Control of *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) with entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) in peach orchards

Carla Ruth de Carvalho Barbosa Negrisoli, Aldomario Santo Negrisoli Jr, Mauro Silveira Garcia, Claudia Dolinski, Daniel Bernardi

1. Introduction

Peach orchards occupy over 20,000 hectares in Brazil, with 50.58% of this area located in Rio Grande do Sul state, where approximately 90% of the Brazilian peaches for canning are produced. *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) is a major pest of temperate fruit trees in southern Brazil, causing losses of 3–5% in peach and apple crops, mainly during the harvesting and implantation of orchards. The larvae damage the fruits by penetrating and shoots, and the severity of the attack depends on the pest and crop maturity. The larvae damage the fruits by penetrating and shoots, and the severity of the attack depends on the pest and crop maturity.
budding, stimulating the emergence of lateral buds, hampering the formation of the crown and, as a result, reducing production (Salles, 1998). The first moths of a new annual cycle come from caterpillars that spend the winter in diapause. Females begin oviposition three days after mating, and lay between 30 and 40 eggs (Botton et al., 2003). The fourth larval instar migrates to branches, and remain, until pupation, in crevices of the bark where branches join, at the base of the fruit stalk on the trunk, or on the ground under canopy (Salles, 1991). Control of G. molesta is hindered by the persistence of larvae in branches and fruits and by the resistance that is derived by the persistence of larvae in branches and fruits and by the resistance development (Salles, 1998). Thus, biological control can mitigate the use of agrochemicals and reduce selection pressure caused to the resistance development of G. molesta and other pests, as well as decrease the harm to natural enemies and other beneficial insects.

Studies have been conducted in the USA and Europe to evaluate the control of tortricids in temperate fruit trees, mainly Cydia pomonella (L) using entomopathogenic nematodes (EPNs) (Lacey et al., 2005, 2006a,b; Unruth and Lacey 2001) and G. molesta (Riga et al., 2006). EPNs are applied on the trunks and soil under the canopy of irrigated plants, and in the storage boxes that serve as shelters for insects from one crop season to another. Given that EPNs have a seeking behavior in cryptic habitats, they are considered as potential control agents of G. molesta not only in peach crop, but also in plum and apple crops. The objective of this study was to select EPNs that are native to Rio Grande do Sul state, in Brazil, and are pathogenic to larvae of G. molesta in the fourth instar in laboratory to evaluate their efficacy in pest control managements in peach trees.

2. Material and methods

The experiments were conducted at the Laboratory of Insect Biology and Biological Control at the Federal University of Pelotas and the Experimental Station of Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul state, Brazil.

2.1. Obtaining G. molesta and entomopathogenic nematodes

Adults of G. molesta obtained from the laboratory colony were placed in PVC tubes (5 cm diameter) coated with PVC film (0.20 mm thick) and fed with a solution of water, honey and methyl propyl (86:13:0.1, v/v) during the oviposition period. The PVC films containing the eggs were collected every five days and immersed into sodium hypochlorite solution (10–15%) for ten seconds. The PVC films containing the eggs were collected every five days and immersed into sodium hypochlorite solution (10–15%) for ten seconds. The eggs were separated, placed in plastic containers of 250 mL (Galvanotek®) containing an artificial diet as described by Arioli et al. (2007) and maintained at 24 ± 2 °C, 70 ± 10% RH and 16 h. After hatching, the plastics were removed from the containers where the larvae developed until adult phase.

Entomopathogenic nematodes were multiplied in vivo on larvae of Galleria mellonella L. (Lepidoptera: Pyralidae), according to Kaya and Stock (1997), and stored in aqueous suspension in a polyurethane sponge in zip-lock bags at 12 °C. During the entire process, a sodium hypochlorite solution 0.01% was used to minimize contaminants (protozoa).

2.2. Laboratory experiments

The laboratory tests were conducted in glass tubes (2.5 × 8.0 cm) containing cardboard strips (2.0 × 6.0 cm), which served as shelters for G. molesta larvae in the fourth instar (ten larvae per strip), pre-acclimatized for 24 h before inoculation of EPNs. One cardboard strip was used per tube, which was considered as a replication, totaling thirty replications (or 300 larvae) per treatment. The EPN strains used were: Heterorhabditis bacteriophora Poinar (1979) RS33, RS56, RS57, RS58, RS72, RS88, RS107, Steinernema glaseri RS38, Steinernema rarum (Doucet, 1986) RS47, RS55, RS69, RS70, RS89, RS90, RS102, RS106 and Steinernema riobrave (Cabanillas, Poinar & Raulston) RS59, all from Rio Grande do Sul state, Brazil (Barbosa-Negrisoli et. al, 2009). In each tube, 50 µL of suspension of each EPN was inoculated, corresponding to 8.33 IJs/cm² (=100 IJs/cardboard strip) and covered with parafilm.

Subsequently, two isolates were selected to determine the lethal concentration (LC50), using the same methodology that was previously described, applying the following concentrations of H. bacteriophora RS33 and/or S. rarum RS69: 0 (control), 6, 12, 24, 48 and 60 IJs/cm². These isolates were selected for presenting high in vivo production (unpublished data), a highly important feature for the production and application on commercial scale. The bioassays were conducted at 25 ± 2 °C, RH 70 ± 10%, photophase of 16 h; the mortality of larvae was registered 72 h after inoculation of EPNs. The experiments were conducted in two separate dates.

2.3. Field experiment

The experiment under field conditions was carried out in a 10-year-old organic peach orchard (‘Vanguarda’ cultivar) with density of 540 trees/ha, without application of insecticides. The two strains of nematodes used, H. bacteriophora RS33 and S. rarum RS69, were selected based on the laboratory results and in vivo production capacity (personal observation). Twenty-four hours prior to the experiments, 30 cardboard sheets (13 × 7 cm) were infested with 30 G. molesta larvae on each. In the field, the cardboard sheets were attached to the main trunk of peach trees (60 cm high), considering each one as a replication (Fig. 1) before the application. The cards were individualized in cotton bags (15 × 10 cm) to prevent the larvae escape and to slow down the drying of EPNs. The cards sprayed with an EPN suspension (backpack sprayer 10L, model 428–01, Guarany®) at a dose of about 12 mL per card (538 IJs/mL, 500 mL/trunk). Inoculum application was carried out late in the afternoon to ensure the action of EPNs, and larvae mortality was observed three days after treatment, considering only those with symptoms of infection by EPNs. The experiment was repeated twice, at different dates.

2.4. Statistical analysis

The experimental design was completely randomized, with ten replications per treatment in the laboratory and thirty replicates in the field. Larvae mortality data were submitted to ANOVA, Tukey’s test for comparison between treatments, and linear regression to determine the lethal concentration of EPNs. Significance level in both tests was 95%.

3. Results

Statistically, there was no difference in mortality of fifteen isolates of larvae of G. molesta in the fourth instar in the laboratory (68–88% of mortality), except for H. bacteriophora RS72 and H. bacteriophora RS88 (28% mortality for both), which was the same as the control group (12% mortality) (F = 20.4, df = 17, p = 0.001, CV = 17.0) (Fig. 2).

Due to their high virulence at the lowest concentration tested, the data were not adjusted for the Probit analysis. Therefore, the lethal concentrations (LC50) of S. rarum RS69 and H. bacteriophora RS33 were 70.5 and 53.8 IJs/cm² and were estimated by the linear
equations ($F = 3.45, 3.83; \ df = 5.5; \ p = 0.034, 0.024; \ CV = 4.3, 4.0$) (Fig. 3).

Application of *S. rarum* RS 69 and *H. bacteriophora* RS33 on cardboard sheets placed on the trunks caused similar mortality of *G. molesta* larvae in the fourth instar in the peach orchard ($F = 0.53, \ df = 1, \ p = 0.47, \ CV = 10.5$) (Fig. 4).

**4. Discussion**

Studies have been carried out to evaluate effectiveness of EPNs to control insect pests in temperate fruit trees, mainly apple, in the USA and Europe. However, only one study has been conducted to assess the effectiveness of EPNs in the control of *G. molesta* in the USA. In the laboratory, *Steinernema carpocapsae*, *Steinernema feltiae*, *S. riobrave* and *Heterorhabditis marelatus* (10 IJs/cm² on cardboard sheets) caused mortality of 63%, 87.8%, 75.6% and 67.1%, respectively, of the pre-pupal larvae of this insect (Riga et al., 2006). In order to control the insects that remain in storage boxes after harvest, the authors also evaluated the efficiency of application of *S. feltiae* (10 and 25 IJs/cm²) in mini storage boxes infested with *G. molesta* in a greenhouse, resulting in mortality of up to 81.6% of larvae at the highest dose of EPN. These results are consistent with the results obtained in the present study, despite the methodological differences.

Regarding doses, technology and the time to apply EPNs in orchards to control tortricids, Unruh and Lacey (2001) studied the application of *S. carpocapsae* on apples. The authors showed that using a hand-gun sprayer (3 million IJs/apple tree), they obtained 78.3% of mortality of *C. pomonella* larvae compared with 34.6% using an air-blast sprayer (5 million IJs/apple tree). In addition, the authors found that application in late afternoon was more efficient than early in the morning. However, there was an exception for orchards irrigated before and after the application, where 100% of mortality insect was achieved in both periods. Therefore, the high mortality of *G. molesta* under field conditions in this study may have been attributed to the dose and time of application.

Another possible way to control *G. molesta* in peach would be to apply EPNs on diapause larvae during the winter. However, for this strategy to be effective, species and/or strains of EPNs adapted to low temperature must be selected, as observed by Lacey et al. (2006b). The authors observed that the mortality of *C. pomonella* caused by EPNs was inversely proportional to temperature. Since *S. feltiae* is more tolerant to cold, it is more efficient than *S. carpocapsae* in the control of *C. pomonella* at 15 °C. Thus, since the efficiency of the nematode depends on its location, further studies should be conducted to evaluate the efficiency of *S. rarum* RS69.
and *H. bacteriophora* RS33 at different temperatures, in order to use EPNs in the control *G. molesta* during the diapause larval period.

Oriental fruit moth produces three to four generations per year and can be found under the bark, in the crevices in tree trunks and in the surrounding soil. It is most susceptible to the control by EPNs during early fall and spring. This points to the possibility of reducing this pest’s population at the beginning of productive period of peaches and, consequently, reducing the use of chemical insecticides (Riga et al., 2006). Finally, the strains of EPNs evaluated in this study can be considered as a new alternative for the biological control of *G. molesta* larvae in IPM programs associated with other control methods. For that purpose, further studies should be performed to evaluate, for example, the best technology for the application of these entomopathogens.

**Acknowledgments**

The authors wish to thank Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) for the scholarship provided to the first author and Embrapa Clima Temperado, Pelotas Brazil, for providing the experimental area.

**References**


Lacey, L.A., Neven, L.G., Headrick, H.L., Fritts, J.R., 2005. Factors affecting entomopathogenic nematodes (Steinernematidae) for control of overwintering codling moth (Lepidoptera: Tortricidae) in fruit bins. J. Econ. Entomol. 98, 1863–1869.


