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First report of *Heterodera glycines* Ichinohe on soybean in Italy

Abstract - The soybean cyst nematode (SCN) *Heterodera glycines* was found in three soybean fields near Pavia (Italy) during the summer 2000. This quarantine nematode was not known in Italy. The soybean plants were smaller, with "yellow draft" disease, the roots were poorly developed and without or with few nitrogen nodules. The population of SCN found was well established, and all stages were recorded. Adult females and juveniles of *H. glycines* had high abundance. An average of 59.5 cysts for 100 cm^3 soil were recorded disease. The density of population ranged from 7900 to 19200 SCN eggs/100 cm^3 soil. This record highlights a probable permanent establishment of this plant parasitic nematode in North Italy.

Riassunto - *Prima segnalazione in Italia di* Heterodera glycines *Ichinohe su soia.* *Heterodera glycines,* nematode cisticolo della soia è stato segnalato, durante l'estate 2000, in tre campi di soya vicino a Pavia (Italia). Questo nematode da quarantena non era mai stato osservato in Italia. Le piante di soia erano mal sviluppate e con giallume fogliare, le radici ridotte, ed i tubercoli assenti o presenti in basso numero. La popolazione di *H. glycines* rinvenuta era bene sviluppata e sono stati osservati tutti gli stadi del nematode. Femmine adulte e stadi giovanili sono stati rinvenuti in gran numero. In media sono state calcolate 59 cisti per 100 cm^3 di suolo. Il numero di uova variava da un minimo di 7900 a un massimo di 19200 per 100 cm^3 di suolo. Questa segnalazione evidenzia come tale nematode fitoparassita potrebbe acclimatarsi nei nostri areali.

Key words: *Heterodera glycines*, soybean, yellow draft, Italy.

INRODUCTION

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the major economic importance parasites of soybean *Glycine max* L. (Noel, 1986; Young, 1996). *H. glycines*, probably original of Japan, Korea and China, where the soybean was historically an important crop, was introduced North Carolina (USA) in 1954. Since then it has been found in more than 25 states of USA, as Illinois, Minnesota, Missouri, Nebraska, and Wisconsin (Riggs & Niblack, 1993). The SNC is actually reported also in the Amur Region of Russia, Canada,

Columbia, Argentina, Brazil, Java, Taiwan, Egypt and recently in Puerto Rico (Noel & Liu, 1998; Smith & Chavarria-Carvajal, 1999; Wang *et al.*, 2000). The SNC nematode cause annual losses of $ 267 million in the only USA (Wang *et al.*, 2000). The importance of this plant parasitic nematode is so great that exist an Soybean Cyst Nematode Coalition (SCNC) of USA, which include Universities and Private Companies, helping the farmer to identify and control this pest.

H. glycines can cause direct, especially reduction of roots, and indirect crop losses. In fact soybean plants growing in SCN infested fields are often infected with other parasitic nematodes (root-knot, lesion, sting, or lance) or fungi that cause diseases such as stem canker, red crown rot, charcoal rot, *Rhizoctonia* root rot, *Fusarium* and *Phytophthora* root rot, charcoal rot (Workneh *et al.*, 1999). In addition, SCN infections may suppress nodule formation and nitrogen fixation by the beneficial bacteria *Bradyrhizobium* sp. (Huang & Barker, 1983; Ko *et al.*, 1991).

However SCN can move through the soil only a few centimetres per year on its own power, *H. glycines* can be spread substantial distances in a variety of way including farm machinery vehicles and tools, wind, water, animals, and farm workers. Consequently, SCN can be spread easily when seed from infested fields is planted in uninfected fields (Riggs & Niblack, 1993). There is even evidence that SCN can be spread even by birds.

In this paper are reported the symptoms of the soybean infection caused by *H. glycines* recorded in Italy. Morphological characteristic of SCN established in Italy and its field abundance are also reported.

MATERIALS AND METHODS

The survey was carried out in July and August 2000 in three fields near Pavia (Lombardia, Italy). Soybean roots were carefully removed from the soil for examination of SCN cysts. Adult females were identified under Leitz (26x) stereo microscope. For a more quantitative analysis 20 samples for hectare were collected, in a zigzag pattern, across the entire area. The soil samples were collected using a cylindrical soil probe to a depth of 15 -20 cm. Combine a well mixed 100 cm^3 soil sample were used for cysts extraction. Cysts of nematode were recovered from dry soil through a combination of decanting and wet-sieving. The technique is a modification of the Cobb's method (1918). After counting the cysts, they were opened and eggs were counted. Egg suspension was brought up to exactly 50 ml with tap water for the counting under stereomicroscope. For juvenile nematodes extraction Bearman funnel technique with sub samples of 100 gr soil was performed.

RESULTS

The symptoms of *H. glycines* injury to soybeans had the appearance in the field of circular- or oval-shaped areas of stunted, yellowed, less vigorous plants; the most severe damage were observed in the centre of the area, with damage decreasing towards the margins (Fig. 1). Such areas were better develop near a field gate, wherever equipment enters a field, and near fences where wind-blown soil was accumulate. The infested areas varied in their size, often showing a sharp dividing line at the edges between stunted and apparently healthy plants. Rows of soybeans grown on SCN-infested were slow to close or fill in with foliage. The area SCN

Fig.1 - Area of *Heterodera glycines* damage in a soybean field.

damage appeared elongated in the direction of tillage operations. (Fig. 1). *H. glycines* caused also the "yellow draft", which can be confused with Iron deficiency chlorosis symptoms however yellowing due to the SCN start at the edges of the leaves, and often affected leaves on the entire plant. The occurrence and severity of the chlorosis recorded was associated with reduction of roots and with suppression of nitrogen nodules formation (Fig. 2).

However the more unmistakable symptom of SCN infection is the presence of adult female nematodes and cysts on the soybean roots. The cysts, appear as tiny, lemon-shaped on the roots, are white initially, but turn yellow and then tan to brown as they mature (Fig. 3 a, b). Other species of genus *Heterodera (H. goettingia, H. trifolii, H. cacti, H. schachtii)* are present in Italy (Lamberti, 1988; Cerioni *et al.*, 1995) that could be confused with *H. glycines,* particularly with *H. schachtii*. In spite of this the other species do not affect particularly the *G. max*. Tacconi (1992) for example reported that sunflower and soybeans are not hosts for *H. schachtii* even after heavy inoculations. Nematode identification to guarantee the exact determination was based on cysts and juveniles morphology (Fig. 3c, d) and morphometric measurements. Table 1 reports morphometric measurements of *H. glycines* juveniles compared with the measurements, from literature, of 2 other similar species of *Heterodera* found in Italy. Cysts and eggs counts are summarized in Table 2. A minimum of 32 cysts/100 cm^3 soil and a maximum of 79 cysts/100 cm^3soil of cyst were recorded. About 12,000 eggs per 100 cm^3 of soil were counted. A cyst contained in about 200 eggs (Fig. 3b).

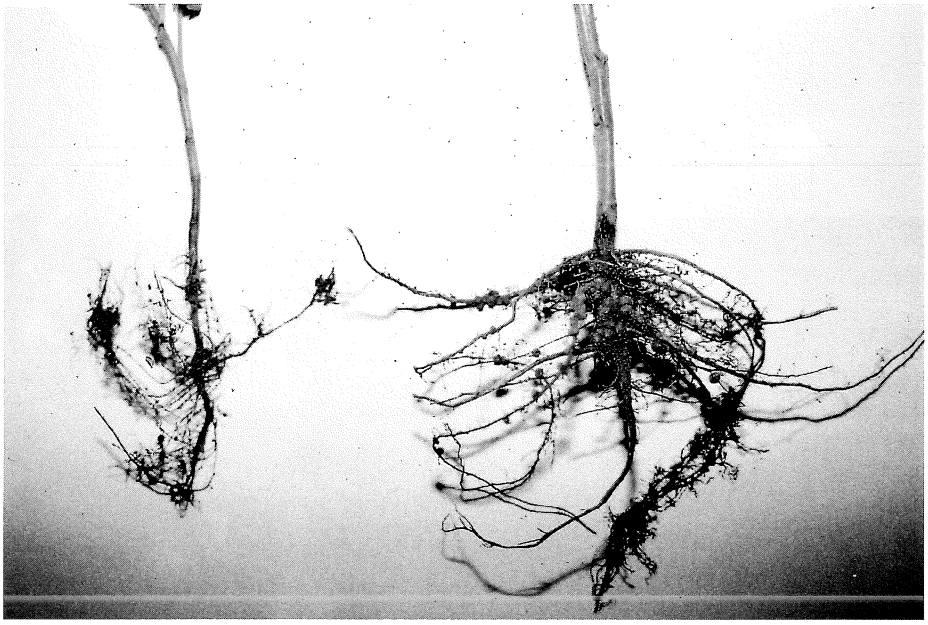

Fig. 2 - Infected and uninfected soybean roots. Note the small size nitrogen nodule of infected root.

Table 1 - Comparative morphological measurements of juveniles of Heterodera glycines *and two similar species present Italy.*

Species	J2 stylet (µm)	J2 tail lenght (µm)	J2 Stylet Knobs
H. glycines	23.0-24.5	40.0-49.0	Subventral rounded
H. schachtii[1]	25.0-27.0	48.0-55.5	Anchor
H. trifolii[2]	25.2-26.3	53.9-56.6	Anchor

1 Ambrogioni *et al.,* 1986.
2 From Ambrogioni & Marinari Palmisano, 1979.

Table 2 - Cysts and eggs of Heterodera glycines *per 100 cm³/soil.*

	Maximum	Minimum	Average
cysts per 100 cm^3soil	79	32	59.5
eggs per cyst	290	183	200.36
eggs per 100 cm^3 soil	19200	7900	12000

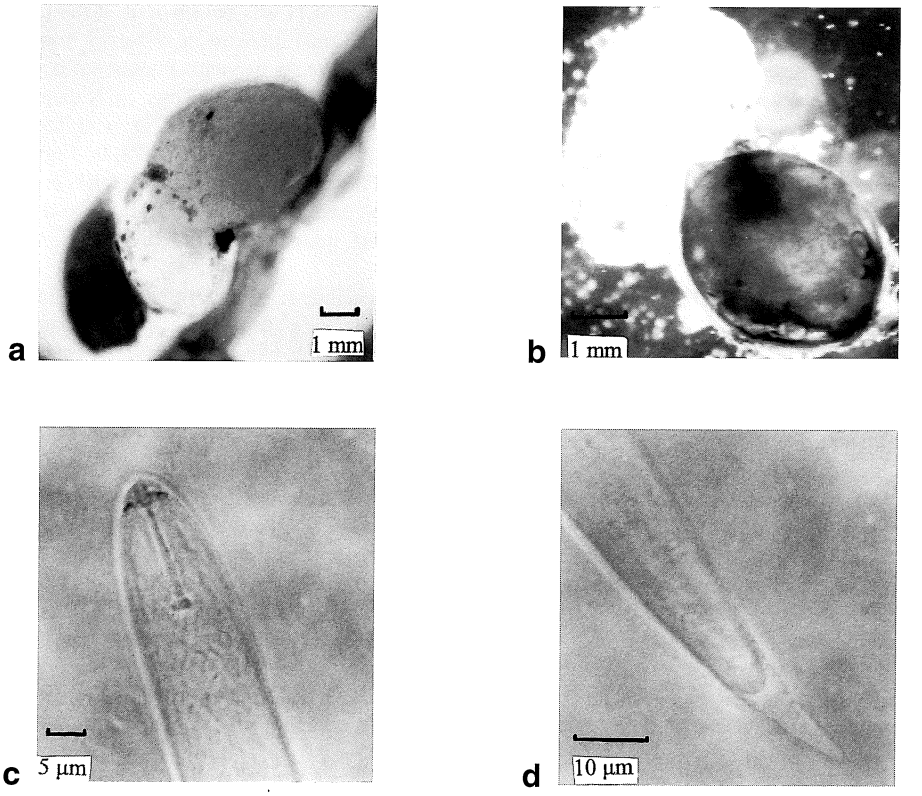

Fig. 3 - Adult female of *Heterodera glycines* with egg filled egg sac (25x) on infected soya root segments (a). Soybean cyst nematode cyst broken open to reveal hundreds of eggs (b). Head and tail (100x) of juveniles tail of juveniles (c, d).

DISCUSSIONS AND CONCLUSIONS

Above-ground symptoms of *H. glycines* damage are not unique to SCN. They often are mistaken for damage from compaction, iron deficiency chlorosis and other nutrient deficiencies, drought stress, herbicide injury, or other plant diseases. SCN injury often has remained undetected for several years because these nondescript symptoms were attributed to other causes. In fact the farmers in Pavia have already noticed the symptoms in the 1998 but believing was a deficiency problem had applied the fertilizer containing Fe, Bo, Mg, Zi and N organic. After an apparent improvement the problems appeared again, and the 2000 was even increased, especially in extension. Alfredo *et al.* (1999) reported that with a nitrogen levels increment, in a greenhouse tests, infested with *H. glycines* soybean growth and yield was significantly decreased by nematode infection, but this effect did not persist long time. Confusing SCN symptoms for nutrition deficiency could lead an underestimation of the problem and an secondary

increment of the *H. glycines* population. The presence of SCN was not obvious at the time of initial soil infestation. The nematode population density must increase in soil until it is sufficient to cause above-ground symptoms on plants or a decrease in yield. Population densities may take several years to reach significant numbers (Ruan *et al.*, 2000). Thus, SCN damage to soybeans recorded in Pavia could be the result of infestations that have been growing for several years. The infestation found is considered dangerous because the presence of 12000 eggs/100 cm^3 soil, injury level is normally considered with 8000 eggs/100 cm^3 soil (Noel, 1986; Wang *et al.*, 2000). The *H. glycines* recorded was well established, this could be also due to the presence of sandy moil and relatively high moisture. In fact, injury usually is more severe in light, sandy soils (Riggs & Niblack, 1993).

To distinct *Heterodera glycines*, on other hosts, especially from Sugar Beet Cyst Nematode, *Heterodera schachtii* other techniques as PCR-RFLP methods are now available (Szalanski *et al.*, 1997). Young (1996) claimed the importance of knowing which race of the nematode is prevalent in a field and demonstrated that the nematode can cause significant yield loss in the absence of visible symptoms of infection. Moreover several authors (Huang & Barker, 1983; Ruan *et al.*, 2000) reported that only some race of *H. glycines* is able to determine the reduction of formation of nodule. Using the modern biomolecular techniques could be helpful to determine from which area the SNC is arrived (Noel & Liu, 1998; Zhang *et al.*, 1998), and determining the race of *H. glycines* was recorded in Italy.

Moreover the host plants of *H. glycines* included more than 30 species of vegetables, ornamental plants and weeds (Ramarao *et al.*, 2000), the presence of SCN in Italy could be dangerous also for other culture. Control methods include: chemical control; rotation; resistant varieties and direct sowing. Though Noel and Edwards (1996) claimed that *H. glycines* grown either in monoculture or rotated with maize in a 2-year rotation. Because of the economical importance of corn and soybean crops in North Italy and severe problems that *H. glycines* could cause further analysis are necessary to determine the presence of this nematodes in other part of Italy.

REFERENCES

AMBROGIONI L., MARINARI PALMISANO A., 1979 - Nematodi del genere Heterodera su colture ortensi e ornamentali in Italia. - Giornate Nematologiche: 89-116.

AMBROGIONI L., D'ERRICO F.P., MARINARI-PALMISANO A., 1986 - Observations on a population of cyst forming nematodes from carnation. - In: Cyst nematodes. Plenum Press, New York, USA: 315-320.

ALFREDO M.M., SEDIYAMA T., PEREIRA P.R.G., CECON P.R., OLIVEIRA R., TEIXEIRA R.C., 1999 - Effect of the cyst nematode, *Heterodera glycines* Ichinohe, and nitrogen fertilization on the agronomic characteristics of soyabean *Glicine max* (L.). - Revista Ceres 46: 279-295.

CERIONI S., MANFREDI M.T., ORECCHIA P., PAGGI P., VINCIGUERRA M.T, ZULLINI A., 1995 - *Nematoda Spiruria*. In: MINELLI A., RUFFO S. & LA POSTA S. (eds), Cecklist delle specie della fauna italiana, 11 Calderini, Bologna.

COBB, N.A., 1918 - Estimating the nematode population of soil. - U.S. Dept. Agr. Bur. Plant Ind. Agr. Tech. Cir. 1:1-48.

HUANG J., BARKER K.R., 1983 - Influence of *Heterodera glycines* on leghemoglobins of soybean nodules. - Phytopathology 73(7): 1002-1004.

KO M.P., HUANG P.Y., HUANG J.S., BARKER K.R., 1991 - Responses of nodulation to various combinations of *Bradyrhizobium japonicum* strains, soybean cultivars, and races of *Heterodera glycines*. - Phytopathology 81(6): 591-595.

LAMBERTI F., 1988 - Parassiti animali: nematodi. - Italia Agricola 1: 49-54.

NOEL G.R., 1986 - The soybean cyst nematode. In Cyst nematodes. Plenum Press, New York, USA: 257-268.

NOEL G.R., EDWARDS D.I., 1996 - Population development of *Heterodera glycines* and soybean yield in soybean-maize rotations following introduction into a noninfested field. - J. Nematol. 28(3): 335-342.

NOEL G.R., LIU Z.L., 1998 - Esterase allozymes of soybean cyst nematode, *Heterodera glycines*, from China, Japan, and the United States. - J. Nematol. 30 (4): 468-476.

RAMARAO V., HARRISON S.K., RIEDEL R.M, 2000 - Weed hosts of soybean cyst nematode (Heterodera glycines) in Ohio. - Weed Technology (14)1: 156-160.

RIGGS R.D., NIBLACK T.L., 1993 - Nematode pests of oilseed crops and grain legumes. - In: EVANS K., TRUDGILL D.L, WEBSTER J.M., Plant Parasitic nematodes in temperate agriculture, (Eds.), CAB International,Wallingford, UK: 209-258.

RUAN W., WANG J., ZHANG F., 2000 - Effects of soyabean cyst nematode (SCN) on soyabean (*Glycine max* (L.)) growth. - C. J. Appl. Envion. Biol. 6(1): 24-27.

SMITH J.R., CHAVARRIA-CARVAJAL J.A., 1999 - First report of soybean cyst nematode (*Heterodera glycines*) on soybean in Puerto Rico. - Plant Disease 83(6): 591-592.

SZALANSKI A.L., SUI D.D, HARRIS T.S., POWERS T.O., 1997 - A method for identifying Soybean Cyst Nematode *Heterodera glycines*, from Sugar Beet Cyst Nematode, *Heterodera schachtii* using PCR-RFLP: Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. - J. Nematol. 29: 253-264.

TACCONI R., 1992 - Riproduzione di *Heterodera schachtii* su alcune piante coltivate. - Atti IV Congr. SIN: 35-38.

WANG J., DONALD P.A., NIBLACK T.L., BIRD G.W., FAGHIHI J., FERRIS J.M., GRAU C., JARDINE D.J., LIPPS P.E., MACGUIDWIN A.E., MELAKEBERHAN H., NOEL G.R. , PIERSON P., RIEDEL R.M., SELLERS P.R., STIENSTRA, W.C., TODD T.C., TYLKA G.L., WHEELER T.A., WYSONG, D.S, 2000 - Soybean cyst nematode reproduction in the north central United States. - Plant Disease 84(1): 77-82.

WORKNEH F., YANG X.B., TYLKA G.L., 1999 - Soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* affected by soil texture and tillage relations.- Phytopathology 89(10): 844-850.

YOUNG L.D., 1996 - Yield loss in soybean caused by *Heterodera glycines*. - J. Nematol. 28(4): 604-607.

ZHANG L., DEAN R.A., KNAP H. T., LEWIS, S.A, 1998 - Diversity among a *Heterodera glycines* field isolate and derived inbreds based on RAPD analysis and reproduction on soybean genotypes. - J. Nematol. 30(4): 477-484.

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