A Rapid Method for Inoculating Soybean Seedlings with *Heterodera glycines*

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ABSTRACT

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A mouth-pipetting method and a newly described method of inoculation of soybean (*Glycine max*) seedlings with resistance to the soybean cyst nematode (SCN) *Heterodera glycines* race 3 were evaluated. Neither method resulted in a change for the index of parasitism in soybean genotypes Peking, Pickett-71, PI 90763, or PI 88788. The auto-dispenser method resulted in a narrow range of cysts (160–184 per plant) produced on the susceptible cultivar Essex and gave a lower standard of deviation than the mouth-pipetting method. The new method is approximately 18 times faster than the mouth-pipetting method and requires only a single individual to conduct the inoculation. An estimated cost of 94% (dollars) can be saved by adopting the auto-dispenser method.

Several inoculation methods have been used to evaluate soybean seedlings (*Glycine max* (L.) Merr.) for resistance to the soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe. Caldwell et al (3) evaluated soybean plants in the greenhouse by growing them in sterilized soil and artificially infesting soil of each plant with SCN. Crushed cysts were placed in the root area in a pot (8 cm diameter) during the transplantation of a single seedling. Triantaphyllou (10) used infected second-stage larvae for inoculation. The larvae were obtained by crushing approximately 500 cysts and placing them on tissue paper supported by a plastic screen inside a petri dish. Soybean seedlings, grown for 15 days in 10-cm-diameter clay pots, were inoculated with 500 freshly hatched larvae with a pipet.

Thomas et al (9) accomplished inoculation with an automatic syringe, which delivered a 2.3-cc aliquot of eggs and larvae. Three aliquots were added to each pot (7.6 cm diameter) filled with sterilized sand. Single seedlings were later transplanted into pots. To prepare inoculum, cysts were rubbed off from roots in water and caught on a 60-mesh sieve. The cysts were broken by forcing them through an 80-mesh sieve. The inoculum was finally collected on a 400-mesh sieve.

Leudders (5) inoculated individual seedlings with a clinical syringe. A magnetic stir bar and stirrer were used to maintain a uniform suspension of inoculum. One thousand eggs were pipetted into fine sand in plastic pipes (2.5 cm diameter × 15.0 cm long). One seedling was transplanted into each pipe and covered with vermiculite. In another method of inoculation (1), two soybean seeds were planted in an 8-cm-diameter pot filled with infested soil. A 100-g sample of soil contained approximately 20 cysts. Recently, an inoculation technique using mouth pipetting (described in Materials and Methods) has been used in evaluating soybean seedlings for SCN resistance (2,6–8).

Most of the reported methods of inoculation are useful for inoculating a relatively small number of soybean seedlings. The method described here was developed to dispense inoculum more rapidly and reliably than existing methods of inoculation to test large numbers of seedlings for reaction to SCN. The new method was also compared with our mouth-pipetting method of inoculation.

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MATERIALS AND METHODS

Inoculum preparation. A population of the SCN was obtained from a field that had a history of race 3 infestation at the Ames Plantation, near Grand Junction, TN (courtesy of L. D. Young, USDA-ARS, Jackson, TN). The population was increased and maintained on a standard susceptible cultivar, Essex, in a greenhouse at the Delta Center, University of Missouri, Portageville. Plants were removed after 30–35 days and the roots were washed with a strong jet of water to dislodge the cysts onto a 60-mesh sieve. White cysts were chosen selectively and crushed to release eggs and larvae. Eggs from such females usually hatched uniformly. Larvae from those eggs often produced desirable infestation of more than 100 cysts on each susceptible test plant within 30 days. The egg suspension was then screened through a 120-mesh sieve to remove debris. The inoculum was diluted with water to 200 eggs and larvae per milliliter.

Growth of seedlings. Soybean seeds were germinated in vermiculite. Plastic micropots (200 mm long × 25 mm diameter) were filled with steam-pasteurized Brosely fine sandy soil. The soil was adjusted to pH 6.5 with $Ca(OH_2)$ before the micropots were filled.

A single 4-day-old seedling with a 15- to 20-mm-long radicle was transplanted into each micropot. Approximately 19–20 micropots were placed in a plastic container (20 cm diameter) and maintained at 27 ± 1 C in a water bath.

Inoculation method. The inoculum was maintained in a reservoir made from a 2-L plastic beverage container with the bottom removed. A hole had been drilled in the lid of the container to accommodate an air hose attached to an aquarium pump. Eggs and larvae were kept in suspension by air bubbling continuously through the air stone. The container was inverted and placed on a ring stand. One tube from an automatic pipetter (Brewer automatic pipetting machine, Scientific Equipment Products, Baltimore, MD) (Fig. 1) was placed in the reservoir and the pipetter was set to remove a 5-ml aliquot of inoculum from the reservoir every 2.5 sec. To assure uniform dispersal of inoculum throughout the system, the delivery tube (a disposable micropipet) was also placed in the reservoir before inoculation and inoculum was recirculated for at least 5 min. Tygon flexible plastic tubing, which was available in our laboratory, was used for the uptake and dispensing tubes.

A total of 12,000 seedlings (600 Essex, 600 each of four host differentials [Peking, Pickett-71, PI 90763, and PI 88788], and 9,000 F_2 and F_3 progenies from several crosses) were inoculated with an automatic pipetter in 10 different tests.

Similar numbers of plants (10 different tests) were also inoculated with a mouth-pipetting method. This method required two individuals to perform inoculation. One person kept the eggs and larvae in suspension in a reservoir. A second person mouth-pipetted a 5-ml aliquot of suspension from the reservoir, containing (1,000 ± 38) eggs, larvae, and inoculated seedlings. The inoculum was delivered close to each root area in an opening (4–5 cm depth) and the opening was covered after inoculation.

Thirty days after inoculation, micropots were soaked in water and plants were gently removed. The roots were washed with a strong jet of water to dislodge the cysts, and the cysts were counted under a stereo microscope. Index of parasitism was used to classify plants as susceptible (number of cysts

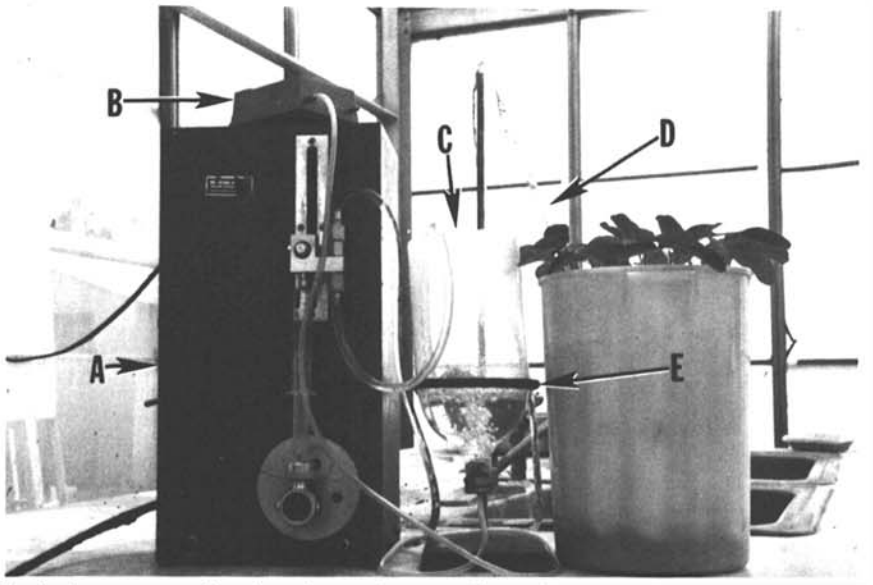

Fig. 1. Apparatus used to inoculate soybean seedlings with eggs and larvae of *Heterodera glycines* race 3: **(A)** auto dispenser, **(B)** aquarium pump, **(C)** a reservoir for inoculum, **(D)** delivery tube with micropots holding soybean seedlings, and **(E)** ring stand.

Table 1. Comparison of two pipetting methods for recovery of cysts of *Heterodera glycines* on *Glycine max* seedlings[a]

Method	Hours required	People required	Cysts			Index of parasitism
			Range	Mean no.	SD	
Mouth pipetting	150.0	2	120–212	166	46	17
Auto pipetting	8.5	1	160–184	172	34	17

[a]Each method was tested in 10 tests on 2,400 host differentials, 9,000 F_2 and F_3 progenies, and 600 Essex plants.

recovered was ≥10% of the number on Essex) or resistant (number of cysts recovered was <10% of the number on Essex) (4).

Data were collected on the time required to inoculate 12,000 seedlings, the range in number of cysts, and the mean number of cysts based on 600 susceptible Essex plants. A standard deviation for number of cysts produced on 600 susceptible Essex plants in each of two methods was calculated. Because each test represented a different soybean cross and results provided a different genetic ratio in F_2 and F_3 progenies, data were obtained only for the time required for their inoculation.

RESULTS AND DISCUSSION

The mean number of cysts obtained on 600 plants of susceptible cv. Essex were comparable in both methods. The host differentials were resistant to SCN race 3 and the means of numbers of cysts obtained on them were nearly zero in both of the methods (*data not shown*). The range of the number of cysts on 600 Essex plants was 120–212 for the mouth-pipetting method and 160–184 for the auto-dispenser method (Table 1).

The two methods of inoculation were also compared for efficiency relative to time required. A total of 12,000 soybean seedlings were inoculated by two individuals in a total of 150 hr with the mouth-pipetting method. With an automatic dispenser, a single individual required just 8.5 hr to complete the task. Estimated labor cost (approximately $5.50 per hour) was $825 for the mouth-pipetting method and only $47 for the auto-dispenser method.

The advantage of the automatic dispenser method of inoculation is improved efficiency, which results from increased rapidity and reliability. In greenhouse tests, inoculation with the mouth-pipetting method required approximately 45 sec per seedling. With the automatic dispenser, a single seedling was inoculated in approximately 2.5 sec. The new method is approximately 18 times faster than the mouth-pipetting method and requires only one person. With inoculation by the automatic dispenser, 25 aliquot samples per test selected at random had equal numbers of eggs and larvae (1,000 ± 8) delivered per seedling. This was confirmed by a narrow range of the number cysts produced on susceptible cv. Essex (Table 1). Neither method changed the index of parasitism (rounded to the nearest whole number), however, a lower standard of deviation resulted from the auto-dispenser method.

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