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Soil Moisture Control and Direct Seeding for Bioassay of *Heterodera glycines* on Soybean[1]

S. SARDANELLI[2] AND W. J. KENWORTHY[3]

Abstract: Soil moisture control during evaluations of *Heterodera glycines-Glycine max* interactions has not been reported routinely as a standardized procedure. A novel soil moisture replacement system was examined in controlled environmental chambers for use in bioassays for female development. The system is compact, lightweight, and has a contained reservoir for moisture supply to multiple test units. Varied soil moisture treatment levels were sustained at or near replacement rates over extended periods of testing. Direct seeding of selected soybean cultivars consistently resulted in 100% seed germination. Subsequent shoot and root growth was successfully restricted to accommodate the size of the system with minimal shoot pruning. Numbers of mature *H. glycines* females extracted from the roots of susceptible soybean cultivars were consistently high. Inoculum levels of either 500 or 1,000 eggs/plant routinely resulted in numbers of females at more than 30% of the initial inoculum. No evidence of nematode contamination of uninfested plants was found at any level of observation. Results demonstrate a potential for the standardization of two additional variables in determining races and for screening cultivars or lines for resistance to *H. glycines*.

Key words: bioassay, direct seeding, *Glycine max, Heterodera glycines,* method, race determination, resistance, screening, soil moisture control, soybean, soybean cyst nematode.

Management of the soybean cyst nematode, *Heterodera glycines* Ichinohe, is a principal factor in the production of soybean, *Glycine max* (L.) Merr. (Wrather et al., 1984). Reported as a pathogen to soybean worldwide, *H. glycines* has been detected in most soybean-producing states in the United States (Schmitt and Riggs, 1989).

Variable development of females of *H. glycines* on soybean cultivars has been reported from existing race test methods (Riggs and Schmitt, 1988; Riggs et al., 1988). Standardization of seeding, inoculation, and temperature regimens in race testing have been studied (Riggs and Schmitt, 1991). Although optimum procedures or levels for these parameters were identified, the numbers of females reported in the tests were variable. The effect of soil moisture on *H. glycines* bioassay performance was not examined.

The lack of consistency or the variation in the numbers of females reported in bioassays of *H. glycines* population levels in soil or in race tests may be related to the following: i) lack of consistency in watering practices (widely fluctuating soil moisture levels), ii) variability in seed germination and subsequent plant growth with direct seeding practices, iii) the possibility of undetected root injury during transplant of test seedlings (Halbrendt, 1992). In an effort to address the above, a soil moisture replacement system was designed, tested, and disclosed (Sardanelli et al., 1995).

The underlying principle of the system is the transport of water via wicking, after establishment of a hydraulic gradient, from an enclosed bottom reservoir upward to the rooting medium. In this paper we report the results of a thorough study conducted to test the system. The main objectives in testing the apparatus were to evaluate: i) the efficacy for soil moisture replacement over time; ii) the practice of direct seeding and quality of subsequent plant growth within the system; iii) *H. glycines* life-cycle completion as manifested in female maturation; iv)

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[2] Laboratory Scientist, Department of Plant Biology, University of Maryland, College Park, MD 20742.

[3] Professor, Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742.

E-mail: ss11@umail.umd.edu

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the system's efficiency in conservation of materials, space, and labor.

Materials and Methods

Moisture replacement system (MRS) construction: The main components of the system included a plant container with wick insert, a holding rack for container support, and an enclosed bottom water reservoir. Two models of the system were used in this study.

Bioassay model: This model of the MRS was used for water transport, plant growth, and bioassay experiments. The main body was a white Polyfoam Freeze Safe stock insulated container (Model #2310-6L, Packers, Wheeling, IL) (Fig. 1A,B). Exterior dimensions were 62.5 × 30 × 20 cm, and interior dimensions were 57.5 × 25 × 15 cm. The upper half was the holding rack, with slots cut with a 3-cm-diam hole saw through the top surface (Fig. 1A). Slots were for container insertion and support. Two slot-spacing patterns were used: i) 27 3-cm-diam. slots with staggered centers 7.5 cm apart, and ii) 36 3-cm-diam. slots evenly spaced with centers 7.2 cm apart.

Each slot supported one grow tube (disposable sterile polypropylene centrifuge tube, tapered bottom end, 50-ml capacity; Fig. 1C) above the water surface of the reservoir at maximum capacity. Wicking made of 0.3-cm-diam. Lehigh nylon rope (Lehigh, Allentown, PA) was coated with 0.35-cm-diam. Cole-Flex irradiated polyolefin heat-shrink tubing (shrinks 50%) (Cole-Flex, W. Babylon, NY). The nylon rope was threaded through the shrink tubing and placed in a 121 °C dry oven for 8 minutes. The treated wicking, 15 cm in length, was inserted through a 0.5-cm-diam. opening in the center bottom of the grow tube. The tubing was stripped from a 2-cm length at each end. The wick was inserted within the tube to a height defined for the particular experiment. The remaining length of the wick extended through the center bottom of the tube and into the reservoir, which was the lower half of the container (Fig. 1B). This portion of the wick was in direct contact with the bottom of the reservoir or a few centimeters higher, depending on the wick height within the grow tube.

The reservoir was lined with a disposable plastic insert and held a maximum volume of 9 liters. When the holding rack was set onto the reservoir and the grow tubes placed in the slots, an airspace of ca. 3 cm lay between the bottoms of the tubes and the water surface in the filled reservoir. For ease in movement, the reservoir was not filled to capacity until the system was situated in the permanent test location. The reservoir was then filled with water through the open slot created by temporary removal of a grow tube.

Culture model: This model was used for the establishment, maintenance, and increase of stock cultures for test inoculum. Modifications from the bioassay model were: i) the holding rack had ten 7-cm-diam. slots—five slots along the length with centers 12.5 cm apart, and two slots per width with centers 15 cm apart; ii) each slot supported one 250-ml plastic beaker with the open top of 7 cm o.d. tapering to a closed bottom of 5.4 cm o.d.; iii) the wicking was 0.45 cm in diameter and coated with 0.5-cm-diam. shrink tubing. The wick was inserted to a height of 6.5 cm within the beaker and extended 11 cm from the bottom of the beaker to the reservoir bottom.

Rooting medium: Greenhouse stock builder's sand was used for cultures and all tests. Sand was steam-sterilized, air-dried, passed through an 850-µm-pore sieve to remove gravel and any extraneous materials, and stored in covered bins. Each batch was analyzed for texture and pH.

Moisture volume percentage (MVP) of the rooting medium: Water uptake, plant growth, and *H. glycines* bioassay evaluations were conducted with an initially established soil moisture content that varied according to treatment. Soil moisture treatment levels were established as the required ratio of water to dry rooting medium by volume (MVP = water volume ÷ dry medium volume × 100; Brady, 1974). To prepare the system for container insertion prior to moistening of the sand, ca. 360 ml tap water was added to the reservoir and the holding rack was seated

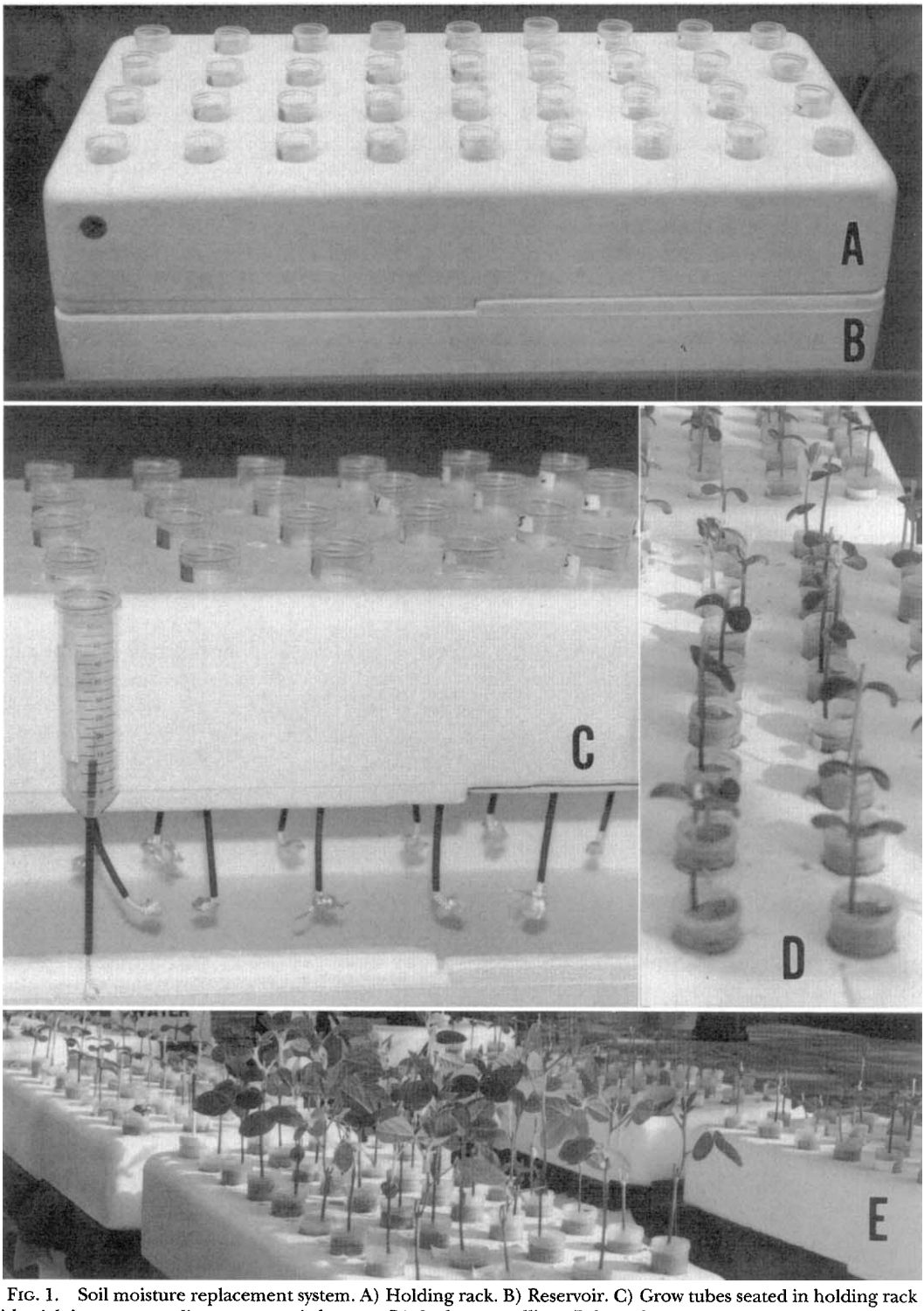

FIG. 1. Soil moisture replacement system. A) Holding rack. B) Reservoir. C) Grow tubes seated in holding rack with wick inserts extending to reservoir bottom. D) Soybean seedlings 7 days after pruning above cotyledons. E) Soybean growth 33 days after soil infestation.

onto the reservoir. As containers were filled with sand they were immediately placed into the holding rack. In water transport and plant growth experiments, dry sand and each grow tube with wick insert were weighed individually prior to setup for calculation of MVP after data collection (Brady, 1974). The weighed volume of dry sand (50 cm^3) was then mixed with the treatment volume of tap water and immediately funneled into the grow tube. In culture and bioassay experiments, the total amount of sand for the entire test was moistened to the desired MVP immediately before the beakers or grow tubes were filled. Moistened sand was added around the central wick with gentle tapping of the beaker or tube at intervals to allow for a uniform settling of the medium. To determine the volume of water held in the wick as opposed to the sand, 35 prepared wicks were weighed individually and then submerged in water prior to use on tubes. To ensure saturation, the wicks were removed each day, free water was blotted away, and weights were determined for a total of 3 days for weight stabilization. The average added water weight was 0.6 g. In addition, dry plant weights were determined at the termination of plant growth experiments and averaged. Wick water weights and (or) dry plant weight were included as a correction factor for determination of MVP for all experiments.

Water transport: Because subjective observations indicated that water was wicked into a moist and not a dry rooting medium, the effect of sand moisture content on subsequent water transport from the bottom reservoir was tested. This experiment was conducted for a total of 36 days in the bioassay model with 27 slots. Wicks were 7.5 cm high within each grow tube in the rack. Treatments consisted of three MVPs (7.5%, 30%, and 50%) with eight replicates. Weighed dry sand (96% sand, 0% silt, 4% clay; pH 8.1) was mixed for even moisture distribution with 3 ml tap water for each individual grow tube. Eight replicates at 7.5%, 30%, and 50% MVP were prepared by respective addition of 0, 12, and 20 ml tap water to the initial 3 ml moistened sand. For each treatment, an additional grow tube was prepared and a thermometer was centered in the bottom. After 8 days on a laboratory bench (means of 21 °C and 32% RH), the system was placed into a controlled environment growth chamber at 27 °C and maintained at a 12:12-hour light:dark period with cool white fluorescent lamps (100 $\mu watt/m^2/sec$). RH varied from 10% to 15% in the growth chambers. All experiments conducted in growth chambers had the same environmental parameters. To monitor the status of MVP over time, visual observations and weights were recorded for each grow tube on days 0, 2, 5, 8, 12, 15, 19, 23, 27, and 36 of the experiment.

Direct seeding and plant growth: Experiments were designed to evaluate the performance of direct seeding, determine the effect of the height of the stripped portion of wick within the grow tube on plant growth and moisture delivery, and accommodate plant growth to the size of the system by shoot pruning. In one experiment with three treatment replicates, the 27-slot rack was used; in a second experiment with four treatment replicates, the 36-slot rack was used. Grow tubes were filled with builder's sand (96% sand, 0% silt, 4% clay; pH 8.1) at an MVP of 30%, based upon results from the moisture percentage experiments. Two wick heights within the grow tubes, 7.5 cm and 5.0 cm, were the main treatments. Tubes were planted with one seed per tube in the direct center at a 2.5-cm depth. Soybean cultivars Essex, Hartwig, Peking, and PI88788 were used. Weights were recorded for each individual grow tube at setup and days 7, 16, 22, and 29 of the experiment to monitor MVP. Plant growth was monitored to determine stage of pruning for control of plant growth. Each system had three randomly placed grow tubes with thermometers inserted in the direct center to half depth. The systems were maintained in separate growth chambers. Fresh and dry root weights for test plants were determined at the end of each experiment.

Foliar discoloration was observed on all seedlings in the plant growth experiments with the previously unused wicking treat-

ment. To clarify probable cause, the experiments were repeated with previously used wicking only.

Stock cultures: An *H. glycines* isolate, previously classified as race 3, was obtained from stock greenhouse cultures initiated by 10 successive single female transfers and maintained on Essex soybean (S. Anand, pers. comm.). This isolate was increased and maintained in the culture model on Essex to provide inoculum for the bioassay experiments. Because of previously observed foliar browning and precipitate formation on the sand surface when plants were grown in tubes with new wicking, the model was preconditioned by running it for 3 weeks before plants were included. The sand was discarded, and beakers were rinsed with tap water and autoclaved. Immediately before seeding, each of the beakers in a model was filled with 240 cm^3 builder's sand moistened to 21% MVP (i.e., 500 ml tap water/2,400 ml sand for 10 beakers). Three seeds of Essex were planted 2.5 cm deep in a triangular pattern ca. 1.5 cm from the wall of the beaker. Seven days after seeding, seedlings were pruned just above the cotyledons. Cultures were initiated with 20 gravid white females from a tap water-filled vial placed in deep watch glasses with 2 ml tap water and broken open with forceps to release eggs. A 2.5-cm depression was made between each two seedlings, and the contents of one watch glass were washed into each depression and covered. Prunings of regrowth were done as needed during the life of the culture. Culture transfer was done every 30 to 33 days with gravid white females collected as in bioassay inoculum preparation.

General bioassay: Wick heights were adjusted to 5.0 cm within the grow tubes, as plant growth experiments demonstrated a greater moisture uptake than water transport experiments. MVP of the builder's sand was established at 24%, based on the plant growth study results. Individual grow tubes were randomly monitored during testing for moisture level. Seeding was done as in plant growth experiments. Test plants were pruned just above the cotyledons on the seventh day after seeding (Fig. 1D). Gravid white females were obtained from 33-day-old stock cultures, and the eggs were released for use as inoculum immediately prior to soil infestation.

For inoculum collection, a culture beaker was removed from the system, submerged on its side in tepid tap water, and allowed to saturate for ca. 40 seconds. The seedlings with the root ball were pulled free, and excess sand was carefully washed from the root mass. Females were massaged from the roots under a stream of tap water onto nested 850-µm- and 149-µm-pore sieves. Washings remaining in the bucket were roiled and poured through the sieves to collect additional females. The females were crushed on the 149-µm-pore sieve with a rubber stopper and rinsed repeatedly with tap water from a wash bottle. The eggs were collected on a 25-µm-pore sieve. Eggs were added to the grow tubes within the growth chamber 7 days after seeding. A 1-ml suspension of eggs was pipetted to a depth of 2.5 cm at ca. 0.5 cm from the base of the test seedling.

Upon termination of the experiments, females were extracted from the roots and the sand as in inoculum preparation, collected on filter paper, and counted (Krusberg et al., 1994). Fresh root weights were determined for test plants at the end of the experiments.

Inoculum density and female development: Evaluations of inoculum density and rate of female development within the system were performed in two different sources of builder's sand to examine the effect of sand source on bioassay response. The two sand batches were 98% sand, 1% silt, 1% clay (pH 6.2) and 97% sand, 1% silt, 2% clay (pH 7.1). Essex was the host cultivar, and each seedling was inoculated with 1,000 eggs. Soybean root systems were harvested at 21, 27, and 33 days after inoculation (DAI) with 10 replications at each harvest.

Cultivar and inoculum level response: An experiment was conducted to evaluate growth response of additional soybean cultivars, *H. glycines* inoculum density, and female maturation within the system. Soil particle analysis was 98% sand, 1% silt, and 1% clay at pH 6.2. Soybean cultivars tested were Essex,

Hartwig, and Lee. Inoculum levels were 500 or 1,000 eggs/tube, and treatments were replicated four times. Soybean roots were harvested 27 (DAI).

Contamination evaluation: Because the system used a common reservoir for all wick transport of water, a test was conducted to address the possibility of contamination within the system. Sand used was as in cultivar and inoculum level testing. Essex was the host, and treatments were the uninfested control and infestation with 1,000 eggs/tube. Each treatment was replicated 15 times. Soybean roots were harvested 30 DAI. At test termination, water remaining in the reservoir was passed through a 25-μm-pore sieve as an additional check for nematode contamination.

General procedures: No additional moisture was added other than the water supplied by wick transport from the original fill of the system reservoir. As a precautionary measure, tap water-washed grow tubes and beakers (with wick inserts) were autoclaved at 121 °C for 30 minutes after each test run. Breeder-selected seed from uniform stock was used in all bioassays and evaluations of plant growth. No plant nutrients were added. For the *H. glycines* evaluations, extracted females were divided by the initial inoculum level to determine relative female development after inoculation. All experiments were repeated once. With the exception of the direct seeding and plant growth experiments, results of the two runs of each test were similar ($P = 0.05$) and data were combined for analysis. Experimental treatments and replications were completely randomized. Data were examined by analysis of variance, followed by mean separation with least significant differences (LSD) (MSTAT-C, Michigan State University, East Lansing, MI).

Results

Water transport: There was no change in moisture level in the 7.5% and 30% MVP soil moisture treatments for the duration of the 36-day experiment ($P \leq 0.01$). The 50% MVP treatment dropped to 41% MVP by day 2 and remained in that range for the remainder of the test. All replicates of the 30% MVP and 50% MVP treatments appeared moist throughout their soil profile during the test. All replicates for the 7.5% treatment were dry to a depth of ca. 1.0–1.5 cm at the end of the test. At all three moisture levels, soil temperatures averaged 2 °C above air temperature.

Direct seeding and plant growth: Both rack patterns of the bioassay system provided similar plant growth data. By the third day after planting, 100% of the seed had germinated. From this point throughout testing, progressive root growth could be seen through the lateral surface of the grow tube. Subsequent seedling growth was uniform. One foliar pruning was done above the unifoliolate node at the unfolding of the first trifoliolate leaf. On the ninth day of testing, browning of the leaf tips began to appear on all test plants in grow tubes with new wicking. Subsequent growth of these plants had no evidence of browning, but a white precipitate was observed on the surface of the sand in the grow tubes. Differences in mean fresh shoot weights for the used and new wicking (1.1 g and 0.9 g, respectively) were not significant. Dry shoot weights averaged 0.2 g. Cultivars were similar in their response to wicking source in both fresh shoot and root weights. However, within each cultivar, the average fresh root weight of 1.7 g for the used wicking treatment was greater than the 0.5-g fresh root weight for the new wicking ($P \leq 0.01$). Root systems were otherwise asymptomatic. Height of the uncovered portion of the wicking at 5.0 cm within the grow tube resulted in higher final MVP than for the 7.5-cm height ($P \leq 0.01$). Moisture levels in the 27-slot pattern were 31% and 23% for the 5.0-cm and 7.5-cm wick heights, respectively. In the 36-slot pattern, moisture levels were 35% for the 5.0-cm wick height and 25% for the 7.5-cm wick height. The sand in all grow tubes was dry from the surface to approximately 0.5 cm deep from the sixth day of testing. The remainder of the soil profile was moist at the termination of the testing. Differences in sand and ambient temperatures were negligible.

The repeat evaluation, where only previously used wicking was tested, had no browning of the test plant foliage, and no white deposit was observed on the sand surface. Average fresh and dry root weights were 1.1 g and 0.1 g, respectively. Other than root performance, results were similar to the first run.

Inoculum density and female development: The greatest numbers of females were extracted from test plants grown in the builder's sand with pH 7.1 at 27 DAI and 33 DAI (Table 1). In both tests, more females were extracted at 33 DAI and 27 DAI than at 21 DAI ($P \leq 0.01$). Mean fresh root weights at test termination were similar for all harvest dates within a test ($P \leq 0.01$). However, average fresh root weights at pH 7.1 were 50% less than those tested at pH 6.2 (Table 1). Root systems in general appeared to be compact and dense with the top pruning done just above the cotyledons. MVP of the builder's sand at test termination was ca. 28%.

Cultivar and inoculum level response: Root growth was similar among cultivars (mean fresh root weight 0.86 g, $P \leq 0.05$). Mean numbers of females extracted from Essex and Lee test plants were more than 30% of the initial inoculum for both inoculum levels. Mature females were not observed on or extracted from Hartwig roots. MVP at test termination remained within the initially established range.

Contamination evaluation: The infested treatments in both tests had mean productions of 385 and 366 mature females for the first and second runs, respectively. No females were extracted from the uninoculated treatment. Mean fresh root weights were 0.67 g for the inoculated plants and 0.85 g for the uninoculated plants. No *H. glycines* juveniles were detected in the sievings from the water remaining in the reservoirs at the termination of testing. Soil moisture replacement was consistent with previous results.

TABLE 1. Effect of builder's sand composition on development of females of *Heterodera glycines* and root weights of *Glycine max* 'Essex' when evaluated in bioassay systems maintained in controlled environmental chambers.

DAI[a]	Extracted females	Percent female development[b]	Root fresh weight (g)
	98% sand, 1% silt, 1% clay; pH 6.2		
21	199 ± 33b	20 ± 3b	1.0 ± 0.2b
27	311 ± 67a	31 ± 7a	1.0 ± 0.1b
33	305 ± 53a	31 ± 5a	1.8 ± 0.2a
	97% sand, 1% silt, 2% clay; pH 7.1		
21	243 ± 44b	24 ± 4b	0.5 ± 0.05a
27	372 ± 36a	37 ± 4a	0.6 ± 0.11a
33	402 ± 35a	40 ± 4a	0.4 ± 0.09b

Values are means ± SE of 20 replicates from two trials. Means in the same column followed by the same letter are not significantly different according to an LSD test ($P \leq 0.01$).

[a] Days after soil infestation.

[b] Extracted females divided by inoculum level of 1,000 eggs/root system.

Discussion

Results of this study indicate that this system is highly useful for bioassay of *H. glycines*. The two primary attributes are soil moisture control and direct seeding of soybean for *H. glycines* bioassay, which provide control possibilities for two additional variables during bioassay evaluations.

The initial water transport test was performed with builder's sand alone. Moisture levels were arbitrarily chosen to observe the potential of the hydraulic conductivity within the system once the particular hydraulic gradient was established. Moisture replacement over time was expected. However, the replacement and maintenance of the three distinct soil moisture levels for the 36-day period indicated a capability for a more precise and long-term application. In plant growth tests, increasing the height of the exposed wick within the grow tube resulted in lower soil moisture levels than at the lower height. Therefore, wick height as well as the initial soil moisture content can be manipulated to maintain desired levels of soil moisture.

Previous testing demonstrated the greatest reproduction by *H. glycines* in soil types with high sand content (Heatherly and Young, 1991; Schmitt et al., 1987). Builder's sand was a highly effective rooting medium for bioassay of *H. glycines* in this system. When the sand was premoistened at the desired level, containers were easily and rap-

idly filled. Direct seeding was quickly accomplished. Because root systems were easily rinsed free of sand at harvest, females were readily observed on the root surface and relatively clean females were extracted for enumeration.

Numerous investigations have focused upon the effects of soil moisture on various aspects of both nematode and host. With saturated or wet soil moisture conditions, the resulting low oxygen levels, carbon dioxide accumulation, and (or) toxin increase were associated with decreases in nematode survival, root invasion, and development rate. These effects were detrimental to plant growth, as well. Conversely, low moisture levels reduced nematode mobility and survival (Rebois, 1973; Wallace, 1971). Singh and Sharma (1995) demonstrated the effects of soil moisture on penetration, development, and reproduction of *Heterodera cajani* on pigeonpea (*Cajanus cajan*) in both growth chamber and greenhouse studies. Moisture treatments of 24% and 32% had the highest reproductive rates. In our study, trials were conducted with initially established moisture levels of both 24% and 30%, and excellent reproduction occurred at each level. Development of mature females of *H. glycines* initial inoculum averaged more than 30% of the initial inoculum on susceptible soybean cultivars. Previously reported race tests, using larger pots and higher inoculum levels, often had female numbers of less than 5% of the initial inoculum on Essex and Lee soybean (Riggs et al., 1988).

This study demonstrated the moisture replacement system as an environment conducive to development of *H. glycines* females. Broader application of this method may enhance future experimentation involving the effects of moisture stress on the interaction of *H. glycines* with soybean (Koenning and Barker, 1995). The role of additional edaphic factors might also be more precisely examined with the incorporation of moisture control. Greater *H. glycines* reproduction has been reported at pH 6.5 and 7.5 than at 5.5 (Anand et al., 1988). In our bioassay system with Essex, greater female development and a lower fresh root weight occurred at pH 7.1 than at pH 6.2.

The air space between the bottom of the grow tubes and the surface of the reservoir was designed to preclude the possibility of an exchange between the grow tube contents and the reservoir water. The contamination trials also demonstrated that second-stage juveniles (J2) did not enter the wicking and move against the hydraulic gradient into the reservoir.

Many bioassay evaluations often involve seedling transplant, wherein the occurrence or extent of root injury is an undetermined variable possibly altering test results. In one study, fewer *H. glycines* J2 entered the roots and fewer females developed in plants when root tips were removed at or before the time of inoculation (Halbrendt, 1992). In tests on postinoculation plant management, the greatest number of females developed on plants that were not transplanted after inoculation (Riggs and Schmitt, 1991). Therefore, one intent in system design was to incorporate direct seeding in order to eliminate effects of root injury on female development. The use of seed harvested within the test year resulted in 100% seed germination with uniformity in emergence and subsequent seedling growth.

Several soybean cultivars were evaluated in this system. Shoot pruning above the cotyledons was practiced in order to maintain manageable shoot and root growth for the size of the system (Halbrendt et al., 1987). In addition, the resultant compact root growth allowed ready observation of females with a hand lens. The inoculum was delivered to a depth of 2.5 cm, and examination at harvest revealed that most of the developed females were within the upper third of the root systems. This concentration of females would improve efficiency in screening for SCN resistance. Pot size had no significant effect on female numbers; therefore, there was no advantage to use of larger pots that take more space (Riggs and Schmitt, 1991). In other studies of shoot pruning (Anand et al., 1988; Halbrendt and Dropkin, 1986; Halbrendt et al., 1987), host-

parasite compatibility was unaffected by shoot pruning as done in these experiments.

The system provided efficient use of resources throughout the protocol for experimentation. The lightweight, compact polyfoam units are easily handled by operators and readily transported between locations. Movement is safely accomplished when the reservoir contains less than 2 liters of water. When top growth is minimized by pruning, the 36-slot rack spacing pattern gives the most efficient use of space. The transparency of the growing containers allows good visibility of both vertical moisture distribution and progress of root growth during bioassay.

No known finished container of similar construction and principle to the container component of this system is in existence. The wick insert seals and retains materials within the container. Also, the wicking provides distance transport of water with no direct contact between the container bottom and reservoir contents. Therefore unlike containers with bottom drainage openings, placement of multiple units into a common reservoir is feasible.

Quantities of sand for the rooting medium and inoculum for testing are minimal in comparison to standard pot testing. Therefore, the amount of labor required for all operations is greatly reduced. Quantities of nematode-contaminated roots and soil that must be sanitized subsequent to evaluation and prior to disposal are also substantially minimized. This fact, coupled with the containment of inoculum within the experimental unit, offers an environmentally sound alternative to bioassay procedures requiring materials on a larger scale.

A few precautions are essential for successful utilization of this system. Because soil moisture replacement is based on the initial establishment of the hydraulic gradient, the moistened rooting medium must be placed around the wick and to the desired height with intermittent firm, but gentle, tapping of the container. This vibration will yield a continuous column of soil. Moisture replacement in columns of soil that are either compacted or interrupted by gaps and large cracks was observed to be ineffective. This was evident in the observable drying of the medium within the transparent containers. The material used for wicking in this study should be preconditioned for at least 3 weeks in advance of experiments to avoid adverse plant response and formation of precipitates. Any alternative wicking should have preliminary trials. Rapid air movement that occurs in close contact with the sand surface in the containers, as can result with blowers in some growth chambers, results in undesirable surface moisture loss. As the soybean hypocotyl emerges and pushes through the soil surface, a layer of dry sand is moved up as well. The surface can be lightly moistened to correct this, but if heavy air movement continues the drying recurs and intensifies with time.

Evaluations are in progress for transfer of this system to greenhouse and laboratory environments for use in *H. glycines* race determination and soybean resistance screening. Should testing demonstrate a positive and consistent bioassay performance in these additional environments, a potential would exist for a broader application and an expansion of resources for experimentation.

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