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EGG PRODUCTION BY *STRELKOVIMERMIS SPICULATUS* (NEMATODA: MERMITHIDAE)

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ABSTRACT. Egg production by the mermithid nematode *Strelkovimermis spiculatus,* a parasite of mosquitoes, was examined over a period of 34 days. Oviposition did not occur in the absence of males. Egg production was best when males were continuously present ($6.4 \times 10^3 \pm 0.9 \times 10^3$ eggs/female). Fewer eggs were produced when males were removed after 7 days ($2.8 \times 10^3 \pm 0.2 \times 10^3$ eggs/female) and oviposition partially recovered after males were returned 11 days later ($4.4 \times 10^3 \pm 0.5 \times 10^3$ eggs/female). The nematodes deposited substantially more eggs in sand ($6.4 \times 10^3 \pm 0.9 \times 10^3$/female) than in water ($1.9 \times 10^3 \pm 0.3 \times 10^3$/female).

Strelkovimermis spiculatus Poinar and Camino was isolated from *Culex quinquefaciatus* Say larvae inhabiting eutrophic water near Buenos Aires, Argentina (Poinar and Camino 1986). Because of its tolerance to high levels of organic pollution and dissolved ions relative to other mermithids such as *Romanomermis culicivorax* Ross and Smith (Camino and Garcia 1991), *S. spiculatus* is being evaluated for biological control of mosquitoes in agricultural wastewater lagoons. *Strelkovimermis spiculatus* is easily maintained in the laboratory in damp sand, where the adult worms undergo their final molt, mate, and deposit their eggs. After a suitable time for embryonation (10–14 days), the eggs hatch when the sand is flooded with water, liberating the preparasites to infect mosquito larvae.

The eggs of *S. spiculatus,* like some other mermithid species parasitizing mosquitoes, do not all hatch on the first flooding of the sand. The sand cultures can be drained and then reflooded days or months later to hatch additional preparasites from the sand (Petersen 1981). Decreasing numbers of preparasites hatch with subsequent flooding and draining cycles. There are several possible causes of the delayed preparasite hatch: egg production over a long period, nonuniform embryonation rates, or a requirement for a number of emersion events (stimulus cycles). The exact percentage that hatch on each flooding cannot be determined because the initial number of eggs is unknown, a problem made worse by the loss of eggs when the sand is drained between cycles. Quantitative experimentation on this question as well as the evaluation of the effect of environmental parameters on egg production, survival, and hatching requires a known number of eggs at the start of an experiment. This work examines the effect of males on the timing of oviposition and production of eggs in 3 substrates: damp sand, saturated sand, and water.

Sand cultures of *S. spiculatus* were maintained at 23 ± 1°C and flooded to obtain preparasites (infectious stages), which were then exposed to second instar *Aedes aegypti* (Linn.) at a rate of 4 preparasites per mosquito larva. Infected mosquito larvae were reared to the 4th instar, at which time the preadult worms emerged. One or 2 days after emergence, 10 female mermithids were placed in 100-ml dessert cups containing either 50 ml deionized water or 40 ml deionized water plus 35 cm^3 sand (medium silica sand-blasting sand, Feldspar Corp., Edgar, FL). The test was replicated once with a different population (culture) of the nematodes. Four mating situations were evaluated in both saturated sand and water: 1) males absent, 2) males continuously present, 3) males present for 7 days, and 4) males present for 7 days, absent for 11 days and then returned. The cumulative production of eggs from saturated sand was compared with a single harvest of eggs from damp sand with the continuous presence of males (standard culture protocol).

Eggs were collected from the sets in deionized water and saturated sand at 3- to 4-day intervals, starting 7 days after the males and females were combined. Adult worms from the water sets were transferred to fresh cups after each collection. The saturated sand sets were washed twice with 30-ml aliquots of water to remove the eggs and adults. Dead females were recorded and removed and then the remainder of the adults were returned to the original sand. When the last count of the water and saturated sand sets were made, eggs were harvested from the sets in damp sand by 2 washes in deionized water. Eggs in three 0.2-ml samples from each cup were counted using a dissecting microscope with dark field lighting. The number of eggs in the 0.6-ml sample was multiplied by the total wash volume and divided by the number of live females to obtain the number of eggs/female.

The body of an egg-depleted female had a characteristic clear or translucent appearance. Eggs were still visible in the body of a female that had not completed oviposition. Females were scored for "depletion" (number of females that completed oviposition/total number of females) at the end of the egg collection period as a measure of completeness of oviposition.

Neither the media (sand or water) nor the presence of males had any effect on the survival of females. The mean ± SE was 79.2 ± 3.1% (n = 24 sets of 10 adults each) at the end of the test.

Table 1. Effect of the presence of males and 2 substrates, water and sand, on the egg production of *Strelkovimermis spiculatus.*

Number of males	Status of males	Eggs/female ($\times 10^3$)[1], $n = 4$: Oviposition in sand	Oviposition in water	Total eggs/female
0	No males	0	0	0
10	Continuous	6.4 ± 0.9	1.9 ± 0.3	4.1 ± 1.0a[2]
10	7 days	2.8 ± 0.2	1.9 ± 0.6	2.4 ± 0.4b
10	7 . . . 18 days[3]	4.4 ± 0.5	2.0 ± 0.4	3.2 ± 0.5ab
Total eggs/female		4.5 ± 0.5A[4]	2.0 ± 0.3B	

[1] The accumulation of all eggs from all samples during the oviposition period.
[2] Lowercase letters, if different, indicate that the sex factor is significant.
[3] Males present for the first 7 days, removed, then returned 11 days later.
[4] Upper case letters, if different, indicate a significant difference in egg production between the substrates.

The average rate of depletion was 26.2 ± 4.0%. There was a low but significant correlation ($|r| = 0.46$, F = 5.75, $P \geq 0.025$) between egg production (total eggs per female) and percentage of depletion. No eggs were produced by virgin females (Table 1); their bodies remained opaque and there were no signs of egg development. Oviposition was lower for females held for only 7 days with the males than for those with which males were continuously present. Returning the males after a hiatus of 11 days significantly increased egg production in sand but not in water (Table 1). The nematodes were often observed to be attached, even at the end of the test, indicating that mating was still occurring. Similar results were obtained by Petersen (1978) for *Octomyomermis muspratti* by adjusting the male : female ratios. Fewer males/female prolonged the oviposition period.

A greater number of eggs were harvested from worms held in saturated sand than from those maintained in deionized water (Table 2). Approximately the same number of eggs was collected from sand whether they were harvested at 3–4-day intervals or only once at 34 days, the last time the other test groups were sampled (Table 1). There was no significant difference in the number of eggs harvested from the standard rearing method and those accumulated from multiple samples in sets with males continuously present in saturated sand.

Table 2. Total numbers of eggs produced under the standard rearing conditions (damp sand), sampled once at 34 days after emergence, compared with production in wet sand or water.

Sampling method Substrate	Eggs per female[1] (mean ± SE, $n = 4$)
Eggs harvested once at 34 days	
Damp sand	6.1 ± 0.7 × 10^3a[2]
Eggs accumulated from multiple harvests	
Saturated sand	6.4 ± 0.8 × 1.0^3a
Water	1.9 ± 0.3 × 10^3b

[1] Ten female mermithids per replicate, 4 replicates.
[2] Different letter indicates significant difference (0.01 level).

Log probit analysis revealed that 50% of the eggs were produced by 19.4 (19.3–19.4) days and 90% by 26.4 (26.3–26.5) days after emergence of the preadult worms from the larval mosquitoes. Preparasites first appeared 24.5 ± 1.5 days postemergence (20.5 days after adding the males or about 14 days after oviposition began) in the 2 saturated sand cultures where the presence of preparasites was recorded. After that, preparasites were observed whenever eggs were collected from the saturated sand but they never exceeded 10% of the number of eggs collected. When eggs were harvested from the damp sand at 34 days after emergence (about 24 days after onset of oviposition), 22.5 ± 4.3% of them hatched immediately.

Oviposition was nearly complete by 35 days after the emergence of the adult worms under these conditions. However, 75% of the females had not completed oviposition. Oviposition rates appear to be equal in the serially sampled saturated sand cultures and the damp sand and lower in water but sampling errors were different for the 3 media. When oviposition occurred in water only, the eggs tended to clump together and adhere to the adults. The preparasites found in the serial washes from the sand cultures demonstrated that not all of the eggs were removed by previous washes.

A major problem for commercialization of mermithid nematodes is the weight of the sand cultures. This problem might be overcome by harvesting the eggs from the sand and placing them in a lighter medium. The time between onset of oviposition and the appearance of the first preparasites suggests a minimum of 14 days for embryonation of *S. spiculatus* eggs. Until this time, eggs can be separated from sand cultures with little hatching. Under the best experimental conditions here, almost 80% of oviposition was complete by this time. Any cultural practice that would promote more rapid or synchronous oviposition and would improve egg yield and reduce handling costs must be considered. Careful attention to mating conditions is important.

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