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Egg laying and development of *Hexamermis serenensis* (Nematoda; Mermithidae) under laboratory conditions

Pedro HERNÁNDEZ-CRESPO and Cándido SANTIAGO-ÁLVAREZ

Dpto. Ciencias y Recursos Agrícolas y Forestales, ETSIAM, Universidad de Córdoba, Ap 3048, 14080 Córdoba, Spain.

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Summary – Egg laying and development of *Hexamermis serenensis* (Nematoda; Mermithidae), a parasite of the locust *Dociostaurus maroccanus* (Orthoptera; Acrididae) has been studied in laboratory. Total fecundity of females was more than 725-1089 eggs, with a maximum rate of 125-130 eggs/day. Eggs were laid at the unicellular stage. Development was followed at 26 °C : bicellular stages were observed 1 day after egg-laying : elongation of the embryo was seen by day 5-6, early coil stage by day 9-10, and hatching occurred by day 26-29. Only 15-20 % of the eggs laid were viable. Infective stages maintained at 4 °C survived 18-66 days, and survived 7-20 days at 15 °C or 26 °C. Results suggest that *H. serenensis* may have only one generation per year in field conditions.

Résumé – ***Ponte et développement d'*Hexamermis serenensis *(Nematoda : Mermithidae) en conditions de laboratoire*** – La ponte et le développement d'*Hexamermis serenenis* (Nematoda : Mermithidae), un parasite du criquet *Dociostaurus maroccanus* (Orthoptera : Acrididae) ont été étudiés au laboratoire. La fécondité totale des femelles a été supérieure à 725-1 029 œufs, avec un taux journalier maximum de 125-130 œufs. Les œufs étaient pondus au stade unicellulaire. Le développement a été étudié à 26 °C : les stades bicellulaires ont été observés un jour après la ponte; l'allongement de l'embryon commençait au cinq-sixième jour, le stade précoce replié au neuf-dixième jour et l'éclosion au vingt-sixième-vingt-neuvième jour. Seulement 15-20 % des œufs pondus étaient viables. Les stades infestants maintenus à 4 °C ont survécu 18-66 jours, et 7-20 jours à 15 ou 26 °C. Les résultats suggèrent que *H. serenensis* ne pourrait avoir qu'une seule génération par an en conditions naturelles.

Key-words : *Dociostaurus maroccanus Hexamermis serenensis,* locust, mermithid, parasite, nematodes.

Hexamermis serenensis Hernández-Crespo & Santiago-Álvarez, 1997 is a parasite of the locust *Dociostaurus maroccanus* and one the most obvious biological agents regulating *D. maroccanus* populations in the locust breeding area of La Serena (Spain) (Hernández-Crespo, 1993). Since this parasite attacks nymph and adult stages, killing its hosts, and since, its host range appears to be restricted to acridoids (Hernández-Crespo & Santiago-Álvarez, 1991), it might represent a good candidate for biological control. Studies on biology and ecology of this species, as well as those conducted on other species of the genus *Hexamermis* (Couturier, 1950 Robinson *et al.*, 1990; Herron & Baker, 1991) and of close related genera (Poinar & Gyrisco, 1962), are of interest in terms of understanding the host-parasite relationship and to assess the potential of using this nematode as a biological control agent against the Moroccan locust. Some other mermithids have been used in insect biocontrol programs (Mongkolkiti & Hosford, 1971; Finney, 1981; Petersen, 1981; Creighton & Fassuliotis, 1983) and mass rearing has been achieved (Poinar, 1979; Creighton & Fassuliotis, 1982). In this paper we report a study on *H. serenensis* development in laboratory including egg laying, embryonic development, egg viability and infective stage survival and parasitization.

Material and methods

Postparasitic juveniles of *H. serenensis* leaving *D. maroccanus* hosts captured at La Serena (Badajoz, Spain) during the spring season of 1991 were transferred to a Petri dish containing clean moist sand and maintained at room temperature to facilitate the final moult to adults. Females were individually transferred to Petri dishes in the same conditions as above, paired with a male and maintained at 26 ± 2 °C and 12 : 12 h photoperiod. Females showing egg-laying behaviour were transferred to crystal-watch glasses containing tap water, and eggs were counted daily. Every day, more than 50 % of the eggs laid were transferred to Petri dishes (60 mm diameter) containing 5 cm^3 of tap water and incubated at 26 °C, in darkness. Embryonic development was recorded daily using a stereoscopic microscope. Terminology and optimal rearing temperature follow that given in Poinar and Gyrisco (1962).

Infective stage survival was studied with newly hatched (less than 36 h) juveniles. Groups of 20 nematodes were exposed at 4, 15, or 26 °C and checked every two days for dead nematodes. Parasitisation assays were performed with laboratory newly hatched first instar *D. maroccanus* nymphs from field collected egg-pods. Fifteen males and fifteen females were exposed to a mixture of recently hatched preparasitic stages and eggs at the last embryonic stage in a cup with clean moist sand maintained at 26 °C for 24 h. The nymphs were then transferred to individual cups and fed with alfalfa leaves.

Results

Three female-male pairs were used for the oviposition studies. Two females began egg laying one month after moulting, the other one did not lay eggs and lived from July 1991 to February 1992 when it was accidentally killed. No eggs were seen in the uterus of this female.

One of the fertile females (Female A) that remained continuously with a male laid eggs over 18 days, with a total fecundity of more than 1089 and at a maximum rate of 130 eggs per day. The rate of egg laying diminished markedly during the final 6 days of oviposition (Fig. 1, A). The other fertile female (Female B) whose male died early before egg-laying laid eggs over 12 days. The total number of eggs laid was more than 725, with a maximum rate of 125 eggs per day. The rate of laying diminished markedly for the final 4 days of oviposition (Fig. 1, B). Both females died a few days after the end of egg-laying; at that time, some eggs still remaining in the uterus.

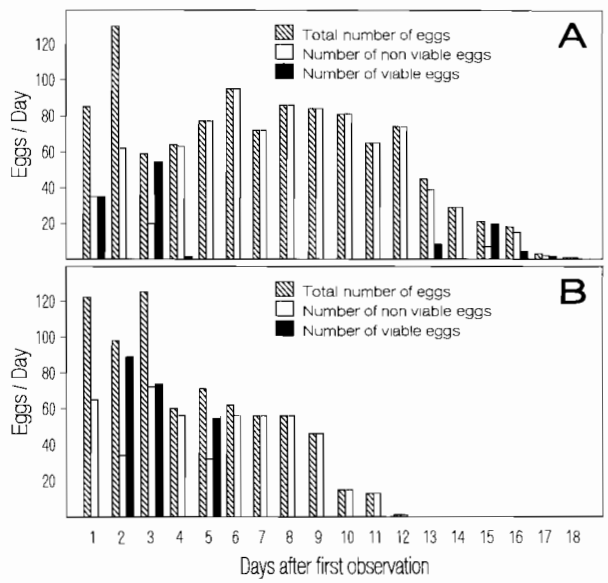

Fig. 1. *Number of eggs laid by females of* Hexamermis serenensis *maintained at 26 °C. A, B : Total number of eggs, number of non viable and viable eggs laid by female A and female B respectively.*

All the eggs were laid at the unicellular stage. First division to the bicellular stage appeared 24-30 h after laying and elongation of the embryo was observed 5-6 days later. The early coil stage was seen 4 days after hatching occurred 26-29 days after laying. Embryonic development was fairly synchronous in the eggs laid on the same day. In contrast, hatching was not synchronous and some nematodes hatched 30 days later than other of the same cohort. Only 15-20 % of the eggs laid by each female showed signs of embryonic development (Fig. 1).

The infective stages survived 18-66 days at 4 °C, and 7-20 days at 15 °C, and at 26 °C.

Two nymphs that died one day after exposition to infective stages were dissected : they contained one and two juveniles of a parasitic stage of the nematode. A parasitic stage that had begun development and measured 2 cm was found in another nymph that died 9 days later.

Discussion

Females of *H. serenensis* can begin to lay eggs 1 month after the last moult and lay more than 1000 eggs under laboratory conditions which is in the line with other species of the genus (Couturier, 1950; Myshachkov, 1980). The fecundity of *H. serenensis* may be different under field conditions given that laboratory females died with eggs remaining in the uterus and that the transfer from soil to tap water greatly reduces the fertility of other species such as *H. truncata* (Myshachkov, 1980).

Couturier (1950) observed that females of *Hexamermis* laid the same number of eggs as *H. serenensis* but over a longer period (8 months) and at a lower rate (20 eggs per day). Temperature can greatly influence the rate of egg laying. The observations of Couturier (1950) were made at 10-28 °C reflecting natural fluctuations and average temperatures in the field. Consequently, field populations of *H. serenensis* may also show a longer egg laying period and lower laying rate than present data suggest.

The longevity of virgin females is greater than that of fertile females actively ovipositing (Couturier, 1950; Myshachkov, 1980; Bedding, 1984). We suggest that the female which lived 5 months longer than the ovipositing females was not inseminated.

The embryonic development of *H. serenensis* is very similar, in the morphology of the embryonic stages and in the duration of each stage, to those described for *Amphibiomermis arvalis* (Poinar & Gyrisco, 1962). The eggs laid are unicellular, as in *Hexamermis cavicola* (Welch, 1963) and *H. truncata* (Myshachkov, 1980).

It is difficult to explain the low viability observed in the eggs laid by the two females, as well as fluctuations in the viability of eggs laid each day. Some aspects of the physiology of reproduction in mermithids remain unclear. Puttler and Thewke (1971) found that not all the

eggs of *A. arvalis* developed in the laboratory and suggested that they were in aestivation. The existence of diapause or aestivation in the eggs of mermithids may be possible but requires further studies.

H. serenensis is found parasitizing acridoids in La Serena (Spain) from April to July (Hernández-Crespo & Santiago-Álvarez, 1991). This large period of parasitism is unlikely to be due to more than one generation of this nematode, because *H. serenensis* develops rather slowly : *i)* the parasitic larval development inside the host may take less than one month as in other species of the genus (Myshachkov, 1980; Herron & Baker, 1991); *ii)* postparasitic larvae emerge from the hosts and develop for two months until the final moult (Hernández-Crespo & Santiago-Álvarez, 1991); *iii)* egg laying begins one month after adult emergence; and *iv)* embryonic development takes 26-29 days. Consequently, the nematode needs at least 5 months to complete its life-cycle in the laboratory, and thus only one generation seems to be possible in field conditions. This is consistent with observations on the life-cycle of *H. truncata* (Myshachkov, 1980) and the closely related species *A. arvalis* (Puttler & Thewke, 1971). The extended period of parasitism in the field may be due to the slow rate of egg-laying by females or by the persistence of infective stages. In fact preparasitic larvae of *H. serenensis* can survive for a long time in tap water in the laboratory.

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References

BEDDING, R. A. (1984). Nematode parasites of Hymenoptera. *In :* Nickle, W. R. (Ed.). *Plant and insect nematodes.* New York, USA, Marcel Dekker : 755-795.

COUTURIER, A. (1950). Biologie d'un *Hexamermis* (Nematodes, Mermithidae) parasite des insectes défoliateurs de l'osier. *Annls Epiphyt.,* 1 : 13-37.

CREIGHTON, C. S. & FASSULIOTIS, G. (1982). Mass rearing of a mermithid nematode, *Filipjevimermis leipsandra* (Mermithida : Mermithidae) on the banded cucumber beetle (Coleoptera : Chrysomelidae). *J. econ. Ent.,* 75 : 701-703.

CREIGHTON, C. S. & FASSULIOTIS, G. (1983). Infectivity and suppression of the banded cucumber beetle (Coleoptera : Chrysomelidae) by the mermithid nematode *Filipjevimermis leipsandra* (Mermithida : Mermithidae). *J. econ. Ent.,* 76 : 615-618.

FINNEY, J. R. (1981). Potential of nematodes for pest control. *In :* Burges, H. D. (Ed.). *Control of pests and plant diseases 1970-1980.* London, UK, Academic Press, 603-620.

HERNÁNDEZ-CRESPO, P. (1963). *La langosta mediterránea,* Dociostaurus maroccanus *(Thunberg), sus enemigos naturales autóctonos y el posible control de sus plagas por medio de microorganismos patógenos.* PhD Thesis, Universidad de Córdoba, Spain, 251 p.

HERNÁNDEZ-CRESPO, P. & SANTIAGO-ÁLVAREZ, C. (1991). On the occurrence of a mermithid on Spanish acridids. *Bulletin Srop/Wprs,* 147 : 208-211.

HERNÁNDEZ-CRESPO, P. & SANTIAGO-ÁLVAREZ, C. (1997). Description of *Hexamermis serenensis* sp. n. (Nematoda : Mermithidae) a parasite of *Dociostaurus maroccanus* (Thunberg) (Orthoptera. Acrididae) in Spain. *Fundam. appl. Nematol.,* 20 : 37-42.

HERRON, G. A. & BAKER, G. L. (1991). The effect of host stage and temperature on the development of *Hexamermis* sp. (Nematoda : Mermithidae) in the Australian plague locust *Chortoicetes terminifera* (Walker) (Orthoptera : Acrididae). *Nematologica,* 37 : 213-224.

MONGKOLKITI, S. & HOSFORD, R. M. Jr. (1971). Biological control of the grasshopper *Hesperotettix viridis pratensis* by the nematode *Mermis nigrescens. J. Nematol.,* 3 : 356-363.

MYSHACHKOV, G. A. (1980). Biology and ecology of the mermithid *Hexamermis albicans* - a parisite of the colorado beetle. *In :* Sonin, M. D. (Ed.). *Helminths of insects.* New Dehli, India, Oxonian Press, 123-128.

PETERSEN, J. J. (1981). Observations on the biology of *Octomyomermis muspratti,* a nematode parasite of mosquitoes. *J. Invert. Pathol.,* 37 : 290-294.

POINAR, G. O. Jr. (1979). *Nematodes for biological control of insects.* Boca Raton, Florida, USA, CRC Press, ix + 277 p.

POINAR, G. O. JR. & GYRISCO, G. G. (1962). Studies on the bionomics of *Hexamermis arvalis* Poinar & Gyrisco, a mermithid parasite of the alfalfa weevil, *Hypera postica* (Gyllenhal). *J. Insect Pathol.,* 4 : 469-483.

PUTTLER, B. & THEWKE, S. E. (1971). Field and laboratory observations of *Hexamermis aryalis* (Nematoda : Mermithidae) a parasite of cutworms. *Ann. ent. Soc. Am.,* 64 : 1102-1106.

ROBINSON, A. F., BAKER, G. L. & HEALD, C. M. (1990). Transverse phototaxis by infective juveniles of *Agamermis* sp. and *Hexamermis* sp. *J. Parasitol.,* 76 : 147-152.

WELCH, H. E. (1963). *Amphimermis bogongae* sp. nov. and *Hexamermis cavicola* sp. nov. from the Australian bogong moth. *Parasitology,* 53 : 55-62.

Hernandez Crespo P., Santiago Alvarez C. (1997)

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