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Effects of Temperature on Development of *Heterodera glycines* on *Glycine max* and *Phaseolus vulgaris*[1]

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Abstract: Soybean cyst nematode resistant 'Fayette' and susceptible 'Williams 79' soybeans (*Glycine max*) and resistant 'WIS (RRR) 36' and susceptible 'Eagle' snap beans (*Phaseolus vulgaris*) were used in determining the effects of host and temperature on the development, female production, sex ratios, and host response to *Heterodera glycines*. Temperatures were maintained constant at 16, 20, 24, 28, and 32 C using water-filled tanks. The most rapid development and greatest female production occurred between 20 and 28 C. The equation $DS = 5(10^{-6})x^2y^2 - 3(10^{-4})x^2y - 2.8(10^{-3})x^2 - 1.94(10^{-2})y^2 + 0.4288x + 1.0220y - 12.7185$, where DS = developmental stage, X = time, and Y = temperature, predicted the developmental stage of the nematode and accounted for 84% of the variation. Male : female ratios did not differ within this range and were generally less than one. At all temperatures the resistant soybean produced the greatest number of necrotic responses to *H. glycines* infection, followed by the resistant snap bean. The susceptible soybean and snap bean produced the fewest necrotic responses.

Key words: *Glycine max*, soybean, *Heterodera glycines*, soybean cyst nematode, hypersensitivity, modeling, *Phaseolus vulgaris*, snap bean, sex ratios, temperature.

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pest of soybean, *Glycine max* (L.) Merr., and has been managed primarily by rotation to nonhost crops or resistant soybean cultivars. In the vegetable growing regions of Illinois, Arkansas, Tennessee, Kentucky, and North Carolina, soybean is often rotated with snap bean (*Phaseolus vulgaris* L.) which may be damaged by SCN (13). Many commercial snap bean cultivars are excellent hosts of SCN, but WIS (RRR) 36 was identified as resistant (12). Other *Phaseolus* species have also been identified as hosts (2,17,18). Although SCN occurs in the major snap bean producing counties of Illinois the effect of this nematode on yields is unknown.

Soil temperature is important in determining the potential population densities of SCN and in predicting the predominate developmental stage of a nematode population for sampling purposes. Degree-day accumulations based on a 10 C base were developed for these purposes (7). SCN development was not related linearly to temperature in one study (20), demonstrating that additive degree-day models are probably inadequate. Greater cyst production occurred at 24 C than at 17 or 31 C, but development was most rapid at 31 C. Minimum and maximum temperatures for development were estimated at 14 and 35 C, respectively. Development slowed between 32 and 35 C.

Factors such as nematode population density, nutritional status, host species or cultivar (22), and soil temperature (20) have influenced sex ratios of some cyst-forming nematodes. Increased male : female ratios (M:F) were a result of few females developing (3,8,9) because of conditions unfavorable for production of syncytia inside roots (19).

Information regarding the effects of temperature on SCN development in susceptible hosts, other than soybeans, or in resistant hosts has not been reported. Nor have reports indicated whether host genotype and temperature interact to affect SCN development. Our objectives were to investigate the effects of temperature and host genotype on SCN development, sex ratios (M:F), and host response using snap bean and soybean as host plants as a means to improve a temperature-based SCN development model.

Materials and Methods

The experimental design was a split-split plot with treatments factorially arranged

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with 5 temperatures, 10 sampling times, 2 lines each of soybean and snap bean, and 4 replications. Temperature tanks were the main plots; they were adjusted to 16, 20, 24, 28, and 32 C (± 0.5). Sampling times 4 days apart represented the subplots. Host genotypes, the sub-subplots, included Fayette and Williams 79 soybeans and Eagle and WIS (RRR) 36 snap beans. Fayette and WIS (RRR) 36 (12) are resistant to races 3 and 4 of SCN, whereas Williams 79 and Eagle are susceptible. The experiment was conducted a second time after rerandomizing temperatures among the tanks.

Two hundred seeds of each cultivar were germinated in rolled, wet paper towels wrapped in plastic wrap and positioned vertically in a 4-liter beaker containing 200 ml of tap water. Seeds were maintained at 24 C until radicles were 5–8 cm long, which required about 3 days. Seedlings were inoculated with second-stage juveniles (J2) of SCN race 3 in wooden inoculation chambers (11). A 3-cm-wide strip of Miracloth was moistened and placed on a layer of vermiculite in the chambers. Seedlings were positioned with the tips of the radicles approximately at the bottom of the Miracloth strip. The radicles were then covered with fine-textured, sterile sand, and another strip of moistened Miracloth was placed over the sand. Two hundred J2 per seedling were pipetted onto the top layer of Miracloth. The boxes were closed and placed in an upright position at 24 C for 36 hours (trial 1) or 30 hours (trial 2).

Infected seedlings were transferred to 800 PVC tubes (20 × 3.75 cm) filled with 110 cm^3 steam sterilized Onarga loamy sand : fine quartz sand (1:1). Four tubes were placed into each of 200 2-liter plastic cups, which were filled with sterilized sand to support the tubes. Forty cups were placed into each temperature tank. Each inoculated seedling was rinsed with water before transplanting to remove J2 adhering to the root surface. One seedling was planted per tube. Each cup contained one seedling of each of the four cultivars.

At every sampling time four plants of each cultivar were removed from each temperature for examination. The inoculated portion of the root system was excised, washed, and stained with acid-fuchsin lactophenol. After destaining, nematodes were counted and developmental stages determined by the method used for *Heterodera schachtii* (15).

TABLE 1. Effects of snap bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) cultivars on numbers of *Heterodera glycines* second-stage juveniles inside roots 4 days after inoculation.

Cultivar	No. of juveniles	
	Trial 1	Trial 2
Snap bean		
WIS (RRR) 36†	16.6 a	15.4 a
Eagle‡	13.9 b	15.3 a
Soybean		
Williams 79‡	10.6 c	12.1 a
Fayette†	9.7 d	13.4 a
CV%	52.2	54.7
R^2	0.23*	0.19*

Data are means of four replications. Within a column, means followed by the same letter are not different after rank transformations of original observations according to Fisher's least significant ($P < 0.05$) difference.
† Resistant to *H. glycines*.
‡ Susceptible to *H. glycines*.
* $P < 0.05$.

Analysis of variance and orthogonal comparisons were conducted on the data using the general linear model procedure. Penetration by J2 among cultivars was compared by analyzing ranks of nematode counts at all temperatures and on all cultivars using four replicates in each trial at the first sampling. Male : female ratios were evaluated at the fourth juvenile stage and transformed to square roots and analyzed to detect differences caused by temperature, line, or temperature × line interactions after combining the trials. In trial 2, hypersensitive responses were counted in each root and the data transformed to $\log_{10}$ for analysis. Numbers of females (stage 7) were transformed to $\log_{10}$ prior to anlaysis. Numbers of females, at the time when numbers peaked at a given temperature, were divided by numbers of J2 at time 1 for the same cultivar, temperature, and replicate and then analyzed. Statistical analysis was calculated by combining data from both trials, thus yielding eight replicates, whenever the error mean squares were not significantly different ($P < 0.25$). The developmental index was calculated as numbers of nematodes at a particular stage of development multiplied by the stage number (0 to 7) summed, and the sum

TABLE 2. Effects of temperature and host genotype on male:female ratios of *Heterodera glycines* at the date of maximum female development for each temperature.

Temperature (C)	Male:female ratios				
	Snap bean		Soybean		
	Eagle†	WIS (RRR) 36‡	Williams 79†	Fayette‡	$\bar{x}$
16	1.39	1.02	0.91	0.77	1.02 a
20	1.25	0.85	0.59	0.70	0.85 ab
24	1.17	0.85	0.57	0.71	0.83 bc
28	1.00	0.65	0.73	0.62	0.75 bc
32	0.72	0.80	0.54	0.59	0.66 c
$\bar{x}$ (cultivars)	1.11+	0.83	0.67	0.68	0.85
$\bar{x}$ (species)	0.97+		0.67		
	CV% = 90.4		$R^2 = 0.23$		

Data are square root transformed means of eight replications.
In column $\bar{x}$, means followed by the same letter are not different according to Fisher's least significant difference ($P < 0.05$).
In bottom two rows, means not followed by + are not different according to Fisher's least significant difference ($P < 0.05$).
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divided by the total number of nematodes in that root. Experimental error was pooled. Parameters for the developmental stage predictive model were selected by forward stepwise regression. In all analyses type III sum of square errors were used for comparison and Fisher's LSD for means were calculated. All transformations were based on plots of residuals to comply with model assumptions.

RESULTS

Numbers of J2 penetrating roots differed ($P < 0.10$) between the two trials. In trial 1, more ($P < 0.05$) J2 penetrated snap bean than soybean roots, whereas no differences were detected in trial 2 (Table 1). WIS (RRR) 36 was penetrated by the greatest number of J2, whereas Fayette was penetrated by the fewest.

Temperature and host genotype influenced the M:F ratio (Table 2). Development of males was generally favored by low temperatures. The M:F was greater ($P < 0.05$) at 16 C than at 24–32 C. No differences were detected among the three middle temperatures. At 32 C, M:F was lower ($P < 0.05$) than at 16 or 20 C. Differences

TABLE 3. Effects of temperature and host genotype on numbers of necrotic responses in roots caused by *Heterodera glycines* 4 days after inoculation.

Temperature (C)	Number of necrotic responses				
	Snap bean		Soybean		
	Eagle†	WIS (RRR) 36‡	Williams 79†	Fayette‡	$\bar{x}$
16	0.23[3]	0.19	0.09	0.68	0.24 a
20	0.05	0.23	0.10	0.73	0.28 a
24	0.13	0.23	0.25	0.63	0.31 a
28	0.09	0.41	0.06	0.73	0.32 a
32	0.17	0.36	0.26	0.70	0.37 a
$\bar{x}$ (cultivar)	0.13 a	0.28 b	0.15 a	0.69 c	0.33
$\bar{x}$ (species)	0.20 a		0.42 b		
	CV% = 91.7		$R^2 = 0.50$*		

Data are $\log_{10}$ transformed means of four replications.
In column $\bar{x}$, means followed by the same letter are not different according to Fisher's least significant difference ($P < 0.05$).
In bottom two rows, means followed by the same letter are not different according to Fisher's least significant difference ($P < 0.05$).
† Susceptible to *H. glycines*.
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* $P < 0.05$.

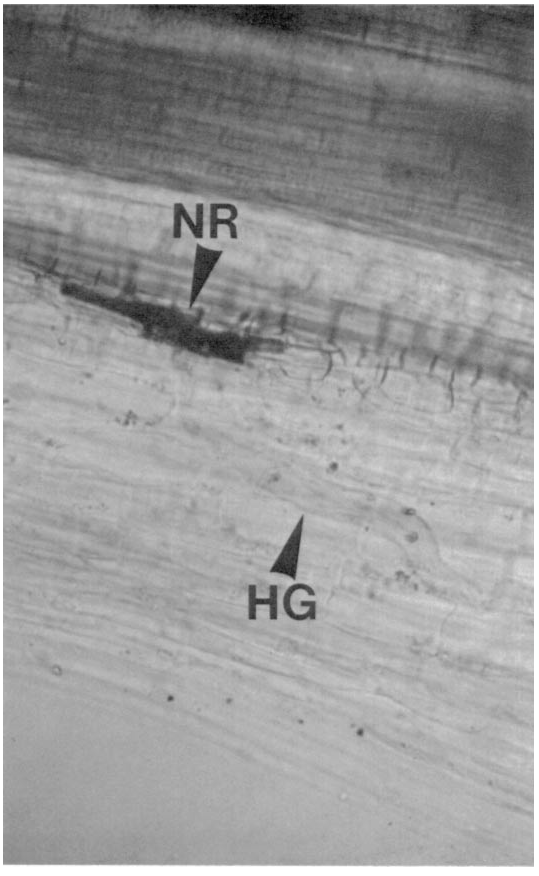

FIG. 1. Necrotic response (NR) of a resistant snap bean *Phaseolus vulgaris* cv. WIS (RRR) 36 root to a *Heterodera glycines* (HG) juvenile 5 days after inoculation at a soil temperature of 28 C.

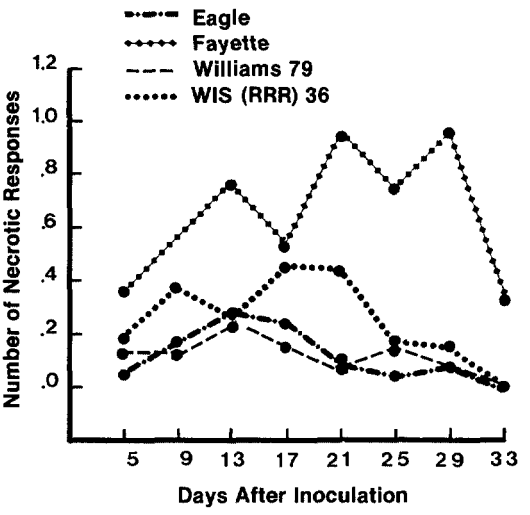

FIG. 2. Log_{10}-transformed numbers of necrotic responses to *Heterodera glycines* in the roots of *Glycine max* cvs. Fayette (resistant) and Williams 79 (susceptible) and *Phaseolus vulgaris* cvs. Eagle (susceptible) and WIS (RRR) 36 (resistant) compared at different times after inoculation.

($P < 0.05$) due to genotype were apparent in both trials. The M:F ratios were greater on snap beans than soybeans ($P < 0.05$), the highest being on the resistant Eagle snap bean.

Necrotic responses to *H. glycines* infection were observed in roots of all four cultivars tested (Fig. 1). The resistant Fayette produced the greatest number of necrotic responses ($P < 0.05$) (Table 3). More necrotic responses were observed in WIS (RRR) 36 than in the susceptible snap bean or soybean. Soybeans produced more necrotic responses, averaged over all temperatures, than snap bean. Necrotic responses of Fayette were most numerous at 21 days (Fig. 2). WIS (RRR) 36 produced most of its necrotic responses by 17 and 21 days, and the two susceptible cultivars by day 13. After day 29 numbers of observed necrotic responses decreased for all cultivars. Furthermore, some nematodes not associated with typical necrotic responses ceased development at the second or third juvenile stage. Mature females were detected in all cultivars at all temperatures but were noticeably smaller in WIS (RRR) 36 than in the other cultivars.

Differences ($P < 0.05$) between total numbers of SCN in stage 7 were significant between temperatures and cultivars (Table 4). The greatest number of females were produced at 20–28 C and the least at 16 and 32 C. More females developed on snap bean than on soybean ($P < 0.05$), and fewer females developed on Fayette than on the other three cultivars.

The effect of temperature on nematode development was significant ($P < 0.05$) (Fig. 3) although there was no temperature × cultivar interaction. The most favorable temperature for development through 21 days was 28 C. At 29 days development was not different at 20–28 C. By 33 days only nematodes at 16 C had not reached an average developmental stage of 7.0. From 5 to 9 days 24 and 32 C were equally favorable; from 13 through 17 days 24 C was more favorable. At 16 C development was slowest at all sampling times after ca. 7 days.

The greatest differences between devel-

TABLE 4. Effects of temperature and host genotype on numbers of *Heterodera glycines* females at the date of maximum female development for each temperature.

Temperature (C)	Numbers of females				
	Snap bean		Soybean		
	Eagle†	WIS (RRR) 36‡	Williams 79†	Fayette‡	$\bar{x}$
16	0.82	0.64	0.45	0.29	0.55 b
20	1.16	1.14	1.01	0.44	0.92 a
24	1.25	1.11	1.13	0.61	1.01 a
28	0.99	1.20	0.97	0.98	1.03 a
32	0.55	0.68	0.15	0.55	0.52 b
$\bar{x}$ (cultivar)	0.99	0.96	0.84	0.52+	0.81
$\bar{x}$ (species)	0.97		0.68+		
	CV% = 41.9		$R^2 = 0.77$*		

Data are $\log_{10}$ transformed means of eight replications.
In column $\bar{x}$, means followed by the same letter are not different according to Fisher's least significant difference ($P < 0.05$).
In bottom two rows, means followed by a + are not different according to Fisher's least significant difference ($P < 0.05$).
† Susceptible to *H. glycines*.
‡ Resistant to *H. glycines*.
* $P < 0.05$.

opmental stages occurred between 13 and 21 days. Adult females developed in 13 days at 28 C, 19–20 days at 20 and 24 C, 27 days at 32 C, and 32 days at 16 C. Adult males were usually detected at 13 days, and occasionally at 9 days, at 28 C.

The equation

$$DS = 5(10^{-6})x^2y^2 - 3(10^{-4})x^2y - 2.8(10^{-3})x^2 - 1.94(10^{-2})y^2 + 0.4288x + 1.0220y - 12.7185 \quad (1)$$

where DS = developmental stage
x = days after inoculation
y = temperature (C)

with an R^2 of 0.84 was generated from the temperature-developmental stage data.

DISCUSSION

The results of this study indicated that equal numbers of *H. glycines* J2 penetrate the roots of resistant and susceptible soybeans and snap beans. Although trends of lower penetration in resistant soybean lines exist (1), because of variation, statistically significant differences have not been shown.

Although the M:F ratio of *H. glycines* was reported to increase at temperatures above 31 C (20), no such trend was observed at 32 C compared with lower temperatures in this study. In fact, the M:F ratio was highest at the lowest temperature studied, 16 C. Therefore, the development of fewer females at 32 C than at lower temperatures was not due to differential death of females as reported previously (3). In this study, some males may have passed through the fourth juvenile stage between samplings, thus diminishing the M:F ratio. Only preadult males were evaluated because of possible migration of adult males from the roots.

Differences in compatibility with SCN observed between soybean and snap bean could be caused by differences in root morphology. *H. glycines* females are more likely to reach maturity in primary rather than secondary or tertiary soybean roots (9). Therefore, maturity may be related to the distance between the infection site and the shoot, with nutrients necessary for formation of syncytia decreasing in availability with increasing distance from the shoot (9). In potato, however, lateral roots are thinner than primary roots and therefore are less able to produce syncytia required for female development of *Globodera rostochiensis* (4,19,23). Furthermore, the M:F ratio may increase directly with nematode population densities (4,9). Since thinner roots provide less space for production of syncytia, the nematode population density at which sex ratios are altered is probably lower in thinner roots. The primary root of snap bean is considerably thinner than the primary root of soybean, accounting for a smaller fraction of the root system. Differences in roots may account for the

differences in M:F ratios between snap beans and soybeans.

Average M:F ratios reported here were less than the 1:1 ratio estimated for *H. glycines* (9), and data are more allied with an earlier report (20) that indicated M:F ratios were less than 1:1 at temperatures below 28 C. However, the ratios may have been artificially lowered in our study if males matured between sample times, which is unlikely. Ratios may have been affected by the low numbers of nematodes used in this study.

Necrotic responses were previously reported in soybean lines that were both compatible and incompatible to *H. glycines* (1). Such responses were observed in conjunction with infections containing both early and late juvenile stages (1,20), as we observed here. Response time of susceptible × resistant backcrossed soybeans is slower than that of the resistant parent (5). The slow response time of Fayette and WIS (RRR) 36 indicates that neither may have the full level of resistance found in their respective parents.

Temperature effects on response of resistant cultivars to infection by *H. glycines* were similar to those reported for *Rotylenchulus reniformis* on soybean (16) and *G. rostochiensis* on potato (6). Production of necrotic responses in WIS (RRR) 36 was greater at 28 C than at 20 C.

Nematodes that ceased development at the J2 or J3 stage may have been victims of degenerating syncytia that were replaced later by parenchyma cells, as observed previously in soybean (5) and clover (10). Nematodes that matured on the resistant lines were small, often with few eggs. Small females and reduced fecundity were associated with *H. glycines* resistance in soybean (14).

The minimum (16 C) and maximum (32 C) temperatures used in this experiment were less favorable for female development than were 20, 24, and 28 C. This broad range of equally favorable temperatures for cyst production suggests a favorable temperature range for maturation rather than a single temperature above or below which numbers of adults decline. These data are consistent with previous reports for *H. glycines* and *H. schachtii* (20,21).

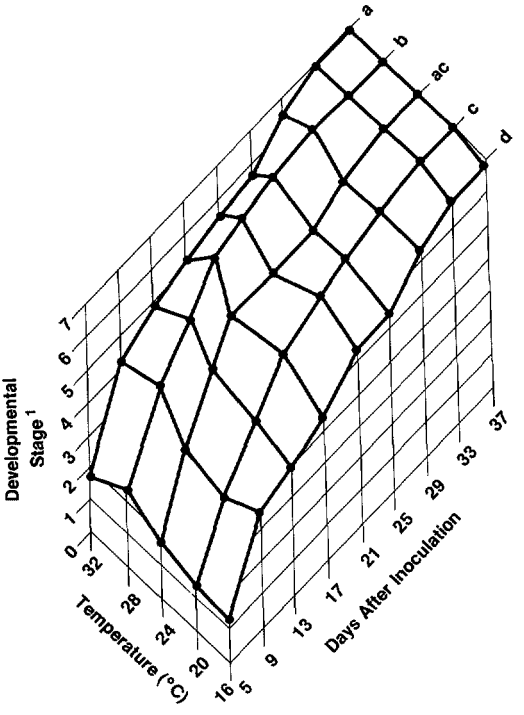

FIG. 3. Effects of temperature and time (days after inoculation) on the average developmental stage (DS) of *Heterodera glycines*. The DS is an average of the DS on four hosts, none of which affected developmental stage ($P < 0.05$). Average developmental stage was calculated as the number of nematodes at each stage, multiplied by the stage number, summed for all stages, and divided by the total number of nematodes in that sample. Temperature curves followed by the same letter are not different ($P < 0.05$) according to Fisher's least significant difference.

Temperature influences the development of cyst-forming nematodes (6,20,21). In this study warmer temperatures favored faster development up to 28 C, above which development slowed. Development time to the adult stage was faster at 32 C than at 16 C. It was reported previously, however, that development at 31 C was faster than at 24 C, but fewer adults developed (20).

Degree-day accumulations for *H. glycines* (20) and *H. schachtii* (21) were not sufficiently accurate to predict the occurrence of specified developmental stages, as reported earlier for *H. glycines* (7). Degree days (base 10 C) needed for the average stage to be the egg-bearing stage (estimated as stage 6.3) ranged from 198 at 16 C to 611 at 32 C. At 24 C, 322 degree days were needed in our study, which was slightly greater than the 308 reported previously

(20); however, those estimates were based on the earliest observation of a particular stage rather than the average stage.

H. glycines development cannot be adequately explained by linear degree-day accumulations. Furthermore, 20–28 C was the optimum range for *H. glycines* development and for maximum production of females. The optimum temperature range for *H. glycines* development was not influenced by cultivar. Neither were necrotic responses nor M:F ratios influenced by temperatures within this range. Equation (1) was generated to predict the developmental stage of *H. glycines* at any specific time, from 5 days after inoculation to maturity, and at constant soil temperatures between 16 and 32 C. This equation will be used as a basis to build a degree-day model for varying temperatures.

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