Distribution and Virulence Phenotypes of *Heterodera glycines* in Missouri

T. L. Niblack, Professor, Department of Crop Sciences, University of Illinois, Urbana 61801; **J. A. Wrather**, Professor, Department of Plant Microbiology and Plant Pathology, University of Missouri Delta Center, Portageville 63873; **R. D. Heinz**, Senior Research Specialist, Department of Plant Microbiology and Pathology, University of Missouri, Columbia 65211; and **P. A. Donald**, Plant Pathologist, USDA-ARS, Jackson, TN 38301

ABSTRACT

Niblack, T. L., Wrather, J. A., Heinz, R. D., and Donald, P. A. 2003. Distribution and virulence phenotypes of *Heterodera glycines* in Missouri. Plant Dis. 87:929-932.

The soybean cyst nematode, *Heterodera glycines*, is the most economically important pathogen of soybean in Missouri. Knowledge of the nematode's distribution and ability to adapt to resistant varieties is important for determining crop losses and establishing research priorities. No previous surveys of Missouri have provided reliable population density and phenotypic diversity data; therefore, we conducted a random survey to obtain both. Two samples from each of 200 fields were collected; 392 samples were processed for extractions of cysts and eggs. Two hundred and forty seven (63%) of the samples had detectable cyst nematode populations, which ranged from 15 to 149,700 eggs per 250 cm³ of soil. The lowest average population densities were observed in the east-central region of Missouri (2,260 eggs per 250 cm³ of soil), and the highest were observed in the northeast (9,238 eggs per 250 cm³ of soil), but among the eight regions sampled, mean population densities did not differ significantly. These population densities were potentially responsible for losses worth over \$58 million in 1999 in Missouri. Race tests were conducted on populations from 183 samples. In order of frequency, races 3, 1, and 2 accounted for 86% of *H. glycines* populations. Nearly 60% of the populations were virulent (able to produce females) on plant introduction (PI) 88788, which is the source of resistance for most *H. glycines*-resistant cultivars. More than a third of the populations were virulent on cv. Peking, another common resistance source. Very few populations were virulent on PI 90763 or PI 437654, suggesting that these sources of resistance should be exploited more frequently.

Additional keywords: area frame probability, race determination, soybean cyst nematode races

The soybean cyst nematode, *Heterodera glycines* Ichinohe, was reported from Missouri in 1959 (5) and is recognized as the most economically important pathogen of soybean (*Glycine max* (L.) Merr.) in the state (18,20). Monetary losses due to this nematode are difficult to estimate because reliable survey data are not available. Previous disease loss estimates for Missouri have not been based on surveys (18,20), and the one previous survey (11) was based on self-selected rather than randomly selected samples. The difficulty is compounded because the state spans a wide range of soybean adaptation from Maturity Group II to Maturity Group V. Differences in the maturity dates and characteristics of cultivars, cultural methods, and growing seasons all may affect the amount of crop loss that occurs. In addition, in central and northern Missouri, yield loss due to *H. glycines* is not necessarily accompanied by symptoms such as stunting and chlorosis

(16); thus, "windshield surveys" are likely to end in underestimation of loss. Crop loss estimates are useful for many purposes such as research prioritization and should be based on random survey data.

Niblack et al. (11) reported that 76% of soybean fields in Missouri were infested with *H. glycines*, and races 1 and 3 were the most common based on samples submitted to the University of Missouri Nematology Laboratory. A random survey by Workneh et al. (19) showed that 71% of soybean fields in Missouri were infested, but no information on races was collected in their survey. Because *H. glycines* can adapt to resistant cultivars and render them ineffective (9), knowledge of the distribution of races of the nematode is essential for soybean breeding and cultivar evaluation programs (21). Thus, our objective was to provide reliable information on *H. glycines* presence, population densities, and ability to parasitize resistant soybean based on randomly collected data. This study represents the first such survey for *H. glycines* races.

MATERIALS AND METHODS

The United States Department of Agriculture National Agriculture Statistics Service area frame probability sampling design (3) was used to select fields to sam-

ple from among those planted to soybean in 1998 in Missouri. Sampling frequency in this design is weighted by production acreage. From November 1998 through March 1999, 200 fields were sampled. A soil sample was collected from each of two 0.25 ha areas separated by at least 100 m within each field (8) for a total of 400 samples.

Each sample was a composite of 20 soil cores (15 cm deep × 2.0 cm diameter) (1). Soil samples were placed in insulated coolers before being transported to the laboratory for analysis. Samples were stored in a cold room between 4 and 10°C until they were processed for *H. glycines* eggs by elutriation and mechanical cyst crushing as reported previously (11). Population densities are reported per 250 cm³ of soil.

All soil samples with detectable populations of *H. glycines* were placed in pots in the greenhouse and planted to *H. glycines*-susceptible soybean cv. Essex to rear sufficient numbers of eggs for race testing. After 30 to 60 days in a water bath at 27°C, cysts were collected from plant roots, and eggs were extracted for use in race tests (12) according to methods described previously (11), except that the resistant plant introduction (PI) 437654 was added to the standard differentials (soybean lines used to differentiate *H. glycines* races) 'Pickett', 'Peking', PI 88788, and PI 90763. Female indices (FI) were calculated for each soybean line as follows: FI = (mean number of females on test soybean line per mean number of females on 'Lee') × 100. Means of three

Fig. 1. Counties in Missouri divided into geographical regions.

Corresponding author: T. L. Niblack
E-mail: tlniblack@uiuc.edu

Accepted for publication 19 March 2003.

replications were rounded to whole numbers. *H. glycines* races were determined according to the standard race test (12).

Data were analyzed with SAS (SAS Institute, Cary, NC) procedures, including general linear models (GLM) for analysis of variance in numbers of eggs and Pearson's product-moment correlations (CORR) for female indices and univariate statistics procedures (MEANS and UNIVARIATE). Geographical regions (Fig. 1) were constructed according to local convention for analysis. No samples were collected from the south-central region because it is an area of low frequency of soybean fields.

RESULTS

Of the 400 samples collected, 392 were processed successfully; eight samples were excluded from the data set due to technical problems in processing. Two hundred and forty-seven (63%) of the samples had detectable populations of eggs from cysts similar in size to *H. glycines* (Table 1). Population densities ranged from 15 to 149,700 eggs per 250 cm³ of soil with an overall mean of 3,450 eggs per 250 cm³ of soil (CV = 314%) or 4,963 eggs per 250 cm³ of soil (CV = 243%). Samples containing no detectable eggs were excluded from the data set. The damage threshold used by the Extension Nematology Laboratory is 500 eggs per 250 cm³ of soil (P. A. Donald, unpublished) representing the state average population density at which yields may be significantly reduced at $P < 0.05$, and 147 samples (60% of infested samples) had egg counts that exceeded the threshold.

Differences in mean numbers of eggs among regions were not significant ($P > 0.05$). The known distribution of *H. glycines* in Missouri (Fig. 2) included Osage County for the first time.

Of 247 samples that had detectable levels of *H. glycines*, 183 produced enough eggs to use for race testing within the study period. In order of frequency, races 3, 1, and 2 were common and collectively accounted for 85.8% of the *H. glycines* populations in Missouri (Table 2). Populations identified as race 2 were more common in the southeast region (Fig. 1) than elsewhere in the state; those identified as race 1 were most frequently found in the northeast, west-central, and central regions; and race 3 populations were most common in the northwest and north-central regions (Table 2).

A majority of *H. glycines* populations in Missouri were able to parasitize PI 88788, whereas few populations were able to parasitize PI 90763 or PI 437654 (Table 3). Female indices on Peking and Pickett were highly correlated (Table 4). With the single exception of the lack of correlation between female indices on PI 88788 and PI 90763, all of the soybean differentials show some degree of correlation in female indices. The converse was true for PI 437654: female indices on that line were significantly correlated only with those on PI 88788.

DISCUSSION

Collected cysts were not identified to species through morphometric or molecular tests. Other cyst nematode species are

known to occur in Missouri, including *H. lespedezae*, *H. schachtii*, *H. trifolii*, and *Cactodera weissi*. Funding constraints prevented more than observation of the cysts as they were extracted from each sample to verify that they were consistent with *H. glycines*. It is not typical (or even common) to expend resources to verify species when the likelihood is high that the assumed identity is correct, as has been the case with most surveys for *H. glycines* (examples include 6,13,17,19). We assumed that cyst nematodes found in soybean fields were *H. glycines*, but we suggest that future surveys include some sort of species verification protocol.

On the basis of our survey, *Heterodera glycines* infest a majority of soybean fields in Missouri regardless of region within the state. At the time of the survey, more than half of the infested fields had *H. glycines* population densities that exceeded the damage threshold of 500 eggs per 250 cm³ of soil. Yield loss due to *H. glycines* has been measured at much lower population densities (10,16), so the utility of using this number as a guideline for interpreting our results is questionable. An action threshold of 1 egg per 250 cm³ of soil (i.e., detection) is used as the basis to recommend rotations with nonhosts and resistant culti-

Table 1. Frequency and population densities of *Heterodera glycines* in Missouri according to regions illustrated in Figure 1

Region	No. of samples	No. of samples positive for <i>H. glycines</i>	<i>H. glycines</i> eggs/250 cm ³ of soil		
			Minimum observed	Maximum observed	Mean of positive samples
Northwest	70	20	100	28,280	5,820
North-central	69	48	20	149,700	8,695
Northeast	58	44	100	81,480	9,238
West-central	26	21	15	15,360	2,805
Central	36	21	40	39,430	5,455
East-central	16	12	50	16,240	2,260
Southwest	16	7	100	15,100	2,840
Southeast	101	74	50	17,000	2,593
Total	392	247			

Table 2. Numbers of *Heterodera glycines* populations identified as each of nine races found in 183 soybean fields in Missouri sorted by regions illustrated in Figure 1

Region	Races								
	1	2	3	4	5	6	9	10	14
Northwest	2	1	14	0	0	0	0	0	0
North-central	12	1	19	1	0	0	1	0	0
Northeast	17	6	11	1	0	0	1	1	0
West-central	7	0	5	0	0	0	0	0	0
Central	7	0	2	1	0	0	2	0	1
East-central	4	1	6	0	0	0	0	0	0
Southwest	1	1	0	0	0	0	0	0	0
Southeast	3	30	4	7	2	4	6	0	1
Total number	54	43	60	7	2	4	10	1	2
Percentage	29.5	23.5	32.8	3.8	1.1	2.2	5.5	0.5	1.1

Fig. 2. Distribution of *Heterodera glycines* in Missouri according to time periods in which the presence of the nematode was confirmed (P. A. Donald, V. L. Dropkin, and T. L. Niblack, University of Missouri, unpublished data).

vars to limit increases in *H. glycines* population densities.

H. glycines field populations are highly variable, not only in population densities, but also in their abilities to develop on and possibly damage resistant soybean plants. This variability is usually assessed as a survey of the frequency of races (6,7,11,14,21). Each race is an average population phenotype defined by its ability (FI ≥ 10 ; “+”) or lack thereof (FI < 10 ; “–”) to develop on all four standard soybean differentials (12). Of the 16 possible races, eight are “+” and eight are “–” on each differential. If one of the purposes of race surveys is to guide soybean breeding programs to use sources of resistance that would be most effective for the largest number of soybean growers, then it is more useful to know the percentage of *H. glycines* populations that are virulent on each source of resistance than to know what races are present. Nematode virulence is defined as “the ability to reproduce on a resistant host” (4). Our results showed that most of the *H. glycines* populations in Missouri are virulent on the most commonly used sources of resistance, PI 88788 or Peking, whereas very few are virulent on PI 90763 or PI 437654. This suggests that sources other than PI 88788 and Peking should be exploited in the future in development of *H. glycines*-resistant cultivars in Missouri.

Another purpose of race surveys appears more straightforward: simply to assess the phenotypic variability of the nematode. However, this cannot be done with race determinations alone, because races are based on the FI obtained on four lines. For example, the relationship between Peking and Pickett (Pickett was selected from a cross with Peking [2]) was reflected in the

high correlation between female indices on the two lines. Tourjee and Niblack (15) showed that these two lines do not differentiate populations of *H. glycines*, therefore, the only reason to include Pickett in a race survey is because it is one of the standard differentials, and a race determination cannot be made without it.

PI 437654 is the most promising source of resistance that has been identified in the last 20 years because it has a high level of resistance to most to *H. glycines* populations and likewise, most *H. glycines* populations are not virulent on it. In our survey, 29 populations were able to produce one or more females on PI 437654, but only 3 had FI > 10 . However, this PI is not resistant to all *H. glycines* populations, and we have been able to select a virulent population (FI = 75; T. L. Niblack and R. D. Heinz, unpublished) from among the Missouri field populations by mass selection through alternating generations on ‘Essex’ and PI 437654. Young (22–24) has noted several instances of *H. glycines* populations virulent on soybean cv. Hartwig, which carries *H. glycines* resistance from PI 437654.

The economic loss due to *H. glycines* infestation cannot be estimated reliably with the information available because losses are dependent on many other factors, including environmental conditions, costs associated with management of the nematode, the price of soybeans, variability in host responses, and interactions between the nematode and other common pathogens such as *Fusarium solani* f. sp. *glycines*, the causal agent of soybean sudden death syndrome. Nonetheless, we must try to provide an estimate because crop loss estimates are used to determine research priorities. Based on our data and estimates of other factors such as those noted above,

H. glycines was responsible for yield reductions that would be valued at approximately \$58 million in 1999. We do not suggest that this is a substitute for reliable economic analysis.

ACKNOWLEDGMENTS

We thank the Missouri Agricultural Experiment Station, the Missouri Soybean Merchandising Council (Research Agreement #99-179), and the United States Department of Agriculture (Special Grant #98-34113-5880) for support of this project. We thank Kamron Colgrove, Joyce Elrod, Dan Oliger, and Robert Wilmsmeyer for technical assistance.

LITERATURE CITED

1. Barker, K. R., Townshend, J. L., Michell, R. E., Norton, D. C. et al. 1978. Determining nematode population responses to control agents. Pages 114-127 in: Methods of Evaluating Plant Fungicides, Nematicides, and Bactericides. E. I. Zehr, G. W. Bird, K. D. Fisher, K. D. Hickey et al. eds. The American Phytopathological Society, St. Paul, MN.
2. Bernard, R. L., Juvik, G. A., Hartwig, E. E., and Edwards, C. J. 1988. Origins and pedigrees of public soybean varieties in the United States and Canada. U.S. Dep. Agric. Agric. Res. Serv. Tech. Bull. 1746.
3. Cotler, J., and Nealson, J. 1987. Area frame design for agriculture surveys. National Agriculture Statistics Service, U.S. Dep. Agric.
4. Hussey, R. S., and Janssen, G. J. W. 2002. Root-knot nematodes: *Meloidogyne* species. Pages 43-70 in: Plant Resistance to Parasitic Nematodes. J. L. Starr, R. Cook, and J. Bridge, eds. CABI Publishing, New York.
5. Jenkins, L. 1963. Nematode Research From 1956-1962. University of Missouri College of Agriculture, Agric. Exp. Stn. Res. Bull. 833.
6. Koenning, S. R., and Barker, K. R. 1998. Survey of *Heterodera glycines* races and other plant-parasitic nematodes on soybean in North Carolina. J. Nematol. (suppl.) 30:569-576.
7. Lehman, P. S., and Dunn, R. A. 1987. Distribution of Florida populations of the soybean cyst nematode with previously undescribed genetic variation. Plant Dis. 71:68-70.
8. Neher, D. A., and Campbell, C. L. 1996. Sampling for regional monitoring of nematode communities in agricultural soils. J. Nematol. 28:196-208.
9. Niblack, T. L. 1992. The race concept. Pages 73-86 in: Biology and Management of the Soybean Cyst Nematode. R. D. Riggs and J. A. Wrather, eds. The American Phytopathological Society, St. Paul, MN.
10. Niblack, T. L., Baker, N. K., and Norton, D. C. 1992. Soybean yield losses due to *Heterodera glycines* in Iowa. Plant Dis. 76:943-948.
11. Niblack, T. L., Heinz, R. D., Smith, G. S., and Donald, P. A. 1993. Distribution, density, and diversity of *Heterodera glycines* in Missouri. J. Nematol. 25:880-886.
12. Riggs, R. D., and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. J. Nematol. 20:392-395.
13. Schmitt, D. P., and Barker, K. R. 1987. Incidence of plant-parasitic nematodes in the coastal plain of North Carolina. Plant Dis. 72:107-110.
14. Sikora, E. J., and Noel, G. R. 1991. Distribution of *Heterodera glycines* races in Illinois. J. Nematol. (suppl.) 23:624-628.
15. Tourjee, K., and Niblack, T. L. 2000. *Heterodera glycines* pathogenicity and fecundity: developing a genetic model of a pathogen's life history traits. J. Nematol. (Abstr.) 32:466.
16. Wang, J., Niblack, T. L., Tremain, J. N., Wie-

Table 3. Univariate analysis of female indices^a (FI) of field populations of *Heterodera glycines* that produced FI ≥ 10 on soybean lines with resistance to *H. glycines*

Soybean line	Number	Percentage	Minimum observed	Maximum observed	Mean	Standard deviation
Pickett	69	37.7	10	115	63	28.5
Peking	62	33.9	11	107	34	18.2
PI ^b 88788	105	57.4	10	112	29	18.0
PI 90763	13	7.1	11	50	19	9.8
PI 437654	3	1.6	10	32	22	11.0

^a Female index = (mean number of females on soybean line per mean number of females on ‘Lee’) $\times$ 100.

^b PI = plant introduction.

Table 4. Correlations coefficients among soybean lines with resistance to *Heterodera glycines* based on female indices (FI)^a from 183 field populations in Missouri

Soybean lines	Pickett	Peking	PI 88788	PI 90763
Peking	0.84 **b			
PI 88788	0.45 **	0.49 **		
PI 90763	0.61 **	0.69 **	0.09 ns	
PI 437654	0.09 ns ^c	0.08 ns	0.27 **	0.02 ns

^a Female index = (mean number of females on soybean line per mean number of females on ‘Lee’) $\times$ 100. Means were obtained from three replications.

^b ** = $P < 0.0001$.

^c ns = not significant, $P > 0.10$.

- bold, W. J., Noel, G. R., Tylka, G. L., Marrett, C. C., Myers, O., and Schmidt, M. E. 2003. The soybean cyst nematode reduces yield without causing obvious aboveground symptoms. *Plant Dis.* 87:623-268.
17. Warner, F., Mather, R., Bird, G., and Davenport, J. 1994. Nematodes in Michigan. I. Distribution of *Heterodera glycines* and other plant-parasitic nematodes in soybean. *J. Nematol.* (suppl.) 26:720-726.
 18. Whitney, G. 1978. Southern states soybean disease loss estimates – 1977. *Plant Dis. Rep.* 62:1078-1079.
 19. Workneh, F., Tylka, G. L., Yang, X. B., Faghihi, J., and Ferris, J. M. 1999. Regional assessment of soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* using area-frame sampling: Prevalence and effects of tillage. *Phytopathology* 89:204-211.
 20. Wraether, J. A., Stienstra, W. C., and Koenning, S. R. 2001. Soybean disease loss estimates for the United States from 1996 to 1998. *Can. J. Plant Pathol.* 23:122-131.
 21. Young, L. D. 1990. Survey of soybean cyst nematode races in Tennessee. *J. Nematol.* (suppl.) 22:672-675.
 22. Young, L. D. 1998. *Heterodera glycines* populations selected for reproduction on Hartwig soybean. *J. Nematol.* (Abstr.) 30:523.
 23. Young, L. D. 1999. Soybeans resistant to *Heterodera glycines* populations attacking Hartwig cultivar. *J. Nematol.* (Abstr.) 31:583.
 24. Young, L. D. 2000. Soybeans resistant to multiple *Heterodera glycines* populations attacking Hartwig cultivar. *J. Nematol.* (Abstr.) 32:472-473.