Control of egg and neonate larvae of *Xylotrechus arvicola* (Coleoptera: Cerambycidae), a new vineyard pest, under laboratory conditions

A. RODRÍGUEZ-GONZÁLEZ[1], H.J. PELÁEZ[2], M. GONZÁLEZ-NÚÑEZ[3] and P.A. CASQUERO[1]

[1] Research Group in Engineering and Sustainable Agriculture, Department of Engineering and Agricultural Sciences, Environment Institute Natural Resources and Biodiversity, University of León, 24071 León, Spain; [2] Freelance, 47008 Valladolid, Spain; [3] Plant Protection Department, National Institute of Agrarian and Food Research and Technology (INIA), Ministry of Economy and Competitiveness, 28040 Madrid, Spain

Corresponding author: Dr Álvaro Rodríguez-González, email alrog@unileon.es

Abstract

Background and Aims: *Xylotrechus arvicola* (Coleoptera: Cerambycidae) is a new vineyard pest. Six insecticides were tested on *X. arvicola* eggs arranged in Petri dishes and in two parts of the vine: branch and trunk.

Method and Results: According to the Abbott formula, on Petri dishes, chlorpyrifos had total ovicidal control, significantly different from that of pyriproxyfen (88.3%), *Beauveria bassiana* (84.3%) and imidacloprid (80.9%). On branches, chlorpyrifos (91.6%), pyriproxyfen (79.1%) and flufenoxuron (75.0%) showed improved toxic effect, and on trunks, chlorpyrifos (83.3%) gave the best control, significantly different from that of imidacloprid (50.0%), pyriproxyfen (45.8%) and flufenoxuron (37.5%). Larval mortality was registered from the seventh to the fourteenth day after treatment: spinosad (50.0%), imidacloprid (45.8%) and *B. bassiana* (33.3%) were the insecticides that showed greater larval mortality on branches. These insecticides also showed greater larval mortality on trunks, but only *B. bassiana* (50.0%) had a greater residual effect on trunks than on branches.

Conclusions: All insecticides evaluated gave better ovicidal control when applied directly on Petri dishes than when applied on branches and trunks, where all insecticides (except chlorpyrifos and imidacloprid) show greater toxic effect when applied on branches. Spinosad and *B. bassiana* have the best larval residual mortality, when applied, respectively, on branches and on trunks.

Significance of the Study: *Beauveria bassiana* is the best insecticide with residual effect on neonate larvae on trunks, where the greater thickness of the rhytidome and cracks favoured the development of this fungus to invade actively the larvae through their shell and proliferate inside.

Keywords*: eggs, insecticides, neonate larvae,* Vitis vinifera, Xylotrechus arvicola

Introduction

Xylotrechus arvicola (Coleoptera: Cerambycidae) is a new grape pest (*Vitis vinifera*) (Ocete et al. 2002, 2010) with a significant capacity to establish in new vineyards (Rodríguez-González 2014); it causes, by action of the larvae, the spread of fungi (*Diplodia seriata, Eutypa lata, Phaeoacremonium aleophilum, Phaeomoniella chlamydospora, Phomosis viticola, Formitiporia mediterranea*) throughout the wood of the vine (García Benavides et al. 2013). After mating, the females of *X. arvicola* lay eggs in cracks or under the rhytidome in the wood of vines. The location of the eggs enables the emerging larvae to bore into the wood and make galleries inside the plant. The most exposed stages of the species are adults, eggs and neonate larvae; however, the eggs are usually protected by the rhytidome or cracks (Peláez et al. 2002). Once inserted in the wood, the larvae are inaccessible when treated with traditional foliar-applied chemicals that are not able to penetrate the vine.

Some of the suitable measures to control *X. arvicola* consist of removing the rhytidome of the vines (Peláez et al. 2006) or pruning affected branches below the area of galleries (Ocete et al. 2004), but these techniques are expensive and not sustainable (Peláez et al. 2006). The renovation of branches in vines damaged by *X. arvicola* is easier with the training system for bush vines than in vines with bilateral cordon training systems (Rodríguez-González et al. 2016b). The absence of studies on the control of *X. arvicola*, beyond preventive treatments (Peláez et al. 2002), or products such as sodium arsenite removed from the market because of health concerns, makes the results obtained with other products even more important, in addition to a possible integrated control of *X. arvicola*.

Currently, there are no efficient tools against this pest, with prophylactic measures being the main control methods (García-Ruiz et al. 2014). It is a priority to choose compounds that control *X. arvicola* with a different mode of action, for example, insecticides that have shown good results in the control of other cerambycid pests, insecticides with a high specificity or insecticides with a low eventual side effect with natural enemies.

- Insecticides that have shown good results with other cerambycid pests, such as chlorpyrifos, which have been used against *Acalolepta vastator* (Coleoptera: Cerambycidae), a cerambycid which has caused serious damage to vineyards in important wine-producing regions (Goodwin 2005) or imidacloprid, which has demonstrated effectiveness against the cerambycid borer *Macropophora accentifer* (Olivier) (Coleoptera: Cerambycidae) in citrus (Machado and Raga 1999).
- Insecticides with a high specifity, such as pyriproxyfen, which has shown ovicidal activity through contact with less than 1-day old *X. arvicola* eggs and ovicidal activity through contact with eggs of different age (García-Ruiz et al. 2014) or

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flufenoxuron, that besides the specificity described for pyriproxyfen, also has residual effect on other development stages of the insect pest *Gonipterus scutellatus* (Santolamazza-Carbone and Fernández de Ana-Magán 2004).
- Insecticides with a low side effect on natural enemies, such as spinosad, which is an insecticide of biological origin used to control some pests that have presented resistance to organophosphates and pyrethroids under field conditions and has shown low toxicity for natural enemies of insect pests (Mori and Gotoh 2001) or the biological control agent *Beauveria bassiana*, which is able to infect and kill other coleoptera cerambycidae, such as *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae), *Acalolepta cervinus* Hope (Coleoptera: Cerambycidae) (Jia-Ning and Rong-Ping 2002) and *Enaphalodes rufulus* (Coleoptera: Cerambycidae) (Meyers et al. 2013).

Therefore, the aim of this study was to evaluate the toxic and residual effect of several insecticides on the eggs and neonate larvae of *X. arvicola* in Petri dishes, and in the branches and trunks of vines.

Materials and methods

Insects and experimental conditions

The eggs were derived from a population of *X. arvicola* maintained in the laboratory on the semi-synthetic diet of Iglesias (SSI) (Iglesias et al. 1989) and from field-caught individuals. The larvae were reared on the SSI diet according to García-Ruiz et al. (2012), and sex was identified after the complete esclerotisation and melanisation of the adults. Once the fatty abdominal reserves were reabsorbed, it was possible to distinguish body colours between the males and the females as described by Moreno (2005). The adult females obtained through the SSI diet were also paired with males obtained through the SSI diet, and if a male died, another was added to allow females to continue laying eggs (García-Ruiz et al. 2012).

Xylotrechus arvicola eggs for the experiments were obtained by the methodology for the management of the development stages described by Rodríguez-González et al. (2016a). All stages of *X. arvicola* prior to testing were maintained in a chamber with controlled environmental conditions of temperature (24 ± 1°C), humidity (60 ± 5%) and subjected to a photoperiod of 16 h light and 8 h dark, with a light intensity of 1000 lux. The eggs were obtained from laying substrates (corrugated strips 12 × 4 cm), introduced in glass jars with a diameter of 80 mm and height of 100 mm. The substrates were reviewed daily, and eggs were extracted and, with the help of a brush, placed in Petri dishes with diameter of 55 mm.

Insecticides

Commercial formulations (De Liñan-Carral and De Liñan-Vicente 2013) of the following insecticides were tested for the activity against *X. arvicola* eggs and larvae: spinosad (Spintor 480 CC, Dow Science Ibérica, Madrid, Spain; 48 g a.i./L) at 25 mL/hL; *B. bassiana* (4.4×10^{10} conidia/g) (GHA strain) (Bassi WP, Massó, Barcelona, Spain; 22 g a.i./100 g) at 125 g/hL; imidacloprid (Confidor 20 LS, Bayer Crop Science, Valencia, Spain; 20 g a.i./L) at 0.10% *v*/v; chlorpyrifos (Cúspide 48, Massó, Barcelona, Spain; 48 g a.i./L) at 0.20% v/v; pyriproxyfen (Atominal 10 EC, Massó, Barcelona, Spain; 10 g a.i./L) at 75 cm^3/hL; and flufenoxuron (Kimlux, Sapec Agro, Valencia, Spain; 10 g a.i./L) at 0.10% v/v. Distilled water was used as carrier in all treatments and as the Control treatment in all trials.

Experiment 1: toxic effect of insecticides on eggs placed in Petri dishes

Five replicates of 20 eggs were used for each of the six insecticides treatments and for the Control. Each replication was placed in a Petri dish. Four holes of 5 mm diameter (20 mm^2) were made in the cover of Petri dishes to avoid the effect of a lethal chamber (and to facilitate the aeration of the treated plate). The impact of the application of the treatments was monitored daily for 7 days after treatment by counting the inhibition of the eggs (the eggs were shrunk or decreased, suppressing the emergence of larvae and whose metamorphosis was altered). A Potter tower of manual loading (Petri dishes are inserted/removed manually by the operator at the beginning/end of each treatment) (Burkard Scientific, Uxbridge, England) with air compressor was used for the application of treatments. Insecticide solution (1 mL) was applied onto Petri dishes at a pressure of 40 kPa in each spray, which produces a deposit 0.004 ± 0.0004 mL/cm^2, which is equivalent to 400 ± 40 L/ha.

Experiment 2: toxic and residual effect of insecticides on eggs and neonate larvae

The experiment used a factorial design with two factors, including two parts of the vine (branch or trunk) and insecticides (seven levels, six insecticides and distilled water as Control) and eight replicates. The vines (cv. Prieto Picudo) were collected from vineyard plots (double cordon Royat) located in DOP Tierra de Leon (European Commission 2007). The vineyard plots were treated with the acaricide fenbutestan (Norvan 55 SC, BASF, Barcelona, Spain). The parts obtained were separated into trunks and branches, grouped in similar sizes and sections: trunks (15 cm long and 5 cm diameter) and branches (15 cm long and 3 cm diameter).

Treatments on branches and trunks of vines (toxic effect)

Xylotrechus arvicola eggs were placed one by one on branches and trunks of vines, using a brush. In order to record the location of the eggs over the following days, their position was marked with a felt pen with white ink. In each experimental unit, 12 eggs were alternatively located in each cm (of trunk or branch) under the rhytidome and in cracks with eight repetitions in each part of the vine. Then, the treatments, six insecticides and a distilled water (Control), were applied by spraying. A manual diffuser (0.10 L) was used to the drip point in order to spray the treatments at the maximum commercial dose. The inhibition of egg hatching was monitored daily during the first 7 days after the application of the treatments. Embryonic development ended 7 days after oviposition under the controlled conditions of temperature (24 ± 1 °C) of the bioassay. The toxicological activity of the active ingredients is mainly produced within 7 days after application (Santolamazza-Carbone and Fernández Ana-Magan 2004, Eken et al. 2006, Poland et al. 2006, Planes et al. 2013).

Treatments on branches and trunks of vines (residual effect)

The residual effect of insecticides on neonate larvae (eggs not inhibited by the toxic effect of insecticides and that were able to hatch) in branches and trunks was evaluated by monitoring daily larval mortality that occurred in the following 7 days (from 7th to 14th day after the application of the treatments). The residual effect was measured counting the paralysed, firm

to touch and darkened larvae, compared to the cream-coloured un-infected larvae in the Control treatment.

Statistical analysis

Experiment 1. A randomly completed experiment general linear model (GLM) procedure, with seven insecticide treatments, and five replicates was subjected to ANOVA. Differences ($P < 0.05$) among insecticide treatments on the same day were examined by mean comparisons using the least significant difference (LSD) test. Analyses of repeated measures using the MIXED procedure were made. Treatments (insecticides), days after treatment (days) and insecticide × day (I × D) interaction were considered as fixed effect, while experimental replicates were considered as random factor. The regression linear coefficients of the I × D interaction were tested using an F-test.

Experiment 2. A factorial experiment (GLM procedure), considering part of vine and insecticide as factors, with two parts of vine, seven insecticide treatments, and eight replicates was subjected to ANOVA. Differences ($P < 0.05$) between parts of vine and among insecticide treatments were examined by mean comparisons using the LSD test. Analyses of repeated measures using the MIXED procedure were made. Treatments (insecticides), days after treatment (days) and insecticide × day (I × D) interaction were considered as fixed effect, while experimental replicates were considered as random factor. The regression linear coefficients of the I × D interaction were tested using an F-test.

The SAS version 9.1.2 software (SAS Institute, Cary, NC, USA) was used for all analyses. The mortality data were corrected with the Abbott's formula (Abbott 1925) in the two experiments described. Mean values and standard errors are given in tables.

Results

Experiment 1: toxic effect of insecticides on eggs placed in Petri dishes

Table 1 shows the significant differences among insecticide treatments when they were applied directly on eggs placed in Petri dishes. The best result among the insecticides was achieved with chlorpyrifos from day 1 until day 7 after treatment, providing a total inhibition of egg hatching. It also showed the highest value of the regression linear coefficient in the I × D interaction, significantly different from the remaining I × D interactions. Pyriproxyfen inhibited 88.3% of eggs treated (according to Abbott formula), differing significantly from chlorpyrifos on day 7. *B. bassiana* and imidacloprid showed good ovicidal effect, inhibiting 84.3 and 80.9% of eggs evaluated (with Abbott formula), respectively, values that were not significantly different to that of pyriproxyfen. The impact of flufenoxuron on day 7 after treatment was significantly lower than that of imidacloprid. Spinosad also strongly inhibited egg hatching, significantly different from the remaining insecticides, 5 days after treatment. The Control treatment differed significantly from the applied insecticides from day 1 after treatment.

Experiment 2: toxic and residual effect of insecticides on egg and neonate larvae

Treatments on branches and trunks of vines (toxic effect). Table 2 shows the significant differences among insecticide treatments when they were applied directly on eggs

Table 1. Toxic effect of insecticides on unhatched *Xylotrechus arvicola* eggs exposed during 7 days on Petri dishes.

Insecticide	Toxic effect—eggs unhatched (%)							Linear regresion coefficient (Insecticide × Day)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Spinosad	12.2 ± 2.4b†	28.0 ± 2.9c	38.5 ± 3.4bc	52.5 ± 3.7c	59.5 ± 4.1c	66.0 ± 4.0a	71.2 ± 3.7d	11.32***±0.5f
Beauveria bassiana	9.7 ± 2.1b	22.0 ± 3.5c	37.0 ± 3.8c	53.2 ± 3.8c	64.0 ± 4.6bc	78.0 ± 2.8b	87.2 ± 2.2b	13.35***±0.5c
Imidacloprid	13.0 ± 2.6b	37.5 ± 2.7b	45.5 ± 2.4b	57.7 ± 2.1BC	65.5 ± 2.6bc	75.7 ± 1.9b	84.5 ± 2.2BC	12.59***±0.5e
Chlorpyrifos	43.7 ± 6.2b	73.7 ± 4.3a	87.5 ± 2.9a	97.0 ± 1.4a	99.5 ± 0.5a	100.0 ± 0.0a	100.0 ± 0.0a	15.53***±0.5a
Flufenoxuron	12.7 ± 2.4b	29.5 ± 2.6bc	43.2 ± 2.4BC	57.2 ± 2.8BC	65.7 ± 3.3BC	74.0 ± 2.1b	81.0 ± 1.9c	12.71***±0.5d
Pyriproxyfen	10.2 ± 2.4b	29.7 ± 3.6BC	44.7 ± 2.8b	61.0 ± 2.7b	70.0 ± 2.5b	79.7 ± 1.5b	90.5 ± 1.6b	13.93***±0.5b
Control	0.0 ± 0.0c	0.0 ± 0.0d	0.75 ± 0.5d	6.0 ± 1.1d	11.5 ± 1.5d	17.2 ± 1.0d	18.7 ± 1.3e	2.88***±0.5f

Linear regression coefficients within a column followed by the same lower case letter are homogeneous ($P > 0.001$). ***Coefficients are significantly different from zero ($P < 0.001$). †Different lower case letters indicate significant differences among insecticides for the same day on Petri dishes.

Table 2. Toxic effect of insecticides on unhatched *Xylotrechus arvicola* eggs when exposed during 7 days after being placed on branches and trunks of vines, and accumulated larvae mortality as a consequence of the residual effect of insecticides 14 days after treatment.

Insecticide	Part of vine	Toxic effect—eggs unhatched (%)								Residual effect—larvae mortality (%)	
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Linear regresion coefficient (Insecticide × Day)	Day 14	Linear regresion coefficient (Insecticide × Day)
Spinosad	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	66.6 ± 6.3aC	62.5 ± 4.2aC	62.5 ± 4.2aB	50.0 ± 6.3aB	41.6 ± 5.5aC	−9.9***±0.9D	50.0 ± 8.8aA	8.4***±0.8B
	Trunk	100.0 ± 0.0aA	100.0 ± 0.0aA	58.3 ± 5.5aB	41.6 ± 5.5bD	33.3 ± 0.0BC	16.6 ± 6.3BC	16.6 ± 6.3BC	−15.7***±1.0E	41.7 ± 10.9aB	7.7***±0.9B
Beauveria bassiana	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	83.3 ± 6.3aAB	50.0 ± 6.3aB	50.0 ± 6.3aB	50.0 ± 6.3aC	−10.7***±0.9E	33.3 ± 6.3bB	7.1***±0.8C
	Trunk	100.0 ± 0.0aA	91.6 ± 5.5aA	62.5 ± 4.2bB	29.1 ± 4.2bDE	12.5 ± 6.1bD	0.0 ± 0.0bD	0.0 ± 0.0bD	−19.0***±1.0G	50.0 ± 8.8aA	9.9***±0.9A
Imidacloprid	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	87.5 ± 6.1aAB	75.0 ± 5.5aBC	58.3 ± 5.5aB	58.3 ± 5.5aB	54.1 ± 6.1aC	−8.9***±0.9C	45.8 ± 8.3aA	8.9***±0.8A
	Trunk	100.0 ± 0.0aA	100.0 ± 0.0aA	83.3 ± 6.3aA	58.3 ± 5.5bC	54.1 ± 6.1aB	50.0 ± 6.3aB	50.0 ± 6.3aB	−9.9***±1.0B	29.2 ± 7.6BC	5.6***±0.9C
Chlorpyrifos	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	95.8 ± 4.2aA	95.8 ± 4.2aA	91.6 ± 5.5aA	91.6 ± 5.5aA	91.6 ± 5.5aA	−1.6 ± 0.9 A	–	0.0 ± 0.8F
	Trunk	100.0 ± 0.0aA	100.0 ± 0.0aA	91.6 ± 5.5aA	87.5 ± 6.1aA	83.3 ± 6.3aA	83.3 ± 6.3aA	83.3 ± 6.3aA	−3.2 ± 1.0 A	–	0.0 ± 0.9F
Flufenoxuron	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	95.8 ± 4.2aA	95.8 ± 4.2aA	87.5 ± 6.1aA	79.1 ± 6.1aA	75.0 ± 5.5aB	−4.4***±0.9B	20.8 ± 6.1aC	4.1***±0.8E
	Trunk	100.0 ± 0.0aA	95.8 ± 4.2aA	87.5 ± 6.1aA	79.1 ± 6.1bAB	54.1 ± 6.1bB	37.5 ± 4.2bB	37.5 ± 4.2bB	−12.0***±1.0D	12.5 ± 6.1aD	2.5 ± 0.9E
Pyriproxyfen	Branch	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	87.5 ± 6.1aAB	79.1 ± 6.1aA	79.1 ± 6.1aA	79.1 ± 6.1aAB	−4.4***±0.9B	33.3 ± 8.3aB	6.3***±0.8D
	Trunk	100.0 ± 0.0aA	95.8 ± 4.17aA	83.3 ± 6.3bA	66.6 ± 6.3bBC	50.0 ± 6.3bB	45.8 ± 6.1bB	45.8 ± 6.1bB	−10.5 ± 1.0C	25.0 ± 8.3aC	4.9***±0.9D
Control	Branch	100.0 ± 0.0aA	95.8 ± 4.17aA	75.0 ± 5.5aBC	45.8 ± 6.1aD	33.3 ± 0.0aC	29.1 ± 4.2aC	0.0 ± 0.0aD	−16.9***±0.9F	0.0 ± 0.0aD	0.0 ± 0.8F
	Trunk	100.0 ± 0.0aA	100.0 ± 0.0aA	54.1 ± 6.1bB	20.8 ± 6.1bE	8.3 ± 5.5bD	8.3 ± 5.5bCD	0.0 ± 0.0aD	−18.8***±1.0F	0.0 ± 0.0aE	2.2 ± 0.9E

Linear regression coefficients within a column followed by the same capital letter are homogeneous ($P > 0.001$). ***Coefficients are significantly different from zero ($P < 0.001$). Different lowercase letters indicate a significant difference between branches and trunks for each insecticide and the same day after treatment. Different capital letters indicate a significant difference among insecticides for the same day after treatment on branches (on one side) and on trunks (on the other side).

placed in grapevine branches and trunks. Best results were achieved with chlorpyrifos, as it inhibited 91.6% of eggs on branches and 83.3% on trunks (according to the Abbott formula) and significant differences between the application of chlorpyrifos on branches and trunks during all period were not found. Chlorpyrifos showed the highest value of the regression linear coefficient, significantly different from all other I × D interactions both on branch and trunk. Pyriproxyfen on branches did not differ significantly from chlorpyrifos. And, its ovicidal effect on trunks was not significantly different from that of imidacloprid and flufenoxuron. A significant difference for the pyriproxyfen toxic effect on eggs located on branches and trunks from day 3 to day 7 after treatment was found. The inhibition of eggs obtained by flufenoxuron was significantly higher on the branches than on the trunks from day 4 to day 7 after treatment. Imidacloprid on branches differed significantly from the three insecticides (chlorpyrifos, flufenoxuron and pyriproxyfen) with improved toxic effect. On trunks, imidacloprid significantly showed lower ovicidal control than chlorpyrifos. A significant difference between the application on branch and on trunk in day 7 was not found. *Beauveria bassiana* showed good ovicidal effect on branches, not significantly different from that of imidacloprid., however, showed no ovicidal capacity in eggs placed in trunks. Thus, the inhibition of eggs was higher on branches than on trunks, with significant differences from day 3 to day 7 after treatment. Spinosad achieved greater inhibition of eggs located on branches than those located on trunks, with a significant difference between the applications. Treatments applied to eggs located on trunks achieved poor inhibition of hatching, which was significantly different from that of chlorpyrifos, imidacloprid, pyriproxyfen and flufenoxuron. The hatching of no eggs was inhibited by the Control treatment applied to branches and trunks of vines.

Treatments on branches and trunks of vines (residual effect). Significant differences were found in the residual effect of insecticides on larvae control when applied to branches and trunks (Table 2). Spinosad was the insecticide with the best residual effect on branches on day 14, controlling 50.0% of hatched larvae. This insecticide also had the second best control on neonate larvae hatched on trunks. Larval mortality obtained on branches and trunks did not differ significantly between the two parts of the vine. The larvae mortality obtained by *B. bassiana* on branches differed significantly from that of spinosad and imidacloprid. The neonate larvae on trunks accumulated a mortality of 50.0% (according to the Abbott formula), so *B. bassiana* was the best insecticide and was significantly different to spinosad. Significant differences for larval mortality between treatment of branches and trunks were found. In addition, *B. bassiana* in the interaction reached the highest value of the regression linear coefficient on trunks, significantly different from all the other I × D interactions. Imidacloprid and spinosad were not significantly different on branches; however, the imidacloprid × day interaction showed the highest value of the linear coefficient on branches, significantly different from the other insecticdes for the I × D interactions. On trunks, imidacloprid achieved a 29.2% larvae mortality, not differing significantly from *B. bassiana* which had the best result. A significant difference between larvae mortality on branches and trunks was found. The larvae mortality achieved by pyriproxyfen on branches differed significantly from that of spinosad and imidacloprid. Pyriproxyfen on trunks differed significantly from *B. bassiana*.

A significant difference between the larvae mortality achieved by pyriproxyfen on branches and trunks was not found. The effect of flufenoxuron on branches was significantly different to that of spinosad and imidacloprid. Also, the larval mortality obtained by flufenoxuron on trunks was significantly different from that of *B. bassiana*. A significant difference in larval mortality obtained by flufenoxuron between branches and trunks was not found. The Control had no impact on larvae mortality on branches and trunks and was the least effective in controlling larvae, which differed significantly from that of the insecticides.

Discussion

The present work reports the toxic effect of six insecticides against *X. arvicola* eggs placed in Petri dishes and the toxic and residual effect of these insecticides against *X. arvicola* eggs and neonate larvae located on trunks and branches of grapevines.

The ovicidal effect demonstrated by spinosad was lower than that of the other insecticides; however, as 71.2% eggs did not hatch in Petri dishes, spinosad can be used to reduce the number of eggs that complete their development and subsequently the emergence of neonate larvae, increasing the ability to control the pest. Spinosad can be an acceptable product when used on branches to reduce the population density of the eggs in order to complete their embryonic development and to increase the control of the pest, considering the moderate harmful effects on other non-target organisms. These effects are attributed to changes in nutrition, behaviour of predators (Tillman and Mulrooney 2000), parasites (Williams et al. 2003) and mode of action, which could provide a margin of safety for these non-target organisms. The rhytidome and the cracks protect against the action of the insecticide; therefore, spinosad does not directly impact the eggs as in the Petri dishes. After the larvae hatch, the contact of spinosad with the treated wood produces the insecticide bioactivation, making changes in the feeding of the larvae, and causing their death in later days. Spinosad achieved the greatest control in the last days of evaluation, 50.0% on branches and 41.7% on trunks. This biological insecticide could potentially affect eggs on embryogenesis development located in different parts of the vines. The lethal and sublethal effects of spinosad make it most beneficial to incorporate it into an IPM program against *X. arvicola*, and it can be compatible with the scarce number of predators discovered until recently for this pest (Peláez et al. 2012).

The activity of *B. bassiana* on eggs placed in Petri dishes was strong, because it inhibited above 87.0% of hatching of the eggs. Such ovicidal control exceeded that described by Ren et al. (2009) when treating *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) eggs and nymphs, where a mortality of 72.8% was achieved, but it was lower than that obtained by Meyers et al. (2013) when treatment of red oak borer *Enaphalodes rufulus* (Coleoptera: Cerambycidae) eggs with *B. bassiana* provided total control. *Beauveria bassiana* achieved better mortality than that produced by conventional insecticides (imidacloprid with 84.5%) or insecticides with ovicidal and larvicidal capacity (flufenoxuron with 81.0%). The high susceptibility of eggs to *B. bassiana* confirmed the ability of entomopathogenic fungi to control *X. arvicola* eggs when they were placed in branches (50.0%). *Beauveria bassiana* reduced hatching and larvae development. This entomopathogenic fungus has the merit that it does not accumulate residues in the field, as can occur with other synthetic insecticides (Zimmermann 2007). *Beauveria bassiana* would be effective in reducing 50% of the egg population avoiding the use of conventional insecticides in the vineyard. The embryonic mortality that *B. bassiana* confers does not occur in the first days after the fungal infection, so it allows the survival of the embryo and the hatching of the larvae. This may explain the low efficiency of the fungus on eggs in treatment of the trunk, where it is more difficult to obtain good contact between the fungus and eggs. Trunks, with a thicker rhytidome and deeper cracks than branches, make the contact of the insecticide with the fungus more difficult, but favouring *B. bassiana* retention and increasing the ability to infect the larvae explain the greater larval mortality on trunks than on branches. *Beauveria bassiana* was the insecticide which showed the fastest residual control of larvae on trunks. *Beauveria bassiana* is highly virulent on larvae of another cerambycid *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) but needs more time to kill adults (Shimazu and Kushida 1983), as the age of larvae is important for the susceptibility to entomopathogenic fungi (Tanada and Kaya 1993). Reay et al. (2007) reported that an aqueous solution of *B. bassiana* applied to New Zealand beech *Nothofagus fusca* (Hook.f.) Oerst. persisted in the field, and fungal conidia satisfactorily penetrated inside the tunnels created by adults of *Treptoplatypus caviceps*, *Platypus apicalis* and *P. gracili* (Curculionidae: Platypodinae). An effective control of neonate larvae of *X. arvicola* by *B. bassiana* decreases the introduction of larvae during the first hours of life, reducing the structural damage that larvae are capable of in the vines. If *B. bassiana* is used in the field in the same way as it has been simulated in our trial, *X. arvicola* adults would be exposed to the fungus while they are feeding and females are laying the eggs in different parts of the vines. Ideal conditions of moisture and temperature for the germination of fungal spores and for the infection of immature stages of *X. arvicola* could be found under the rhytidome or in the cracks. The rhytidome and the cracks or wounds in the vines may mean that fungal spores are retained, and they could reach more easily the immature stages of *X. arvicola.* Entomopathogenic fungi are considered safer for the environment than conventional insecticides and can be used in sensitive areas where predators and parasitoids of pests have a crucial role. This type of fungus may persist and proliferate in the environment, having the ability to be transmitted in other pest populations (Liu and Bauer 2006). *Beauveria bassiana* is considered an insurance against beneficial insects, such as predators, parasitoids and honeybees in the field (Brinkman and Fuller 1999, Cottrell and Shapiro-Ilan 2003, Dunkel and Jaronski 2003) which makes it more attractive than conventional insecticides (Liu and Bauer 2006).

Imidacloprid is used against many stages of development of insect pests that cause damage worldwide. Imidacloprid strongly inhibited eggs placed in Petri dishes with 84.5% not hatching, but demonstrated a lower ovicidal control at this stage than that described by Bostanian et al. (2010) for *Neoseiulus fallacis* (Acari: Phytoseiidae) eggs where 100% of treated eggs was inhibited because imidacloprid has demostrated high toxicity against all stages of phytoseiid predatory mites (James 2003). Its toxic effect was also observed on eggs that were placed in branches (54.1%) and on trunks (50.0%). Larvae mortality is lower on trunks (29.2%) than on branches (45.8%) where it showed the highest value of the I × D interaction, probably because this systemic insecticide does not easily reach the xylem when it is applied on trunks because of its thicker rhytidome. Toxin and antifeedant activity (paralyses action in which the insects feed) of imidacloprid was demonstrated by Elbert et al. (1991), and it is not sufficient to suppress the appetite of larvae in contact with the treated wood to prevent the insertion of the larvae and to make

galleries inside the plant. The effects induced in different insect families to low exposure of this toxin cause in the insects different performance in their bodies (Calabrese and Baldwin 1998, 2003, Forbes 2000). Other trials should be conducted at other stages of development of the insects, which can show other toxic responses of the insect pest to this systemic insecticide (Terriere 1984). The injection of systemic insecticides such as imidacloprid into the trees has been effective in preventing attacks of other beetles such as *Hypocryphalus mangiferae* (Coleoptera: Scolytidae) (Poland et al. 2006, Saeed et al. 2011).

The significant inhibition of eggs confirmed the efficacy of chlorpyrifos at this stage development of *X. arvicola*. Chlorpyrifos provided total control (100% eggs unhatched) on all *X. arvicola* eggs evaluated when placed on Petri dishes. The mode of action is relatively fast because of nerve toxins that also produce serious side effects against non-target and/or beneficial insect populations (Corso 1988). The mode of action of chlorpyrifos means that high mortality was obtained in the first days after application, as was also demonstrated on eggs (branch and trunk) by the highest value of the regression linear coefficient in the I × D interaction, decreasing the residual activity at the time when larvae hatched in wood. Organophosphorus insecticides are among the most commonly used pesticides in the world (Tong et al. 2014). Significant ovicidal control also coincides with serious side effects caused against non-target insect populations. Although chlorpyrifos shows a high level of efficiency in a large number of species, it is also known to cause mortality in beneficial populations (Corso 1988). Currently, attempts are being made to look for natural insecticides that do not cause problems with beneficial populations, in order to replace chlorpyrifos and traditional chemical insecticides such as pyrethroids and organophosphates.

Flufenoxuron affects the final stage of embryogenesis, where chitin forms in the mouth of the embryo, thus preventing it from acquiring the suitable rigidity to hatch the egg (Wilson and Cryan 1997). The ovicidal insecticide flufenoxuron obtained acceptable values on eggs placed in Petri dishes, with a 81% of eggs unhatched, a value higher than that obtained by Santolamazza-Carbone and Fernández de Ana-Magan (2004) against *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) eggs, where a mortality of 74.5% was obtained in 15 days of evaluation. Pascual et al. (2012) also described the inhibitory capacity of flufenoxuron, feeding during 3 days fly olive females *Bactrocera oleae* Rossi (Diptera: Tephritidae) with diets in which the fertility rate was not altered but inhibition of eggs collected during 7 days was 66% and 73.1% in the first 2 days. Inhibition on branches (75.0%) and trunks (37.5%) showed the susceptibility of *X. arvicola* eggs to these insecticides. Larvae mortality obtained by the residual effect was acceptable on branches (20.8%) and low on trunks (12.5%) once these were exposed from day 7 after application. The insect growth regulators (IGRs) are known to be more toxic to the immature stages than to the adults of the herbivorous insects, including beetles (Staal 1975, Peleg 1983, Parrella and Murphy 1998). These insecticides may be considered for use in an IPM against immature stages of the beetle, except in those countries belonging to the European Union, where the use of flufenoxuron is prohibited.

Pyriproxyfen showed better ovicidal capacity than that of flufenoxuron. Pyriproxyfen obtained an inhibition of 90.5%, similar to that obtained by García Ruiz (2009) who applied pyriproxyfen to *X. arvicola* eggs from 0 to 24 h after laying of the eggs (90% of inhibition), or that described by Abo-Elghar et al. (2003) to treat *Callosobruchus maculatus* Fabricius 1775 (Coleoptera: Bruchidae) 0–24 h old eggs on bean seeds, with an inhibition of 91.9%. Pyriproxyfen showed great ovicidal capacity when it was applied on branches (79.1%), having less capacity on trunks (45.8%), where the greater thickness of the rhytidome and the deeper cracks make contact between the insecticide and eggs more difficult. These insecticides may be a remarkable tool when performing integrated control over the borers of those insect species that develop most of their life cycle inside the host plants and in which one of the most sensitive stages, such as the egg, occurs outside the host plant. Larvae mortality obtained by the residual effect of pyriproxyfen confirms the sensitivity of the immature stages of *X. arvicola* to this insecticide. Pyriproxyfen has a wide range of action and good results on various insect families. Mendel et al. (1994) described that the application of pyriproxyfen on pine needles before or after the oviposition of six different species of insects (which include two species of beetles) inhibited completely the hatching of eggs. Pyriproxyfen has a short residual effect over time, and the timing of its application must be precise, because of its poor stability in the field, and the application must always be aimed at the places where the possibility of reaching the development stage of pest is maximum (García Ruiz 2009).

From the results obtained, some alternative insecticides evaluated (*B. bassiana*, imidacloprid, pyriproxyfen) killed more than half of the egg population, enabling insecticides to be used for integrated control of *X. arvicola*, because of the mortality of eggs on Petri dishes and on branches and trunks of vines. It is known that the adult emergence curve can have fluctuations in the field because of environmental factors. Having a clearer knowledge of the biology of this species, we would have more information on the behaviour of females when making oviposition and on the dispersal of neonate larvae from *X. arvicola* eggs hatched in vineyard wood, which would permit the application of the insecticide when eggs and larvae were exposed.

Chlorpyrifos and pyriproxyfen were the insecticides that offered best ovicidal control in the two experiments because of their mode of action and toxicity. The downside to the significant efficacy of chlorpyrifos is the severe side effects caused to other non-target insect populations; as a result, their use, when the law allows it, can be justified only in vineyards with a high level of infestation. The timing for application of pyriproxyfen in the vineyard would be at maximum adult emergence or a spike in the population of adults, as they would have the ability to control their egg and larvae hatching of adults in flight. Spinosad, *B. bassiana* and imidacloprid, offer good ovicidal control on Petri dishes and the poorest ovicidal control on branches and trunks, but their residual capacity produces the best results in larvae control, which makes these insecticides, or a combination of them, a great tool for population control of *X. arvicola* at low density, and should be included in an IPM for *X. arvicola.*

All insecticides evaluated had the best ovicidal control when applied directly on Petri dishes, compared to that achieved when they were applied to branches and trunks, where the rhytidome and cracks protected the eggs. The protection was greater on trunks, which resulted in lower inhibition and mortality for all insecticides evaluated, excluding the residual effect of the biological control agent *B. bassiana*, which becomes the best insecticide with residual effect on neonate larvae on trunks. The capacity of the entomopathogenic fungi to invade actively the eggs through their shell and to proliferate inside them make them a highly effective tool for their control.

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