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The Effect of Resistant Soybean on Male and Female Development and Adult Sex Ratios of *Heterodera glycines*[1]

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Abstract: To determine whether currently used sources of resistance (soybean Plant Introductions [PI] 548402, 88788, 90763, 437654, 209332, 89772, and 548316) influence sex ratios in *H. glycines,* four inbred lines of the nematode characterized by zero or high numbers of females on resistant soybean were used to observe the number of adult males produced. Nematodes were allowed to infect soybean roots for 5 days in pasteurized sand. Infected plants were washed and transferred to hydroponic culture tubes. Males were collected every 2 to 3 days up to 30 days after infestation (DAI), and females were collected at 30 DAI. Resistance that suppressed adult females also altered adult male numbers. On PI 548402, 90763, and 437654, male numbers were low and close to zero, whereas on PI 88788, male numbers were higher ($\alpha = 0.05$). In a separate experiment, the same PIs were infected by an inbred line that tested as an HG Type 0 (i.e., the numbers of females that developed on each PI were less than 10% of the number that developed on the standard susceptible soybean cultivar Lee). In this experiment, male numbers were similar to female numbers on PI 548402, 90763, 437654, and 89772, whereas male numbers on PI 88788, 209332, and 548316 were higher than those of females ($\alpha = 0.05$). In all experiments, the total number of adults that developed to maturity relative to the number of second-stage juveniles that initially penetrated the root was less on resistant than on susceptible soybean ($P \leq 0.05$), indicating that resistance influenced *H. glycines* survival and not sexual development.

Key words: Glycine max, Heterodera glycines, resistance, sex determination, sex ratios, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines,* is an economically important parasite of soybean (*Glycine max*) that can be a challenge to control. The use of host resistance along with rotation of non-hosts is recommended (Riggs and Niblack, 1999). Assays for host resistance involve enumeration of adult females that develop on resistant soybeans relative to a susceptible standard (Golden et al., 1970; Niblack et al., 2002; Riggs and Schmitt, 1988). Male development has been largely ignored. Similar to other amphimictic cyst nematode species in the family Heteroderidae, *H. glycines* females cause the majority of damage to plants; they feed longer than males and are associated with more extensive syncytia. Females must feed from the second juvenile stage (J2) to the adult stage (about 21 days), whereas males feed only as J2 and J3 (about 9 days) (Endo, 1964, 1992). Females of the closely related *Heterodera schachtii,* the sugarbeet cyst nematode, required 29 times more food than males (Muller et al., 1981), and syncytia induced by females were 3.7 times larger than those induced by males (Caswell-Chen and Thomason, 1993).

Interest in the sex determination mechanisms of amphimictic cyst nematodes such as *Heterodera* and *Globodera* spp. is due not only to basic biology questions but also to the possibility of developing improved nematode control methods that operate by interfering with female development. A number of factors have been reported to affect adult sex ratios within *Heterodera* and *Globodera* spp., including infection density (Ellenby, 1954; Koliopanos and Triantaphyllou, 1972; Steele, 1974), temperature (Melton et al., 1986; Ross, 1964), host nutrition (Grundler et al., 1991; Johnson and Viglierchio, 1969), and host resistance (Bridgeman and Kerry, 1980; Halbrendt et al., 1992; Johnson and Viglierchio, 1969; Muller, 1985). Both environmental sex determination (ESD) and genetic sex determination (GSD) have been proposed to explain these variations.

In general, individuals capable of ESD develop into males or females based on conditions at certain developmental stages, such as temperature (Bull et al., 1982), nutritional status (Hirschmann and Triantaphyllou, 1972; Petersen, 1971; Triantaphyllou and Hirschmann, 1972), and space (Christie, 1929). Intersexes, or individuals that develop sexual characteristics of both males and females, may occur. Unbalanced sex ratios occur due to differential development toward one sex or the other caused by manipulation of experimental conditions, with no evidence of differential death based on sex. In contrast, organisms with GSD develop according to their genotypic sex, which is determined upon fertilization. Intersexual individuals are rare, and unbalanced adult sex ratios are due to differential survival based on sex. *Caenorhabditis elegans,* previously known to have GSD, was recently found to have ESD in very specific situations; it occurred under conditions of poor nutrition but only in the progeny from sexual reproduction between males and hermaphrodites (Prahlad et al., 2003). Post-embryonic sexual transformation from hermaphrodite to male occurs by loss of the paternal X chromosome.

Although the amphimictic cyst nematodes can have sex ratios different from 1, absolute evidence of ESD or GSD has not been presented. Both sexes are obligate endoparasites; neither will develop past the J2 without feeding on a susceptible host. Therefore, J2 that penetrate the root are potentially affected by every condi-

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tion that affects the host plant. These influences can have a differential effect on survival or development of males and females because of their different nutritional requirements. Under stress conditions, the production of high numbers of adult males in conjunction with low numbers of adult females led to the interpretation that J2 were triggered to develop as males instead of females by an ESD mechanism (Ellenby, 1954; Grundler et al., 1991; Trudgill et al., 1967). Other researchers concluded that differential death of females only makes it appear that more males than females occurred because more males survive to become adults (Bridgeman and Kerry, 1980; Johnson and Viglierchio, 1969; Koliopanos and Triantaphyllou, 1972; Leudders, 1987).

Under optimum conditions, males and females of *H. glycines* usually occur in equal numbers. The sex ratio changes that occur under stress conditions are not bidirectional; the production of mainly adult males has been reported, but production of mainly females has not (Endo, 1965; Halbrendt et al., 1992; Koliopanos and Triantaphyllou, 1972; Leudders, 1987; Ross, 1958, 1964). In early studies on resistant soybean, males were reported to occur in the absence of any female development; however, higher percentages of males were associated with higher numbers of degenerated juveniles (Ross, 1964). Leudders (1987) noted that host resistance genes may affect males and females differently, and that resistance genes in the soybean plant introduction (PI) 88788 may not affect males. Studies on the soybean cultivar 'Peking' generated conflicting results; Endo (1965) found no males when females failed to develop, whereas Ross (1958) reported that males were common. In initial attempts to select a population that could reproduce on soybean PI 437654, a few males were found but no females (Leudders and Anand, 1989). Handoo and Anand (1993) reported that PI 437654 had partial resistance to males; they found males but no females on PI 437654, and the number of males was lower than on the susceptible cultivar Essex. The response of soybean resistant to *H. glycines* infection varies, depending on host source of resistance (Endo, 1965; Handoo and Anand, 1993; Kim and Riggs, 1992; Kim et al., 1987; Mahalingham and Skorupska, 1996; McCann et al., 1982; Young, 1982). Stage-related resistance was reported that did not affect males and females equally (Halbrendt et al., 1992). The objective of this study was to investigate the effect of soybean resistance on male development when resistance is strongly effective against females.

Materials and Methods

Heterodera glycines populations: All *H. glycines* populations were inbred for multiple generations by single-cyst descent (Leudders, 1985) (Table 1). Lines were named according to Bird and Riddle (1994). Bioassays were conducted annually for 3 consecutive years according to protocols described previously (Colgrove et al., 2002; Niblack et al., 2002) to determine the stability of female production on resistant soybean PIs. Female production on a given PI was assessed as the female index (FI): the percentage of females that develop on a resistant soybean accession relative to the susceptible soybean 'Lee'.

Table 1. Selection characteristics of *Heterodera glycines* inbred lines (selected by single-cyst-descent).

Inbred line	Selection host	Number of generations
TN9	*Lycopersicon esculentum* 'Tiny Tim'	96
TN10	*L. esculentum* 'Roma'	96
TN16	*Glycine max* Plant Introduction 209332	69
TN19	*G. max* 'Hartwig'	49

Inoculum and seedling preparation: The nematode lines were increased for 30 days on susceptible soybean in pasteurized very fine sandy loam soil (75% sand) in plastic beakers maintained in a water bath at 27 °C. Females were harvested from the roots with a high-pressure water spray over nested 710 and 250-µm-aperture sieves and ground with a rubber stopper to release eggs on the 250-µm sieve. The eggs were concentrated by sugar centrifugation (454 g sucrose/l water) and rinsed into a 40-ml centrifuge tube. The volume was brought to 40 ml with water, and the number of eggs per ml was determined. The suspension was adjusted to 1,500 eggs/ml.

The susceptible soybeans Lee and 'Essex' and the resistant soybeans PI 548402, 88788, 90763, and 437654 were used for the first group of experiments with TN9, TN16, and TN19. In the second set of experiments with TN10, the same soybeans were used as were resistant soybeans PI 209332, 89772, and 548316. Each experiment was repeated once. Seeds were germinated in sterilized germination paper at 27 °C for 2 days when radicles were 2 to 3 cm long.

Infection, staining, and hydroponic culture of test plants: Plastic containers were used for cultivation of test plants. Each container held 23 (100 cm^3) polyvinyl chloride tubes filled with a pasteurized very fine sandy loam (75% sand). A 2- to 3-cm-deep hole was made in the center of each soil-filled tube with a 7-mm-diam. dowel. Each hole was infested with 1,500 eggs in 1 ml water of the appropriate *H. glycines* line. Seedlings of the appropriate soybean were planted in the holes, and the soil around each seedling was washed in with a water bottle. Containers were moved to the greenhouse and maintained in 27 °C water tables for 5 days. For the first experiments, 20 of each soybean/*H. glycines* line were planted, 10 for hydroponics and 10 for staining. For the second set, eight of each line were planted, four for hydroponics and four for staining. Five days after soil infestation (DAI), infected soybean plants were

washed carefully from the soil and placed in water-filled plastic beakers for further processing.

Half of the plants of each *H. glycines* line-soybean combination were arbitrarily selected for root staining with acid fuchsin (Byrd et al., 1983). Roots in glycerin were stored at 4 °C until counted. The number of J2 within each root system was determined at ×64 magnification with a stereomicroscope.

The remaining plants were transferred to hydroponic vessels filled with approximately 400 ml water. The glass vessels, designed specifically for catching males, were 5.1 cm diam., 28 cm long, with a funnel at the bottom with clamped rubber tubing attached (Leudders, 1987). Vessels were placed in racks and submerged in a 27 °C water bath in the greenhouse. Vessels were aerated through submerged 2-mm-diam. polyvinyl chloride tubing in part to facilitate sinking of males to the bottom of the vessels as they exited the roots.

Beginning at 10 DAI, and every 2 to 3 days thereafter to 30 days, the clamps at the bottom of hydroponic vessels were released to allow the contents to drain into a 25-μm-aperture sieve; sieve contents were rinsed into 40-ml tubes. The tube contents were rinsed into counting dishes and males, and any females found were enumerated at ×40 magnification with a stereomicroscope. Females were harvested from the roots at 30 DAI with a water spray as described earlier for inoculum preparation, and counted. Percentage adult survival was calculated as (total adults at 30 DAI)/(J2 within roots at 5 DAI) ×100.

Statistical analysis: The chi-square test was used to test the hypothesis that males constituted 50% of the adults produced in all soybean-*H. glycines* combinations; at $\alpha = 0.05$. J2 infection rates within and between tests were compared with *F*-tests ($P \leq 0.05$) with the general linear models (GLM) procedure of SAS (SAS Institute, Inc., Cary, NC). Statistics of variation are not included for FI values (Table 2) because FI are calculated from means and the variability measured in the components (female counts) is therefore lost.

TABLE 2. Female indices[a] of *Heterodera glycines* inbred lines on *Glycine max* plant introductions (PI).

Inbred line	Year[b]	PI 548402	PI 88788	PI 90763	PI 437654	PI 209332	PI 89772	PI 548316
TN9	1	70	0	7	0	nt[c]	nt	nt
	2	80	2	5	0	nt	nt	nt
	3	47	0	5	7	nt	nt	nt
TN10	1	12	1	3	0	1	9	0
	2	9	7	3	0	9	7	1
	3	12	4	1	0	7	10	2
TN16	1	0	126	0	0	nt	nt	nt
	2	0	77	0	0	nt	nt	nt
	3	0	113	0	0	nt	nt	nt
TN19	1	nt	nt	nt	nt	nt	nt	nt
	2	35	18	44	17	nt	nt	nt
	3	85	79	59	27	nt	nt	nt

[a] Female Index = (mean of females on soybean PI/mean of females on susceptible check 'Lee') ×100. Data are calculated from means of three replications.

[b] For TN9, TN16, and TN19, year 1 = 1998, 2 = 1999, 3 = 2000. For TN10, year 1 = 2001, 2 = 2002, 3 = 2003.

[c] nt = not tested.

RESULTS

Characteristics of H. glycines populations: For TN line × PI combinations in which FI were high, the interaction was termed "compatible" and for those in which FI were low (less than 10%) or zero, "incompatible" (Acedo et al., 1984) (Table 2). For example, TN9 and TN10 were incompatible with PI 88788, whereas TN16 and TN19 were compatible (Table 2).

TN10 originally tested as an HG Type 0 (data not shown), which is defined as a *H. glycines* population that has FI of less than 10% on the seven HG Type indicator lines listed in Table 2 (Niblack et al., 2002). In the bioassays performed for this study, FI were greater than 10% on PI 548402 twice, and equal to 10% on PI 89772 once. These results changed the HG Type designation for this population (1 in 2000, 0 in 2001, and 1.6 in 2002), but it was the only inbred line available that was generally incompatible with all seven PI lines and therefore, of interest, to reflect a "wild type" without the ability to parasitize resistant soybean yet inbred enough to reduce variability in bioassays.

J2 penetration and survival: The number of J2 of TN9, TN16, and TN19 within soybean roots at 5 DAI differed ($P \leq 0.05$) between experiments; therefore, data were not pooled. In general, the number of J2 did not differ ($P \leq 0.05$) between soybean lines within each test, whereas the number of adults differed ($P \leq 0.05$) among soybean lines. The percentage survival in compatible *H. glycines*-soybean interactions ranged from 41.1% to 112.0% (Table 3). In incompatible combinations, survival ranged from 1.8% to 16.9%. The highest survival in incompatible interactions was for TN9 on PI 88788. Results from the two tests with TN10 did not differ ($P \leq 0.05$) for J2 or survival; therefore, data were pooled. Percentage survival was lower ($P \leq 0.05$) in incompatible combinations than in compatible ones (Table 4).

Sex ratios: Results of the first and second experiments for TN9, TN16, and TN19 were not pooled due to differences ($P \leq 0.05$) between tests (Table 3). Males were produced in every test, in every combination, as were females except for TN16 on PI 437654 in test 1. In compatible interactions, male and female numbers were not different according to chi-square tests ($\alpha = 0.05$), except for TN16 on Lee in test 2. In incompatible interactions, male-female ratios were always different from 1 ($\alpha = 0.05$). Data from the two tests on TN10 did not differ ($P \leq 0.05$) and were pooled. Males were produced on all soybean lines (Table 4). Male-female ra-

TABLE 3. Development and survival of *Heterodera glycines* inbred lines on soybean lines differing for interaction type.

Inbred line	Test[a]	Soybean	Interaction type[b]	No. males[c]	No. females[c]	Male:female ratio	Survival (%)[d]
TN9	1	Lee	C	76.0 a	67.0 a	1.1	73.3 a
		PI 88788	I	26.2 b	0.6 b	43.7*[g]	16.9 b
		Mean		51.1ns	33.8[f]		47.9 ns
TN9	2	Lee	C	120.4 a	110.6 a	1.1	99.6 a
		PI 88788	I	27.8 b	0.2 b	111.3*	11.3 b
		Mean		74.2ns	55.5[f]		64.7 ns
TN16	1	Lee	C	77.7 a	43.6 a	1.8*	111.1 a
		Essex	C	70.6 a	45.0 a	1.6	112.0 a
		PI 548402	I	3.8 c	0.1 c	38.0*	3.9 c
		PI 88788	C	39.6 b	30.1 b	1.3	55.2 b
		PI 90763	I	1.9 c	0.2 c	9.5*	2.6 c
		PI 437654	I	1.9 c	0.3 c	6.3*	1.8 c
		Mean		32.5[f]	19.9[f]		46.7 ns
TN16	2	Lee	C	110.9 a	74.1 a	1.5	66.6 ab
		Essex	C	95.9 ab	64.9 ab	1.5	108.5 a
		PI 548402	I	16.8 c	0.1 c	168.0*	6.2 b
		PI 88788	C	70.5 b	57.2 b	1.2	79.2 ab
		PI 90763	I	8.3 c	0.2 c	41.5*	3.2 b
		PI 437654	I	6.1 c	0.0 c	6.1/0[e]*	2.8 b
		Mean		51.4[f]	32.8[f]		48.4 ns
TN19	1	Lee	C	91.0 a	68.6 a	1.3	75.9 ab
		Essex	C	49.1 c	48.3 bc	1.0	41.1 c
		PI 548402	C	69.2 b	55.5 ab	1.3	87.5 a
		PI 90763	C	22.0 d	30.0 d	0.7	49.9 bc
		PI 437654	C	40.7 cd	38.6 cd	1.1	52.1 bc
		Mean		54.4[f]	48.2[f]		61.3[f]
TN19	2	Lee	C	125.5 a	132.4 a	1.0	92.5 ab
		Essex	C	99.4 a	80.9 b	1.2	99.1 a
		PI 548402	C	102.9 a	80.3 b	1.3	65.6 ab
		PI 90763	C	60.1 b	68.7 b	0.9	60.8 b
		PI 437654	C	102.6 a	90.1 b	1.1	74.3 ab
		Mean		98.1[f]	39.9[f]		78.4[f]

[a] Data from two tests were not combined due to differences between tests.
[b] Interaction type is based on performance in annual tests for the past 3 years as shown in Table 2. C indicates a compatible interaction (high female development); I indicates incompatible (low female development) with FI of 0 or near 0.
[c] Numbers of males and females produced per plant. Data are means of 10 replications.
[d] Percentage survival = (total adults at 30 days after soil infestation)/(J2 within roots at 5 days after infestation) × 100. Means followed by the same letter within tests are not different (LSD at $P \leq 0.05$).
[e] Exact male/female ratio; numerical value cannot be calculated.
[f] Means differed between tests 1 and 2 ($P \leq 0.05$); ns = means not significantly different between tests. Means followed by the same letter within tests are not different (LSD at $P \leq 0.05$).
[g] * = sex ratio different from 1:1 (chi-square test at $\alpha = 0.05$).

tios differed from 1 ($\alpha = 0.05$) on PI 88788, 209332, and 548316 but not on PI 548402, 437654, and 89772.

DISCUSSION

Previous studies of cyst nematodes within the family Heteroderidae showed that the male:female ratio is affected by conditions such as the state of host resistance, nutrition, and infection density (Ellenby, 1954; Grundler et al., 1991; Johnson and Viglierchio, 1969; Koliopanos and Triantaphyllou, 1972; Steele, 1974; Steele and Savitsky, 1980). In many studies, heterogenous nematode populations were used in which differences in male and female numbers may have been amplified by the variability inherent in cyst nematode populations (Aeny and Riggs, 1993; Anand et al., 1995; Colgrove et al., 2002; Riggs et al., 1988; Young, 1989; Zhang et al., 1998). In our study, the effect of soybean resistance on the male:female ratio was investigated with inbred lines of *H. glycines* for which FI were low (incompatible) or high (compatible) on specific resistant soybeans. In incompatible combinations, low female numbers would be expected, and higher numbers of males could be assumed to be due to a differential male-female effect.

Because the sex of J2 cannot be determined at present, and because both males and females are biotrophic, infection must be assessed in sex ratio studies. In general, *Heterodera* spp. do not infect resistant and susceptible hosts differentially (Acedo et al., 1984; Endo, 1965, 1991; Halbrendt et al., 1992; Handoo and Anand, 1993; Kim et al., 1987; Lelivelt and Hoogendoorn, 1993); however, the numbers of individuals that penetrate can be variable (Halbrendt and Dropkin, 1986) even under controlled conditions. We observed that the number of J2 within roots at 5 DAI did not differ among soybean lines except in one experiment. The cause of this difference is unknown. In the second experiment of the same combination, J2 penetration was

TABLE 4. Development and survival of *Heterodera glycines* inbred line TN10 on soybean lines differing for interaction type.

Soybean	No. males[a]	No. females[a]	Interaction type[b]	Male/female ratio	Survival[c] (%)
Lee	64.3 a	60.0 a	C	1.1	52.8 a
Essex	46.5 b	58.3 a	C	0.9	59.1 a
PI 548402	5.5 f	4.8 b	I	2.0	5.3 bc
PI 88788	12.8 de	3.2 b	I	4.9*[d]	9.1 bc
PI 90763	2.2 f	2.0 b	I	1.0	1.3 bc
PI 437654	0.3 f	0.0 b	I	0.3/0[e]	0.1 c
PI 209332	20.7 c	3.3 b	I	6.6*	10.4 b
PI 89772	6.5 ef	6.3 b	I	1.2	6.6 bc
PI 548316	15.2 cd	1.5 b	I	10.5*	6.5 bc

[a] Numbers of males and females produced per plant. Means followed by the same letter are not different (LSD at $P \leq 0.05$). Data are means of six replications.

[b] Interaction type is based on performance in a greenhouse bioassay in 2002. C indicates a compatible interaction (high female development); I indicates incompatible (low female development).

[c] Percentage survival = [(total adults)/total initially penetrated J2] × 100. Means followed by the same letter within tests are not different (LSD at $P \leq 0.05$).

[d] * = sex ratio different 1:1 (chi-square test at $\alpha = 0.05$).

[e] Exact male/female ratio; numerical value cannot be calculated.

not different among soybean lines. Overall, J2 penetration did differ between experiments even though inoculum densities and all other conditions were standardized. This suggests that each individual batch of eggs used for inoculum had its own characteristic hatching and infection rate, unaffected by resistance or other conditions. Even though the absolute numbers of nematodes differed between tests, the patterns of relationships to host resistance were consistent.

Mortality of nematodes after infection was also a function of unknown factors in addition to host resistance. We observed survival rates of 41.1% to 112.0% in compatible combinations. Some of this variation, in particular the rates exceeding 100%, were due to experimental error proceeding from unavoidable use of separate plants for infection and adult development assays. In addition, the number of J2 in roots may not reflect the number that initiate feeding sites; some of those stained may still be in the process of migrating and may not have been successful in locating an initial feeding cell. Several researchers have reported that a percentage of cyst nematode juveniles typically fail to develop to maturity (Acedo et al., 1984; Dropkin and Halbrendt, 1986; Endo, 1965; Halbrendt and Dropkin, 1986; Halbrendt et al., 1992; Johnson and Viglierchio, 1969; Koliopanos and Triantaphyllou, 1972; Lelivelt and Hoogendoorn, 1993; Melton et al., 1986). Halbrendt et al. (1992) reported that the normal mortality rate in *H. glycines*-susceptible soybean ranged from 23% to 51%. These effects were not sex-specific as sex ratios were near 1:1 in compatible combinations much of the time (Bridgeman and Kerry, 1980; Halbrendt et al., 1992; Koliopanos and Triantaphyllou, 1972; Lelivelt and Hoogendoorn, 1993; Steele, 1974). Failure of juveniles to develop probably involves many causes. For example, in a study of feeding behavior, Wyss (1992) found that some J2 died because they failed to reinsert the stylet into the syncytium during feeding or damaged the syncytium attempting to feed. A syncytium of another nematode could obscure the chemical stimuli that the nematode is following, causing it to revert to quiescence (Doncaster and Seymour, 1973). Dead *H. glycines* were observed that were not associated with a tissue response or reaction that would indicate syncytium induction, in both susceptible and resistant soybean (Endo, 1965). Mortality, or survival, may be affected by numerous as-yet nonmeasurable factors.

Sex-specific mortality may also affect infection success. The requirements for success of females are much higher than for males. For example, in sugar beet, males of *H. schachtii* were seen to develop with only their heads embedded (Steele, 1971). Because males feed only as J2 and J3, whereas females feed from J2 to adults, the period of vulnerability to developmental effects is much shorter for males. The syncytia of males begin degenerating as early as day 9, whereas intact syncytia of females were observed for longer than 21 days, even after eggs were seen (Endo, 1992). Crowding may have a greater impact on females (Steele, 1974). Syncytia of females reach maximum size during the J4 stage (Golinowski et al., 1996), whereas those of males begin to degrade at the J4 stage (Sobczak et al., 1997). Ross (1964) observed that higher percentages of males were always associated with higher numbers of degenerated juveniles. Most factors, including environmental conditions and general condition of juveniles at the time of infection, or any condition that might stress developing nematodes, probably have a greater effect on females.

In previous studies, adult male numbers differed from those of females on soybean with resistance to *H. glycines* (Endo, 1965; Halbrendt and Dropkin, 1986; Halbrendt et al., 1992; Handoo and Anand, 1993; Leudders 1987; Leudders and Anand, 1989; Ross 1958, 1964). Leudders (1987) indicated that many males developed on PI 88788 when the resistance was effective against females and speculated that PI 88788 resistance may not be effective against males. In our study, male development in incompatible combinations (TN9 and TN10) was reduced by the resistance in PI 88788 but to a lesser degree than by the other resistant lines; overall, the number of males was about 30% of that on the susceptible checks.

Our results add to the growing body of evidence that soybean resistance to *H. glycines* can be classified into two main groups: the PI 88788 group, including PI 209332 and 548316; and the PI 548402 (Peking) group, including PI 90763, 89772, and, perhaps, PI 437654. Peking is more or less equivalent to the accession PI 548402 (R. L. Nelson, pers. comm.). In a number of studies that measured the reduction in the number of females on resistant soybean, differentiation of the resistance response corresponded with these groups

(Anand and Brar, 1982; Leudders and Anand, 1989; McCann et al., 1982; Young, 1982). One dissimilarity between the groups concerns male and female development. Halbrendt et al. (1992) found that resistance was juvenile stage-related and therefore did not affect males and females equally in all resistant hosts. On 'Pickett' soybean, derived from Peking, the resistance response halted juvenile development at the J2 and J3 stages; on PI 209332, J3 and J4 were mainly affected. On PI 89772, all stages of development were affected. Overall, resistance that affected J2 was associated with reduction in male numbers. In our test of the *H. glycines* line TN10, the general pattern of development was consistent with these observations. The reactions of PI in the PI 548402 group were similar in that male numbers were close to those of females. Those in the PI 88788 group were more similar to each other than to the PI 548402 group in that more males developed than females. These results indicate that the mechanism of resistance in the PI 548402 group affects J2 and J3 primarily, whereas the mechanism in the PI 88788 group mainly affects J3 and later stages.

Studies of the histological and ultrastructural responses of *H. glycines* resistance further support this conclusion. Features unique to each group were observed in Peking and Peking × Lee crosses (Endo, 1965; Kim and Riggs, 1992), 'Forrest' and 'Bedford' (derived from Peking and from Forrest × PI 88788, respectively) (Kim and Riggs, 1992; Kim et al., 1987), and PI 437654 (Mahalingham and Skorupska, 1996). In Peking, the major response, apparent 5 DAI, was modification of cell walls followed by necrosis and disruption of the syncytium (Endo, 1965; Kim et al., 1987; Kim and Riggs, 1992). Because males as well as females must feed as J2 and J3 for survival, these observations and the report by Halbrendt et al. (1992) of Pickett's J2-related resistance could together explain why few males develop on PI 548402. In Bedford, wall depositions and necrosis were not evident; instead, nuclear degeneration at 5 DAI and cytoplasmic degradation at 10 to 15 DAI were observed (Kim and Riggs, 1992; Kim et al., 1987). Bedford's resistance is due to PI 88788 resistance gene(s) in its background (Anand and Brar, 1982; Kim and Riggs, 1992; Kim et al., 1987; Young, 1982). Because males feed only until 9 DAI, the PI 88788-associated resistance response appears to allow some males to develop to the J4 stage, which is mature enough to complete development without feeding (Endo, 1992). The similarity between PI 209332 and 88788 and the finding that PI 209332 allowed development to the J3 and J4 (Halbrendt et al., 1992) would collectively explain why more males survive on PI 88788 than on soybeans with PI 548402-type resistance. In PI 437654, features of both the Peking and PI 88788 resistance responses were observed (Mahalingham and Skorupska, 1996). In our study, the observation of similar male and female development on PI 437654 indicated that J2 and J3 stages were affected by the earlier Peking-like component of the PI 437654 response.

Most of the evidence, including our study, suggests that soybean resistance is not a factor in determination of sex in *H. glycines*. On resistant soybean, as under other conditions of nematode stress, males and females face differential selection pressure because males and females have different requirements for survival. In our study, even though resistance had large effects on the total numbers of adults that were produced, the sex ratios were 1 in compatible *H. glycines*-soybean combinations in all but one case, which could be due to experimental error. In ESD mechanisms, the total number of adults is not affected as strongly. For example, when sexual transformation occurs in *C. elegans,* male numbers are greater than the expected 50% even when survival of larvae is greater than 98% (Prahlad et al., 2003). Our results suggest that high male:female ratios occurred due to differential death of males and females. We conclude that under optimum environmental conditions, sex in *H. glycines* is determined genetically regardless of host resistance.

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