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Size Differences Among Root-knot Nematodes on Resistant and Susceptible Alyceclover Genotypes[1]

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Abstract: The influence of plant resistance on the size of individual root-knot nematodes was determined in greenhouse experiments. Five genotypes of alyceclover were inoculated with second-stage juveniles of *Meloidogyne incognita* race 3 or *M. arenaria* race 1. Plants were harvested at selected intervals and stained for detection of the nematodes, which were dissected from the roots. Length, width, and sagittal-sectional area of each animal were measured using an image-analysis system, and areas of nematodes in all stages were compared at different times and across alyceclover lines. Nematodes feeding on roots of resistant lines were consistently smaller than those on susceptible plants, with significant differences in growth detected after the final molt. Similar results were observed with both nematode species.

Key words: alyceclover, *Alysicarpus vaginalis*, growth rate, image analysis, *Meloidogyne arenaria*, *Meloidogyne incognita*, resistance.

The effects of postinfectional resistance of plants on invading nematodes are often widespread and complex (3). This resistance affects the reproductive capabilities of the nematodes (6). Melakeberhan and Ferris (6) hypothesized that nematode energy expenditures may also be different in resistant and susceptible host plants. Using computer algorithms (9) to calculate the volumes of nematodes, they found differences in the size of egg masses, but not in the size of adult females between resistant and susceptible cultivars. Total size of females plus egg masses was much less in resistant plants, on which egg production was lower (6).

Preliminary observations of root-knot adult females (*Meloidogyne* spp.) within the roots of alyceclover (*Alysicarpus vaginalis* (L.) DC. Ann.) suggested a size difference between nematodes feeding on resistant roots and those feeding on susceptible roots (8). Five breeding lines of alyceclover (FL-1, FL-3, FL-4, FL-5, and FL-100) displayed different degrees of resistance to *M. incognita* race 3 (Kofoid & White, 1919) Chitwood, 1949 and *M. arenaria* race 1 (Neal, 1889) Chitwood, 1949 (8). Degrees of resistance reported were based on the average number of adult females per plant in the roots 36 days after inoculation. Thus, the *Meloidogyne–Alysicarpus* system may be an excellent model for testing the hypothesis that nematode size may vary with degree of host susceptibility. The objective of this study was to determine if final adult size differed within *M. incognita* race 3 and *M. arenaria* race 1 on various lines of alyceclover.

Materials and Methods

Arredondo fine sand (95.5% sand, 2.0% silt, 2.5% clay), obtained from a field plot near the University of Florida, was autoclaved at 150 C for 2 hours. One hundred 7-day-old plants of each of five lines of alyceclover (FL-1, FL-3, FL-4, FL-5, and FL-100) were transplanted to 80-cm^3 disposable plastic cups. The plants were grown in a temperature-controlled growth room at 26 C with a 14-hour photoperiod.

Nematode populations were maintained on tomato plants (*Lycopersicon esculentum* Mill. cv. Rutgers) in the greenhouse for 2 months before egg collection. Eggs were extracted from the roots (5) and placed on circles of Nitex (Tetko Inc., Briarcliff Manor, NY) polyamide nylon fiber fabric (20-μm pores). Nematodes used in inoculation all hatched and were collected within the same 24-hour period. Ten days after trans-

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planting, each alyceclover plant was inoculated with approximately 100 infective juveniles of *Meloidogyne incognita* race 3 or of *Meloidogyne arenaria* race 1. Five plants of each line were left uninoculated as controls.

After 6, 12, 24, and 72 hours and every 3 days thereafter for a 36-day period, five plants of each line were carefully removed from the soil. Excised roots were washed and stained (1) and stored in a glycerin-lactic acid solution (2.0 ml glycerol : 1.0 ml lactic acid : 2.0 ml water). All nematodes within each root system were dissected from the roots under a dissecting microscope. The extracted nematodes were stored in glycerin acidified with lactic acid and grouped according to host line, date, and replicate number.

The nematodes were measured on the BQ System IV (Bioquant; R&M Biometrics, Nashville, TN), a computerized image-analysis system. A phototube, connected to a dissecting microscope, projected an image of the field of vision to a black and white video monitor. A computer mouse was used to trace the outline of the nematode on the video screen. Bioquant software on an IBM PC converted the measurements from screen to actual measured sizes. Parameters measured were length, width, and sagittal-sectional area for each stage of the nematode on each alyceclover line. Each nematode was placed into one of four general categories: 1) second-stage juveniles (J2) that had penetrated but not yet established a feeding site, 2) swollen second-stage juveniles (SJ2) that had established a site but not yet molted, 3) third-stage and fourth-stage juveniles (J34), and 4) adult females.

Means and variances were computed for all measurements of each stage. Analyses of variance (2) were performed on measurements of 36-day-old females to ascertain if differences existed between peak sizes of nematodes across breeding lines, and on adults of all ages to determine size variability. Means were compared with Kramer's modification of Tukey's test at $P \leq 0.05$, since unplanned comparisons of means with unequal sample sizes were required.

Results and Discussion

Means and standard deviations for the lengths, widths, and sagittal-sectional areas of 1,195 *Meloidogyne incognita* race 3 and 1,597 *Meloidogyne arenaria* race 1, of all life stages, are given in Tables 1 and 2. Data were consistent, with very low variances despite small sample sizes for some groups studied. Low penetration rates and poor extraction efficiency of early stages from the roots were responsible for these small sample sizes. The results indicate that Bioquant may be a sufficiently precise system to use for nematode measurements.

Variances of adult measurements were larger than those of juveniles for both nematode species. Since data on all females are included in the averages (Tables 1, 2), regardless of age, the larger variances are due to the tremendous variability in size of the adults as they develop in the root system from small, newly molted adult females to larger, mature egg-producing females.

For *M. incognita* race 3, alyceclover line FL-100 was most susceptible, followed by FL-5, FL-3, FL-1, and the most resistant FL-4 (8). FL-4 was most susceptible to *M. arenaria* race 1, followed by FL-5, FL-100, FL-1, and the most resistant line FL-3 (8). Mean measurements of *M. incognita* in the earlier stages of development were fairly consistent across lines (Table 1). Differences in sizes due to effects of alyceclover lines were barely distinguishable until the J34 and adult stages.

Lengths in the early developmental stages of *M. incognita* were similar between categories of development, with a large increase in size occurring at the final molt to the adult stage (Table 1). Increases in both width and sagittal-sectional area occurred between all consecutive developmental stages, with the most significant change in growth between the J34 and adult stages.

Measurements of *M. arenaria* showed similar trends to *M. incognita* (Table 2). Lengths, widths, and sagittal-sectional ar-

TABLE 1. Means and standard deviations of length, width, and sagittal-sectional area of life-cycle stages† of *Meloidogyne incognita* race 3 on five lines of alyceclover.

Alyceclover line	J2	SJ2	J34	Adult
		Sample size		
FL-1	4	7	30	92
FL-3	4	6	36	113
FL-4	0	0	11	28
FL-5	2	27	100	211
FL-100	17	23	131	353
		Length		
FL-1	0.323 ± 0.005 a	0.322 ± 0.065 a	0.313 ± 0.050 a	0.480 ± 0.111 ab
FL-3	0.321 ± 0.020 a	0.325 ± 0.021 a	0.333 ± 0.066 a	0.527 ± 0.135 bc
FL-4			0.343 ± 0.104 a	0.414 ± 0.086 a
FL-5	0.357 a	0.332 ± 0.059 a	0.345 ± 0.074 a	0.531 ± 0.137 c
FL-100	0.300 ± 0.071 a	0.319 ± 0.076 a	0.338 ± 0.078 a	0.557 ± 0.144 c
		Width		
FL-1	0.015 ± 0.004 a	0.025 ± 0.011 a	0.067 ± 0.021 a	0.265 ± 0.092 a
FL-3	0.010 ± 0.008 a	0.039 ± 0.014 a	0.067 ± 0.022 a	0.308 ± 0.108 b
FL-4			0.071 ± 0.011 a	0.218 ± 0.072 a
FL-5	0.018 a	0.038 ± 0.008 a	0.068 ± 0.019 a	0.273 ± 0.103 a
FL-100	0.016 ± 0.008 a	0.042 ± 0.022 a	0.075 ± 0.030 a	0.305 ± 0.113 b
		Area		
FL-1	0.004 ± 0.000 a	0.006 ± 0.002 a	0.018 ± 0.007 a	0.092 ± 0.043 ab
FL-3	0.003 ± 0.002 a	0.009 ± 0.004 a	0.020 ± 0.008 a	0.115 ± 0.052 c
FL-4			0.019 ± 0.009 a	0.066 ± 0.032 a
FL-5	0.004 a	0.010 ± 0.004 a	0.020 ± 0.008 a	0.103 ± 0.050 b
FL-100	0.003 ± 0.002 a	0.010 ± 0.007 a	0.022 ± 0.012 a	0.116 ± 0.053 c

Measurements are in millimeters for length and width and in millimeters squared for sagittal-sectional area. Means of each measurement within the same column with the same letter are not different ($P \geq 0.05$) according to Kramer's modification of Tukey's test.

† J2 = second stage, SJ2 = swollen second stage, J34 = third and fourth stage.

eas showed a gradual increase from one life stage to the next. The mean width of *M. arenaria* doubled between the J2 and SJ2 and between the SJ2 and J34 stages and quadrupled between the J34 and adult stages.

Differences in size across alyceclover lines were significant for both *M. arenaria* and *M. incognita* (Tables 1, 2). Adult females of *M. incognita* on line FL-4 were consistently smaller ($P \leq 0.05$) than those on FL-3 and FL-100 across measurement parameters (Table 1). Adult females of *M. arenaria* on lines FL-1 and FL-3 had shorter lengths and smaller sagittal-sectional areas than those on FL-4, FL-5, and FL-100; widths of adults on FL-1 were also smaller ($P \leq 0.05$) than those of adults on these other three lines (Table 2).

Only minimal growth occurred between the J2 and J34 stages of both nematodes. The third and fourth molts of root-knot nematode occur within the J2 cuticle; the juveniles are unable to feed on their hosts during these stages (4). The abrupt increase in size between the J34 and adult stages occurred only when the nematode resumed feeding as an adult. Host line did not appear to affect the growth of the nematodes in the juvenile stages, with the exception of SJ2 parameters in *M. arenaria* (Table 2), but the considerable variation over genotype in size of adult females within each species appears to be related to the variation in resistance exhibited by the host plant.

Growth of adult females is shown for *M. incognita* (Fig. 1) and *M. arenaria* (Fig. 2), as mean sagittal-sectional areas as a function of time in degree days (DD, 10 C base)

TABLE 2. Means and standard deviations of length, width, and sagittal-sectional area of life-cycle stages† of *Meloidogyne arenaria* race 1 on five lines of alyceclover.

Alyce-clover line	J2	SJ2	J34	Adult
		Sample size		
FL-1	39	42	4	50
FL-3	58	24	9	12
FL-4	18	68	111	239
FL-5	13	76	103	244
FL-100	23	65	131	268
		Length		
FL-1	0.372 ± 0.046 a	0.379 ± 0.036 a	0.400 ± 0.047 a	0.521 ± 0.122 a
FL-3	0.360 ± 0.050 a	0.374 ± 0.041 a	0.337 ± 0.062 a	0.463 ± 0.109 a
FL-4	0.360 ± 0.051 a	0.420 ± 0.074 b	0.431 ± 0.071 a	0.581 ± 0.116 b
FL-5	0.380 ± 0.073 a	0.394 ± 0.047 a	0.411 ± 0.083 a	0.595 ± 0.128 b
FL-100	0.391 ± 0.047 a	0.392 ± 0.060 a	0.412 ± 0.071 a	0.588 ± 0.114 b
		Width		
FL-1	0.016 ± 0.005 a	0.030 ± 0.011 a	0.064 ± 0.021 a	0.244 ± 0.118 a
FL-3	0.018 ± 0.008 a	0.032 ± 0.007 ab	0.062 ± 0.018 a	0.219 ± 0.113 ab
FL-4	0.018 ± 0.005 a	0.037 ± 0.011 b	0.066 ± 0.024 a	0.286 ± 0.102 b
FL-5	0.020 ± 0.005 a	0.036 ± 0.010 b	0.060 ± 0.019 a	0.291 ± 0.096 b
FL-100	0.020 ± 0.004 a	0.036 ± 0.013 b	0.063 ± 0.020 a	0.296 ± 0.096 b
		Area		
FL-1	0.004 ± 0.002 a	0.010 ± 0.004 ab	0.023 ± 0.007 a	0.086 ± 0.048 a
FL-3	0.004 ± 0.001 a	0.008 ± 0.003 a	0.020 ± 0.009 a	0.069 ± 0.049 a
FL-4	0.004 ± 0.002 a	0.013 ± 0.007 c	0.024 ± 0.010 a	0.107 ± 0.045 b
FL-5	0.004 ± 0.002 a	0.011 ± 0.004 ab	0.022 ± 0.010 a	0.112 ± 0.047 b
FL-100	0.004 ± 0.001 a	0.012 ± 0.006 bc	0.023 ± 0.008 a	0.111 ± 0.043 b

Measurements are in millimeters for length and width and in millimeters squared for sagittal-sectional area. Means of each measurement within the same column with the same letter are not different ($P \geq 0.05$) according to Kramer's modification of Tukey's test.

† J2 = second stage, SJ2 = swollen second stage, J34 = third and fourth stage.

from inoculation. Females from all lines appeared at approximately the same time for *M. incognita* race 3 (192 DD postinoculation) with the exception of females on FL-100, which first appeared at 240 DD postinoculation. Females of *M. arenaria* race 1 appeared at 240 DD postinoculation, with the exception of females on FL-1, which appeared 96 DD earlier, and on FL-3, which appeared 48 DD later.

The sizes of nematodes within the roots of each line were consistent at first appearance of adult females in the root systems. After approximately 288 DD and 336 DD postinoculation for *M. incognita* and *M. arenaria*, respectively, the size of nematodes was more variable. Increases in size of females of both nematodes appeared to level off at approximately 432 DD, although some fluctuation continued to occur.

Growth of *M. incognita* race 3 adults increased dramatically once the final molt of the nematode occurred (Fig. 1). This, coupled with data in Table 1, demonstrates that suppression of nematode growth on a resistant line occurs in the adult stage rather than in the juvenile stages. Since most growth of the nematodes occurs as an adult, host resistance affecting growth should have its most significant effect on this stage. Fluctuation of growth of nematodes on FL-1 as shown in Figure 1 could be due to contamination of FL-1 seed stock with FL-100 late in the seed-selection process (D. D. Baltensperger, pers. comm.).

Growth of adult females of *M. arenaria* (Fig. 2) followed the same general patterns as *M. incognita*. Nematodes on susceptible genotypes FL-4, FL-5, and FL-100 all showed consistent growth increases over time. Growth of nematodes on FL-3 was

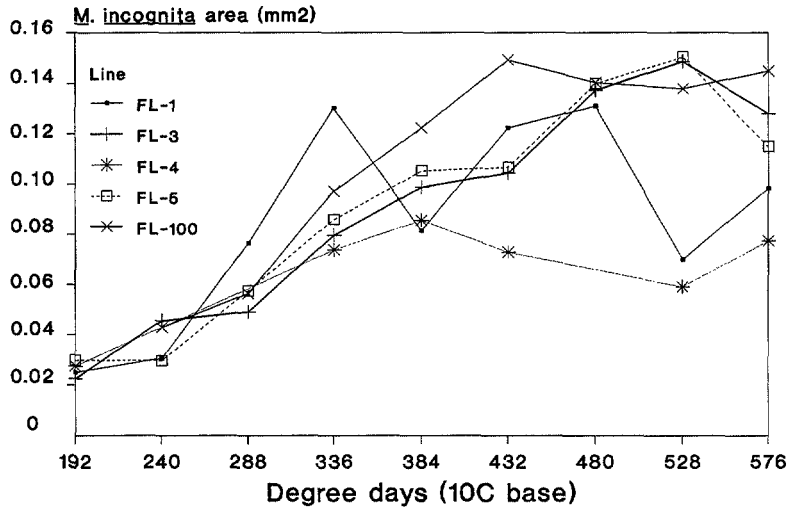

FIG. 1. Sagittal-sectional areas of *Meloidogyne incognita* race 3 adult females on five lines of alyceclover at different times from day of inoculation.

less than that on other lines. Data for nematode growth on FL-1 fluctuated widely, again possibly due to contaminated seed stock.

Mean sagittal-sectional areas of 36-day-old females of *M. incognita* on alyceclover lines FL-1 and FL-4 were not significantly different, with adult sizes of 0.098 and 0.077 mm², respectively (Table 3). However, these two lines, the two most resistant to *M. incognita*, produced nematodes smaller ($P \leq 0.001$) than those from lines FL-3, FL-5, and FL-100. When data on sagittal-sectional area were pooled across all adult degree-day classes, females on FL-4 were smaller ($P \leq 0.001$) than those on all other lines, and females on FL-1 were smaller than those on FL-3 and FL-100 (Table 1).

After 36 days, sagittal-sectional areas of adult females of *M. arenaria* ranged from 0.119 to 0.206 mm² on all lines. No differences ($P \leq 0.05$) were detected among mean areas of 36-day-old females (Table

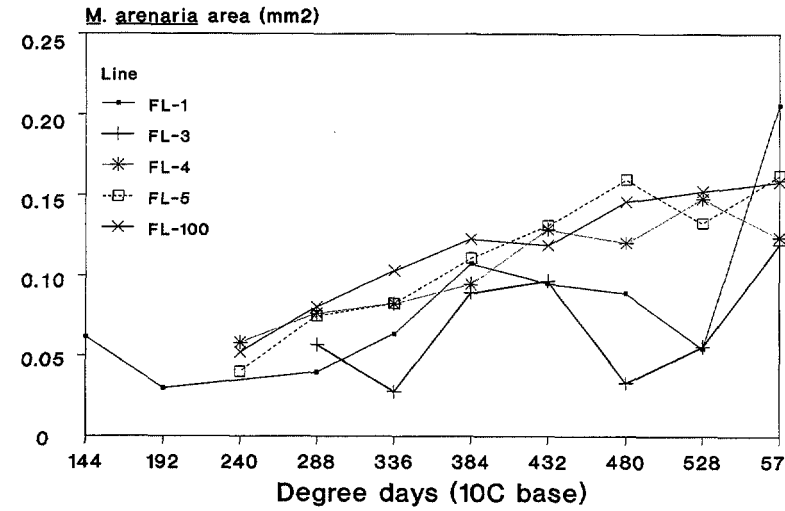

FIG. 2. Sagittal-sectional areas of *Meloidogyne arenaria* race 1 adult females on five lines of alyceclover at different times from day of inoculation.

TABLE 3. Cross-sectional areas (mm^2) of 36-day-old adult females of two species of *Meloidogyne* on five lines of alyceclover.

Line	*M. incognita* race 3	*M. arenaria* race 1
FL-1	0.098 a	0.206 a
FL-3	0.128 bc	0.119 a
FL-4	0.077 a	0.124 a
FL-5	0.115 bc	0.168 a
FL-100	0.145 c	0.158 a

Means with the same letter within a column are not significantly different according to Kramer's modification of Tukey's test ($P \leq 0.05$).

3). When females harvested across all days were considered, however, nematodes on FL-1 and FL-3 were different ($P \leq 0.001$) in size from those on FL-4, FL-5, and FL-100 (Table 2). This difference may not be discernable in older females because of extremely low sample sizes of 36-day-old females on FL-1 and FL-3, the most resistant lines to this nematode. A representative sample may not have been measured if nematodes on these lines were not mature. Thus, a high error mean square value could have contributed to the difficulty in separation.

The results suggest that host resistance limited adult size in resistant lines. Related work (8) suggests that the resistance expressed by alyceclover does not appear to affect either the sex ratio of the nematodes or the reproductive output of the individual adults, contrary to observations on other plants (6,7). Individual fecundity was actually higher for nematodes on plants that supported fewer adult females (8), which may indicate an allocation of available nutrients toward reproductive success, and away from growth.

Since few males developed in this system (8) and since there was no difference in growth among juvenile nematodes on resistant and susceptible alyceclover plants, it may be that only the adults are significantly affected by this host-resistance mechanism. At this point, sex differentiation has occurred, and the nematodes must develop as females, albeit stunted ones. It may also be possible that nematodes in various stages of development require different nutritional factors which may be lacking in the resistant plants.

Size differentiation between nematodes on resistant and susceptible host plants is a known phenomenon on other plant species (6) and should be investigated as a potentially important resistance mechanism.

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