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A Modified Screening Test for Determining *Heterodera glycines* Resistance in Soybean[1]

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Abstract: A modified version of a standard greenhouse bioassay for determining soybean cyst resistance in soybean plants is described. White plastic laundry tubs served as microplots for rearing large numbers of nematodes in a confined space; up to 3 million eggs of each generation were collected per tub. Before screening, SCN populations were evaluated on susceptible and resistant soybean to characterize female development; these were periodically retested. Screening tests took place in Todd planter flats (120 plants per flat). Test plants were inoculated with 1,200 eggs per plant and evaluated for resistance 33–37 days after inoculation. The plants were pruned at the cotyledonary node which resulted in a greatly reduced root system. Staining the roots in Toluidine Blue created contrast with the white females and facilitated counting. Greenhouse space was conserved, and the labor to set up and maintain the screening test was reduced.

Key words: bioassay, cyst nematode, *Glycine max*, *Heterodera glycines*, screening, soybean.

Identification of soybean (*Glycine max* (L.) Merr.) resistant to the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) can be accomplished only by bioassay of nematode and host. The standard criterion for determining resistance is low numbers of females developing to maturity as compared to the number recovered from a known susceptible soybean genotype (2). The bioassay requires only that comparable numbers of mature eggs or infective juveniles be inoculated to healthy root systems under conditions favorable to penetration and development.

Several bioassay techniques have been reported. Lauritis et al. (4) demonstrated that excised soybean roots in culture will provide the same resistant or susceptible response to SCN as intact plants of the same cultivar grown in soil. Halbrendt (unpubl.) showed that the adventitious roots of soybean leaf cuttings also displayed the same response to SCN as the plant from which they were taken. Halbrendt and Dropkin (3) described a technique for maintaining severely pruned, SCN-infected soybean under hydroponic conditions that resulted in the same resistant and susceptible responses previously identified for SCN-soybean combinations. Soybean breeders typically screen large numbers of breeding lines in greenhouse pots to identify resistant progeny. The soybeans are grown in SCN infested soil, and the roots lining the pot are examined for the presence of mature females at the end of one generation.

The primary objective of this study was to design a reliable greenhouse test for SCN resistance that reduces space and labor relative to the standard greenhouse test. Our method was a modified greenhouse bioassay which employed pruning to greatly reduce the size of plants and roots. Our studies also examined an efficient method of rearing and recovering SCN inoculum.

Materials and Methods

Inoculum: Five populations of *Heterodera glycines* were established in the greenhouse. The South Carolina Gene Pool Population (SCGP) was initiated with eggs recovered from 'Braxton' soybean grown 35 days in naturally infested field soil taken from four locations in South Carolina. Similarly, eggs recovered from Braxton grown in two other soil samples provided the inoculum to establish the Ebenezer population (Eb), and the Youngblood population (Yb). These three populations were constantly maintained on Braxton and 'Essex' soybean. 'Pickett' and 'Peking' soybean were later grown in these same soil samples and the eggs recovered from each were

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used to initiate the Pickett (Pick) and Peking (Pek) populations, respectively; these were maintained on their selecting hosts.

The SCN populations were maintained in white plastic laundry tubs 52 × 52 × 31 cm deep. The drain of each tub was fitted with a double layer of nylon window screen and covered with an inverted clay saucer before filling with soil. A mixture of sand and sandy loam soil, 1:1 (v:v), that had been fumigated previously with methyl bromide was infested with nematode eggs. The tubs were filled and one-half of each tub was planted with 18–21 seedlings. Two weeks later the remaining half of each tub was planted, and approximately 35 days were allotted for nematode development. Females with eggs were collected every second or third week from each tub. No further reinfestation of the soil was necessary; however, the soil required periodic dilution to permit soybean growth. Approximately every other generation, the soil was diluted by 10–25%.

Roots were dug from the soil and gently rinsed before spraying them with a strong jet of water over a 430-μm-pore (25-mesh) sieve nested over a 131-μm-pore (80-mesh) sieve. Females and a small amount of debris were recovered from the 80-mesh sieve. Eggs were liberated from females by grinding nematodes and debris on a 110-μm-pore (100-mesh) sieve with a rubber stopper and collecting the washing. The eggs were separated from this mixture by centrifugation in a sugar gradient (1) and incubated 1–2 days at 27 C before they were used as inoculum.

Screening: Todd planter trays (Speedling Incorporated, Sun City, FL) containing 128 chambers per tray were filled with clean river bottom sand previously sifted through hardware cloth (0.64-cm aperture). A Pasteur pipette was used to deliver a 1-ml water aliquot of 1,200 eggs 5 cm deep into each planting chamber. One soybean seedling (2.5–5-cm radicle) germinated 3 days at 27 C in regular weight seed germination paper (Anchor Paper, St. Paul, MN) was transplanted to each chamber. Each tray contained three controls and 21 soybean lines with five replicates. The soybean selected as controls varied with each screening test, so as to include the parental cultivars of the lines being screened, but always included a susceptible, a race 3 resistant, and a race 4 resistant cultivar. The sand was covered with a layer of vermiculite and given a light watering. Trays were placed on plastic-lined benches in the greenhouse and maintained at 27 C. During summer months, moist paper towels were placed over the seedlings for the first day or two. Nematode penetration seemed to improve if the top layer of vermiculite and sand was allowed to become slightly dry during the first few days in the greenhouse. After appearance of unifoliate leaves, the stem was severed above the cotyledonary node. Subsequent growth appearing from the cotyledonary node was also removed as new leaves appeared. Most plants required two prunings at 6–7 and 12–14 days postinoculation.

Soybean plants were harvested 33–37 days postinoculation by first shaving excess roots from the tray bottom and then inserting a short metal rod to loosen the plants. A gentle pull on the hypocotyl removed the root and sand together without dislodging females. Roots were rinsed in water to remove adhering sand and placed in a solution of Toluidine Blue that contained 1 part stock : 80 parts 10% glacial acetic acid for a minimum of 30 minutes up to 24 hours. The stock solution of Toluidine Blue contained 0.5 g Toluidine Blue and 1.0 g sodium borate in 100 ml distilled water. After staining, roots were kept in water. Since SCN do not take up the stain, white females stood out against the blue roots which facilitated counting. Nematode counts were made by holding the roots against a black background under an illuminated magnifying lens. The stain was refreshed after each screening test with ca. 5 ml stock solution and 5 ml glacial acetic acid per liter of used stain and reused.

Inoculum collection: An experiment to determine the optimal time for collection of inoculum was set up with the SCGP population. Braxton soybean was grown 27,

34, and 48 days in infested soil, and eggs were extracted from females that developed on each age group of plants. Aliquots of 1,100 eggs from each batch of eggs were inoculated to 40 Braxton soybean seedlings (eight replicates of five subsamples) according to our method for screening. Thirty-three days postinoculation, the roots were processed and female counts taken. The mean was calculated for each replicate and for the total plants of each egg harvest date.

Experimental variability: A second experiment with the SCGP population demonstrated the variability of female counts encountered. One hundred Braxton and one hundred 'Centennial' soybean (20 replicates of five plants) were inoculated with 1,200 eggs. Thirty-three days after inoculation roots were processed; the mean number of females and standard deviation was calculated for each replicate.

Results and Discussion

Plastic laundry tubs hold a large volume of soil, provide good drainage and resist heat buildup in the greenhouse. These features provided excellent conditions for rearing large populations of the soybean cyst nematode with efficient use of bench space. Egg inoculum was more difficult to recover from soybeans grown only 27 days in infested soil than from older plants because females were smaller and more difficult to dislodge. The lower number of females appearing at the end of a bioassay with eggs from 27-day-old plants suggests that fewer mature eggs were present than among eggs recovered from females developing on older plants (Table 1). Eggs obtained from females on 48-day-old plants did not produce a greater number of females at the end of the bioassay than did those from 34-day-old plants, suggesting that the percentage of mature unhatched eggs remained relatively constant. Because there is no advantage to rearing inoculum for a longer period of time, we have adopted the practice of harvesting eggs after approximately 35 days of soybean growth. We have also found that incubating eggs at 27 C for 1–2 days before inoculation increases the number of females that develop in the bioassay, presumably because more eggs become fully mature.

Table 1. Mean number of females counted from Braxton soybean 33 days after inoculation with 1,100 eggs (eight replicates of five plants).

Age of soybean providing inoculum	Range of means among reps		Mean of total
	Low	High	
27 days	22	33	28
34 days	31	52	37
48 days	28	50	38

The variability in female numbers that was typical of resistant and susceptible SCN-soybean combinations with our technique and SCN populations is presented in Table 2. Standard deviations calculated for the mean values from five plants show that counts may deviate considerably from the mean. Variability in female numbers seems inherent in SCN-soybean bioassays (3), and therefore detection of soybean lines that contain SCN resistance depends largely upon the degree to which the SCN populations are affected by resistance. The level of SCN resistance expressed by a resistant

Table 2. Mean number of females and standard deviation (SD) from a susceptible (Braxton) and race 3 resistant (Centennial) soybean cultivar 33 days after inoculation with 1,200 SCN eggs (20 replicates of five plants).

Soybean	Range of means among reps				Mean of total (100 plants)	
	Low		High			
	Mean	SD	Mean	SD	Mean	SD
Braxton	13.4	6.8	28.2	9.4	19.2	10.9
Centennial	2.8	2.6	6.6	3.2	4.6	3.2

TABLE 3. Reliability of screening tests based on the percent of accurate determinations of susceptible and resistant soybean cultivars infected with known SCN populations.

	Susceptible	Resistant
SCGP population (race 3)		
Number of times tested	100	185
% accurate determinations	99%	100%
Combined results Eb, Yb, Pek, and Pick populations (race 4)		
Number of times tested	148	74
% accurate determinations	99%	95%

Each determination is based on the mean value from five plants.

soybean is determined by the frequency of specific virulence genes in the SCN population used in the bioassay (5). Centennial soybean for example is resistant if tested with race 3 SCN but susceptible if tested with race 4 SCN. We used the level of resistance expressed by the resistant soybean parent compared with susceptible controls as the criterion for identifying resistant soybean progeny. Our SCGP SCN population was affected by race 3 resistance and produced female counts on race 3 resistant soybeans that were 10–27% that of susceptible controls. A similar level of resistance was detected with SCN populations Eb, Yb, Pek, and Pick to race 4 resistance but not to race 3 resistance.

An estimate of the reliability of this resistance screening technique has been made by recording the number of times that resistant and susceptible controls have actually been determined resistant and susceptible on the basis of nematode counts (Table 3). The SCGP population provided an excellent bioassay for race 3 resistance, accurately demonstrating resistance in 100% of the race 3 resistant controls and only 1% error in identifying susceptible controls. The combined results of all race 4 resistance screening tests using either the Eb, Yb, Pek, or Pick populations for the bioassay resulted in determinations of race 4 resistant controls with 95% reliability and susceptible controls with 99% reliability.

We consider our greenhouse screening technique to be an efficient and reliable method for detecting resistance in soybean breeding lines. SCN populations used in the program are first characterized for their ability to develop on different sources of SCN resistance and are periodically rechecked to insure consistent performance. Pruning soybean results in a greatly reduced root system that facilitates counting of females. Additional benefits derived from pruning include conservation of greenhouse space and potting medium, reduced labor to set up a screening test, and a reduction in the amount of watering necessary to maintain the plants.

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