Effect of Insecticides Sprayed on Leaves and Applied via Soil to *Aphis illinoisensis* Shimer, 1866 (Hemiptera: Aphididae) on Grapevines

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High infestations by the grapevine aphid *Aphis illinoisensis* (Hemiptera: Aphididae) have been observed in vineyards in southern Brazil, retarding plant growth and causing premature berry drop. This study evaluated the effect of insecticides on the control of the species in two experiments carried out in a greenhouse. The first control experiment for *A. illinoisensis* was conducted with seedlings of *Vitis vinifera* var. 'Cabernet Franc' to assess the effect of azadirachtin (Azamax®) at dosages of 2.4 and 3.6 mL a.i. (active ingredient)/100 L of water, with reapplication seven days after the first application (DAFA). To compare its effect, the neonicotinoids imidacloprid (Provado 200 SC®) and thiamethoxam (Actara 250 WG®) were sprayed at dosages of 8 mL or g a.i./100 L of water in foliar application without reapplication. The second experiment compared the effect on *A. illinoisensis* by spraying these neonicotinoids at dosages of 8 mL or g a.i./100 L in foliar applications and of 0.05 mL or g a.i./100 L applied in the soil. Evaluations were performed at 0, 1, 5, 7, 10 and 14 DAFA. Imidacloprid and thiamethoxam effectively controlled *A. illinoisensis* in both forms of application [soil and foliar], while azadirachtin at the dosage of 3.6 ml a.i./100 L reapplied seven days after the first application provided 55.7% control. In conclusion, *A. illinoisensis* can be controlled effectively by employing neonicotinoids in the soil, while azadirachtin can be an alternative to reduce infestation pressure.

INTRODUCTION

Viticulture is an important economic and social activity in Brazil, both directly and indirectly, and also is important for the cultivation and processing industry and for tourism (Mello, 2014). In all wine-producing regions of the world, pests and diseases pose a major obstacle to the expansion of grapevine cultivation, affecting the quantity and the quality of the product (Kuhn & Nickel, 1998). In recent years, the incidence of infestations by the grapevine aphid *Aphis illinoisensis* Shimer, 1866 (Hemiptera: Aphididae) in vineyards in southern Brazil has increased significantly. The species is originally from North America, but it also occurs in Central and South America and was introduced into Europe in 2005 (Tsitsipis *et al*., 2005).

The feeding insects retard plant growth and shoot formation, and cause leafroll in new leaves; when they reach high levels, the aphids can be found in clusters, causing the berries to drop (Baker, 1917). In Brazil, *A. illinoisensis* reached harmful levels for grapevine culture only recently (Baronio *et al*., 2014), and this has made it necessary to apply insecticides for pest control, especially when the attack occurs when the plants are producing shoots and soon after flowering, or if the insect is damaging the clusters (Zaaqiq, 2007). However, no information is available about the effect of insecticides in controlling this pest in Brazil (Agrofit, 2014). Because of this lack of information, producers spray mainly pyrethroid and organophosphorus insecticides, which, because of their low selectivity for natural enemies, induce outbreaks of secondary pests, especially the broad mite, *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) (Botton *et al*., 2004), the two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) (Valadão *et al*., 2012), and the mealybug *Pseudococcus longispinus* (Targioni-Tozetti, 1867) vector of grapevine leafroll-associated virus (GLRaV-3) in Brazil (Kuniyuki *et al*., 2005).

One alternative to control aphids on grapevines is the neonicotinoids imidacloprid and thiamethoxam, which are

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already used for the management of the grape phylloxera *Daktulosphaira vitifoliae* (Fitch, 1856) (Hemiptera: Phylloxeridae) and *Eurhizococcus brasiliensis* Wille, 1922 (Hemiptera: Margarodidae) and are applied as a foliar spray as well as a soil drip or irrigation (Botton *et al*., 2004; 2013; Nondillo *et al*., 2014). The neonicotinoid insecticides imidacloprid and thiamethoxam are currently recommended for the control of *D. vitifoliae* in spray form at rates of 8 mL and 7.5 g a.i./100 L water (Botton *et al*., 2004), and for *E. brasiliensis*, applied to the soil at doses from 0.12 to 0.35 g a.i./plant (Botton *et al*., 2013), respectively. Based on the results it appears that this form of treatment is also effective for the control of *A. illinoisensis*, and in this case it was consider that application to the soil can be an alternative to preserve non-target organisms, particularly pollinators (Oliveira *et al*., 2013; Rossi *et al*., 2013).

Another alternative is azadirachtin, which is present in neem plants (*Azadirachta indica* A. Juss.) and recently was registered for the control of the red-banded thrips, *Selenothrips rubrocinctus* (Giard, 1901), in vineyards (UPL-Brasil, 2015). Azadirachtin inhibits feeding, is a repellent and affects metamorphosis, preventing the normal development of insect pests in different crops (Schmutterer, 1990; Mordue & Nisbet, 2000; Martinez & Van Emden, 2001). Another advantage of azadirachtin-based insecticides is their low toxicity to humans (Isman, 2006). They therefore can be used in organic production systems and/or when the attack occurs in the pre-harvest period due to the absence of residues and the rapid degradation of the active ingredient (Mordue *et al*., 2010; Agrofit, 2014; IBD Certifications, 2014).

This study aimed to evaluate the effect of azadirachtin, imidacloprid and thiamethoxam sprayed on leaves and applied via soil for the control of *A. illinoisensis* in vineyards.

MATERIALS AND METHODS

The experiments were carried out in a greenhouse at Embrapa Uva e Vinho in Bento Gonçalves, State of Rio Grande do Sul, Brazil. *A. illinoisensis* adults were collected in commercial vineyards of fine table grapes (*Vitis vinifera*) 'Itália', grown in a trellis system (1.8 x 1.25 m) under plastic cover in Caxias do Sul, State of Rio Grande do Sul (29º08'01"S; 51º06'06"W). The insects were transported to a greenhouse at the Entomology Laboratory of Embrapa Uva e Vinho, where they were inoculated on seedlings of *V. vinifera* cv. 'Cabernet Sauvignon' cultivated in a greenhouse. The insects were kept isolated on plants inside the greenhouse between April and October 2013, in a rearing stock kept in cages isolated from the plants used in the experiments conducted in the greenhouse.

Two experiments were conducted, evaluating azadirachtin, applied in the form of a foliar spray, and imidacloprid and thiamethoxam, both sprayed and applied to the soil (Table 1). *V. vinifera* cv. 'Cabernet Franc' plants grown on the rootstock 'Paulsen 1103' were cultivated individually in polyethylene pots (1.7 L) containing a mixture of soil, organic substrate (Mecplant®) and vermiculite in a ratio of 3:1:1. One month after the onset of sprouting, each plant was placed in a cage with 10 females of *A. illinoisensis* aged seven to 14 days, which were kept in a cage made of acetate sheets containing eight holes (4 cm in diameter). The holes were covered with nonwoven fabric that was glued over them, while the upper part was closed with the same tissue, using rubber bands, to prevent the insects from escaping.

Ten days after the grapevine sprouts had been infested with *A. illinoisensis* adults using a camel-hair brush, a preliminary count was made of the number of live insects (nymphs and adults) present on the leaves and shoots with the aid of a pocket magnifying glass (10 x magnification)

TABLE 1
Commercial product, active ingredient, dose, chemical group and toxicity class of insecticides used in the experiments to control *Aphis illinoisensis* on grapevines in a greenhouse.

Commercial product	Active ingredient	Dose		Chemical group	Toxicity class
		a.i.	c.p.		
		Experiment 1			
Azamax®*	Azadirachtin	2.4	200	Tetranotriterpenoid	III
Azamax®*	Azadirachtin	3.6	300	Tetranotriterpenoid	III
Provado 200SC®	Imidacloprid	8	40	Neonicotinoid	III
Actara 250WG®	Thiamethoxam	8	32	Neonicotinoid	III
Control	-	-	-	-	-
		Experiment 2			
		Foliar application[1]			
Provado 200SC®	Imidacloprid	8	40	Neonicotinoid	III
Actara 250WG®	Thiamethoxam	8	32	Neonicotinoid	III
		Soil application[2]			
Provado 200SC®	Imidacloprid	0.05	0.25	Neonicotinoid	III
Actara 250WG®	Thiamethoxam	0.05	0.20	Neonicotinoid	III
Control	-	-	-	-	-

[1]grams or mL of active ingredient (a.i.) or commercial product (c.p.) per 100 litres of water, sprayed to runoff point;

[2]grams or mL of a.i. or c.p. per plant; * reapplication of insecticide seven days after the first application.

before the treatments were applied. In the first experiment, the insecticides (Table 1) were sprayed until the runoff point, using a Jacto PJH manual backpack sprayer with 20 L capacity, a working pressure of 6 kgf/cm^2 and a Teejet TXA800015VK spray tip. In the control treatment, only water was sprayed. The treatments with azadirachtin were reapplied seven days after the first application (DAFA).

In the second experiment the foliar spray of neonicotinoid insecticides was the same as in the first experiment, and the aim was to compare the effect of the spraying with the effect of the application of the same active ingredients (a.i.) in the soil, at a dose of 0.05 g per plant, diluted in 50 mL of water and showered on the surface of the base of the plants with a graduated beaker. Each treatment was repeated 10 times, in a fully randomised design, considering each plant as one replicate.

The effect of the treatments on *A. illinoisensis* was evaluated by recording the number of live insects (nymphs and adults) per plant at 1, 5, 7, 10 and 14 DAFA. The evaluation was performed with the aid of a pocket magnifying glass (10 x), considering dead aphids as those that showed no perceptible movement and an opaque and dehydrated body.

The efficacy provided by the insecticide for *A. illinoisensis* control was calculated using the Abbott equation (Abbott, 1925). The mean number of surviving insects was compared among the treatments by Tukey's test ($p < 0.05$), using Statistica 7.0 software (Statsoft, 2004).

RESULTS

In both experiments, the mean number of adults and nymphs of *A. illinoisensis* per plant before the application of the insecticides ranged from 136 to 148 in the first experiment (Table 2), and 236 to 254 insects per plant in the second experiment (Table 3). There was no significant difference ($p < 0.05$) among the treatments, demonstrating the uniformity of the artificial infestation.

In the first experiment, in the evaluation conducted at 1 DAFA, azadirachtin (2.4 mL a.i./100 L) reduced the aphid population by 29.2, differing significantly from the control (Table 2). The results of this experiment show that, one day after the application, azadirachtin caused lower mortality, even in the treatment with a 50% higher dosage.

In the evaluation conducted at 5 DAFA, the plants treated with azadirachtin were more heavily infested than in the first evaluation (Table 2). However, compared to the control treatment the infestation was reduced by 44.4 and 29.5% at doses of 2.4 and 3.6 mL a.i./100 L of azadirachtin respectively (Table 2).

At 7 DAFA, azadirachtin decreased the infestation of *A. illinoisensis* by 15.4 and 26.1% (2.4 and 3.6 mL a.i./100 L) respectively, with a significant effect compared to the control only at the higher dose (Table 2). Thus, even when increasing the dose of azadirachtin there was no significant reduction in the *A. illinoisensis* population at 7 DAFA. At 10 DAFA, or three days after the second application (DASA) of azadirachtin, the insect population was lower in the treatments with azadirachtin compared with the assessment at 7 DAFA, with reductions in infestation by 46.6 and 46.9% in the treatments with 2.4 and 3.6 mL a.i./100 L of water respectively, differing from the control treatment.

In the final evaluation of the first experiment, performed at 14 DAFA, azadirachtin showed reductions in infestation of 46.1 and 55.7% (2.4 and 3.6 mL a.i./100 L water respectively), differing from the control treatment (Table 2).

TABLE 2

Mean number (± SE) of live insects per plant and corrected mortality percentage (%M) of *Aphis illinoisensis* in different periods after insecticide application to *Vitis vinifera* var. 'Cabernet Franc' plants in a greenhouse.

	Dose[3]	PC[4]	Days after the first application (DAFA)			
			1		5	
Treatment	a.i.	N[5]	N	%M[6]	N	%M
Azamax® [2] (Azadirachtin)	2.4	146 ± 22a[1]	107 ± 9.4b	29.2	164 ± 14b	44.4
Azamax® [2] (Azadirachtin)	3.6	141 ± 20a	120 ± 9.9ab	20.7	207 ± 24b	29.5
Provado 200 SC® (Imidacloprid)	8	142 ± 19a	10,8 ± 3.1c	92.9	0.0 ± 0.0c	100
Actara 250 WG® (Thiamethoxam)	8	136 ± 17a	102.9 ± 7.9b	32.1	2.7 ± 0.7c	99.1
Control		148 ± 20a	151.6 ± 15a	-	294 ± 29a	-

	Days after the first application (DAFA)					
	7		10		14	
Treatment	N	%M	N	%M	N	%M
Azamax® [2] (Azadirachtin)	272 ± 19ab	15.4	174 ± 21b	46.6	212 ± 25b	46.1
Azamax® [2] (Azadirachtin)	238 ± 16b	26.1	173 ± 17b	46.9	174 ± 11b	55.7
Provado 200 SC® (Imidacloprid)	0.0 ± 0.0c	100	0.0 ± 0.0c	100	0.0 ± 0.0c	100
Actara 250 WG® (Thiamethoxam)	0.7 ± 0.7c	99.8	0.0 ± 0.0c	100	0.0 ± 0.0c	100
Control	322 ± 29a	-	326.3 ± 24a	-	392.9 ± 26a	-

[1] Means followed by lowercase letters in the same column do not differ by Tukey's test at 5% probability; [2] Azadirachtin was reapplied seven days after the first application; [3] Dose (g or mL a.i./100 L water) in leaf application; [4] PC: Pre-treatment count; [5]N: Mean number of *A. illinoisensis* per plant; [6] Corrected mortality (%M) (Abbott, 1925).

TABLE 3
Mean number (± SE) of live insects per plant and corrected mortality percentage (%M) of *Aphis illinoisensis* in different periods after insecticide applications to the leaves and soil of *Vitis vinifera* var. 'Cabernet Franc' plants in a greenhouse.

	Dose[2]	PC[3]	Days after the first application (DAFA)			
			1		5	
Treatment	a.i.	N[4]	N	%M[5]	N	%M
			Foliar application			
Provado 200 SC® (Imidacloprid)	8	245.8 ± 26a[1]	38.1 ± 7c	88.1	0.3 ± 0.1b	99.9
Actara 250 WG® (Thiamethoxam)	8	243.5 ± 19a	219 ± 19b	31.6	5.9 ± 1.4b	98.5
			Soil application			
Provado 200 SC® (Imidacloprid)	0.05	236.2 ± 24a	231.5 ± 22b	27.7	36.5 ± 15b	90.6
Actara 250 WG® (Thiamethoxam)	0.05	242 ± 21a	215 ± 26b	32.8	5.9 ± 1.4b	98.5
Control		241.9 ± 17a	320.2 ± 24a	-	386.5 ± 29a	-

	Days after the first application (DAFA)					
	7		10		14	
Treatment	N	%M	N	%M	N	%M
			Foliar application			
Provado 200 SC® (Imidacloprid)	0.0 ± 0.0b	100	00 ± 00b	100	00 ± 00b	100
Actara 250 WG® (Thiamethoxam)	0.5 ± 0.4b	99.9	00 ± 00b	100	00 ± 00b	100
			Soil application			
Provado 200 SC® (Imidacloprid)	3.8 ± 2.9b	99.0	00 ± 00b	100	00 ± 00b	100
Actara 250 WG® (Thiamethoxam)	0.0 ± 0.0b	100	00 ± 00b	100	00 ± 00b	100
Control	372.5 ± 31a	-	348.5 ± 26a	-	390.1 ± 42a	-

[1] Means followed by lowercase in the same column do not differ by Tukey's test at 5% probability; [2] Dose (g or mL a.i./100 L water) in leaf application or (g or mL a.i./plant) via soil; [3] PC: Pre-treatment count; [4] N: Mean number of *A. illinoisensis* per plant; [5] Corrected mortality (%M) (Abbott, 1925).

However, although the aphid population was reduced, the level of infestation in plants with two applications of azadirachtin, even with the 50% increase in concentration, remained fairly high, averaging 174 to 212 insects per plant, whereas in the control treatment the infestation level was more than 390 insects per plant (Table 2).

In the case of the neonicotinoids, in the evaluation conducted at 1 DAFA, imidacloprid in foliar application decreased the infestation of *A. illinoisensis* by 92.2%, differing significantly from both thiamethoxam (32.1%) and the control (Table 2). In the subsequent evaluations, at 5, 7, 10 and 14 DAFA, the neonicotinoids in the foliar application provided infestation reductions of more than 99%, with 100% control at 10 and 14 DAFA. The levels of reduction were equivalent, thus confirming the efficacy of these insecticides for pest control.

In view of the observed efficacy of the neonicotinoids against *A. illinoisensis* in foliar application, the effect of these insecticides in the soil was also evaluated and compared to the powdered form (Table 3).

The evaluation conducted at 1 DAFA showed that imidacloprid and thiametoxam applied to the soil reduced the infestation by 32.8 and 27.7% respectively, which was equivalent to the foliar application of thiametoxam (31.6%). Imidacloprid in foliar application showed a better effect than the other treatments, reducing infestation by 88.1% in the same period (Table 3).

Five days after the application of the neonicotinoids, thiamethoxam provided a 98% reduction of the infestation in both foliar and soil applications. This did not differ from imidacloprid, which provided population reductions of 90.6 and 99.9% in the soil and on leaves respectively (Table 3). At the subsequent evaluations (7, 10 and 14 DAFA), the insecticides provided excellent control of this insect population, regardless of the form of application, with 100% control at 10 and 14 DAFA (Table 3).

DISCUSSION

The reduction in infestation observed after the application of azadirachtin was also observed by Bernardi *et al*. (2012), who evaluated the effect of insecticides on the control of the strawberry aphid, *Chaetosiphon fragaefolli* (Cockerell, 1901) (Hemiptera: Aphididae). Costa *et al*. (2010) found that azadirachtin (as Neemazal®; 10 mL a.i. /100 L water) afforded 91% control of *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae) two days after application to seedlings of the cowpea *Vigna ungiculata* (L., 1753). However, it should be noted that the amount used for the control of *A. craccivora* was approximately five times higher than that used in this study.

Bernardi *et al*. (2012) observed a 28% reduction in the fecundity of females, regardless of the dosage used, compared to the control treatment, 15 days after the first application on *C. fragaefoli*. Whereas the azadirachtin dosages evaluated

by these authors were similar to the dosages used in this study, the smaller effect on *A. illinoisensis* could be due to the lower susceptibility of the species, as the test of Bernardi *et al*. (2012) used direct contact of the insecticide on the aphids, and the effect of direct contact of this compound on aphids has been demonstrated in *A. craccivora* in beans (Stark & Rangus, 1994). This reinforces the observation that the efficiency of azadirachtin depends on the target species.

In a study evaluating the effect of aqueous extracts of azadirachtin powder from seeds (neem) at concentrations of 0.4 and 1.4 g a.i./100 L water on *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), Santos *et al*. (2004) found that females fed since birth with cotton-leaf discs treated at a dose of 1.4 g a.i./100 L did not generate progeny, while the control increased the production of nymphs 35-fold.

In this study, azadirachtin affected the colour and the mobility of the aphids, leaving the nymphs dark and nearly immobile. In addition to the observed effects, azadirachtin can cause physiological changes that affect growth, metamorphosis and reproduction, including feeding inhibition (Mordue & Nisbet, 2000).

Bernardi *et al*. (2012), while evaluating the effect of insecticides on *C. fragaefoli* control in strawberry, observed an 83% reduction in aphid infestation with the application of thiamethoxam, with more superior efficiency than on *A. illinoisensis* in the same evaluation period. Thus, imidacloprid applied to the leaves showed the greatest shock effect on the grapevine aphid, even compared with the same dose of the active ingredient of thiamethoxam.

Studies on the control of *A. illinoisensis* were performed by Zaaqiq (2007), who evaluated organophosphorus insecticides (diazinon and chlorpyriphos) and the pyrethroid cypermethrin to control this species and demonstrated that all insecticides were effective in pest control. However, 60 days after the last application, the plants treated with cypermethrin showed high levels of insect infestation – higher than in the plants treated with chlorpyriphos and diazinon. This effect, according to the author, was due to the negative impact of cypermethrin on natural enemies, thus increasing *A. illinoisensis* infestation. In this case, although the organophosphorus insecticides were effective in controlling the pest, they are not authorised for use in viniculture in Brazil (Agrofit, 2015).

The application of neonicotinoid insecticides are effective for *A. illinoisensis* control in vine seedlings, both in foliar and soil application. The pollinators that are present in flowering weeds in the vineyards may be affected negatively occur with foliar application, thus restricting this method of treatment. Grapevines are self-pollinated, resulting in a reduced presence of bees in the crop, while there is no significant effect of cropping system (conventional or organic) on the richness and abundance of pollinators (Brittain *et al*., 2010a). Even so, due to the fact that the foliar application of neonicotinoids can have a negative effect on the richness of some pollinators in vineyards (Brittain *et al*., 2010b), we suggest the soil application of these chemicals for pest control.

CONCLUSIONS

The insecticides thiamethoxam (Actara 250 WG®, 8 g a.i./100 L of water by foliar spray or 0.05 g a.i./plant in the soil) and imidacloprid (Provado 200 SC®, 8 mL a.i./100 L water or 0.05 mL a.i./plant in soil application) are effective in the control of *Aphis illinoisensis* in vineyards.

The insecticide azadirachtin (Azamax, 2.4 and 3.6 mL a.i./100L water), with reapplication seven days after the first application, is not effective in the control of *Aphis illinoisensis* in vineyards.

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