OBSERVATIONS ON THE HATCHING OF EGGS LAID BY FEMALES OF DIFFERENT AGE OF THE ROOT-KNOT NEMATODES *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*

E.A. Tzortzakakis[1]

Nematology Lab, Plant Protection Institute, N. AG. RE. F., Hellenic Agricultural Organization – DEMETER, PO BOX 2228, Heraklion, 71003, Crete, Greece

Received: *5 January 2012*; *Accepted*: *10 April 2012*.

Summary. An experiment was undertaken to investigate the hatching pattern of eggs laid within 48 h by single females of different age of the root-knot nematodes *Meloidogyne incognita* and *M. javanica*. Three groups of females, young, middle aged and old were used. To obtain the eggs, root pieces of tomato containing a single female were used. The females were deprived of their egg masses and the root pieces transferred to small sieves immersed in water, inside watch glasses, and incubated in the dark at 25 °C for 48 h for the females to lay new eggs. The batches of new eggs were incubated at 25 °C and the hatching test continued for 24 days. No more eggs hatched during a further 14-day incubation period. Between 93 and 98% of the eggs hatched and there was no indication that a high percentage of eggs laid by old females were in diapause.

Keywords: Diapause, egg laying, hatching pattern.

Several factors have been investigated to obtain insights on the diapause (arrested development of eggs) in root-knot nematodes (*Meloidogyne* spp.). In a study dated more than 30 years ago (de Guiran, 1980), it is stated that eggs in diapause appeared at the very beginning of egg-laying, that their percentage increased with the age of the mother and was the largest proportion of the eggs laid after ten weeks. In that work, intact egg masses containing eggs of different age laid during the life span of the nematode female were used. However, it is not known whether freshly laid eggs from old females have more of an arrested development compared to those laid by young females. The aim of this work is to compare the percentage of hatching of eggs newly laid by females of different age, excluding the eggs which had been deposited earlier in the gelatinous matrix.

Egg masses from single female lines of *M. incognita* and *M. javanica*, reared in potted tomatoes, were removed, put on a sieve in a watch glass and incubated at 25 °C for two days to obtain juveniles. Thereafter, the same egg masses were kept in a fridge (at 5 °C) to arrest egg development and incubated again as before 20 and 40 days later, to obtain new batches of juveniles required for the inoculation of tomato plants. This was necessary to obtain, at the same time of plant uprooting, nematode females of different age. Therefore, tomato seedlings of similar size were transplanted into 300 ml pots filled with sterilized commercial compost soil at c. 20 day intervals and each time inoculated with the juveniles coming from the same extraction sieve. After inoculation, all of the tomatoes were reared in a growth room, at 24-26 °C and 16 h photoperiod, and uprooted the same day, so as to obtain females of the same nematode population but of different ages. Previous work had indicated that the egg laying of *M. javanica* reared at 26 °C commenced between 18 and 21 days from inoculation of the juveniles and continued for at least a further 40 days (Tzortzakakis and Trudgill, 1996). Based on these results, we considered as young, middle aged and old, the females which were removed from the roots 20, 40 and 60 days after juvenile inoculation.

After uprooting, the roots of the inoculated plants were gently washed and cut into small segments containing single galls with one female in each. To ensure that, in the plants uprooted 60 days after inoculation, the females were from the first generation of the nematode and not young ones from the second generation, only transparent females with large egg masses were selected for the study.

The egg mass was removed from each female with a fine needle and the root tissue was carefully pared away to expose the posterior end of the female, leaving the remaining body embedded inside the root tissue. The root pieces were then transferred to small Petri dishes containing a thin layer of 1% water agar. The Petri dishes were incubated in the dark in a box at 25 °C. After 48 h, the females were removed leaving a batch of newly laid eggs, ranging from 5 to 50 per female, on the agar surface (Tzortzakakis and Trudgill, 1996).

These groups represented eggs laid by females at the beginning, the middle and the end of the egg laying period. The new eggs laid on the water agar by females of ages 20, 40 and 60 days were incubated at 25 °C. The Petri dishes were examined daily, till the appearance of the first juvenile, to estimate the minimum time required for embryogenesis, which was similar to that de-

[1] Corresponding author: etzortza@her.forthnet.gr

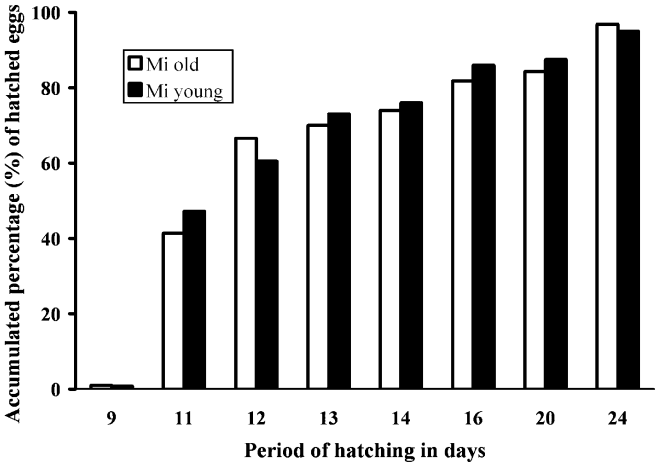

Fig. 1. Accumulated percentage of emerged juveniles, during an incubation period of 24 days, from eggs laid by old (55 days) and young (20 days) females of *Meloidogyne incognita* on sieves immersed in watch glasses with water. The total number of eggs (accumulated percentage of hatching in parentheses) was: *Mi* old = 192 (96.9%) and *Mi* young = 123 (95.6%).

scribed for both nematode species (Tzortzakakis and Trudgill, 2005).

The first juvenile of *M. incognita* appeared one day before that of *M. javanica* and hatching started the same day, independently of the origin of the eggs (laid by young, middle aged and old females). Attempts to estimate the whole hatching period in these Petri dishes failed, as many juveniles penetrated the agar and were not easily visible and, also, saprophytic fungi usually grew in the dishes and made observation difficult.

For that reason, another experiment was set up in which we used small extraction dishes, aiming to estimate the length of the hatching period and the number of juveniles emerging from eggs laid by females 20 (for both tests), 40 (for 2nd test), 55 (for 1st test) and 60 (for 2nd test) days old. These extraction dishes contained sieves of 1-cm-diameter and were made of 0.5 cm high rings cut from a disposable plastic syringe with a 38 μm aperture nylon mesh welded onto the bottom (Antoniou, 1986). Each sieve was put in a 2-cm-diameter watch glass filled with sterile distilled water. The watch glasses were placed in a 5-cm-diameter plastic Petri dish and covered with the lid to reduce evaporation. Several root pieces with individual females of the same age were put on the mesh of the same sieve and incubated for 48 h at 25 °C, when the females were removed. The number of eggs laid on the mesh by these females could not be estimated accurately, as they were deposited in small individual batches and attempts to disperse them could have damaged them. The dishes in the incubator were removed at given dates to estimate hatching. For each count, the contents of the watch glass were rinsed into a counting dish, filled again with water and the sieve returned to the incubator. The dishes remained in the incubator for a further two weeks, when no more juveniles were observed. At the end of the hatching period, the watch glasses were checked for presence of juveniles and the unhatched eggs remaining on the mesh counted. Then, the total number of eggs initially deposited on the mesh was estimated by summing the number of emerged juveniles and the unhatched eggs. Finally, the percentage of eggs hatched at each observation date was calculated. Two hatching tests were performed in the watch glasses as described and these data are presented in the figures.

In the first test, groups of *M. incognita* females 20 (young) and 55 (old) days old, were left to lay eggs for 48 h on the sieves and the hatching pattern recorded. After a 24 days period no further emergence of juveniles was observed (Fig. 1). The watch glasses were inspected for a further 14 days and percentages of 3% of eggs of old *M. incognita* females and 5% of young *M. incognita* females remained unhatched.

In the second test, females 20 (young), 40 (middle aged) and 60 (old) days old from *M. incognita* and *M. javanica* laid eggs as described before. The hatching pattern was similar for both nematode species and up to the 15th day eggs laid by the young females hatched earlier (Fig. 2). No more juveniles emerged after 21 days. The watch glasses were inspected for a further 14 days and only 2-7% of the eggs of both species, independently of the female age, remained unhatched.

In the work described here, the egg laying of *M. incognita* and *M. javanica* in three different periods of their life span (beginning, middle and end of the egg laying period) was simulated and the hatching pattern of eggs produced at each stage was studied. The results

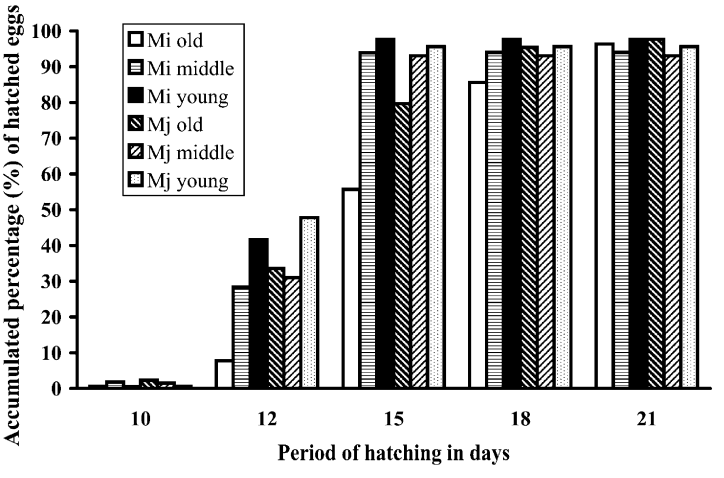

Fig. 2. Accumulated percentage of emerged juveniles, during an incubation period of 21 days, from eggs laid by old (60 days), middle aged (40 days) and young (20 days) females of *M. incognita* (*Mi*) and *M. javanica* (*Mj*), on sieves immersed in watch glasses with water. The total number of eggs (accumulated percentage of hatching in parentheses) was: *Mi* old = 167 (96.4%), *Mi* middle aged = 169 (94%), *Mi* young = 214 (97.7%), *Mj* old = 217 (97.7%), *Mj* middle aged = 87 (93.1%) and *Mj* young = 161 (95.6%).

were variable at the beginning of hatching but at the end of the hatching period, which spanned 21 to 24 days, similar percentages of unhatched eggs were recorded independently of the female age. Although statistical analysis could not be used, due to lack of replicates, the percentages of unhatched eggs differed by up to 5% between young, middle aged and old females. Eggs had been laid in batches and were loosely stacked together, probably due to traces of gelatinous matrix excreted from the vulva. That differs from what happens in natural conditions, where all laid eggs are well protected inside a well formed gelatinous matrix. It is concluded that the young, middle aged and old females lay eggs with similar and low percentages of eggs in diapause. The increased percentage of eggs in diapause laid by old females observed by de Guiran (1980) is probably due to their retention inside the gelatinous matrix of the egg mass and subsequent long exposure to external factors. External stresses, such as soil drying and saturation and temperature dropping, may induce diapause in eggs (de Guiran, 1980; Huang and Pereira, 1994).

LITERATURE CITED

Antoniou M., 1986. Simple plastic sieves, and hair loops, for handling nematodes, cysts, egg masses and eggs. *Nematologia Mediterranea*, *14*: 161-162.

De Guiran G., 1980. Factors induisant chez *Meloidogyne incognita* un bloccage du développement des œufs considéré comme une diapause. *Revue de Nématologie*, *3*: 61-69.

Huang S.P. and Pereira A.C., 1994. Influence of inoculum density, host and low-temperature period on delayed hatch of *Meloidogyne javanica* eggs. *Journal of Nematology, 26*: 72-75.

Tzortzakakis E.A. and Trudgill D.L., 1996. A thermal time based method for determining the fecundity of *Meloidogyne javanica* in relation to modelling its population dynamics. *Nematologica*, *42*: 347-353.

Tzortzakakis E.A. and Trudgill D.L., 2005. A comparative study of the thermal time requirements for embryogenesis in *Meloidogyne javanica* and *M. incognita*. *Nematology*, *7*: 313-315.