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Effect of Inoculation Temperature and Soybean Genotype on Root Penetration and Establishment of *Heterodera glycines*

M.R. da Rocha,[1] T. R. Anderson,[2] T. W. Welacky[2]

Abstract: In order to study the effect of resistance and temperature on soybean cyst nematode development, soybean cultivars Bell (PI88788 resistance) and S20-20 (susceptible) were inoculated by transplanting into *H. glycines* infested soil maintained at 20, 25, 30 or 35°C. After 10 days, seedlings were either transferred to hydroponic culture to study male development or transplanted into sand to study female development. The mean number of males on S20-20 (2524/g of dry root) did not differ significantly from the mean number on Bell (2418/g of dry root). Numbers of males and females were highest on seedlings incubated at 30°C during inoculation. The average length of males on Bell (1065 μm) was slightly but not significantly less than males on S20-20 (1126 μm). Short, abnormal males, < 800 μm in length, with spicules and stylets were observed in populations from Bell. The mean number of females was significantly higher on S20-20 (818/g of dry root) than on Bell (216/g of dry root) regardless of inoculation temperature. The mean number of eggs per female was significantly higher on S20-20 (253/cyst) than on Bell (60/cyst). The male to female ratio was highest at 35°C.

Key words: adult-development, *Glycine max*, *Heterodera glycines*, host-parasitic relationship, hydroponic system resistance, temperature.

Soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) causes significant yield losses in soybean production (*Glycine max* [L.] Merr.) worldwide (Wrather et al., 2001). Planting resistant cultivars has been one of the primary control measures to minimize yield losses.

Temperature is an important factor affecting *H. glycines* development. Alston and Schmitt (1988) described the relationship between temperature and *H. glycines* embryonic development between 15°C and 30°C as a linear function. The basal temperature threshold for egg development was approximately 5°C. Thermal optimum for embryogenesis and hatch with low mortality was 24°C. Development proceeded to first-stage juveniles at 10°C and to second-stage juveniles (J2) at 15 to 30°C. Hatch occurred at 20°C to 30°C. At 36°C, development proceeded only to the four-cell stage. Thus, the upper threshold for egg development was estimated to be between 30°C and 36°C. Mortality during embryogenesis is high at temperatures above 30°C (Schmitt, 1991). Temperatures near 24°C favor hatching and below 16°C or above 36°C inhibit hatching (Riggs and Schmitt, 1989). Under field conditions egg hatch declines sharply in autumn when soil temperatures decrease from 21°C to 10°C (Ross, 1963). *H. glycines* juveniles do not develop beyond the second-stage in soybean roots growing at a constant temperature of 10°C in the greenhouse. Development increases linearly between 10 and 24°C and decreases at 30°C (Schmitt and Riggs, 1989). Penetration of soybean roots by *H. glycines* J2 occurs four times faster at 28°C than at 22°C. Low (14°C) and high (35°C) temperatures limit penetration and development, and completely inhibit reproduction of *H. glycines* (Hamblen et al., 1972).

In the genus Heterodera, unbalanced sex ratios have been attributed to genetic and environmental factors. Sex of *H. rostochiensis* Wollenweber is reported to be environmentally controlled (Trudgill, 1967; Ross and Trudgill, 1969). Under crowded conditions, *H. rostochiensis* juveniles change course in sexual differentiation and develop into males. In contrast, unbalanced sex ratios in *H. schachtii* Schmidt and *H. glycines*, are believed to be the result of differential death rates of male and female juveniles under adverse environmental conditions (Johnson and Viglierchio, 1969; Koliopanos and Triantaphylou, 1972).

High male to female ratios have been observed in *H. glycines* exposed to high soil temperatures, particularly during the early stages of juvenile development (Ross, 1964). Increases in the percentage of males, however, were always accompanied by increases in the percentage of degenerated juveniles in the roots. This suggested that high death rate of female juveniles was the cause of the increased male to female ratio.

According to Colgrove and Niblack (2005) soybean resistance is not a factor in determination of sex in *H. glycines*. They concluded that under optimum environmental conditions, sex in *H. glycines* is determined genetically regardless of host resistance. The objective of this study was to confirm the effect of inoculation temperature and the host resistance of PI 88788 on infection and subsequent development of *H. glycines* males and females.

Materials and Methods

Seeds of the soybean cultivars Bell with resistance to *H. glycines* from PI 88788 (Nickell et al, 1990) and S20-20 (Northrup King Ltd) susceptible to *H. glycines* were germinated in rolled paper towels at 25°C and constant light. After 3 days, six healthy seedlings were

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transplanted to 15-cm fiber pots containing *H. glycines*, race 3 infested field soil and autoclaved sand in a 50/50 mixture. The inoculum concentration in the final mixture was 20,000 eggs per 100 g of dry soil. Pots were placed in growth chambers at constant temperatures of 20, 25, 30 or 35°C. Growth chambers were adjusted to provide a day length of 14 hours and light intensity of 800 μmol/s/m^2. After 10 days exposure to the temperature treatments and inoculum, soybean seedlings were removed and divided into two groups.

To assess the number of *H. glycines* males that infected and exited the roots of the two soybean cultivars, two seedlings from each pot were grown hydroponically for an additional 10 days after washing the roots under a stream of water to remove all soil and nematodes on the root surface. Seedlings were suspended using cotton plugs in the necks of glass bottles (200 mL) filled with sufficient distilled water to immerse the roots. The water in each bottle was aerated continuously from a central source by fine tubing and bubblers. The bottles, containing 2 seedlings each, were maintained at 23°C to 25°C with a 14 hour day length and light intensity of 240 μmol/s/m^2. After 10 days, seedlings were removed and roots were air dried for 48 hr at 60°C and weighed to determine dry weight. Excess water in each bottle was removed carefully by suction leaving approximately 5 ml of solution containing nematodes and sediment. Nematodes were examined and enumerated with a stereoscopic microscope at 30 × magnification. Twenty *H. glycines* males were randomly selected from each sample and measured for total length at 45 × magnification with an ocular micrometer. Males less than 800 μm in length were considered abnormally short (DaRocha et al., 2001).

To determine the number of *H. glycines* females that infected and initiated development at each temperature, roots of four seedlings from each pot were washed with water to remove soil and surface nematodes and transplanted to 15-cm pots containing autoclaved sand. The seedlings were grown for 30 days under greenhouse conditions at approximately 22°C to 28°C with supplemental lighting to provide a minimum of 14 hours of daylight. At harvest, shoots were discarded and roots were placed on an 850-μm-pore sieve nested over a 180-μm-pore sieve. Females were dislodged from roots with a high pressure water spray. Roots were dried as described above and weighed. Females collected from the lower sieve were counted under a stereoscopic microscope at 15 × magnification. Ten females were randomly selected from each sample and crushed with a tissue grinder to release eggs, which were counted in a Peters counter slide at 45 × magnification.

The experiment was conducted twice in a completely randomized design and treatments were replicated five times. Data were analyzed as a 4 × 2 factorial with temperature and cultivar as factors. Numbers of *H. glycines* were expressed on a dry root weight basis, transformed to $Log_{10}(x+1)$ values and analyzed with SAS (Cary, NC) procedures including general linear model (GLM) and regression (REG). Unless stated otherwise, all tests of significance were made at $P < 0.05$.

Results and Discussion

Analysis of the combined experiments indicated that the 2 experiments did not differ significantly; therefore, the data were treated as 1 experiment with 10 replications.

Temperature during the first 10 days of infection and early development of *H. glycines* had a significant effect on the total number of males ($F = 69.81$, $P < 0.01$), average male length ($F = 62.94$) ($P < 0.01$), number of abnormal males ($F = 40.80$) ($P < 0.01$), number of females ($F = 192.33$) ($P < 0.01$) but not the number of eggs per female ($F = 0.31$) ($P = 0.82$). The cultivars Bell and S20-20 had a significant effect on average male length ($F = 17.48$) ($P < 0.01$), number of abnormal males ($F = 5.01$) ($P < 0.05$), number of females ($F = 64.07$) ($P < 0.01$), and number of eggs per female ($F = 81.02$) ($P < 0.01$) but not the total number of males ($F = 0.66$) ($P = 0.89$). Temperature × cultivar interaction was non significant ($F = 1.71$) ($P < 0.16$).

The relationships between temperature and number of males and females per g of dry root were described by a quadratic model (Fig. 1). Total number of males was maximum at 27.6°C and 27.4°C on Bell and S20-20 respectively (Fig. 1A). The number of abnormal males was maximum at 28.8°C and 28.5°C on Bell and S20-20 respectively (Fig. 1B). The number of females was highest at 26.8°C and 26.4°C on Bell and S20-20, respectively (Fig. 1C).

The number of *H. glycines* males and females observed in this study may have been influenced by the effect of temperature on hatching, penetration, or feeding site establishment during infection. Tefft et al. (1982) found no differences in hatch numbers at temperatures between 20°C and 36°C but substantially less hatching at 16°C. Alston and Schmitt (1988) concluded that hatching occurs between 20°C and 30°C but not at 36°C. The highest temperature in the current study was 35°C which is slightly less than the reported maximum threshold that limits hatching; therefore, it is possible that the maximum temperature evaluated in this study inhibited hatching. Although temperature treatments were applied only during the first 10 days of these experiments, it is possible that the treatments influenced early development once feeding sites were established. Melton et al. (1986) reported effects of temperature and genotype on *H. glycines* development with no temperature × cultivar interaction with greatest numbers of females produced at 20°C to 28°C and least at 16°C and 32°C. Sipes & Schmitt (1989) found that the most rapid development occurs from 24°C to 28°C. Whereas Palmateer et al. (2000) observed greater

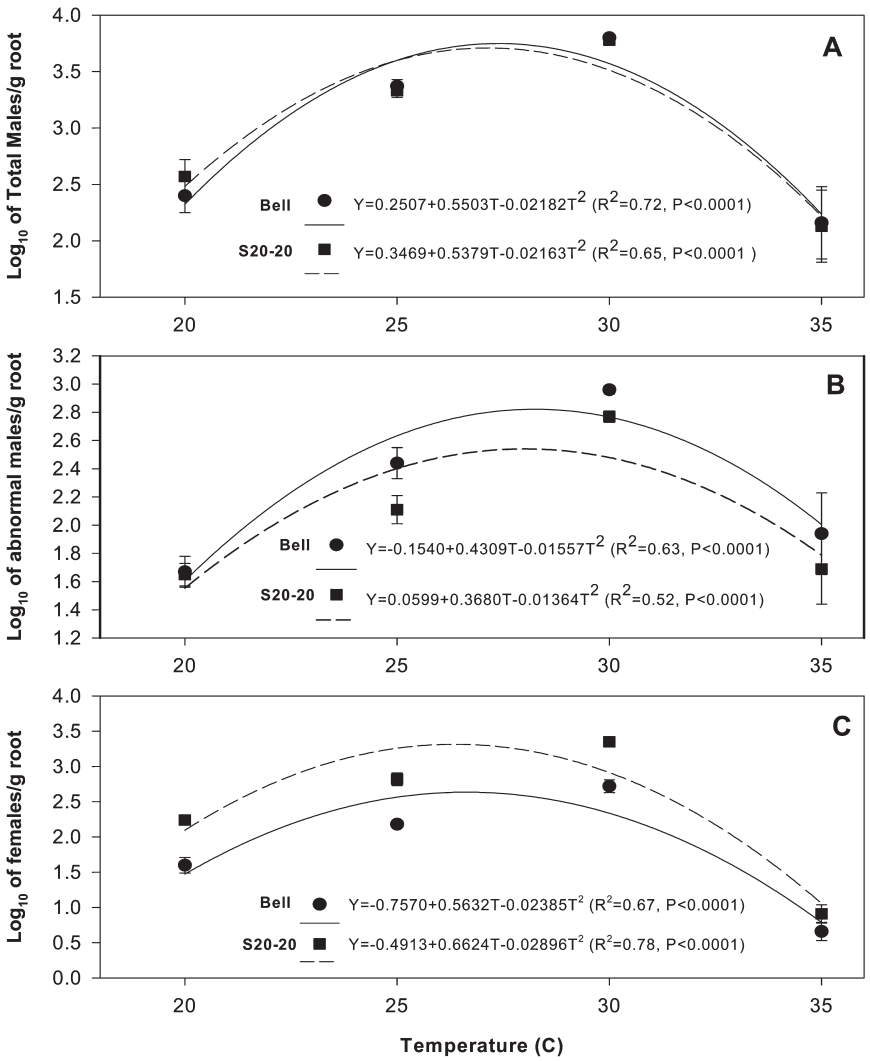

FIG. 1. Effect of initial exposure to different temperatures on SCN adult male and female population on soybean cultivars Bell and S20-20; A – Total males/g of root; B – Abnormal males/g of root; C – Females/g of root. Data transformed into $\log_{10}(x+1)$.

production of females, cysts, eggs and juveniles at 27°C than at 20°C or 30°C.

In our study, infection and early development of *H. glycines* male and female numbers were lowest at 20°C and 35°C (Fig. 1). Hamblen et al. (1972) reported that 28°C was more favorable to root penetration than 22°C, and observed little penetration and no maturation at 35°C. They never recovered males at 14°C and 35°C. Melton et al. (1986) observed that minimum (16°C) and maximum (32°C) temperatures were less favorable for female development. They found that temperatures warmer than 16°C favored faster development up to 28°C, above which development slowed. It was reported previously, however that development at high temperatures is faster, but fewer adults developed (Ross, 1964).

Soybean cultivar affected female numbers (Fig. 1C). Mean numbers of *H. glycines* females on Bell and S20-20 were 226 and 818 females per g of dry root, respectively. The inability to establish feeding sites on the resistant cultivar probably caused the development of the lower number of females on Bell in our studies.

Luedders (1987) noted that host resistance genes may affect males and females differently, and that resistance genes in soybean PI 88788 may not affect males. This was confirmed by Colgrove and Niblack (2005) whose results indicated that the mechanism of resistance in PI 88788 has the greatest effect on J3 and later development stages because males feed for up to 9 days after infection. The PI 88788 mechanism of resistance allows some males to develop to the J4 stage, which is mature enough to complete development without additional feeding.

Number of eggs per female was not affected by temperature during early establishment but was affected by soybean genotype. Average numbers of eggs per female were 253 and 60 for S20-20 and Bell, respectively. The PI 88788 resistant reaction in Bell may be initiated early in the infection process and be characterized by nuclear degeneration followed by degradation of the cytoplasm

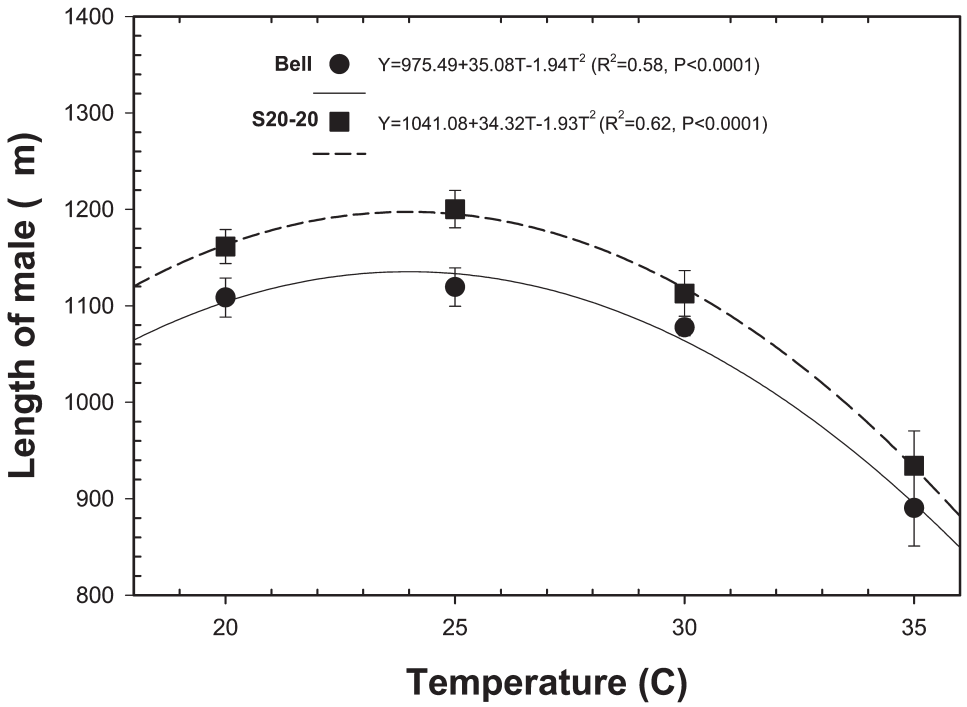

FIG. 2. Effect of initial exposure to different temperatures on SCN adult male length (μm) on soybean cultivars Bell and S20-20.

of the root cells that would normally form the syncytium, resulting in a decrease in available nutrients (Kim and Riggs, 1992) and fewer eggs per female. Temperature during infection and early development of females did not affect egg production in this study. Palmateer et al. (2000) observed that constant temperatures of 20°C resulted in inconsistent results with standard race testing and that at 27°C and 30°C numbers of females, eggs and juveniles were more consistent. Our experiments indicate that infection and early establishment are reduced at a temperature of 20°C.

PI 88788 resistance did not affect the total number of males (Fig. 1A) but resistance caused noticeable morphological changes in SCN males. The mean number of abnormally short males on Bell was greater than on S20-20 (Fig. 1B). Average length of *H. glycines* males on Bell was always less than the length of those on S20-20 (Fig. 2). Temperature during infection and early development influenced the length of males from both resistant and susceptible cultivars. *H. glycines* male length increased from 20°C to 24°C and was less at 35°C. According to Robbins (1992), normal male length varies from 1,135 μm to 1,625 μm. In the present study, the mean male length on Bell was 890 μm and the mean male length on S20-20 was 933 μm at 35°C. At 25°C the frequency of longer males was high on both cultivars and the mean length on S20-20 was 1,226 μm (range: 660 to 1,485 μm) the greatest length observed in this study. The mean length of males that developed on S20-20 was 1,116 μm and on Bell was 1,062 μm. It is probable that high temperature during infection as well as PI 88788 resistance reduces feeding site establishment of females and to a lesser extent establishment of *H. glycines* males as observed previously (Colgrove and Niblack, 2005). However, unfavorable temperatures and PI88788 resistance can affect male development as demonstrated by the abnormally short males observed in this study.

Based on female indices and egg production, the resistance of PI 88788 was effective within the range of temperatures evaluated during the initial stages of infection. Considering male indices and length, inoculation temperature had minimal effect on the resistance of PI88788. Inoculation at 35°C decreased the establishment of both males and females but the number of eggs/ mature female was not reduced. Because this was a field population it is possible that some individuals differed in virulence or tolerance to temperature during infection. The highest mean male to female ratio (43.82) occurred at 35°C. The highest ratio occurred on Bell at 35°C (68.70) (Table 1). From the results of

TABLE 1. SCN male to female ratios on cultivars Bell and S20-20 following inoculation at 20, 25, 30 and 35°C.

	Cultivars		
Temperature	S20-20	Bell	Mean
20°	2.72	6.22	4.47
25°	3.23	15.65	9.44
30°	2.98	10.10	6.54
35°	18.95	68.70	43.82
Mean	6.97	25.17	16.07

the current study, it was concluded that both inoculation temperature and PI 88788 resistance influence the male to female ratio.

Optimum temperature for *H. glycines* male and female development range from 26°C to 28°C. Temperature significantly affects the length of males with highest temperatures resulting in higher number of abnormally short males. Besides reducing the number of females and the eggs per female, resistance also affects male length.

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