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Development of *Heterodera glycines* Life Stages as Influenced by Temperature[1]

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Abstract: The effects of temperature on rates of development of *Heterodera glycines* egg and juvenile stages were examined as a basis for predicting generation times of the nematode on soybean. The relationship of temperature to *H. glycines* embryonic development between 15 and 30 C was described by a linear model. The calculated basal temperature threshold was 5 C. Thermal optimum for embryogenesis and hatch with low mortality was 24 C. Development proceeded to first-stage juvenile at 10 C and to second-stage juvenile at 15–30 C. Hatch occurred at 20–30 C. At 36 C, development proceeded to the four-cell stage, then the eggs died. The range of diurnal soil temperature fluctuation and accumulated degree-days between 5 and 30 C ($DD_{5/30}$) had an impact on rate of development of juveniles in soybean roots. From early June to early July, *H. glycines* required 534 ± 24 $DD_{5/30}$ (4 weeks) to complete a life cycle in the field. During the midseason (July and August), life cycles were completed in 3 weeks and 429 ± 24 $DD_{5/30}$ were accumulated. Late in the season (September to November), declining soil temperatures were associated with generation times of 4 weeks and slower rates of development.

Key words: development rate, ecology, *Glycine max*, hatching rate, *Heterodera glycines*, mortality rate, soybean cyst nematode, threshold temperature.

Seasonal population fluctuations of *Heterodera glycines* Ichinohe on soybean, *Glycine max* (L.) Merr., are largely regulated by population densities in the field at planting and developmental and reproductive potentials of the nematode (2). Nematode development and reproduction are influenced by many factors, including environmental conditions (1,8,9,12,21), soil factors such as texture and pH (13,15), host suitability (11,26), and management practices such as applications of pesticides and fertilizers (13,22). A model (4) developed to simulate the effects of many of these variables on *Meloidogyne* sp. population dynamics on grape was modified to include a temperature data-base on egg development and death rates to improve its predictive potential (6,7). Temperature, a regulator of nematode metabolic rates, is used to drive simulation of nematode population changes (5,16).

Second-stage juveniles (J2) of *H. glycines* fail to emerge from cysts incubated below 16 C or at 36 C, and emergence is optimum at 24 C (23). Egg hatch declines sharply in autumn when soil temperatures decrease from 21 to 10 C (20). At constant temperatures of 31, 24, and 17 C, *H. glycines* life cycles are completed in 18, 22, and 37 days, respectively. Penetration of soybean roots by *H. glycines* J2 occurs four times faster at 28 C than at 22 C (9). Low (14 C) and high (35 C) temperatures limit penetration and development and completely inhibit reproduction (9). On soybean root explants grown under gnotobiotic conditions at 25 C, the life cycle of *H. glycines* is completed by 21 days after inoculation (14).

The objective of this research was to determine the effect of temperature on development of *H. glycines* life stages on soybean.

Materials and Methods

Embryonic development: Two experiments were conducted in the laboratory to determine the effects of temperature on embryonic development and egg hatch of *Heterodera glycines* race 1. Mature cysts, collected from 2–3-month-old greenhouse soybean cultures, were crushed with a tissue grinder to release the eggs. Two-celled eggs were used as the beginning stage.

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In the first experiment, single eggs were placed in BPI watch glasses containing distilled water, and the watch glasses were enclosed in glass petri dishes (100 mm d × 15 mm high) to minimize evaporation. The eggs were incubated at 10, 15, 20, and 25 C and were examined microscopically every 2 days until all eggs hatched or development ceased. Stages of development were classified as two-cell, four-cell, eight-cell, multicell, first-stage juvenile (J1) and J2 within the egg, emerged J2, and dead eggs. Eggs were considered dead when contents became dark and granular. Treatments were replicated within temperature chambers 10 times and the experiment was repeated once.

A second experiment included incubation of single eggs at 10, 16, 24, 30, and 36 C. Treatments were replicated within chambers eight times, and the experiment was performed once. All other procedures were as described for the first experiment.

Models of egg development were derived from the temperature data-base generated in the two experiments. Two approaches were used (6): 1) The rates of development, death, and hatch per day were expressed as a function of temperature, and 2) development was represented in terms of accumulated degree-days (DD). The daily rates of development and hatch were calculated by the proportion of development or hatch completed per day from the two-cell stage to J2 within the egg and from J2 within the egg to hatch, respectively. Mortality levels were described by the relationship $M = 1 - (1 - m)^n$, where M is the proportion of mortality after n days, m = daily mortality rate expressed as a proportion of the initial population, and n = number of days over which death occurs. This equation accounts for the decreasing population to which the daily mortality rate was applied.

Development of juvenile stages: The influence of soil temperature on development of *H. glycines* race 1 juvenile stages in soybean roots was determined for 17 weekly periods in a naturally infested field at the Central Crops Research Station near Clayton, North Carolina, in 1984. The soil was a Varina loamy sand (85% sand, 9% silt, 6% clay). Soil temperature was monitored at a depth of 15 cm with a remote thermograph (Weathertronics, Sacramento, CA).

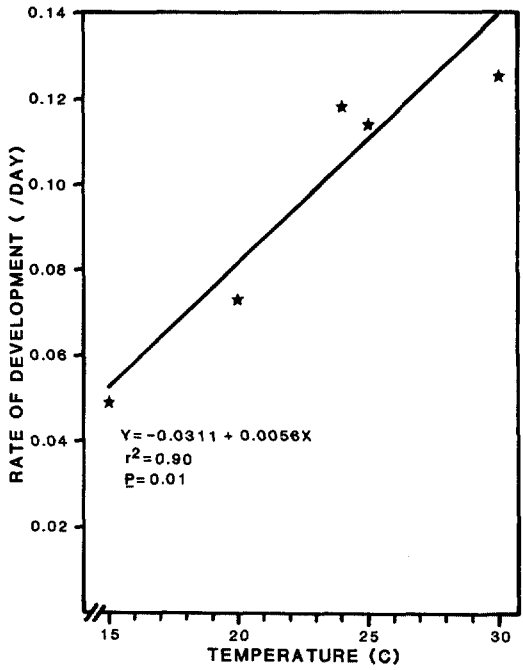

FIG. 1. Relationship between rate of *Heterodera glycines* egg development (per day) and temperatures from 15 to 30 C. Rate of development = proportion of development completed per day by eggs from two-cell stage to J2 within the egg.

'Coker 156' soybeans were planted at weekly intervals from 23 May to 7 November 1984. Also once a week, roots from approximately 2 m of row were dug from each planting to a depth of 30 cm. Whole root systems from five randomly selected plants were stained using a NaOCl-acid fuchsin technique (3), and numbers and developmental stages of *H. glycines* were determined. Developmental stages identified were vermiform J2, swollen J2, third-stage juvenile (J3), fourth-stage juvenile (J4), male, and female with eggs. Males still within J4 cuticles were counted as mature males. Females without eggs were counted as J4. When adults were found from a given planting date, no more plants were removed from those plants. Models were de-

TABLE 1. Rates of development, mortality, and hatch of *Heterodera glycines* eggs at constant temperatures.

Temperature (C)	Rate per day		
	Development†	Mortality‡	Hatch§
10	—‖	0.026	0.000
15	0.049	0.000	0.000
16	0.100	0.000	0.000
20	0.073	0.000	0.110
24	0.118	0.014	0.395
25	0.114	0.010	0.275
30	0.125	0.117	0.338
36	Death	1.000	0.000

† Rate of development = proportion of development completed per day by eggs from the two-cell stage to J2 within the egg.
‡ Rate of mortality = m in the equation for proportion of mortality after n days, $1 - (1 - m)^n$.
§ Rate of hatch = proportion of hatch completed per day by eggs starting in the J2 stage.
‖ Development proceeded only to J1, and therefore is not included in calculations for development to J2 within the egg.

veloped to characterize nematode maturation development in terms of accumulated DD.

RESULTS

Embryonic development: Data from the two experiments were combined. The daily rate of development of eggs from the two-cell stage to J2 within the egg was linearly related to temperature between 15 and 30 C (Fig. 1, Table 1). Egg development proceeded only to J1 at 10 C. At 36 C, egg cells divided once to the four-cell stage and then died. Therefore, development at 10 and 36 C could not be included in calculations for completion of development to J2. The temperature-dependent rate of development between 15 and 30 C was described by the equation

$$R = 0.0056T - 0.0311,$$
$$r^2 = 0.90, P = 0.01$$

where R = predicted rate of embryonic development (proportion of development completed per day) and T = 15 C ≤ temperatures ≤ 30 C. The basal threshold for development, determined by solving the regression equation for the x-intercept, was 5.55 C. In a preliminary experiment, 8 of 10 two-celled eggs placed at constant 5 C completed development to the multicell stage within 12 days, and three of these eggs hatched when placed at 24 C up to 15 additional days. Although mortality was very high, these data support the use of 5 C as a basal threshold for development. The upper threshold for development was approximated to be between 30 and 36 C.

Mortality did not occur at 15, 16, or 20 C (Table 1). Mortality was less than 3% at 10, 24, and 25 C, increased to 11.7% at 30 C, and was 100% at 36 C. Mortality of two-celled and four-celled eggs occurred within

TABLE 2. Accumulated sum of degree-days, assuming a basal threshold for development of 5 C (DD_5), for *Heterodera glycines* eggs maintained at constant temperatures.

Temperature (C)	Accumulated DD_5					
	Four-cell	Eight-cell	Multicell	J1	J2	Hatch
Experiment 1†						
10	11 ± 1	24 ± 3	46 ± 2	75 ± 5	—‡	—
15	16 ± 0	37 ± 2	65 ± 5	110 ± 4	169 ± 1	—
20	22 ± 7	36 ± 6	60 ± 0	126 ± 6	190 ± 2	269 ± 2
25	22 ± 2	40 ± 4	78 ± 6	129 ± 1	180 ± 0	216 ± 16
Experiment 2§						
10	10 ± 2	22 ± 1	40 ± 4	74 ± 3	—	—
16	20 ± 2	27 ± 3	44 ± 5	92 ± 4	115 ± 2	—
24	22 ± 2	37 ± 4	76 ± 2	118 ± 4	160 ± 2	226 ± 2
30	18 ± 1	38 ± 2	49 ± 3	150 ± 1	200 ± 1‖	250 ± 3‖
36	62 ± 0‖	—	—	—	—	—

† Values are means of 10 replicates and two experimental trials.
‡ — = development did not proceed to the stage indicated.
§ Values are means of eight replicates.
‖ Less than 50% of the eggs developed to the stage indicated.

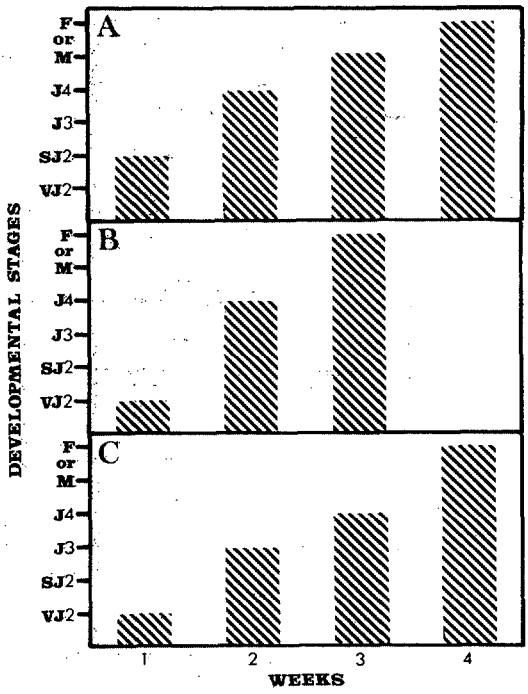

FIG. 2. Developmental stages of *Heterodera glycines* juveniles in soybean roots at 1, 2, 3, and 4 weeks after planting during early, mid and late season in a Varina sandy loam at Central Crops Research Station, North Carolina, 1984. VJ2 = vermiform second-stage juvenile, SJ2 = swollen second-stage juvenile, J3 = third-stage juvenile, J4 = fourth-stage juvenile, M = male, and F = female with eggs. A) Planting dates between 23 May and 27 June. B) Planting dates between 5 July and 15 August. C) Planting dates between 11 September and 10 October.

2–6 days from inception of the experiment. Although completion of egg development to the multicell stage occurred within 2 days at 25 and 30 C, mortality of multicell stage eggs was not observed until 10–20 days. Death of J1 or J2 within the egg was not observed at any temperature studied during these experiments.

Eggs hatched at 20, 24, 25, and 30 C but not at or below 16 C nor at 36 C during the 40-day observation period (Table 1). The optimum temperature for hatch was 24 C.

Egg development at the eight temperatures was computed in terms of accumulated degree-days above 5 C (DD_5) (Table 2). Degree-day requirements for completion of stages were similar among temperatures, with some tendency to require more

TABLE 3. Accumulated sum of degree-days between 5 and 30 C ($DD_{5/30}$) and number of weeks required to complete *Heterodera glycines* life cycle on soybean for 17 weekly planting dates at Central Crops Research Station, North Carolina, 1984.

Soybean planting date	Accumulated $DD_{5/30}$ to complete life cycle	Number of weeks to complete life cycle	Soil temperature during life cycle (C)†		
			Minimum	Mean	Maximum
May 23	415	3	12	25	32
30	432	4	12	27	32
Jun 6	510	4	22	28	36
13	548	4	21	27	36
21	539	4	20	26	34
27	558	4	20	26	34
Jul 5	409	3	20	26	32
12	405	3	20	26	32
18	418	3	20	26	32
25	445	3	20	26	32
Aug 1	453	3	22	27	32
7	443	3	22	26	31
15	415	3	22	26	30
Sep 11	338	3	15	20	26
20	375	4	13	19	25
Oct 3	405	4	13	20	24
10	401	4	12	19	24

† Minimum, mean, and maximum soil temperatures recorded at 2-hour intervals at a depth of 15 cm during life cycle period.

DD_5 at warmer temperatures. The DD_5 for development to J2 and for development and hatch were 157 ± 44 and 236 ± 35, respectively. Development to J1 required fewer DD_5 at the lower temperatures (10, 15, and 16 C) than at 20 C and above. At 30 C, only 32% of the eggs developed to J2 and hatched.

Development of juvenile stages: DD_5 was also selected as a basal threshold for completion of a life cycle from penetration of soybean roots by J2 to production of new eggs by mature females. The range of diurnal temperature fluctuation had an impact on the DD_5 and time requirements for completion of a life cycle (Table 3). An upper temperature threshold for development of 30 C was selected because it allowed for the least variability in DD_5 calculations for completion of *H. glycines* life cycles during the season. At temperatures above 30 C, development was assumed to cease and no DD_5 accumulated. (Here-

after, degree-days accumulated between 5 and 30 C will be referred to as $DD_{5/30}$.)

Early in the season, from 23 May to 5 June, the minimum soil temperature recorded during a *H. glycines* life cycle was 12 C and the maximum temperature was 32 C (Table 3). During this period, generation times were 3 or 4 weeks and 424 ± 8 $DD_{5/30}$. In June, minimum soil temperatures ranged from 20 to 22 C and maximum temperatures from 34 to 36 C. The life cycle during this period required 4 weeks and 534 ± 24 $DD_{5/30}$ (Fig. 2A). In the midseason, from 5 July to 3 September, minimum and maximum soil temperatures were 20–22 C and 30–32 C. During this period, life cycles were completed in 3 weeks and 429 ± 24 $DD_{5/30}$ (Fig. 2B). Late in the season, from 11 September to 6 November, when soil temperatures declined, generation times increased to 4 weeks (Fig. 2C). Minimum soil temperatures were 12–15 C, and maximum temperatures were 24–26 C. Although life cycles were longer, accumulated $DD_{5/30}$ were less (372 ± 33) than during the midseason.

Discussion

The rates of some physiological processes of poikilothermic organisms such as nematodes are controlled by temperature. *H. glycines* is a nematode that can function over a wide temperature range. This function was evidenced by the strong linear relationship of development rates of *H. glycines* eggs to temperatures between 15 and 30 C. This relationship between temperature and embryonic development is similar in *Meloidogyne* spp. (6,25). Based on experimental data and extrapolation by linear regression, the basal temperature threshold for egg development was estimated to be approximately 5 C, much cooler than the earlier estimate of 14 C (21). Basal developmental thresholds are 6.74 C for *M. hapla* (25), 8.26 C for *M. incognita* (25), and 10.11 C for *M. arenaria* (6). Differential developmental thresholds for the different species are probably genetically based and may be important in overwinter survival and related to differences in their geographic spatial patterns (27).

The thermal optimum for embryogenesis and hatch in *H. glycines* was between 24 and 30 C. Low mortality of eggs and rapid development and hatch at 24 and 25 C are supporting evidence for these temperatures being optimal. Juvenile emergence from cysts is greatest at 24 C (23). The minimal temperature threshold for hatch under the conditions of this experiment was 20 C. *Meloidogyne incognita* J2 can migrate through soil and penetrate roots at temperatures above but not below 18 C, which is considered as an activity threshold; however, development can occur at lower temperatures (19). The concept of an activity threshold indicates that while temperature is sufficiently high for development, it may still be below a level allowing for sufficient muscular activity for such events as hatch, migration, and penetration of roots. This nematode may have evolved a mechanism that prohibits hatching below temperatures that would not be suitable for soybean growth (18). Stimuli from host root exudates (24), moisture (17), and a minimal temperature threshold may be the most important factors regulating hatch in field soils, especially in spring when temperatures begin to rise.

Since there was no mortality in J1 and J2 within the egg and early embryonic stages had high mortality levels, it seems likely that overseasoning would occur with the more advanced developmental stages.

In general, $DD_{5/30}$ was a satisfactory predictor of juvenile development in roots. In June, soil temperatures above 30 C seemed to be detrimental, especially to development of advanced juvenile and adult stages, resulting in greater $DD_{5/30}$. Penetration, development, and reproduction of *H. glycines* is limited at low (14 C) and high (33 C) temperatures (9). In this study, 429 ± 24 accumulated $DD_{5/30}$ were required for completion of a life cycle during the midseason when temperatures were in the optimum range. On soybean root explants grown under gnotobiotic conditions at

constant 25 C, a life cycle of *H. glycines* requires 21 days (14), or 420 $DD_{5/30}$. Completion of *H. glycines* life cycle on soybeans grown in temperature-controlled water tanks required 259, 308, and 378 DD_{10} at 17, 24, and 31 C, respectively (21), or 444, 418, and 468 converting to $DD_{5/30}$.

Late in the season, fewer $DD_{5/30}$ required to complete a life cycle (372 ± 33) were associated with lower soil temperatures (12–26 C) than during the midseason (20–32 C). The discrepancy between mid and late season $DD_{5/30}$ requirements may suggest a lower basal and (or) upper threshold for development. Development of *Heterodera rostochiensis* J2–J4 in host roots was more rapid at 23.9 C than at 18.3 C; however, eggs were formed first at 18.3 C (8). In addition, *H. glycines* has been shown to increase rates of reproduction late in the season on reproductive soybeans (10). Perhaps an increase in rate of development is associated with this increased reproductive capacity.

Modeling approaches used for studies of nematode population dynamics have generally resulted in models describing an integration of many effects during an average season (4). The approach of this study has been to model nematode development as influenced by a single variable, temperature. The hypothesis was that temperature, as a regulator of the nematode's metabolic rate, could be used as the primary driving force for model simulation of developmental rates. Further experimentation and validation is needed to improve reliability and predictive ability of the model's simulation of *H. glycines* population fluctuations in the field.

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