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A Revised Classification Scheme for Genetically Diverse Populations of *Heterodera glycines*[1]

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Abstract: Heterodera glycines, the soybean cyst nematode, is a major yield-limiting pathogen in most soybean production areas worldwide. Field populations of *H. glycines* exhibit diversity in their ability to develop on resistant soybean cultivars. Since 1970, this diversity has been characterized by a bioassay used to assign a race classification to a population. The value of the race scheme is reflected in the number and quality of resistant soybean cultivars that have been developed and released by soybean breeders and nematologists working in concert. However, the race scheme also has been misapplied as a means of studying *H. glycines* genotypes, in part due to the use of the term "race." For fungal and bacterial pathogen species, "race" can theoretically be applied to individuals of a population, thus allowing inference of individual genotypes. Application of a race designation to an individual egg or second-stage juvenile (J2) of *H. glycines* is not possible because a single J2 cannot be tested on multiple hosts. For other nematode species, "race" is defined by host ranges involving different plant species, whereas the *H. glycines* race test involves a set of lines of the same plant species. Nonetheless, because *H. glycines* populations vary in genetic diversity, and this variation has implications for management strategies, a mechanism is needed for documenting and discussing population differences. The HG Type scheme described herein avoids the implication of genetic uniformity or predictability in contrast to the way the race scheme has been used.

Key words: Heterodera glycines, HG type, nematode, races, soybean cyst nematode.

The success of modern agriculture is due, in part, to the biological diversity available in nature. This variation provides the resources from which we can make selections for genetic improvement of crops. Soybean (*Glycine max* [L.] Merr.) cultivars have multiple genes that control resistance to *Heterodera glycines* Ichinohe, 1952 (Anand, 1994). Use of a wide array of cultivars with different sets of genes or alleles for *H. glycines* resistance has allowed production of high yields in infested fields. This same practice has facilitated expression of *H. glycines* genes involved in host compatibility through selection pressure. For example, we assume that soybean growers in China selected soybean lines that provided acceptable levels of production in soils with a problem that was ultimately recognized as the disease (yellow dwarf) caused by *H. glycines*. As a result, many "land races" of soybean carried resistance to *H. glycines,* including populations that were genetically diverse.

An early example of the recognition of genetic diversity in cyst nematodes was the placement of *H. glycines* as a subspecies of *H. schachtii* (Fujita and Miura, 1934). After *H. glycines* had been described as a separate species (Ichinohe, 1952), Ross (1962) reported that isolates of *H. glycines* from North Carolina differed from Tennessee populations in that those from North Carolina developed on the soybean Plant Introduction (PI) 88788 but those from Tennessee did not. During the next several years, reports of genetic diversity of *H. glycines* came from Virginia (Miller, 1967, 1969, 1970; Miller and Duke, 1966; Smart, 1964), Tennessee (Golden and Epps, 1965), Arkansas (Riggs et al., 1968), and Japan (Sugiyama et al., 1968). Evidence of genetic diversity among populations of the nematode continues to accrue (Anand et al., 1994; Niblack et al., 1993; Rao-Arelli et al., 1991; Sikora and Noel, 1991), along with evidence of similar diversity within populations (Colgrove et al., 2002; Zhang et al., 1998). Because *H. glycines* was and continues to be a serious pest of one of the world's major agricultural crops, efforts to counter its impact through host resistance were implemented.

A major step needed for breeding resistance to *H. glycines* was the development of a classification scheme that would separate the major genetic groups for host compatibility within this species. To provide a means of assessing this type of variability, a group of scientists (nematologists and soybean breeders) met in 1969 and proposed a race test for *H. glycines* populations based on comparative development of females on four differ-

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ential soybean lines (Golden et al., 1970) (Table 1, races 1 through 4). The susceptible standard was the cultivar Lee. Almost immediately, the four-race scheme was shown to describe inadequately the extensive genetic diversity of *H. glycines* populations that existed in soybean production areas (Epps and Duclos, 1970; Miller, 1970). This diversity could be classified into many groups (or types) by increasing the number of differentials. Riggs et al. (1981) differentiated up to 25 different "races" on 12 resistant soybean lines, demonstrating that the set of four differentials used by Golden et al. (1970) did not account for all of the variability for host compatibility in *H. glycines*.

An immediate consequence of a classification scheme limited to four races was the discovery of populations that were different from those four. The dilemma was partially resolved by the description of two new races (Chen et al., 1988; Inagaki, 1979). However, the problem was magnified by the incorrect classification of some populations as race 4. These and other problems associated with race determination, which were summarized by Niblack (1992), ultimately led to the description of all possible 16 races (Riggs and Schmitt, 1988) according to the system developed by Golden et al. (1970) (Table 1, races 5 through 16). This expansion was made with full recognition that four of the races were illogical and not likely to be found (Niblack, 1992): 'Pickett' was selected from a cross with 'Peking'; thus, a nematode population that reproduced on Peking but not on Pickett (i.e., races 11, 12, 13, and 16) was highly unlikely. Discriminant function analysis of the results of more than 250 race tests showed that Peking and Pickett did not differentiate most *H. glycines* populations (Tourjee and Niblack, 2000). Populations identified as race 6, i.e., "+" on Pickett and "–" on the three Plant Introductions (PI), could as easily be differentiated on any number of cultivars derived from one of the three PI sources of resistance; the difference is only that Pickett, and no other cultivar, was agreed upon as a differential.

TABLE 1. Races of the soybean cyst nematode, *Heterodera glycines*, according to the race determination schemes[a] of Golden et al. (1970) and Riggs and Schmitt (1988).

Race	Pickett	Peking	PI 88788	PI 90763
1	–	–	+	–
2	+	+	+	–
3	–	–	–	–
4	+	+	+	+
5	+	–	+	–
6	+	–	–	–
7	–	–	+	+
8	–	–	–	+
9	+	+	–	–
10	+	–	–	+
11	–	+	+	–
12	–	+	–	+
13	–	+	–	–
14	+	+	–	+
15	+	–	+	+
16	–	+	+	+

[a] Race determination is made on the basis of the pattern of "+" and "–" ratings for each race. A "+" rating is given if the number of females produced by an *H. glycines* population on each soybean differential is equal to or greater than 10% of the number produced on the standard susceptible cultivar Lee. If the number of females is less than 10%, a "–" rating is given.

The race test was intended as a means of separation of *H. glycines* populations—not genotypes within populations, and not as a means of assessing resistance in soybean cultivars. No one has been successful in developing genetic or biochemical markers for *H. glycines* races because races are population averages, not individual genotypes. In contrast, Dong et al. (1997) demonstrated the existence of major genes in *H. glycines* inbred lines associated with the ability to develop on PI 88788, Peking, and PI 90763. Their inbred nematode lines have strain designations (Bird and Riddle, 1994), rather than race designations, to reflect their homogeneity for the trait of interest.

The race designation of a population of *H. glycines* does not necessarily predict its behavior on soybean cultivars, regardless of typical cultivar labeling. For example, a cultivar that is labeled as "resistant to race 3" may be susceptible to populations classified as race 3 (Wiebold et al., 2001) if resistance is defined solely on the basis of suppression of *H. glycines* development. This issue was partially addressed by separating the plant effect into levels of resistance and susceptibility (Schmitt and Shannon, 1992).

The term "race" is inappropriate as a population descriptor. For pathogens in other phylogenetic kingdoms, "race" can be applied, actually or theoretically, to individuals of a population, thus allowing inference of individual genotype. This inference is not possible with the *H. glycines* race scheme because of the nature of the test as an average population phenotype. For other nematode species, such as *Meloidogyne incognita* and *Rhadopholus similis,* "race" is defined by host ranges involving different host plant species, whereas the *H. glycines* race test involves a set of lines of the same plant species. Nonetheless, *H. glycines* populations do vary in genetic diversity, and this variation can have implications for management strategies (Cloud et al., 1988; Colgrove et al., 2002; Noel and Edwards, 1996; Riggs et al., 1977, 1988; Young, 1984). For this and other reasons, a mechanism for documenting and discussing *H. glycines* population differences is necessary.

Classification of *H. glycines* populations has at least four important uses: (i) as a mechanism for assessing and documenting differences among field populations or population change over time, (ii) for use by nematologists and soybean breeders for producing *H. glycines*-resistant cultivars, (iii) as a means of describing populations used for screening; and (iv) as an aid to making management recommendations to growers. No particular system of population classification can serve

all four purposes without specificity in applying the system and interpreting the results. Thus, we propose not only the classification scheme itself but also rules for conducting the test, recommendations for reporting and interpreting the results, suggestions for adapting the test, a mechanism for addition of new indicator lines, and suggestions for relating results of the test to soybean producers. The description of a revised scheme that is pertinent, practical, adaptable, and yet will relate to the past system is the objective of this publication.

The HG Type Test

The genetic diversity present and being incorporated into elite soybean lines (Rao-Arelli et al., 1997) has allowed description of certain "types" of *H. glycines* populations that can adversely affect even highly resistant cultivars. Sufficient variation in *H. glycines* types now has been described that the race scheme no longer is adequate to define the populations. As did a group of nematologists looking at the same issue in 1990 (Report of the Infraspecific Designations Committee, Society of Nematologists, Nematology Newsletter 36:3), we found other commonly used biological terms to be inappropriate, and settled on "HG Type." The "HG" part of the term represents the first letters of the genus and species names of the nematode and is included to avoid confusion with taxonomic uses of the term "type," and "type" itself has few confusing implications.

Determining HG Type: Indicator lines. In race testing for *H. glycines* and other pathogens, the cultivars used for testing are referred to as differentials. The phrase "indicator lines" is used for the HG Type test to avoid the inference that all the lines have different panels of resistance genes. There are at least 118 sources of resistance to *H. glycines* in the USDA Soybean Germplasm Collection (Arelli et al., 2000), but a test designed to differentiate field populations of *H. glycines* should be limited to those sources that have actually been deployed in the field. The indicator lines are listed in chronological order according to their registrations in *Crop Science* (Table 2) as sources of *H. glycines* resistance in soybean germplasm lines or cultivars. The first three indicator lines occur in the same order in which they were used in the race scheme, which preserves compatibility with previous publications on *H. glycines* populations. Any new source of resistance can be added to the list in Table 2 whenever it is registered as a germplasm line or cultivar. The Society of Nematologists has established an ad-hoc committee on HG Types to provide a mechanism for expediting changes to the system described herein. Because many of the seven lines used in the HG Type test (Table 2) may share one or more genes for resistance, and because the test is based on population averages, the lines do not absolutely differentiate *H. glycines* genotypes.

Table 2. Indicator lines for HG Type classification of genetically diverse populations of *Heterodera glycines*.

Number	Indicator line	Reference
1	PI 548402 (Peking)	Brim and Ross, 1966
2	PI 88788	Hartwig and Epps, 1978
3	PI 90763	Hartwig and Young, 1990
4	PI 437654	Anand, 1992a
5	PI 209332	Anand, 1992b
6	PI 89772	Nickell et al., 1994a
7	PI 548316 (Cloud)	Nickell et al., 1994b

Determining HG Type: Standard susceptible. The choice of a standard susceptible cultivar for a nematode population bioassay is important because all of the results are based on the numbers of females that develop on it. The calculation for determining races, resistance levels, HG Types, and other population descriptors is the Female Index, where FI = (mean number of females on a test soybean line) / (mean number of females on the standard susceptible) × 100.

The standard susceptible cultivar for the original race scheme was Lee. Reports, both published (Riggs and Schmitt, 1991) and unpublished, of variation within Lee and Lee 68 militate against retaining either as the susceptible check for the HG Type test. Retaining either cultivar would not necessarily preserve compatibility with the race scheme because Lee may have been reported as the standard susceptible even when Lee 68 or Lee 74 was used. Other susceptible cultivars, such as Essex, Williams 82, and Hutcheson (Palmateer et al., 2000; Niblack and Arelli, unpubl.) have been used or tested as standards for race testing, and some PI lines were shown to have significantly higher female development than Lee (Aeny and Riggs, 1993). In two tests of susceptible cultivars against isolates of races 1, 2, 3, and 5 in a growth chamber at 28 °C, Lee 68 had more females than did Lee 74 after 28 days, but the difference was not significant and the numbers on Lee 74 were less variable (Riggs and Schmitt, 1991). On plants that were not evaluated until 35 days, Lee 74 had a few more females than Lee 68 but not statistically so, and the variability also was not different. In many cases, different populations of *H. glycines* show differing abilities to develop and reproduce on the susceptible cultivar (Tables 3,4). This fact cannot be addressed with a population-testing scheme involving lines with resistance genes, but the standard susceptible can and should be stipulated. We chose Lee 74 as the standard susceptible because it has not exhibited the variation seen with Lee or Lee 68, and because it may provide compatibility for some research programs. The actual number of females produced on Lee 74 in a test should be reported.

Determining HG Type: The 10% rule. A more difficult problem than either the name or the standard susceptible is the criterion for determining which hosts are compatible and which are not. A virulent isolate of any

TABLE 3. HG Type determinations for field and greenhouse populations of *Heterodera glycines*.

Name[a]	Source	No. females on Lee 74	FI[b] 1 PI 548402	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 PI 548316	HG Type
RR101	Inbred	653	0.0	5.6	0.0	0.0	4.7	0.0	12.0	7
RR103	Inbred	362	0.5	2.6	0.0	0.1	37.8	0.1	6.6	5
RR104	Inbred	158	98.7	11.9	36.1	0.0	0.0	46.1	43.9	1.2.3.6.7
RR105	Inbred	80[c]	5.5	138.2	0.0	0.0	105.5	0.0	118.2	(2.5.7)[c]
RR106	Inbred	82[c]	33.5	0.0	41.5	0.0	0.4	0.0	11.5	(1.3.7)[c]
RR109	Inbred	174	17.9	10.4	12.3	0.0	0.0	11.6	1.7	1.2.3.6
MO0601	Field	365	3.3	49.5	0.0	0.0	0.0	0.3	77.7	2.7
MO0701	Field	212	0.0	11.3	4.2	0.2	0.0	0.0	16.6	2.5.7
MO0801a	Field	143	0.3	2.8	3.2	0.0	0.0	0.6	21.0	7
MO0801b	Field	280	6.8	32.2	9.0	0.5	3.8	3.1	64.7	2.5.7
MO0901	Field	367	0.8	4.6	0.0	0.0	0.5	0.0	93.0	7

[a] Field populations are given names based on location, lab number, or the whim of the investigator. Inbred populations are named according to published rules (Bird and Riddle, 1994).
[b] Female Index (FI) = (average number of females on indicator line)/(average number of females on Lee 74) × 100.
[c] Note the provisional HG Type designations (in parentheses) for populations producing fewer than 100 females on Lee.

TABLE 4. Incomplete HG Type determinations for selected inbred lines and field populations of *Heterodera glycines*.

Name[a]	Source	No. females on Lee74	FI[b] 1 PI 548402	2 PI 88788	3 PI 90763	4 PI 437654	5 PI 209332	6 PI 89772	7 PI 548316	HG Type
AR56	Field	7.0	0.0	3.6	32.1	0.0	7.1	7.1	nt[c]	nd[d]
AR62	Field	315.3	26.1	4.7	14.4	0.0	6.5	16.2	nt	1.3.6–
JW230	Field	269.3	0.1	1.0	0.0	0.0	4.8	0.1	nt	0–
JW312	Field	291.0	0.1	4.4	0.1	0.0	5.7	0.0	nt	0–
JW367	Field	240.8	40.2	54.7	2.0	0.0	53.9	2.4	nt	1.2.5–
Can01	Field	162.0	1.1	8.3	0.6	0.0	2.8	1.2	nt	0–
IA904	Field	114.3	0.0	8.3	0.0	0.0	4.8	0.0	nt	0–
IA914	Field	199.3	0.0	14.9	0.0	0.0	11.7	0.3	nt	2.5–
KS02	Field	288.0	28.6	10.9	14.5	0.0	5.6	18.9	nt	1.2.3.6–
LA06	Field	131.5	17.5	8.7	4.8	0.0	3.2	12.7	nt	1.6–
OP50	Inbred	231.8	107.2	40.5	70.3	0.0	97.4	105.2	nt	1.2.3.5.6–
PA02	Inbred	147.3	48.4	48.0	2.7	0.0	58.6	3.9	nt	1.2.5–
PA03	Inbred	75.0	0.3	1.7	0.7	0.0	1.0	0.7	nt	nd
PA14	Inbred	207.5	66.9	2.4	34.5	0.0	5.2	5.2	nt	1.3–
LY1	Inbred	129.8	53.4	57.6	65.7	34.1	63.6	39.1	nt	1. . .6–

[a] Field populations are given names based on location, lab number, or the whim of the investigator. Inbred populations are named according to published rules (Bird and Riddle, 1994).
[b] Female Index (FI) = (average number of females on indicator line)/(average number of females on Lee 74) × 100.
[c] nt = not tested.
[d] nd = not determined because the number of females on Lee 74 was too low.

pathogen likely can be selected from a population that produces even one reproductive unit on a given resistant host. However, *H. glycines* females sometimes develop at a low rate on incompatible hosts (i.e., the progeny of the females are not able to reinfect and develop on the resistant host); thus, a "cutoff" FI lower than 10 probably would allow many false positives. The designers of the original race scheme (Golden et al., 1970) agreed that populations with FI < 10 would not be able to maintain themselves, at least within the confines of a single growing season. Retention of the FI cutoff at 10 for a positive host compatibility rating maintains correspondence with the race scheme, but this alone does not fully justify its use; however, no other number can be justified based on statistical analysis of race test data (Niblack, unpubl.). The requirement to publish FI values along with HG Type designations should alleviate some objections to the FI 10 cutoff. Note also that the use of an FI of 10 as the cutoff has no effect on assessment of resistance in soybean cultivars because the HG Type scheme is intended only for use in describing *H. glycines* populations. Those assessing resistance based on FI can choose any number as their cutoff, unencumbered by the rules for HG Type testing.

Use of the FI to classify *H. glycines* populations is retained for several reasons. First, compatibility with the race scheme is desirable. Second, the use of eggs for calculation of indices is inherently more variable re-

gardless of whether total egg counts or eggs per cyst are used (Niblack, Wang, and Arelli, unpubl.). Third, counts of any stage other than the adult female are much more difficult to perform and to interpret than are female counts. For example, infection levels by second-stage juveniles (J2) varied among resistant soybean lines from significantly lower to significantly higher than susceptible checks and each other (Halbrendt et al., 1992). The meaning of male counts is not clear. At the very least, the term "female index" reflects the actual life stage counted.

Determining HG Type: Naming conventions. The HG Type of a population is determined by a bioassay very similar to that used for race determination, but conducted according to a standardized set of rules outlined and justified in the next section. A replicated set of soybean indicator lines (Table 2) and the standard susceptible, Lee 74, are infested with equal numbers of *H. glycines* eggs extracted from a population. After 30 days the females that have developed are extracted from the roots and soil (Niblack et al., 1993) and counted with the aid of a microscope, and the FI is calculated. An indicator associated with an FI $\geq$ 10 is considered a suitable host of the population because of the relatively short time required, theoretically, to build the population on a resistant line that allows $\geq$10% level of reproduction at the start. The HG Type classification of a population is simply a list of the numbers from Table 2 that correspond to the suitable hosts. For example, a population that produces FI $\geq$ 10 on PI 548402 (Peking), PI 88788, and PI 89772 is an HG Type 1.2.6. A population that produces no FI $\geq$ 10 is an HG Type 0 (zero). This naming convention is compatible with the nematode strain-naming convention (Bird and Riddle, 1994).

HG Types should never be reported in scientific or technical journals by themselves. In every case, the FI should be reported in the text or in a table, whichever is most appropriate. This convention is required to avoid the inference that all populations given the same HG Type are equivalent.

In some cases, it may not be necessary or possible to complete the entire HG Type test. When a test is incomplete, all indicator hosts not tested should be reported and the HG Type designation should have a "–" suffix. For example, a population can be referred to as HG Type 1.2.3–. The suffix serves as a signal to the reader that the test was incomplete. The citation of this paper (e.g., Niblack et al., 2002) or Table accompanying the test in a Materials and Methods (or equivalent) section of a report should make clear which indicator lines were used.

Standardization of HG Type Testing

An unavoidable consequence of the "disease pyramid" (i.e., a pedagogical device illustrating that disease is a function of four variables: host suitability, pathogen virulence, environmental conditions, and time) is that bioassays are subject to environmental influences. *Heterodera glycines* race determination, in particular, was shown to be influenced by several environmental factors (Colgrove et al., 2002; Johnson et al., 1993; Riggs et al., 1988; Riggs and Schmitt, 1991) as well as by inoculum level (Wang et al., 1998). Therefore, standardization of the conditions of a bioassay is essential.

Seed source and seedling preparation. The source for seed of the indicator lines and Lee 74 should be the curator of the USDA Soybean Germplasm Collection, or the curator's designee. The current curator, Randall Nelson, agrees with this restriction and has made arrangements to provide seed to researchers. No substitution should be made for an HG Type test indicator line, but other lines and cultivars can be added to the test. In fact, addition of other cultivars and lines probably would be desirable when tests are done for the purpose of making cultivar recommendations (see the section on cultivar recommendations). If tests cannot contain all the necessary indicator lines, then the naming convention for incomplete tests should be used (see the section on naming conventions). Indicator lines important to the interpretation of a study's results should never be omitted or substituted.

Surface-disinfested seed should be germinated in sterile germination paper beginning 3 days before the test is set up. Just before soil infestation with eggs + J2 prepared as described in the next section, seedlings should be chosen for uniformity and transplanted singly to appropriate containers.

Inoculum. Extra care should be taken with field sampling to ensure a reasonable estimate of the population (Barker and Campbell, 1981; Francl, 1986; Schmitt et al., 1990). Cysts should be extracted from soil and roots and crushed to release eggs and J2 (Riggs and Schmitt, 1991). Treatment of the inoculum with antimicrobial substances has not been shown to influence the outcome of race tests although it may influence egg hatching rates (Charlson, 2000; Walk, 1996). Judicious use of such substances might be necessary when a field population is known or suspected to be heavily infested with nematode-parasitic organisms, and should be reported. Preparation of the eggs + J2 suspension should take place on the day after the plants are inserted into the pots. The suspension of eggs + J2 should be constantly gently agitated during the process of soil infestation with 20 eggs + J2/cm^3 soil. An HG Type test should not be run on a field sample that has an inadequate number of eggs.

Containers and soil. Containers should be large enough to accommodate a 30-day-old soybean plant without excessive root restriction. Containers with volumes between 100 and 500 cm^3 have been used successfully. They should also be suitable for placement in a water bath or growth chamber. A pasteurized sandy

loam soil (75% sand) has been found most suitable for *H. glycines* bioassays. Pure coarse sand and media containing a high percentage of organic matter are unsuitable.

Experimental design and environmental conditions. Experimental units, each unit consisting of one container with one seedling, should be placed in a greenhouse or growth chamber in a standard arrangement, such as a completely randomized or randomized complete block. Each test should contain at least three replications, and be repeated once. The soil temperature should be maintained at a constant 27 to 28 °C (Alston and Schmitt, 1988; Hamblen et al., 1972; Riggs and Schmitt, 1991) under 16-hour days. In a recent study, race determination was inconsistent at 20 °C but was consistent at 27 and 30 °C (Palmateer et al., 2000). In greenhouse studies, this may necessitate the use of a water bath and artificial supplemental lighting. Lighting intensity and watering cannot be specified precisely but, obviously, too little or too much of either will be deleterious to the test.

Data collection. The test should be maintained for 28 to 30 days. Shoots can be removed and discarded. Each experimental unit should be soaked gently in a bucket of water to loosen soil to avoid dislodging females. Roots should be placed on nested 850-µm-diam. aperture over 250-µm-diam. aperture (20- over 60-mesh) sieves. All females should be dislodged from the roots with a combination of water spray and manipulation. Most of the females will be retained on the 60-mesh sieve if the 20-mesh is rinsed into it carefully. Females should be prepared for counting by an appropriate method and enumerated at up to × 60 magnification.

The number of females on Lee 74 should be extracted and counted first. If fewer than 100 females (average) are observed on Lee 74, the test should probably be discarded and repeated. In any event, the number should be reported along with the FI calculated for the indicator lines.

Examples of HG Type Tests

Complete tests. Bioassays were conducted during 2000 on five field populations of *H. glycines* in Missouri under the conditions specified for the HG Type test in the preceding section, and on six inbred lines in Arkansas under the conditions described for the HG Type test, except that the inbred lines were grown on a greenhouse benchtop instead of in a water bath or incubator (Table 3). Two tests yielded fewer than 100 females on Lee 74; thus, the HG Type designation for these tests is provisional (given in parentheses).

Incomplete tests. Bioassays were conducted on 15 *H. glycines* field populations and inbred lines from August through November 2000 under the conditions described for the HG Type test, except that the infestation level was 10 eggs + J2/cm^3 soil (Table 4). None of the tests were complete because they did not include PI 548316 (Cloud). The cultivar Pickett was included in the test (data not shown), and race designations would have shown KS02, LY1, and OP50 all to have "+" ratings on Pickett and therefore to be race 4, with the inference that they do not differ in virulence, which is clearly not the case.

Uses and Misuses of the HG Type Test

We cannot emphasize strongly enough that schemes for classifying genetically diverse populations (i.e., field populations) of *H. glycines* should be used for only that purpose and none other. Use of the HG Type test, like the race test, does not affect strain-naming conventions such as that described by Bird and Riddle (1994). Geneticists working on either the soybean or the nematode (or both) should use named, well-characterized strains. Also, use of the HG Type test does not affect schemes used to assess resistance, such as that recommended by Schmitt and Shannon (1992) or in use by private soybean breeding programs; therefore, it does not eliminate the race test upon which these programs are based. Use of phrases such as "resistance to HG Type 5" is a misapplication of the test because HG Type 5 is defined only by its ability to develop on one indicator line. A summary of the standardized procedures for conducting the HG Type test is presented (Table 5).

As with the race scheme, two populations with the same HG Type designation may not behave in the same way because they can easily differ in characteristics not measured by the test. Thus, use of the HG Type test to infer genotypes is not appropriate. For example, research "to identify genetic markers for HG Type 3," implying that all HG Type 3 populations are genetically identical, is a misapplication of the test. However, all HG Type 3 populations should have the genes that enable them to parasitize PI 90763 above the 10% level.

Adaptations. If assessment of resistance is a misapplication of the test, then how can this system be used in breeding programs? Very simply, given our current state of knowledge about interactions between the host and pathogen at the genetic level, screening should be done with named strains or populations that have the desired characteristics. For example, if the source of resistance in a soybean line is PI 88788, then it is logical to screen with any *H. glycines* population that does not have a "2" in its HG Type designation. As positive controls, or just for comparison, screening with populations that *do* have a "2" also is logical. Use of FI values (rather than just race or HG Type designations) allows statistical inspection of the data. This approach can be easily integrated with marker-assisted selection to verify resistance in advanced generations. However, resistance levels in screening are not necessarily predictive of performance in the field, for which trials in *H. glycines*-infested fields are still necessary.

TABLE 5. Summary of standardized conditions for the HG Type test.

I. Sampling
A. Ensure a representative sample of the population.
B. Subsample eggs after cysts are extracted and crushed.
II. Indicator lines
A. Use seed from USDA Soybean Germplasm Collection only.
B. Prepare seed for germination in germination paper 3 days before installing the test.
C. Select seedlings for uniformity and lack of disease symptoms just before transplantation.
D. Transplant seedlings into pasteurized sandy loam soil (75% sand) just before soil infestation.
III. Infestation mixture
A. Prepare infestation mixture (eggs + J2) just before soil infestation.
B. Agitate infestation mixture gently before and during soil infestation procedure.
C. Infest each experimental unit (soil in container with one seedling) with 20 eggs + J2/cm^3 soil.
D. Do not overwater after inoculum application!
IV. Experimental design
A. Use a completely randomized or randomized complete-block arrangement.
B. Replicate treatments at least 3 times; run experiment twice.
V. Environmental conditions
A. Maitain 27 to 28 °C root zone temperature.
B. Provide 16-hour days.
C. Water judiciously.
VI. Data collection
A. Allow test to run 28 to 30 days after soil infestation.
B. Soak experimental units to release soil from roots.
C. Dislodge females from roots with water, on nested 20- over 60-mesh sieves.
D. Prepare suspensions of females for counting, and enumerate under ×60 magnification.
E. Extract and count females on Lee 74 first. If lower than 100, discard test and run again.
F. Calculate FI and assign HG Type designation (Table 2).
VII. Reporting
A. Report actual number of females observed on Lee 74.
B. Report FI on all indicator lines tested in text or table. Specify lines not tested. If test is incomplete, add "–" to the HG Type designation.
C. Name inbred lines or special isolates according to Bird and Riddle (1994).

The HG Type also may be used to make cultivar recommendations to growers. However, cultivars with the same genetic sources of resistance do not necessarily have the same levels of resistance in the field. Based on our experience with race testing, the HG Type scheme may not be directly applicable to grower recommendations. However, if growers have the *H. glycines* populations in a field "HG Type," they will know that a cultivar that has resistance from the same indicator plants as in the population's HG Type name are not likely to have the resistance needed. A useful approach would be to test all new cultivars in comparison with the HG Type indicator plants. Any cultivar that has a similar FI $\geq$10 should not be used in a field that has an *H. glycines* population that is compatible with that indicator line. Most of the *H. glycines*-resistant cultivars have either PI 548402 (Peking), PI 88788, or both in their backgrounds (Diers et al., 1997; Tylka, 2001). A grower-friendly adaptation of the HG Type approach would include a bioassay of the grower's *H. glycines* population on the following: (i) PI 88788 and PI 548402, (ii) any other indicator lines that were the sources of resistance in available cultivars, and (iii) a selection of commercially available resistant cultivars. However, this type of test could be so large that running any number of them would require large amounts of space and time. Of course, other considerations such as field history and *H. glycines* population density would be important to interpreting the results, but this type of bioassay would be more likely than an HG Type designation to tell the grower something useful.

Heterodera glycines field populations probably adapt to cultivars rather than to their sources of resistance because cultivars have genes other than those from the *H. glycines*-resistant parent and may not have all of the genes for resistance that were present in the resistant parent; therefore, the approach described in the preceding paragraph would be useful. However, this procedure requires at least two important elements that are missing in many soybean production areas today. First, it requires a well-informed technical person (extension specialist, clinic director, technical representative, etc.) with appropriate facilities and resources. Second, the sources of resistance should be acknowledged on the labels of all commercially available cultivars.

Conclusions and Recommendations

The *H. glycines* race scheme served a critical role to many members of the soybean community, including breeders, nematologists, growers, consultants, extension specialists, and farm advisers, to name a few. Even now, it has a vital function in development of germplasm and recommendations to growers regarding cultivar selection and rotation options. The relative ease of the testing protocol, combined with the universal acceptance by both public and private breeders, has made the *H. glycines* race scheme robust and practical. However, the race scheme is inadequate to describe the genetic variation we know to exist in *H. glycines* field populations. To better describe population variation and to expand the flexibility of the classification system, we propose the HG Type Test.

The HG Type test bears many of the hallmarks of the race test, including both protocols and interpretations, and is an evolution of this successful system. The HG Type system uses three of the four resistant soybean genotypes currently used as indicator hosts (Peking [=PI 548402], PI 88788, and PI 90763), and both systems describe nematode populations based on development of adult females. In both systems, reproduction of 10% or more on a resistant cultivar, when compared

with Lee 74, results in a designation of compatibility. Importantly, the guidelines for reporting HG Type test results stipulate that actual female numbers on Lee 74 are reported along with the FI. This will provide an indication of both allele frequency in the population and the risks associated with planting a resistant cultivar.

Similar to any evolving technology (i.e., software), the HG Type test provides many new features compared with the race test. Significantly, all of the indicator hosts in the HG Type test are currently in use or have been used as sources of resistance in released soybean germplasm. Each indicator host has been given its own unique identifier number such that an HG Type designation of HG Type 1.2.4 will easily and unambiguously indicate the sources of resistance with which the tested population is compatible. For management and recommendation purposes, it will be readily apparent that compatible sources of resistance will be less effective than incompatible ones at reducing *H. glycines* reproduction. The simplicity of interpreting an HG Type designation is one of its key strengths. A second, new feature of the HG Type test is its ease of expandability as new soybean germplasm is released and deployed. Guidelines have been established to add these new indicator hosts to the system by simply adding a new number to the designation. The addition of new indicator lines will not invalidate or make less useful the results of previous tests. (In contrast, an expansion of the race scheme would result in doubling of potential races every time a new differential was added, complicating interpretation dramatically.) Another new feature of the HG Type test is the elimination of Pickett as an indicator host. The use of Pickett has always been questioned because Peking is an indicator host and is also the sole source of resistance in Pickett.

Included in the many features of the race test that have been retained in the new system are some longstanding limitations. A major source of variability in characterizing *H. glycines* populations is sampling error. Additionally, the range of host compatibility of a single nematode cannot be tested on multiple hosts, due to the obligate nature of nematode parasitism. Neither the HG Type test nor the race test can account for these issues, nor can any other bioassay. Even if it were feasible to collect multiple, single infective juveniles from the field to test individually on indicator hosts, the inherent heterogeneity and allele frequency variation would skew the interpretation of results. Finally, the utility of the HG Type test, like that of its predecessor, is limited by the fact that there may be multiple nematode populations that are similar with respect to reproduction on the indicator host, but will vary with respect to their compatibility with released soybean cultivars.

Although the focus of this system is the nematode, the host side of the interaction cannot be ignored. The complexity of *H. glycines* resistance in soybean, combined with the significant heterogeneity of nematode populations, makes strict conclusions both difficult and questionable. Clearly, however, there are numerous sources of resistance to *H. glycines* in soybean germplasm. As more becomes known about resistance genes, and indeed about alleles of currently known genes, the HG Type test may evolve to incorporate this knowledge. Although the nematode appears to carry single genes that enable it to reproduce on resistant soybean genotypes, the nature of soybean resistance, or how multiple genes may interact to confer varying levels of resistance, is not at all clear.

Soybean breeders have used the *H. glycines* race designation to great advantage, and have developed numerous high-yielding resistant varieties suited for particular environments. HG Type testing will expand their abilities to explore their elite germplasm, while also retaining the advantages of the race system. To modify the HG Type test for resistance testing, few additional things need to be done. The number of recommended replications should be increased to accommodate for typical experimental variation but, more importantly, several HG Type populations with the same designation, but distinct geographical origin, should be employed. This will enable breeders to examine the response of their germplasm to nematodes carrying the same virulence genes, but different interacting genes that can affect host compatibility. This is extremely important because very little is currently known about modification of nematode virulence by independent genes.

The HG Type test immediately supersedes the race test for describing *H. glycines* populations. The guidelines for conducting these tests must be followed as closely as possible. All deviations from the standardized HG Type test conditions (Table 5) should be described in detail when reporting test results. Numbers of females on Lee 74, the FI on all tested lines, and a list of lines not tested (if applicable) should accompany every report of HG Type designation. An extremely important proviso of this system is that HG Type designations are population descriptions, not genotypes of individual nematodes. Inbred lines of *H. glycines* should be given strain names according to the accepted nomenclature. Additionally, these strains should be made available by depositing type samples with the collections maintained at the University of Arkansas and the University of Illinois.

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