Characterization of the Resistance to *Meloidogyne arenaria* in an Interspecific *Arachis* Spp. Hybrid[1]

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ABSTRACT

Seedlings of TP-135, an interspecific hybrid derived from four *Arachis* spp. and resistant to *Meloidogyne arenaria* race 1, and the susceptible cultivar Tamnut 74 were inoculated with 2,500 freshly hatched juveniles of *M. arenaria*. Plants were harvested at 7, 14, 21, and 35 days after inoculation (DAI) and the roots treated with acid fuchsin to stain infecting nematodes. Adult females with eggs were detected in roots of Tamnut 74 at 21 DAI, producing 1,395 eggs/g roots at 35 DAI. Most nematodes remained as second-stage juveniles and no nematode was observed to develop beyond the third or fourth juvenile stage in roots of TP-135 by 35 DAI. In other experiments, seedlings of Tamnut 74 and root cuttings of TP-135 were each inoculated separately with 3,000 eggs of 10 geographically diverse populations of *M. arenaria* race 1. All populations of the nematode had greater ($\underline{P}$ = 0.01) reproduction on Tamnut 74 than on TP-135. Based on these data, we conclude that although the mechanism of resistance in TP-135 to *M. arenaria* is most similar to that of the wild species *A. cardenasii*, it is not identical to that of any of the nematode-resistant parental species. Furthermore, we believe that the resistance will be effective against a range of populations of the nematode.

Key Words: *Arachis batizocoi, A. cardenasii, A. chacoensis, A. hypogaea*, host resistance, *Meloidogyne arenaria*, root-knot nematode, and wild *Arachis* species.

Meloidogyne arenaria is an economically important pathogen of peanut estimated to be present in 26% of the peanut fields in Texas (13) and is widely distributed in the southeastern United States (3, 6, 7). Although no agronomically acceptable peanut cultivar resistant to *M. arenaria* is available, resistance has been identified in different *Arachis* spp. (1, 4). Nelson *et al.* (8) reported that numerous wild *Arachis* species were resistant to *M. arenaria*. Additionally, they found that two interspecific hybrids were resistant to the nematode. One of these resistant hybrids (TP-129) was the F_1 of the cross

[*A. batizocoi* K-9484 X (*A. cardenasii* GKP-10017 X *A. chacoensis* GKP-10602)]^{4x}.

The other resistant hybrid (TP-135) was the first backcross generation from

A. hypogaea cv. Florunner X TP-129

with Florunner as the recurrent parent.

Nelson *et al* (9) also reported that the mechanisms of resistance for two of the wild species parents of TP-135 are different. Resistance in *A. batizocoi* is due to a reduction in the total number of invading nematodes which reach maturity and produce eggs, along with an increase in the time required for the nematodes to reach maturity. In *A. cardenasii*, resistance is due to an almost complete inhibition of nematode development. Necrosis of host root-cells at the site of nematode invasion also was observed in *A. cardenasii*. Other studies have shown that the resistance of *A. chacoensis*, the third nematode resistant species in the parentage of TP-135, to *M. arenaria* is similar to that of *A. batizocoi* (Starr, unpublished data).

One objective of this research was to determine the mechanism of resistance in the resistant hybrid TP-135. Additionally, because our previous experiments to identify resistance to *M. arenaria* in *Arachis* spp. have used a single population of the pathogen (#82-4), we wanted to determine if the resistance in TP-135 would be effective against other, geographically diverse, populations of *M. arenaria*.

Materials and Methods

Seeds of the susceptible culitvar Tamnut 74 and the resistant hybrid TP-135 (F_4 progeny of the first backcross generation) were germinated in moist paper towels at 24 C. After 3 days, seedlings with radicles of uniform length (3-5 cm) were transplanted singly into 470-cm^3 plastic cups containing a pasteurized sand-peat mixture (6:1, v:v). Each seedling was inoculated 7 days later by pipeting a suspension of 2,500 freshly hatched second-stage juveniles (J2) of *M. arenaria* into depressions in the potting mix around the base of each seedling. Nematode inoculum was obtained by the method of Vrain (12) from a population of *M. arenaria* race 1 (#82-4) isolated from peanut in Texas and cultured on tomato (*Lycoperscion esculentum* Mill. cv. Rutgers) in a greenhouse. The inoculated plants were placed in a controlled environment at 28 C daytime and 24 C nighttime temperatures with a 14-hr day length (218 uE $m^{-2}s^{-1}$).

Three plants of each genotype were selected for evaluation at 7, 14, 21, and 35 days after inoculation (DAI). Penetration of roots and postinfectional development of the nematodes were determined by microscopic examination of roots stained with acid fucshin as described previously (9) using the method of Byrd *et al.* (2). The experiment was repeated once and the data from the two experiments were combined and analyzed with the SAS GLM procedure (10).

To determine the effectiveness of the resistance in TP-135 against other populations of nematodes, ten populations of *M. arenaria* race 1 were obtained from Alabama, Florida, Georgia, North Carolina, South Carolina, and Texas (Table 2), and cultured on Rutgers tomato. All nematode populations were received under the auspices of the Animal and Plant Health Inspection Service permit no. 891305. Inoculum, in the form of eggs, was obtained from permanent cultures of each population by the NaOCl method (5).

Seeds of Tamnut 74 were germinated in moist paper towels and transplanted to 12.7-cm diameter pots filled with the sand-peat potting mixture when the radicles were 3-5 cm long. Because of the limited quantities of seed of TP-135 available, cuttings were taken from main branches of established plants, placed in 4.3-cm diameter peat pellets, and incubated in moisture chambers at 28 C. Previous experiments provided evidence that the reaction of root-cuttings to *M. arenaria* was identical to that of plants from seed (Starr, unpublished data). Cuttings rooted after 10-14 days and were transplanted into 12.7-cm pots filled with the sand-peat soil mixture. Five days after transplanting, Tamnut 74 seedlings and TP-135 cuttings were inoculated by pipeting a suspension of 3,000 eggs of each nematode population separately into depressions in the soil around the base of the plants. Plants were maintained in the greenhouse until harvested at 56 DAI. The roots were washed free of soil, weighed, and then treated with 0.5% NaOCl to extract nematode eggs (5).

The experimental design was a split-plot with nematode populations as the main plots and *Arachis* genotypes as the subplots. There were five replications of each treatment and the experiment was conducted twice. Data on eggs per gram of fresh root weight were subjected to analysis of variance using the SAS GLM procedure (10).

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Table 1. Root weights and population densities of *Meloidogyne arenaria* in roots of Tamnut 74 (T-74) peanut and the interspecific hybrid TP-135 at different times after inoculation.

Days after	Fresh root wt		Nematodes/g root	
Inoculation	T-74	TP-135	T-74	TP-135
7	1.7	0.2 *	36	81 *
14	3.2	0.3 *	16	204 *
21	2.6	0.7 *	16	25
35	4.5	1.2 *	17	21

* Indicates a significant difference between Tamnut 74 and TP-135 at $P = 0.05$.

Table 2. Reproduction, measured as eggs per gram of fresh root weight, of populations of *Meloidogyne arenaria* race 1 on the susceptible cultivar Tamnut (T-74) and the nematode-resistant interspecific hybrid TP-135.

Population and	Exp 1		Exp 2	
Source	T-74	TP-135	T-74	TP-135
89-4 Florida	2,020	0	369	0
89-1 South Carolina	1,800	0	302	2
89-2 South Carolina	1,270	0	611	5
89-6 Alabama	1,100	0	54	1
89-5 Georgia	340	0	214	0
89-3 Florida	300	0	315	13
89-10 Texas	170	0	-	-
89-9 Georgia	150	0	-	-
89-7 North Carolina	20	0	29	3
82-4 Texas	-	-	198	1
$LSD_{0.05}$	1,500	NS	NS	NS

Values are means of 5 replications; the experiment was terminated at 56 days after inoculation.

Results

Fresh root weights of Tamnut 74 were greater ($P = 0.01$) than those of TP-135 at each harvest date in experiments on mechanism of resistance (Table 1). TP-135 had more ($P = 0.05$) nematodes per gram roots at 7 and 14 days after inoculation than did Tamnut 74 (Table 1) but not at 21 or 35 days after inoculation.

Nematode development was faster on Tamnut 74 than on TP-135. Adult females were detected in roots of Tamnut 74 at 14 DAI and the first eggs were detected at 21 DAI (Fig. 1). By 35 DAI, *M. arenaria* had produced a mean of 1,395 eggs/g fresh root weight on Tamnut 74. In contrast, no nematode had developed beyond the third to fourth juvenile stage (J3-4) by 35 days after inoculation in the roots of TP-135 (Fig. 1) and egg production was not observed. No evidence of root necrosis was observed in association with nematode infections of Tamnut 74 or TP-135.

In the greenhouse experiments on reproduction of different populations of *M. arenaria* on Tamnut 74 and TP-135, there were differences ($P = 0.01$) between the two host genotypes for all nematode populations. Reproduction was higher on Tamnut 74 in all instances, with little or no reproduction being observed on TP-135 (Table 2).

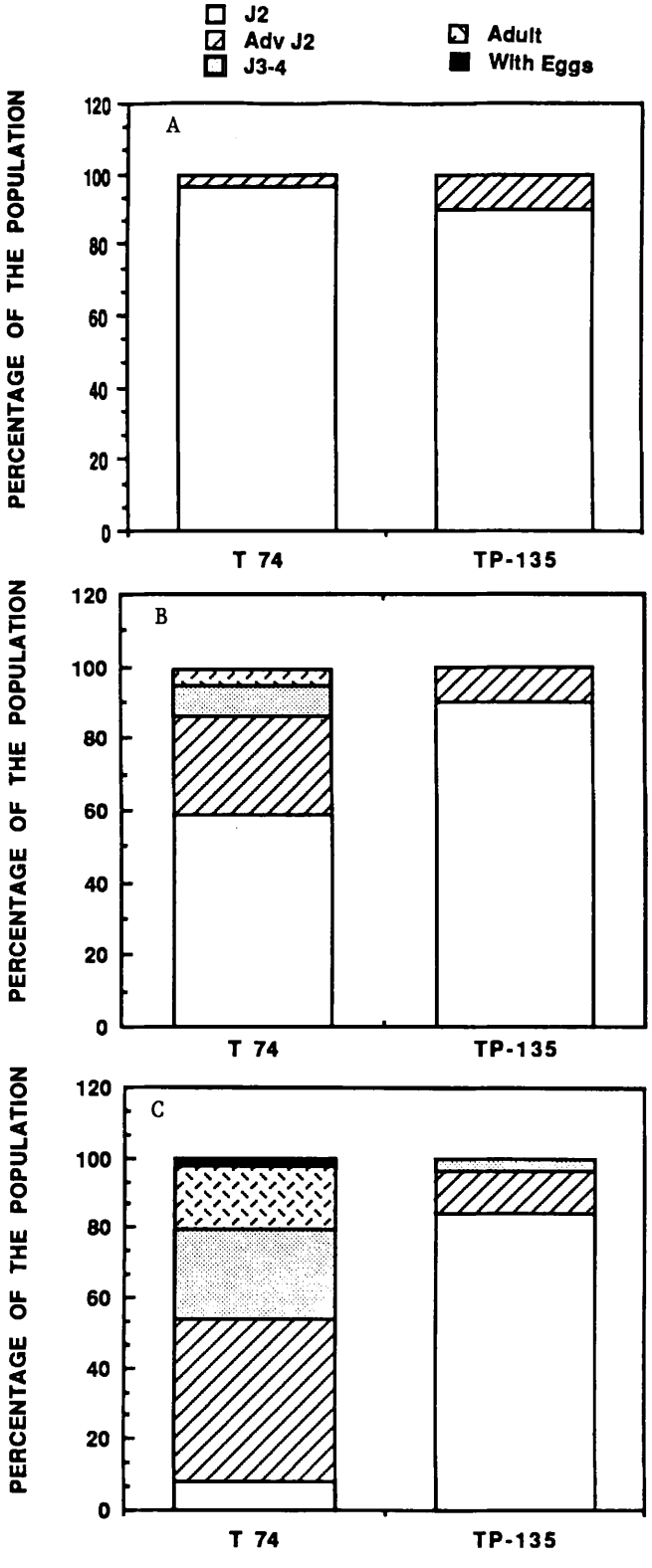

Fig. 1. Development of Meloidogyne arenaria on the susceptible cultivar Tamnut 74 and the resistant interspecific hybrid TP-135.

A: 7 days after inoculation.
B: 14 days after inoculation.
C: 21 days after inoculation.

J2 = vermiform, motile second-stage juveniles; Adv J2 = swollen, nonmotile J2; J3-4 = third or fourth stage juveniles; Adults = adult females; and With Eggs = adult females with eggs.

Differences ($\underline{P} = 0.05\%$) in nematode reproduction among the different populations on Tamnut 74 were noted in the first experiment with a similar, but nonsignificant, trend observed in the second experiment (Table 2). Maximum temperatures did not exceed 32 C during experiment 1 (February-March 1990) whereas during experiment 2 (May-June 1990) maximum temperatures exceeded 41 C on numerous occasions.

Discussion

The interspecific hybrid TP-135 has three wild *Arachis* spp. in its parentage; each of these wild species is resistant to *M. arenaria* race 1 (8). The resistance of TP-135 was most similar to that of *A. cardenasii* (9) in that there was an almost complete inhibition of nematode development in TP-135. We did not, however, observe any host necrosis associated with infection of roots of TP-135 by the nematodes as previously was observed in the resistance response of *A. cardenasii* to infection by *M. arenaria*. The greater number of nematodes in the roots of TP-135 than in roots of Tamnut 74 at 7 and 14 DAI is believed to be due to the smaller size of roots of TP-135. In the small cups used for these experiments, if roots of both species are equally attractive to the nematodes, one would expect relatively greater numbers of nematodes per g roots associated with smaller root systems.

Because the resistance of TP-135 was not identical to the resistance of any of the resistant wild parents (9, unpublished data), we have concluded that the hybrid has resistance genes from more than one parent being expressed. If this is the case, then the resistance of TP-135 should be broadly based and stable. Studies are in progress to determine the number of genes which condition resistance in the wild species parent and in TP-135. In preliminary tests, progeny (F_1) from crosses of susceptible *A. hypogaea* genotypes with TP-135 exhibited reactions to *M. arenaria* ranging from highly resistant to susceptible (unpublished data).

That TP-135 was highly resistant to all of the 10 populations of *M. arenaira* against which it was tested suggests that the resistance will be effective against a range of populations of this species of root-knot nematodes. The differences in total nematode reproduction in these two greenhouse experiments are believed to be due to the high temperatures experienced in the second greenhouse experiment. Thomason and Lear (11) reported that maximum reproduction of *M. arenaria* occurs at 28-32 C and that reproduction decreases at temperatures higher than 32 C. That some egg production was observed on TP-135 in these experiments but not in experiments on the mechanism of resistance was probably due to 1) the greater number of samples examined (5 versus 3 per experiment) and 2) the longer incubation period (56 versus 35 days). The variation in reproduction of the nematode populations on the susceptible Tamnut 74 also suggests that there is some variation in aggressiveness in the pathogen population.

The resistance of TP-135 is the first report (8) of high levels of resistance to *M. arenaria* in germplasm which can be readily crossed with agronomically acceptable peanut genotypes. Based on the data presented herein, we believe it will be possible to develop agronomically acceptable peanut cultivars with a high level of resistance to *M. arenaria* using the resistance present in the interspecific hybrid TP-135.

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