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MASTER‘S THESIS

„Laboratory trials to reduce the nymphal hatching of the American grapevine leafhopper (*Scaphoideus titanus*) with selected substances“

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Affirmation:

I herewith assure that I wrote the present thesis independently, the thesis has not been partly or fully submitted as graded academic work and that I have used no other means as the ones indicated. I have indicated all parts of the work in which sources are used according to their wording or to their meaning.

Date	Signature

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1 Introduction

Scaphoideus titanus (Ball 1932), commonly named the American grapevine leafhopper (AGVL), is a leafhopper belonging to the family of Cicadellidae that originates in North America. It was observed for the first time in 1925 in North America and probably introduced on an American grapevine to Europe in the 1950s (Bonfils and Schvester, 1960; Ball, 1932). Today it is found in North America and 18 countries in Southern and Central Europe (EPPO, 2018c; Mazzoni et al., 2009b). In Austria, the AGVL was found for the first time in Styria in 2004 and has spread since then to Burgenland and Lower Austria (Rebschutzdienst, 2018; Strauss et al., 2014). It is an univoltine insect which overwinters as egg under the bark of *Vitis* sp. *Vitis* sp. is the main food plant and the only plant species on which this leafhopper can complete its whole life cycle (Vidano, 1964; Schvester et al., 1962). The AGVL itself does not cause any major damage to the grapevine plant, but is the main vector of the Flavescence dorée (FD) phytoplasma (Boudon-Padieu, 2002). By feeding on the phloem it takes up the Flavescence dorée phytoplasma and transmits it in a persistent manner (Chuche et al., 2017; Chuche et al., 2014). The AGVL cannot transmit the FD phytoplasma vertically (Bressan et al., 2005a). After 4-5 weeks of latency, they are able to infect plants throughout their whole life (Mazzoni et al., 2009b). Nymphs stay on the same plant and therefore play an insignificant role in the transmission of the disease, in contrast to the adults (Lessio and Alma, 2006). Flavescence dorée is a quarantine disease, widespread in Europe (Fig. 11) and was observed for the first time in Austria in 2009 (Reisenzein and Steffek, 2011; Steffek et al., 2011; Duduk et al., 2004; EPPO, 2018a; EC, 2000). FD causes rolling and yellowing of leaves (white cultivars), dark red discoloration (red cultivars), short internodes, green canes, lack of lignification and inhibits growth and maturation of berries (Vidano, 1964; Belli et al., 2010). Symptoms may involve the entire plant or only selected branches. Infected plants may either die or recover, but they remain less productive for several years after the infection (Morone et al., 2007). FD in vineyards can only be controlled by preventive measures such as planting of healthy propagation material, removing of potential FD host plants (e.g. *Clematis vitalba*) and by control of the vector AGVL with insecticides. Especially preventive measures should not only be executed in managed vineyards, but also in abandoned vineyards. The only method to eliminate the FD phytoplasma is hot water treatment of grapevine propagation material (EPPO, 2012). If FD occurs in a vineyard, the diseased plants have to be removed or the vineyard has to be cleared (BAES, 2018; Rebschutzdienst; AGES, 2018). Until now FD did not cause any major damage in Austria, but if the disease spread continued it could cause extensive yield losses such as in Italy or Serbia (Belli et al., 2010; DPP, 2006). In Austria currently five different substances are authorized for the control of AGVL in integrated production and one substance for organic viticulture (EASY-CERT-services-GmbH, 2018). The main plant

protection product applied against AGVL was Applaud 25 SC®, but the authorization for this product ended in 2017 (BAES, 2018). Since Applaud 25 SC is no longer available, the need for an alternative plant protection product or measure to control *S. titanus* increased. The present study had the objective to find a plant protection treatment, which reduces AGVL nymphal hatch and as a further consequence the overall intensity of infestation at the beginning of the growing season (BBCH 13-19[1]) (Lorenz et al., 1994). In order to answer the research question an extensive literature search and laboratory efficacy trials were carried out.

[1] Newsletters by Rebschutzdienst Krems Langenlois from May 2016 and May 2017

2 Extensive literature search

2.1 Materials and methods

2.1.1 Materials

2.1.1.1 Description of the sources of information searched

2.1.1.1.1 Scientific literature

The scientific literature search was executed with the electronic database Ovid which included records and available full-text journals from Books@Ovid, AGRICOLA, AGRIS, CAB Abstracts and Ovid MEDLINE. The scientific literature also included information from websites (e.g. https://www.eppo.int/, https://www.cabi.org/).

2.1.1.1.2 Grey literature

The grey literature included grower's literature, full-text from conference papers, IOBC-WPRS-Bulletins and Euphresco project (https://www.euphresco.net/projects/portfolio) reports (Schaub et al., 2010; Chuche et al., 2011). The grower's literature included the monthly Rebschutzdienst-Informationsblätter from May 2016 to December 2017 and Leitlinie für den integrierten Weinbau 2017-2018.

2.1.1.1.3 Language restrictions

Records in English, German, Italian, Spanish, French and Portuguese were evaluated.

2.1.2 Methods

The extensive literature search was carried out according to the EFSA guidance document on systematic review methodology (EFSA, 2010).

2.1.2.1 Search strategies

The search terms and sets were developed to answer the question: "which chemical substance or pesticide can reduce the egg and/or nymphal hatching of *Scaphoideus titanus*?"

All search terms consisted of the name of the test substance and the new or old name of the insect order. The name of the substance was either the ISO common name or synonyms. To form the search terms Boolean operators and truncations were used. The search terms were combined to search term sets.

2.1.2.1.1 Limits applied to the search

Except language restrictions the search was not limited by any other parameter.

2.1.2.2 Precise search strategy for electronic database search

A pre-search with the following search term sets was conducted:

Search term set 1: ((control* and larv* and (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 2: ((control* and larv* and lab* (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 3: ((control* and nymph* and (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 4: ((control* and nymph* and lab* (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 5: ((larvicid* (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 6: ((ovicid* (scaphoideus and titanus)) or scaphoideus and littoralis) or (american grapevine leafhopper)

Search term set 7: (ovicid* and (auchenorrhyncha* or leafhopper* or cicadellida* or jassidae*))

Search term set 8: (ovicid* and heteroptera*)

Based on the results from this pre-search the following search with optimized search terms was conducted.

Search terms:

The search terms consisted of the name of chemical agents, which were found in the pre-search and the insect order Hemiptera or the suborder Homoptera. Since the literature search was not limited by publication dates, it was necessary to include the suborder Homoptera for older records.

Search term 1: (mineral and oil and insecticid* and Hemipter*)

Search term 2: (kaolin clay* and Hemipter*)

Search term 2.1: (kaolin* and Hemipter*)

Search term 2.2: (aluminium silicate* and Hemipter*)

Search term 3: (azadirachtin* and Hemipter*)

Search term 4: ((etofenprox* or ethofenprox*) and Hemipter*)

Search term 5: ((chlorpyriphos* or chlorpyrifos*) and Hemipter*)

Search term 6: (spirodiclofen* and Hemipter*)

Search term 7: (spirotetramat* and Hemipter*)

Search term 8: (mineral and oil and insecticid* and Homopter*)

Search term 9: (kaolin clay* and Homopter*)

Search term 9.1: (kaolin* and Homopter*)

Search term 9.2: (aluminium silicate* and Homopter*)

Search term 10: (azadirachtin* and Homopter*)

Search term 11: ((etofenprox* or ethofenprox*) and Homopter*)

Search term 12: ((chlorpyriphos* or chlorpyrifos*) and Homopter*)

Search term 13: (spirodiclofen* and Homopter*)

Search term 14: (spirotetramat* and Homopter*)

Exclusion term:

One exclusion term was used to reduce the number of irrelevant results.

Exclusion term: NOT (scale insect* or aphid* or fung* or nematod* or mite* or beneficial* or pherom* or predato* or parasit* or hymenoptera* or diaspidida* or mirida* or anthocorida* or aphidida* or pseudococcida* or aphelenida* or coleopter* or dipter* or thysanopter* or coccoide* or psyllid*)

Search term Sets:

Each search term was combined with the exclusion term to form a search term set or search string.

Table 1: Search strategy for azadirachtin.

1. Search term 3: (azadirachtin* and Hemipter*)
2. Exclusion term: NOT (scale insect* or aphid* or fung* or nematod* or mite* or beneficial* or pherom* or predato* or parasit* or hymenoptera* or diaspidida* or mirida* or anthocorida* or aphidida* or pseudococcida* or aphelenida* or coleopter* or dipter* or thysanopter* or coccoide* or psyllid*)
3. Search term Set 3: (azadirachtin* and Hemipter*) NOT (scale insect* or aphid* or fung* or nematod* or mite* or beneficial* or pherom* or predato* or parasit* or hymenoptera* or diaspidida* or mirida* or anthocorida* or aphidida* or pseudococcida* or aphelenida* or coleopter* or dipter* or thysanopter* or coccoide* or psyllid*)

2.1.2.3 Set-up of the EndNote libraries

An EndNote (version X 8.2) library was created. In the library the group set “whitefly” and the group set “planthopper” were set up. Each of those group sets had the same sub groups consisting of the different treatment groups (Fig. 1). The records were deduplicated in the Ovid database and exported to the EndNote library. There the records were checked for completeness and missing information was added manually. Afterwards the records in the EndNote library were deduplicated again. The references were moved into their respective EndNote library group set and group: e.g. records found with the search term azadirachtin were either moved into the group set “whitefly” or into the group set “planthopper” and there in the group “azadirachtin”.

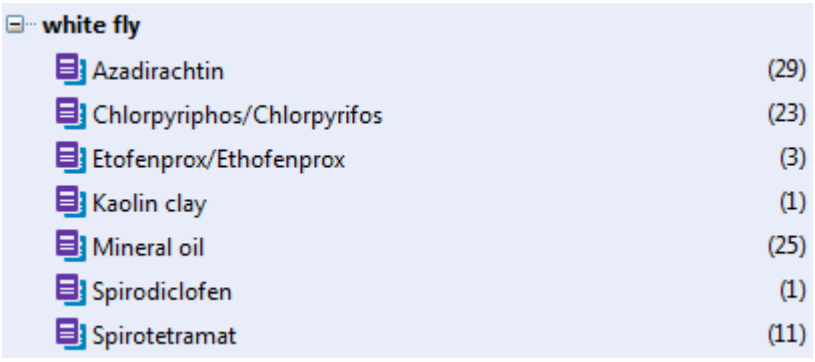


Figure 1: Detail of the EndNote library, group set "whitefly" with their treatment groups.

Author	Year	Title	Rating
Akbar, M. F.; Haq, M. A.; Nikhat, Yasmin; Khan, M. F.; Azmi, M. A.	2011	Efficacy of bio-insecticides ...	★★★★
Akbar, M. F.; Rana, H. U.; Khan, M. F. U.	2015	Management of Bemicia ta...	★★★★
Kumar, A.; Singh, R.	2014	Bioefficacy of some insecti...	★★★★★
Bhavani, B.; Rao, C. V. N.	2013	Management of sugarcane...	★★★★★
Bleicher, E.; Goncalves, M. E. de C.; Silva, L. D. da	2007	Effects of neem derivatives ...	★★★★★

Figure 2: Detail of the EndNote library, rating of references from the group azadirachtin.

2.1.2.4 The selection process/selection criteria

In EndNote the records were evaluated according to the following rating system. A rating system was applied by using the option to rate references between zero and five stars.

The rating process was split into two steps. In the first step the records were rated from 1 star to 3 stars. During the second step all 3 stars ratings were rated again with either 4 or 5 stars.

Table 2: Star based rating for the EndNote library.

* star	Irrelevant
** stars	Incomplete
*** stars	Potentially relevant
**** stars	Unclear
***** stars	Relevant

The 1-star rating “irrelevant” was used when the record did not contain information about the target organisms (planthoppers, whitefly) or when the record only contained information about the irrelevant development stage (e.g. adults).

The 2-stars rating “incomplete” was used when the record lacked formal aspects (e.g. keywords or abstract).

The 3-stars rating “potentially relevant” was used when the record contained information about the target organism or the relevant development stage (eggs or nymphs).

The 4-stars rating “unclear” was used when the record did not specify if the information referred to juvenile stages or adults.

The 5-stars rating “relevant” was used when the record was referring to the relevant target organism in the relevant development stage and it contained all formal aspects such as title, abstract and keywords.

Author	Year	Title	Rating
Suri, K. S.; Singh, Gursharan	2011	Insecticide-induced resurgence of th...	★
Surjaram,; Sobita, Simon; Kamal, Tanwar	2014	Evaluation of plant extracts against L...	★
Sushil, Kumar; Raghvani, B. R.; Bhatt, R. I.	2005	Bioefficacy of newer insecticides aga...	★
Sutaria, V. K.; Motka, M. N.; Jethva, D. M.; Ramoliya, D. R.	2010	Field efficacy of insecticides against j...	★
Sutherland, J. P.; Baharally, V.; Permaul, D.	2002	Use of the botanical insecticide, nee...	★

Figure 3: Detail of an EndNote library with 1-star rating.

Author	Year	Title	Rating
Abdul Latif, A. Z.; Nik Mohd Noor, N. S.; Chang, P. M.; Habibud...	1992	Buprofezin and etofenprox, two new ...	★★
Chambre d' Agriculture de l' Aude, Carcassonne	1992	Golden flavescence control in grape...	★★
French, J. V.; Meagher, R. L. Jr.	1992	Citrus blackfly: chemical control on ...	★★
Heyde, J. von der	1985	On effects of neem extracts on plant...	★★
Liao, Shichun; Lin, Mingzhen; Lu, Zhixin	1991	Effects of ethofenprox [Trebon] on ri...	★★

Figure 4: Detail of the EndNote library with 2-stars rating.

Author	Year	Title	Rating
Mochida, O.; Basilio, R. P.; Valencia, S. L.; Macatula, R. F.	1987	Ethofenprox (Trebon), a novel insecti...	★★★
Mishra, H. P.	2006	Chemical management of the white ...	★★★
Medeiros, F. A. S. B.; Bleicher, E.; Menezes, J. B.	2001	Effect of mineral oil and neutral dete...	★★★
Marques, M. de A.; Quintela, E. D.; Mascarin, G. M.; Fernandes, P...	2014	Management of Bemisia tabaci bioty...	★★★
Marcano, R.; Gonzalez, E.	1993	Evaluation of insecticides for the con...	★★★

Figure 5: Detail of the EndNote library with 3-stars rating.

Author	Year	Title	Rating
Rigo, G.; Mori, N.	1997	Containment of populations of gree...	★★★★
Roshan, D. R.; Raju, S. V. S.; Singh, K. N.	2016	Relative efficacy of acetamirpid+fipr...	★★★★
Rumine, P.; Burchi, G.	2005	Soilless gerbera: control of the green...	★★★★
Samal, T.; Patnaik, H. P.	2008	Field efficacy of insecticides against ...	★★★★
Sandeep, Kumar; Sundar, Pal; Gore, Lal; Singh, D. K.; Umrao, R. S.	2014	Bio-efficacy of insecticides and bio-...	★★★★

Figure 6: Detail of the EndNote library with 4-stars rating.

Author	Year	Title	Rating
Flint, H. M.; Parks, N. J.	1989	Effect of azadirachtin from the neem...	★★★★★
Garrido, A.; Busto, T. del; Tarancon, J.	1982	Effect of some pesticides in the labor...	★★★★★
Ge, DaQing; Jiang, XingYin; Wang, Yan; Li, JunHu; Duan, Qiang	2011	Toxicity of spirotetramat to Bemisia t...	★★★★★
Girish, V. P.; Balikai, R. A.; Mallapur, C. P.	2016	Efficacy of newer insecticide molecu...	★★★★★
Grassi, A.; Ri, M. dal	2006	Asymmetrasca (Empoasca) deceden...	★★★★★

Figure 7: Detail of the EndNote library with 5-stars rating.

2.1.2.4.1 Reasons for exclusion of records

The exclusion was based on the title, keywords, abstract and full paper, if this information was available.

Records were excluded for reasons not related to their content if they were written in a language other than the specified ones, if they were not available or if they were missing essential parts such as the abstract.

Records were excluded for reasons related to their content if the target organism was irrelevant, the described chemicals were not authorized in Austria according to the Austrian plant protection products register or Austrian fertilizer law 1994 (Düngemittelgesetz 1994 BGBl.Nr. 513/1994 idgF) and fertilizer regulation 2014 (Düngemittelverordnung BGBl Nr. II 100/2004 idgF), if the type of application was irrelevant e.g. soil application or if the host plant type was not comparable to grapevine, such as arable crops and vegetables (e.g. rice or eggplant).

2.1.2.4.2 Reasons for inclusion of records

Records were included if they contained one or more of the following aspects: the target organism planthopper or whitefly, the relevant mode of application, an authorization as Plant protection product or fertilizer in Austria as described above or the relevant host plant.

2.1.2.4.3 Study selection

The final selection of the records was based on a discussion with the two supervisors as additional reviewers according to the quality criteria of independent literature review (EFSA, 2010). The full papers were reviewed.

2.2 Results

A total number of 954 records were found in the database Ovid after deduplication. 578 records had a 1-star rating (60.6%), 9 a 2-stars rating (0.9%), 169 a 3-stars rating (17.7%), 99 a 4-stars rating (10.4%) and 99 a 5-stars rating (10.4%).

Table 3: Number of hits after deduplication for each search term set (exclusion term not shown).

Search term sets (exclusion term not shown)	Number of hits after deduplication
1 (mineral and oil and insecticid* and Hemipter*)	69
2 (kaolin clay* and Hemipter*)	0
2.1 (kaolin* and Hemipter*)	16
2.2 (aluminium silicate* and Hemipter*)	0
3 (azadirachtin* and Hemipter*)	190
4 ((etofenprox* or ethofenprox*) and Hemipter*)	85
5 ((chlorpyriphos* or chlorpyrifos*) and Hemipter*)	425
6 (spirodiclofen* and Hemipter*)	1
7 (spirotetramat* and Hemipter*)	27
8 (mineral and oil and insecticid* and Homopter*)	17
9 (kaolin clay* and Homopter*)	0
9.1 (kaolin* and Homopter*)	3
9.2 (aluminium silicate* and Homopter*)	0
10 (azadirachtin* and Homopter*)	38
11 ((etofenprox* or ethofenprox*) and Homopter*)	23
12 ((chlorpyriphos* or chlorpyrifos*) and Homopter*)	55
13 (spirodiclofen* and Homopter*)	0
14 (spirotetramat* and Homopter*)	5

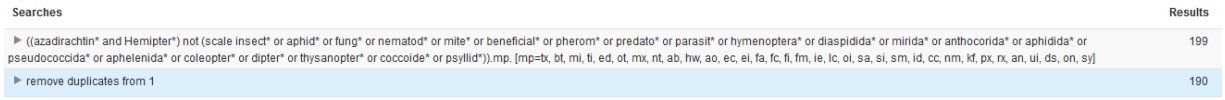


Figure 8: Detail from the Ovid search engine after the deduplication of the search term set 3.

The differences between the number of 5-stars ratings and the percentage of 5-stars ratings for each EndNote library group were high. The chlorpyrifos-group had the highest number of 5-stars rated records (n=28), but the least percentage of 5-stars ratings from the total records. The groups azadirachtin (n=26) and mineral oil (n=25) followed with a similar high number of 5-stars ratings and a higher percentage of 5-stars ratings. The groups kaolin (n=3), etofenprox (n=10) and spirotetramat (n=6) had a lower amount of 5-stars ratings. The spirodiclofen-group had the lowest number of 5-stars ratings with one record, but the highest percentage of 5-stars ratings.

Table 4: Number and percentage of 5 stars ratings of each EndNote library group.

Group	Number of 5-stars ratings in the groupset “whitefly“	Number of 5-stars ratings in the groupset “planthopper”	Percentage of 5-stars ratings within each group
Mineral oil	18	7	29.0
Kaolin	1	2	15.8
Azadirachtin	20	6	11.4
Etofenprox	3	7	9.4
Chlorpyrifos	7	21	5.8
Spirodiclofen	1	0	100.0
Spirotetramat	6	0	18.8

3 Laboratory trials

3.1 Materials and methods

3.1.1 Materials

3.1.1.1 Test organism (*Scaphoideus titanus* Ball)

Figure 9: Adult *Scaphoideus titanus*.

3.1.1.1.1 Taxonomical Classification

Different taxonomical classifications are existing for *Scaphoideus titanus* (CABI, 2018; EPPO, 2018c).

The EPPO classification was chosen because EPPO is the Regional Plant Protection Organization in the European and Mediterranean region which thus releases only validated information (EPPO, 2018b).

Table 5: Taxonomy of the American grapevine leafhopper (EPPO, 2018b).

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Order	Hemiptera
Suborder	Auchenorrhyncha
Family	Cicadellidae
Genus	Scaphoideus
Species	*Scaphoideus titanus*

3.1.1.1.2 Morphology

S. titanus eggs are elongated with a length of 1.3mm and a width of 0.3mm. The head side is slim and the back end is rounded. The color is whitish to light yellowish and it is turbid. During the development the egg grows to a length of 1.5mm and forms a bulge at the head side. The color changes to a creamy-yellow or ochre brown (Schvester et al., 1962; Vidano, 1964).

The juvenile development comprises five instars. The shape of the instars characteristically shows a pointed head and a compacted thorax and abdomen. All five instars exhibit a black, rhombus-shaped mark on each side of the last abdominal segment. The size of the instars starts with 1 - 1.8 mm for the first instar, increasing by 1 mm per each succeeding instar and finally reaching 5 mm for the fifth instar. Female nymphs are bigger than male nymphs. The first three instars are white and the forth instar either white or yellowish, with brown coloured first and second segments and brown dotted fifth and sixth segments of the abdomen. The nymphs of the fifth instar are yellowish with ochre coloured spots spread across three terga. The first, second, fifth and sixth abdominal segments are almost completely brown to blackish (Linder, 2016; Chuche and Thiéry, 2012; Schvester et al., 1962; Bernard et al., 1988; Vidano, 1964).

Male adults measure 4.5 – 5.2 mm, female adults 5.1 - 6 mm (Quartau et al., 2001; Barnett, 1976; Ball, 1932; Linder, 2016). Both genders have a distinct vertex with black crosslines between the eyes. Females have three to four crosslines and males two to three. Another characteristic on the head is a brown to reddish spot dorsal between the eyes. Two big tawny crosslines are situated at the pronotum and one on the mesoscutum. The scutellum has a pair of dots located on the basal angles. The legs are overall white. The elytra are opalescent and for the biggest part ocher or brownish. They have brown nerves, white spots and a distal, blackish band. The sterna of the thorax and abdomen are creamy grey. The third distal abdominal sternum of females is black (Vidano, 1964; Ball, 1932).

3.1.1.1.3 Biology

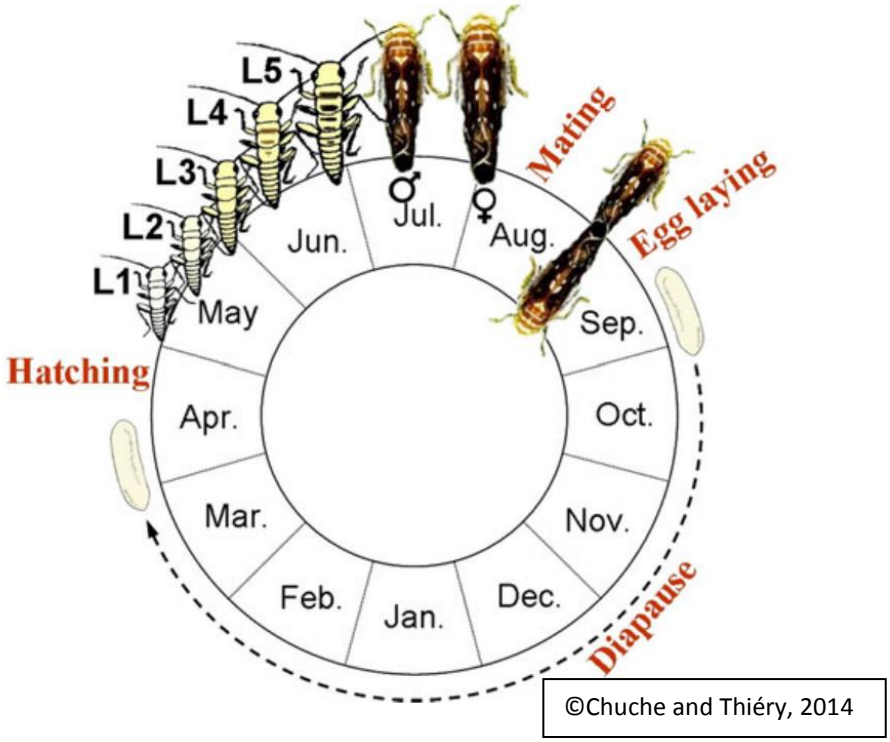


Figure 10: General life cycle of *Scaphoideus titanus (Chuche and Thiéry, 2014)*.

S. titanus is univoltine and can complete its life cycle only on grapevine, i.e. *Vitis vinifera* in Europe and *Vitis labrusca* and *Vitis riparia* in North America (Vidano, 1964; Maixner et al., 1993). Other plants such as *Vicia fabae*, *Prunus* sp. or *Malus* sp. can be used as temporary hosts (Barnett, 1976; Caudwell et al., 1970). Instars and adults live mostly on the lower side of the leaves and feed there by puncturing the veins, feeding on a mixed dietary of phloem and xylem sap (Chuche et al., 2011; Chuche et al., 2017; Bonfils and Schvester, 1960). Especially the nymphs prefer to stay in the lower and inner parts of the grapevine. AGVL overwinters as egg, mostly under the bark of two year old wood around the nodes (Bagnoli and Gargani, 2011). The diapause of the eggs lasts between six and eight months and does not require cold temperature to be broken, but the hatching is affected by it. Eggs that are hold at a temperature of 20°C can hatch four weeks earlier are bigger and hatch over a longer time period as compared to eggs kept at 5°C (Chuche and Thiéry, 2009). Nymphal hatching starts with the beginning of May in France, mid of May in Switzerland and Italy, end of May in Austria and usually lasts between four to six weeks, up to eight weeks (Boudon-Padieu, 2002; Linder, 2016; Rebschutzdienst, 2018). The development duration of the different instars is influenced by the temperature (Chuche and Thiéry, 2009). The first and second instar each last seven days, the following three instars each last 15 days at a temperature of 20 to 22°C. But each of the three last instars can also develop in one week if the temperature is between 27 and 30°C (Bernard et al., 1988). All nymphal stages are mobile and walk or jump to leaves. They settle at the plant where they hatched (Maixner et

al., 1993; Lessio and Alma, 2006). The nymphs have a lifespan of 35 to 55 days (Rahola et al., 1997). The adults appear from July to end of September in Austria, August to September in Switzerland, mid-July to early October in Italy and from end of July to September in Romania (Strauss et al., 2014; Bosco et al., 1997; Chireceanu et al., 2011). The adults, especially the males, are able to fly and move to other plants (Lessio et al., 2009), however often not higher than 2.4m and not much further than 24m away from the vineyard (Lessio and Alma, 2004a). *S. titanus* is nocturnal and has the highest flight activity - which is negatively correlated with increasing humidity and decreasing temperatures- between the late afternoon (18:00) and the early morning (08:00). Seasonal temperature dependent flight peaks were found to occur between mid-July and mid-August in Italy (Lessio and Alma, 2004b). Males start to emit calling signals 24 hours after they have hatched, females not earlier than six days after emergence (Mazzoni et al., 2009a). Males can mate several times, females however only once (Mazzoni et al., 2009b). The female adults start to lay eggs from August to September in the excoriated bark of the grapevine, ten days after they have finished the juvenile stages. Mated females carry between eight and 24 eggs and virgin females around one egg (Vidano, 1964; Linder and Jermini, 2007; Eriksson et al., 2012). The adults have a lifespan of 40 days. All stages of *S. titanus* can occur at the same *Vitis* sp. plant at the same time (Chuche and Thiéry, 2012; Schvester et al., 1962).

3.1.1.1.4 Damage (Symptoms) and economic importance

S. titanus does not cause direct damage to the grapevines, but is the main vector of the Flavescence dorée phytoplasma that causes severe damage to several grapevine cultivars (Rahola et al., 1997; Boudon-Padieu, 1996). *S. titanus* nymphs and adults transmit this disease causing microorganism to *Vitis* sp. by feeding first on infested and then on healthy *Vitis* sp. plants. Flavescence dorée phytoplasma can also be transmitted by other Auchenorryncha species such as *Dictyophara europaea* from *Clematis sp.* to *Vitis sp*. But those insects usually do not feed on *Vitis sp*. and therefore a transmission is unlikely (Filippin et al., 2009). Flavescence dorée causes various symptoms on grapevines including delaying or lack of bud break, lack of lignification in new shoots, yellowing of leaves in white cultivars, reddening of leaves in red cultivars, drying of inflorescence and berries and an overall reduction in yield and quality of the grapes (Albetis et al., 2017). If the grapevine survives the disease it will continue to have a reduced yield with lower quality (Chuche and Thiéry, 2014). Although differences in the susceptibility of grapevine cultivars regarding the FD phytoplasma exist, no resistant grapevine varieties are known (Morone et al., 2007; Bellomo et al., 2007; Jagoueix-Eveillard et al., 2012). Some cultivars such as Chardonnay have the ability to recover and are less susceptible to FD, even though the recovered plants can show symptoms again after a symptomless year (Bellomo et al., 2007). The susceptibility of

cultivars has an influence on the titer FD phytoplasma and acquisition by the AGVL (Bressan et al., 2005b).

3.1.1.1.5 Control of *Scaphoideus titanus*

The control of the *S. titanus* begins with the monitoring process. This is usually done by direct counting of nymphs on leaves and surveillance of adults with yellow sticky traps. The use of different insecticides against AGVL starts with the appearance of the third nymphal instar (Rebschutzdienst, 2018). Currently no insecticides are authorized for the application against AGVL in organic viticulture (EASY-CERT-services-GmbH, 2018). The 8 insecticides currently authorized in Austria in integrated viticulture against AGVL contain as active ingredient either spirotetramat, chlorpyrifos, fenpyroximate or indoxacarb (BAES, 2018). Natural enemies such as the egg parasitoid *Anagrus atomus* or predatory mites are not sufficient to suppress the development of AGVL (Linder, 2016; Kreiter, 2000; CABI, 2018).

3.1.1.1.6 Geographical distribution

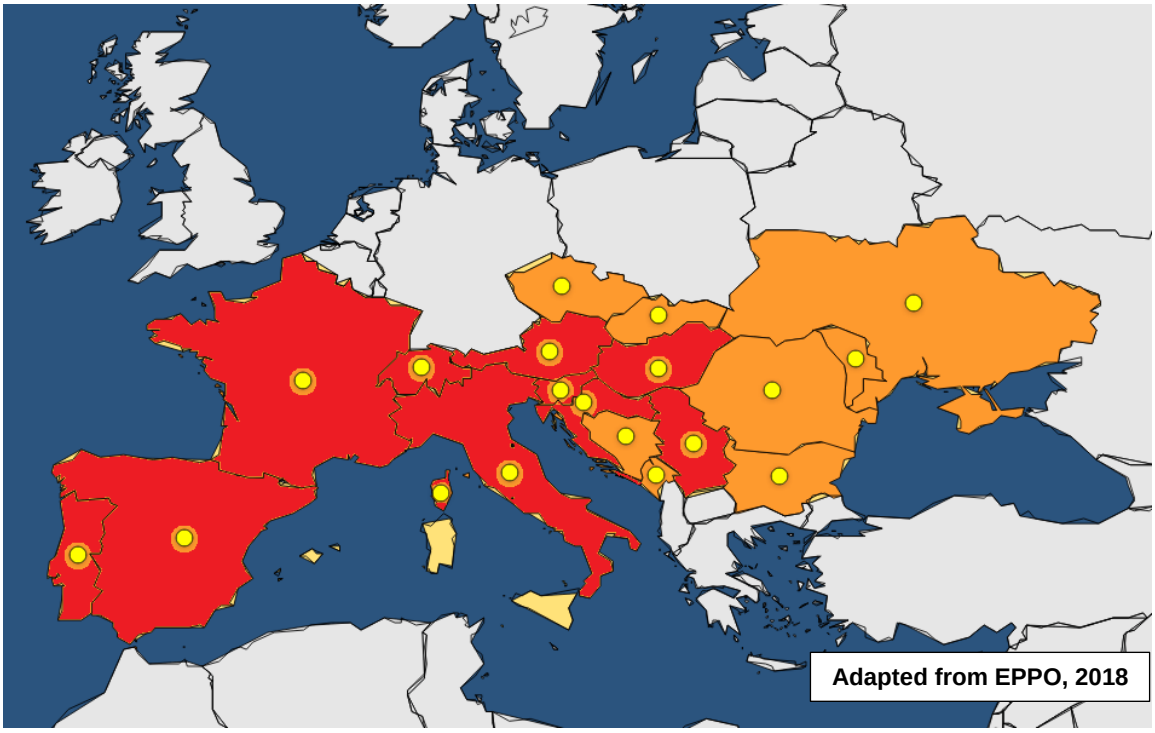


Figure 11: Distribution of *Scaphoideus titanus* (orange) and Flavescence dorée together with *Scaphoideus titanus* (red).

S. titanus was observed for the first time in North America in 1925 (Ball, 1932). It was introduced into Europe by one major introduction in the late 1950ties and by a second, later introduction into Switzerland. This could be proved by the molecular identification of *S. titanus* individuals from different origin. *S. titanus* appears to be genetically more diverse in North America, than in Europe (Papura et al., 2012). The first insects in Europe were observed 1958 in France (Bonfils and Schvester, 1960). A few years later, 1964 they were

found in Italy (Vidano, 1964) and 1968 in Switzerland (Chuche and Thiéry, 2014). They continued to spread onwards from 1983 into Slovenia (Seljak, 2008), Spain (Rahola et al., 1997; Batlle et al., 1997), Portugal (Quartau et al., 2001), Austria (Steffek et al., 2007), Serbia (Duduk et al., 2004), Bulgaria (Avramov et al., 2011), Bosnia and Herzegovina (Delic et al., 2007), Croatia (Dér et al., 2007b), Hungary (Dér et al., 2007a), Romania (Chireceanu et al., 2011), Montenegro (Radonjić, 2012), Slovakia (Tóthová et al., 2015), Moldova (Chireceanu et al., 2017), Czech Republic (EPPO, 2016) and Ukraine (Mirutenko et al., 2018). *S. titanus* is currently present in 18 countries in Europe (Fig. 11).

3.1.1.2 Test substances

The test substances were selected according to the results of the extensive literature search, considering their mode of action, the range of target organisms and their authorization status. All information presented in table 6 refers to Austrian standards and is derived from the same references as in the respective descriptions for the individual test substances. Test substances were applied either as emulsifiable concentrate (EC) or as suspension concentrate (SC). In the column “Application rate [per ha]” only information referring to viticulture was included. The spray volume applied in the laboratory trial corresponded 400 l/ha for each treatment in compliance with the recommendation for the BBCH growth stage 17-19/55 (Weinbauverband, 2018).

Table 6: Selected test substances for the laboratory trials.

Common name (ISO)	Trade name	Type of pesticide	Austrian plant protection register number	Formu -lation	Active ingredient [g/l]	Max. application rate [per ha]	Concentration test substance in laboratory trial [%]	Authorized in viticulture/ against *S. titanus*
Azadirachtin[2]	NeemAzal-T/S	Insecticide	2699-0	EC	10.0	3.00 kg	0.375	Yes/No
Etofenprox	Trebon 30 EC	Insecticide	3395-0	EC	287.5	/	0.100	No/No
Aluminium silicate (kaolinite)[3]	/	Insecticide	/	/	/	/	1.750	No/No
Paraffin oil	Austriebsspritz mittel 7 E	Acaricide, insecticide	1739-0	EC	836.5	/	2.000	Yes/No
Spirodiclofen	Envidor	Acaricide, insecticide	3351-0	SC	240.0	0.48 l	0.064	Yes/No
Spirotetramat	Movento 100 SC	Insecticide	3021-0	SC	100.0	0.70 l	0.140	Yes/Yes

[2] Azadirachtin A has no ISO-name.
[3] Pure aluminium silicate was used.

3.1.1.2.1 Azadirachtin

Azadirachtin A is a synthetic insecticide with indistinct mode of action which belongs to the group of triterpenes and is derived from the seeds of the neem tree (*Azadirachta indica*) (Insecticide-Resistance-Action-Committee, 2018; Kühne and Friedrich, 2010a). This insecticide is ingested by the target organism through feeding and impacts the endocrine system. The substance blocks the production of the moulting hormone ecdysone and juvenile hormones. This leads to a reduction of feeding, molting and fecundity (BVL, 2011). Azadirachtin A can be used in viticulture, fruit growing, vegetable crops, arable crops and ornamentals both in integrated and in organic production and is marketed under various commercial product names (EASY-CERT-services-GmbH, 2018). Target organisms are Colorado potato beetle (*Leptinotarsa decemlineata*), European winter moth (*Operophtera brumata*), *Melolontha sp*., grapevine phylloxera (*Viteus vitifoliae*), *Aleyrodes sp*., *Bradysia sp*., *Aphiphidae*, *Lygus sp*., carrot moth (*Depressaria daucella*), *Thysanoptera*, *Crioceris*, young nymphs of sucking insects except *Heteroptera*, young nymphs of leaf-mining insects and young nymphs of biting insects (BAES, 2018).

3.1.1.2.2 Etofenprox

Etofenprox is a synthetic insecticide which belongs to the group of pyrethroids. It is used as a contact insecticide that can also be taken up by feeding. Etofenprox causes hyperexcitation by interacting with the sodium channels which disturb the neurons of the nervous system (EFSA, 2008). Etofenprox can be used in integrated production of arable crops. Target organisms are cabbage seed weevil (*Ceutorhynchus obstrictus*), rape beetle (*Brassicogethes aeneus*), rape stem weevil (*Ceutorhynchus napi*) and cabbage stem weevil (*Ceutorhynchus pallidactylus*) (EASY-CERT-services-GmbH, 2018; BAES, 2018).

3.1.1.2.3 Aluminium silicate (kaolinite)

In the context of the present study Aluminium silicate will be referred to as Kaolinite. Currently no kaolinite-based pesticide is registered in Austria and it is not listed in the IRAC Mode of Action Classification (Insecticide-Resistance-Action-Committee, 2018; BAES, 2018). It falls under the fertilizer law 1994 (Düngemittelgesetz 1994 BGBl.Nr. 513/1994 idgF) and fertilizer regulation 2014 (Düngemittelverordnung BGBl Nr. II 100/2004 idgF). In Switzerland kaolinite is available as the insecticide Surround WP®, where it is registered for viticulture, fruit production and arable crops. Target organisms are pear psylla (*Cacopsylla pyri*), walnut husk fly (*Rhagoletis completa*), spotted-wing drosophila (*Drosophila suzukii*) and rape beetle (*Brassicogethes aeneus*) (Stähler,

2017). For the present study the indication of Surround WP® and the pest alert No. 4/16 by the chamber of agriculture Styria were used to find the appropriate concentration for this trial. Kaolinite was bought from a ceramic supplier[4] in form of a pure powder.

3.1.1.2.4 Paraffin oil

Paraffin oil belongs to the group of mineral oils. It is used as a contact-acaricide and contact-insecticide. The oil creates a film which covers all hibernating insect stages. The covered stages are starved by oxygen and suffocate (University-of-Hertfordshire, 2018; Kühne and Friedrich, 2010b). This pesticide is registered in Austria to be used in organically managed and integrated viticulture and fruit production (EASY-CERT-services-GmbH, 2018). Target organisms are juvenile stages of hibernating pests and European red mite (*Panonychus ulmi*) (BAES, 2018).

3.1.1.2.5 Spirodiclofen

Spirodiclofen is the only synthetic acaricide and insecticide which belongs to the group of tetronic acid (EFSA, 2009). It is effective by contact and inhibits acetyl CoA carboxylase, lipid synthesis and growth (EPA, 2005; Insecticide-Resistance-Action-Committee, 2018). In Austria Spirodiclofen can be used in viticulture, fruit production, vegetable crops, ornamentals and hop. Target organisms are mites (Acari), apple rust mite (*Aculus schlechtendali*), *Psylla sp*. and *Aculus sp*. (BAES, 2018).

3.1.1.2.6 Spirotetramat

Spirotetramat is a synthetic insecticide which belongs to the group of tetronic and tetramic acid derivatives. Spirotetramat inhibits acetyl CoA carboxylase, lipid synthesis, growth of juvenile insects and reproduction of adult insects (Insecticide-Resistance-Action-Committee, 2018; EPA, 2008). In Austria this insecticide can be used in viticulture, fruit production, arable crops, vegetable crops, ornamentals and hop. Target organisms are Aphididae, Aleyrodidae, common cotton thrips (*Thrips tabaci*), cabbage crowngall fly (*Contarinia nasturtii*), *Dasineura spp*., Coccoideaceae, *Psylla sp*., Acaridae, *Cicada sp*., grapevine phylloxera (*Viteus vitifoliae*) (BAES, 2018).

[4] Keramikbedarf Ing. Skokan, Austria, 1120 Vienna.

3.1.1.3 Test plant material

As test plant material two-year-old canes of Isabella (*Vitis vinifera* × *Vitis labrusca*) vines were used. The canes were collected on 01.02.2018 from a 20 years old vineyard in Sicheldorf (South East Styria, Austria). The vineyard sized 0.094 ha and consisted of five rows of Isabella vines, trained according to the Guyot-system (Bauer et al., 2013). Monitoring with yellow sticky traps during 2017 revealed the presence of *S. titanus*. The canes for the laboratory trials were collected from the first four rows (Fig. 12) of the trial site. In each row all two year old canes were cut, placed separately in plastic bags and kept in a cold storage room at 6.6°C and 73.5% rel. humidity. The temperature and relative air humidity inside the bags was 11.0°C respectively 92%. In total 331 canes were collected (row 1: 85; row 2: 78; row 3: 79; row 4: 89).

Figure 12: Vineyard with rows from which the canes for the present study were cut (red).

3.1.1.4 Test units

For the bioassay two different types of test units were assembled: the test box and the test cage. The test boxes were only used for the pre-trials and the test cages were used for the pre-trials and the main trial.

The number of AGVL ranged between 275 and 1727 per kg cane (Table 7).

Table 7: Description of canes used for the test cages and boxes.

Trial	Type of test unit	Number of test units	Number of canes	Number of *S. titanus* nymphs per kg cane[5]	Ø length of canes [cm]	Ø number of nodes per cane	Ø weight of canes [g]
Pre-trial A	Cage	4	16	877-1727	25.90	4.00	13.2
Pre-trial B	Box	1	8	940	27.40	2.10	15.6
Pre-trial C	Cage	3	12	0	35.25	3.75	20.2
Pre-trial C	Box	1	17	801	24.30	2.20	8.7
Main-trial	Cage	84	336	275-1485	26.00	4.00	15.6

3.1.1.4.1 Test box

A test box consisted of a closable polypropylene (PP) box[6] (30cm x 35cm x 16cm), a cloth[7] with a mesh size of 0.25mm x 0.25mm, vermiculite, a metal frame and a water filled tube with a *Vitis* sp. leaf. The lid of the box had a rectangular cutout which was closed by the cloth to prohibit AGVL from exiting. The cutout enabled airflow and circulation of humidity. The bottom of the box was covered with vermiculite, which stored humidity. On top of the vermiculite was a metal frame, on which the test canes were placed. The metal frame prevented the canes from direct contact with the wet vermiculite. Attached to the metal frame was a water filled tube with a *Vitis* sp. leaf. The leaf was used to monitor the nymphs that hatch from the canes (Schaub et al., 2010).

[5] For the calculation see table 15A
[6] ROTHO Aufbewahrungsbox "App my Box" mit Deckel 18 Liter, Pagro product number 160260.
[7] Curtain Teresia, IKEA® Austria product number 502.323.33.

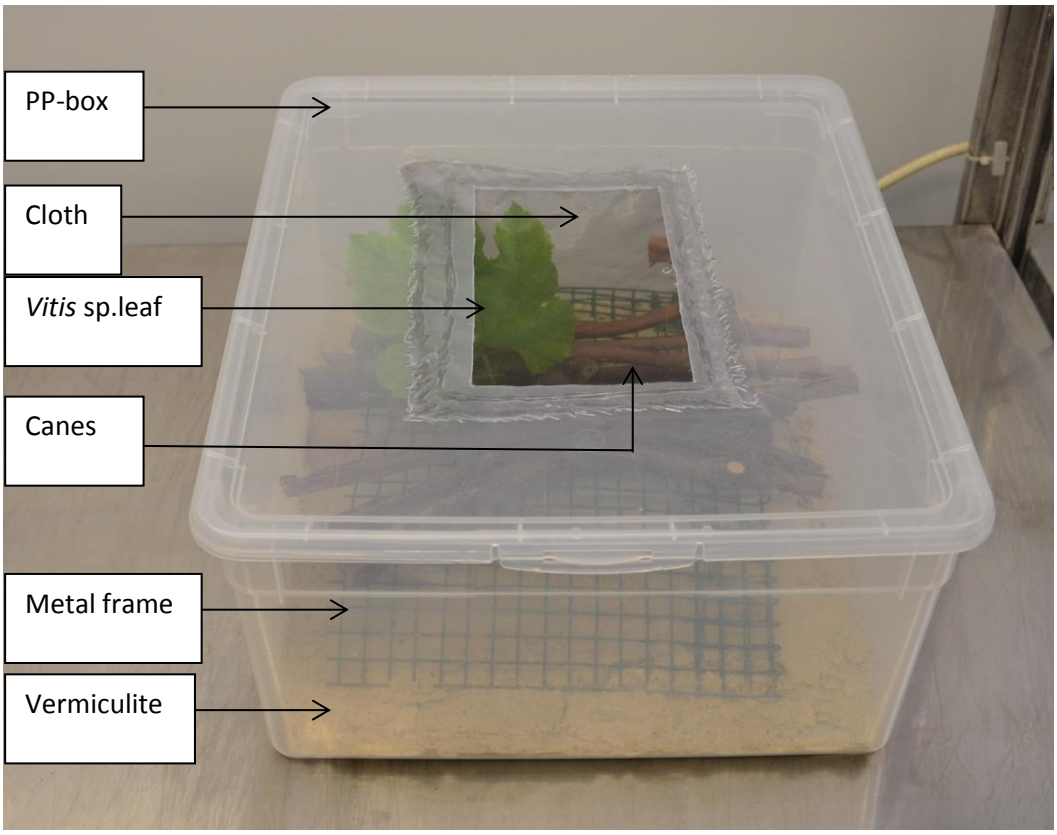


Figure 13: Test box from pre-trial B.

3.1.1.4.2 Test cage

The test cage consisted of a circular metal frame, a polyvinyl chloride cylinder, a cloth on the top side, a yellow sticky trap and an insulating board on the bottom side. The canes were fixed on the horizontal and vertical beams of the circular metal frame (height: 50cm; Ø 16cm). If a cane was too long it was cut into two smaller pieces with two nodes each and both were fixed onto the same cage and counted as one cane. The metal frame was surrounded by a cylinder of polyvinyl chloride (height: 55 cm; Ø 19cm). The cylinder circumference overlapped 3 cm and was fixed together on the bottom and top side with a stapler. To ensure that the cylinder was closed, the overlapping area was sealed with a durable and temperature resistant adhesive tape. On top of the plastic cylinder was a cloth with a mesh size smaller than the *S. titanus* nymphs (see 3.1.1.4.1). The cloth was attached to the cylinder by several rubber bands and tightened so that insects could not escape. On the bottom side of the cage was an insulating board. The insulating

board was made out of Styrofoam and measured 25 x 33 cm[8]. On top of that base was a yellow sticky trap[9]. The sticky traps were 25 x 32 cm odorless and insecticide-free.

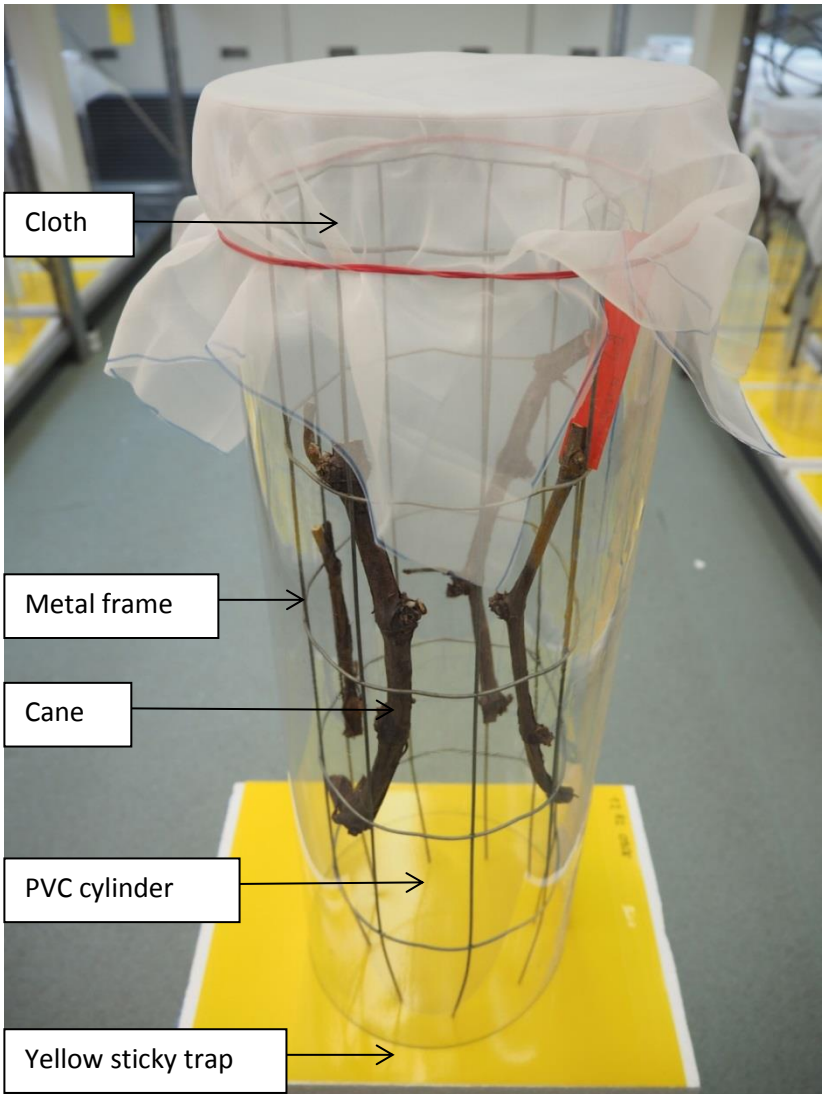


Figure 14: Test cage from the main trial.

3.1.2 Methods

Three pre-trials with the two types of test units (test box or test cage see 2.2.1.4) were conducted to determine the hatching period of *S. titanus* and to investigate the influence of the different test unit types and the different trial dates on the nymphal hatching.

[8] Dämmplatte Precit®, Hornbach productnumber 4000226.
[9] Gelbtafeln Neudorff®, Neudorff productnumber 00319.

The test units were kept in a climate chamber at 24°C, 75- 80% relative humidity and a photoperiod of L:D 16:8 (Caudwell et al., 1970; Privet et al., 2007).

Table 8: Information about the different trials regarding duration of cold storage, duration of trial and assessment method.

Trial	Duration cold storage [days]	Start of trial	Duration of trial [days]	Type of test unit	Assessment method of *S. titanus*
Pre-trial A	1	02.02.2018	110	Cage	Sticky trap and leaf
Pre-trial B	65	06.04.2018	85	Box	Leaf
Pre-trial C	78	19.04.2018	72	Box	Leaf
Pre-trial C	78	19.04.2018	72	Cage	Sticky trap and leaf
Main-trial	95	07.05.2018	96	Cage	Sticky trap

3.1.2.1 Pre-trials

3.1.2.1.1 Pre-trial A

The test canes were cold stored for 1d before the trial start (Table 8). They were fixed onto the metal frames and wetted with a hand sprayer[10] before placing them into the test cages (n=4), since it was not possible to wet them afterwards. The cages were then moved into the climate chamber. Canes from the different vineyard rows were placed in corresponding test cages. Canes measured on average 25.9 cm and had on average four nodes. To monitor AGVL nymphs, a plastic tube with water with one *Vitis* sp. leaf was put into each cage. The leaves were changed when they started to wilt. For the evaluation of the nymphal hatch, the leaves were checked for the presence of nymphs in one to three day intervals over a period of 110 days. When nymphs were found, they were removed from the leaf by using a portable exhauster with a net at the cone-end to capture the nymphs. The nymphs were then removed from the exhauster with a fine brush and put into alcohol to count them again by using a stereomicroscope with amplification 10 x 0.65. The control with the stereomicroscope was done to ensure that the nymphs were caught by the exhauster and did not jump off the leaf. The yellow sticky traps on the bottom of the test cages were checked for *S. titanus* nymphs the first time 83 days after trial start. The trial was ended after no nymph appeared on the leaf for 17 days. The two different evaluation methods were used to find the first AGVL nymphs as early as possible, since it was unsure when the nymphs will move from the leaf onto the sticky trap.

[10] Pressure sprayer solo comfort line 402®, 2 liter.

3.1.2.1.2 Pre-trial B

The test canes (n=8) were cold stored for 65 days before the trial started (Table 8). They were laid onto the frame inside the test box (Fig. 13), wetted with a hand sprayer until the vermiculite appeared saturated and moved into the climate chamber. The humidification was repeated twice a week until the first nymphs appeared on the leaf. The canes inside the test box were all from the 2nd vineyard row. Cane length measured on average 27.4 cm and had on average 2.1 nodes per cane (Table 7). The monitoring was done with a *Vitis* sp. leaf as described in pre-trial A. The leaf was checked for nymphs for the first time after 21 days. The monitoring continued for a period of 85 days during which the controlling took place with a one to three day interval. The trial was ended after no nymph appeared on the leaf for seven days.

3.1.2.1.3 Pre-trial C

The test canes were cold stored for 78 days before the trial started (Table 8). Four test units were used, including one test box and three test cages. The canes were all from the 4th vineyard row and were either fixed onto the cage frames (test cages) or laid onto the frame (test box), wetted and moved into the climate chamber. The canes (n=17) from the test box measured Ø 24.3 cm and had on average 2.2 nodes per cane. A *Vitis* sp. leaf was used to monitor the AGVL nymphs as described in pre-trial A. The leaves were checked after eight days in a one to three day interval over a period of 72 days. The canes (n=12) from the test cages measured Ø 35.25 cm and had on average 3.75 nodes per cane. The monitoring of *S. titanus* inside the cages was done as described in pre-trial A. The yellow sticky traps were checked for the first time after 25 days and then with a one to three day interval for 72 days.

3.1.2.2 Main-trial

The main trial started the 7th of May 2018 by moving the canes out of the storage room and spraying them with water until runoff and then moving them into the climate chamber. In the climate chamber they were put into the cages, but without yellow sticky traps.

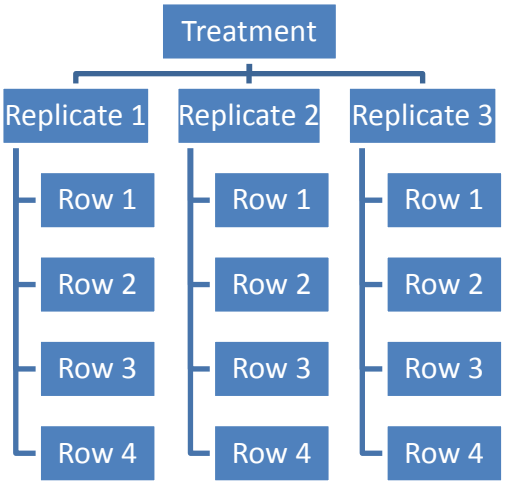
3.1.2.2.1 Test design

The test canes for the main trial were cold stored for 95 days after the sampling before the trial start. The main trial was carried out in test cages (Fig. 15). Each test cage contained four canes only from one of the four different rows as described in pre-trial A.

Figure 15: Test cages in the climate chamber during the main-trial.

Each treatment consisted of four cages with three replicates for a total of 12 cages per treatment (Table 9). With seven treatments including the control this results in a total of 84 test cages and 336 canes. The canes had an average length of 26cm with 4 nodes each.

Table 9: General test design of a treatment in the main-trial.



3.1.2.2.2 Calculation of the spray volume

The spray volume was calculated to simulate an application that would be used under field conditions during the BBCH growth stages 17-19/55. To determine the amount of spray mixture that was applied by using the hand sprayer, pre- trials with water were carried out (not shown). The trials showed that for the most consistent amount of applied liquid the tank had to be opened to release the pressure, filled up to 2 liters and pump the handle with 30 strokes. This procedure has to be repeated for each application. By this spraying procedure the amount of liquid during a 30 second application duration is around 155 ml. The amount of spray mixture required for each cage is 156 ml.

The 156 ml per test cage were calculated as follows:

First the number of vines per hectare was calculated, based on the assumed planting distance of one meter and a row distance of three meters (Hanni and Andergassen, 2004), resulting in approximately 3.333 vines/ha. The cane length of the two-year-old wood was assumed to be around 0.80 meter because of the planting distance and the Guyot vine training system. This leads to 2.666 meters effective cane length. The recommended field application rate during the growth stages BBCH 0 to 61 is between 100 and 800 liter (Weinbauverband, 2018; Weinbauverband, 2013). The optimal time of application would be mid-May before the nymphal hatch starts, when the vines are in the growth stages BBCH 17-19/55 according to the newsletters by Rebschutzdienst Krems Langenlois from May 2016 to August 2017. For this BBCH stage 400l/ha are recommended, which results for the 2.666 meters effective cane length per ha, in 39 ml per 26 cm. This equals 156 ml per test cage with four canes of 26 cm length each.

3.1.2.2.3 Application of test-substances

Table 10: Application dates.

Task	Date	Interval between next application	Days since beginning of trial
First application	24.05.2018	12	17
Second application	05.06.2018	16	29
Third application	21.06.2018	/	45

The cages were moved as treatment-groups from the climate chamber to the application laboratory. All the applications throughout the trial were conducted by the same person to reduce variation through handling. The applications were made 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. The pressure sprayer was moved vertically along the cage and after each vertical repeat it was moved horizontally to cover about 180° of the cage. After the first 15 seconds of the application the cages were turned 180° to ensure that all sides get sprayed equally. At the end of the application the cages were moved back into the climate chamber.

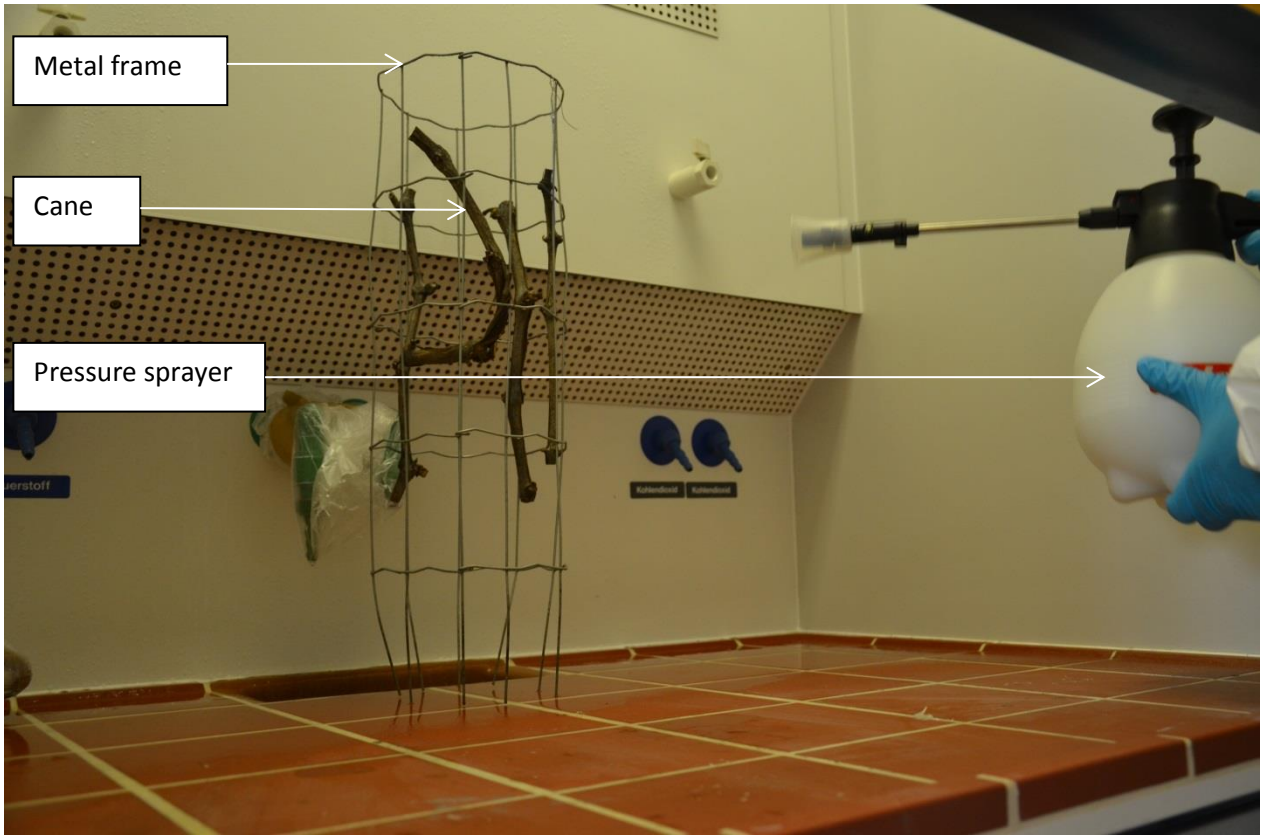


Figure 16: Depiction of the spray process under the fume hood (metal frame did not touch the wall).

3.1.2.2.4 Evaluation

The first yellow sticky traps were placed under the cages immediately after the first application; 17 days after trial start (DAS). The yellow sticky traps were changed 29 DAS (second application), 45 DAS (third application), 56 DAS and 80 DAS. The yellow sticky traps from 80 DAS were removed after 95 DAS.

The yellow sticky traps were examined by using a stereomicroscope with an amplification 10 x 0.65.

Only nymphs inside the area on the yellow sticky traps which was delimited by the polyvinyl chloride frame of the test cages were evaluated. A nymph was only counted if it was clearly determined as AGVL nymph by its color, shape and the two black dots on its abdomen. The control of all traps was done by one person and randomly selected traps were additionally checked by a second person.

3.1.2.3 Data analysis

All statistical analyses were calculated with the software IBM® SPSS® Statistics Version 22.0.0.0 64-bit edition.

The significance level was 5% and for the post hoc analysis Tukey's test was used.

The effect treatment on the mean number of *S. titanus* nymphs was examined by using an analysis of variance (ANOVA). To calculate the effect of the row on the treatment a multilevel model (mixed model) was used.

The effects of row and respectively treatment on the mean weight of the canes was tested by using an ANOVA (Field, 2013).

3.2 Results

3.2.1 Pre-trials

3.2.1.1 Pre-trial A

The nymphal hatching period of AGVL lasted 33 days (66 DAS to 99 DAS). The total number of caught nymphs from the four test cages was 285 (cage 1: 16.5%, cage 2: 20.4%, cage 3: 28.1%, cage 4: 35.1%), which includes 183 nymphs caught on yellow sticky traps and 102 nymphs extracted by exhauster (Table 11).

Table 11: Mean number of *S. titanus* nymphs caught per cage on yellow sticky traps and extracted with exhauster at evaluation dates when nymphs were observed.[11]

Date	n *S. titanus* on yellow sticky trap				n *S. titanus* extracted with exhauster			
	Cage 1	Cage 2	Cage 3	Cage 4	Cage 1	Cage 2	Cage 3	Cage 4
09.04.2018	/	/	/	/	0	0	0	1
10.04.2018	/	/	/	/	0	0	0	1
20.04.2018	/	/	/	/	6	3	1	2
23.04.2018	/	/	/	/	3	8	7	18
24.04.2018	/	/	/	/	0	0	0	5
25.04.2018	7	9	20	31	0	2	5	4
26.04.2018	/	/	/	/	6	0	4	3
27.04.2018	/	/	/	/	5	2	0	0
30.04.2018	/	/	/	/	0	3	1	2
02.05.2018	16	24	31	21	1	3	3	1
03.05.2018	0	2	3	6	0	0	0	0
04.05.2018	1	1	1	0	1	0	1	0
09.05.2018	1	1	3	5	0	0	0	0
Total number	25	37	58	63	22	21	22	37
Mean ± Std.	2.08 ± 4.81	0.50 ± 1.50	3.08 ± 7.06	0.48 ± 1.44	4.83 ± 10.00	0.50 ± 1.46	5.25 ± 10.1	0.84 ± 2.87

[11] / = no evaluation.

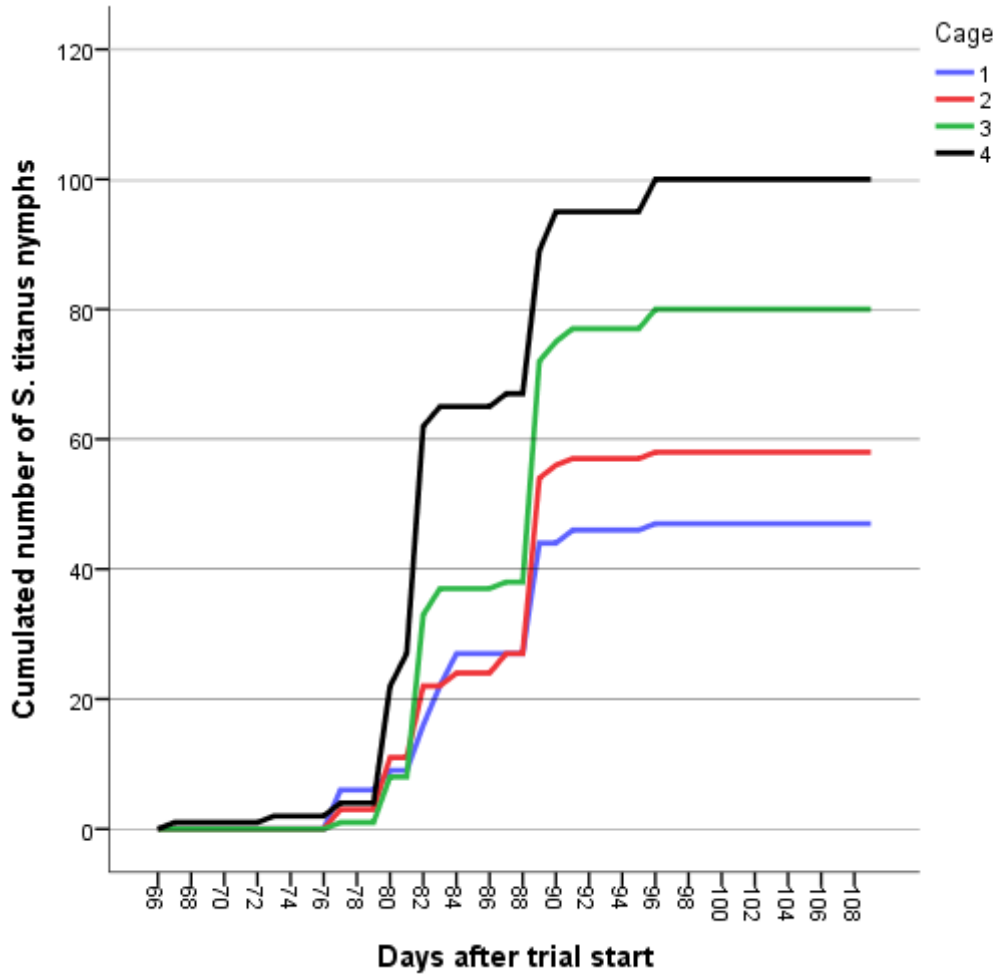


Figure 17: Cumulative sum of *S. titanus* nymphs caught on the yellow sticky traps and extracted with the exhauster from the cages of pre-trial A.

3.2.1.2 Pre-trial B

The nymphal hatching period of AGVL lasted 41 days, from 28 DAS until 69 DAS. The total number of caught nymphs from the box was 117.

3.2.1.3 Pre-trial C

The nymphal hatching period of AGVL in the test-box lasted 38 days, from 26 DAS until 64 DAS. 118 AGVL nymphs were caught during that period.

During the 71 days that pre-trial C was running no nymphs hatched in the test-cages.

The comparison of the cumulated number of nymphs from the box of pre-trial B and pre-trial C showed a similar development of the cumulative number and peak of AGVL nymphs. The major

difference was that the nymphs of pre-trial B hatched approximately 10 days earlier than the nymphs of the test-box in pre-trial C (Fig. 18; Table 14A).

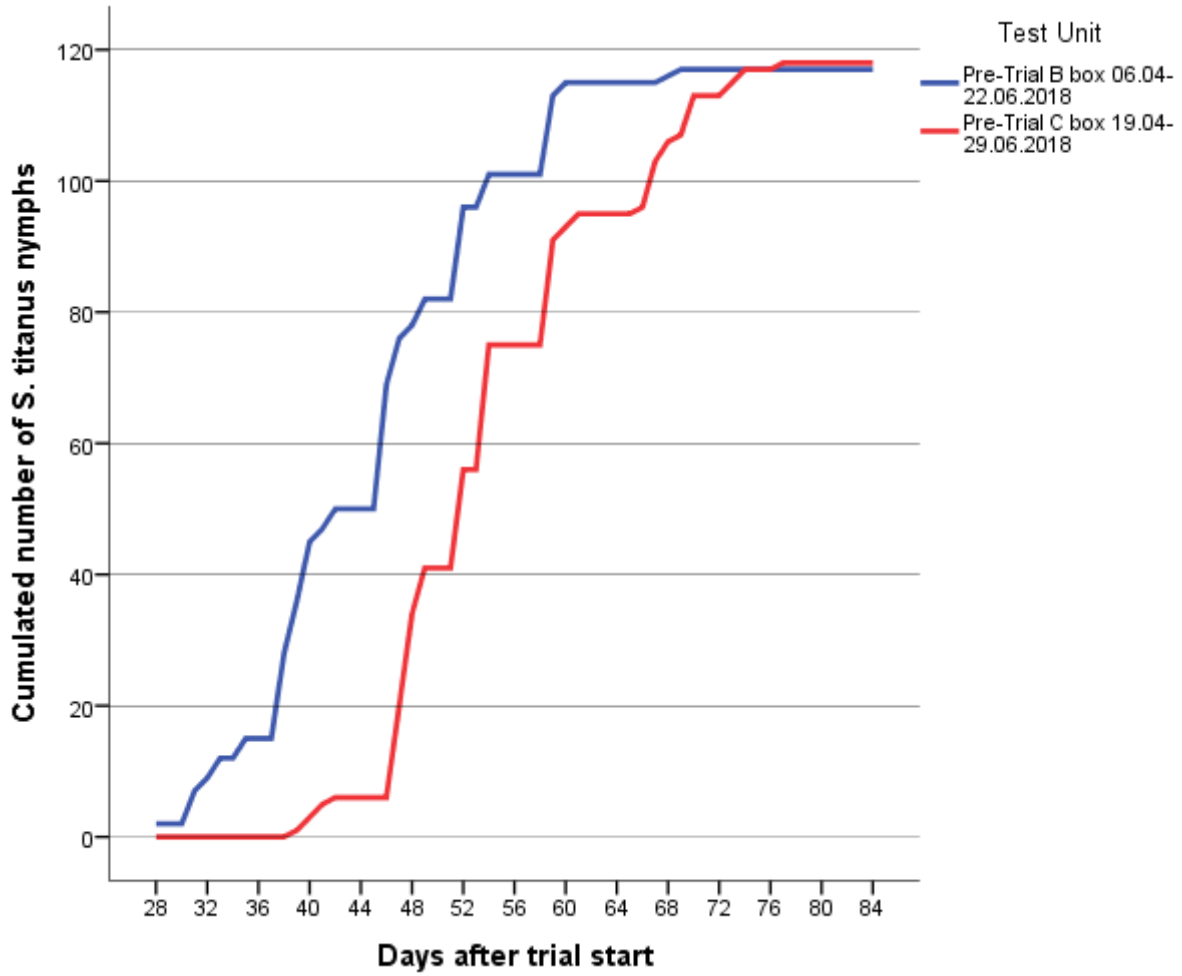


Figure 18: Cumulative sum of *S. titanus* nymphs extracted with the exhauster from the boxes of pre-trial C.

3.2.2 Main trial

The first nymphs of the main trial hatched between 29 DAS and 45 DAS. The last nymphs hatched between 80 DAS and 95 DAS. Thus the hatching period was between a minimum of 25 and a maximum of 66 days long.

The different treatments had a statistically significant (α=0.05) influence on the mean number of hatched AGVL nymphs compared to the mean number of hatched AGVL nymphs in the water treated control. The mean number of hatched AGVL nymphs in the treatments etofenprox and spirotetramat were significantly lower compared to the mean number of hatched AGVL nymphs in the treatments kaolinite, spirodiclofen and the control (Table 3). The etofenprox treatment

resulted in a nymphal hatch decrease of 100% compared to the control (n nymphs=623). Spirotetramat resulted in a decrease of nymphal hatch of 99% (n nymphs=1) compared to the control. The mean number of hatched AGVL nymphs in the treatment paraffin oil was significantly lower to the mean number of hatched AGVL nymphs in the control and resulted in a 83% (n nymphs =105) reduction of nymphal hatch compared to the control. The mean number of hatched AGVL nymphs in the azadirachtin treatment was significantly lower compared to the mean number of hatched AGVL nymphs in the control and lead to a reduction of 72% (n nymphs =172) in nymphal hatch compared to the control. The mean number of hatched AGVL nymphs in the treatment spirodiclofen was significantly different compared to the mean number of hatched AGVL nymphs in the treatments etofenprox, spirotetramat and control and caused a reduction of 64% (n nymphs =222) in the nymphal hatch of AGVL as compared to the control. The mean number of hatched AGVL nymphs in the treatment kaolinite was significantly different to the mean number of hatched AGVL nymphs in the treatments etofenprox, spirotetramat and control. It had the lowest reduction of nymphal hatch in comparison to the control with 47% (n nymphs =298, Fig. 20).

Table 12: Comparison of the mean numbers of hatched *S. titanus* nymphs in the different treatments (significance: α=0.05)[12].

	Aza-dirachtin	Etofenprox	Kaolinite	Paraffin oil	Spiro-diclofen	Spiro-tetramat	Control (water)
Azadirachtin	/	N. sig.	N. sig.	N. sig.	N. sig.	N. sig.	*
Etofenprox	N. sig.	/	*	N. sig.	*	N. sig.	*
Kaolinite	N. sig.	*	/	N. sig.	N. sig.	*	*
Paraffin oil	N. sig.	N. sig.	N. sig.	/	N. sig.	N. sig.	*
Spiro-diclofen	N. sig.	*	N. sig.	N. sig.	/	*	*
Spiro-tetramat	N. sig.	N. sig.	*	N. sig.	*	/	*
Control (water)	*	*	*	*	*	*	/

[12] * = statistically significant. N. sig. = statistically not significant.

The first (29 DAS) and last (95 DAS) evaluation date were excluded for the following observations because no nymphs were observed.

Over the three evaluation dates (45 DAS, 56 DAS, 80 DAS) the median of the number of *S. titanus* nymphs for etofenprox and spirotetramat did not change. For the treatments azadirachtin, paraffin oil and spirodiclofen the median of the number of *S. titanus* nymphs rose from the first to the second evaluation date and declined from the second to the third evaluation date. The median of the number of *S. titanus* nymphs for the control (water) and kaolinite was rising during the evaluation dates (Fig. 19).

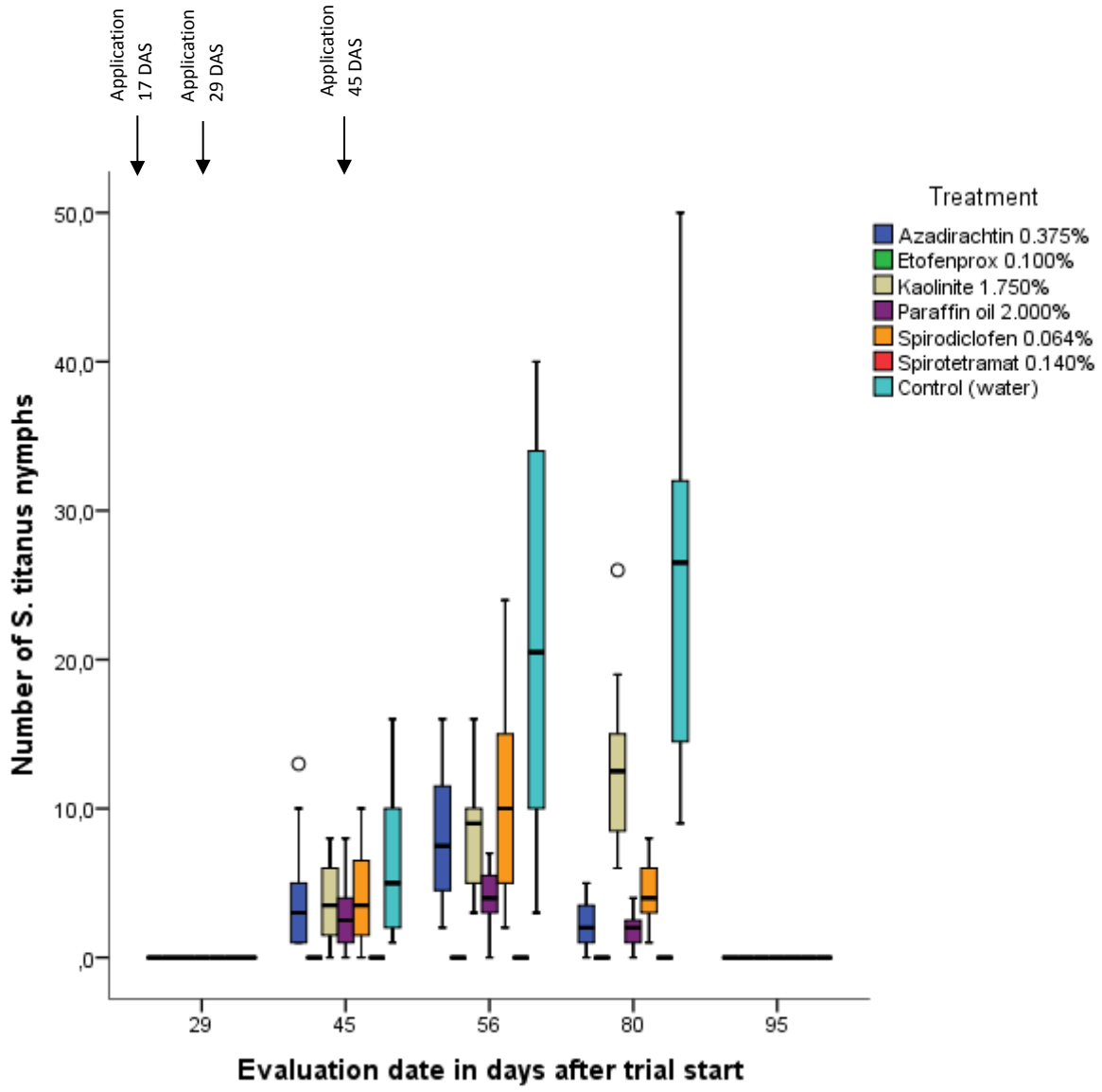


Figure 19: Number of *S. titanus* nymphs in the different treatments during the main trial per evaluation date after 1, 2 or 3 three applications (17 DAS, 29 DAS and 45 DAS).

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 hatched AGVL at the end of the trial. Except etofenprox and spirotetramat with 0 respectively 1 hatched AGVL, the treatments resulted in a 2.1 to 5.9 fold lower cumulative sum of AGVL in relation to the control indicating the efficacy of the different treatments in relation to the control. No statement can be made for the efficacy after the different numbers of applications since no nymphs hatched until the day of the second application (29DAS) (Fig. 20).

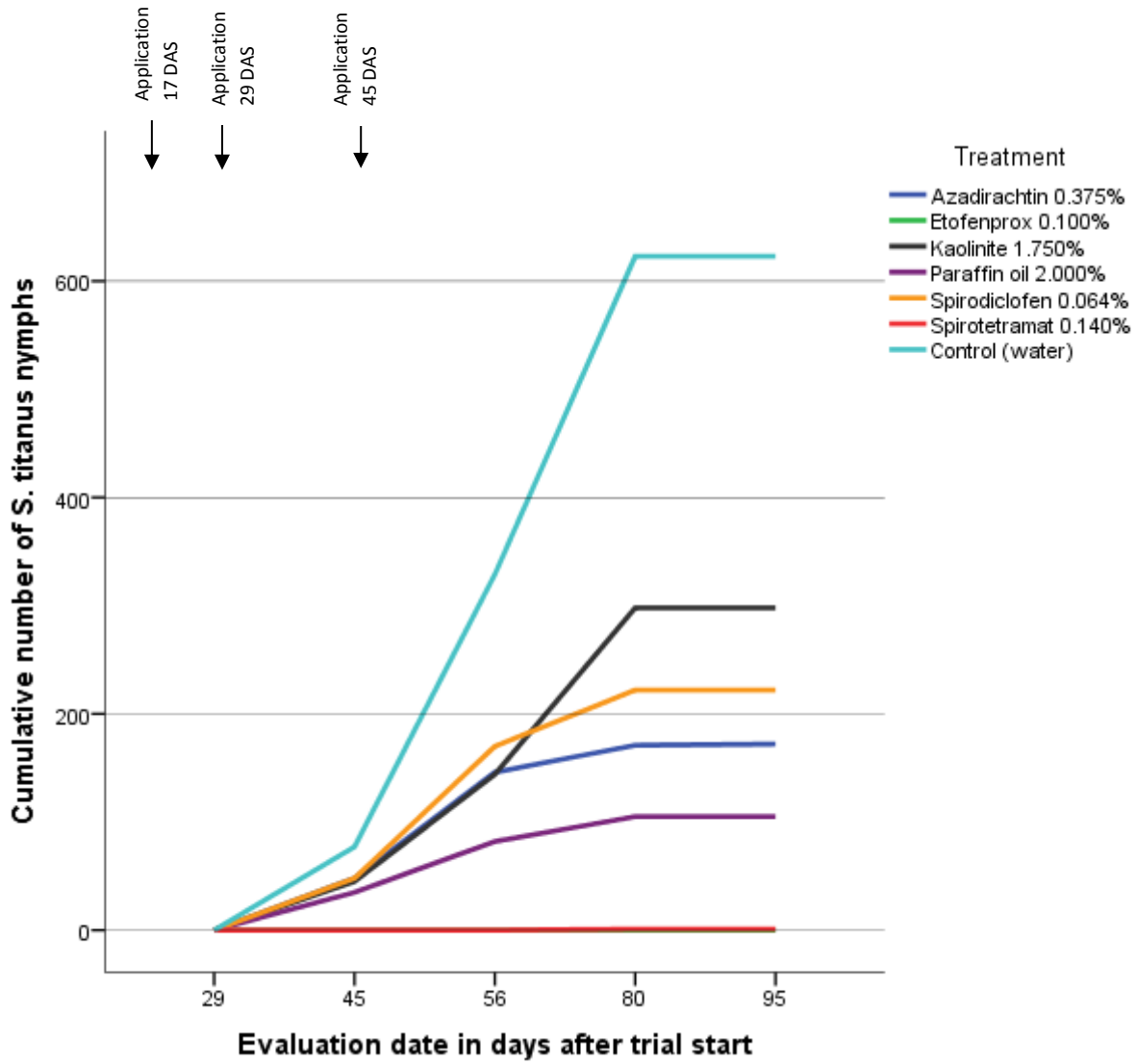


Figure 20: Cumulative sum of *S. titanus* nymphs in the different treatments during the main trial per evaluation date after 1, 2 or 3 three applications (17 DAS, 29 DAS and 45 DAS).

3.2.3 Additional results

The number of AGVL nymphs (Table 11, Table 15A) and the weight of the canes (Table 7) were used to calculate AGVL nymphs per cane weight. This was then converted to AGVL nymphs per kg cane (Table 15A).

4 Discussion

In the present study laboratory trials were carried out to test the reducing effect of different pesticides on the nymphal hatch of *Scaphoideus titanus*. The trials were conducted with a new test-cage-method and pesticides were directly applied on grapevine canes. Beforehand an extensive literature search took place to assess the efficacy of different pesticides.

4.1 Extensive literature search

An ELS which was conducted to identify potential substances that can reduce the nymphal hatch of AGVL, showed that very few publications exist on this topic.

4.1.1 Material and methods

To increase the probability to find more potential test-substances, whiteflies as an organism with similar development stages to AGVL were included as search term in the ELS.

The search terms which were combined with the exclusion search term helped to reduce the number of inappropriate hits, but still 61% of records were deemed irrelevant after the first evaluation step. The number of irrelevant records could have been reduced further with stricter exclusion terms, but this would have also increased the risk of excluding relevant records. Most records were irrelevant because they focused on adult leafhoppers instead of immatures, but if adults would have been included in the exclusion term, records that discuss adults and nymphs would have been excluded too. Therefore, the current more general exclusion term was chosen.

The second evaluation step was difficult to apply because most titles and abstracts did not specify if the record contains information about eggs, nymphs or adults. The records used unspecific terms such as “individuals” or “population”, which did not specify the development stage of the insects.

4.1.2 Results

The extensive literature search showed that it was difficult to find relevant records as only 10% of the records could be rated as relevant according to the selection criteria. Of those 10% more records comprised information about whiteflies (56) than about planthoppers (43). The records which were deemed relevant contained information about the topic, but only one record contained information about an experiment targeting AGVL eggs in a vineyard (Constant, 2005). The other records about AGVL were instead targeting the nymphs. Those records were still

deemed useful since a high efficacy against newly hatched nymphs would also be sufficient to keep populations low.

As the publication period was not limited the quality of the records had a great variance, with more recent publications having an overall higher quality. Including older publication years also caused a problem with the availability of certain records. But the lack of limitation by publication year was necessary due to the low number of relevant records.

The trials described in the different records showed highly different results for the same substances with regard to the efficacy. It was difficult to compare the efficacy of the substances described in the different records because of different test organisms and test plants. In some field trials yellow sticky traps were used as evaluation method and those can have a high variance in the number of catches and do not show the actual population and reduction of the insect. The application method was also an important factor. The most suitable application method to compare with the trial of the present study would be a potter spray tower. A lot of trials used the runoff-method[13] or leaf-dip-method[14] and were not suitable for a comparison (Jamieson et al., 2010; Jafarbeigi et al., 2012). Leaf dip trials have a better coverage and show a higher efficacy for substances that require a good coverage such as paraffin oil (see 1.2.1.4.4 paraffin oil) compared to a trial with a spraying application (Uygun et al., 2011; Stansly and Liu, 1994). Several trials also used a combination of multiple pesticides and were not suitable for comparison (Khajuria et al., 2015; Ray et al., 2011; Kumari et al., 2009).

All substances of the present study, (see 2.2.1.2 Test substances), were used due to the mostly positive, but varying efficacy from the records for each test-substance. Some records had to be excluded because they used a combination of multiple pesticides. No direct comparability to the present test design was possible for the substances.

The extensive literature search also showed that only few studies were aimed to reduce the nymphal hatch and even less the egg survivability. Furthermore most of the used substances are currently not registered in Austria (BAES, 2018). Nearly all of the trials were conducted during winter or when 3rd, 4th, 5th instar nymphs or adults occurred and these treatments resulted mainly in low efficacy.

The extensive literature search revealed that CAB Abstracts had a higher quality of records than Agricola as less parameters were missing. Often duplicates from CAB Abstracts and Agricola

[13] The solution is applied onto the surface until it runs off.
[14] The test organism or plant is dipped into the solution for a short period of time.

showed up in the EndNote library. This was because for certain records Agricola did not manage to export the information in the corresponding slots. This means that some records from Agricola were at first missing information such as author, publication year and journal. This information was then found under the field “notes” and had to be manually changed in the respective row.

4.2 Trials

Pre-trials were conducted to determine the beginning and duration of the nymphal hatch in order to determine the best date for the application of the pesticides which is just before the hatching starts. The subsequent main-trial was carried out to test the impact of the different treatments on the nymphal hatch of AGVL.

4.2.1 Material and methods

The plant-material was taken from a vineyard where the presence of AGVL was monitored with yellow sticky traps in the year previous to the present study and which showed a high number of AGVL individuals. The yellow sticky traps showed a difference in the numbers of AGVL depending on the vineyard row. The canes which were cut for the laboratory study were not uniform and had to be standardized for the trial to reduce the variability. For the adaptation of the plant-material the number of nodes per cane was considered as important factor, since the AGVL adults prefer to lay their eggs close to the nodes (Bagnoli and Gargani, 2011). Other important parameters were the length and weight of the cane, since those three parameters influence the egg number the most. The trials showed that the row and weight had no statistically significant impact on the number of AGVL on standardized canes.

The disadvantage of using canes from a field is the different number of AGVL present on each cane. The eggs are inserted into the bark and difficult to locate as the bark needs to be removed. This process is very labor intensive and could harm the eggs and makes it difficult to differentiate if certain eggs were already dead or were harmed during the removal of the bark.

The test units used during the present study exhibited different advantages and disadvantages.

The test-boxes had the advantage that bigger quantities of canes and therefore more *S. titanus could* be placed into one test-unit at a time. Another advantage was that the moistening of the wood, which prevents the dehydration of the eggs, was possible during the trial. Moreover, it was also easier to access the canes directly during the trial. Additionally vermiculite was added to the test boxes to keep the moisture high (Bressan et al., 2005b) (Bressan et al., 2005a)

(Lessio et al., 2009). The disadvantage was that during the extraction with the exhauster nymphs could jump away or be overseen.

In the present study a never before described test unit was used. The extensive literature search and other available full articles did not show any similar methods of the test cage (see 2.2.1.4.2).

An advantage of the test-cage was that it enables the simulation of field conditions by using canes. Another advantage of the test-cages was that the yellow sticky traps on the bottom of the cages allowed a standardized, continuous assessment of the number of hatched AGVL nymphs. A disadvantage of the cage method is that the moistening of the canes in the cages is not as simple as in the boxes, because the water would run off on the yellow sticky traps. In order to solve this problem and prevent the eggs from dehydration the relative air humidity (75-80%) was increased as compared to other trials (50-80%). The other parameters such as temperature (24°C) and day-night-cycle (16:8) corresponded to the first trial of Caudwell and adaptions by other researchers (23-26°C) (Caudwell et al., 1970; Privet et al., 2007; Galetto et al., 2014; Caudwell, 2008).

4.2.2 Results

4.2.2.1 Hatching of *S. titanus* nymphs

The first assessment of the number of *S. titanus* nymphs was done right before extracting them with the exhauster. This assessment was then validated with a second assessment of the extracted nymphs under a stereoscope. The two-step assessment showed that no nymphs jumped away before being extracted. They only moved when the exhauster came close to them and they responded by slowly walking away.

A comparison of both methods to assess the number of hatched AGVL nymphs showed that more nymphs were caught on the yellow sticky traps than extracted by the exhauster. This can be explained in two ways. The first one is that the AGVL migrated from the *Vitis* sp. leaf to the yellow sticky traps before they could be extracted. The second one is that they stayed on the surface of the cane until they migrated to the yellow sticky trap. The difference in the number of caught AGVL is probably due to the fact that the control of the leaves is a control at an exact moment while the yellow sticky traps capture the nymphs over a longer time period. Since the assessment method by yellow sticky trap caught more AGVL and monitors over a longer time period with less intervening in the trial, it was chosen as the assessment method for the main-trial.

Three trials of the present study (pre-trial B, the box of pre-trial C, main trial) lasted close to 4-6 weeks before the first nymph hatched, which is comparable to the findings of Lessio et al. (2009), but longer than for most trials reported in the literature with close to 3 weeks after trial start for the first nymph to hatch (Chuche et al., 2014; Caudwell, 2008; Bressan et al., 2005b; Privet et al., 2007; Maggi et al., 2013). On the contrary in pre-trial A of the present study it took 9 weeks until the first nymph hatched, which was probably caused by the diapause due to the earlier start of pre-trial A compared to the other trials of the present study. The diapause is only broken after 3 months at a temperature of 3-4°C (Caudwell, 2008).

In the cages of pre-trial C no nymphs hatched. This even though the setup of this trial was the same as used for pre-trial A and the main trial, where nymphs hatched. Furthermore pre-trial C, was conducted in the same climate chamber as the other trials and partially temporally overlapping with them. Because of those similarities it is unclear why no nymph hatched.

The hatching of the AGVL in the different pre-trials lasted between 33 and 41 days. The exact hatching duration of the main-trial could not be observed since it was measured with yellow sticky traps over time intervals. Therefore, the hatching duration in the main-trial lasted between 25 and 66 days. In other trials the nymphal hatching started between 15 and 30 DAS and lasted usually close to 20 days which is much shorter than the duration in the present study (Chuche et al., 2014; Caudwell, 2008; Maggi et al., 2013; Privet et al., 2007; Bressan et al., 2005b).

The temperature during the winter has influence on the duration of the hatch, with temperatures below the 5°C threshold causing a shorter hatching duration (Chuche and Thiéry, 2009). The mean temperature in the vineyard of the present study was below the temperature threshold from November to January, when the canes were cut (ZAMG, 2018). Therefore the hatching duration of the present study should have been shorter compared to the other trials mentioned earlier. At the moment no reason is known for the longer hatching duration with a cold winter below the temperature threshold.

4.2.2.2 Magnitude and meaning of number of AGVL per kg cane

The results of the present trial showed the presence of a minimum of 801 AGVL/kg canes and a maximum of 1727 AGVL/kg canes. A rearing trial showed 27 eggs on 180g of two year old wood canes which would be equivalent to 150 AGVL/kg cane (Privet et al., 2007). The high number of AGVL/kg cane increased the meaningfulness of the present study, because it led to nymphs hatching in all test-units of the control during the hatching period and increased the differences between the efficacies of the different test-substances. The high number of the AGVL/kg cane

also represents the population better and increases the power of the different post-hoc tests (Field, 2013).

The difference of the number of AGVL/kg in the present study between the different rows could be caused by different factors such as the different exposure to wind, which inhibits the emission of calling signals or plant density, of which *S. titanus* prefers more dense planting (Fig. 12) (Mazzoni et al., 2009a; Lessio and Alma, 2004a).

The ratio of AGVL/kg from the trial cages was in the same range as the ones from the box trials. Therefore, the type of test unit had no major impact on the rearing ability of AGVL. The yellow sticky traps of the cages were easy to exchange and the lack of additional humidification, such as spraying the canes in the boxes, did not lead to a decrease of AGVL hatch.

4.2.2.3 Efficacy

Based on the test design and the relative late hatch of the nymphs in the present study it is not possible to make a statement on the difference in the reduction of the nymphal hatch of AGVL after the different numbers (one, two or three) of applications. The efficacy results show the reduction in the nymphal hatch of AGVL after three applications. In the present study etofenprox respectively spirotetramat had a very high efficacy on AGVL nymphs with a reduction of the hatching rate by 100% respectively 99 % compared to the control with water. The other treatments ranked in the reduction of nymphal hatch as follows: paraffin oil (83%), azadirachtin (72%), spirodiclofen (64%) and kaolinite (47%).

The extensive literature search did not result in any trials that had a similar test method. Therefore the efficacy results of the current trial cannot be directly compared to other trials as reported in the references. Nonetheless the records show efficacies on similar infraorders and families such as planthopper and whiteflies.

Trials with etofenprox against nymphs of another Auchenorrhyncha species and against whitefly eggs and nymphs showed an efficacy range of 91-100% (Grassi and Ri, 2006; Soad et al., 2005).

The ELS revealed no other trials with spirotetramat against AGVL eggs or nymphs. In trials against whiteflies spirotetramat showed a high toxicity against nymphs, but inconsistent toxicity for eggs (Ge et al., 2011; Kovarikova et al., 2017; Cameron et al.).

Paraffin oil trials against different other planthopper species showed no efficacy for eggs and for nymphs between 66-97% efficacy depending on the species (Cornale et al., 1998; Dardar et al., 2013; Mahmoudi et al., 2014). Trials with whiteflies also showed a high variance of efficacy, for eggs the efficacy was between 22-88% and for nymphs the efficacy was between 46-82% (Jamieson et al., 2010; Lokender et al., 2016; Garrido et al., 1982; Degani et al., 1985; Marques et al., 2014; Stansly and Liu, 1994; Rao et al., 1990).

Azadirachtin caused an egg mortality of 24-26% against another planthopper species (Deepak and Choudhary, 1999). For whiteflies azadirachtin caused an egg mortality around 60% and an nymphal mortality between 28-100% (Kumar et al., 2005; Farnisi et al., 2014; Kumar and Singh, 2014; Kumar and Poehling, 2007; Lawand et al., 1992; Lokender et al., 2016; Silva et al., 2003; Pandya, 2005; Prabhat and Poehling, 2006; Bhavani and Rao, 2013; Bleicher et al., 2007; Flint and Parks, 1989; Uygun et al., 2011; Stansly and Liu, 1994; Jamieson et al., 2010; Parmar et al., 2004; Price et al., 1991).

Spirodiclofen reached an efficacy of 80% in a trial against whitefly nymphs (Vasquez-Martinez et al., 2016). The extensive literature did not show results for spirodiclofen-trials with planthopper in the egg- or nymph-stage.

Trials with kaolinite against nymphs of another leafhopper species showed an efficacy of over 80% and trials against whiteflies resulted in a nymphal mortality of 92% (Mahmoudi et al., 2014; Tubajika et al., 2011).

4.3 Outlook

During the present study a new test unit and test method were developed 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 offered the possibility of a standardized, continuous assessment of the number of hatched AGVL nymphs. The trials revealed that after three applications all test-substances had a reducing effect on the nymphal hatch of AGVL, with etofenprox and spirotetramat being the most promising.

Due to the test design with three applications, the potentially cumulative effect of the test substance residues and the late hatch of the AGVL nymphs the impact of different numbers of applications on the nymphal hatch could not be evaluated in the present study, but 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 17-19/55) against *S. titanus* nymphs than the usually recommended one (BBCH 19/73-81) is effective and fits well between necessary applications against other pests occurring in the vineyards (Fig. 21A) (Rebschutzdienst, 2018; Weinbauverband, 2018). This plant protection measure could provide an additional tool in the control of *S. titanus* and spread of Flavescence dorée.

5 Summary

The overall aim of the present study was to find a plant protection treatment, which effectively reduces the nymphal hatch of the American grapevine leafhopper (*Scaphoideus titanus*) at the start of the growing season.

In the first part of the study an extensive literature search according to the EFSA guidance document on systematic review methodology was carried out to identify candidate pesticides (EFSA, 2010). The information sources searched included for scientific literature the electronic database Ovid, information from pertinent websites (e.g. EPPO) and grey literature (e.g. grower's literature, IOBC-WPRS bulletins). The search was not limited by publication year, but by language. Search terms consisted of the names of potential chemical agents and the insect order Hemiptera or the suborder Homoptera. The search resulted in a total of 954 records, 10.4% of them relevant, which were rated in the EndNote library considering the target organism, its developmental stage and the formal aspects of the record. The extensive literature search resulted in the selection of azadirachtin, etofenprox, aluminium silicate (kaolinite), paraffin oil, spirodiclofen and spirotetramat as test-substances.

In the second part of the present study laboratory trials were carried out to evaluate the effect of the selected pesticides on the nymphal hatch of *S. titanus* under controlled conditions at 24°C, 75- 80% rel. humidity and a photoperiod of L:D 16:8.

As test plant material two-year-old canes of Isabella (*Vitis vinifera* × *Vitis labrusca*) vines from a vineyard with presence of *S. titanus* in the year 2017, were collected.

In the laboratory trials two different types of test units - an adapted test box and a newly developed test cage - were used. The test boxes were only used for the pre-trials and the test cages were used for the pre-trials and the main trial. The pre-trials were conducted to determine the beginning and the duration of the larval hatch of *S. titanus* in the different test units.

The test cage was developed to allow the use of infested canes in a vertical position during application and a standardized assessment of the number of hatched *S. titanus* nymphs over the whole hatching period with yellow sticky traps.

The pre-trials and the main-trial mainly resulted in a nymphal hatching start 4-6 weeks after the trial start and a duration of the nymphal hatching period of *S. titanus* between 3 to 9 weeks.

In order to provide a sufficient number of *S. titanus* individuals per test unit the number of AGVL per weight of two-year old canes was counted and converted to AGVL/kg canes. The amount varied between the trials from a minimum of 801 AGVL/kg canes to a maximum of 1727 AGVL/kg canes.

The six test-stubstances etofenprox (Trebon 30 EC), spirotetramat (Movento 100 SC), paraffin oil (Austriebsspritzmittel 7 E), azadirachtin (NeemAzal-T/S), spirodiclofen (Envidor) and aluminium silicate (pure) resulted in a reduction of the nymphal hatch of *S. titanus* in relation to the control (water) of 100%, 99%, 83%, 72%, 64% and 47% respectively.

Subsequent trials could be carried out to evaluate the impact of lower numbers of applications on the nymphal hatch in the laboratory and could additionally be repeated in the greenhouse or in the vineyard to confirm the efficacy of the tested substances under more field related conditions.

The present study suggests that a plant protection treatment against *S. titanus* nymphs at an earlier application date (BBCH 17-19/55) than the usually recommended one (BBCH 19/73-81) is effective and fits well between necessary treatments against other pests occurring in the vineyards (Fig. 21A) (Rebschutzdienst, 2018; Weinbauverband, 2018). This plant protection measure could provide an additional tool in the control of *S. titanus* and the prevention of the spread of Flavescence dorée.

6 Zusammenfassung

Das Ziel dieser Studie war es ein Pflanzenschutzmittel zu finden, das den Nymphenschlupf der Amerikanischen Rebzikade (*Scaphoideus titanus*) zu Beginn der Vegetationsperiode effektiv unterdrückt.

Im ersten Teil der vorliegenden Studie wurde eine umfassende Literaturrecherche gem. EFSA guidance document on systematic review methodology (EFSA, 2010) durchgeführt, um potentiell geeignete Testsubstanzen zu identifizieren. Die Informationsquellen umfassten wissenschaftliche peer reviewed Publikationen in der elektronischen Datenbank Ovid und Informationen von relevanten Webseiten (z.B. EPPO) sowie weitere wissenschaftliche Literatur (z.B. IOBC-WPRS Bulletins, weinbauliche Informationszeitschriften). Die Literaturrecherche wurde nicht durch das Publikationsjahr, aber durch die Sprache eingegrenzt. Die Suchbegriffe setzen sich aus dem Namen der potenziellen chemischen Testsubstanz und der Insektenordnung Hemiptera oder der Unterordnung Homoptera zusammen. Die Suche resultierte in 954 Literaturzitaten (10,4% davon relevant), die in der EndNote Bibliothek anhand der Übereinstimmung mit dem Zielorganismus, des Entwicklungsstadiums und den formalen Aspekten der Literaturzitate für die weitere Verwendung eingestuft wurden. Die umfassende Literaturrecherche resultierte in der Auswahl von Azadirachtin, Etofenprox, Aluminium-Silikat (Kaolin), Paraffinöl, Spirodiclofen und Spirotetramat als Testsubstanzen für die Laborversuche.

Im zweiten Teil der vorliegenden Studie wurden Laborversuche durchgeführt um den Effekt der ausgewählten Testsubstanzen auf den Nymphenschlupf von *S. titanus* unter kontrollierten Bedingungen bei 24°C, 75- 80% rel. Luftfeuchtigkeit und einer Photoperiode von L:D 16:8 zu untersuchen.

Als Versuchspflanzenmaterial wurden zweijährige Triebe der Sorte Isabella (*Vitis vinifera* × *Vitis labrusca*) von einem im Jahr 2017 mit *S. titanus* befallenen Weingarten gesammelt.

In den Laborversuchen wurden zwei verschiedene Arten von Testeinheiten – eine adaptierte Testbox und ein neuentwickelter Testkäfig – verwendet. Die Testboxen wurden nur für die Vorversuche verwendet, die Testkäfige hingegen für die Vorversuche und den Hauptversuch. Die Vorversuche wurden durchgeführt, um den Beginn und die Dauer des Nymphenschlupfs von *S. titanus* in verschiedenen Testeinheiten festzustellen. Der Testkäfig wurde entwickelt um die Verwendung von befallenem Rebholz in vertikaler Position während der Applikation und eine standardisierte Auswertung der Anzahl von geschlüpften *S. titanus* Nymphen über den gesamten Schlupfzeitraum mit Gelbfallen zu ermöglichen.

Die Vorversuche und der Hauptversuch resultierten in einem Beginn des Nymphenschlupfs innerhalb von 4-6 Wochen nach dem Beginn des Versuchs und einer Dauer des Nymphenschlupfs von *S. titanus* zwischen 3 bis 9 Wochen.
Um eine ausreichende Anzahl von *S. titanus* Individuen pro Testeinheit zu gewährleisten, wurde die Anzahl von AGVL pro Gewichtseinheit von zweijährigen Trieben ausgezählt und in AGVL/kg Triebe umgerechnet. Die Anzahl variierte zwischen den Versuchen von mindestens 801 AGVL/kg Triebe zu maximal 1727 AGVL/kg Triebe.
Die sechs Testsubstanzen Etofenprox (Trebon 30 EC), Spirotetramat (Movento 100 SC), Paraffinöl (Austriebsspritzmittel 7 E), Azadirachtin (NeemAzal-T/S), Spirodiclofen (Envidor) und Aluminium-Silikat (Reinstoff) resultierten in einer Reduktion des Nymphenschlupfs von *S. titanus* im Vergleich zur Kontrolle (Wasser) von 100%, 99%, 83%, 72%, 64% und 47%.

Weiterführende Versuche könnten durchgeführt werden, um den Effekt einer geringeren Anzahl von Applikationen auf den Nymphenschlupf im Labor zu untersuchen und zusätzlich im Glashaus oder im Weingarten um die Wirksamkeit der getesteten Substanzen unter mehr Freiland ähnlichen Bedingungen zu bestätigen.

Die vorliegende Studie lässt darauf schließen, dass eine Pflanzenschutzbehandlung gegen *S. titanus* Nymphen zu einem früheren Applikationsdatum (BBCH 17-19/55) als das üblicherweise empfohlene (BBCH 19/73-81) effektiv ist und sich gut zwischen die nötigen Behandlungen gegen andere im Weingarten auftretenden Schädlinge einfügt (Fig. 21A) (Rebschutzdienst, 2018; Weinbauverband, 2018). Diese Pflanzenschutzmaßnahme könnte ein zusätzliches Hilfsmittel in der Bekämpfung von *S. titanus* und der Prävention der Ausbreitung von Flavescence dorée bereitstellen.

7 References

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8 List of illustrations

9 List of tables

10 Annex

Table 13A: Development of S. titanus larval hatching in the test boxes and test cages from the different pre-trials (see 3.1.2) at evaluation dates when larvae were observed.

DAS	Pre-trial B – test box	Pre-trial C – test box	Pre-trial C – test cages
26	0	1	0
27	0	2	0
28	2	2	0
29	0	1	0
31	5	0	0
32	2	0	0
33	3	0	0
34	0	14	0
35	3	14	0
36	0	7	0
38	13	0	0
39	8	15	0
40	9	0	0
41	2	19	0
42	3	0	0
46	19	16	0
47	7	2	0
48	2	2	0
49	4	0	0
52	14	0	0
53	0	1	0
54	5	7	0
55	0	3	0
56	0	1	0
57	0	6	0
59	12	0	0
60	2	2	0
61	0	2	0
64	0	1	0
68	1	0	0
69	1	0	0
Total	117	118	0
Mean ± Std.	2.34±4.30	2.07±4.56	0

Table 14A: Number of observed S. titanus on yellow sticky traps per cage over the five intervals.

	Number of *S. titanus* nymphs				
Cage	24.05 - 05.06	05.06 - 21.06	21.06 - 02.07	02.07 - 16.07	16.07 - 10.08
1.1 R1	0	13	16	0	0
1.1 R2	0	1	11	2	0
1.1 R3	0	3	5	1	0
1.1 R4	0	1	2	2	0
1.2 R1	0	3	9	1	0
1.2 R2	0	4	5	3	0
1.2 R3	0	1	4	5	0
1.2 R4	0	1	3	0	0
1.3 R1	0	10	6	1	0
1.3 R2	0	2	11	4	1
1.3 R3	0	6	14	4	0
1.3 R4	0	3	12	2	0
2.1 R1	0	0	0	0	0
2.1 R2	0	0	0	0	0
2.1 R3	0	0	0	0	0
2.1 R4	0	0	0	0	0
2.2 R1	0	0	0	0	0
2.2 R2	0	0	0	0	0
2.2 R3	0	0	0	0	0
2.2 R4	0	0	0	0	0
2.3 R1	0	0	0	0	0
2.3 R2	0	0	0	0	0
2.3 R3	0	0	0	0	0
2.3 R4	0	0	0	0	0
3.1 R1	0	6	9	14	0
3.1 R2	0	0	4	6	0
3.1 R3	0	1	3	12	0
3.1 R4	0	0	3	9	0
3.2 R1	0	8	10	6	0
3.2 R2	0	6	10	13	0
3.2 R3	0	5	12	14	0
3.2 R4	0	2	9	8	0
3.3 R1	0	7	16	19	0
3.3 R2	0	3	10	26	0
3.3 R3	0	4	6	11	0
3.3 R4	0	3	7	16	0
4.1 R1	0	3	4	0	0
4.1 R2	0	2	4	2	0

	Number of *S. titanus* nymphs (continued)				
4.1 R3	0	8	6	1	0
4.1 R4	0	4	7	2	0
4.2 R1	0	3	5	3	0
4.2 R2	0	2	0	2	0
4.2 R3	0	7	7	4	0
4.2 R4	0	1	0	1	0
4.3 R1	0	0	4	2	0
4.3 R2	0	1	2	1	0
4.3 R3	0	4	4	3	0
4.3 R4	0	0	4	2	0
5.1 R1	0	1	3	3	0
5.1 R2	0	2	3	2	0
5.1 R3	0	8	16	3	0
5.1 R4	0	0	2	6	0
5.2 R1	0	10	24	6	0
5.2 R2	0	2	10	4	0
5.2 R3	0	7	14	8	0
5.2 R4	0	5	7	4	0
5.3 R1	0	6	16	3	0
5.3 R2	0	3	10	4	0
5.3 R3	0	0	10	8	0
5.3 R4	0	4	7	1	0
6.1 R1	0	0	0	0	0
6.1 R2	0	0	0	0	0
6.1 R3	0	0	0	0	0
6.1 R4	0	0	0	0	0
6.2 R1	0	0	0	1	0
6.2 R2	0	0	0	0	0
6.2 R3	0	0	0	0	0
6.2 R4	0	0	0	0	0
6.3 R1	0	0	0	0	0
6.3 R2	0	0	0	0	0
6.3 R3	0	0	0	0	0
6.3 R4	0	0	0	0	0
7.1 R1	0	8	22	16	0
7.1 R2	0	16	40	33	0
7.1 R3	0	12	35	24	0
7.1 R4	0	3	10	9	0
7.2 R1	0	16	33	32	0
7.2 R2	0	2	23	50	0

	Number of *S. titanus* nymphs (continued)				
7.2 R3	0	2	16	31	0
7.2 R4	0	2	10	14	0
7.3 R1	0	7	35	32	0
7.3 R2	0	1	3	9	0
7.3 R3	0	7	19	29	0
7.3 R4	0	1	6	15	0

Table 15A: Number of *S. titanus* nymphs per kg cane from the different trials.

Trial	Test-unit	Number of *S. titanus* nymphs per kg cane
Pre-trial A	Cage 1	877
	Cage 2	1261
	Cage 3	1507
	Cage 4	1727
	Mean ± Std.	1343 ± 364
Pre-trial B	Box 1	940
Pre-trial C	Box 1	801
Main trial	Cage 1	698
	Cage 2	1271
	Cage 3	733
	Cage 4	374
	Cage 5	1086
	Cage 6	1485
	Cage 7	600
	Cage 8	618
	Cage 9	911
	Cage 10	275
	Cage 11	637
	Cage 12	348
	Mean ± Std.	753 ± 373

The sum of hatched AGVL nymphs during the whole trial duration was calculated for each test unit in the pre-trials and for each test-cage in the control group of the main trial. Those sums were then divided by the weight of the corresponding canes in gramm to obtain the number of AGVL nymphs per gramm cane. The resulting “number of AGVL nymphs per gramm cane” was then converted to “number of AGVL nymphs per kg cane” by multiplying with one thousand.

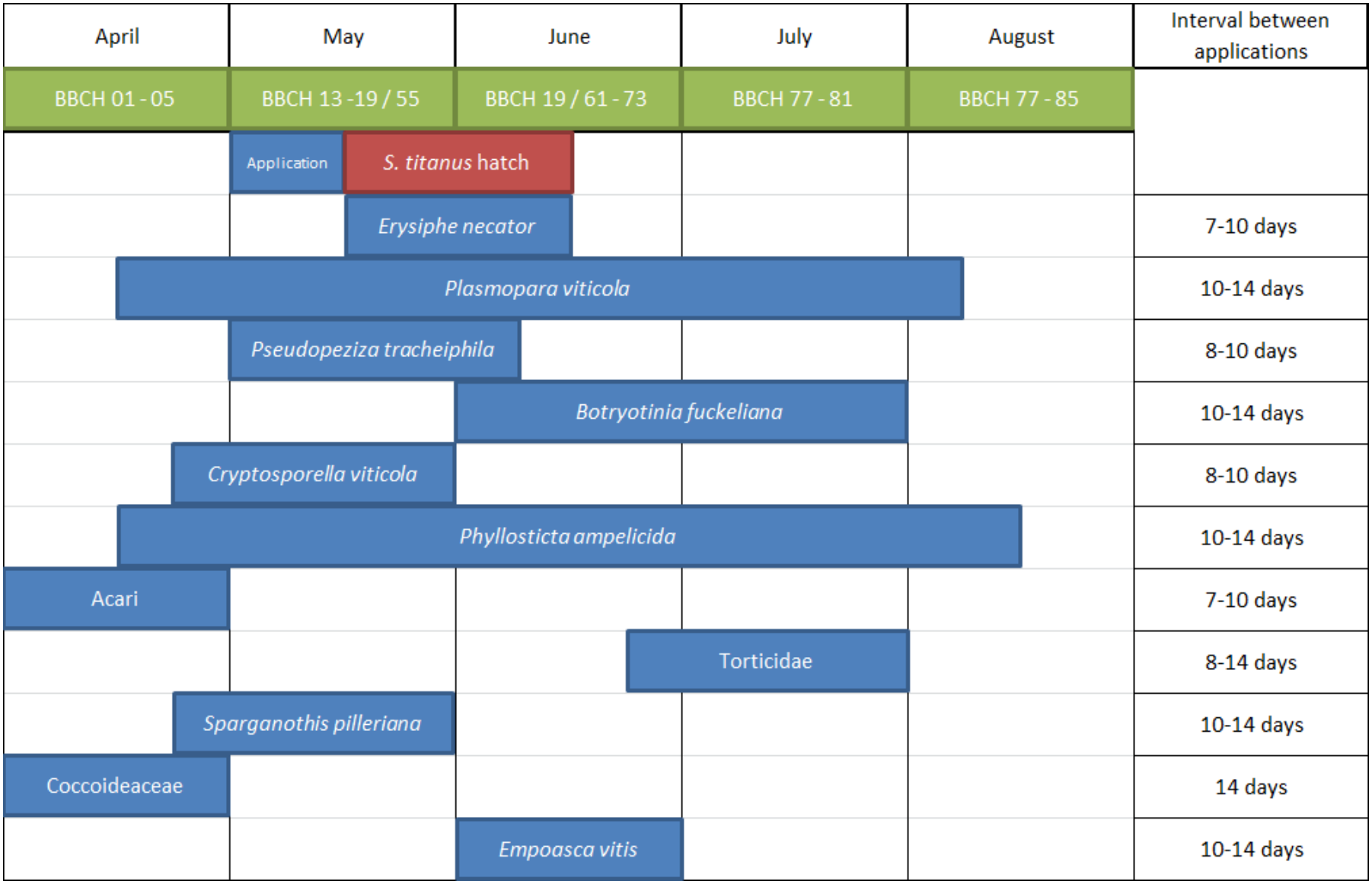

Figure 21A: Recommended application periods (blue bars) and intervals between applications of pesticides against common grapevine pests (Weinbauverband, 2018; Weinbauverband, 2013).