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Population density and soil antagonists of *Meloidogyne hapla* infecting kiwi in southern Italy

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Summary – The development of a *Meloidogyne hapla* population parasitising kiwifruit *(Actinidia deliciosa)* and the associated community of soil antagonists were studied at Metaponto, Italy. The highest nematode densities, $105 \pm 69.3 \times 10^2$ eggs/g of roots and 93.8 ± 88.8 females/g of roots were observed in November and September 1990, respectively. Four species of nematode capturing fungi *(Arthrobotrys dactyloides, A. oligospora, Monacrosporium salinum* and *M. bembicodes),* two fungal egg parasites (*Verticillium chlamydosporium,* mycelia sterilia) and the endoparasite *Verticillium balanoides* were isolated from soil or *M. hapla* eggs. Other nematode antagonists present in the soil included the aquatic fungus *Catenaria anguillulae* and the predacious nematode *Mylonchulus brachyuris.* The monthly average rates of *M. hapla* egg parasitism decreased from $17.6\ \% \pm 14.3$ in June 1990 to $4.2\ \% \pm 2.2$ in November 1990. For all the replications and months, the *M. hapla* eggs density and the rate of colonized eggs were inversely correlated ($r = -0.509$; $P < 0.001$). The total number of colonized eggs and eggs density were positively correlated ($r = 0.7943$; $P < 0.001$). The complex of antagonists was not associated with suppression of the *M. hapla* population below the economic injury level during the period of examination, whereas tests realized using increasing soil amounts revealed the occurrence of a soil mycostatic effect on nematophagous fungi when a 2.30 cm^3 or higher inoculum level per plate was used.

Résumé – ***Densité de population et antagonistes telluriques de* Meloidogyne hapla *attaquant le kiwi en Italie méridionale*** – Le développement d'une population de *Meloidogyne hapla* attaquant le kiwi *(Actinidia deliciosa)* et la communauté d'antagonistes présente dans le sol ont été étudiés à Metaponto, Italie. Les plus fortes densités du nématode ($105 \pm 69{,}3 \times 10^2$ œufs/g de racine et $93{,}8 \pm 88{,}8$ femelles/g de racines) ont été observées pendant les mois de novembre et septembre 1990, respectivement. Quatre espèces de champignons prédateurs *(Arthrobotrys dactyloides, A. oligospora, Monacrosporium salinum* et *M. bembicodes),* deux champignons parasitant les œufs *(Verticillium chlamydosporium* et mycelia sterilia) ainsi que l'endoparasite *Verticillium balanoides* ont été isolés à partir du sol ou des œufs de *M. hapla.* Les autres antagonistes présents dans le sol incluent le champignon aquatique *Catenaria anguillulae* et le nématode prédateur *Mylonchulus brachyuris.* Le pourcentage mensuel du parasitisme des œufs de *M. hapla* a diminué de $17{,}6\ \% \pm 14{,}3$ en juin à $4{,}2\ \% \pm 2{,}2$ en novembre. En considérant l'ensemble des répétitions et des mois, la densité des œufs de *M. hapla* et le taux de colonisation des œufs sont négativement corrélés ($r = -0{,}509$; $P < 0{,}001$). Le nombre total d'œufs colonisés et la densité des œufs sont, par contre, corrélés positivement ($r = 0{,}7943$; $P < 0{,}001$). Pendant la période d'observation, l'action de l'ensemble des antagonistes n'a pas conduit à une diminution de la population de *M. hapla* en dessous du seuil de dommage économique, tandis que des essais réalisés en utilisant des quantités de sol progressivement croissantes démontrent un effet mycostatique du sol sur les champignons nématophages à partir de 2,30 cm^3 de sol par boîte de Petri.

Fungal parasitism of root-knot nematode (*Meloidogyne* spp.) eggs has been reported for several host-parasite associations throughout the world (Morgan-Jones *et al.,* 1981; Gaspard *et al.,* 1990; De Leij & Kerry, 1991). Efficient control appears related to fungi density and virulence as the presence of fungal parasites does not prevent the development of root-knot nematodes infestations (Gaspard *et al.,* 1990; Gomes Carneiro & Cayrol, 1991). Acceptable levels of natural control were observed only over long-term nematode and parasite associations or when the nematode population was exposed to non-optimal feeding or developmental conditions (Stirling *et al.,* 1979; Kerry, 1990).

The objectives of the present study were to determine the population dynamics of *Meloidogyne hapla* in a kiwi orchard, to assess the occurrence of fungal antagonists in soil and to evaluate the level of antagonism under the agronomic and climatic conditions of Southern Italy.

Materials and methods

Field observations were carried out at monthly intervals between June and December, 1990, in a seven years old kiwi orchard (cv. Hayward) at Metaponto, Italy. Soil (85 % sand, 5 % silt, 10 % clay; pH = 7.83) and root samples were taken from twelve randomly selected vines over the whole orchard. Samples were taken 30-40 cm away from the trunk of each vine at a depth of 20-30 cm from three sites, separated 120° from each other. A 100 cm^3 aliquot of the homogenized soil sample was processed by Cobb's sieving and decanting technique using 420 and 45 µm mesh sieves, and *M. hapla* juveniles and predatory nematodes were counted using a 1 ml Hawksley counting chamber. Roots were gently washed, weighed and 3 to 5 g were examined under a dissecting microscope to count females. Eggs were extracted by shaking an average of 3 g of roots in 200 ml of

a 1 % NaOCl solution in a 500 ml jar for 3 min (Hussey & Barker, 1973). Total number of eggs and the number of dead and possibly parasitized eggs were counted in a 1 ml counting chamber at 125 ×. The number of eggs per egg mass was determined by gently crushing an average of 20 egg-masses on microscope slides with a cover slip, and counting the eggs at 125 ×. An aliquot of the extracted eggs was collected in a few ml of distilled water, poured onto 1.5 % Difco water-agar plates (WA) containing 0.1 % streptomycin and 0.01 % penicillin and observed under a dissecting microscope for fungal colonization. Eggs colonized with fungi were isolated and transferred to 1.5 % WA plates with 10 % potato dextrose agar (PDA) for fungal isolation and identification. To assess the parasitic capability of the fungi recovered from the eggs, some of the isolates were inoculated on WA with healthy *M. hapla* eggs extracted as previously described (Hussey & Barker, 1973). The eggs on the plates were examined after one week incubation at 20-24 °C. Three replications per treatment were used.

A semi-quantitative method (Mankau, 1975) and a soil sprinkling technique (Duddington, 1955) were used to isolate nematode trapping fungi (NTF). To estimate density of NTF, 0.23, 0.46, 2.30, 4.60 and 6.90 cm^3 of soil from each replicated sample collected in June and September 1990 were placed in three replicated series of Petri dishes containing 1.5 % Difco WA. Plates were sealed and incubated at 20-24 C for 30 days and the presence of NTF conidia, conidiophores and trapping organs was observed at 80 and 125 ×.

Results

Kiwi roots were severely galled in all replicates at each monthly sampling. Densities of *M. hapla* juveniles and females increased from June until November and September, respectively, and gradually decreased until December (Fig. 1). The number of eggs/g of root increased from $15 \pm 14.5 \times 10^2$ in June to $105 \pm 69.3 \times 10^2$ in November. Rate of *M. hapla* eggs colonized with fungi decreased from 17.6 % ± 14.3 in June to 4.2 % ± 2.2 in November. The density of *M. hapla* eggs and the rate of colonization for all the replicated measurements and

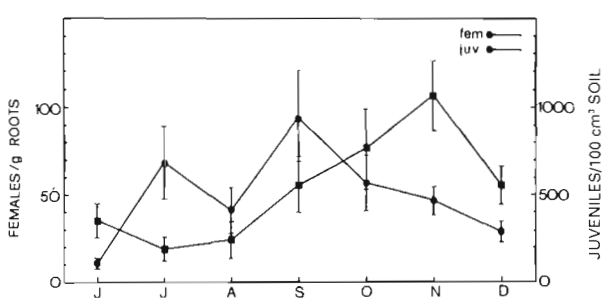

Fig. 1. Monthly densities of *Meloidogyne hapla* juveniles and females (n = 12).

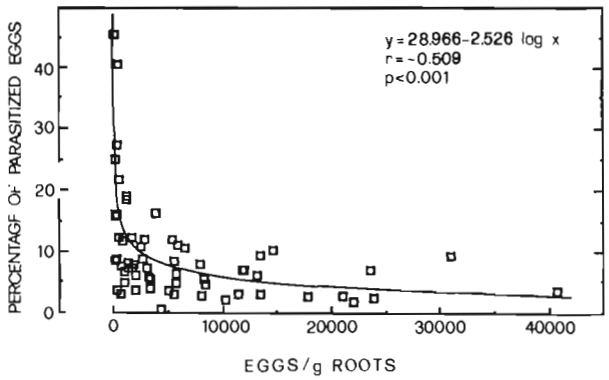

Fig. 2. Relationship between density of *Meloidogyne hapla* eggs and percentage of eggs with fungal parasites.

months were inversely correlated (Fig. 2). The total number of colonized eggs for all months and replications and the eggs density were positively correlated ($r = 0.7943$; $P < 0.001$). Average number of eggs per egg mass ranged from 137 ± 49 (September) to 232 ± 64 (October). Two egg parasites, *Verticillium chlamydosporium* Goddard and a mycelia sterilia, were isolated from colonized eggs. Rates of colonization ranged from 8.8 % and 3.2 % (June) to 2.1 % and 0.75 % of total eggs, respectively.

In the *in vitro* tests, *V. chlamydosporium* and mycelia sterilia parasitised only unembryonated *M. hapla* eggs from which they were reisolated at each replication. The latter species in pure culture showed a very slow growth rate and never sporulated on PDA.

The species of NTF recovered from preliminary tests were *Arthrobotrys dactyloides* Drechsler, also isolated from egg masses, *A. oligospora* Fres., *Monacrosporium bembicodes* (Drechsler) Subram. and *M. salinum* Cooke & Dickinson. The endoparasite *Verticillium balanoides* (Drechsler) Dowsett was isolated from free living nematodes together with the aquatic fungus *Catenaria anguillulae* Sorokin. The agar plate method revealed a soil inhibitory effect on the isolation of NTF when the inoculum level per plate was 2.30 cm^3 or higher (Fig. 3).

The predacious nematode *Mylonchulus brachyuris* (Bütschli) Andrássy was recovered at each monthly sampling at densities between 18 (August) and 62 (October) specimens/100 cm^3 of soil. Densities in October and November were correlated with the *M. hapla* juveniles densities ($r = 0.7603$ and $r = 0.6702$ respectively; $P < 0.05$).

Discussion

During the period of study the *M. hapla* population remained the main nematode pest at densities far beyond any tolerable plant damage treshold. Although the experimental protocol did not allow a direct evaluation

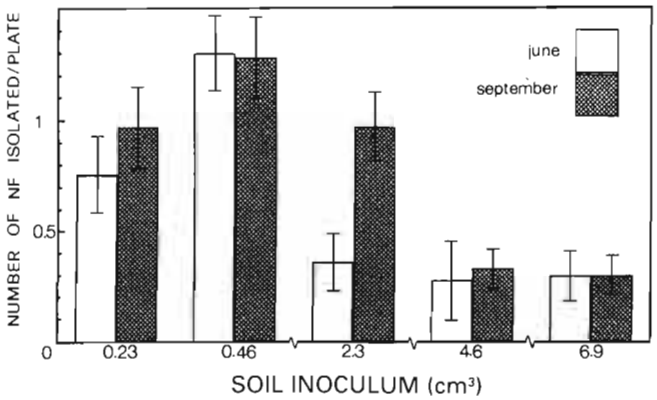

Fig. 3. Recovery of nematode-trapping fungi from water-agar plates at different soil inoculum levels (n = 36).

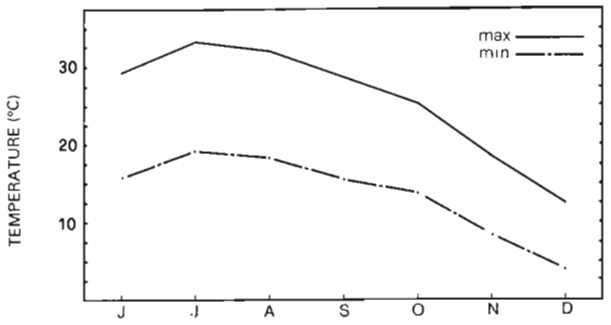

Fig. 4. Average monthly temperatures at Metaponto (June-December 1990).

of the role played by soil antagonists, no suppressiveness of economic significance was evident in the field. An inverse density dependent relationship occurred between the nematode density and the eggs colonizing fungi. Fungal activity interested a limited number of eggs relatively constant in time and yielded progressively lower rates of colonization when their density increased (Fig. 2). A density dependent relationship was probably occurring between the *M. brachyuris* and the *M. hapla* populations.

M. hapla has an optimal temperature range of 20-25 °C and a minimum temperature treshold of 8.25 °C. In temperate regions it is able to reproduce on susceptible hosts with several (10-14) generations per year, reaching high field densities during colder months (Lahtinen *et al.,* 1988; Pinochet *et al.,* 1990). The temperature range reported for optimal growth of *V. chlamydosporium* is 18-25 °C and at 10 °C its growth is largely reduced (Kerry *et al.,* 1986).

Studies on fungi parasitic to root-knot nematode eggs showed that parasitism increased when nematodes were exposed to suboptimal developmental conditions (Stirling, 1978; Stirling *et al.,* 1979). These circumstances were not present in the field studied and the reduced efficacy of *V. chlamydosporium* and other antagonists was related to the nematode fast reproduction in feeding and climatic conditions which favoured its development (Kerry *et al.,* 1986) (Fig. 4).

The NTF species detected are common soil inhabitants in most agricultural soils. A soil fungistatic effect (Mankau, 1962), a weak competition efficiency with the other soil micro-organisms and/or among germinating conidia or microcolonies can account for the *in vitro* inhibition of microcolonies development (Cayrol, 1988).

Eggs of *Meloidogyne incognita, M. arenaria* and *Heterodera avenae* have been reported to be parasitised by different *V. chlamydosporium* isolates at all developmental stages (Morgan-Jones *et al.,* 1981; Irving & Kerry, 1986; Gaspard *et al.,* 1990). Our isolate was observed to parasitise in one week only unembryonated *M. hapla* eggs, confirming that immature eggs are more readily susceptible to hyphal penetration (Irving & Kerry, 1986).

Effective antagonism by *V. chlamydosporium* and other parasitic fungi occurs in Northern Europe against cyst nematodes under monoculture conditions (Kerry, 1990). Parasitism by *V. chlamydosporium* of *Meloidogyne* spp. has also been observed, but no isolate was effective in reducing root-knot nematode numbers (De Leij & Kerry, 1991; Gaspard *et al.,* 1990). *V. chlamydosporium* exhibits a wide range of variability in parasitic efficiency and other biological features and also this diversity might have affected the level of parasitism in the field (Irving & Kerry, 1986; Kerry *et al.,* 1986). This is the first report of this fungus in Italy and the first record of *M. hapla* eggs colonization in the field.

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