Biological manifestation of resistance to soybean cyst nematode development in 'Hartwig' soybean

Z. A. Handoo* and S. C. Anand†
Department of Agronomy, University of Missouri-Columbia, Portageville, MO 63873, USA

'Hartwig' soybean [*Glycine max* (L.) Merr.] is the first cultivar reported to be resistant to all known races of soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe; however, the nature of resistance in this cultivar is not known. Resistance to SCN in Hartwig and its donor parent, PI 437654, was studied from observations of the developmental stages of the nematode in the plant roots and compared with the development in susceptible 'Essex'. Soybean seedlings (emerging roots) were exposed to a wild population of SCN for 8 h and transferred to hydroponics. Adult males and females were extracted and the number of immature juveniles in the roots was recorded from stained roots. A greater number of juveniles of SCN penetrated the roots of Hartwig than roots of PI 437654 or Essex. Several immature juveniles were observed in the roots of Hartwig and PI 437654; however, no mature females developed on either of these two lines. There were no immature juveniles in Essex. Many adult males developed in Hartwig and PI 437654. Complete resistance to female development operated in both Hartwig and PI 437654, whereas partial resistance to male development was observed in PI 437654. Hartwig soybean seems to attract more SCN juveniles, resulting in greater initial penetration than occurs in PI 437654 or Essex.

Keywords: *Heterodera glycines*; *Glycine max*; soybean cyst nematode; soybean; resistance

Traditionally, yield losses due to soybean cyst nematode (SCN) have been minimized through the development and use of resistant cultivars. The soybean plant introductions 'Peking' and PI 88788 have been used extensively as sources of resistance to SCN (Anand, 1991). Recently, a new soybean cultivar, 'Hartwig', was released that derives its resistance from PI 437654 (Anand, 1992). Hartwig originated from the cross Forrest (Hartwig and Epps, 1973) × PI 437654 (Anand *et al.*, 1988) and is resistant to all known races of SCN.

Ross (1958) studied the host–parasite relationship of SCN in resistant soybean roots and observed a few third-stage SCN juveniles in resistant Peking, but found no fourth-stage juveniles and adult females in resistant plants. He also noticed several fourth-stage male juveniles and adult males in resistant parents. SCN development in the soybean roots was described by Endo (1965). In susceptible plants, females feed until fully developed, whereas males cease feeding after two moults (Ichinohe, 1961). Acedo, Dropkin and Luedders (1984) observed varying degrees of mortality of nematodes in resistant lines, depending upon the genetic make-up of the SCN population. A new technique for evaluating *H. glycines* has been developed by Halbrendt, Lewis and Snipe (1992) that enables each SCN juvenile entering the root to be accounted for. The objective of the research described here was to study the developmental pattern of SCN in resistant Hartwig and PI 437654 and to compare it with that in a susceptible line.

Materials and methods

The development of SCN in two resistant soybean lines, PI 437654 and Hartwig, and in a susceptible line, Essex, were studied against an SCN gene pool using the technique described by Halbrendt *et al.* (1992). In brief, 50 000 eggs of *H. glycines* were collected, mixed with moist sand, placed in a cup 4 cm deep and stirred thoroughly. The radicle tips of 3-day-old seedlings of each line were submerged in the sand to a depth of 1.0 cm. The cup was covered with a plastic sheet and incubated at 27°C for 8 h. Seedlings were then lifted from the cup, sand was washed from the roots, and the seedlings were transferred to a hydroponic system. These soybean seedlings were maintained in test tubes (20 × 3 cm) filled with aerated sterilized water. A single layer of sterile cheese-cloth fastened to the top of the tube held the seedlings in place. The test tubes were incubated at 27°C. Plants were maintained in a 16 h daylight regime.

To control root growth, seeding shoots were pruned upon transfer to the hydroponic system: ~50–75% of each cotyledon was cut off with a sterile surgical blade leaving the apical meristem intact. As the apical meristem elongated, it was pruned back to the cotyledonary node.

*Present address: Nematology Lab, USDA, BARC-W, Beltsville, MD 20705, USA; †to whom correspondence should be addressed

0261-2194/93/05/0371-02

Counts of protruding females were made after 20 days, and the females were then removed from the roots. Adult males were retrieved by sieving the fluid of the test tubes and were counted. Infected root portions (2.5 cm length) of each plant were stained for 16 h in 1:1 glacial acetic acid and ethanol containing 0.0175% acid fuchsin, and replaced with clearing agent containing one part lactic acid, one part glycerol and one part distilled water, autoclaved for 10 min and then firmly pressed between two glass slides. The immature juveniles and adult males in each root were counted using a microscope.

There were five seedlings of each soybean line in a replication and the experiment had six replications. The test was repeated and the results were pooled for statistical analysis.

Results and discussion

The number of adult males, adult females and immature juveniles in each soybean line is presented in *Table 1*. There were more than twice as many adult males in Essex as in PI 437654. The number of adult males that developed in Hartwig was almost the same as the number that developed in Essex. Eight adult females matured in Essex, whereas no adult females were found in either PI 437654 or Hartwig. All the SCN juveniles that penetrated Essex developed into adult males or females, as no immature juveniles were found in the roots. Several juveniles, predominantly at the J_3 stage and a few at stages J_2 and J_4, were observed in the two resistant soybean lines. The number of immature juveniles in Hartwig was almost twice that in PI 437654. The total numbers of juveniles (immature juveniles in the roots and adult males and females) that penetrated the roots of Essex and PI 437654 were the same; however, twice that number of juveniles penetrated the roots of Hartwig.

As the total number of juveniles entering the roots of Essex and PI 437654 was the same, it is apparent that PI 437654 did not offer any resistance to SCN penetration of roots. The number of adult males recovered from PI 437654 was significantly less than that from Essex, indicating that some male juveniles that penetrated PI 437654 roots did not develop into adults. These results support earlier findings (Ross, 1958; Endo, 1965) of a limited number of males developing in resistant soybean lines. The total number of juveniles that entered the roots of Hartwig was significantly greater than that in Essex or PI 437654. It appears that Hartwig either stimulated SCN egg hatch or that juveniles were more attracted to the roots of this line. Hartwig had almost twice as many immature juveniles and adult males as were found in PI 437654. The absence of any mature females in Hartwig and PI 437654 indicated that both soybean lines had complete resistance to female development, whereas PI 437654 had partial resistance to male development.

Table 1. Total number of adult males, adult females and immature juveniles of SCN in five soybean roots of three soybean cultivars

Soybean	Adult males	Adult females	Immature juveniles	Total
Essex	18.4	8.0	0.0	26.4
PI 437654	7.0*	0.0	17.8	24.8
Hartwig	15.4	0.0	32.2*	47.6*
l.s.d. (0.05)	8.2		9.6	16.1

*Significant at 5% level of probability

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Received 22 September 1992
Revised 7 January 1993
Accepted 8 January 1993