Susceptibility of larvae of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) to entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidae)

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Susceptibilidad de larvas de *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) al nematodo entomopatógeno *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidae)

RESUMEN. *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) es vector de los agentes etiológicos de la fiebre amarilla y del dengue. Una alternativa al control químico de este vector es el uso de agentes biológicos. Los nematodos entomopatógenos son efectivos en el control de plagas. La infectividad y el ciclo de vida de un aislado argentino de *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) en larvas de *A. aegypti* se registró por primera vez bajo condiciones de laboratorio. Para cada unidad experimental, 30 larvas de mosquito de segundo estadio fueron expuestas a 8 dosis del nematodo (0:1, 1:1, 5:1, 15:1, 100:1, 500:1, 750:1, 1500:1). Los juveniles infectivos (JIs) utilizados fueron multiplicados sobre *Galleria mellonella* (Lepidoptera: Pyralidae). La continuidad infectiva de los JIs obtenidos de *A. aegypti* fue probada aplicándolos en una dosis de 100:1 sobre larvas del mosquito. Las tasas de mortalidad fueron de 0% a 84%. El número de nematodos desarrollados dentro de la larva de mosquito, la mortalidad larval y los nuevos JIs se incrementaron con el aumento de la dosis de nematodos. Los resultados indican que *H. bacteriophora* es capaz de infectar larvas de *A. aegypti*, se desarrolla y produce nuevos JIs, permitiendo la continuidad de su ciclo de vida.

PALABRAS CLAVE. *Heterorhabditis.* Ciclo biológico. Mosquitos. Infectividad. Control biológico.

ABSTRACT. *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) is a vector of etiological agents of yellow fever and dengue. An alternative to chemical control of this vector is the use of biological agents. Entomopathogenic nematodes are effective in pest control. The infectivity and life cycle of an Argentinean isolate of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) on *Aedes aegypti* larvae is registered for the first time under laboratory conditions. For each experimental unit, 30 second-instar larvae were exposed to 8 nematode doses (0:1, 1:1, 5:1, 15:1, 100:1, 500:1, 750:1, 1500:1). The infective juveniles (IJs) used were multiplied on *Galleria mellonella* (Lepidoptera: Pyralidae). The infective continuity of IJs obtained from *A. aegypti* was tested applying the nematodes in a 100:1 dose on mosquito larvae. Larval mortality rates ranged from 0% to 84%. The larval mortality, the num-

ber of nematodes developed inside mosquito larvae and the number of new IJs increased with the increase of the nematodes dose. The results indicated that *H. bacteriophora* is able to infect *A. aegypti* larvae, and these nematodes can develop and produce new IJs, allowing the continuity of its life cycle.

KEY WORDS. *Heterorhabditis.* Biological cycle. Mosquitoes. Infectivity. Biological control.

INTRODUCTION

Aedes aegypti (Linnaeus) (Diptera: Culicidae) is the most important vector for the transmission of etiological agents of yellow fever, dengue and dengue hemorrhagic fever (DHF) in America (Nelson, 1986; Salvatella-Agrelo, 1996; Simmons *et al.*, 2012). The historic direction of *Aedes* mosquito dispersion in South America has been towards higher latitudes and from tropical to sub-tropical areas, in particular in the Southern Region. Nowadays, the current geographical distribution of *A. aegypti* in Argentina has been expanded (Rossi *et al.*, 2006; Díaz-Nieto *et al.*, 2013). Insecticides are used in the reduction of larval and adult *A. aegypti* populations; however, the control success is limited by resistance to chemical products (Georghiou *et al.*, 1987; Mekuria *et al.*, 1991; Seccacini *et al.*, 2008; Bisset-Lazcano *et al.*, 2009). Alternative control strategies were implemented such as biological agents, environmental management and the use of insecticides of biological origin, providing modes of innovative action and reducing the risk of cross resistance (Pérez-Pacheco *et al.*, 1998; Parra *et al.*, 2007). Currently, several assays were carried out using, for instance, mermithid nematodes against *A. aegypti* and other mosquito larvae (Santamarina-Mijares, 1994; Santamarina-Mijares *et al.*, 2000; Achinelly *et al.*, 2004, Achinelly & Micieli, 2013; Sanad *et al.*, 2013). Furthermore, entomopathogenic nematodes (EPNs) of the genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae) provide an environmentally safe and economically reasonable alternative against a variety of important insect pests and some of them could be used commercially for biocontrol (Ehlers, 1996; Cagnolo *et al.*, 2010; Mbata & Shapiro-Ilan, 2010; Yan *et al.*, 2013; Zadji *et al.*, 2013). However, few studies were developed to demonstrate effectiveness of EPNs against mosquitoes (but see Poinar & Kaul, 1982; Molta & Hominick, 1989;

Cagnolo & Almirón, 2010). Recent assays have found that *H. bacteriophora* (Poinar) kills *Culex quinquefasciatus* mosquitoes faster and in a more effective way than other *Heterorhabditis* and *Steinernema* species (Zohdy *et al.*, 2013).

The infectivity, reproduction and size of progeny of EPNs may be related to nematode behavior, adaptation to a given host (ability to overcome the defense mechanism of host) (Kaya, 1990) and different developmental stages and size of the host (Murdoch *et al.*, 1997; Morgan & Hare, 1997; Boff *et al.*, 2000). Steinernematids and heterorhabditids are terrestrial organisms that move in the interstitial water between the soil particles (Begley, 1990; Cagnolo & Almirón 2010). However, the aquatic habitat offers an excellent environment for nematode survival (Pandii *et al.*, 2010). Cagnolo & Almirón (2010) demonstrated than IJs: (infective juveniles) can swim actively looking for the mosquito larvae. Moreover, mosquito larva movement favors the suspension of IJs into the water. Particularly, *A. aegypti* larvae collect detritus from the aquatic substratum and this feeding behavior leave the insects more exposed to nematodes than other mosquito species (Pandii *et al.*, 2010). This could make *A. aegypti* larvae easy targets for control with EPNs.

Heterorhabditid nematodes are symbiotically associated with the bacterium *Photorhabdus luminescens* (Boemare *et al.*, 1993). Bacteria are transmitted into the haemocoel of the host insects by a developmentally-arrested third juvenile stage, the infective juvenile. Reaching the host haemocoel, the IJs initiate the development and release of their symbiotic bacteria (Smart, 1995; Toledo *et al.*, 2006). The host dies within 48 hours after infection (García del Pino, 1994; Smart, 1995), although sometimes, it can continue its development for a while, causing the melanization of the nematode as a defensive reaction (Poinar & Kaul, 1982).

There is no research done until today describing the life cycle of the genus *Heterorhabditis* in *A. aegypti*. This information is fundamental to un-

derstand their persistence, reproductive potential and effect on insect host populations, and to develop predictive models for control programs (Ricci *et al*., 1996; Oğuzoğlu Ünlü & Özer, 2003). A comprehensive knowledge of the life cycle of the nematode and the reproduction potential in mosquitoes is needed as a prerequisite for genetic studies aimed at enhancing the nematode performance under field conditions (Zioni *et al*., 1992). Our study was performed in order to evaluate the infectivity and life cycle of *Heterorhabditis bacteriophora* on *A. aegypti* larvae.

MATERIALS AND METHODS

Mass rearing of mosquitoes and nematodes

Aedes aegypti larvae and nematodes used in the experiment were obtained from colonies maintained at the Laboratory of Parasitology (Facultad de Ciencias Exactas, Físicas y Naturales, UNC). Mosquito larvae were collected from artificial containers and kept in the laboratory according to conventional rearing techniques, with larvae at a standardized density and level of nutrition 1,000 immatures/3 liters of water and fed on a 1:1 mixture of liver powder and brewer's yeast (Gerberg *et al*., 1994). Females were fed 5-7 days after emergence on mouse blood and then held for 6 days at 27-29 °C and 85-90% relative humidity under a photoperiod of 14:10 h light: dark. A 10% sugar solution was provided continuously.

The nematodes came from the breeding of strain detected in 2005 in Cordoba Capital, Argentina (Cagnolo & Carranza, 2007). They were multiplied on *Galleria mellonella* (Lepidoptera: Pyralidae) larvae, because of its ideal size and susceptibility to most known EPNs (Hyrsl, 2011), which allow the standardized mass production of the EPNs (Ehlers & Shapiro Ilan, 2005). The host used was reared in aerated plastic boxes at room temperature (25 ± 1 °C) on an artificial medium containing 15.4% corn flour, 15.4% wheat flour, 15.4% three-cereals mixture, 10.6% powdered milk, 5.4% brewer's yeast, 13.5% glycerin and 24.3% honey, following standard techniques (Stanuszek, 1974; Poinar, 1975; Cagnolo *et al*., 2004).

The IJs obtained were stored at 10 °C in tissue culture flasks for 20 days before being used for experiments (Kung *et al*., 1990). During the storage, the IJs were not fed because they can utilize their own food reserves as an energy source (Smart, 1995; Burnell & Stock, 2000). Before the assays, viability was confirmed by observing nematode activity (rapid wiggling) under a binocular microscope (Laznik *et al*., 2010).

Infectivity of *H. bacteriophora* on *A. aegypti* larvae

Infection experiments were carried out using cohorts of 30 second-instar larvae put in plastic trays (10.5 x 12 x 4 cm), containing 80 ml of water and maintained at 26 °C. The concentration of the IJs used in these experiments ranged from 0 to 45000 IJs per tray (i.e. parasite: host ratios of 0:1 (control), 1:1, 5:1; 15:1, 100:1, 500:1, 750:1 and 1500:1). The culture flasks with stored IJs were diluted to a final volume of 250 ml with sterile water. An estimation of the number of IJs present in 1 ml of each solution was carried out by counting the nematodes under a magnifying glass (Arcano®, X40). This technique was repeated 10 times for each solution. A total of 120 mosquito larvae per each dosage were analyzed.

Once each parasitized mosquito larva died, it was transferred individually to a Petri dish containing 3 ml of boiled water, in order to make daily observations of each mosquito larva and the development of nematodes inside it. Daily observations and nematode counting were made for each mosquito larva under stereomicroscope during a 30-day period after infection. We recorded the number of dead larvae and the time from nematode-host exposition to the larval death. We determined the developmental time of hermaphrodite nematodes, the time until the emergence of juvenile out of the hermaphrodite female body and the time until the IJ emergence from the larval host. We recorded the total number of IJs that entered the mosquito larvae, the number of hermaphrodites developed, the number of melanized nematodes by the insect defensive reaction, the number of generations produced inside the insect and the total number of new IJs originated by the nematode reproduction inside the host. The different stages of the life cycle such as hermaphroditic females, females and males were identified by morphological characters (according to Zioni *et al*., 1992).

Infectivity and continuity of cycle of *H. bacteriophora* IJs obtained from *A. aegypti* host

The *H. bacteriophora* IJs obtained from *A. aegypti* host in the previous experiment were

stored in plastic boxes (12 x 25 cm) at 10 ºC, in order to use them later to determine the *H. bacteriophora* capacity for maintaining the infectivity and continuity of the life cycle in *A. aegypti* host. These characteristics were assessed infecting the mosquito larvae with IJs obtained from *A. aegypti* host and comparing them to mosquito larvae infected with IJs obtained from *G. mellonella* host, which is widely used as a highly susceptible model organism in laboratories studying EPNs. For the infections we used a 100:1 dose, because its application on the mosquito host generated the highest number of IJs (Table I).

Statistical analysis

We used Shapiro-Wilk's test for normality and Levene's test for homogeneity of variance. When data distribution was not normal, they were previously transformed. A one-way analysis of variance (ANOVA) and Fisher LSD test were performed to compare mean values of larval mortality percentage, hermaphrodite nematode produced, melanized nematode, IJs that entered the mosquito larvae and new IJs produced into *A. aegypti*. Data obtained were statistically analyzed using probit analysis, after a test for correlation between dosage and mortality using the PASW Statistic 18.0.0.

A linear regression between the mean number of melanized nematodes and the mean number of IJs that entered the mosquito larvae was performed. Mean values of infectivity of nematodes produced from mosquito larvae with IJs obtained from *G. mellonella* and *A. aegypti* host were compared by Student's *t*-test.

RESULTS

#### Infectivity of *H. bacteriophora* on *A. aegypti* larvae

Aedes aegypti larval mortality varied between 0% and 84%, with significant differences between the doses tested (F = 31.46, P <0.0001, df = 7). There was an increasing mortality in mosquito larvae with increasing doses. The higher percentages of parasitism 77.5%, 78.3% and 84.2% corresponded to the dose 1500:1, 500:1 and 750:1, respectively (Fig. 1).

A significant (r^2 = 0.94) linear regression was

obtained with the probit-transformed mortality of the larvae against the logarithm of the nematode doses. The mean lethal dose (LD_{50}) of *H. bacteriophora* predicted from the probit analysis was 184 per mosquito larva (Fig. 2).
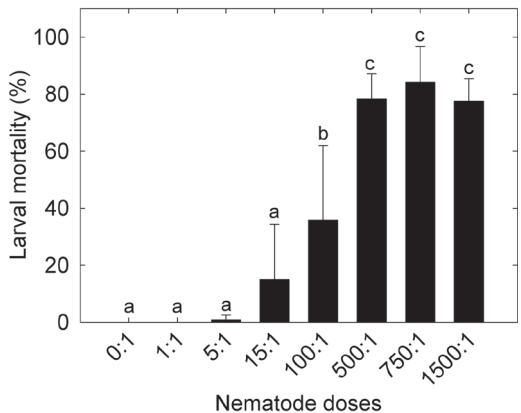
Fig. 1. Mean percentage (± Standard deviation) of mortality of *Aedes aegypti* larvae for each nematode dose. Different letters between columns indicate significant differences (P < 0.05).
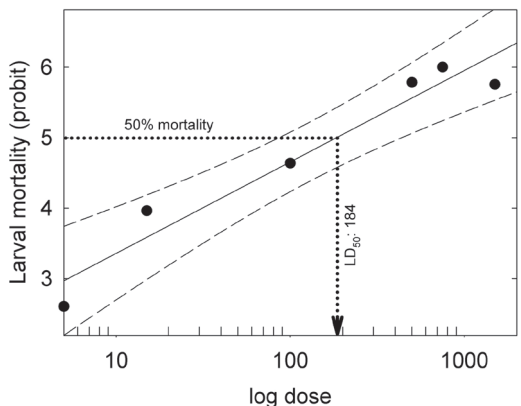
Fig. 2. Relationship between mortality of *Aedes aegypti* larvae and the dose of infective juveniles nematodes. Solid line is the linear regression fitted to the data (r^2 = 0.94; P < 0.05) and dash lines are the confidence limits at 95% of the mean.

The mortality of mosquito larvae started to be registered 48 hours after exposition to the nematodes. Because the contact with nematodes was not interrupted, re-infections by IJs and death of larvae, in the third- and fourth-instar, were recorded for 216 hours (9 days).

The cycle of *H. bacteriophora* inside *A. aegypti* larvae lasted 3 to 8 days since the death of

the insects (Fig. 3). A greater number of nematodes got into the host at higher doses, although this number was significantly higher since dose 100:1 (F = 28.01, *P* <0.0001, df = 7) (Table I).

The IJs which got into the mosquito larvae developed to adult individuals (hermaphrodite females) between the first and third day after the death of the larvae. Hermaphrodite females were identified by presents full of eggs and their vulva was protruding. Hermaphrodite nematodes produced from the IJs were registered since the dose 15:1 and significant differences were observed between doses (F = 6.64, *P* < 0.001, df = 7) (Table I). Hermaphrodite development was registered in 73.1%, 69.0%, 64.8%, 63.0% and 53.1% of the parasitized larvae, corresponding to the doses of 15:1, 1500:1, 750:1, 100:1 and 500:1, respectively.

Melanization was observed since dose 15:1 and the number of melanized nematodes increased with higher doses (F = 20.93, *P* <0.0001, df = 7), but this number did not differ significantly at the higher doses 100:1, 500:1, 750:1 and 1500:1 (Table I). We found melanized nematodes in the abdomen (50.3%), thorax (39.0%), head (6.4%) and neck (4.3%) of the mosquito larvae. A positive linear relationship between the mean number of melanized nematodes and the mean number of nematodes that entered the host was found (r^2= 0.95, *P* <0.001) (Fig. 4).

The endotokia matricida phenomenon (*Heterorhabditis* females death because of the intra-uterine development of IJs, Johnigk & Ehlers 1999) was recorded by observation of hermaphrodite individuals after 1-4 days, and its duration varied from a few hours to two days. Juvenile nematodes that left the maternal body at the end of the endotokia matricida emerged from the body of the mosquito immediately after or within 5 days. A single generation of nematodes was observed in the 70% of the parasitized larvae, while a second generation was observed in a variable percentage of larvae according to the dose (Fig. 5). Generation of new IJs was observed at the end of the parasitic cycle, with the higher production being registered at the 100:1 and 750:1 doses, which did not differ significantly (F = 5.38, *P* < 0.001, df = 7) (Table I).

Infectivity and continuity of cycle of *H. bacteriophora* IJs obtained from *A. aegypti* host

The mortality percentages of *A. aegypti* larvae, the number of hermaphrodite nematodes produced, the number of melanized nematodes, the number of IJs that entered the mosquito larvae, the number of new IJs obtained in either the first or second generation of nematodes and the time from nematode-host exposition to the larval death (time of death) did not differ significantly between IJs obtained from both *G. mellonella* and *A. aegypti* host in a 100:1 dose on *A. aegypti* larvae (*P* > 0.05, Table II).

Table I. Mean number and (±) standard deviation of adult hermaphrodite nematodes, melanized nematodes produced, IJs that entered the mosquito larvae and new infective juveniles obtained in either the first or second generation of nematodes (new IJs) produced into larvae of *Aedes aegypti*. Means followed by the same letter are not significantly different (*P*< 0.05, Fisher LSD test).

Dose (Parasite/host)	Hermaphrodite nematodes	Melanized nematodes	IJs that entered the host	New IJs
0:1	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
1:1	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
5:1	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
15:1	0.53 ± 0.50[ab]	1.07 ± 0.93[b]	1.60 ± 1.39[b]	15.54 ± 31.08[ab]
100:1	1.10 ± 0.44[bc]	2.08 ± 0.62[c]	3.18 ± 0.22[c]	168.18 ± 164.79[c]
500:1	1.06 ± 0.49[bc]	2.27 ± 0.36[c]	3.33 ± 0.27[c]	47.27 ± 39.47[bc]
750:1	1.50 ± 0.51[c]	2.48 ± 0.02[c]	3.98 ± 0.51[c]	109.89 ± 118.84[c]
1500:1	1.89 ± 1.01[c]	2.20 ± 0.27[c]	4.09 ± 0.75[c]	62.82 ± 24.96[bc]

Table II. Mean number and (±) standard deviation of mortality percentages of *A. aegypti* larvae, hermaphrodite nematodes produced, melanized nematodes, IJs that entered the mosquito larvae, new infective juveniles obtained in either the first or second generation of nematodes (new IJs) and the time from nematode-host exposition to the larval death (time of death) for mosquito larvae exposed to 100:1 dose of IJs obtained from both *G. mellonella* and *A. aegypti* host. Means followed by the same letter are not significantly different (*P*< 0.05, t-test).

	Mortality of *A. aegypti* larvae (%)	Hermaphrodite nematodes	Melanized nematodes	IJs that entered the host	New IJs	Time of death (days)
Infection of mosquito with IJs obtained from *G. mellonella*	35.84 ± 26.16[a]	1.10 ± 0.44[a]	2.08 ± 0.62[a]	3.18 ± 0.22[a]	168.18 ±164.79[a]	3.80 ± 0.59[a]
Infection of mosquito with IJs obtained from *A. aegypti*	62.22 ±18.36[a]	2.15 ± 1.60[a]	1.75 ± 0.12[a]	3.90 ± 1.67[a]	99.08 ± 57.24[a]	6.73 ± 3.47[a]
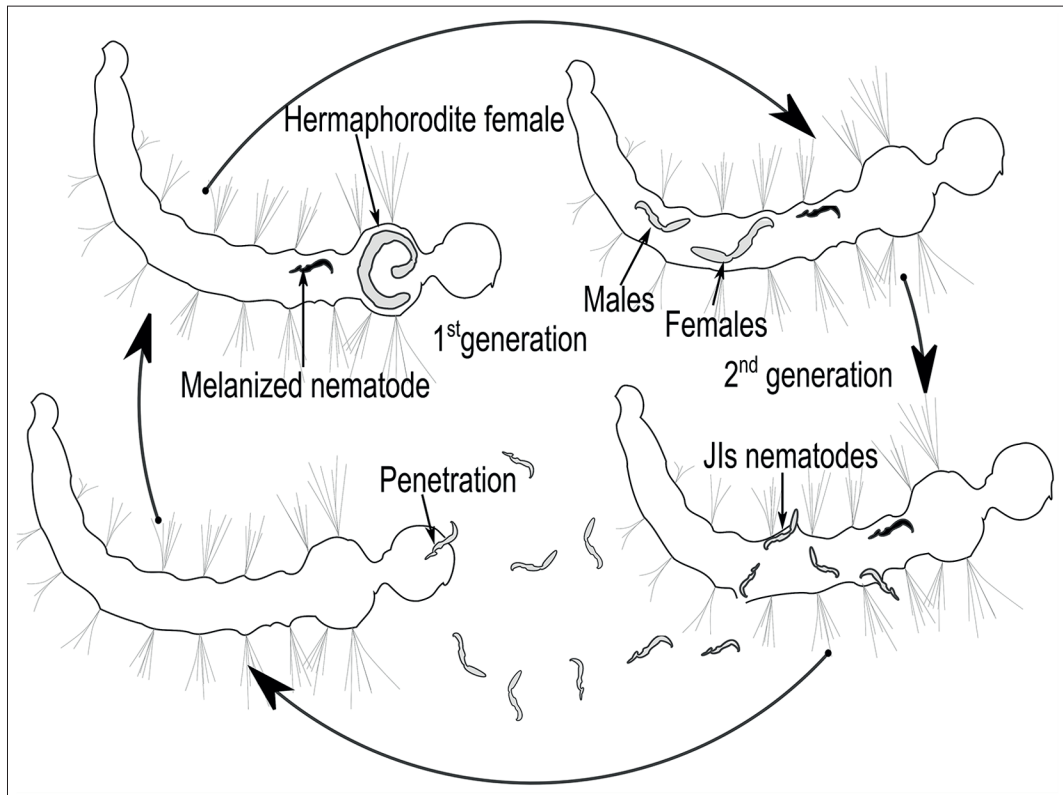
Fig. 3. Schematic diagram showing the life cycle of *Heterorhabditis bacteriophora* inside *Aedes aegypti* larvae.

DISCUSSION

The parasitism and life cycle of *Heterorhabditis bacteriophora* on *A. aegypti* is registered for the first time under laboratory conditions. We found that *A. aegypti* is highly susceptible to various doses of this native nematode strain, reaching 84% mortality at 750:1. These results are consistent with those found for this mosquito species in its third larval stage with

other strains of EPNs such as *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann) and *Steinernema feltie* (Filipjev) for which parasitism rates were high and positively correlated with the dose of applied nematodes (Molta & Hominick, 1989). Similar mortality was found by Poinar & Kaul (1982) in *Culex pipiens* hosts. The LD_{50} of 184 IJs per mosquito larvae was consistent with the results obtained by Molta & Hominick (1989) and Poinar & Kaul (1982).

Mortality of *A. aegypti* was detected between the second and ninth day after the nematode-host exposition, it was independent from the dose, and observed in larvae of third and fourth stage. Similar results were found in *Steinernema carpocapsae* (Weiser) parasitizing *Culex gelidus* (Theobald) (Pandii *et al*., 2010). The total number of nematodes which entered the mosquito larvae increased as the dose increased and this was consistent with the study of Poinar & Kaul (1982) and Zohdy *et al*. (2013).

The life cycle of *H. bacteriophora* on *A. aegypti* host lasted from 3 to 8 days at 26-28 ℃, measured since infection until the emergence of new IJs. However, other studies in *G. mellonella* host found that the emergence of the *H. bacteriophora* nematode is slower (about 12-18 days) (García del Pino, 1994; Burnell & Stock, 2000; Baliadi *et al*., 2009), because the time to first emergence increases with the increase of the host size (Boff *et al.,* 2000).

The number of melanized nematodes was higher when the number of nematodes inside the mosquito larvae was increased. These contrasts with what was observed in *H. heliothidis, S. feltie*, in which it was found that melanization reaction decreases with increasing number of nematodes entering dipterans as *A. aegypti* and *Tipula oleracea* (Linnaeus) (Molta & Hominick, 1989; Peters & Ehlers, 1997). With other strains of nematodes, it was noticed that there is a greater likelihood that IJs are melanized when they are present in a number below or equal to seven (Molta & Hominick, 1989) and nematodes can develop and release the bacteria into haemocele when they attain a certain number of melanized nematode (Poinar & Kaul, 1982). Petersen & Willis (1974) suggested that melanization of nematodes occurs in the haemocele, yet many melanized nematode hosts also have one or more developing nematodes. The biggest number of melanized nematodes can be found
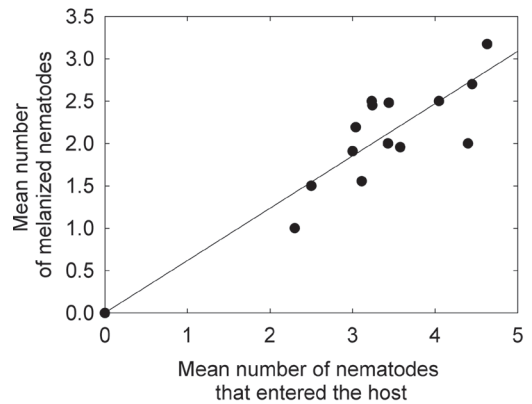
Fig. 4. Mean number of melanized nematodes in relation to mean number of nematodes that entered *Aedes aegypti l*arvae. The line is the linear regression fitted to the data (r^2= 0.95, *P* <0.001).
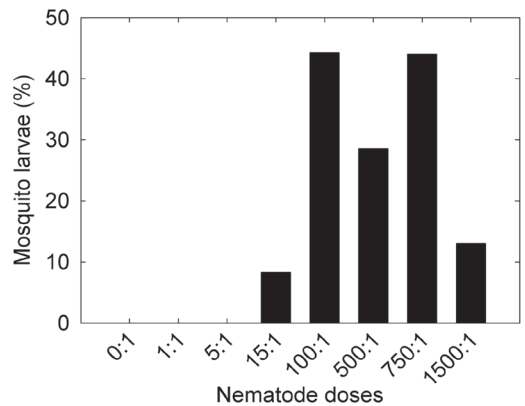
Fig. 5. Percentage of mosquito larvae in which a second generation of nematodes in each dose tested is developed.

in the abdomen and thorax of the mosquito larvae, although Pandii *et al.* (2010) found than the nematode infestation was greatest in the thorax and the head of host.The non- melanized IJs continued their development within the host. The nematodes molted to fourth instar larvae and the formation of hermaphrodite individuals was subsequently observed between the first and the third day after the death of the diptera. Similar results were obtained by Baliadi *et al.* (2009), who reported *H. bacteriophora* hermaphrodites developed 4 days after nematode inoculation in *G. mellonella.*

The second generation was developed in less than half (45%) of parasitized larvae. In studies of Baliadi *et al.* (2009) nematode reproduction in *G. mellonella* continued over two or three generations until the nutrient status of the insect cadavers deteriorated whereupon adult

development was suppressed.

At the end of the parasitic cycle, the generation of new IJs was observed and their number varied depending on the dose. With all the doses tested, IJ production was found, except for 1:1 and 5:1 dose. Similar results were found in *Steinernema rarum* (Doucet), parasitizing *Culex apicinus* (Philippi) larvae, where with the highest doses tested (100:1; 400:1), the nematodes infected the mosquito larvae, established inside them, and multiplied producing a new generation of IJs (Cagnolo & Almirón, 2010). The successful reproduction of *H. bacteriophora* in all of the studied doses was possible because this species can survive at higher nematode densities than the other ones (Zohdy *et al.*, 2013).

In our work, the largest number of individuals was obtained with doses 100:1 (168.2 IJ / larvae) and 750:1 (109.9 IJ / larvae). At both doses, there was a higher percentage of second generation of nematodes, which would explain the higher reproductive potential in those doses. Other studies that consider the production of new IJs of *H. bacteriophora* were in *G. mellonella* as a host, and the number obtained was much higher (Wang & Bedding, 1996; Burnell & Stock, 2000; Oğuzoğlu Ünlü & Özer, 2003; Baliadi *et al.*, 2009). It is known that IJ production can vary depending on host species and the body dimensions of the host larvae (Lindegren *et al.*, 1993; Boff, 2001). Nutritional deficiency seems a key factor for IJ development via endotokia matricida (Baliadi *et al.,* 2001). *Aedes aegypti* is a smaller species than *G. mellonella*, and then the amount of nutrients available for the development and reproduction of nematodes is also smaller (Dutky *et al.,* 1964, Boff, 2001). Boff *et al.* (2001) demonstrated that IJs originating from small hosts show the highest infectivity against all sizes of host species. These authors also found significant differences in infectivity between IJs originating from different host species. Then the conditions in which IJs are grown have a severe impact on their later infectivity and performance (Boff, 2001).

In laboratory studies, we found that the infection of mosquito larvae with IJs produced into a susceptible host model like *G. mellonella* and IJs generated from *A. aegypti* hosts produced new IJs of similar number and infectivity. Once these nematodes are applied, they not only could control the population density of mosquito larvae, but could also maintain the continuity of their biological cy-cle. This aspect should be taken into account for future studies on the field release of EPNs; because long term persistence in the environment depends on the IJ ability for maintaining its infectivity and productivity (Padmanaban, 2012).

Moreover, 100:1 and 750:1 doses had the highest generation of new IJs and since 500:1 dose an increase in mortality was observed. The 750:1 dose would be the most appropriate to be applied in future biological control trials because at this concentration, with a smaller number of applied nematodes, they could effectively reduce the number of mosquito larvae. At the same time this number will be sufficient to allow the propagation of new infective forms. However, these laboratory bioassays do not provide an assurance of field efficacy, so, field application studies at greater scales should be done. Nevertheless, they guaranteed the promising value of using *H. bacteriophora* in controlling the aquatic larvae.

The relevance of this work resides in the demonstration of the pathogenic capability of an *H. bacteriophora* isolate against *A. aegypti* mosquito larvae. *Heterorhabditis bacteriophora* successfully completed its life cycle within the host larvae until the adult stage and IJs emergence in few days, being this species able to reach higher IJs number with lower number of nematodes that entered the mosquito larva. Characteristics of infection strategies, host mortality and particular biological cycle give *H. bacteriophora* a promising horizon on the possibility of using these nematodes for mosquito larva control.

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