Development of a Population of *Heterodera glycines* Race 5 at Four Soil Temperatures in Minnesota

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ABSTRACT

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Development of *Heterodera glycines* race 5 was most rapid at 30 C, populations were largest at 25 C, and some development took place at 20 and 15 C when nematode-infected soybeans were grown in temperature tanks. A maximum of four generations of this nematode could theoretically be produced within one growing season in southern Minnesota.

Additional key words: *Glycine max*, life cycle, soybean cyst nematode

Heterodera glycines Ichinohe, the soybean cyst nematode (SCN), causes a destructive disease of soybeans (*Glycine max* (L.) Merr.). In 1985, the SCN was known to occur in 24 states including Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Minnesota, Mississippi, Missouri, New Jersey, North Carolina, Ohio, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and Wisconsin (9,15; C. P. Schulze, New Jersey Department of Agriculture, *personal communication*). In 1979, soybean losses caused by this pathogen were estimated at 1.54×10^6 t in the United States (10).

The SCN was first reported in Minnesota in August 1978 in a soybean field in the southwest quarter of section 26, Emerald Township, Faribault County, near the town of Frost. In 1979 and 1980, the nematode was found in seven other counties in Minnesota including Blue Earth, Brown, Cottonwood, Freeborn, Kandiyohi, Martin, and Waseca (8). In 1985, additional infestations were found in Dodge and Mower counties. All of these counties, with the exception of Kandiyohi, are in southern Minnesota. At present, SCN is known to occur in 10 of the 75 counties where soybeans ($\geq$ 405 ha/yr) are grown. Race determination tests were made on populations found at many of the sites, and all but the Frost isolate were designated race 3, the most common race in the United States. The Frost isolate most closely corresponds to the race found in Japan (4) that has been classified as race 5 (8).

After establishing syncytia in the root, SCN is able to feed and obtain an uninterrupted supply of food and water until it matures and dies. The rate at which the nematode develops is highly dependent on soil temperature (6).

Research pertaining to the effect of temperature on the development, i.e., the rate at which the nematode completes its life cycle, of SCN has been done primarily on race 3. Ichinohe (3) reported generation times of 24 and 40 days at 23.3 and 17.6 C, respectively. He estimated

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the threshold temperature, the lowest temperature at which the nematode could complete its life cycle, to be 10 C. Skotland (13) recovered second-stage, second-generation juveniles from soil held at 23 C, 21 days after inoculation of soybean roots. Four or five generations were estimated to develop each year in North Carolina (13). Ross (11,12) first detected adult nematodes 12 and 24 days after inoculation at 24 and 17 C, respectively, and estimated the threshold temperature to be about 14 C. Hamblen et al (2) reported the presence of white females after 14 days at 28 or 31 C but not until 58 days at 14 C. Second-generation infective juveniles were present after 22 days at 28 C but were never found at 14, 33, or 35 C.

The objectives of this study were to determine the number of days necessary for the nematode to complete its life cycle at different temperatures and to predict the number of generations produced during a growing season in Minnesota. An abstract of an earlier experiment has been published (14).

MATERIALS AND METHODS

Seeds of Hodgson 78, a maturity group 1 soybean cultivar susceptible to SCN race 5, were planted in shallow flats containing pasteurized silt-loam soil. Fourteen days after planting, the seedlings were washed free of soil and their roots trimmed to a length of 2.5 cm. Each soybean seedling was transplanted into a plastic cone (21 × 4 cm) without drainage holes (Ray Leach Conetainer Co., Canby, OR). Each cone contained the following layers of materials: 29 cm^3 of vermiculite, 29 cm^3 of sand, and 29 cm^3 of SCN-infested soil on which the root-trimmed soybean seedling was placed. The field soil, Harpster silty clay loam (37.5% sand, 35% silt, and 27.5% clay), was sieved (6-mm^2 screen) to remove large pieces of organic material and mixed thoroughly in a cement mixer. An additional 29 cm^3 of SCN-infested soil was placed over the roots, and sand was added to fill the cone to within about 1.5 cm from the top. The cones were placed directly into trays in the temperature tanks and watered as needed.

The infested soil contained about 1,500 second-stage juveniles. The SCN population (race 5) came from the infested field site near Frost, MN. This soil was stored in a cold room at 4 C before use. The numbers of second-stage juveniles in the soil were estimated by the Cornell pie-pan extraction technique (7). Samples consisting of 58 cm^3 of field soil were extracted at room temperature (24 C) for 5 days. The extracted juveniles were concentrated in 100 ml of water and processed as described by MacDonald et al (8).

The recovery of juveniles from pie-pan extractions over a period of days showed that greatest recovery took place on the fifth day. This greatest recovery period was correlated with greatest infection potential. In a separate study, a nematode stain (1) was applied to roots of infected soybeans after each day during a 5-day infection period in infection cones. Results confirmed that most infection took place after 3–5 days (M. E. Sortland, *unpublished*).

After a 5-day infection period, the seedlings were lifted out of the cones and any adhering soil was rinsed off the roots with tap water. The infected seedlings were transplanted directly into sand (Mississippi River sand [97.5% sand, 2.5% silt]) in 10.5-cm plastic pots. Four of these pots were embedded in sand in each plastic tank container (26.5 cm deep × 22 cm in diameter with a 4-cm layer of vermiculite at the bottom). Twelve of the container units were placed in each Cornell temperature tank. This life cycle study was done at 15, 20, 25, and 30 C, which represented the range of average soil temperatures that occur where SCN race 5 was discovered. Six plants selected at random from each temperature tank were harvested every 2 days for 16 days starting 5 days after seedlings were transplanted at 30 and 25 C and starting 9 and 11 days after seedlings were transplanted at 20 and 15 C, respectively. Mercury-halide lamps (1,600 lux) provided light for a 14-hr period. The soybeans were watered as needed, and a nutrient solution (Peter's Plant Food, 2.8 g/L, 18-18-18, NPK, Robert B. Peters Co., Allentown, PA) was applied to soil after transplanting seedlings. The plants were harvested by lifting them out of the sand and dipping them in water to remove adhering sand. The root systems were then vigorously kneaded 10 times in a bucket partially filled with water. The resulting suspension was poured through both a 25-mesh sieve (707-μm openings) to collect extraneous debris and a 200-mesh sieve (74-μm openings) to collect white females and cysts. The 200-mesh sieve allowed collection of young female nematodes too small to be collected on the 60-mesh sieve that is normally used in such studies. Material collected on the 200-mesh sieve was washed into a beaker. The suspension was sieved two more times through the 200-mesh sieve.

The female nematodes in each beaker were concentrated in 100 ml of water. Two 8-ml subsamples from this suspension were collected with a syringe, and this suspension was placed in each of two counting dishes. Because 16 ml of the 100-ml suspension was examined, the numbers presented represent about 16% of the population that developed in or on the six root systems. The female nematodes were counted with a dissecting scope at 24×. The female nematodes were categorized according to their stage of development: 1) immature with pliable body wall; 2) maturing with thickened, hardened body wall (white female); 3) mature with gelatinous matrix; 4) mature with egg mass; and 5) mature containing eggs (brown cyst with eggs). Empty brown cysts were not counted because of unreliable counts that result when "floaters" are not observed. This experiment was repeated three times, but in the first two experiments, the infection period did not take place in the temperature tanks, so only results from the third experiment are reported.

RESULTS

Immature white females with pliable body walls were first detected after 10 days in the temperature tanks at 25 and 30 C. At 15 and 20 C, this stage was first detected after 32 and 16 days, respectively. Eggs were produced after 20 and 18 days at 25 and 30 C, respectively, after 28 days at 20 C, and one cyst containing eggs was present after 24 days at 15 C (Table 1).

White female production. More immature white female nematodes (stage 1) were present 12, 14, and 18 days after

Table 1. Effects of four root-zone temperatures on number of days for female nematodes to reach each stage of development and number of *Heterodera glycines* race 5 females recovered

	Female stage				
Temperature (C)	Immature (1)	Mature (2)	Mature with gelatinous matrix (3)	Egg mass (4)	Cyst with eggs (5)
	Days (no.)[a]				
30	10[b]	14	14	20	18
25	10[b]	16	16	20	20
20	16[b]	20	20	28	28
15	32	32	...[c]	...	24
	Nematodes (no.)[d]				
30	124	121	28	48	48
25	94	183	53	50	49
20	9	72	32	3	11
15	1	2	0	0	1

[a] First day observed, including 5-day infection period.
[b] First harvest day.
[c] Not observed.
[d] Each value represents the total number of females, counted in two 8-ml subsamples, recovered in each stage present on roots of 48 plants over a 16-day period of eight harvests.

inoculation of roots at 30, 25, and 20 C, respectively, than at any other time. One immature nematode was recovered by day 32 at 15 C (Fig. 1).

Peaks of maturing white female development (stage 2) were observed at 18, 20, and 28 days at 30, 25 and 20 C, respectively. This stage of nematode development was just beginning at 32 days at 15 C (Fig. 1).

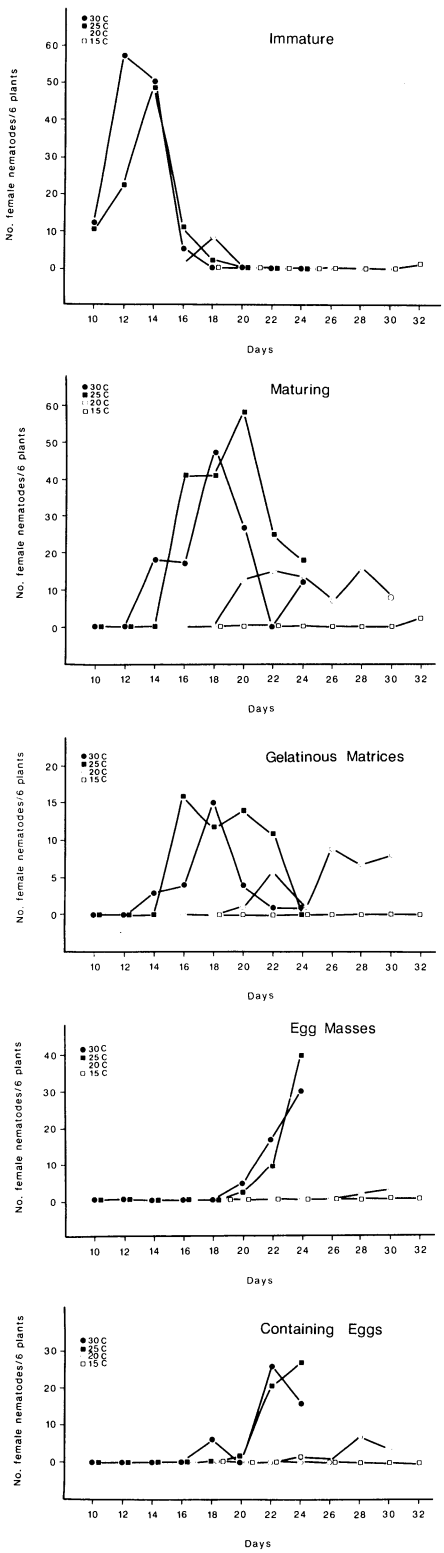

Fig. 1. Effect of root-zone temperature on number of *Heterodera glycines* race 5 females present during each of five life cycle stages. Each value represents the number of females, counted in two 8-ml subsamples, recovered from roots of six plants.

Gelatinous matrix formation. More females with gelatinous matrices (stage 3) were present 18, 16, and 26 days after inoculation of roots at 30, 25, and 20 C, respectively, than at any other harvest time. Gelatinous matrices were not produced by females at 15 C during the 32-day observation period (Fig. 1).

Egg production. The experiment ended before a peak was reached in egg mass production (stage 4) and cysts containing eggs (stage 5) (Fig. 1). More nematodes infected and survived and developed in roots growing at a root-zone temperature of 25 or 30 C than at 20 or, especially, 15 C. Development of the females at 25 and 30 C was about equally rapid, with 35 and 34%, respectively, of the white females in or past the gelatinous matrix stage by the end of the experiment (Table 1).

DISCUSSION

The soil temperature in southern Minnesota during the growing season ranges from 15 to 25 C at 10 cm deep. The average soil temperature throughout the soybean-growing season was calculated by averaging the average daily temperature at 10 cm over a 3-yr period from 15 May through 30 September from data collected at the Southern Experiment Station, Waseca, MN. The average soil temperatures per time period were as follows: 15–20 May, 15 C; 21–31 May, 20 C; 1–30 June, 21 C; 1–31 July, 25 C; 1–31 August, 23 C; 1–12 September, 22 C; and 13–30 September, 19 C. On the basis of the temperatures used in this study, the life cycle (juvenile to egg) of SCN race 5 can be completed within 20–28 days at soil temperatures of 25 and 20 C.

The temperature requirements of SCN race 5 agree with most values obtained for race 3 according to Ichinohe (3) and Skotland (13). In our study, white females (maturing female nematodes, stage 2) were produced more rapidly (14 days) at 30 C than they were in the study by Hamblen et al (2) in which this stage of development was present after 18 days at 31 C. Egg masses were produced slightly earlier (day 20) at 25 C than reported by Ross (11), where they were produced after 22 days at 24 C.

At 20 C, mature females (stage 2) were present at day 20 and egg mass production began at day 28. Considering race 3 adult female nematodes were first observed after 24 days at 17 C and after 12 days at 24 C, it appears that race 5 has a similar life cycle to race 3 at the lower soil temperatures (11).

Eggs laid in a gelatinous matrix can hatch immediately if environmental conditions are favorable (5). These unprotected eggs and the juveniles that subsequently emerge from them are the most vulnerable stages of the nematode. Consequently, this stage of the nematode would be appropriate for control with nematicides. If a postemergence nematicide could be developed with timed-release properties that coincide with egg hatch, nematode populations would increase slowly if at all.

The greatest number of egg masses was produced at 25 C, followed closely by 30 C. At these temperatures, the production of egg masses coincided with that of cysts with eggs. However, because more eggs were contained in cysts than in egg masses at 20 C, it appears that cool temperatures stretch out the life cycle.

The single cyst containing eggs recovered on day 24 at 15 C was probably a contaminant from a higher temperature collection.

If the results from this greenhouse study are used to estimate the life cycle of SCN race 5 in a field near Waseca (though extrapolations of greenhouse tests to the field are not always accurate), the following estimations can be made: at planting time (15–20 May), the soil temperature at 10 cm averages 15 C and gradually increases to 20 C by 31 May. If race 5 (or possibly any race) of the SCN were present, some females would probably begin laying eggs in egg masses around 17 June after becoming visible on the roots by 9 June. The application of a nonphytotoxic nematicide around this time (17 June) could protect plants from reinfection. Otherwise, if egg mass eggs hatched immediately, another generation of SCN race 5 could be produced by 7–15 July when the soil temperature averages 21–25 C, a next generation by 17 July to 4 August when soil temperatures average 23–25 C, and possibly another generation by 20–28 August when soil temperatures average 23 C. These calculations are based on the minimal number of days estimated for one generation to occur and also assume that hatching occurs immediately after egg formation, which is probably not entirely realistic.

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