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Optimizing laboratory production of *Strelkovimermis spiculatus* (Nematoda: Mermithidae) with a discussion of potential release strategies for mosquito biological control

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ABSTRACT

We assessed *Strelkovimermis spiculatus* preparasite (J2) infectivity and postparasite (J4) production in rearing pans using a laboratory host, second-instar *Aedes aegypti* mosquitoes, and evaluated first the production efficiency by testing increasing J2 concentrations (preparasite:host ratios). At 10 000 host larvae/rearing pan, we obtained the maximum yield of J4s (3.50 g), number of J4s/larva (3.1), and parasitism frequency (94.9 ± 9.5%) at a 10:1 J2/larva ratio. This 10:1 ratio, however, resulted in a significantly male-skewed sex ratio, whereas at ratios between 4:1 and 7:1 females predominated and parasite frequencies were still acceptable. Next, postparasite-inoculation methods were compared under culture conditions reflecting two categories of mosquito habitat (permanently flooded or desiccated cultures). The J4s were left to develop into adults, mate, and oviposit in the substrate. The flooded-culture J2s hatched at week 3 and after 30 weeks gave a total production of 6.2 × 104 per pan. Maximum J2 yield in desiccated cultures occurred at the first flooding (5 weeks), but emergence continued through drying flooding cycles for 10 weeks, giving a total J2 production of 4.5 × 105 per pan. The J2s infected mosquito larvae under both conditions. *S. spiculatus* preparasites or postparasites would serve as mosquito bioregulatory agents in temporary or permanent mosquito habitats. Relevant strategies are discussed.

1. Introduction

Mermithids have proved to be effective in parasitizing natural populations of mosquito larvae (Achinelly and Micieli, 2009; Perez-Pacheco et al., 2005, 2009; Petersen and Willis, 1972; Platzer, 2007; Santamarina Mijares et al., 2000). Infective preparasitic (second-stage) juveniles (J2) hatch from eggs when potential mosquito breeding sites are flooded and then actively seek out and penetrate the larvae of the mosquito host. The third-stage juvenile (J3) develops within the mosquito larva in 6–8 days, at which time the postparasitic juveniles (J4) emerge, killing the host. The J4 develop into adults; mature adults mate and then lay eggs in the aquatic environment to complete the cycle (Platzer, 2007).

Petersen and Willis (1972) were the first to release preparasites (J2) and postparasites (J4) of *Romanomermis culicivorax* experimentally in natural mosquito populations. Since then, numerous field trials have demonstrated the effectiveness of the J2 and J4 against mosquito larvae (Achinelly and Micieli, 2009; Kerwin and Washino, 1985; Perez-Pacheco et al., 2005, 2009; Petersen et al., 1978; Santamarina and Perez-Pacheco, 1997; Walker et al., 1985). Petersen et al. (1978) have proposed several advantages to postparasite inoculation into mosquito breeding sites since these stages can be applied when habitats are not flooded, as long as the soil remains moist, or inoculated into standing water without the presence of early-instar mosquitoes; under both conditions the parasites are able to complete the life cycle. In contrast, preparasites can be applied to standing water only, and there must be susceptible mosquito instars present at the time of application (Walker et al., 1985).

Strelkovimermis spiculatus Poinar and Camino 1986 (Nematoda: Mermithidae) is a parasite isolated initially from *Ochlerotatus albifasciatus* (Macquart) larvae in temporary ponds of the Buenos Aires province, Argentina (Poinar and Camino, 1986), and subsequently from other mosquito species (Campos et al., 1993; García and Camino, 1990; García et al., 1994).

The breeding sites of *O. albifasciatus* are flooded by rain. Mosquito larvae hatch at the same time as *S. spiculatus* eggs when their common habitat is flooded. Epizootics produced by this nematode are commonly observed in natural populations of *O. albifasciatus*, since *S. spiculatus* is a natural enemy of the immature insect stages (Campos and Sy, 2003). Other mosquito species have been recorded in these environments when they have remained flooded for a longer period of time than usual, and enzootic levels of parasitism by this mermithid have been registered in those species as well (García et al., 1994; Maciá et al., 1995).

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To date, nothing is known about the effectiveness of a deliberate inoculation of preparasites and postparasites of *S. spiculatus* into either permanent or temporary mosquito-breeding sites in order to effect a natural form of regulation by this nematode on the survival of mosquito larvae. We conducted experiments in the laboratory to assess the degree of infectivity by this mermithid to mosquito larvae and the levels and timing of its preparasite and postparasite production. We determined the J2 concentration producing a maximum postparasite production as well as the preparasite production after the addition of postparasites under two conditions (cultures permanently flooded or desiccated). An optimization of infection and production efficiencies in the laboratory under field-simulating conditions in this way should facilitate the practical application of these nematodes as bioregulatory agents against wild-mosquito populations.

2. Materials and methods

2.1. Source and maintenance of mosquito larvae

Larvae of the mosquito *Aedes aegypti* were used in our bioassays because *O. albifasciatus*, the natural host of *S. spiculatus*, has never been colonized in the laboratory. Mosquito larvae were obtained from colonies maintained at the Centro de Estudios Parasitológicos y de Vectores (CEPAVE) and were handled according to standard techniques described by Micieli et al. (2001).

2.2. Laboratory production of S. spiculatus

2.2.1. Production of postparasites (J4) of S. spiculatus from the preparasites (J2) added to A. aegypti larvae

The basic methodology described by Camino and Reboredo (1996) was used for rearing *S. spiculatus* in the laboratory. Bioassays were conducted to assess the concentration of J2/host larva to produce the maximum efficiency of postparasite production i.e., the percentage of J2 mermithids able to parasitize, develop, and finally emerge as J4 from the mosquitos. Second-instar larvae of *A. aegypti* (n = 10 000) were exposed for 24 h to the nematode *S. spiculatus* at ratios of 1:1, 2:1, 3:1, 4:1, 5:1, 7:1, 10:1 J2s/larva and reared in 21-cm–diameter plastic containers containing 500 ml of chlorine-free water (pH: 7.4, conductivity: 767 μmhos/cm) at room temperature. The preparasites were obtained by flooding sand cultures (for 24 h) containing nematode eggs with chlorine-free water. The nematode inoculum was obtained as follow. The water of the flooded cultures containing the preparasites was collected in a common container. After serial dilutions, the number of J2 was counted under a stereoscopic microscope and the concentration of the original suspension calculated by standard procedures (Kaya and Stock, 1997). Each of the tests, itself performed in triplicate, was repeated six times. Triplicate control groups with no J2 present were used for each *S. spiculatus* concentration. The mosquito larvae, fed on commercial rabbit chow, developed readily up to the fourth instar. The percentage of parasitism; the number of emerged nematodes per host larva; the sex ratio; and the postparasite-production efficiency were determined by placing 25 *A. aegypti* larvae into the wells of test plates and counting the number of postparasites that emerged. All bioassays were conducted at 27 °C ± 1 °C under a 12:12-h (light–dark) photoperiod.

2.2.2. Production of preparasites (J2) of S. spiculatus from postparasites (J4)

2.2.2.1. Addition of postparasites of S. spiculatus to permanently flooded nematode cultures. Adults of *S. spiculatus* from various emergence trays were pooled, washed, and weighed. Then 1 g (approximately 22 mermithids/cm^2) was placed in plastic containers (10 × 15 × 3 cm) each with a bed of sterile sand (2 cm deep) and 100 ml of chlorine-free water. Nematodes were introduced at a sex ratio of 2:1 (♀/♂) to allow for the possibility of their becoming established and recycling within the model environment. Cultures were maintained flooded, and the number of hatched J2s counted weekly after serial dilution from an initial aliquot of 1 ml. This procedure was repeated until no further hatching of J2 occurred in the cultures.

Fifty second-instar *A. aegypti* larvae were exposed to the *S. spiculatus* nematodes at a ratio of 10:1 J2 mermithids/larva to determine the infectivity of the hatched J2s in the cultures. The tests were conducted in plastic cylinders (8 cm in diameter) containing 100 ml of chlorine-free water. When the number of hatched J2s was fewer than 500, the same ratio of 10:1 J2/larva was used. Throughout the experiment the volume of water and number of mosquito larvae were adjusted to keep the density per ml constant. Each test was done in at least triplicate. A control group was used with no nematodes present. The exposed mosquito larvae were examined 24 h later by phase-contrast microscopy and the infection rate and mean number of nematodes per larva recorded. Since the larvae of *A. aegypti* are usually quite transparent in the earlier stages, the actively moving first parasitic stage of the mermithid is readily detectable upon removal of the larvae with a glass pipette and viewing them by phase-contrast microscopy in the following way. The larvae are placed on a slide in water under a cover slip and a gentle compression subsequently applied to the cover slip. The nematodes can then be discerned simply from their movement without harming either the parasites or the mosquito larvae.

2.2.2.2. Addition of postparasites of S. spiculatus to nematode cultures subjected to desiccation. Plastic containers (10 × 15 cm) with sterile sand (2 cm deep) and 100 ml of chlorine-free water were inoculated with 1 g of nematodes (2:1 ♀/♂). The cultures were covered and stored for 1 week before the excess water was poured off, carrying with it any dead nematodes. After an additional 4 weeks the cultures were flooded with 100 ml of chlorine-free water to obtain the J2. The number of J2 mermithids was determined by serial dilutions 12 h later. The excess of water was then removed and the cultures stored for an additional week before a second flooding. This procedure was repeated weekly until the cultures no longer produced J2.

After each of the succesive floodings, 50 s-instar *A. aegypti* larvae were exposed to *S. spiculatus* at a ratio of 10:1 J2/larva to determine the infectivity of the hatched J2 nematodes. The bioassay was carried out in plastic cylinders (8 cm in diameter) with 100 ml of chlorine-free water. Each test was performed in triplicate. A control group was used with no nematodes present. The exposed mosquito larvae were examined by phase-contrast microscopy 24 h later and the infection rate and average number of nematodes per larva recorded as described above. The concentration of J2 mermithids and mosquito larvae per ml was maintained constant in the bioassays.

2.3. Statistical analyses

Parasitism, the number of nematodes per larva, and the efficiency data were analyzed by the one-and two-way ANOVA and the Tukey comparison-of-the-means tests (Sokal and Rohlf, 1985).

3. Results

3.1. Production of postparasites (J4) of S. spiculatus from the preparasites (J2) added to A. aegypti larvae

The levels of parasitism of *A. aegypti* larvae by *S. spiculatus* ranged from 48.0 ± 14.6% to 94.9 ± 9.5% for the different concentrations

of J2/larva and the number of nematodes present within each larva ranged from 1.2 to 3.1. The mean yield of J4 mermithids ranged between 1.05 and 3.50 g/rearing tray and increased in proportion to the level of parasitism (Table 1). The maximum yield per tray (3.50 g), number of nematodes per larva (3.1), and level of parasitism (94.9 ± 9.5%) were obtained at a ratio of 10:1 J2/larva. At this concentration the number of male nematodes produced was increased. The highest efficiency (i.e., number of J4 postparasites per J2 preparasite, expressed as a percent) was 32% at a ratio of 1:1 J2 stages/larva. Significant differences (F: 10.34; g.l: 6.42; $p < 0.001$) were found in the yield of J4s among the different J2:larval ratios (Tukey, $p < 0.05$). The main differences were between the ratio of 10:1 J2s/larva and any of the others. (Table 1).

3.2. Production of preparasites (J2) of S. spiculatus from postparasites (J4)

3.2.1. Production of preparasites (J2) of S. spiculatus in permanently flooded nematode cultures

The preparasites of *S. spiculatus* hatched on the third week with a total number of 1130 J2 nematodes emerging during that week. The preparasites hatched asynchronously each week thereafter to give a maximum level of 10,000 J2 mermithids by the eighth week. Although J2 emergence was registered throughout a total of 30 weeks, less than 6% of the total production occurred after the fifteenth week (Fig. 1). The total number of infective stages over the thirty weeks was 6.2 × 104.

Preparasites of *S. spiculatus* always retained their ability to infect *A. aegypti* larvae, with infection percentages continually falling between 70% and 100% and the number of J4 nematodes per larva ranging between 1.4 and 4.0 (Table 2). No significant differences were observed in the degree of parasitism or in the number of nematodes/larva present over the different individual weeks from the third week on ($p < 0.05$).

Table 2
Infectivity of the preparasitic stage (J2) in *Strelkovimermis spiculatus* cultures subjected to permanent flooding.

Week	Parasitism (%)	Number of J4 per larva
1	97.0 ± 3.0	2.7 ± 0.4
2	92.6 ± 7.3	3.6 ± 1.0
3	80.0 ± 14.7	2.1 ± 0.7
4	81.3 ± 5.2	1.5 ± 0.1
5	93.7 ± 9.5	2.9 ± 0.8
6	87.3 ± 9.5	2.8 ± 1.4
7	98.1 ± 3.2	2.6 ± 1.8
8	85.0 ± 15	2.9 ± 1.9
9	86.0 ± 14	2.4 ± 0.8
10	91.2 ± 8.7	2.5 ± 1.1
11	92.1 ± 7.8	3.4 ± 1.5
12	80.0 ± 0.5	2.3 ± 3.2
13	70.0 ± 0.3	2.8 ± 1.5
14	94.6 ± 5.3	2.0 ± 0.02
15	100	1.3 ± 0.4
16	78.3 ± 4.1	2.0 ± 0.1
17	73.0 ± 3.1	1.4 ± 0.2
18	83.0 ± 6.1	1.6 ± 0.1
19	70.0 ± 3.2	1.0 ± 0.3
20	100 ± 4.1	2.0 ± 0.9
21	82.0 ± 6.2	2.2 ± 0.5
22	75.0 ± 3.1	2.3 ± 1.2
23	80.0 ± 0.8	2.0 ± 1.1
24	83.0 ± 0.3	1.5 ± 1.1
25	91.6 ± 7.1	2.5 ± 1.9
26	100 ± 9.1	4.0 ± 0.4
27	83.0 ± 4.1	1.5 ± 0.9
28	91.6 ± 2.1	2.5 ± 1.2
29	100	4.0 ± 0.8
30	100	1.5 ± 0.7

Table 1
Effect of increasing parasite:host ratios (J2 *Strelkovimermis spiculatus*:second instar *Aedes aegypti* larvae) on parasite production parameters in mosquito rearing pans with 10 000 mosquitoes each.

J2/Host	Parasitism (%)	N[a]	J4 (g)	Range (g)	Efficiency[b] (%)	N° J4[c]	Sex ratio[d]
1:1	48.0 ± 14.6	1.2 ± 0.3	1.05 a	0.7–1.9	32	2 900	1.0:1.0
2:1	53.0 ± 24.3	1.2 ± 0.2	1.10 a	0.8–1.8	16	3 100	1.0:1.0
3:1	72.0 ± 15.0	1.3 ± 0.2	1.38 a	1.0–1.9	13	4 000	1.4:1.0
4:1	81.1 ± 8.1	1.7 ± 1.0	2.15 b	1.5–2.5	16	6 200	2.6:1.0
5:1	87.5 ± 10.9	2.0 ± 0.7	2.18 b	1.3–3.1	13	6 300	1.2:1.0
7:1	83.6 ± 6.8	1.9 ± 0.0	3.08 b	2.5–3.8	13	8 900	1.3:1.0
10:1	94.9 ± 9.5	3.1 ± 1.2	3.50 c	2.5–4.0	10	9 700	1.0:2.5

The same letter indicates no significant difference (Tukey, $p > 0.05$).
[a] N: Number of J4 (postparasitic) nematodes per host larva.
[b] Efficiency: percentage of J2 larvae emerging as J4 postparasites.
[c] N° J4: total yield of J4 mermithids per pan.
[d] Sex ratio: female/male.

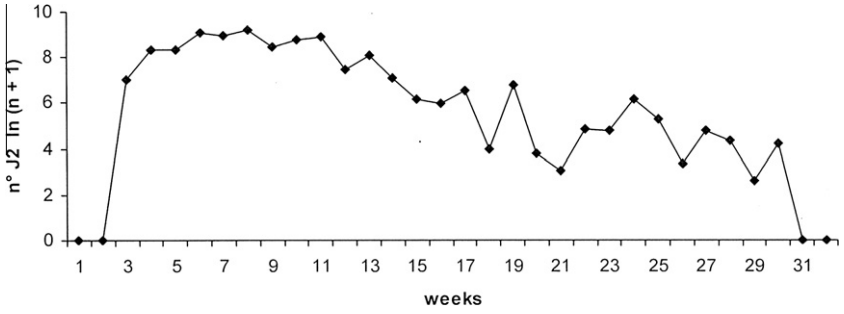

Fig. 1. Production of preparasites (J2) in *Strelkovimermis spiculatus* cultures permanently flooded.

3.2.2. Production of preparasites (J2) of S. spiculatus in nematode cultures subjected to desiccation

The maximum hatch of 2.1 × 105 J2s in cultures of *S. spiculatus* subjected to desiccation was obtained at the first flooding when the cultures were 5 weeks old (Fig. 2). These cultures then produced a total of 4.5 × 105 J2s hatched at the end of 10 weeks. Of the total number of preparasites obtained, 99% hatched in cultures flooded at up to the third week. Parasitic activity was observed throughout all the floodings, with parasitism levels ranging between 90.0 ± 14% and 100% and the number of nematodes/mosquito larva between 1.8 and 5.7 (Table 3). No significant differences were observed in the degree of parasitism or in the number of J2 nematodes/larva registered over the various weeks of the experiment ($p < 0.05$).

Table 3
Infectivity of the preparasitic stage (J2) in *Strelkovimermis spiculatus* cultures subjected to desiccation.

Week	Parasitism (%)	Number of nematodes per larva (J4)
1	94.3 ± 5.6	1.8 ± 0.33
2	94.1 ± 7.9	2.1 ± 0.7
3	95.0 ± 5.7	2.1 ± 0.5
4	97.0 ± 4.7	3.0 ± 1.6
5	100	3.3 ± 1.3
6	100	5.7 ± 0.5
7	90.0 ± 14.1	1.8 ± 0.6
8	95.0 ± 8.7	1.9 ± 0.8
9	8.1 ± 3.2	2.4 ± 1.8
10	93.3 ± 11.5	1.9 ± 0.8

4. Discussion

The establishment of a cost-effective control program using this nematode parasite is limited by the methods of mass rearing in vivo. Therefore, the development of appropriate procedures for the breeding, growth, and maintenance of *S. spiculatus* is needed. Accordingly, we evaluated the efficiency of postparasite production by testing increasing J2 concentrations (i. e., through varying the preparasite:host ratios). At 10 000 host larvae per rearing pan (a 10:1 ratio of J2/larva), we obtained maxima with respect to the yield of J4s, the number of J4s/larva, and parasitism frequency. This 10:1 ratio, however, resulted in a significantly male-skewed sex ratio. The sex of mermithid nematodes is influenced by the host sex, species, diet, and size as well as by the number of nematodes per larva (Petersen, 1972; Petersen and Willis, 1970). High levels of infection caused by some mermithid parasites can produce multiple parasitism so as to increase the number of males and thus potentially reduce the production of J2s (Camino, 1988; Camino and Reboredo, 1994; Petersen, 1972). In *S. spiculatus* laboratory trials, Camino (1988) obtained a sex ratio of 1:1 when the number of postparasites emerged per mosquito larva was between 1 and 5. When this number was increased to 6, the proportion of male nematodes became progressively greater until only males were observed at 10 nematodes/host larva. In the mermithid *R. culicivorax*, when the average number of emerged nematodes/larva exceeded three, the male:female ratio increased rapidly (Petersen, 1972). Therefore, higher proportion of female adults were produced when the level of parasitism was lower than 100% (Petersen, 1973). The maximum yield of J4s per tray with *R. culicivorax* was attained from a parasite:host ratio of 12:1 and a 94.8% parasitism of the host population (Petersen, 1978). Taher et al. (2008) determined that the highest infection rate and maximum production of nematode eggs for *S. spiculatus* was obtained at a ratio of 3:1 J2s/larva. Rodríguez-Rodriguez et al. (2003) evaluated the infective capacity of *S. spiculatus* for larvae of *Culex pipiens* under laboratory conditions in terms of the mean values for infection and the percentage of larval mortality and concluded that a dose of 7:1 would be optimum for mass rearing at high levels of parasitism (100%). Camino and García (1991), however, attained the same percentage of parasitism in *C. pipiens* larvae (100%), but only at the high ratio of 15:1 J2s/larva. In order to improve the mass rearing of *S. spiculatus*, we thus propose ratios of J2s:larvae that range between 4:1 and 7:1 as being adequate to obtain an optimum mass of nematodes (2.15–3.08 g) along with levels of parasitism at between 81.1 and 87.5%, efficiencies between 13% and 16%, and a female-predominant sex ratio.

In this study we also performed bioassays in order to evaluate different methods for introducing *S. spiculatus* into the specific ecological contexts in which the mermithid would be most likely to serve as an effective bioregulatory agent for the particular mosquito habitat present. Augmentation is defined as the deliberate release of a vector's natural enemies into its habitats with the express purpose of reducing the pest population there. The two principal augmentation strategies are inoculation and inundation. An inoculative release of a pathogen into an ecosystem is defined as the introduction of low numbers of a vector's natural enemies with the expectation of their reproducing within the environment to effect a long-term suppression of the vector. An inundative release refers to the release of a high number of parasitic organisms into an environment so as to produce an immediate decline in the vector population present (Woodring and Davidson, 1996). Mermithid mosquito parasites in particular could be used in an inoculative rather than an inundative strategy for bioregulation by introducing them into breeding sites having a substrate that would facilitate nematode burrowing, mating, and oviposition (e.g., pools, ponds, drainage ditches). Such an environment would permit the introduction of *S. spiculatus* in either the pre-or postparasites at inoculative-release levels, and in either instance the mermithid would first become established in the environment and then be

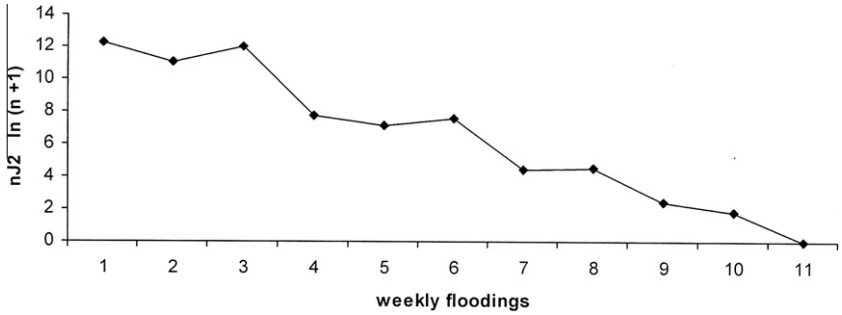

Fig. 2. Production of preparasites (J2) in *Strelkovimermis spiculatus* cultures subjected to desiccation.

recycled as a continuous and renewable bioregulator. Two key parameters of a given parasite population have to be considered before an inoculative release: the degree of parasitism that will ensue and the resulting sex ratio – the former in order to obtain a high level of larval mortality and the latter to maintain an adequate number of females to ensure continued high levels of oviposition.

Before introducing *S. spiculatus* as preparasites into a mosquito population, the concentration of J2s to be released must be determined from a previous sampling of the habitat that ascertains the mosquito numbers present. In our study high levels of parasitism were reached at the ratio of 10:1 J2/larvae. Similar data were registered for *A. aegypti* larvae at the same ratio with *R. culicivorax* (80–88%; Santamarina Mijares et al., 2000) or with *Romanomermis iyengari* (85–93%; Pérez-Pacheco et al., 2004; Santamarina Mijares and Perez Pacheco, 1998). Rodríguez-Rodriguez et al. (2005) proposed the use of a ratio of 10:1 J2/larvae to evaluate the effectiveness of *R. iyengari*, *R. culicivorax*, or *S. spiculatus* under natural conditions in artificial containers. In none of these studies, however, was the resulting sex ratio reported. Since we observed with *S. spiculatus* that this higher ratio produced an excessive number of males under our experimental conditions with *A. aegypti* larvae, we would recommend the use of ratios ranging between 4:1 and 7:1 J2s/larva for field trials with wild mosquito populations.

The introduction of the preparasites (J2) or postparasites (J4) involves a prior consideration of whether the habitat of the mosquito larvae in question is subject to either temporary or permanent flooding, conditions that would either facilitate or inhibit the nematode's establishment and recycling. Accordingly, in permanently flooded cultures we observed that from 1 g of total *S. spiculatus* nematodes, the J2 were present by the third week after inoculation. This timing agrees with the findings of Camino and Reboredo (1996), who reported a total interval of 35 days from the introduction of the postparasites to the initiation of oviposition by the resulting adult females. In our experiments, preparasites were observed until the thirtieth week, while the highest number of J2 mermithids was registered in the fifteenth week. Thus, a mere inoculation of J4 postparasites under these conditions would produce infection within the host populations between 1 and 3 months after release, though at this later period the levels would likely be low. For this reason, repeated applications would be required at fifteen-week intervals in order to increase and extend the levels of parasitism. Monthly applications of preparasites of *R. iyengari* on a larval *Anopheles pseudopunctipennis* population were able to control its further development. Those nematodes were also able to recycle and persisted for up to 4 months (Pérez-Pacheco et al., 2005). Likewise, *R. culicivorax* maintained viability in the same habitat for 3 months under natural conditions (Pérez-Pacheco et al., 2009). The introduction of postparasites of *R. culicivorax* in experimental ponds managed to last for 15 months (Platzer and Eby, 1980).

Future applications of *S. spiculatus* to mosquito breeding sites are needed, however, to determine the survival of this species of nematode under field conditions.

We observed a lower total number of J2 in cultures subjected to permanent flooding compared with those intermittently flooded. This difference could be a consequence of the nonsynchronous hatching of nematode eggs that can remain unhatched but viable for several months in permanently flooded cultures. By contrast, the periodic flooding with intervening dry periods simulated in the temporarily flooded cultures of our experiments would promote egg hatches. Accordingly, the eggs of the nematode *R. culicivorax* are capable of what has been referred to as installment hatching (Walker et al., 1985) previously defined by Gillet (1955) for mosquitos as a partial hatch of viable eggs upon exposure to periodic flooding. This characteristic has also been observed in floodwater mosquito species such as *O. albifasciatus*, the natural host of *S. spiculatus* (Campos and Sy, 2006). Prolonged mating and hatching would optimize the potential for nematode recycling in sites where multivoltine mosquito species are breeding. Future studies are needed in order to ascertain the hatching patterns of batches of *S. spiculatus* eggs under these different environmental conditions. Our results point to *S. spiculatus* as a suitable candidate for the inoculation of preparasites or postparasites into the habitats of floodwater mosquitos or permanent breeding sites since the nematodes could become easily established and readily recycled within those environments.

We conclude that even though the stocks of *S. spiculatus* need to be cultured on a mass scale in mosquito larvae under laboratory conditions, this mermithid would still be an excellent candidate for use as a mosquito-bioregulatory agent through strategies employing the introduction of J2s and J4s into the appropriate environment for enhancing their subsequent natural replication in the field.

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