Investigation on the biology of *Meloidogyne artiellia*

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Summary

Investigations were undertaken in Italy to study the biology of *Meloidogyne artiellia.* The development of the nematode was investigated in small pots in growing chambers set at 10, 15, 20, 25, or 30° and in microplots sown with chickpea. The decline of the nematode after harvest of chickpea was studied in microplots under rainfall conditions, irrigated only once on 17 June 1985, or every two weeks. Hatch of eggs from egg masses collected all over spring and incubated in distilled water at 25° and the ability of second stage juveniles to undergo anhydrobiosis, was also investigated. Second stage juveniles invaded chickpea roots at all tested temperature, but development was very slow at 10 and 30°, the most favorable temperatures being in the range 20-25°, at which females developped after an accumulation of 230-240 day degrees above 10°. Out doors females with egg masses appeared in April. New eggs hatched readily, but juveniles did not invade chickpea roots from late April onwards. After harvest of chickpea the nematode population declined to 55-59 % at the end of July, to 13 % by mid November and to 3 % after 15 months. Most of the soil population in summer was of second stage juveniles which survived as coiled anhydrobiotic specimens.

Résumé

Recherches sur la biologie de Meloidogyne artiellia

Des recherches ont été conduites pour connaître la biologie de *Meloidogyne artiellia.* Le développement du nématode a été étudié en utilisant des pots disposés dans des cellules de croissance, à 10, 15, 20, 25 et 30°, et en microparcelles ensemencées avec des pois chiches. La diminution de la population du nématode après la récolte du pois chiche a été mesurée dans des microparcelles sans irrigation, irriguées une seule fois, le 17 juin, ou irriguées toutes les deux semaines. L'éclosion des œufs à partir de masses d'œufs prélevées au printemps et incubées dans de l'eau distillée à 25°, ainsi que la capacité des larves de deuxième stade à survivre en anhydrobiose ont été également mesurées. Les larves de deuxième stade pénètrent dans les racines du pois chiche à toutes les températures testées, mais le développement du nématode reste lent à 10 et 30°. Aux températures les plus favorables de 20 à 25°, les femelles se développent après une sommation de 230-240 degrés-jour, au-dessus de 10°. Dans les microparcelles, les femelles avec masses d'œufs se développent en avril. Les nouveaux œufs éclosent rapidement, mais les larves de deuxième stade ne pénètrent pas dans les racines du pois chiche après le 9 avril et jusqu'à la fin de la culture. Après la récolte du pois chiche, la densité de la population du nématode se réduit à 55-59 % fin juillet, 13 % à mi-novembre et 3 % après 15 mois. La plus grande partie de la population du nématode dans le sol est composée de larves de deuxième stade en anhydrobiose.

Meloidogyne artiellia Franklin was first reported from oats in England (Franklin, 1961). Later this nematode was found to be the causal agent of severe yield losses of wheat in Greece (Kyrou, 1969) and, especially of chickpea, *Cicer arietinum* L., in Spain (Tobar-Jimenez, 1973), Italy (Greco, 1984), and Syria (Mamluk, Augustin & Bellar, 1983; Greco *et al.*, 1984) where 12 % of stunted chickpeas were infested with the nematode. Investigations on the host range of this root-knot nematode (Di Vito, Greco & Zaccheo, 1985) indicated that, with a few exceptions, it reproduces well on cereals, cruciferae, and leguminosae. Many of these crops are widely cultivated in the mediterranean basin and severe damage can be expected in infested fields. However, the only information on the biology of *M. artiellia* is that of Ekanayake and Di Vito (1985) who found that eggs readily hatched in the range 15-25°, but less frequently at 10 and at 30°, and that there was no diapause and no cool pre-treatment was required. Because of the paucity of information on the biology of *M. artiellia,* experiments were undertaken in Italy to investigate : *i)* the effect of temperature on development; *ii)* the development within the roots of field grown chickpea; *iii)* the population decline in the soil; *iv)* the hatching of eggs collected in different periods of the year; and *v)* the ability of the nematode to undergo anhydrobiosis during summer months.

Materials and methods

Effect of temperature on development

Three hundred plastic pots each containing 170 cm^3 of sandy soil infested with 2 000 second stage juveniles of *M. artiellia* were sown on 5 November 1985 with two pregerminated chickpea cv. Ghab 1 seeds per pot. The pots were then equally divided among temperature-controlled cabinets at 10, 15, 20, 25 or 30 ± 1° in which they were left to grow for 66 days. Four pots were randomly selected from each cabinet after 1, 2, 4, 6, 8, 10, 13, 16, 20 days, then at weekly intervals for a further 35 days and, finally, 11 days later. The roots of each plant were washed free of adhering soil and the nematodes were extracted by Coolen's method (1979) and counted.

DEVELOPMENT WITHIN THE ROOTS OF FIELD GROWN CHICKPEA

Twenty microplots made of concrete bottomless tiles (cm 30 × 30 × 50) were filled with 40 dm^3 soil treated four months earlier with 500 l/ha of a fumigant containing 80 % DD + 20 % methylisothiocyanate to which was added sufficient soil infested with an Italian population of *M. artiellia* (reared on chickpea cv. Ghab 1 in a greenhouse at 18-25°) to give a population density of six eggs/cm^3 soil. Each microplot was sown with nine seeds of chickpea cv Ghab 1 on 14 December 1984. Four whole chickpea roots were collected every ten days from February to April 1985 and then weekly until the plants died in June. The roots were washed free of soil and the nematodes were extracted and counted, as described before. The occurrence of egg masses was also recorded and the eggs per egg masses estimated by dissolving the gelatinous matrix in a 1 % sodium hypochlorite solution (Hussey & Barker, 1973). Soil temperature at 20 cm deep and soil moisture content of the microplots were recorded.

EGG HATCH

The aim of this study was to ascertain whether increase of soil temperature and reduction of soil moisture, which usually occur in the mediterranean basin from mid spring, affect egg hatch. Egg masses were detached from the roots at weekly intervals from 19 April 1985 until 3 June 1985. Five batches of 25 each, were incubated (Greco, Brandonisio & De Marinis, 1982) in distilled water at 25° for three weeks. The water was changed weekly when counts of emerging second stage juveniles were made. At the end of the hatching test the egg masses were dissolved as described above to estimate numbers of unhatched eggs and thereby calculate percentage hatch.

POPULATION DECLINE IN THE SOIL

The summer months are usually dry in mediterranean countries, although with occasional local storms. To investigate the population changes occurring in the absence of host crops, soil samples were collected from the microplots every two weeks from 17 June 1985 until 18 November 1985 and then bimonthly until 22 September 1986. Twenty cores of soil were taken from each microplot with a probe (1.5 cm diam and 30 cm long). Three irrigation regimes were used : two microplots were irrigated only on 17 June 1985 immediately after the initial soil sampling; two were irrigated every two weeks; and two were not irrigated. During the experiment natural rainfall occurred in mid-August. Nematodes were extracted from two 500 cm^3/soil sub-samples per microplot by Coolens's modified method (Di Vito, Greco & Carella, 1985). Eggs and second stage juveniles were counted separately.

ANHYDROBIOSIS

To investigate whether *M. artiellia* undergoes anhydrobiosis during the dry and hot summer period, soil samples were taken when the soil was dry (moisture content 7,5 % = pF 5) and second stage juveniles extracted by centrifugation in a 1.25 M sugar solution, equivalent to 1.165 sp. gr., (Freckman, Kaplan & Van Gundy, 1977) or in a magnesium sulphate solution of the same specific gravity. Four soil samples (100 g each) were mixed with 300 ml of either tested solutions, centrifugated at 2 300 revs/mn for 5 mn and the supernatant sieved through a 5 µm sieve to reduce the volume and numbers of coiled anhydrobiotic juveniles were counted.

Four more sub-samples each chemical solution were processed as above, but the extracting solutions were soon washed away after centrifugation by sieving through a 5 µm sieve and collecting nematodes in water. Nematodes were gathered in beakers, counted and then poured on sives of 45 µm. The sieves were then placed in Petri dishes with water to the level of the sieves and placed in a cabinet at 15°. The numbers of active juveniles that moved through the sieves into the water below were counted at 2, 6, 24, 48, 72 and 96 hours.

Results

EFFECT OF TEMPERATURE ON DEVELOPMENT

Second stage juveniles of *M. artiellia* invaded chickpea roots at all temperatures tested. However, the time taken to invade the roots decreased, with increase in temperature, to a minimum of two days at 20-25°, and then increased (Fig. 1).

More nematodes were recovered from the roots at 15-25° (450-602) (Fig. 2) than at 10° (318) or 30° (64). Also, at 30° the nematodes took longer to invade the roots than at 15-25° (10 days after planting), suggesting that the higher temperature is not suitable for the development of *M. artiellia.*

Further development of the nematode also was clearly affected by temperature. At 10°, third stage juveniles occurred 35 days after root invasion, fourth stage females and males at 42 and 53 days respectively, after root invasion, and adults were not seen by the end of the

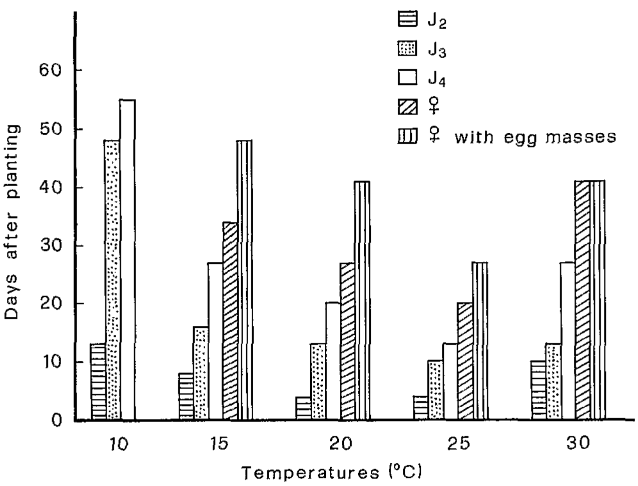

Fig. 1. Days after planting chickpea required by *Meloidogyne artiellia* to develop different life cycle stages at various temperatures.

experiment. Thus, 10° is about the basal threshold temperature for the development of *M. artiellia.* At 15-25°, third stage, fourth stage, and adults usually occurred 3-5, 10-12, and 14-18 days respectively after root invasion. At 30° only eight females were found 33 days after root invasion. Females started to produce egg masses 14, 14, and 7 days after they appeared, at 15, 20, and 25°, respectively (Fig. 1). At 30° a few eggs (3-4/female) were found when females were first observed.

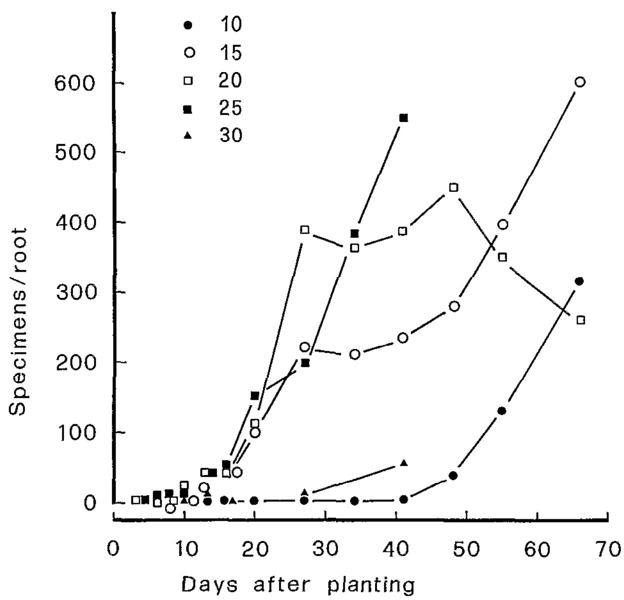

Fig. 2. Total numbers of *Meloidogyne artiellia* specimens recovered from roots of chickpea grown at different temperatures.

Based on these results, the accumulated day degrees, above 10°, required by *M. artiellia* to develop adults are 130 at 15°, 230 at 20°, 240 at 25°, and 620 at 30°.

Development within the roots of field grown chickpea

Chickpeas emerged by the end of January. At this time soil moisture was not a limiting factor for the development of *M. artiellia,* but soil temperature at 20 cm depth was below 10° until the end of February, and only in April rose over 15°. However, in the top 10 cm of soil temperatures could occasionally have been higher because of radiation from the sun. This is probably the reason for the appearance of both second and third stage juveniles inside chickpea roots on 20 February (Fig. 3). Fourth stage females appeared by early March (1 %), and adult females (18 %), fourth stage (12 %) and adult males (5 %) were present on 25 March. Females started to lay eggs by 9 April, but only in substantial numbers from 19 April (427 eggs/egg mass), after which the egg content of the egg masses increased continuously to 600 by mid May and 1 043 at the end of the experiment. From 9 April until the chickpeas matured most of the specimens were adults, and second stage juveniles were not found within the chickpea roots until harvest. Therefore it is concluded that *M. artiellia* usually completes one generation during the growing season of the chickpea crop.

Egg hatch

Egg hatch occurred from all egg masses irrespective of the time they were collected (Fig. 4), confirming that there is no diapause and no cool pre-treatment is required. However, the percent eggs hatched during the first week of incubation was only 3 % from egg masses collected on 19 April, but increased with the age of egg masses to 92 % on 3 June. Most of the egg hatched, however, during the first two weeks, and only a few juveniles (2-10 %) emerged during the third week. Total

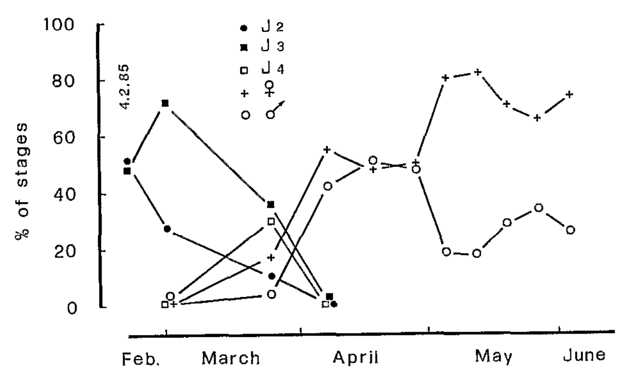

Fig. 3. Per cent of *Meloidogyne artiellia* life cycle stages recovered from roots of chickpea grown in microplots in 1985.

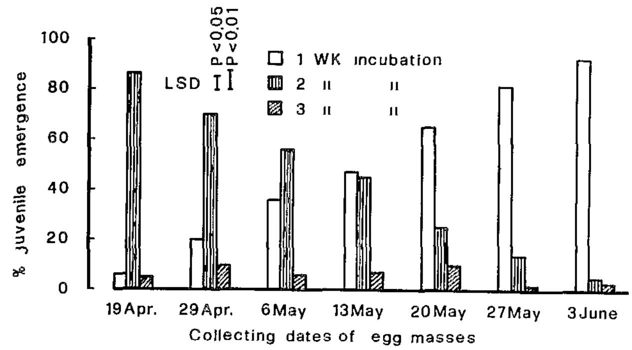

Fig. 4. Per cent of *Meloidogyne artiellia* second stage juveniles emerged from egg masses collected periodically from 19 April to 6 June 1985 and incubated in distilled water at 25°, for three weeks.

egg hatch varied between 96.4 % for the egg masses collected on 19 April 1985 and 99.4 % for those collected on 3 June 1985.

POPULATION DECLINE IN THE SOIL

After harvest of the chickpea soil populations of *M. artiellia* declined rapidily until mid August and then more slowly in all microplots. During the summer months, population densities were similar in the irrigated microplots and only slightly higher in those not irrigated but exposed to natural rainfall. Population declined to 55-59 % at the end of July and to about 13 % by mid November when most of the winter crops would usually be sown. In September 1986 the soil population was only 3 % of that in June 1985. On 17 June 1985, the population of *M. artiellia* was composed of 46, 57 and 78 % of second stage juveniles in the microplots under rainfall, irrigated every two weeks or only on 17 June, respectively (Fig. 5). Thereafter because of egg hatch,

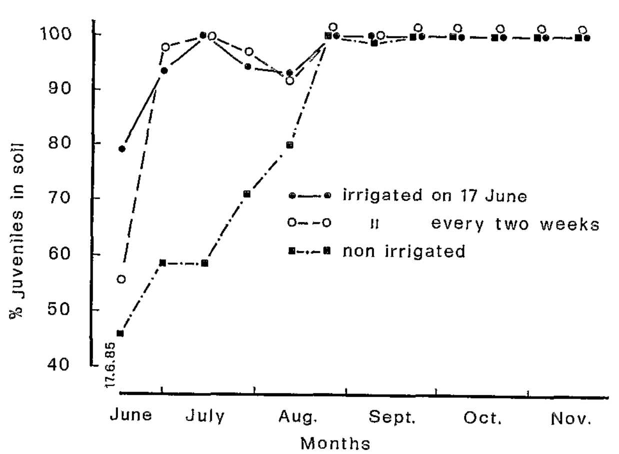

Fig. 5. Per cent of *Meloidogyne artiellia* juveniles on the total population in the soil from microplots kept fallow, rainfed, irrigated only an 17 June or every two weeks.

the percentage of juveniles in the soil population increased more rapidily in the irrigated microplots than in those left under rainfall conditions. A heavy rain occurred in early August, and stimulated egg hatch. Therefore, from the end of August 1985, the soil population of *M. artiellia* consisted only of second stage juveniles in all microplots.

ANHYDROBIOSIS

Extraction of *M. artiellia* from the soil with sugar or magnesium sulphate solutions revealed the presence of coiled second stage juveniles indicating anhydrobiosis. The percent coiled juveniles was 39 % when extracted with 1.25 M sugar solution and significantly ($P \leqslant 0.05$) higher (64 %) with magnesium sulphate solution, suggesting that the latter can successfully be used for the extraction of anhydrobiotic nematodes. Anhydrobiotic second stage juveniles soon recovered when the extracting solutions were washed away : some juveniles became active after two hours, 60-67 % were active after 24 hours with totals of 83-86 % after 96 hours. There was no significant difference between the two solutions in the percentage of second stage juveniles which became motile.

Discussion

Meloidogyne artiellia seems less temperature dependent than *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood, but similar in its temperature-related development to *M. hapla* Chitwood, *M. chitwoodi* Golden, O'Bannon, Santo & Finley, and *M. naasi* Franklin (Van Gundy, 1985). The estimated basal temperature for development is also lower than that of the first group of root-knot nematodes and similar to that assumed for *Heterodera schachtii* Schmidt (Greco, Brandonisio & De Marinis, 1982), or reported for *H. ciceri* Vovlas, Greco & Di Vito (Kaloshian, 1986) and *H. carotae* Jones (Greco & Brandonisio, 1987). Accumulated day degrees required by *M. artiellia* to develop adult females, were nearly the same (230-240) at 20-25°, which were the most favourable temperatures, but they were variable when temperatures were not optimal. This phenomena was also observed with *H. schachtii* (Thomason & Fife, 1962). At 30°, the nematode required more day degrees to develop females because under stress and probably many juveniles which invaded the roots did not develop further. Unlike *M. naasi*, which shares with *M. artiellia* cereals as good hosts, no diapause occurred and no cool pre-treatment was necessary for egg hatch. More than 96 % of eggs hatched in three weeks and many second stage juveniles emerged from the eggs as soon as embryogenic development was completed. However, although these juveniles could invade host roots if soil temperature and soil moisture content were favorable, under field condition chickpea roots might be unsuitable for the nematode. This could explain why no root invasion occurred from late April onwards. Therefore, only one generation would be completed by the nematode during a growing season of chickpea, even though root invasion occurs in winter and egg masses are formed by early April.

After harvest of the host crops, the soil population of *M. artiellia* declined rapidly during the summer, and then slowly, and can persist for more than one year in the soil. Since many eggs may hatch before harvest of the host plant, survival during summer is dependent on anhydrobiotic second stage juveniles. Anhydrobiosis has been reported to be common for some nematode genera (Demeure, Freckman & Van Gundy, 1979*a, b)*, but among root-knot nematodes it has been observed only in *M. javanica* (Towson & Apt, 1983). In the mediterranean area anhydrobiosis has also been reported for *Pratylenchus thornei* Sher & Allen (Glazer & Orion, 1983), and probably is the normal way experienced by many nematodes, to survive dry and hot months in the area.

Winter is rather cool in many semiarid areas of the mediterranean basin in which snow and frost are rather frequent in inland areas. *Meloidogyne artiellia* does not seems to suffer from cold stress as severe damage to crops is experienced in inland area of Syria. Storing in water suspension eggs and juveniles of *M. artiellia* obtained by the sodium hypochlorite method (Hussey & Barker, 1973), and refrigerated at 10°, showed that 35 % and 7 % of the juveniles were still motile after 13 and 26 months later, respectively.

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