IMPACT OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, ON POTATO DURING TWO DIFFERENT GROWING SEASONS

G. Russo[1], N. Greco[2], F.P. d'Errico[1] and A. Brandonisio[2]

[1] *Dipartimento di Entomologia e Zoologia Agraria, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy*
[2] *Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., Via Amendola 122-D, 70126 Bari, Italy*

Summary. An investigation was conducted in southern Italy to ascertain the effect of the root-knot nematode, *Meloidogyne incognita*, on potato during the autumn and spring growing seasons. The experiments were conducted in microplots containing 25.6 dm^3 soil infested with initial population densities (*Pi*) of 0, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 eggs and juveniles of the nematode/cm^3. Plant height was monitored during the growing seasons and top weights of plants, tuber yields and nematode population densities were recorded at harvest. The effect of the nematode on growth and yield of potato was severe during the autumn season but almost negligible during the spring season. A tolerance limit of potato to *M. incognita* of 1.2 eggs and juveniles/cm^3 soil was only estimated for the autumn season, when a maximum tuber yield loss of 80% occurred at $Pi \geq 128$ eggs and juveniles/cm^3 soil. Plant fresh top weight and plant height had patterns similar to that of tuber yield. The number of tubers was not significantly affected by the nematode but the average weight per tuber was reduced at $Pi \geq 64$ eggs and juveniles/cm^3 soil during the autumn season and at *Pi* of 128 eggs and juveniles/cm^3 soil during the spring season. The post-harvest soil population density of *M. incognita* declined strongly in the autumn season and increased slightly in the spring season, when a maximum reproduction rate of 9.1-fold was observed. At harvest, tubers did not show symptoms of nematode attack. The nematode was not found in the tubers harvested in December but symptoms of nematode attack and many nematodes inside the tubers collected in June became obvious 4-5 months after harvest.

Key words: Nematode dynamics, *Solanum tuberosum*, tolerance limit, yield loss.

In southern Italy and the Mediterranean region, potato (*Solanum tuberosum* ssp. *tuberosum* L.) is an important crop. The mild winter along the coastal area means that potatoes can be grown during different periods of the year. However, to avoid market competition with potatoes planted in inland areas and non-Mediterranean European countries, cultivation of early or extra-seasonal potatoes, for the fresh local and European markets, is preferred. Therefore, potatoes are mainly planted in late summer to be harvested in late autumn, mid-autumn to be harvested in early spring or in mid to late winter to be harvested by late spring.

In Italy, potato cyst nematodes, *Globodera rostochiensis* (Woll.) Behrens and *G. pallida* Stone are known to be widespread and to cause severe yield loss to the winter/spring sown potato crops (Greco *et al.*, 1982; 1993). However, infestations of root-knot nematodes have also been observed on potatoes planted in late August (Marinari Palmisano, 1967; d'Errico, 1984). In December 2003, severe infestations and damage to potato tubers by a population of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were observed in November-December in small areas in the Campania region, on potatoes planted in late August-September. This and the other major root-knot nematodes, *M. arenaria* (Neal) Chitw., *M. hapla* Chitw. and *M. javanica* (Treub) Chitw., are also common in Italy and the Mediterranean region (Lamberti, 1979) and are known to affect potatoes (Lamberti, 1979; Jatala *et al.*, 1982; Brodie *et al.*, 1993; Vovlas *et al.*, 1994; Grammatikaki *et al.*, 1999; Scurrah *et al.*, 2005; Vovlas *et al.*, 2005).

To design an appropriate management strategy, information is required on the extent of yield loss the nematode may cause and on its dynamics. Therefore, two microplot experiments were conducted to ascertain the effects of increasing population densities of *M. incognita* on potato grown during the autumn and spring growing seasons.

MATERIALS AND METHODS

The experiments were conducted at Portici (near Naples), from September 2004 to June 2005. The population of the nematode was from infected potato tubers collected at Acerra (province of Naples) and reared on tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers in a glass-house maintained at 25 ± 3 °C.

First experiment. The nematode population was increased from early July to mid-September 2004. When large egg masses of the nematode had formed, the tomato plants were uprooted and the roots washed in running tap water. Roots were cut into pieces about 0.5 cm long and dipped into a pail of tap water to enable them to be easily mixed. The roots were then separated from the water, excess water was removed by drying between paper tissues, and the roots were weighed. To estimate the nematode population available, four 5-g root samples were processed by the hypochlorite method of Hussey and Barker (1973), the egg and second stage juvenile (J_2) suspension diluted appropriately and the nematodes in three 1-ml aliquots per sample were counted under a dissecting microscope. The roots were then mixed with sterilized sand and used as inoculum. Appropriate amounts of this inoculum (19 kg containing 8,412 eggs and J_2s/g) were added to the soil of each microplot (25.6 dm^3) on 23 September 2004 and thoroughly mixed, along with 10 g of a NPK (12-12-12) fertilizer, using a cement mixer. The population densities of the nematode tested were 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 eggs and J_2s/cm^3 soil. To monitor changes in the nematode population in the absence of a crop, ten microplots were infested with 61.6 eggs and J_2s/cm^3 soil and left fallow. From each of these microplots, a 0.5-kg soil sample was collected at the time of inoculation and 8, 15, 29, 44, 58, 72 and 87 days later and processed by Coolen's method (Coolen, 1979) as modified by Di Vito *et al.* (1985).

Microplots were 30.5 cm diameter, 40 cm long and 3 mm wall thickness grey plastic tubes sunk into the soil up to 35 cm. There were 140 microplots (ten replicates per inoculum level) arranged according to a randomized block design, contiguous along the row and 80 cm apart between rows. The soil used in the experiment (sand 83.8%, silt 13.4%, clay 2.8%, pH 7.7) was from a pasture and had never been cropped to potato before. Of the plant parasitic nematodes found, the soil contained only a few specimens of *Tylenchus* sp. and *Pratylenchus* sp. per 50 cm^3 soil. These nematodes are not considered important pathogens of potato in Italy.

After inoculation, each microplot was planted (23 September, 2004) with a tuber of uniform size of potato cv. Spunta and irrigated. Thereafter, irrigation and weeding was carried out as and when required. Ammonium nitrate (27% N) (2 g/microplot) was applied on 6 November. A solution of copper product was sprayed every 10-15 day on the aerial plant parts, from early November onwards, depending on climatic conditions, to prevent damage by potato late blight [*Phytophthora infestans* (Mont.) De Bary].

The height of potato plants in each microplot was measured weekly from plant emergence (8 October) until 10 December but only that on 4 December is reported as this is the last date on which all shoots were still measurable.

The potatoes were harvested on 23 December, 2004 and tubers and aerial plant parts in each microplot were weighed separately. Tubers were also counted, stored at room temperature until the following summer and, from time to time, checked for the presence of symptoms of nematode attack. A 2-kg soil sample was collected from the top 30 cm of each microplot. Eggs and J_2s of *M. incognita* were then extracted from 500 cm^3 sub-samples using the method of Coolen (1979) as modified by Di Vito *et al.* (1985) and counted.

Air temperatures were also recorded during the growing season.

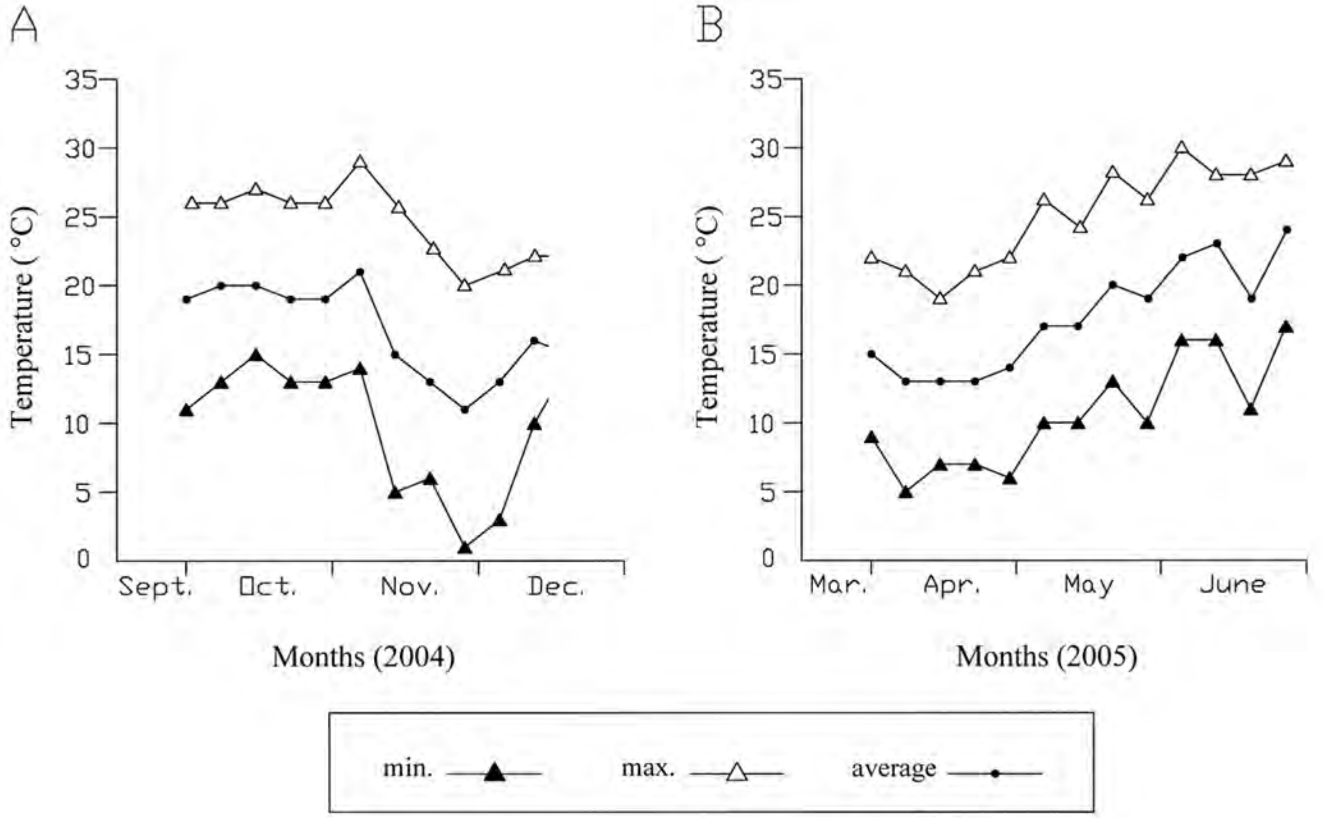

Fig. 1. Mean maximum, mean minimum, and average daily temperatures recorded during the autumn (A) and spring (B) seasons.

Second experiment. The procedure was essentially the same as the previous experiment. The nematode population was increased on tomato from early January to mid-March 2005 and an initial population density treatment of 0.06 egg and J_2/cm^3 soil was added to the experimental design while that of 256 eggs and J_2s/cm^3 was omitted. The nematode population density in the microplots left fallow was of 4.2 eggs and J_2s/cm^3 soil and each nematode level was replicated 8-fold. Potato cv. Spunta was planted on 23 March and harvested on 23 June 2005. Ammonium nitrate was applied at the rate of 2 g/microplot twice during the growing season. Plant height was measured from 11 April to 23 May.

Statistical analysis. The data were subjected to analysis of variance and LSD at $P < 0.05$ was calculated to compare the means.

RESULTS

First experiment. Mean air temperatures (Fig. 1) were in the range 19-21 °C throughout October and then remained in the range 11-16 °C until harvest. Maximum temperatures were 26-29 °C until the first ten days of November and then dropped to 21-23 °C until harvest, while minimum temperatures were in range 13-15 °C until early November, dropped to 1-5 °C in November and rose again to 11-12 °C in December.

Potatoes emerged in about 10 days after planting and their height increased continuously until 4 December. No differences could be observed between plants in the plots infested with 0-8 eggs and J_2s of *M. incognita*/cm^3 soil. At larger population densities, potato growth decreased with the increase of the nematode density and a delay of plant emergence occurred in the plots inoculated with the largest (256 eggs and J_2s/cm^3 soil) population of the nematode. On 4 December, the height of potatoes was significantly reduced in microplots inoculated with $Pi \geq 32$ eggs and J_2s/cm^3 soil and was about half or one third that of the plants in microplots infested with 0-8 nematodes/cm^3 soil at *Pi* of 128 or 256 eggs and J_2s/cm^3 soil, respectively (Table I).

The weight of potato tubers (Table I) was rather similar in the microplots inoculated with 0-1 eggs and J_2s/cm^3 soil but larger (although not significantly) than that of the plots infested with 2-32 nematode eggs and J_2s /cm^3 soil. A significant decrease in tuber yield occurred at $Pi \geq 64$ eggs and J_2s/cm^3 soil, with tuber weights of 37%, 31% and 19% of that at *Pi* 0-1 eggs and J_2s/cm^3 soil in the plots infested with 64, 128 and 256 eggs and J_2s/cm^3 soil, respectively. Because of the variability of the data at $Pi \leq 4$ eggs and J_2s/cm^3 of soil, it was not possible to fit all of the data to Seinhorst's model $y = m + (1\text{-}m)z^{Pi-T}$ (Seinhorst, 1965; 1986). To estimate the tolerance limit of potato to the nematode, the model was fitted only to data of tuber yield obtained at $Pi \geq 8$ eggs and J_2s/cm^3 of soil (Fig. 2). In this model, y is the relative yield (the yield at a given *Pi* divided by the average yield at $Pi \leq T$) and is $y = 1$ at $Pi \leq T$; m is the minimum relative yield (the minimum value of y, usually that at the largest *Pi*); z is a constant with z^{-T} usually = 1.05; *Pi* is the nematode population density and T is the tolerance limit (the value of *Pi* above which yield loss begins to occur). A tolerance limit (T) of potato to *M. incognita* of 1.2 eggs and J_2s/cm^3 soil and a minimum yield (m) of 0.2 at *Pi* 128 eggs and J_2s /cm^3 soil were estimated.

Table I. Effect of increasing soil population densities of *Meloidogyne incognita* at planting (*Pi*) on growth and yield of potato cv. Spunta and on nematode population at harvest (*Pf*). Autumn 2004 crop.

Pi (eggs and juveniles/ cm^3 soil)	Plant height (cm) on 4 December	Fresh plant top weight (g)	Tuber yield per microplot (g)	Tubers per microplot	*Pf* (eggs and juveniles/ cm^3 soil)
0	58.6	226.6	164.4	6.8	
0.125	59.9	248.4	214.2	7.6	0.01
0.25	58.5	207.2	171.6	6.2	0.01
0.5	57.6	161.3	148.6	5.6	0.07
1	55.9	195.8	186.4	7.2	0.04
2	53.0	118.0	113.2	5.1	0.04
4	55.4	158.2	110.0	5.6	0.4
8	57.4	176.5	155.3	6.4	2.9
16	52.7	122.6	112.4	4.6	3.8
32	48.1	134.4	113.7	4.8	9.1
64	39.6	86.1	65.0	5.2	11.5
128	31.7	49.0	54.7	4.8	4.9
256	20.4	36.7	34.4	4.1	2.3
LSD at $P \leq 0.05$	8.74	59.62	62.60	2.34	4.45

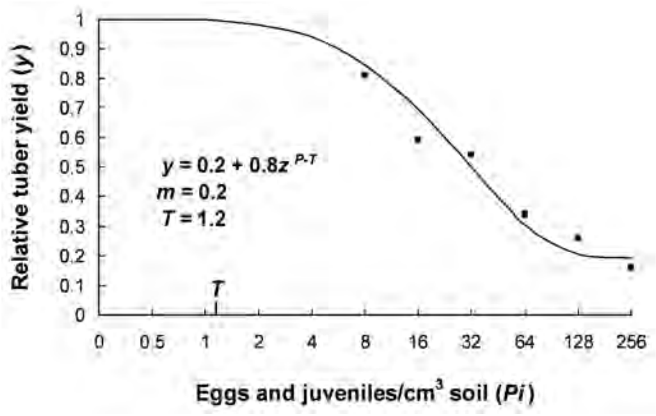

Fig. 2. Relationships between population densities of *Meloidogyne incognita* at planting (*Pi*) and relative yield (*y*) of potato tuber during the autumn 2004 growing season.

At harvest, the fresh weight of the plant tops did not differ in the microplots with *Pi* 0-8 eggs and J_2s/cm^3 soil, while a decline was observed at larger *Pi*s, which, however, became significant only at ≥ 64 eggs and J_2s/cm^3 soil. In general, the effect of the nematode was little on the number of tubers per microplot (Table I), which was in the range 5.1-7.6 at *Pi* 0-8 eggs and J_2s/cm^3 soil and declined slightly (4.1-5.2) at larger *Pi*. Tuber size remained fairly constant at *Pi* 0-32 eggs and J_2s/cm^3 soil (22-28 g per tuber), but dropped to 8-12 g per tuber at larger *Pi*.

The nematode population in the microplots left fallow had declined slightly after one week (16%), markedly after two weeks (76%) and had nearly disappeared by December. A general and strong decline of the nematode population was observed at harvest in the microplots planted to potato (Table I), with *Pf/Pi* ratios in the range 0.01-0.36. At harvest, heavily galled roots were observed, especially at *Pi* ≥64 eggs and J_2s/cm^3 soil, but egg masses of the nematodes were few and small. Observation of the tubers did not reveal the presence of any swellings or blisters nor of the nematodes inside them, even in tubers produced in the microplots with the largest *Pi*. Moreover, no surface symptom was found during the periodical observation of these tubers stored at room-temperature until the next summer.

Second experiment. In the spring experiment, the temperature (Fig. 1) increased progressively, averaging 13-17 °C until the end of April, 17-22 °C in May and 19-24 °C in June. In the same periods, maximum temperatures were 19-22 °C, 24-30 °C and 28-29 °C and minimum temperatures were 5-10 °C, 10-16 °C and 11-17 °C, respectively.

Potatoes emerged in two weeks and increased in height until the end of May, but no clear difference could be observed between treatments throughout the growing season. However, although no clear difference in fresh top weight at harvest of potatoes could be seen in microplots at *Pi* of 0-32 eggs and J_2s/cm^3 soil, a significant 25-27% suppression of growth was observed at *Pi* 64-128 eggs and J_2s/cm^3 soil when compared with the general average of the top weights at *Pi* of 0-32 eggs and J_2s/cm^3 soil (Table II).

No effect of the nematode population densities was observed on potato tuber yield although a slight decrease (11-16%) was observed at *Pi* ≥ 64 eggs and J_2s/cm^3 soil if compared with the general average at all smaller values of *Pi*. The number of potato tubers (13.1-17) per microplot and weight per tuber (57-82 g), al-

Table II. Effect of increasing soil population densities of *M. incognita* at planting (*Pi*) on growth and yield of potato cv. Spunta and on nematode population at harvest (*Pf*). Spring 2005 crop.

Pi (eggs and juveniles/ cm^3 soil)	Plant height (cm) on 23 May	Fresh plant top weight (g)	Tuber yield per microplot (g)	Tubers per microplot	*Pf* (eggs and juveniles/ cm^3 soil)
0	74.9	490.4	815	14.3	
0.062	68.2	425.6	1100	14.6	0.4
0.125	66.6	484.2	1200	16.1	0.7
0.25	69.0	367.5	1172	14.3	1.0
0.5	65.9	377.6	998	13.1	1.4
1	62.5	387.6	1157	16.1	1.4
2	65.2	402.4	1168	15.6	5.2
4	68.5	395.0	1180	14.4	7.2
8	62.7	389.4	1149	16.6	15.5
16	66.9	392.5	1089	16.6	57.5
32	68.1	365.5	1109	17.0	291.3
64	63.1	304.5	986	14.3	375.7
128	69.4	297.4	933	20.3	671.1
LSD at P ≤ 0.05	7.73	129.13	231.34	4.35	103.54

though variable, were basically similar at *Pi* 0-64 eggs and J_2s/cm^3 soil but a significant increase in their number (20.3) and reduction of the weight per tuber occurred at *Pi* 128 eggs and J_2s/cm^3 soil.

In the microplots left fallow, the population density of the nematode declined rapidly during the first (52%) and second (72%) weeks after planting, declined slightly until the end of May and had nearly disappeared by harvest (23 June). In the infested microplots, the population density of the nematode increased, reaching a maximum of 671 eggs and J_2s/cm^3 soil at *Pi* 128 eggs and J_2s/cm^3 soil. The reproduction rate of the nematode decreased from 6.7 to 1.4 with the increase of *Pi* from 0 to 1 egg or J_2/cm^3 soil and was rather variable at larger values of *Pi*, with a maximum value of 9.1. At harvest, potato roots showed clear galling and the severity appeared to increase with the increase of *Pi*. However, most of the galls were small and devoid of or with only small egg masses. Observation of the tubers did not reveal any symptom of nematode attack at harvest but blisters were observed the following October-November (Fig. 3A) on the same tubers stored at room temperature. Transverse sections of these stored tubers revealed several nematode specimens at different stage of development and egg masses (Fig. 3B).

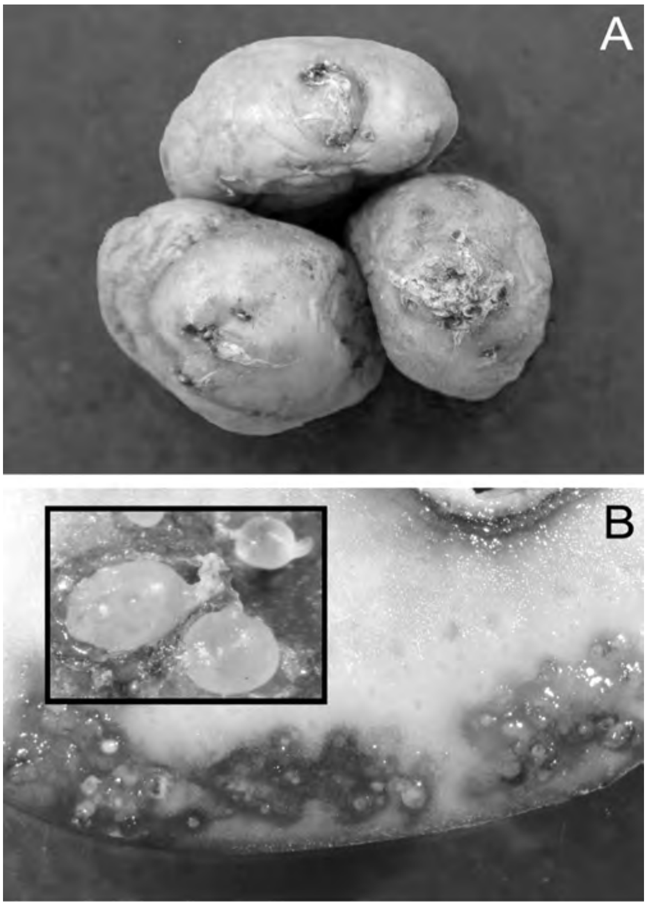

Fig. 3. Small potato tubers collected in June 2005 and observed the following October-November. A: Entire tubers with blisters caused by *M. incognita* attack; B: sections of the same tubers showing necrotic areas and clusters of females, with an enlargement in the insert, that seems to have originated from a single female.

DISCUSSION

Root-knot nematodes, *Meloidogyne* spp., are the most damaging plant parasitic nematodes worldwide. *Meloidogyne hapla* and *M. chitwoodi* Golden, O'Bannon, Santo *et* Finley are severe parasites of potato in Europe and North America (Brodie *et al.*, 1993; Scurrah *et al.*, 2005) and *M. fallax* Karssen in a few countries in Europe (Waeyenberge and Moens, 2001). Also, the warm season species *M. incognita*, *M. javanica* and *M. arenaria* damage potato in tropical and sub-tropical areas (Brodie *et al.*, 1993; Scurrah *et al.*, 2005). Damage by these species of root-knot nematodes has also been reported in the Mediterranean area (Lamberti, 1979; Vovlas *et al.*, 2005). In southern Italy, damage to potato (poor plant growth and severe root galling) was observed in early autumn but symptoms of nematode infection were never observed on the tubers even though they were found to be infected. The observation of damaged potato tubers in late autumn-early winter in the Campania region of southern Italy was thought to be an indication of the aggressiveness to potato and tolerance to rather cool temperature of the nematode population present in that area. In potatoes planted in September, we observed reduction of plant growth and yield but no symptoms of nematode attack were seen on the tubers soon after harvest nor even by the next summer on stored tubers. This would indicate that the damage to tubers observed earlier in the field is not common and almost certainly was due to an unusually warm autumn. In potatoes planted in March, a small reduction of plant growth and yield occurred only at the largest inoculum level, but symptoms of nematode attack on the tubers, although absent at harvest, became obvious by the next autumn on the stored tubers. However, early potatoes produced in southern Italy are marketed immediately and, therefore, their commercial acceptability is not reduced. As expected, the yield of potatoes of the March-June season was much greater than that of potatoes grown in the September-December season.

The tolerance limit of potato to *M. incognita* estimated in our study (1.2 eggs and juveniles/cm^3 of soil) is larger than that of 50 eggs/250 cm^3 soil for *M. hapla* (Brodie *et al.*, 1993) and 0.50-0.64 eggs and J_2s/cm^3 soil for *M. javanica* in pot experiments in the glasshouse (Vovlas *et al.*, 2005). Santo and O'Bannon (1981) reported the pathogenicity of *M. chitwoodi* and *M. hapla* on potato under controlled conditions. However, Santo *et al.* (1981) investigated the effect in the field of 10, 100 and 250 eggs/250 cm^3 soil of both nematodes on potato cv. Russet Burbank, in Washington State (USA), and concluded that a threshold level may occur for *M. hapla* but not for *M. chitwoodi*. All this suggests that the tolerance limit can be greatly affected by environmental conditions, potato cultivar and nematode species.

The dynamics of the nematode were probably affected by the different temperatures during the two cropping seasons. In the September planting, the nematodes invaded potato roots and induced the formation of galls. However, by the time the tubers had begun to form, the temperature had dropped to a level suppressing nematode infection, thus allowing the tubers to escape invasion. Moreover, the nematode could not complete a single generation and, therefore, its soil population density fell much below that at planting. On the spring crop, although the nematodes invaded the potato roots at a relatively late growth stage, a small proportion were able to complete their life cycles, thus slightly increasing the soil population density. Also, by the time the tubers started to form the temperature had increased, so they became infected and allowed the nematode to continue developing after harvest and to cause tuber swellings, as observed by Jatala *et al.* (1982). This indicates that, as for the potato cyst nematodes *G. rostochiensis* and *G. pallida*, the use of potato tubers harvested in late spring for planting in summer is risky as it can spread the nematode.

Finally, we feel that while there is very little to no chance for *M. incognita* to damage spring potatoes in southern Italy, and probably in all the Mediterranean basin, summer-sown potatoes can be severely damaged if planting is in mid-August to early September, when soil temperature is conducive to nematode infection and damage.

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