SHORT COMMUNICATION

DIFFUSION AND EFFECTIVENESS OF THE NEMATOPHAGOUS FUNGUS *HIRSUTELLA RHOSSILIENSIS* IN CONTROL OF THE CYST NEMATODE *HETERODERA DAVERTI* UNDER FIELD CONDITIONS

G. Del Sorbo[1], F. Marziano[1] and F. P. D'Errico[2]

[1]Dipartimento di Arboricoltura, Botanica e Patologia vegetale – Sezione di Patologia vegetale, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (Napoli), Italy
[2]Dipartimento di Entomologia e Zoologia agraria, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (Napoli), Italy

SUMMARY

An epidemiological survey on 176 soil samples, collected from carnation fields in the Vesuvius area (Naples, Italy) revealed, in more than 90% of samples, the occurrence of second stage juveniles of the cyst nematode *Heterodera daverti* parasitized by the nematophagous fungus *Hirsutella rhossiliensis*. The diffusion of the fungus over the area of cultivation of carnation could account for the substantial decrease in severity of nematode attacks, which were observed over the last five years. The influence of various factors on *H. rhossiliensis*-mediated suppressiveness in soils subjected to repeated treatments with fumigants is discussed.

Key words: biological control, soil suppressiveness, carnation, nematophagous fungi.

The first report of severe epiphytotics caused by the cyst nematode *Heterodera daverti* Wouts *et* Sturhan in cultivations of carnation in the Vesuvius area (Naples, Italy) dates back to 1983 (Ambrogioni *et al*., 1986). In the following years, *H. daverti* became the most diffuse and harmful phytoparasitic nematode on carnation, fully replacing the previously reported species: the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitwood and *Paratylenchus dianthus* Jenkins (D'Errico *et al*., 1985; D'Errico *et al*., 1987). One reason for the turnover among nematode species could be the repeated use of nematicides which fully control *M. incognita* and *P. dianthus* but not *H. daverti* whose eggs are retained within a cyst and also laid outside within a gelatinous matrix which confer a degree of protection to them. The soil type in the mentioned area is particulary suitable for the development of *H. daverti* (D'Errico *et al*., 1987), which proved not to be controlled by a single treatment even with high dosages of fumigant nematicides before set up of the crop, and required repeated applications with non-fumigant nematicides during cultivation. In soil samples from a glasshouse cultivation of carnation in Ercolano (Naples, Italy) the hyphomycete fungus *Hirsutella rhossiliensis* Minter *et* Brady was found associated to the second stage juveniles of the nematode. This fungus is commonly reported as a parasite of several species of phytophagous nematodes (Stirling, 1991) and studied as potential biocontrol agents. Over the last years severe attacks of *H. daverti* on carnation cultivations were no more observed. To gain insight on the role of the fungus in suppressing the nematode, an epidemiological study was undertaken.

The presence of *H. daverti* and *H. rhossiliensis* was monitored in 176 glasshouses by collecting soil samples (10 samples per glasshouse), in the period April-September 2002 in the area of cultivation of carnation around Vesuvius (Naples, Italy), where treatments with various soil fumigants (including methyl bromide, dazomet, vapam) are a common practice. Nonetheless, in this area severe attacks of the nematode *H. daverti* have been reported in the late 80s and early 90s. After collection, ten soil samples from each glasshouse were pooled and extensively mixed. Each pool was labeled and stored at 7°C until examinations. To collect all life stages of the nematode 500 g of soil from each pool were processed according to the centrifugation method of Jenkins (1964). The nematode suspensions were observed under a microscope to ascertain the presence of parasitized or healthy juveniles of *Heterodera daverti*. Microscopical observations were done on at least 100 juveniles per soil pool. In all cases, the parasitizing fungus was identified as *H. rhossiliensis* on the basis of analytical keys.

Second stage juveniles of *H. daverti* clearly parasitized by *H. rhossiliensis* were found in 159 out of the 176 soil samples examined (90.34%). The percentage of juveniles of *H. daverti* parasitized by *H. rhossiliensis* ranged between 88 and 100% (average 95.5%). Non infected juveniles of *H. daverti* were found in 14 out of the remaining 17 soil samples. The occurrence of only healthy juveniles of *H. daverti* was found in fields where the control of attacks of *H. daverti* usually requires application of non-fumigant nematicides during carnation cultivation cycle. In 3 soil samples no *H. daverti* was found. No other species of nematode was found in any of the soil samples analysed.

Several studies (Sturhan and Schneider, 1980; Müller, 1982; Müller, 1985; Jaffee and Zehr, 1982; Jaffee and Zehr, 1983a; Jaffee and Zehr, 1985, Juhl, 1985; Cayrol

Corresponding author: G. Del Sorbo
Fax: +39.081.2539379
E-mail: delsorbo@unina.it

and Frankowski, 1986; Eayre *et al*., 1987; Jaffee *et al*., 1989; Jaffee and Muldoon, 1989) demonstrate the efficiency of *H. rhossiliensis* in reducing soil populations of *Heterodera* spp., *Criconemella xenoplax* (Raski) Luc & Raski and *Ditylenchus dipsaci* (Kühn) Filipjev. The constant association of *H. rhossiliensis* with phytoparasitic nematodes (Stirling, 1991) pushed researchers to study its biology, saprophytic fitness and application perspectives in biological control.

The strong parasitic aptitude of *H. rhossiliensis* against *H. daverti* in laboratory and field tests was reported by Marziano *et al*. (1995). In the present study, we report the occurrence of a high incidence of parasitism of *H. rhossiliensis* on juveniles of *H. daverti*, whose populations are contained, in the large majority of the carnation fields examined, below limits determining the presence of symptoms of nematode attack. In these situations, there is no need for nematicide applications for the control of *H. daverti*. More investigations are needed to elucidate the reasons for the lack of parasitized forms of the nematode in a small fraction (7.9%) of the soil samples examined.

The poor saprophytic fitness of *H. rhossiliensis* (Ciancio, 1988) constitutes a serious drawback to its field performances depending on its requirement for particular nutrients (*i.e.* juveniles of nematodes) and poor competitive capacity in comparison with other soil fungi. In soils treated with fumigants, living juveniles of *H. daverti*, originating from the fraction of eggs that are not killed by fumigants, could constitute the small and exclusive ecological niche for survival of the nematophagous fungus. In all cases, the time needed for host devitalization is proportional to the number of spore, which adhere to the body of the nematode (Cayrol and Frankowski, 1986). The onset of fungal sporulation on the body of the nematode is normally observed 2-3 days after its invasion by fungal hyphae and lasts about 20 days, leading to production of about 700 conidia per nematode (Jaffee and Zehr, 1983b). The overall nematicide efficacy of *H. rhossiliensis* depends on successful and abundant sporulation on nematodes. The activity of *H. rhossiliensis* is negatively influenced by fungicides used in spray applications to protect carnation from foliar diseases. However, this effect seems to be temporary and not to depend on intrinsic resistance of the fungus but rather on the dilution of the fungicides in the liquid phase of the soil, on their degradation, adsorption on soil particles and non-homogeneous distribution in the soil (Pullen *et al*., 1990). The frequent occurrence of parasitized *H. daverti* juveniles in fumigated soils is one example of modifications of soil microenvironment following repeated fumigation treatments having benefic impact on nematode control.

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