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Effect of resistant and susceptible soybean cultivars on the development of male and female *Heterodera glycines* Ichinohe

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Soybean cyst nematode, *Heterodera glycines* Ichinohe, is one of the major phytopathological problems affecting soybeans, *Glycine max* (L.) Merr., in the major producing countries and the use of resistant cultivars and crop rotation have been the main methods of control adopted to reduce the nematode population in infested soils. Purpose of this study was to evaluate the effect of resistant (BRSGO Ipameri and BRSGO Chapadões) and susceptible (BRSGO Araçu, BRSGO Jataí, BRSGO Luziânia, BRS Favorita RR, BRS Valiosa RR, BRS Silvânia RR) soybean cultivars on the development of *H. glycines* males and females during two successive years (2007 and 2008). In the trial of 2008 the plants were divided in three plots, with the last one having the roots stained to count the juveniles and to evaluate survival rate. Resistant cultivars always maintained a small number of females and males, except for cultivar BRSGO Ipameri that had a high count of males. Only cultivars BRS Favorita RR and BRS Silvânia RR had a sex ratio of 1:1. All other susceptible cultivars had, in general, greater number of males than females. Survival rate was nil on both resistant cultivars, and varied from 6.75 to 35.00% on the susceptible cultivars.

Key words: *Glycine max*, cyst nematode, sex ratio, hydroponics.

INTRODUCTION

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a major disease of soybean, *Glycine max* (L.) Merr. in the main producing countries of this legume, such as the United States, Brazil and Argentina (Wrather et al., 1997).In Brazil, *H. glycines* was first identified in the cropping season 1991 /1992 (Lima et al., 1992; Lordello et al., 1992; Monteiro and Morais, 1992), and now occurs in ten states with the races 1, 2, 3, 4 (Dias et

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al., 2009), 4+ (Dias et al., 1998) 5, 6, 9, 10, 14 (Dias et al., 2009) and 14+ (Dias et al., 1999) been identified.

Losses caused by *H. glycines*, depending on its incidence, can be greater than those caused by any other disease affecting soybeans (Wrather et al., 1997). Yield decreases caused by *H. glycines* in the United States, the years 2003, 2004 and 2005 were 2.9, 3.47 and 1.93 million tons of the grain, respectively, highlighting the disease severity (Wrather and Koenning, 2006).

Genetic resistance is one of the major management strategies for the control of soybean cyst nematode. In general, on resistant cultivars, *H. glycines* juveniles are incapable of establishing feeding sites, due to the deterioration of syncytium, which occurs soon after infection, leading to nematode death in the root tissue (Kim et al., 1987; Kim and Riggs, 1992). Nematode females are responsible for most damage due to the formation of syncytium and by feeding from J2 to adulthood, while the males feed only as J2 and J3 (Endo, 1964, 1965). Therefore, sex determination in *H. glycines* is fundamental, since females cause most damage to soybeans.

In ideal conditions, the sex ratio of H. glycines is 1:1 (Endo, 1965; Koliopanos and Triantaphyllou, 1972; Luedders, 1987; Halbrendt et al., 1992). However, several factors can affect this proportion, such as inoculum density (Koliopanos and Triantaphyllou, 1972; Stelle, 1975; Evans and Fox, 1977), temperature (Melton et al., 1986), host nutritional status (Grundler et al., 1991) and genetic resistance (Endo, 1965; Luedders, 1987; Halbrendt et al., 1992; Colgrove and Niblack, 2005). Sex determination in this species is under strong genetic control, and is determined in the zygote instead of by environmental conditions during juvenile development. Stress conditions can reflect in greater mortality of a given sex and, consequently, in a sex ratio other than 1:1 (Koliopanos and Triantaphyllou, 1972). Most reports of unbalanced sex ratio are related to differential female death (Evans and Fox, 1977; Colgrove and Niblack, 2005).

Soybean cultivars with syncytium degeneration occurring four to five days after infection, present a small number of males (Endo, 1965). Luedders (1987) found that resistance genes against this nematode can affect males and females differentially, as occurs with PI 88788, but does not seem to affect male development. Halbrendt et al. (1992) also did not find changes in male development in PI 209332. However, in the same study, they observed that male development is severely affected in PI 89772 and in cultivar Pickett. Those authors stated that resistance of PI 209332 affects the development of J_3 and J_4, resistance of cultivar Pickett affects the development of J_2 and J_3, while resistance of PI 89772 affects all developmental stages. Since *H. glycines* males feed only during the stages J_2 and J_3, the genotypes with resistance affecting the end of the nematode cycle (J_3 and J_4), exert lesser influence on male development.

Colgrove and Niblack (2005) found sex ratio 1:1 for PIs 548402, 90763, 437654 and 89772, and increased male number for PIs 88788, 209332 and 547316. Those authors found that the greater proportion of males occurred due to differential death of males and females, contributing to the hypothesis that sex determination are a genetic characteristic of nematode *H. glycines* unaffected by environmental factors. Thus, this study evaluated the development of *H. glycines* males and females on resistant and susceptible soybean cultivars recommended for the Cerrados region in Brazil.

MATERIALS AND METHODS

The population of *H. glycines* race 14 used in the experiments was collected from a naturally infested field in the county of Campo Alegre (GO), and sequentially multiplied in a greenhouse, using the susceptible cultivar BRSGO Luziânia. *H. glycines* race 14 was chosen due to its wide spread in Brazil. Two experiments were conducted under greenhouse conditions, at Universidade Federal de Goiás (16°35'47.36"S; 49°16'48.01"W; 726 m above sea level), using six commercial soybean cultivars susceptible to *H. glycines*: BRSGO Araçu, BRSGO Jataí, BRSGO Luziânia, BRS Favorita RR, BRS Valiosa RR, BRS Silvânia RR and two resistant cultivars: BRSGO Ipameri and BRSGO Chapadões. These soybean cultivars are recommended for planting at the central part of Brazil (Cerrados region).

Experiment 1

The experiment was conducted from March to April 2007, in a completely randomized design with eight treatments and five replications. The eight soybean cultivars were germinated in a germination chamber, at Universidade Federal de Goiás (16°35'47.36"S; 49°16'48.01"W; 726 m above sea level), and four seedlings were transplanted to 1,400-cm^3 plastic pots, containing naturally infested soil, with an average initial population of 157 cysts of the nematode per 100 cm^3, with a mean of 225 eggs per cyst. The pots containing the seedlings were maintained over a wet sand bed on the benches. Two seedlings were removed from the pots ten days after transplanting, and transferred to a hydroponics system, and maintained there for 19 days, to collect *H. glycines* males. The other plants remained in the pots until completing 30 days, when they were removed to evaluate the number of females present in the root system and the number of eggs per female.

Experiment 2

The experiment was done in January and February 2008, in a completely randomized design, with eight treatments and four replications. The eight soybean cultivars were germinated in sand in the greenhouse and six seedlings were transplanted to 1,400 cm^3 clay pots, containing naturally infested soil, with an average initial population of 118 cysts of the nematode per 100 cm^3, with a mean of 150 eggs per cyst. The pots containing the seedlings were maintained over a wet sand bed on the benches. Two seedlings were removed from the pots ten days after transplanting, and transferred to a hydroponics system to collect the males. On the following day, two other seedlings were removed and the root system stained, using the clearing technique with NaOCl and staining with acid fuchsin (Byrd et al., 1983), to quantify the juveniles and determine the survival rate. The other two plants

Table 1. Number of male and female individuals, number of eggs per female and sex ratio of *H. glycines*, in a trial conducted in 2007.

Soybean cultivars	2007			
	Number females	Number eggs/female	Number males	Sex ratio
BRSGO Jataí	467[a]	217[a]	1.003[a]	2,15*
BRSGO Luziânia	528[a]	285[a]	818[ab]	1,55*
BRSGO Araçu	304[a]	178[ab]	879[abc]	2,88*
BRS Favorita RR	270[a]	248[a]	212[bc]	0,78
BRS Valiosa RR	1.184[a]	183[ab]	839[ab]	0,71*
BRS Silvânia RR	401[a]	231[a]	334[abc]	0,83
BRSGO Ipameri	3[c]	15[c]	201[c]	62,98*
BRSGO Chapadões	19[b]	36[b]	51[d]	2,74*
CV (%)[1]	10.01	12.43	7.77	

Averages in a column followed by the same letter do not differ significantly (Tukey P < 0.05). Data were transformed into log (x+1) for statistical analysis purposes.*Sex ratio different from 1:1 (χ^2 test at α=0.05).[1]Coefficient of variation.

remained in the pots until completing 32 days, when the number of females and the number of eggs per female were evaluated.

The hydroponics system was set with 500 cm^3 PET flasks and an air compressor adjusted to 40 PSI, with fine hoses connected to the flasks to maintain constant airing of the water. Nematode collection started 10 days after transferring the seedlings, and was done every three days. The water removed from the flasks was transferred to glass bottles and taken to the laboratory for decanting for two hours. Subsequently, the water volume was reduced, with the aid of a vacuum pump, to 20 m*l* and then homogenized, and the males present in the suspension counted, using the Peter's slide. Each sample was counted three times under an optical microscope (magnification of 50 *x*).

The number of females was evaluated by removing the plants from the pots and rinsing the root system under running water over a set of 20 and 60 mesh sieves. The material retained in the 20 mesh sieve was discarded and that retained in the 60 mesh screen was filtered with filter paper over a plastic screen (Andrade et al., 1995) and counted under the stereoscope (magnification of 15 *x*). Ten females were arbitrarily picked and broken over a set of 100 mesh and 400 mesh sieves, and the eggs recovered in water on the 400 mesh sieve and quantified under the stereoscope, using Peters' slide (magnification of 50 *x*).

The stained roots were placed in Petri dishes and the number of juveniles was quantified under the stereoscope (magnification of 15 *x*). The number of juveniles in the stained roots and the number of females present in the root system of the plants maintained in the pots were used to determine the survival rate [(females present in the root system / number of juveniles in the stained roots) *x* 100] (Congrove and Niblack, 2005).

The chi-square (χ^2) test was used to confirm the hypothesis that the males of *H. glycines* comprise 50 % of the adult population (sex ratio 1:1). The data were transformed into logx + 1 and submitted to the analysis of variance. The averages were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Significant differences for the number of *H. glycines* females in the roots among the cultivars were observed for both experiments, in 2007 and 2008 (Tables 1 and 2). The commercial cultivars resistant to *H. glycines*, race 14, BRSGO Ipameri and BRSGO Chapadões, confirmed the expected performance in both experiments, presenting smaller numbers of females (Tables 1 and 2). All other cultivars performed as susceptible to the nematode.

In 2007, the number of eggs per female on the cultivar BRSGO Ipameri differed statistically from the other cultivars presenting the lowest number. The cultivar BRSGO Chapadões had low number of eggs per female but did not differ statistically from BRS Araçu and BRS Valiosa RR. In 2008 both resistant cultivars, BRSGO Ipameri and BRSGO Chapadoes, had low development of eggs per female differing from the susceptible cultivars (Tables 1 and 2).

A high number of *H. glycines* males were found in the roots of plants from the hydroponic system in both experiments (Tables 1 and 2). In 2007, the cultivar BRSGO Chapadões differed from all the other cultivars presenting the lowest number of males. Cultivar BRSGO Ipameri only differed from BRS Valiosa RR, BRSGO Luziânia and BRSGO Jataí (Table 1). In 2008 the cultivar BRSGO Chapadões presented the lowest number of males differing from cultivars BRS Favorita RR, BRS Valiosa RR and BRS Silvânia RR (Table 2).

The sex ratio between males and females in 2007 (Table 1), is near 50% only for cultivars BRS Favorita RR and BRS Silvânia RR, by the χ^2 test (5%). In 2008 all cultivars had the sex ratio different from 1:1. The resistant cultivars had sex ratios extremely high due to the absence or very low development of females. The survival rate, done only for the 2008 experiment, varied from 0% to 35.00 % (Table 2). All root systems were colonized by the nematode however, females developed only in the root system of susceptible cultivars.

The greater number of females found in 2007 than in 2008 may be explained by the greater initial inoculum concentration in the first experiment and, also, by the inoculum condition, which had been collected from the field, presenting greater virulence in 2007 than the population maintained in the greenhouse for several

Table 2. Number of male and female individuals, number of eggs per female, sex ratio and survival rate of *H. glycines*, in a trial conducted in 2008.

Soybean cultivars	2008				
	Number females	Number eggs/female	Number males	Sex ratio	Survival rate (%)[1]
BRSGO Jataí	118[a]	195[a]	627[ab]	5.32*	8.17[b]
BRSGO Luziânia	231[a]	261[a]	435[ab]	1.88*	35.00[a]
BRSGO Araçu	76[a]	163[a]	840[ab]	11.06*	6.75[b]
BRS Favorita RR	213[a]	264[a]	1048[a]	4.91*	26.25[a]
BRS Valiosa RR	132[a]	250[a]	942[a]	7.14*	10.25[ab]
BRS Silvânia RR	116[a]	262[a]	866[a]	7.46*	12.25[ab]
BRSGO Ipameri	1[b]	14[b]	970[ab]	970.75*	0.00[c]
BRSGO Chapadões	0[b]	0[b]	248[b]	248.5/0*	0.00[c]
CV (%)[2]	13.4	20.3	6.38		12.74

Averages in a column followed by the same letter do not differ significantly (Tukey P < 0.05). Data were transformed into log (x+1) for statistical analysis purposes; [1]Survival percentage (number of females present in the root system / number of juveniles in the stained roots) *x* 100. Averages followed by the same letter do not differ significantly (Tukey P < 0.05); [2]Coefficient of variation;*Sex proportion different from 1:1 (χ^2 test at α=0.05).

generations until the next year. Brito et al. (1999) and Koenning (2000) also observed that the increase in the initial concentration of inoculum of *H. glycines*, tends to increase the number of females present in the root of soybean system.

Genetic resistance to the soybean cyst nematode affects the number of male individuals, as highlighted by the evaluations of these trials. Cultivar BRSGO Chapadões, presented the smallest number of males in both experiments. However, cultivar BRSGO Ipameri, which is also resistant to *H. glycines*, race 14, had greater number of males than BRSGO Chapadões. This difference in values is probably due to the source of resistance of each of these cultivars. Cultivar BRSGO Chapadões had PI 437654 as genetic background, while cultivar BRSGO Ipameri had PI 88788 (Dias et al., 2007). Studies done by Luedders (1987) and Colgrove and Niblack (2005) demonstrated that greater proportions of males are found in PI 88788 than in PI 437654, as a function of differential mortality of males and females.

Halbrendt et al. (1992) also found effect of the source of resistance on the development of *H. glycines* male individuals which confirms the results found in this study for both resistant cultivars. These authors confirmed that the resistance that affects the development of J3 and J4, as the resistance from PI 209332, provides higher male development than the sources of resistance that affects the stages J2 and J3, like the resistance from cultivar Pickett. This occurs due to the feeding period of the males. *H. glycines* males only feed during the stages J2 and J3 while females feed from J2 until adult (Endo, 1964, 1965). Therefore, the sources of resistance that are not effective during the early stages of development result in lower mortality of males.

It was expected to find a sex ratio of 1:1 in all susceptible soybean cultivars, since, according to Luedders (1987), Halbrendt et al. (1992) and Colgrove and Niblack (2005), resistance is one of the stress factors that inhibits *H. glycines* female development and thus causes a differential death among male and female. In general, the number of males found, especially in the resistant cultivars, was greater than the number of females. Koliopanos and Triantaphylou (1972) found that under greater population densities, a trend of forming more male than female individuals existed, especially with the inoculation of 5,000 eggs and J_2. Considering that the initial population in the substrate used was greater than 5,000 eggs and J_2, it may explain the greater proportion of *H. glycines* males found.Survival rate was 0% for both resistant cultivars, which is near the values found by Colgrove and Niblack (2005) for the plant introductions PI 88788 and PI 437654. In contrast, the susceptible cultivars had survival rates varying from 6.75 to 35.00%. These values corroborate those found by Evans and Fox (1977) and Colgrove and Niblack (2005) for the soybean cultivar used as susceptibility standard, Lee. Colgrove and Niblack (2005) found survival rates varying from 41.00 to 112.00% for several resistance sources evaluated in their experiments. Halbrendt et al. (1992) reported mortality rate varying from 23.00 to 51.00% and stated that high mortality percentage is normal among juveniles.

The developments of *H. glycines* males do not get the same proportion as the development of females in the same soybean cultivar. The results presented in this study suggest that the differential development of males and females occur as a function of resistance sources of soybean cultivars. The Brazilian resistant cultivars BRSGO Ipameri and BRSGO Chapadoes are effective on controlling *H. glycines* by the reduction of female development although allowing high male development.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Andrade PJM, Asmus GL, Silva JFV (1995). Um novo sistema para detecção e contagem de cistos de *Heterodera glycines* recuperados de amostras de solo. Fito. Br. 20(Suplemento):358.

Brito CH, Sediyama T, Pozza EA, Dias WP (1999). Níveis de inóculo e época para avaliação de populações de *Heterodera glycines* Ichinohe em soja. Ciênc. Agrotec. 23(4):836-840.

Byrd DW, Kirpatrick T, Barker KR (1983). An improved technique for clearing and staining plant tissue for detection of nematodes. J. Nemat. 15(1):142-143.

Colgrove AL, Niblack TL (2005). The effect of resistant soybean on male and female development and adult sex ratios of *Heterodera glycines*. J. Nemat. 37(2):161-167.

Dias WP, Silva JFV, Kiilhl RAS, Hiromoto DM, Abdelnoor RV (1998). Quebra da resistência da cv. Hartwig por populações do nematoide de cisto da soja (*Heterodera glycines*). Pesq. Agropec. Br. 33(6):971-973.

Dias WP, Silva JFV, Hiromoto DM, Kiihl RAS (1999). Ocorrência de uma segunda população do nematoide de cisto da soja (NCS) "quebrando" a resistência da cv. Hartwig no Brasil. In: Congresso Brasileiro de Soja I, Resumos, Londrina, P 462.

Dias WP, Silva JFV, Garcia A, Carneiro GES (2007). Nematoides de importância para a soja no Brasil. In: Yuyama MM, Suzili S, Camacho AS (eds.), Boletim de Pesquisa de Soja 2007. Fundação MT, Rondonópolis, pp. 173-184.

Dias WP, Silva JFV, Carneiro GES, Garcia A, Arias CAA (2009). Nematoide de cisto da soja: biologia e manejo pelo uso da resistência genética. Nemat. Br. 33(1):1-16.

Endo BY (1964). Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. Phytopathology 54:79-88.

Endo BY (1965). Histological responses of resistant and susceptible soybean varieties, and backcross progeny to entry and development of *Heterodera glycines*. Phytopathology 55:375-381.

Evans DM, Fox JA (1977). The sex ratio of *Heterodera glycines* at low population densities. J. Nemat. 9(3):207-210.

Grundler F, Betka M, Wyss U (1991). Influence of changes in the nurce cell system (syncytium) on sex determination and development of the cyst nematode *Heterodera schachtti*: total amounts of proteins and amino acids. Phytopathology 81(1):70-74.

Halbrendt JM, Lewis SA, Shipe ER (1992). A technique for evaluating *Heterodera glycines* development in susceptible and resistant soybean. J. Nemat. 24(1):84-91.

Kim KS, Riggs RD (1992). Cytopathological reactions of resistant soybean plants to nematode invasion. In: Riggs RD, Wrather JA (eds.), Biology and Management of the Soybean Cyst Nematode. APS Press, St. Paul, pp. 157-168.

Kim YH, Riggs RD, Kim KS (1987). Structural changes associated with resistance of soybean to *Heterodera glycines*. J. Nemat. 19(2):177-187.

Koenning SR (2000). Density-dependent yield of *Heterodera glycines* resistant and susceptible cultivars. J. Nemat. 32(4):502-507.

Koliopanos CN, Triantaphyllou AC (1972). Effect of infection density on sex ratio of *Heterodera glycines*. Nematologica 18:131-137.

Lima RD, Ferraz S, Santos JM (1992). Ocorrência de *Heterodera* sp. em soja no Triangulo Mineiro. In: Congresso Brasileiro de Nematologia XVI, Resumos, Lavras. P 81.

Lordello AI, Lordello RRA, Quaggio JA (1992). *Heterodera* sp. reduz produção de soja no Brasil. In: Congresso Brasileiro de Nematologia XVI, Resumos, Lavras. P 81.

Luedders VD (1987). Selection against *Heterodera glycines* males by soybean lines with genes for resistance. J. Nemat. 19(4):459-462.

Melton TA, Jacobsen BJ, Noel GR (1986). Effects of temperature on development of *Heterodera glycines* on *Glycine max* and *Phaseolus Vulgaris*. J. Nemat. 18(4):468-474.

Monteiro AR, Morais SRAC (1992). Ocorrência do nematoide de cisto da soja, *Heterodera glycines*, Ichinohe, 1952, prejudicando a cultura da soja no Mato grosso do sul. In: Congresso Brasileiro de Nematologia XVI, Resumos, Lavras. P 82.

Stelle AE (1975). Population Dynamics of *Heterodera schachtti* on Tomato and Sugarbeet. J. Nemat. 7(2):105-111.

Wrather JA, Anderson TR, Arsyad DM, Gai J, Ploper LD, Porta-Puglia A, Ram HH (1997). Soybean disease loss estimates for the top 10 soybean producing countries in 1994. P. Disease 81(1):107-110.

Wrather JA, Koenning SR (2006). Estimates of disease effects on soybean yields in the United States 2003 to 2005. J. Nemat. 38(2):173-180.