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Effect of temperature, photoperiod, flooding, and drying on the hatching pattern of the eggs of *Strelkovimermis spiculatus* (Nematoda: Mermithidae)

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ABSTRACT

We assessed the number of *Strelkovimermis spiculatus* preparasites obtained from a known initial number of nematode eggs and the effect of abiotic conditions (temperature, photoperiod, flooding–drying) on the number of emerged preparasites. Two egg groups were maintained: one continuously flooded, another with flooding–drying cycles (every 15, 30, 60 days). Each egg group was studied at 25 °C and 14:10 (L:D) and 16 °C and 12:12 (L:D). The flooded eggs contained a higher overall percentage of *S. spiculatus* preparasites compared to the wet–dry-cycle eggs. The conditions of continuous flooding at 16 °C and 12:12 (L:D) produced the maximum percent of emerged J2s (30 ± 15%). Preparasites were recorded by 7 (25 °C) and 14 (16 °C) days, suggesting this period as the minimum time for embryonic development. The preparasite-emergence time observed from the same flooded-egg batch (98 and 112 days at 25 °C and 16 °C, respectively) suggested a nonsynchronous hatching, possibly through nonuniform egg embryonation. The time of exposure to drought in the assays did not significantly affect the total average percentage of J2s obtained at 25 °C and 14:10 (L:D), whereas at 16 °C the number of emerged J2s diminished with a prolongation of the drying period. The oviposition period was also recorded only at 16 °C and 12:12 (L:D): *S. spiculatus* eggs were detected at 12.6 days after postparasite emergence, and oviposition was complete at 51 days under those conditions. We propose a flooding schedule to optimize the mass-rearing of *S. spiculatus*.

1. Introduction

The mermithid *Strelkovimermis spiculatus* Poinar and Camino (1986) was discovered in Argentina after infecting larvae of the floodwater mosquito *Ochlerotatus albifasciatus* (Macquart) (Poinar and Camino, 1986). *S. spiculatus* produces epizoites in natural populations of *O. albifasciatus*, constituting a bioregulatory agent of this culicid in nature (Micieli and García, 1999; Campos and Sy, 2003). This nematode, as with other mermithids parasiting mosquitoes, has been indicated as not constituting an ideal biological control agent because of the need for mass rearing *in vivo* and the inherent population dynamics of the species (Federici, 1995; Kerry and Hominick, 2002). Nevertheless, the mass rearing in developing countries would be a viable mechanism for controlling mosquitoes that are disease vectors on a small scale (Santamarina Mijares and Perez Pacheco, 1997). An evaluation of the effect of environmental parameters on preparasite behavior and on egg production and hatching under culture conditions could provide crucial information for an improved mass rearing of this mermithid and would also contribute to a better understanding of this parasite's host cycle in nature.

S. spiculatus is maintained in the laboratory in damp sand where the adults mate and deposit their eggs. After the time of embryonation, the eggs hatch when the sand is flooded with water to liberate the J2 stage infective to mosquito larvae. The sand cultures can be drained and then reflooded days or months later, at which time additional preparasites can be observed (Camino and Reboredo, 1996).

The present study analyzed the effects of specific abiotic parameters (temperature, photoperiod, the flooding–drying cycle) on the number of preparasites obtained from a known initial number of nematode eggs and determined the time during which oviposition occurred. We prescribe here a flooding schedule for optimizing the mass rearing of *S. spiculatus*.

2. Materials and methods

2.1. Experimental design

S. spiculatus eggs were obtained from colonies maintained at the Centro de Estudios Parasitológicos y de Vectores (CEPAVE). The basic methodology for rearing *S. spiculatus* in the laboratory involved the procedures previously described by Camino and Reboredo (1996). In the experiment, 24 h after the oviposition of *S. spiculatus* two groups of eggs were studied: one maintained

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continuously under flooding conditions, the other subjected to different numbers of sequential floodings and drying-out periods. Each egg group was also studied under two conditions: First, eggs were maintained in the laboratory at 25 °C and in a 14:10 (L:D) photoperiod, simulating the summer in natural habitats of *O. albifasciatus* in the Pampean biogeographic province (30–39° S) of the Neotropical region, Buenos Aires province, Argentina. Second, the eggs were kept at 16 °C and in a 12:12 (L:D) photoperiod, simulating the conditions during the spring and fall in these natural habitats. Each experimental condition was performed in sextuplicate from the same batch of eggs and the experiment was also repeated in its entirety on three separate occasions. The tests were conducted in the wells of multiwell trays containing sterile sand (15.5 g.) and 0.10 ml chlorine-free water. One hundred eggs were placed in the center of the damp sand in each well. The permanently flooded eggs were maintained with chlorine-free water (10 ml) during the entire experiment and the number of J2s counted twice a week until no further J2s were detected. The preparasites were removed from the containers, counted, and evaluated for infectivity during each observation. The eggs subjected to drying out were treated as above but were maintained without free water for three different time periods: 15, 30, and 60 days. After each drying out the trays were flooded with chlorine-free water, and 24 h later the preparasites were counted and evaluated for infectivity. After flooding each well, the water was removed by leaving the lid off the tray to facilitate evaporation; once the sand was left in a merely moistened state, the lid was replaced. Finally, the containers were sealed with parafilm to maintain a constant level of moisture. This means of removing the water insured that no eggs would be carried off with it. The J2s' infectivity was evaluated upon their exposure to early larval stages of *Aedes aegypti* at a ratio of between 1:1 and 5:1 (J2s:mosquito larvae) depending on the number of J2s available during each evaluation.

The time during which the ovipositions took place was recorded under the 16 °C–12:12 (L:D.) condition. The postparasite females ($n = 20$) from the same assays were maintained in Petri dishes containing distilled water until reaching the adult stage. Daily observations were made; and every time eggs were detected, the adult worms were placed in a new Petri dish to screen for new eggs. The experiment was repeated three times.

2.1.1. Data analyses

Statistical analyses were conducted through the use of InfoStat version 2010 (Di Rienzo et al., 2010). To express the relative abundance of *S. spiculatus* preparasites in the initial number of eggs, Williams's mean was used. An initial analysis by the one-way ANOVA test and a *post hoc* analysis by the Least-Significant-Difference (LSD) test were performed to compare the mean percent values among treatments, after an arc-sine (X/100) transformation. The log-probit analysis was performed to determine the period of time (days) required to reach 50% and 90% of the emerged J2s.

3. Results

3.1. Effect of temperature and photoperiod on the egg-hatching pattern

Significant differences were detected in the emersion rate of preparasites between both experimental conditions of temperature and photoperiod ($F = 7.10$; $df = 1/592$; $P = 0.0079$), with the highest average value being observed at the lower temperature under continuously flooded conditions. When the eggs were subjected to the cycles of flooding and drying out, no significant differences were detected in the percentage of emerged preparasites between the two different conditions of temperature and photoperiod at flooding intervals of either 15 or 60 days ($F = 0.09$; $df = 1/430$; $P = 0.7698$ and $F = 0.12$; $df = 1/142$; $P = 0.7252$, respectively). The assays performed on the eggs subjected to a flooding every 30 days, however, did show differences in the percentage of J2s emerged between both experimental conditions ($F = 7.73$; $df = 1/250$; $P = 0.0059$), indicating a significantly higher average value at 25 °C.

3.2. Effect of flooding and drying out on the egg-hatching pattern

3.2.1. Eggs permanently flooded

Under continuous flooding conditions, the total percentage of emerged J2s was 30 ± 15% (mean ± SD, $n = 1800$) at 16 °C, while 14 ± 7% (mean ± SD, $n = 1800$) of the preparasites had emerged at 25 °C (Fig. 1). The preparasites were detected from 7 to 14 days on and up to 98 and 112 days after the beginning of experiment at 25 °C and 16 °C, respectively (Fig. 2). The log-probit analysis revealed that 50% of the preparasites were observed by an average of 28.9 (28.3–29.4) days and 90% by an average of 43.6 (42.7–44.5) days after oviposition in the assays maintained at 16 °C; while 50% of the preparasites were observed by an average of 33.2 (31.9–34.4) days and 90% by an average of 61.8 (60.1–63.8) days after oviposition at 25 °C. The J2s were always able to infect *Ae. aegypti* larvae under laboratory conditions of exposure.

3.2.2. Eggs under conditions of flooding and drying out

In assays subjected to the flooding–drying cycles at of 16 °C and 12:12 (L:D) photoperiod, the total percentage of emerged J2s was 8.4 ± 6.0% at 15 days, 1.9 ± 2.2% at 30 days, and 2.6 ± 1.5% at 60 days (Fig. 1). Significant differences among the treatments (15, 30, or 60 days) were registered ($F = 3.89$, $df = 2/411$, $P = 0.0211$), with the highest mean value at the interval of 15 days and the lowest at 30 days. No differences were found at 25 °C among the flooding–drying cycles ($F = 1.07$; $df = 2/411$; $P = 0.3436$). The total percentage of emerged preparasites from eggs flooded every 15 days was 7.8 ± 7.4%; every 30 days, 6.6 ± 7.2%; and every 60 days, 2.2 ± 2.6% (Fig. 1).

After a flooding every 15 days at 16 °C, the preparasites were detected for the first time during the first immersion (*i.e.*, at Day 15) and for the last time during the ninth (*i.e.*, at Day 135), whereas at 25 °C J2s were present during only the eighth flooding (*i.e.*, at Day 120; Fig. 3A).

After a flooding every 30 days at 16 °C, preparasites were recorded during 5 of the immersions, with the last emergence occurring at Day 150; by contrast at 25 °C J2s were present during 4 floodings, and the last emergence was registered at Day 120, after which time no further preparasites were observed. The maximum number of J2 emergence occurred at Day 60 at either temperature and photoperiod (Fig. 3B).

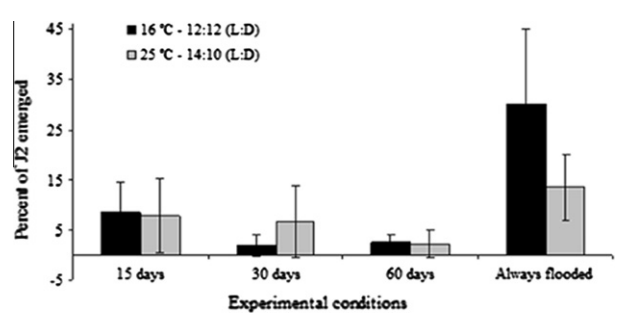

Fig. 1. Total percentage of preparasites of *S. spiculatus* out of the initial number of eggs at 16 °C and 12:12 (L:D) photoperiod (black bars) and at 25 °C and 14:10 (L:D) photoperiod (gray bars) under the experimental conditions: flooded and with different numbers of flooding–drying out cycle (15, 30 and 60 days).

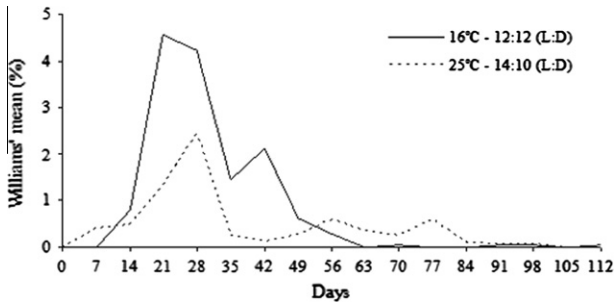

Fig. 2. Percentage of preparasites of *S. spiculatus* out of the initial number of eggs at 16 °C and 12:12 (L:D) and at 25 °C and 14:10 (L:D) photoperiod, under the experimental condition of flooding.

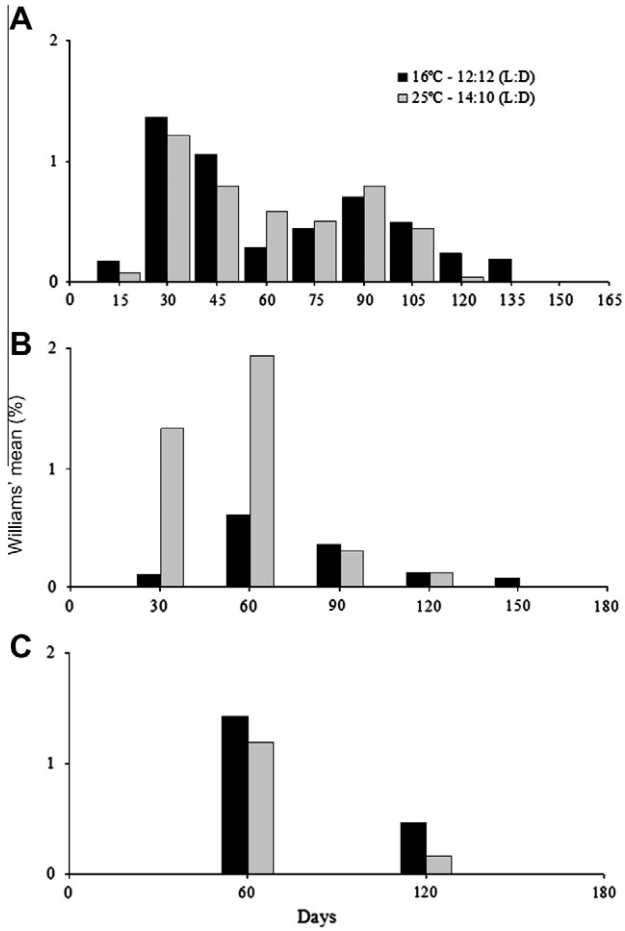

Fig. 3. Relative abundance (percent) of *S. spiculatus* preparasites out of the initial number of eggs subjected to cycles of flooding–drying of 15 (A), 30 (B) and 60 (C) days under both experimental conditions of temperature–photoperiod: 16 °C—12:12 (L:D) (black bars) and 25 °C—14:10 (L:D) (gray bars).

After a flooding every 60 days at either temperature and photoperiod, preparasites were present during 2 of the immersions, with a maximum number occurring at Day 60 and the last emergence of preparasites at Day 120 (Fig. 3C). The J2s were always able to infect *Ae. aegypti* larvae in laboratory exposures.

3.3. Period of oviposition

Oviposition at 16 °C and 12:12 (L:D) began at 12.6 ± 0.47 (mean ± SD, $n = 3$) days after postparasite emergence and continued up to 51.3 ± 3.29 (mean ± SD, $n = 3$) days.

3.4. Flooding schedule for optimizing the mass rearing of S. spiculatus

A flooding schedule for optimizing the mass rearing of this mermithid in order to obtain the maximum number of preparasite stages is proposed as follow: On the basis of only one period of copula at 25 °C and 14:10 (L:D) photoperiod, the cultures have to be flooded twice: The first flooding would occur at about six weeks after the postparasites are buried in the sand (*e.g.*, 43 days: 13 days between postparasite emergence and the start of oviposition plus 30 days: the J2 50%-hatch time). The second flooding would take place at about 6 weeks later (*e.g.*, at 90 days: 30 days between J4 emergence and the end of oviposition plus 60 days: the 90%-J2 hatch time).

4. Discussion

The aquatic habitats where this parasite develops and infects mosquito-host populations are ephemeral or semipermanent pools that typically undergo a wide variation in water levels throughout the year involving a periodic flooding and drying out (Maciá et al., 1995). The present investigation has acquired new information on the hatching pattern of *S. spiculatus* eggs that indicates possible strategies of this parasite within the context of the transient nature of these habitats. The parasitism of *S. spiculatus* in *Oc. albifasciatus* was found to be seasonal, with peaks restricted to spring and fall (Maciá et al., 1995; Micieli and García, 1999). Accordingly, a temperature of 16 °C (a spring-fall condition) would seem to be more appropriate for egg-hatching than one of 25 °C because the hatching pattern of *S. spiculatus* eggs with a constant water stimulus involved a greater emergence of J2s at 16 °C, with the implication being that water temperature played an influential role in the emergence of nematode juveniles. By contrast, a temperature of 25 °C and a 14:10 (L:D) photoperiod appeared to be the favorable condition when eggs were buried in the sediment, since at that temperature the frequency of flooding and drying out did not affect the yield of J2s; whereas at 16 °C the number of emerged J2s diminished with a prolongation of the drying period.

Eggs of *S. spiculatus* were detected at 12.6 days after postparasite emergence, and oviposition became complete at 51 days under our experimental conditions. Undeen et al. (1996) found that the 50% of *S. spiculatus* eggs were produced by 19.4 days after the emergence of the preadult nematodes and that egg production was complete by 35 days.

The period of time between the beginning of our experiment—about 24 h after oviposition—and the appearance of the first preparasites in the flooded assays suggested a minimum of 7 days at 25 °C and 14 days at 16 °C for the embryonation of *S. spiculatus* eggs. Camino (1985) had cited embryonic-development periods from the time of oviposition to the emergence of J2s of 11 days at 12 °C, 8 days at 20 °C, and 5 days at 28 °C; while Undeen et al. (1996) working with eggs of *S. spicultatus* suggested a minimum of 14 days.

The long period of time over which preparasites continued to emerge from the same group of nematode eggs in the flooded assays furthermore pointed to a asynchronous hatching of the eggs, possibly owing to a nonuniform embryonation. A higher total percentage of preparasitic stages of *S. spiculatus* was recorded when eggs were always maintained under flooded conditions compared to the assays subjected to different flooding–drying cycles, thus suggesting that drying very likely adversely affected egg viability.

The J2s were always able to infect *Ae. aegypti* larvae in laboratory exposures, even the very last ones that were found to emerge at 150 days. Petersen (1980) found significant differences in the infectivity of preparasites of *R. culicivorax* with respect to culture

age, with the parasitism for 10 week-old cultures being significantly higher than the level for 20 week-old cultures.

The identification of parameters that affect the mermithid egg-hatching pattern constitutes a key parameter for improving the mass rearing of these nematodes and is accordingly a crucial consideration in planning future releases of the parasite into mosquito-breeding habitats.

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References

Camino, N.B., 1985. Estudio del desarrollo embrionario y de las larvas pre-parásita y parásita de*Strelkovimermis spiculatus* (Nematoda: Mermithidae) en larvas de Culex pipiens fatigans Wied. (Diptera: Culicidae). CIRPON. Rev. Invest. 3, 15–23.

Camino, N.B., Reboredo, G.R., 1996. Producción de *Strelkovimermis spiculatus* Poinar y Camino 1986 (Nematoda: Mermithidae). Neotrópica 42, 47–50.

Campos, R.E., Sy, V.E., 2003. Mortality in immatures of the floodwater mosquito *Ochlerotatus albifasciatus* (Diptera: Culicidae) and effects of parasitism by *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in Buenos Aires province Argentina. Mem. Inst. Osw. Cruz. 98, 199–208.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2010. InfoStat 2010. Grupo InfoStat. FCA, Universidad Nacional de Córdoba, Argentina.

Federici, B.A., 1995. The future of microbial insecticides as vector control agents. J Am Mosq Control Assoc. 11, 260–268.

Kerry, B.R., Hominick, W.M., 2002. Biological control. In: Lee, D.L. (Ed.), The Biology of Nematodes. Taylor and Francis, London, pp. 483–509.

Maciá, A., García, J.J., Campos, R.E., 1995. Bionomía de *Aedes albifasciatus* y *Ae. crinifer* (Diptera: Culicidae) y sus enemigos naturales en Punta Lara. Buenos Aires. Neotrópica. 41, 43–50.

Micieli, M.V., García, J.J., 1999. Estudios epizootiológicos de *Strelkovimermis spiculatus* Poinar y Camino, 1986 (Nematoda: Memithidae) en una población natural de *Aedes albifasciatus* Macquart (Diptera: Culicidae) en la Argentina. Miscellània Zoològica 22, 31–37.

Petersen, J.J., 1980. The effect of culture age on the infectivity of preparasites of the mosquito parasite *Romanomermis culicivorax*. Mosq. News 40, 640–641.

Poinar Jr., G.O., Camino, N.B., 1986. *Strelkovimermis spiculatus* n. sp. (Mermithidae: Nematoda) parasitizing *Aedes albifasciatus* Mac. (Culicidae: Diptera) in Argentina. J. Nematol. 18, 317–319.

Santamarina Mijares, A., Perez Pacheco, R., 1997. Reduction of mosquito larval densities in natural sites after introduction of *Romanomermis culicivorax* (Nematoda: Mermithidae) in Cuba. J. Med. Entomol. 34 (1), 1–4.

Undeen, A.H., White, S.E., Fukuda, T., 1996. Egg production by *Strelkovimermis spiculatus* (Nematoda: Mermithidae). J Am Mosq Control Assoc. 12, 736–738.