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Laboratory trials to reduce egg hatching of the American grapevine leafhopper (*Scaphoideus titanus*) with selected insecticides

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Abstract

Laboratory trials were carried out under controlled conditions (24 °C, 75% RH, 16L:8D) to evaluate the effect of six selected insecticides on egg hatching of *Scaphoideus titanus* Ball (Hemiptera Cicadellidae). Standardized cuttings of two-year-old Isabella (*Vitis vinifera* × *Vitis labrusca*) canes were used as test plant material. A newly developed test cage allowed the treatment of infested canes in a vertical position and a standardized assessment of the number of *S. titanus* nymphs on yellow sticky traps over the whole hatching period. The six test substances etofenprox, spirotetramat, paraffin oil, azadirachtin, spirodiclofen and aluminium silicate (kaolinite) resulted in a reduction of egg hatching of *S. titanus* of 100%, 99%, 83%, 72%, 64% and 47% in relation to the control (water) respectively. The present study suggests that plant protection treatments against the immobile stage of *S. titanus* at an earlier application date (BBCH-scale 13-17) than the usually recommended one in Austria (BBCH 65-77) could effectively reduce *S. titanus* population at the start of the growing season and lead to an improved suppression of the vector-assisted spread of Flavescence dorée phytoplasma.

Key words: *Scaphoideus titanus*, Cicadellidae, vector, laboratory trial, Flavescence dorée phytoplasma, etofenprox, spirotetramat, paraffin oil, azadirachtin, spirodiclofen, aluminium silicate, kaolinite.

Introduction

Scaphoideus titanus Ball (Hemiptera Cicadellidae), commonly named the American grapevine leafhopper (AGVL), originates in North America but was introduced to Europe, where it was first recorded in France in the 1950s (Bonfils and Schvester, 1960). Since then, AGVL spread to the Eastern and Southern wine growing areas of Europe and regionally became invasive. Until now, *S. titanus* has been reported from 18 countries in Europe (Ball, 1932; Bonfils and Schvester, 1960; EPPO, 2018b). In Austria, the AGVL was recorded for the first time in Styria in 2004 and has spread since then to Burgenland and some parts of Lower Austria where the largest wine-growing areas are located (Strauss *et al*., 2014; Rebschutzdienst, 2018).

S. titanus is univoltine and overwinters as egg laid under the bark of *Vitis* sp., the only host plant on which it can complete its whole life cycle in Europe (Schvester *et al*., 1962; Vidano, 1964). AGVL is the main vector of the quarantine pest Flavescence dorée (FD) phytoplasma (EC, 2000). It acquires FD phytoplasmas by feeding on the phloem and transmits them to healthy grapevines in a persistent propagative manner (Boudon-Padieu, 2002; Chuche *et al*., 2014; 2017). AGVL becomes infective after a latency period of 1-5 weeks, depending on the developmental stage, with adults being able to infect plants within few days and throughout their whole life (Chuche and Thiéry, 2014; Alma *et al*., 2018). AGVL cannot transmit the FD phytoplasma vertically to the progeny (Bressan *et al*., 2005a). In contrast to the adults, the nymphs are poorly mobile and therefore play a negligible role in the spread of FD phytoplasma (Lessio and Alma, 2006). Flavescence dorée is widespread in Europe and was detected for the first time in Austria in 2009 in Styria and 2015 in Burgenland in few grapevines (Duduk *et al*., 2004; Reisenzein and Steffek, 2011; Steffek *et al*., 2011; EPPO, 2018a). FD-infected plants may either die or recover, but they remain less productive for several years after the infection (Morone *et al*., 2007). FD did not cause any major damage in Austria so far, but if the disease continues to spread it could cause extensive yield losses such as e.g. in Italy or Serbia (DPP, 2006; Belli *et al*., 2010). The suppression of *S. titanus* is crucial for prevention of FD spread because FD cannot be controlled directly. In Austria, regional regulations were adopted in Styria and Burgenland, where FD-infected grapevines had been detected and infected zones and buffer zones were put in place. Measurements to eradicate FD and to control the vector comprise a systematic monitoring in the main Austrian wine-growing areas, laboratory testing to detect FD phytoplasma in symptomatic grapevines, wild host plants and *S. titanus*, uprooting of infected grapevines or the whole vineyard and obligatory treatments of *S. titanus* in the demarcated areas. In wine-growing areas where only *S. titanus* occurs, vector control is recommended. The first treatment against *S. titanus* is applied when the first third instar appears (middle of June, BBCH-scale 65) based on the yearly monitoring. A second treatment is performed approximately 14 days later, depending on the number of nymphs. In general, this treatment approach is effective but also has disadvantages: the yearly monitoring to determine the right time for spraying is time-consuming and because of the mobility of the nymphs, only a part of the *S. titanus* population is treated. In Austria, fewer plant protection products are registered against *S. titanus* in comparison to some other European countries (BAES, 2020) and in addition, the authorization of buprofezin, which was

mainly used, ended in 2017. Research for new and effective control methods is necessary, because only few effective products against *S. titanus* are registered in Austria at present. Additionally, chlorpyrifos-methyl, which was also commonly used to control *S. titanus* in Austria, will probably no longer be approved in the EU (EFSA, 2019). Thus, the objective of the present study was to find a plant protection product, which effectively reduces AGVL egg hatching and as a further consequence the overall intensity of AGVL infestation at the beginning of the growing season (BBCH 13-17) (Lorenz *et al*., 1994). Laboratory trials were carried out to evaluate the effect of six selected insecticides on the egg hatching of *S. titanus*.

Materials and methods

An extensive systematic literature search (ELS) was carried out to identify candidate pesticides (EFSA, 2010). The electronic database Ovid was used to search for scientific literature, pertinent websites (e.g. EPPO) and other published studies (e.g. grower's literature, IOBC-WPRS bulletins) were used as additional information sources. Search terms consisted of the names of potential chemical agents and the insect order Rhynchota or the suborder Homoptera. The resulting records were evaluated for their relevance with a rating system, based on the target organism, the mode of application and the authorization status of the candidate pesticides in Austria.

Based on the ELS, the six test substances for laboratory trials were selected (table 1). As test plant material, two-year-old Isabella (*Vitis vinifera* × *Vitis labrusca*) canes were collected at the beginning of February 2018, standardized (average length of 26 cm, 4 nodes) and kept in a cold storage room at 6.6 °C and 73.5% RH. The canes were derived from four different rows of a 20-year-old vineyard in South East Styria, Austria, with confirmed presence of *S. titanus* in the previous growing seasons.

For the bioassays, either adapted test boxes according to Bagnoli and Gargani (2011) or newly developed test cages (figure 1) were used as test units. All test units were kept in a climatic chamber at 24 °C, 75% RH and a photoperiod of 16L:8D (Caudwell *et al*., 1970). The test boxes and the test cages were used in pre-trials to determine the beginning and the duration of egg hatching in the different test units as well as the number of *S. titanus* per kg cane.

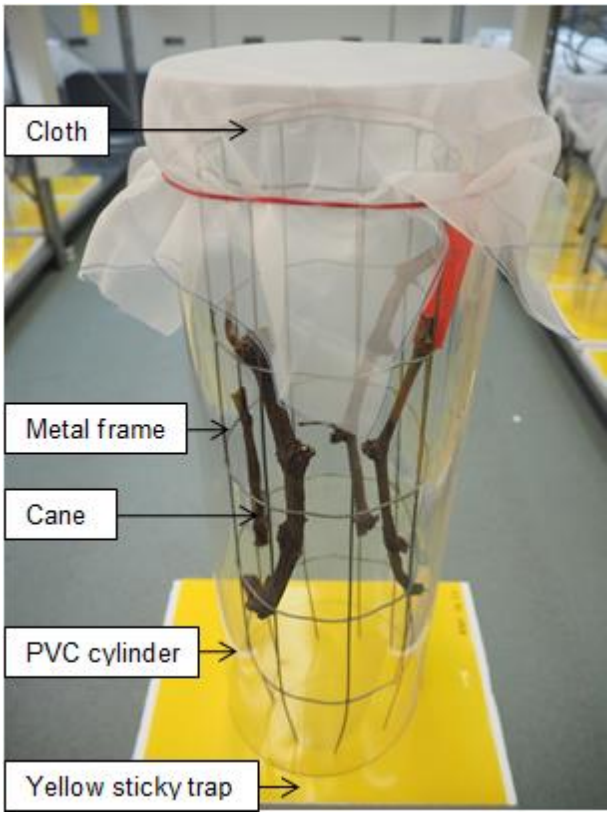

Figure 1. Test cage used for the main-trial.

The newly developed test cage which was used for the main-trial consisted of a circular metal frame surrounded by a sealed polyvinyl chloride cylinder (height: 55 cm; ⌀ 19 cm) with a cloth (mesh size: 0.25 mm × 0.25 mm) ventilation coverage on top and a yellow sticky trap and an insulating board on the bottom. The sticky traps were odourless and insecticide-free. The canes were fixed on the horizontal and vertical beams of the circular metal frame (height: 50 cm; ⌀ 16 cm).

The test canes for the main-trial were cold stored for 95 days before the start of the trial (07 May 2018), when they were put into the climatic chamber and placed into the cages without yellow sticky traps. At the application day, the cages were moved as treatment-groups from the climatic chamber to the application laboratory. The ap-

Table 1. Insecticides used in the laboratory trial.

Active substance	Trade name	Austrian plant protection register number	Active ingredient [g/l]	Concentration in laboratory trial [%]	Total number test cages (n replicates*n row)
Azadirachtin	NeemAzal-T/S	2699-0	10.0	0.375	12 (3*4)
Etofenprox	Trebon 30 EC	3395-0	287.5	0.100	12 (3*4)
Aluminium silicate (kaolinite)[1]	/	/	/	1.750	12 (3*4)
Paraffin oil	Austriebsspritzmittel 7 E	1739-0	836.5	2.000	12 (3*4)
Spirodiclofen	Envidor	3351-0	240.0	0.064	12 (3*4)
Spirotetramat	Movento 100 SC	3021-0	100.0	0.140	12 (3*4)

[1]regulated in Austria under the fertilizer law 1994 (Düngemittelgesetz 1994 BGBl.Nr. 513/1994 idgF) and fertilizer regulation 2014 (Düngemittelverordnung BGBl Nr. II 100/2004 idgF).

plications were done with a different hand sprayer of the same type for each treatment in order to avoid any mixture of test substances. The spraying distance was approximately 40 cm between the tip of the sprayer and the cage.

Treatments were carried out with a hand sprayer for 30 seconds. After the first 15 seconds of the application the cages were turned 180° to ensure that all sides were sprayed equally. The spray volume of 155 ml per test cage with four canes of 26 cm length each corresponded to 400 l/ha in the field (recommendations for BBCH growth stage 17-19/55, Österreichischer Weinbauverband, 2018). This is based on the assumption of 2.666 m effective cane length/ha from 3.333 vines/ha at a planting distance of 1 m and the Guyot vine training system. Each treatment consisted of four cages with three replicates for a total of twelve cages per treatment. In each test cage four canes only from one of the four different rows in the vineyard were placed to consider possible row effects on the mean number of AGVL. After the application, the cages were moved back into the climatic chamber. In total three applications were made: 1st application, 24 May 2018, 17 days after trial start (DAS), 2nd application, 5 June 2018, 29 DAS and 3rd application, 21 June 2018, 45 DAS.

The yellow sticky traps were placed under the cages immediately after each application and changed before the next application. In addition the yellow sticky traps were changed 56 DAS (2 July 2018) and 80 DAS (16 July 2018) and used until the end of the trial (95 DAS, 10 August 2018). The yellow sticky traps were examined for *S. titanus* nymphs by using a stereomicroscope with an amplification 10 × 0.65. Only nymphs inside the area on the yellow sticky traps, which was delimited by the polyvinyl chloride cylinder of the test cages, were evaluated. A nymph was only counted if it was clearly determined as an AGVL nymph (Della Giustina *et al*., 1992). The control of all yellow sticky traps was done by one person and randomly selected traps were additionally checked by a second person.

The statistical analysis was carried out with SPSS® Statistics Version 22.0.0.0.

The effect of the different treatments on the mean number of *S. titanus* nymphs was compared by an analysis of variance (ANOVA). The significance level was 5% and for the post-hoc analysis Tukey's HSD test was used. A multilevel model (mixed model) was used to analyse the effect of the different rows on the mean number of AGVL in each treatment group.

Results

Parameters such as the beginning and duration of hatching as well as the number of *S. titanus* nymphs per cane were important factors for the precise timing of the treatments and interpretation of the results and were therefore evaluated. In the pre-trials, egg hatching started 26-66 DAS and lasted until 64-99 DAS, resulting in a hatching duration of 33-41 days. In the main-trial, egg hatching started 29-45 DAS and lasted until 80-95 DAS, resulting in a hatching duration of 25-66 days. No statistically significant impact of the four rows on the mean number of AGVL in each treatment group was found. A range from 275 AGVL/kg canes to 1727 AGVL/kg canes was determined in all trials.

The different treatments had a statistically significant ($\alpha = 0.05$) influence on the mean number of AGVL nymphs compared to the mean number of AGVL nymphs in the water treated control (table 2). The mean number of AGVL nymphs in the treatments etofenprox and spirotetramat was significantly lower compared to the mean number of AGVL nymphs in the treatments aluminium silicate (kaolinite), spirodiclofen and the control. The etofenprox treatment resulted in an egg hatching decrease of 100% compared to the control (n nymphs = 623). Spirotetramat resulted in a decrease of egg hatching of 99% (n nymphs = 1) compared to the control. The mean number of AGVL nymphs in the treatment paraffin oil was significantly lower to the mean number of AGVL nymphs in the control and resulted in a 83% (n nymphs = 105) reduction of egg hatching compared to the control. The mean number of AGVL nymphs in the azadirachtin treatment was significantly lower compared to the mean number of AGVL nymphs in the control and led to a reduction of 72% (n nymphs = 172) in egg hatching compared to the control. The mean number of AGVL nymphs in the treatment spirodiclofen was significantly different compared to the mean number of AGVL nymphs in the treatments etofenprox, spirotetramat and control and caused a reduction of 64% (n nymphs = 222) in egg hatching of AGVL as compared to the control. The mean number of AGVL nymphs in the treatment aluminium silicate (kaolinite) was significantly different to the mean number of AGVL nymphs in the treatments etofenprox, spirotetramat and control. It had the lowest reduction of egg hatching in comparison to the control with 47% (n nymphs = 298) (figure 2, table 2).

Except for etofenprox and spirotetramat with 0 and 1

Table 2. Number of *S. titanus* nymphs after treatment (observation period 2018).

Treatment	5-21 June	21 June - 2 July	2-16 July	Total	Mean ± SD
Azadirachtin	48	98	26	172	57.3 ± 30.1*
Etofenprox	0	0	0	0	0 ± 0*
Aluminium silicate (kaolinite)	45	99	154	298	99.3 ± 44.5*
Paraffin oil	35	47	23	105	35.0 ± 9.8*
Spirodiclofen	48	122	52	222	74.0 ± 34.0*
Spirotetramat	0	0	1	1	0.3 ± 0.5*
Control (water)	77	252	294	623	207.7 ± 94.0

* statistically significant ($p < 0.05$) compared to control.

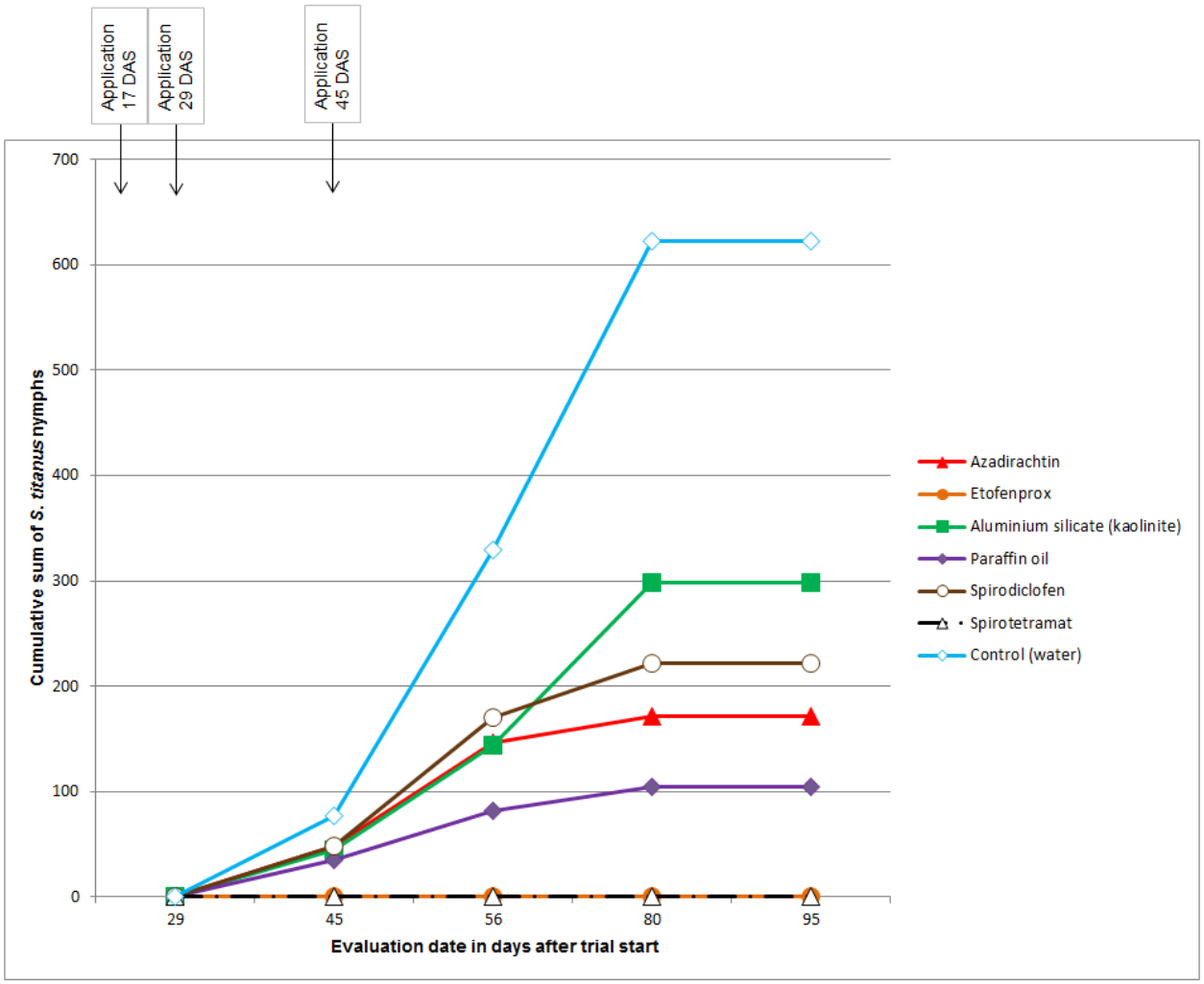

Figure 2. Cumulative sum of *S. titanus* nymphs in the different treatments per evaluation date after 1, 2 or 3 applications (17 DAS, 29 DAS and 45 DAS).

S. titanus nymph respectively, the overall cumulative sum of *S. titanus* nymphs increased over the duration of the trial, with the control (water) resulting in the highest amount of nymphs at the end of the trial. The treatments with aluminium silicate, spirodiclofen, azadirachtin and paraffin oil resulted in a 2.1- to 5.9-fold lower cumulative sum of *S. titanus* nymphs in relation to the control indicating the efficacy of the different treatments. Based on the test design and the relative late hatching of the eggs in the present study, it is not possible to make a statement on the difference in the reduction of egg hatching of AGVL after the different numbers (one, two or three) of applications. The efficacy results show the reduction in egg hatching of AGVL after three applications.

Discussion and conclusions

A new test cage was developed, as the test boxes exhibited some disadvantages for the planned efficacy trials, although they admitted placing high quantities of canes and *S. titanus* in one test unit at the same time and easy access during the trial. The substantial disadvantages were that the application of the test substances and the exact evaluation of their effect could not be carried out in a way that allowed either to simulate field conditions or to obtain precise hatching results.

The newly developed test cage allowed the use of infested canes in a vertical position during application and the whole trial period similar to the growth on the grapevine in the field. Additionally a standardized continuous assessment of the number of hatched *S. titanus* eggs over the whole hatching period, with yellow sticky traps on the bottom of the cages, was ensured.

In the present study (two pre-trials and the main-trial) it took up to 4-6 weeks before the first eggs hatched, which is longer than the 3-4 week period reported in the literature (Bressan *et al*., 2005b; Caudwell, 2008; Maggi *et al*., 2013; Chuche *et al*., 2014). However, in one pre-trial of the present study it took as long as 9 weeks until the first eggs hatched, which was probably caused by the diapause due to the earlier start of that pre-trial compared to the other trials. The diapause is only broken after 3 months at a temperature of 3-4 °C (Caudwell, 2008).

The hatching duration of *S. titanus* eggs in the different trials in the present study lasted between 25 and 66

days, which is comparable to the hatching periods of 20-107 days reported in other studies (Bressan *et al*., 2005b; Caudwell, 2008; Chuche and Thiéry, 2009; 2012; Maggi *et al*., 2013; Chuche *et al*., 2014).

The results showed that three applications of the tested substances effectively reduced egg hatching of AGVL. It should be emphasized that these efficacies were obtained despite the high numbers of *S. titanus* nymphs of 753 AGVL/kg cane in the present study.

In this research, etofenprox and spirotetramat had a very high efficacy on AGVL with a reduction of egg hatching rate by 100% and 99% respectively, compared to the control with water. High nymphal mortality (92.1-100%) was demonstrated using etofenprox against *Asymmetrasca decedens* (Paoli) by Grassi and Ri (2006). No comparable efficacy trials with spirotetramat against Auchenorrhyncha were found in the literature. Paraffin oil trials against planthopper species showed no efficacy against the eggs, whereas the mortality against the nymphs ranged between 66-97% depending on the species (Cornale *et al*., 1998; Dardar *et al*., 2013; Mahmoudi *et al*., 2014). Azadirachtin caused an egg mortality of 24-26% against *Pyrilla perpusilla* (Walker) (Deepak and Choudhary, 1999). Comparable trials about the efficacy of spirodiclofen against Auchenorrhyncha eggs or nymphs were not found in the literature. Trials with kaolinite against nymphs of *Ommatissus lybicus* de Bergevin showed an efficacy of over 80% (Mahmoudi *et al*., 2014).

A new test unit and test method were developed during the present study, which allowed on the one hand to better simulate field conditions with regard to vertical position of the test canes during application and, on the other hand, it offered the possibility of a standardized, continuous assessment of the number of hatched AGVL eggs. The trials revealed that after three applications, all test substances had a reducing effect on *S. titanus* egg hatching, with etofenprox and spirotetramat being the most effective. Yet, it cannot be concluded, if these reducing effects were solely ovicidal or larvicidal because *S. titanus* eggs had started to hatch at the time when the third treatment was conducted.

The potentially cumulative effect of the test substance residues could not be evaluated in the present study due to the test design with three applications and the late hatching of the AGVL nymphs. The impact of different numbers of applications on egg hatching should be addressed in further trials with regard to the feasibility of the proposed control method.

Additionally, the effect of the test substances on the longevity of the hatched nymphs and other fitness parameters such as size, weight and mobility could be assessed, as they might influence the survival rate of the AGVL nymphs. Furthermore, it would be advisable to repeat the laboratory trials in the greenhouse (semi-field-test) and in the field to confirm the efficacy of the substances under more field related conditions.

The present study suggests, that an earlier application date (BBCH 13-17) against *S. titanus* than the usually recommended one (BBCH 65-77) effectively reduces egg hatching and fits well between necessary applications against other pests e.g. Acari, *Erysiphe necator* and *Plasmopara viticola* occurring in the vineyards (Rebschutzdienst, 2018; Österreichischer Weinbauverband, 2018). By this, the *S. titanus* population and the vector-assisted spread of Flavescence dorée phytoplasma could effectively be suppressed at the start of the growing season when the vector is still immobile.

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