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Distribution, Density, and Diversity of *Heterodera glycines* in Missouri[1]

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Abstract: Between May, 1988, and July, 1992, the University of Missouri Plant Nematode Diagnostic Laboratory collected data on *Heterodera glycines* populations in Missouri by offering diagnostic services to soybean farmers. A greenhouse bioassay (free), egg count ($10/sample), and race determination test ($25) were conducted by request on soil samples submitted to the lab. Each test was offered for a specific purpose: the bioassay for fields not known to be infested; the egg count for population monitoring in fields with known infestations; and the race test for fields with a history of resistant cultivars. Of 818 samples submitted for bioassay on a *H. glycines*-susceptible soybean cultivar, 13 (1.6%) contained brown cysts but no white females, and 364 (45%) contained white females after 35 days in the greenhouse. Of 6,193 egg counts, 39% were either free of *H. glycines* or contained fewer than 500 eggs/250 cm^3 soil, the action threshold for Missouri. The remaining 61% ranged from 500–400,000 eggs/250 cm^3 soil (mean = 10,617). Eleven races were detected, with races 3 (45%) and 1 (23%) the most common. The data show that *H. glycines* is widespread in Missouri (with confirmed infestations in 80 of 114 counties), that most infested fields have population densities above the action threshold, and that there is considerable genetic diversity among *H. glycines* field populations.

Key words: bioassay, distribution, *Glycine max, Heterodera glycines,* nematode, race, race determination, soybean, survey.

The soybean cyst nematode, *Heterodera glycines*, was reported from southeastern Missouri in 1956 (Fig. 1) (9). The seven-county "Bootheel" was under quarantine restrictions until 1972 in an attempt to control further spread of the nematode (9). In 1971, the first infestations were found north of the Missouri River (J. A. Wrather, pers. comm.), which roughly separates the southern two-thirds from the northern third of the state. By 1987, 57 (of 114) counties throughout Missouri had confirmed *H. glycines* infestations, including all of the top 10 soybean production counties (5). Even though *H. glycines* infestations were relatively common in northern Missouri, it was not thought to seriously constrain soybean production (E. W. Palm, pers. comm.). The probable reason for this lack of concern was that the presence of the nematode was not generally correlated with the typical symptoms of stunting and chlorosis often associated with infestations in the Bootheel and the rest of the southeastern United States (V. H. Dropkin, pers. comm.).

In order to document the potential for soybean yield losses due to *H. glycines* north and west of the Bootheel, we not only conducted field trials (e.g., 13), but also recognized that we needed to know more about the nematode's distribution than just whether an infestation had been confirmed in a particular county. Lacking the resources to conduct a systematic survey of the state, we opened a plant nematode diagnostic laboratory and advertised its availability to state extension personnel. Its expressed purpose was to offer a service to farmers, not limited to soybean producers, that was not otherwise readily available. Adjunct research purposes were to document new infestations of *H. glycines*, determine the population densities of infested fields, and document the genetic diversity of field populations.

Materials and Methods

In May 1988, state extension personnel were apprised that the Plan Nematode Di-

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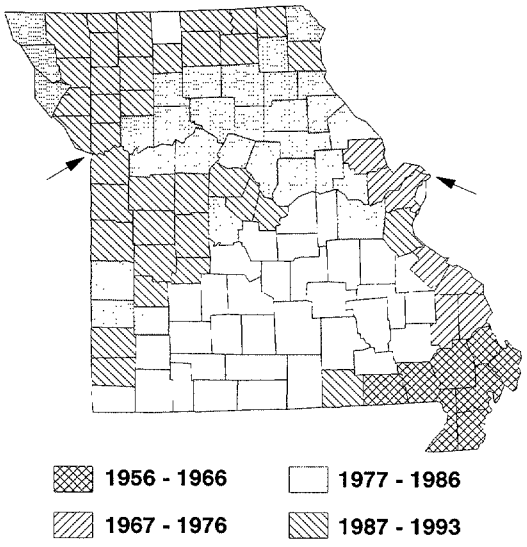

FIG. 1. Distribution of confirmed infestations of *Herterodera glycines* in Missouri by county. Arrows indicate eastern and western state boundary intersections of the Missouri River, arbitrarily separating northern from southern Missouri. The crosshatched area includes the "Bootheal."

agnostic Laboratory would offer five diagnostic tests to Missouri farmers submitting soil samples: i) a bioassay to detect the presence of *H. glycines*, specifically for soybean farmers who did not know whether the nematode was present in a field; ii) an egg count to determine the population density of *H. glycines*, for fields with confirmed infestations; iii) a race determination test, for soybean farmers growing *H. glycines*-resistant cultivars; iv) a "complete nematode profile" in which all vermiform plant-parasitic nematodes were identified to genus and counted, for producers of any crop; and v) species identifications for root-knot (*Meloidogyne* spp.) and lesion (*Pratylenchus* spp.) nematodes, where needed for crop management recommendations. Methods and results from the complete nematode profile through 1991 were reported previously (6), and similar data were collected through July 1992. *Meloidogyne* and *Pratylenchus* species identifications were obtained for few soybean field samples and will not be discussed further.

Along with the soil sample, the Plant Nematode Sample Submission Form (Fig. 2) was submitted. Instructions for taking soil samples for each type of test were printed on the reverse of the form. When samples could not be processed immediately, they were held in cool storage (10–15 C) for a maximum of 10 days. Submitters received management recommendations with the results of the tests, which were conducted according to the following procedures.

Bioassay: The *H. glycines* bioassay was offered free to all soybean growers. Each sample was mixed thoroughly, and as much soil as possible was put into a 400-cm^3 styrofoam cup with drainage holes in the bottom. Five seeds of soybean cultivars Essex or Williams 82 (both *H. glycines*-susceptible) were planted. The cups were maintained in a greenhouse for ca. 35 days, at which time the root balls were removed and examined for the presence of white to yellow female cyst nematodes. If females were not observed, the roots were gently washed and then subjected to a

Plant Nematode Sample Submission Form

Submit samples and all three copies of this form to:
Plant Nematology Laboratory Department of Plant Pathology, 103 Waters Hall, Columbia MO 65211
See the back of this form for instructions on collecting and shipping soil samples or plant specimens for nematode analysis

Date: _____ Lab use Fee:
County _____ Code:

Sample information: Complete parts A and B below for each sample.

Submitted by: _____ Grower _____
Address: _____ Address _____
Phone: _____ Phone _____

A. _____

Field name or sample number	Acreage sampled	Current crop or variety	Test desired (see back)	Lab use only
1.				
2.				
3.				
4.				

B. _____

Field name or sample number	Soil type*	Symptoms observed	
1			
2.			
3			
4			

*1=light, 2=medium, 3=heavy; use other description if necessary.

MP 655

FIG. 2. Facsimile of the form required for submission of soil samples to the University of Missouri Plant Nematode Diagnostic Laboratory.

high-pressure spray on nested 710-μm-pore (20 mesh) over 250-μm-pore (60 mesh) sieves. Debris collected on the 250-μm-pore sieve was inspected at 40× magnification for the presence of females. Results were reported as positive (females observed) or negative (no females observed).

Egg count: Heterodera glycines egg counts were conducted for $10 per soil sample. Because *H. glycines* egg counts in Iowa and Missouri field tests were more highly correlated with yield than were cyst counts, and because counts of second-stage juveniles (J2) had extremely high variability (150–300% coefficient of variation) (8; unpubl.), eggs were chosen as the most informative life stage to enumerate for making recommendations. The sample was mixed thoroughly and a 100-cm^3 subsample (measured by water displacement) was removed. The original sample was usually retained in cool storage in case we encountered problems during or after processing. The subsample was elutriated with a semiautomatic apparatus (University of Georgia Science Instrument Shop, Athens, GA) (2) in which the cysts were collected on a 250-μm-pore sieve. Eggs were extracted from the cysts by a modified mechanical method (1). The cysts were placed in a 100-ml polypropylene test tube and the water level was increased to 40 ml. A stainless steel bit with 1-mm helical ridges (University of Missouri Science Instrument Shop, Columbia, MO) attached to a variable speed stirrer was used to crush the cysts: the stirrer's rheostat was turned to 6,000 rpm while the test tube containing the cysts was held gently against the rotating bit for 60 seconds. Contents of the tube were washed through nested 75-μm-pore (200 mesh) over 25-μm-pore (500 mesh) sieves. Eggs collected on the 25μm-pore sieve were stained with acid fuchsin (14). After staining, the egg suspensions were standardized to 100 ml, stirred, and a 5 ml "dipper" (University of Missouri Science Instrument Shop, Columbia, MO) was used to collect a subsample for counting at 60× magnification. Results were reported as number of eggs per 250 cm^3 soil.

Race determination: Race determinations for *H. glycines* were conducted for $25 per sample. Cysts and eggs were extracted from the soil samples as described for the egg count test (except that the eggs were not stained before counting) to confirm that sufficient eggs to complete a race test were present in the sample. Additional subsamples were processed if necessary to obtain at least 21,000 eggs. Eggs and debris from the extraction procedure were concentrated in tap water and cleaned by sucrose centrifugation: the concentrated eggs and debris were layered on top of a sucrose solution (454 g sucrose in 1 liter tap water) in a 50-ml centrifuge tube and spun at ca. 1,500*g* for 4 minutes; the band of clean eggs was removed with a syringe and rinsed in tap water. Eggs were rinsed into a 250-ml beaker, and sufficient tap water was added to dilute the suspension to 1,000 eggs/ml. Eggs were kept in suspension through the inoculation process by gentle stirring. Seed of soybean cultivars Lee 74, Essex, and each of the soybean race differential lines ('Pickett', 'Peking', PI 88788, and PI 90763) were germinated in moist, sterilized germination paper. Essex was a susceptible check not included in the calculations of female indices (FI). After 48 hours, three seedlings of each soybean line were chosen for uniformity and planted singly in 2.5-cm-d by 20-cm-long polyvinylchloride tubes containing a sandy loam soil (74% sand, 16% silt, 10% clay) previously sterilized with methyl bromide and free of *H. glycines*. One 3-cm-deep hole was made in the sand next to each seedling with a pencil-sized probe, infested with 1 ml of the egg suspension, and covered. All tubes composing a test were packed vertically together with sand in a single plastic 6.3-liter crock. The crock was suspended in a water bath maintained at 27 C. Thirty to 35 days later, the females produced on each soybean line were extracted as described for cysts in the egg count test, counted, and used to calculate FI (= I [index] per reference 10) for race determination (10). The resulting FI were reported along with the race determina-

tion and the number of eggs per 250 cm^3 soil in the original sample.

Results

Between May 1988 and July 1992, the number of Missouri counties with confirmed infestations of *H. glycines* increased from 57 to 80 (Fig. 1). Soil samples collected from soybean fields were also submitted from 7 of the remaining 34 "uninfested" counties. The large area in south-central Missouri in which there are no known infestations (Fig. 1) is mostly Ozark Mountain terrain.

Bioassay: Submissions for the *H. glycines* bioassay totaled 818. Thirteen of these had equivocal results: females were not observed on the roots of the bioassay plant, but one or more brown cysts were observed in the debris obtained after processing the roots. Of the remaining 805 samples, 364 (45%) were positive.

Egg count: A total of 6,193 samples were submitted by soybean growers. Several thousand more were processed for extension personnel or private companies conducting local demonstration or research trials, but those were not included in the summary (Table 1). The range of egg counts was 0–400,000/250 cm^3 soil. A total of 4,701 (76%) samples had detectable numbers of eggs, of which the average was 10,617/250 cm^3 (SD = 20,660).

Race determination: A total of 359 race determinations were conducted, of which 293 (Table 2) were from soybean growers rather than researchers. Eleven races were detected, with race 3 most common. Average egg counts for the same populations on which the race determinations were conducted (i.e., the same soil sample) were 24,202 eggs/250 cm^3 soil (SD = 31,617), with a range of 980–128,000. There was no apparent relationship between egg count and race, nor between geographic location (northern Missouri, Bootheel, etc.) and race.

Table 1. Frequency distribution of soil samples from Missouri soybean fields among classes of *Heterodera glycines* egg counts per 250 cm^3 soil.

Egg count in 250 cm^3 soil	Number of samples	Percentage of samples
0	1,489	24
1–499	949	15
500–1,499	714	12
1,500–4,999	1,024	17
5,000–14,999	1,047	17
15,000–24,999	415	7
25,000–49,999	385	6
50,000–99,999	125	2
>100,000	45	<1

Discussion

There are three caveats for interpreting the data presented here: first, we did not collect soil samples systematically; second, we had no control over how the samples were collected, stored before transportation, or transported; and third, we did not identify cysts from each sample to species. We do not believe the third caveat is a serious problem, even though other species of *Heterodera* may be found in soybean fields in Missouri (6). In controlled studies, we have not observed any other cyst nematodes to be as well adapted to soybean as is *H. glycines* (Niblack, unpubl.). In 13 bioassay tests (1.6%), brown cysts were observed when no white females were found on the roots; these could have been cysts from other species, but this was not a common occurrence. Samples with low egg counts (Table 1) could also have been due to other species. The presence of *H. glycines* in Missouri has been confirmed several times by inspection of vulval cones and J2 (3), and we are confident that when cyst densities in soybean fields are high, the predominant species present is *H. glycines*.

The absence of a systematic design for collection of samples precludes overgeneralization of the results. For example, we cannot derive an estimate of total infested soybean acreage from the data. Further, areas in which extension personnel, consultants, or others were active in collecting samples or encouraging sample collection were overrepresented in the data, and

TABLE 2. Results of *Heterodera glycines* race determination tests conducted by the Univesity of Missouri Plant Nematode Diagnostic Laboratory, 1988–1992†.

Race‡	Number of tests	Percentage of all tests	Female index‡			
			Pickett	Peking	PI 88788	PI 90763
1	68	23	2.5 (0.0–9.4)	0.3 (0.0–7.7)	19.2 (10.0–70.0)	0.3 (0.0–7.7)
2	15	15	84.5 (25.5–150.7)	22.4 (12.3–76.8)	31.8 (15.3–52.3)	4.5 (0.6–9.7)
3	131	45	1.6 (0.0–9.4)	0.3 (0.0–4.3)	4.3 (0.0–9.4)	0.3 (0.0–6.0)
4	18	6	107.2 (28.6–312.1)	35.5 (11.5–67.1)	23.0 (13.6–38.9)	18.0 (10.7–36.4)
5	22	8	42.9 (10.1–104.1)	3.9 (0.0–9.0)	24.1 (10.0–66.7)	1.5 (0.0–8.8)
6	23	8	45.4 (9.9–132.9)	4.3 (0.0–8.6)	6.6 (0.0–8.0)	2.7 (0.0–8.6)
14	8	3	85.1 (12.1–150.0)	36.2 (12.7–100.0)	6.9 (0.0–9.6)	21.0 (10.6–50.0)

† Races 7, 8, 9, and 15 were also found; races 7 and 8 were each found once, race 9 four times, and race 15 twice. These were included in the denominator for calculating "percentage of all tests."

‡ Female indices and races were determined according to Riggs and Schmitt (12). The single number under each soybean differential is the mean of all tests listed under "number of tests," and the range in parentheses are the maximum and minimum values observed.

therefore expression of the results by county (or other geographical unit) would be misleading. Nonetheless, we can make some interesting observations. Although the bioassay was offered free to encourage submission of samples from soybean producers who did not know whether they had infested fields, only 818 submitted samples for the free test, compared with 6,193 who submitted samples for egg counts. Nearly half of the bioassays were positive, which suggests that numerous soybean growers in Missouri are unaware that they could be losing money due to *H. glycines*.

Our lack of control over how the samples were collected is a benefit as well as a problem for interpretation, in that we can explain extremely high numbers by citing poor sampling technique. These samples were likely taken from "hot spots" and indicate a need to educate growers about soil sampling for nematodes. Samples with unexpectedly high egg counts are only a small percentage of the total, and the rest of the counts in samples that contained detectable levels of *H. glycines* (76%) are probably reasonably representative of reality. Poor short-term storage conditions after collection, or damage during transportation, could have resulted in failure to detect an infestation or a misleadingly low egg count, thus the totals in the lower egg count classes could be inflated.

Our classes of egg counts are artificial and useful only for breaking the data into comprehensible units, except for the first class (1–499); based on field data, the action threshold for *H. glycines* in Missouri is 500 eggs/250 cm^3 soil, irrespective of soil type or other local conditions (unpubl.). Thus, 61% of the samples had counts that exceeded the threshold, and these samples did not reflect a pattern relative to geographical origin. Although 61% cannot be considered an absolute value, it illustrates the potential for yield loss due to *H. glycines* in Missouri in infested fields.

The race determinations are similarly informative. All but race 10 and the four "impossible" races (7) are found in Missouri. This test was intended and advertised for growers who had been using resistant cultivars on infested fields, but our information on which cultivars were actually grown is incomplete. Compared with

surveys of *H. glycines* races conducted in other states, our results are most similar to those obtained in our eastern neighbor, Illinois (12). Illinois had large percentages of races 1 (27%) and 3 (64%), but races 6 and 14 were not found. Another neighboring state, Tennessee, had high percentages of races 2 (14%), 5 (38%), and 6 (19%), but races 1 and 14 were not found (15). The range and percentages of races found in Missouri is more reflective of these states than of North Carolina (11) or Florida (4). The predominance of race 1 populations in Missouri and Illinois may be related to the fact that nearly all of the adapted *H. glycines*-resistant cultivars had PI 88788 in their pedigrees; the difference between race 3 and race 1 is the FI on PI 88788 (10).

Knowledge of *H. glycines* race distributions is particularly important for soybean breeders and agronomists. For example, the emphasis placed on breeding for resistance to races 4 and 14 is not justified due to their low frequency of occurrence in all the aforementioned surveys. In addition, there is probably a need for additional research on the importance of the range in FI for soybean management: a race 1 population with a FI on PI 88788 of 70 will probably behave differently than a race 1 with a FI of 10 when grown at similar population densities on a cultivar with PI 88788 in its pedigree.

Taken as a whole, the data reported herein are useful in spite of the problems in interpretation. When this data collection effort began, *H. glycines* was not thought to be important or widespread in northern or southwestern Missouri. We now know that all but one county with annual soybean production exceeding 100,000 bushels (5) is known to have *H. glycines*-infested fields. We have established that there is considerable genetic diversity among Missouri populations of *H. glycines*, as measured by race determination tests, and that the diversity is not limited to the Bootheel, where the nematode has been known for nearly four decades. Data such as ours can serve as the basis for further study, as illustrations in farmer education efforts, and as the justification for providing nematode diagnostic services. As has been done in many parts of the Southeast, soybeans can be produced profitably in the presence of *H. glycines*, but optimal management decisions can be made only if the nematode's presence is known and populations are monitored.

As an epilogue, we should point out that the prices charged did not cover the expenses involved in conducting these tests. We were subsidized by grants and Missouri Experiment Station funds because the information is essential to a state in which the major row crop is soybean. Also, unless considerable additional resources are put into accounting, a diagnostic lab cannot count on collecting all or even most of what is owed.

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