Note

EFFECT OF MINI-SPRINKLER IRRIGATION SYSTEM ON *Heterorhabditis baujardi* LPP7 (NEMATODA: HETERORHABDITIDAE) INFECTIVE JUVENILE

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ABSTRACT: Entomopathogenic nematodes (EPNs) are currently being used as successful biological control agents of soil-dwelling insect pests. Previous field and greenhouse studies demonstrated that application techniques and non-biotic factors (temperature and pressure) have a significant effect on EPNs efficacy. The objective of this study was to evaluate the influence of an irrigation spray application system on the viability, infectivity and host search capability of *Heterorhabditis baujardi* LPP7 (Nematoda: Heterorhabditidae) infective juveniles (IJ). Two assays were proposed. Their viability was evaluated under the microscope after the IJ passed through the irrigation system. Infectivity on *Galleria mellonella* larvae, and host search capability, as evidenced by larval mortality, were evaluated in containers (Experiment 1). In the field (Experiment 2), mortality of *G. mellonella* larvae was evaluated under different nematode concentrations (0, 100,000, 300,000 and 500,000 IJ per tree). No differences were recorded on the viability, infectivity and host search capability of the IJ in Experiment 1. In Experiment 2, differences were recorded among the different concentrations used ($p < 0.05$), and a higher mortality was observed at the highest nematode concentration (28.3% and 37% in each one of the two experiment repetitions). This irrigation system did not affected adversely the viability, infectivity and host search capability of *H. baujardi* LPP7.
Key words: biological pest control, application technology, viability, infectivity, search capability

EFEITO DO SISTEMA DE IRRIGAÇÃO POR MICROASPERSÃO EM JUVENIS INFECTANTES DE *Heterorhabditis baujardi* LPP7 (NEMATODA: HETERORHABDITIDAE)

RESUMO: Nematóides entomopatogênicos (NEPs) vêm sendo usados com sucesso como agentes do controle biológico de pragas de solo. Estudos anteriores mostraram que técnicas de aplicação e fatores abióticos (temperatura e pressão) afetam a eficiência dos NEPs em testes de campo e casa-de-vegetação. O objetivo deste trabalho foi avaliar a influência de condições geradas por um sistema de irrigação por microaspersão, na viabilidade, infectividade e na capacidade de busca de hospedeiros nos juvenis infectantes (JI) de *Heterorhabditis baujardi* LPP7 (Nematoda: Heterorhabditidae). Dois experimentos foram propostos. A viabilidade dos juvenis infectantes (JI) foi avaliada no microscópio imediatamente após sua passagem pelo sistema de irrigação. A infectividade e a capacidade de busca pelo hospedeiro em larvas de *Galleria mellonella* foram avaliadas em vasos (Experimento 1). A campo (Experimento 2), foi avaliada a mortalidade de larvas de *G. mellonella* sob diferentes concentrações do nematóide (0, 100.000, 300.000 e 500.000 JI por árvore). A viabilidade, infectividade e a capacidade de busca dos nematóides após a passagem pelo sistema não foi diferente da testemunha. No Experimento 2, houve diferença entre os tratamentos ($p < 0.05$) e se observou maior mortalidade na maior concentração de nematóides, com mortalidade de 28,3% e 37% em cada uma das duas repetições do experimento. Este sistema de micro-aspersão não afeta a viabilidade, infectividade e capacidade de busca dos JIs de *H. baujardi* LPP7 até o inseto-alvo.
Palavras-chave: controle biológico de pragas, tecnologia de aplicação, viabilidade, infectividade, capacidade de busca

INTRODUCTION

Heterorhabditis Poinar (Nematoda: Heterorhabditidae) is an obligatory pathogen of insects, known as entomopathogenic nematode (EPN). Once the 3rd-stage infective juveniles (IJ) locate a host

(cruiser or ambusher foraging strategy), they can enter through body openings (i.e. mouth, anus and spiracles) or through the cuticle, reach the hemocoel and release the symbiotic bacteria held in their intestines (*Photorhabdus* spp.) (Poinar-Jr., 1990). Infected hosts die within 48-72 h, and the nematodes feed on the symbiotic bacteria and insect tissues (Grewal & Georgis, 1999; Lewis et al., 1992). These nematodes have a potential use as biological control agents specially towards soil-dwelling pests and can even substitute insecticides due to their safety, easy application and host search capability (Capinera & Epsky, 1992).

The majority of mini-sprinkler equipments do not generate enough pressure to cause physical damage to nematodes, but it is not known if the passage through spray systems would affect their host search capability (Grewal, 1998). Irrespective of the suitability of an insect species as a target-host, the application of a given EPN will not succeed if it is not delivered in a manner that enables access to the host. However, the technical aspects of biopesticide applications in the field have often been neglected. Effective delivery of EPNs can only be achieved with consideration of the available application technologies together with comparative analysis of the advantages and limitations of the biological control agents. Consequently, it is important to understand how each EPN species/ strain can be affected when applied through a particular equipment (Shapiro-Ilan et al., 2006).

This study demonstrates the effects resulting from the passage of a cruiser EPN, *H. baujardi* LPP7 Phan, Subbotin, Nguyen & Moens, through a surface spray irrigation system that uses mini-sprinklers, evaluated on the basis of its viability, infectivity and host search capability at the infective juvenile stage in soil containers and under field conditions.

MATERIAL AND METHODS

Laboratory and field assays were carried out in an experimental guava (*Psidium guajava* L.) orchard in Campos dos Goytacazes, Rio de Janeiro State, Brazil (21°45' S, 41°18' W), from June to August, 2006. The EPN *H. baujardi* LPP7 was isolated from the Rain Forest of the Rondônia State, Brazil. It was reared *in vivo* in the laboratory on larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) according to standard growth procedures. The harvested infective juvenil (IJ) suspensions were stored in tissue culture bottles for less than a week at 16ºC. Prior to the experiments, the area was assessed for native nematodes using the *Galleria* baiting technique (Woodring & Kaya, 1988).

The irrigation system was composed by a single stage centrifugal pump (0.66 Mega Nm - ¼ horse power), 3500-rpm rotor, 146.8 kPa (21.3 psi), four lateral lines (35 m each), a smaller water reservoir to mix nematodes (50 L), a larger water reservoir to collect water (300 L), and mini-sprinklers with one-mm fan nozzles (20-35 psi), outflow between 44 and 56 L h^{-1} and 2.8 m effective watering angle (Figure 1). Strainers were removed for nematode application to prevent the juveniles from eventual damage.

Christiansen's Uniformity Coefficient (CUC) was determined prior to the assays. This coefficient describes the uniformity of the water depth applied by an irrigation system (Mantovani et al., 2006). Soil and air temperatures were also measured during both experiments.

Experiment 1 - The experimental area was composed by four lateral lines, with seven mini-sprinklers at each line (total of 28 sprinklers and plants) (Figure 1). A water suspension of IJ (100,000 IJ L^{-1}) was applied through the system. Nematode viability was evaluated from 500-mL samples collected from twelve sprinklers. A 500-mL sample of nematode suspension that

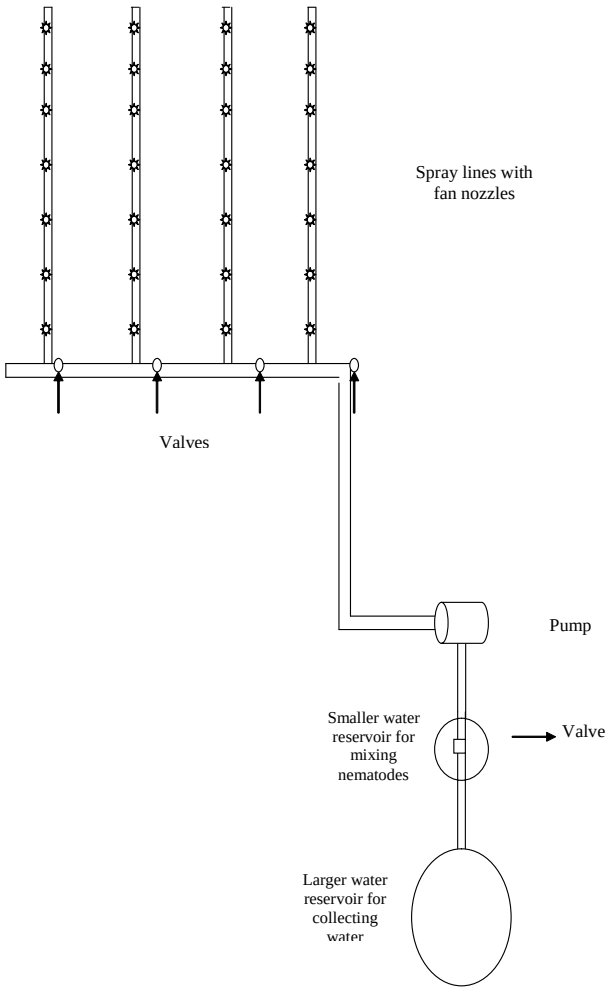

Figure 1 - Schematic diagram of the experimental layout.

did not pass through the irrigation system was used as control. For data recording samples were subsequently taken to a laboratory kept at 27 ± 1ºC (Figure 2 A-B).

Viability - Nematodes in each sample were estimated by collecting 100-µL sub-samples with a micro-dispenser from a thoroughly mixed suspension to a counting slide. Five sub-samples per sample were counted, observing and recording under the microscope the number of dead and live nematodes. Nematodes were considered dead when lied on a straight position and did not respond to repeated prod.

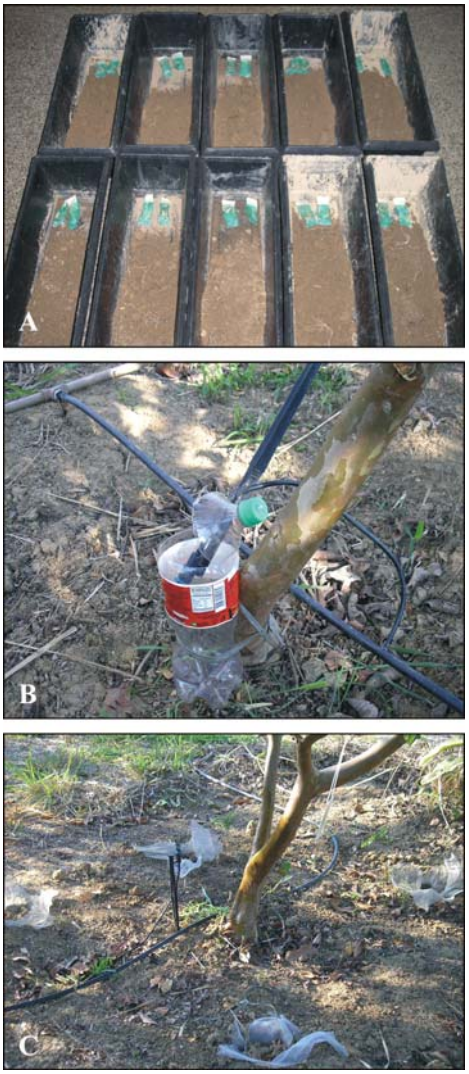

Figure 2 - A) Plastic containers filled with sterile soil with metal screen envelopes each containing ten *Galleria mellonella* larvae, before covering the envelopes. B) Samples collected from sprinklers with nematode suspension. C) Experimental area with four pits, 80 cm apart, around a guava tree. The pits were lined with metal screen and ten *G. mellonella* larvae were placed in each.

Relative viability of the EPNs (V%) was determined as: V% = L × 100/ (L + D) where: *L* = number of living IJ and *D* = number of dead IJ.

Infectivity - Nematode infectivity in relation to *G. mellonella* larvae was determined using 100 IJ mL^{-1} on 9-mm Petri dishes lined with filter paper (Whatman Nº1). Eight Petri dishes with ten larvae each were used for treatment nematodes, and eight for the control. The Petri dishes were kept in a controlled chamber at 25 ± 1ºC and 60 ± 10% relative humidity and 16:8 (L:D) photoperiod. Larval mortality was evaluated 72h after the infection.

Host Search Capability - Capability of the IJ to search for insect-hosts was evaluated in six plastic containers (42 × 25 × 12 cm) filled with 2 kg of sterile soil (580 g kg^{-1} sand, 230 g kg^{-1} silt and 190 g kg^{-1} clay). Five metal screen envelopes (6 × 5 cm), each containing ten *G. mellonella* larvae, were placed at one end of each container, 5 cm under soil surface. The concentration of 10,000 IJ per 30 mL of water was added 40 cm apart from the position at which the larvae had been placed. The insect mortality was evaluated ten days after the IJ application. The same procedure was performed for the control (Figure 2A).

Experiment 2 - In the same experimental area, four soil pits (20 cm × 20 cm × 20 cm) 80 cm apart from each other were dug around each guava tree. The pits were lined with metal screen and ten *G. mellonella* larvae were placed in each. The larval mortality due to different *H. baujardi* LPP7 IJ water concentrations (0, 100,000, 300,000 and 500,000 JI/tree) was evaluated. Seven plants were used for each treatment (total of 28 plants) and each plant received 1 L of water containing the nematode concentration. The applications were made from the lowest to the highest concentration and during applications all other lateral line valves, but the one that was receiving the specific concentration, were closed. The water reservoir with nematodes was in constant agitation to prevent nematodes from settling. Larval mortality was evaluated five days after application. Experiment 2 was repeated twice, subsequently, here referred as 'first' and 'second' applications. After the first application the reservoir and spray lines were washed thoroughly to avoid nematode trapping into the hose due to sedimentation (Figure 2C).

Data of both experiments were first transformed into arcsine and then submitted to analysis of variance. Comparisons of means were performed using the Tukey test, ($p < 0.05$) using the program SAEG, version 9.0 (UFV, 1987).

RESULTS AND DISCUSSION

The Christiansen's Uniformity Coefficient (CUC) was 94.6%, indicating that the area had received water in an adequate and uniform way. The average air temperature during this period was 17.4°C and water temperature after passing through the system was on average 25.6°C.

In Experiment 1 there were no differences in the means of viability compared to the control (F = 1.14; df = 1, 22; p = 0.29) (Table 1). Therefore, for *H. baujardi* LPP7 the pressure of 146.8 kPa (21.3 psi) is perfectly tolerable and does not affect the IJ viability. With regard to *G. mellonella* larvae mortality, there were again no differences between the control and the irrigation treatment (F = 0.69; df = 1, 14; $p > 0.05$) (Table 1), in agreement with Hayes et al. (1999), who observed that the infectivity of *S. carpocapsae* on *G. mellonella* larvae was not affected (up to 95% mortality) when the IJ passed through a sprinkler equipment. Nilsson & Gripwall (1999) did not find reduction in the viability of *S. feltiae* (Filipjev) after spraying with a backpack sprayer and a high-pressure sprayer. Moreover, Klein & Georgis (1994) did also not record adverse effects for *Steinernema* spp. and *H. bacteriophora* after flow through several different pumps, nozzle types, and strainers. Fife et al. (2003) reported viability and infectivity differences among EPN species in relation to pressure differential treatments. They recommended 1380 kPa (200 psi) for *H. megidis* Poinar and 2000 kPa (290 psi) for *S. carpocapsae* (Weiser) Wouts et al. and *H. bacteriophora* Poinar. Garcia et al. (2005) pointed out that *S. glaseri* (Steiner) Wouts et al. kept its viability under pressure of 1379 MPa. These data suggest that low pressure equipment in general do not affect the viability and infectivity of IJ. On the other hand, it seems that each nematode species/strain might have its own recommended pressure. More studies with *H. baujardi* LPP7 have to be performed to establish the threshold pressure.

This was the first time that IJ search capability was evaluated after the IJ were passed through an irrigation system. No differences were recorded between treatments. Nematodes placed on the extreme of the container were able to move along 40 cm of soil to find and infect the *G. mellonella* larvae (F = 0.34; df = 1, 8; $p > 0.05$) (Table 1). This indicates that the irrigation system used did not affect the host search capability of *H. baujardi* LPP7.

Since the mini-sprinkler equipment did not affect the viability, infectivity and host search capability of *H. baujardi* LPP7 IJ in containers, it was decided to conduct Experiment 2 under field conditions. In this assay, the mortality was assessed in the field by removing the metal screen and counting the infected larvae. For the first application, differences were found between treatments (=concentrations) and control, but not among treatments (F = 10.77; df = 3, 24; $p < 0.05$) (Table 2). For the second application, there were differences between the control and treatments, and among treatments (F = 39.14; df = 3, 24; $p < 0.05$). Differently from the first application, the highest mortality values were found for the two highest concentrations (300,000 and 500,000) used (Table 2), which did not differentiate among themselves but from the lower concentration (100,000). This demonstrated that there is no posi-

Table 1 - Mortality of *Galleria mellonella* larvae by *Heterorhabditis baujardi* LPP7 infective juveniles applied through a spray irrigation system.

Treatment	Viability	Infectivity	Mortality after host search
	---------- % ----------		
Infective juveniles without passing through irrigation system	91.8 a*	83.7 a	85 a
Infective juveniles after passing through irrigation system	89.5 a	77.5 a	82 a

*Means in same column followed by the same letter do not differ (Tukey test, $p < 0.05$).

Table 2 - Mortality of *Galleria mellonella* larvae by infective juveniles of *Heterorhabditis baujardi* LPP7 applied through a spray irrigation system. (IJ = infective juvenile)

Treatment	Concentrations	First application	Second application
	IJ per tree	---------- % ----------	
T1 (Control)	0	6.1 a*	3.9 a
T2	100,000	24.8 b	21.4 b
T3	300,000	28.3 b	31.9 c
T4	500,000	25.0 b	37.0 c

*Means in same column followed by the same letter do not differ (Tukey test, $p < 0.05$).

tive correlation between the number of IJ and larval mortality and that, for economic reasons, the concentration 300,000 should be preferred.

The differences between the mortality data determined in the containers (85%, Experiment 1) and in the field (37%, Experiment 2) can be explained, among other factors, by the soil composition. In the containers, the soil was sandy, while in the field it was loamy (sand 240, silt 470 and clay 290 g kg^{-1}). Soil texture affects nematode movement and survival (Kaya, 1990). Generally, compared with lighter soils, soils with higher clay content restrict nematode movement due to potentially reduced aeration, resulting in reduced nematode survival and efficacy (Georgis & Poinar-Jr., 1983).

The low mortality in the field could be related to nematode losses along lateral lines, but samples at the nozzles were taken randomly to check on the number of nematodes exiting the system and this number was around the expected.

Changes in the water temperature may occur with time. Within one hour, the water temperature in a tank can increase from 22 to 43°C for the centrifugal pump, and to 27°C for both the diaphragm and roller pumps. The volume of liquid in the reservoir plays an important role since the smaller the volume, the more water passes through the pump during a pumping period (Klein & Georgis, 1994; Fife et al., 2003). However, the temperature of the water coming from the sprinklers was around 25°C, and the mortality shown in Experiment 1 supports that water temperature was not a problem in the Experiment 2.

Compared to the first application, the higher mortality of the highest concentrations in the second application of Experiment 2 could be explained by the persistence of few IJ in the soil. From experience, we knew that the persistence of the IJ in the field area would not be more than one week, due to the heavy characteristics of the soil. Nematodes require the first 25-65 cm of the soil to be well-irrigated to allow their movement and persistence. More over, Shetlar (1999) had shown the need of applying IJ within a large quantity of water, so that they would not suffer from desiccation. Although the area was kept without irrigation in between the two applications to avoid IJ persistence, it is impossible to affirm that they all had died at the moment of the second application.

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REFERENCES

CAPINERA, J.L.; EPSKY, N.D. Potential for biological control of soil insects in the Caribbean basin using entomopathogenic nematodes. **Florida Entomologist**, v.75, p.525-532, 1992.

FIFE, J.P.; DECKSEN, R.C.; OZKAN, H.E.; GREWAL, P.S. Effects of pressure differentials on the viability and infectivity of entomopathogenic nematodes. **Biological Control**, v.27, p.65-72, 2003.

GARCIA, L.C.; RAETANO, C.G.; WILCKEN S.R.S.; RAMOS, H.H.; LEITE, L.G.; FILHO, A.B.; MOSCARDI, F. Pressurização da calda de pulverização na viabilidade de microrganismos entomopatogênicos. **Engenharia Agrícola,** v.25, p.783-790, 2005.

GEORGIS, R.; POINAR-JR., G.O. Effect of oil texture on the distribution and infectivity of *Neoplectana carpocapsae* (Nematoda: Steinernematidae). **Journal of Nematology**, v.15, p.308-311, 1983.

GREWAL, P.S. Formulation of entomopathogenic nematodes for storage and application. **Japanese Journal of Nematology**, v.28, p.68-74, 1998.

GREWAL, P.S.; GEORGIS, R. Entomopathogenic nematodes. In: HALL, F.R.; MENN, J.J. (Ed.) **Methods in biotechnology**, Totowa: Humana Press, 1999. vol.5, p.271-299.

HAYES, A.E.; FITZPATRICK, S.M.; WEBSTER, J.M. Infectivity, distribution and persistence of the entomopathogenic nematode *Steinernema carpocapsae* All Strain (Rhabditida: Steinernematidae) applied by sprinklers or boom sprayer to dry-pick cranberries. **Journal of Economic Entomology**, v.92, p.539-546, 1999.

KAYA, H.K. Soil Ecology. In: KAYA, H.K. (Ed.) **Entomopathogenic nematodes in biological control of insect pests**. Boca Raton: CRC Press, 1990. p.93-115.

KLEIN, M.G.; GEORGIS, R. Application techniques for entomopathogenic nematodes. In: INTERNATIONAL COLLOQUIUM ON INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL, 6., Montpellier, 1994. **Proceedings.** Montpellier: Montpellier University, 1994. p.483-484.

LEWIS, E.E.; GAUGLER, R.; HARRISON, R. Entomopathogenic nematode host finding: response to host contact cues and ambush foragers. **Parasitology**, v.105, p.309-319, 1992.

MANTOVANI, E.C.; SALASSIER, B.; PALARETTI, L.F. **Irrigação:** princípios e métodos. Viçosa: Ed. UFV, 2006. 358p.

NILSSON, U.; GRIPWALL, E. Influence of application technique on the viability of the biological control agents *Verticillium lecanii* and *Steinernema feltiae*. **Crop Protection**, v.18, p.53-59, 1999.

POINAR-JR., G.O. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In*:* GAUGLER, R.; KAYA, H.K. (Ed.) **Entomopathogenic nematodes in biological control**. Boca Raton: Academic Press, 1990. p.23-61.

SHAPIRO-ILAN, D.I.; GOUGE, D.H.; PIGGOTT, S.J.; FIFE, J.P. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. **Biological Control**, v.38, p.124-133, 2006.

SHETLAR, D.J. Application methods in different cropping systems. In: WORKSHOP ON OPTIMAL USE OF INSECTICIDAL NEMATODES IN PEST MANAGEMENT, New Brunswick, 1999. **Proceedings**. New Brunswick: Ohio State University, 1999. p.31-36.

UNIVERSIDADE FEDERAL DE VIÇOSA - UFV. **SAEG:** Sistema de Análises Estatísticas e Genéticas; versão 9.0. Viçosa: UFV, 1987. 150p.

WOODRING, J.L.; KAYA, H.K. **Steinernematid and heterorhabditid nematodes:** a handbook of techniques. Fayettsville: Arkansas Agricultural Experimental. Station, 1988. 30p.

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