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Effect of Harvest Timing on *Heterodera glycines* Race and HG Type Characterization

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Abstract – Rocha, M.R.; T. Anderson & T. Welacky, 2004. Effect of harvest timing on *Heterodera glycines* race and HG type characterization.

Three populations of *Heterodera glycines* developed on soybean cultivars Ina, Hammer and S20-20, respectively resistant, tolerant and susceptible to the nematode, were subjected to a race test and HG type test. These populations were maintained during six consecutive plantings of the soybean cultivars. The tests were conducted under greenhouse conditions using the soybean differentials Peking, PI88788, PI90763, PI437654, Pickett and the susceptible checks Lee 74 and S20-20. In order to evaluate the female index (number of females on differentials/number of females on susceptible check) x 100) plants were harvested at 35 and 42 days after inoculation (DAI). Harvest timing resulted in different races and HG type identification from the same inoculum source. These results suggest that incubation period can cause significant effect on the race/HG type test results interpretation.

Keywords: *Glycine max*, *Heterodera glycines*, race test, HG type test, soybean cyst nematode.

Resumo: Rocha, M.R.; T. Anderson & T. Welacky, 2004. Efeito do momento da avaliação sobre a caracterização de raça e tipo de *Heterodera glycines*.

Três populações de *Heterodera glycines* desenvolvidas nas cultivares de soja Ina, Hammer e S20-20, respectivamente resistente, tolerante e suscetível ao nematóide, foram submetidas ao teste de caracterização de raça e tipo HG. Estas populações foram mantidas durante seis plantios consecutivos das cultivares de soja. Os testes foram conduzidos sob condições de casa de vegetação em Harrow, Ontário, Canadá, utilizando as diferenciadoras Peking, PI88788, PI90763, PI437654, Pickett e, como padrão de suscetibilidade, as cultivares Lee 74 e S20-20. Para avaliar o índice de fêmeas [(número de fêmeas nas diferenciadoras/ número de fêmeas na cultivar suscetível)x 100], as plantas foram colhidas aos 35 e 42 dias após inoculação. O momento da avaliação resultou em diferentes identificações de raça e tipo HG para a mesma fonte de inóculo. Estes resultados sugerem que o momento da avaliação podem exercer efeito significativo na interpretação dos resultados na identificação de raça e/ou tipo de *Heterodera glycines*, constituindo mais um importante fator a ser considerado na padronização destes testes.

Palavras-chave: *Glicine max*, *Heterodera glycines*, raça, tipo HG, nematóide de cisto da soja.

Introduction

Soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) reproduces by amphimixis, which provides an opportunity for genetic variation between individuals or populations through genetic recombination. A host plant favorable for reproduction of a few specific individual nematode genotypes will differentially increase these genotypes to form a population that has host specialization (Sidhu & Webster, 1981). Host specific physiological strains of *H. glycines* were defined as races by Golden et al. (1970) and became widespread with the complete characterization

scheme (Riggs & Schmitt, 1988). Now it has been redefined as HG type (Niblack et al., 2002).

Significant variability in race determination has been found in tests conducted in different laboratories and several studies have reported the sources of variation and recommended race tests to be conducted under standardized procedures (Riggs et al., 1988; Riggs & Schmitt, 1991; Wang et al., 1998 and Palmateer et al., 2000).

One factor that may contribute to the variation in race determination results is lack of genetic uniformity in seeds of soybean differentials (Riggs & Schmitt, 1991). Other variations may occur due to inability to obtain completely uniform inoculum or to recover all nematodes that penetrated roots (Riggs et al., 1988). These authors stated that the five cultivars and lines should be purified by single seed culture, tested for reaction to *H. glycines* races, and maintained at a single location in a manner that would assure purity. Inoculum should consist of eggs and (or) second-stage juveniles (J2) added just before or just after transplanting. Mature female counts should be made 30 days after inoculation. Temperature of the test medium should not be below 20°C or above 33°C for an extended period of time (Riggs et al., 1988).

Palmateer et al. (2000) observed that at 20°C a *H. glycines* population confirmed as race 3 was designated as race 5 based on egg indices and as race 6 based on juvenile indices. They found that race designations based on female, egg and juvenile indices were inconsistent at 20°C. Race designations at 27 and 30°C were consistent for all three indices, which indicates that counting females, eggs or juveniles should be equally reliable when race determinations are conducted at these two temperatures.

Wang et al. (1998) found that the infestation density affected the race identification. An infestation density of 4,000 eggs and J2/ pot was best for race identification. The differentiation was inconsistent at the 1,000-infestation density, and densities higher than 4,000 had reduced numbers of females on Lee 74 and relatively high numbers on the differentials, which resulted in poor race differentiation with some races.

Among other factors affecting the race determination, Riggs & Schmitt (1991) tested several susceptible checks, including Lee 74, and the results indicated that the susceptible cultivar used could greatly affect the apparent race of a population of *H. glycines*. They also compared two harvest timings and found that the number of females produced per plant was lower at 35 days than at 28 days even though the soil was screened to extract females and cysts that were dislodged from the roots when the soil was removed (Riggs & Schmitt, 1991).

The purpose of this study was to evaluate the effect of harvest timing on *H. glycines* race and HG type designation.

Materials and Methods

A soybean cyst nematode field population initially identified as race 3 was cultivated with soybean cultivars S20-20, Hammer and Ina for six consecutive plantings under greenhouse conditions. Each planting period was long enough to allow one nematode generation (40 days). Cultivars S20-20, Hammer and Ina are rated as susceptible, tolerant and resistant to *H. glycines*, respectively. Inoculum obtained after consecutive plantings was multiplied on susceptible cultivar S20-20 for three generations in order to increase population.

For inoculum preparation plants were removed from stock population pots and roots were washed under high-pressure water on an 850-µm-pore sieve nested over a 180-µm-pore sieve to dislodge the females. Females collected from the lower sieve were placed on a match of 150-µm-pore and 38-µm-pore sieves and gently crushed with a rubber cork to release eggs. The eggs collected from the 38-µm-pore sieve were poured into a becker and the inoculum concentration was determined by counting eggs in a Peters slide under stereoscopic microscope at x45 magnification.

Seeds of differentials Peking, PI88788, PI90763, PI437654 and Pickett, and the susceptible checks Lee 74 and S20-20, were germinated in rolled paper towels for three days before planting. Seedlings were selected for uniformity and lack of disease symptoms. The seedlings were transplanted to 150 cm^3 cones filled with autoclaved sand, set in a bucket also filled with sterilized sand and maintained in water table with temperature adjusted to 28°C, under greenhouse conditions and 14-hour day length.

Inoculum consisted of a suspension of eggs and J2 obtained from soybean cultivars S20-20, Hammer and Ina at a concentration of 4,800, 4,800 and 2,100 eggs and J2/plant, respectively. Seedlings were inoculated just after transplanting. Inoculum suspension was kept agitating during inoculation procedure.

Experiments were arranged in a completely randomized design and the treatments were replicated 10 times. Five replications were harvested at 35 days after infestation (DAI) and five replications were harvested at 42 DAI. Cones containing plants were soaked in water to release soil from roots. Shoots were discarded and roots were placed on a match of 850 over 108-µm-pore sieves. Females were dislodged from roots by washing under high-pressure water. Females collected from the lower sieve were counted under stereoscopic microscope at x30 magnification. Actual number of females was used to calculate Female Index as follows: FI = [(number of females developed on differentials / number of females developed on susceptible check Lee 74) x 100]. Races were

designated according to Riggs & Schmitt (1988) and HG type according to Niblack *et al.* (2002).

Results and Discussion

The three *H. glycines* populations obtained from soybean cultivars S20-20, Hammer and Ina were identified as race 3 when harvested at 42 days after inoculation (DAI), either using Lee 74 as a susceptible check or S20-20 (Tables 1, 2, 3), except for the population on Hammer that was identified as race 1 when identification was based on Lee 74 (Table 2). However, when plants were harvested at 35 DAI in a different race designation resulted for all three populations compared to what was found at 42 DAI. The same trend was observed for the HG type test (Table 4).

The inoculum source obtained from consecutive plantings of susceptible soybean cultivar S20-20 was identified as race 1 at 35 DAI and race 3 at 42 DAI regardless of the susceptible check used in the test (Table 1). The HG type designation for the same population was 2- at 35 DAI and 0- at 42 DAI based on both susceptible checks (Table 4).

The number of females obtained from the population developed on the susceptible cultivar S20-20 was very low when the experiment was harvested at 35 DAI (Table 1). According to Niblack et al. (2002) the females on Lee 74 must be extracted and counted first. If numbers are lower than 100 the test should be discarded and run again. However, the other two populations obtained from soybean cultivars Hammer (Table 2) and Ina (Table 3) had a higher number of females. The seven days between the two evaluations provided a great increase in the female number on roots for all three populations.

At 35 DAI inoculum obtained from consecutive plantings of soybean cultivar Hammer (Table 2) was identified as race 15 and 1 when the differentials were compared to the susceptibles Lee 74 and S20-20, respectively. The same population was identified as race 1 and 3 when plants were harvested at 42 DAI.

The *H. glycines* population developed on 'Ina' (Table 3) was identified at 35 DAI as race 16 based on Lee 74 as a susceptible check and as race 1 based on S20-20. Harvesting at 42 DAI the population was identified as race 3.

The HG type designation for both populations obtained from cultivars Hammer and Ina (Table 4) were also different at 35 and 42 DAI either considering Lee 74 or S20-20 as susceptible check.

These results agree with Riggs & Schmitt (1991) who found that the susceptible cultivar used could greatly affect the apparent race of a population of *H. glycines*.

Table 1 – Average number of *H. glycines* females developed on soybean differential lines and cultivars, female index (FI) and race designation for the isolate obtained from soybean cultivar S20-20.

Differentials	35 DAI		42 DAI	
	Females	FI*	Females	FI*
Lee 74	69.8		760	
Peking	0.2	0.29	5.8	0.76
PI88788	14.4	20.63	27.4	3.60
PI90763	0	0	22.0	2.89
PI437654	0	0	4.6	0.60
Picket	3.4	4.87	14.8	1.95
RACE		1		3

*FI = [(number of females developed on differentials)/ (number of females developed on Lee 74)] x 100

Table 2 – Average number of *H. glycines* females developed on soybean differential lines and cultivars, female index (FI) and race designation for the isolate obtained from soybean cultivar Hammer.

Differentials	35 DAI		42 DAI	
	Females	FI*	Females	FI*
Lee 74	255.6		1168	
Peking	20.2	7.9	52.8	4.52
PI88788	91.2	35.68	139.2	11.92
PI90763	41.8	16.35	60.4	5.17
PI437654	13.6	5.32	43.2	3.70
Picket	39.4	15.41	63.8	5.46
RACE		15		1

*FI = [(number of females developed on differentials)/ (number of females developed on Lee 74)] x 100

Table 3 – Average number of *H. glycines* females developed on soybean differential lines and cultivars, female index (FI) and race designation for the isolate obtained from soybean cultivar Ina.

Differentials	35 DAI		42 DAI	
	Females	FI*	Females	FI*
Lee 74	219.2		797.6	
Peking	31.4	14.32	14.2	1.78
PI88788	45.6	20.80	35.5	4.45
PI90763	31.2	14.23	30.0	3.76
PI437654	14.0	6.39	12.4	1.55
Picket	4.25	1.94	12.8	1.60
RACE		16		3

*FI = [(number of females developed on differentials)/ (number of females developed on Lee 74)] x 100

Table 4 – Incomplete HG type determinations for *Heterodera glyicines* populations obtained from six consecutive plantings of soybean cultivars S20-20, Hammer and Ina.

			Female Index (FI)							HG type	
	Females on Lee 74	Females on S20-20	1	2	3	4	5	6	7		
Name			PI 548402	PI 88788	PI 90763	PI 437654	PI 209332	PI 89772	PI 548316	based on Lee 74	based on S20-20
S20-20 (35 DAI)	69.8	119.2	0.29 (-)	20.6 (+)	0 (-)	0 (-)	nt*	nt	Nt	2-	2-
S20-20 (42 DAI)	76.0	982	0.76 (-)	3.6 (-)	2.89 (-)	0.60 (-)	nt	nt	Nt	0-	0-
Hammer (35 DAI)	255.6	488	7.9 (-)	35.7 (+)	16.4 (+)	6.28 (-)	nt	nt	Nt	2.3-	2-
Hammer (42 DAI)	1168	1565	4.52 (-)	11.9 (+)	5.17 (-)	3.70 (-)	nt	nt	Nt	2-	0-
Ina (35 DAI)	219.2	347.6	14.3 (+)	20.8 (+)	14.2 (+)	6.39 (-)	nt	nt	Nt	1.2.3-	2-
Ina (42 DAI)	797.6	1762.4	1.78 (-)	4.45 (-)	3.76 (-)	1.55 (-)	nt	nt	nt	0-	0-

Female Index (FI) = (average number of females on differentials) / (average number of females on Lee 74) x 100.
*nt = non tested

In all three populations the race and HG type designations were different when harvested at 35 or 42 DAI. It is suggested by many authors that the test should be harvested at 28 to 30 days after soil infestation. Not many references were found for stuies of the effect of harvest timing, but Riggs & Schmitt (1991) observed lower number of females at 35 days than at 28 days. The reason for the greater number of females extracted at 28 days than at 35 days was not known. If the roots alone had been processed at 35 days, the difference might have been caused by some females coming free in the soil, when the roots were extracted; however, when the females were extracted from both roots and soil, fewer were obtained at 35 days than at 28 days. In our study the number of females on roots increased greatly on the susceptible checks from 35 to 42 DAI, which may be explained by the longer period available for the nematodes to fully develop into adult females. Because the female index is a relative value determined by the actual number of females produced on differential hosts compared to the number of females produced on a standard susceptible host, the variation in number of females produced on differentials and/or susceptible will affect the FI value and consequently the race designation.

It is important to mention that inoculating with J2, females can appear on the roots 13 days after inoculation when the temperature is maintained at 28° C, according to Melton et al. (1986). These authors found that the most favorable temperature for development of *H. glycines* was 28° C. Alston & Schmitt observed that the life cycles were completed in 3 to 4 weeks depending on the temperature ranging from 15 to 30° C. Although the inoculation in this study was made with a suspension of mainly eggs but also containing J2, it is possible that at 42 DAI we have counted females from the second and third generation. In this case, when the temperature is maintained at 28° C, it is also possible to have 2 generations at 28 to 30 DAI, which is the timing recommended for evaluating race tests.

Considering that the initial population from the field was previously identified as race 3 before the six consecutive plantings, if we take the results from the first harvest timing as the final results, we conclude that after six consecutive plantings of the cultivars S20-20, Hammer or Ina, respectively susceptible, tolerant and resistant to *H. glycines*, the race shift occurred. The race 3 changed into race 1 on cultivar S20-20, race 15 or 1 on 'Hammer' and race 16 or 1 on 'Ina'. However, if we consider the second harvest timing (42 DAI) we would find no race shifts except for the population developed on soybean cultivar Hammer, and only when the race test was based on Lee 74 as a susceptible check.

The results obtained at 42 DAI suggest that six consecutive plantings of soybean cultivars with different levels of resistance did not enable the race shift in the *H. glycines* population.

In conclusion, this study raises one more factor that may interfere with the results of race or HG type test and reaffirms the need to standardize procedures and carefully interpret the results.

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