Evaluation of efficacy of 18 strains of entomopathogenic nematodes (Rhabditida) against Planococcus citri (Risso, 1813) (Hemiptera: Pseudococcidae) under laboratory conditions

Carla Ruth de Carvalho Barbosa Negrisolia, Aldomario Santo Negrisoli Júnior, Marcos Botton, Mauro Silveira Garcia, Daniel Bernardi

Federal University of Alagoas, Chemistry Institute, Maceió, AL, Brazil
Embrapa Coastal Tablelands, UEP Rio Largo, AL, C.P. 2013, ZipCode 57061-970, Brazil
Embrapa Grape & Wine, Bento Gonçalves, RS, Brazil
Federal University of Pelotas, FAEM, Pelotas, RS, Brazil
Superior Agriculture School, University of São Paulo, Piracicaba, SP, Brazil

Entomopathogenic nematodes are pathogenic to Planococcus citri.
Heterorhabditis bacteriophora RS33 are the most virulent nematode to P. citri.
Entomopathogenic nematodes are potential agents to control P. citri in vineyards.

Abstract
Planococcus citri (Risso, 1813) (Hemiptera: Pseudococcidae) is an important plant virus vector in grapevine crops in Brazil and other countries. The mealybug grows in roots and leaves of the grapes. Entomopathogenic nematodes (EPNs) are efficient control agents against insects associated to the soil and could be applied with the same equipment used for chemical insecticides. The aim of this study was to select effective EPNs for controlling P. citri females in laboratory conditions (25 ± 1 °C, UR 60 ± 10%). We tested 17 native [Steinernema rarum (6 strains), Steinernema glaseri, Steinernema feltiae, Steinernema riobrave, Steinernema sp., Heterorhabditis bacteriophora (7 strains)] and only one exotic strain (Steinernema carpocapsae ALL). The bioassays were done on Petri dishes infested with females of P. citri, which were sprayed with EPNs juveniles. The strain with larger pathogenicity and virulence in laboratory was H. bacteriophora RS33 (from 69.0% to 92.2% of mortality), native of Rio Grande do Sul.
The mealybugs (Hemiptera: Pseudococcidae) are considered the main causal agent for virus spreading in vineyards [Grapevine leafroll virus 3 (GLRaV-3), Grapevine virus A (GVA) and Grapevine virus B (GVB)] in South Africa, Argentina, Australia, Chile, Spain, United States, Italy, New Zealand, Portugal and Uruguay (Walton and Pringle, 2004; Charles et al., 2006). These insect vectors cause death of plants in the field directly (damaging the fruit and causing appearance of sooty mold) and indirectly, by transmitting viruses. (Morandi Filho et al., 2009).

In Brazil, the main species of mealybug in vineyards is Planococcus citri (Risso, 1813) (Morandi Filho et al., 2009). This species develops on leaves and roots of grapes, making it difficult to be controlled. The most used form of control is treatment with lime sulfur (4% Be), which is not effective for reducing high infestations. For this, a wait of 40 days should be required for using mineral or vegetal oils (Botton et al., 2003).

The use of entomopathogenic nematodes (EPNs) for P. citri control in the vineyards can be a viable strategy, because the environment taken by the mealybug, especially when it is on the roots, is quite similar to the one required by the EPNs. Because of this, Andaló et al. (2004b) and Alves et al. (2009a) observed that Heterorhabditis spp. and Steinernema spp. caused high mortality of Dysmicoccus texensis females (Tinsley, 1900) (Hemiptera: Pseudococcidae) under laboratory conditions. Moreover, Alves et al. (2009b) found that Heterorhabditis sp. (strains JPM3 and CCA) presented host seeking behavior in sand column, causing high mortality of the mealybug in laboratory, greenhouse and field, in levels equivalent to the chemical control. It is important to mention that D. texensis is, like P. citri, is associated to the soil, which reinforces the possibility of controlling these plagues with EPNs. This way, the aim of this work is to evaluate 10 strains of entomopathogenic nematodes against female P. citri under laboratory conditions. Entomopathogenic nematodes were isolated from different physiographic regions of Rio Grande do Sul, Brazil (Barbosa-Negrisoli et al., 2009), representing an important source of agents for biocontrol of pests occurring mainly in southern Brazil. In this study, some isolates were evaluated against P. citri in the laboratory, aiming future application for controlling this pest in vineyards.

The experiments were conducted at the laboratories of Federal University of Pelotas and Embrapa Clima Temperado, Rio Grande do Sul, Brazil. Initially, the mealybugs were collected in roots of grapevine crops, which were multiplied in pumpkin cultivar ‘Cabocha’ (Cucurbita maxima Duchesne), maintained at room temperature (25 ± 3 °C) in plastic boxes (43 x 24 x 18 cm) covered with voile. When the pumpkins became unavailable for feeding insects, due to its decomposition, new fruits were placed beside them, encouraging the migration of nymphs to the new substrate. Eighteen strains of entomopathogenic nematodes (EPNs) were used in the experiments, of which 17 were native [Steinernema raraum (RS47, RS57, RS79, RS90, RS102, RS106), Steinernema glaseri (RS38), S. feltiae (RS76), S. riobrave (RS59), Steinernema sp. (RS69), Heterorhabditis bacteriophora (RS33, RS56, RS57, RS58, RS72, RS88, RS107)], and for comparison purposes, an exotic strain (Steinernema carpocapsae ALL) was also tested (Table 1). The nematodes were produced in Galleria mellonella L. (Lepidoptera: Pyralidae) larvae as proposed by Kaya and Stock (1997) and stored in polyurethane sponge at 12 °C. G. mellonella larvae were reared on artificial diet, according to Parra (1998).

The first experiment aimed to select efficient EPNs strains against females of P. citri. The experimental arena consisted of Petri dishes (9 cm diameter, 1.5 cm height) containing 1 cm water agar layer (1%) covered by 2 mm layer of sterilized sand (125–250 µm). A piece of pumpkin shell (9 cm² by 1 cm height) was placed over the substrate in the Petri dish over which 40 P. citri females were released. Nematodes were spread using a spraying equipment (Potter-Precision Laboratory Spray Tower, Burkard Scientific Ltd, UK) calibrated at 10 lb/in² and 2.0 ± 0.1 mL of aqueous suspension per dish, at a concentration of 400 IJs (=6.2 infective juveniles [IJ]s per cm²). Then, the dishes were covered and incubated (25 ± 1 °C, UR 60 ± 10%) for four days. The experimental design was randomized, with 18 treatments (EPNs isolates) and the control (water). Data were submitted to ANOVA and compared by Tukey test at 5% probability.

Based on mortality rates obtained in the first experiment, three most virulent strains were selected to determine their lethal concentration (LC50 and LC90) further on. The experimental method was similar to the description above, using the following concentrations: 0 (control), 400, 800, 1200, 1600 and 2000 IJs per Petri dish. The experimental design was random, with 3 treatments (EPNs isolates) and the control (sterile water). Mortality means were subjected to the Probit analysis to determine the lethal concentrations (Polol Plus 1.0, Probit and Logit Analysis, LeOra Software®).

A third bioassay was designed to evaluate the efficiency of H. bacteriophora RS33, selected as the most virulent, to spray on squash infested with P. citri. Pumpkins (10 cm diameter) were cut in half and placed in Petri dishes (15 cm diameter, 1.5 height) containing liquid paraffin (to avoid liquid accumulation). Afterwards, layers of water-agar and sand (as in the previous bioassay) were poured over the solidified paraffin. The application of nematodes was performed with hand pre-compression sprayer (PCP 424.00, Guarany®, 1L) at a pressure of about 10 lb/in², with a volume of 4.0 ± 0.1 mL of aqueous suspension per dish, at a concentration of 2.49 IJs per dish (=14 IJs/cm²), based on the lethal concentration (LC90). The experimental design was randomized, with 2 treatments (EPN isolate) and the control (sterile water). Larvae mortality caused by infective juveniles in bioassays was confirmed by symptoms of infection (change in color and viscosity of the insect hemolymph caused by the presence of the nematode symbiotic bacteria) and/or the presence of nematodes after 96 h exposure. Data were submitted to ANOVA and compared by Tukey test at 5% probability.

Only 10 EPNs strains were pathogenic to females of P. citri in laboratory, from the 18 tested (F = 21.0; P = 0.0001, df = 18) (Fig. 1). Although no significant differences were found, we selected H. bacteriophora RS33, Steinernema feltiae RS76, H. bacteriophora RS7 that caused 92.2%, 73.2% and 68.2% mortality of P. citri, respectively. H. bacteriophora RS33 showed the highest virulence on the females of P. citri, presenting LC50 equal to 7.7 (1.07–11.9) and LC90 equal to 22.4 (15.6–57.1) IJs per insect. Strains H. bacteriophora RS72 and S. feltiae RS76 were the less virulent ones (Table 2). Based on these results, the strain H. bacteriophora RS33 was selected for the subsequent bioassay.

Results of the third assay confirmed efficiency of the isolated H. bacteriophora RS33 (69.0% mortality compared to control, with 9.4%) (F = 342, P = 0.001, df = 1), indicating these strains as the most promising ones for controlling P. citri.

The results obtained with P. citri in this study are similar to those observed by Andaló et al. (2004a), Alves et al. (2009b) and Alves and Moino (2009), that demonstrate the potential use of EPN to control Pseudococcidae and especially D. texensis in coffee crops. Future studies should be conducted to evaluate efficiency of H. bacteriophora RS33 in the field.

Moreover, there is a possibility to use a mixture of chemical insecticides and EPNs, as carried on by Andaló et al. (2004a). These authors assessed the compatibility of EPNs with pesticides used in coffee crops and concluded that viability and infectivity of IJs were not affected after exposition to some insecticides such as imidacloprid and thiamethoxam, normally used against D. texensis (Souza et al., 2007). Another possibility would be to mix nematodes with...
other types of pesticides, such as fungicides (Laznik et al., 2012) or other (chemical and/or biological) products which could be used simultaneously, reducing costs of phytosanitary control (Negrisoli et al., 2010). This way, Morandi Filho et al. (2009), when evaluating the effect of growth regulators and neonicotinoids against *P. citri*, showed that acetamiprid, imidaclorpid and thiamethoxam caused 82%, 94% and 82% of mortality in grapevine, respectively. This demonstrates that the combination of two methods, in this case, biological and chemical controls, can be an effective alternative to significantly reduce the population of these insects in coffee and grape crops.

**References**


