1 nematode/100 cm^3 soil (Abdel-Momen and Starr, 1997; Koenning and Barker, 1992; McSorley et al., 1992). The Texas Agricultural Experiment Station released the cultivar COAN in 1999 with resistance to *M. arenaria* (Simpson and Starr, 2001). COAN has yields that range from 25% to 210% greater than susceptible cultivars in *M. arenaria*-infested fields (Church et al., 2000).

The resistance in COAN is derived from *Arachis cardenasii* and segregates as a single dominant gene that has been mapped to linkage group 1 (Burow et al., 2001; Choi et al., 1999). Choi et al. (1999) reported that nematode development was retarded in the breeding line (TP262-3-5) from which COAN was selected. Further, they did not detect any host necrosis associated with invading nematodes in COAN that would indicate the occurrence of a hypersensitive host response (rapid cell death in response to a challenge by a pathogen) (Leach, 2001) as a component of the resistance mechanism. However, the procedure used in that study (root clearing with NaOCl followed by treatment with acid fuchsin to stain the nematodes) may not have been adequate to detect necrosis of a few host cells.

The objectives of this study were to (i) evaluate the behavior of *M. arenaria* J2 following root penetration on the resistant peanut cultivar COAN and the susceptible peanut cultivar Florunner, and (ii) examine more closely the host response to infection to determine if a necrotic, hypersensitive response is expressed in the root cells of COAN immediately surrounding the anterior region of the nematode.

Materials and Methods

Penetration and emigration of second-stage juveniles: Twelve seeds of peanut cultivars COAN (resistant) and Florunner (susceptible) were surface-sterilized by rinsing in 0.6% NaOCl for 1 minute and then rinsed in sterile distilled water. The seeds were then germinated in moistened germination paper (Anchor Paper, St. Paul, MN) at 25 °C for 5 days. Seedlings were then transplanted to 180-cm^3 cups containing a soil mixture of 6 parts sand to 1 part peat and were incubated at 28 °C. After 7 days each plant was inoculated with approximately 2,000 freshly hatched J2 (Vrain, 1977). Two days after inoculation (DAI), six plants of each cultivar were harvested, and the roots were washed and stained with acid fuchsin (Byrd et al., 1983) to determine the number of nematodes present in each root system. The remaining six plants of each cultivar were transferred, after washing the soil from the roots, to individual 180-cm^3 plastic containers filled with distilled water and fitted with plastic tubing attached to a small air-pump. Air was bubbled through the water to keep the roots well oxygenated and healthy. The J2 that emigrated from the roots into the water of each container were recovered daily from 3 to 7 DAI and counted. At 7 DAI the roots of these remaining plants were stained with acid fuchsin as before. This experiment was repeated three times.

To determine if differences in size of the root system or number of potential infection courts could be a factor in root penetration, seeds of COAN and Florunner

Received for publication 1 July 2002.

[1] Supported by grants from the Texas Peanut Producers Board, the Texas Higher Education Board Advanced Technology Program (000517-0233-1999) and by USDA-NRICGP grant no. 97-35300-4584.

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The authors wish to thank Helga Sittertz-Bhatkar from the Microscopy and Imaging Center at Texas A&M University for help with the staining and embedding of root tissue.

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This paper was edited by Patricia Timper.

were germinated as described previously. Six 5-day-old plants of each cultivar were collected from the germination paper, the roots of each plant were blotted dry and weighed, then the number of root tips counted. An additional six plants of each cultivar were then transplanted to 180-cm^3 cups containing the sand-peat potting mix. These later plants were grown for an additional 10 days at 28 °C then harvested to determine root weight and number of root tips. This experiment was repeated once.

Statistical analysis: The effects of trial, DAI, and host genotype on the number of J2 in the roots at 2 and 7 DAI, and on number of J2 emerging daily from each peanut root system, were subjected to analysis of variance using the SAS (SAS Institute, Inc., Cary, NC) general linear model procedure. Similarly the effect of genotype on root weight and number of root tips per root system were subjected to analysis of variance.

Hypersensitivity reaction: Seeds of Florunner and COAN were germinated in moistened germination paper as described, and after 3 weeks seedlings were transferred to 36-liter plastic tubs containing the sand-peat potting mix, at 15 seedlings/tub. One individual root tip from each seedling was placed singly inside a 1.5-ml microfuge tube containing fine sand. The individual root tips of 30 COAN and 30 Florunner plants were inoculated by adding 50 freshly hatched *M. arenaria* J2 to each microfuge tube. Plants were grown at 28 °C with a 14-hour light, 10-hour dark regime. Five plants from each cultivar were harvested at 2 DAI, 4 DAI, 6 DAI, 8 DAI, and 10 DAI. Samples were fixed for 6 hours at room temperature in a mixture of 2% glutaraldehyde, 2% paraformaldehyde, 2% acrolein, and 1.5% dimethyl sulfoxide in 0.133 M sodium cacodylate buffer (pH 7.4) in a modification of the procedure of Kalt and Tandler (1971). After rinsing in 0.1 M sodium cacodylate, tissue was post fixed in 1% osmium tetroxide. Following fixation, dehydration, and ethanol replacement with propylene oxide, samples were embedded in a mixture of Araldite and Embed 812 (Epon-812) embedding medium (Mollenhauer, 1964) and polymerized in an oven at 45 °C for 24 hours and at 60 °C for another 24 hours. Longitudinal and transverse sections, 17-µm-thick, were obtained from root apices and examined with bright field and interference contrast optics. Data were collected on the number of J2 associated with necrotic host cells at each sample time, on the position of the J2 in the roots, and on the percentage of J2 that had grown from the vermiform stage to the swollen J2 stage.

Results

Penetration and emigration of second-stage juveniles: In each trial, the number of J2 present in the root system at 2 DAI and 7 DAI was higher in Florunner than in COAN ($P < 0.05$) (Fig. 1). Because the cultivar by trial interaction was not significant, the data from the three separate trials were combined for analysis (Table 1). At 7 DAI swollen J2 were observed with a mean of 8.0 swollen J2/root system for Florunner compared with 1.3 swollen J2/root system for COAN. Necrotic tissue was not observed in roots where vermiform or swollen J2 were present in this experiment. In all three trials, more J2 ($P < 0.05$) emigrated from the roots of COAN than from the roots of Florunner. These differences were significant ($P < 0.05$) at all sample dates, except for 3 DAI (Fig. 2).

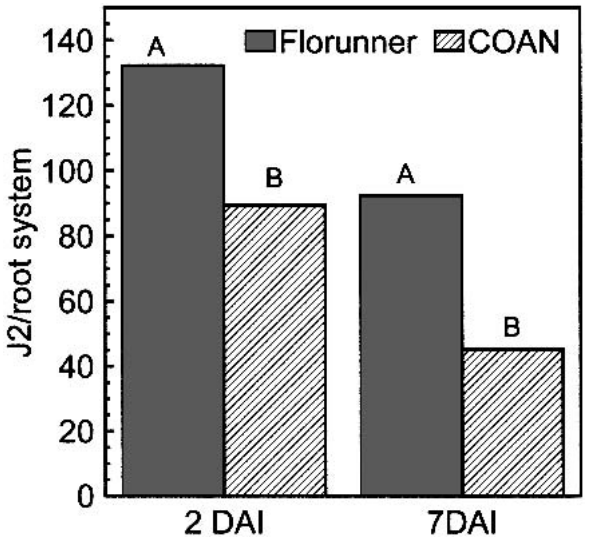

Fig. 1. Number of *Meloidogyne arenaria* vermiform juveniles in roots of peanut cultivars COAN (resistant) and Florunner (susceptible) at 2 and 7 days after inoculation (DAI). Data from three trials of the experiment were combined for analysis. Different letters over bars from the same sample date indicate significant differences at $P = 0.05$.

For 5-day-old seedlings, the mean fresh root weight (1.6 g) and mean number of roots tips (218.0) for Florunner was not different from COAN (1.76 g and 229.0 root tips). Similarly for 15-day-old seedlings, equivalent to 10 DAI, the values for root weights (2.37 g vs. 2.32 g) and root tips (379.7 vs. 380.8) for COAN and Florunner, respectively, were similar.

Table 1. Analysis of variance of number of *Meloidogyne arenaria* juveniles (J2) per root system and number of J2 emigrating from the roots for peanut cultivars Florunner (susceptible) and COAN (resistant).

Source of variance	dF	F	Pr > F
	J2/root system		
Cultivar	1	22.84	0.001
Experiment	2	9.79	0.001
DAI	1	19.74	0.001
Cultivar * Experiment	2	0.25	0.782
	Total emigration		
Cultivar	1	38.60	0.001
Experiment	2	27.08	0.001
Cultivar * Experiment	2	0.93	0.408

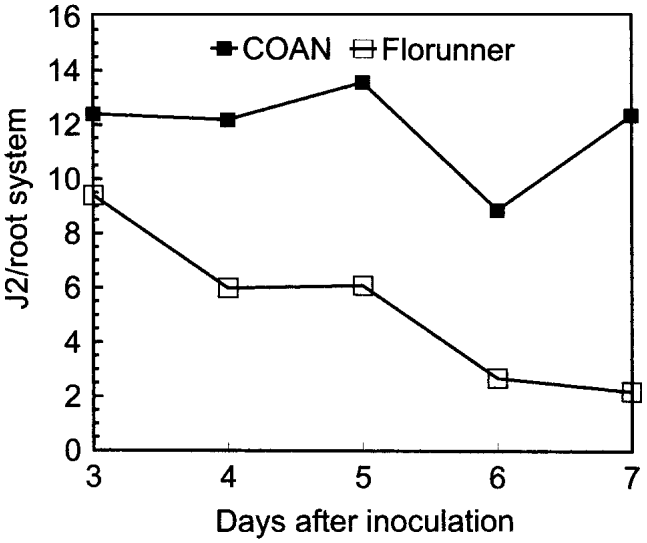

FIG. 2. Number of *Meloidogyne arenaria* vermiform juveniles emigrating from roots of peanut cultivars COAN (resistant) and Florunner (susceptible) at different days after inoculation (DAI). Data from three trials of the experiment were combined for analysis. Numbers of J2 emigrating from roots of COAN were different ($P = 0.05$) from those emigrating from Florunner on all sample dates except 3 DAI.

Hypersensitive reaction: Sections cut from the root apices (0.5 to 1.0 cm long) showed that, at 48 hours after inoculation, penetration of juveniles of *M. arenaria* into cortical tissues of COAN and Florunner had occurred. Galling was not observed on the roots of either peanut cultivar at any sample date. In most root samples containing nematodes, cell necrosis was occasionally observed in the tissue surrounding the area through which the nematode had migrated. However, in only 2 of 90 instances in COAN was host necrosis observed near the anterior region of a nematode. Necrosis was observed in Florunner in less than 1% of the infection sites. Most of the J2 were within the root cortex in COAN. Only 1 of 90 J2 observed in COAN was associated with the vascular cylinder whereas, 72% of the observed J2 were associated with the vascular cylinder in Florunner, mainly from 6 DAI to 10 DAI.

DISCUSSION

COAN and Florunner are near-isogenic lines with resistance to *M. arenaria* in COAN developed by a backcross breeding program with Florunner as the recurrent, susceptible parent (Simpson and Starr, 2001). Data from this study show that penetration and subsequent development of *M. arenaria* in roots were affected by peanut genotype. The resistance gene in COAN seems to have three different effects on *M. arenaria*. The first effect of the resistant genotype COAN was a reduction in root penetration relative to the susceptible Florunner. The difference in number of juveniles entering the roots of the two peanut cultivars was not due to differences in number of root tips or the overall size of the root system of these two cultivars. These results differ from those reported for *M. incognita* in soybean (Herman et al., 1991), cotton (Minton, 1962), alfalfa (Griffin and Elguin, 1977; Reynolds et al., 1970), and tomato (Hadisoeganda and Sasser, 1982) where the number of J2 entering the root was not different between resistant and susceptible genotypes. It is possible that early emigration (before 2 DAI) affected the recorded penetration rate (Call et al., 1996; Herman et al., 1991; Pedrosa et al., 1994, 1996; Timper et al., 2000). The second effect observed was that most J2 failed to establish a feeding site and emigrated from the roots. It is possible that the J2 were repelled by mechanisms similar to those observed in *Brassica nappus* (Potter et al., 1999) and soybean (Ibrahim and Lewis, 1986). Thirdly, the difference in observed percentage of J2 that were swollen is consistent with the previous report of Choi et al. (1999) that nematode development is delayed for J2 that establish a feeding site in COAN relative to the rate of development in Florunner.

The general absence of host necrosis near the J2 during the early stages of the host-parasite interaction in resistant plants confirms the observations by Choi et al. (1999) and indicates that the resistance conditioned by the single, dominant resistance gene in COAN is not due to a hypersensitive reaction. This is similar to the resistance to *M. incognita* in soybean (Pedrosa et al., 1996). It is likely that in some instances necrosis caused by mechanical damage has been mistaken in the past for a hypersensitive reaction.

The differential location of the nematodes (vascular vs. cortical tissue) in Florunner and COAN suggests that resistance is expressed when nematodes are in the root cortex. The resistance in COAN may be due to a constitutive trait and not by an active response by the host. It is possible that COAN may contain one or more factors that act as a repellent or to inhibit feeding activities, both of which may result in increased rates of emigration. Further, these putative factors may be produced constitutively and thus resistance may not require an active host response to the nematode. However, the absence of observable host responses does not negate the possibility of an active response that was not detected by the experiments reported in this study.

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