Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i3.16034

Biological characterization of the colombian isolate *Heterorhabditis* sp. SL0708 (Rhabditida: Heterorhabditidae)

María Camila Mejía Torres[1*] and Adriana Sáenz Aponte[2]

*[1]Pontificia Universidad Javeriana, Carrera 7a, 43-82, Ed. Jesús Emilio Ramírez-54, Bogotá, Colombia. [2]Laboratorio de Control Biológico, Unidad de Ecología y Sistemática, Departamento de Biología, Pontificia Universidad Javeriana, Bogotá, Colombia. *Author for correspondence. E-mail: mariacamila30@gmail.com*

ABSTRACT. *Heterorhabditis* sp SL0708 (Rhabditida: Heterorhabditidae) is a native entomopathogenic nematode from Alcalá, Valle del Cauca (Colombia), a natural enemy of insects that can be used for controlling *Plutella xylostella* L, *Conotrachelus psidii* Marshall and *Delia platura* (Meigen); however its biological characterization is unknown. In order to know about the infective capacity of the isolate, tests were made with last instar larvae of *Galleria mellonella* (Lepidoptera: Pyrallidae) on penetration, one by one, exposure time, dose response and foraging strategy. The average infecting juveniles (IJ) entering larvae was 3 (1.5%). In the one-on-one test, larvae mortality ranged between 8.3 and 16.7%. There was no significant difference ($p < 0.05$) between treatments and in number of dead larvae in the exposure time and dose response assays. *Heterorhabditis* sp. SL0708 presented a cruiser foraging strategy, which indicates that it can be used for sessile or cryptic hosts.

Keywords: entomopathogenic nematodes, infective juvenile, penetration, Heterorhabditidae, virulence.

Caracterização biológica do isolamento colombiano *Heterorhabditis* sp. SL0708 (Rhabditida: Heterorhabditidae)

RESUMO. *Heterorhabditis* sp.SL0708, nematoide entomopatogênico nativo de Alcalá, Valle del Cauca (Colômbia), é um inimigo natural que pode ser usado para o controle de *Plutella xylostella* L, *Conotrachelus psidii* Marshall e *Delia platura* (Meigen), porém, a sua caracterização biológica é desconhecida. Assim, ensaios de penetração, um a um, tempo de exposição, exposição a diferentes doses e estratégia de forrageio foram realizados com lagartas de último ínstar de *Galleria mellonella* (Lepidoptera: Pyrallidae). A média dos juvenis infectantes (IJs) que ingressaram na lagarta foi 3 (1,5%). No ensaio um a um a mortalidade das lagartas oscilou entre 8,3 e 16,7%. Não foram encontradas diferenças significativas entre os tratamentos e o número de lagartas mortas no tempo de exposição e dose. *Heterorhabditis* sp., apresentou uma estratégia de forrageio de cruzeiro, o que indica que pode ser usado para hospedeiros com hábitos sésseis ou crípticos.

Palavras-chave: nematóides entomopatogênicos, juvenis infectantes, penetração, Heterorhabditidae, virulência.

Introduction

The most important entomopathogenic nematodes (EN) in biological pest control correspond to the Steinernematidae and Heterorhabditidae families, whose members are mutually associated with bacteria from the *Xenorhabdus* and *Photorhabdus* genera that cause septicemia and other types of lethal afflictions in their hosts (ADAMS et al., 2006). Infective juveniles (IJs) penetrate the natural openings (mouth, anus, spiracles) or go through the cuticle and release the bacteria into the host hemocel, causing death (HAZIR et al., 2004; MORTON; GARCÍA-DEL-PINO, 2009). One or two generations are presented at the moment of emergence; the IJs abandon the cadaver and disperse controlling thus the insects in the area where they are (KAYA, 1990; SÁENZ, 2005).

Other attributes of this promising group of biological controllers to manage insect pests is the high virulence and rapid action when killing the host; the IJ does not feed, it is morphologically and physiologically adapted to survive for long periods in the soil in the absence of its host. Infective Juveniles have high reproductive potential and show numerical response with respect to the host; they can be bred massively in the laboratory; have a broad range of action, although some are not very specific; they have high resistance to chemicals and to adverse environmental conditions; EN like their bacteria are innocuous to human and domestic animals; they do not damage plants because they are specific to insects; some species can be reproduced without the presence of the male and are exempt from registration for commercialization in Europe and the United States (GAUGLER, 2002; SÁENZ; OLIVARES, 2008).

The EN field efficacy for pest control is affected by factors associated with the nematode (range of invasion, time of exposure, foraging, and release of bacteria), the bacteria (range of multiplication and establishment), the host (susceptibility, behavior, and immune response), and the environment (location of the host, temperature, humidity, pH, soil composition and texture) (GREWAL et al., 2005). These factors are determinant for the selection of the EN species and/or strains as control agents and, especially, studies are required to recognize the new isolates that can be used in comprehensive pest management programs.

In Colombia, different EN species have been registered (MELO et al., 2009; SÁENZ; LÓPEZ, 2011) among which there is the isolate from Alcalá, Valle del Cauca, *Heterorhabditis* sp. SL0708 (SÁENZ; LÓPEZ, 2011) whose life cycle is known, but its virulence is not. Because of such, the objective of this study was to establish the biological characterization focused on the IJ activity related with penetration, time of exposure, one-on-one, exposure to different dosages and foraging.

Material and methods

General conditions of the assays

The assays were conducted at 25ºC under darkness in the biological control at Pontificia Universidad Javeriana (PUJ). For reactivation, multiplication of IJs and development of the bio-assays, last instar *Galleria mellonella* (Lepidoptera: Pyrallidae) larvae were used, obtained from Bioagro S. A.

Penetration of IJs

Forty larvae were exposed for 48h to 0 and 200 IJs in ½-oz plastic containers with 2 g of sterile river sand at field capacity. After the incubation period, larvae were washed with distilled sterile water to remove the IJs present in the cuticle and the percentage of mortality was recorded. Each larva was dissected under stereomicroscope, following the recommendations of Glazer and Lewis (2000); the number of nematodes was counted and the percentage of penetration was calculated

$$P = \frac{N \times 100}{T}$$

where:

N: number of nematodes found in each cadaver;

T: number of nematodes applied in each experimental unit). The assay was repeated twice.

Time of exposure

The larvae were exposed to 200 IJs for 0.5, 1, 2, 3, 6, 9, and 12h in 140 ½-oz plastic containers with 2 g of sterile river sand at field capacity. After exposure time, larvae were washed with sterile distilled water to remove nematodes present in the cuticle and were incubated in 5 cm Petri dishes with sterile filter paper until completing 48h. The percentage of mortality was calculated; larvae were dissected and the number of nematodes present in the cadavers was counted. This assay was repeated twice.

One-on-one assay

A total of 48 larvae were exposed to 0 and 1 IJ in Falcon multiwell culture plates with sterile filter paper. At 72h, the number of dead larvae was counted, the surface was washed with distilled sterile water to remove the IJs present in their cuticle, and the larvae were dissected. The percentage of mortality was calculated from the number of larvae with presence of IJs. The assay was repeated twice.

Exposure to different dosages

Larvae was exposed to 0, 1, 5, 15, 50, 100, and 200 IJs in 140 ½-oz plastic containers with 2 g of sterile river sand at field capacity. This was incubated and the mortality of larvae was recorded at 24, 48, 72, and 96h. This assay was repeated twice.

Foraging

Twenty plastic cylinders (4 cm high; 4.5 cm diameter) with 80 g sterile river sand at field capacity were partially divided into four 1-cm parts. On the upper part, 1000 IJs were added. Each segment was separated with removable metal sheets at the end of the assay to count the number of IJs per section and establish the displacement along the cylinder.

A larva was added at the base of 10 cylinders and to impede vertical displacement, a metallic mesh 4.5 cm diameter was placed at 2 cm. Each cylinder was covered and incubated in darkness for 72h. At the end of the incubation, larvae were removed and washed to remove the nematodes present in the cuticle. Dead larvae were dissected to count the number of penetrating IJs and live larvae were incubated for 48h.

To establish IJ displacement on the cylinder, each section of sand was processed in Baermann funnels and an IJ count was performed. This assay was repeated twice.

Statistical analysis

The penetration assay calculated the percentage of IJs entering the larvae. The one-on-one and foraging strategy assays calculated the average of IJs present in each count. The assays of time of exposure and exposure to different dosages were organized on a completely randomized design (CRD) with seven treatments; their analysis was made via ANOVA and a Tukey's test at 0.05%.

Results and discussion

Mortality was registered at 50% at 48h in *G. mellonella* and an average of 3 IJs/larva, corresponding to 1.5% penetration. The penetration measured the percentage of IJs entering the host and it is determined by the location of the larvae on the substrate (surface or buried) (GREWAL et al., 1994; KAYA et al., 1993), substrate temperature and texture (soil with high proportion of sand are most favorable, allowing IJ movement) (BERNAL et al., 1998; MUKUKA et al., 2009), the host to which the nematodes are exposed (BOFF et al., 2001; ROSA et al., 2002), time of exposure and capacity of IJs to enter via the insect's mouth, anus, spiracles, or cuticle (SÁENZ; LÓPEZ, 2011). For the *Heterorhabditis* sp. SL0708 native nematode, the range of penetration is between 1 and 8 IJs, corresponding to that reported by Glazer and Lewis (2000) for Heterorhabditidae and to that established for this same host by Caroli et al. (1996). However, Caroli et al. (1996) for HP88 and NJ strains from *H. bacteriophora* (Poinar) in the *Tenebrio molitor* L hosts (Coleoptera: Tenebrionidae), *Spodoptera exigua* (Hubner) and *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), established that the range of penetration reaches up to 12.3 IJs within 48h. This difference in the range of penetration can be determined by the time of exposure needed by an IJ to enter the host and overcome the insect's barriers.

No significant difference was detected among exposure times (df = 6, 133; f = 0.74, p = 0.620), and upon performing dissections IJs were found in exposed larvae as of two hours. Twelve hours of exposure were not sufficient to reach 50% larvae mortality (Figure 1).

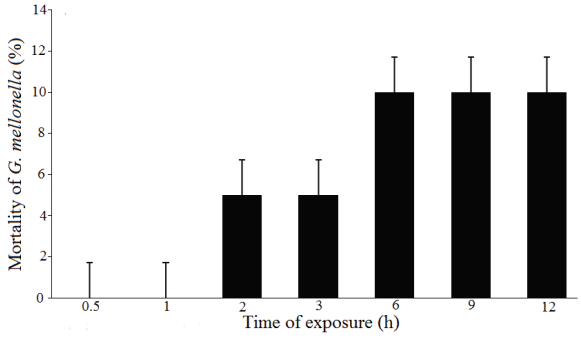

Figure 1. Mortality of the last larval instar of *Galleria mellonella* exposed to *Heterorhabditis* sp. SL0708 during six exposure times. Error bars were calculated using the standard deviation of each treatment.

Heterorhabditis sp. SL0708 need two hours of exposure to enter the *G. mellonella* and possibly more than 12 h of exposure to increase the number of IJs entering the host and causing a percentage of mortality above 50%. This is because the mortality percentage of *G. mellonella* larvae increases gradually with time of exposure. Similar mortality results were obtained by Chung et al. (2010) with *H. bacteriophora* Jeju and Hamyang strains, with 80% mortality as of 36 h in this same host. For *Pseudaletia unipuncta* (Gueneé) (Lepidoptera: Noctuidae), the percentage of mortality can be lower when exposed to *H. bacteriophora* Az29 (40 and 60%), Az32 (18 and 25%) (ROSA et al., 2002).

The one-on-one assay registered a range of mortality between 8.3 and 16.7% and in the dosages evaluated 100 and 200 IJs presented the greatest mortality between 24 and 48 hours, while in those with lower dosages (1, 5, 15 IJs) mortality had not exceeded 30% during the times of evaluation. There were no significant differences for the sampling hours among dosages (df = 6, 133; f = 124.56, p = 1.000), but there were significant differences in the number of dead larvae per treatment (df = 6, 133; f = 30.6, p = 0.0001), (Figure 2) in the one-on-one assay, and where the virulence of the *Heterorahbditis* sp. SL0708 nematode, measured by the mortality of the insect, was not above 17%.

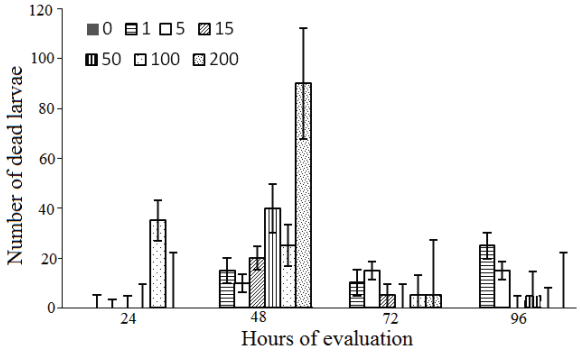

Figure 2. Mortality of *Galleria mellonella* exposed to seven dosages of *Heterorhabditis* sp. SL0708 IJs. Error bars were calculated using the standard deviation of each treatment.

These results agree with Ricci et al. (1996), who established this same percentage of mortality with *H. bacteriophora* in *G. mellonella,* but it is a lower percentage to that reported by Enright and Griffin (2005) who recorded 50% mortality with *H. megidis* during more than 72 hours. Gaugler et al. (2000) evaluated a ratio of 5:1 with *H. bacteriophora* and *G. mellonellao O,* obtaining a percentage of mortality at 70%. This difference in the percentage of mortality may be determined by the capacity of the IJs entering the strains studied to which the hosts are exposed, the host species, and the ratio of the number of IJs present in the substrate. Besides the aforementioned, the competition by the IJs to enter, the immune and behavioral response of the host are other characteristics that may probably influence on

the variability in the percentage of mortality in the assays of high dosages (100-200 IJs) and low dosages (5, 10, 15, and 50 IJs), (CHUNG et al., 2010; KOPPENHÖFER et al., 2006).

The *Heterorhabditis* sp.SL0708 IJs have displaced 4 cm along the sand column from the point of inoculation in the presence or absence of the host and there were significant differences in the number of IJs per segment (df = 1, 18; f = 102.69, p = 0.0001). In the columns without larvae, a great number of IJs was recovered, especially in the first two segments and in columns with larvae in the last segment (Figure 3).

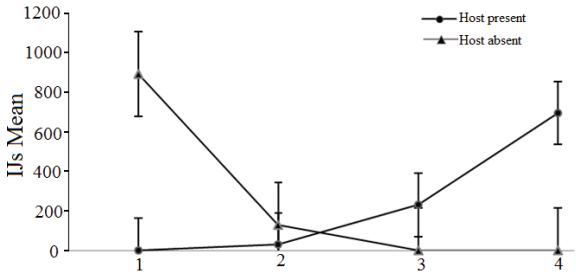

Figure 3. Average of *Heterorhabditis* sp. SL0708 IJs recovered in each cylinder segment. Error bars were calculated using the standard deviation of each treatment.

The search for the host by the IJs is determined by the type of foraging they may present, which is important in selecting species or strains of entomopathogenic nematodes for pest control. Models describing this behavior are based on the response to stimuli by the IJs (chemotaxis, mechanotaxis, and thermotaxis) and how it searches for and finds the target host (cruiser or ambusher), (LEWIS et al., 2006). This study bore in mind the second model and established that *Heterorhabditis* sp. SL0708 shows displacement with or without the presence of the host, which indicates that this is a cruiser nematode. This result agrees with that reported for different species of heterorhabditis like *H. bacteriophora, H. megidis,* and *H. mexicana* Nguyen (CAMPBELL; GAUGLER, 1997; ENRIGHT; GRIFFIN, 2005; KAYA, 1990; LEWIS; SHAPIRO-ILAN, 2002; MORTON; GARCÍA DEL-PINO, 2009; SHAPIRO-ILAN et al., 2005; SHAPIRO-ILAN et al., 2009).

Cruiser IJs move through the substrate and are attracted or not by signals that indicate the location of a host. The movement of cruisers is relatively linear, typical of the range of IJ search in the absence of signals from the host. The range of movement is carried out to broaden the search area.

During the search, cruisers IJs typically respond to volatile signals or to signals dissolved in the water available in the substrate, liberated by the host or its surrounding environment. This response may be considered part of the process of locating the host's habitat and locating the host within its habitat (LEWIS et al., 2006). However, the displacement of the nematode is limited by humidity, composition and granulometry of the substrate, temperature, age of the nematode, and its energy reserve (FITTERS; GRIFFIN, 2006; WENNEMANN et al., 2004).

Conclusion

The bio-assays used with *Heterorhabditis* sp. SL0708 revealed the potential of behavioral responses, as a specific criterion to learn the biological characterization of IJs and it is an important factor for selecting species or strains of entomopathogenic nematodes as biological pest control agents. Also, it is necessary to run ecology assays (effect of temperature on the viability, infection, and reproduction of the nematode, humidity of the substrate, time of storage) of *Heterorhabditis* sp. SL0708, to establish if environmental conditions affect their entomopathogenic potential and compare its virulence against other hosts in contrast with other EN species.

Acknowledgements

To Colciencias and Pontificia Universidad Javeriana for funding this research, which is part of the Project Evaluating the goods and services of biodiversity for the sustainable development of Colombian rural landscapes: *Complejo Ecorregional Andes del Norte*, from the *Centro de Investigaciones y Estudios en Biodiversidad y Recursos Genéticos* (CIEBREG). We also thank to the owners and personnel in charge of Ramada, Floresta, and Topacio productive systems for permitting access to the study zones.

References

ADAMS, B.; FODOR, A.; KOPPENHÖFE, H.; STACKEBRANDT, E.; STOCK, S. P.; KLEIN, M. Biodiversity and systematic of nematode-bacterium entomopathogens. **Biological Control**, v. 37, n. 1, p. 32-49, 2006.

BERNAL, S.; GONZÁLEZ, N.; ANDINO, M. Influencia de la temperatura del suelo en el parasitismo de *Heterorhabditis* spp. Suelo pardo con carbonatos. **Centro Agrícola**, v. 25, n. 2, p. 16-19, 1998.

BOFF, M. I. C.; WIEGERS, G. L.; SMITS, P. H. Host influences on the pathogenicity of *Heterorhabditis megidis*. **BioControl**, v. 46, n. 1, p. 91-103, 2001.

CAMPBELL, J. F.; GAUGLER, R. R. Inter-Specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? **Fundamental and Applied Nematology**, v. 20, n. 4, p. 393-398, 1997.

CAROLI, L.; GLAZER, I.; GAUGLER, R. Entomopathogenic nematode infectivity assay: comparison of penetration rate

into different hosts. **Biocontrol Science and Technology**, v. 6, n. 2, p. 227-233, 1996.

CHUNG, H. J.; LEE, D. W.; YOON, H. S.; LEE, S. M.; PARK, C. G.; CHOO, H. Y. Temperature and dose effects on the pathogenicity and reproduction of two Korean isolates of *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae). **Journal Asia Pacific Entomology**, v. 13, n. 4, p. 277-282, 2010.

ENRIGHT, M.; GRIFFIN, C. Effects of *Paenibacillus nematophilus* on the entomopathogenic nematode *Heterorhabditis megidis*. **Journal of Invertebrate Pathology**, v. 88, n. 1, p. 40-48, 2005.

FITTERS, P. F. L.; GRIFFIN, C. T. Survival, starvation, and activity in *Heterorhabditis megidis* (Nematoda: Heterorhabditidae), **Biological Control**, v. 37, n. 1, p. 82-88, 2006.

GAUGLER, R. **Entomopathogenic nematology**. Wallingford: CABI Publishing, 2002.

GAUGLER, R.; GREWAL, P.; KAYA, H.; SMITH-FIOLA, D. Quality assessment of commercially produced entomopathogenic nematodes. **Biological Control**, v. 17, n. 1, p. 100-109, 2000.

GLAZER, I.; LEWIS, E. Bioassays for entomopathogenic nematodes. In: NAVON, A.; ASHER, K. R. S. (Ed.). **Bioassays of entomopathogenic microbes and nematodes**. Wallingford: CABI Publishing, 2000. p. 229-247.

GREWAL, P. S.; SELVAN, S.; GAUGLER, R. Thermal adaptation of entomopathogenic nematodes niche breadth for infection establishment and reproduction. **Journal of Thermal Biology**, v. 19, n. 4, p. 245-253, 1994.

GREWAL, P. S.; EHLERS. R. U.; SHAPIRO-ILAN. D. I. **Nematodes as bio-control agents**. Wallingford: CABI Publishing, 2005.

HAZIR, S.; KAYA. H. K.; STOCK. P.; KESKIN. N. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. **Turkish Journal of Biology**, v. 27, n. 4, p. 181-202, 2003.

KAYA, H. K. Soil ecology. In: GAUGLER, R.; KAYA, H. K. (Ed.). **Entomopathogenic nematodes in biological control**. Florida: CRC Press, 1990. p. 93-115.

KAYA, H. K.; BURLANDO, T. M.; THURSTON, G. Two entomopathogenic nematode species with different search strategies for insect suppression. **Environmental Entomology**, v. 22, n. 4, p. 859-864, 1993.

KOPPENHÖFER, A.; GREWAL, P.; FUZY, E. Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turf grass in North America. **Biological Control**, v. 38, n. 3, p. 397-404, 2006.

LEWIS, E. E.; CAMPBELL, J.; GRIFFIN, C.; KAYA, H.; PETERS, A. Behavioral ecology of entomopathogenic nematodes. **Biological Control**, v. 38, n. 1, p. 66-79, 2006.

LEWIS, E. E.; SHAPIRO-ILAN, D. Host cadavers protect entomopathogenic nematodes during freezing. **Journal of Invertebrate Pathology**, v. 81, n. 1, p. 25-32, 2002.

MELO, E. L.; ORTEGA. C. A.; SUSURLUK. A.; GAIGL. A.; BELLOTTI A. C. Poblaciones de nemátodos entomopatógenos (Rhabditida) en cuatro departamentos de Colombia. **Revista Colombiana de Entomología**, v. 35, n. 1, p. 28-33, 2009.

MORTON, A.; GARCIA-DEL-PINO. F. Ecological characterization of entomopathogenic nematodes isolated in stone fruit orchard soils of mediterranean areas. **Journal of Invertebrate Pathology**, v. 102, n. 3, p. 203-213, 2009.

MUKUKA, J.; STRAUCH, O.; HOPPE, C.; EHLERS. R-U. Fitness of heat and desiccation tolerant hybrid strains of *Heterorhabditis bacteriophora* (Rhabditidomorpha: Heterorhabditidae). **Journal of Pest Science**, v. 83, n. 3, p. 281-287, 2009.

RICCI, M.; GLAZER, I.; CAMPBELL, J. F.; GAUGLER, R. Comparison of biossays to measure virulence of different entomopathogenic nematodes. **Biocontrol Science and Technology**, v. 6, n. 2, p. 235-246, 1996.

ROSA, J. S.; CABRAL, C.; SIMÕES, N. Differences between the pathogenic processes induced by *Steinernema* and *Heterorhabditis* (Nemata: Rhabditida) in *Pseudaletia unipuncta* (Insecta: Lepidoptera). **Journal of Invertebrate Pathology**, v. 80, n. 1, p. 46-54, 2002.

SÁENZ, A. Importancia de los nemátodos entomopatógenos para el control biológico de plagas en palma de aceite. **Palmas**, v. 26, n. 2, p. 41-57, 2005.

SÁENZ, A.; LÓPEZ, J. C. Ciclo de vida y patogenicidad de un aislamiento nativo de *Heterorhabditis* sp., (Rhabditida: Heterorhabditidae). **Revista Colombiana de Entomología**, v. 37, n. 1, p. 43-47, 2011.

SÁENZ, A.; OLIVARES, C. Capacidad de búsqueda del nemátodo entomopatógeno *Steinernema* sp. SNIO 198 (Rhabditida: Steinernematidae). **Revista Colombiana de Entomología**, v. 34, n. 1, p. 51-56, 2008.

SHAPIRO-ILAN, D. I.; MBATA, G. N.; NGUYEN, K. B.; PEAT, S. M.; BLACKBURN, D.; ADAMS, B. J. Characterization of biological control traits in the entomopathogenic nematode *Heterorhabditis georgiana* (Kesha strain) and phylogenetic analysis of the nematode's symbiotic bacteria. **Biological Control**, v. 51, n. 3, p. 377-387, 2009.

SHAPIRO-ILAN, D. I.; STUART, R. J.; MCCOY, C. W. Characterization of biological control traits in the entomopathogenic nematode *Heterorhabditis mexicana* (MX4 strain). **Biological Control**, v. 32, n. 1, p. 95-103, 2005.

WENNEMANN, L.; SHANKS, C. H.; SMITH, K. A. Movement of entomopathogenic nematodes in soils of *Fragaria* spp. **Communications in Agricultural and Applied Biological Sciences**, v. 69, n. 3, p. 347-357, 2004.

Received on February 13, 2012.

Accepted on February 7, 2013.