

***Heterodera glycines* in Indiana: I. Reproduction of Geographical Isolates on Soybean Differentials¹**

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Abstract: Four of five geographical isolates of *Heterodera glycines* from Indiana classified as Race 3 using standard differentials showed many differences when classified using another group of differentials comprised of five soybean breeding lines and cultivars. Two isolates from northern Indiana produced cysts on more of the differentials tested than did three isolates from southern Indiana, suggesting that potential resistant lines should be tested on a range of *H. glycines* populations originating from the areas for which cultivars are being developed.

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

Heterodera glycines Ichinohe was discovered in southern Indiana in 1973 and more recently in northern areas of the state. Current knowledge that soybean cyst nematode (SCN) is widespread in northern Indiana has intensified a demand for resistant cultivars better suited to a short growing season than those presently available. Patterns of discovery of SCN (4,7,9), together with preliminary data on differences in the protein patterns of selected isolates (5), suggest that *H. glycines* may be indigenous to Indiana and that geographically separated populations may be very different. As part of a larger project to investigate variability among isolates in behavior, morphology, and protein patterns, and to facilitate development of SCN-resistant soybeans useful to Indiana growers, we selected five isolates of *H. glycines* from geographically separated areas of Indiana for detailed comparative study. Here we report comparisons of isolates from southern Indiana with those from northern Indiana in their ability to reproduce on known soybean differentials.

MATERIALS AND METHODS

Isolates of SCN were collected from separate fields in five Indiana counties and maintained in the greenhouse in the original field soil planted with *Glycine max* (L.)

Merr. cv. Williams. Counties from which isolates were collected included White and Benton in northern Indiana and Vigo, Posey, and Vanderburgh in southern Indiana.

To prepare inoculum, cysts were obtained from soil of a greenhouse culture of SCN by sieving and decanting. Ten milliliters of commercial bleach (5.25% sodium hypochlorite = NaOCl) were added to a 20-ml suspension of cyst material to dissolve the cyst walls and gelatinous matrix to release eggs. After 6 minutes of stirring (on an electric stir plate), the mixture was poured over a series of sieves as follows: 150- μ m-pore to collect intact cysts and large debris, 53- μ m-pore to collect smaller debris, and 38- μ m-pore to collect eggs. Sodium hypochlorite treatment was repeated for cysts caught on the 150- μ m-pore sieve. Prior tests showed that egg hatch was stimulated by NaOCl treatment, inasmuch as more second-stage juveniles were recovered from treated eggs than from eggs from mechanically crushed cysts. For eggs from cysts recovered from soils with high organic matter content, a modified sugar flotation technique was next used in which eggs in water were centrifuged for 3 minutes at 750 g to remove floating debris, a 37.5% aqueous sugar solution added, the mixture centrifuged at 310 g for 1 minute, the supernatant containing the eggs poured through a 38- μ m-pore sieve, and the eggs rinsed with water sprayed gently up through the sieve from the bottom.

The following 16 soybean cultivars were used as differentials to screen each SCN population: Peking, Custer, Old Dominion, Pine Dell, Pickett, Williams, Lee, PI 88788, PI 90763, PI 91684, PI 209332, PI 84611, PI D75-10710, PI 84751, PI

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TABLE 1. Development of five isolates of *Heterodera glycines* from Indiana on selected soybean differentials. Data from all experiments (3–5 tests per isolate).

Differential	Isolate				
	Benton (north)	White (north)	Vanderburgh (south)	Vigo (south)	Posey (south)
Peking	– (0.0)	– (0.3)	– (0.0)	– (0.0)	– (0.0)
Custer	– (0.0)	– (0.4)	– (0.0)	– (0.0)	– (0.0)
Old Dominion	+ (14.7)	+ (52.2)	+ (12.2)	+ (15.9)	+ (14.3)
PI 88788	– (0.4)	– (0.4)	– (0.0)	– (0.0)	– (0.0)
PI 90763	– (4.3)	– (0.0)	– (5.1)	– (0.1)	– (0.0)
PI 91684	+ (140.8)	+ (159.8)	+ (88.3)	+ (125.2)	+ (50.3)
PI 209332	– (3.3)	– (0.0)	– (0.0)	– (1.4)	– (0.0)
PI 84611	+ (30.1)	+ (17.6)	+ (13.6)	+ (24.1)	+ (27.2)
PI D75-10710	– (2.5)	– (0.1)	– (2.8)	– (0.0)	– (0.0)
PI 84751	– (0.0)	– (0.0)	– (0.0)	– (0.0)	– (0.1)
Williams	+ (145.6)	+ (144.6)	+ (100.9)	+ (76.2)	+ (100.1)
Lee	+ (100.0)	+ (100.0)	+ (100.0)	+ (100.0)	+ (100.0)

+ = number of cysts \geq 10% of the number on Lee; – = number of cysts < 10% of the number on Lee.

Numbers in parentheses indicate percentage of development of cysts on differentials compared with development on Lee.

79693, and PI 87631. Seeds were germinated in vermiculite or sand, and 2-week-old seedlings were inoculated with nematodes. For inoculation, a seedling was placed in a sand-loam (2:1) potting soil mixture in a 500-ml pot and ca. 2,000 (the exact number varied slightly between tests) eggs and juveniles in 1 ml aqueous suspension poured directly over the roots. The roots were covered with additional soil mixture and watered immediately. In each test each cultivar was replicated 10 times. Pots were arranged in a randomized block design. Tests were carried out on a greenhouse bench at 20–24 C or in root zone temperature tanks at 24 C. Each SCN isolate was tested 3–5 times on each differential.

Cysts were recovered from the soil in each pot 30 days after inoculation. Roots placed on a 710- μ m-pore sieve were sprayed with pressurized water to dislodge cysts. Cysts for counting, obtained from soil and roots, were collected on ruled filter paper by means of a Buchner funnel and vacuum pump (12). After excess water was removed, the filter paper was transferred to a glass plate on the stage of the stereoscopic microscope. An index of mature females (11) was calculated as follows:

$$\text{Index} = \frac{\text{number of cysts on test differential}}{\text{number of cysts on Lee}} \times 100$$

The response of an isolate (“+” or “–” =

susceptibility or resistance for the particular soybean differential) was based on the number of cysts recovered from the susceptible cultivar Lee, according to the criteria of Golden et al. (6).

RESULTS

All five Indiana SCN isolates received the same rating response on 12 soybean lines that included four of the five original standard differentials (6); Pickett was the lone exception (Table 1). There was a range in numbers of cysts produced by the different SCN isolates on a particular soybean differential. For example, the five isolates were rated “+” on Old Dominion, but the means and ranges of the number of cysts recovered as a percentage of the total recovered from Lee varied from 12.2% (10.1–13.2%) for the Vanderburgh isolate to 52.2% (49.2–53.2%) for the White isolate. The number of cysts on PI 91684 varied from 50.3% (6.7–81.9%) for the Posey isolate to 159.8% (123.9–315.7%) for the White isolate. Reaction of all isolates was rated “–” on PI 90763, but numbers of cysts, as compared to numbers on Lee, ranged from 0.0% for Posey and White to 5.1% (1.3–8.1%) for Vanderburgh.

A second group of five differentials separated the five isolates into four groups (Table 2). These differentials included Pickett, one of the original five standard differentials (6). The Vigo and Posey isolates comprised a discrete group, by virtue

TABLE 2. Soybean differentials used for separating five isolates of *Heterodera glycines* from Indiana. Data from all experiments (3-5 tests per isolate).

Differential	Isolate				
	Benton (north)	White (north)	Vanderburgh (south)	Vigo (south)	Posey (south)
Pine Dell	- (4.5)	- (3.7)	+ (13.6)	+ (16.9)	+ (12.3)
PI 79693	- (4.1)	- (2.3)	- (0.0)	+ (27.5)	+ (10.2)
PI 87631	+ (22.4)	- (1.7)	- (2.4)	- (2.0)	- (0.1)
Pickett	- (8.1)	+ (24.5)	- (0.0)	- (1.1)	- (0.0)
Lee	+ (100.0)	+ (100.0)	+ (100.0)	+ (100.0)	+ (100.0)

+ = number of cysts ≥ 10% of the number on Lee; - = number of cysts < 10% of the number on Lee. Numbers in parentheses indicate percentage of development of cysts on differentials compared with development on Lee.

of their “+” rating on PI 79693, in contrast to the “-” rating on PI 79693 for the other three isolates. Except for its inability to reproduce on PI 79693, the third southern isolate (Vanderburgh) behaved similarly to Vigo and Posey on this group of five differentials. The two northern Indiana isolates showed additional differences, however. Both of these isolates (Benton and White) were rated “-” on Pine Dell, in contrast to the “+” ratings of the three southern isolates. In addition, Benton alone was rated “+” on PI 87631 and White alone was rated “+” on Pickett.

DISCUSSION

Establishment of guidelines for characterization of four races of SCN (6) was probably a necessary first step toward managing this important pest, but classifying natural populations into discrete races is proving to be an elusive concept (11). The standard differentials no longer appear to be adequate, and reassessment is needed (1-3,5,9-11,13,14). Although four of the five Indiana isolates we studied must still be called Race 3, based on their behavior on the standard soybean differentials, four different response patterns are evident when the five are tested using a different set of differentials.

A further complication is the arbitrary rating scheme for designating resistance and susceptibility of a differential. For example, reproduction of the Vanderburgh and Benton isolates on PI 90763 (5.1% and 4.3%, respectively), although low, might result in rapid field buildup of SCN on a cultivar with resistance inherited from this line.

The White County SCN isolate cannot

be categorized as one of the four recognized races based on the reproduction of this isolate on the standard soybean differentials. In reproducing on Pickett, it is similar to an isolate from Camden, North Carolina (14) and to one from Minnesota (9). However, the White County isolate reacted somewhat differently from both on PI 88788. The Minnesota isolate was rated “+” on PI 88788 with 17% reproduction, compared with susceptible Essex (9); reproduction of the North Carolina isolate was 4.8% of that on Lee, although it was rated as “-”, according to standard practice (14). The reproduction of our White County isolate on PI 88788 was only 0.4% of that on Lee.

Continuing to categorize SCN populations into four races, based on the standard differentials, may be misleading even when it is possible, although the current race concept is said to be useful to plant breeders (11). Adding new race designations only as new cultivars are introduced with new levels of resistance has been recommended (11). A somewhat different approach is taken by Luedders (8) who thinks of the degree of resistance or susceptibility as an “association phenotype” that is determined by genes in both the host plant and the nematode. Thus an “association phenotype” between a particular isolate of SCN and a given cultivar is unique to those genotypes. A different association phenotype might obtain between the same host cultivar and another genotype of SCN, even though the SCN isolates are assigned to the same race, on the basis of arbitrarily selected criteria.

We found some indication of regional similarity in the behavior of the Indiana

isolates on the soybean lines tested, in that the responses of the three isolates from southern Indiana were more similar to each other than they were to the two northern isolates. Furthermore, we found that the two isolates from northern Indiana produced cysts on more of the differentials tested than did the three isolates from southern Indiana, which might indicate that development of resistant cultivars suitable for northern soybean growing regions may pose more difficulties than has development of cultivars for use in the south. Because reproductive rates of natural SCN populations on resistant lines seem to vary widely, it would be important to a breeding program to test potential resistant lines on several populations from the areas for which new cultivars are being developed, rather than to rely on tests with populations that happen to share the same standard race designation but are from different geographic areas.

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