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CYST NEMATODES OF THE GENUS *HETERODERA* AND *GLOBODERA* IN SICILY

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Lombardo S., Colombo A., Rapisarda C. – Cyst nematodes of the genus *Heterodera* and *Globodera* in Sicily.

A survey was conducted in Sicily, from 2007 to 2009, to obtain insights on specific composition, frequency and geographical distribution of cyst nematodes associated to crops of agricultural economic importance.

Soil and root samples were collected mainly from crops showing chlorosis, poor growth and reduced yield. Cysts and larvae were extracted and identified to species level by comparative examination of morphological and morphometric characters. Confirmation of the species identity was also made by molecular analysis for some species.

Seven species of the genus *Heterodera* Schmidt, 1871 and two of the genus *Globodera* Skarbilovic, 1959 were found. They were *H. avenae* Wollenweber, 1924, *H. bifenestra* Cooper, 1955, *H. carotae* Jones, 1950, *H. goettingiana* Liebscher, 1892, *H. hordecalis* Andersson, 1974, *H. latipons* Franklin, 1969 and *H. schachtii* Schmidt, *G. rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *G. pallida* (Stone, 1973) Behrens, 1975.

All species dectected are known to occur in Europe and the Mediterranean basin, but *H. avenae*, *H. hordecalis*, and *H. latipons* had not been reported from Sicily and *H. bifenestra* is a new report for Italy.

KEY WORDS: cyst nematodes, host crops, Sicily.

INTRODUCTION

The economic importance of cyst nematodes in the Mediterranean area is continuously increasing, particularly with reference to major host crops, due to crop intensification and specialization.

Cyst nematodes attack many species of food plants, frequently causing severe yield losses. Therefore, there is the need to investigate their distribution, life-cycle and economic impact.

Information on the presence of *Heterodera* and *Globodera* species in Sicily was scarce and scattered. Moreover, farmers and professionals are unaware of cyst nematode attacks as the symptoms these pathogens cause on the aerial parts of the infected plants are not specific and are confused with those caused by other stresses.

Therefore, a survey was conducted to obtain insights on species composition and distribution of cyst nematodes associated with the decline of the main crops in Sicily, in order to suggest sound strategies for their control.

MATERIAL AND METHODS

The survey was conducted during 3 years (2007-2009) in the main crop growing areas of Sicily. Samples were taken mainly from fields showing chlorotic and poorly growing plants and reduced production (fig. I) and, therefore, were suspected to be attacked by nematodes. Each sampled area was of about one hectare and each soil sample was composed of 30 subsamples collected with a small spade in the plant rhizosphere, to a depth of 5-20 cm, after removing the top 5 cm soil. The entire sample was thoroughly mixed and and a 2 kg of it was kept in a plastic bag and taken to the laboratory and stored at 6 °C until processing. Each soil sample was air dried and processed with a Fenwick can to extract cysts. Cysts were dissected and second-stage juveniles collected, killed by gentle heat, fixed in triethanolamine-formalin (TAF) solution and mounted in anhydrous glycerol on permanent slides. Also, cysts were cleaned and the posterior cones cut and mounted in Canada balsam to observe the perineal patterns.

The same scheme was followed to collect root samples from the same fields. Roots were gently washed free of adhering soil and a portion was observed under a stereomicroscope (25×) to ascertain the presence of nematode females, which were subsequently collected. When the cysts were present, they were dissected as previously mentioned and larvae collected.

The identification to species level was based on morphology of cysts and their perineal patterns and on morphology of 30 second-stage juveniles per cyst, with special emphasis to total body length, length of the tail (both total length and the length of jaline tail portion) and

Fig. I – Field damage caused to cereals by *Heterodera avenae* group in Sicily.

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shape and length of the stylet. Data deriving from these morphometric measurements were compared with those of other populations of various *Heterodera* and *Globodera* species deposited in the USDA Nematode Collection at Beltsville, Maryland, USA.

To identify cryptic species or confirm the identification of new records for Sicily, molecular analysis was also made of some nematode population. This was conducted at the Nematology Laboratory of the United States Department of Agriculture (USDA), in Beltsville, Maryland (USA) by applying PCR ITS 1 and 2 on samples and on rDNA's genes, which is a genome region frequently used as a marker for differentiating closely related species (SUBBOTIN, 2000).

RESULTS

The survey was conducted mainly on sandy soils and in crops of potato, carrot, peas, field beans, vetch, barley, durum wheat, cabbage, chrysanthemum, oats and spinach. Fields with spontaneous vegetation were also sampled (Table 1).

Seven species of the genus *Heterodera* and two of the genus *Globodera* were detected during the survey. The seven species of the genus *Heterodera* Schmidt, 1871 were: *H. avenae* Wollenweber, 1924; *H. bifenestra* Cooper, 1955; *H. carotae* Jones, 1950; *H. goettingiana* Liebscher, 1892; *H. hordecalis* Andersson, 1974; *H. latipons* Franklin, 1969, *H. schachtii* Schmidt, 1871. The two species of the genus *Globodera* Skarbilovic, 1959 were *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *G. pallida* Stone, 1973. All species are known to occur in Europe and the Mediterranean basin, but *H. avenae*, *H. hordecalis*, *H. latipons* had not been reported from Sicily and *H. bifenestra* is a new report for Italy.

The findings in Sicily of the above mentioned species belonging to the "*H. avenae* species-group" are of relevant importance and brief notes will be given hereafter on their

Table 1 – Collecting data of cyst nematodes in Sicily during the survey.

N	Location	Species	Host plant	Symptom
1	Giarre (CT)	*G. rostochiensis* *G. pallida*	Potato	Poor production
2	Riposto (CT)	*G. rostochiensis* *G. pallida*	Potato	Poor production
3	Cassibile (SR)	*G. rostochiensis* *G. pallida*	Potato	Total loss of production
4	Vittoria (RG)	*H. carotae*	Carrot	Poor production
5	Caltagirone (CT)	*H. goettingiana*	Pea	Yellowing
6	Resuttano (CL)	*H. goettingiana*	Bean	Yellowing
7	Resuttano (CL)	*H. goettingiana*	Vetch	Yellowing
8	Catania	*H. schachtii*	Cabbage	Total loss of production
9	Caltagirone (CT)	*H. schachtii*	Chrysanthemum	/
10	Chiaramonte Gulfi (RG)	*H. bifenestra* *H. latipons* *H. hordecalis*	Oats Wheat	Yellowing
11	Mazzarone (CT)	*H. latipons*	Barley	Yellowing
12	Caltagirone (CT)	*H. avenae*	Wheat	Yellowing
13	Caltagirone (CT)	*H. hordecalis* *H. latipons*	Oats Wheat Barley	Yellowing
14	Caltagirone (CT)	*H. hordecalis* *H. latipons*	Wheat Barley	Yellowing
15	Paternò (CT)	*H. hordecalis*	Uncultivated	/
16	Catenanuova (EN)	*H. hordecalis* *H. latipons*	Wheat	Yellowing
17	Comiso (RG)	None	Spinach Carrot Wheat Cabbage	/
18	Caltagirone (CT)	None	Oats Wheat Barley	Yellowing
19	Piana di Catania (CT)	None	Wheat	Yellowing
20	Catenanuova (EN)	None	Wheat	Yellowing

distribution, as well as morphological and biological features.

Cysts and second-stage juveniles of *H. latipons* were abundant in both soil and root samples of cerealas. Cysts appeared full of eggs and juveniles. Adult females of this species, recorded for the first time in Italy in 1975 in the Veneto region (TACCONI, 1976), are lemon-shaped; some individuals are smaller or larger compared to the typical shape. Females are pearl white in colour and later turn gradually to dark brown cysts; they show a distinct neck and a prominent vulval cone. The cuticle is covered by a thin sub-crystalline layer. Vulval cones (fig. II, 1) are characterized by a short vulval slit (6-9 m), two different translucent areas called fenestrae, a strong underbridge with a pronounced thickening in the middle and a bifurcation at both ends. As already reported by TACCONI (1976), the cysts studied showed few bullae. Moreover second-stage juveniles of this Sicilian population are vermiform with a rounded and conical tail (tail length 48-57 m), a well developed stylet (23-25 m long) with basal rounded knobs having a typical anchor shape. All other morphological details agree with the typical characters of *H. latipons* reported in the original description (FRANKLIN, 1969) and by HANDOO (2002).

Morphological characters of *H. hordecalis* (ANDERSSON, 1974) are almost similar to those of *H. latipons*. Nevertheless, some fine distinctive features occur between *H. latipons* and *H. hordecalis*, such as the vulval slit (fig. II, 3), which is longer (17-25 m) in *H. hordecalis* than in *H. latipons* (6-9 m). Moreover *H. hordecalis* differs from *H. latipons* for the less sclerotized underbridge. In the material collected in Sicily, the mentioned distinctive morphological details agree with the typical characters of *H. hordecalis* (ANDERSSON, 1974).

Cysts of *H. avenae* collected during the survey were dark brown to black, ambifenestrate, provided with bullae and prominent underbridge, with a shorter vulval slit (9-10 m long) (fig. II, 2). Second-stage juveniles were 530-553 m long, with a stylet 24-26 m long, whose knobs were shallowly concave anteriorly; the tail measured 50-56 m and the hyaline tail terminus was 30-36 m long.

All other morphological details agree with the typical characters of *H. avenae* (HANDOO, 2002).

The Sicilian population of *H. bifenestra*, according to the description made by Andersson (ANDERSSON *et al.,* 1983), showed very small cysts (about 360 µm long and 270 µm wide), with vulval cone (fig. II, 4) having a double fenestra (20-32 m wide and 39-58 m long) and two circular translucent semifenestrae, very distant each other; bullae and underbrige are absent; vulval slit 10-16 m. The above mentioned morphological and morphometric data were consistent with those given by HANDOO (2002).

Information on the other cyst nematode species, such as location, host plant on which they were detected and symptoms is given in Table 1.

DISCUSSION

The present study broaden the knowledge on cysts nematodes in Sicily, both widening the areas or sites of detection for species whose occurrence was already known in the territory and, above all, increasing the list of nematode species found in this region (fig. III). As to the

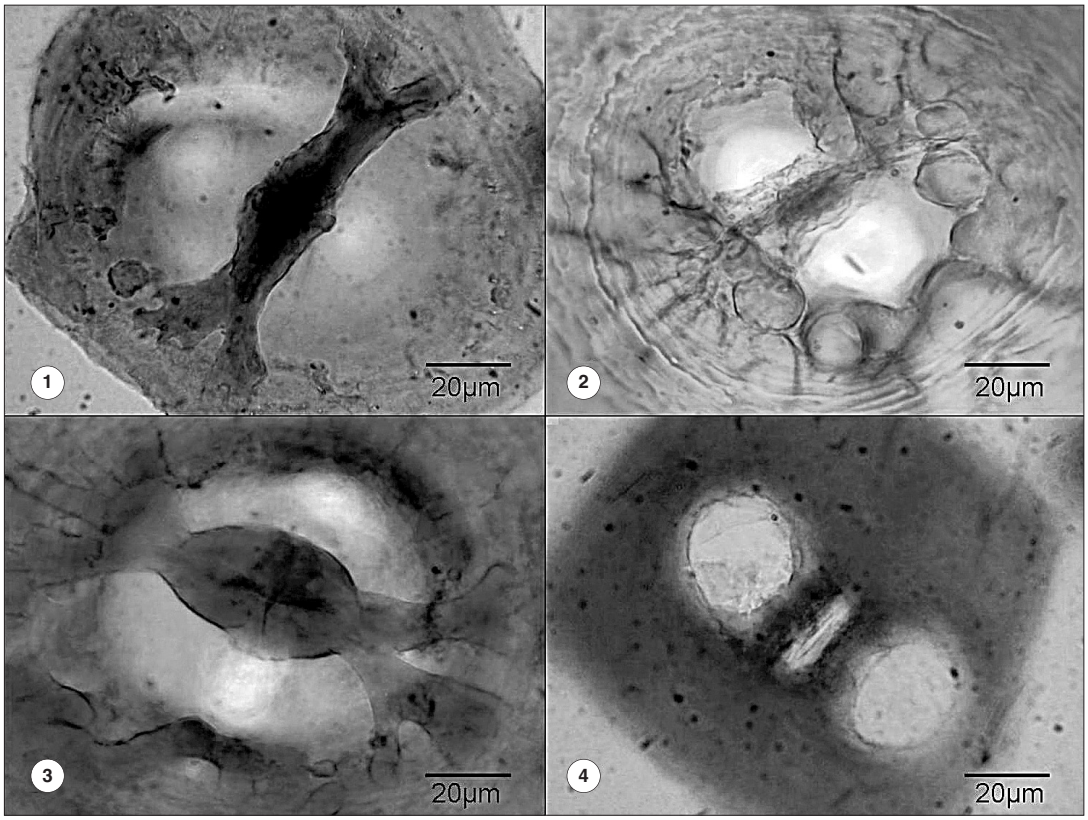

Fig. II – Microscopic views of perineal patterns of vulval cones from cyst of *Heterodera* spp. 1. *H. latipons*; 2. *H. avenae*; 3. *H. hordecalis*; 4. *H. bifenestra*.

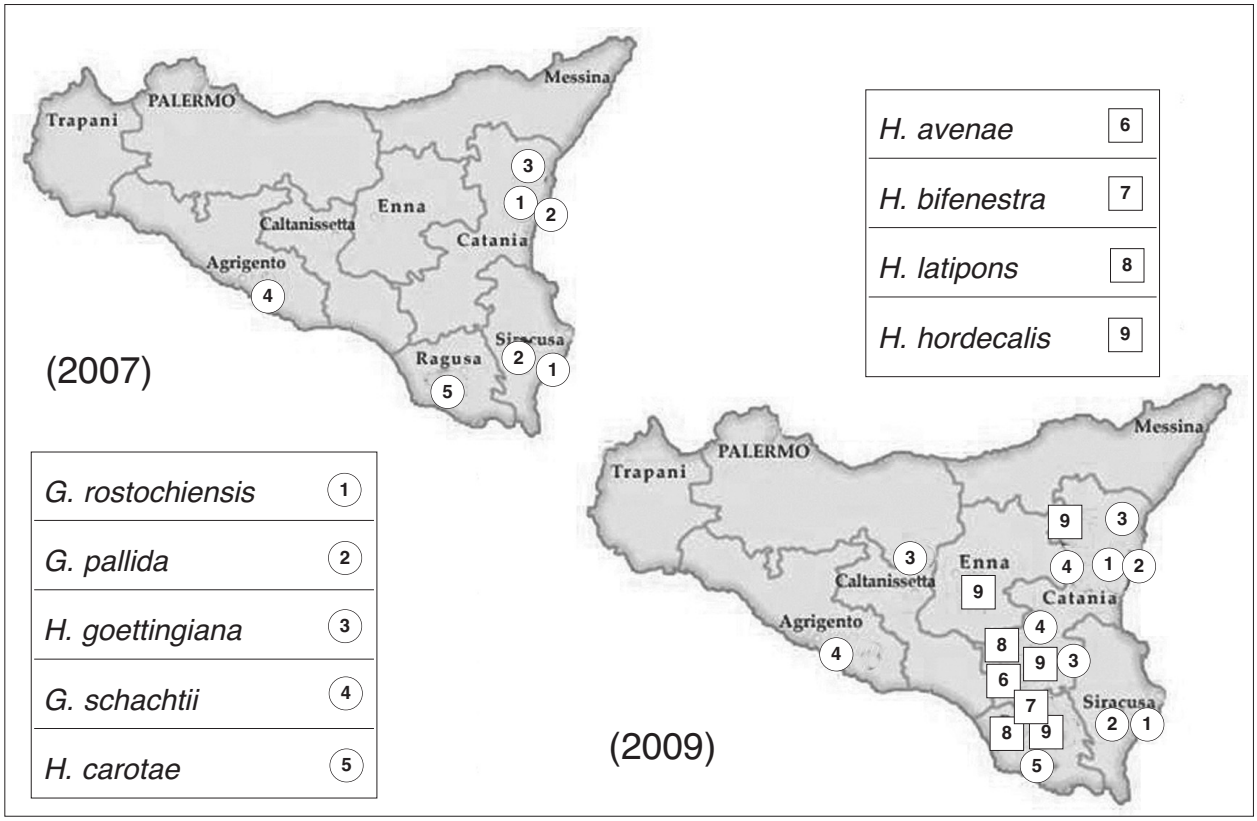

Fig. III – Occurrence and distribution of cyst nematodes in Sicily in 2007 (left) and 2009 (right).

latter aspect, the number of species of cyst nematodes occurring in Sicily has been nearly doubled by this study, especially through the findings of several representatives of the *Heterodera avenae* species-group.

It is susrprising that so many species of cyst nematodes have remained undetected till now in Sicily, in spite of their strong phytosanitary impact on their host plants. This is most likely due to the fact that the symptoms caused by cyst nematodes on host plants, apart from being not specific, are not obvious when the nematodes occur at low densities in the soil: indeed, only when *Heterodera* populations increase above the tolerance limit of the hos plant (POTTER *et al.,* 1993) infection becomes sufficiently obvious to concern farmers and plant pathologists about the consequent damage and economic losses.

All the above stress the importance of extending the study to areas, such as all western side of Sicily, which have not been properly investigated so far.

RIASSUNTO

NEMATODI CISTICOLI DEL GENERE *HETERODERA* E *GLOBODERA* IN SICILIA

In Sicilia, dal 2007 al 2009, è stata condotta un'indagine sulla presenza e la diffusione dei nematodi cisticoli al fine di poter disporre di un quadro più preciso sulle specie presenti e sulle relative perdite di produzione.

L'indagine è stata condotta su campioni di terreno e di radici prelevati in aree coltivate e non, effettuando prelievi in corrispondenza di evidenti manifestazioni di sintomi attribuibili ad attacchi di nematodi cisticoli. Successivamente, in laboratorio è stata eseguita l'estrazione delle cisti e delle larve e la successiva identificazione mediante esame comparativo dei dati morfologici e morfometrici. Ulteriore conferma tassonomica è stata ottenuta tramite analisi molecolare.

Dall'esame dei campioni studiati è emersa la presenza di sette specie appartenenti al genere *Heterodera* Schmidt, 1871 – e più precisamente: *H. avenae* Wollenweber, 1924, *H. bifenestra* Cooper, 1955, *H. carotae* Jones, 1950, *H. goettingiana* Liebscher, 1892, *H. hordecalis* Andersson, 1974, *H. latipons* Franklin, 1969 e *H. schachtii* Schmidt, 1871 – e due al genere *Globodera* Skarbilovic, 1959 – *G. rostochiensis* (Wollenweber, 1923) Behrens, 1975 e *G. pallida* (Stone, 1973) Behrens, 1975.

Tutte le specie riscontrate sono note per l'Europa e il bacino del Mediterraneo; tuttavia, alcune di esse (*H. avenae, H. hordecalis, H. latipons*) non erano fino a oggi state rinvenute in Sicilia e una (*H. bifenestra*) è nuova per l'Italia.

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