



Population suppression of phylloxera gallicolae and radicolae forms on grapevines with the use of synthetic insecticides

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ABSTRACT: The grapevine phylloxera *Daktulosphaira vitifoliae* (Fitch, 1856) is the main insect pest of viticulture globally. Infestations can occur in the aerial part of the plant (gallicolae form) and roots (radicolae form). In this study, the effect of insecticides on the populations suppression of the gall and root forms of phylloxera one vine was evaluated. For the gallicolous form, the thiamethoxam (Actara 250 WG[®], 40g c.p./100L⁻¹), flupiradifurone (Sivanto[®] Prime 200 SL, 75mL c.p./100L⁻¹), and sulfoxaflor (Closer[®] SC, 40mL c.p./100L⁻¹) were evaluated in the field, under natural infestation, using rootstock plants ‘Paulsen 1103’ (*Vitis berlandieri* x *V. rupestris*). For the root stage, an experiment was carried out in a greenhouse using rooted seedlings of ‘Cabernet Sauvignon’ (*Vitis vinifera*) grown in pots artificially infested with 200 phylloxera eggs per plant. After 80 days of infestation, the thiamethoxam (0.2g p.c./plant), flupiradifurone (0.8mL p.c./plant), sulfoxaflor (0.3mL/plant) and imidacloprid (Proved 200 SC, 0.7 mL/plant) were applied via drench. For the gallicolae form, an application of the flupiradifurone provided a gall reduction of 90% at 28 days after the first application (DAFA). While for thiamethoxam, 3 applications were needed at weekly intervals to maintain the same level of control. For sulfoxaflor, a second application at 14 DAFA was necessary to provide a level of control above 90%. For the root stage, the insecticides sulfoxaflor and imidacloprid showed the best results, with 96 and 89% of control over nymphs and adults, respectively. The insecticides flupiradifurone and sulfoxaflor are suitable for the chemical control of phylloxera in the vine.

Key words: *Daktulosphaira vitifoliae*, management, galls, Sulfoximines, Butenolides.

Supressão populacional de fase gálica e radícula da filoxera em videira com a utilização de inseticidas sintéticos

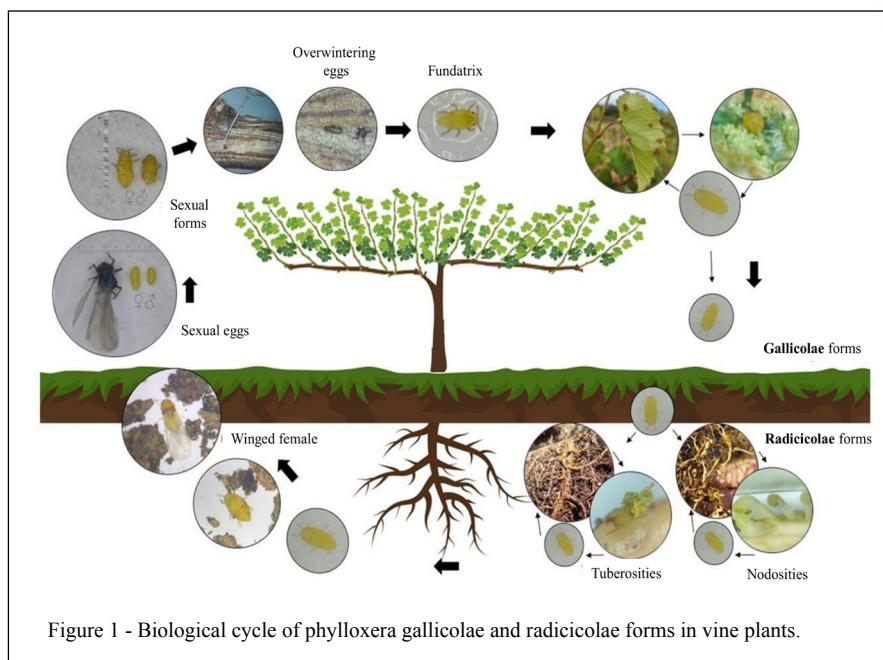
RESUMO: A filoxera-da-videira *Daktulosphaira vitifoliae* (Fitch, 1856) é o principal inseto-praga da viticultura mundial. As infestações podem ocorrer na parte aérea (forma gálica) e raízes (forma radícula). Neste trabalho foi avaliado o efeito de inseticidas na supressão de populações da forma gálica e radícula da praga em videira. Para a fase gálica, os inseticidas thiamethoxam (Actara 250 WG[®], 40g p.c./100L⁻¹), flupiradifurona (Sivanto[®] Prime 200 SL, 75mL p.c./100L⁻¹) e sulfoxaflor (Closer[®] SC, 40mL p.c./100L⁻¹) foram avaliados a campo, sob infestação natural, utilizando plantas do porta-enxerto ‘Paulsen 1103’ (*Vitis berlandieri* x *V. rupestris*). Para a fase radícula foi conduzido um experimento em casa-de-vegetação a partir de mudas enraizadas de ‘Cabernet Sauvignon’ (*Vitis vinifera*) cultivadas em vasos infestados artificialmente com 200 ovos da filoxera por planta. Após 80 dias da infestação, os inseticidas thiamethoxam (0,2g p.c./planta), flupiradifurona (0,8mL p.c./planta), sulfoxaflor (0,3mL/planta) e imidacloprido (Provado 200 SC, 0,7 mL/planta) foram aplicados via drench. Para a fase gálica, uma aplicação do inseticida flupiradifurona proporcionou uma redução de galhas de 90% aos 28 dias após a primeira aplicação (DAPA). Enquanto que para o thiamethoxam, foram necessárias três aplicações em intervalos semanais para manter o mesmo nível de controle. Para o sulfoxaflor, foi necessária uma segunda aplicação aos 14 DAPA, para proporcionar um nível de controle acima de 90%. Para a fase radícula, os inseticidas sulfoxaflor e imidacloprido apresentaram os melhores resultados, com 96 e 89% de controle sobre ninfas e adultos respectivamente. Os inseticidas flupiradifurona e sulfoxaflor são alternativas aos neonicotinoides (imidacloprido e thiametoxam) para a supressão populacional da filoxera na cultura da videira.

Palavras-chave: *Daktulosphaira vitifoliae*, manejo, galhas, Sulfoxaminas, Butenolidas.

INTRODUCTION

Grapevine phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera, Phylloxeridae) is considered one of the main pests of grapevine leaves and roots in Brazil (GRANETT et al., 2001; ANDZEIEWSKI et al., 2022). Damage is caused on the leaves through the formation of galls (gallicolae form) (Figure 1), which leads to leaf distortion, necrosis, and early defoliation, causing reductions

in production and fruit quality (FORNECK et al., 2019; YIN et al., 2019). However, infestation can also occur in plant roots (radicolae form) (Figure 1), producing nodosities in rootlets and tuberosities in older roots (GRANETT et al., 2001) which directly affects the transport and absorption of nutrients and water, leading to plant decline (BENHEIM et al., 2012), as well as promoting a reduction in the host plant’s resistance, increasing susceptibility to fungal infection (EDWARDS et al., 2007).



The use of resistant rootstocks and chemical control with the use of insecticides based on neonicotinoids is the most used strategy for the management of the gall-forming form of phylloxera (YIN et al., 2019). The constant use of this chemical group over a season (3 to 5 applications) may provide pest resistance; a fact not yet verified in Brazil. Studies aimed at the availability of insecticides with different modes of action are essential to preserve the activity of insecticidal molecules in the field (NAUEN et al., 2015). The present study evaluated the potential use of insecticides belonging to the chemical class butenolides and sulfoximines in the management of gall and root phylloxera in grapevines.

MATERIALS AND METHODS

Population suppression of phylloxera's gallicolae form

The experiment was carried out in the field, using five-year-old plants of the rootstock 'Paulsen 1103' (*V. berlandieri* × *V. rupestris*) grown at 1.0 × 2.6 m spacing with a history of phylloxera infestation, in the municipality of Bento Gonçalves, RS, Brasil (Latitude 29°09'56" S, Longitude 51°32'3" W). The experimental design was randomized blocks with six replications, each repetition consisting of 4 plants. The insecticides (Table 1) were applied at weekly intervals with an electric backpack sprayer with a capacity of 18 liters to the point of run-off,

applying an approximate volume of spray of 460 L.ha⁻¹. Evaluations were carried out before the application of the treatments (0 days) and at 7, 14, 21, and 28 Days After the First Application (DAFA). Plants without insecticide application were used as a control. For each repetition, the total number of phylloxera galls present on the first two leaves on 20 randomly chosen shoots was evaluated, without prior marking. Through these data, the percentage of Infestation reduction (%IR) was calculated in relation to the control. When the IR was equal to or less than 70%, a new application of the respective insecticide was performed.

Population suppression of phylloxera's radicolae form

Grapevine cuttings of 'Cabernet Sauvignon' (approximately 28 cm long and 7 mm in diameter) were collected in the field during the winter period (July 2019 – average local temperature of 12.3 °C) when the plants were in dormancy (without the presence of leaves). Then, the cuttings were stored in a cold chamber (temperature between 2 ± 2 °C and air humidity > 95%) for approximately two months. After this time, the cuttings were removed from the chamber and hydrated for 12 hours in pure water before planting (September 2019). Planting was carried out in plastic pots (3L) (one plant per pot) containing organo-mineral compost consisting of 2 parts of soil (corrected to pH 5.6-5.8), 1 part of

Table 1 - Insecticides evaluated for the management of *Daktulosphaira vitifoliae*.

Active ingredient (a.i.)	Commercial product (c.p.)	Concentration ^a [Formulation]	Foliar dose ^b		Soil dose ^c		Chemical group
			a.i.	c.p.	a.i.	c.p.	
Thiamethoxam ⁱ	Actara	250 [WG]	7.50	30	0.05	0.2	Neonicotinoids [4A]
Flupyradifurone ⁱⁱ	Sivanto Prime	200 [SL]	15	75	0.16	0.8	Butenolides [4D]
Sulfoxaflor ⁱⁱⁱ	Closer	240 [SC]	9.6	40	0.07	0.3	Sulfoxamines [4C]
Imidacloprid ^{iv}	Provado	200 [SL]	-	-	0.14	0.7	Neonicotinoids [4A]

ⁱSyngenta Proteção de Cultivos Ltda, São Paulo, SP, Brazil; ⁱⁱBayer S.A, São Paulo, SP, Brasil; ⁱⁱⁱDow AgroSciences Industrial Ltda, Barueri, SP, Brasil;

^aConcentration in g a.i. kg⁻¹ or mL⁻¹ [WG = dispersible granules, SL = soluble concentrate, SC = concentrated suspension];

^bDose in g a.i. kg⁻¹ or mL⁻¹ for 100L of water;

^cDose in g a.i. kg⁻¹ or mL⁻¹ for 1 plant.

Canadian peat-based substrate, and 0.5 of vermiculite (fine granulometry). After five months (February 2020), 200 phylloxera eggs from maintenance breeding were inoculated per plant. The eggs were inoculated close to the root system of the plants, following the methodology proposed by HERBERT et al. (2008). After 80 days, the period necessary for infestation and multiplication of insects in the roots (HERBERT et al., 2008), the application of insecticides (treatments) was carried out by drench (Table 1) in a spray volume of 500 mL of spray solution per pot. Only water was applied as a control treatment. The experimental design was completely randomized, with 12 replications (plants) per treatment. After 30 days after application (DAA), the number of eggs, nymphs and adults of *D. vitifoliae* present in the roots was counted. For this, two samples of roots per pot were randomly collected (three grams of root per sample) using pruning shears. Subsequently, the roots were placed in identified Falcon tubes (50 mL) and stored in a freezer (-15 °C). After 24 hours, the tubes were removed from the freezer and the sampled roots went through a washing and filtering process, adapting the methodology proposed by HERBERT et al. (2008). After this process, the eggs, nymphs, and adults collected were counted with the aid of a stereoscopic microscope (5× magnification).

Statistical analysis

Generalized linear models of the exponential family of distributions (NELDER & WEDDERBURN, 1972) were used for the analysis of the variables studied (HINDE & DEMÉTRIO, 1998). When significant differences were detected among treatments, multiple comparisons (Tukey's test, $P < 0.05$) were performed using

the ght function in the Multicomp package, with adjustment of p-values. All analyses were performed using the statistical software "R" version 2.15.1 (R DEVELOPMENT CORE TEAM, 2012). The (%) of IR was calculated using the ABBOTT formula (1925).

RESULTS

Population suppression of phylloxera's gallicolae form

In the pre-evaluation performed before the application of insecticides, there was no significant difference ($F = 0.31$; d.f. = 3, 279; $P = 0.8186$) among treatments, indicating uniformity in phylloxera infestation on leaves (Table 2). In the first evaluation, performed 7 DAFA (Days After the First Application), there was a significant reduction in the number of galls on the plants after the application of thiamethoxam, flupyradifurone, and sulfoxaflor (Table 2), providing an IR of 67.5%, 96.1%, and 94.4%, respectively (Table 2). Due to the insecticide thiamethoxam providing $IR < 70\%$ at 7 DAFA, a new application of the product was carried out. In the evaluation carried out 14 DAFA, an increase in the formation of galls on grapevine plants was observed with the use of sulfoxaflor, a fact that resulted in an $IR = 29.8\%$, differing statistically from the insecticide flupyradifurone ($IR = 77\%$) (Table 2). However, after the second application of thiamethoxam and sulfoxaflor there was a significant reduction in the number of galls per leaf, with an IR greater than 88% for the two treatments (Table 2). The lowest number of galls per leaf and; consequently, the highest rate of IR was observed for the insecticide flupyradifurone in a single application (28 DAFA – $IR = 97.4\%$) (Table 2).

Table 2 - Mean number (\pm standard error) and percentage of infestation reduction (% IR) of *Daktulosphaira vitifoliae* galls on pointers of rootstock 'Paulsen 1103' in the field.

Active ingredient	Number of applications	Pre-evaluation	-----7 DAFA-----		----14 DAFA----		----21 DAFA----		----28 DAFA----	
			X \pm SE ¹	X \pm SE	%IR ²	X \pm SE	%IR	X \pm SE	%IR	X \pm SE
Thiamethoxam	2	21.7 \pm 1.5A	10.9 \pm 1.0B	67.5	9.6 \pm 1.1C	70.2	0.7 \pm 0.2C	97.7	3.0 \pm 0.6B	88.9
Flupyradifurone	1	23.0 \pm 1.6A	1.3 \pm 0.3C	96.1	7.4 \pm 1.0C	77.0	3.3 \pm 0.6B	89.1	0.7 \pm 0.1C	97.4
Sulfoxaflor	2	22.2 \pm 1.6A	1.7 \pm 0.4C	94.4	22.6 \pm 0.9B	29.8	0.5 \pm 0.1C	98.3	1.3 \pm 0.3BC	95.2
Control	-	23.7 \pm 1.4A	33.5 \pm 1.1A	-	32.2 \pm 1.0A	-	30.4 \pm 1.0A	-	27.1 \pm 0.9A	-
<i>F</i>		0.31	391.6		140.6		603.4		474.8	
<i>d.f.</i>		3, 279	3, 279		3, 279		3, 279		3, 279	
<i>P</i>		0.8186	< 0,0001		< 0,0001		< 0,0001		< 0,0001	

¹Average number \pm SE. Means followed by the same letter in the column do not differ by Tukey's test at a 5% significance level.

²Percentage of infestation reduction (%IR) calculated by the formula of ABBOTT (1925).

Population suppression of *phylloxera's radicolae* form

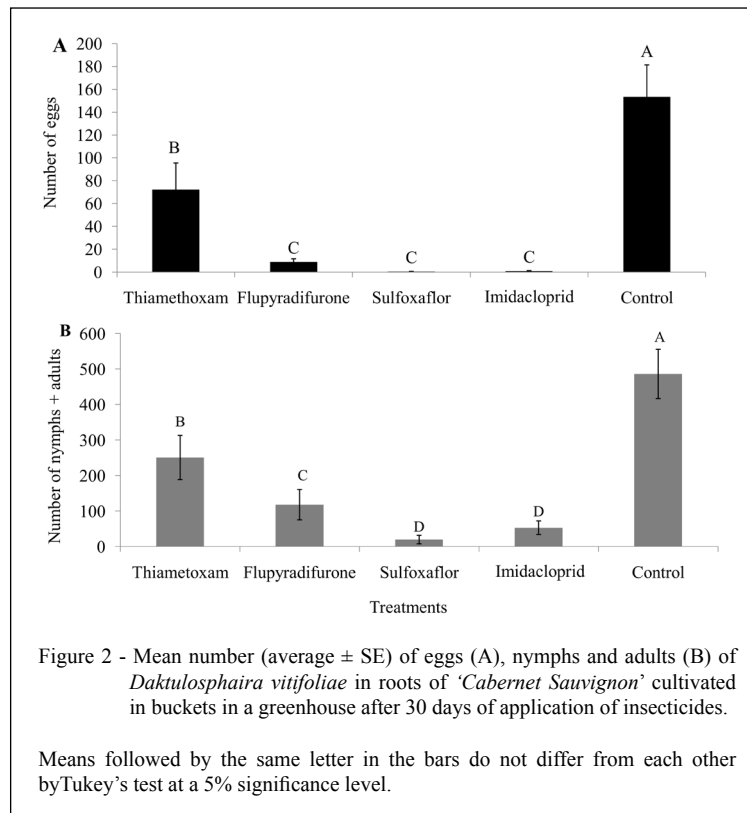
At 30 DAFA, the insecticides flupyradifurone (8.75 eggs), sulfoxaflor (0.37 eggs), and imidacloprid (0.83 eggs) showed a significantly ($F = 4.12$; $d.f. = 4, 168$; $P < 0.0001$) lower number of eggs in relation to the insecticide thiamethoxam (72.08 eggs) and the control (153.2 eggs) (Figure 2A). The smallest numbers of nymphs and adults were observed in plants treated by drench with the insecticides sulfoxaflor (19.5 insects) and imidacloprid (53.0 insects) (Figure 2B), differing statistically ($F = 8.11$; $d.f. = 4, 168$; $P < 0.0001$) from the insecticides thiamethoxam (250.8 insects) and the control (486.2 insects) (Figure 2B).

DISCUSSION

Spraying chemical insecticides belonging to the neonicotinoid group (thiamethoxam and imidacloprid) is the most efficient way for the management of phylloxera according to several studies (AL-ANTARY et al., 2008; HERBERT et al., 2008). For the radicolae form, the management of phylloxera is considered more complex because the insecticides used in the management have a low displacement of the chemical in the soil (BENHEIM et al., 2012). According to SLEEZER et al. (2011), grapevine phylloxera can be reported on roots as deep as 1.2 m in the soil profile. In contrast, when pest infestations occur on the leaves (gallicolae form), the insects are more exposed to the action of the products (GRANETT et al., 2001). Among the insecticide options that do not

belong to the neonicotinoid chemical group for the management of phylloxera, spirotetramat stands out (Movento™) and fenpropathrin (Danitol™) (SLEEZER et al., 2011; BENHEIM et al., 2012; YIN et al., 2019). Spirotetramat is derived from tetrionic acid and acts mainly by ingestion by inhibiting lipogenesis which leads to a decrease in growth regulators and fertility (BOSTANIAN et al., 2012). Fenpropathrin is a fourth generation pyrethroid and acts as a modulator of sodium channels (YIN et al., 2019). Studies have shown satisfactory efficiency of the use of spirotetramat and fenpropathrin (infestation reduction greater than 80%) in the population density of phylloxera when used in the form of application by drench and foliar (spirotetramat) and via foliar application (fenpropathrin) (SLEEZER et al., 2011; KOCSIS & ANDOR, 2014). However, the insecticide is not yet available in the Brazilian market.

In the present study, a high efficiency of the sulfoxaflor insecticide was observed for both the gall-forming and root form of phylloxera, with a population suppression of 95.98% of the gallicolae form of the pest. However, for the suppression of the gallicolae form, two applications were necessary to achieve a reduction in gall infestation above 90%. The sulfoxaflor (Closer™), from the chemical group of sulfoximines, showed promising results in the control of the gallicolae form when compared with neonicotinoid and spirotetramat (BACCI et al., 2018). The sulfoxaflor, even though it belongs to the mode of action of neonicotinoids, interacts with nicotinic acetylcholine receptors in a different way (ZHU et al., 2011; WATSON et al., 2017).



The sulfoxaflor is the first compound used to protect crops that contain a fraction of sulfoxamine and; therefore, presents differences in its susceptibility to metabolic enzymes associated with resistance to some insecticides (SPARKS et al., 2012; SIVITER et al., 2019). As with neonicotinoids, sulfoxaflor acts on nicotinic acetylcholine receptors, but with a distinct combination of attributes. These distinctions showed notable differences in the frequency and degree of cross-resistance between sulfoxaflor and other insecticides. Studies have shown that high levels of resistance to sulfoxaflor can be selected in the laboratory, through increased metabolism by specific cytochrome P450 (WATSON et al., 2021). However, approximately 82% of studies examining cross-resistance to sulfoxaflor observed limited or no cross-resistance. Also, no direct correlation with resistance to neonicotinoids such as Imidacloprid and Acetamiprid (WATSON et al., 2017). In this way, it can be a valuable tool for the management of sucking insects and to prevent or delay the evolution of pest resistance.

Similar results were obtained with the use of the insecticide flupyradifurone when used as

a foliar application. However, when applied via soil, flupyradifurone showed an intermediate efficiency of control. Although the insecticide flupyradifurone shares the same mode of action as neonicotinoids (targets nAChRs), the molecule is chemically different and it still exhibits distinct structural properties relevant to the target of action fact that disfavors the evolution of resistance (PILLI et al., 2010; NAUEN et al., 2015). Until now, flupyradifurone has been reported to be very powerful for controlling a variety of notorious insect pests, such as *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) (NAUEN et al., 2015; LIANG et al., 2019). According to YIN et al. (2019), after uptake in the plant by the leaves, flupyradifurone is translocated, systemically, providing toxicity to insects that are feeding on both the adaxial and abaxial part of the leaves. When used in the form of a drench, flupyradifurone has greater mobility in sandy loam soils than in clayey soils, since greater adsorption of the product molecules occurs in clayey soils, reducing the potential for mobility of the compound to leaching (SARKAR & MUKHERJEE, 2021). This point must be taken into account for the management of phylloxera, since in the traditional viticultural areas of southern Brazil, on

average, orchards are cultivated in soils with a high proportion of clay and; consequently, have drainage restrictions (SARKAR & MUKHERJEE, 2021). This factor may contribute positively to the use of sulfoxaflor to control root phylloxera.

In addition, flupyradifurone did not demonstrate toxicological effects on forager bees (HESSELBACH & SCHEINER, 2018). In contrast, recent studies warn that sulfoxaflor can present adverse effects on pollinating species similar to Neonicotinoid (SIVITER et al., 2019). This point is considered of paramount importance for the management of phylloxera, since the chemical control of the gallicolae form of the pest occurs during the flowering phase of the vine crop, a period in which there is a high incidence of bees in the orchards.

CONCLUSION

The insecticides flupyradifurone and sulfoxaflor are suitable for the chemical control of phylloxera in the grapevine crop.

For the management of the aerial part of the plant, an application of flupyradifurone was satisfactory to cause suppression of the pest population for 28 days. While for sulfoxaflor, two applications were needed to obtain an infestation reduction above 90%.

The insecticides sulfoxaflor and imidacloprid efficiently control the root form of phylloxera in grapevines.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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