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# Efficacy of entomopathogenic nematodes (Nemata: Rhabditida) to control Brazilian apple leafroller *Bonagota salubricola* (Meyrick, 1937) (Lepidoptera: Tortricidae)

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ABSTRACT

The Brazilian apple leafroller, *Bonagota salubricola* (Meyrick, 1937) (*Bonagota cranaodes*) (Lepidoptera: Tortricidae), is one of the main pest problems in apple orchards is Brazil. The purpose of this work was to evaluate *B. salubricola* larval and pupal susceptibility to different species of entomopathogenic nematodes, isolated in Rio Grande do Sul state in Brazil, under laboratory and field (apple orchard) conditions. Bioassays for isolates selection and determination of lethal concentration were performed in tubes of 1.5 ml (eppendorf), each containing one *B. salubricola* third instar larvae and filter paper. Field experiments were performed in commercial orchard, with application of 100 infecting juveniles (IJs)/cm$^2$ for each apple plant previously infected with five *B. salubricola* larvae covered with plastic trays containing thin cloth. Nematodes *H. bacteriophora* RS107 and *H. bacteriophora* RS57 had $LC_{50}$ of 13 and 4.5 IJs/larvae (4.3 and 1.5 IJs/cm$^2$), respectively. In the field, *H. bacteriophora* RS107 and *H. bacteriophora* RS57, applied with sorbitol as an adjuvant, reached 70.2 and 61.1% larval mortality, respectively. The results showed that both isolates had biological activity against *B. salubricola* under laboratory and field conditions.


## 1. Introduction

Brazilian apple leafroller, *Bonagota salubricola* (Meyrick, 1937) (*Bonagota cranaodes*) (Lepidoptera: Tortricidae), is the main pest affecting apple orchards in the states of Santa Catarina and Rio Grande do Sul, Brazil (Kovaleski, 1992; Lorenzato, 1984). A population peak occurs with higher intensity in the beginning of summer until the end of autumn (Lorenzato, 1984; Coracini et al., 2007), but the insect remains active for multiple generations during the whole year (Botton et al., 2000a). Due to its polyphagous behavior the species may be found, even during the cooler months of the year, in different host plants as in Plum (*Prunus domestica* L.), Pear (*Pyrus* sp. L.) and Alamo trees (*Populus* sp. L.), rapeseed (*Brassica napus* L.), clover (*Trifolium* sp. L), hydrangeas (*Hydrangea* sp. L.), honeysuckle (*Lonicera japonica* Thunb.) and rose plants (Fam. Rosaceae), or even in mummified apple fruits and leaves remaining in the apple trees after harvest (Kovaleski, 1992; Betancourt et al., 2004). The main damage occurs when the larvae scratches apple peel, reducing fruit marketability (Kovaleski, 1996). The production losses can reach 3–5% in Rio Grande do Sul state, Brazil, beyond the disequilibrium caused to the apple-eco-system and the environmental impact due to the use of insecticides (Kovaleski et al., 1984).

Female *B. salubricola* oviposition occurs on the upper side of apple leaves. The larvae feeding on leaves and fruits (Lorenzato, 1984; Kovaleski, 1995; Coracini et al., 2007). Last instar larvae make a partial cut of the pedicel, drying the leaves, promoting leaf rolling that serves as a shelter for the pupa until the adult stage (Kovaleski, 1996). *Bonagota salubricola* control is basically made by the use of phosphated insecticides (Botton et al., 2000b), that may affect beneficial fauna negatively and result in the presence of toxic residues in the fruits. Leafroller larvae and other Lepidoptera species are susceptible to microbial control (Lacey and Shapiro-Ilan, 2008), especially with *Bacillus thuringiensis* (Lorenzato, 1984) and entomopathogenic nematodes (EPNs) (Koppenhöfer, 2000; Grewal et al., 2005; Shapiro-Ilan et al., 2005; Georgis et al., 2006). *Steinernema carpocapsae* (Weiser, 1955), one of the first commercialized species of EPNs, was originally isolated from larvae of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the most important apple pest in U.S.A. (Lacey and Unruh, 2005). Under this context, EPNs may be considered as important biological control agents against lepidopteran pests, including those that attacks leaves and trunks (Arthurs et al., 2004), forraging in criptic

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environments (Unruh and Lacey, 2001), as in rolled leaves by *B. salubricola* larvae.

Entomopathogenic nematodes have been investigated for the control of leafroller species. Bélair et al. (1999) and Kaya and Reardon (1982) tested *S. carpocapsae* All, *Steinernema feltiae* UK, *Steinernema glaseri* 326 and *Steinernema riobrave* 335 against *Choristoneura rosaceana* (Harris) and *Choristoneura occidentalis* Freeman, infesting apple and *Abies grandis* (Dougl.). However, the desiccation is considered the major limiting factor to the application of EPNs in the aerial plant parts (Head et al., 2004). This limitation has been diminished by the use of adjuvant substances added to the application mixture (Glazer et al., 1992).

During the years of 2005 and 2006 native EPN species from Rio Grande do Sul, Brazil were investigated, aiming the selection of efficient strains to control native pests of this region (Barbosa-Negrisoli et al., 2009). In the present work the efficiency of different EPNs species over *B. salubricola* larvae and pupa was evaluated in laboratory and field (apple orchard) conditions.

## 2. Materials and methods

### 2.1. Entomopathogenic nematodes (EPNs) multiplications and Bonagota salubricola rearing

Insect rearing, EPN multiplication and bioassays were performed at the Laboratório de Biologia de Insetos e Controle Biológico of the Departmento de Fitossanidade at the Faculdade de Agronomia "Eliseu Maciel" of the Universidade Federal de Pelotas/UFPel, Pelotas, Brazil.

Entomopathogenic nematodes (EPNs) (Table 1) were multiplied on last instar *Galleria mellonella* larvae, according the methods of Kaya and Stock (1997). Infective juveniles were stored in plastic bags (zip-lock like) containing poliuretane sponge blocks. In order to reduce microbial contaminant proliferation, EPNs were maintained in environment controlled chamber at 12 °C and 24 h scotophase, using distilled water with 0.01% sodium hypochlorite solution during the whole process.

Apple leafroller *B. salubricola* reared on artificial diet in a controlled room at 25 ± 2 °C, 70 ± 10% relative humidity (RH) and 14 h photoperiod, according to the methods of Parra et al. (1995).

### 2.2. Selection of EPNs strains to control B. salubricola in laboratory conditions

The experiment was performed in environmental controlled chambers at 25 ± 1 °C, RH 70 ± 10% and photoperiod of 12 h. The experiment was repeated twice, 20 insects (10 larvae and 10 pupae) for each nematode strain and 20 replicates. For each strain of EPN (Table 1), a suspension of 50 μL (100 infective juveniles) in water was used for inoculation of one third instar larvae or pupae of *B. salubricola* in a 1.5 mL eppendorf tube containing filter paper (1.5 × 2 cm). Evaluation of larval and pupal mortality was performed after 72 h, confirming infection by the nematodes through the observation of color symptoms and dissecation seven days after inoculation. Data of insect mortality were combined and submitted to analysis of variance (ANOVA); differences between treatment means were estimated by Tukey's HSD test at $P < 0.05$ probability.

Determination of lethal concentration ($LC_{50}$ and $LC_{99}$) of *Heterorhabditis bacteriophora* RS107 and *Heterorhabditis bacteriophora* RS57 over *B. salubricola*

For the three native EPNs strains from Rio Grande do Sul state, Brazil, that showed higher mortality of *B. salubricola* third instar larvae and pupae, only *H. bacteriophora* RS107 and *H. bacteriophora* RS57 were submitted to a bioassay to determinate the larval and pupal lethal concentration ($LC_{50}$ and $LC_{99}$). The experiment was repeated twice, constituting 10 replicates with 10 individuals each replicate, for each one of the selected strains. Insects were exposed to EPNs in the same way as the previous experiment, using concentrations of 10; 20; 40; 60 and 80 IJs/tube (2.0 $cm^2$) over larvae and pupae in a controlled environment chamber at 25 ± 1 °C, RH 70 ± 10% and 12 h photoperiod. Control larvae and pupae were sprayed with distilled water. Evaluation of larval and pupal mortality was performed after 72 h, confirming infection by the nematodes through the observation of color symptoms and dissecation seven days after inoculation. Data of *B. salubricola*

**Table 1**
Source of the entomopathogenic nematodes strains used in the experiments.

| EPNs species | Strain | Collector and source origin |
|---|---|---|
| *Heterorhabditis bacteriophora* (Poinar, 1976) | RS33 | Barbosa-Negrisoli,[a] Capão do Leão, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | CCA | Aguillera,[b] Araras, São Paulo, Brazil |
| *H. bacteriophora* | RS88 | Barbosa-Negrisoli, Julio de Castilhos, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | RS107 | Barbosa-Negrisoli, Arroio Grande, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | RS72 | Barbosa-Negrisoli, Rosário do Sul, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | RS57 | Barbosa-Negrisoli, Lagoa Vermelha, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | RS56 | Barbosa-Negrisoli, Bom Jesus, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | RS58 | Barbosa-Negrisoli, Lagoa Vermelha, Rio Grande do Sul, Brazil |
| *H. bacteriophora* | HP88 | Gaugler,[c] New Jersey, EUA |
| *Heterorhabditis indica* (Poinar, Karunakar and David, 1992) | IBCB 5 | Leite,[d] Itapetininga São Paulo, Brazil |
| *Steinernema carpocapsae* (Weiser, 1955) | St[a] Rosa | Aguillera, Sta Rosa do Viterbo, São Paulo, Brazil |
| *Steinernema feltiae* (Filipjev, 1934) | RS76 | Barbosa-Negrisoli, Cacequi, Rio Grande do Sul, Brazil |
| *Steinernema glaseri* (Steiner, 1929) | CCA | Aguillera, Sta Rosa do Viterbo, São Paulo, Brazil |
| *S. glaseri* | RS38 | Barbosa-Negrisoli, Coxilha, Rio Grande do Sul, Brazil |
| *Steinernema rarum* (Doucet, 1986) | RS90 | Barbosa-Negrisoli, Canguçu, Rio Grande do Sul, Brazil |
| *S. rarum* | RS55 | Barbosa-Negrisoli, Muitos Capões, Rio Grande do Sul, Brazil |
| *S. rarum* | RS89 | Barbosa-Negrisoli, Canguçu, Rio Grande do Sul, Brazil |
| *S. rarum* | RS106 | Barbosa-Negrisoli, Cidreira, Rio Grande do Sul, Brazil |
| *S. rarum* | RS70 | Barbosa-Negrisoli, Dom Pedrito, Rio Grande do Sul, Brazil |
| *S. rarum* | RS102 | Barbosa-Negrisoli, São José do Herval, Rio Grande do Sul, Brazil |
| *S. rarum* | RS47 | Barbosa-Negrisoli, Planalto, Rio Grande do Sul, Brazil |
| *Steinernema riobrave* (Cabanillas, Poinar & Raulston, 1994) | RS59 | Barbosa-Negrisoli, Lagoa Vermelha, Rio Grande do Sul, Brazil |
| *Steinernema* sp. | RS69 | Barbosa-Negrisoli, Dom Pedrito, Rio Grande do Sul, Brazil |
| *Steinernema* sp. | RS92 | Barbosa-Negrisoli, Canguçu, Rio Grande do Sul, Brazil |

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