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Influence of Temperature on Susceptibility of Cvs. Tifguard and Georgia-06G Peanut to *Meloidogyne arenaria*

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Abstract

Tifguard was released in 2008 as a peanut cultivar with a high level of resistance to *Meloidogyne arenaria*. Our objective was to determine the role of temperature on infection and development of *M. arenaria* in Tifguard compared to that in the nematode susceptible cultivar, Georgia-06G. Temperature affected the rate of nematode infection and development in both Tifguard and Georgia-06G ($P \leq 0.05$). In Georgia-06G, egg-laying females were observed 25, 20 or 25 days after inoculation at 28°C, 31°C, and 34°C, respectively. There were greater numbers of nematodes entering roots and acceleration of development in response to 31°C compared with that at 28°C. There was, however, a decrease in the number of nematodes entering roots and their development was retarded at 34°C compared with that occurring at 31°C. Although second-stage juveniles penetrated Tifguard roots, they did not develop further at 28°C or 31°C; however, at 34°C both females, males, and a few egg-laying females of *M. arenaria* were observed. The optimum temperature for nematode infection and development was 31°C in Georgia-06G. In summary, it is unlikely that high soil temperatures would lessen the effectiveness of the nematode resistance gene in Tifguard.

Key words

Arachis hypogaea, management, *Meloidogyne arenaria*, resistance, root-knot nematode, temperature

Meloidogyne arenaria race 1, the peanut root-knot nematode, is considered as one of the most important soil-borne pathogens affecting peanut, *Arachis hypogaea* in the southern USA. This species is prevalent in Alabama, Florida, Georgia, and Texas (Ingram and Rodriguez-Kabana, 1980; Starr et al., 2002; Dickson and DeWaele, 2005). The suppression of peanut yields caused by this nematode can reach 80% or greater in heavily infested fields (Dickson and Hewlett, 1988).

Resistant cultivars potentially provide the most economical and effective means of managing nematode diseases on agricultural crops. Before 2001 there were no peanut cultivars with root-knot nematode resistance, yet a number of *Arachis* spp. were observed to be highly resistant to the peanut root-knot nematode (Baltensperger et al., 1986; Nelson et al., 1989; Holbrook and Noe, 1990). Genetic material from a wild peanut species, *Arachis cardenasii* was introgressed into cultivated peanut resulting in the first root-knot nematode resistant cultivar, COAN (Simpson and Starr, 2001). The second nematode resistant peanut cultivar, NemaTAM was released in 2002. This cultivar had the same resistance level as COAN, but had greater yield potential (Simpson et al., 2003). The resistance gene in COAN was reported to have three different effects on *M. arenaria* (Bendezu and Starr, 2003). The first was to reduce the number of the second-stage juveniles (J2) of *M. arenaria* that penetrated roots; second, the resistance gene prevented most J2 from developing further, and third, the resistance gene delayed further development. No hypersensitive reaction (HR) was observed in the early stage of *M. arenaria* infection in COAN roots, which is considered as a common defense mechanism produced by

resistant plants to disease pathogens (Bendezu and Starr, 2003).

Neither of these two cultivars was considered for planting in the southeastern U.S. because of the prevalence and severity of tomato spotted wilt virus (TSWV) and other important fungal diseases in this peanut growing region (Holbrook et al., 2008). In 2008, a runner-type peanut cultivar (*Arachis hypogaea* L. subsp. hypogaea var. hypogaea), Tifguard was released by the USDA-ARS and the Georgia Agricultural Experiment Station (Holbrook et al., 2008). The breeding population was generated by hybridizing C-99R (Gorbet and Shokes, 2002), a peanut cultivar with good field resistance to TSWV (Wells et al., 2002) with COAN. Tifguard was reported to have a high level of resistance to both TSWV and *M. arenaria* (Holbrook et al., 2008).

Temperature is an important factor influencing the expression of resistance genes in plants to parasitic nematodes. For example, it was observed that susceptible alfalfa plants were infected with greater numbers of J2 of *Meloidogyne hapla* than resistant plants, but the magnitude of this difference decreased when temperature increased (Griffin, 1969; Griffin and Elguin, 1977). In addition, half of the resistant alfalfa plants grown at 32°C had galled roots, whereas no galled roots were observed at 28°C. Similarly, the number of adult *Meloidogyne incognita* and *M. hapla* increased in resistant and susceptible common bean as the soil temperature increased from 25°C to 30°C (Irizarry et al., 1971). Increased temperature also affected nematode resistance in soybean and tomato cultivars (Dropkin, 1969; Holtzmann, 1965). Greater penetration and development of *M. incognita* was observed in resistant tomato roots at 30 and 34.5°C than at 20 and 25°C. In other studies on tomato, decreased hypersensitive necrotic response to nematode infection was reported at temperatures near or above 30°C (Paulson and Webster, 1972). The *Mi*-1 gene in tomato confers resistance to root-knot nematodes; however, it is reported in some studies to become nonfunctional when the temperature is at or above 28°C (Williamson, 1998). Changes in the response of resistant plants to root-knot nematode infection by high soil temperature has been reported under both greenhouse and field conditions (Philis and Vakis, 1977; Tzortzakakis and Gowen, 1996; Noling, 2000). It appears that heat stress affects the function of resistance genes in host plants (Ammiraju et al., 2003). On the other hand, it is well known that root-knot nematodes are dependent on temperature for vital physiological activities. Temperature affects root-knot nematode migration, development, and the nematode's ability to sense host roots (Tyler, 1933). The optimal temperature for root-knot nematode development was reported to be 28°C, above which development was reduced with no development occurring at 36.5°C (Tyler, 1933).

In a comparison of the number of the J2 of *M. arenaria* in different peanut genotypes at 2 and 7 days after inoculation (DAI), higher numbers of J2 penetrated the roots of the susceptible peanut cv. Florunner than the resistant COAN (Bendezu and Starr, 2003). Since the recent release of root-knot nematode resistant peanut cultivars, there have been no studies on how temperature affects the number and development of *M. arenaria* in resistant peanut. The objectives of this study were to determine the effect of temperature on i) the number of *M. arenaria* that penetrated roots, and ii) the nematode's development in resistant and susceptible peanut.

Materials and methods

Nematode origin: Meloidogyne arenaria was isolated from infected peanut roots from an infested farm located in Levy County, FL, and maintained on the susceptible tomato cultivar (*Solanum lycopersicum* L. cv. Agriset 334) in the greenhouse. Polyacrylamide gel electrophoresis was used as an aid in identification of the species based on malate dehydrogenase and esterase phenotypes (Esbenshade and Triantaphyllou, 1985). Eggs were extracted from infected tomato roots with the 0.25% NaOCl (Hussey and Barker, 1973) method as modified (Bonetti and Ferraz, 1981). A modified Baermann funnel method was used for egg hatching (Rodriguez-Kabana and Pope, 1981). Second-stage juveniles collected after a period of 48 hr were used as inoculum.

Penetration and development of Meloidogyne arenaria

Peanut seed of Tifguard and Georgia-06G were provided by the peanut breeder Corley Holbrook, USDA-ARS, Tifton, GA. Tomato cv. Agriset 334 was used as a susceptible plant to ensure inoculum viability. Peanut seeds were surface-treated by soaking in 0.6% NaOCl for 1 min followed by rinsing in sterile distilled water (Bendezu and Starr, 2003). They were placed in a petri dish with a piece of moistened paper towel for germination at 28°C for 5 days. Seedlings were transplanted into 250 ml plastic cups filled with autoclaved sand and grown at 28°C for 7 days. Holes were punched into the cup bottoms to provide drainage. All seedlings were inoculated with 2,000 freshly-hatched J2 of *M. arenaria*, and then transferred to environmental

growth chambers set at either 28, 31, or 34°C with a 12-hr photoperiod. The seedlings of each cultivar were completely randomized within the chambers and were watered daily.

Data collection

Three plants were removed at 5-day intervals following inoculation (DAI), washed in tap water, dried with a paper towel, and weighed. Roots were fixed, stained and cleared for microscopic examination (Byrd et al., 1983). The number and images of nematodes in the roots were recorded based on their developmental stages as follows: (i) J2; (ii) J2 with swollen body (late-J2); (iii) third and fourth stage juveniles; (iv) female; (v) egg-laying female; and vi) male. Red food coloring (20%) was used to stain egg masses once they were observed on roots (Thies et al., 2002). The experiment was repeated once. In Experiment 1, harvest ceased at 30 DAI, whereas in Experiment 2, harvest was extended to 40 DAI to provide more time to determine if egg masses of *M. arenaria* would form on Tifguard roots. Root-knot nematode egg masses were hand-picked (three egg masses per repetition per cultivar) from both peanut cultivars once they were observed at 40 DAI.

Statistical analysis

The effects of temperature, host genotype, and DAI on the number of different developmental stages of *M. arenaria* per gram of root tissue were subjected to analysis of variance using R programming software (R 3.1.2 with R studio). The means were compared by Tukey's honest significant difference test. The data from tomato were not included in the statistical analyses. Values were subjected to arcsin ($\sqrt{x}$) transformation before analysis of variance. The number of eggs per egg mass was subjected to mean separation by student's *t*-test.

Marker analysis for AdSNP92

In the first experiment, a young leaflet from any Tifguard plant with root-knot nematode infection was collected and genotyped with nematode resistance marker AdSNP92 (Chu et al., 2016). Samples that tested negative for the nematode resistance marker were excluded from analysis. In the second experiment, all of the Tifguard seeds were genotyped with marker AdSNP92, thereby assuring three replicates for each treatment.

Results

Penetration of Meloidogyne arenaria

The root systems from Georgia-06G were generally 1 to 1.5-fold greater in size than that from Tifguard ($P \leq 0.05$) (Table 1). Thus, the number of each developmental stage of *M. arenaria* per gram of roots was calculated.

Table 1. Analysis of variance of root weight, number of *Meloidogyne arenaria* per gram of roots of peanut cultivars Georgia-06G and Tifguard.

Source of variance	*F* value
	Root weight
Cultivar	2.744e-06***
Temperature	0.6528
DAI[a]	<2.2e-16***
Cultivar × Temperature	0.7204
Cultivar × DAI	1.896e-08***
Temperature × DAI	0.8505
Cultivar × Temperature × DAI	0.7657
	M. arenaria per gram of roots Experiment 1
Cultivar	0.0009106***
Temperature	0.0059029**
DAI	<2.2e-16***
Cultivar × Temperature	0.0374213*
Cultivar × DAI	0.0005185***
Temperature × DAI	4e-07***
Cultivar × Temperature × DAI	0.0197020*
	M. arenaria per gram of roots Experiment 2
Cultivar	6.651e-07***
Temperature	0.0001727***
DAI	<2.2e-16***
Cultivar × Temperature	0.0109771*
Cultivar × DAI	3.001e-09***
Temperature × DAI	<2.2e-16***
Cultivar × Temperature × DAI	6.126e-06***

[a]Days after inoculation.

Both peanut cultivars and temperature affected the number of nematodes in roots ($P \leq 0.05$) (Table 1). In both experiments, relative to the number of J2 found in roots at 5 and 10 DAI, the number of J2 in Tifguard and Georgia-06G were decreased at the end of the experiments at all three temperatures (Figs. 1, 2). There were greater numbers of J2 infecting roots in both experiments at 5 DAI in response to temperatures above 28°C. In Experiment 1, at day 5 the initial penetration of *M. arenaria* in Georgia-06G was greater at 31°C than at 28°C or 34°C ($P \geq 0.05$) (Fig. 3). Similarly, in Experiment 2, there were greater numbers of *M. arenaria* found in Georgia-06G roots at 5 DAI between 28 and 31 or 34°C, but there was no difference in numbers between 31°C and 34°C ($P \geq 0.05$) (Fig. 3). The number of J2 in Tifguard was greater at 31°C, but no differences occurred between 31°C and 34°C in both experiments (Fig. 3). In Experiment 1, there were higher numbers of J2 in Tifguard roots at 34°C than in Georgia-06G, whereas a higher number of J2 was observed in Georgia-06G roots at 28°C ($P \leq 0.05$) (Fig. 3). There was no difference in nematode numbers found in the susceptible and resistant peanut roots at 31°C; however, differences were found in the amount of penetration between both cultivars at 28°C and 34°C ($P \leq 0.05$) (Fig. 3).

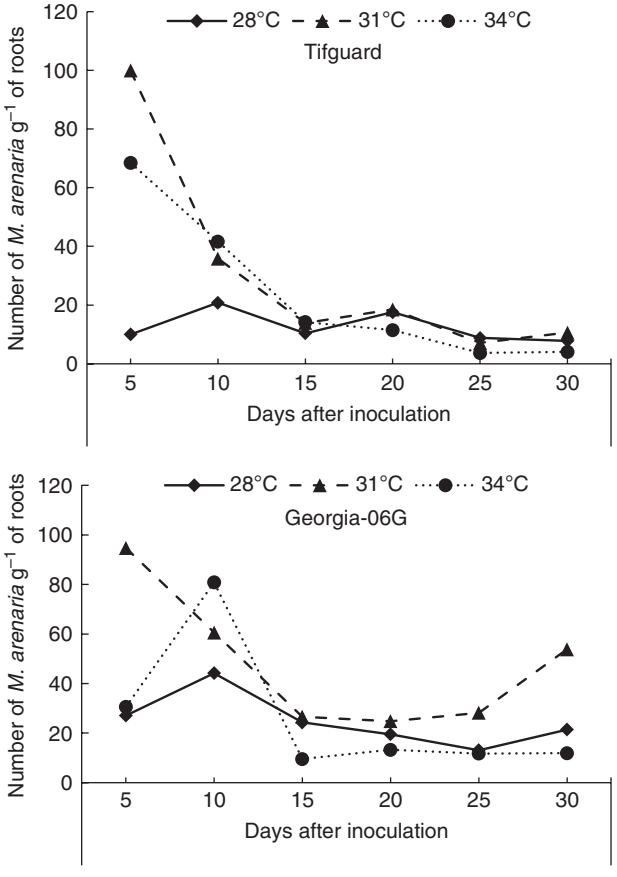

Figure 1: Mean number of *Meloidogyne arenaria* in roots of Tifguard and Georgia-06G at 5-day intervals over a 30-day period in Experiment 1.

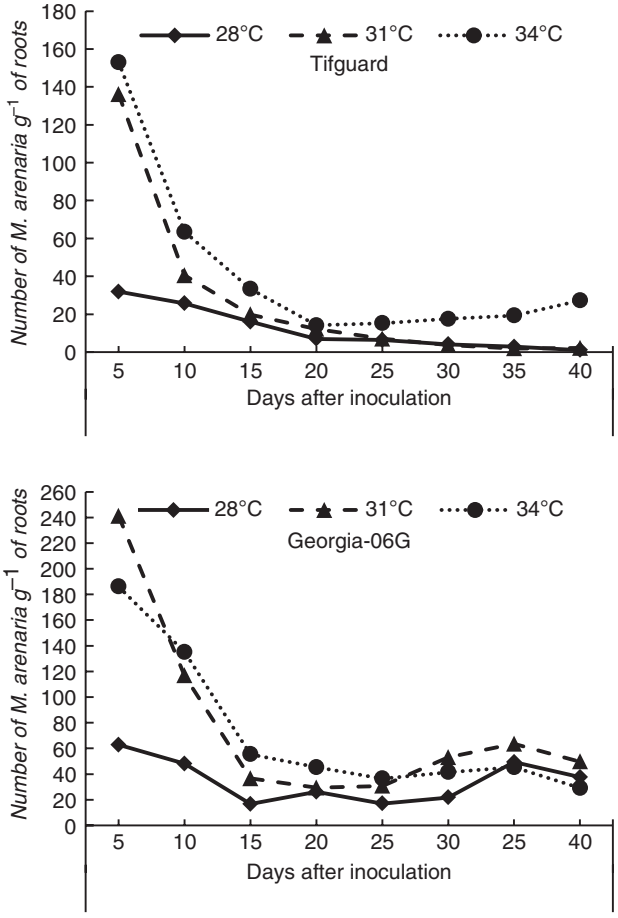

Figure 2: Mean number of *Meloidogyne arenaria* in roots of Tifguard and Georgia-06G at 5-day intervals over a 40-day period in Experiment 2.

Development of Meloidogyne arenaria

In both experiments, females of *M. arenaria* were observed in Tifguard roots only at 34°C (Figs. 4, 6A), whereas no development of *M. arenaria* occurred in Tifguard roots at 28°C or 31°C. Although females were found in Tifguard at 34°C, no egg-laying females were observed in Experiment 1 over the 30-day period; whereas an average of 26 females with small egg masses per gram of roots were found in Experiment 2 at 40 DAI (Figs. 5, 6B).

In both experiments egg-laying females were first observed in Georgia-06G at 25 and 20 DAI at 28°C

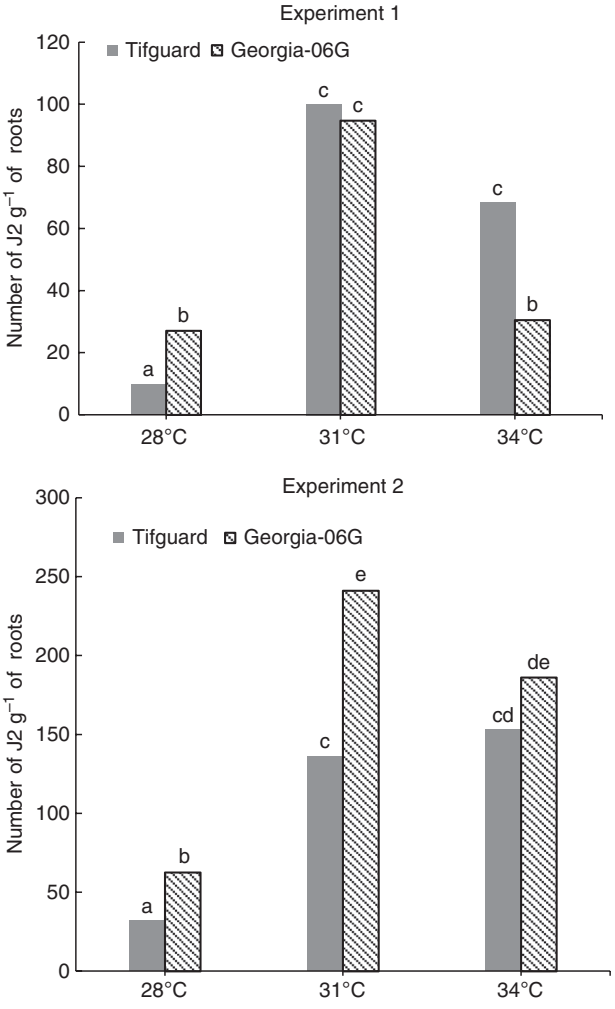

Figure 3: Number of second-stage juveniles (J2) per gram of root system of the resistant cultivar Tifguard and the susceptible cultivar Georgia-06G 5 days after inoculation in Experiments 1 and 2. Different letters over bars from the same experiment indicate significant differences at $P \leq 0.05$.

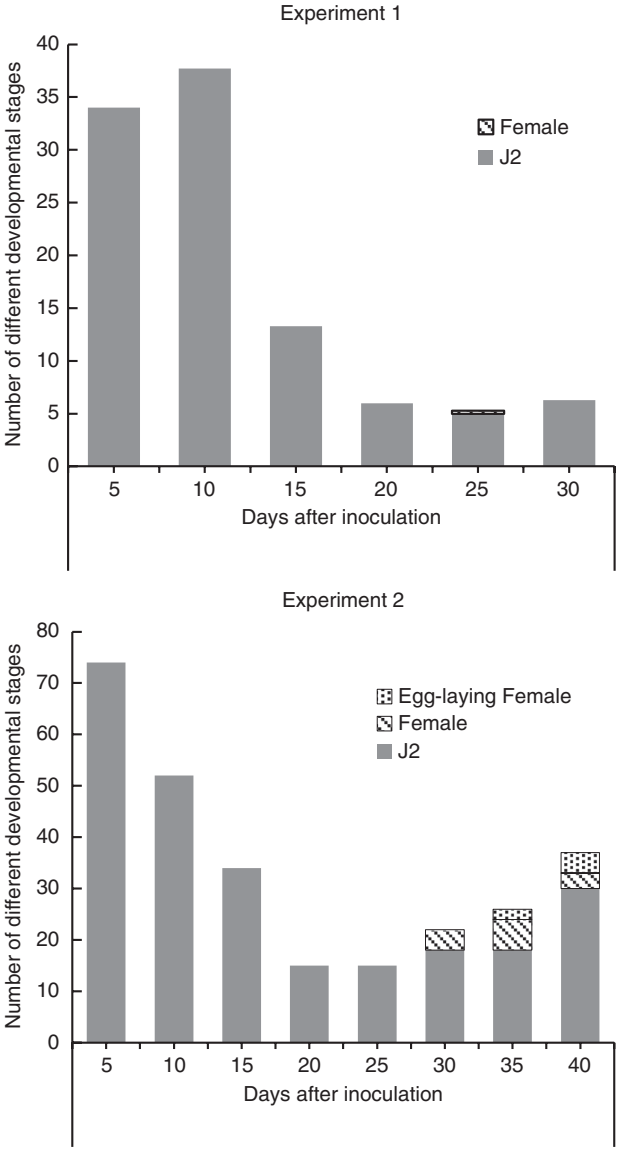

Figure 4: Number of *Meloidogyne arenaria* second-stage juveniles (J2) and females 30 days after inoculation (Experiment 1), and J2, females, and egg-laying females 40 days after inoculation (Experiment 2) on Tifguard.

and 31°C, respectively. However, in both experiments there was a delay in the development of nematodes at 34°C compared with that at 31°C. The number of egg-laying females was less in Georgia-06G roots at 34°C, whereas the highest number was found at 31°C. The numbers were similar to those occurring at 28°C in Experiment 1 (Fig. 5). In Experiment 2, highest number of egg-laying females was observed at 28°C and 31°C at 40 DAI ($P \leq 0.05$) (Fig. 5).

Necrotic lesions were formed at the site where J2 were attempting to feed in Tifguard roots in response to *M. arenaria* infection at all sampling dates. Cell necrosis was found in the tissue surrounding the swollen J2 after which the J2 apparently stopped further development (Fig. 6).

Reproduction of Meloidogyne arenaria

Numbers of eggs per egg mass obtained from Tifguard roots maintained at 34°C were different from those obtained from Georgia-06G at 40 DAI ($P \leq 0.05$) (Fig. 7).

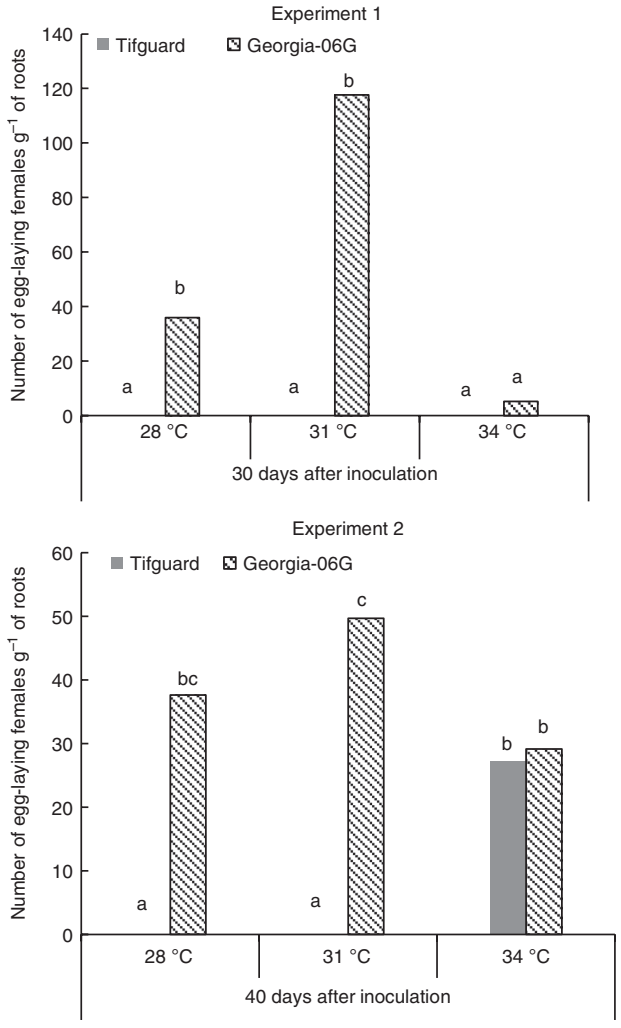

Figure 5: Number of egg-laying females per gram of roots of the resistant cultivar Tifguard and susceptible cultivar Georgia-06G at 30 or 40 days after inoculation in Experiments 1 and 2, respectively. Bars followed by different letters from the same experiment indicate significant differences at $P \leq 0.05$.

The average number of eggs per egg mass isolated from Tifguard roots maintained at 34°C was 13, whereas the number from Georgia-06G was 244 (Fig. 7).

Discussion

Temperature effects on development

There was a strong effect of peanut cultivar on *M. arenaria* infection. Tifguard was reported to be

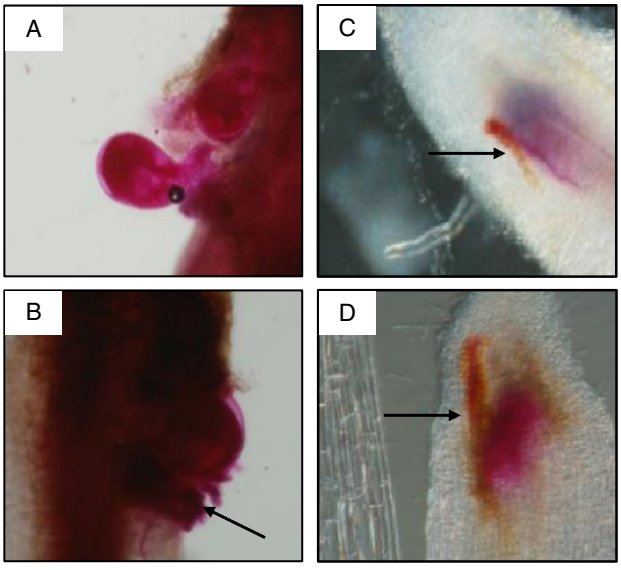

Figure 6: Mature egg-laying females and necrotic lesions formed around *Meloidogyne arenaria* infection sites in Tifguard roots. A. Mature females at 35 days after inoculation (DAI) at 34°C; B. Egg-laying female at 40 DAI at 34°C (arrow points to egg mass); C. Arrow points to necrotic lesion at 5 DAI; D. Arrow points to necrotic lesion at 40 DAI.

highly resistant to *M. arenaria* infection (Holbrook et al., 2008). Although nematode development occurred faster in Georgia-06G, there was only a small rate of development at 34°C in Tifguard. In Georgia-06G the first egg-laying females were observed at 20 or 25 DAI, whereas J2 were unable to develop after penetrating Tifguard roots (except at 34°C).

Temperature is considered to be an important factor on egg hatching, nematode migration, root invasion and development in host roots (Tyler, 1933). At 31°C more J2 entered Tifguard and Georgia-06G roots compared with that at 28°C and 34°C. In addition, final numbers of *M. arenaria* infecting Georgia-06G also were influenced by higher temperatures. In this study, the temperature of 31°C was optimal for J2 to penetrate and parasitize the susceptible peanut cultivar Georgia-06G. This result was different from Tyler's where 28°C was reported to be the optimal temperature for root-knot nematode penetration and infection. Moreover, only 6 out of 26 root systems were reported to be infected at 33°C to 35°C by the root-knot nematode species used (Tyler, 1933), whereas all Georgia-06G and tomato plants were galled and had egg masses at 34°C in this study. This discrepancy may be because of different root-knot

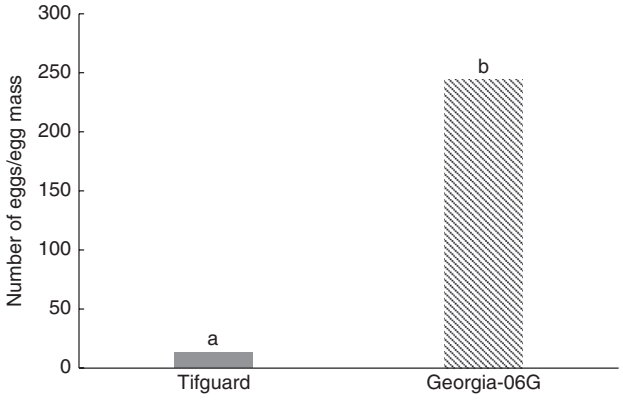

Figure 7: Number of *Meloidogyne arenaria* eggs per egg mass from roots of the peanut cultivars Tifguard (resistant) and Georgia-06G (susceptible) at 34°C at 40 days after inoculation. Different letters over bars indicate significant differences at $P \leq 0.05$.

nematodes species and also because of different host plants being tested.

Function of resistant genes

Based on the data from this study, the number of egg-laying females and eggs per egg mass were much less in Tifguard than that in Georgia-06G. This confirms results reported by Garcia et al. (1996) regarding the functions of *Mae* and *Mag* genes found in the wild type *Arachis* spp., which were resistant to *M. arenaria*. These two genes were discovered to be linked to *Rma*, which is the dominant root-knot nematode resistance gene that was introgressed into cultivated peanut (Nagy et al., 2010). *Mae* was reported to suppress egg production of *M. arenaria*, whereas *Mag* is reported to inhibit formation of galls (Garcia et al., 1996).

Hypersensitive reaction

The presence of host necrosis near the site where J2 were observed in the early stages of the host-parasite interaction was contradictory to the observation by Choi et al. (1999) and Bendezu and Starr (2003). This HR mechanism has been documented in the case of the *Mi* resistance gene in tomato to root-knot nematode infection (Paulson and Webster, 1972; Williamson, 1998). After recognition of the nematode by the plant bearing a single resistance gene, host defenses respond and are activated, leading to necrotic cells surrounding the nematode.

A resistant plant provides an effective and environmentally safe means to manage root-knot nematodes, thus understanding the mechanisms involved in resistance is critical for the sustainable management of *M. arenaria* on peanut. In summary, this research demonstrates that soil temperature does not inhibit the expression of resistance in Tifguard against *M. arenaria*, but high soil temperature may favor nematode development in Tifguard.

Acknowledgments

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