A Technique for Screening Peanut for Resistance to *Meloidogyne arenaria*

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ABSTRACT

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Two hundred ninety-three peanut accessions were screened for resistance to the peanut root-knot nematode using visual galling and egg-mass ratings. Staining with phloxine B greatly expedited the egg mass screening process. No high level of resistance was observed in any of the accessions evaluated.

Development of a peanut cultivar resistant to the peanut root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood, would reduce losses in peanut-producing regions throughout the southeastern United States. In Florida alone, R. A. Dunn (*unpublished*) estimated a loss in 1981 of more than $2.2 million in peanut production caused by *M. arenaria*.

Selection and development has yielded 93 cultivars in 15 major crops resistant to *M. arenaria* (3); however, there is no known source of resistance to this nematode that can be used in breeding cultivated peanuts. Minton and Hammons (8) screened 512 peanut entries on the basis of galling severity and reported that all entries were susceptible to *M. arenaria*.

Resistance to plant-parasitic nematodes is commonly defined as a reduction or inhibition of nematode reproduction (3,9,10). Fassuliotis (3) noted that because galling occurs in most susceptible plants infected with root-knot nematode, this is often the sole measurement of resistance during screening experiments. He advised, however, that galling alone does not indicate nematode reproduction and may lead to erroneous measurements of resistance.

The objectives for this research were 1) to develop a fast and accurate screening technique based on nematode reproduction and 2) to use this technique in the field and in the greenhouse to examine peanut germ plasm for resistance to the peanut root-knot nematode, *M. arenaria*.

MATERIALS AND METHODS

Seed from 293 peanut plant introductions (PIs) were provided by the USDA Southern Regional Plant Introduction Station, Experiment, GA or the Department of Agronomy, University of Florida, Gainesville. *M. arenaria* inoculum for greenhouse screening was obtained from a culture established and maintained on Rutgers tomato, (*Lycopersicon esculentum* Mill.) by D. W. Dickson (Department of Entomology and Nematology, University of Florida, Gainesville). The culture came from an infested peanut field in Levy County, FL.

Inoculum was extracted using a modification of the sodium hypochlorite (NaOCl) method developed by Hussey and Barker (7). Tomato roots with egg masses were washed clean of soil and agitated in a 20% solution of commercial bleach for 30 sec. After the tomato roots were removed and placed in a beaker of water, the NaOCl solution was quickly passed through nested 200- and 500-mesh sieves to collect the freed eggs. Eggs collected on the 500-mesh sieve were rinsed in tap water to remove residual NaOCl and poured into a beaker. Roots were then rinsed two more times with water to remove additional eggs.

Single peanut plants were grown in 10-cm pots filled with steam-sterilized sandy soil. Four holes about 5 cm deep and 1 cm wide were made in the soil around each peanut seedling 10 days after emergence. Ten milliliters of inoculum of 5,000, 7,500, or 10,000 eggs and juveniles were

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Table 1. Correlation of egg mass and gall indices from peanut plants in screening experiments for resistance to *Meloidogyne arenaria*

Experiment location and inoculum level[a]	Correlation coefficient	Significance level ($P =$)
Greenhouse (5,000)	0.696	<0.01
Greenhouse (7,500)	0.325	<0.01
Greenhouse (10,000)	0.688	<0.01
Field	−0.210	<0.01

[a] Eggs and juveniles per plant.

applied into each hole with a pistol pipet. Four replicates were used at the intermediate level of inoculum and two replicates were used at other levels.

Peanut plants were uprooted and washed clean of soil after allowing at least 40 days for development of egg-laying females. The roots were then placed in 1,000-ml beakers containing about 300 ml of 0.05% phloxine B solution for 3–5 min (5). This stained egg masses bright red (2). After staining, plant roots were visually rated by two methods, one estimating the number of galls and the other the number of egg masses. Each plant was assigned galling and egg-mass values based on the following index: 0 = no galls or no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = more than 100 galls or more than 100 egg masses per plant.

The field planting of 260 PIs was made on a farm in Levy County, FL, known to have a heavy infestation of the peanut root-knot nematode. The soil was an arredondo fine sand (91, 4.4, 4.6% sand, silt, clay; pH 7.9, 1.0% organic matter). Seeds were planted in a randomized complete block design with six replicates. A single plot consisted of 10 seeds planted in a 91-cm row, with 76 cm between rows. Production practices were based on University of Florida Cooperative Extension Service recommendations.

Individual plants were harvested with a shovel 148 days after planting. Care was taken to retain as much of the taproot as possible. Roots and pods were washed in a large bucket of water and transferred to smaller buckets containing phloxine B stain solution. After staining for 3–5 min, the plants were removed and visually rated for egg-mass production with the same index used in the greenhouse experiments. Because field-grown plants had much higher levels of galling than greenhouse-grown plants, an index based on percentage of galled tissue was used to facilitate the rating procedure. The amount of galling on pegs and pods was recorded using the index: 0 = no galling, 1 = 1–9% of peg and pod surface galled, 2 = 10–19%, 3 = 20–39%, 4 = 40–59%, 5 = more than 60% of the peg and pod surface galled.

RESULTS AND DISCUSSION

None of the 293 peanut PIs screened for resistance to *M. arenaria* had an egg mass or gall index of 2 or less whether screened in the field or in the greenhouse. Thus, none of the peanut genotypes showed a high level of resistance. Two hundred seventy-three of the accessions had not been tested previously, whereas 15 of the accessions had previously been found susceptible on the basis of galling severity by Minton and Hammons (8).

Egg mass and gall indices showed a significant positive correlation in the greenhouse, although at the intermediate inoculum level, the correlation was low (Table 1). In the field, the two indices showed a weak but significant negative correlation, indicating little relationship between number of egg masses and percentage of galled surface area on pegs and pods. These correlations must be viewed with caution because all genotypes proved susceptible. The correlation between gall and egg mass indices that would occur in a resistant peanut accession is unknown.

Fassuliotis and others (3,9,10) have indicated resistance in plants to nematodes should be based on the inhibition of nematode reproduction. A measurement of galling alone may not show a corresponding amount of reproduction, as shown by the inconsistent relationship between numbers of egg masses and galling in this study. Galling can occur in some host plants in the absence of nematode growth and reproduction (6), whereas others have found galling may scarcely occur in other hosts even though nematodes grow and reproduce normally (1,4).

Because the egg-mass staining technique is relatively quick and inexpensive, it is suggested that future screening of peanut germ plasm for resistance to *M. arenaria* include an assessment based on nematode reproduction. A complete list of the peanut accessions tested can be obtained from D. A. Knauft or D. W. Dickson.

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