DISTRIBUTION OF *HETERODERA GLYCINES* IN PARAGUAY AND ITS EFFECT ON THE GROWTH OF SOYBEAN

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Summary. *Heterodera glycines* was identified from a total of 13 fields in the main soybean producing areas of Paraguay in a 2004-2005 survey. Among these fields, eight, three and two were located in the districts of Canindeyu, Alto Parana and Caaguazu, respectively, and seven of them are new records of the nematode in the country. Severe growth reduction of soybean was observed in a field at San Alberto, Alto Parana, where the average height of soybean plants was less than 60% of that of apparently non-damaged plants. The infestation by nematode females of root systems of stunted plants was severe compared to the light infestation of roots in the surrounding area. An average of 28,000 eggs per 100 g of soil was found in the rhizosphere of the stunted area. Results suggest that *H. glycines* is potentially a serious threat to soybean production in Paraguay.

Key words: Distribution, pathogenicity, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is one of the most widespread and destructive pests of soybean (*Glicyne max* [L.] Merr.) (Schmitt *et al.*, 2005). The occurrence of this nematode was reported for the first time in South America in Colombia in 1983 (Norton *et al.*, 1983). It has now become an important problem in soybean production in Argentina and Brazil (Wrather *et al.*, 2001). In Paraguay, the occurrence of *H. glycines* was reported in 2002 (Centurión *et al.*, 2004), but no growth reduction of soybean was observed at that time. As soybean is the most important crop in Paraguay, a survey was undertaken to ascertain the distribution of the nematode in the main soybean producing areas of the country and assess its potential to damage soybean.

MATERIALS AND METHODS

Distribution of H. glycines. Soil samples were collected from 59 fields in the main soybean producing areas in Paraguay in September 2004 (before sowing), and in March and May 2005 (after harvest). The previous crop in these fields was soybean. A soil sample of approximately 5 kg was collected with a shovel from the top 0-15 cm soil from 10 points in each field. GPS coordinates of sampling points in each infested field are listed in Table I.

Each soil sample was thoroughly mixed and part of it was air dried. Nematode cysts were extracted, using a flotation sieving technique (Shepherd, 1986), from 50 g of dried soil with three repetitions except as indicated in Table I. The extracted cysts were counted and the presence of eggs was ascertained. The nematode was identified on the basis of shape and vulval cone structure of the cysts (Mulvey and Golden, 1983).

Growth reduction of soybean. An area of stunted plants was observed in January 2005 in a soybean field at San Alberto (San Alberto 1 in Table I), Alto Parana, that had been cropped with soybean for many years. In this field the soybean cv. Monsoy 7204 had been sown without tillage on October 22, 2004 and was at the seed-producing stage when sampled.

To asses the effects of the nematode on the growth of soybean, a total of 32 and 36 soybean plants were randomly dug up with a shovel from 10 points in the stunted area and in an area with no apparent stunting. The sampling points in the non-stunted area were at least 10 m from plants in the stunted area. The plants sampled from the two areas were put in two separate large plastic bags to avoid drying. Within an hour after sampling, the heights of the plants were measured and degrees of root infestation rated, according to the number of nematode females on the root systems, as follows: no infection, 0; light, 25; moderate, 50; considerable, 75; and heavy, 100 (Aiba, 2004).

Soils collected at each of the 10 points where plants had been removed from the stunted and non-stunted areas were separately combined and referred to as rhizosphere soil samples. The soils that remained in the two plastic bags in which soybean plants were temporarily stored were also collected and referred to as 'root surface' soil. Soil samples were thoroughly mixed without drying and cysts extracted by the sugar solution flotation technique. The procedure was as follows: *a)* ten grams of soil were put in a 100-ml beaker; *b)* 60 ml of sugar solution with a specific gravity of 1.2 was

Table I. Sampling points where cysts of *Heterodera glycines* were detected in the main soybean producing areas of Paraguay, during the 2004-2005 survey.

State and sampling points	Date of the survey	GPS - X	GPS - Y	Cysts in 50 g soil[1]	Previous or (present) crop
CANINDEYU					
Troncal 6-1	Sep. 8	764551	7320453	7.4[b]	Soybean (*Phaseolus* sp.)
*La Paloma 2	May 2	746902	7328858	0.1[c]	Soybean
*Yhovy 2	May 2	702086	7311623	66.3	Soybean
*Yhovy 3	May 2	718236	7315232	2	Soybean
Troncal 4-1	Nov. 30	719570	7304710	3.7	Soybean
*Troncal 4-2	March 8	717627	7284507	3.3	Soybean
*Troncal 2-1	March 8	719229	7264694	18.3	Soybean
*Troncal 2-1	March 8	706563	7223667	13.9	Soybean
ALTO PARANA					
San Alberto 1	Sep. 8	708761	7206150	12.6[b]	Soybean (Wheat)
*San Alberto 3	Sep. 8	708545	7234694	1.8[b]	Soybean (Oat)
Yguaz 1	Sep. 7	692564	7175144	0.4[c]	Soybean (Fallow)
CAAGUAZU					
Campo 2-1	March 10	623180	7184137	1.3	Soybean
Campo 9-1	March 10	638649	7169615	2.7	Soybean

* Fields in which *H. glycines* was detected for the first time during this survey.
[1] Values are averages of three replicates except [b] and [c], which are averages of five and eleven replicates, respectively.

added; *c)* the soil and sugar solution was stirred vigorously with a glass bar; *d)* after settling for one minute, sugar solution and all floating materials were poured through a 20-mesh sieve nested onto a 60-mesh sieve; *e)* cysts and soil debris caught on the 60-mesh sieve were gathered in a 100-ml beaker. The process from *b)* to *e)* was repeated twice. The cysts and debris in the 100-ml beaker were spread on a filter paper and the cysts were collected with forceps and counted under a dissecting microscope. Eggs and juveniles contained within the cysts were then counted after crushing cysts with a glass homogenizer.

RESULTS AND DISCUSSION

Distribution of H. glycines. The nematode was recovered from a total of 13 fields (Table I). Among these fields, seven are new records of *H. glycines* in the country (Table I).

In Canindeyu, *H. glycines* was detected for the first time in six out of the 25 fields surveyed, i.e. a frequency of 24%. However, the nematode was also detected in two fields from which it had been reported previously. Several cysts were extracted from some of these fields (Table I). The results suggest that *H. glycines* may be distributed over large areas in this district. The nematode was also detected from three fields in Alto Parana and two fields in Caaguazu districts, respectively, but not in the Itapua district.

It is not clear when, from where and how this nematode was introduced into Paraguay. However, the fact that this nematode was detected in places that are far from each other, and at rather high densities in several of them, suggests that it might have been introduced some time ago and through two or more routes into Paraguay. In Brazil, *H. glycines* has spread rapidly since its first detection (Silva, 1999). Therefore, continuous monitoring of the nematode may be necessary in Paraguay.

Growth reduction of soybean. In the stunted area, the average height of soybean plants was 48 cm, less than 60% of that of plants (85 cm tall) from the non-stunted

Table II. Comparison of population densities of *H. glycines* (per 100 g wet soil) in the stunted and non-stunted areas of a soybean field at San Alberto, Alto Parana.

Area	Root surface soil[1]		Rhizosphere soil[2]	
	Cysts	Eggs	Cysts	Eggs
Stunted	2,326	128,120	686	28,354
Non-stunted	102	4,526	118	7,734

[1] Soil remaining in the plastic bags in which soybean plants were stored.
[2] Soils collected from the 10 points where plants were removed from the field.

area. Chlorosis was not obvious at the time of survey, but browning of leaf margins (Wrather *et. al*., 1984) was observed on the soybean plants in the stunted area. Female infestation index was severe (60.1) on the root-systems of stunted plants and rather light (19) on the roots of the apparently non-stunted plants.

In the soil, the nematode population density was also extremely high in the stunted area (Table II), where more than 2,000 cysts and 128,000 eggs were extracted from 100 g of soil collected from the surface of roots, more than 20-fold the densities found in the non-stunted area. A similar trend was observed in the rhizosphere soil. This would indicate that *H. glycines* was the main causal agent of the observed soybean stunting.

The occurrence of *H. glycines* in several districts (Table I) and the severe growth reduction observed suggest that this nematode is potentially a serious threat to soybean production even in the "Terra rossa" soil type and subtropical climate conditions in Paraguay. Therefore, more insights are necessary on the ecology and pathogenicity of the nematode to allow the implementation of proper management measures.

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