Journal of Nematology 29(1):90–103. 1997.

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

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Abstract: *Phaseolus vulgaris* lines with heat-stable resistance to *Meloidogyne* spp. may be needed to manage root-knot nematodes in tropical regions. Resistance expression before and during the process of nematode penetration and development in resistant genotypes were studied at pre- and postinoculation temperatures of 24 °C and 24 °C, 24 °C and 28 °C, 28 °C and 24 °C, and 28 °C and 28 °C. Resistance was effective at all temperature regimes examined, with fewer nematodes in roots of a resistant line compared with a susceptible line. Preinoculation temperature did not modify resistance expression to later infections by root-knot nematodes. However, postinoculation temperatures affected development of *Meloidogyne* spp. in both the resistant and susceptible bean lines tested. The more rapid development of nematodes to adults at the higher postinoculation temperature of 28 °C in both bean lines suggests direct temperature effects on nematode development instead of on resistance expression of either of two gene systems. Also, resistance was stable at 30 °C and 32 °C.

Key words: common bean, host plant resistance, *Meloidogyne arenaria, Meloidogyne incognita,* nematode development, *Phaseolus vulgaris,* resistance mechanisms, root-knot nematode, temperature.

More than 77% of the world production of common bean (*Phaseolus vulgaris* L.) occurs in tropical developing countries of Latin America and Africa (Pachico, 1989). The majority of bean production in these areas occurs during cool, dry seasons or at high altitudes where temperatures range from 16 °C to 24 °C, which are close to the optimum temperatures for *P. vulgaris* growth (Allen et al., 1989; Van Schoonhoven and Voysest, 1989). However, higher-than-optimal soil temperatures often occur in bean-growing regions, thereby increasing the stress on bean plants. Cultivars with heat-stable resistance to *Meloidogyne* spp. may be needed for management of root-knot nematodes in these situations.

The effects of high soil temperature on the expression of resistance to root-knot nematodes have been reported for several crop plants (Ammati et al., 1986; Carter, 1982; Dropkin, 1969), including common beans (Fassuliotis et al., 1970; Mullin et al., 1991; Omwega et al., 1990). Often, resistance to nematodes decreases as temperature increases (Carter, 1982; Dropkin, 1969). Fassuliotis et al. (1970) found that roots of both a susceptible bean cultivar (Black Valentine) and a resistant breeding line (B3864) showed light galling at a lower temperature (16 °C), and more galls appeared as temperatures increased to 28 °C. Production of females and egg masses also increased with the increase in temperature, but the numbers of mature females that developed in the roots of the resistant lines at the higher temperatures were lower than those in the susceptible cultivar. Also, based on the number of mature females, bean lines Alabama No. 1 and PI 165435 lost their resistance to *M. incognita* and *M. hapla,* but not *M. arenaria,* when soil temperature increased from 25 °C to 30 °C (Mullin et al., 1991). Omwega et al. (1990) also found that resistance to *M. incognita* race 2 was lost at 30 °C in Alabama No. 1 and PI 165435, but remained partially effective in PI 165426. Resistance to *M. incognita* race 1 and *M. javanica* in other bean lines was stable at temperatures of 24 °C to 30 °C; however, *M. javanica* populations increased as temperatures increased from 26 °C to 30 °C. Mullin et al. (1991) reported that bean line A211 was resistant to *M. incognita* at 16 °C and 22 °C, but susceptible at 24 °C and above. They found that short incubation times (<16 days) of inoculated plants at 30 °C, before low-temperature incubation, resulted in loss

Received for publication 12 December 1995.

[1] Florida Agricultural Experiment Station Journal Series No. R-04897. A portion of a Ph.D. dissertation by the first author.

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of resistance as measured by nematode reproduction and root galling. Incubation of inoculated plants at 21 °C for >16 days, before high-temperature incubation, resulted in retention of resistance expression during a high-temperature treatment.

Effect of temperature on resistance expression has been reported mostly as gall ratings, numbers of adults, and reproduction measured as egg mass and total egg production (Fassuliotis et al., 1970; Mullin et al., 1991; Omwega et al., 1990). Previous workers have not studied the effect of temperature on resistance expression either before or during the process of nematode penetration and nematode development in resistant bean lines. The objectives of this study were to determine if preinoculation conditioning temperatures modify resistance before nematode infection and to evaluate the effect of postinoculation incubation temperatures on resistance expression in different genotypes of *P. vulgaris.*

Materials and Methods

All experiments were conducted at the University of Florida in Gainesville, Florida, during 1995. The protocol for all experiments was similar.

Gene system 1 and gene system 2: Separate experiments were conducted to evaluate different temperature combinations on each resistance gene system. Each experiment used Black Valentine as the susceptible genotype, together with a resistant genotype containing one of the two resistance gene systems. *Gene system 1:* A susceptible common bean cultivar (Black Valentine) and a cultivar resistant to *M. incognita* race 2 (Nemasnap) were used in this experiment. Treatment combinations on Black Valentine or Nemasnap inoculated with *M. incognita* race 2 were arranged in a completely randomized design with four replications per treatment and seven harvest dates. *Gene system 2:* The experimental design of the second experiment was the same as with gene system 1. Treatment combinations were imposed on susceptible Black Valentine and a line resistant to *M. arenaria* race 1 (G1805).

All experiments were conducted in growth chambers with 10 hours of light and 14 hours of dark. The temperatures used in all experiments as the preinoculation conditioning temperature or the postinoculation incubation temperature were 24 °C and 28 °C; 24 °C is within the range at which resistance is stable, and 28 °C is within the range at which resistance is reportedly modified but still sufficiently low for bean plant growth not to be too heat-stressed (Omwega et al., 1990). Therefore, the pre- and postinoculation temperature regimes established were 24 °C and 24 °C, 24 °C and 28 °C, 28 °C and 24 °C and 28 °C and 28 °C (±0.5 °C). All plants were grown for a total of 99 accumulated heat units (degree days over 10 °C base) before inoculation to ensure that all the plants were of similar physiological age at time of inoculation. All chambers were maintained at a constant relative humidity level of 75% (± 2%).

Germinated seeds with radicles 1 to 3 cm long were planted singly in a CYG Seed Growth Pouch (Mega International, Minneapolis, MN) and watered with approximately 15 ml of water. Arbitrarily chosen pouches were inserted in pairs in manila folders, for vertical support, and placed in growth chambers set at the four temperature regimes. Each bean plant in its pouch was inoculated with a 2- to 2.5-ml water suspension, with 400 second-stage juveniles (J2), over the roots. The pouches were returned to the growth chambers and kept horizontal for 24 hours to ensure the inoculum remained in the root zone before returning the pouches to an upright position for the remainder of the experiment.

At 1,2,3,7,14,21, and 28 days after inoculation (DAI), four plants of each genotype and temperature regime were harvested. The plants were cut at the root line, and fresh weights of the top and root system were measured. The roots were stained with acid fuchsin (Byrd et al., 1983). Total numbers of nematodes in the stained root systems were recorded at each harvest date, and individuals were assigned to one of four developmental stages based on readily visible characteristics (Sydenham, 1995). The

first developmental stage (vermiform) included vermiform, non-swollen, second-stage juveniles; the second developmental stage (swollen) included swollen, sausage-shaped, second-stage juveniles; the third developmental stage (globose) included swollen, partially globose individuals with conical tails; the final developmental stage (adult) included fully globose adults with or without egg masses.

Both experiments were repeated once. Data from both trials of each experiment were analyzed separately using the PROC ANOVA procedure (SAS, release 6.09, SAS Institute, NC). To determine the effects of the pre- and postinoculation temperatures on nematode penetration and development, data from the respective temperature regimes were pooled for analysis. All the replications for each temperature regime were within one chamber, making it impossible to separate the effects of temperature from position. However, due to the stability and consistency of the temperature and humidity levels (± 0.5 °C and ± 2%, respectively) in all the growth chambers used in these experiments, position effects were not considered.

High-temperature experiments 1 and 2: To examine the effects of high temperature on resistance expression of gene system 1 and gene system 2, two additional experiments were conducted in growth chambers set at 30 °C and 32 °C. Each experiment used Black Valentine as the susceptible genotype, together with either Nemasnap and *M. incognita* race 2 (gene system 1; experiment 1) or G1805 and *M. arenaria* race 1 (gene system 2; experiment 2). The treatment combinations were arranged in completely randomized designs with three replications per treatment and two harvest times, 13 and 22 DAI. All plants were grown for 5 days before inoculation with 400 J2 of either *M. incognita* race 2 or *M. arenaria* race 1. Plants were grown in seed pouches. Plant maintenance, inoculation, harvest, and data collection procedures were conducted as described above. Numbers of nematodes in plant roots were analyzed as previously described in this section.

Results

Gene system 1: For both Black Valentine and Nemasnap, the effects of the four temperature regimes on *Meloidogyne incognita* are presented as total nematode populations (Fig. 1). Linear regression analyses performed on nematode populations at the four temperature regimes showed similar significant ($P > 0.05$) relationships on Black Valentine but not on Nemasnap (Fig.1). The effects of the pre- and postinoculation temperatures are presented as the distribution of nematodes among the developmental stages (Figs. 2,3).

The total populations of *M. incognita* present in roots of susceptible Black Valentine plants were similar among the four temperature regimes ($P > 0.05$; Fig. 1). By 14 DAI, the populations within the roots of Black Valentine plants grown at all temperature regimes were approximately 50% of the initial inoculum level (Fig. 1). The total populations of *M. incognita* within the roots of resistant Nemasnap were more variable among the four temperature regimes (Fig. 1). At 1 DAI, more nematodes were present in the roots of plants grown at the 28 °C and 28 °C temperature regime than in roots of plants grown at the other temperature regimes ($P < 0.05$). By 2 DAI, the populations of *M. incognita* had peaked to maximum levels of less than 40% of the initial inoculum. At 28 DAI, the mean numbers of nematodes, pooled over all four temperature regimes, were higher ($P < 0.05$) in Black Valentine (222) than in Nemasnap (72). At all four temperature regimes in the second trial, similar numbers of *M. incognita* were observed in Black Valentine and Nemasnap roots as in the first trial; these data confirm the results of trial 1.

At 28 DAI, a second generation of vermiform J2 had penetrated the roots of both bean genotypes grown at the higher post-inoculation temperature of 28 °C. Roots of plants grown at the lower post-inoculation temperature of 24 °C had not been penetrated by J2 by this time (Fig. 3). The population data for second-generation J2 at 28 DAI were not plotted in Fig. 1 to emphasize

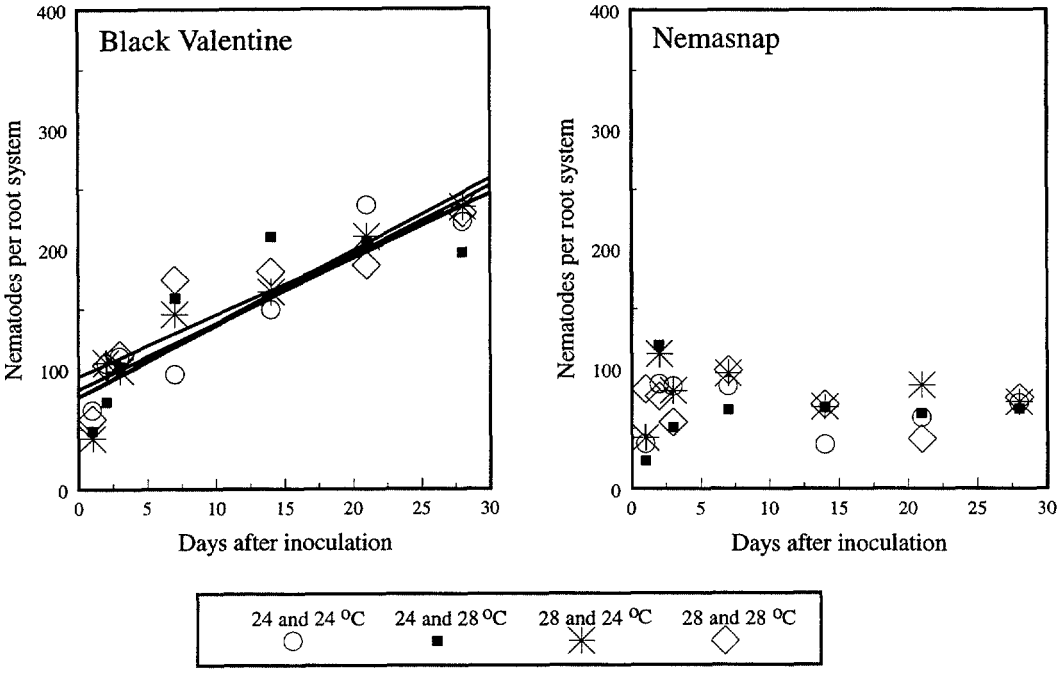

FIG. 1. Total populations of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean at four temperature regimes of preinoculation and postinoculation temperatures of 24 °C and 24 °C, 24 °C and 28 °C, 28 °C and 24 °C, and 28 °C and 28 °C (gene system 1). Data are means of four replications at each harvest time. Population data for the second generation of vermiform juveniles are not plotted for Black Valentine and Nemasnap plants 28 DAI. Regression equations for nematode numbers (y) over time (x) on Black Valentine: 24 and 24 °C: $y = 5.84x + 77.64$ ($R^2 = 0.88$, $P < 0.05$); 24 and 28 °C: $y = 5.03x + 83.36$ ($R^2 = 0.72$, $P < 0.05$); 28 and 24 °C: $y = 6.04x + 77.55$ ($R^2 = 0.89$, $P < 0.05$); 28 and 28 °C: $y = 5.08x + 94.06$ ($R^2 = 0.88$, $P < 0.05$). Linear regressions not significant for Nemasnap data at any temperature regime.

that the total numbers of first-generation nematodes in the roots were similar in plants grown under all temperature regimes.

From 14 to 28 DAI, males of *M. incognita* were observed in the roots of both Black Valentine and Nemasnap plants at all temperature regimes. Pre- and postinoculation temperatures of 24 °C and 28 °C had no consistent effect on the numbers of males in the susceptible or the resistant plants (Table 1).

Preinoculation conditioning temperatures did not greatly affect the populations of *M. incognita* in roots of Black Valentine and Nemasnap plants (Fig. 2). Preinoculation conditioning temperatures had no effect on the numbers of nematodes penetrating the roots of Black Valentine plants during the first 3 DAI (Fig. 2). Total numbers of nematodes in the roots of Black Valentine plants at all harvest times were similar between the two temperatures ($P > 0.05$). Preinoculation conditioning temperatures had little effect on nematode penetration and development in the roots of Nemasnap plants. At 1 DAI, 50% more nematodes had penetrated the roots of plants conditioned at the higher preinoculation temperature than at the lower temperature ($P < 0.05$; Fig. 2). Total nematode numbers were similar at all other harvest times between the two temperatures ($P > 0.05$). Similar results were obtained in the second trial.

Postinoculation incubation temperatures had greater effects than preinoculation conditioning temperatures on the development of *M. incognita* in the roots of both bean genotypes (Fig. 3). At 7 DAI, 98% more nematodes had developed to the globose stage in roots of Black Valentine plants grown at the higher temperature than at the

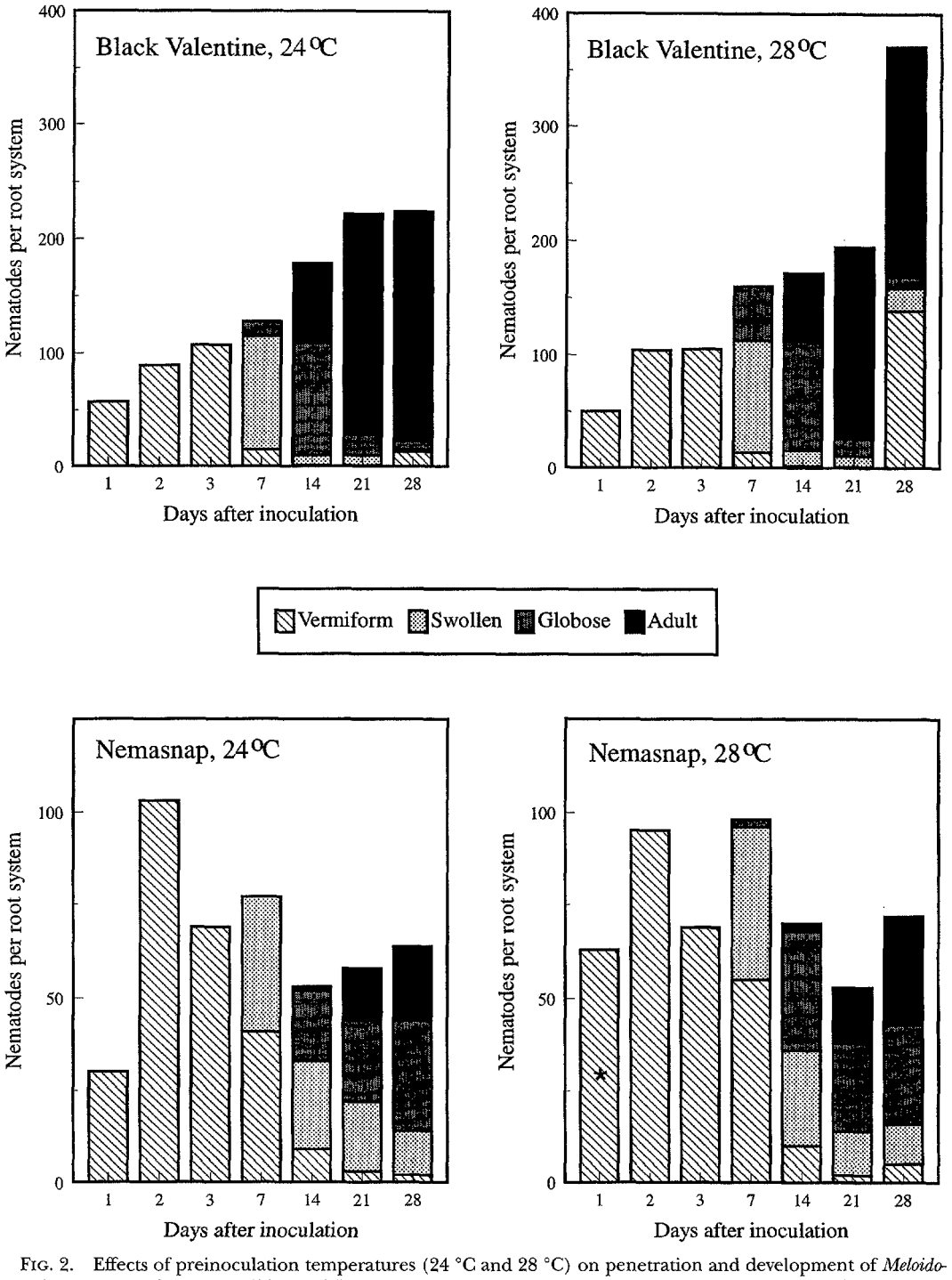

FIG. 2. Effects of preinoculation temperatures (24 °C and 28 °C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean genotypes (gene system 1). Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time. At each harvest day within each genotype, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other preinoculation temperature.

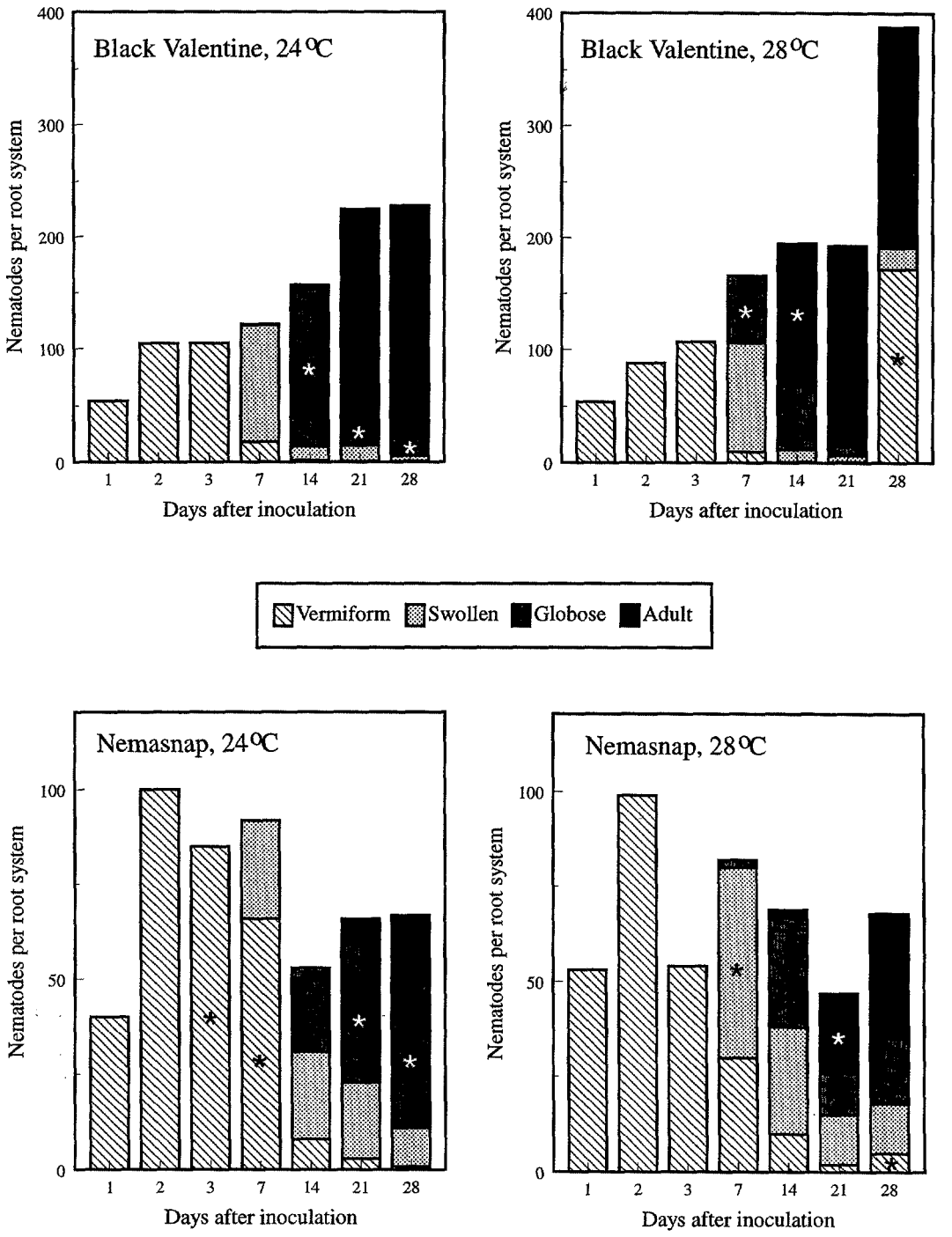

FIG. 3. Effects of postinoculation temperatures (24 °C and 28 °C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean genotypes (gene system 1). Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time. At each harvest day within each genotype, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other postinoculation temperature.

TABLE 1. Numbers of males of *Meloidogyne incognita* race 2 in root systems of susceptible Black Valentine (Black Val.) and resistant Nemasnap common bean (gene system 1) incubated at the preinoculation conditioning temperatures and postinoculation incubation temperatures of 24 °C and 28 °C.

Germplasm	Harvest day	Numbers of males of *M. incognita* race 2			
		Preinoculation temperature		Postinoculation temperature	
		24 °C	28 °C	24 °C	28 °C
Black Val.	14	0	1	0	1
	21	2	1	1	2
	28	0	1	1	0*
Nemasnap	14	1	1	0	1
	21	4	8	5	6*
	28	5	4	5	3

Data are means of four replications.

Within each pre- and post-inoculation time, germplasm and harvest time, asterisks (*) indicate significantly different ($P < 0.05$) numbers of males in roots grown at 28 °C compared with numbers of males in roots grown at 24 °C.

lower temperature ($P < 0.05$). At 14 DAI, 97% more adults were present in plants incubated at 28 °C than at 24 °C ($P < 0.05$). At 14 and 21 DAI, 60% more nematodes remained in the globose stage in roots of Black Valentine plants incubated at the lower temperature than in plants incubated at the higher temperature. At 28 DAI, 87% more nematodes remained in the globose stage in roots incubated at 24 °C than in roots incubated at 28 °C ($P < 0.05$), although, at this time, large numbers of adults were present in the roots of plants incubated at both temperatures. Also at 28 DAI, roots of Black Valentine plants incubated at 28 °C were penetrated by a second generation of vermiform J2; this did not occur in roots of plants incubated at 24 °C.

Both penetration into and development of *M. incognita* in the roots of resistant Nemasnap were affected by postinoculation incubation temperatures. At 3 DAI, 36% more juveniles penetrated roots incubated at 24 °C than at 28 °C ($P < 0.05$; Fig. 3). The effects of the postinoculation incubation temperatures on the development of *M. incognita* in Nemasnap plants were more pronounced. At 7 DAI, 50% more nematodes were present in the vermiform stage in roots of plants incubated at the lower temperature than in roots of plants incubated at the higher temperature ($P < 0.05$; Fig. 3). Also at this time, 50% more nematodes were present in the globose stage in plants incubated at 28 °C than in plants incubated at 24 °C ($P < 0.05$). At 14 DAI, adults had developed in roots of Nemasnap plants incubated at 28 °C but were absent from roots incubated at 24 °C; at 21 DAI, there were 71% more adults at 28 °C than at 24 °C ($P < 0.05$). At 21 and 28 DAI, 68% more nematodes still remained in the globose stage in roots incubated at the lower temperature compared with the numbers of globose nematodes in roots incubated at the higher temperature ($P < 0.05$). Also at 28 DAI, a second generation of vermiform J2 had penetrated roots of plants incubated at 28 °C; these were absent from roots of plants incubated at 24 °C. The data from the second trial confirm the results of the first trial.

Gene system 2: For both Black Valentine and G1805, the effects of the four temperature regimes on *Meloidogyne arenaria* are presented as total nematode populations (Fig. 4). Linear regression analyses performed on nematode populations at the four temperature regimes showed a significant relationship on Black Valentine at 28 °C and 24 °C only but not at the other temperature regimes, and on G1805 (Fig. 4). The effects of the pre- and postinoculation temperatures are presented as the distribution of nematodes among the developmental stages (Figs. 5,6).

Total populations of *Meloidogyne arenaria* within the roots of susceptible Black Valentine plants, at all the harvest times, were not different among the four temperature regimes ($P > 0.05$; Fig. 4). By 14 DAI, approximately 50% of the initial inoculum had entered and developed in root systems of the susceptible plants grown under all four temperature regimes (Fig. 4). Total populations of *M. arenaria* present in the roots of resistant G1805 plants also were similar among the four temperature regimes ($P > 0.05$; Fig. 4). By 3 DAI, numbers of *M. arenaria* had reached their maximum levels; less than 30% of the initial inoculum had entered in the roots of the resistant G1805 plants

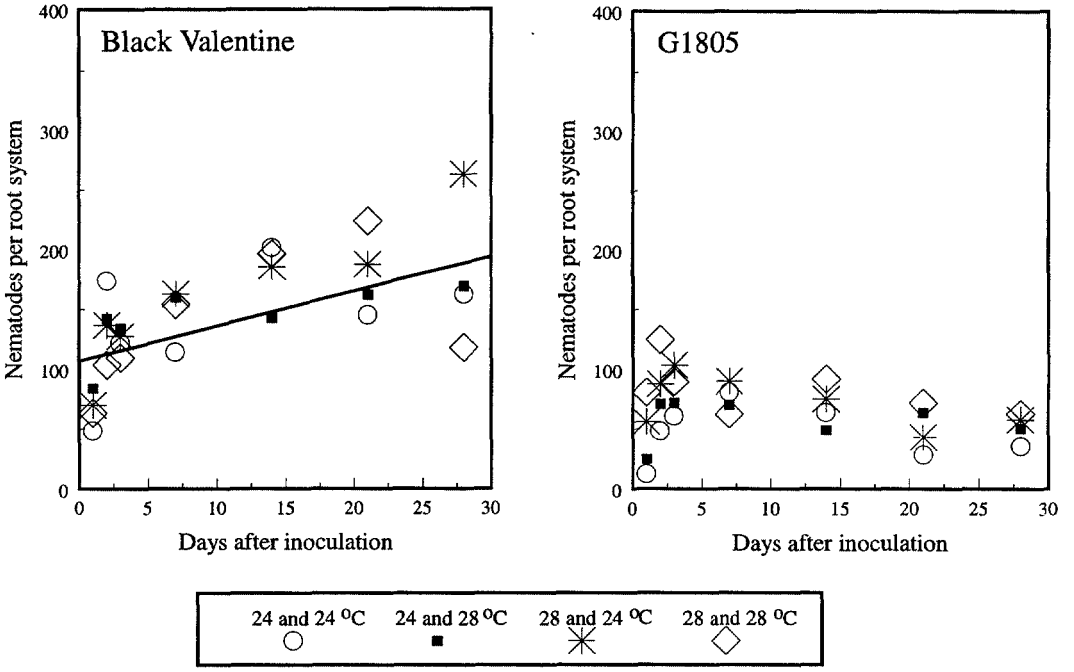

FIG. 4. Total populations of *Meloidogyne arenaria* race 1 on susceptible Black Valentine and resistant G1805 common bean at four temperature regimes of preinoculation/postinoculation temperatures of 24 °C and 24 °C, 24 °C and 28 °C, 28 °C and 24 °C, and 28 °C and 28 °C (gene system 2, trials 1 and 2). Data are means of four replications at each harvest time. Population data for the second generation of vermiform juveniles are not plotted for Black Valentine plants 28 DAI. Regression equations for nematode numbers (y) over time (x) on Black Valentine: 28 and 24 °C: $y = 5.26x + 105.09$ ($R^2 = 0.84$, $P < 0.05$). Linear regressions not significant for Black Valentine data at 24 °C and 24 °C, 24 °C and 28 °C, 28 °C and 28 °C, or for G1805 data at any temperature regime.

grown under all temperature regimes. After these times, the numbers of nematodes within the root systems began to decline, reaching levels below 10% of the initial inoculum level. Overall, the populations of *M. arenaria* in the roots of resistant G1805 plants were lower than the populations in the roots of Black Valentine plants. At 28 DAI, the mean total numbers of nematodes, pooled over all four temperature regimes, were higher in Black Valentine (219) than in G1805 (52) ($P < 0.05$).

At 28 DAI, a second generation of vermiform J2 had penetrated the roots of susceptible Black Valentine plants grown at the higher post-inoculation temperature; these were absent from the roots of Black Valentine plants grown under the lower postinoculation temperature (Fig. 6). The population data for second-generation J2 at 28 DAI were not plotted in Fig. 4 to emphasize that the total numbers of first-generation nematodes in Black Valentine roots were similar in plants grown under all temperature regimes.

Males of *M. arenaria* were not observed in roots of Black Valentine or G1805 plants at any of the temperature regimes. At all four temperature regimes in the second trial, similar numbers of *M. arenaria* were observed in Black Valentine and G1805 roots as in the first trial; these data confirm the results of trial 1.

Preinoculation conditioning temperatures did not greatly affect the populations of *M. arenaria* in roots of Black Valentine and G1805 plants (Fig. 5). Total numbers of nematodes and their rates of development in roots of Black Valentine plants conditioned at 24 °C and 28 °C were similar ($P > 0.05$). The effect of preinoculation conditioning temperature on nematode penetration and development in G1805 roots was variable and limited. At 1 and 2 DAI, more

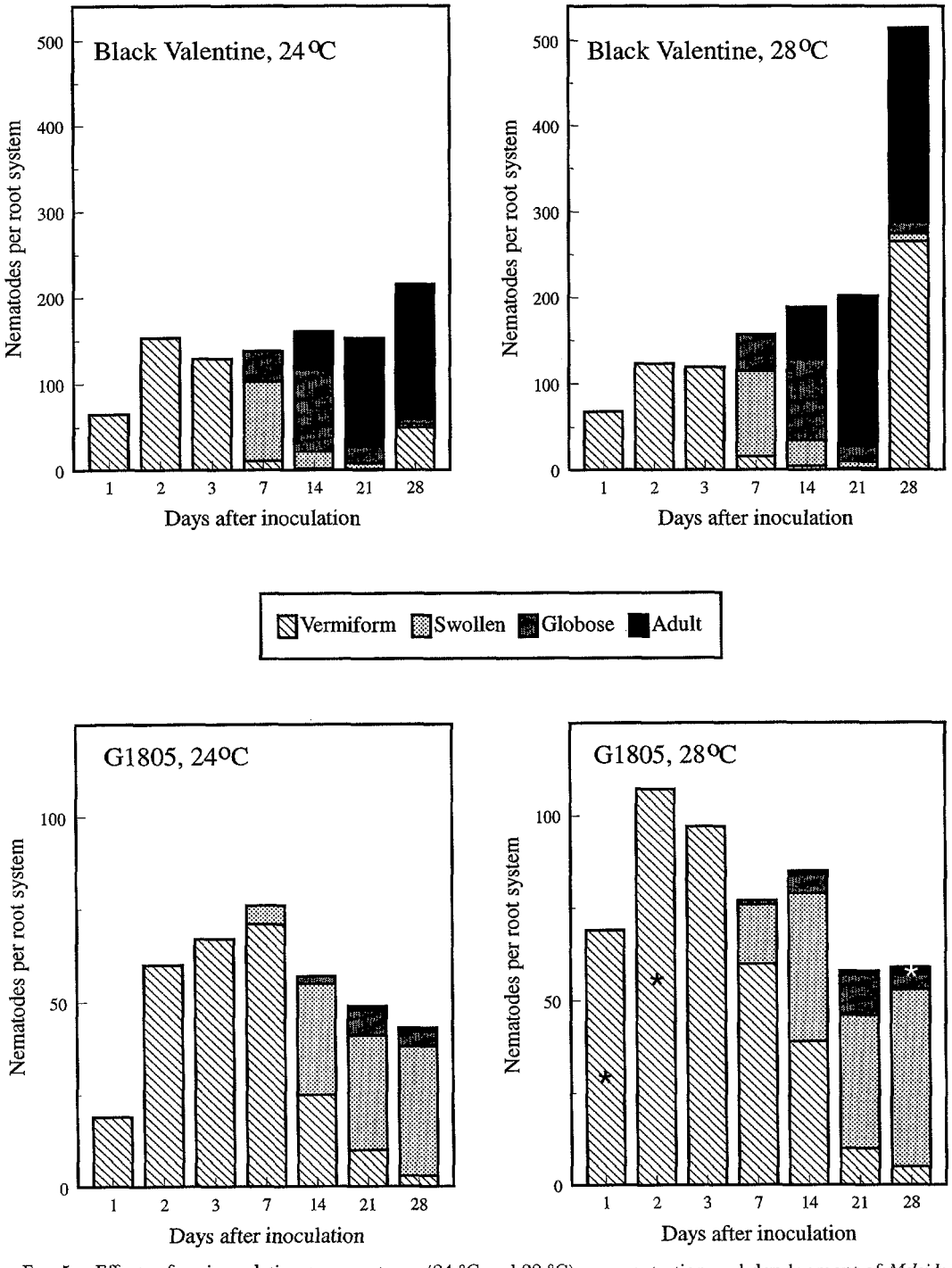

FIG. 5. Effects of preinoculation temperatures (24 °C and 28 °C) on penetration and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean genotypes (gene system 2). Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time. At each harvest day within each genotype, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other preinoculation temperature.

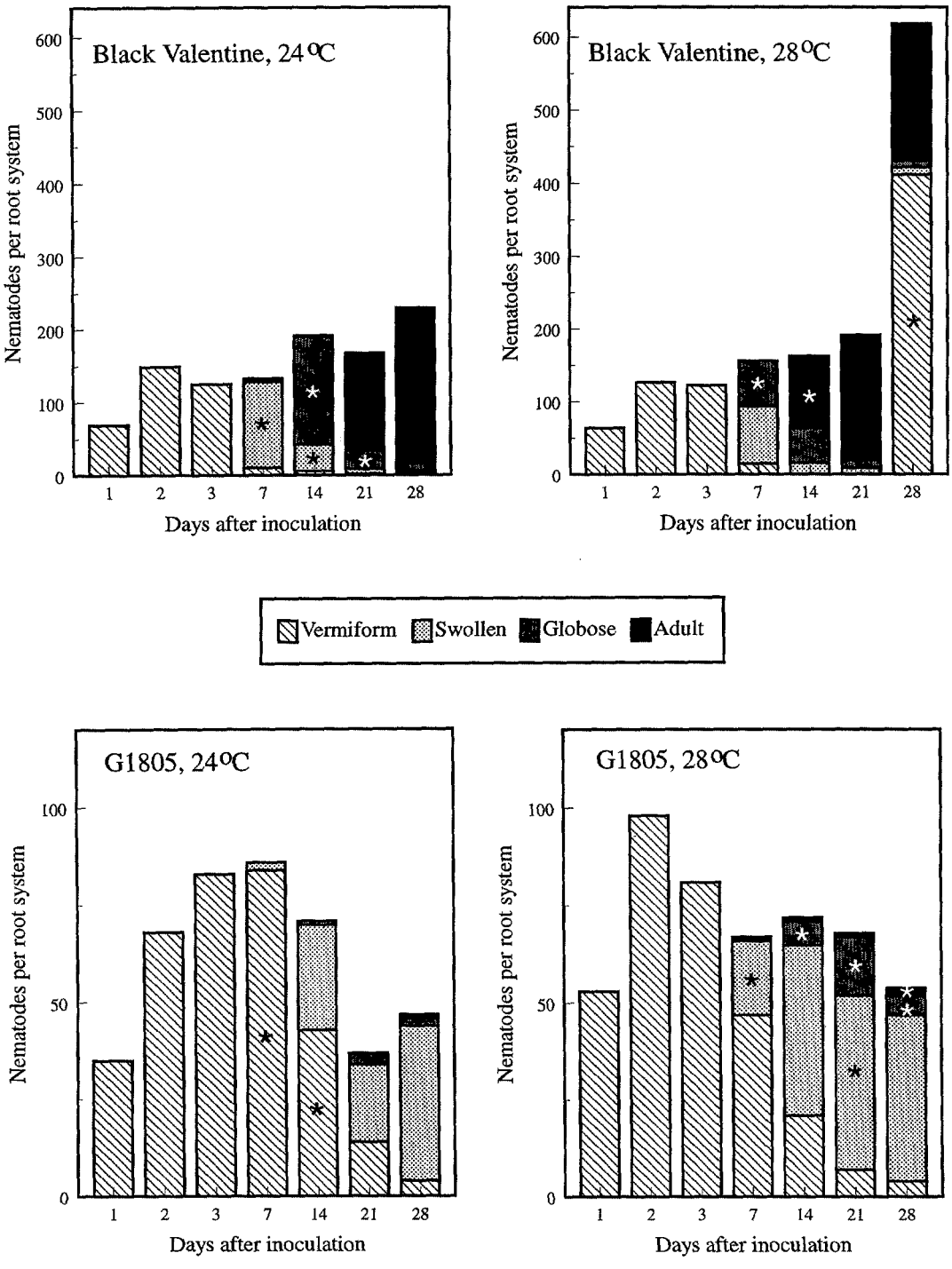

FIG. 6. Effects of postinoculation temperatures (24 °C and 28 °C) on penetration and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean genotypes (gene system 2). Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time. At each harvest day within each genotype, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other postinoculation temperature.

nematodes penetrated the roots of plants preconditioned at 28 °C than at 24 °C ($P < 0.05$; Fig. 5). Also at 28 DAI, 50% more nematodes reached the adult stage at 28 °C compared with 24 °C ($P < 0.05$; Fig. 5).

Postinoculation incubation temperatures had a pronounced effect on the development of *M. arenaria* in the roots of both Black Valentine and G1805 plants. The numbers of vermiform juveniles that penetrated the roots of Black Valentine plants 1, 2, and 3 DAI were not influenced by postinoculation temperature ($P > 0.05$; Fig. 6). However, in Black Valentine roots, at 7 DAI, 94% more had developed to the globose stage in the roots of plants incubated at the higher temperature than in plants incubated at the lower temperature. At 14 DAI, adults were present in roots of plants incubated at 28 °C but not at 24 °C ($P < 0.05$); also at this time, there were 62% more swollen and 65% more globose stages remaining (did not develop into adults) in Black Valentine roots grown at 24 °C than at 28 °C ($P < 0.05$; Fig. 6). At 21 DAI, numbers of nematodes still present in the globose stage were 90% higher in plants incubated at 24 °C than at 28 °C ($P < 0.05$). At 28 DAI, a second generation of J2 had penetrated the roots of plants grown at 28 °C. This second generation was absent from plants grown at 24 °C (Fig. 6).

Postinoculation incubation temperatures affected development of *M. arenaria* in roots of resistant G1805 plants (Fig. 6). Similar to Black Valentine roots, numbers of vermiform juveniles penetrating G1805 roots, 1, 2, and 3 DAI were not influenced by postinoculation incubation temperature ($P > 0.05$; Fig. 6). At 7 and 14 DAI, there were 50% more vermiform J2 in plants incubated at the lower temperature than at the higher temperature ($P < 0.05$); also at 7 DAI, 92% more nematodes had developed to the swollen stage at 28 °C than at 24 °C ($P < 0.05$; Fig. 6). At 14, 21, and 28 DAI, 75% more nematodes had developed to the globose stage at 28 °C than at 24 °C ($P < 0.05$). Adult nematodes were present at higher levels at 28 °C than at 24 °C at 28 DAI, although actual numbers of adults were still low, two at 28 °C and one at 24 °C (Fig. 6).

High-temperature experiment 1: Growth of both Black Valentine and Nemasnap plants was affected by the high incubation temperatures of 30 °C and 32 °C. The mean fresh weights of Black Valentine plants, pooled over both harvest times, were greater at 30 °C (7.63 g) than at 32 °C (3.89 g) ($P < 0.05$). The mean plant weights of Nemasnap, pooled over both harvest times, were not different between 30 °C (4.37 g) and 32 °C (2.96 g) ($P > 0.05$; data not shown). At both harvest times and temperatures, the majority (>65%) of the *M. incognita* populations within the roots of Black Valentine and Nemasnap plants were present as adults (Table 2). At these times, only few nema-

TABLE 2. Numbers of *Meloidogyne incognita* race 2 in four developmental stages in root systems of susceptible Black Valentine (Black Val.) and resistant Nemasnap common bean (gene system 1) incubated at temperatures of 30 °C or 32 °C.

Incubation temperature	Harvest day	Germplasm	Developmental stages of *M. incognita* race 2				
			Vermiform	Swollen	Globose	Adult	Total
30 °C	13	Black Val.	0	4	20	118	142
		Nemasnap	0	2	9	31	42
	22	Black Val.	1	1	5	169	176
		Nemasnap	0	4	3	13	20*
32 °C	13	Black Val.	0	3	6	67	76
		Nemasnap	0	1	4	14	19
	22	Black Val.	0	0	0	77	77
		Nemasnap	0	0	1	9	10*

Data are means of three replications.

Asterisks (*) indicate significantly different ($P < 0.05$) total numbers of nematodes in roots of Nemasnap compared with the total numbers in roots of Black Valentine at the same temperature and harvest time.

todes were present in immature stages in both genotypes at both temperatures. However, at 22 DAI at both 30 °C and 32 °C, fewer nematodes were observed in the roots of Nemasnap plants than in the roots of Black Valentine plants ($P < 0.05$).

High-temperature experiment 2: Growth of Black Valentine and G1805 plants was affected by the high incubation temperatures of 30 °C and 32 °C. The mean fresh weights of Black Valentine plants, pooled over both harvest times, were greater at 30 °C (8.62 g) compared with that at 32 °C (5.93 g) ($P < 0.05$). The mean plant weights of G1805, pooled over both harvest times, also were greater at 30 °C (5.89g) than at 32 °C (4.18 g) ($P < 0.10$; data not shown). At both harvest times and temperatures, the majority (>64%) of the *M. arenaria* population in roots of Black Valentine plants were present as adults (Table 3). Development of the nematodes to adults in roots of G1805 plants occurred at both 30 °C and 32 °C, but the counts were low (Table 3). At 13 and 22 DAI at both temperatures, the total populations of *M. arenaria* in roots of G1805 plants were smaller than those in the roots of Black Valentine plants ($P < 0.05$).

Discussion

At all four temperature regimes, resistance gene system 1 in Nemasnap was effective against *Meloidogyne incognita,* and resistance gene system 2 in G1805 was effective against *M. arenaria.* Resistance was demonstrated by the lower numbers of nematodes in the roots of the resistant genotypes compared with the total numbers in the roots of the susceptible cultivar Black Valentine. These results confirm a previous report (Fassuliotis et al., 1970). Counts of both *M. incognita* and *M. arenaria,* in Nemasnap and G1805, respectively, reached maximum levels by 3 DAI and declined thereafter. However, counts in roots of Black Valentine continued to increase for the duration of the experiments, indicating that nematodes entered the roots of the susceptible genotype over an extended period of time. The decline in population levels in the roots of the resistant genotypes may be due to an inability to establish normal feeding sites (Huang, 1985).

Temperature and nutrient stress in plants can cause sex reversal in *Meloidogyne* spp., resulting in a higher proportion of males in the population (Triantaphyllou, 1973). Also, fewer males of *Meloidogyne* spp. develop in plants grown at higher temperatures (Davide and Triantaphyllou, 1967). Males of *M. incognita* developed in the roots of Black Valentine and Nemasnap plants grown at all temperature regimes; however, pre- and postinoculation temperatures of 24 °C and 28 °C had no consistent effect on the number of males in the roots of susceptible or resistant plants. Thus, it is not possible to determine if temperature stress on the

Table 3. Numbers of *Meloidogyne arenaria* race 1 in four developmental stages in root systems of susceptible Black Valentine (Black Val.) and resistant G1805 common bean (gene system 2) incubated at temperatures of 30 °C or 32 °C.

Incubation temperature	Harvest day	Germplasm	Developmental stages of *M. arenaria* race 1				
			Vermiform	Swollen	Globose	Adult	Total
30 °C	13	Black Val.	2	19	25	82	128
		G1805	0	10	1	1	12*
	22	Black Val.	1	3	19	95	118
		G1805	0	3	2	0	5*
32 °C	13	Black Val.	0	8	22	43	73
		G1805	1	2	7	5	15*
	22	Black Val.	0	1	1	74	76
		G1805	0	2	2	6	10*

Data are means of three replications.

Asterisks (*) indicate significantly different ($P < 0.05$) total numbers of nematodes in roots of G1805 compared with the total numbers in roots of Black Valentine at the same temperature and harvest time.

plants reduced development of males or increased the process of sex reversal.

The effects of preinoculation conditioning temperatures on resistance expression in Nemasnap and G1805 plants to a later infection of *M. incognita* and *M. arenaria,* respectively, were infrequent and variable. Although Mullin et al. (1991) have reported an effect of high and low early-incubation temperatures and duration on resistance expression, their work was conducted on nematode-infected plants. There is no evidence from our experiments that preinoculation temperature directly affects resistance expression to a later infection by root-knot nematode.

Effects of postinoculation incubation temperature were more pronounced on nematode development than were preinoculation conditioning temperature effects. In all genotypes and in all tests, development of *M. incognita* and *M. arenaria* to adults was more rapid at the higher postinoculation temperature of 28 °C compared with development at 24 °C. Because this occurred in both resistant and susceptible genotypes, postinoculation incubation temperature probably has a greater effect on nematode development than on resistance expression of either gene system 1 or gene system 2.

Gene system 1 resistance in Nemasnap to *M. incognita* race 2 was stable at temperatures up to 32 °C; however, development to mature adults with egg masses increased as temperature increased from 24 °C to 32 °C. Omwega et al. (1990) demonstrated that resistance to *M. incognita* race 2 in two of three genotypes containing gene system 1 (Alabama No. 1 and PI 165435) was lost at 30 °C, and gradually reduced in all three lines (Alabama No. 1, PI 165426 and PI 165435) as temperature was increased to 30 °C. Another resistant line, A211, has been shown to be susceptible to *M. incognita* at 24 °C when comparing egg mass and total egg production with those on a susceptible cultivar (Mullin et al., 1991). These differences between results may be explained by the epistatic effects on the effect of temperature on resistance expression (Canto-Sáenz, 1985). The similar temperature effects on resistance expression between PI 165426 and Nemasnap may be due to the fact that PI 165426 was used as the resistant parent in the development of the cultivar Nemasnap (Wyatt et al., 1983).

The results of these studies with gene system 2 in G1805 demonstrated that resistance to *M. arenaria* was not reduced or lost at temperatures up to 32 °C. Omwega et al. (1990) also reported stable resistance at 24 °C to 30 °C in G1805 to *M. incognita* race 1 and *M. javanica,* even though reproduction of *M. javanica* increased as temperature increased from 28 °C to 30 °C.

Regardless of the resistance mechanisms involved at temperatures of 28 °C and higher, these are not the optimum temperatures for bean growth. In practice, most common beans are produced in regions where the temperatures range from 16 °C to 24 °C, within the range at which resistance is stable (Fassuliotis et al., 1970; Omwega et al., 1990). However, because temperatures in bean-growing areas can reach higher-than-optimum levels during part of the growing season, germplasm with heat-stable resistance to root-knot nematodes may be necessary in *Meloidogyne* spp. management.

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