Journal of Nematology 18(2):173–177. 1986.

Heterodera glycines in Indiana: II. Morphology of Geographical Isolates[1]

J. Faghihi, J. M. Ferris, and V. R. Ferris[2]

Abstract: Although much morphometric overlap occurs among five geographical isolates of *Heterodera glycines* in Indiana, significant differences in means exist among the isolates for various comparisons of second-stage juveniles. By using combinations of means, most of the isolates can be distinguished from the rest: e.g., the Vanderburgh County isolate (southern Indiana) has the longest esophagus, tail, and tail terminus; the Vigo County isolate (also from the south) has the shortest esophagus; the White County isolate (northern Indiana) has the shortest tail and tail terminus and the greatest total length; the Benton County isolate (north) is the shortest. Morphological similarities and differences do not appear to be coordinated with reproductive behavioral patterns we observed in the northern versus the southern isolates.

Key words: soybean cyst nematode, *Glycine max*, soybean, *Heterodera glycines*, Indiana, morphology, races, geographical isolate.

Various attempts have been made to distinguish geographically separated populations and (or) races of *H. glycines* Ichinohe on the basis of morphology (5,6). Golden and Epps (2) reported significant differences in tail length of three such populations and in the length and shape of the hyaline part of the tail terminus of second-stage juveniles. Golden et al. (3) suggested that tail length might be used to differentiate among races of soybean cyst nematode (SCN). Koliopanos and Triantaphyllou (4) also found significant differences in length of tail and tail terminus of second-stage juveniles of four populations. We compared behavioral, morphological, and protein differences among five isolates of *H. glycines* from Indiana. Three of the isolates were from southern Indiana, where SCN has been known to occur since 1973. Two of the isolates were from northern Indiana where SCN was discovered more recently. We have reported differences in the ability of the isolates to reproduce on soybean breeding lines and cultivars (1). Our objective in this study was to compare morphologically the five Indiana isolates.

Materials and Methods

Isolates of SCN, the same as those of Faghihi et al. (1), were maintained in the greenhouse in the original field soil planted with *Glycine max* (L.) Merr. cv. Williams. Counties from which the isolates were collected, plus a letter to indicate whether the county is in the north (N) or south (S) are as follows: Posey (S), Vanderburgh (S), Vigo (S), Benton (N), and White (N).

Data were analyzed by one-way analysis of variance (one-way ANOVA) and Duncan's multiple-range test. Student's *t*-test was used to analyze data collected from cysts and eggs. The assumption of normality was checked using the Shapiro-Wilk test for normality. Specific details for handling each life stage were as follows:

Second-stage juveniles: Several hundred randomly selected cysts were mechanically ruptured and the contents placed in a Baermann funnel. Second-stage juveniles were collected after 24 and 48 hours, killed and fixed in 4% formalin, mounted on slides, and measured using a camera lucida. The following measurements were made on 100 specimens from the White County (White) and Vigo County (Vigo) isolates and 40 specimens from Benton County (Benton), Posey County (Posey), and Vanderburgh County (Vanderburgh) isolates: total length, stylet length, esophagus length, distance of excretory pore from anterior end, width of widest part of body, tail length, length of tail terminus, and width of widest part of tail terminus. In addition, the width of the esophageal bulb, and the length and width of the genital primordium were measured for the White (N) and Vigo (S) isolates.

Males: Male specimens obtained by incubation of infected roots from green-

Received for publication 25 April 1985.

[1] Journal paper 10272, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907. Part of a Ph.D. Thesis, Purdue University, by the senior author. Supported in part by the Indiana Crop Improvement Association and Public Varieties of Indiana.

[2] Department of Entomology, Purdue University, West Lafayette, IN 47907.

We thank C. Hicks for statistical advice.

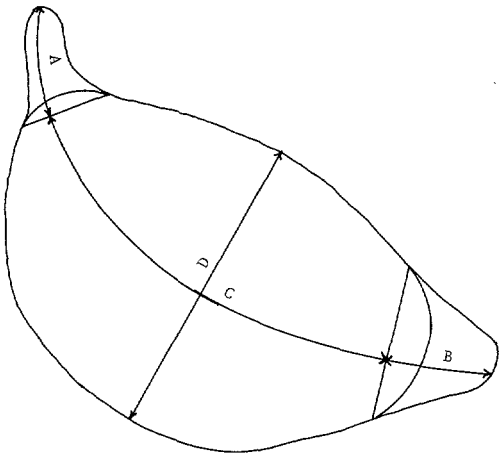

FIG. 1. Diagram of cyst of *Heterodera glycines* showing method for obtaining standardized measurements. A = neck length. B = cone length. A + B + C = total length. D = width.

house cultures were killed, fixed, and measured in the same manner as the juveniles. The following measurements were made on 100 specimens from White and Vigo and 40 specimens from Benton, Posey, and Vanderburgh: total length, stylet length, length of esophagus, distance of excretory pore from anterior end, width of widest part of body, and tail length. In addition, width of the median bulb and length of spicule were also measured for the White (N) and Vigo (S) isolates.

Cysts and eggs: Cysts and eggs were measured from the White (N) and Vigo (S) isolates only. Measurements of randomly selected cysts were difficult to make because of the nonuniform shapes of cysts. Further, published literature references were not explicit as to procedures followed by previous researchers. The following scheme was developed to improve the consistency of measurements (Fig. 1): 1) The outline shape of a cyst was traced with a camera lucida. 2) To standardize measurements of neck length, a French curve was used to continue the contours of the main body in this region and a straight line was drawn between the points where the sketched curve intersected the outline of the main body on either side. 3) The measurement for neck length (A, Fig. 1) was made from the straight line to the anterior end of the body. 4) A similar procedure was used to standardize measurements of cone length (B, Fig. 1). 5) Total length was estimated by measuring the distance along a curve following the mid-line of the body, rather than measuring a straight line between lip region and tail tip.

To determine the number of eggs per cyst, each cyst was mechanically ruptured to release its egg contents after measurements of the cyst were completed. Length and width of randomly selected eggs were measured using the camera lucida.

RESULTS

Second-stage juveniles: Second-stage juveniles from the five Indiana SCN isolates separate into 2–5 groups by using selected significant differences in morphometric characters (Table 1). On the basis of total length, the five isolates can be separated into three partially overlapping groups. Mean length of juveniles from the White isolate differs from that of all other isolates. A difference in mean length occurs between the Vanderburgh and Benton isolates, but neither differs from the Vigo and Posey isolates. Small differences in length of stylet exist, with the Posey isolate different from all others. The Vigo plus White isolates differ from the Vanderburgh plus Benton isolates. Using esophagus length, the Vigo, White, and Vanderburgh isolates can be differentiated from all others. The Posey and Benton isolates cannot be separated from each other by this measurement, but they can be separated from the other three isolates. Using the distance of the excretory pore from the anterior end, the five isolates can be divided into three groups, with the Vigo isolate separated from all others, the White plus Posey isolates forming a second group, and the Benton plus Vanderburgh isolates a third.

Only the Vanderburgh and White isolates can be differentiated on the basis of tail length. The Vanderburgh and Vigo isolates differ from the rest in length of tail terminus but cannot be distinguished from each other by this measurement. Similarly, the White, Benton, and Posey isolates cannot be separated from each other using the length of the tail terminus.

In the ratio of total length over esophagus length, the White and Vigo isolates differ from each other and from all the rest; however, no differences occur among the Benton, Vanderburgh, and Posey isolates. Using the ratio of total length over

TABLE 1. Summary of characteristics of second-stage juveniles showing significant differences ($P = 0.01$) among five isolates of *Heterodera glycines* from Indiana.

Characteristic	Number of groups	Composition of groups*				
		1	2	3	4	5
Total length (μm)	3	416.8 a	450.9 c	432.9 b	424.8 ab	423.6 ab
Stylet length (μm)	3	24.7 c	23.8 b	24.5 c	23.7 b	23.3 a
Esophagus length (μm)	4	173.3 c	163.8 b	178.7 d	160.2 a	170.0 c
Excretory pore opening (μm)†	3	96.9 c	93.1 b	97.9 c	91.3 a	93.4 b
Tail length (μm)	2	43.2 ab	41.6 a	44.3 b	42.8 ab	42.9 ab
Tail terminus length (μm)‡	2	22.5 a	22.1 a	24.5 b	24.4 b	22.6 a
Total length/esophagus length	3	2.41 a	2.76 c	2.42 a	2.66 b	2.50 a
Total length/stylet length	4	16.9 a	19.1 d	17.6 b	17.9 bc	18.2 c
Total length/distance of excretory pore	5	4.32 a	4.84 e	4.42 b	4.65 d	4.54 c
Total length/tail terminus	3	18.6 b	20.6 c	17.7 a	17.6 a	19.0 b

Based on one-way ANOVA of data from 40–100 juveniles per isolate.

Data in rows followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple-range test.

* 1 = Benton isolate (north). 2 = White isolate (north). 3 = Vanderburgh isolate (south). 4 = Vigo isolate (south). 5 = Posey isolate (south).

† Distance from anterior end to opening of excretory pore.

‡ Distance from beginning of hyaline area to posterior end of body.

length of stylet, the Benton and White isolates differ significantly from each other and from the rest. The Vanderburgh and Posey isolates differ from each other, but the values for each overlap those of the Vigo isolate. In the ratio of total length over distance of the excretory pore opening from the anterior end, all five Indiana isolates can be differentiated by small but consistent differences. Using the ratio of the total length over tail terminus length, the White County isolate can be separated from the rest, the Vigo plus Vanderburgh isolates form a distinct group, and the Benton plus Posey isolates form another group.

Males: Males of the five Indiana isolates can be separated into three groups on the basis of significantly different morphometric comparisons, but composition of the groups varies depending on the particular comparison used (Table 2). The Vigo isolate can be separated from all other isolates by stylet length, the Posey plus White isolates form a second group, and the Vanderburgh plus Benton isolates comprise a third. Marked differences in esophagus length separate the extremes (males of the Benton and White isolates), but much overlap exists among the isolates. Vigo males differ in tail length from all the other isolates. The noticeably shorter tails of these males can be readily observed under the microscope, even without measurements. Males of the Vanderburgh plus Posey isolates form a second group based on tail length, and males of the Benton plus White isolates comprise a third.

The groupings overlap for distance of the excretory pore opening from the anterior end. Males of the White isolate differ from males of all other isolates, except Benton. Benton and Vigo males cannot be separated, but the Benton males differ from males of the Posey and Vanderburgh isolates.

Based on the ratio of the total length over width, the Vanderburgh and Posey males differ from each other and from males of all other isolates. No differences exist among the Vigo, White, and Benton isolates. The Vigo and White isolates differ from each other and from the other isolates in the ratio of the stylet length over body width, but males of the other isolates do not differ from each other. Small, but distinct differences in the ratio of esophagus length over stylet length separate the Benton and Posey males, but the values for the other three isolates do not differ from each other. The Vigo and Vanderburgh males are distinct in the ratio of tail length over distance of excretory pore opening from the anterior end, but no differences exist among Posey, Benton, and White isolates.

Cysts and eggs: Significant differences were found between the White and Vigo isolates in the total length of cysts, the width of eggs, and the ratio of total length over width of cysts and eggs (Table 3). Neck

TABLE 2. Summary of characteristics of males showing significant differences ($P = 0.01$) among five isolates of *Heterodera glycines* from Indiana.

Characteristic	Number of groups	Composition of groups* 1	2	3	4	5
Stylet length (μm)	3	27.5 c	26.7 b	27.4 c	25.7 a	26.3 b
Esophagus length (μm)†	3	178.9 a	193.7 c	188.1 bc	191.3 c	180.4 ab
Excretory pore opening (μm)‡	3	130.9 ab	130.2 a	138.3 c	134.1 bc	137.4 c
Tail length (μm)§	3	7.83 c	7.66 c	6.72 b	5.49 a	6.98 b
Total length/width‖	3	44.5 b	44.3 b	41.3 a	44.2 b	48.1 c
Stylet length/width	3	1.02 c	0.98 b	1.02 c	0.94 a	1.03 c
Esophagus length/stylet length	3	6.46 a	7.26 c	6.88 c	7.43 c	6.86 b
Tail length/distance of excretory pore	3	0.059 c	0.059 c	0.048 b	0.041 a	0.050 c

Based on one-way ANOVA of data from 40–100 males per isolate.
Data in rows followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple-range test.
* 1 = Benton isolate (north). 2 = White isolate (north). 3 = Vanderburgh isolate (south). 4 = Vigo isolate (south). 5 = Posey isolate (south).
† Distance from base of esophagus to anterior end.
‡ Distance from anterior end to opening of excretory pore.
§ Distance from anal opening to posterior end.
‖ Measured at widest part of body.

length and cone length of cysts are also different. The mean number of eggs per cyst (200+) does not differ significantly between the two isolates.

DISCUSSION

Previous workers (5) have found considerable overlap in morphometric measurements of SCN populations. We also found such overlap, but in spite of this, significant differences in means were found for a number of morphometric comparisons of the five Indiana isolates. A problem is that large numbers of individuals must be measured to obtain reliable data, and this reduces the usefulness of morphometrics as an important tool for distinguishing among isolates. However, in special cases morphometrics might be used in conjunction with other measures of difference.

Morphometrics of second-stage juveniles seem to have more utility for differentiating our Indiana isolates than do morphometrics of other life stages. We cannot use tail length to separate our five isolates, as did Golden et al. (3) for their races, except to distinguish between the White (shortest tail and tail terminus) and the Vanderburgh (longest tail and tail terminus) isolates. The White isolate has the longest total length, whereas the other isolate from northern Indiana (Benton) has the shortest total length. These extremes are reflected in the comparisons of ratios of total length over the length of the esophagus, stylet, and the distance of the excretory pore from anterior end; hence these particular morphometrics might be useful in separating the White and Benton isolates from each other. Certain isolates are consistently located at one extreme or the other for several morphometric characters. In addition to having the longest tail and tail terminus, the Vanderburgh isolate has the longest esophagus and distance of excretory pore from anterior end. The Vigo isolate has the shortest esophagus and distance of excretory pore from anterior

TABLE 3. Measurements (in μm) of randomly selected cysts (n = 50) and eggs (n = 100) from two Indiana isolates of *Heterodera glycines*.

Measurement	Cyst Vigo (south)	Cyst White (north)	Egg Vigo (south)	Egg White (north)
Length (μm)	717.6**	764.3	103.8	103.5
Width (μm)	440.3	434.2	43.9**	46.6
Length/width	1.6**	1.8	2.4**	2.2
Neck length (μm)	71.3*	80.1		
Cone length (μm)	100.2*	106.8		
Width/cone length	4.5	4.1		
Number of eggs/cyst	204.8	219.6		

* Significant difference ($P = 0.05$).
** Significant difference ($P = 0.01$).

end; these measurements might be used to separate these two southern isolates.

We were unable to find any morphometric characteristics that distinguish isolates from southern counties from those of northern counties, and the morphometric similarities and differences do not coincide in any way with our reported observations (1) on similarities in ability of certain of these isolates to reproduce on various breeding lines and cultivars of soybeans.

Morphometrics of males are less useful for separating Indiana SCN isolates than are measurements of second-stage juveniles. The male stylet and tail are shortest in the Vigo isolate and the longest in the Benton isolate. Similar differences between these two isolates are found in comparisons of ratios of these characters over body width and distance of excretory pore from anterior end. Morphometrics of cysts are difficult to use because variability in cyst shape makes comparisons unreliable. By following a consistent scheme of measurement, a few significant differences were found between cysts of White and Vigo isolates (the only two isolates compared). Morphometrics associated with eggs appear to be of little use in differentiating among isolates.

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