

Effect of Colombian Strains of *Steinernema colombiense* (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) Against *Eurhizococcus colombianus* (Hemiptera: Margarodidae) and *Aeneolamia* sp. (Hemiptera: Cercopidae)

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Effect of Colombian strains of *Steinernema colombiense* (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) against *Eurhizococcus colombianus* (Hemiptera: Margarodidae) and *Aeneolamia* sp. (Hemiptera: Cercopidae)

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A new species of entomopathogenic nematode, *Steinernema colombiense* sp. nov. (Rhabditida: Steinernematidae), was described from the Central Andean Region of Colombia (López-Núñez et al. 2008). Phylogenetic data placed *S. colombiense* sp. nov. in the same clade as *S. carpocapsae* (Weiser), *S. monticolum* Stock, Choo & Kaya, *S. siamkayai* Stock, Somsook & Reid, and *S. scapterisci* Nguyen & Smart, although *S. colombiense* can be differentiated based on several morphometric features of the infective juvenile (IJ) and the 1st-generation male. Because little information on the use of entomopathogenic nematodes against agricultural pests in Colombia exists, we compared *S. colombiense* and a native strain of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), against 2 root-feeding insects that are difficult to control with insecticides.

Related to scale insects, the ground pearl, *Eurhizococcus colombianus* Jakubski (Hemiptera: Margarodidae), infests fruit crops, such as blackberry *Rubus glaucus* Benth (Rosales: Rosaceae) (Kondo & Gómez 2008; Rodríguez & Gómez 2008). Immature stages resemble shiny pearl-like cysts, and their feeding results in a decline in host vigor (Foldi 2005). The spittlebug *Aeneolamia varia* (F.) (Hemiptera: Cercopidae) is an important pest of pastures and sugarcane in the Neotropics (Lucía Cuarán et al. 2012; Gómez 2007). Immature spittlebugs feed on the root sap, whereas adults feed on leaves, injecting a toxin that causes necrosis or "frog hopper burn."

Steinernema colombiense (strain SNIO198) and *H. bacteriophora* (strain Fresno HNIO100) were isolated from the western slopes of the Central Andean highlands (López-Núñez et al. 2007) and reared on *Galleria mellonella* L. (Lepidoptera: Pyralidae) at the National Coffee Research Center (Cenicafé), Manizales, Colombia, according to the methods of Realpe-Aranda et al. (2007). Experiments were conducted at "Tesorito" farm at the University of Caldas, Manizales, Colombia. The farm was 2,280 m asl, with an average temperature of 17 °C, 180 cm annual rainfall, and 78% RH.

In the 1st experiment, reproductive branches of blackberry were propagated vegetatively by "tip layering" bushes in 2 L plastic

containers filled with sterile potting mix. The mix was fumigated with Dazomet (Bazamid® 98%, BASF Corp., Bogotá, Colombia) at 35 g/m² applied 60 d before propagation. After 30 d, when branches had developed roots and new foliage, they were cut from the mother plant. To establish infestations, 6 adult ground pearls per container (collected from nearby blackberry crop) were placed 5 cm underground, close to the roots. After 30 d, roots were examined to confirm that ground pearls had started reproducing, and populations were standardized with 4 adult females and 10 first instars (crawlers) per plant. Any additional insects were removed with a paintbrush, and soil was replaced around the roots.

Three concentrations of nematodes (10³, 10⁴, and 10⁵ IJ/mL) were prepared according to methods of Kaya & Stock (1997). Nematodes were applied as a drench in 500 mL sterile water per container. Nematode viability (> 95% activity) was confirmed under a microscope. Controls received 500 mL water. An insecticide standard consisted of 5 g carbofuran (Furadan® 3G, Bayer Crop-Science, Bogotá, Colombia) per plant. There were 5 replications per treatment conducted under semi-field conditions, i.e., plants were maintained outside at 15 to 20 °C, 70 to 80% RH, and 5.5 cm of rainfall during the observation period. After 5 d, roots were removed and ground pearl survivorship quantified in the laboratory. Mobility and response to stimulation with a paintbrush (leg movement) were used to assess individuals. Dead individuals were placed in White traps (White 1927) and maintained at 26 °C for 48 h in darkness to observe nematode reproduction.

For the 2nd experiment, stolons of kikuyu grass, *Pennisetum clandestinum* Hochst. ex Chiov. (Poales: Poaceae) were planted in 1 L pots filled with sterile potting mix and placed in a greenhouse (average 19 °C, 80% RH, and a 10:14 h L:D photoperiod). After 30 d, when new roots and foliage had developed, plants were infested with immature *Aeneolamia* sp. spittlebugs collected from nearby kikuyu grass. Five nymphs (2nd and 3rd instars) were placed per pot. Nematode treatments were applied after 1 wk when the spittlebugs were feeding. Treatments were *S. colombiense* and *H. bacteriophora*

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ra applied at 10^5 IJ/mL in a 300 mL drench. There were 6 replicates, and control plants were treated with water. Insect mortality was assessed after 12 d, based on response to stimulation via paintbrush. Dead spittlebugs were placed in a White trap (25 °C for 20 d in darkness) to evaluate nematode reproduction.

Data from both experiments were evaluated using analysis of variance (ANOVA) with means separated via Tukey's HSD test at $P \leq 0.05$. Percentage mortality was corrected with the Schneider-Orelli formula (Schneider-Orelli 1947).

Results showed significant treatment effects for immature *E. colombianus* ground pearls ($F = 167.5$, $df = 7$, $P < 0.0001$) due to carbofuran, whereas no mortality was observed from nematodes 5 d post-treatment (Table 1). Treatment differences were observed against adults ($F = 6.8$, $df = 7$, $P < 0.0001$). Here, significant mortality was caused by the high rate of *S. colombiense*, whereas carbofuran was relatively less effective. Mortality of adults exposed to *H. bacteriophora* strain Fresno was not significantly different from controls, suggesting that this strain was less pathogenic than *S. colombiense*. Nematode reproduction was corroborated using White traps where IJ progeny were recovered, though not quantified.

In the 2nd experiment, both nematodes induced mortality of immature spittlebugs under greenhouse conditions ($F = 252.1$, $df = 2$, $P < 0.001$) (Fig. 1). The parasitism for *S. colombiense* was $> 70\%$ but significantly lower compared with *H. bacteriophora*, which killed all spittlebugs by 12 d post-inoculation. Nematode reproduction from White traps was also higher for *H. bacteriophora* (415 ± 24 IJ per nymph) compared with *S. colombiense* (37 ± 3 IJ per nymph). Spittlebugs from controls did not show symptoms of parasitism.

To date, few studies have investigated the susceptibility of ground pearls to insect pathogens. Due to their feeding behavior on roots, we hypothesized that *E. colombianus* might be a good target for entomopathogenic nematodes that naturally occur in soil environments. In our study, only moderate control of the non-feeding adults was achieved with *S. colombiense* at the highest tested rate, whereas *H. bacteriophora* was ineffective. The latter result was unexpected because *H. bacteriophora* is a "cruiser" known to be active within the soil profile (Grewal et al. 1994a). The cooler conditions during tests may have been unsuitable for *H. bacteriophora* (Grewal et al. 1994b). The foraging behavior of *S. colombiense* has not been reported. It may be that *E. colombianus* was not attractive to these nematodes, or that the IJ stages had difficulty penetrating the waxy cysts.

Much better control by both nematodes, especially *H. bacteriophora*, was achieved against spittlebug nymphs. The relative superiority of *H. bacteriophora* over several *Steinernema* species against the sugarcane spittlebug (*A. varia*) nymphs was noted in 2 previous studies (Moreno Salguero et al. 2012; Rosero-Guerrero

et al. 2012). These authors suggested that the "tooth" possessed by *Heterorhabditis* species (Bedding & Molyneux 1981) may allow them to better penetrate the cuticle of the spittlebug compared with *Steinernema* species, which lack the tooth.

Information on native entomopathogenic nematodes in Colombia is still scarce. As several distinct geographical regions are represented, further surveys may reveal additional species that might be adapted and developed for biological control strategies against important agricultural pests. We thank National Coffee Research Center, Cenicafe, for supplying nematodes and University of Caldas for providing facilities.

Summary

Two entomopathogenic nematodes, *Steinernema colombiense* sp. nov. (strain SNIO198) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (strain Fresno HNI0100) (Rhabditida: Heterorhabditidae), were isolated from the Colombian Andes and cultured in the laboratory. In semi-field tests against the ground pearl *Eurhizococcus colombianus* Jakubski (Hemiptera: Margarodidae) on blackberry, neither species was pathogenic to crawlers and only *S. colombiense* was moderately infective against adults at 10^5 IJ/ml. Higher activity for both nematodes was observed against immature spittlebugs *Aeneolamia* sp. (Hemiptera: Cercopidae) infesting kikuyu grass, with *H. bacteriophora* and *S. colombiense* killing 100% and 75% of insects, respectively, in our tests. Our results demonstrate the potential use of native strains of entomopathogenic nematodes as biological control agents in Colombia.

Key Words: entomopathogenic nematode; ground pearl; spittlebug; biological control

Sumario

Dos especies nativas de nematodos entomopatógenos, *Steinernema colombiense* sp. nov. (cepa SNIO198) (Rhabditida: Steinernematidae) y *Heterorhabditis bacteriophora* Poinar (línea Fresno HNI0100) (Rhabditida: Heterorhabditidae), fueron aisladas de los andes Colombianos y cultivadas en laboratorio. En la evaluación bajo condiciones de semi-campo contra la perla de tierra *Eurhizococcus colombianus* Jakubski (Hemiptera: Margarodidae) en mora, ninguna de las especies fue patogénica contra los estados inmaduros y solamente *S. colombiense* fue moderadamente infectiva contra los adultos at 10^5 IJ/ml. Mayor actividad de ambos entomo nematodos fue observada contra los estados inmaduros del salivazo *Aeneolamia* sp. (Hemiptera: Cercopidae) infectando pasto kikuyo con *H.*

Table 1. Mortality (%) of ground pearl *Eurhizococcus colombianus* life stages treated with 2 Colombian strains of entomopathogenic nematodes at 3 concentrations, and 1 concentration of carbofuran insecticide, compared with controls.

Treatment	Rate (IJ/ml)	Immature stage	Adult female
<i>Steinernema colombiense</i> strain SNIO198	10^3	0.0b	5.0bc
	10^4	0.0b	15.0bc
	10^5	0.0b	40.0a
<i>Heterorhabditis bacteriophora</i> strain Fresno	10^3	0.0b	0.0c
	10^4	0.0b	5.0bc
	10^5	0.0b	15.0bc
Carbofuran	5 g/plant	66.0a	25.0ba
Control (water)	n/a	0.0b	0.0c

Different letters in a column indicate significant differences (ANOVA and Tukey's HSD test at $P < 0.05$); n/a = not applicable.

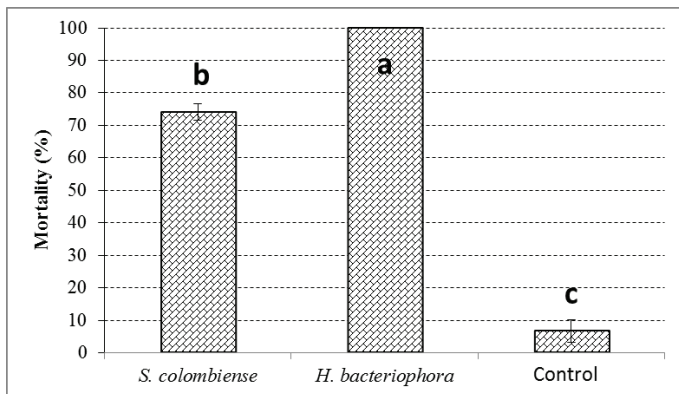


Fig. 1. Percentage mortality (mean \pm SE) of *Aeneolamia* sp. nymphs treated with *Steinernema colombiense* and *Heterorhabditis bacteriophora* strain Fresno at 12 d after inoculation under greenhouse conditions. Different letters indicate significant differences (ANOVA and Tukey's HSD test at $P < 0.05$).

bacteriophora y *S. colombiense* controlando en nuestra evaluación 100% y 75% de los insectos respectivamente. Nuestros resultados demuestran el uso potencial de cepas nativas de nematodos entomopatógenos como agentes de control biológico en Colombia.

Palabras Claves: nematodos entomopatógenos; perla de tierra; salivazo; control biológico

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