Cowpea Resistance to Root Knot Caused by *Meloidogyne incognita* and *M. javanica*

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ABSTRACT

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Cowpea cultivars were tested for suitability as hosts for *Meloidogyne incognita* and *M. javanica* by comparing the numbers of eggs produced on root systems. Reproduction by both nematode species was lower on cultivars Magnolia Blackeye, California Blackeye No. 5, Mississippi Silver, and Mississippi Purple than on other cultivars tested. *M. javanica* produced 10–20 times more eggs on Magnolia Blackeye, Mississippi Silver, and Mississippi Purple. Some variability in cultivar suitability for the four *M. incognita* races was also found. Most notably, California Blackeye No. 3 was resistant to race 4 but Queen Ann was not. The results support the hypothesis that root-knot resistance in cowpea is ineffective for a California population of *M. javanica*.

Root knot is an important cowpea disease that occurs wherever the crop is produced in the United States (3). All four root-knot nematode species most commonly encountered in agricultural soils can attack cowpea, but *Meloidogyne incognita* is most frequently associated with the crop (3,4). In California, however, *M. javanica* was the prevalent species on cowpea in surveys in the Chino and San Joaquin valleys (I. J. Thomason, *unpublished*), possibly because one of the main cultivars grown in California is more resistant to *M. incognita* than to *M. javanica*.

Root-knot-resistant cowpeas have been sought since 1902 and several resistant cultivars are now available (4). Resistance to four root-knot species attacking cowpea has been reported (6). In a test of 44 cultivars and lines in California, none was resistant to *M. javanica* (14), although most were resistant to *M. incognita acrita*. Recently, *M. incognita* resistance in the cultivar Mississippi Silver was shown to be inherited as a single dominant gene (4). This same gene was also found in the cultivars Iron and Colossus, and it conferred resistance to *M. javanica, M. hapla,* and *M. incognita*. Cowpea resitance to root knot may vary with the root-knot species, race, and host cultivar. Mississippi Silver was resistant to each of the four races of *M. incognita*, both *M. arenaria* races, and *M. javanica* and *M. hapla*.

Fery and Dukes (4) have suggested that failure to find *M. javanica* resistance in cowpea in California could be caused by pathogenic variability in the nematode, environmental effects on resistance, inoculum density optima, or resistance criteria. We present results that support the view that the failure is due to pathogenic variability and suggest that *M. javanica* in California can at least partly overcome root-knot resistance in cowpea.

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MATERIALS AND METHODS

Inocula. Cultures of *M. incognita* (isolate 6A) and *M. javanica* (isolate J7c54) were started with single egg sacs taken from greenhouse populations at the University of California at Riverside. *M. incognita* was originally isolated from fig roots (field 6A, University of California Experimental Station, Riverside). *M. javanica* was originally isolated from cowpea plants growing in Chino, CA, in 1954. Each species was maintained in the greenhouse on tomato, pepper, or lima bean.

Isolates of four *M. incognita* races, originally obtained from North Carolina State University, were also maintained on tomato and pepper in the greenhouse.

Nematode inocula were prepared by extracting eggs from culture plant roots by the blender/bleach technique (8) or by collecting (every 24 hr) juveniles hatched from infected roots incubated in aerated water at 27 C. Root-knot juveniles were collected on a 26-μm pore sieve. The residue was placed in Baermann funnels to separate root debris and select for active individuals.

Experiments. In all experiments, plants were grown in twice-steam-sterilized blow sand. Nutrients were provided by weekly watering with Hoagland's solution (7) or by placing or mixing pellets of Osmocote controlled-release fertilizer (formula 18-6-12, Sierra Chemical Co., Milipitas, CA) with the sand.

The first four experiments were conducted in a growth cabinet (air temperature 27 C, photoperiod 15 hr). Seeds were germinated in vermiculite, and when seedlings were 2 wk old, they were transplanted to sand in PVC tubes (7.5 cm tall × 3.5 cm i.d.) (11). Six tubes were grouped on a clay brick, which represented one block of six cultivars. There were 16 blocks in a galvanized tray containing a uniform water level. Two days after transplanting, each plant was inoculated with 5,000 eggs.

Resistance of three cultivars commonly grown in California was evaluated in the greenhouse using seed from a commercial source. Plants were grown in sand in molded fiber pots (Western Pulp Products, Corvallis, OR) placed on plant-propagation mats (Pro-Gro Supply Co., Butler, WI) set to maintain the soil temperature at 27 C. Each seedling was inoculated with 5,000 *M. incognita* eggs when 2 wk old. Pots were arranged in a completely random design with 10 replicates. Nematode development as measured by eggs produced per root system was determined 6 wk after inoculation.

Reproduction of *M. javanica* and *M. incognita* on the cultivar Mississippi Silver was compared at four inoculum levels (50, 500, 5,000, or 10,000 eggs per plant). Seedlings were grown in PVC tubes and placed on a nylon mesh screen. Redwood strips were used to create cells in which tubes were placed. There were 10 replicates of each treatment arranged in a completely random design on the screen. The experiment was conducted in the growth cabinet set as described before. Eggs were extracted from roots 6 wk after inoculation.

Resistance of Magnolia Blackeye and Mississippi Purple to *M. javanica* and *M. incognita* was compared in the greenhouse. CBE 3 was included as a susceptible control. Plants were grown in 450-cm^3 Styrofoam cups in which holes had been punched through the bottoms for drainage. Uniform 2-wk-old plants were selected and inoculated with 700 juveniles of either species. The eggs were extracted after 61 days of incubation. There were eight replicates arranged in a completely random design.

Reproduction of four races of *M. incognita* on 12 cowpea cultivars was tested in two growth-cabinet experiments using the PVC tubes on the brick system described before. Each plant was inoculated with 5,000 eggs of one of the four races. In the first experiment, there were five replicates arranged in a split-plot design, with blocks (five) and races (four) as the main plots and cultivars (six) as the subplots. In the second experiment, there were 10 blocks.

Egg extractions. At the conclusion of the experiment, roots were washed out of the sand, blotted dry, weighed, and stored in 10% (v/v) formalin-tap water solutions until extraction. Eggs were extracted by macerating the root systems in 10% (v/v) commercial bleach-tap water solution for 60 sec at full speed in a blender. The resulting suspension was poured through nested 500- and 26-μm pore sieves. Residue retained on the 26-μm pore sieve was stained with boiling acid-fuschin lactophenol for 60 sec and counted. Statistical tests were always performed on counts of eggs per root system transformed to $\log_{10}$ in order to eliminate the correlation between means and variances (9). Pertinent orthogonal contrasts were used for trend-line comparisons (9).

RESULTS

Significant differences in nematode reproduction by cultivar were found in experiments in the growth chamber (Table 1). Reproduction on CBE 5, Mississippi Silver, Mississippi Purple, and Magnolia Blackeye was significantly lower than on other cultivars tested. Reproduction by *M. incognita* on Mississippi Purple was significantly lower than on Magnolia Blackeye. One of the two selections of CBE 5 tested was not resistant to either nematode species.

M. incognita reproduction was lower on CBE 5 and Magnolia Blackeye than on CBE 3 in an experiment in the greenhouse (Table 2). *M. javanica* produced more eggs than *M. incognita* on Mississippi Purple, Magnolia Blackeye, and CBE 3 (Table 3). *M. javanica* reproduction was reduced from P_f/P_i = 141 on CBE 3 to P_f/P_1 = 77–78 on Mississippi Purple and Magnolia Blackeye. *M. javanica* reproduction on Mississippi Silver exceeded that of *M. incognita* over all four inoculum densities (Table 4). Statistical analysis of the data showed that both the position and shape of the second-order curves fitted on $\log_{10}$ inoculum density differed with the nematode species (P=0.05). At P_i = 5,000 eggs, about 10 times more eggs were produced by *M. javanica* than by *M. incognita*.

The race × cultivar interaction was not statistically significant in the first experiment with *M. incognita* races (P=

Table 1. Reproduction of *Meloidogyne incognita* (isolate 6A) and *M. javanica* (isolate J7c) on cowpea

	P_f/P_i[a]		Eggs/mg root tissue	
Cultivar	***M. javanica***	***M. incognita***	***M. javanica***	***M. incognita***
Experiments 1 and 2				
CBE 5 (source 2)	11.2 b	1.2 c	25.4 ab	1.0 c
CBE 5 (source 1)	24.6 a	33.4 b	30.7 a	16.7 b
Chinese Red	9.5 b	43.7 b	24.2 ab	31.4 ab
Arlington	12.8 b	53.7 ab	22.3 b	39.9 a
Groit	21.9 a	71.3 a	28.4 ab	41.0 b
Grant	26.6 a	38.9 b	26.4 ab	21.3 b
Experiments 3 and 4				
Magnolia Blackeye	6.1 b	0.8 c	30.3 b	0.3 c
Mississippi Silver	8.0 b	0.6 cd	39.9 b	0.3 bc
Mississippi Purple	6.8 b	0.5 d	11.0 b	0.4 b
CBE 3	55.6 a	8.8 b	50.1 a	3.2 a
Queen Ann	40.4 a	9.3 ab	31.7 a	2.5 a
Chino 3	53.0 a	13.8 a	48.0 a	3.9 a

[a] P_f/P_i = eggs extracted from a root system/5,000 eggs inoculated. Means within an experiment (one species and six cultivars) followed by common letters do not differ (P = 0.05) according to Duncan's new multiple range test performed on egg numbers per root system transformed to $\log_{10}$ (n = 16).

Table 2. Reproduction and gall ratings for *Meloidogyne incognita* on three cowpea cultivars

Cultivar	P_f/P_i[a]	Eggs/mg root tissue	Gall index[b]
CBE 5	0.6 a	0.40	1.1
MBE	0.7 a	0.22	1.1
CBE 3	16.9 b	7.20	4.0

[a] P_fP_i = eggs extracted from a root system/5,000 eggs inoculated. Means followed by common letters do not differ (P = 0.05) according to Duncan's new multiple range test performed on egg numbers per root system transformed to $\log_{10}$ (n = 10).

[b] Gall index: 1 = 0–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% roots with galls.

0.05) (Table 5); however, there was a tendency for greater reproduction by race 1 on Groit, indicating that some degree of race × cultivar specificity existed. Reproduction was low only on CBE 5. The relative ranking of the other cultivars differed somewhat from that found in the initial growth-chamber experiments (Table 1).

The race × cultivar effect was barely significant ($P = 0.05$) in the second experiment. Mississippi Silver and Magnolia Blackeye supported only limited reproduction of all four races. Mississippi Silver and CBE 5 were significantly more resistant to races 1, 2, and 3 than CBE 3 or Queen Ann (Table 5). Race 4 reproduction was significantly lower on CBE 3 than on Queen Ann. Even though race 2 reproduction differed with the cultivar, the level of reproduction by race 2 was low on all cultivars (Table 5).

DISCUSSION

On the basis of nematode reproduction, cowpea cultivars Magnolia Blackeye, Mississippi Purple, and Mississippi Silver were more resistant to *M. incognita* than to *M. javanica* in our experiments. This finding disagrees with earlier reports that resistance to root knot is independent of the nematode species (4–6).

We recovered two eggs per milligram of root tissue from Mississippi Silver plants inoculated with *M. incognita* (isolate 6A) eggs in the inoculum density experiment and 33 *M. javanica* eggs per milligram. Others have reported somewhat higher figures on this and other cultivars (4; I. J. Thomason, *unpublished*). The lower levels of reproduction in our experiments may be due to a shorter incubation period (1.5 vs. 3 mo), inoculum and extraction efficiencies, root size at the time of inoculation, and container size effect on root growth. Still, our results indicate that *M. javanica* J7c reproduction by *M. javanica* isolate J7c54 on cultivars considered *M. incognita*-resistant approaches levels on cultivars considered *M. incognita*-susceptible. The inoculum density experiment indicated that *M. javanica* has a much greater maximum reproductive (12) rate and equilibrium population density (12) on Mississippi Silver than *M. incognita*. These differences could be due to more reproducing females per root system, and/or more eggs per female.

Resistance to *M. javanica* may have been lower in our experiments because we used egg production as the compatibility criteria, not gall or egg mass numbers. Little resistance was found (14) to a progenitor of the *M. javanica* population we used, however, so we suggest the results are evidence of pathogenic variability in the nematode. Root-knot species-specific resistance in cowpea has been reported previously. In Nigeria, ACC 64298 was resistant to a local and to a North Carolina population of *M. incognita* but not to *M. incognita* or *M. javanica* populations from Columbia (10).

The conclusion that root-knot nematode resistance in cowpea is less effective toward *M. javanica* (isolate J7c54) is based on comparisons with *M. incognita* (isolate 6A), and therefore could have been influenced by pathogenic variability in the *M. incognita* populations. There was some evidence of physiological specialization among the *M. incognita* races in the second experiment with the four *M. incognita* races; however, except for races 1 and 3 on CBE 3 and races 1, 3, and 4 on Queen Ann, the differences were minor. It would be interesting to study the inheritance of resistance to these races in CBE 3, Queen Ann, and Mississippi Silver and determine whether *M. incognita* race-specific resistance genes exist in cowpea.

Viewed from the perspective of the gene-for-gene theory (1,2,15), these *M. incognita* race × cultivar interactions present the opportunity to identify genes determining race × cultivar specificity. *M. incognita* races, however, are named for their compatibility with tobacco and cotton (13). Unless the virulence genes in *M. incognita* that specify compatibility with tobacco and cotton cultivars also specify compatibility with cowpea

Table 3. Reproduction of *Meloidogyne javanica* (isolate J7c) and *M. incognita* (isolate 6A) on three cowpea cultivars

	P_f/P_i[a]		Eggs/mg root tissue	
Cultivar	***M. javanica***	***M. incognita***	***M. javanica***	***M. incognita***
Magnolia Blackeye	78.0	9.3	3.37	0.50
Mississippi Purple	76.9	9.9	3.21	0.42
CBE 3	141.1	86.8	5.61	2.99

[a] P_f/P_i = eggs extracted from a root system/700 juveniles inoculated. Effects of nematode species, cultivar and interaction were significant ($P = 0.001$) in an analysis of eggs per root system transformed to $\log_{10}$ ($n = 6$). P_f/P_i ratios that differ by 1.62 times are significantly different ($P = 0.05$) according to the LSD test.

Table 4. Effect of initial egg inoculum density on the reproduction of *Meloidogyne javanica* (isolate J7c) and *M. incognita* (isolate 6A) on Mississippi Silver cowpea

	P_fP_i[a]		Eggs/mg root tissue	
P_i (eggs)	***M. javanica***	***M. incognita***	***M. javanica***	***M. incognita***
50	226.7	9.9	3.8	0.1
500	113.2	8.3	17.3	0.9
5,000	26.6	2.1	33.3	2.0
10,000	27.1	1.3	48.1	2.2

[a] P_f/P_i = eggs extracted from a root system/number of eggs inoculated. In analysis of variance of egg counts per root system transformed to $\log_{10}$, the change in $\log_{10} P_f$ with $\log_{10} P_i$ was quadratic, and both the position and shape of the curve relating $\log_{10} P_f \log_{10} P_i$ for *M. javanica* differed ($P = 0.05$) from the curve for *M. incognita*.

Table 5. Reproduction of *Meloidogyne incognita* on cowpea as affected by nematode race and host cultivar

	Race (P_f/P_i)[a]				
Cultivar	**1**	**2**	**3**	**4**	**Cultivar mean**
Experiment 1					
CBE 5 (source 1)	15.6	29.7	16.7	15.1	19.2 ab
CBE 5 (source 2)	6.5	7.0	1.7	1.2	4.1 d
Grant	23.1	15.6	19.3	14.0	18.0 ab
Arlington	8.5	13.7	6.2	20.7	12.3 c
Chinese Red	21.6	12.6	7.9	16.1	14.6 bc
Groit	63.6	24.7	26.8	34.3	37.3 a
Experiment 2					
Mississippi Silver	2.1	0.4	0.6	1.0	1.0 b
Mississippi Purple	0.4	0.2	0.4	0.4	0.4 c
Magnolia Blackeye	0.3	0.2	0.4	1.4	0.6 bc
CBE 5 (source 3)	0.6	0.4	0.4	0.7	0.5 c
CBE 3	11.5	3.0	0.8	1.9	6.6 a
Queen Ann	12.6	1.2	7.1	6.8	6.9 a

[a] P_f/P_i = eggs extracted from a root system after 6 wk/5,000 eggs inoculated. In experiment 1 ($n = 5$), only the cultivar main effect in analysis of variance of $\log_{10}$-transformed data was significant ($P = 0.001$). In experiment 2 ($n = 10$), the race × cultivar interaction was also significant ($P = 0.05$). Cultivar means were separated by Duncan's new multiple range test. Means within an experiment followed by common letters do not differ ($P = 0.05$). Race × cultivar means in experiment 2 that differ by a factor of 3.17 are significantly different ($P = 0.05$) according to the LSD test.

cultivars, within-race variability could occur. Race 3 and Colossus may present such an example. Colossus was susceptible to one population of race 3 but not to another (4). If these disagreements are not artifacts of the compatibility criteria, the purity of Colossus seed, or the accuracy of race identification, they demonstrate that races have meaning only for the host cultivars on which they are defined.

An earlier survey of root-knot nematodes in California indicated that *M. javanica* was more common on cowpea than *M. incognita*. Surveys designed to identify the species, to determine the distribution of the *M. javanica* J7c54 isolate, and to assess the pathogenic variability in *M. incognita* are now needed. This information will help in the use of host cultivar resistance to control root knot in California.

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