Use of soil insecticides to control the Brazilian ground pearl (Hemiptera: Margarodidae) in vineyards

Uso de insecticidas de suelo para el control de la perla de la tierra brasileña (Hemiptera: Margarodidae) en viñedos

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Abstract: The Brazilian ground pearl (BGP), *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae), is one of the most important grape pests in Brazil. Granular and water dispersible granule formulations, doses of application, times of treatment, and application technology of the soil insecticides imidacloprid and thiamethoxam were evaluated to control BGP in infested vineyards. Thiamethoxam and imidacloprid effectively reduced BGP by 90% in established vineyards. No difference in insect control was observed between the formulations of thiamethoxam. Multiple applications of thiamethoxam in November and January were more effective than a single treatment delivered in November. Doses higher than 0.2 g a.i./plant of imidacloprid and thiamethoxam reduced BGP infestations in 3-year-old vineyards. The granular formulation of thiamethoxam was more effective when applied around the root system than when delivered in six points around the trunk using a hand-operated granular spreader. Drench application of imidacloprid and thiamethoxam using a hand-operated hydraulic sprayer was effective for insecticide treatment, allowing regulation of the amounts of water and active ingredients applied per plant.

Key words: *Eurhizococcus brasiliensis*. Neonicotinoids. Imidacloprid. Thiamethoxam.

Resumen: La perla de la tierra brasileña (BGP), *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae), es una de las especies más importantes de la vid en el Brasil. Las formulaciones granulado y gránulos dispersables en agua, las tasas de aplicación, los tiempos de tratamiento, y métodos de aplicación de los insecticidas de suelo imidacloprid y tiamethoxam, fueron evaluados para controlar BGP en viñedos infestados. Tiamethoxam e imidacloprid efectivamente redujeron BGP por 90% en los viñedos establecidos. No se observó diferencia en el control de insectos entre formulaciones del tiamethoxam. Múltiples aplicaciones de tiamethoxam en noviembre y enero fueron más efectivas que un solo tratamiento dado en noviembre. Los dosis superiores a 0.2 g i.a./ planta de imidacloprid y tiamethoxam redujeron infestaciones de BGP en viñedos de tres años de edad. La formulación de granulado tiamethoxam fue más eficaz cuando se aplicó en todo el sistema de raíces que cuando se hizo en seis puntos alrededor del tronco utilizando una dispersadora manual granular. La aplicación de imidacloprid y tiamethoxam humedecida utilizando un pulverizador manual de accionamiento hidráulico fue eficaz para el tratamiento de insecticida, permitiendo la regulación de las cantidades de agua y los ingredientes activos aplicados por planta.

Palabras clave: *Eurhizococcus brasiliensis*. Neonicotinoides. Imidacloprid. Thiamethoxam.

Introduction

Brazilian ground pearl (BGP) *Eurhizococcus brasiliensis* (Hempel, 1922) (Hemiptera: Margarodidae) is one of the major grape pests occurring in Brazil (Hickel 1994; Soria and Dal Conte 2000; Botton *et al.* 2004). Similar species such as *Margarodes vitis* (Philippi, 1884) (Hemiptera: Margarodidae) are registered for Chile (Gonzalez *et al.* 1969), *Eurhizococcus colombianus* Jakubsky, 1965 (Hemiptera: Margarodidae) for Colombia (Kondo 2001; Quiñones *et al.* 2008; Rodrígues and Gómez 2008) and *M. capensis* Giard, 1897, *M. greeni* Brain, 1915, *M. prieskaensis* (Jakubski, 1965), *M. trimeni* Giard, 1897 and *M. vredendalensis* De Klerk, 1983 in South Africa (De Klerk 1987; Foldi 2005). BGP is a soil scale native to southern Brazil, and its immature stages feed on the roots of more than 80 species of plants (Botton *et al.* 2004). Scale reproduction is parthenogenetic facultative with one generation per year, producing crawlers from November to March (Botton *et al.* 2003). Infested plants show a gradual decline in vigor that becomes more severe with time. Plant decline and death are the result of scale sap suction in the roots. Shoots become shorter and thinner, with smaller leaves, followed by death of the cordons, finally the entire vine dies. The duration of this process varies but vines can be kill within four years. Great economic hardship occurs in the vineyards where growers must abandon grape cultivation and move to new areas free of the pest.

Pest spread occurs mainly in the roots of contaminated vegetative material and machinery used in infested vineyards (Botton *et al.* 2004). After contamination in the field, pest dispersion usually starts in patches that gradually become larger because of the migration of nymphs in the soil. This movement is assisted by Argentine ants *Linepithema humile* (Mayr, 1868) (Hymenoptera: Formicidae) which tend nymphs, helping the pest to colonize new roots (Hickel 1994). Measures to reduce BGP damage are difficult to implement because of scale polyphagy, subterranean development, an apodous feeding nymphal instar called a cyst and the defensive strategy of constructing a separate protective layer around their body from their own liquid excreta (Foldi 2005).

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Current pest management techniques use resistant root-stocks (VR 43-43 and VR 39-16 - *V. rotundifolia* x *V. vinifera*) (Botelho *et al.* 2005), organic matter to complement plant nutrition, cover crop management inside vineyards to reduce pest dispersal and application of insecticides (Botton *et al.* 2003; Dalbó *et al.* 2007). Chemical control is an important component in BGP management; however, previous trials conducted to select effective products were not successful (Gonzalez *et al.* 1969; De Klerk 1987; Soria and Braghini 1999; Soria and Grigoletti Júnior 1999). Some experiments have shown that phosphine and methidathion applied on roots of propagative material are alternatives to control cysts and avoid insect spread (Dalbó and Crestani 1988; Hickel *et al.* 1997; Hickel and Schuck 2005). In established vineyards, foliar applied vamidothion was the principal chemical product used for BGP management (Soria and Braghini 1999; Teixeira *et al.* 2002), however, this product has been withdrawn from the Brazilian market.

Neonicotinoid insecticides showed promising results for BGP control when applied to new plantings (Teixeira *et al.* 2002) and when used with resistant root-stocks and soil management (Dalbó *et al.* 2007). Both imidacloprid and thiamethoxam have shown excellent activity against sucking insects in grape and other fruit crops and show promise as alternatives for grape pest management. In this work, granulated and water dispersible granules formulations, doses of application, times of treatment, and application technique of the soil applied insecticides imidacloprid and thiamethoxam were evaluated to control BGP in commercial vineyards.

Material and Methods

Insecticides, product application, and insect sampling. Commercial formulations of imidacloprid (Confidor 700 GRDA®, Bayer Crop Science) and thiamethoxam (Actara 10 GR® and Actara 250 WGR®, Syngenta) were evaluated. Actara 10 GR® is a granular formulation with 10 g/kg (1%) of thiamethoxam, whereas Actara 250 WGR® and Confidor 700 GRDA® are granules micro dispersible in water containing 250 g/kg (25%) of thiamethoxam and 700 g/kg (70%) of imidacloprid, respectively.

Imidacloprid and thiamethoxam were diluted in 2 liters of water and applied as a drench with a watering can to the soil around the trunk. Before application, the soil around the plants was cleared to avoid insecticide absorption by surrounding vegetation. Granulated thiamethoxam was manually distributed uniformly around the plants incorporating the grains into the soil following application.

The number of BGP cysts per plant was evaluated in July of the year after the first insecticide application (November). Soil around the plants was dug and all plants were removed together with their roots. The radicular systems with insects were placed on a tray and counted. The month of July was chosen for evaluation because most of BGP population is in pearl-like cysts at that time of the year, which facilitates counting (Teixeira *et al.* 2002). All experiments were installed in naturally BGP infested areas located in the hills of the Rio Grande do Sul State, Brazil where high infestations of the pest are found.

Effect of formulations and splitting doses in new grape plantings. The trial was conducted using own rooted plants of 'Paulsen 1103' (*Vitis berlandieri x V. rupestris*) rootstocks which was field planted in a naturally infested BGP area located in Garibaldi, Rio Grande do Sul State, Brazil. Cuttings were potted, grown in a greenhouse, and field planted in September using a 1.5 x 2.5 m (line x row) spacing. The treatments evaluated were: Thiamethoxam (1% and 25%) at 0.1 g a.i./ plant in November, thiamethoxam (1% and 25%) at 0.1 g a.i./ plant split in two applications of 0.05 g a.i./ plant in November and January. Control plots were left untreated.

Evaluation of insecticides in established vineyards. The trials were conducted using 3-year-old vineyards located in Bento Gonçalves and Caxias do Sul, Rio Grande do Sul State, Brazil. The first vineyard consisted of a 'Cabernet Sauvignon' (*Vitis vinifera*) grafted on 'Paulsen 1103' with a vine spacing of 1.5 x 2.5 m and the second an own rooted 'Isabella' (*V. labrusca*) planted in the same spacing.

The insecticides evaluated were a) thiamethoxam (1%) at 0.2 and 0.4 g a.i./plant, and b) imidacloprid (70%) 0.21 and 0.42 g a.i./plant, where both insecticides were applied in November. An untreated control plot was included for comparison.

Effect of application technology in adult vineyards. In this trial, we evaluated the effect of thiamethoxam and imidacloprid applied in November using two application techniques in a 20-year-old 'Isabella' (*V. labrusca*) vineyard planted in a 2.0 x 3.0 m spacing. The treatments evaluated were a) thiamethoxam (1%) at 0.6 g a.i./plant applied manually around plants, b) the same treatment applied using a hand-operated granular machine (6 points - 10 g/point) around plants within a 50 cm distance from the trunk, and c) imidacloprid (70%) and thiamethoxam (25%) at 0.6 g a.i./ plant applied using a hand-operated mechanized sprayer distributing 6 liters of water per plant. This hand-operated mechanized drench sprayer was developed to regulate the amount of water per plant according to the size of the root system, where digital flow switch allows the operator to calculate the amount of water per plant during application. A control without treatment was included for comparison.

Experimental design and statistical analysis. All trials were designed using a randomized complete block design with four replications using four plants per plot with the exception of five plants per plot in the application technology. Analysis of variance and Tukey test were used to compare the data means. Insecticide efficiency was calculated using the Abbott (1925) formula.

Results

Effect of formulations and splitting doses in new plantings. A split application 0.05 g a.i./ plant of thiamethoxam in both November and January provided better BGP control ($\geq$ 90%) when compared with a single treatment in November (60-72%) using the same amount of active ingredient (0.1 g) per plant (g.l.= 6, F= 92.3429* P << 0.001) (Table 1). Granular and water dispersible in water formulations of thiamethoxam were equivalent for pest control when used in the same dose and time of application (Table 1). In control plants, an average of 23.9 ± 4.94 BGP cysts per plant was registered.

Table 1. Number of *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) cysts per plant and control after application of different thiamethoxam formulations in new Paulsen 1103 plantings.

Treatment	Dose (g a.i./plant)	Number of application	Application (month)	Number cysts per plant (X ± EP)[a]	Population reduction (%)
Thiamethoxam (1%)	0.05	1	November	7.6 ± 2.93 b	68.2
Thiamethoxam (1%)	0.05	2	November and January	2.0 ± 0.84 a	91.6
Thiamethoxam (1%)	0.10	1	November	6.9 ± 2.55 b	71.1
Thiamethoxam (25%)	0.05	1	November	8.9 ± 3.64 b	62.8
Thiamethoxam (25%)	0.05	2	November and January	2.5 ± 1.09 a	90.0
Thiamethoxam (25%)	0.10	1	November	7.9 ± 3.96 b	66.9
Control	-	-	-	23.9 ± 4.94 c	-

[a] Means in the same column followed by a different letter are significantly different Tukey test at P ≤ 0.05.

Evaluation of insecticides in established vineyards. In Garibaldi (g.l.= 4, F= 3.18*, p= 0.04) and Bento Gonçalves (g.l.= 4, F= 3.63*, P = 0.02), application of imidacloprid and thiamethoxam markedly decreased BGP density compared to untreated controls in established vineyards independent of dose and formulation (Table 2). When imidacloprid (70%) and thiamethoxam (1%) were applied at similar doses, both insecticides gave equivalent BGP control. In the first trial at Garibaldi, imidacloprid treatment decreased the infestation by 83.6% and 93.4% with no differences between the doses of 0.21 and 0.42 g a.i./ plant (Table 2). The same results were observed in the second trial at Caxias do Sul where imidacloprid reduced the infestation by 84.7% and 94.4% for the lower and higher doses, respectively (Table 2). For thiamethoxam, the population reduction was 86.8% and 98.6% (trial I) and 87.1% and 96.7% (trial II) for the lower and higher doses of AI per plant, respectively (Table 1). No differences in BGP control were observed between the higher and lower doses of imidacloprid and thiamethoxam.

Effect of application technology in adult vineyards. Manual incorporation and machine application of granular thiamethoxam reduced the BGP population when compared with untreated plots (g.l. = 4, F = 17.27*, P = 0.000003) (Table 3). Control plots showed an infestation of 135.8 ± 24.25 cysts per plant. However, homogeneous hand distribution of granules around the trunk with subsequent incorporation gave better results than machine application in six single spots placed around each plant (Table 3). Imidacloprid and thiamethoxam (0.6 g a.i./ plant) applied as a drench using a hand-operated sprayer provided a pest reduction of 86.9% and 86.5%, respectively, equivalent to the results obtained by hand spread of the granules and better than spot application of granules.

Discussion

Imidacloprid and thiamethoxam have been successfully used to manage different sucking insect species (Dominiak *et al.* 1996; Cowles *et al.* 2006), mainly mealybugs and sharpshooters, in vineyards of different countries (Daane *et al.* 2006; Tubajika *et al.* 2007). In this experiment, we reduced BGP populations in established vineyards and confirmed the results from Teixeira *et al.* (2002) in new plantings. Because of the univoltine development, where BGP crawlers emerge from November to March (Botton *et al.* 2003), better results were observed when insecticide doses per plant were divided into multiple applications, in November and January, instead of a single treatment in November. However, because of the difficulty of soil application and high labor costs, a single application in November using higher doses is considered to be satisfactory. In addition, the pre-harvest interval defined for grapes in Brazil (45 days for thiamethoxam and 60 days for imidacloprid) makes a second treatment in January impractical because of pesticide residue constraints.

In general there was a positive relationship between pest control and the neonicotinoid dose per plant in 3-years-old vineyards. In Brazilian vineyards, there is a large variation of plant spacing used by growers; with a density of 2000 to

Table 2. Effect of insecticides on *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) population after a single application in November in a 'Cabernet sauvignon' grafted on 'Paulsen 1103' (Trial I) and 'Isabella' own rooted (Trial II) vineyard with three years after planting.

Treatment	Dose (g a.i/plant)	Trial I (Garibaldi) Nº cysts per plant (X ± EP)[a]	Trial I (Garibaldi) Population reduction (%)	Trial II (Bento Gonçalves) Nº cysts per plant (X ± EP)[a]	Trial II (Bento Gonçalves) Population reduction (%)
Imidacloprid (70%)	0.21	23.4 ± 8.36 a	83.6	9.5 ± 4.84 ab	84.7
Imidacloprid (70%)	0.42	9.4 ± 2.20 a	93.4	3.5 ± 2.52 a	94.4
Thiamethoxam (1%)	0.20	18.8 ± 7.73 a	86.8	6.3 ± 1.03 ab	89.9
Thiamethoxam (1%)	0.40	2.0 ± 0.85 a	98.6	2.0 ± 1.14 a	96.8
Control	-	142.9 ± 62.46 b	-	62.1 ± 29.46 b	

[a] Means in the same column followed by a different letter are significantly different by Tukey test at P ≤ 0.05.

Table 3. Number of insects per plant and control of *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) after one application of insecticides (0.6 g a.i./plant) in November using different technologies in a own rooted 'Isabella' 20 years old vineyard.

Treatment	Application technology	Nº cysts per plant (X ± EP)[a]	Population reduction (%)
Thiamethoxam (1%)	Hand operated granulator with six points around trunk	49.4 ± 6.88 a	63.6
Thiamethoxam (1%)	Hand distribution of granules	21.6 ± 4.98 b	84.1
Thiamethoxam (25%)	Drench (6 liters of water per plant)	18.3 ± 5.56 b	86.5
Imidacloprid (70%)	Drench (6 liters of water per plant)	17.8 ± 3.97 b	86.9
Control	-	135.8 ± 24.25 c	-

[a] Means in the same column followed by a different letter are significantly different by Tukey test at $P \leq 0.05$.

3000 vines per hectare common for processing grapes (vine and juice) and 800 to 1000 vines/ ha for table grapes. Vineyard spacing must be considered when determining doses of neonicotinoids for BGP control. Here, the higher dose evaluated (0.6 g a.i./ plant), equivalent to 600 or 1800 g a.i/ ha, effectively controls BGP in table and processing vineyards, respectively.

The dose/ha for table grapes is near the maximum effective dose of imidacloprid evaluated for other grape sucking insects in other countries (Castillo *et al.* 2004; Byrne and Toscano 2006). As insect infestation normally occurs in foci inside vineyards, high doses can be used for treatment of initial infestations or localized population control in vineyards.

Several factors may contribute to reduced field efficacy of soil applied insecticides to control BGP. In these experiments, soils were >40% clay and 3% organic matter and were not irrigated. Better results are expected with neonicotinoids in sandy soils in younger vineyards that are drip irrigated (Ramakrishnan *et al.* 2000; Byrne and Toscano 2006; Daane *et al.* 2006). In drip-irrigated vineyards, roots are more concentrated and are located in a restricted soil portion when compared with vineyards that are not irrigated. Imidacloprid and thiamethoxam are systemic insecticides (Maienfisch *et al.* 2001a, 2001b; Cole and Horne 2006) and their effectiveness against a pest depends on uniform insecticide uptake by the roots. Because of a low contact activity of imidacloprid with BGP cysts (Hickel *et al.* 2001), effective uptake is important for insect control. The extensive root development by size and depth in non-irrigated vineyards may be a constraint because the insecticide may not reach all active roots, reducing insecticide absorption and the effectiveness of BGP control. This hypothesis was confirmed with better BGP control when granular thiamethoxam was uniformly spread around the root system and when insecticides were drenched, compared to spot application of thiamethoxam around the roots. The choice of a granular formulation or the drench system for neonicotinoid insecticide applications will depend mainly on the availability of water, which is limited in some regions.

The results shown in these experiments are from one-year trials where effective pest reduction was achieved. However, the levels of BGP infestation that kill a grapevine were not evaluated. Large differences in pest infestation between vineyards, regions and cultivars are commonly observed. This means that a pest control of 90% may be ineffective for plant protection considering the age of the vineyards and the level of infestation. The interaction between BGP, resistant rootstocks and chemical control in infested areas, as well as the relationship between soil fungi (*Fusarium* and *Cylindrocarpon*) and BGP damage caused by stylets into the grape roots must be investigated.

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