Time Period Between Infection of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) and Soil Application of *Galleria mellonella* (Lepidoptera: Pyralidae) Cadavers on the Emergence of Infective Juveniles

Eleodoro E. Del Valle[1], Claudia Dolinski[2]*, Eduardo L.S. Barreto[2] & Ricardo Moreira Souza[2]

[1]Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, Esperanza (Santa Fe) Argentina.
[2]Laboratório de Entomologia e Fitopatologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, Parque Califórnia, 28015-620 Campos dos Goytacazes (RJ) Brazil.
*Autora para correspondência: claudia.dolinski@censanet.com.br

Recebido para publicação em 09 / 09 / 2010. Aceito em 1 / 4 / 2011
Editado por Mário Inomoto

Summary - Del Valle, E.E., C. Dolinski, E.L.S. Barreto & R.M. Souza. 2013. Time period between infection of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) and soil application of *Galleria mellonella* (Lepidoptera: Pyralidae) cadavers on the emergence of infective juveniles.

It was evaluated the effect of different time periods between the infection and soil application of the *Galleria mellonella* larva cadavers, infected with *Heterorhabditis baujardi* LPP7, on the emergence of the infective juvenile (IJs) nematodes. The time period of six to ten days between infection and soil application resulted in higher emergence of IJs.
Key words: entomopathogenic nematode, insect host cadaver, biological control.

Resumo - Del Valle, E.E., C. Dolinski, E.L.S. Barreto & R.M. Souza. 2013. Período de tempo entre infecção de *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) e aplicação de cadáveres infectados de *Galleria mellonella* (Lepidoptera: Pyralidae) no solo sobre a emergência de juveniles infectantes.

Foi avaliado o efeito de diferentes tempos entre a infecção e aplicação no solo de cadáveres de larvas de *Galleria mellonella*, infectadas com *Heterorhabditis baujardi* LPP7, sobre a emergência de juveniles infectantes (JIs). O período de seis a dez dias entre infecção e aplicação no solo resultou em maior emergência de JIs.
Palavras-chaves: nematoides entomopatogênicos, cadáveres infectados, controle biológico.

Content

Entomopathogenic nematodes (EPN) belonging to the *Heterorhabditis* and *Steinernema* genera are obligate parasites of insects. Species of *Steinernema* and *Heterorhabditis* possess a symbiotic association with pathogenic bacterias *Xenorhabdus* spp. and *Photorhabdus* spp., respectively (Poinar, 1990). Infective juveniles (IJ), which are the only free-living stage of EPN, enter the host insect through its natural apertures (oral cavity, anus and spiracles) or punching a hole through the cuticle, using a structure like a teeth located in its cephalic region. After penetrating the insect hemocel, IJ release symbiotic bacteria, which cause host death, and also provide a base for the nematodes nutrition and defense against secondary infections (Poinar, 1990). These nematodes complete their development and live for two or three generations inside their host. When food is depleted, IJs exit from host cadaver searching for new hosts (Grewal & Georgis, 1998).

Application of EPN by insect host cadavers has recently become an alternative to the use of chemicals for the control of agricultural pests that have a life stage in the soil, especially for the small and medium growers. The cost of production is very low, because it avoids or decreaes concentration, formulation and stocking costs of IJ required in production involving

aqueous suspension (Shapiro-Ilan *et al.,* 2001).

IJ emerged from insect cadaver formulation have a high amount of nutritional reserves, therefore have a great ability to search for hosts and survival on unfavorable environment. In addition, IJs emerging from infected cadavers showed higher infectivity and survival when compared with those from aqueous suspension application (Shapiro & Lewis, 1999; Shapiro-Ilan *et al*., 2003). In Brazil, some research has taken place with insect cadaver formulations in recent years. In field conditions, Del Valle *et al.* (2008a) observed that IJs of *Heterorhabditis baujardi* LPP7 emerging from cadavers presented a migratory capacity of more than 120 cm from the application point, and that an increase in number of insect cadavers applied led to a higher homogeny of IJ distribution in the application areas. Field applications of cadavers infected with *H. baujardi* LPP7 showed efficient control of the fourth instar larvae of *Conotrachelus psidii* (Del Valle *et al*., 2008b). This work emphasized the persistence and infectivity of IJs originating from insect cadavers after three months of application.

Many characteristics related to the biology and physiology of the EPN applied as insect cadavers are still unknown. Among these characteristics, understanding the consequences on IJ emergence from different intervals between infection and soil application is crucial to establish an ideal application moment in programs of pest control with EPN.

The objective of the present work was to evaluate the influence of different time periods between the infection and soil application of the *Galleria mellonella* cadavers, infected with *H. baujardi* LPP7, on the emergence of the infective juvenile nematodes.

Insect cadavers were obtained by the contact of seventh instar larvae of *G. mellonella,* in groups of five, with 1,000 IJs / m*l* of *H. baujardi* LPP7 in a Petri dish (15 cm diameter) containing a filter paper disc (Whatman nº. 1) on the dish base. Petri dishes were incubated in germination chamber at 25 ºC and 80% humidity. After 6, 8, 10 and 12 days, the *G. mellonella* larvae cadavers infected with *H. baujardi* LPP7 were individually placed in 200 m*l*-plastic cups filled with 120 g of soil from a commercial guava orchard (58 % sand, 23 % clay and 19 % silt; pH 4, 6 and 2.43 % of organic matter). The insect cadavers were placed at 1 cm from soil surface.

Cups were watered at 48-hour intervals in order to maintain content as close as possible to field capacity during the experimental period; they were kept in a germination chamber at 25 ºC and 80 % humidity. Nematode extraction was performed twenty days after infecting the *G. mellonella* larvae according to the method of Jenkins (1964). Live infective juveniles were differentiated from other nematode species based on their characteristic locomotion, size and bucal morphology and counted in a dissecting microscope with the aid of a Peters slide.

The experiment was performed in a completely

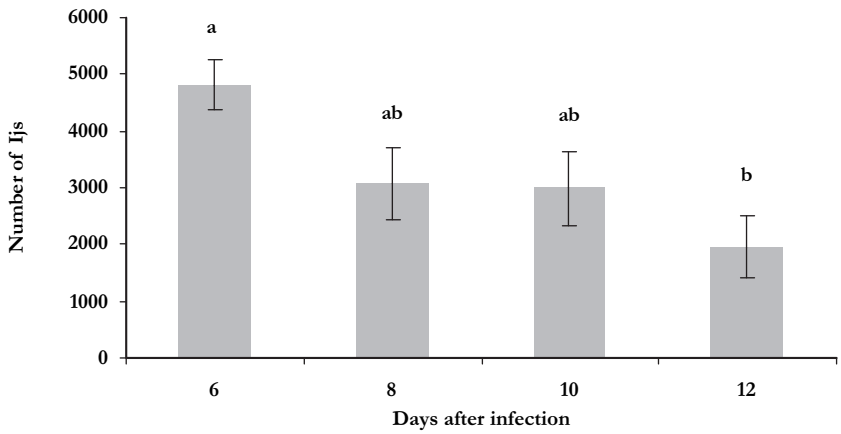

Figure 1 - Number of infective juveniles recovered from soil after cadaver application of *Galleria mellonella* infected with *Heterorhabditis baujardi* LPP7 at 6, 8, 10 and 12 days after infection. Means followed by different letters are statistically different as determined by Tukey test ($P < 0.05$). Bars show standard error.

randomized design with 20 replicates for each treatment. Data was submitted to variance analysis and differences between treatment means compared by the Tukey test ($P < 0.05$).

Time period elapse between *G. mellonella* larvae infection and soil application of the insect cadavers significantly influenced the emergence of IJ into soil ($F = 4.35$; $P = 0.0069$) (Figure 1). Time periods of 6 days showed significantly higher soil emergence of IJ than 12 days.

Long incubation period of insect cadavers reduces IJ emergence (Koppenhöfer *et al.*, 1995). When time between insect infection and cadaver application in the soil increases, the effects of cadaver desiccation become evident, as the cadaver cuticle shrink and the entire insect cadaver loses water, the emergence of IJ decreases. Additionally, as time after infection increases, insect cadavers lose internal turgor, becaming susceptible to physical ruptures (Kaya & Gauger, 1993). These physical damages may halt or prevent normal nematode development and IJ production due to external contamination and/or leakage of internal material (Stuart *et al.*, 2006). The further reason is probably the cause for the low IJ emergence observed at 12 days after infecting insect cadavers (1,951.7 ± 538.2).

In conclusion, periods of 6 days after infection with *H. baujardi* LPP7 and soil application of cadavers of *G. mellonella* resulted in the highest emergence of IJs into the soil. Therefore, this period is recommended for field applications with *H. baujardi* LPP7.

Literature Cited

DEL VALLE, E.E., C. DOLINSKI & R.M. SOUZA. 2008a. Dispersal of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) applied to the soil as infected host cadavers. International Journal of Pest Management, 54: 115-122.

DEL VALLE, E.E., C. DOLINSKI, E.L.S. BARRETO, R.M. SOUZA & R.I. SAMUELS. 2008b. Efficacy of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) applied in *Galleria mellonella* (Lepidoptera: Pyralidae) insect cadavers to *Conotrachelus psidii* (Coleoptera: Curculionidae) larvae. Biocontrol Science & Technology, 18: 33-41.

GREWAL, P.S. & R. GEORGIS. 1998. Entomopathogenic nematodes. In: HALL & J.J. MENN (ed). Biopesticides: Uses and Delivery. Humana Press Inc., p. 15- 271.

JENKINS, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Report, 48: 692.

KAYA, H.K. & R. GAUGLER. 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38: 181-206.

KOPPENHÖFER, A.M., H.K. KAYA, & S.P. TAORMINO. 1995. Infectivity of entomopathogenic nematodees (Rhabditida: Steinernematidae) at different soil depths and moistures. Journal of Invertebrate Pathology, 65:193-199.

POINAR Jr., G.O. 1990. Taxonomy and biology of Steinernematidae and Heterorhabitidae. In: R. GAUGLER & H.K. KAYA (ed). Entomopathogenic Nematodes in Biological Control. CRC Press, p. 23-61.

SHAPIRO, D.I. & E.E. LEWIS. 1999. Comparisons of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. Environmental Entomology, 28: 907-911.

SHAPIRO-ILAN, D.I., E.E. LEWIS, R.W. BEHLE & M.R. McGUIRE. 2001. Formulation of entomopathogenic nematode-infected-cadavers. Journal of Invertebrate Pathology, 78: 17-23.

SHAPIRO-ILAN, D.I., E.E. LEWIS & W.L. TEDDERS. 2003. Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension. Journal of Invertebrate Pathology, 83: 270-272.

STUART, R.J., M.E. BARBERCHECK, P.S. GREWAL, R.A.J. TAYLOR. & C.W. HOY. 2006. Population Biology of Entomopathogenic Nematodes: Concepts, Issues and Models. Biological Control, 38: 80-102.