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Effects of Resistance in *Phaseolus vulgaris* on Development of *Meloidogyne* Species[1]

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Abstract: Use of resistant *Phaseolus vulgaris* germplasm has a potential role in limiting damaging effects of *Meloidogyne* spp. on bean production. Effects of two genetic resistance systems in common bean germplasm on penetration and development of *Meloidogyne spp.* were studied under growth room conditions at 22°C to 25°C. Nemasnap (gene system 1) and G1805 (gene system 2) were inoculated with second-stage juveniles (J2) of *M. incognita* race 2 and *M. arenaria* race 1, respectively; Black Valentine was used as the susceptible control. Up to 7 days after inoculation, there were no differences in numbers of *M. incognita* J2 penetrating roots of Black Valentine and Nemasnap; subsequently, more nematodes were present in Black Valentine roots ($P < 0.05$). More nematodes reached advanced stages of development in Black Valentine than in Nemasnap roots ($P < 0.05$). Total numbers of *M. arenaria* were greater in Black Valentine than in G1805 roots from 14 days after inoculation ($P < 0.05$). Advanced stages of development occurred earlier and in greater numbers in Black Valentine plants than in G1805 plants. In these studies, resistance to M. *incognita* race 2 and *M. arenaria* race 1 in bean germplasm, which contain gene system 1 and gene system 2, respectively, was expressed by delayed nematode development rather than by differential penetration compared with susceptible plants.

Key words: common bean, development, gene system, *Meloidogyne* spp., nematode, penetration, *Phaseolus vulgaris,* resistance, root-knot nematode.

Root-knot nematodes (*Meloidogyne* spp.) can reduce yields of common bean (*Phaseolus vulgaris* L.) by 50% to 90% (1,11,12). Bean germplasm with resistance to root-knot nematodes has been identified (6,15). Use of resistant germplasm may provide an economic and environmentally safe alternative to nematicides for managing root-knot nematodes in beans.

Resistance to root-knot nematodes in *P. vulgaris* is controlled by one of two genetic systems (13,15). Gene system 1, found in Alabama No. 1, Nemasnap, and PI 165426, conditions resistance to *M. incognita* (Kofoid & White) Chitwood races 2, 3, and 4, and to some race 1 populations. Resistance in Alabama No. 1 and Nemasnap is controlled by the interaction of two or more independent recessive genes (3,6); resistance in PI 165426 is controlled by one dominant and one recessive gene (13). Gene system 2, found in Mexican accessions G2618 and G1805, confers resistance to some populations of *M. incognita* race 1, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood races 1 and 2. Resistance in gene system 2 is controlled by a single dominant gene (13,14). Some resistant cultivars were developed before the specific nature of these gene systems was understood. Use of cultivars with resistance conditioned by either gene system 1 or gene system 2 may be limited in production areas where mixed populations of *Meloidogyne* spp. occur.

Resistance in plants generally can be described as mechanisms that prevent or restrict nematode reproduction, whereas tolerance is the ability of the plant to withstand nematode injury and is independent of resistance (19). Mechanisms of resistance can be divided into pre- and post-infectional mechanisms (8). Resistance mechanisms in *P. vulgaris* have been reported only in lines containing gene system 1 (2,6). Barrons (2) reported that penetration of roots by root-knot nematode juveniles was similar in both a susceptible (Kentucky Wonder) and a resistant (Alabama No. 1) cultivar, but that galls did not form in the resistant cultivar. Fassuliotis et al. (6) found no differences in the penetration of roots of susceptible and resistant plants by *M. incognita* juveniles. However, differences in number, size, and rate of

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formation of giant cells and galls were observed in the roots of the susceptible and resistant lines. The effects of resistance on development of root-knot nematodes in the two gene systems have not been reported. The objectives of this study were to evaluate the effects of the two genetic resistance systems on nematode infection and development, and to determine the effects of nematode infection on growth of resistant and susceptible bean genotypes.

Materials and Methods

The isolate of *M. incognita* race 2 was obtained from a tobacco (*Nicotiana tabacum* L.) field, and the isolate of *M. arenaria* race 1 was obtained from a peanut (*Arachis hypogea* L.) field, both in north-central Florida. The *M. incognita* race 2 isolate was increased on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.), and the *M. arenaria* race 1 isolate was increased on 'Florunner' peanut under greenhouse conditions. Nematode eggs were extracted from 2-month-old plants with NaOCl (9). Hatched second-stage juveniles (J2) were collected from Baermann funnels over 72 hours to obtain populations of similarly aged infective juveniles.

Separate experiments were conducted with each resistance gene system. Inoculated plants were used to follow nematode penetration and development, and uninoculated plants served as controls to compare plant growth with inoculated plants.

Gene System 1: Two common bean cultivars, susceptible Black Valentine and Nemasnap, resistant to *M. incognita* race 2, were evaluated in this experiment. Fifty plants of each cultivar were inoculated with J2 of *M. incognita* race 2, and an equal number of plants of each cultivar served as uninoculated controls. The treatments were arranged in a completely randomized design. Each treatment consisted of five replicates with 10 harvest dates.

Gene System 2: The experimental design of the second experiment was similar. Fifty plants of susceptible Black Valentine and G1805, resistant to *M. arenaria* race 1, were inoculated with J2 of *M. arenaria* race 1. An equal number of plants were left uninoculated. The treatments were arranged in a completely randomized design. Each treatment consisted of five replicates of 10 harvest dates. Both experiments were conducted at the same time, and the uninoculated Black Valentine plants served as a common susceptible treatment for both experiments.

Germinated bean seeds with radicles 1 to 3 cm long were planted singly in 10-cm-diam. pots filled with steam-sterilized Kendrick sand (86% sand, 2% silt, 12% clay). Seven days after planting, one-half of the plants were inoculated with freshly hatched J2 of either *M. incognita* race 2 (Experiment 1) or *M. arenaria* race 1 (Experiment 2). One thousand J2 suspended in 10 ml of water were pipetted into three equidistant 5-cm-deep holes around the root zone of each plant. Inoculation holes were filled with steam-sterilized soil, and pots were watered immediately to moisten the soil. The remaining plants were similarly treated with sterile water and served as controls for each harvest date. Plants were fertilized biweekly with NPK 15:30:15. The experiments were carried out in a growth room maintained with a 10-hour daylength and mean day and night temperatures of 25°C and 22°C, respectively.

At 1, 2, 3, and 7 days, and every 7 days thereafter for 49 days after inoculation, five inoculated and five uninoculated plants of each germplasm were harvested. The plants were cut at the soil line, and fresh and dry weights of the stems, petioles, leaf blades, and pods were recorded; areas of leaf blades also were measured. The roots were washed free of soil, blotted dry with paper towels, and weighed. Whole root systems of inoculated plants harvested 1, 2, and 3 days after inoculation were stained with acid fuchsin (4). The root systems of the uninoculated plants were dried and weighed. Root systems of plants harvested 7, 14, and 21 days after inoculation were divided into two approximately equal portions; one portion was stained and the other was dried and

weighed. Root systems of plants harvested 28, 35, 42, and 49 days after inoculation were divided into three portions; one portion was stained, one was dried and weighed, and root-knot nematode eggs were extracted from the third portion using 2% NaOCl (9). All root systems were rated for galling using an index of 0 to 5, where 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100 galls per root system (18).

Total numbers of nematodes in the stained portions of the root systems were recorded at each harvest date, and individuals were assigned to one of four developmental stages (17). Individuals in the first developmental stage (vermiform) were vermiform, non-swollen, second-stage juveniles; individuals in the second developmental stage (swollen) were swollen, sausage-shaped J2; individuals in the third developmental stage (globose) were swollen, partially globose juveniles with conical tails; and the final developmental stage (adult) included fully globose females with or without egg masses.

Average individual fecundity (eggs per female) was calculated from the total number of extracted eggs per root system divided by the total number of females per plant. Viability of eggs was measured as percentage hatch in water after incubation for 10 days at 25°C. Percentage hatch = (number of juveniles/number of eggs) × 100.

Both experiments were repeated. The final harvest was made 42 days after inoculation for the second trial because plants had senesced by 49 days after inoculation in the first trial. Data from both trials of each experiment were analyzed separately by analysis of variance using SAS software (6.09, SAS Institute, Cary, NC); the model was a completely randomized design. Plant, egg, hatch, and fecundity data were transformed with $\log_{10}(x + 1)$ before analysis. Data from each harvest date were analyzed separately; nematode developmental stages and plant-growth parameters were compared between the germplasm treatments.

Results

Gene system 1: In the first trial, there were no differences in the total numbers of *M. incognita* race 2 in the susceptible and resistant systems up to and including 14 days after inoculation (Fig. 1). Beyond this time, more nematodes were present in the roots of Black Valentine plants ($P < 0.05$). At 7 days after inoculation, more nematodes had reached the swollen stage of development in the susceptible Black Valentine roots than in the resistant Nemasnap roots ($P < 0.05$). Also, beginning with day 14 and continuing through 21 days after inoculation, more nematodes had reached the globose stage in the roots of Black Valentine than in the roots of Nemasnap ($P < 0.05$). More adults were present in Black Valentine roots than in Nemasnap roots on 21, 28, 42, and 49 days after inoculation ($P < 0.05$). At 49 days after inoculation, vermiform stages were present in the roots of Black Valentine, indicating that development of *M. incognita* race 2 had progressed to a second generation. Development of nematodes to the adult stage was delayed in Nemasnap compared to that in Black Valentine. By 14 days after inoculation, 63% of nematodes in Black Valentine roots had already developed to the globose stage; at this time, only 5% of nematodes in Nemasnap roots had developed to this stage. The swollen stage persisted longer in resistant Nemasnap roots, with 22% of nematodes still present in this stage at 35 days after inoculation compared with only 2% in Black Valentine roots. Although from day 35 and beyond, high percentages of nematodes on both cultivars were adults (96% in Black Valentine, 71% in Nemasnap), total numbers were much higher in Black Valentine roots than in Nemasnap roots (Fig. 1). In the second trial, rates of development similar to the first trial were observed in Black Valentine and Nemasnap, although total populations were slightly lower (data not shown).

In the first trial, galls on the root systems of Black Valentine were visible at 14 days

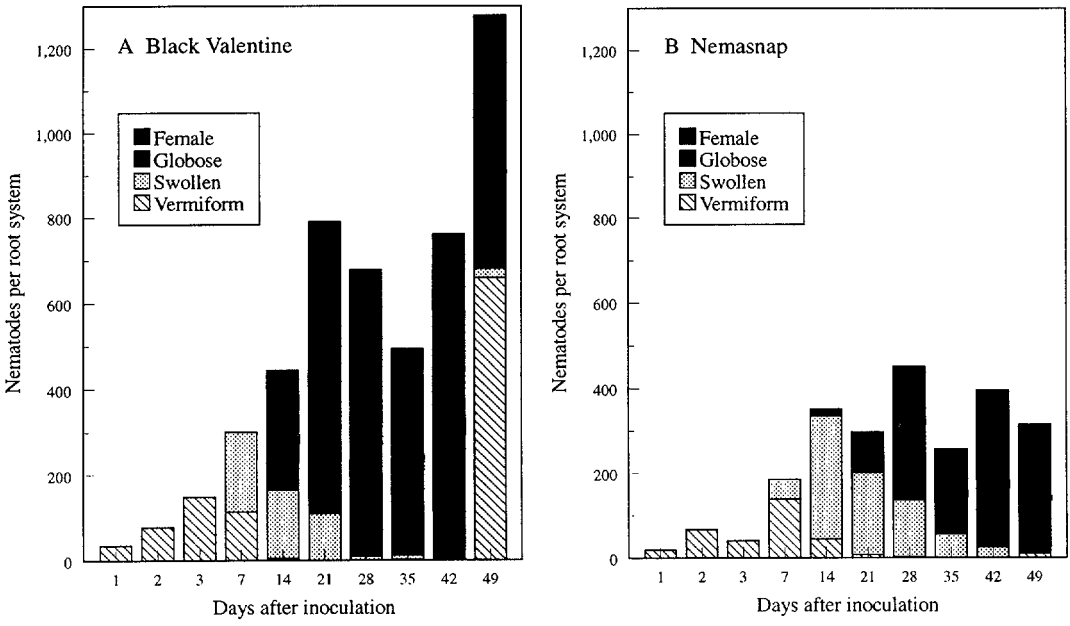

FIG. 1. Penetration and development of *Meloidogyne incognita* race 2 on bean germplasm (gene system 1). A) Black Valentine (susceptible). B) Nemasnap (resistant).

after inoculation. At this time, the mean gall rating was 4.8 (data not shown). Galls were first visible on the roots of Nemasnap plants at 21 days after inoculation (mean gall rating = 0.8). The gall rating on Nemasnap was highest (3.4) at 42 days after inoculation. Forty-two days after inoculation, greater numbers of males ($P < 0.05$) were observed in roots of Nemasnap plants than in roots of Black Valentine (16 males and 0 males, respectively; data not shown). At 35 days after inoculation, numbers of eggs per root system were greater in Black Valentine roots than in Nemasnap roots ($P < 0.05$) (Table 1). However, there were no differences in fecundity of females (eggs per female) in Black Valentine and Nemasnap (Table 1). Results were similar for the second trial.

Plant growth in both trials was not affected by nematode inoculation. Measurements of total plant dry weights and leaf areas did not differ consistently for nematode-inoculated and uninoculated plants (17).

Gene system 2: In the first trial, total numbers of *M. arenaria* race 1 were greater in the roots of Black Valentine than in the roots of G1805 from 14 days through 49 days after inoculation ($P < 0.05$) (Fig. 2). At 7 and 14 days after inoculation, more nematodes had developed to the swollen

TABLE 1. Numbers of *Meloidogyne incognita* race 2 eggs from root systems of Black Valentine and Nemasnap bean plants, numbers of juveniles hatched from eggs after 10 days, and numbers of eggs per female (Trial 1).

	Eggs/root system		Hatched juveniles		Eggs/female	
Days to harvest	Black Valentine	Nemasnap	Black Valentine	Nemasnap	Black Valentine	Nemasnap
28	201	0	0	0	0	0
35	10,200	1,740[a]	646	104	40	12
42	16,200	4,920	3,420	889	23	13

Data are means of five replications.
[a] Log10-transformed data differ from number on Black Valentine at $P < 0.01$.

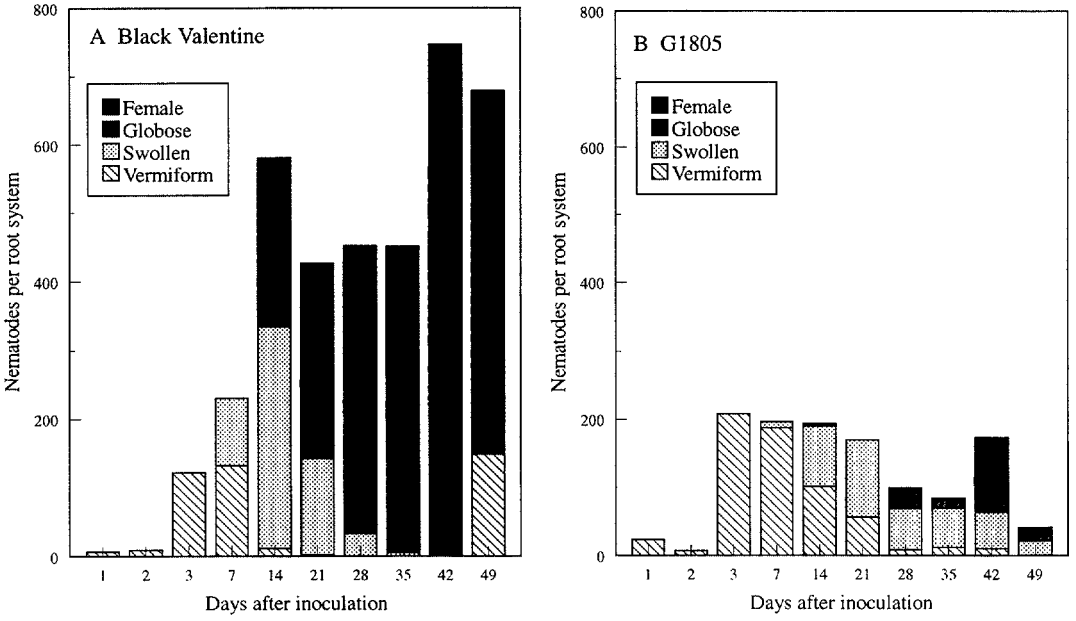

FIG. 2. Penetration and development of *Meloidogyne arenaria* race 1 on bean germplasm (gene system 2). A) Black Valentine (susceptible). B) G1805 (resistant).

stage in Black Valentine roots than in G1805 roots ($P < 0.05$). At 14, 21, and 28 days after inoculation, more nematodes had reached the globose stage in Black Valentine roots than in G1805 roots ($P < 0.05$). Also, at 35, 42, and 49 days after inoculation, more females were present in susceptible roots than in resistant roots ($P < 0.05$). Second-generation J2 were present in the roots of Black Valentine plants, but not in G1805 plants, by 49 days after inoculation. Development of nematodes to the adult stage was delayed in G1805 compared to that in Black Valentine. At 21 days after inoculation, no nematodes in the roots of G1805 plants had developed beyond the swollen developmental stage; at this time, 65% of the nematodes in the roots of Black Valentine plants already had reached the globose stage, and 2% were adults. At 49 days after inoculation, only 30% of the nematodes in roots of G1805 plants were adults compared with 78% in Black Valentine plants. The actual numbers of nematodes reaching the adult stage in G1805 were small compared with Black Valentine (Fig. 2). In the second trial, total numbers and rates of development of *M. arenaria* race 1 in both bean genotypes were similar to those observed in trial 1 (data not shown).

Galls were visible on the root systems of Black Valentine 14 days after inoculation. At this time, the mean gall index was 3.6; the index reached 5.0 by 35 days after inoculation (trial 1, data not shown). Galls were first visible on the roots of G1805 plants at 21 days after inoculation (mean gall index = 0.8). The highest gall index on G1805 roots was 2.0 at 35 days after inoculation; even at this time, the galls were small in size (≤3 mm in width). Forty-two and 49 days after inoculation, greater numbers of males ($P < 0.05$) were present in the roots of G1805 plants than in roots of Black Valentine plants (10 males and 0 males, respectively; data not shown). Numbers of eggs per root system, at 35 and 42 days after inoculation, and numbers of hatched J2, at 42 days after inoculation, were greater for Black Valentine roots than for G1805 roots ($P < 0.05$) (Table 2). However, there were no differences in fecundity of females in Black Valentine and G1805 roots (Table 2). These results were confirmed in the second trial.

TABLE 2. Numbers of *Meloidogyne arenaria* race 1 eggs from root systems of Black Valentine and G1805 bean plants, numbers of juveniles hatched from eggs after 10 days, and numbers of eggs per female (Trial 1).

Days to harvest	Eggs/root system		Hatched juveniles		Eggs/female	
	Black Valentine	G1805	Black Valentine	G1805	Black Valentine	G1805
28	54	0	0	0	0	0
35	1,780	594[a]	16	0	5	49
42	11,000	90[a]	4,080	0[a]	11	7

Data are means of five replications.
[a] Log10-transformed data differ from number on Black Valentine at $P < 0.01$.

Plant growth in both trials was not consistently affected by nematode inoculation as measured by total plant dry weights, leaf areas, or energy analyses (17). Plants under both inoculation regimes grew similarly and were not affected by nematode inoculation.

DISCUSSION

Resistance to *M. incognita* race 2 and *M. arenaria* race 1 in bean germplasm containing gene systems 1 and 2, respectively, was expressed by delayed nematode development rather than by differential penetration compared with a susceptible bean cultivar. Similar numbers of J2 penetrated roots of resistant and susceptible genotypes, indicating that physical and chemical root barriers do not prevent penetration of roots in resistant Nemasnap and G1805. Penetration of roots of resistant and susceptible common bean plants by equal numbers of root-knot nematode juveniles also has been reported (2,6); similar observations have been made in resistant and susceptible corn (20), cotton (5), and tobacco (16).

The numbers of *M. incognita* race 2 and *M. arenaria* race 1 in Black Valentine roots increased over time, indicating that J2 continued to enter roots of the susceptible genotype over an extended period of time. Second-stage juveniles of *M. incognita* race 2 also continued to enter the roots of the resistant Nemasnap plants over time; however, this occurred at a much reduced rate compared with the susceptible Black Valentine plants. Since numbers of *M. arenaria* in roots of G1805 did not exceed the numbers that had penetrated by 3 days after inoculation, it is possible that some of the J2 were unable to establish feeding sites and emigrated from the roots, as observed in other plant species (7,16).

Development of *M. incognita* race 2 in Nemasnap and *M. arenaria* race 1 in G1805 was delayed compared with development of these nematodes in susceptible Black Valentine plants, indicating that a postinfectional mechanism may retard nematode development. The resistance response against *M. arenaria* race 1 in bean germplasm G1805 (gene system 2) appears to be more restrictive than the response against *M. incognita* race 2 observed in Nemasnap (gene system 1). Few nematodes in G1805 roots developed beyond the swollen stage. This may be due to the failure of giant cell development because we frequently observed areas of discolored and somewhat collapsed tissues around vermiform and swollen nematodes. This evidence suggests that a hypersensitive reaction occurred which inhibited normal or further giant cell development, resulting in arrested nematode development. The small number of nematodes that did advance to globose and adult stages were present in small root galls, indicating that some giant cell initiation and development had occurred normally in a few cases.

Development of *M. incognita* race 2 was delayed in Nemasnap roots compared with development in Black Valentine roots, and fewer nematodes advanced to the mature stages in Nemasnap than in Black Valentine roots. However, in comparison to *M. arenaria* race 1 in G1805 roots, discolored

and collapsed tissues were not observed surrounding nematodes in Nemasnap roots, and a larger number of nematodes developed to the globose stage. It appears that once nematodes have reached the globose stage in the roots of both resistant germplasms, development to adults will occur. A similar observation has been reported with *M. incognita* and resistant cotton genotypes (10). The critical stage in Nemasnap seems to be at 21 to 28 days after inoculation, when development from swollen to globose stages occurs. In G1805, development to globose stages does not occur until 28 to 35 days after inoculation, at which time the numbers of *M. arenaria* race 1 decline somewhat, probably due to the death and decay of the nematodes that cannot continue development.

We speculate that the single dominant gene controlling resistance in plants containing gene system 2 is effective early in the nematode-plant interaction, and is expressed as a hypersensitive reaction in response to the attempts of nematodes to establish feeding sites. The interaction of the two or more recessive genes controlling resistance in gene system 1 plants also was reported to result in a hypersensitive reaction with giant cell dissolution early in nematode-plant interaction (6). Our studies indicate that this resistance resulting from gene system 1 is less efficient than that expressed by the single dominant gene in germplasm containing gene system 2.

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