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Aggressiveness and Reproduction of Four *Meloidogyne arenaria* Populations on Soybean[1]

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Abstract: Aggressiveness and reproduction differed among four geographical populations of *M. arenaria* on six soybean cultivars in field microplots. These differences were consistent over 3 years. The populations did not differ in virulence; i.e., population by cultivar interactions were not significant. Perineal pattern morphology, the North Carolina differential host test, chromosome counts of immature oocytes, and esterase phenotypes confirmed that the four populations were *M. arenaria*. Three populations were host race 2 and one population was host race 1.

Key words: aggressiveness, chromosome, electrophoresis, esterase, *Glycine max*, *Meloidogyne arenaria*, microplot, peanut root-knot nematode, race, reproduction, resistance, soybean.

Populations of *Meloidogyne* spp. may differ in their ability to reproduce and to suppress crop yield. For example, populations of *Meloidogyne incognita* (Kofoid & White) differed in the amount of disease they caused on tomato (*Lycopersicon esculentum* Mill.), soybean (*Glycine max* (L.) Merr.), cowpea (*Vigna unguiculata* (L.) Walp.), cotton (*Gossypium hirsutum* L.), and tobacco (*Nicotiana tabacum* L.) (9,16). The following terms, defined by Van der Plank (21), are used to characterize nematode–host relationships. Virulence is defined as occurring when races interact differentially with host cultivars, whereas aggressiveness occurs when races do not interact differentially. Knowledge of variation in disease induction and reproduction is important in cultivar selection, crop rotation, and plant breeding programs for nematode management (17).

Meloidogyne arenaria (Neal) Chitwood is an important pest of soybean. The objective of this study was to determine whether populations of *M. arenaria* differed in reproduction, number and morphology of host galls, and suppression of growth and yield of soybeans. To insure that the populations were all *M. arenaria*, perineal patterns, chromosome number, host range, and esterase phenotypes were examined.

Materials and Methods

Reproduction and suppression of crop growth and yield: Composite soil samples containing one of three *M. arenaria* race 2 populations were collected from Govan, Florence, and Pelion, South Carolina. A composite sample containing one *M. arenaria* race 1 population was collected from Florida. For each geographical population, soil cores taken from one field were bulked in an effort to maintain the genotypic diversity present in each field population. Soil from each field was placed in 1-liter plastic containers, planted with tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), and placed in water baths at 25–30 C. The populations of *M. arenaria* were cultured on successive generations of Rutgers tomato for 4–5 months.

Plant growth, yield suppression, and reproduction of these four *M. arenaria* populations were evaluated in 120 microplots (76 cm d, 122 cm deep) in a Troup loamy sand (82% sand, 6% silt, 12% clay) (3). Microplot soil was fumigated each year before planting with 90 ml of a mixture of methyl bromide (98%) and chloropicrin (2%). Microplots were then covered with plastic which was removed after 1 week. The soil was turned and left undisturbed for 3 weeks before adding nematodes, mycorrhizae, rhizobia, and soybean seed. Eggs of the four *M. arenaria* populations were extracted from galled root tissue of 40-day-old Rutgers tomato (17). Three liters of soil was removed from each microplot and infested with 24,000 eggs of *M. arenaria* and

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5,000 chlamydospores of the mycorrhizal fungus, *Glomus macrocarpus* Tul. & Tul. Macronutrients (N, P, K) and the nitrogen-fixing symbiont, *Bradyrhizobium japonicum* (Kirchner) Jordan 137, also were added based upon soil fertility analysis (15). After thorough mixing on a tarp, the 3 liters of soil and additives were placed in each microplot at planting.

Nematode inoculum delivered to each microplot was measured. A 250-cm^3 sample of soil from each infested microplot was collected, placed in a 10-cm pot, and planted with a Rutgers tomato seedling. After 40 days, plants were removed, and egg masses were stained in phloxine B (150 mg/liter) for 15 minutes (5) and counted.

Seeds of one of six soybean cultivars—Centennial, Cobb, Braxton, Gordon, Perrin, and Kirby—reported to have differential tolerance to *M. arenaria* (13,18) were sown in a single row per microplot. Two weeks after germination, plant rows were thinned to 12 seedlings per microplot. Microplots were irrigated with equal amounts of water as needed for soybean survival.

Numbers of *M. arenaria* in soil were measured by extracting (12) and counting juveniles collected in a 250-cm^3 sample consisting of four composite cores per microplot. Soil samples were taken from the root zone at V6 and R3 soybean growth stages (8). Shoot lengths of four randomly selected plants per microplot were measured at the V6 growth stage. Gall (22) and egg-mass (17) indices were determined, and root fresh weights were recorded for the two end plants in each microplot at the R3 growth stage. Shoots from these and two randomly selected plants per microplot were harvested, dried, and weighed. Seed yield was recorded at maturity for the eight remaining plants in each microplot.

The experimental design was a split plot with whole plot treatments being soybean cultivars arranged in a randomized complete block design replicated five times. Subplot treatments consisted of microplots inoculated with one of four *M. arenaria* populations.

Perineal patterns: At least 20 perineal patterns per population were examined at 250× magnification after staining with acid fuchsin. Patterns obtained during preparation of chromosome mounts also were examined.

Host range: The North Carolina differential host test (17) was performed by inoculating three replications of each differential host in 10-cm-d pots with 10,000 eggs obtained by the NaOCl method (17). Hosts were grown for 50 days. Root systems were examined and rated for galls (22) and egg masses (17). Egg masses were stained in phloxine B (150 mg/liter) for 15 minutes (5) and viewed through a magnifying lens.

Chromosome numbers: Chromosomes of the South Carolina populations but not the Florida population were examined and counted after staining with propionic orcein (20) or DAPI (4,6-diamidine-2-phenylindol-dihydrochlorid) fluorescent stain (1). Populations to be stained were cultured for 45–50 days at 25–30 C on Rutgers tomato seedlings. Before staining, the number of immature oocytes at metaphase was increased by incubating individual root galls in a 0.05% colchicine solution at 25 C for 2–3 hours (4).

Root galls were rinsed with tap water, and females were separated from root tissue and transferred to a 0.9% KCl solution to prevent rupturing. Three or four females were placed on a clean microscope slide and dissected at 40× magnification to remove ovaries. Smears were dried at least 15 minutes (usually overnight) before preparation as described by Triantaphyllou (20). Slides were sealed with a paraffin-lanolin mixture (1:1) and scanned at 100× magnification for metaphase plates in polar orientation. Phase contrast optics, green light filter, and camera lucida drawing tube aided in visualization and counting at 1,000× magnification.

For DAPI staining, ovarial smears were dried in a similar manner and specimens were hydrolyzed in a 1 N HCl for 10 minutes and incubated in a fixative (20). One or two drops of DAPI (1.0 μg/ml) in McIlvaine's buffer (0.1 M citric acid, 0.2 M sodium phosphate; pH 4.0) was added to

each slide. A coverslip was placed carefully on top and sealed with a paraffin–lanolin mixture. Slides were stored in a light-proof box to protect specimens from ultraviolet light. They were examined with a Leitz Dialux 20 compound microscope equipped with epiilluminator source, ultraviolet light emitter, and 40× and 100× Neofluor objectives. Photographs were taken within 30 minutes to avoid fading of fluorescent material after exposure to ultraviolet light. Only the DAPI preparations were used in making chromosome counts of nematode populations. Chromosomes were counted in metaphase plates of immature oocytes because these plates are larger than those found in germinal zone ovarial cells.

Esterase phenotypes: Esterase phenotypes of the four *M. arenaria* populations were compared with thin-slab polyacrylamide electrophoresis. Young females with small, white egg masses were teased from galled root tissue and placed in 0.9% KCl solution in a BPI dish to prevent rupturing. A filter paper square (2–3 mm²) was placed in each well of a porcelain reaction plate which had been thoroughly chilled on a bed of ice. Each square was moistened with a chilled solution of 20% glucose and 2% Triton X-100 applied with a microhematocrit tube. Females were crushed on the filter paper squares with a small glass pestle. Each square was transferred to a small, labeled, plastic petri dish on ice. Covered petri dishes containing 2–4 squares were placed immediately in cold storage (−80 C) and examined within 3–4 months.

Gels were prepared 1 day before electrophoresis as described by Esbenshade and Triantaphyllou (6) except that 1) TEMED (N,N,N,N-tetramethylethylenediamine) was added to each gel but not to stock solutions A2 or B, 2) ammonium persulfate was added to solution G at 0.14 g/100 ml water, and 3) the potassium phosphate buffer in the esterase stain solution was at a concentration of 0.1 M. Filter paper samples were placed in wells of each stacking gel after diluted, chilled bridge buffer was added to the apparatus. Electrophoresis (6) was performed on a gel slab (10 cm × 7 cm × 0.7 mm thick) in a Bio-Rad Mini-PROTEAN II electrophoresis unit.

TABLE 1. Egg masses† of *Meloidogyne arenaria* on tomato-bioassay plants in microplots containing six soybean cultivars and four populations of *M. arenaria*.

	1985	1986	1987
	Cultivar		
Centennial	51	38	42
Cobb	39	48	27
Braxton	46	43	31
Gordon	50	61	30
Perrin	20	39	36
Kirby	48	35	35
	Nematode population		
Govan	44	34	24 a
Pelion	41	50	46 b
Florence	60	47	16 a
Florida	25	45	48 b

Means followed by the same letter or no letter in columns are not different ($P = 0.05$) according to LSD means analysis.

† Number of egg masses on greenhouse-cultured Rutgers tomato plants grown in soil from individual microplots infested with 24,000 eggs of *M. arenaria* and planted with soybean. For cultivar analysis, all nematode populations were grouped together; for nematode population, all cultivars were combined.

Esterase standards included a nonspecific pig liver esterase preparation from Sigma Chemical Corporation (St. Louis, MO) and single-female samples of a *M. incognita* population from snapdragon (*Antirrhinum majus* L.). Bromphenol blue was incorporated into the pig liver standard and used as a tracking dye during electrophoresis. Voltage was maintained at 80 for the first 10 minutes and at 230 for the remainder of the separation period (45–50 minutes). Gels were stained with Fast Blue RR Salt (Sigma Chemical Corporation) bound to the substrate alpha-naphthyl acetate, fixed, and stored in plastic bags containing a 7% acetic acid solution. Forty-eight single-female samples per population were electrophoresed in all possible pair-wise combinations.

RESULTS

Reproduction and suppression of crop growth and yield: Equivalent infection of bioassay plants indicated that eggs were distributed in similar amounts in microplots in 1985 and 1986. In 1987, lower egg-mass numbers were recorded for the Govan and

TABLE 2. *Meloidogyne arenaria* juveniles, egg-mass index, and root-gall index as affected by four *Meloidogyne arenaria* populations over 3 years.

Population	Juveniles/250 cm³ soil		Gall† index	Egg-mass† index
	V6	R3		
		1985		
Govan	910 a	3,103 a	6.5 a	4.9 a
Pelion	443 b	1,872 b	5.3 b	4.9 a
Florence	672 ab	1,460 b	6.2 a	5.1 a
Florida	87 c	340 c	3.4 c	3.2 b
		1986		
Govan	1	40,193 a	6.9 a	4.0 a
Pelion	0	28,863 b	5.6 b	4.8 b
Florence	1	28,202 b	5.9 b	4.7 b
Florida	2	3,635 c	2.6 c	2.6 c
		1987		
Govan	509 a	11,935 a	7.2 a	4.3 a
Pelion	339 ab	14,094 a	6.2 b	4.8 b
Florence	234 bc	10,996 a	6.6 b	4.7 b
Florida	87 c	1,429 b	3.3 c	3.2 c

Means followed by the same letter or no letter in columns for each year are not different ($P = 0.05$) according to LSD means analysis.

† Gall index based upon gall size and portion of root covered, 1–10 rating; egg-mass index based upon number of egg masses on root surface, 1–5 rating. Data were averaged across six cultivars.

TABLE 3. Plant height, shoot dry weight, root fresh weight, and seed yield of soybean as affected by four *Meloidogyne arenaria* populations over 3 years.

Population	Plant height (cm)	Shoot dry wt. (g)	Root fresh wt. (g)	Seed yield (g)
		1985		
Govan	36	11 ab	24 a	20 a
Pelion	35	11 ab	20 ab	21 a
Florence	34	9 a	23 a	15 a
Florida	36	12 b	16 b	53 b
		1986		
Govan	44	31 a	23 a	2 a
Pelion	44	52 b	38 b	14 b
Florence	44	46 b	31 ab	12 b
Florida	46	86 c	33 b	64 c
		1987		
Govan	48	29 a	21	2 a
Pelion	47	48 b	27	4 a
Florence	48	42 b	27	2 a
Florida	46	80 c	23	15 b

Data were averaged across six cultivars. Means followed by the same letter or no letter in columns for each year are not different ($P = 0.05$) according to LSD means analysis.

Florence *M. arenaria* populations than for those from Pelion and Florida (Table 1).

Analysis of variance indicated the absence of a cultivar by population interaction for any of the variables measured. Therefore, data have been averaged across cultivars. Gall and egg-mass indices over a 3-year period demonstrated differences among *M. arenaria* populations. Root-gall index was greatest for the Govan population, intermediate for the Pelion and Florence populations, and lowest for the Florida population (Table 2). Low egg-mass indices for Govan were due to many egg masses being concealed by subsequent galling of root tissue. Such large, coalescing galls produced by the Govan population resulted in a high gall index (Table 2) and root fresh weight (Table 3), especially on the cultivars Centennial and Cobb (data not shown). Juvenile numbers were higher for the Govan than for the other populations (Table 2). Very few juveniles were recovered during the V6 sampling of 1986 (Tables 2, 4).

Seed yield was lowest with the Govan population, highest with the Florida population, and intermediate with the Pelion and Florence populations (Table 3). The Govan population suppressed shoot dry weight more than did the other popula-

TABLE 4. Number of chromosomes in metaphase plates of immature oocytes from three populations of *Meloidogyne arenaria* from South Carolina.

Plates counted	Chromosomes/plate
Govan	
2	30–34
3	35–39
7	40–44
1	45–49
1	50–54
Pelion	
3	30–34
7	35–39
2	40–44
1	45–49
2	50–54
Florence	
2	30–34
0	35–39
2	40–44
1	45–49
1	50–54

tions in 1986 and 1987 (Table 3). Plant height was similar among nematode populations (Table 3) and among soybean cultivars except in 1987, when Cobb was smaller than the other cultivars (data not shown).

There was little tolerance to *M. arenaria* in Centennial and Cobb cultivars, whereas considerable tolerance was present in Gordon, Perrin, and Kirby. Braxton was intermediate in tolerance (data not shown).

Perineal patterns, host ranges, chromosome number, and esterase phenotypes: Perineal patterns of the four populations were those of *M. arenaria*. Lateral incisures were gently sloped. Dorsal arches were very low and rounded, forming "shoulders," and all cuticular annuli were relatively smooth and not wavy in appearance. Differential host tests showed reactions typical of *M. arenaria* race 2 for the three South Carolina populations and of *M. arenaria* race 1 for the Florida population.

Chromosomes were easier to count with the DAPI fluorescent staining procedure than with the propionic-orcein procedure. The DAPI procedure was more selective in staining chromosomes and provided more contrast and depth of focus than the propionic-orcein method. A total of 35 metaphase plates were counted and judged to be accurate within five chromosomes because chromosomes were well spread, not overlapping, and in the same focal plane (Table 4). All three South Carolina populations had chromosome counts ranging from 31 to 54 (Fig. 1). Most spreads in all the populations ranged from 38 to 42.

Esterase phenotypes were the same among and within the Govan, Pelion, Florence, and Florida populations. All phenotypes were of the two-banded *M. arenaria* form (Fig. 2).

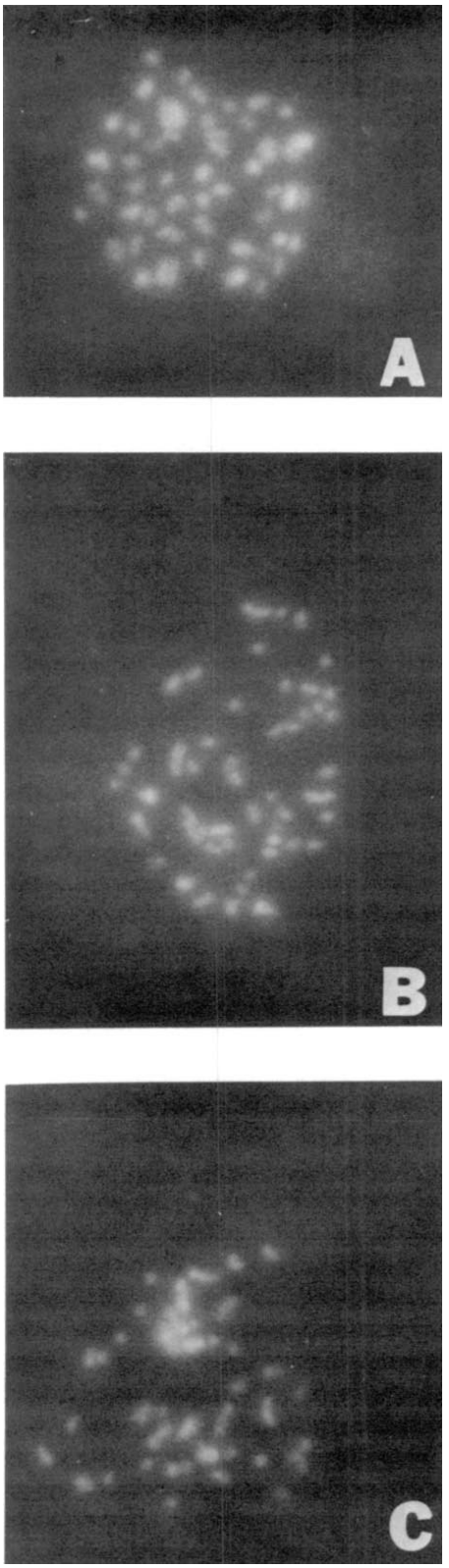

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FIG. 1. Metaphase plate of an immature oocyte of three populations of *Meloidogyne arenaria* race 2. A) Specimen from Govan population with 45–49 chromosomes. B) Specimen from Pelion population with 50–54 chromosomes. C) Specimen from Florence population with 40–44 chromosomes.

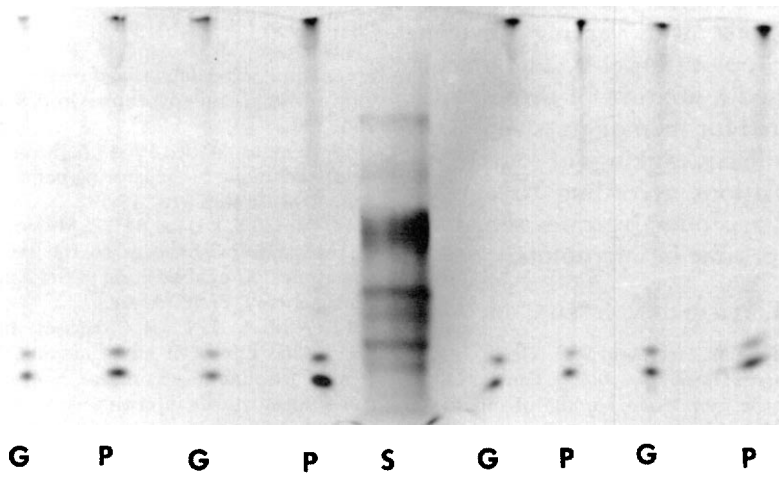

FIG. 2. Single female pair-wise comparisons of esterase phenotypes of Govan (G) and Pelion (P) populations of *Meloidogyne arenaria* on polyacrylamide gel with nonspecific pig esterase standard (S).

DISCUSSION

Reproduction and disease induction on soybean differed among populations of *M. arenaria*. The ranking of different populations with respect to these variables was similar across six soybean cultivars. Therefore, the populations differed in aggressiveness rather than in virulence (21). These results confirm those of previous tests (2).

Differences in reproduction and aggressiveness of *M. arenaria* populations will affect the success of management tactics. For example, more aggressive populations may require longer rotations with nonhosts. Unfortunately, there is no rapid means of determining aggressiveness; aggressiveness was not associated with variance in any of the morphological, cytogenetic, or biochemical characteristics examined in this study. Our results indicate that aggressiveness can be maintained in the greenhouse for at least 3 years. Furthermore, the aggressiveness observed in microplots was correlated with that observed in the field (data not shown).

Low juvenile population densities during the V6 sampling of 1986 reflect the possibility that the majority of second-stage juveniles had infected the soybean roots but had not yet reproduced. Delay in reproduction could have been due to extremely hot temperatures and drought conditions which stressed the soybean hosts early in the growing season. Differences in plant height would have been more pronounced had measurements been taken later in the growing season, allowing more time for pathogen–host interactions to develop.

Chromosomes are difficult to count if they are not separated from each other. Therefore, all metaphase plates that were counted and recorded were those in which chromosomes were well spread. Determination of an exact number of chromosomes was not possible even in the best preparations, but accuracy within five chromosomes was deemed possible.

Meloidogyne arenaria has three chromosomal forms (diploid, hypotriploid, and triploid) which may coexist in a genetically diversified, parthenogenetic population. Our method of establishing a greenhouse culture from a composite of many field soil samples would preserve such diversity. The presence of several chromosomal forms in a single population could contribute both adaptability and stability in a host–parasite relationship. A population derived from a composite of many field samples more closely approximates the genotypic diversity of a natural field population than does a population derived from a single egg mass.

Earlier work documented stability in re-

production of a mixture of three *M. incognita* populations over varying environments in microplot tests (14). The inocula used contained a mixture of three populations selected for their aggressiveness on soybean. Similarly, rankings of four *M. arenaria* populations according to aggressiveness and reproductive rates remained the same over time in microplots.

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