INVESTIGATIONS ON *DITYLENCHUS DIPSACI* DAMAGING CARROT IN ITALY

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Summary. Investigations were undertaken in southern Italy from September to the following March on the epidemiology of an Apulian and a Sicilian population of *Ditylenchus dipsaci*, on carrot under field conditions. The host status of various plants to the Sicilian population of the nematode was also assessed. In Apulia, nematode infection on carrot sown in early September started at end of October on the leaves and in early December on the tap roots. Leaf death and tap root rot were observed the following January on mature plants. On carrot sown one month later, nematode infection occurred by the beginning of November and greatly suppressed plant growth. In Sicily, on carrot sown in early October, infection was observed on aerial plant parts at end of November and on tap roots in mid December. Leaf death and tap root rot occurred at the end of January. Early symptoms of nematode attack were straddled leaves, multi-bud plant crowns and discoloration of the tops of tap root. The portion of the plant most affected by *D. dipsaci* was that 2-4 cm below and above ground. The Sicilian population of the nematode reproduced on carrot, broad bean, oats, rye, Italian rye grass, celery, *Ammi majus* and *Galium aparine*.

Carrot (*Daucus carota* L.) is among the most widely cultivated vegetables. In Italy about 10000 ha (Anonymous, 2001), mainly concentrated in five regions, are devoted to this crop. Among the nematodes reported in association with it (Potter and Olthof, 1993), in Europe the most important are the carrot cyst nematode, *Heterodera carotae* Jones, and several species of root-knot nematodes, *Meloidogyne* spp. The stem and bulb nematode *Ditylenchus dipsaci* (Kuehn) Filipjev, is reported to reproduce on about 450 plant species (Hooper, 1972). In England, Goodey (1931) reported carrot as a new host for *D. dipsaci* and described symptoms of infection, but Hooper (1972) did not report carrot among the vegetables most affected by the nematode. However, Kaai (1972) investigated the effect of nematicides on the nematode on carrot in the Netherlands, indicating that this pathogen caused severe damage. Other authors report carrot as a host, but without mentioning damages (Edwards and Taylor, 1963; Hooper and Southey, 1978), or resistant (Barker and Sasser, 1959) to *D. dipsaci*. All this would indicate that in general *D. dipsaci* is not a severe pathogen to carrot. However, in Italy Benintende *et al.* (1994) and Schillirò *et al.* (1995) reported that damage caused by *D. dipsaci* to carrot in Sicily was so severe (Fig. 1) to warrant the use of control measures (Cartia *et al.*, 1997; Greco and Cartia, 2000). In 1999 damage to carrot due to *D. dipsaci* was also observed in another Italian region (Apulia), in an area in which onion is known to be severely affected by the nematode. However, information on epidemiology and host plants of the Italian populations of *D. dipsaci* attaking carrots was lacking. Therefore, investigations were undertaken to obtain insights on these aspects of the nematode biology.

MATERIALS AND METHODS

The investigations were conducted in fields in which carrots had been severely damaged by *D. dipsaci* in the preceding growing season.

Investigations on epidemiology and dynamics. Two fields were selected, one of clay soil infested with 115 nematode fourth stage juveniles/100 cm^3 soil at Ispica (Sicily) and one of sandy soil infested with 50 nematodes/100 cm^3 soil at Margherita di Savoia (Apulia).

At Ispica, carrot cv. Nelson was sown on 2 October 1999. After germination (20 October) the field was divided in two parts and plant and rhizosphere soil samples were collected fortnightly from 29 October 1999 to 21 March 2000.

At Margherita di Savoia the selected field was also

Fig. 1. A carrot field in Sicily showing a large patch severely damaged by the stem and bulb nematode *Ditylenchus dipsaci*.

divided in two parts but one was sown on 3 September and one on 1 October 1999 with carrot cv. Bolero. After plant germination, two plant samples and two rhizosphere soil samples were collected at weekly intervals from each part of the field.

In both locations, temperatures were recorded with thermographs at 5 to 15 cm depth in the soil and at ground level in the air. The soil moisture content of each soil sample was also determined and referred to as per cent of soil dried weight. Plant sample size varied from a few grams soon after germination to large as plant size increased. At harvest some severely infested carrots were left to dry in the shade to ascertain the survival of the nematode until the next season.

The carrot crop was rainfed at Margherita di Savoia and at Ispica received supplementary sprinkler irrigation.

Soil samples were processed by Coolen's (1979) method and nematode specimens counted. Plants were washed free of the adhering soil and nematodes were extracted separately from tap roots and aerial parts. Until the plants reached 5-7 cm tall the entire tap root or aerial part was used to extract nematodes. When the plants reached 10 cm height only the first 10 cm of the aerial parts and the top 2-3 cm of the tap roots were selected. The leaves were cut into 0.5 cm long pieces and tap roots into slices less than 1 mm thick. Then all plant material was mixed and two 10 g sub-samples were put on 100 µm mesh sieves of 10 cm diameter. These sieves were arranged in larger glass Petri dishes in which tap water was added to nearly submerge the plant material. All dishes were maintained at 20 °C for 48 hours. The nematode water suspension in the Petri dishes was collected every 24 hours, concentrated by sieving on a 5 µm sieve and nematode specimens counted.

At each sampling date, note was also taken of the symptoms of nematode infestation.

Host range. The investigation was undertaken only with the Sicilian population of the nematode. A field plot of 80 m^2 next to that used for the previous study was divided into 20 plots each of 1 m^2. These were sown or planted on 28 October 1999 with the plant species recorded in Table I. The plants were selected because they are known to be typical host plants of particular races of the nematodes or because of their importance in areas were *D. dipsaci* is spread or suspected to be host of Mediterranean populations. There were four rows per plot, each planted with a different plant species. These were distributed randomly among plots and each was planted in four rows, on different plots. For broad bean, strawberry, potato and bulbous plants there were 5-8 plants per row. For the remaining plant species, the number of seeds that were sown varied according to species.

Because infestation of *D. dipsaci* is affected by environmental conditions and plant stage, some of the plants in each row were harvested on 22 February and the remaining on 21 March, 2000. Sub-samples were then collected from each plant species and rows, cut in small pieces, mixed and nematodes extracted as mentioned before from sub-samples of 10 g.

To assess to host status of wild plants occurring in the area, another piece of land, contiguous to that used for the cultivated host study, was left uncultivated to allow wild plants to growth. These were collected at the same time of the cultivated plants, grouped according to species and nematodes extracted as already specified.

RESULTS

Investigations on epidemiology and dynamics

Plant germination occurred in 15-20 days and the first samples were collected after 10 more days.

In Sicily. Maximum soil temperature at 5 cm depth (Fig. 2) was in the range 13-19 °C in November-December, dropped to 11-14 °C in January-February and rose to 15-20 °C in March. Soil moisture content (Fig. 4) remained in the range 21-31% for most of the growing season. Nematodes in the aerial plant parts were undetectable on 29 October, a few were found throughout November and larger numbers thereafter, especially from January onwards. Tap roots (Fig. 5) were not infected until mid November; thereafter nematode numbers remained at a low level throughout December and increased greatly from 12 January onwards when populations were of the same magnitude as those in the leaves. In the soil nematode populations remained at a rather low level (10-61 specimens/100 cm^3 soil) throughout the carrot crop growing season (Fig. 5), even when severe symptoms occurred on both leaves and tap roots. No symptoms of nematode attack were observed until the end of November, but on 13 December enlargement of the bud at crown level (Fig. 8), presence of straddled leaves and some discoloration of the tap root top (Fig. 9) were observed. Later, infected plants besides the main bud started to produce lateral adventitious buds (Fig. 10). Severe symptoms were observed only by the end of January when both the basal aerial plant part and the tap root top started to rot thus leading to plant death (Fig. 11). Rotting was severe on the tap root top, decreased with increasing distance from the ground level and was negligible at more than 3-4 cm depth (Fig. 12).

In Apulia. Maximum soil temperature at 5 cm depth (Fig. 3) was above 20 °C until the end of October, in the range 20-15 °C in November, 14-10 °C in December-February and increased to 15-21 °C in March. At the same depth minimum temperatures were less than maximum temperatures of about 6-8 °C until November, 5-6 °C in December-February and 9-11 °C in March. At 15 cm depth maximum temperature were about 2 °C less and minimum temperature 2 °C more than those at 5 cm depth. Air temperature at ground level (Fig. 3)

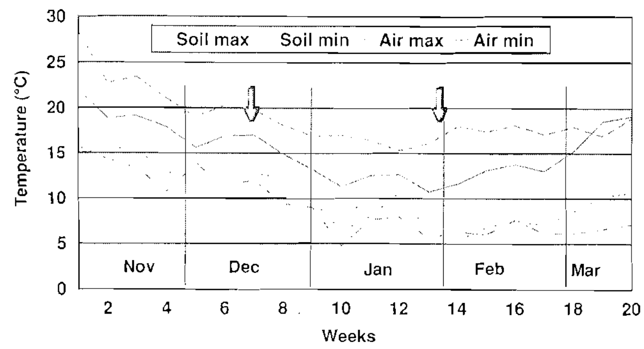

Fig. 2. Maximum and minimum soil (5 cm depth) and air (ground level) temperatures recorded at Ispica (Sicily) during the carrot growth season. Arrows indicate dates when early and late symptoms of nematode attack became obvious.

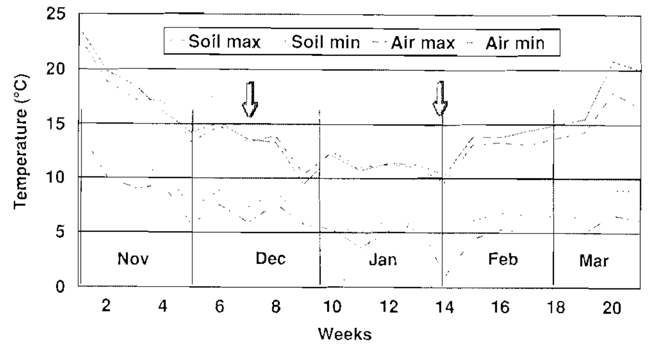

Fig. 3. Maximum and minimum soil (5 cm depth) and air (ground level) temperatures recorded at Margherita di Savoia (Apulia) during the observation period. Arrows indicate dates when early and late symptoms of nematode attack became obvious.

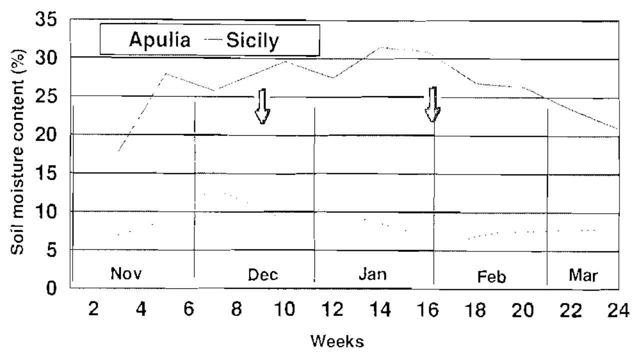

Fig. 4. Soil moisture content at Ispica (Sicily) and Margherita di Savoia (Apulia) during the experiment. Arrows indicate dates when early and late symptoms of nematode attack became obvious.

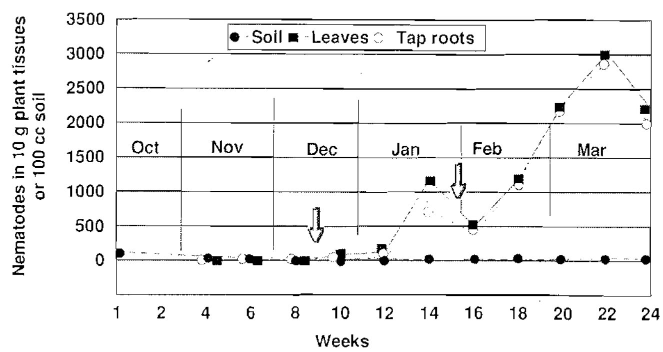

Fig. 5. Dynamics of *D. dipsaci* populations in carrot plant tissues and soil at Ispica (Sicily). Carrot sown in early October. Arrows indicate dates when early and late symptoms of nematode attack became obvious.

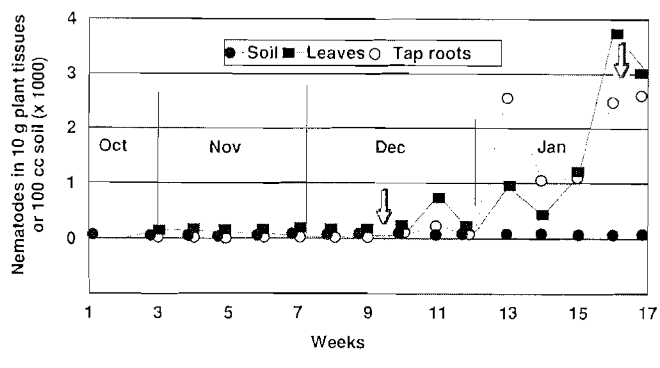

Fig. 6. Dynamics of *D. dipsaci* populations in carrot plant tissues and soil at Margherita di Savoia (Apulia). Carrot sown in early September. Arrows indicate dates when early and late symptoms of nematode attack became obvious.

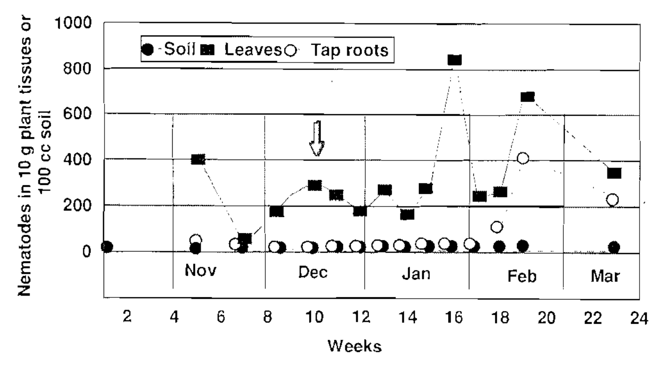

Fig. 7. Dynamics of *D. dipsaci* populations in carrot plant tissues and soil at Margherita di Savoia (Apulia). Carrot sown in early October. Arrow indicates date when symptoms of nematode attack became obvious.

was ± 0.5-1 °C that at 5 cm depth. Soil moisture content (Fig. 4) was in the range 6-13% of dry soil weight. However, weather conditions in November and December were rainy.

In carrots sown in September nematodes were extracted from the leaves from the end of October onwards (Fig. 6). They remained at a rather low level throughout December but increased continuously until the end of January. Nematodes in tap roots (Fig. 6) were not detected until 22 November, in small numbers until December but increased in January when they reached nearly the same level as those in the aerial plant parts. The first symptoms of nematode infestation on the aerial plant parts first occurred on 13 December, when plants had 5-6 true leaves and tap roots were of 0.5-0.8 cm diameter. They consisted of basal bud enlargement

Fig. 8. Small carrot plants showing enlarged crowns (arrowed) caused by attack of *D. dipsaci*. In the middle, a healthy plant with no symptoms.

Fig. 9. Small carrot plants showing enlarged crowns, straddled leaves and light discolorations (arrowed) of the tap root tops, caused by infestation of *D. dipsaci*.

and some spotted discoloration of the tap root top (Fig. 9). These symptoms became more obvious in early January, when the carrots were approaching maturity. At this plant stage the presence of multi-bud carrots was evident (Fig. 10). Tap roots started to rot by the end of January (Fig. 11) when carrots are usually, at least partly, harvested.

Separate examination of 10 g of leaf petioles and leaf laminas revealed that the number of nematodes was nearly the same (120-140) on 6 December, but were 369 in the petioles and 10 in the lamina on 13 December, 625 in the petioles and 93 in the laminas on 20 December and 1113 in the petioles and only 48 in the laminas on 3 January.

On 13 December nematodes were also extracted separately from plants with and without disease symptoms (enlargements of basal buds) attributed to nematode infestation. No nematodes were extracted from aerial parts or tap roots of plants without symptoms. In the plants with obvious symptoms nematodes in 10 g were 6,323 in the basal buds and only 9 in the tap roots of the same plants. At the same date two plants, one showing clear symptoms of the nematode attack and another without symptoms, were selected and nematodes extracted from three 2-cm tall section of the aerial part (0-2, 2-4, 4-6) and two 1.5-cm parts of the tap roots (0-1.5 and 1.5-3 cm depth) and referred to as nematodes in 10 g. In the plant without symptoms nematode in the aerial parts were 45 in the 0-2 cm section, 25 in the 2-4 cm section and absent in the top 4-6 cm. No nematodes were extracted from the tap root. In the plant with symptoms, nematodes in the aerial part were 19,531 in the 0-2 cm section and the same (1160) in the 2-4 cm and 4-6 cm sections, while in the tap root there were much fewer nematodes: 410 in the 0-1.5 cm section and absent in the 1.5-3 cm section.

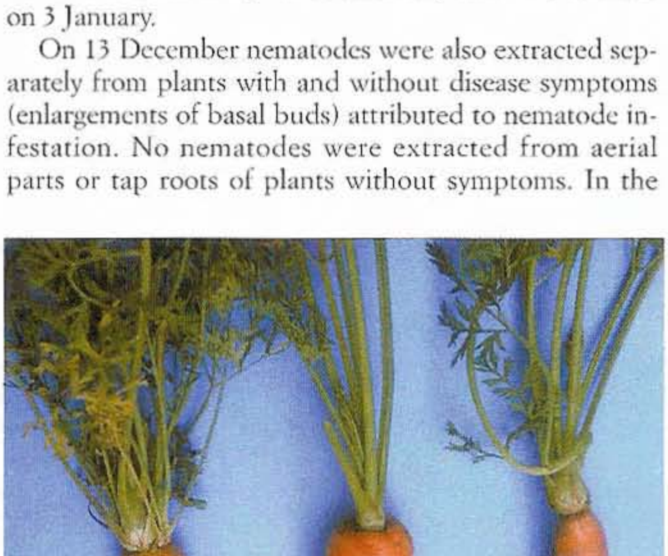

Fig. 10. Symptoms of *D. dipsaci* attack on adult carrot plants. Note the enlargement of the buds at crown level (left and right carrots), the presence of adventitious buds (left plant) and the absence of these symptoms on the middle healthy plant.

Temperatures and soil moisture content for carrots sown at the beginning of October were the same as for those sown in September (Figs 3,4). However, the growth of carrots sown in October was much slower than that sown in early September, probably because of the drop of the temperature and of the much severe effect of the nematode attack. Notwithstanding, observations continued until mid March when these carrots remained too small and unmarketable. In these carrots (Fig. 8) nematodes were found at the beginning of November in both aerial parts (415 specimens/10 g) and tap roots (47 specimens/10 g) (Fig. 7). Thereafter nematodes were extracted always from the aerial parts although in rather small numbers (maximum 857 specimens/10 g on 24 January). Nematodes in the tap roots were few and not always present until January; they were in the range 109-414 thereafter. Although many plants died, symptoms of nematode attack on late sown carrots were not as obvious as on early sown carrots and the only ones were the presence of basal enlargement of the buds and straddled leaves that started on 20 December and became evident by the beginning of January (Fig. 8).

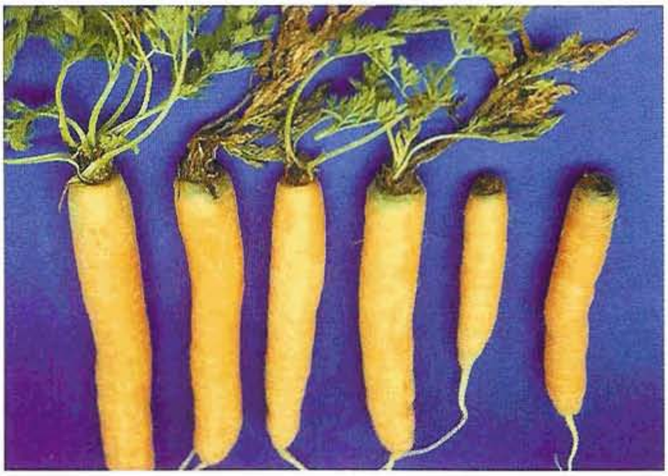

Fig. 11. Late symptoms of *D. dipsaci* attack on carrots. Note the presence of rot of the tap roots (right) and death of leaves.

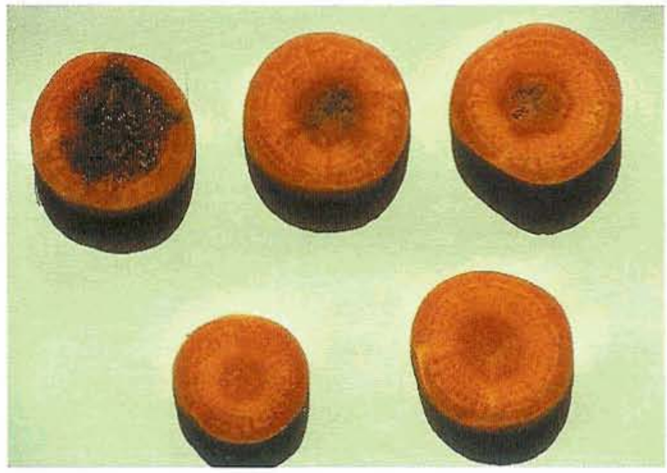

Fig. 12. Sections of a carrot tap root (clockwise from top to tip) showing rotting. Note that severity of rotting is more on the top section and decreases in deeper sections.

Examination of dried severely infested carrot plants six months after harvest, revealed the presence of only a few living fourth stage nematodes.

Host range

Because of heavy rains that occurred at the end of November, the soil remained wet throughout December and this may have affected nematode infection. As a consequence the number of nematodes in plant tissues were much less than expected. Moreover, germination and growth of *Trifolium* spp. (not reported), sugarbeet and strawberry was poor.

The first observation (22 February) (Table I) revealed the presence of large numbers of *D. dipsaci* specimens (max 2005/10 g) in all broad bean samples. Infestation was also observed in several samples of carrot, celery, onion, oats, rye, Italian rye grass, and sugar beet but not in garlic, strawberry, hard wheat and potato. Observations made one month later (21 March) confirmed the host status of the mentioned plant species. Moreover, a few nematodes were also extracted from strawberry. At this time symptoms of nematode infestation were obvious on broad bean (basal necrosis) and carrot (swollen buds). Although the number of nematodes in their tissues were rather low, all celery plants showed cracked and distorted basal parts of the external leaves and production of new lateral and distorted buds (Fig. 13). These symptoms were similar to those described by Stahl (1960) but different from those reported by Vovlas *et al.* (1993). Therefore, whether they are induced by the nematode infection it cannot be stated with certainty.

Among the wild plants (Table II) observed in February, the most infested were oats (*Avena fatua* L.) and Italian rye grass (*Lolium perenne* L.). Some of these plants exhibited typical symptoms (basal swellings) of nematode attack and up to 2,930 nematodes/10 g tissues were extracted from wild oat and 16,130/10 g tissues from wild Italian rye grass. A few nematodes were extracted from *Diplotaxis erucoides* (L.) DC., *Brassica nigra* (L.) Koch., *Galium aparine* L. and none from *Veronica hederifolia* L., *Fumaria officinalis* L., *Anthemis arvensis* L. and *Papaver rhoeas* L. On 21 March, wild carrot (*Daucus carota* L.) vas not infested while *Ammi majus* L. (of the same botanical family) contained 212 nematodes/10 g aerial part and showed symptoms similar to those of celery (Fig. 14).

DISCUSSION

Our results clearly show that nematode infestation and severity increased when temperature dropped to autumn average and concomitantly with a raining period, especially in Apulia, thus confirming previous findings in the same area (Greco *et al.*, 1974) and elsewhere (Weischer and Steudel, 1972). The parts of the carrot plant most affected are those next to ground level, with infection occurring first on aerial parts (mainly leaf petioles) and only later on tap roots.

Field observations indicate that *D. dipsaci* presents a more severe problem in Sicily than in Apulia, both regions with a long history of carrot cultivation. In Sicily carrot is sown in early October and harvested the following March-April and it rotates mainly with wheat or summer crops, unaffected by the nematode. In Apulia carrot is mainly sown in August and harvested from December to February and is rotated with early potato (not affected) and onion which is severely damaged by the nematode. On this basis one would expect more damage to carrot in Apulia than in Sicily. Although the different aggressiveness of the nematode populations occurring in the two geographical regions may account for the observed differences, we feel that these are mainly due to different environmental conditions during the carrot growing seasons. In Apulia, August and September are rather hot and dry and, therefore, unsuitable for nema-

Fig. 13. Celery plant with cracked and distorted basal part of the external leaves and production of new lateral and distorted buds, probably caused by infestation of *D. dipsaci*.

Fig. 14. Plant of *Ammi majus* infested by *D. dipsaci* and showing disease symptoms similar to those of celery.

tode infection and reproduction. Here nematode infection would occur only at the end of October and carrot rot only at the end of the next January when most of the carrot is already been harvested and, therefore, symptoms and damages overlooked. On late sown carrots, in October in this investigation, the nematode infection would occur when carrots are at very early growth stage. These carrots would not be harvested before the following February-March thus exposing the crop to the nematode for a longer period, and resulting in more severe damage. In this case carrots would hardly develop and many plants would die without showing typical symptoms of nematode attack and, therefore, the observed damage would be attributed to a different agent, unless plants are examined for the presence of *D. dipsaci*. In Sicily, environmental conditions are favourable to nematode infection for most of the carrot growing period and, therefore, the final stage of the nematode severity (tap root rot) is achieved well before carrot harvest.

Some symptoms of the nematode attack described here are similar to those described on carrot leaves by Goodey (1931) who did not report tap root rot probably because he examined only rather young carrots. Tap root rot was, however, reported by Benintende *et al.* (1994) and Schillirò *et al.* (1995). We did not observe local gall-like swellings of the leaf petioles as reported by Goodey (1931). Besides symptoms that appear at late stage of the nematode attack, we realise that other symp-

Table I. Host status of several cultivated plant species for a population of *Ditylenchus dipsaci* attacking carrot in Sicily.

Plant species			Nematodes in 10 g tissue	Host?
Botanical name	Common name	Cultivar		
Daucus carota L.	Carrot	Bolero	2219	Yes
Apium graveolens L.	Celery	Maggiolino	184	Yes
Allium sativum L.	Garlic	Bianco veneto	0	No
Allium cepa L.	Onion	Bianca di marzo	36	Yes
Vicia faba L.	Broad bean	Supersimonia	2923	Yes
Medicago sativa L.	Alfalfa	Equipe	21	Yes
Fragaria x ananassa Duch.	Strawberry	Tudla	29	Yes
Avena sativa L.	Cultivated oat	Ava	15	Yes
Lolium multiflorum Lam.	Italian rye grass	Menichetti	23	Yes
Secale cereale L.	Rye	Picasso	922	Yes
Triticum durum Desf.	Durum wheat	Simeto	0	No
Beta vulgaris L.	Sugar beet	Suprema	13	Yes
Solanum tuberosum L.	Potato	Spunta	0	No
Hyacinthus orientalis L.	Hyacinth	Lady derby	0	No
Iris hybrida hollandica Hort.	Iris	White excelsior	3	?
Narcissus pseudonarcissus L.	Narcissi	Garbiner	0	No
Tulipa gesneriana L.	Tulip	Orange bouquet	0	No

Table II. Host status of several wild plants for a population of *D. dipsaci* attacking carrot in Sicily.

Plant species	Max N° nematodes in 10 g tissue	Host ?
Avena fatua L.	2930	Yes
Lolium perenne L.	16130	Yes
Ammi majus L.	212	Yes
Galium aparine L.	112	Yes
Brassica nigra (L.) Koch.	7	Yes/No
Diplotaxis erucoides (L.) D.C.	3	Yes/No
Anthemis arvensis L.	0	No
Fumaria officinalis L.	0	No
Papaver rhoeas	0	No
Veronica hederifolia L.	0	No

toms, such as partial discoloration of the tap root top (reported by Goodey, 1931) and straddled leaves (Fig. 9), are associated with nematode infection at an early stage of carrot growth. Because infestation occurs first in the leaves and later moves into the roots, observation of these early symptoms can be useful to diagnose the nematode infection and thus suggest nematicidal treatments to prevent or to limit the nematode infection of the tap roots. In general, symptoms on carrots are similar to those caused by *D. dipsaci* on sugar beet (Dunning, 1957). Moreover, the soil nematode population of *D. dipsaci* during the carrot growing season is rather low even if the carrots are heavily infested. Therefore, visual observation of carrot plants and extraction of nematodes from them must be considered as the most reliable method to diagnose the nematode infection.

The host range study indicates that the Sicilian population of *D. dipsaci* can infest broad bean, carrot, oats, rye, Italian rye, sugar beet and celery. Although environmental conditions (wet soil) were not suitable for germination and growth of small seed plant species, nevertheless the results indicate that the Sicilian population of the nematodes appears adapted to graminaceous (oats, rye, Italian rye) and umbelliferous (carrot, celery, *Ammi majus*) hosts and that onion and broad bean appear as good hosts for many nematode populations occurring in southern Italy. The investigations also indicate that there are no substantial differences between Sicilian and Apulian populations of *D. dipsaci* although the latter did not substantially reproduce on oats (Lamberti and Greco, 1974). Moreover, the very good reproduction of the nematode observed especially on wild oats and Italian rye grass clearly emphasise the role of wild plants in maintaining nematode populations to damaging levels and hence the need for their control.

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