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Grape leafhopper management with emphasis on monitoring techniques, cultural practices and insecticide use

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Abstract

Viticulture is expanding worldwide due to its economic and social relevance. Climate change is allowing grapevine cultivation in new areas of the World representing another factor influencing the future of viticulture. Viticulture relies heavily on pesticides due to the high sensitivity of grapevines to pests and diseases, but pesticide use is a matter of discussion because of their effects on human health and the environment. Therefore, the European Commission has prohibited the use of some active ingredients. Among them some were considered extremely effective against leafhoppers.

Grape leafhoppers outbreaks are still occurring in European vineyards despite insecticide use. *E. vulnerata* is an emerging pest in North-eastern Italy, affecting both organic and conventionally managed vineyards. Insecticides seems to not be very effective on this pest and may be harmful to natural enemies of leafhoppers. The increasing occurrence of *Scaphoideus titanus* in Northern Italy is also linked to Flavescence dorée issues, potentially due to the effectiveness of available insecticides. Relationships between leafhoppers and the natural vegetation surrounding vineyards are also discussed. Finally, the effects of agronomic and cultural practices on leafhopper abundance in vineyards have been poorly explored.

We investigated the relationships between woody vegetation and vineyard colonization by leafhoppers (Typhlocybinae) and their egg parasitoids. A close relationship between *E. vulnerata* and egg parasitoids belonging to the genus *Anagrus* was found close to the woody vegetation during spring.

We tested the effect of kaolin applications on *E. vulnerata* populations and its side effects on predatory mites and leafhopper parasitoids. Kaolin proved to be an effective tool for leafhopper control and its side-effects were acceptable.

The effects of leaf removal and insecticide application were evaluated on grape leafhoppers (*Empoasca vitis*), predatory mites and phytophagous mites. Leaf removal seldom reduced leafhopper and predatory mite populations. Insecticide applications reduced leafhopper and predatory mites density levels.

The effectiveness of monitoring strategies of *S. titanus* is crucial for appropriate control measures against this pest. We investigated this topic in fragmented viticultural areas, frequently involved in FD outbreaks. *Scaphoideus titanus* populations showed spatial aggregation patterns revealed by an accurate monitoring approach. Actual control strategies seemed insufficient to control this pest. The adoption of accurate monitoring techniques coupled with the correct timing of insecticide applications and the choice of effective active ingredients improved the control of this pest.

The potential influence of woody vegetation on Flavescence dorée epidemiology was investigated by studying the spatial distribution of the main vector of phytoplasmas associated to this disease, i.e., *S. titanus.* The aggregation patterns of *S. titanus* were poorly related to woody vegetation. Observations on *Orientus ishidae* suggested an opposite trend. A relation between *S. titanus* distribution and the presence of symptomatic plants was also suggested.

An evaluation of the efficacy of conventional and organic insecticides against *S. titanus* in field and semi-field trials was performed. Pyrethroids obtained good results in controlling this pest compared to neonicotinoids and butenolides. A decline in the pyrethroid residual activity was observed when these insecticides were used at high temperatures. This phenomenon must be taken into account in the context of climate change.

Chapter 1

Introduction and aims

Viticulture is one of the most valuable agricultural activities in many European countries (Salinari *et al*., 2006; Rivera *et al*., 2007; Shahini *et al*., 2010; Priori *et al*., 2014; Fraga *et al*., 2017; Pertot *et al*., 2017; Smits and Gary, 2018; Ostojcic and Udiljak, 2020), and this activity is expected to expand due to climate change (Kenny and Harrison 1992; Neumann and Matzarakis 2011; Gaál *et al*., 2012). Furthermore, after the reform of the Common Wine Market Organization (CMO) (European Commission, 2007), some European countries such as Italy, France, Spain, and Romania have reconverted several rural abandoned lands to viticulture (Zambon *et al*., 2018). Viticulture relies heavily on agrochemicals (Raclot *et al*., 2009; Komárek *et al*., 2010; El Azzi *et al*., 2013; Glavan *et al*., 2013; FAO, 2014; Serpa *et al*., 2017) due to the high sensitivity of grapevines to pests and diseases (Pertot *et al*., 2017). Thus, combining the increase in land use devoted to viticulture and the intense use of pesticides which characterizes viticulture, an increase in the use of agro-chemicals is observed in Europe. However, the use of insecticides is undesired for environmental and human health concerns (European Commission, Farm to Fork Strategy), thus implementing Integrated Pest Management (IPM) strategies is necessary to reduce pesticide use.

Fungicides are the main responsible for this high pesticide use, since they are necessary to reduce the risk of severe diseases (Delière *et al*., 2015; Pertot *et al*., 2017). Apart fungicides, insecticides are the most used pesticides in viticulture (Pertot *et al*., 2017). Insecticides are required to control the huge amount of pests that threaten grapevine yields and to achieve optimal quality and quantity standards. Among insect pests, the most relevant are grapevine moths, such as *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera Tortricidae), which causes direct damage to bunches and favor the development of grey mould (*Botrytis cinerea*) (Fermaud and Giboulot, 1992). Mealybugs (Hemiptera: Pseudococcidae), are responsible for significant direct damages that may affect the quantity and quality of grapes and are vectors of grapevine virus (Petersen and Charles, 1997; Sforza *et al*., 2003; Cabaleiro and Segura 2006; Charles and Froud, 2009; Daane *et al*., 2012). Leafhoppers (Hemiptera Cicadellidae) are common in European vineyards; *Zygina rhamni* Ferrari and *Erasmoneura vulnerata* (Fitch) are both mesophyll feeders (Vidano 1958; Duso *et al*., 2019) while *Empoasca vitis* (Göthe) is a phloem feeder. Leafhoppers can impact transpiration rate, mesophyll conductance and net photosynthesis (Candolfi *et al*., 1993). However, the most economically important pest in European vineyards is *Scaphoideus titanus* Ball, the main vector of the phytoplasmas associated with Flavescence dorée (FD) (Chuche and Thiery 2014). The risk posed by invasive species is also significant (Levine and D'Antonio 2003; Hulme 2009) as demonstrated by the introduction of *Antispila oinophylla* van Nieukerken & Wagner, *Drosophila suzukii* (Matsumura), *Halyomorpha halys* (Stål) and *Popillia japonica* Newman in Italy (van Nieukerken *et al*., 2012; Cini *et al*., 2012; Maistrello *et al*., 2016; Pavesi *et al*., 2014). Among mites, spider mites (Acari: Tetranychidae) can reduce bunch quality by reducing plant's growth and affecting the plant photosynthesis rate (Candolfi *et al*., 1991).

Recently, outbreaks of *S. titanus* and *E. vulnerata* have been detected in organic and conventional farms in North-eastern Italy (Duso *et al*., 2020; Contaldo *et al*., 2021). Among factors affecting these outbreaks the impact of insecticides could be the most significant. FD outbreaks occurred in Veneto in the 1990s but were mitigated by the use of effective insecticides against *S. titanus* (Posenato *et al*., 1996a, b; Sancassani *et al*., 1997; Bertaccini *et al*., 1998; Martini *et al*., 1999). The most commonly used active ingredients in the 1990s belonged to the organophosphates (Girolami *et al*., 2002; Posenato *et al*., 2001; Pavan *et al*., 2005), then neonicotinoids such as thiamethoxam proved to be successful in controlling leafhoppers (Žežlina *et al*., 2013; Matko *et al*., 2013; Zidaric *et al*., 2013). The use of some neonicotinoids like thiamethoxam, clothianidin, and imidacloprid was first restricted in 2013 and then prohibited in 2018 by the EU commission (Jactel *et al*., 2019; Medina *et al*., 2020). Organophosphates were also banned in the EU and used for the last time in viticulture in 2020. Recent FD outbreaks could be related to the low effectiveness of available insecticides against *S. titanus*. The occurrence of resistant strains has been evocated but not yet demonstrated. On the other hand, *E. vulnerata* outbreaks were likely induced by the extensive use of organophosphates (in particular chlorpyriphos-methyl) poorly effective against the pest while dangerous for beneficials (Duso *et al*., 2020; Tirello *et al*., 2021). Considering these issues but also the need to reduce pesticide use in the European Union by 2050, this thesis focused on the following topics:

1) **Spatial and temporal distribution of grapevine leafhoppers and their egg parasitoids in vineyards in North-eastern Italy**

   A complex of leafhopper species (Cicadellidae Typhlocybinae) is reported in many viticultural areas of North-eastern Italy. Recently, the American leafhopper *E. vulnerata* has been recorded with an increased frequency together with the native *E. vitis* and *Z. rhamni*. Outbreaks of the first species have been reported in organic and conventional farms. Chemical control has been the traditional approach to manage grapevine leafhoppers with negative implications for the human health and the environment. Moreover, insecticide resistance has been evocated as a risk. Alternatives to insecticides are requested and biological control remains an option. Mymarid parasitoids (Hymenopetra Mymaridae) are the most effective natural enemies of these leafhoppers but their occurrence seems to be strictly related to the woody vegetation surrounding vineyards. Mymarids overwinter inside the eggs of leafhoppers associated to the natural vegetation, while grape leafhoppers overwinter as adults in the woody vegetation. These relationships have been poorly studied in vineyards colonized by *E. vulnerata*. Ad hoc experiments were performed in four agroecosystems comprising organically managed vineyards. Moreover, specific studies on the preference of parasitoids (*Anagrus* sp.) for *E. vulnerata* or *Z. rhamni* were conducted.

2) **Kaolin as an alternative to insecticides in controlling the grape leafhopper *Erasmoneura vulnerata***

   Viticulture is facing new challenges due to climate change and a social demand for environmentally friendly management techniques. This has compelled winegrowers to implement plant protection practices that limit the environmental impact while maintaining profitability. In this context, the use of kaolin is considered with particular interest, as it provides several advantages: it reduces sunburns and water stress and shows insecticidal properties. Kaolin can reduce insect adhesion to the treated surfaces and cause dehydration of small insects; it acts as a repellent and interferes with insects' oviposition. We planned a number of field trials in order to test the effectiveness of kaolin against *E. vulnerata* and evaluate its side-effects on parasitism rate by Mymarids and predatory mites. Observations were carried out in four vineyards during three growing seasons.

3) **Effect of leaf removal and insecticide applications on population densities of leafhoppers and mites associated to grapevines**

Cultural practices in viticulture are becoming increasingly significant due to their role in mitigating the impact of climate change on grape quality. It has been demonstrated that some cultural practices may also affect the arthropod fauna in vineyards. Therefore, we tested the effects of leaf removal, insecticide applications and their interaction on grapevine leafhoppers (*E. vitis*), as well as on phytophagous and predatory mites. Observations were carried out in two vineyards during three growing seasons.

4) The importance of *Scaphoideus titanus* monitoring for appropriate pest control measures in fragmented viticultural areas

Gaps in *S. titanus* monitoring techniques, vineyard fragmentation and the low effectiveness of the most used insecticides (in organic and conventional viticulture) are suspected to be among the main reasons of recent FD outbreaks in North-eastern Italy, namely in the Veneto region. We conducted an intensive monitoring of *S. titanus* populations in 85 vineyards located in the territory of Conegliano Valdobbiadene Prosecco Superiore DOCG (Treviso district, Veneto region) to analyze the *S. titanus* phenology and identify the most critical situations. At the same time, the spatial and temporal distribution of *S. titanus* was studied in four areas previously reported as very infested and characterized by fragmentation or the presence of organic vineyards. Observations were carried out for two subsequent growing seasons. In the second growing season control strategies were changed and thus we evaluated their effects.

5) Does woody vegetation influence the spatial distribution of *Scaphoideus titanus* in vineyards? Analysis of four case-studies in North-eastern Italy

Past studies have revealed the presence of *S. titanus* in American and European woodlands, in particular when wild grapevines were present. Several plant species (e.g., *Clematis vitalba*, *Alnus glutinosa*, *Alnus incana, Corylus avellana, Salix* spp. and *Ailanthus altissima)*, frequently included in groves and hedgerows surrounding vineyards, are potential reservoirs of phytoplasmas that could contribute to the epidemiology of FD. Moreover, other potential vectors of phytoplasmas associated with FD, e.g., *Dictyophara europaea*, *Orientus ishidae*, and *Phlogotettix cyclops* can colonize these host plants. The proximity of woody vegetation to vineyards has been evocated as an additional factor favoring the recent FD outbreaks. We investigated whether the spatial and temporal distribution of *S. titanus* in vineyards is influenced by the presence of the nearby woody vegetation. Observations were carried out in four agro-ecosystems during 2 subsequent growing seasons.

6) Efficacy of conventional and organic insecticides against *Scaphoideus titanus*: field and semi-field trials

Serious FD issues detected in recent years in Northern Italy could be related to the use of less effective insecticides against *S. titanus* compared to those available in the 1990s (mainly organophosphates) that have been banned for use in the European Union. Trials aimed at evaluating the efficacy of the most used conventional and organic insecticides in the control of *S. titanus* have been performed in field conditions. The residual activity of a number of insecticides was also evaluated in semi-field and field conditions.

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Chapter 2

Spatial and temporal distribution of grapevine leafhoppers and their egg parasitoids in vineyards in North-eastern Italy

Manuscript in preparation as: Spatial and temporal distribution of grapevine leafhoppers and their egg parasitoids in vineyards in North-eastern Italy

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Prazaru Stefan Cristian contributed to data collection, performed statistical analysis, and the manuscript drafting.

Abstract

Vineyards in the Northern Italy can be colonized by three species of leafhoppers i.e., *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari, and *Erasmoneura vulnerata* (Fitch) which represent important pests. Semi-natural areas located in the proximity of cultivated plots can play a role in the distribution of these pests within the vineyard during different phases of the season. Woody vegetation outside vineyards is also important for the occurrence of the natural enemies of leafhoppers and in particular for the Mymarid parasitoids. Here we investigated the effect of woody vegetation on the spatial and temporal distribution of the three leafhoppers and their parasitoids. A sentinel plant experiment was also performed to study the interaction among *E. vulnerata*, *Z rhamni* and their egg parasitoids. Results showed that vineyard colonization by *E. vulnerata* adults is strongly affected by woody vegetation, while this phenomenon was less important for the other leafhoppers. The woody vegetation also influenced the vineyards colonization by the parasitoids *Anagrus atomus*. During summer the edge effect related to woody vegetation was more important for *A. atomus* than for the leafhoppers. Associations between the distribution of *A. atomus* and *E. vulnerata* were observed in the same period. Sentinel plant experiment showed that grapevines infested by both *E. vulnerata* and *Z. rhamni* eggs were attractive towards *A. atomus* adults. The results obtained here can be used in the definition of habitat management practices for sustainable viticultural systems.

Introduction

The viticulture industry is experiencing extensive growth globally, both in hectares and economic value. Along with this increased economic relevance, the demand for more sustainably grown products is also increasing (Stanley Howell, 2001). Therefore, pest management in viticulture must be redefined. For this purpose, the set-up of new IPM programs to control harmful insects has been largely stimulated in the last years (Daane *et al*., 2018).

Leafhoppers are economically important pests, and among them, *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari, and *Erasmoneura vulnerata* (Fitch) are frequently detected in Italian vineyards (Vidano, 1963; Duso *et al*., 2019). The green leafhopper *E. vitis* is a polyphagous species that can infest vineyards and orchards (apple trees and kiwifruit) (Schvester *et al*., 1962; Vidano, 1963; Baggiolini *et al*., 1968; Pavan and Picotti, 1993). *Empoasca vitis* is a phloem-sucking insect that causes symptoms such as vein browning, chromatic alterations and desiccation of leaf margins, and early leaf drop at high population densities (Vidano, 1963; Carle and Moutous, 1965; Baggiolini *et al*., 1968). Symptoms are associated with a reduction in photosynthesis, mesophyll conductance, and transpiration rate (Candolfi *et al*., 1993) and can result in economic damage due to yield losses and reduced sugar content in berries (Moutous and Fos, 1971; Baillod *et al*., 1993; Gremo *et al*., 1994; Pavan *et al*., 2000). The green leafhopper overwinters as an adult on evergreen plants and can complete up to four generations a year in wine-growing areas of southern European (Schvester *et al*., 1962; Vidano, 1963; Pavan *et al*., 1988; Cerutti *et al*., 1988; Mazzoni *et al*., 2001; Böll and Herrmann, 2004). *Zygina rhamni* is typical of Mediterranean regions of Europe (Hoch, 2007) and is considered the autochthonous Italian leafhopper (Vidano, 1958). This species is oligophagous, overwinters in woody vegetation near the vineyards, mostly on *Rubus* spp., on which can complete a generation before migrating towards vineyards, where it completes three generations per year (Pavan, 2001; Mazzoni *et al*., 2008). *Zygina rhamni* feeds on mesophyll cells, causing a progressive reduction in the amount of chlorophyll, which reduces the rate of photosynthesis and can lead to early leaf drop (Vidano, 1963). The American leafhopper *E. vulnerata* was first found in Europe in 2004 (Duso *et al*., 2005). Nymphs and adults feed on mesophyll cells, causing symptoms similar to those of *Z. rhamni* (Duso *et al*., 2020; Tirello *et al*., 2021). *Erasmoneura vulnerata* overwinters as adults on evergreens as well in protected sites, completes three generations per year and has been associated with outbreaks in 2016 in wine-growing areas of the Veneto region (Duso *et al.,* 2019). Currently, it is distributed in Northern Italy and other European countries such as Switzerland, Slovenia, Serbia, and Romania (Seljak, 2011; Chireceanu *et al*., 2020; Rizzoli *et al*., 2020; Sciban and Kosovac, 2020).

These leafhoppers may be associated with significant outbreaks, and pesticide application is the typical approach to reduce their densities. However, in organic viticulture, most insecticides authorized against leafhoppers showed moderate efficacy (Tacoli *et al*., 2017; Duso *et al*., 2020; Tirello *et al*., 2021). The release of generalist predators proved to be a complementary tool (Prazaru *et al*., 2021). The egg parasitoids of the *Anagrus* genus (Hymenoptera: Mymaridae) are the most effective natural enemies of leafhoppers and planthoppers worldwide (Triapitsyn, 2015). In European vineyards, *Anagrus atomus* L., *A. avalae* Soyka and *Stethynium triclavatum* Enock have been frequently recorded (Vidano *et al*., 1988; Cerutti *et al*., 1991; Chiappini *et al*., 1996; Böll and Herrmann, 2004; Pavan and Picotti, 2009). *Anagrus atomus* is suspected to be a complex of species with *A. atomus* and *A. parvus* Soyka *sensu* Viggiani (2014) frequently reported in vineyards (Chiappini *et al*., 1996; Floreani *et al*., 2006; De Leon *et al*., 2008; Zanolli *et al*., 2016). According to Triapitsyn *et al.* (2020), these two species belong to the same taxon, i.e., *Anagrus atomus*.

Anagrus spp. are polyvoltine, reproducing continuously during the growing season (Cooper, 1993; Picotti and Pavan 1993;); the egg parasitism rate of grapevine leafhoppers can exceed 50% (Vidano *et al*., 1988; Cerutti *et al*., 1991; Picotti and Pavan 1993; Pavan *et al*., 1997; Pavan and Picotti 2009).

The fact that mymarid parasitoids overwinter outside the vineyards and the use of pesticides can affect the success of the biological control of leafhoppers. Mymarids are known to overwinter as eggs in the eggs of leafhopper species laid in autumn in the woody vegetation both in North America (Doutt and Nakata, 1973; Kido *et al*. 1984; Williams, 1984; Lowery *et al*. 2007) as well as in Europe (Cerruti *et al*., 1991; Zanolli *et al*., 2011). Vineyard colonization by mymarids is favored by the woody vegetation surrounding them and based on that, conservation biological control strategies have been proposed in North America against *Erythroneura* spp. (Corbett & Rosenheim, 1996; Murphy *et al*., 1998; Williams & Martinson, 2000). The possibility of improving biological control of grapevine leafhoppers by *A. atomus* using habitat management approaches has also been suggested in European vineyards (Cerutti *et al*., 1991; Van Helden and Decante, 2001; Van Helden *et al*., 2003; Boller *et al*., 2004; Ponti *et al*., 2003, 2005; Böll *et al*., 2006; Viggiani *et al.,* 2006; Zanolli *et al*., 2011, 2013; Lo Pinto and Agrò, 2014). The persistence of *Anagrus* spp*.* in vineyard systems is threatened by pesticides and thus a better understanding of vineyard colonization patterns can contribute to promoting conservation biological control in an IPM context (Williams and Gill 1996; Pavan *et al*., 1997; Martinson *et al*. 2001).

Here we investigated the positive effect of semi-natural habitats constituted by woody vegetation surrounding vineyards in a multiple leafhopper pests' context. We performed experiments aimed at testing the following two hypotheses: (i) the surrounding woody vegetation influence the spatial and temporal distribution of mymarids in vineyards; (ii) the distribution of mymarids is associated with that of adult leafhoppers (and their oviposition).

An additional experiment investigated the relationships between the American *E. vulnerata* and the native egg parasitoids. Previous investigations showed that *E. vulnerata* eggs could be parasitized by *Anagrus* spp. in Italian vineyards (Duso *et al*., 2020). In these systems, *E. vulnerata* coexists with native leafhoppers and probably shares the same egg parasitoids. The preference of European Mymarids for native or American leafhoppers has not been explored. Therefore, sentinel potted grapevines previously infested by *E. vulnerata* and *Z. rhamni* were exposed in vineyards to investigate their attractiveness towards mymarids.

Materials and Methods

Spatial and temporal distribution of leafhoppers and their parasitoids

Four organic vineyards located in Northern Italy (Veneto region) and colonized by the above mentioned leafhoppers were selected for this study in the growing season of 2021. Two vineyards were located in Vicenza province (municipalities: Lonigo 45°23'17''N, 11°23'17''E and Gambellara 45°27'39''N, 11°20'29''E) and two in Verona province (municipalities: Soave 45°25'19''N, 11°14'52''E and Fittà 45°27'14"N, 11°15'9"E). These vineyards comprised the cultivar Garganega and were trained according to Pergola veronese system in Gambellara and Soave, and Guyot system in Lonigo and Fittà. Vineyards were rectangular, and woody vegetation was present on the south border of the fields. The woody vegetation consisted of linear hedgerows or small groves comprising mainly of hazelnut (*Corylus* spp.), elm (*Ulmus* spp.), ailanthus (*Ailanthus* spp.), ash (*Fraxinus* spp.), ivy (*Hedera* spp.) and brambles (*Rubus* spp.). In June, two mandatory insecticide applications (pyrethrins) against *S. titanus* were performed in these vineyards.

In each vineyard, samplings were performed following a regular grid design based on a Cartesian plane where the x-axis corresponded to the side bordering the woody vegetation, and the y-axis indicated the distance from the vegetation. Sampling points were placed on a regular grid of about 15 x 15 meters. The number of sampling points depended on the different sizes of vineyards as follow:

1) Lonigo: 42 sampling points, 7 coordinates on the x-axis and 6 on the y-axis.
2) Soave: 28 sampling points, 7 coordinates on the x-axis and 4 on the y-axis.
3) Fittà: 25 sampling points, 5 coordinates on the x-axis and 5 on the y-axis.
4) Gambellara: 36 sampling points, 6 coordinates on the x-axis and 6 on the y-axis.

Samplings were performed in all vineyards using yellow sticky traps (20 x 12.3 cm, Super Color, Serbios, Rovigo, Italy) placed on the selected sampling points. Samplings started on the 19th of April and ended at the end of September, and traps were replaced every 14-15 days. They were observed under a stereomicroscope (Stemi 508, Carl Zeiss Microscopy GmbH, Jena, Germany), and all the adults of *E. vulnerata, Z. rhamni,* and *E. vitis* were counted. Mymarids were also sampled by counting the adult captures on traps used for leafhoppers. Moreover, 50 leaves from each sampling point were collected, and leafhopper nymphs were counted.

Exposure of sentinel potted grapevines to mymarids

The relationships between *E. vulnerata* and *Z. rhamni* and their mymarid parasitoids were studied in an experiment performed in the four vineyards in the growing season of 2021. The experiment was carried out using sentinel potted grapevines where eggs of the two leafhopper species were present at the time of grapevine exposure in the field. *Erasmoneura vulnerata* and *Z. rhamni* were reared on Garganega potted grapevines (20 cm diameter, filled with 50% topsoil and 50% brown peat), inside two different insect-proof screenhouses located in the facilities of the University of Padova (DAFNAE). From July 13 to October 4, 48 grapevines were taken from both *E. vulnerata* and *Z. rhamni* screen-houses every two weeks, checked to exclude the presence of leafhopper motile stages and then moved into the four vineyards where they were distributed regularly. Additional 48 grapevines per screen-house were checked to detect parasitism using emergence cages (below described). Before their exposure, grapevines were caged with tulle fabric in order to avoid oviposition by leafhoppers occurring in the vineyards. However, the tulle mesh was sufficient to allow *Anagrus* spp. adults to enter inside. After two weeks of permanence in vineyards, the grapevines were cut just above the ground and transferred to the DAFNAE laboratories where shoots were placed inside an emergence cage, consisting of a dark 18 L plastic tank. The screw cap of the tank was replaced with a transparent plastic tube where the emerged parasitoids were attracted by light. The plastic tubes were replaced daily, and the adult parasitoids were collected, mounted on slides, and identified at the species level under a compound microscope. Subsequently, the total DNA of *Anagrus* spp. parasitoids was extracted using the salting-out protocol (Patwary *et al*., 1994). Barcoding analysis based on PCR and Sanger sequencing of cytochrome c oxidase subunit 1 (COI) fragment was performed as described by Martinez-Sañudo *et al*. (2019). Furthermore, we verified the occurrence of leafhoppers in the tubes.

Statistical analyses

Data on leafhoppers and their parasitoids were analyzed to depict spatio-temporal patterns in their distribution within the vineyards. Two different approaches were used. Firstly, we evaluated the effect of distance from the woody-vegetation (i.e., 0 m, 15 m, 30 m and 45 m). Secondly, we investigated spatial patterns in the leafhopper and their parasitoid distribution in the vineyards using a spatial explicit modeling approach based on SADIE (Perry *et al*., 1999).

Spatial analysis: first approach

Data collected during the three periods were analyzed with a repeated measures linear mixed model with the MIXED procedure of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC, USA). Three different periods were considered: spring (from mid-April to early June); early to mid-summer (from mid-June to early August); late-summer (from mid-August to late-September), and the abundance of leafhoppers on leaves (nymphs) or traps (adults) were considered response variables in separate analyses. In both analyses, the distance from the woody vegetation (i.e., 0 m, 15 m, 30 m, and 45 m), the date of sampling, and their interaction were considered sources of variation and tested with an F test ($\alpha = 0.05$). Comparisons between distances on each date were performed using a pairwise *t*-test ($\alpha = 0.05$), Bonferroni-adjusted on the least-square means. The degrees of freedom were estimated using the Kenward–Roger method, which can calculate non-integer values for error terms. The vineyard was considered as a random effect term in the model. Before the analysis, data were checked for model assumptions and transformed to log (n + 1). Untransformed data are shown in the figures. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods.

Spatial analysis: second approach

We applied SADIE red-blue analysis (Perry *et al*., 1999) to determine spatial patterns in the distribution of leafhoppers and their parasitoids within the selected vineyards. This methodology identifies areas with relatively high-density counts (patches) or relatively small or zero counts (gaps) and calculates for each sampling point the indexes of clustering (vi; vj) that measure the local contribution to either patch or gap, respectively. For each variable (trap captures and nymph on leaves) clustering significance ($\alpha = 0.05$) was provided by comparing the vi and vj mean values with their corresponding values under the null hypothesis (Perry *et al*., 1999). A two-dimensional map showing the spatial distribution of local clustering indexes (vi; vj) for each variable was generated using linear kriging with SURFER (Golden Software 191 Inc., CO). Datasets from the red-blue analysis were used to evaluate the similarity among the spatial patterns between leafhoppers and Mymarids and among the three leafhoppers species. A specific algorithm was used to derive an overall index of spatial association (X_k), which significance (Px) was established through a randomization test (Perry and Dixon, 2002). This test determines whether the spatial patterns of two variables are associated (Px < 0.025), unassociated (0.025 ≤ Px ≤ 0.975) or dissociated (Px > 0.975). The potential outcomes can be: both gaps or both patches association, or one patch, one gap, dissociation (Perry, 1998). Regarding relationships between leafhoppers and Mymarids we considered only significantly positive associations occurring in the same sampling period (day).

Exposure of sentinel grapevines to Mymarids

The attractiveness of *E. vulnerata* or *Z. rhamni* eggs towards mymarids was analyzed with a repeated measures linear mixed model with the MIXED procedure of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC, USA). We considered the number of parasitoids collected as the response variable. In this analysis, leafhopper species, the date of sampling, and their interaction were considered sources of variation and tested with an F test (α = 0.05). Comparisons on each date were performed using a *t*-test (α = 0.05) on the least-square means. The degrees of freedom were estimated using the Kenward–Roger method, which can calculate non-integer values for error terms. The vineyard was considered as a random effect term in the model. Before the analysis, data were checked for model assumptions and transformed to log (n + 1). Untransformed data are shown in the figures. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods.

Results

Leafhopper and *Anagrus* sp. adults at different distances from the woody vegetation

Empoasca vitis, *Z. rhamni* and *E. vulnerata* adults were regularly detected on traps during the 2021 growing season (Figure 1). Data suggest that *E. vulnerata* dominated over *E. vitis* and *Z. rhamni* in summer. *Anagrus* sp. adults were also detected during the entire season, and they were more abundant in late summer (Figure 1).

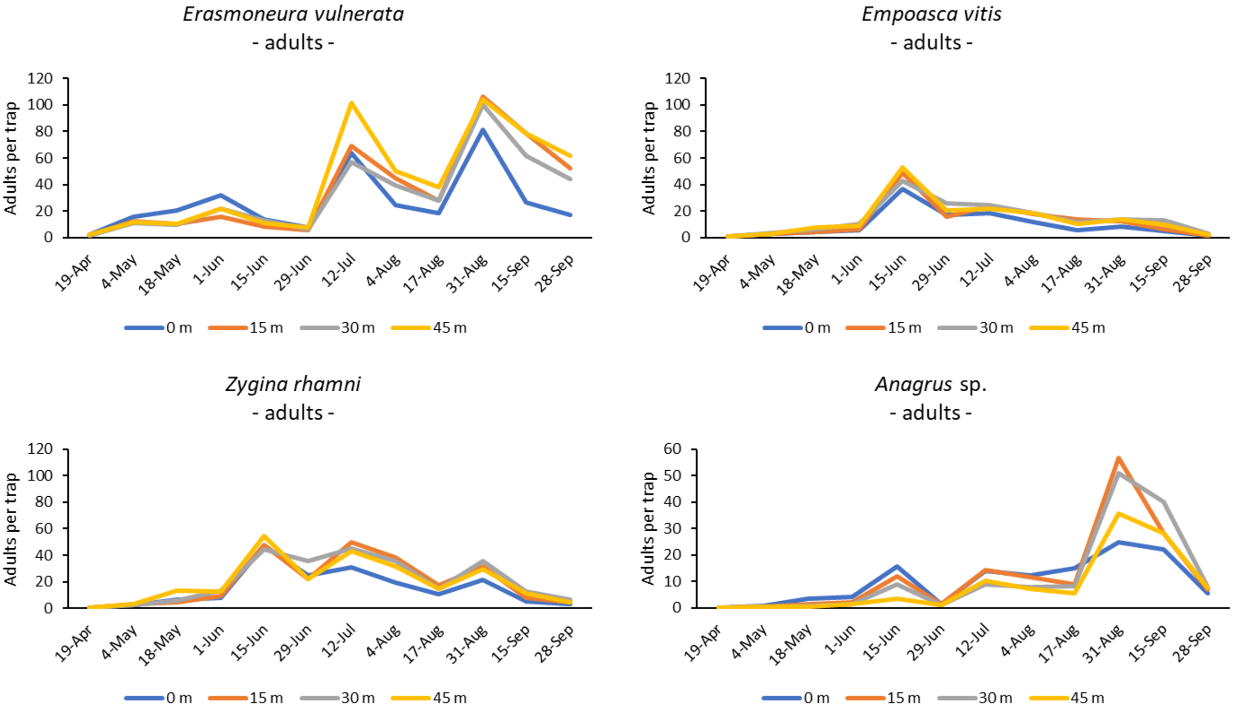

Figure 1 Adult captures of *E. vulnerata, E. vitis, Z. rhamni* and *Anagrus* sp. from April to the end of September 2021 on yellow sticky traps.

During spring, there were more adults of *E. vulnerata* close to woody vegetation than inside the vineyards (F= 3.78; df = 3,294; P = 0.011; Figure 2a), while there were no differences for *E. vitis* and *Z. rhamni* (Figures 2b, 2c). The effect of time was significant for the three leafhopper species (F= 24.94; df = 3,294; P < 0.0001; F = 14.9; df = 3,258; P < 0.0001; F = 21.47; df = 3,239; P < 0.0001; respectively for *E. vulnerata, E. vitis* and *Z. rhamni*). *Anagrus* sp. was more abundant near the woody vegetation (F = 8.47; df = 3,102; P < 0.0001; Figure 2d). The effect of the time was also significant (F = 6.49; df = 3,102; P = 0.0005).

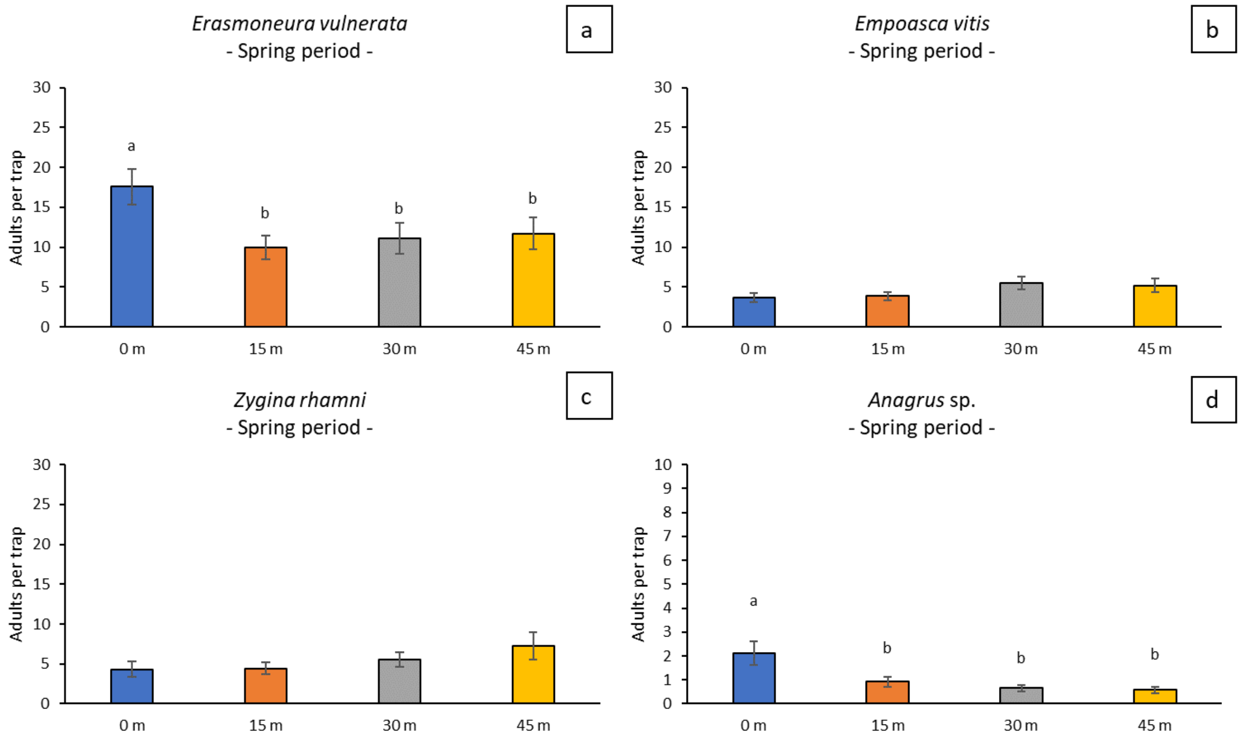

Figure 2 Mean number (± SE) of leafhopper adults and their parasitoids captured on traps during spring (four dates) at different distances from the woody vegetation, different letters indicate significant differences according to *t-test* on the least square means with Bonferroni correction (α = 0.05).

From early to mid-summer, *Z. rhamni* was more abundant inside the vineyard than close to woody vegetation, but at intermediate distances (F = 3; df = 3,377; P = 0.031; Figure 5c) while there were no differences for *E. vitis* and *E. vulnerata*; Figures 3a, 3b, 3c). The effect of time was significant for the three leafhopper species (F = 54.49; df = 3,325; P < 0.0001; F = 20.94; df = 3,374; P < 0.0001; F = 9.18; df = 3,377; P < 0.0001; respectively for *E. vulnerata, E. vitis, Z. rhamni*. *Anagrus* sp. was still abundant close to the woody vegetation (F = 4.40; df = 3,317; P = 0.005; Figure 5d). The effect of time was also significant (F = 45.57; df = 3, 317; P < 0.0001).

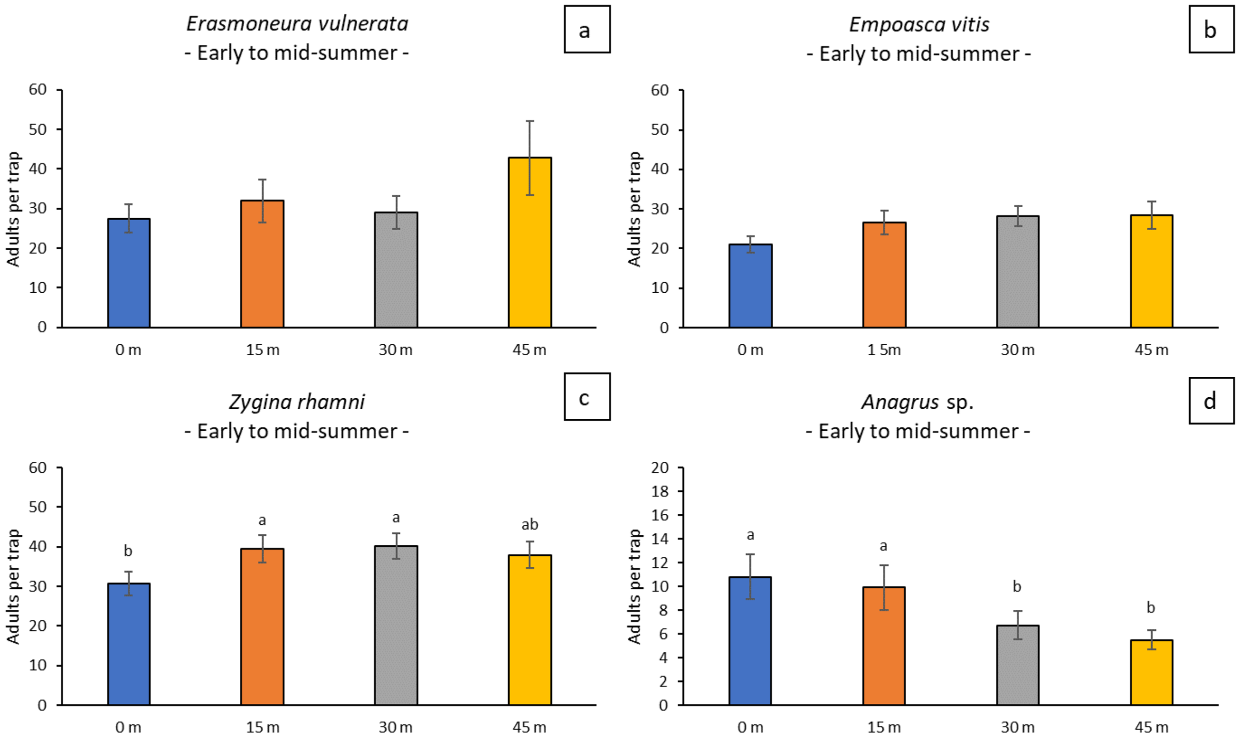

Figure 3 Mean number (± SE) of leafhopper adults and their parasitoids captured on traps during early to mid-summer (four dates) at different distances from the woody vegetation, different letters indicate significant differences according to *t-test* on the least square means with Bonferroni correction (α = 0.05).

In late summer, the three leafhoppers were more abundant in the vineyards than close to the woody vegetation (*E. vulnerata*: F = 2.81; df = 3,35; P= 0.039; *E. vitis*: F = 4.22; df = 3,327; P = 0.006; *Z. rhamni:* F = 3.94; df= 3,345; P= 0.009; Figures 7a, 7b, 7c). The effect of time was significant (F = 10.66; df = 3,350; P < 0.0001; F = 25.84; df = 3,327; P < 0.0001; F= 33.55; df = 3,345; P < 0.0001; respectively for *E. vulnerata, E. vitis,* and *Z. rhamni*: Figure 4a, 4b, 4c). *Anagrus* sp. was also more abundant inside the vineyard(F = 4.40; df = 3,317; P = 0.039; Figure 4d). The effect of time on *Anagrus* sp. abundance was significant (F = 21.47; df = 3, 317; P < 0.0001, Figure 4d).

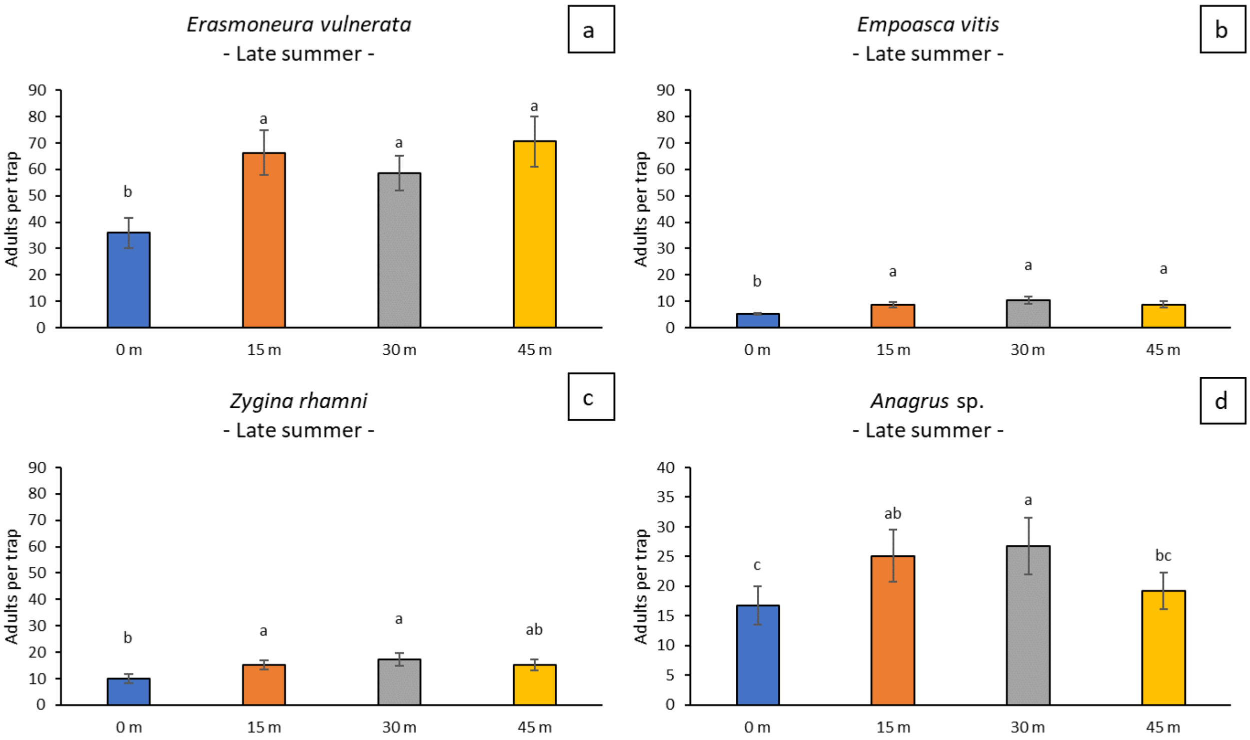

Figure 4 Mean number (± SE) of leafhopper adults and their parasitoids captured on traps during late summer (four dates) at different distances from the woody vegetation, different letters indicate significant differences according to *t-test* on the least square means with Bonferroni correction (α = 0.05).

Leafhopper nymph abundance at different distances from the woody vegetation

Erasmoneura vulnerata nymphs clearly dominated over *Z. rhamni* and *E. vitis* (Figure 5). In spring (15th of June), *E. vulnerata* nymphs appeared to be higher close to woody vegetation, but this result was not supported by the statistical analysis (F = 1.24, df = 3, 24, P = 0.318). In summer (July-September), *E. vulnerata* spread inside the vineyards, and no differences in terms of nymph abundance at the different distances were found (F = 0.93, df = 3, 120, P = 0.435); the effect of time was significant (F= 14.66, df= 3, 144, P< 0.0001). Regarding *E. vitis* and *Z. rhamni* nymphs, no effects of the distance from the woody vegetation were observed neither in spring (respectively, F = 0.60, df = 3, 789, P = 0.615; F = 0.12, df = 3, 789, P = 0.947) nor in summer (respectively, F = 0.84, df = 3, 120, P = 0.475; F= 0.14, df = 3, 120, P = 0.938) (Figure 5).

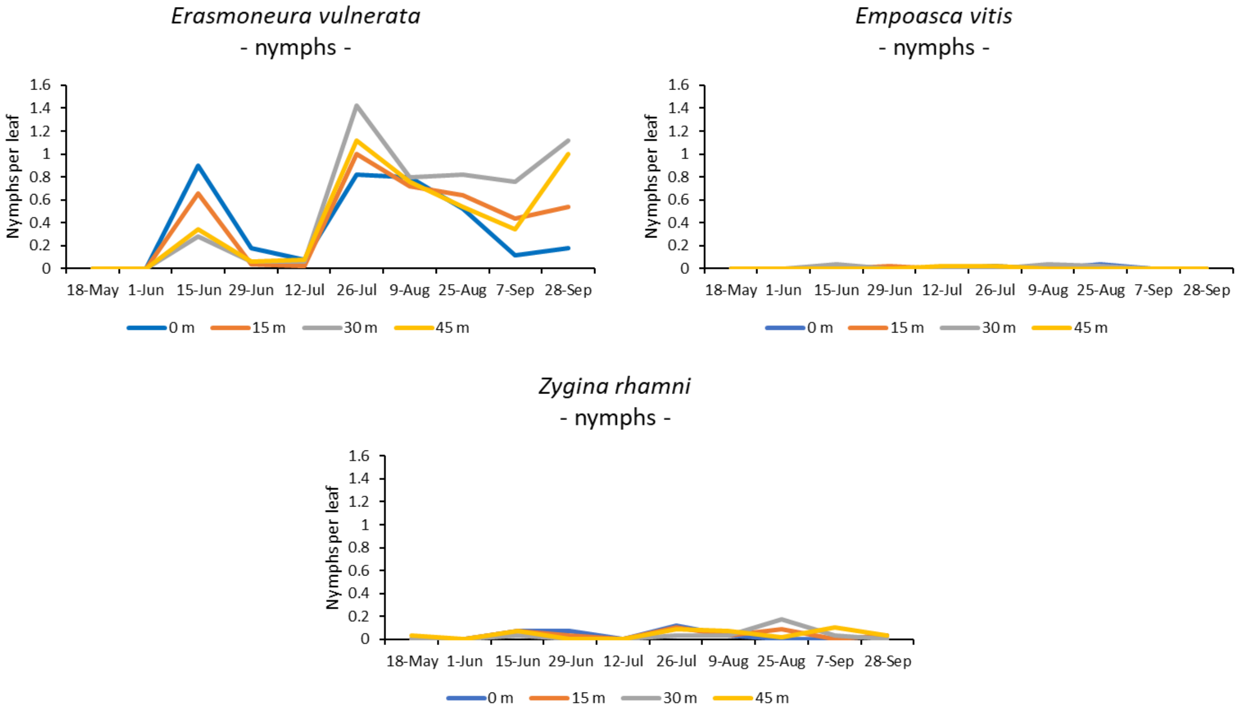

Figure 5 Nymph abundance of the three leafhopper species at different distances from woody vegetation during the growing season.

Spatio-temporal distribution of leafhoppers and *Anagrus* sp. in vineyards

Soave

Erasmoneura vulnerata

The distribution of *E. vulnerata* was aggregated on different sampling dates: 19th April, 4th May, 18th May, 1st June, 17th August, and 28th September (Table 1). In spring, *E. vulnerata* patches were found on the west border and close to the woody vegetation, then patches were localized in various parts of the vineyard (Figure 6).

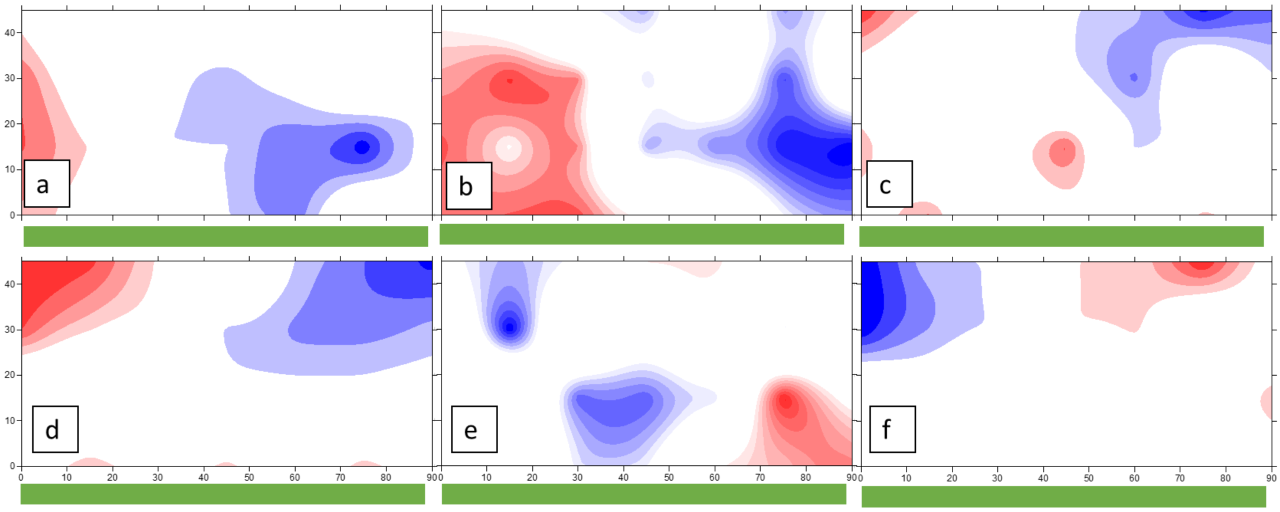

Figure 6 Spatial distribution of *E. vulnerata* adults on six sampling dates: 19th April (a), 4th May (b), 18th May (c), 1st June (d), 17th August (e), and 28th September (f). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 1 Results of the SADIE analysis for *E. vulnerata* in Soave vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
19-apr	-1.535	1.571	0.0203	0.0159
4-May	-2.04	1.765	0.0005	0.0022
18-May	-1.675	1.509	0.0065	0.0209
1-June	-1.554	1.773	0.0156	0.0034
17-August	-1.506	1.58	0.0193	0.0471
28-September	-1.518	1.563	0.0184	0.0468

Empoasca vitis

Empoasca vitis distribution showed significant aggregation on different dates: 18thJune, 29th June, 12th July, 4th August, 17th August, and 31st August (Table 2). No spatial patterns emerged during spring, later patches were frequently found on the North-eastern border of the vineyard, while gaps on the west border and close to wood margins (Figure 7).

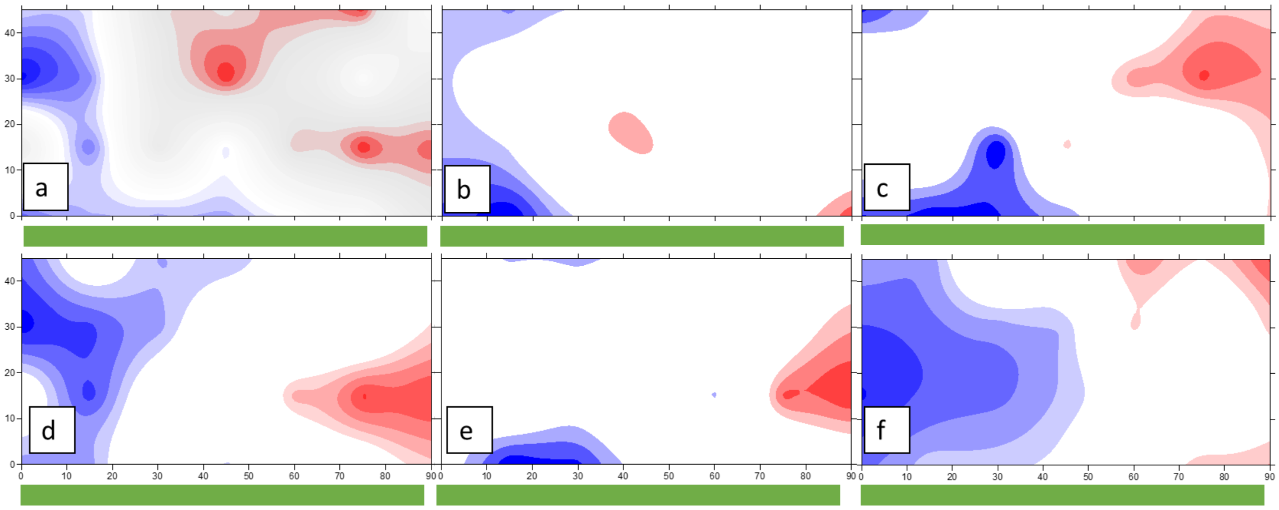

Figure 7 Spatial distribution of *E. vitis* adults on six sampling dates: 18th June (a), 29th June (b), 12th July (c), 4th August (d), 17th August (e), and 31st August (f). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 2 Results of the spatial pattern analysis for *E. vitis* in Soave vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-June	-1.664	1.57	0.0085	0.0085
29-June	-1.508	1.514	0.0085	0.0171
12-July	-1.704	1.904	0.0001	0.0001
04-August	-1.94	1.527	0.0085	0.0001
17-August	-1.503	1.519	0.0171	0.0171
31-August	-1.998	2.182	0.0001	0.0001

Zygina rhamni

The distribution of *Z. rhamni* exhibited aggregation on different dates: 18th June, 29th June, 12th July, 4th August, 17th August, 31st August and, 28th September (Table 3). There were no spatial patterns during the vineyard colonization period. In summer, patches were located on the east border and in the center of the vineyard while gaps on the west and south-west borders (Figure 8).

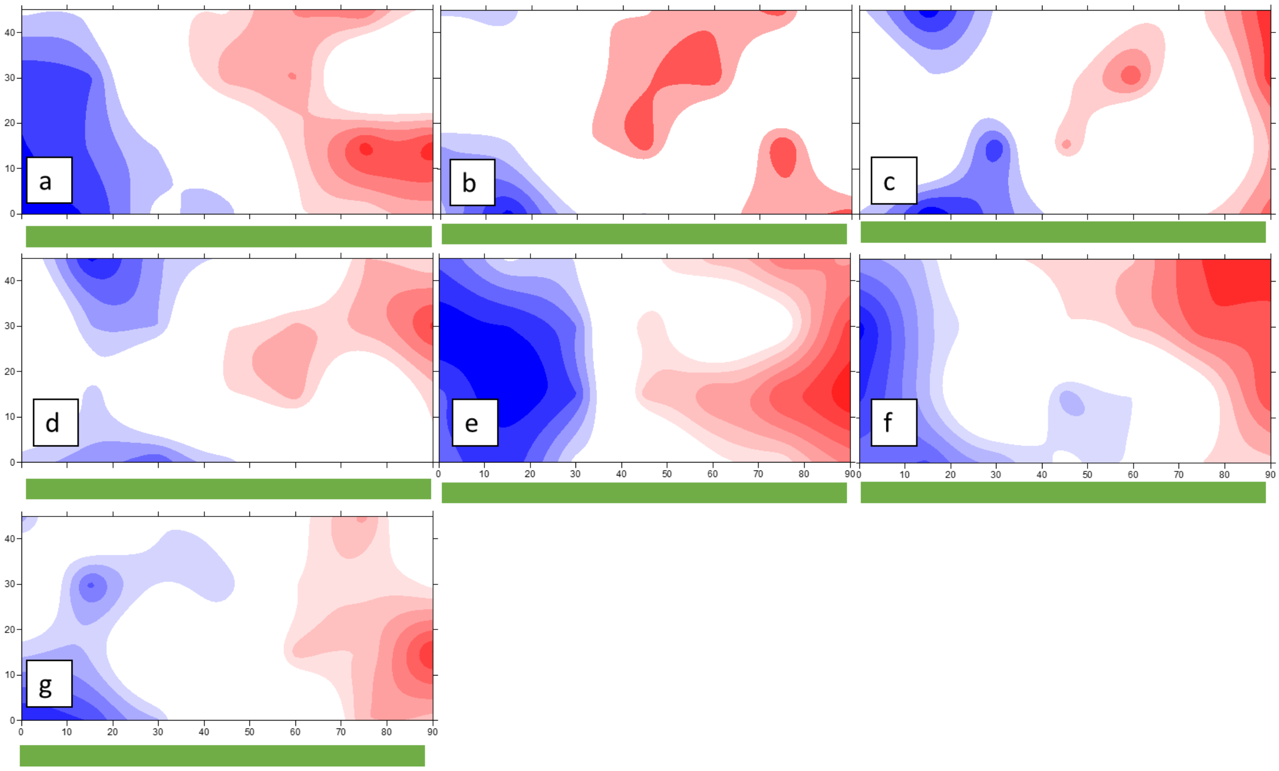

Figure 8 Spatial distribution of *Z. rhamni* adults on seven sampling dates: 18th June (a), 29th June (b), 12th July (c), 4th August (d), 17th August (e), 31st August (f), and 28th September. Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 3 Results of the spatial pattern analysis for *Z. rhamni* in Soave vineyard showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test ($\alpha = 0.05$)

Date	vj	vi	Pvj	Pvi
18-June	-1.908	2.096	0.0003	0.0002
29-June	-1.503	1.663	0.0198	0.0049
12-July	-1.784	1.689	0.002	0.001
04-August	-1.829	1.697	0.0025	0.0045
17-August	-2.482	2.624	0.0001	0.0001
31-August	-2.473	2.243	0.0001	0.0002
28-September	-2.003	1.878	0.0002	0.001

***Anagrus* sp.**

Anagrus sp. distribution was aggregated only on the 1st of June. A patch (vi= 1.972, Pvi= 0.0005) was extended on the west side of the vineyard, while gaps (vj= -2.068, Pvj= 0.0005) were observed on the opposite side (Figure 9).

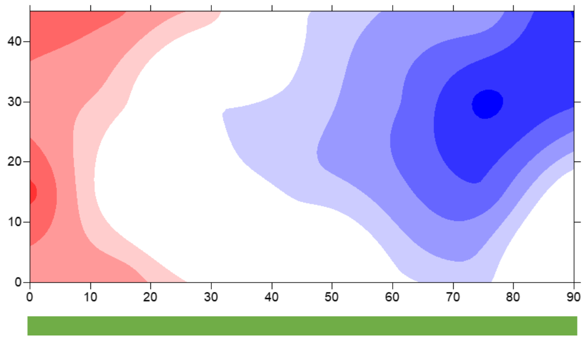

Figure 9 Spatial distribution of *Anagrus* sp. on the 1st June. Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Fittà

Erasmoneura vulnerata

The distribution of *Erasmoneura vulnerata* was aggregated on different dates: 4th May, 18th May, 1st June, 31st August, and 29th September (Table 4). In spring, leafhopper patches were close to wood margins and gaps on the opposite side (Figure 10). A reverse situation occurred in late summer.

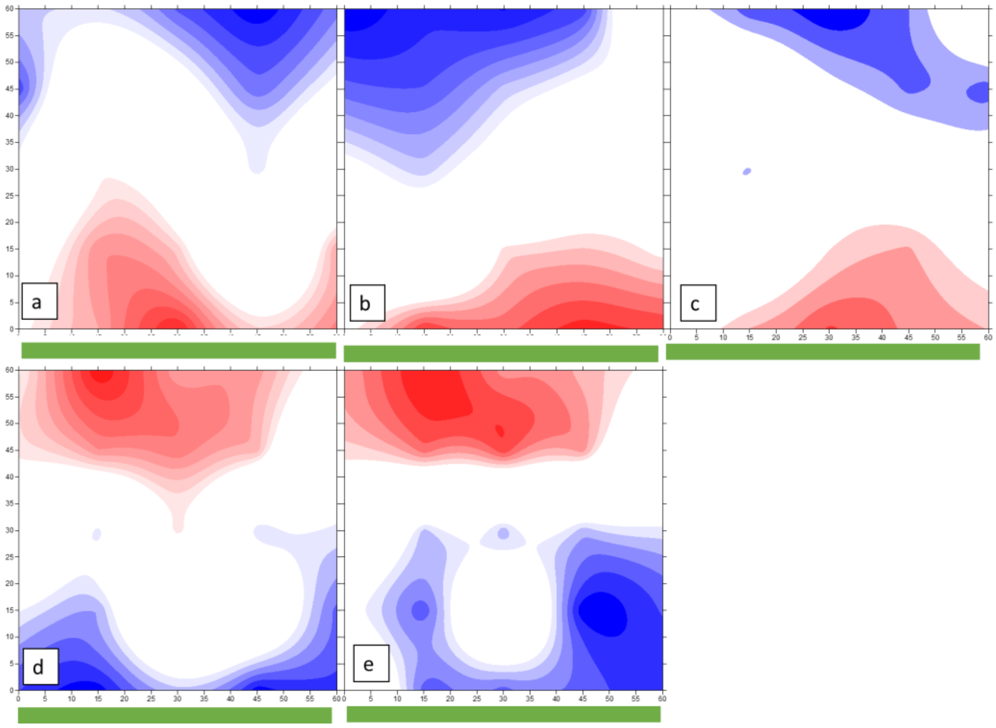

Figure 10 Spatial distribution of *E. vulnerata* adults on five sampling dates: 4th May (a), 18th May (b), 1st June (c), 31st August (d), and 28th September (e). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 4 Results of the spatial pattern analysis for *E. vulnerata* in Fittà vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-June	-1.908	2.096	0.0003	0.0002
29-June	-1.503	1.663	0.0198	0.0049
12-July	-1.784	1.689	0.002	0.001
04-August	-1.829	1.697	0.0025	0.0045
17-August	-2.482	2.624	0.0001	0.0001
31-August	-2.473	2.243	0.0001	0.0002
28-September	-2.003	1.878	0.0002	0.001

Empoasca vitis

Empoasca vitis distribution exhibited significant aggregation on three dates: 15th June, 12th July, and 28th September (Table 5). These days, clustering into patches and gaps was significant, except for the 28th of September when no clustering into gaps area was found (Table 5). Patches were localized on the east border of the vineyard (Figure 11).

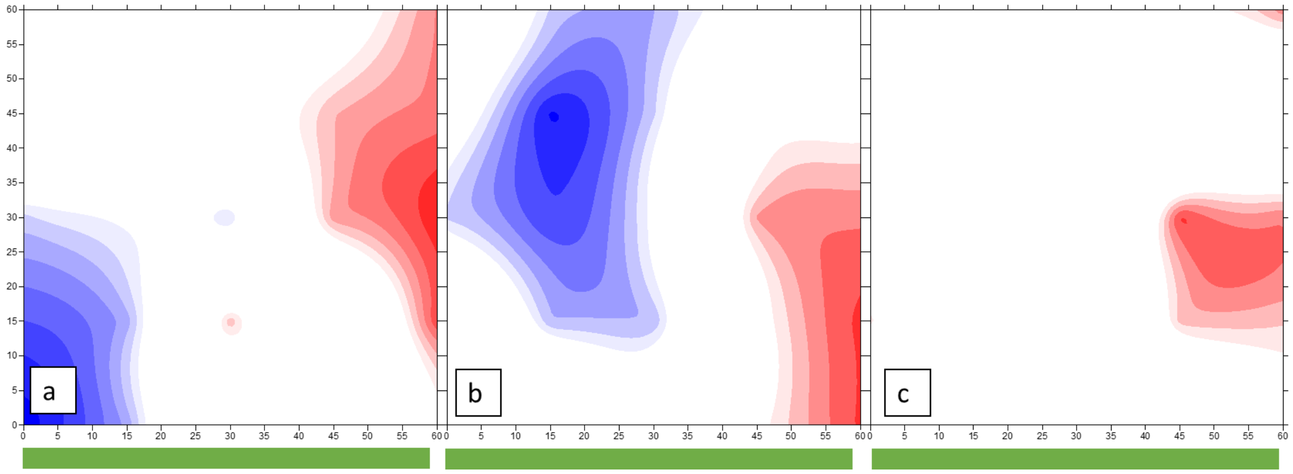

Figure 11 Spatial distribution of *E. vitis* adults on three sampling dates: 15th June (a), 12th of July (b), and 28th of September (c). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 5 Results of the spatial pattern analysis for *E. vitis* in Fittà vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-June	-1.542	1.536	0.004	0.0059
12-July	-1.527	1.505	0.0042	0.0313
28-September	-1.297	1.501	0.0431	0.0064

Zygina rhamni

Zygina rhamni exhibited no significant patterns in its distribution, neither clustering into gaps nor patches.

Anagrus sp.

Anagrus sp. showed significant aggregation in the distribution on different dates: 18th May, 1st June, 15th June, and 28th June, with significant clustering into patches and gaps (Table 6). In spring, patches were present near the woody vegetation, then moved inside the vineyards (Figure 12).

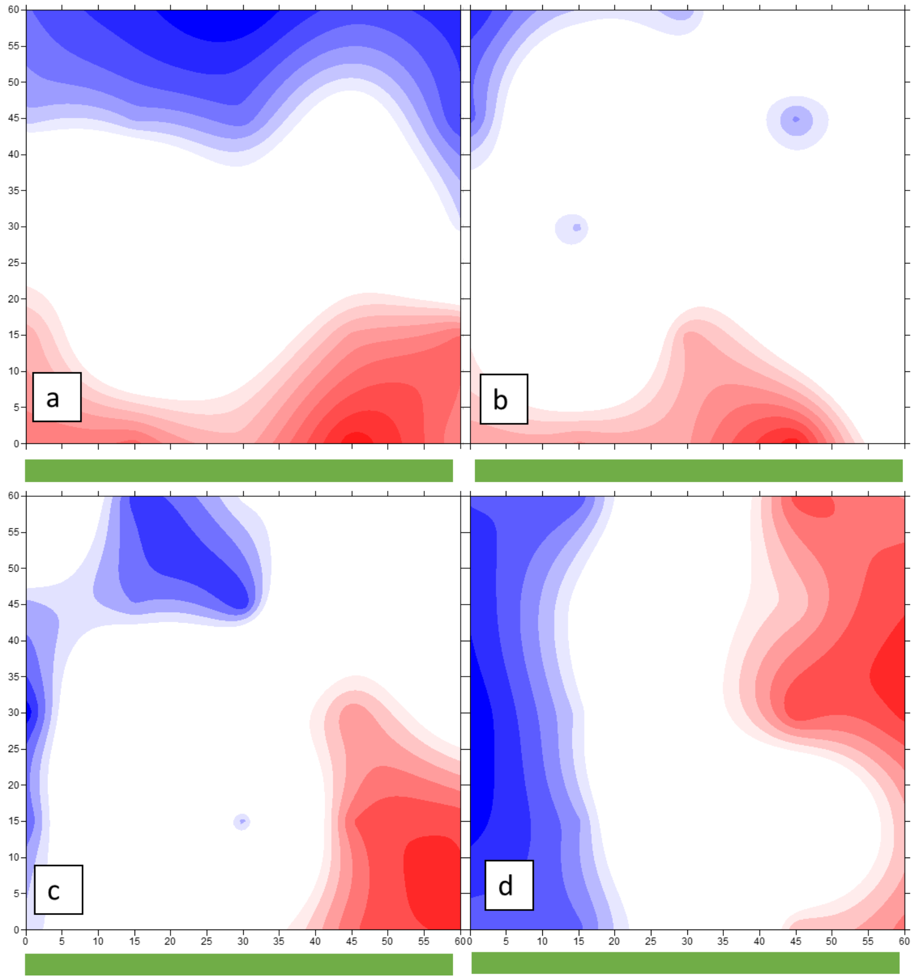

Figure 12 Spatial distribution of *Anagrus* sp. on four sampling dates: 18th May (a), 1st June (b), 15th June (c), and 28th June (d). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 6 Results of the spatial pattern analysis for *Anagrus* sp. in Fittà vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-May	-1.991	1.691	0.0001	0.0001
1-June	-1.549	1.547	0.0275	0.0092
18-June	-1.621	1.538	0.0018	0.0117
28-September	-1.83	1.635	0.0001	0.0003

Gambellara

Erasmoneura vulnerata

Erasmoneura vulnerata distribution exhibited a significant aggregation only on the 4th of May (Pa=0.0002 Ia=1.934), with both clustering into patches (vi=1.862, Pvi <0.0001) and into gaps (vj=-1.936, Pvj<0.0001) were significant. On this sampling date, patches of *E. vulnerata* were localized on the west border of the vineyard, in particular close to wood margins, while gaps were located along the opposite border (Figure 13).

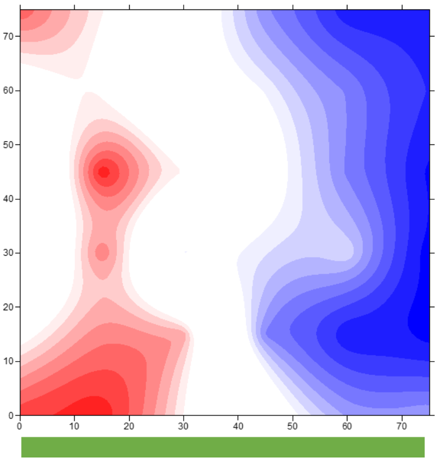

Figure 13 Spatial distribution of *E. vulnerata* adults on the 4th May. Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Empoasca vitis

The distribution of *E. vitis* was aggregated on three dates: 4th May, 31st August, and 28th September (Table 7). Patches were localized on the west border of the vineyard, gaps on the east border of the vineyard (Figure 14). Patches were close to wood margins in the first two dates.

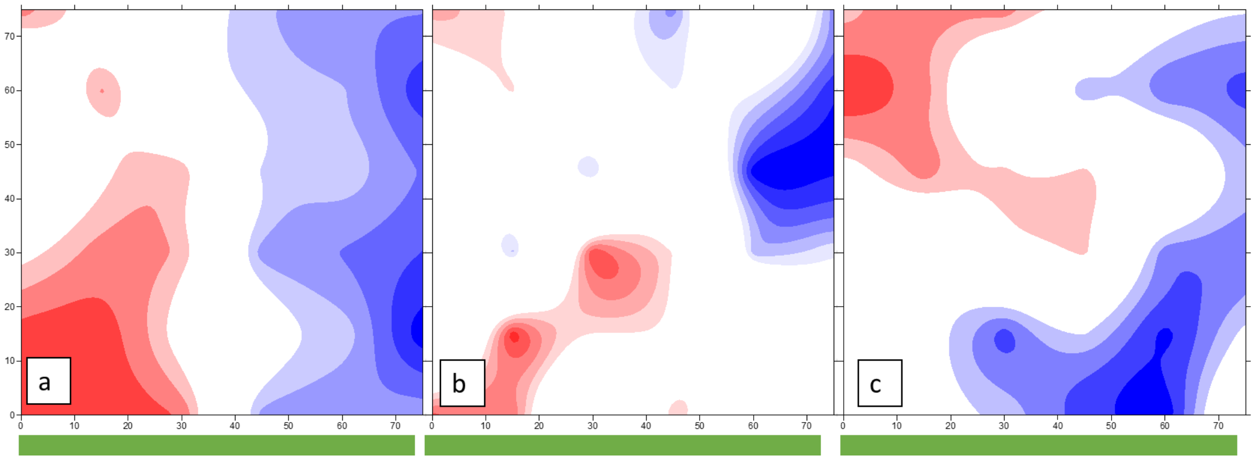

Figure 14 Spatial distribution of *E. vitis* adults on three sampling dates: 4th of May (a), 31st of August (b), and 28th of September (c). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 7 Results of the spatial pattern analysis of *E. vitis* in Gambellara vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test ($\alpha = 0.05$)

Date	vj	vi	Pvj	Pvi
4-May	-1.999	2.005	0.0001	0.0001
31-August	-1.401	1.524	0.0168	0.0052
28-September	-1.897	1.55	0.0001	0.0042

Zygina rhamni

Zygina rhamni distribution was aggregated on two dates: 4th May, and 31st August (Table 8). In these dates, patches partially involved wood margins. Interestingly, the spatial pattern of *Z. rhamni* in May was similar to that of *E. vitis* (Figure 15).

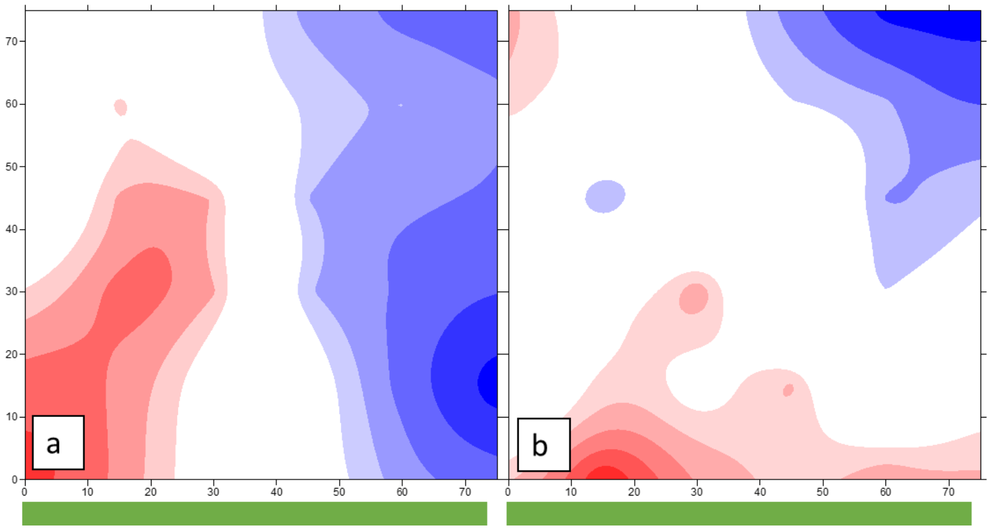

Figure 15 Spatial distribution of *Z. rhamni* adults on two sampling dates. 4th of May (a), and the 31st of August (b). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 8 Results of the spatial pattern analysis of *Z. rhamni* in Gambellara vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
4-May	-2.008	1.925	0.0001	0.0001
31-August	-1.57	1.731	0.0027	0.0002

Anagrus sp.

The distribution of *Anagrus* sp. populations was aggregated on two dates: 4th May, and 4th August (Table 9). During spring, patches were extended along the west border of the vineyard, while in summer, they were localized close to the woody vegetation (Figure 16).

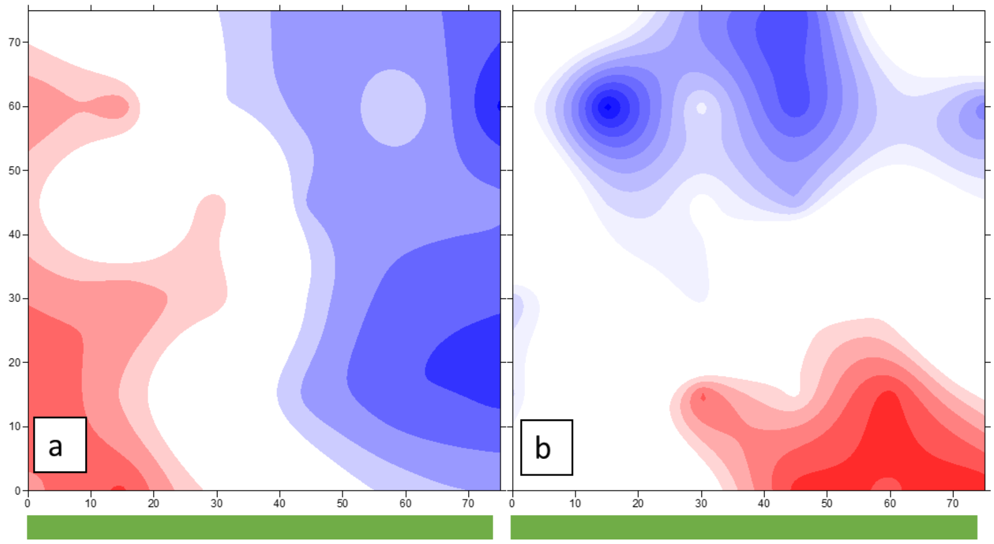

Figure 16 Spatial distribution of *Anagrus* sp. during two sampling dates the 4th May (a), and 4th August (b). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 9 Results of the spatial pattern analysis of *Anagrus sp.* in Gambellara vineyard showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
4-May	-2.042	2.129	0.0001	0.0001
04-August	-1.58	1.571	0.0027	0.0023

Lonigo

Erasmoneura vulnerata

Erasmoneura vulnerata distribution was significantly aggregated on various dates: 4th May, 29th June, 12th July, 4th August and 31st August (Table 10). In this vineyard, *E. vulnerata* patches were distributed far from the woody vegetation, close to a vineyard of cv. Glera was seriously infested by the leafhopper (Figure 17).

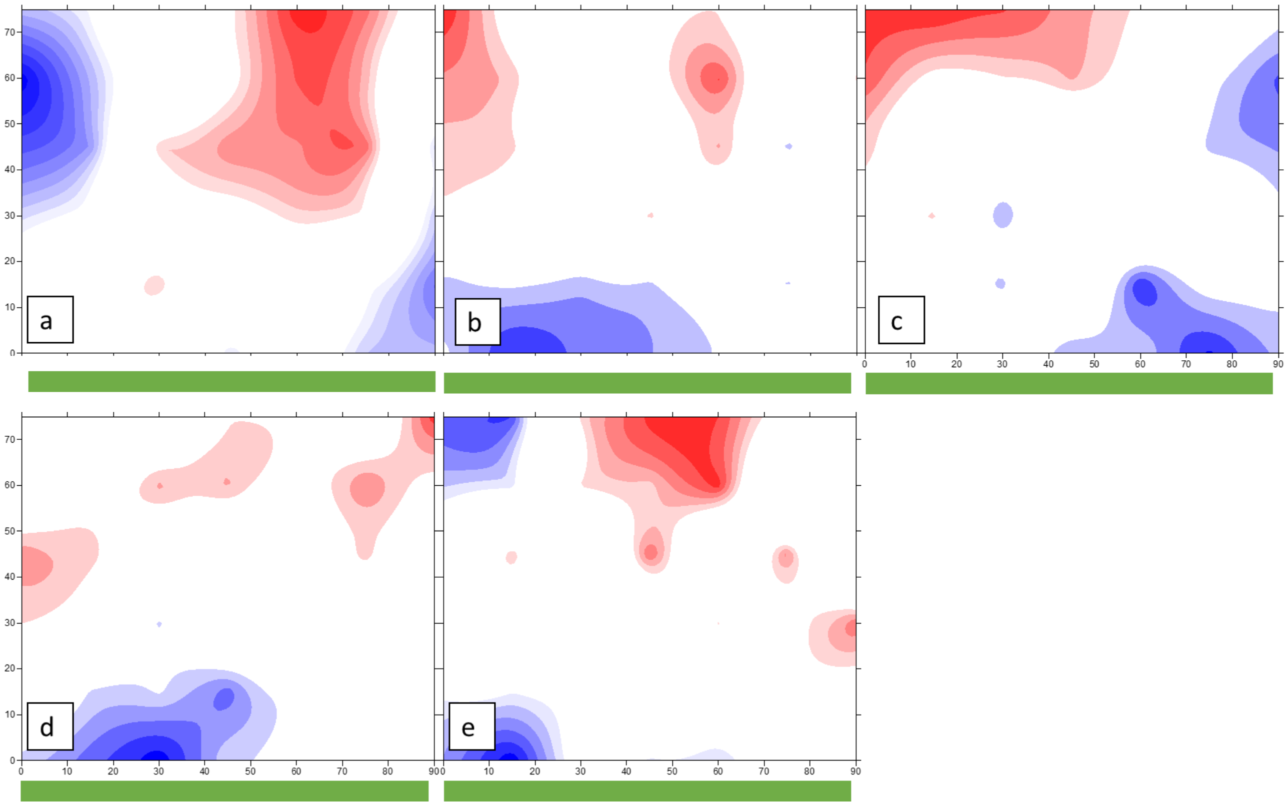

Figure 17 Spatial distribution of *E. vulnerata* adults on five sampling dates: 4th of May (a), 29th of June (b), 12th of July (c), 4th of August (d), and 31st August (e). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 10 Results of the spatial pattern analysis of *E. vulnerata* in Lonigo vineyard showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05).

Date	vj	vi	Pvj	Pvi
4-May	-1.595	1.512	0.0037	0.0159
29-June	-1.544	1.558	0.0116	0.0121
12-July	-1.584	1.641	0.0042	0.0025
04-August	-1.534	1.559	0.0144	0.0106
31-August	-1.501	1.535	0.0335	0.0154

Empoasca vitis

Empoasca vitis distribution showed significant aggregation on 18th May, 1st June, 15th September, and 28th September (Table 11). During spring patches were distributed first along the west border, then on the east border of the vineyard. In late summer adults moved from the middle of the vineyard towards areas close to wood margins (Figure 18).

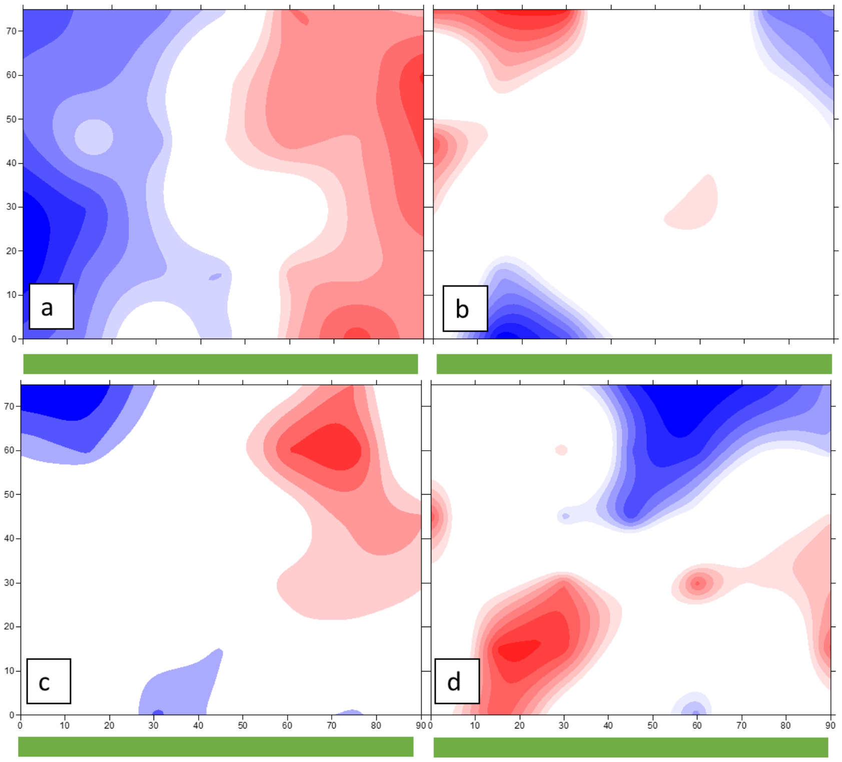

Figure 18 Spatial distribution of *E. vitis* adults on four sampling periods: 18th May (a), 1st June (b), 15th September (c), and 28th September (d). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 11 Results of the spatial pattern analysis of *E. vitis* in Lonigo vineyard showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-May	-2.699	2.32	0.0001	0.0001
1-June	-1.566	1.528	0.0293	0.0372
15-September	-1.516	1.564	0.0181	0.0126
28-September	-1.573	1.53	0.0035	0.0055

Zygina rhamni

Zygina rhamni distribution showed significant aggregation on 4th May, 18th May, 31st August, and 15th September (Table 12). In spring patches did not confirm a definite trend, while in late summer they were localized in the North-eastern borders of the vineyard (Figure 19).

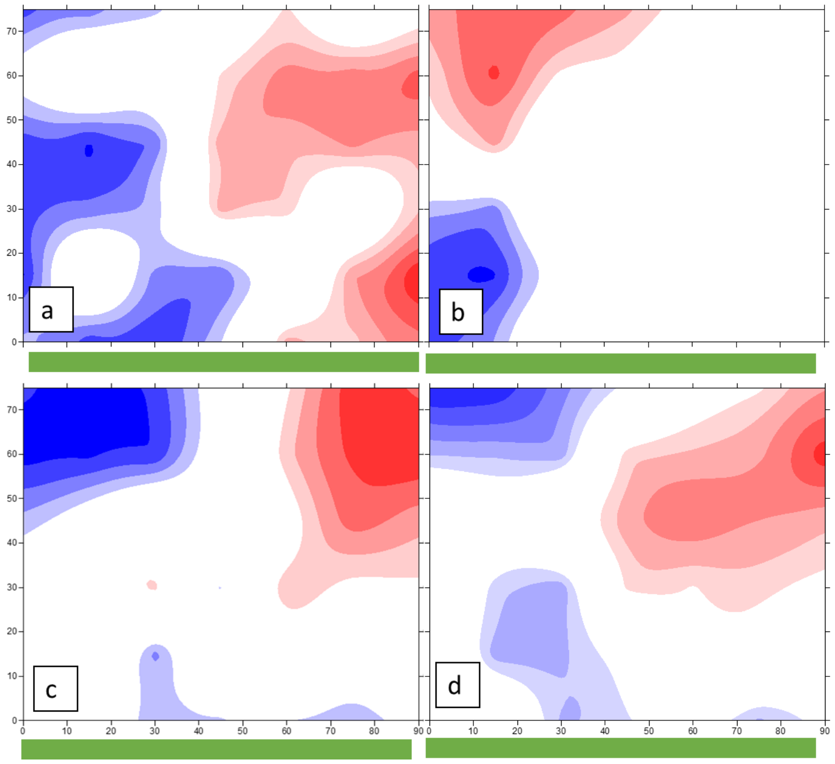

Figure 19 Spatial distribution of *Z. rhamni* adults on four sampling periods: 4th May (a), 18th May (b), 31st August (c), and 15th September (d). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 12 Results of the spatial pattern analysis of *Z. rhamni* in Lonigo vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Date	vj	vi	Pvj	Pvi
18-May	-2.017	2.162	0.0001	0.0001
1-June	-1.505	1.525	0.0218	0.0094
31-August	-1.752	1.646	0.001	0.0015
15-September	-1.902	1.647	0.0001	0.0008

Anagrus sp.

Anagrus sp. distribution showed a significant aggregation on 18th August and 28th September, without a definite trend (Table 13; Figure 20).

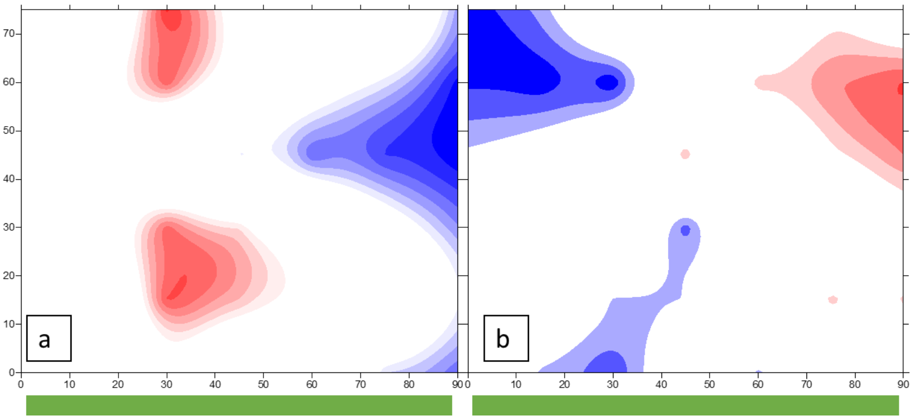

Figure 20 Spatial distribution of *Anagrus* sp. adults on two sampling periods: 18th August (a) and 28th September (b). Maps of clustering indexes where red areas are patches with interpolated cluster index vi > 1.5, and blue areas are gaps with interpolated cluster index vj < -1.5. Green bars represent wood margins.

Table 13 Results of the spatial pattern analysis of *Anagrus* sp. in Lonigo vineyard, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05).

Date	vj	vi	Pvj	Pvi
17-August	-1.509	1.509	0.0163	0.0173
28-September	-1.584	1.501	0.0039	0.0013

Spatial association between leafhoppers and *Anagrus* sp.

Erasmoneura vulnerata

Among the three leafhopper species, the distribution of *E. vulnerata* exhibited the highest number of associations with the distribution of *Anagrus* sp. and these associations were recorded in all the vineyards, unlike the other species. Associations between *E. vulnerata* and *Anagrus* sp. occurred in three out of four vineyards in spring (i.e., during vineyard colonization and the emergence of the first-generation adults) and in all vineyards during summer (Table 14).

Table 14 Results of significant spatial association analysis between *E. vulnerata* and *Anagrus* sp. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability Px < 0.025 are reported.

Vineyard	*Anagrus* sp.	*E. vulnerata*	X_k	P_x
Lonigo	17-Aug	17-Aug	0.3918	0.0081
Lonigo	31-Aug	31-Aug	0.3315	0.0188
Lonigo	15-Sep	15-Sep	0.5038	0.0014
Lonigo	28-Sep	28-Sep	0.3364	0.0197
Soave	18-May	18-May	0.4353	0.0131
Soave	1-Jun	1-Jun	0.7496	< 0.0001
Soave	29-Jun	29-Jun	0.4214	0.0206
Soave	12-Jul	12-Jul	0.4585	0.0114
Fitta	18-May	18-May	0.8099	< 0.0001
Fitta	1-Jun	1-Jun	0.6714	< 0.0001
Fitta	15-Jun	15-Jun	0.4987	0.0124
Fitta	4-Aug	4-Aug	0.4874	0.012
Gambellara	4-May	4-May	0.7784	< 0.0001
Gambellara	12-Jul	12-Jul	0.3796	0.0165
Gambellara	15-Sep	15-Sep	0.3781	0.0162

Empoasca vitis

The distribution of *Empoasca vitis* was associated with the distribution of *Anagrus* sp. in three out of four vineyards. *Empoasca vitis* and *Anagrus* sp. were associated in spring in two out of three vineyards, and in the three vineyards in summer (Table 15).

Table 15 Results of significant spatial association analysis between *E. vitis* and *Anagrus* sp. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability Px < 0.025 are reported.

Vineyard	*Anagrus* sp.	*E. vitis*	X_k	P_x
Lonigo	19-apr	19-apr	0.3849	0.0114
Lonigo	17-Aug	17-Aug	0.4571	0.0046
Fitta	12-Jul	12-Jul	0.4408	0.0245
Fitta	28-Sep	28-Sep	0.3936	0.0245
Gambellara	19-apr	19-apr	0.3933	0.0177
Gambellara	4-May	4-May	0.8544	< 0.0001
Gambellara	12-Jul	12-Jul	0.5493	0.0031
Gambellara	4-Aug	4-Aug	0.4518	0.0112
Gambellara	28-Sep	28-Sep	0.4534	0.0081

Zygina rhamni

The distribution of *Zygina rhamni* was associated with the one of *Anagrus* sp. only in two vineyards, both in spring and summer (Table 16).

Table 16 Results of significant spatial association analysis between *Z. rhamni* and *Anagrus* sp. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability Px < 0.025 are reported.

Vineyard	*Anagrus* sp.	*Z. rhamni*	X_k	P_x
Lonigo	1-Jun	1-Jun	0.2948	0.05
Lonigo	17-Aug	17-Aug	0.3571	0.0205
Lonigo	15-Sep	15-Sep	0.3538	0.0161
Lonigo	28-Sep	28-Sep	0.431	0.0023
Gambellara	4-May	4-May	0.8733	< 0.0001
Gambellara	12-Jul	12-Jul	0.6146	0.0009

Spatial association among the leafhopper species

Erasmoneura vulnerata* and *Empoasca vitis

Considering a total of 48 possible associations/dissociations (12 dates x 4 vineyards), the associations between these two species were the least frequent, occurring only eight times. The distributions of these two species was dissociated in five cases (Table 17).

Table 17 Results of significant spatial associations or dissociations analysis between *E. vulnerata* and *E. vitis*. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability $Px < 0.025$ (associated) and with probability $Px > 0.975$ (dissociated) are reported.

Vineyard	Date	Association index (X)	Associated probability (P)
Lonigo	**19-Apr**	**0.3772**	**0.0077**
Lonigo	15-Jun	-0.2574	0.9759
Lonigo	**31-Aug**	**0.3774**	**0.0078**
Lonigo	**15-Sep**	**0.3486**	**0.0184**
Soave	**18-May**	**0.5313**	**0.0042**
Soave	15-Jun	-0.3311	0.9766
Soave	12-Jul	-0.5909	0.9986
Soave	4-Aug	-0.4984	0.9946
Fitta	**19-Apr**	**0.6192**	**0.0008**
Fitta	28-Sep	-0.4362	0.986
Gambellara	**4-May**	**0.8744**	**< 0.0001**
Gambellara	**12-Jul**	**0.3673**	**0.0151**
Gambellara	**17-Aug**	**0.5344**	**0.0006**

Erasmoneura vulnerata and Zygina rhamni

The association between these two species occurred in nine out of 48 cases among the four vineyards, with most of them in summer. In contrast, dissociations were observed only once in the Soave vineyard (Table 18).

Table 18 Results of significant spatial associations or dissociations analysis between *E. vulnerata* and *Z. rhamni*. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability Px < 0.025 (associated) and with probability Px > 0.975 (dissociated) are reported.

Location	Date	Association index (X)	Associated probability (P)
Lonigo	**4-Aug**	**0.3402**	**0.0205**
Lonigo	**17-Aug**	**0.3648**	**0.0153**
Lonigo	**28-Sep**	**0.4592**	**0.0027**
Soave	**4-May**	**0.6563**	**0.0002**
Soave	12-Jul	-0.4663	0.9866
Soave	**15-Sep**	**0.4492**	**0.0158**
Fitta	**31-Aug**	**0.4718**	**0.0147**
Fitta	**15-Sep**	**0.4378**	**0.0223**
Gambellara	**4-May**	**0.8927**	**< 0.0001**
Gambellara	**12-Jul**	**0.34**	**0.022**

Empoasca vitis* and *Zygina rhamni

These two species were frequently associated (30 associations out of 48 cases) during the entire season in all the vineyards; no significant dissociations occurred between them (Table 19).

Table 19 Results of significant spatial associations or dissociations analysis between *E. vulnerata* and *Z. rhamni*. throughout the growing season in the four vineyards. In the table, values of the index of spatial association (X_k), with probability Px < 0.025 (associated) and with probability Px > 0.975 (dissociated) are reported.

Location	Date	Association index (X)	Associated probability (P)
Lonigo	**4-May**	**0.3705**	**0.0134**
Lonigo	**18-May**	**0.7971**	**< 0.0001**
Lonigo	**1-Jun**	**0.4969**	**0.0004**
Lonigo	**15-Jun**	**0.5462**	**0.0001**
Lonigo	**29-Jun**	**0.6431**	**< 0.0001**
Lonigo	**12-Jul**	**0.3972**	**0.0107**
Lonigo	**4-Aug**	**0.5262**	**0.0011**
Lonigo	**17-Aug**	**0.6307**	**0.0001**
Lonigo	**31-Aug**	**0.6854**	**< 0.0001**
Lonigo	**15-Sep**	**0.6639**	**< 0.0001**
Soave	**15-Jun**	**0.7834**	**< 0.0001**
Soave	**29-Jun**	**0.7245**	**< 0.0001**
Soave	**12-Jul**	**0.824**	**< 0.0001**
Soave	**4-Aug**	**0.457**	**0.0069**
Soave	**17-Aug**	**0.5008**	**0.0065**
Soave	**31-Aug**	**0.7673**	**< 0.0001**
Soave	**28-Sep**	**0.4738**	**0.013**
Fitta	**18-May**	**0.5083**	**0.025**
Fitta	**1-Jun**	**0.5896**	**0.0038**
Fitta	**15-Jun**	**0.7481**	**0.0004**
Fitta	**12-Jul**	**0.54**	**0.0112**
Fitta	**4-Aug**	**0.6613**	**0.0015**
Fitta	**17-Aug**	**0.4554**	**0.0121**
Fitta	**15-Sep**	**0.677**	**0.0038**
Gambellara	**4-May**	**0.9641**	**< 0.0001**
Gambellara	**15-Jun**	**0.6603**	**< 0.0001**
Gambellara	**29-Jun**	**0.3671**	**0.0213**
Gambellara	**12-Jul**	**0.8779**	**< 0.0001**
Gambellara	**17-Aug**	**0.7072**	**< 0.0001**
Gambellara	**31-Aug**	**0.6802**	**< 0.0001**

Exposure of sentinel potted grapevines to Mymarids in vineyards

Mymarid adults emerged from sentinel grapevines infested with both *E. vulnerata* and *Z. rhamni* (Figure 21). Morphological and molecular identifications highlighted that mymarids emerged from *E. vulnerata* or *Z. rhamni* eggs belonged to *A. atomus.* The number of *A. atomus* adults emerged from the two types of sentinel potted grapevines was not significantly different (F= 1.04, df= 1, 658, P= 0.3082). The effect of time was significant due to an increase in mymarids numbers in late summer (F= 13.01; df= 6, 658, P < 0.0001).

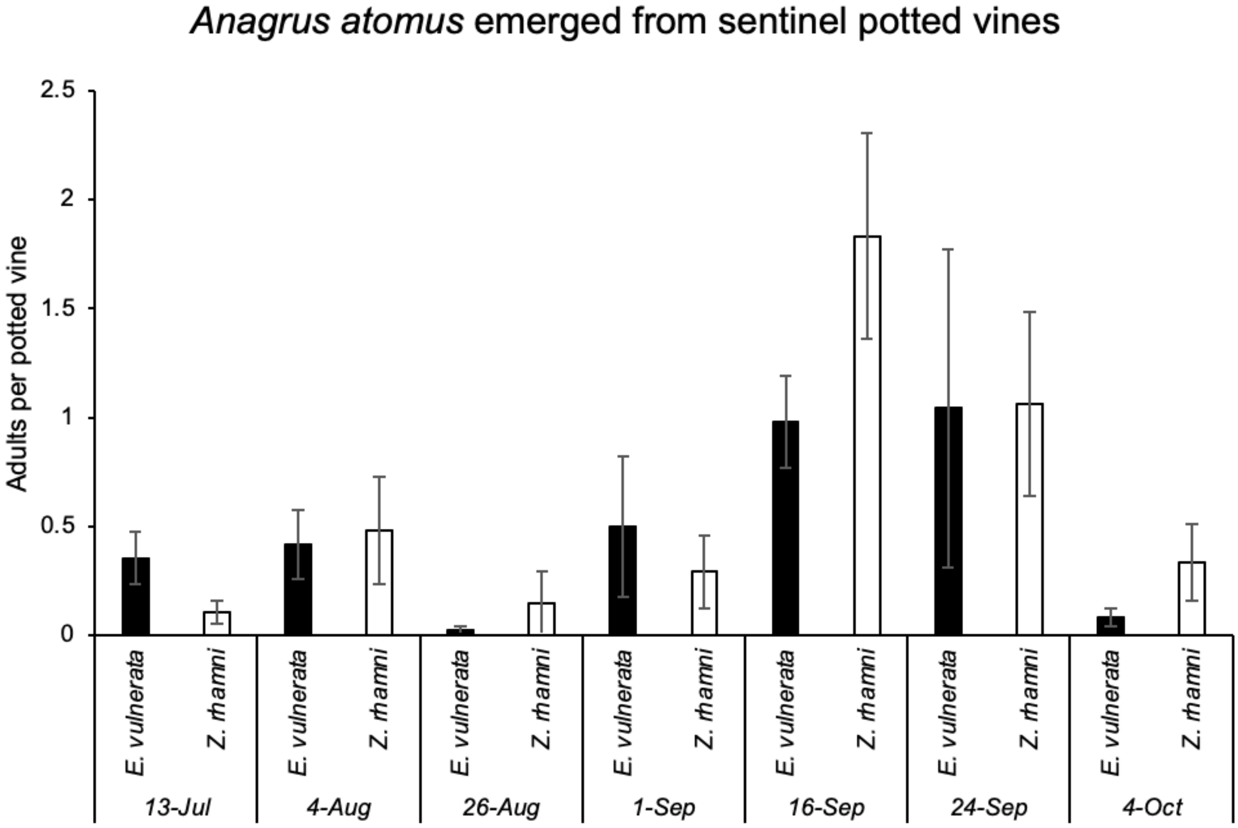

Figure 21 Number of *Anagrus atomus* emerged from sentinel eggs during the selected dates.

Discussion

Vineyard colonization

Among leafhoppers considered in this study, *E. vulnerata* was more abundant than *E. vitis* and *Z. rhamn*i over the sampling period, including the colonization phase, as shown by both adult captures on traps and nymph populations on leaves. It is known that adults of the three leafhopper species overwinter on spontaneous or ornamental woody plants with persistent or semi-persistent leaves (Vidano, 1963; Cerutti *et al*., 1991; Duso *et al*., 2019) and an edge effect was expected in adult colonization in relation to the woody vegetation close to vineyards. In the present study, this effect was significant only for *E. vulnerata* adults, confirming previous trends (Duso *et al.*, 2020). In particular, distribution maps showed higher adult captures on the border close to the spontaneous woody vegetation in three out of four vineyards. The vineyard not involved in this phenomenon (i.e., Lonigo, cv. Garganega) was contiguous to a vineyard of cultivar Glera already colonized by *E. vulnerata* adults at the bud-break of Garganega grapevines. In fact, sprouting takes place about two weeks earlier in the Glera than in the Garganega cultivar (D. Tomasi, personal communication based on data collected during 1964–2010 in the ampelographic collection of CRA-VE of Spresiano and Susegana, Treviso province, Italy). We can assume that, at the sprouting, the overwintered *E. vulnerata* adults colonized the Glera vineyard and from this moved to the Garganega vineyard: this is also suggested by the edge effect reported in the distribution map of May 4. The present study also investigated the edge effect on nymphs, but the data did not support a statistically significant effect.

For *E. vitis* and *Z. rhamni* adults, an aggregation was recorded only in Gambellara vineyard during vineyard colonization close to the woody vegetation. This vineyard showed the highest captures of the two species compared to the remaining vineyards. The absence of an edge effect in the other three vineyards may be due to a greater distance from the preferred overwintering sites or to lower overwintered adult populations. The absence of this effect was also observed in a previous study (Duso *et al*., 2020), where the adult population densities of the two species were even lower than those reported in this work. In other studies, an edge effect in vineyard colonization was already observed for *Z. rhamni* (Mazzoni *et al*., 2008), but not for *E. vitis* (Decante and Van Helden, 2008). The absence of edge effects for *E. vitis* and *Z. rhamni* nymphs may be associable with their low densities.

It is known that *A. atomus* overwinters in eggs of various leafhopper species laid in autumn inside leaves of various host plants, mostly with semipersistent leaves such as *Rubus* spp. and *Rosa* spp. (Cerutti *et al*., 1991; Remund and Boller, 1996; Arnò *et al*., 1987; Matteucig and Viggiani, 2008; Mazzoni *et al*., 2008; Zanolli and Pavan, 2011). Therefore, an edge effect was also expected *for A. atomus* adults colonizing vineyards from the woody vegetation. This study highlighted this effect. In fact, the spatial and temporal distribution analysis showed adult aggregation patterns in spring in three out of the four vineyards.

Vineyard distribution patterns in summer

In summer, the three leafhopper species showed aggregation patterns frequently distant from the woody vegetation. Aggregation of *E. vitis* associated with plant vigor was reported in France vineyards (Decante and Van Helden, 2008; Decante *et al*., 2009). This association is consistent with the leafhoppers (*E. vitis* and *Z. rhamni*) preference for egg laying on more vigorous plants and shoots (Pavan and Pavanetto, 1989, Vidano *et al*., 1988; Mazzoni *et al.,* 2008; Pavan and Picotti, 2009). A comparative analysis of distribution maps revealed that patches of *E. vulnerata* did not overlap with those of *E. vitis* and *Z. rhamni*, while the latter two were frequently associated. This result was corroborated by an association analysis among the three leafhopper species, which showed that *E. vitis* and *Z. rhamni* frequently occupied different areas of the vineyards compared to *E. vulnerata*, while they coexist with each other. The ratios between the populations of the three species in the four vineyards, considering both adults and nymphs, suggest that *E. vulnerata* is more competitive than *E. vitis* and *Z. rhamni* (see also Duso *et al*., 2020). even if the prevalence of the former may have been favored by the presence of natural enemies more effective against the last two species.]

Regarding *A. atomus*, adults were still close to the natural vegetation in early summer but moved inside vineyards in mid- and late summer. In these periods, we found associations between *A. atomus* and leafhoppers, particularly with *E. vulnerata*. The latter association suggests that *A. atomus* is attracted by vineyard areas colonized by high numbers the American leafhopper females, thus exhibiting an adaptation to this new species.

Experiments with sentinel vines

Experiments with sentinel grapevines showed that those infested by both *E. vulnerata* and *Z. rhamni* eggs attracted *A. atomus* adults. The statistical analysis showed a significant effect of time on *A. atomus* emergence, probably related to the seasonal abundance of parasitoids in these agroecosystems, since in most of the four vineyards *A. atomus* numbers increased in late summer. The data did not prove a preference for one of the two leafhoppers, but show that mymarid females can parasitize the eggs of the exotic *E. vulnerata*.

Conclusions

In these case-studies natural and semi-natural overwintering sites near vineyards promoted *E. vulnerata* colonization in spring. Considering the severe damage that the leafhopper can cause in spring, adult colonization should be carefully monitored, and appropriate control measures can be locally adopted according to the results of our study (e.g., kaolin application mostly in organic vineyards). In the summer, *E. vulnerata* populations tend to aggregate in some areas of the vineyard, which must be promptly identified. It should be crucial to investigate whether these aggregations can be associated with areas with more vigorous grapevines or particular microclimates. Factors influencing the competition among *E. vulnerata* and the native leafhoppers require to be investigated.

Natural populations of *A. atomus* were attracted by sentinel plants infested with *E. vulnerata* and parasitized their eggs, indicating that the parasitoid is adapting to the American leafhopper. Even the associations between adults of the leafhopper and the parasitoid in summer indicate that *A. atomus* is attracted to the areas of the vineyard where the leafhopper is abundant, and this phenomenon has positive implications for an effective biological control. Considering the spring colonization pattern of *Anagrus sp.*, further research should be conducted on the possibility of increasing the parasitoids' population through woody vegetation manipulation. This will enable better biological control of leafhoppers.

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Chapter 3

Kaolin as an alternative to insecticides in controlling the grape leafhopper *Erasmoneura vulnerata*

Manuscript in preparation as: Kaolin as an alternative to insecticides in controlling the grape leafhopper *Erasmoneura vulnerata*

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Prazaru Stefan Cristian contributed to data collection, statistical analysis, and the manuscript drafting.

Abstract

Recently, *E. vulnerata*, that was first reported in Italy, has been reported in other European viticulture areas, suggesting that the current issues associated with this species in Italy may spread throughout Europe. The most effective control strategies against *E. vulnerata* are those based on conventional insecticides. Organic control strategies mostly rely on pyrethrum, but its effectiveness seems limited, considering the outbreaks in North-eastern Italy in organic managed vineyards. Therefore, the control of *E. vulnerata* on organic farms must be pointed out. In this study large scale trials were planned in several organically managed vineyards located in North-eastern Italy. We evaluated kaolin's impact on *E. vulnerata* and the effect on beneficial arthropods, particularly Acari Phytoseiidae and the egg parasitoids Hymenoptera Mymaridae. A decline in the leafhopper nymph population was observed in three out of four vineyards, while adults in two out of four. Kaolin affected predatory mite populations, but not permanently, and did not affect mymarid parasitism rates.

Introduction

In the last decades, viticulture has received a significant boost in economic growth compared to other cultivations (Brostrom and Brostrom 2009). At the same time, viticulture is facing new challenges due to climate change and the demand for environmental-friendly management techniques. The European Commission recently unveiled its Farm to Fork strategy that imposes restrictions on pesticide use (European Commission 2020). This has compelled winegrowers to implement agricultural practices that reduce environmental impact while maintaining profitability (Wery and Langeveld 2010). In this framework, the search for alternatives to insecticides is crucial.

In this context, the development of particle film technology based on kaolin is considered with a particular interest. Kaolin is a white, inert, and non-toxic clay aluminosilicate applied as water formulation on cultivated plants to create a coating protective film (Sharma *et al*., 2015). The use of this technology in grapevine cultivation provides several advantages. Kaolin-based particle film is applied on leaves because of its reflectance proprieties to reduce sunburns and water stress (Boari *et al*. 2015, Brillante *et al*. 2016, Ferrari *et al*. 2017). Kaolin applications have also been proposed against various pests such as *Homalodisca coagulata* (Say) (Wood and McBride 2001, Puterka *et al*. 2003, Barker *et al*. 2006, Tubajika *et al*. 2007); the grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (Sleezer *et al*. 2011); grapevine leafhoppers such as *Zygina rhamni* Ferrari, *Empoasca vitis* (Göthe) and *Scaphoideus titanus* Ball (Tacoli *et al*. 2017a, Tacoli *et al*. 2017b); the tortricid moth *Lobesia botrana* (Denis and Schiffermüller) (Pease *et al*. 2016, Tacoli *et al*. 2019); the spotted wing *Drosophila suzukii* (Marsumura) (Linder *et al*., 2020; González-Núñez *et al*., 2021; Dam *et al*., 2022) and also the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (D'Aquino *et al*., 2011; Campos-Rivela *et al*., 2021). It has been observed that kaolin can reduce insect adhesion to the treated surfaces (Puterka *et al*., 2005; Salerno *et al*., 2020; Salerno *et al*., 2021) and cause dehydration in small insects (Bengochea *et al*., 2013). Moreover, kaolin application can act as a repellent barrier interfering with host-plant attractiveness reducing insect feeding and oviposition (Glenn *et al*. 1999, Vincent *et al*. 2003, Barker *et al*. 2006, Lapointe *et al*. 2006, Valizadeh *et al*., 2013; Tacoli *et al*. 2017a, 2017b).

Here we tested the effect of kaolin-based applications for the management of the leafhopper *Erasmoneura vulnerata* (Fitch), an invasive pest of grapevine in Europe. This leafhopper, native to the Nearctic region, was recorded in North-eastern Italy in 2004, and for a decade has been considered a minor pest of grapevines (Duso *et al*., 2005; Duso *et al*., 2019). In 2016, the first *E. vulnerata* outbreaks were recorded in Italian vineyards (Duso *et al*., 2020a). These studies showed that *E. vulnerata* overwinters as adults in natural and artificial sites close to vineyards and completes three generations per year. This species is very competitive towards native leafhoppers and can reach significant densities in commercial vineyards. Organic vineyards are particularly exposed to *E. vulnerata* infestations, likely due to the moderate impact of naturally derived insecticides.

Recently, *E. vulnerata* has been reported in other European countries (Seljak *et al*. 2016, Chireceanu *et al*. 2020, Rizzoli *et al*. 2020, Šćiban and Kosovac,2020), suggesting that current issues related to this species in Italy could involve other viticultural areas. Among strategies used to control *E. vulnerata* those based on conventional insecticides gave promising results in small-scale trials (Tirello *et al*., 2021). In these trials, the efficacy of naturally derived insecticides was lower than that of conventional insecticides. Therefore, the control of *E. vulnerata* in organic farms must be pointed out. On the other hand, insecticide use should be reduced according to the objectives of EU policy. An increasing number of active ingredients widely used in vineyards have been banned in Europe due to toxicological and environmental issues; thus, alternatives to insecticides should be identified. Strategies based on the exploitation of biocontrol agents have also been explored. Inoculative releases of two predators, i.e., *Chrysoperla carnea* (Stephens) or *Orius majusculus* (Reuter) reduced *E. vulnerata* densities by about 30% in open field experiments (Prazaru *et al*. 2021). Promising results in controlling *E. vulnerata* have been obtained in small scale-trials using kaolin (Tirello *et al*. 2021). These results suggested further evaluating the possibility of developing control strategies based on kaolin. Large scale trials were planned in a number of vineyards located in North-eastern Italy. Moreover, in the light of incorporating kaolin applications in IPM programs in vineyards, the effect on beneficial arthropods, particularly natural enemies, should be considered (Duso *et al*. 2020b). Here we evaluated the side effects of kaolin on two groups of beneficials of importance for grapevine cultivation, the predatory mites belonging to the Acari Phytoseiidae and the egg parasitoids Hymenoptera Mymaridae.

Materials and methods

The effects of kaolin on *E. vulnerata* populations were evaluated in four organic vineyards located in Verona and Treviso provinces (Veneto region, North-eastern Italy) during three growing seasons (2019-2021). In 2019, trials were carried out in a hilly vineyard in Verona province (PO vineyard, Monteforte d'Alpone, cv. Garganega, pergola veronese training system, planting space 3.50 m × 0.80 m). In 2020, trials were carried out in two vineyards in the Verona province, one in the plain (SP vineyard, San Pietro in Cariano, cv. Corvina, doppia pergola veronese training system, planting space 3,20m x 0,80m), and the second in hilly areas (SU vineyard, Soave, cv. Garganega, Guyot training system, planting space 2.30 m × 0.9 m). An additional trial was carried out in the plain of Treviso province (GA vineyard, Ponte di Piave, cv. Glera, Bellussi training system, planting space 6m x 4m). The 2021 trials were performed in the GA vineyard only. In all vineyards, moderate to high populations of *E. vulnerata* had been reported in the season preceding the trials. In each vineyard, we tested different strategies of kaolin application (Surround WP by SERBIOS, dose 4 kg/100L) as reported in Table 1. In each trial, an untreated control was included for comparison. In 2019, kaolin was applied twice against the first and the second generations of *E. vulnerata*. In 2020 and 2021 the timing of kaolin applications depended on leafhopper abundance over the growing seasons.

Table 1. Characteristics of trials carried out in the three growing seasons.

Year	Vineyard	Treatments	Application dates
2019	PO	Kaolin Control	June 24, July 1, July 24, July 31 -
2020	SP	Kaolin Control	June 23, July 1 -
	SU	Kaolin Control	May 16, June 16, August 8 -
	GA	Kaolin Control	June 12, June 22, August 1, August 8 -
2021	GA	Kaolin Control	May 20, May 27 -

Each treatment comprised four replicates of at least 2-3 rows. Sampling was conducted every 10-15 days throughout the growing season. A total of 40-80 leaves per treatment (10-20 leaves per replicate) were removed at each sampling date and transferred to the laboratory, where leafhoppers and predatory mites were identified to genus or species level under a dissecting microscope (Stemi 508, Carl Zeiss Microscopy GmbH, Jena, Germany). Leafhopper adults were monitored using yellow sticky traps (SUPER COLOR yellow, SERBIOS, 20x12 cm). In each vineyard, four traps per treatment (one trap per replicate) were placed after sprouting and renewed at each sampling date. In some trials, the emergence holes of leafhopper first instar nymphs, and egg parasitoids (Hymenoptera Mymaridae) were recorded to calculate the parasitism rates (ratio between parasitoid emergence holes and the total of observed emergence holes).

Statistical analyses

The effects of kaolin on the abundance of *E. vulnerata* on leaves or traps were analyzed with a repeated measures linear mixed model with the MIXED procedure of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC, USA). Similar procedures were applied to evaluate the side-effects of kaolin on predatory mites and the parasitism rate by the Mymaridae. Data obtained in each field trial were analyzed separately. In this analysis, treatments (kaolin vs. control), date of sampling, and their interaction were considered sources of variation and tested with an F test (α = 0.05). Comparisons between treatments on each date were performed using a *t*-test (α = 0.05) on the least-square means. The degrees of freedom were estimated using the Kenward–Roger method, which can calculate non-integer values for error terms. Before the analysis, data were checked for model assumptions. The model was run on data transformed to log (n + 1), while the arcsine of the square root was applied to data on parasitism rate. Untransformed data are shown in the figures. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods.

Results

Effects of kaolin on *E. vulnerata* nymphs and adults

PO vineyard (2019)

In this vineyard, kaolin was applied four times against the first and the second generations of *E. vulnerata*. *Erasmoneura vulnerata* nymph densities increased in July when kaolin reduced their density in the treated plots (F = 6.2; df = 1, 11.6; P = 0.029; Figure 1). The effect of time was also significant (F = 3.25; df = 8, 44.5; P = 0.005) in contrast with the interaction treatment*time (F = 0.95; df = 8, 44.5; P = 0.484).

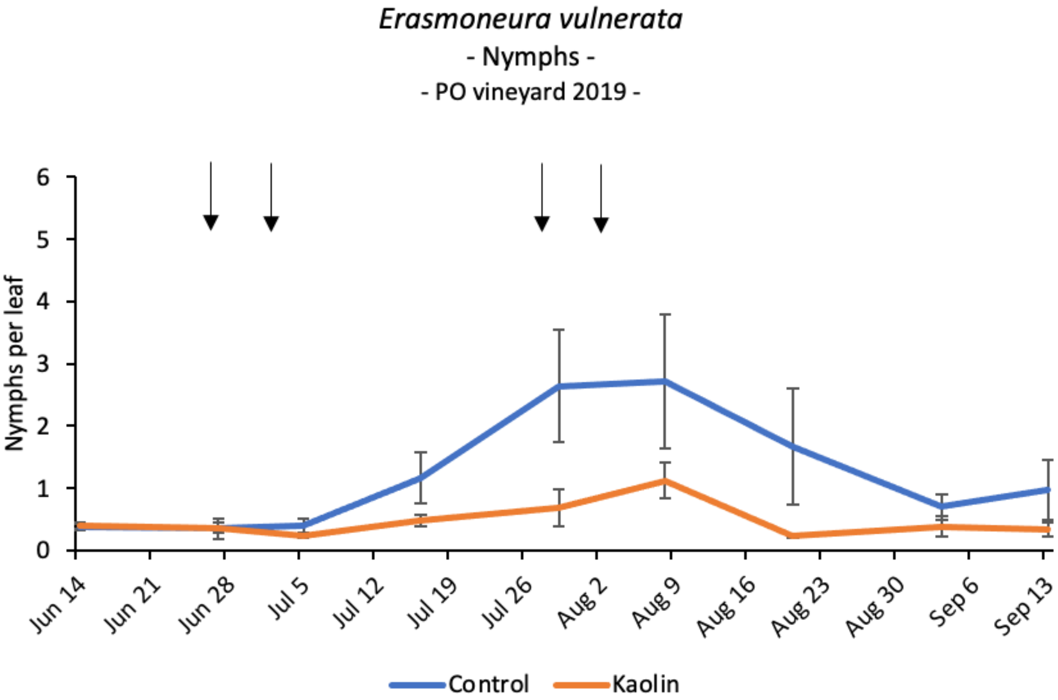

Figure 1. Seasonal abundance of *E. vulnerata* nymphs (mean ± std. err.) in PO vineyard during 2019. Kaolin was applied four times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Erasmoneura vulnerata adults' captures were detected from the end of July onwards (Figure 2). Their densities appeared to be higher in the control plots, but the effect of treatment and the interaction treatment*time were not significant (respectively: F= 0.68; df = 1, 4.30; P= 0.453; F= 1.83; df = 5, 11.13; P= 0.187; Figure 2). The effect of time was significant (F= 4.29; df = 5, 11.3; P= 0.02).

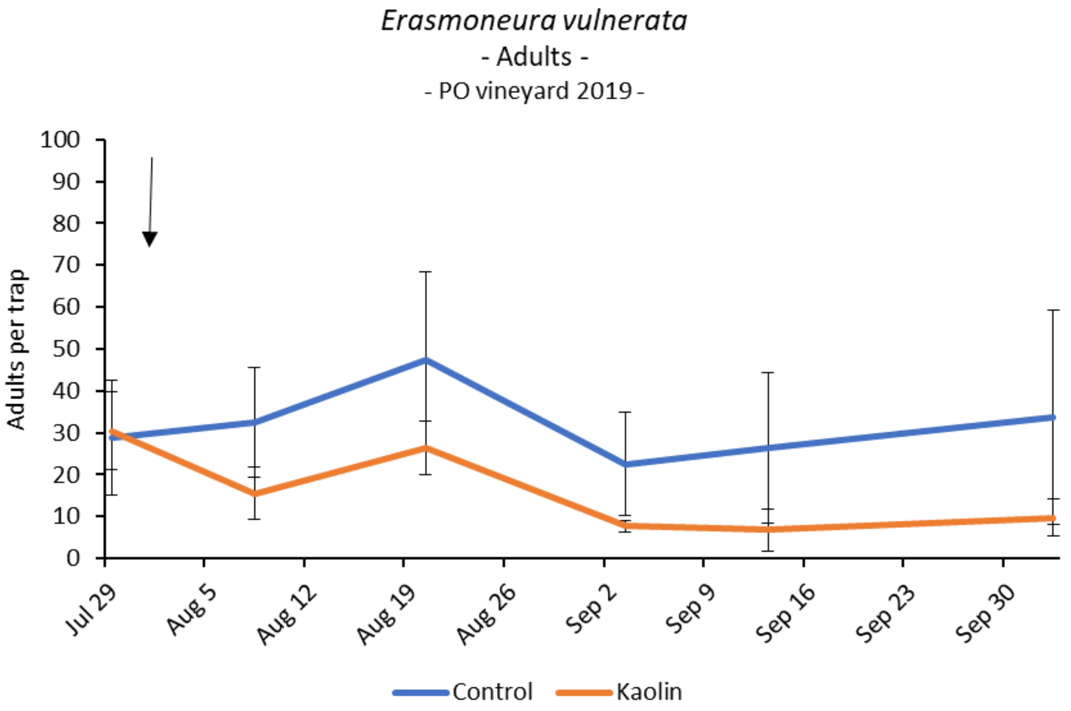

Figure 2. Seasonal abundance of *E. vulnerata* adults (mean ± std. err.) in PO vineyard during 2019. Kaolin was applied four times but arrows indicate only the application made on July 31. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates

SP vineyard (2020)

In this vineyard kaolin was applied twice against the first generation of *E. vulnerata.* The effect of treatment, time and their interaction were significant (respectively, F= 29.4; df= 1, 13.2; P< 0.0001; F= 3.83; df = 7, 38.2; P= 0.003; F= 2.68; df= 7, 38.2; P= 0.023) with nymph densities being lower in kaolin than in control plots, particularly during August (Figure 3).

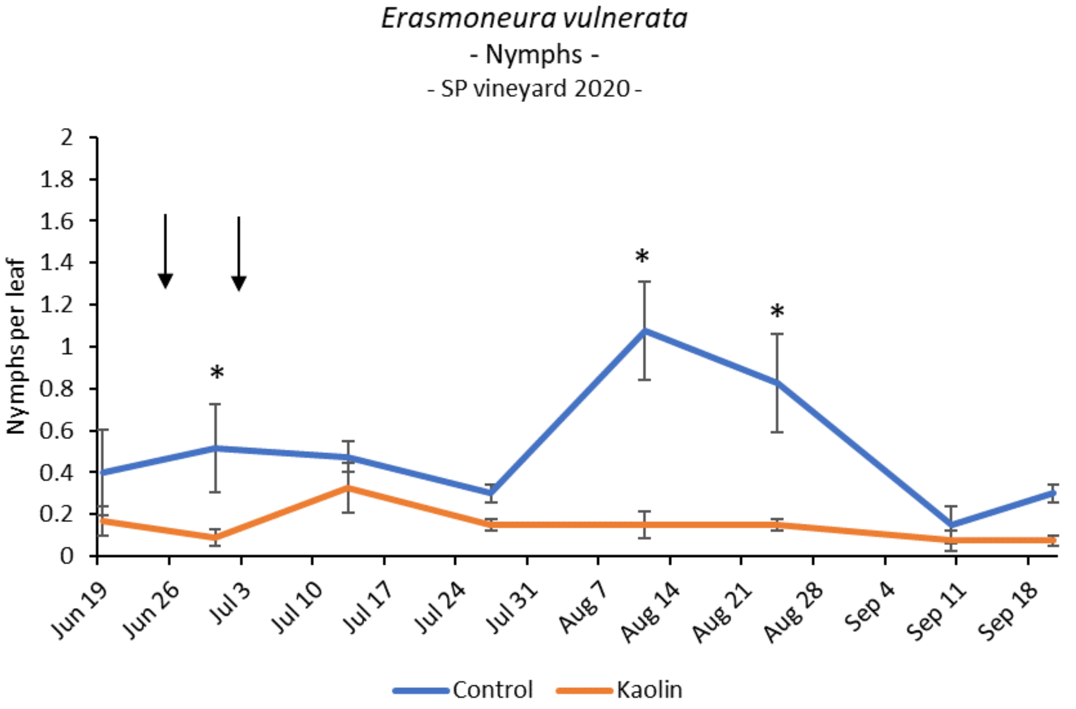

Figure 3. Seasonal abundance of *E. vulnerata* nymphs (mean ± std. err.) in SP vineyard during 2020. Kaolin was applied two times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates

Concerning *E. vulnerata* adults, their presence was observed from the end of June. The effects of treatment and time were significant (respectively: F = 17,75; df = 1, 19; P < 0.001; F = 7.21; df = 7, 38.5; P < 0.0001) while their interaction was not significant (F = 0.9; df = 7, 38.5; P = 0.515). There were more adults in the control than in kaolin-treated plots (Figure 4).

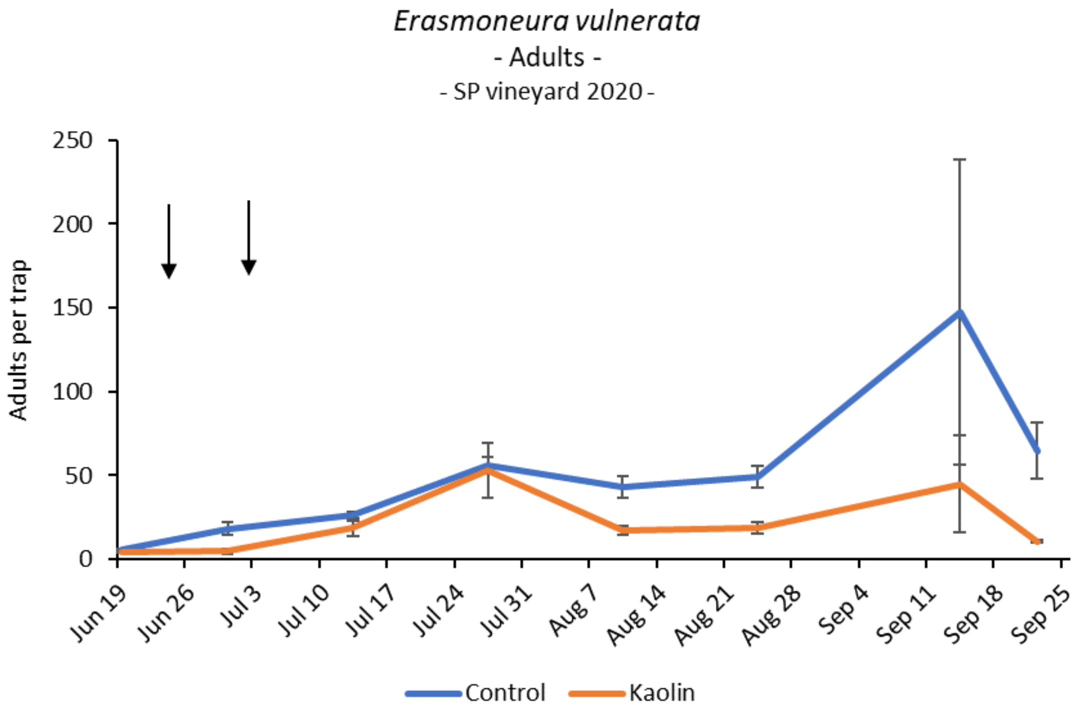

Figure 4. Seasonal abundance of *E. vulnerata* adults (mean ± std. err.) in SP vineyard during 2020. Kaolin was applied two times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates

SU vineyard (2020)

Kaolin was applied three times, the first against *E. vulnerata* overwintered adults, the second against the first generation, and the third against the second generation. The effects of treatment, time and their interaction were significant (respectively: F = 127.69; df = 1, 60.5; P < 0.0001; F = 73.19; df = 8, 65.7; P < 0.0001; F = 117.31; df = 8, 65.7; P < 0.0001), with a higher number of *E. vulnerata* nymphs found in control compared to kaolin treated plots, particularly from August onwards (Figure 5).

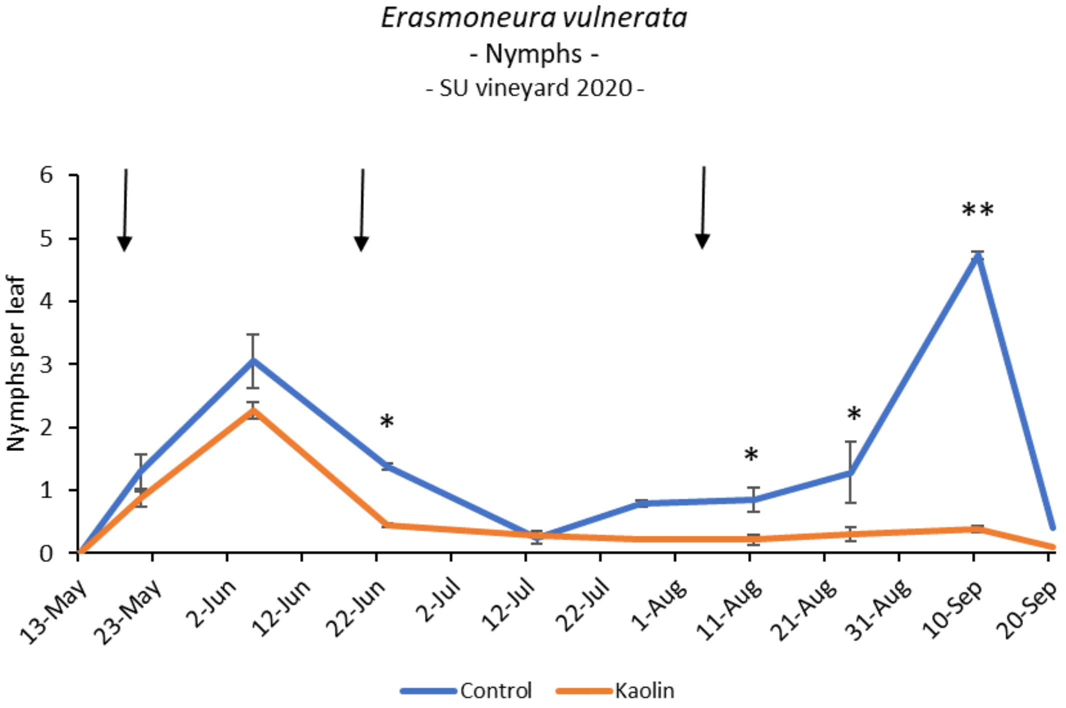

Figure 5. Seasonal abundance of *E. vulnerata* nymphs (mean ± std. err.) in SU vineyard during 2020. Kaolin was applied three times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates

Erasmoneura vulnerata adult densities were significantly affected by kaolin application, especially in late summer (Figure 6). In fact, the effect of treatment, time and their interaction were significant (respectively: F = 11.54; df = 1, 8.65; P = 0.008; F = 10.27; df = 8, 27.7; P< 0.0001; F = 4; df = 8, 27.7; P= 0.003).

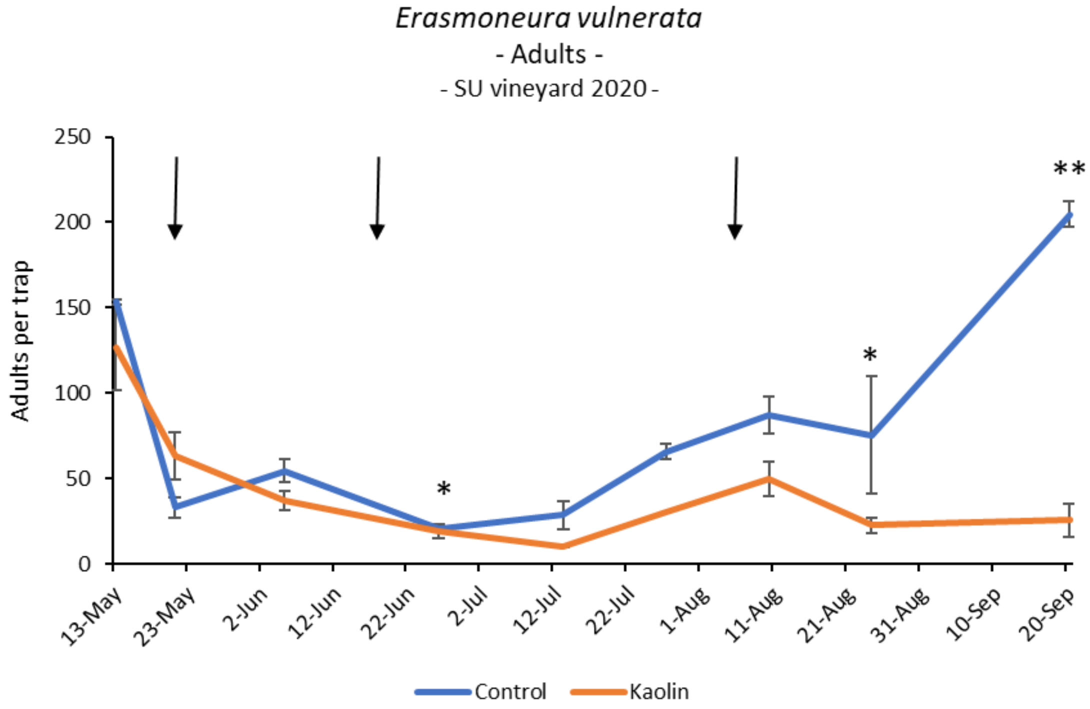

Figure 6. Seasonal abundance of *E. vulnerata* adults (mean ± std. err.) in SU vineyard during 2020. Kaolin was applied three times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates

GA vineyard (2020)

Kaolin was applied four times, against the first and the second generations of *E. vulnerata.* The effect of the time was significant (F = 48.27; df = 10, 98.9; P < 0.0001), kaolin applications significantly reduced nymph densities (F = 62.78; df = 1, 55.3; P < 0.0001) and their effects were particularly significant during June and August (F = 4.93; df = 10, 98.9; P < 0.0001; Figure 7).

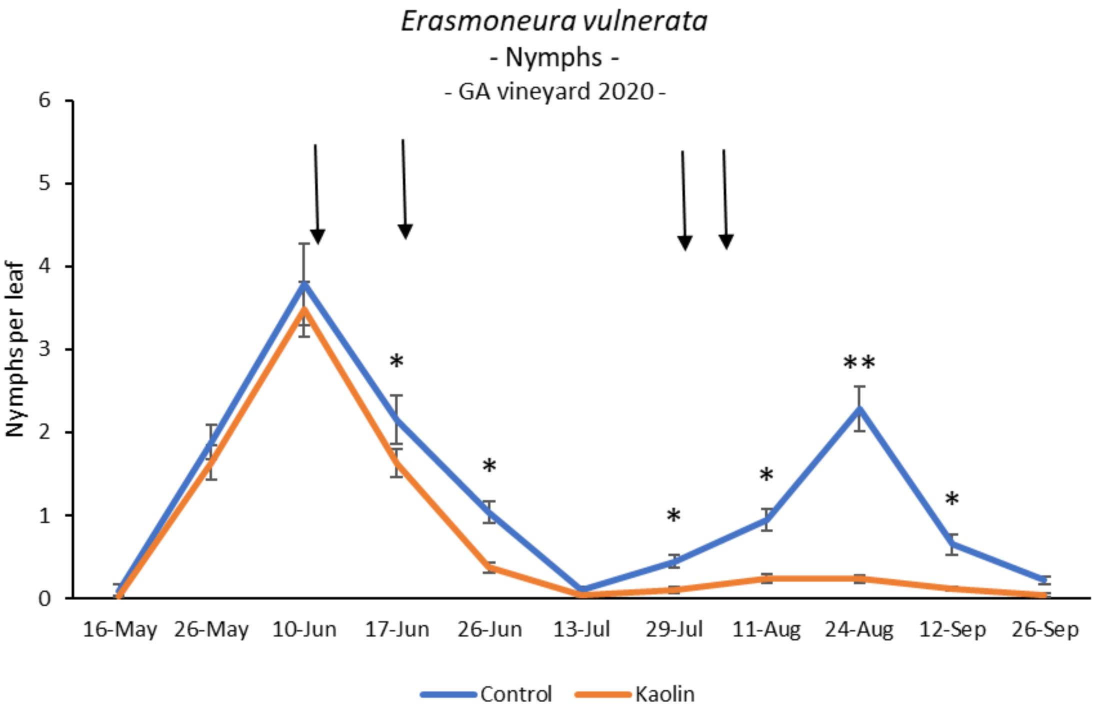

Figure 7. Seasonal abundance of *E. vulnerata* nymphs (mean ± std. err.) in GA vineyard during 2020. Kaolin was applied four times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Regarding adults, there was a significant variation during time (F=11.41; df= 9, 62.3 ; P< 0.0001), but kaolin applications (F=4.44; df= 1, 11.6; P = 0.058) and interaction treatment*time (F=0.98; df= 9, 62.3; P=0.464) were not associated to significant effects (Figure 8).

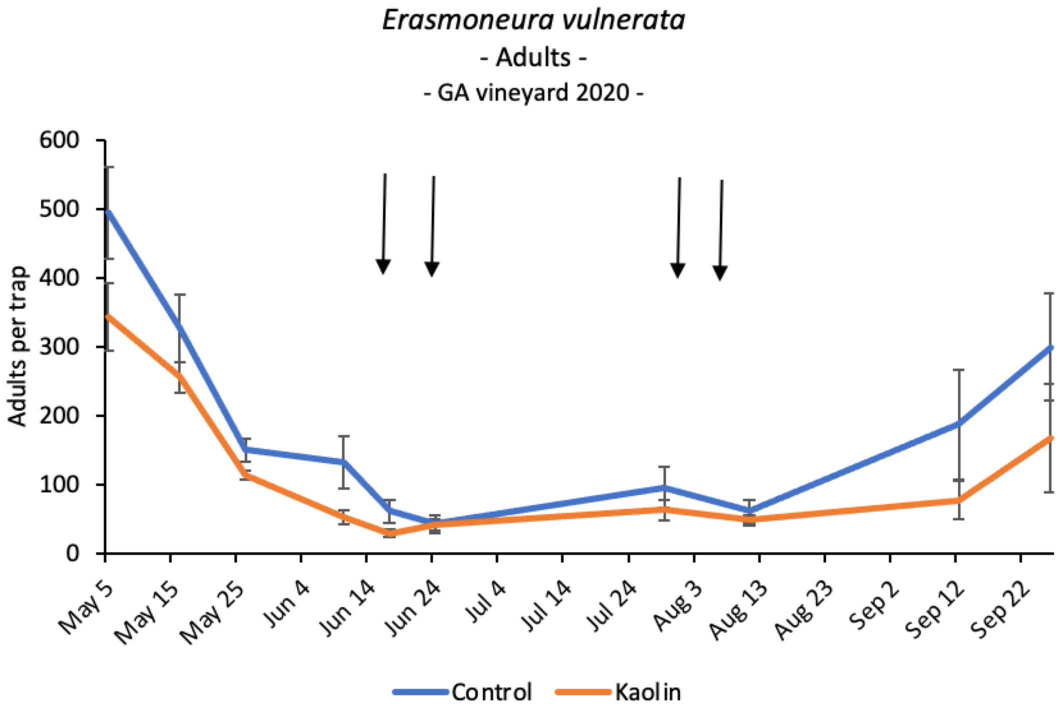

Figure 8. Seasonal abundance of *E. vulnerata* adults (mean ± std. err.) in GA vineyard during 2020. Kaolin was applied four times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

GA vineyard (2021)

Kaolin was applied earlier than in previous trials, i.e., in the vineyard colonization phase by overwintered adults (Figure 9). Kaolin applications significantly reduced *E. vulnerata* nymphs over the growing season (F = 80.79; df = 1, 12.4; P < 0.0001), with an effect that emerged in June and August in particular (time: F = 42.77; df = 8, 43.2; P < 0.0001; time*treatment: F = 7.58; df = 8, 43.2; P < 0.0001).

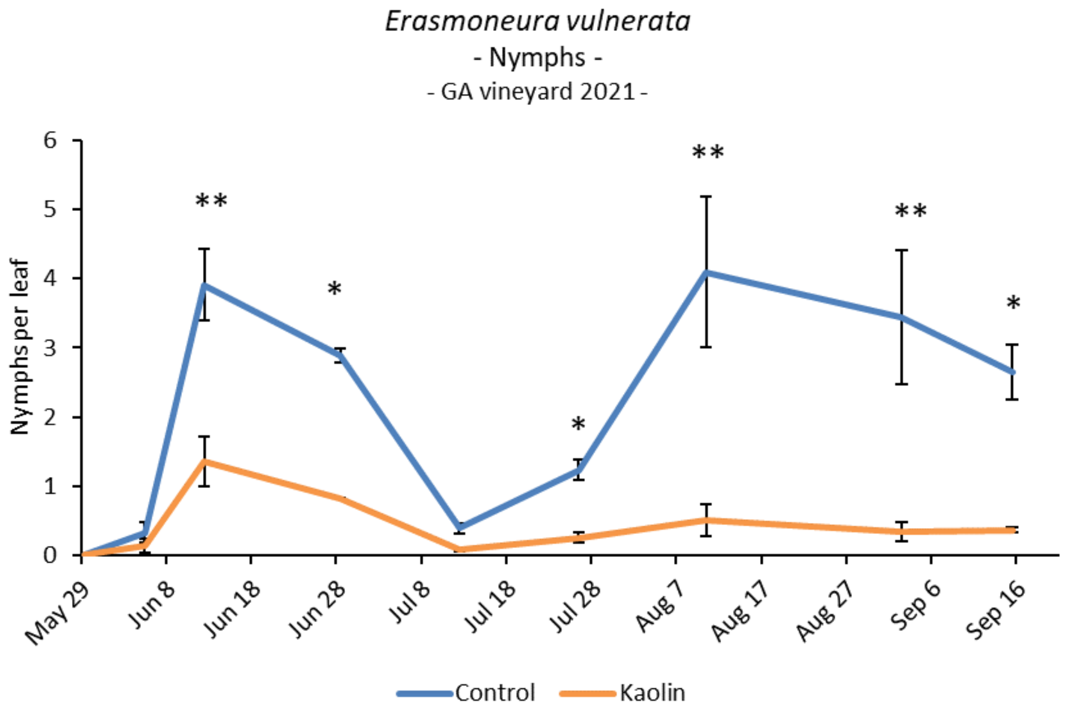

Figure 9. Seasonal abundance of *E. vulnerata* nymphs (mean ± std. err.) in GA vineyard during 2021. Kaolin was applied two times against the overwintered adults of *E. vulnerata* (kaolin was applied against wintering adults). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Kaolin applications did not affect *E. vulnerata* adult numbers (F=0.6; df= 1, 19; P= 0.449). Only a significant variation during time was observed (F=19.76; df= 6, 44.5; P< 0.0001), not the interaction treatment*time (F=0.53; df= 6, 44.5; P= 0.781; Figure 10).

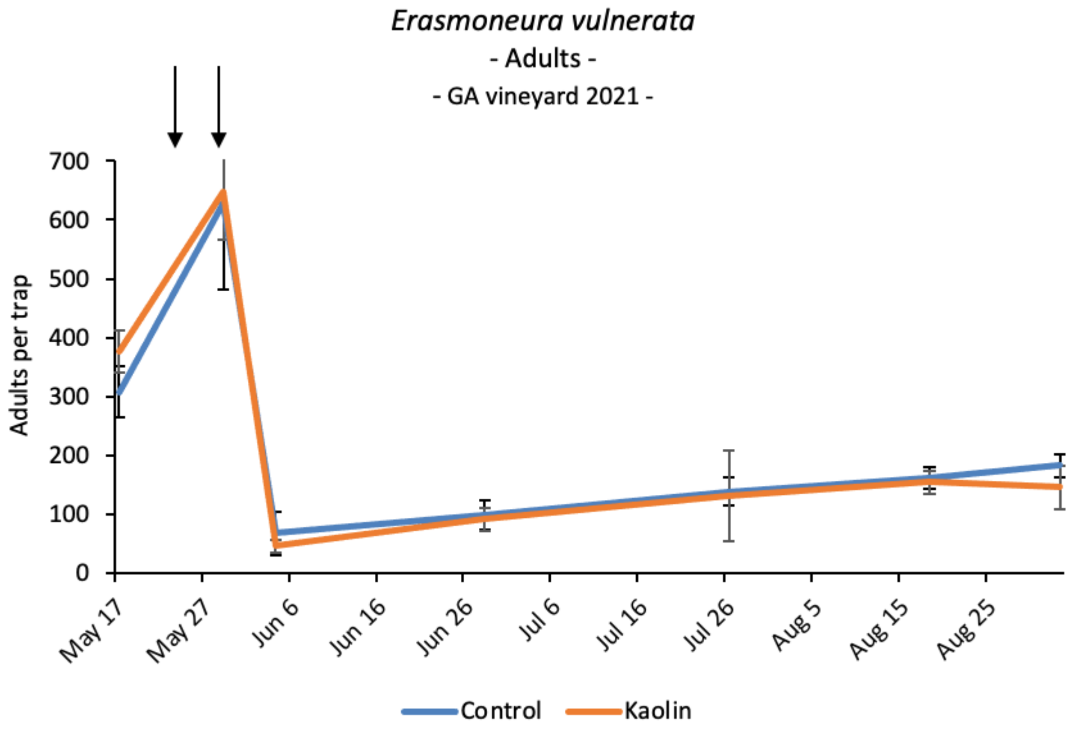

Figure 10. Seasonal abundance of *E. vulnerata* adults (mean ± std. err.) in GA vineyard during 2021. Kaolin was applied two times (arrows indicate application dates). Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Side-effects of kaolin on leafhopper parasitism

The occurrence of egg parasitoids belonging to the Hymenoptera Mymaridae (*Anagrus* spp.) was widely detected in vineyards in the 2019 and 2020 growing seasons (parasitism was not investigated in GA vineyard during 2021). The parasitism rate was calculated as the ratio between parasitoid emergence holes and the total of observed emergence holes (leafhopper nymphs + parasitoid adults). Kaolin applications did not affect the parasitism rate by *Anagrus* spp. (Figure 11, Table 2). The effect of time was significant in all vineyards because of the variation of parasitism throughout the season (Table 2).

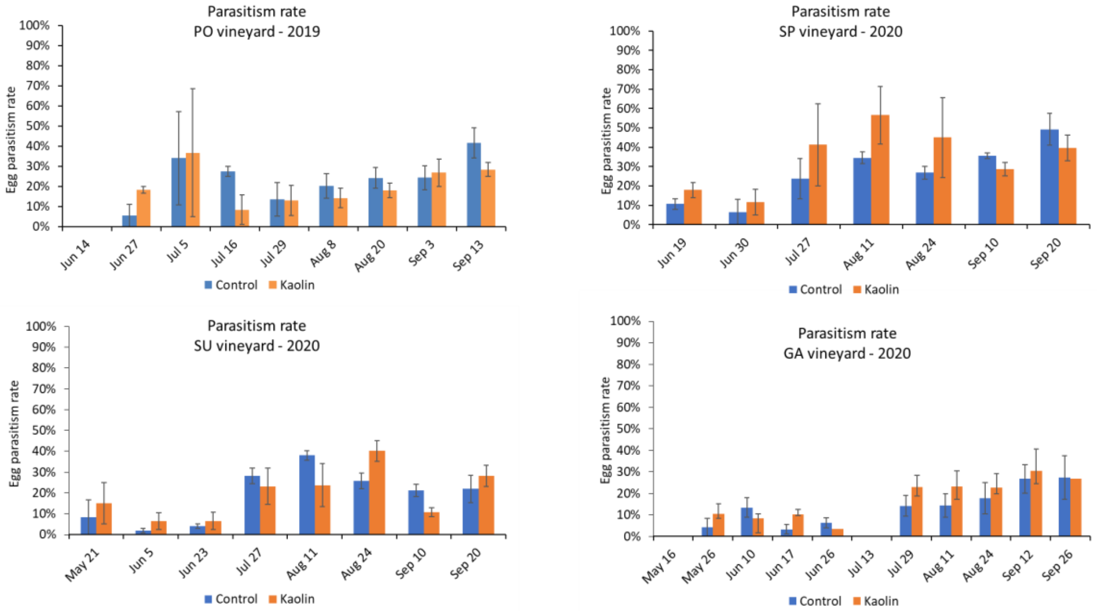

Figure 11. Parasitism rate (mean ± std. err.) by *Anagrus* spp. observed in kaolin treated and untreated plots in vineyards under investigation. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Table 2. Results of linear mixed model on leafhopper parasitism rate observed in the investigated vineyards.

Vineyard	Effect	DF	F value	P value
PO – 2019	treatment	1, 18.2	0.6	0.4489
PO – 2019	time	7, 27.1	2.92	0.0206
PO – 2019	time*treatment	7, 27.1	0.2	0.9828
SP – 2020	treatment	1, 15.6	1.45	0.2463
SP – 2020	time	6, 34.4	2.92	0.0206
SP – 2020	time*treatment	6, 34.4	0.42	0.8603
SU – 2020	treatment	1, 23.3	0.08	0.7818
SU – 2020	time	7, 41.6	8.92	<0.0001
SU – 2020	time*treatment	7, 41.6	0.81	0.5859
GA – 2020	treatment	1, 17.3	3.07	0.0976
GA – 2020	time	8, 41.9	4.35	0.0007
GA – 2020	time*treatment	8, 41.9	0.6	0.7734

Side-effects of kaolin on predatory mites

PO vineyard (2019)

Predatory mites belonging to the species *Kampimodromus aberrans* (Oudemans) were commonly detected in this vineyard with significant variation during time (F= 6.25; df= 9, 59.2; P< 0.0001; Figure 12). Predatory mite densities were not affected by kaolin applications (F= 0.01; df= 1, 59.2; P= 0.920) nor by the interaction treatment*time (F= 1.37; df= 9, 59.2; P= 0.225).

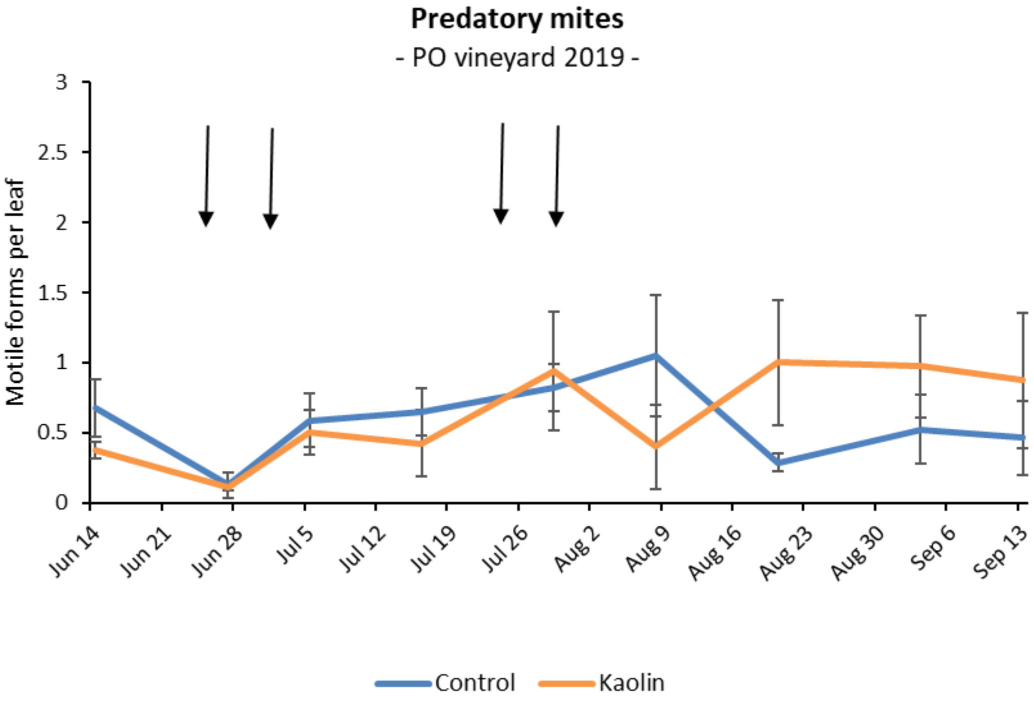

Figure 12. Seasonal abundance of predatory mites (mean ± std. err.) observed in kaolin treated and untreated plots in PO vineyard during 2019**.** Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

SP vineyard (2020)

SP vineyard was also colonised by *K. aberrans*. Kaolin applications did not affect predatory mite numbers (F= 2.65; df= 1, 50; P= 0.110) but the interaction time*treatment was significant (F= 3.36; df= 7, 50; P= 0.005): predatory mite densities appeared to be lower on kaolin treated plots for most of sampling dates but the opposite situation emerged in late season (Figure 13).

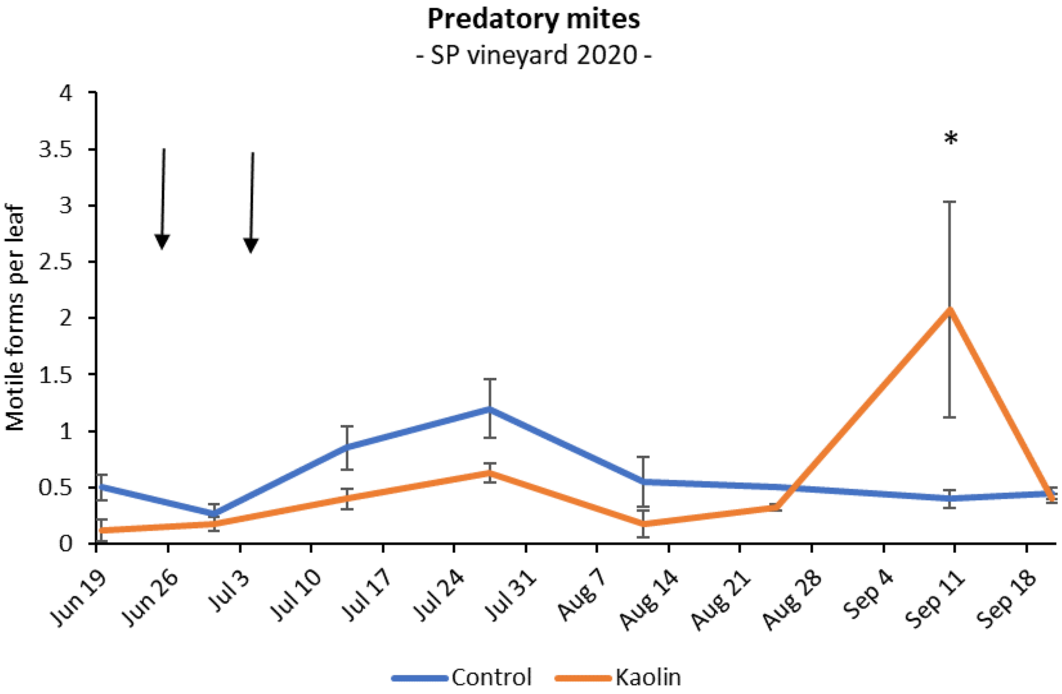

Figure 13. Seasonal abundance of predatory mites (mean ± std. err.) observed in kaolin treated and untreated plots in SP vineyard during 2020. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

SU vineyard, 2020

SU vineyard was colonised by *K. aberrans*. Kaolin applications reduced predatory mite numbers (F = 11.92; df = 1, 21.4; P = 0.002), and their densities were lower as compared to the control, in particular from May to July (time: F = 30.18; df = 9, 58.2; P < 0.0001; time*treatment: F = 2.81; df = 9, 58.2; P = 0.008; Figure 14).

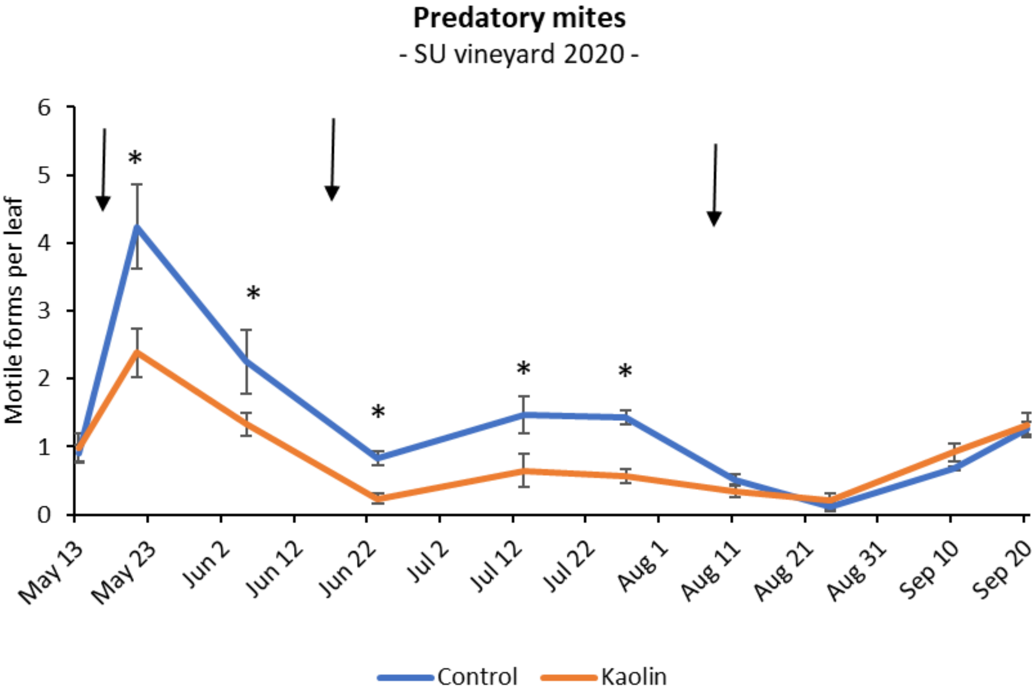

Figure 14. Seasonal abundance of predatory mites (mean ± std. err.) observed in kaolin treated and untreated plots in SU vineyard during 2020. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

GA vineyard (2020)

Amblyseius. Andersoni (Chant) and *K. aberrans* colonised GA vineyard. The effects of treatment, time and their interaction were significant (respectively: F = 9.43; df = 1, 26; P = 0.005; F = 16.73; df = 10, 88.9; P < 0.0001; F = 2.71; df = 10, 88.9; P = 0.006). Kaolin applications reduced predatory mite densities, but this effect was clear from mid-summer onwards (Figure 15).

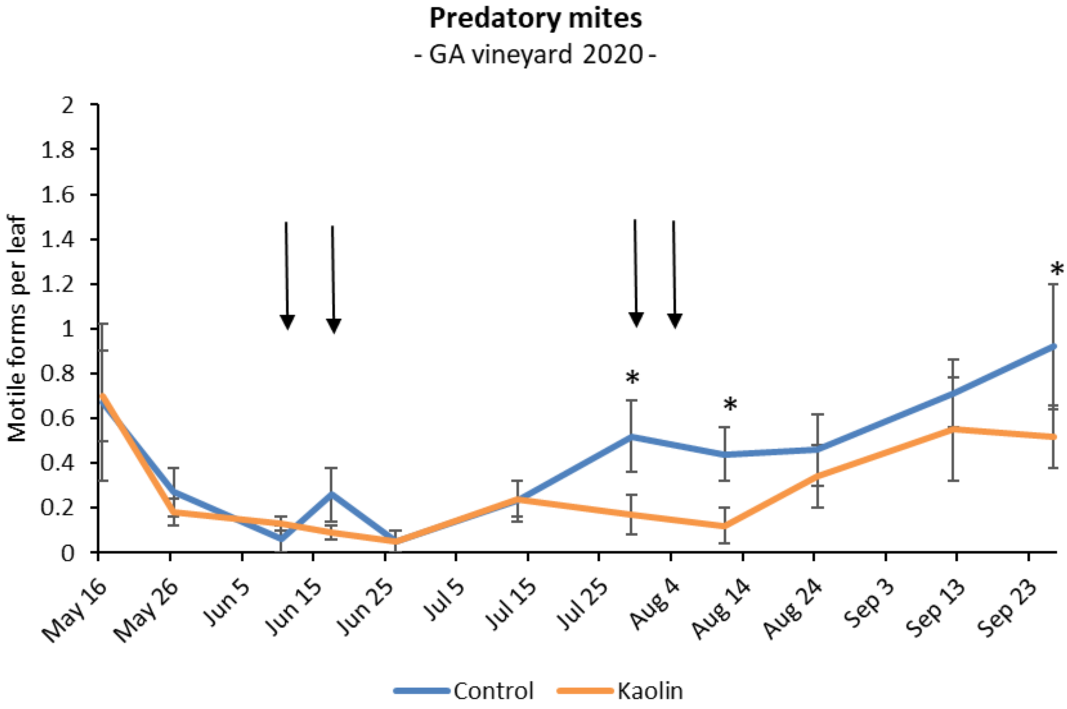

Figure 15. Seasonal abundance of predatory mites (mean ± std. err.) observed in kaolin treated and untreated plots in GA vineyard during 2020. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

GA vineyard (2021)

Kaolin applications did not affect predatory mite numbers. Only the effect of time was significant (F= 4.61; df= 8, 43.9; P= 0.0004), in contrast with the effects of treatment (F= 0.09; df= 1, 9.57; P= 0.770) and the interaction treatment*time (F= 0.95; df= 8, 43.9; P= 0.490) (Figure 16).

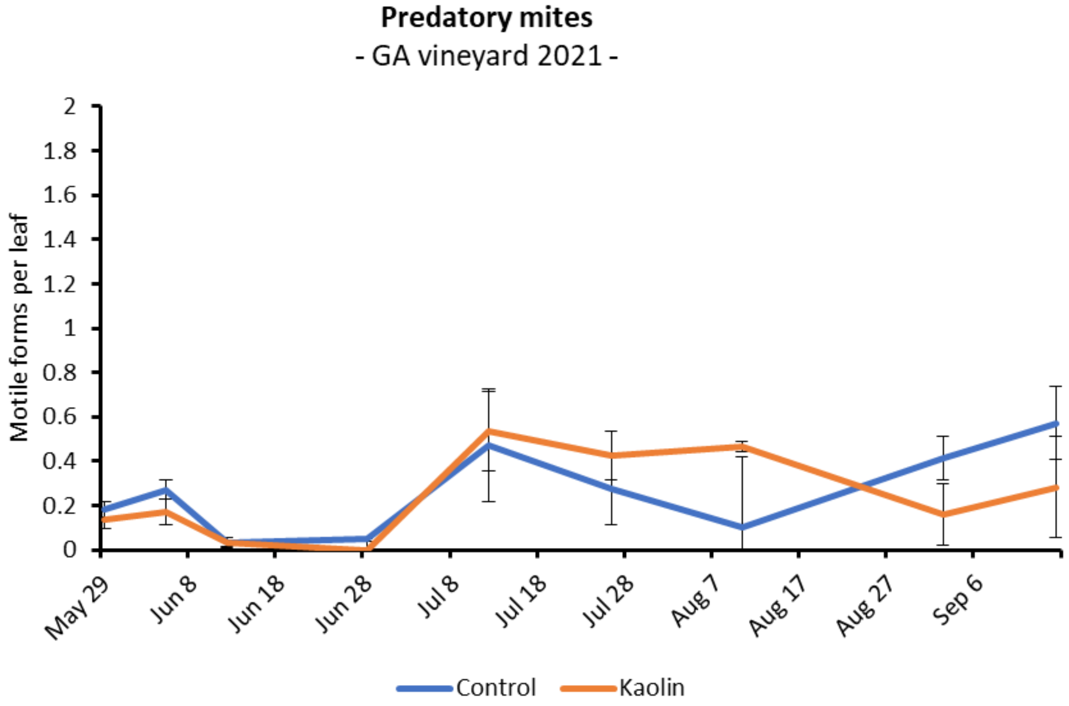

Figure 16. Seasonal abundance of predatory mites (mean ± std. err.) observed in kaolin treated and untreated plots in GA vineyard during 2021. Asterisks indicate significant differences at the *t-test* (α = 0.05) on the least square mean for the same sampling dates.

Discussion

The present study shows that particle film technology applications based on kaolin treatments are useful for managing the leafhopper *E. vulnerata*. These results align with preliminary research on the same (Tirello *et al*., 2021), and other grapevine pests (Tacoli *et al*. 2017a, 2017b). In all the experiments, kaolin application significantly reduced *E. vulnerata* nymph densities on grapevine plants. In our trials, kaolin was applied two to four times starting from mid-May until the beginning of August, and in all cases, a significant reduction of nymph numbers was observed. Reduction in nymph density was observed during the application period of kaolin but also for more than a month later. In this regard, SP and SU vineyards represent two interesting case-studies. In both vineyards, kaolin's effect emerged, particularly when nymph populations peaked in the control. Our results suggest that kaolin affects the nymphs directly and hampers the oviposition by *E. vulnerata* adults*.* Previous studies on the effect of kaolin on leafhopper-infesting grapevines showed an increase in nymph mortality, and the primary mechanism was a reduction in their feeding activity (Tacoli *et al*., 2017a and b; Tirello *et al*., 2021) while an effect on oviposition has been observed on the glassy-winged sharpshooter *Homalodisca vitripennis* Germar (Puterka *et al*., 2003; Tubajika *et al*., 2007). Moreover, kaolin-based particle film technology can create a persistent barrier (Sharma *et al*., 2015) that can interfere with the activity of *E. vulnerata* nymphs over a prolonged period. Particle films can also act as pest repellent (Sharma *et al*., 2015; Puterka *et al*., 2000; 2003), constituting an additional mechanism determining the effects on *E. vulnerata* nymphs. The positive effects observed in terms of reducing the nymph's population were also observed on *E. vulnerata* adults, but this effect was not constant across experiments. Indeed, a significant reduction of *E. vulnerata* adults was observed in two experiments, but the captures of *E. vulnerata* adults tend to be lower in kaolin-treated plots than in control. A reduced effect on adults is likely associated with the greater mobility of these life stages and their capacity to move between treated and untreated, attracted by yellow sticky cards. It should be stressed that despite the inconstant effect of kaolin applications on adult numbers, the infestation level measured by the number of nymphs per leaf was always reduced. However, due to the higher mobility of the adult stages and the promising results of these trials, the efficacy of kaolin on adult stages should be evaluated on bigger plots to prevent edge effects.

The use of kaolin was not associated to a reduction of the parasitism of leafhopper eggs. *Erasmoneura vulnerata* was completely dominant over native leafhoppers (> 95% in all vineyards considering nymphs) and thus we can assume that most of parasitism refers to this species. Therefore, the impact of Mymarids on this leafhopper was not disrupted by kaolin applications. Leafhopper eggs were constantly parasitized by *Anagrus* spp*.,* with a parasitism level reaching a maximum of about 55% in the SP vineyard in August 2020 in kaolin-treated plots, highlighting the possible integration of particle film technology with biological control that can be based on conservative and augmentative strategies (Duso *et al*., 2020a; Prazaru *et al*., 2021; Zanettin *et al*., 2021).

The impact of kaolin on predatory mites was also not dramatic. Only two out of five experiments observed a significant reduction in beneficial mite populations. The reduction was about 40% in SU vineyard and about 27% in GA vineyard (2020) and seems associated to repeated kaolin applications. Indeed, in both the vineyards where kaolin affected predatory mites, its applications lasted until August. The moderately harmful effect of kaolin on predatory mites inhabiting grapevines is not new and was associated with reduced fecundity (Tacoli *et al*., 2017a). Kaolin was also associated with the reduction of spider mites (Knight *et al.,* 2001; Lalancette *et al.,* 2005; Arbabi *et al.,* 2020), thus the reduction of prey may potentially impact predatory mites. It should be noted that in our experiments the presence of spider mites was never observed. However, in the case of generalist predatory mites (*sensu* McMurtry *et al*. 2013) that are the largely dominant species in European vineyards (Duso *et al*., 2012), these negative effects could be mitigated by habitat management practices that promote the availability of non-arboreal pollen (Pozzebon *et al*., 2014; Zanettin *et al*., 2021; Malagnini *et al*., 2022).

The use of kaolin in viticulture also has positive effects on plant physiology. It can reduce the average temperature of the grapevine canopy by 4 to 6 °C, thus facilitating the maintenance of an efficient photosynthetic activity, reducing physiological damage and dehydrated berries (Frioni *et al*. 2019). Using kaolin is beneficial in reducing the incidence of high temperatures affecting the grapevine's physiological processes, such as the biosynthesis of anthocyanins, which are crucial for obtaining high-quality wines (Movahed *et al*. 2016). For these advantages, the use of kaolin in viticulture is increasing.

In conclusion, the application kaolin-based particle film technology represents a valid tool for the management of *E. vulnerata* that can be used in integrated pest management strategies. This technology is up-and-coming for organic agriculture, which was demonstrated to be more efficient than available tactics (Tirello *et al*., 2021). Moreover, kaolin application can have positive effects for the management of other grapevine pests and the improvement of physiological processes that enhance grapevine production. These aspects fulfill the requirement of EU targets to improve sustainability in agricultural production.

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Chapter 4

Effect of leaf removal and insecticide applications on population densities of leafhoppers and mites associated to grapevines

Manuscript in preparation as: Effect of leaf removal and insecticide applications on population densities of leafhoppers and mites associated to grapevines

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Prazaru Stefan Cristian contributed to data collection, performed statistical analysis, and the manuscript drafting.

Abstract

In this study, we tested the effects of leaf removal, insecticide application and their interaction on leafhoppers, phytophagous and predatory mites occurring in vineyards. Leaf removal was carried out on the fruit-zone using a two-heads pulsed air leaf remover, while insecticides were applied with a tunnel air-assisted sprayer. Insecticide application reduced leafhopper and predatory mite density levels. In a number of case-studies leaf removal reduced leafhopper and predatory mite densities. In a vineyard phytophagous mite populations increased some weeks after leaf removal and insecticide application. The impact of leaf removal on pests and beneficials in vineyards is discussed.

Introduction

About 650.000 ha of land is used for grapevine growing in Italy (Eurostat Statistics, 2022). More than 90% is devoted to wine grape production with a net worth of about 7.3 billion USD in terms of wine export in 2018 (Pomarici *et al*., 2021). In all the 20 regions of Italy grapevine cultivation also influences tourism (Torquati *et al*., 2015). Several factors affect grapevine cultivation ranging from insect pest infestations to diseases associated to plant pathogenic fungi and viruses. Grapevine protection practices have economic and environmental consequences. Challenging pathogens common to most grapevines in Italy as well as in most European countries include downy and powdery mildews, respectively *Plasmopara viticola* (Berk *et* Curt.) Berl. et de Toni and *Erysiphe necator* Schwein. Most of pesticide applications carried out in European vineyards are devoted to the control of these diseases (Pertot *et al*., 2017). Among insect pests we can mention grapevine moths, in particular *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera Tortricidae), because they cause direct damage to bunches and favor the proliferation of grey mould (*Botrytis cinerea*) (Fermaud and Giboulout, 1992; Pertot et., al 2017). Leafhoppers (Hemiptera Cicadellidae) are common components of grapevine fauna; the most common include *Empoasca vitis* (Göthe) which alters photosynthesis, transpiration rate, and mesophyll conductance (Candolfi *et al*., 1993), *Zygina rhamni* Ferrari and *Erasmoneura vulnerata* (Fitch) which are mesophyll feeders (Vidano, 1958; Duso *et al*., 2019). The most economically important pest affecting North Italian vineyards is the leafhopper *Scaphoideus titanus* Ball, the main vector of the phytoplasma associated to Flavescence dorée (Chuche and Thiery 2014). Mealybugs can reduce dramatically yield and are important vectors of grapevine viruses (Petersen and Charlers, 1997; Sforza *et al*., 2003; Cabaleiro and Segura, 2006; Charles and Froud, 2009; Daane *et al*., 2012). Finally, spider mites (Acari: Tetranychidae) can compromise the net photosynthesis reducing plant growth and bunch quality (Candolfi *et al*., 1991). To achieve the optimal qualitative and quantitative production standards, the use of insecticides in viticulture is intense and sometimes mandatory, e.g., insecticides against *S. titanus* in some Italian regions (Barba *et al*., 2006). However, the use of insecticides is undesired for environmental and human health concerns (European Commission, Farm to Fork Strategy).

Cultural practices may have a potential in mitigating the impact of grape diseases and pests in viticulture. A number of studies have demonstrated that leaf canopy management practices have a significant impact on diseases and pests in vineyards; therefore, they could help to reduce pesticide use (Chellemi and Marois 1992; English *et al*., 1993; Moyer *et al*., 2016). Chellemi and Marois (1992) conducted a two-year experiment and reported a significant reduction of berries infected by powdery mildew (from 15.3% to 4.2%) in plots where basal leaves were removed in the first experimental year; this reduction doubled in the second year. Leaf removal combined with three fungicide applications obtained a satisfactory control of damaged fruits as compared with plots receiving 11-12 fungicide applications. English *et al*. (1993) showed that leaf removal reduced the incidence of Botrytis bunch rot up to 47%. Moyer *et al*., (2016) has demonstrated that the application of leaf removal integrated with biopesticides enhanced the efficacy of the latter. In other studies, the positive effects of leaf removal in controlling diseases and increasing grape quality have been extensively documented (Gubler *et al*., 1987; Percival *et al*., 1994; R'Houma *et al*., 1998; Valdéz-Gomez *et al*., 2008; Diago et., 2010).

The impact of leaf removal on grapevine arthropod pests has been less explored. Literature has focused on the effects of this technique on *L. botrana* populations and consequently on the reduction of *B. cinerea* spread in vineyards. Vartholomaiou *et al*. (2008), in a two-year experiment, found that shoot and/or leaf removal had a negative impact on *L. botrana* density. Summer pruning significantly reduced *L. botrana* infestation levels in grapevines under investigation in both years, compared to control plots (Vartholomaiou *et al*., 2008). Pavan *et al*. (2016) applied leaf removal 10 days before the start of the second-generation flight of *L. botrana* and found that larval infestation declined by about 50%. A positive effect was also recorded on the third generation of *L. botrana* (Pavan *et al*., 2016). Kiaeian *et al*. (2018) demonstrated that the increase in temperature due to sunlight exposure associated with bunch-zone leaf removal reduced the infestation of the European grapevine moth (Kiaeian *et al*., 2018). Tacoli *et al*. (2019b) compared the effects of leaf removal, kaolin and *B. thuringensis* on the moth population. Leaf removal, kaolin and Bt application resulted in a significant decline in the moth infestation level compared to the control. Although Bt was the most effective, the efficacy of Bt and kaolin when combined with leaf-removal was similar (Tacoli *et al*., 2019a).

In San Joaquin Valley (California, USA) vineyards, leaf removal proved to be an effective strategy to eliminate early season insecticide applications against leafhoppers, with positive implications for beneficial insects, when this practice was applied to contrast bunch rot (Stapleton *et al*., 1990). In contrast, Tacoli *et al*. (2017), testing the effect of kaolin spraying on different canopy zones combined or not with leaf-removal in bunch zone, did not find any effect of the latter mentioned practice on *E. vitis* population densities neither when combined with kaolin application (Tacoli *et al*., 2017).

The effects of cultural practices on beneficials have been less investigated. A study conducted by Prishmann *et al*. (2006) reported no effects of leaf removal on predatory mite population densities (Prishmann *et al*., 2006). In another study, leaf removal initially decreased phytoseiid numbers but this effect was not irreversible: predatory mite populations recovered to acceptable levels at the end of the season also in plots where leaf-removal and kaolin treatments were combined (Tacoli *et al*., 2019b).

In this study we investigated the effect of mechanical fruit-zone leaf removal, combined or not with insecticides, on the population densities of leafhoppers and mites occurring in two vineyards during three growing seasons

Materials and Methods

Experimental sites

This study was performed during the 2019, 2020 and 2021 growing seasons in two conventional farms located in the province of Treviso, Veneto region, North-eastern Italy. The first farm (A) is in Mogliano Veneto and the second (B) in Susegana municipalities. In 2019 the trials were conducted only in the farm A, in 2020 in both farms, in 2021 only in the farm B. All trials were carried out on Glera cultivar. In farm A, grapevines were trained with VSP (Vertical Shoot Position trellis) system while in farm B with the Sylvoz system. Vineyards having a surface of about 3 hectares (farm A) and about 4 hectares (farm B) and planted, respectively, in 2011 and 2012 were considered for trials.

Experimental design

A factorial experimental design was applied. The factors considered were mechanical leaf removal (LR) and insecticide application (T) obtaining four treatments: 1. No leaf-removal, no insecticides (CTRL-NT), 2. No leaf-removal, insecticides (CTRL-T), 3. Leaf-removal, no insecticides (LR-NT), 4. Leaf-removal, insecticides (LR-T). Each treatment comprised four replicates of about 50 vines. In both vineyards, leaf removal was carried mechanically using a two-heads pulsed air leaf remover (OLMI, Mod. Two head bilateral, Castiglione d'Asti, Italy). The forward speed was about 5 km/h and 0.8 bar operating pressure. The leaf removal was carried out as close as possible to the peak of the first generation of *E. vitis*, in order to reduce the total amount of laid eggs and the second leafhopper generation. Insecticide application was done using a tunnel air-assisted sprayer Model "Drift Recovery", Friuli, Agricolmeccanica, Udine, Italy. Insecticide applications were always performed after leaf removal (Table 1). Leaf removal and insecticide application were carried out based on weather conditions (no rain at least for 2 days before and after treatments) and machines availability.

Year	**2019**		**2020**		**2021**	
Farm	**Leaf removal**	**Insecticide application**	**Leaf removal**	**Insecticide application**	**Leaf removal**	**Insecticide application**
A	19 Jun 2019	20 Jun 2019 Tau-fluvalinate (30ml/hl)	16 Jun 2020	17 Jun 2020 Tau-fluvalinate (30ml/hl)	-	-
B	-	-	18 Jun 2020	18 Jun 2020 Acetamiprid (150ml/hl)	2 Jul 2021	2 Jul 2021 Acetamiprid (150ml/hl)

Table 1. Dates of leaf removal and insecticide applications in the two farms and the three growing seasons

Sampling

Leafhoppers and mites (phytophagous and predators) samplings were conducted before and after leaf removal and insecticide application. A total of 40 leaves per treatment (10 leaves per replicate) were collected from the fruit-zone every approximately 14 days, from June to August. Each leaf was immediately checked for leafhoppers and all the individuals were counted and eliminated. Subsequently the leaves were inserted into a bag and transferred to the laboratory to check the abundance of grapevine arthropods under a dissecting microscope.

Point Quadrat Analysis (PQA)

In order to determine the absence of differences in canopy structure before mechanical leaf removal and the presence of differences after mechanical leaf removal, a Point Quadrat Analysis was conducted. Canopy insertions were done with a rigid steel rod horizontally channelled into the canopy ten times through holes drilled at 10 cm intervals on a 1-meter wooden rod. For each treatment, the pipeline was repeated four times at randomly chosen position throughout the vineyard at fruit-zone of the canopy. For each insertion, contacts with leaves and clusters were annotated as well as canopy gaps. Leaf layer number (LLN), fraction of canopy gaps (%CG) and fraction of interior leaves (%IL) were then calculated according to Smart and Robinson (1991) (Smart, 1991). PQA was made on the same dates of leaf sampling before and after leaf removal.

Statistical analyses

Data were analyzed using a repeated measures linear mixed model with the MIXED procedure of SAS® (ver. 9.4; SAS Institute Inc., Cary, NC, USA). Data obtained from each farm were analyzed separately. The number of insects or mites per leaf was considered as a response variable with repeated measures made at different times. Leaf removal, Insecticide application, sampling time, and interaction were considered sources of variation in the model and tested using an *F-test* ($\alpha = 0.05$). Multiple comparisons of the abundance of insects or mites on different treatments were performed using *t*-test ($\alpha = 0.05$) on the least-square means. The degrees of freedom were estimated with the Kenward–Roger method, which can calculate non-integer values for error terms. Before the analysis, data were checked for model assumptions. The model was run on data transformed to log (n + 1), while untransformed data are shown in the figures. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods. Regarding, Point Quadrat data: Leaf layer number (LLN), fraction of canopy gaps (%CG) and fraction of interior leaves (%IL) on the canopy were considered as response variables and were analyzed using analysis of variance (ANOVA) with the MIXED procedure of SAS and differences among treatments were evaluated with an *F*-test ($\alpha = 0.05$).

Results

Farm A – 2019

The leafhoppers found in leaf samples were represented by nymphs of *E. vitis* and *Z. rhamni* but *E. vitis* was clearly dominant (more than 90% of the total individuals). Anyway, their densities were relatively low and thus the total number of leafhoppers was considered in the statistical analyses (Table 2). No differences were found before leaf removal and insecticide application (F = 0.03; d.f. = 1, 12; *p* = 0.876). Later, leafhopper densities were reduced by the insecticide application while the effect of leaf removal was not significant (Figure 1; Table 2). The effect of time and the interaction time*insecticide were significant as leafhopper population densities showed remarkable fluctuations over the growing season (Figure 1; Table 2).

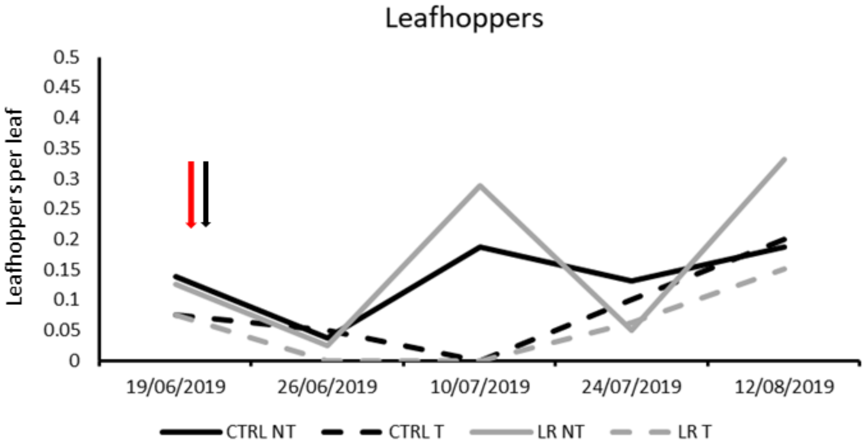

Figure 1. Seasonal abundance of leafhoppers in treatments under comparison in Farm A during 2019 (the red arrow indicates leaf-removal application, the black arrow insecticide application).

Table 2. Results of the F-test for leafhoppers occurring in Farm A during 2019.

Effect	DF num	DF den	F value	P-value
Time	**5**	**69**	**18.66**	**<.0001**
Insecticide	**1**	**69**	**6.61**	**0.012**
Leaf removal	1	69	0.99	0.323
Insecticide*Leaf removal	1	69	1.77	0.188
Time*Insecticide	**5**	**69**	**7.10**	**<.0001**
Time*Leaf removal	5	69	2.32	0.052
Time*Insecticide*Leaf removal	5	69	1.04	0.395

Mite communities were represented mostly by predatory mites belonging to the family Phytoseiidae, in particular *Amblyseius andersoni* (Chant) and *Kampimodromus aberrans* (Oudemans). The total number of phytoseiid mites was considered in the statistical analyses (Table 3). No differences among treatments were found before leaf removal and insecticide application (F = 0.06; d.f. = 1, 12; *p* = 0.817). Then, leaf removal and insecticide application did not affect phytoseiid numbers. Only the effect of time was significant (Table 3).

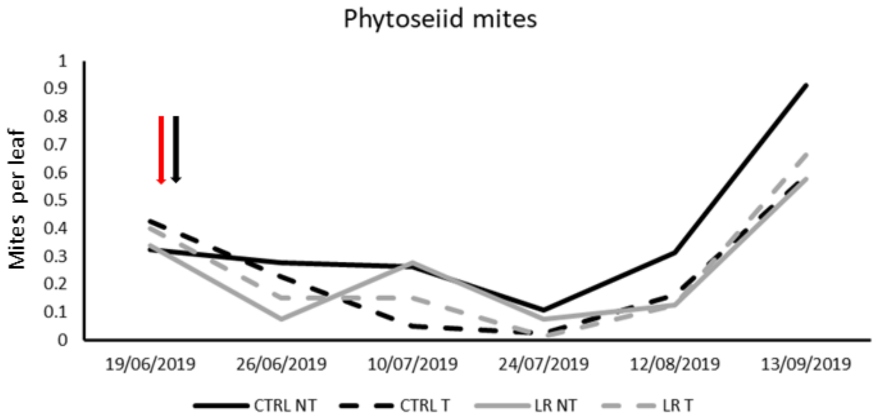

Figure 2. Seasonal abundance of phytoseiid mites in treatments under comparison in Farm A during 2019 (the red arrow indicates the leaf-removal application, the black arrow insecticide application).

Table 3. Results of the *F*-test on phytoseiid mites occurring in Farm A during 2019.

Effect	DF num	DF den	F value	P-value
Time	**5**	**72**	**22.34**	**<.0001**
Insecticide	1	72	3.35	0.071
Leaf removal	1	72	2.55	0.115
Insecticide*Leaf removal	1	72	2.96	0.089
Time*Insecticide	5	72	1.33	0.260
Time*Leaf removal	5	72	0.89	0.492
Time*Insecticide*Leaf removal	5	72	0.53	0.755

Farm A – 2020

Among leafhoppers, *E. vitis* confirmed to be the most frequent species while a few *Z. rhamni* individuals were found. Their total numbers were considered in statistical analyses. No differences were found before leaf removal and insecticide application (F = 3.12; d.f. = 1, 12; $p = 0.103$). The effects of leaf removal, and insecticide application were significant but not their interaction. The effect of time was also significant. Both insecticide application and leaf removal reduced significantly leafhopper densities (Table 4; Figure 3).

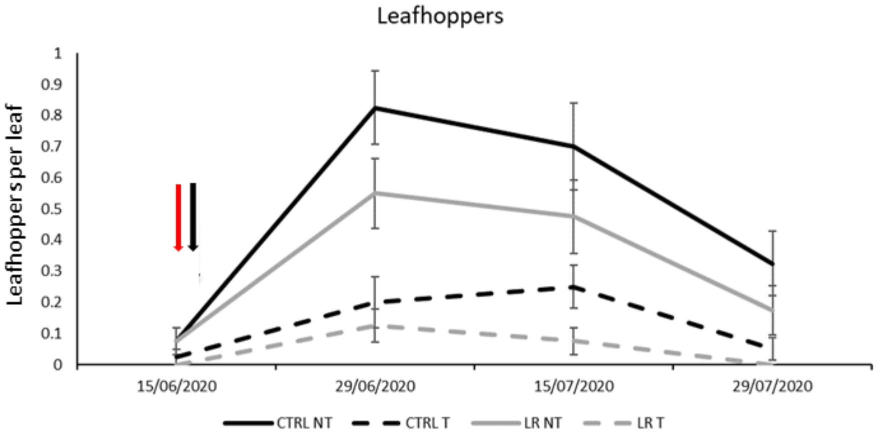

Figure 3. Seasonal abundance of leafhoppers in treatments under comparison in Farm A during 2020 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 4. Results of the *F*-test for leafhoppers occurring in Farm A during 2020.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**27.10**	**<.0001**
Insecticide	**1**	**48**	**76.77**	**<.0001**
Leaf Removal	**1**	**48**	**11.91**	**<.0001**
Insecticide*Leaf Removal	1	48	1.32	0.256
Time*Insecticide	**3**	**48**	**8.55**	**<.001**
Time*Leaf Removal	3	48	1.43	0.246
Time*Insecticide*Leaf Removal	3	48	0.45	0.721

Amblyseius andersoni and *K. aberrans* confirmed to be the most frequent predatory mites and their total numbers were considered in the statistical analysis. No differences among treatments were observed before leaf removal and insecticide application (F = 1.70; d.f. = 3, 12; *p* = 0.177). Then, the effect of time, leaf removal, and insecticide application were significant (Table 5). The interaction time*insecticide application was also significant. Both leaf removal and insecticide application reduced predatory mite densities (Figure 4; Table 5).

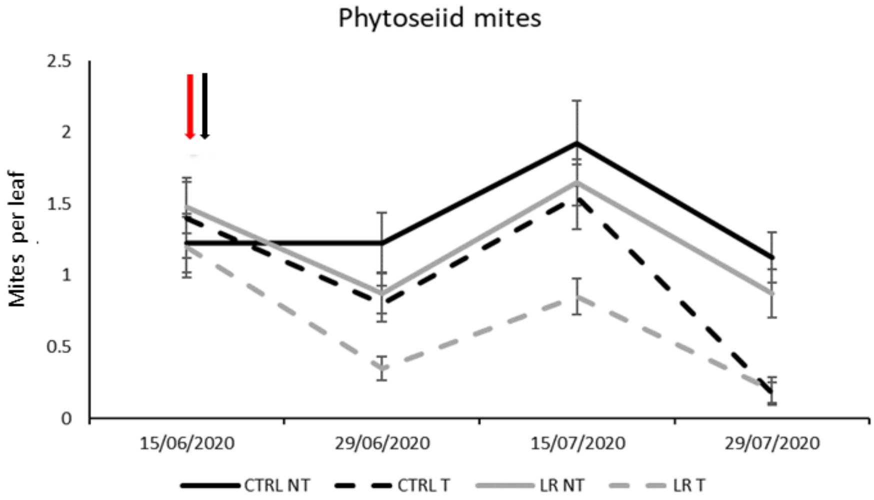

Figure 4. Seasonal abundance of phytoseiid mites in treatments under comparison in Farm A during 2020 (the red arrow indicates the leaf-removal application, the black arrow insecticide application).

Table 5 Results of the *F-test* on phytoseiid mites occurring in Farm A during 2020.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**17.20**	**<.0001**
Insecticide	**1**	**48**	**28.87**	**<.0001**
Leaf removal	**1**	**48**	**5.09**	**0.029**
Insecticide*Leaf removal	1	48	0.81	0.372
Time*Insecticide	**3**	**48**	**2.99**	**0.040**
Time*Leaf removal	3	48	1.04	0.385
Time*Insecticide*Leaf removal	3	48	0.65	0.587

In Farm A, before leaf removal, the means for leaf layer number (LLN) and internal leaves (IL) were not significantly different with values of about 6 and 68% for LLN and IL, respectively (Table 6). Leaf removal performed on June 15 caused substantial reduction in canopy density as reported for point quadrat derived parameters that significantly differed compared to the control (CTRL). Two weeks later, at end of June, a significant reduction of about 2.3 leaf layers and a decrease of about 5% of the fraction of IL was observed. One month later, at the end of July, the differences in LLN and fraction of IL between LR and CTRL treatments were maintained and even increased with a reduction of about 3.3 and 17.5% for LLN and fraction of IL, respectively. While LR was observed significantly impacting canopy density, both the two treatments did not show any difference in the fraction of canopy gaps (CG) with null values observed on all the sampling dates throughout the season.

Table 6. Results of the *F-test* for the LR effect on canopy density parameters on Farm A during 2020.

Site	Year	Date	Treatment	LLN	CG (%)	IL (%)
(A)	2020	15-Jun	CTRL	5.98	0.00	67.87
			LR	6.11	0.00	67.80
			F value	0.047	-	0.0005
			P-value	0.8332	-	0.983
		29-Jun	CTRL	7.38	0.00	72.69
			LR	5.08	0.00	66.86
			F value	**24.28**	-	**10.97**
			P-value	**0.0026**	-	**0.016**
		15-Jul	CTRL	5.8	0.00	67.01
			LR	4.75	0.00	58.25
			F value	2.92	-	2.43
			P-value	0.1383	-	0.170
		29-Jul	CTRL	7.78	0.00	74.59
			LR	4.48	0.00	57.08
			F value	**27.72**	-	**44.30**
			P-value	**0.0019**	-	**0.0006**

CTRL = no leaf removal treatment; LR = leaf removal; LLN = Leaf layer number; CG= fraction of canopy gaps; IL = fraction of interior leaves.

Farm B – 2020

In this vineyard *E. vitis* was still the most frequent species and just a few individuals of *Z. rhamni* were detected. The total leafhoppers densities were low and thus their total numbers were considered in the analysis of data (Table 7). No differences among treatments were found before leaf removal and insecticide application (F = 1; d.f. = 3, 12; *p* = 0.426). Later, insecticide application, time and their interaction resulted in significant differences. Insecticide application reduced significantly leafhopper densities (Figure 5, Table 7). Leaf removal appeared to reduce leafhopper numbers, but statistics was not significant (p = 0.057).

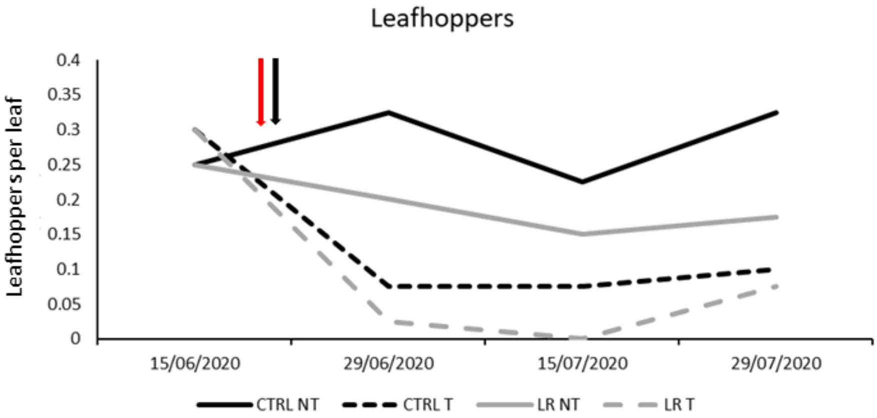

Figure 5. Seasonal abundance of leafhoppers in treatments under comparison in Farm B during 2020 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 7. Results of the *F-test* on leafhoppers occurring in Farm B during 2020.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**4.62**	**0.006**
Insecticide	**1**	**48**	**13.71**	**0.001**
Leaf removal	1	48	3.80	0.057
Insecticide*Leaf removal	1	48	0.61	0.439
Time*Insecticide	**3**	**48**	**3.25**	**0.029**
Time*Leaf removal	3	48	0.43	0.732
Time*Insecticide*Leaf removal	3	48	0.23	0.877

Both predatory and phytophagous mites occurred in the vineyard (Figures 6, 7). Among the former, *A. andersoni* and *K. aberrans* were detected and their total numbers were considered in the statistical analysis (Table 9). No differences were found before leaf removal and insecticide application (F = 1.28; d.f. = 3, 12; *p* = 0.326). Later, phytoseiid densities were reduced by insecticides but not by leaf removal (p = 0.053); the effect of time was also significant as phytoseiid densities changed over time (Table 8, Figure 6).

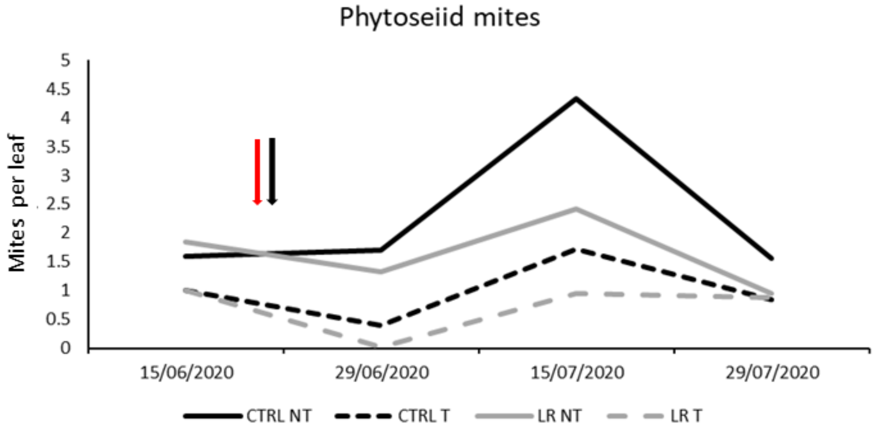

Figure 6. Seasonal abundance of phytoseiid mites in treatments under comparison in Farm A during 2020 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 8. Results of the *F-test* on phytoseiid mites occurring in Farm B during 2020.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**8.68**	**0.0001**
Insecticide	**1**	**48**	**5.45**	**<.0001**
Leaf removal	1	48	3.93	0.053
Insecticide*Leaf removal	1	48	0.02	0.901
Time*Insecticide	3	48	2.50	0.071
Time*Leaf removal	3	48	0.95	0.422
Time*Insecticide*Leaf removal	3	48	0.52	0.668

Phytophagous mites were represented by the spider mite *Panonychus ulmi (*Koch). No differences were found before treatment application (F = 3.36; d.f. = 3, 12; *p* = 0.055). The effect of leaf removal and time were significant as well as their interaction (Table 9). Higher *P. ulmi* numbers were found in the leaf removal plots compared to the control but in one date only (Figure 7). In contrast, insecticide application did not cause significant effects.

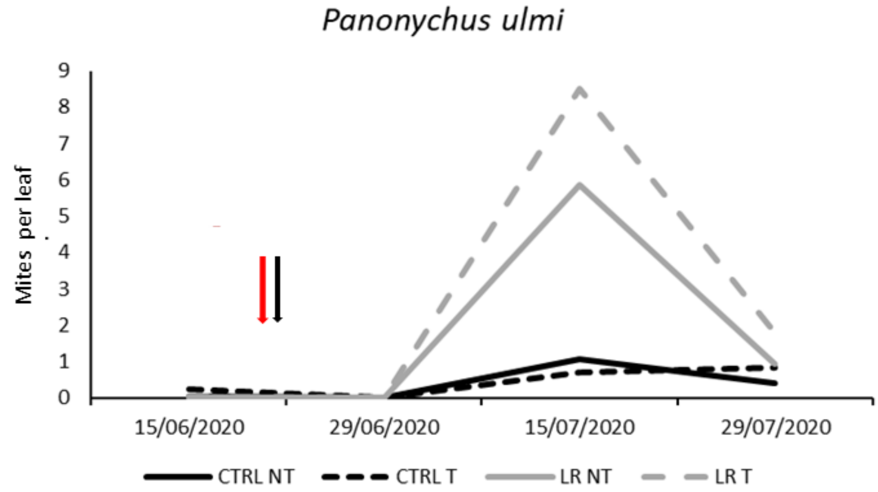

Figure 7. Seasonal abundance of *Panonychus ulmi* in treatments under comparison in Farm B during 2020 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 9. Results of the *F-test* for the *P. ulmi* on Farm B during 2020.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**30.39**	**<.0001**
Insecticide	1	48	2.35	0.133
Leaf removal	**1**	**48**	**15.50**	**<.001**
Insecticide*Leaf removal	1	48	0.49	0.486
Time*Insecticide	3	48	0.50	0.683
Time*Leaf removal	**3**	**48**	**10.12**	**<.0001**
Time*Insecticide*Leaf removal	3	48	0.65	0.589

Seasonal variations of point quadrat derived parameters in Farm B are reported in Table 10. As observed in Farm A, no differences were observed on canopy density parameters in mid-June (pre-LR) with mean values of 3.3-3.5 and 43.8-45.4% for LLN and fraction of IL, respectively. At the end of June, after leaf removal (June 15), a reduction in canopy density was observed with mean values of point quadrat derived parameters that significantly differed among treatments. A significant difference of about -1 and -14% of LLN and fraction of IL, respectively, was observed in LR against the CTRL. Two weeks later, on July 15, CTRL canopy showed more than doubled values of LLN and fraction of IL than LR treatment with values of +2.18 and +40% for LLN and %IL, respectively. A delayed secondary shoots development in the fruiting zone of the canopy probably occurred in LR canopies against CTRL, recovering leaf density with no differences in point quadrat derived parameters two weeks later in the last sampling date at the end of July. As observed for the vineyard trained as VSP in Farm A, even in Farm B with the Sylvoz system, no differences were observed in the fraction of CG among treatments while, differently from the higher LLN measured in both treatments in the VSP canopies, on Sylvoz system as increasing trend in fraction of canopy gaps was visible, even if not significant.

Table 10. Results of the *F-test* for the LR effect on canopy density parameters on Farm B during 2020.

Site	Year	Date	Treatment	LLN	CG %	IL %
(B)	2020	15-Jun	CTRL	3.35	5.00	43.81
			LR	3.53	0.00	45.38
			F value	0.45	3	0.267
			P-value	0.5275	0.1340	0.624
		29-Jun	CTRL	3.00	0.00	36.01
			LR	2.06	2.78	22.05
			F value	**16.94**	1.00	2.92
			P-value	**0.0062**	0.36	0.138
		15-Jul	CTRL	4.03	0.00	52.14
			LR	1.85	2.50	11.35
			F value	**142.81**	1.00	**93.95**
			P-value	**<0.0001**	0.36	**0.0001**
		29-Jul	CTRL	3.38	0.00	47.42
			LR	2.50	2.50	32.97
			F value	3.30	1.00	4.37
			P-value	0.1194	0.3559	0.081

CTRL = no leaf removal treatment; LR = leaf removal; LLN = Leaf layer number; CG= fraction of canopy gaps; IL = fraction of interior leaves.

Farm B – 2021

Regarding leafhoppers, no differences were found among treatments before leaf removal and insecticide application (F = 0.77; d.f. = 3, 12; p = 0.876). Later, only insecticide application exerted a significant impact upon leafhoppers densities (Figure 8; Table 11).

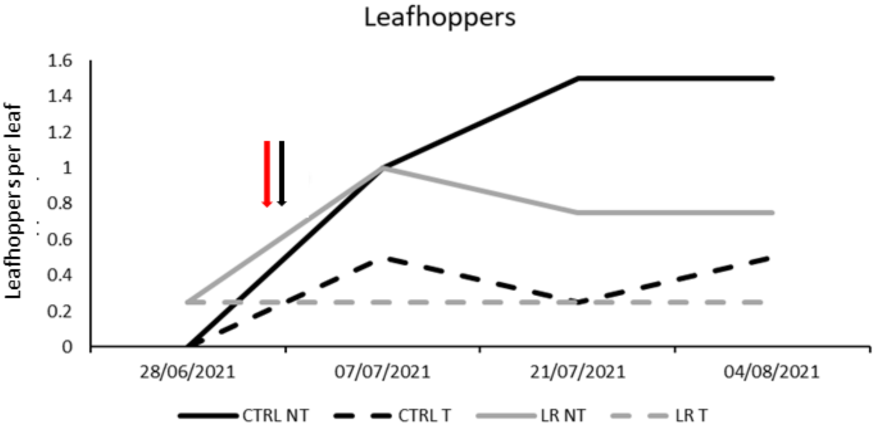

Figure 8. Seasonal abundance of leafhoppers in treatments under comparison in Farm B during 2021 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 11. Results of the *F-test* for the leafhoppers on Farm B during 2021.

Effect	DF num	DF den	F value	P-value
Time	3	48	5.82	0.100
Insecticide	**1**	**48**	**8.10**	**0.006**
Leaf removal	1	48	0.90	0.347
Insecticide*Leaf removal	1	48	0.40	0.530
Time*Insecticide	3	48	0.97	0.416
Time*Leaf removal	3	48	0.70	0.557
Time*Insecticide*Leaf removal	3	48	0.33	0.801

In the first part of the growing season mite communities were dominated by phytoseiid mites (*A. andersoni* and *K. aberrans*). No differences were found among treatments before leaf removal and insecticide application (F = 1.90; d.f. = 3, 12; *p* = 0.1425). Later, both leaf removal and insecticide application affected phytoseiid mite densities (Figure 9; Table 12).

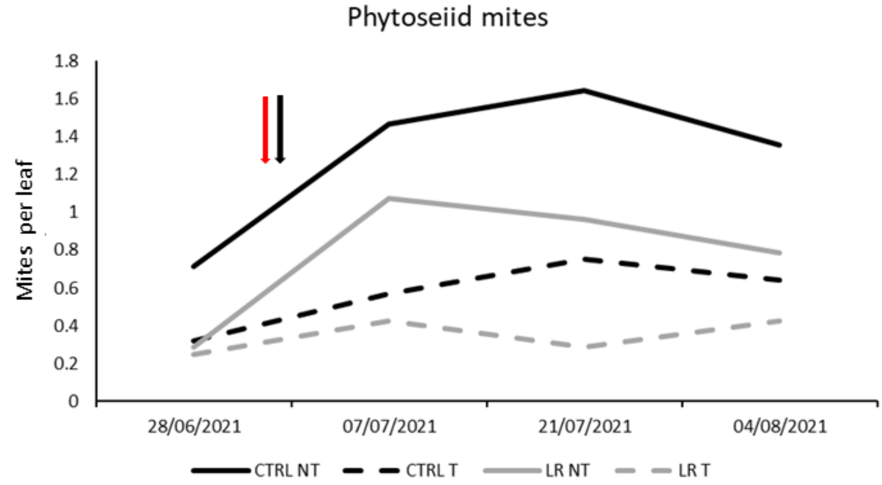

Figure 9. Seasonal abundance of phytoseiid mites in treatments under comparison in Farm B during 2021 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 12. Results of the *F-test* for the phytoseiid mites on Farm B during 2021

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**4.13**	**0.011**
Insecticide	**1**	**48**	**23.6**	**<.0001**
Leaf removal	**1**	**48**	**9.77**	**0.003**
Insecticide*Leaf removal	1	48	1.54	0.220
Time*Insecticide	3	48	1.26	0.297
Time*Leaf removal	3	48	0.39	0.790
Time*Insecticide*Leaf removal	3	48	0.02	0.995

Tetranychid populations (*P. ulmi*) increased over the growing season (Figure 10). No differences among treatments were found before leaf removal and insecticide application (F = 0.10; d.f. = 3, 12; *p* = 0.959). Later, the effect of the insecticide application and time were significant (Figure 10; Table 13) as tetranychid densities increased more in insecticide treated than in untreated plots (Figure 10; Table 13).

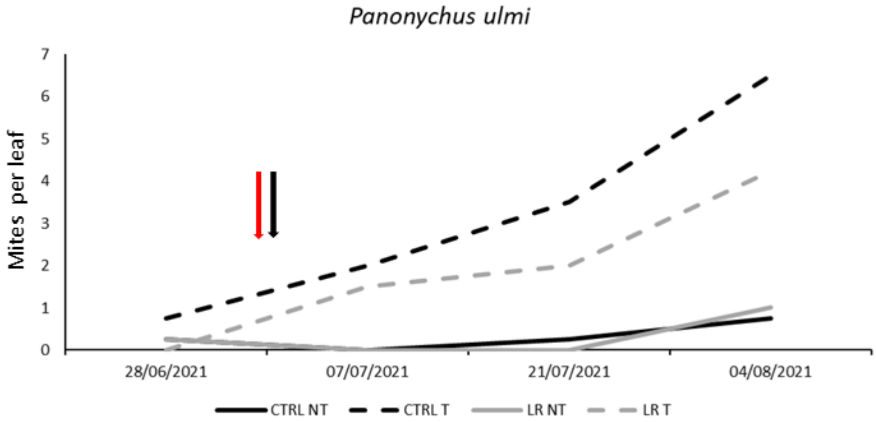

Figure 10. Seasonal abundance of *Panonychus ulmi* in treatments under comparison in Farm B during 2021 (the red arrow indicates the leaf-removal application, the black arrow indicates insecticide application).

Table 13. Results of the *F-test* for the *P. ulmi* on Farm B during 2021.

Effect	DF num	DF den	F value	P-value
Time	**3**	**48**	**6.18**	**0.001**
Insecticide	**1**	**48**	**21.19**	**<.0001**
Leaf removal	1	48	1.63	0.207
Insecticide*Leaf removal	1	48	1.63	0.207
Time*Insecticide	**3**	**48**	**3.48**	**0.023**
Time*Leaf removal	3	48	0.14	0.935
Time*Insecticide*Leaf removal	3	48	0.21	0.891

The results of the canopy density derived parameters between control and LR treatments obtained in Farm B in 2021 are reported in Table 14. As reported in the previous vintage, similar values of point quadrat derived parameters were measured in the Sylvoz system on June 28 before LR with mean values of about 3 and 35-45 % for LLN and fraction of IL, respectively, which did not differ among treatments. Upon LR was performed on July 2, a considerable canopy density reduction was observed on July 7 with mean values of about -1.5 and -22% for LLN and fraction of IL, respectively. Afterwards, LR treatment maintained a sparser canopy throughout the entire season until the last sampling date on August 4. While in CTRL treatment almost stable values were measured until August with LLN values of about 3.3-3.4 and values of fraction of IL of 40-45 %, after a substantial drop registered on July 7 and 21, a progressive leaf regrowth was observed in the fruiting zone of the LR canopy in terms of LLN and fraction of IL. Nonetheless, significantly different values were observed in LR treatment against CTRL one for LLN and IL point quadrat parameters until the last sampling date. No differences were observed for fraction of CG among treatments in all the sampling dates.

Table 14. Results of the *F-test* for the LR effect on canopy density parameters on farm B during 2021.

Site	Year	Date	Treatment	LLN	CG %	IL %
(B)	2021	28-Jun	CTRL	3.33	0.00	45.89
			LR	3.05	0.00	35.80
			F value	1.42	-	3.66
			P-value	0.2779	-	0.104
		7-Jul	CTRL	3.425	0.00	45.71
			LR	1.88	2.50	23.62
			F value	**40.32**	1.00	**28.39**
			P-value	**0.0007**	0.3559	**0.002**
		21-Jul	CTRL	3.35	0.00	40.35
			LR	1.55	0.00	16.07
			F value	**22.09**	-	**10.07**
			P-value	**0.0033**	-	**0.019**
		4-Aug	CTRL	3.45	0.00	47.62
			LR	2.8	0.00	37.18
			F value	**9.57**	-	**11.49**
			P-value	**0.021**	-	**0.015**

CTRL = no leaf removal treatment; LR = leaf removal; LLN = Leaf layer number; CG= fraction of canopy gaps; IL = fraction of interior leaves.

Discussion

Leafhopper densities were affected by insecticide applications in all the case-studies. Insecticide formulations were based on acetamiprid and tau-fluvalinate active ingredients, both considered effective against *E. vitis* and other leafhoppers in previous investigations (Duso *et al*., 2019; Wei *et al*., 2015; Yadav *et al*., 2015; Prabhavathi *et al*., 2016; Hemadri, 2018). Our results are consistent with those of findings reported in other areas and suggest that the populations considered in this study did not evolve resistance to these compounds.

The effect of leaf removal on leafhoppers was significant only in one out of four case-studies and in this case leaf removal reduced leafhopper population densities. A similar trend was observed in another trial, but statistics did not fully support this effect (p = 0.057). The analyses performed on the canopy (except for the 2019 season) showed that leaf removal was applied correctly: leaf layer numbers and fractions of interior leaves significantly decreased in leaf removal plots. *Empoasca vitis* prefers to colonize leaves located inside the canopy (Smart *et al*., 1990; Kok, 2013; Duso *et al*., 2014; Schmidt-Jeffris *et al*., 2021; Shahbaz *et al*., 2019). Pavan and Picotti (2009) demonstrated that the number of eggs is proportional to leaf density and thus a negative effect of leaf removal on egg laying is expected. The lack of such evidence in some experiments could be due to the low population densities recorded. Moreover, Glera is a vigorous cultivar that produces an important canopy offering many shelter areas to leafhoppers, even above the fruit zone. To achieve a stronger effect on leafhopper population, leaf removal should be repeated but this is not possible due to the impact of this practice on the grape quality (English *et al*., 1989; Ani'c *et al*., 2021).

Insecticide applications affected predatory mite populations in three out four case-studies. In the remaining one, populations densities were very low. The impact of acetamiprid and tau-fluvalinate on predatory mites has been reported in a number of papers and our results are consistent with those published in these papers (Duso *et al.,* 2014; Shahbaz *et al.,* 2019; Schmidt-Jeffris *et al.,* 2021). Acetamiprid and tau-fluvalinate have been applied against *S. titanus*, the main vector of phytoplasmas associated to Flavescence dorée disease, and the number of insecticide applications is still increasing in North-eastern Italy. The side-effects of these and other insecticides on predatory mites can favour spider mite infestations. In two out of four case-studies *P. ulmi* occurred at moderate levels. In one of them, spider mites reached higher densities on insecticide treated plots than in the control.

Leaf removal was associated to a decline in predatory mite numbers in two out of four case-studies. Tacoli *et al.* (2019b) reported that phytoseiid mite populations occurring on leaf removal plots firstly decreased but then recolonized the canopy. Leaf removal practice decreases relative humidity levels inside the canopy (English *et al*., 1989; Ani'c *et al*., 2021) and predatory mites (in particular *A. andersoni*) require moderate to high relative humidity for moulting and egg hatching (Mori and Chant., 1966; Helle, 1985; Walzer *et al*., 2007; Gomez-Moya *et al*., 2018; Solano-Rojas *et al*., 2022). In a case-study, spider mites were more abundant in leaf removal plots. One can argue that factors inhibiting predatory mites (leaf removal) can favour their prey. However, in this case-study the negative effect of leaf removal on predatory mites was not fully significant (p = 0.057). The limited number of case-studies suggests that further investigations are needed to evaluate the effects insecticides and leaf removal on mites and especially their potential interactions.

Conclusions

Probably due to the limited leafhopper infestation level and the great impact of insecticide treatments on leafhoppers and predatory mites no significant interactions emerged in this study. Anyway, a clear tendency of reducing leafhoppers and predatory mites of leaf removal emerged, this has to be taken into consideration, especially in those areas where an increase in pest pressure requires an increase in insecticide use.

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Chapter 5

The importance of monitoring strategies of *Scaphoideus titanus* and appropriate pest control measures in fragmented viticultural areas

Manuscript in preparation as: The importance of monitoring strategies of *Scaphoideus titanus* and appropriate pest control measures in fragmented viticultural areas

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Abstract

Flavescence dorée (FD) is a grapevine quarantine disease that causes serious damage in European vineyards. The phytoplasma associated with FD is transmitted mainly by the leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). The monitoring of *S. titanus* populations is crucial to define its phenology and population levels, to establish the optimal timing of insecticide applications, and, to evaluate the efficacy of control strategies. At this purpose, in the territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG (Treviso district, Veneto Region), where severe FD outbreaks were reported in the last years, *S. titanus* populations (nymphs and adults) were monitored during two growing seasons (2021 and 2022). In four fragmented areas (small vineyards belonging to different owners) with a high incidence of FD, the spatial distribution of nymphs and adults during the same vegetative seasons was studied using SADIE. The phenology of *S. titanus* was anticipated in 2022 compared to 2021 due to higher temperatures. The population size in the two years was slightly different for nymphs, whereas a dramatic decline in adult populations was observed in the second year due to the use of pyrethroids instead of systemic insecticides, against nymphs. The study of spatial distribution showed patches and gaps of both nymphs and adults in the four areas. In 2021, when insecticides were only partially effective, nymph and adult patches were overlapping, whereas in 2022, when effective insecticides against nymphs were used, patches were poorly related. Overlaps were observed between the adult patches in late summer of 2021 and the nymph patches in spring of 2022, suggesting that adult monitoring in the previous year provides a good indication of the spatial distribution of nymphs in the following year. In one of the fragmented areas, high populations of *S. titanus* persisted for two subsequent growing seasons because in both years chemical control applied by a few winegrowers were not fully effective. FD must be managed at a territory level since, especially in fragmented viticulture, few infested vineyards can compromise what has been done in the well-managed vineyards.

Introduction

Flavescence dorée (FD) is a serious grapevine disease that affects European vineyards (Bradshaw *et al*., 2016; Jeger *et al*., 2016). This disease is associated with a phytoplasma vehiculated by *Scaphoideus titanus* Ball, a leafhopper originating from North America, first detected in France (Bonfils and Schvester 1960; Vidano, 1964, 1966) and now present in the most prestigious wine-growing areas of Europe (Chuche and Thiéry, 2014; Jeger *et al*., 2016; Mirutenko *et al*., 2018). *Scaphoideus titanus* is an univoltine species that overwinters as eggs; hatching occurs from mid-May until the end of June and adults emerge from late June onwards (Schvester *et al*., 1962; Vidano, 1964). The biology of *S. titanus* has been recently reviewed (Chuche and Thierry, 2014) but this topic still attracts the interest of specialists (Alma *et al*., 2018; Bocca *et al*., 2020).

Flavescence dorée was first observed in France in the Bordeaux region (Caudwell, 1957), subsequently it has spread all over Europe from Portugal to Romania (Chuche and Thiéry, 2014), and recently it has been detected in the Caucasian region (Gnezdilov and Orlov, 2022). FD strongly impacts both yields and grape quality, and frequently causes the death of vines. As no cure is available for infected plants, the most effective control strategy relies on planting of healthy grapevines, rouging of symptomatic ones and applying insecticides against *S. titanus* (Caudwell, 1981; Boudon-Padieu, 2003; Chuche and Thiéry, 2014; Ay and Gozlan, 2020; Mori *et al*., 2020; Bertaccini *et al*., 2022). In Italy, mandatory control measures such as insecticide applications against *S. titanus* and the rouging of symptomatic grapevines must be taken in areas where FD is present (D.M. n. 32442 of 31/05/2000). In some Italian wine-growing areas (e.g., Veneto region), the first FD emergence in the 1990s was successfully controlled by a strict application of these rules (Posenato *et al*., 1996, Sancassani *et al*., 1997; Bertaccini *et al*., 1998; Martini *et al*., 1999). In the subsequent years, FD status was less critical and control strategies were focused on insecticide applications based on vector monitoring in the context of IPM (Posenato *et al*., 2001; Sancassani *et al*., 2007; see also Bur n. 122, 25 ottobre 2019, Regione Veneto). Chemical control was mainly based on the availability of organophosphates, very effective against both grape berry moths and leafhoppers. In the 2000s, neonicotinoids, in particular thiamethoxam, became very popular in controlling *S. titanus* (Lessio *et al*., 2011; Žežlina *et al*., 2013). In 2013, the EU commission restricted the use of some neonicotinoids (including thiamethoxam) in order to provide mitigation measures for pollinators' protection and prohibited them in 2018 (Jactel *et al*., 2019; Medina *et al*., 2020). Organophosphates have been progressively banned in Europe (the last OPs were applied in viticulture in 2019). In the last years new outbreaks of FD have been recorded in the Veneto region as well as in other Italian and European areas. Various factors could be potentially involved, e.g., the role of multiple vectors (e.g., Casati *et al*., 2017; Jermini *et al*., 2019; Malembic-Maher *et al*., 2019; Mori *et al*., 2020), the moderate efficacy of some insecticides devoted to the control of *S. titanus* (see Chapter 7 of this thesis), the spread of more virulent FD strains (Contaldo *et al*., 2021). The last authors pointed out that the timeliness and the accuracy with which prevention practices, monitoring and insecticide applications should be applied by winegrowers represent risk factors in FD spread. Moreover, a study on the biology of *S. titanus* proved that some parameters (i.e., adult longevity and fecundity) were underestimated (Alma *et al.,* 2018). The prolongation of the adult inoculation period, and a temporal expansion of the risk of infecting the vineyards suggest reconsidering vector management in vineyards (Bocca *et al*., 2020). Monitoring *S. titanus* (and other vectors of FD phytoplasmas) remains crucial in IPM tactics.

Recently, a large FD outbreak has been recorded in the territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG (Treviso district, Veneto Region) where viticulture is highly fragmented, and vineyards are frequently surrounded by woody vegetation. In order to better understand the causes of these outbreaks, we gathered information on the potentially involved factors highlighting suspected gaps in both *S. titanus* control measures (e.g., the timing of insecticide applications) and the adoption of cultural practices against FD (e.g., rouging of symptomatic grapevines). For this reason, intensive monitoring activity of *S. titanus* populations was planned in 85 vineyards of this territory to collect data on the leafhopper phenology and abundance. Moreover, in four areas of this territory, characterized by high incidence of FD, the spatial and temporal distribution of *S. titanus* was analyzed to localize the outbreak areas and develop vector control strategies in a territorial perspective.

Materials and Methods

The phenology and abundance of *Scaphoideus titanus* in the selected vineyards

The study was conducted in the territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG (San Pietro di Feletto and Refrontolo municipalities, Treviso district, Veneto Region). A total of 86 vineyards belonging to 23 farms were sampled in order to collect data on *S. titanus* phenology and abundance in 2021 and 2022 growing seasons. One of these vineyards was removed in winter 2021 and thus 85 vineyards were sampled in 2022. Weekly sampling was performed from late May to early October. At each sampling, 50 leaves per vineyard were visually inspected and all the nymphs were counted and collected with an aspirator to identify their instar in the laboratory according to current keys (Della Giustina *et al.*, 1992). When the first 5th instar nymphs were found, three yellow sticky traps (Serbios SRL, Badia Polesine, Rovigo, Italy) were placed in each vineyard; traps were inspected every weak to count adults and replaced every two weeks. Leaf sampling stopped when *S. titanus* nymphs were not found in two subsequent sampling dates.

Spatial and temporal distribution of *Scaphoideus titanus*

In four areas with high incidence of FD (so-called "Area 1, 2, 3, 4"), ranging from 6 to 30 ha and comprising part of the 85 vineyards, the spatial and temporal distribution of *S. titanus* was studied. The sampling points were intensified to identify the vineyards with the greatest abundance of the vector, since these could represent a potential source of infected vectors for other vineyards in the same area. Concerning vineyard management, the "Area 1" comprised three organic (1o; 2o and 3o) and eleven conventional (1c; 2c; 3c; 4c; 5c; 6c; 7c; 8c 9c, 10c and 11c) vineyards (total 12 ha). Within "Area 1" these groups of vineyards have the same owner, thus the same management practices: 1o, 2o, 3o; 1c, 2c, 5c; 4c, 9c; 7c, 8c, 10c; 6c, 11c; 3c. The "Area 2" consisted in 14 conventional (1c; 2c; 3c; 4c; 5c; 6c; 7c; 8c; 9c; 10c; 11c; 12c; 13; 14c) vineyards (total 11 ha). Within "Area 2" these groups of vineyards have the same owner, thus the same management practices: 1c, 3c, 9c, 10c, 12c; 11c,13c; 4c; 6c; the remaining five vineyards have different owners. The "Area 3" comprised two organic vineyards (1o and 2o) having two different owners (total 6 ha). The "Area 4" was composed by 10 conventional (1c; 2c; 3c; 4c; 5c; 6c; 7c; 8c; 9c and 10c) vineyards (total 30 ha). Within "Area 4" these groups of vineyards have the same owner, thus the same management practices: 1c, 3c; 2c, 4c, 5c,7c; 6c, 8c, 9c, 10c. In each area, about 45 sampling points, comprising 10 vines, were previously selected and uniformly distributed inside using the software Google Earth Pro. *Scaphoideus titanus* nymphs were weekly monitored on 10 leaves per sampling point (one leaf per grapevine) following the above procedure. For *S. titanus* adult monitoring, a sticky trap was placed in each sampling point, inspected every week to count adults, and replaced every two weeks.

Pest control measures

Insecticide application was carried out according to the Veneto Region's regulatory measures indicating the timing and type of insecticides against *S. titanus*. Farms were free to select the active ingredients among those suggested by the Region. In 2021, systemic insecticides (acetamiprid and flupyradifurone) and only seldom the pyrethroid tau-fluvalinate were applied in most of conventional vineyards. Pyrethrins were used by organic winegrowers (at least two applications). One to four insecticide applications were made by the different farms. In 2022, regional regulatory control measures were tighter compared to the previous season, and they indicated two mandatory applications against nymphs using first systemic insecticides (e.g., acetamiprid, flupyradifurone and sulfoxaflor) and then pyrethroids (e.g., tau-fluvalinate, acrinathrin, etofenprox and deltamethrin) in conventional vineyards. Three applications of pyrethrins were mandatory in organic vineyards. In most vineyards, three insecticide applications were made in 2022.

Data analysis

We applied SADIE red-blue analysis (Perry *et al*., 1999) to determine spatial patterns in the distribution of S. titanus nymphs and adults within the selected areas. This methodology identifies zones with relatively high-density counts (patches) or relatively small or zero counts (gaps) and calculates for each sampling point the indexes of clustering (vi; vj) that measure the local contribution to either patch or gap, respectively. For each variable (nymph on leaves or adults per trap) clustering significance ($\alpha = 0.05$) was provided by comparing the vi and vj mean values with their corresponding values under the null hypothesis (Perry *et al*., 1999). A two-dimensional map showing the spatial distribution of local clustering indexes (vi; vj) for each variable was generated using linear kriging with SURFER (Golden Software 191 Inc., CO). Distribution maps were created using cumulative date on nymphs before and after mandatory insecticide applications, as well as on adults caught from July to early August (early summer) or from early August to October (late summer).

Results

The phenology and abundance of *Scaphoideus titanus* in the selected vineyards

In 2021, *S. titanus* was first detected in late May (Figure 1). The first instar nymphs peaked on the 10th of June while the second instar nymphs one week later. The third and fourth instar nymphs peaked in the same sampling date (the 24th of June). Then, the peak of the fifth instar nymphs was recorded on the 8th of July. Adults were first detected on the 15th of July and their captures peaked on the 13th of August (Figure 1). Adults were detected until early October.

In 2022, the first instar nymphs were detected earlier than in 2021 with a peak on the 27th of May. The second and third instar nymphs peaked on the 10th of June, the fourth instar nymphs one week later. Finally, fifth instar nymphs peaked on 24th of June, two weeks before than in 2022. Adults were first detected on the 1st of July. Their captures peaked in late July, reaching lower densities than in the previous season (Figure 1).

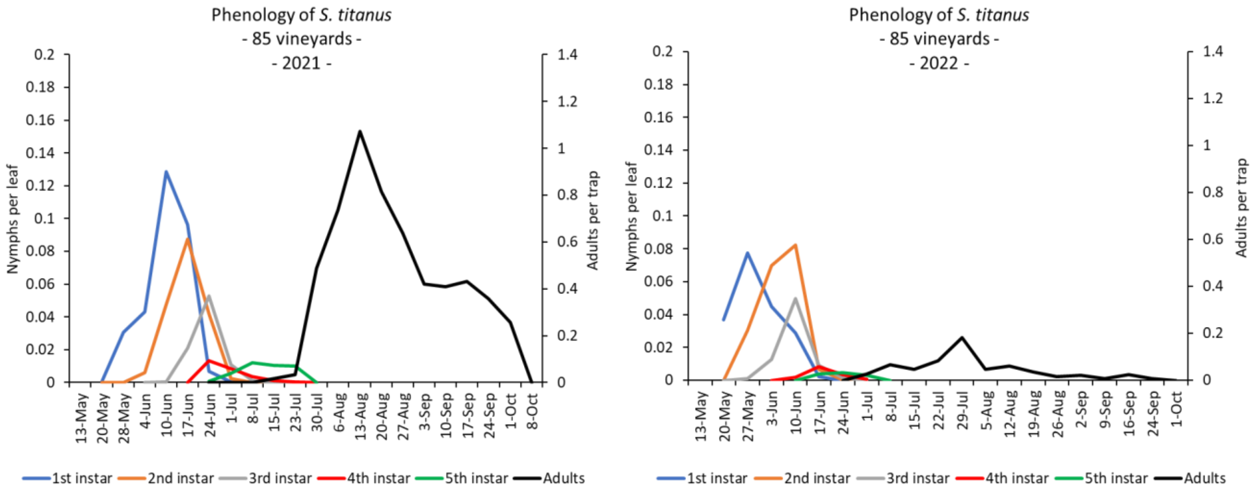

Figure 1 Phenology of *Scaphoideus titanus* considering the selected 85 vineyards (mean of data) during the 2021 and 2022 growing seasons.

The incidence of *S. titanus* in the two growing seasons is reported in Figure 2. *Scaphoideus titanus* nymphs were not detected in 9 vineyards in both growing seasons. The most infested vineyards (> 50 cumulate nymphs per 50 leaves) decreased from 16 (2021) to 10 (2022). Regarding adults, *S. titanus* was not detected on traps in 15 out of 85 vineyards in 2021; this figure increased to 70 vineyards in 2022 (Figure 3). The most infested vineyards (> 11 cumulate captures per trap) decreased from 11 (2021) to 1 (2022).

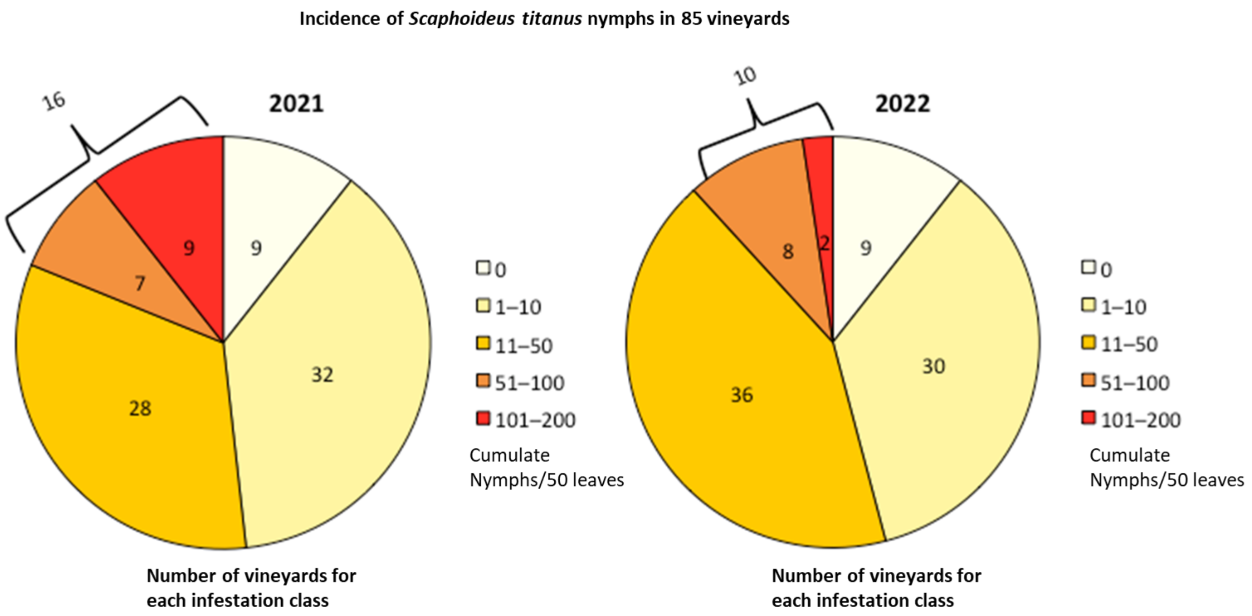

Figure 2 Incidence of *S. titanus* nymphs in the 85 vineyards included in the monitoring project, in the 2021 and 2022 growing seasons.

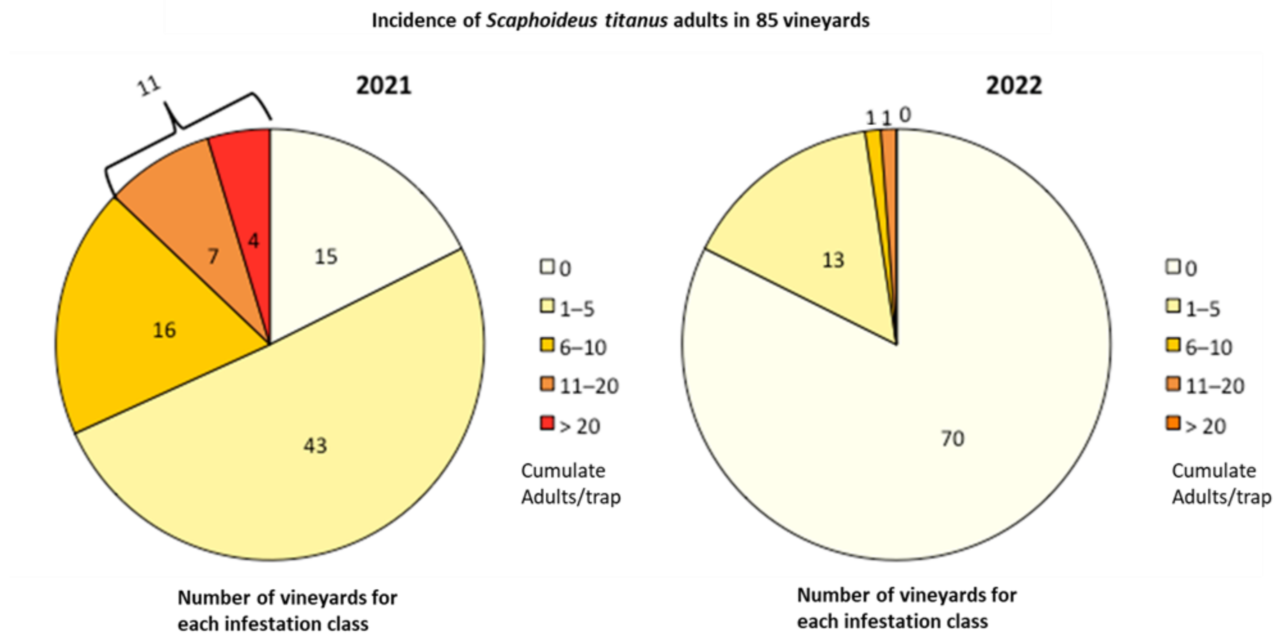

Figure 3 Incidence of *S. titanus* adults in the 85 vineyards included in the monitoring project, in the 2021 and 2022 growing seasons.

The spatial distribution of *S. titanus* in the selected areas

Area 1

The phenology of *S. titanus* in this area was similar compared to the average of all the vineyards above reported, but leafhopper densities were higher (Figure 4). In 2021 two major peaks of adults were detected, on the 23rd of July and on the 13th of August. A clear decline in leafhopper densities was recorded in 2022 with respect to 2021 (Figure 4).

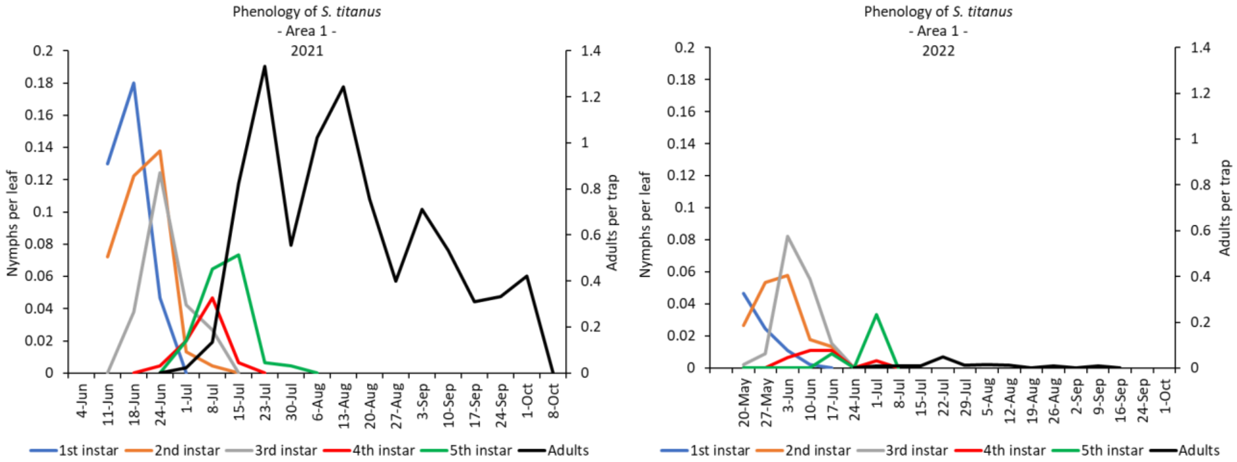

Figure 4 Phenology of *S. titanus* in Area 1 during the 2021 and 2022 growing seasons.

Before the mandatory insecticide applications, nymph distribution in the 2021 growing season was aggregated. Significant patches were found close to conventional vineyards 1c, 2c and 4c while gaps were localized around the vineyards 8c and 11c (Figure 5; Table 1). Patches of nymphs were detected even after insecticide application in the area between vineyards 4c and 5c. Early summer patches of adults reflected previous ones while late summer adult patches were localized in different areas, in particular around organic vineyards. In the 2022 growing season, patches of nymphs were similar to those reported for adults in the late summer of the previous years. After insecticide applications patches were still observed close to 1o, 3o and 9c vineyards. In summer, patches of adults were localized around a number of organic and conventional vineyards.

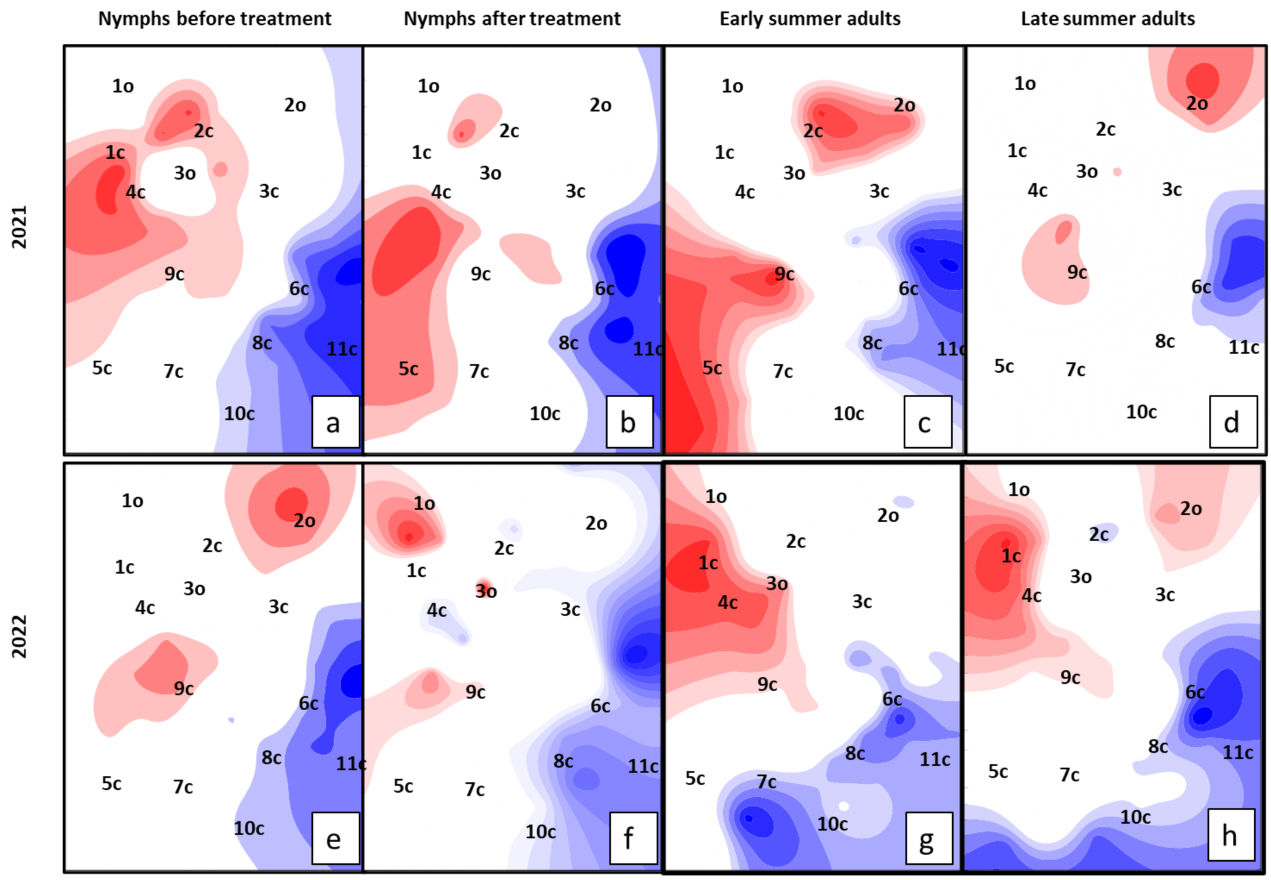

Figure 5 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in the two growing seasons (2021 – 2022) in Area 1 before and after insecticide applications. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers.. Numbers indicate the different vineyards (c = conventional; o = organic).

Table 1 Results of the spatial pattern analysis of *S. titanus* nymphs and adults in Area 1, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Area	Year	Figure	Stage	vj	vi	Pvj	Pvi
1	2021	5a	nymphs before treatments	-1.990	1.626	0.000	0.004
1	2021	5b	nymphs after treatments	-1.694	1.511	0.002	0.013
1	2021	5c	early summer adults	-1.542	1.568	0.011	0.039
1	2021	5d	late summer adults	-1.384	1.536	0.041	0.047
1	2022	5e	nymphs before treatments	-1.578	1.539	0.007	0.040
1	2022	5f	nymphs after treatments	-1.586	1.527	0.006	0.023
1	2022	5g	early summer adults	-2.155	1.762	0.000	0.002
1	2022	5h	late summer adults	-2.242	2.053	> 0.0001	0.000

Area 2

In 2021, the abundance of *S. titanus* appeared to be much lower than in Area 1 and thus the leafhopper phenology was less representative (Figure 6). Despite relatively low numbers of nymphs, adults were regularly detected throughout the growing season. The most relevant peak of captures was detected on the 6th of August. In 2022, nymph densities were comparable with those recorded in the previous season while adult numbers were dramatically reduced (Figure 6).

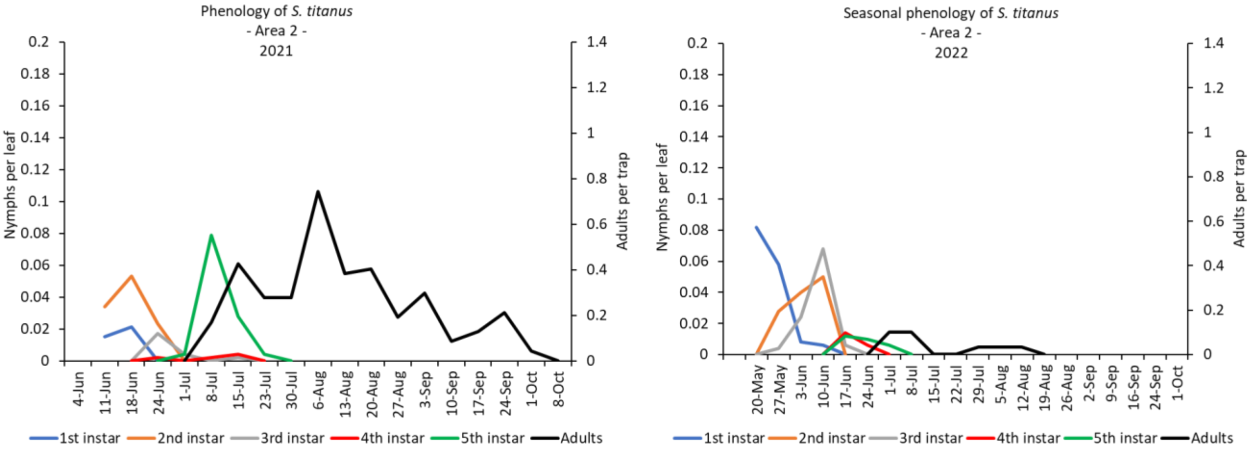

Figure 6 Phenology of *S. titanus* in Area 2 during the 2021 and 2022 growing season.

During the 2021 growing season, patches of nymphs before insecticide applications were localized where viticulture was very fragmented (9c and 13 c vineyards) (Figure 7; Table 2). Neither patches nor gaps of nymphs were observed after insecticide applications. In early summer, adults exhibited patches in vineyards 3c, 9c and 13c. In late summer patches were observed close to vineyard 2c. In the 2022 growing season, only nymphs before insecticide applications showed patches (2c, 11c, 13c) and gaps. After insecticide applications neither nymphs nor adults exhibited any aggregative pattern, due to the low number of individuals present (see Figure 6).

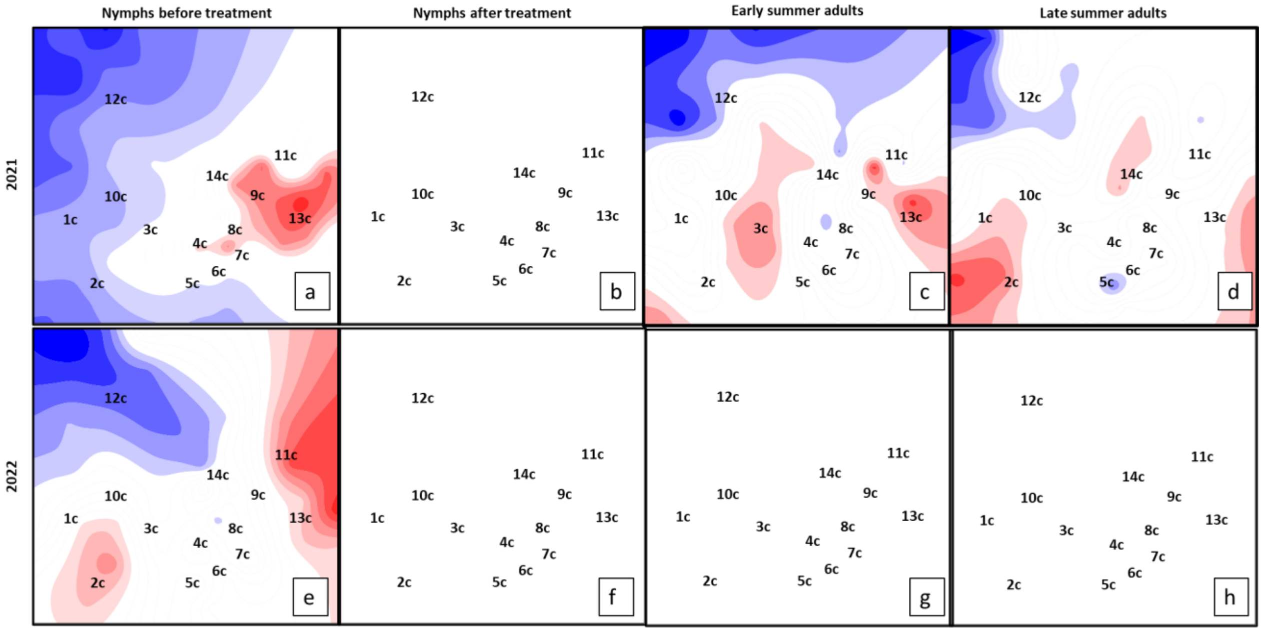

Figure 7 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in the two growing seasons (2021 – 2022) in Area 2 before and after insecticide application. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers.. Numbers indicate the different vineyards (c = conventional; o = organic).

Table 2 Results of the spatial pattern analysis of *S. titanus* nymphs and adults in Area 2, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Area	Year	Figure	Stage	vj	vi	Pvj	Pvi
2	2021	7a	nymphs before treatment	-1.505	1.548	0.035	0.024
2	2021	7b	nymphs after treatment	-2.024	2.647	0.001	> 0.0001
2	2021	7c	early summer adults	-1.628	1.697	0.004	0.003
2	2021	7d	late summer adults	-1.501	1.520	0.015	0.024
2	2022	7e	nymphs before treatment	-1.782	1.823	0.002	0.001
2	2022	7f	nymphs after treatment	-0.980	0.868	0.456	0.809
2	2022	7g	early summer adults	-1.378	1.290	0.043	0.071
2	2022	7h	late summer adults	-1.306	1.433	0.063	0.029

Area 3

In 2021, although few nymphs were found in the spring, adult captures were relatively high with a peak on the 13th of August (Figure 8). In 2022 nymph densities were much higher than in 2021 and their phenology reflected trends previously reported. Two peaks of adults were detected on the 29th of July and 24th of September, respectively.

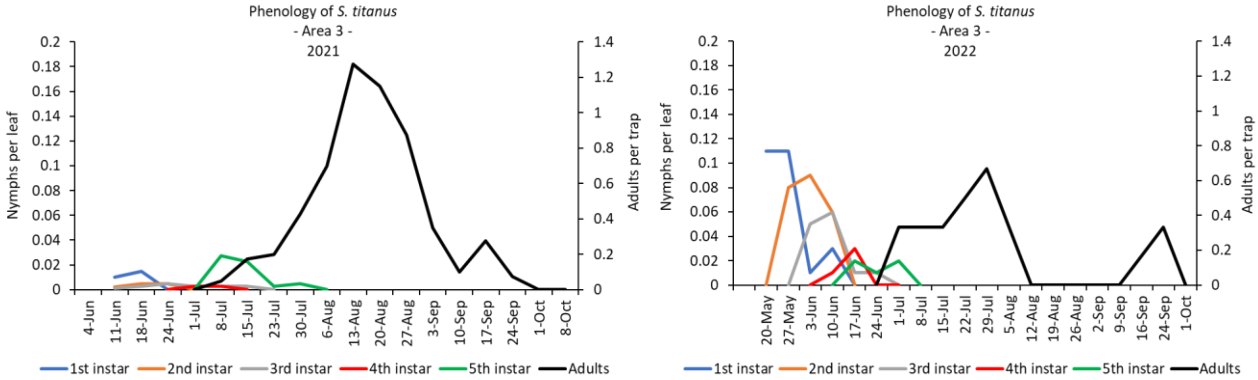

Figure 8 Phenology of *S. titanus* in Area 3 during the 2021 and 2022 growing seasons.

Patches of nymphs appeared only in vineyard 2o in 2021, while adult patches were observed in both vineyards. A small patch of adults was found in vineyard 1o, possibly caused by immigrant individuals (Figure 9; Table 3). In the 2022 growing season, patches of nymphs were found in vineyard 2o where adult patches were detected in the previous year. Regarding adults a random distribution occurred in the whole area.

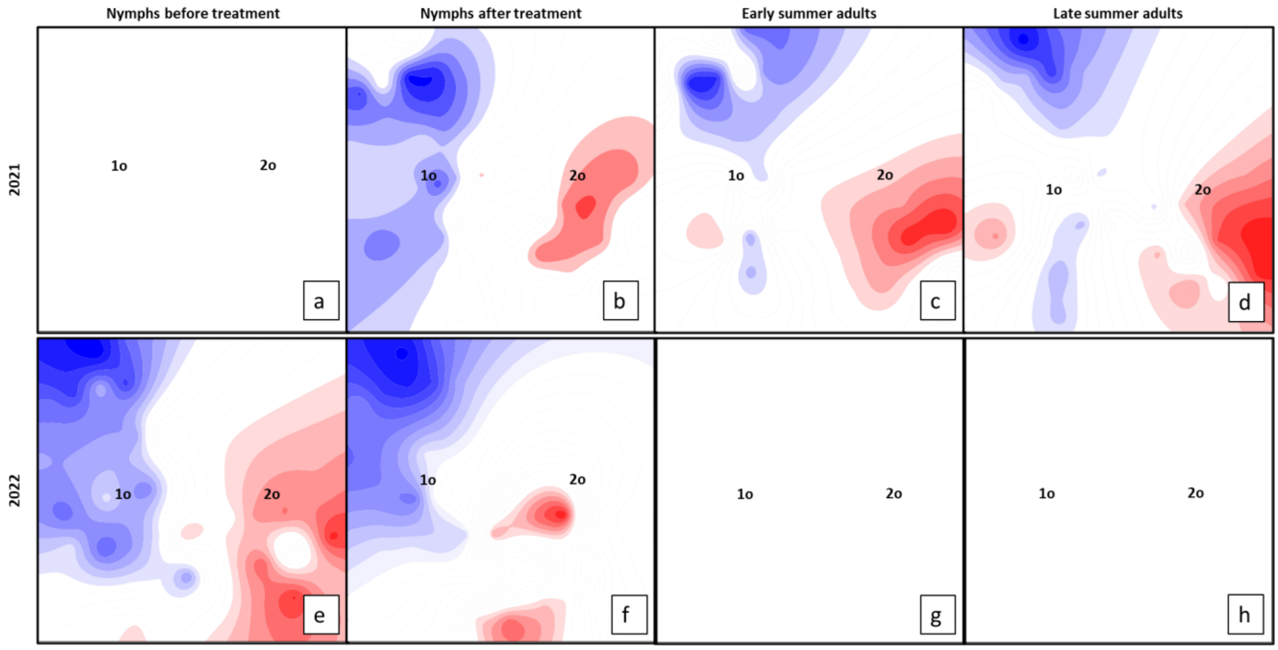

Figure 9 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in the two growing seasons (2021 – 2022) in Area 3 before and after insecticide application. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers.. Numbers indicate the different vineyards (c=conventional; o=organic).

Table 3 Results of the spatial pattern analysis of *S. titanus* nymphs and adults in Area 3, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05).

Area	Year	Figure	Stage	vj	vi	Pvj	Pvi
3	2021	9a	nymphs before treatment	-1.824	1.804	0.002	0.001
3	2021	9b	nymphs after treatment	-1.201	1.346	0.154	0.065
3	2021	9c	early summer adults	-2.087	1.633	0.000	0.009
3	2021	9d	late summer adults	-2.025	1.939	0.001	0.001
3	2022	9e	nymphs before treatment	-2.826	2.380	> 0.0001	> 0.0001
3	2022	9f	nymphs after treatment	-1.595	1.593	0.013	0.012
3	2022	9g	early summer adults	-1.406	1.366	0.049	0.057
3	2022	9h	late summer adults	-1.465	1.257	0.035	0.107

Area 4

During the 2021 growing season, few nymphs were found but the most significant phenological phases (i.e., the appearance of fourth and fifth instar nymphs) confirmed general trends (Figure 10). Adults peaked on the 13th and the 27th of August. In 2022, leafhopper densities were dramatically reduced after insecticide application and rare adults were detected.

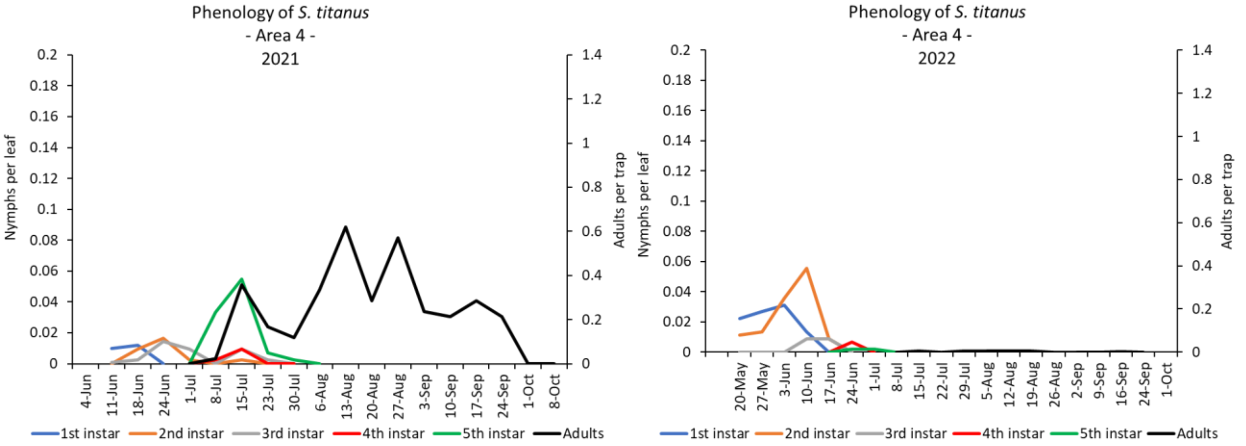

Figure 10 Phenology of *S. titanus* in Area 4 during the 2021 and 2022 growing seasons.

During 2021 no patches or gaps were detected neither for nymphs nor for adults (Figure 11; Table 4). In the 2022 growing season, nymphs before treatment showed large patches in vineyards 1c, 2c and 3c. While gaps were detected on the opposite side of the area. After insecticide applications only adults exhibited aggregative patterns around vineyards 7c and 9c, while gaps were localized where nymphs were previously aggregated (Figure 11) (Table 3).

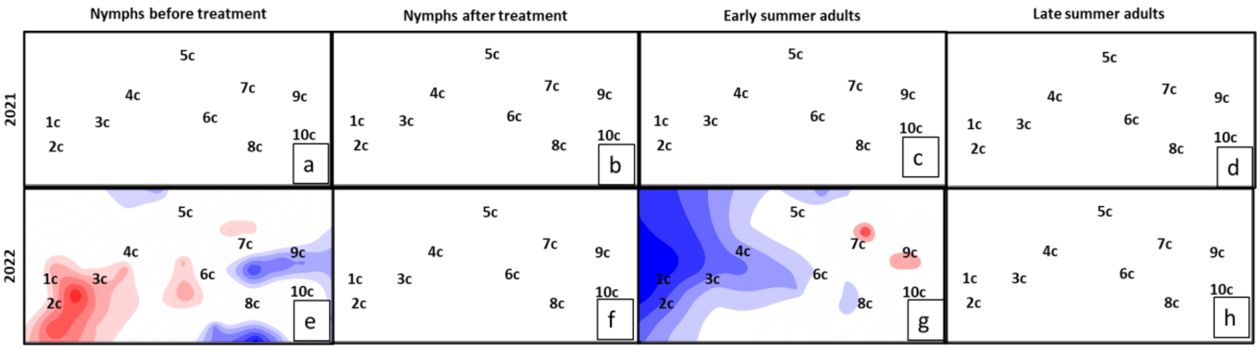

Figure 11 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in the two growing seasons (2021 – 2022) in Area 4 before and after insecticide application. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers.. Numbers indicate the different vineyards (c = conventional; o = organic).

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Table 4 Results of the spatial pattern analysis of *S. titanus* nymphs and adults in Area 4, showing average indexes of clustering into patch (mean vi) and into gap (mean vj) with associated probability (P) that were significant at randomization test (α = 0.05)

Area	Year	Figure	Stage	vj	vi	Pvj	Pvi
4	2021	11a	nymphs before treatment	-1.134	1.099	0.239	0.279
4	2021	11b	nymphs after treatment	-1.048	0.957	0.330	0.472
4	2021	11c	early summer adults	-1.400	1.282	0.065	0.113
4	2021	11d	late summer adults	-1.187	0.939	0.173	0.523
4	2022	11e	nymphs before treatment	-1.920	1.624	0.003	0.019
4	2022	11f	nymphs after treatment	-0.916	1.067	0.590	0.311
4	2022	11g	early summer adults	-1.672	1.856	0.013	0.004
4	2022	11h	late summer adults	-1.014	1.154	0.384	0.188

Discussion

The phenology and abundance of *S. titanus* in the selected vineyards

In 2021 the phenology of *S. titanus* was detectable in a number of infested vineyards where peaks of the different instars were clearly distinguishable. Adult captures were recorded from July to October with a peak in mid-August. In 2022, first instar nymphs peaked two weeks earlier than in 2021. This advance was confirmed for subsequent instar nymphs and adults and explained by higher temperatures occurred in 2022 (METEO VENETO – ARPAV, data of Weather Station of Conegliano) in agreement with phenological models based on temperatures (Rigamonti *et al*., 2011; Maggi *et al*., 2013; Falzoi *et al*., 2014). The population structure of *S. titanus* has been clearly reproduced despite the application of insecticides.

The most significant difference between the two growing seasons concerned adult captures, that were dramatically reduced in 2022 compared to 2021. We can assume that changes in control strategies from 2021 (prevalent use of systemic insecticides belonging to IRAC 4 group) to 2022 (prevalent use of pyrethroids) affected *S. titanus* abundance. In fact, the high efficacy of deltamethrin, etofenprox and acrinathrin has been proved (Prazaru *et al.,* 2023*,* trials reported in chapter 7). Furthermore, it has already been shown that adult captures are significantly reduced by the use of the most effective insecticides against nymphs compared to the least effective ones (Pavan *et al.,* 2012). Probably due to this reason, the number of vineyards without adult captures has increased from 15 in 2021 to 73 in 2022. Considering that FD symptoms usually appear in the year following the inoculation of the phytoplasma by the vector (Caudwell, 1990) and there is a relationship between the presence of *S. titanus* in the previous year and the infection rate (Morone *et al*., 2007), the reduction in adult captures should lead to a reduction in the incidence of new symptomatic grapevines in 2023.

The spatial distribution of *S. titanus* in the selected areas

In most of the case studies the spatial distribution of *S. titanus* was aggregated, confirming previous results (Bosco *et al.*, 1997; Lessio *et al.*, 2011). It is known that other leafhoppers may have aggregative spatial patterns (Allsop and Bull 1990; Delrio *et al.* 2001; Maixner, 2003; Beers and Jones 2004; Mori *et al.,* 2016). The approach used in studying the spatial and temporal distribution of *S. titanus* allowed us to identify the most significant aggregations in vineyards of definite areas. These areas had been selected because of their issues with FD and high fragmentation (small vineyards belonged to different owners) that hindered the simultaneous application of insecticides by winegrowers and favor adult migration among vineyards. To this purpose, it is known that *S. titanus* may disperse at least up to 300 m (Lessio *et al.,* 2014). Timing in insecticide applications before the project was very variable among the vineyards within the same area giving more chances to *S. titanus* (particularly adults) to escape insecticide effects. In 2021, winegrowers applied insecticides within a restricted timing (suggested by regional regulatory measures, BUR Regione Veneto) but results in keeping *S. titanus* at low levels were not completely satisfactory probably due to the moderate efficacy of some insecticides. Adults captures in summer of 2021 reflected this situation. Changes in active ingredients suggested by the regional rules in 2022 determined a good control of the leafhopper, confirming that pest management strategy is the most important factor affecting *S. titanus* population density (Lessio *et al*., 2011).

The approach followed in the monitoring of *S. titanus* in the selected areas and the creation of distribution maps allowed to identify a good overlapping between the adult patches in late summer of 2021 and the nymph patches in spring of 2022.

In some areas, during the same growing season, *S. titanus* adult patches did not overlap with those of nymphs suggesting different efficacy of insecticides applied against nymphs among vineyards within the same areas or the possible colonization of adults coming from contiguous vineyards. If *S. titanus* adults colonizing vineyards from outside are infectious, the presence of vineyards with high levels of adult populations, because insecticides were not applied or their control of the vector was suboptimal, may represent a risk for the vineyards belonging to the same area (Pavan *et al.,* 2012; Lessio *et al*., 2015).

Conclusions

Based on the results obtained, fragmentation represents a serious barrier to *S. titanus* control. In this study, the "Area" with the highest number of aggregations of *S. titanus* in both growing seasons was "Area 1", which is characterized by having many winegrowers and both organic and conventionally managed vineyards. Contrary to the other "Areas" where the vineyards were all conventionally managed or organically managed, thus allowing better timing for pesticide applications. The situation of "Area 1" was the most problematic as outbreaks persisted for two consecutive growing seasons. *Scaphoideus titanus* outbreaks were localized in conventional and organic vineyards suggesting that insecticide use in these vineyards was not effective.

The reasons for the apparent failure of insecticide control in some vineyards must be carefully investigated, as these vineyards can represent a source of infectious vectors for the contiguous vineyards, nullifying the positive effects of the insecticide control that the latter have implemented against the nymphs. Effective control of FD in areas of fragmented viticulture can be achieved only through strategies involving all vineyards and winegrowers.

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Chapter 6

Does woody vegetation influence the spatial distribution of *Scaphoideus titanus* in vineyards? Analysis of four case-studies in North-eastern Italy

Manuscript in preparation as: Does woody vegetation influence the spatial distribution of *Scaphoideus titanus* in vineyards? Analysis of four case-studies in North-eastern Italy

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Prazaru Stefan Cristian contributed to data collection, performed statistical analysis, and the manuscript drafting.

Abstract

Flavescence dorée (FD) is a grapevine quarantine disease that causes serious damage in European vineyards. The phytoplasma associated with FD is mainly transmitted by *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) but other vectors, such as *Orientus ishidae* (Matsumura) (Hemiptera: Cicadellidae), may be involved. FD-phytoplasma can be inoculated in healthy grapevines by vectors developed inside vineyards, but infective adults can also colonize vineyards from outside. The role of American grapevines growing naturally in woody vegetation as a source of infectious *S. titanus* is well known, while the real risk associated with broad-leaved trees (e.g., hazel, alder and *Salix* spp.) that can host both *O. ishide* and FD-phytoplasma is still a matter of discussion. Many winegrowers noticed that the disease is more widespread in parts of the vineyard adjacent to groves or at vineyard margins. For this reason, the influence of woody vegetation as a potential source of infectious *S. titanus* and *O. ishidae* was studied during 2021 and 2022 in a vine-growing area of North-eastern Italy involved in FD outbreaks. Four rectangular vineyards with groves bordering on one or two sides were considered. The presence of broad-leaved trees hosting *O. ishidae* and the absence of American grapevines occurring naturally in the woody vegetation allowed us to verify the role of natural vegetation in FD outbreaks even in the absence of potential sources of infective *S. titanus*. In each vineyard, at least 28 sampling points were established and in both growing seasons *S. titanus* nymphs (on leaves) and *S. titanus* and *O. ishidae* adults (captured on yellow sticky traps) were sampled. In three out of four vineyards, symptomatic grapevines were mapped before harvest. The spatial distribution of leafhoppers and symptomatic plants was studied using SADIE (Spatial Analysis by Distance Indices). The spatial distribution of *S. titanus* was not associated with groves bordering vineyards but rather with nearby vineyards harboring high populations of the vector. *O. ishidae* was instead aggregated along vineyard borders where groves were present. The spatial distribution of symptomatic grapevines was never in contradiction with that of S. *titanus.* The spatial distribution of symptomatic grapevines recorded in 2022 was in two out of three cases clearly related to the spatial distribution of adults of *S. titanus* recorded the year before. There was no a relationship between the spatial distribution of symptomatic grapevines and that of *O. ishidae*. Therefore, in the vine-growing area under study, outbreaks of FD cannot be attributed to the vegetation surrounding the vineyards and to the colonization by *O. ishidae* adults.

Introduction

Scaphoideus titanus Ball (Hemiptera: Cicadellidae), a neartic leafhopper, is the main vector of *Candidatus* Phytoplasma vitis, a phytoplasma belonging to 16SrV group. This phytoplasma is the causative agent of Flavescence dorée (FD), one of the most serious diseases in European vineyards (Bertaccini and Duduk, 2013; Angelini *et al*., 2018; Malembic-Maher *et al*., 2020; Debonneville *et al*., 2022). In Europe, *S. titanus* was detected for the first time in France (Bonfils and Schvester, 1960) and then in Italy (Vidano, 1964). Nowadays, *S. titanus* is present in many European countries (Chuche and Thiéry, 2014; Gnezdilov and Orlov, 2022). *Scaphoideus titanus* is strictly ampelophagous and acquires phytoplasmas by feeding on infected vines. Nymphs require about 4-5 weeks to become infective, adults only 1-2 weeks (Alma *et al.,* 2018). *Scaphoideus titanus* is monovoltine and overwinters as eggs; egg hatching starts in May and lasts for about two months depending on winter temperatures (Vidano, 1964; Chuche and Thiery, 2014). Females survive up to 70 days (Bocca *et al*., 2020) and for this reason *S. titanus* adults can be observed up to early November resulting in long periods of oviposition and FD phytoplasma inoculation (Pavan *et al*., 1987; Alma *et al*., 2018).

Insecticide application is the most effective control measure against *S. titanus* and consequently for maintaining the disease at acceptable levels. However, insecticides applied against the vector in vineyards may not be sufficient to control FD due to the outside source of infectious *S. titanus* adults (Pavan *et al*., 2012; Lessio *et al*., 2015). Sources can be represented by American grapevines growing in natural vegetation (i.e., groves, hedgerows and ditch slopes) and abandoned vineyards with high levels of both *S. titanus* populations and FD phytoplasmas (Caudwell *et al*., 1994; Pavan *et al*., 2005, 2012; Lessio and Alma, 2006; Lessio *et al*., 2007; Eveillard *et al*., 2016). Even cultivated vineyards, in which the vector was not controlled effectively, are a danger to contiguous vineyards (Pavan *et al*., 2012). The risk associated with external sources is due to the mobility of *S. titanus* adults (even to over 300 m) (Lessio *et al*., 2014) which can colonize vineyards from outside (Maixner *et al*., 1993; Beanland *et al*., 2006).

Besides *S. titanus,* even *Dictyophara europaea* (L.) and *Orientus ishidae* (Matsumura) were reported as vectors of FD phytoplasmas related to grapevines (Filippin *et al*., 2009 ; Lessio *et al*., 2016). *Dictyophara europaea* was able to transmit FD phytoplasmas from *Clematis vitalba* L. to potted grapevines (Filippin *et al*., 2009). *Orienthus ishidae* was also capable to transmit FD phytoplasmas after acquiring them from infected broad beans (Lessio *et al.,* 2016). *Alnus glutinosa* L., *Corylus avellanae* L and *Salix* spp. were found infected by FD phytoplasmas as well as adults of *O. ishidae* collected on these plants (Casati *et al*., 2017; Krstić *et al*., 2018; Strauss and Reisenzein, 2018; Malembic-Maher *et al.,* 2020 Rizzoli *et al*., 2021). Based on the above information, natural vegetation could represent a risk for the spread of FD in vineyards. However, if in the case of the binomial *S. titanus*/American grapevines in natural vegetation the danger is demonstrated, there is no evidence for the binomials *D. europaea*/*C. vitalba* and *O. ishidae*/*A. glutinosa-C. avellanae-Salix* spp. for (Linder *et al*., 2014; Rizzoli *et al*., 2021). The presence in the natural vegetation of different plant and leafhopper species potentially involved in the epidemiology of FD, suggested that groves close to vineyards can cover a role in the recent FD outbreaks detected in North-eastern Italy. Considering that the role of groves with American grapevines growing wild is already known, the study was conducted in a wine-growing area of North-eastern Italy in which this potential source of infectious vectors was virtually absent . The study involved four vineyards located in the territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG where serious FD outbreaks were observed since 2019. In these vineyards, bordered by natural groves or secondary stands, the influence of these vegetation structures on the spatial and temporal distribution of *S. titanus* and *O. ishidae* was investigated.

Materials and Methods

Vineyards

Scaphoideus titanus populations were monitored in four conventional vineyards located in North-eastern Italy (Treviso district, San Pietro di Feletto municipality, acronyms RUA, SMF, SPF1 and SPF2). Vineyards were cultivated with Glera cultivar, trained with the Sylvoz system, with a planting space of 1.5 m x 3 m. in the vineyards fungicides and insecticides, were regularly applied, including those against *S. titanus* according to Italian rules (D.M. N. 32442 – 31/05/2000). For each vineyard, a rectangular portion (1 to 3 ha size) bordered by groves on one or two sides was considered. Groves comprised typically hornbeams, hazelnuts, maples, elders, ash trees, oaks and conifers.

RUA vineyard (Figure 1a) had a percentage of FD symptomatic plants around 25%. It was bordered on two sides (east and south) by a grove. The southwestern border of the vineyard is adjacent to a house fence, which means that the last row can only be treated from one side. On the other hand, the North-eastern side borders a vineyard with relatively high populations of *S. titanus* and FD incidence. The grove bordering the southern side of the vineyard is mainly dominated by hornbeams, which were planted for ornamental purposes. In contrast, the eastern border has a more diverse vegetation, with elms, hazelnuts, and conifers being the predominant species, and a few chestnut trees interspersed throughout.

SMF vineyard (Figure 1b) had a percentage of FD symptomatic plants around 5%. It was bordered on two sides (east and south) by a grove. The other sides of the vineyard were contiguous to other vineyards belonging to the same owner and characterized by similar levels of both *S. titanus* and FD. However, on the opposite side of the grove part which borders the vineyard to the south, there was a vineyard infested by *S. titanus*. The tree composition of the grove adjacent to this vineyard was largely dominated by elms, hazelnuts, elders and brambles. There were no chestnuts or conifers in this grove. SPF1 vineyard (Figure 1c) had a percentage of FD-symptomatic grapevines around 75%. A grove bordered the vineyard on the south side and partly on the east side as well. On the west side, the vineyard was bordered by an organic vineyard and on the east part by a conventional vineyard. The grove was dominated by ash trees, hazels, elders, brambles and a few specimens of fig trees were present. There were no conifers nor chestnuts.

SPF2 vineyard had a percentage of FD symptomatic grapevines around 50% (Figure 1d). It was bordered on two sides (south and west) by a grove. This vineyard was bordered on the east side by a conventional vineyard with low populations of *S. titanus* and on the North-western side by a conventional vineyard with high pest population levels. The grove surrounding this vineyard was the largest among those considered, and was dominated by hazels, elms, and brambles. A few ash trees and chestnuts were present, mostly in the inner part of the grove. No conifers were recorded.

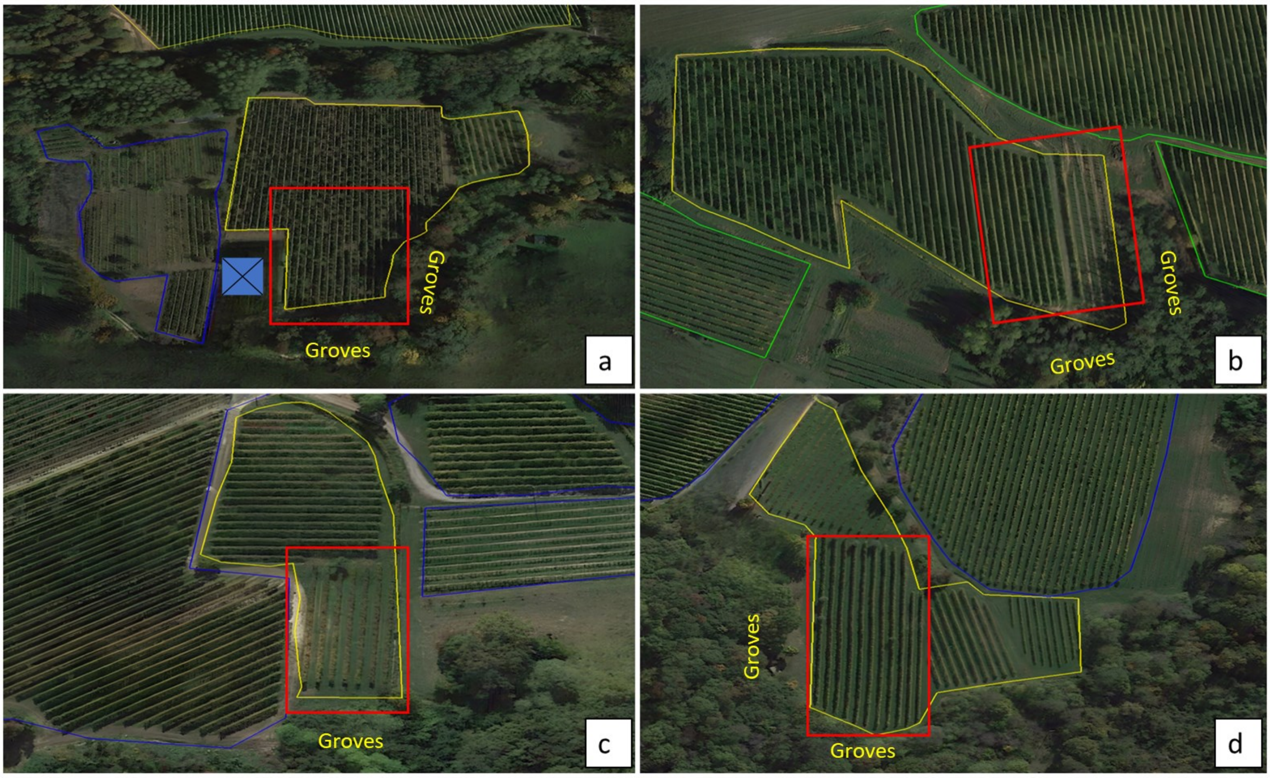

Figure 1 Vineyard Rua (a), Vineyard SMF (b), Vineyard SPF1 (c), Vineyard SPF2 (d); yellow contours indicate the entire vineyards, red contours indicate where the cartesian plane was created, blue contours indicate vineyards belonging to different owners, green contours indicate different vineyards belonging to the owners of the investigated vineyards.

Insect sampling

A cartesian plane has been set in each vineyard, the side adjacent to stands was considered as the X-axe, and a number of transects (depending on the vineyard) along the Y-axis were defined. In this way, 32, 28, 31, and 30 sampling points were created for RUA, SPF1, SPF2, and SMF vineyards, respectively.

Scaphoiudeus titanus populations were sampled from May until October during the 2021 and 2022 growing seasons. Nymphs were sampled weekly from May to July examining 10 leaves per sampling point. When the first 5th instar nymphs of *S. titanus* were found, a yellow sticky trap (Serbios, 20 x 20 cm) was placed in each sampling point to detect the occurrence of adults. Traps were checked weekly for adults of *S. titanus* and renewed every two weeks. On the same traps the adults of the alternative vector *O. ishidae* were also counted.

Mapping of FD-symptomatic grapevines

The spatial distribution of FD symptomatic grapevines in three out of the selected vineyards (RUA, SMF, SPF1) was also investigated. In both growing seasons grapevine surveys were done during late August-early September. All grapevines were classified as symptomatic or asymptomatic depending on the presence of typical FD symptoms (e.g., rolling of leaves, sectorial discolorations of the blades, partial or total lack of lignification of canes and shoots). grapevines were monitored contemporary by two people, one per each side of the row. Samples of leaves of ten symptomatic plants chosen at random were taken from each vineyard and brought to the laboratory, in order to confirm the positivity of the grapevines to FD in both growing seasons.

Data analysis

We applied SADIE red-blue analysis (Perry *et al*., 1999) to determine spatial patterns in the distribution of *S. titanus* nymphs (on leaves) and adults (per trap) within the selected vineyards. This methodology identifies areas with relatively high-density counts (patches) or relatively small or zero counts (gaps) and calculates for each sampling point the indexes of clustering (vi; vj) that measure the local contribution to either patch or gap, respectively. For each variable (nymph on leaves or trap captures) clustering significance ($\alpha = 0.05$) was provided by comparing the vi and vj mean values with their corresponding values under the null hypothesis (Perry *et al*., 1999). A two-dimensional map showing the spatial distribution of local clustering indexes (vi; vj) for each variable was generated using linear kriging with SURFER (Golden Software 191 Inc., CO).

Results

Leafhopper species on yellow sticky traps

In the four sampled vineyards, by far the most abundant leafhopper captured on yellow sticky traps was *S. titanus*. Adults of *O. ishidae* were also captured in all the vineyards and in some cases numbers allowed to elaborate distribution maps. Other leafhoppers potentially involved in FD spread, such as *Phlogotettix cyclops* Mulsant & Ray and *Hishimonus hamatus* Kouc, were occasionally captured (Strauss and Reisenzein, 2018; Belgeri *et al*., 2022).

RUA vineyard

In 2021 moderate densities of *S. titanus* nymphs occurred in this vineyard (0.14 nymphs per leaf per sampling date, from June 6 to July 15). Their distribution (cumulative numbers) revealed aggregation patterns. A large patch was found along the west side of the vineyard involving only marginally the south side contiguous to the grove (vi = 1.970, Pvi = 0.0002). Another patch was localized close to the North-western edge of the vineyard near to the contiguous vineyard infested by *S. titanus*. Significant gaps were located along the east side of the vineyard bordering the grove (vj = - 1.638, Pvj = 0.003) (Figure 2a). *Scaphoideus titanus* adults were detected regularly on traps (0.21 adults per trap per week from July to September). The distribution of *S. titanus* adults was aggregated showing a patch in the south-western area of the vineyard, partially bordering the grove (vi= 1.592, Pvi = 0.026). This patch was quite overlapping to one of the two patches of nymphs. Significant gaps were found inside the vineyard and close to the eastern side of the vineyard bordering the grove (vj= - 1.589, Pvj< 0.0001) (Figure 2b).

Regarding 2022, *S. titanus* nymphs reached higher densities compared to the previous season (0.28 nymphs per leaf per sampling date, from May 25 to July 14) and their distribution was still aggregated. Significant patch involved the south-western area of the vineyard, partially bordering the grove (vi = 1.738, Pvi = 0.001). The patch corresponded to the adult patch recorded in the previous year. Gaps were localized along the north and east sides of the vineyard, the latter bordering the grove (vj = - 1.641, Pvj = 0.003; Figure 2c). Captures of *S. titanus* were low (0.03 adults per trap per week from July to September) and showed a random distribution without significant patches and gaps (respectively vi = 0.911, P = 0.667 and vj = - 0.904 P = 0.687) (Figure 2d).

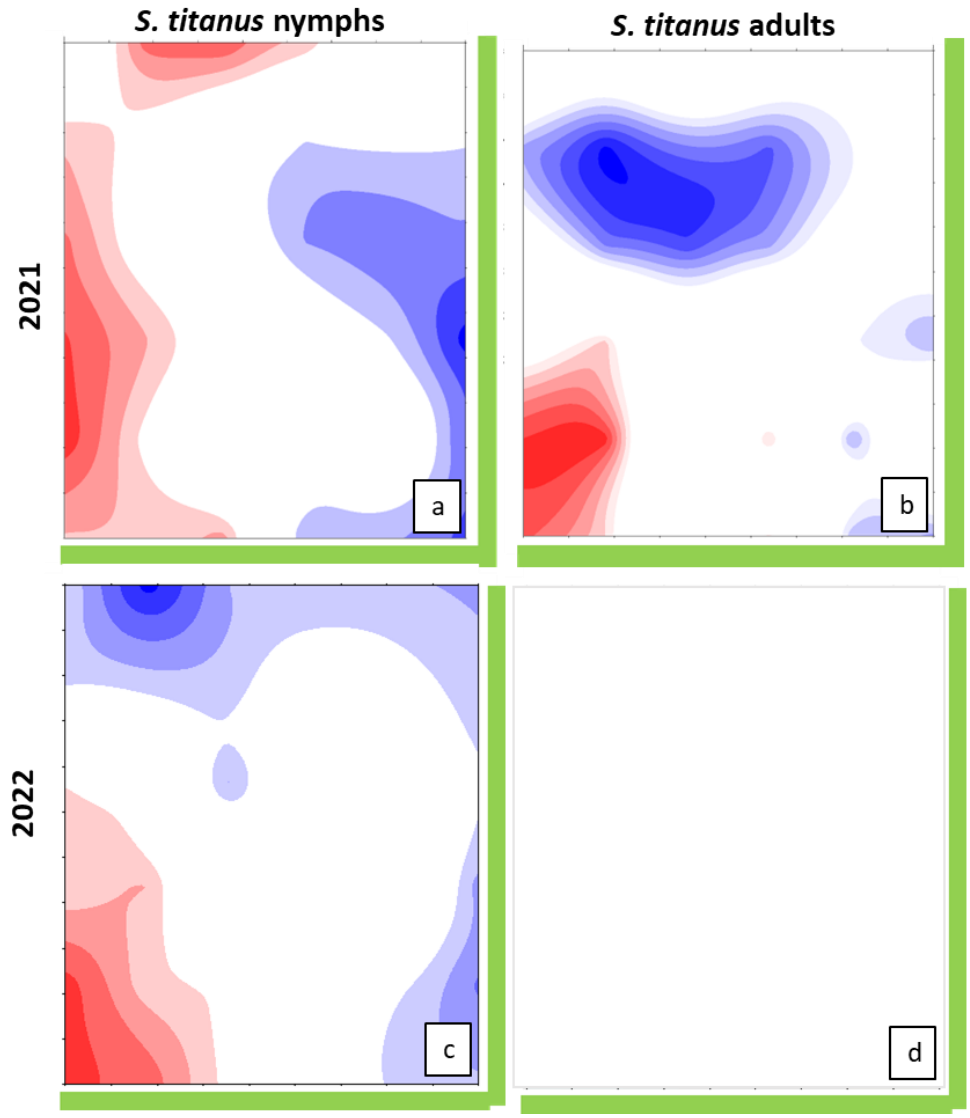

Figure 2. Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in RUA vineyard during the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. Green lines represent groves margins.

In 2021, *O. ishidae* adults were also detected on traps (0.12 adults per trap per week from July to September) without showing aggregation patterns. Lower numbers of *O. ishidae* were detected in 2022 (0.02 adults per trap per week from July to September), again they didn't exhibit an aggregative pattern and, thus no significant patches or gaps were found (respectively vj= 1.327, P= 0.0421; vi=-1.322, P= 0.441).

The distribution of FD symptomatic grapevines in the 2021 growing season resulted aggregated. The most significant patches were found in the North-western edge (vi= 1.579, Pvi = 0.003) while gaps were along the eastern area of the vineyard bordering the grove both on the east and south sides (vj = -1.505, Pvj = 0.007; Figure 3a). The patches coincides with one of the two patches of nymphs. The distribution of symptomatic grapevines in 2022 was also aggregated, with significant patches located along the western area of the vineyard (vi= 1.569, Pvi = 0.003). The patches in 2022 were in the western area of the vineyard as observed for *S. titanus*. As in 2021, gaps were located along the western area of the vineyard (vj= - 1.569, Pvj= 0.013; Figure 3b).

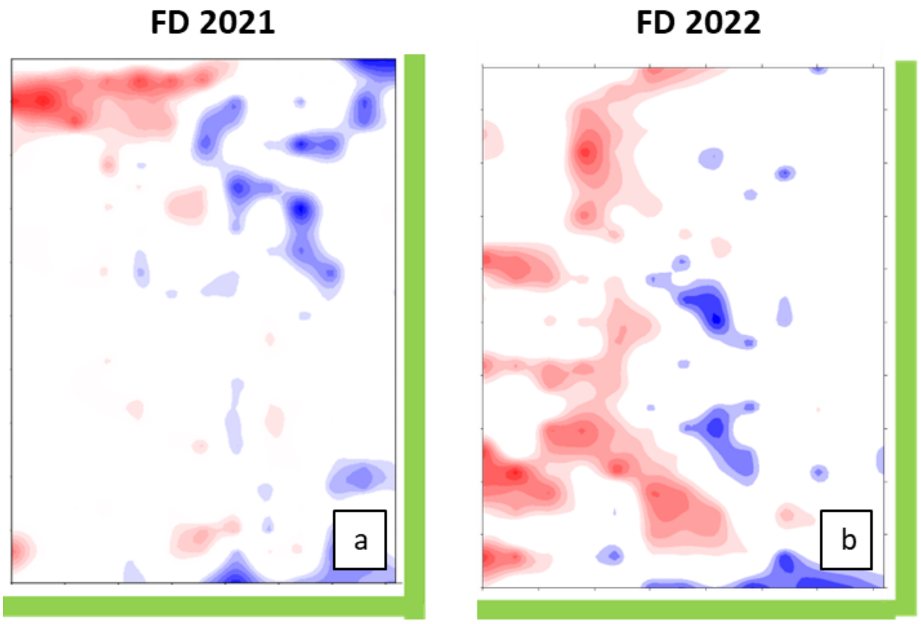

Figure 3 Spatial distribution of FD symptomatic grapevines in RUA vineyard in 2021 and 2022. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white areas random distribution of symptomatic vines. Green lines represent groves margins.

SMF vineyard

Low to moderate densities of *S. titanus* nymphs were detected in the 2021 growing season (0.09 nymphs per leaf per sampling date from June 17 to July 22). Their spatial distribution showed aggregation patterns with a significant patch along the west side of the vineyard involving only marginally the south side bordering the grove (vi = 1.970, Pvi = 0.0002). This patch was connected by a corridor without tree vegetation to the vineyard with high populations of *S. titanus* located beyond the grove. Significant gaps were found along the west side of the vineyard partially bordering the grove (vj = - 1.638, Pvj = 0.003; Figure 4a). *Scaphoideus titanus* adults were regularly detected in the summer of 2021 (0.09 adults per trap per week from July 15 to October 1). Their distribution was aggregated with patches along the west side of the vineyard partially involving the south side bordering the grove (vi= 1.59, Pvi = 0.003). Gaps were located along the east side bordering the grove (vj= - 1.847, Pvj= 0.0005; Figure 4b). In 2022, *S. titanus* nymph populations reached lower densities compared to the previous year (0.07 nymphs per sampling date from May 25 to June 30). The spatial distribution of nymphs (cumulative numbers) basically reflected that of adults in 2021 showing significant patches along the west side of the vineyard (vi= 1.684, Pvi = 0.002). Gaps were located on the opposite side (vj= - 1.662, Pvj= 0.003; Figure 4c). *Scaphoideus titanus* adults reached low densities in the summer of 2022 (0.005 adults per trap per week from July to September) and their distribution was not aggregated, so no significant patches and gaps were found (respectively: vi= 1.128, P= 0.215 and vj= - 1.125, P= 0.204; Figure 4d).

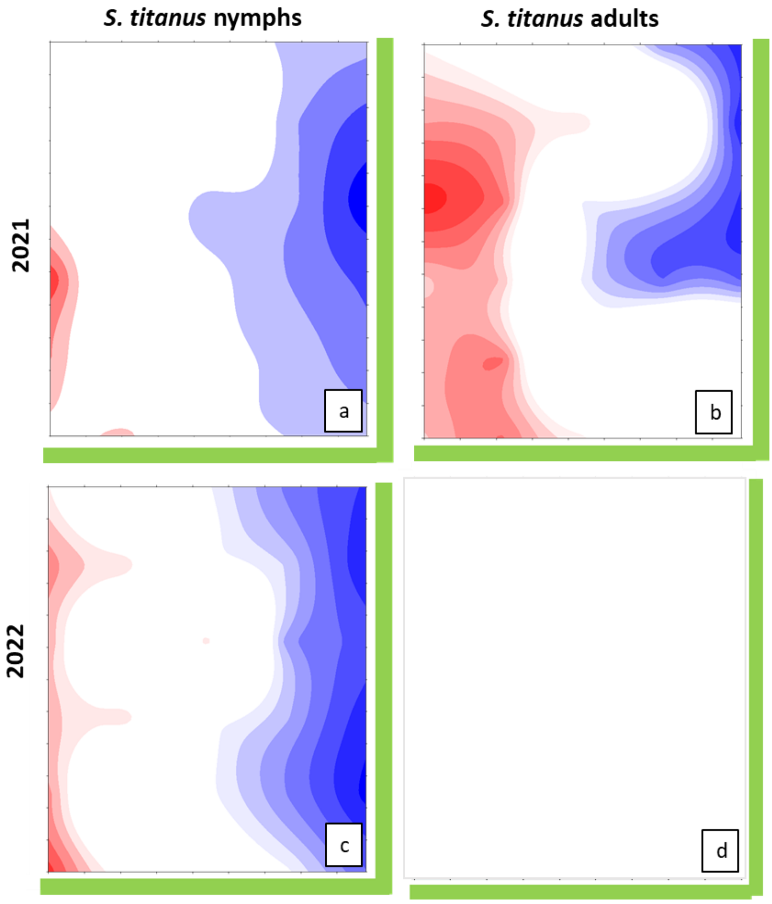

Figure 4 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in SMF vineyard in the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. Green lines represent groves margins.

Orientus ishidae adults were detected in eight sampling dates of 2021 (0.09 adults per trap per week from July to September). Their distribution resulted aggregated with a significant patch close to the conjunction of south and east sides of the vineyard bordering the grove, and another inside the vineyard but in any case, close to south side of the vineyard completely bordering the grove (vi= 1.533, Pvi = 0.007). Significant gaps were recorded in the north edge of the vineyard (vj= - 1.677, Pvj= 0.002; Figure 5a). One year later, higher captures of *O. ishidae* were detected (0.14 adults per trap per week from July to September). Their distribution confirmed to be aggregated with significant patches on the north-eastern edge of the vineyard close to the grove (vi= 1.505, Pvi = 0.012). Significant gaps were localized on the left side of the vineyard (vj= - 1.507, Pvj= 0.012; Figure 5b).

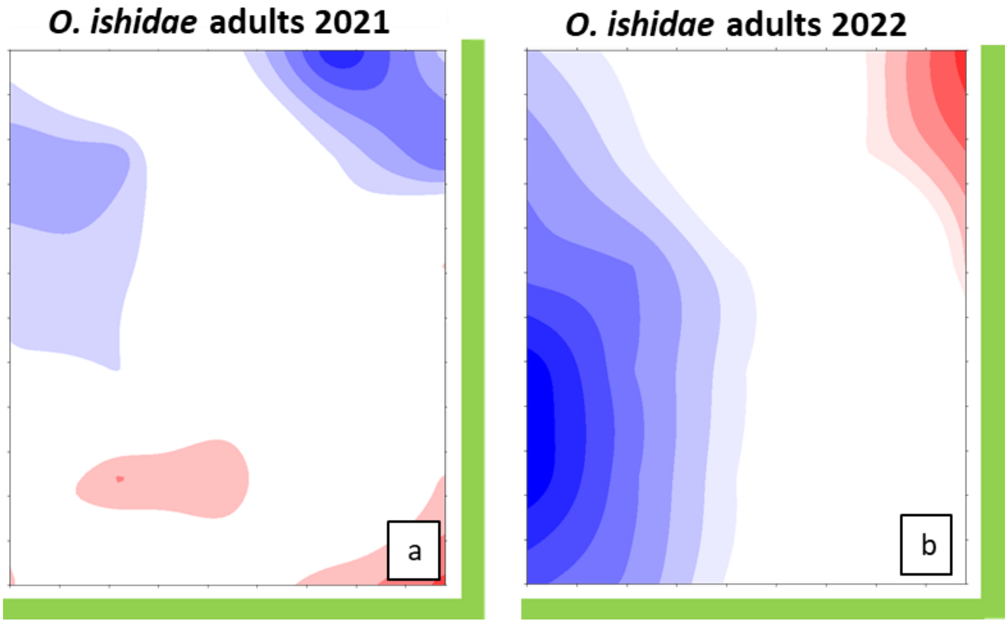

Figure 5 Spatial distribution of *O. ishidae* adults (cumulative numbers) in SMF vineyard in the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. Green lines represent groves margins.

The distribution of symptomatic grapevines in the 2021 growing season resulted in an aggregation pattern. The most significant patch was found on the south-western edge of the vineyard, close to the grove margins while minor patches were localized inside the vineyard (vi = 2.283, Pvi < 0.0001). Gaps were distributed in various areas (vj = - 2.239, Pvj < 0.0001; Figure 6a). In 2022, the distribution of symptomatic grapevines still showed an aggregation pattern. Most of the patches were along the west side of the vineyard (vi = 2.306, Pvi < 0.0001) while gaps from the middle of the vineyard towards grove margins (vj = - 2.356, Pvj < 0.0001; Figure 6b). The patches in 2022 were in the same part of the vineyard as observed for the patches of *S. titanus* adults in 2021.

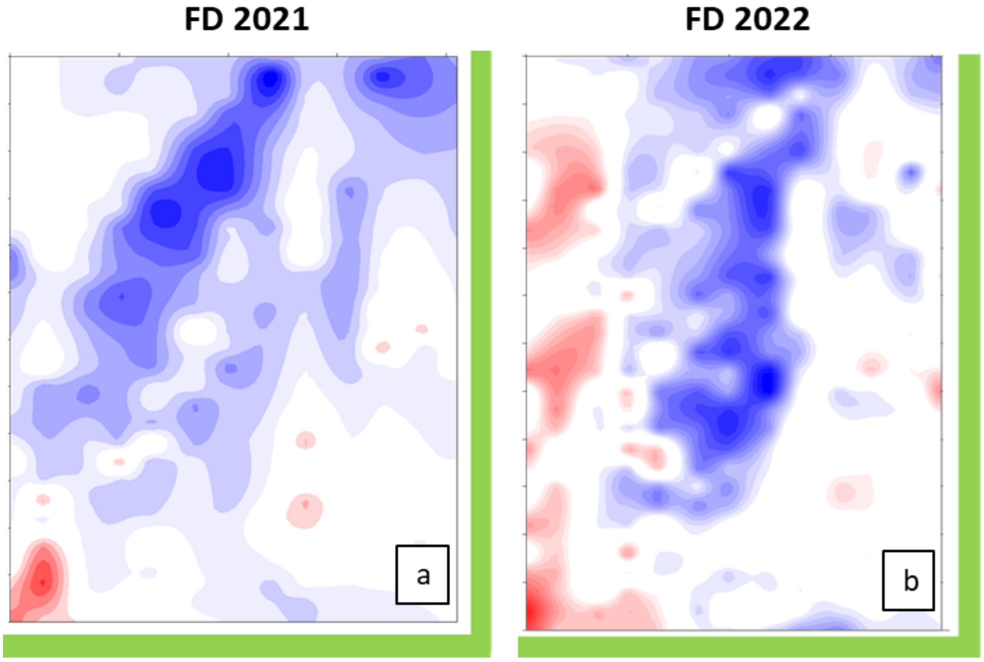

Figure 6 Spatial distribution of FD symptomatic grapevines in SMF vineyard in 2021 and 2022. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of symptomatic vines. Green lines represent groves margins.

SPF1

In 2021, *S. titanus* nymph populations reached moderate to high densities (0.32 nymphs per leaf per sampling date, from June 9 to July 15). Nevertheless, their distribution did not show aggregation patterns and no patches nor gaps were found (respectively: vi= 1.080, Pvi= 0.245 and vj= - 1.156, P= 0.163; Figure 7a). *Scaphoideus titanus* adults were detected regularly throughout the growing season (0.15 adults per trap per week from July to September) and their distribution resulted aggregated. Significant patches were found on the north-western area of the vineyard (vi= 1.668, Pvi< 0.0001) while significant patches were detected on the south-eastern area of the vineyard bordering the grove on the south side (vj= - 2.167, Pvj= 0.0002; Figure 7b). One year later, *S. titanus* nymph populations reached lower densities compared to the previous season (0.11 nymphs per leaf per sampling date, from May 25 to July 5). Their distribution resulted aggregated with significant patches along the west side and on the north-eastern edge of the vineyard (vi = 2.219, Pvi < 0.0001). Significant gaps close to grove margins (vj = -2.179, Pvj < 0.0001; Figure 7c). Adults captures in 2022 were very low (0.02 adults per trap per week, from June 30 to September 20) and adult distribution was not aggregated. No significant patches or gaps were found (respectively vi = 1.149, Pvi = 0.173 and vj = - 1.177, Pvj = 0.151; Figure 7d).

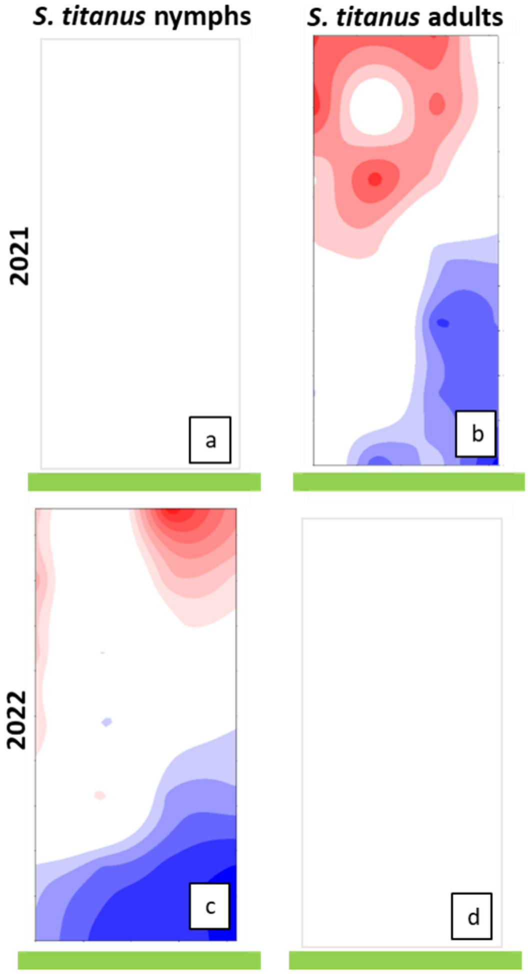

Figure 7 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in SPF1 vineyard in the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. The green line represents groves margins.

Orientus ishidae was detected throughout the 2021 growing season (0.27 adults per trap per week from July to September) and its population revealed an aggregation pattern. Significant patches were found on the south part of the vineyard bordering the grove (vi= 2.325, Pvi< 0.0001) while gaps were on the opposite side (vj = - 2.021, Pvj = 0.0005; Figure 8a). In 2022 *O. ishidae* was detected at lower levels than the previous year (0.04 adults per trap per week from July to September) and its distribution was not aggregated. No significant patches and gaps were found (respectively vi = 0.861, Pvi = 0.743 and vj = - 0.861, Pvj = 0.788; Figure 8b).

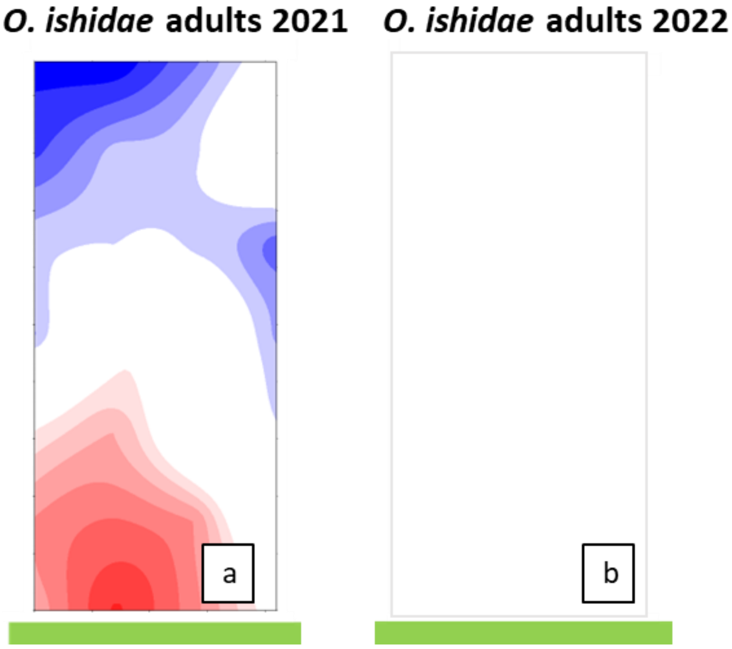

Figure 8 Spatial distribution of *O. ishidae* adults (cumulative numbers) in SPF1 vineyard during the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ - 1.5) and white area random distribution of leafhoppers. The green line represents groves margins.

Regarding symptomatic grapevines, their distribution in 2021 revealed an aggregation pattern. Significant patches were found from the central to the northern part of the vineyard (vi= 1.929, Pvi= 0.001) while gaps in the southern part close to the groves margins (vj = -1.706, Pvj = 0.004; Figure 9a). Similar trends in terms of aggregation patterns of symptomatic grapevines emerged in 2022 when patches (vi = 1.829, Pvi = 0.001) and gaps (vj = - 2.027, Pvj = 0.0008) were localized far and close to the grove, respectively (Figure 9b). In 2022, patches corresponded to those of *S. titanus* adults in 2021, whereas that of *O. ishidae* in 2021 was observed in the opposite side.

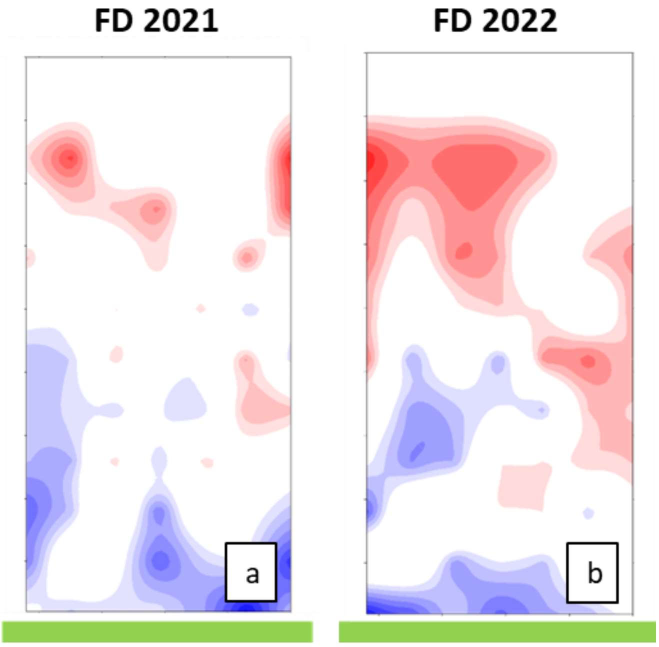

Figure 9 Spatial distribution of FD symptomatic grapevines in SPF1 during the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of symptomatic vines. The green line represents groves margins.

SPF2

In 2021, *S. titanus* nymph populations reached relatively high densities (0.47 nymphs per leaf per sampling date, from June 9 to July 15) and their distribution was aggregated. Patches were localized in the north-western edge of the vineyard (vi = 1.669, Pvi = 0.0007), partially bordering the grove on the west side but above all contiguous to the vineyard infested by *S. titanus* (see figure 1 and the description of the agroecosystem). Gaps were present along the east side distant to the grove (vj = - 1.895, Pvj = 0.007) (Figure 10a). *Scaphoideus titanus* adults were frequently detected in 2021 (0.27 adults per trap per week, from July to September) and their distribution was aggregated. Patches of adults were localized on the north-western edge of the vineyard like those of nymphs, but with a greater extension along the west side of the vineyard (vi = 1.588, Pvi = 0.026); on the other hand, gaps were in south-western edge completely delimited by the grove vegetation (vj = - 1.528, Pvj = 0.026) (Figure 10b). In 2022, *S. titanus* nymph populations reached moderate densities (0.17 nymphs per leaf per sampling date). Their distribution was aggregated with patches localized in the north-western part of the vineyard and gaps on the opposite part, as the before growing season (respectively: vi = 1.756, Pvi = 0.003; vj = - 1.878, Pvj = 0.001; Figure 10c). Nymph patches in 2022 were similar to those of adults in the previous year. Captures of *S. titanus* adults were lower than in the previous season (0.02 adults per trap per week, from July to September). Their distribution was not aggregated and thus no patches nor gaps were noticed (respectively: vi = 1.078, Pvi =0.204 and vj= - 0.867, Pvj = 0.215; Figure 10d).

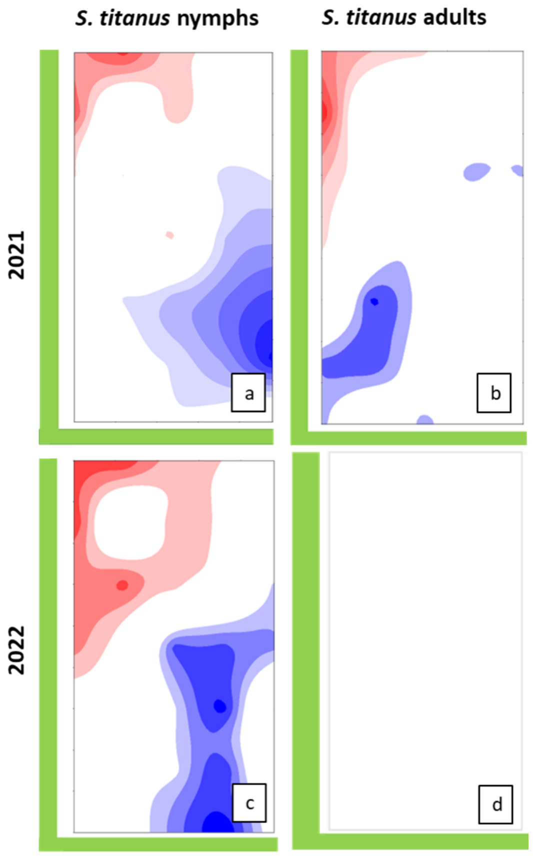

Figure 10 Spatial distribution of *S. titanus* nymphs and adults (cumulative numbers) in SPF2 vineyard during the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. The green line represents groves margins.

The occurrence of *O. ishidae* in this vineyard in 2021 was regularly detected (0.17 adults per trap per week from July to September). Their distribution was aggregated. The largest patch was localized in the middle of the vineyard (vi= 1.572, Pvi = 0.011); gaps were localized along the north and east sides, those not bordered the grove (vj = - 1.541, Pvj = 0.048; Figure 11a). In 2022, the spatial distribution of *O. ishidae* (0.097 adults per trap per week, from July to September) was aggregated and characterized by a patch (vi = 1.853, Pvi = 0.0005) on the conjunction of the south and west sides of the vineyard, those bordered by the grove. In contrast, gaps were localized in the north-eastern area of the vineyard far from the grove margins (vj = - 1.910, Pvj = 0.001; Figure 11b).

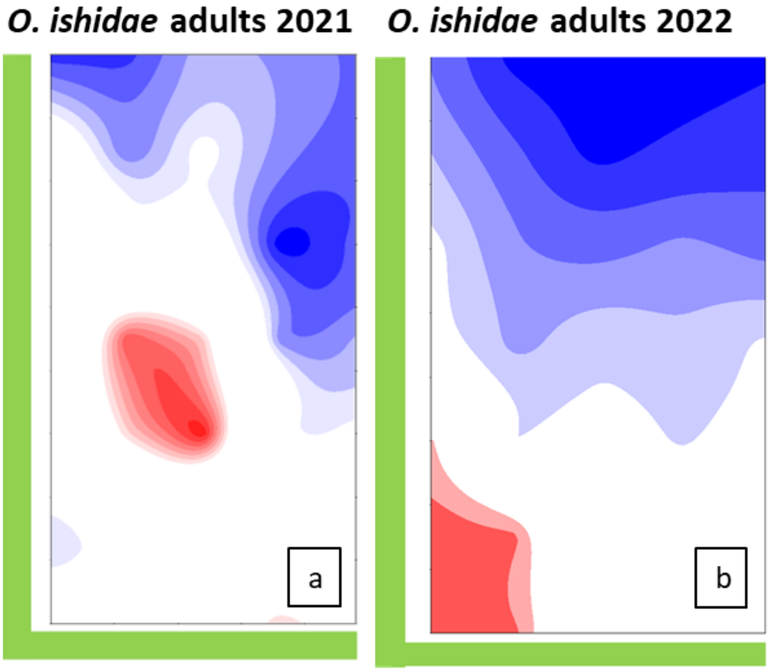

Figure 11 Spatial distribution of *O. ishidae* adults (cumulative numbers) during the 2021 and 2022 growing seasons. Red areas indicate aggregation (patches, vi ≥ 1.5), blue areas dispersion (gaps, vj ≤ -1.5) and white area random distribution of leafhoppers. The green line represents groves margins.

Discussion

Mechanisms underlying the epidemiology of FD have been widely discussed and various authors have pointed out on the complexity of relationships among host plants, phytoplasmas and vectors (e.g., Purcell, 1982; Malembic-Maher *et al*., 2020). At the landscape level, a positive role of groves in promoting FD spread was reported (Adrakey *et al*., 2022). At the vineyard level, groves were found to favor the spread of FD when they hosted naturally growing American grapevines (Pavan *et al*., 2012; Lessio *et al*., 2015). In this study we tried to understand if groves have a role in FD spread when naturally growing American grapevines were virtually absent. A role of groves in the epidemiology of FD was suggested by studies on the interactions between alternative vectors (e.g., *D. europaea* and *O. ishidae*) and their typical host plants (clematis, hazel, *Salix* spp. and alder) that were also capable to host FD phytoplasmas (Filippin *et al*., 2009; Linder *et al*., 2014; Krstic *et al*., 2016, 2018, 2019; Casati *et al*., 2017; Jermini *et al*., 2017, 2019; Desqué *et al*., 2019; Jarausch *et al*., 2019; Lessio *et al*., 2019; Malembic-Maher *et al.,* 2019, 2020; Cvrković *et al*., 2021; Rizzoli *et al*., 2021).

The territory of the Consortium Conegliano Valdobbiadene Prosecco Superiore DOCG, recently included in Unesco's sites. Is dominated by a mosaic structure of the landscape, which is a result of historical and ongoing environmental and land use practices. The plots dedicated to vineyards coexist with forest patches, small groveslands, hedges, and rows of trees that serve as corridors connecting different habitats (https://whc.unesco.org/en/list/1571). While the fragmented structure of this territory is considered a value, the proximity of the groves to the vineyards is often considered by winegrowers and viticultural technicians a favorable element for the spread of FD, but there is no evidence that this is true.

Groves and vector spatial distribution

In the current study, among FD phytoplasma-vectors, we detected regularly *S. titanus* and less frequently *O. ishidae*. *Scaphoideus titanus* is monophagous on grapevines and its occurrence on grove vegetation is expected when American grapevines grow naturally inside hedgerows or groves (Maixner *et al*., 1993; Beanland *et al*., 2006; Lessio *et al.,* 2007; Pavan *et al*., 2012). On the other hand, *O. ishidae* is strictly related to arboreal plants frequently comprised inside hedgerows or groves (Lessio *et al*., 2016, 2019; Parise, 2017).

We analyzed the spatial distribution of *S. titanus* considering cumulative numbers of nymphs and adults in four vineyards involved in recent FD outbreaks. The spatial distribution of nymphs and adults recorded in 2021 and 2022 did not show aggregations that could be clearly associated with the proximity of groves. In one case patches were clearly far from the grove (SPF1 vineyard). In the remaining three cases (RUA, SMF and SPF1 vineyards), the patches slightly involved the grove margins, while gaps occurred in parts of vineyards largely bordering the groves. This suggests that in the absence of wild American vines, groves did not serve as a source of *S. titanus* and did not seem to promote the aggregation of the vector in neighboring parts of the vineyard.

In at least two cases (Rua and SMF vineyards), patches were distributed on an area contiguous to vineyards managed by other growers and characterized by high *S. titanus* populations and FD incidence. This result confirms that the presence of cultivated vineyards, where the control of the vector was ineffective, can represent a danger for contiguous vineyards in which *S. titanus* populations were effectively controlled (Pavan *et al*., 2012).

In three out four vineyards (SMF, SPF1 and SPF2) *O. ishidae* adults showed aggregative patches clearly related to the groves. This result was expected due to the polyphagy of this leafhopper (Lessio *et al*., 2016; Parise, 2017; Cvrković *et al*., 2021) and the presence of their host plants in the groves.

Temporal variations in the density and spatial distribution of *S. titanus* nymphs and adults

In the summer of 2021, *S. titanus* adults were abundant; their distribution was aggregated, and patches localization was similar to that of nymphs in the three vineyards in which nymph patches were recorded (Rua, SMF, SPF2). This suggests that insecticide application against nymphs was not effective. In fact, nymph densities in 2022 were still moderate to high and their aggregative distribution patterns frequently reflected those of adults in 2021. In the summer of 2022, *S. titanus* adults detected on traps were much lower than those reported in the previous season, and their distribution was not aggregated. This suggests that chemical control applied against nymphs in 2022 was more effective than in the previous growing season. Pyrethroids were applied more frequently in 2022 than in 2021 (at least twice vs. once) and with different active ingredients (acrinathrin, etofenprox or deltamethrin vs. tau-fluvalinate) and this variation is probably involved in *S. titanus* control (see Chapter 7; Prazaru et al., 2023).

Spatial distribution of FD-symptomatic grapevines

The distribution of symptomatic grapevines was not influenced by groves in any of the three considered vineyards. The distribution substantially mirrors that of *S. titanus* and, in particular, a good relationship was observed between the distribution of adults in 2021 and that of symptomatic grapevines in 2022. This result indirectly confirms that symptoms first occur in the year following the inoculation (Caudwell, 1990; Morone *et al*., 2007) and that *S. titanus* adults are very effective in the transmission (Alma *et al*., 2019).

On the other hand, the distributions of symptomatic grapevines and *O. ishidae* were inconsistent, suggesting that this vector plays a minor role in FD epidemiology in agreement with other studies (Desqué *et al*., 2019; Rizzoli *et al*., 2021). This result is also consistent with the very low transmissibility rate of FD-phytoplasmas using *O. ishidae* (Lessio *et al*., 2016; Desqué *et al*., 2019). Therefore, our study clearly highlighted that the FD epidemic that occurred in the study area cannot be attributed to the high incidence of groves and in particular to infectious *O. ishidae* adults colonizing vineyards coming from groves. This occurred even if *O. ishidae* adults collected in the study area were found infected by FD-phytoplasmas (data not reported).

Therefore, when American grapevines growing naturally are absent in groves, they don't represent a risk for cultivated vineyards. Instead, it is indirectly confirmed that infected vineyards, where *S. titanus* control is not carried out adequately, are the most dangerous source of infectious vectors.

Conclusion

When natural American grapevines are absent in groves, the latter are not a risk for cultivated vineyards. Groves can be a source of *O. ishidae*, but in the case studies reported in this work this phenomenon did not represent a real danger for vineyards. Therefore, an effective FD control would be favored by the removal of American grapevines growing naturally in them.

The primary role of *S. titanus* in the epidemiology of FD was confirmed. In this context, the present study highlighted once again that infected vineyards, where *S. titanus* control is not carried out adequately, are a dangerous source of infectious vectors. The data relating to the abundance of *S. titanus* adults in 2022 highlighted the importance of using effective insecticides against nymphs. Given the good overlapping between patches of *S. titanus* adults captures in 2021 and those of FD incidence in 2022, we can reasonably be confident that the reduction of the vector populations in 2022 will lead to a slowdown in the increase of the disease in 2023.

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Chapter 7

Efficacy of conventional and organic insecticides against *Scaphoideus titanus*: field and semi-field trials

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Prazaru Stefan Cristian contributed to data collection, performed statistical analysis, and contributed to manuscript drafting.

Abstract

Scaphoideus titanus is the main vector of phytoplasmas associated to Flavescence dorée (FD), one of the most serious threats to viticulture in many European countries. To minimize the spread of this disease, compulsory control measures against *S. titanus* were decided in Europe. In the 90ties the repeated application of insecticides (mainly organophosphates) proved to be an effective measure to control the vector and the related disease in north-eastern Italy. These insecticides and most of neonicotinoids were recently banned from European viticulture. Serious FD issues detected in the recent years in northern Italy could be related to the use of less effective insecticides. Trials aimed at evaluating the efficacy of the most used conventional and organic insecticides in the control of *S. titanus* have been performed in semi-field and field conditions to test this hypothesis. In efficacy trials, carried out in four vineyards, etofenprox and deltamethrin proved to be the best conventional insecticides, while pyrethrins were the most impactful among organic insecticides. Insecticide residual activity was evaluated in semi-field and field conditions. Acrinathrin showed the most significant residual effects in both conditions. In semi-field trials most of pyrethroids were associated to good results in terms of residual activity. However, these effects declined in field conditions probably due to high temperatures. Organic insecticides showed poor results in terms of residual efficacy. Implications of these results in the context of Integrated Pests Management in conventional and organic viticulture were discussed.

Introduction

The leafhopper *Scaphoideus titanus* Ball is the main vector of Grapevine flavescence dorée phytoplasma (FDP) (Schvester *et al*., 1962; Mori *et al*., 2002), belonging to the elm yellows group (16SrV) (Bertaccini *et al*., 2022). Flavescence dorée (FD) is considered one of the most important diseases in European vineyards and is currently causing serious damage to grapevine yield and quality in many European regions (Belli *et al*., 2010; Dermastia *et al*., 2017; Malembic-Maher *et al*., 2020). Originating from North America, *S. titanus* was detected first in France (Bonfils and Schvester, 1960) then in Italy (Vidano, 1964). In the following years, *S. titanus* spread to many other European countries, from Portugal to Romania (Chuche and Thiéry, 2014). More recently, it has been detected in the North Caucasus (Gnezdilov and Orlov, 2022). Monophagous on grapevines, *S. titanus* can acquire phytoplasmas as nymphs by feeding on infected plants. The latency period is about 4-5 weeks and the infective adults can transmit phytoplasmas to healthy plants for the rest of his life even across large areas (Chuche and Thiéry, 2014; Lessio *et al*., 2011). Moreover, *S. titanus* can acquire the phytoplasma as adult, becoming infective within 1-2 weeks (Alma *et al*., 2018). *Scaphoideus titanus* develops one generation per year and overwinters as eggs. Egg hatching starts in May and lasts for about two months depending on winter temperatures (Chuche and Thiéry, 2009). Females with mature eggs can be found from July (when oviposition starts) till late October (Vidano, 1964; Pavan *et al*., 1987; Bocca *et al*., 2020). Adult females live an average of 60 days, some of them 70-100 days, while adult male longevity is shorter (Bocca *et al.*, 2020). The short latency period combined with the long survival implies that adults have a long inoculation period.

FDP is a quarantine pathogen and its control is regulated by mandatory measures including the rouging of infected grapevines and the chemical control of *S. titanus* (Belli *et al*., 2010). An area-wide and prompt monitoring of the vector is the pre-requisite to design a rational control strategy, moreover the vector sampling is a key action for pest management as it allows us to identify the best timing for insecticide applications. With a high density of *S. titanus*, two insecticides applications are required: the first one against third instar nymphs (before they become infectious) and the second one after two weeks, to suppress new hatched nymphs (Pavan *et al*., 2005). Chemical control must be integrated with cultural measures such as the removal of branches from winter pruning and suckers. The elimination of pruning remains reduces the stock of eggs in the vineyard (Trivellone *et al*., 2015), while the removal of suckers growing along the vertical trunk could contribute to decrease *S. titanus* population levels, frequently higher on suckers than within the canopy (Schvester *et al*., 1962; Bernard and Du Fretay, 1988). The presence of abandoned vineyards and American grapevines growing spontaneous in woodland, can represent a source of infectious vectors for surrounding vineyards (Caudwell, 1981; Pavan *et al*., 2012; Lessio *et al*., 2015) and should be removed before the appearance of adults which can fly up to 300 m (Lessio *et al*., 2014).

One of the most severe FD outbreaks in Europe took place in the Veneto region, north-eastern Italy, in the 90ties (Martini *et al*., 1999). Chemical control measures against *S. titanus* gave satisfactory effects in controlling the spread of the disease (Girolami *et al*., 2002; Pavan *et al*., 2005; Pavan *et al*., 2012). Insecticides used for this purpose (e.g., organophosphates, some chitin-inhibitors) were characterized by a long residual effect often associated with contact activity. The most used active ingredients were fenitrothion, chlorpyriphos-ethyl, chlorpyrifos-methyl, buprofezin and flufenoxuron (Posenato *et al*.,2001). More recently, the neonicotinoid thiamethoxam was largely employed with satisfactory results (Žežlina *et al*., 2013; Matko *et al*., 2013; Zidaric *et al*., 2013). In the last years all these active ingredients have been removed from the European market (in particular, chlorpyrifos-ethyl, chlorpyrifos-methyl and thiamethoxam were applied until 2019 in vineyards) because of concerns for human health or the environmental impact. At the same time other insecticides such as the pyrethroids etofenprox and lambda-cyhalothrin were classified as candidates for substitution by the European Commission (Regulation (EC) No 1107/2009) (European Parliament, 2009) and their use in the IPM guidelines developed in many Italian regions was restricted. In the last three years, the main active ingredients considered in IPM guidelines in Italy for the control of *S. titanus* have been acetamiprid, flupyradifurone, and tau-fluvalinate. In this context issues with FD increased in northern Italy and changes in chemical control products were suspected to be involved in this event.

In Italian organic viticulture few active ingredients are allowed for the control of *S. titanus.* Pyrethrins are commonly used but their low persistence makes the execution of repeated applications necessary (Pavan *et al*., 2005; Gusberti *et al*., 2008; Mori *et al*., 2014; Tacoli *et al*., 2017). Their effects may be enhanced when used in combination with adjuvants. The effectiveness of other products based on azadirachtin, potassium salts of fatty acids or *Beauveria bassiana* is considered to be from moderate to low (Caruso and Mazio, 2004; Mori *et al*., 2014). It is not surprising that FD is a key problem for organic farms.

In this work we evaluated the effectiveness of insecticides authorized in Italy for the control of *S. titanus* in conventional and organic viticulture. Specific trials, both in semi-field and field conditions, were performed to evaluate the efficacy and the residual activity of the insecticides on nymphs and adults. Investigations on the residual effects of insecticides are important for nymph control due to the long egg-hatching period, and for adult control because FD epidemics are sustained by infected adults colonizing vineyards from the surrounding areas (Pavan *et al*., 2012; Lessio *et al*., 2015). The results of these trials could help to plan adequate control strategies against *S. titanus* nymphs and adults, complying with the European Union's achievement of sustainable use of pesticides (Directive 2009/128/EC).

Materials and Methods

Experimental sites and insecticides used in conventional viticulture

The efficacy of insecticides used in conventional viticulture was evaluated in two vineyards located in the Veneto region (north-eastern Italy) in the 2022 growing season. The first vineyard (SPF) was located at San Pietro di Feletto (45°54’43’’N, 12°14’12’’E, 214 m a.s.l.) and the second (FM) at Fumane (45°32’34’’N, 10°52’39’’E, 289 m a.s.l.). SPF vineyard was characterised by cultivar Glera, Sylvoz training system, 3.5 m x 1.65 m of planting space. FM vineyard was characterised by cultivar Corvina, Pergola training system, 3.0 m x 0.8 m of planting space. The same protocol and insecticide formulations were used in both vineyards (Table 1). No insecticides other than the comparison ones were applied in the experimental vineyards during the field trials. The experimental plan was a randomized block design with four replicates per treatment represented by plots of 600 m^2 (SPF) and 850 m^2 (FM). Insecticides were applied on June 10 in SPF and on June 15 in FM, when the third nymphal instars of *S. titanus* were dominant. Insecticides were sprayed using a farmer atomizer with pressure10 bar, driving speed of 6.0 km/h, Albuz core disc nozzles in SPF vineyard, pressure 15 bar, driving speed of 6.5 km/h, MFT core disc nozzles in FM vineyard. The application volume was 10 hL/ha, with the exception of flupyradifurone (8 hL/ha) due to producer instructions. Samplings were carried out before and 7 days after insecticide application by examining 100 basal leaves per replicate (5 leaves per grapevine) for a total of 400 leaves per treatment. In Verona vineyard, sampling occurred also on suckers developed along grapevine trunk counting the *S. titanus* nymphs present on 50 suckers per replicate for a total of 200 suckers per treatment.

Table 1. Characteristics of insecticides investigated in the different experimental trials.

Management	Formulation	Active ingredient	Dose (mL or g/hL)	Experimental trial
Conventional	Decis EVO	Deltamethrin	50	FTE, SRAT*
Conventional	Closer	Sulfoxaflor	40	FTE, SRAT*
Conventional	Epik SL	Acetamiprid	150	FTE, SRAT*
Conventional	Sivanto Prime	Flupyradifurone	60	FTE, SRAT*
Conventional	Mavrik Smart	Tau-fluvalinate	30	FTE, SRAT*
Conventional	Trebon UP	Etofenprox	50	FTE, SRAT*
Conventional	Rufast	Acrinathrin	60	SRAT*
Conventional	Karate Zeon	Lambda-cyhalothrin	25	SRAT*
Organic	Biopiren Plus	Pyrethrins	160	FTE SRAT*
Organic	Biopiren Plus + Mago	Pyrethrins + ethoxylated sorbitan monooleate	160 + 150	FTE*
Organic	Naturalis	*Beauveria bassiana*	150	FTE, SRAT*
Organic	Flipper	Potassium salts of fatty acids	1500	FTE*
Organic	Neemik TEN	Azadirachtin	390	FTE, SRAT*
Organic	Surround WP	Kaolin	2500	FTE*

*FTE: Field efficacy trial; SRAT: Semi-field residual activity trial:

Experimental sites and insecticides used in organic viticulture

The efficacy of insecticides used in organic viticulture was evaluated in two vineyards located in Veneto region in the 2022 growing season (Table 1). The first vineyard (SAR) was located at Sarmede (45°58’22’’N, 12°22’45’’E, 103 m a.s.l.) and the second (STA) at Stallavena (45°32’15’’N, 11°00’07’’E, 219 m a.s.l.). The vineyard (SAR) was characterised by cultivar Glera, Sylvoz training system, 3.5 m x 1.65 m of planting space, the vineyard (STA) was characterised by cultivar Corvina, Pergola training system 4.0 m x 1.0 m of planting space. The protocol was similar to that previously described for conventional vineyards but in this case, insecticides were applied twice, the first time on June 6 (in SAR) or June 9 (in STA), when the second instar nymphs were dominant, and the second time on June 13 (SAR) or June 15 (STA). The application volume was 10 hL/ha using a farmer atomizer, pressure 8 bar, driving speed of 6,0 km/h, Albuz core disc nozzles in both vineyards. According to producer instructions, the pH of solution was 6.5-6.8 for pyrethrins and pyrethrins + ethoxylated sorbitan monooleate, 5.5 for Azadirachtin and for potassium salts of fatty acids the water hardness was < 20 French degrees (°fH). The experimental plan was a randomized block design with four replicates per treatment represented by plots of 600 m^2 (SAR) and 1700 m^2 (STA). In the trial performed in SAR vineyard samplings before and after insecticide application were done on basal leaves (100 per replicate) while in the STA vineyard suckers (50 per replicate) were considered for sampling.

Insecticide residual activity in semi-field conditions

Semi-field trials to evaluate insecticide residual activity on *S. titanus* nymphs and adults were carried out in the experimental farms of the University of Padova (Agripolis, Legnaro) and the University of Verona (Villa Lebrecht, San Pietro in Cariano) in the 2022 growing season. These trials were carried out on irrigated one-year potted grapevines cultivar Chardonnay kept under shading net. Shoot thinning was performed to obtain at least two grapevine shoots per vine. The effects of eleven products containing active ingredients authorized in conventional or organic viticulture were evaluated (Table 1); a control treated with tap water was included for comparison. In Verona trials, acrinathrin was not tested, and azadirachtin and *B. bassiana* were evaluated only against nymphs. Treatments comprised respectively three (Verona) or four (Padova) replicates each represented by a potted vine. Trials were performed on nymphs (second and third instars) or adults of *S. titanus*. Insecticides were applied in mid-June for nymph trials and in July for adult trials. Potted grapevines were sprayed with a compression sprinkler (8 L volume), spacing them from each other to avoid drift effects. A shoot of each potted grapevine was inserted 3 days after treatment into a tulle sleeve (1.0 m x 0.6 m) and 10 nymphs or adults per replicate were released into the tulle sleeves, carefully closed around the stem of the shoot to avoid insect escaping. Samplings were carried out 3 days after caging. At this purpose, grapevine shoots inside the tulle sleeves were cut and carefully inspected, counting dead and alive individuals. A second release of 10 *S. titanus* nymphs or adults was made 7 days after insecticide applications, confining them on another shoot as above. After 3 days of confining, dead and alive individuals were counted following the procedure previously described. Leafhoppers used for these trials were collected from organic vineyards located in San Pietro di Feletto (TV) and Stallavena (VR), respectively fron trials at the University of Padova and the University of Verona.

Insecticide residual activity in field conditions

The residual activity of several conventional insecticides was evaluated in a vineyard located in San Pietro di Feletto (45°52’19’’N, 12°15’40’’E, 148 m a.s.l) during 2021 and 2022 growing seasons (Table 1). This vineyard was characterised by cultivar Glera, Sylvoz training system, 3.5 m x 1.65 m of planting space. The occurrence of *S. titanus* was negligible. Four insecticides (acetamiprid, acrinathrin, flupyradifurone and tau-fluvalinate) were tested in both growing seasons, deltamethrin, etofenprox and sulfoxaflor in 2022 only. An untreated control was included for comparison. Insecticides were applied according to the maximum dose per hectare indicated in the product labels against leafhoppers. Each treatment comprised four replicates represented by three rows 40-60 m long. In both seasons, two trials were carried out, the first devoted to evaluating the insecticide residual activity against nymphs, the second against adults. For nymphs, insecticide applications were performed on June 21, 2021, and on June 10, 2022, while for adults on August 2, 2021, and on July 13, 2022. Three days from insecticide application, 10 nymphs or adults, collected from nearby vineyards, were confined on a shoot with a tulle sleeve (1 m x 0.60 m). Treatments comprised 4 replicates, each represented by a shoot for a total of 40 individuals per treatment. Three days after caging, dead and alive individuals were counted, cutting the shoots and pouring the tulle sleeve contents into a basin for a careful check. The same procedure was performed 7 days after insecticide applications.

Statistical analysis

Data obtained from each trial were analyzed using a generalized linear mixed model with the GLIMMIX procedure of SAS® (ver. 9.4; SAS Institute Inc., Cary, NC, USA). In field trials, the number of *S. titanus* per basal leaf or sucker after treatment was used as the dependent variable, both in organic and conventional vineyards while the type of insecticide was considered as the factor of variation. In the conventional insecticide efficacy trial, the sampling unit and experiment arrangement was identical, thus data from both fields were used to run the model and vineyard was considered as a random effect term in the model, in order to contribute at the error calculation. In the organic insecticide trial data from the two field were analyzed separately due to the different sampling unit (i.e., basal leaves in SAR and suckers in STA). In trials aimed at evaluating insecticide residual activity, the number of dead individuals was considered as dependent variable. The factor of variation (type of insecticide) was tested using an *F-test* ($\alpha = 0.05$). Comparisons of the mean numbers of *S. titanus* per leaf or sucker in open field trials and dead *S. titanus* in residual activity trials in different treatments were performed using *t*-test ($\alpha = 0.05$) on the least-square means. The degrees of freedom were estimated with the Kenward–Roger method, which can calculate non-integer values for error terms. Before the analysis, data were checked for model assumptions. The model was run on data transformed to log (n + 1), while untransformed data are shown in the figures. The effectiveness of the insecticides was calculated according to the Henderson and Tilton formula (1955) in field trials and with the Abbott formula (1925) for the residual activity trials.

Results

Efficacy of conventional insecticides against S. titanus nymphs in open field (basal leaves)

No differences among treatments were detected prior to insecticide applications (F = 0.52; d.f. = 6, 48; p = 0.788). Insecticide applications significantly reduced *S. titanus* nymph populations (F = 21.76; d.f. = 6, 48; p < 0.0001). All insecticides differed from the control, but deltamethrin and etofenprox were more effective than acetamiprid and flupyradifurone; sulfoxaflor and tau-fluvalinate showed intermediate effects (Figure 1).

Henderson and Tilton efficacy agreed with statistical analysis showing the highest efficacy values for deltamethrin and etofenprox (> 90%), the lowest values for flupyradifurone and acetamiprid (< 50%) and intermediate values for sulfoxaflor and tau-fluvalinate (Figure 1).

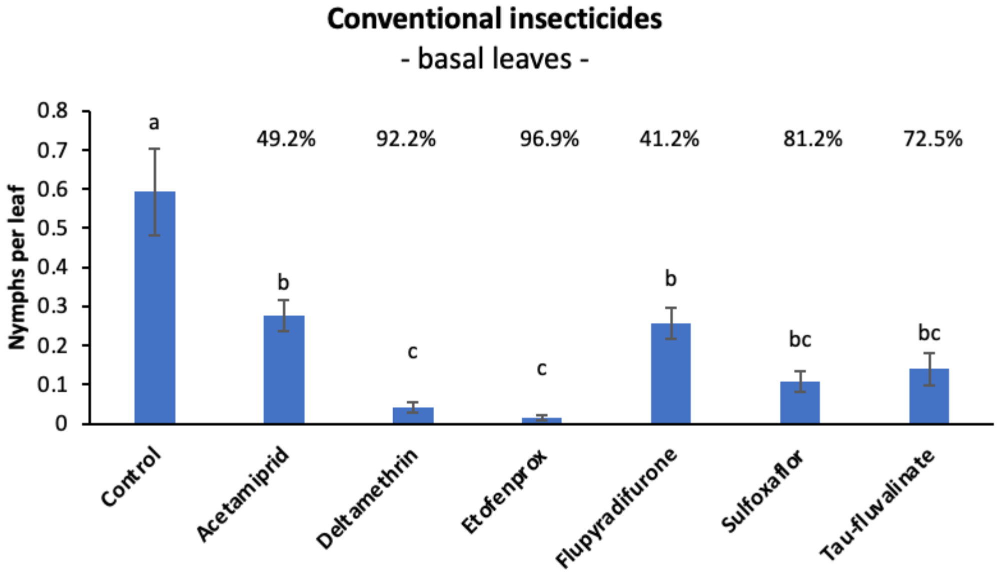

Figure 1. Effects of six insecticides on *S. titanus* nymphs (mean ± SE) evaluated at 7 days from their application (SPF and FM vineyard). Different letters indicate significant differences at the *t*-test (α = 0.05). For each insecticide the Henderson and Tilton efficacy (%) is also reported.

Efficacy of conventional insecticides against S. titanus nymphs in open field (suckers)

Before insecticide application there were no differences among treatments (F = 0.58; d.f. = 6, 21; p = 0.743) while insecticide application caused significant effects on *S. titanus* nymphs (F = 14.41; d.f. = 6, 21; p < 0.0001). All insecticides differed from the control and among them deltamethrin was more effective than acetamiprid (Figure 2). The remaining insecticides caused intermediate effects.

Henderson and Tilton efficacy agreed with statistical analysis showing the highest efficacy value for deltamethrin (around 90%), the lowest efficacy value for acetamiprid (around 60%) and intermediate values for the other insecticides (Figure 2).

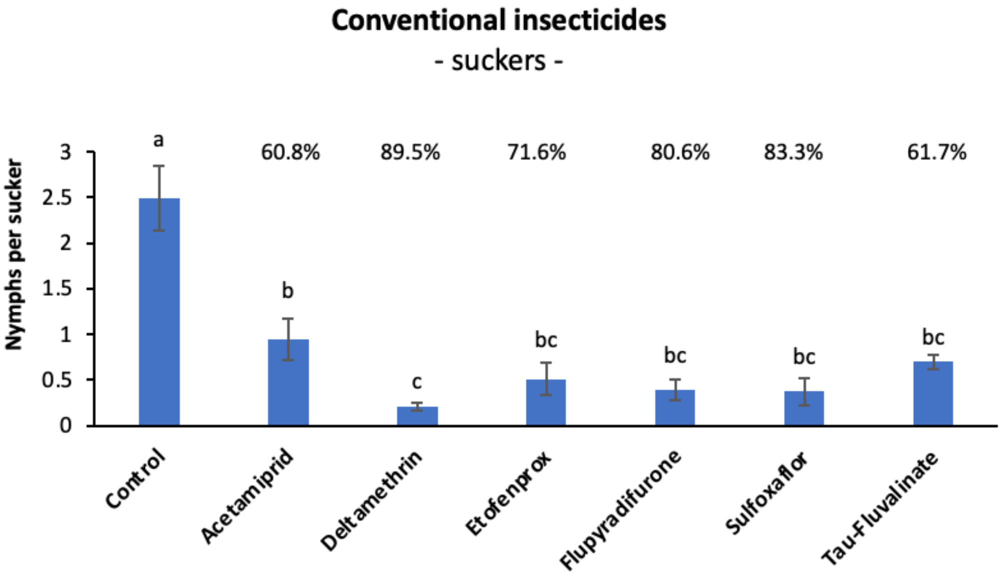

Figure 2. Effects of six insecticides on *S. titanus* nymphs (mean ± SE) evaluated at 7 days from their application (FM vineyard). Different letters indicate significant differences at the *t*-test (α = 0.05). For each insecticide the Henderson and Tilton efficacy (%) is also reported.

Efficacy of organic insecticides against S. titanus nymphs in open field (basal leaves)

Before insecticide application, no differences among treatments were found (F = 0.03; d.f. = 6, 21; p = 0.999). Later, insecticide application affected *S. titanus* nymph densities (F = 4.65; d.f. = 6, 20.04; p = 0.004). Only pyrethrin-based insecticides and kaolin differed significantly from the control without differing each other. Azadirachtin and *B. bassiana* were significantly less effective than the two pyrethrin-based insecticides (Figure 3).

Henderson and Tilton efficacy agreed with statistical analysis showing the highest efficacy values for pyrethrin-based insecticides (around 70%) and the lowest efficacy for azadirachtin (< 10%). The remaining products showed intermediate efficacy levels (Figure 3).

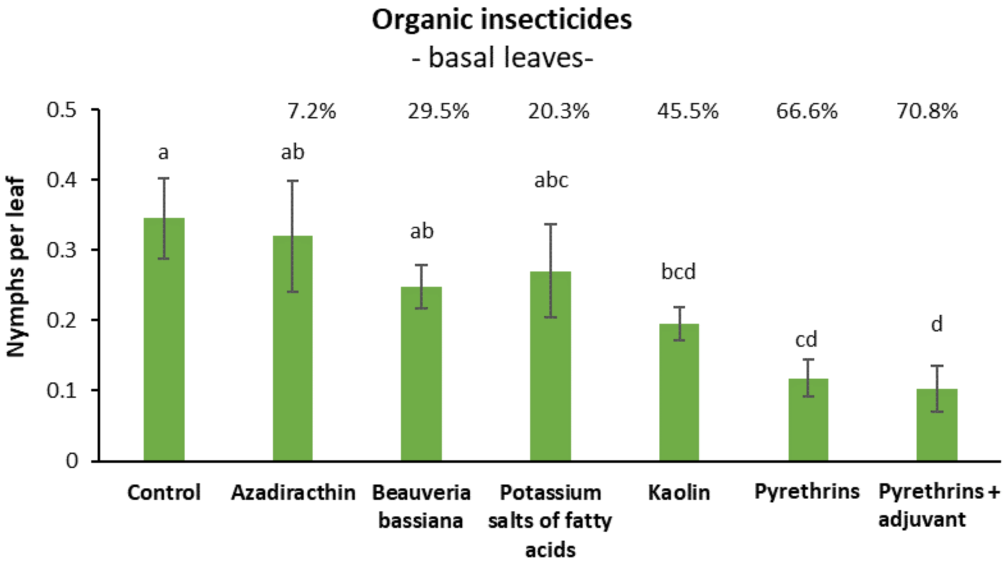

Figure 3. Effects of two applications of five insecticides or kaolin on *S. titanus* nymphs (mean ± SE) evaluated at 14 days from the first application (SAR vineyard). Different letters indicate significant differences at the *t*-test (α = 0.05). For each product the Henderson and Tilton efficacy (%) is also reported.

Efficacy of organic insecticides against S. titanus nymphs in open field (suckers)

When suckers were sampled prior to insecticide application there were no differences among treatments (F = 0.42; d.f. = 6, 21; p = 0.859). Later, insecticide application caused significant effects on *S. titanus* nymphs (F = 6.61; d.f. = 6, 21; p = 0.0005). All products except potassium salts of fatty acids differed significantly from the control. The effects of pyrethrins + adjuvant were more impactful than those of *B. bassiana* and kaolin (Figure 4).

Henderson and Tilton efficacy agreed with statistical analysis showing the highest efficacy values for pyrethrin-based insecticides (> 80%) and azadirachtin (> 70%), and a moderate efficacy for kaolin and *B. bassiana* (> 50%) (Figure 4).

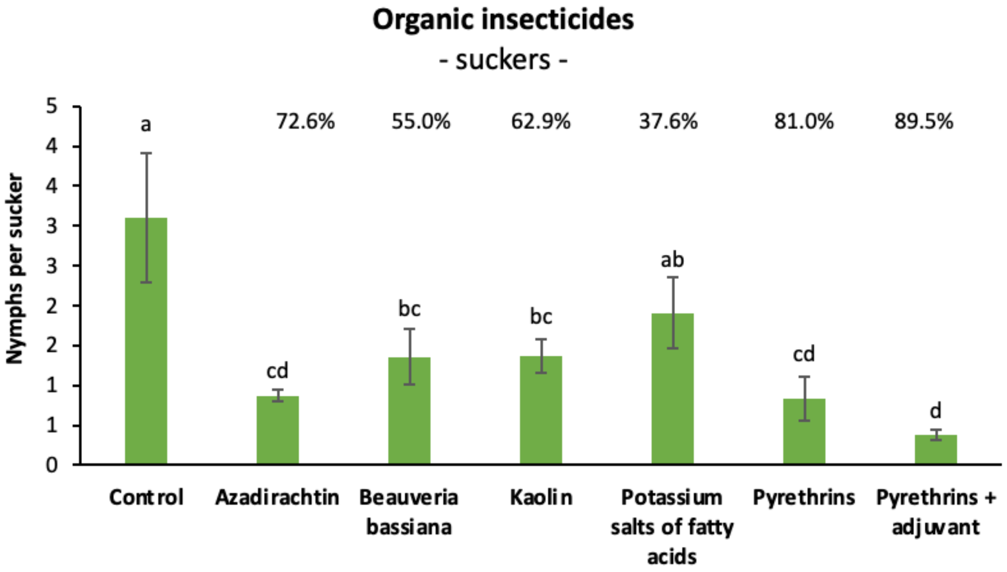

Figure 4. Effects of two applications of five insecticides or kaolin on *S. titanus* nymphs (mean ± SE) evaluated at 14 days from the first application (STA vineyard). Different letters indicate significant differences at the *t*-test (α = 0.05). For each product the Henderson and Tilton efficacy (%) is also reported.

Insecticide residual activity in semi-field trials

Against *S. titanus* nymphs

In the trial carried out at the University of Padova, *S. titanus* nymphs were significantly affected by insecticides when confined on plants 3 and 7 days after their application (3 days: F = 22.03; d.f. = 11, 36; $p < 0.0001$; 7 days: F = 15.41; d.f. = 11, 36; $p < 0.0001$). At three days, only conventional insecticides determined higher mortality rates compared to the control. Among them, acetamiprid was less effective than acrinathrin, that caused the highest mortality level (> 80%) (Figure 5). At seven days, all pyrethroids (acrinathrin, deltamethrin, etofenprox, lambda-cyhalothrin and tau-fluvalinate), acetamiprid and sulfoxaflor differed significantly from the control and among them the more effective were acrinathrin and lambda-cyhalothrin.

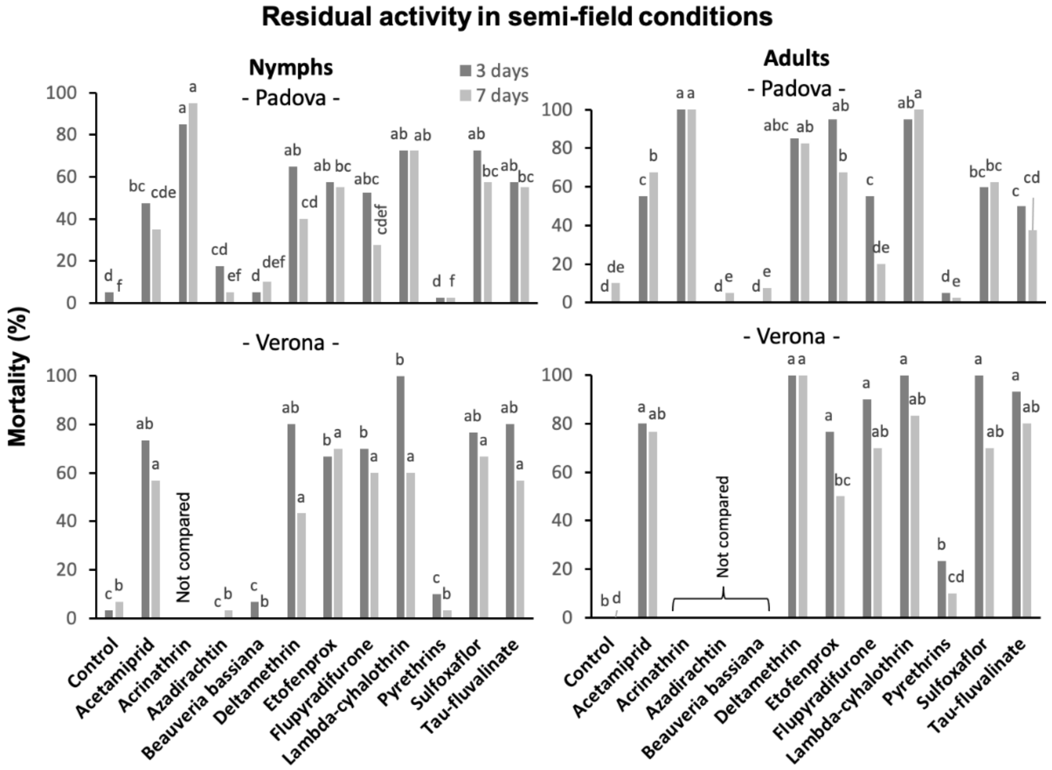

Figure 5. Mortality rates of *S. titanus* nymphs and adults confined on leaves three and seven days after insecticide application in semi-field conditions in Padova and Verona Universities. Different letters above the columns with the same color indicate significant differences at the *t*-test (α = 0.05).

In the trial carried out at the University of Verona, *S. titanus* nymphs were significantly affected by insecticides when confined on plants three and seven days after their application (3 days: F = 44.44; d.f. = 10, 22; $p < 0.0001$; 7 days: F = 26.7; d.f. = 10, 22; $p < 0.0001$). At three days, only conventional insecticides determined mortality rates significantly higher than in the control. Etofenprox and flupyradifurone were less effective than lambda-cyhalothrin (Figure 5). At seven days, only conventional insecticides determined mortality rates significantly higher compared to the control and, among them, no significant differences were observed.

Based on Abbott mortality, the two trials (Padova and Verona universities) showed the poor efficacy of organic insecticides (i.e., azadirachtin, *B. bassiana* and pyrethrins) (Table S1). In contrast, they stress the efficacy of pyrethroids (i.e., acrinathrin, deltamethrin, etofenprox, lambda-cyhalothrin and tau-fluvalinate) and sulfoxaflor. Acetamiprid and flupyradifurone showed a good efficacy only in the University of Verona trial. Comparing Abbott values at three and seven days, a reduction in efficacy from the first to second caging was observed for deltamethrin and flupyradifurone in the University of Padova trial and for deltamethrin, lambda-cyhalothrin, tau-fluvalinate and acetamiprid in the University of Verona trial.

Against *S. titanus* adults

In the trial carried out at the University of Padova, *S. titanus* adults were significantly affected by insecticides when confined on plants three and seven days after their application (3 days: F = 30.87; d.f. = 11, 36; $p < 0.0001$; 7 days: F = 40.25; d.f. = 11, 36; $p < 0.0001$). At three days, only conventional insecticides determined mortality rates significantly higher than in the control. Among them, acetamiprid, flupyradifurone, tau-fluvalinate and sulfoxaflor were less effective than acrinathrin, that caused a mortality of 100%, and the first three were also less effective than etofenprox and lambda-cyhalothrin (Figure 5). At seven days, flupyradifurone and tau-fluvalinate did not differ anymore from the control; among the other insecticides, acrinathrin and lambda-cyhalothrin were more effective than acetamiprid, etofenprox and sulfoxaflor.

In the trial carried out at the University of Verona, *S. titanus* adults were significantly affected by insecticides when confined on plants three and seven days after their application (3 days: F = 30.22; d.f. = 8, 18; $p < 0.0001$; 7 days: F = 16.67; d.f. = 8, 18; $p < 0.0001$). At three days, all insecticides except pyrethrins determined mortality rates significantly higher than in the control without differences among them (Figure 5). At seven days, this result was confirmed for all insecticides except etofenprox that still differed from the control but was less effective than deltamethrin.

Based on Abbott mortality, the two trials (Padova and Verona Universities) agreed with the absence of efficacy of pyrethrins and the good efficacy of pyrethroids (i.e., deltamethrin, etofenprox, lambda-cyhalothrin and tau-fluvalinate) and sulfoxaflor. Acrinathrin showed an excellent residual activity in the trial in which it was used. Acetamiprid and flupyradifurone showed a good efficacy only in the University of Verona trial. Comparing Abbott values at three and seven days, a reduction in efficacy from the first to the second release was observed for etofenprox and flupyradifurone in both trials and for tau-fluvalinate in the University of Padova trial (Table S2).

Insecticide residual activity in field trials

Against *S. titanus* nymphs

In the trial carried out in 2021, insecticides showed significant effects on *S. titanus* nymphs confined on shoots three and seven days from their application (3 days: F = 72.89; d.f. = 4, 15; p < 0.0001; 7 days: F = 12.47; d.f. = 4, 15; p < 0.0001). At three days only acrinathrin and tau-fluvalinate differed from the control with the first active ingredient significantly more effective than the second (Figure 6). At seven days, only acrinathrin was still significantly different from the control.

In the trial carried out in 2022, insecticides showed significant effects on *S. titanus* nymphs confined on shoots three and seven days from their application (3 days: F =21.53; d.f. = 7, 24; p < 0.0001; 7 days: F = 5.47; d.f. = 7, 24; p < 0.001). At three days all insecticides differed significantly from the control, but the pyrethroids acrinathrin, deltamethrin and etofenprox were more effective than the remaining active ingredients; sulfoxaflor and tau-fluvalinate were more effective than acetamiprid (Figure 6). At seven days, only acrinathrin was still significantly different from the control as well as from all other insecticides.

Based on Abbott's mortality, only acrinathrin showed remarkable residual effects on nymphs both at three (nearly 90%) and seven days (approximately 60%) from insecticide application. Deltamethrin and etofenprox had a good efficacy but only at three days (84% and 60%, respectively). The other active ingredients did not exceed the 30% of efficacy at three days and showed practically a lack of efficacy at seven days.

Against *S. titanus* adults

In the trial carried out in 2021, insecticides showed significant effects on *S. titanus* adults confined on shoots three and seven days from their application (3 days: F = 10.29; d.f. = 4, 15; $p < 0.001$; 7 days: F = 8.09; d.f. = 4, 15; $p < 0.001$). At three days only acrinathrin and acetamiprid differed from the control without significant differences between them, even if the mortality recorded for the first insecticide was almost double (Figure 6). At seven days, only acrinathrin was still significantly different from the control.

In the trial carried out in 2022, insecticides did not show significant effects on *S. titanus* adults confined on shoots three and seven days from their application (3 days: F = 1.27; d.f. = 7, 24 p = 0.304; 7 days: F = 0.61; d.f. = 7, 24 p = 0.740).

Abbott's mortality on adults was high only for acrinathrin in 2021 trial (around 75% both at three and seven days) (Table S4). In the 2022 trial no insecticides caused a mortality exceeding 20%.

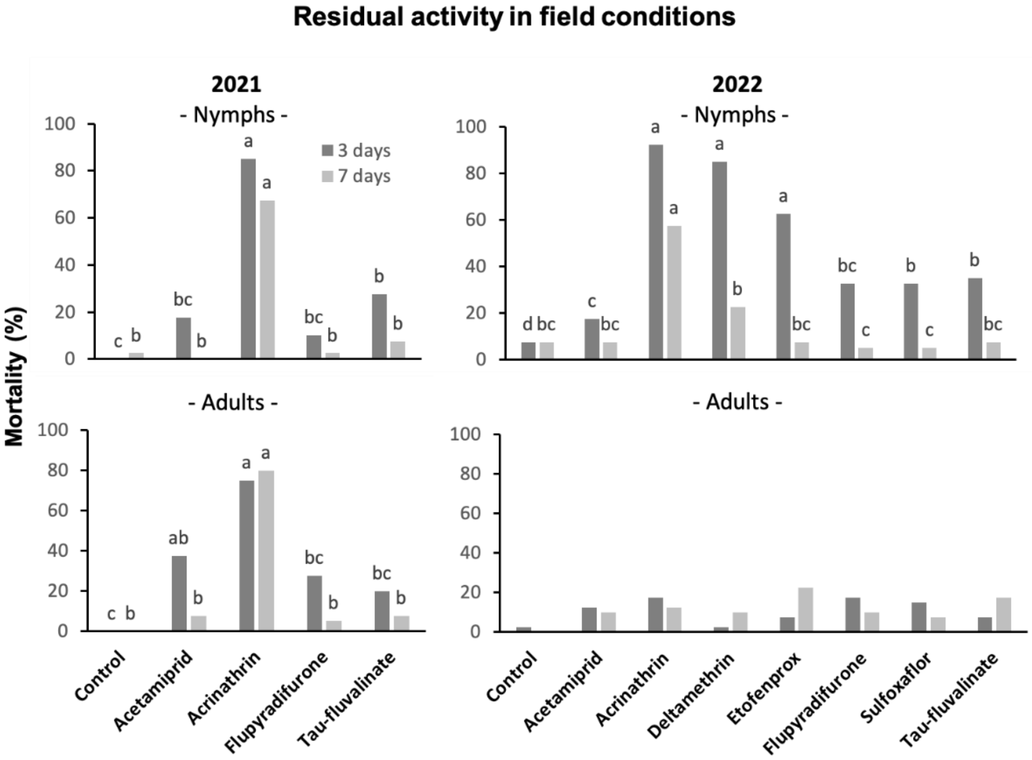

Figure 6. Mortality of *S. titanus* nymphs and adults confined on leaves 3 and 7 days after insecticide application in field conditions in 2021 and 2022. Different letters above the columns with the same color indicate significant differences at the *t*-test (α = 0.05).

Discussion

In this study different approaches were used to evaluate the efficacy of insecticides authorized in Europe for the control of *S. titanus* in conventional and organic vineyards. We carried out field trials against nymphs using a procedure that reflected a winegrowers' realistic scenario. In these trials leafhopper nymphs were potentially exposed to insecticides through topical, residual and ingestion (particularly important for systemic insecticides such as neonicotinoids, sulfoximines and butenolides) routes. Semi-field trials were designed to evaluate the mortality of nymphs and adults exposed to aged residues in controlled conditions to disentangle the effect of residual exposure from the other routes of exposure. Finally, field tests were planned to assess the impact of insecticide residual activity in realistic conditions.

The efficacy of conventional insecticides against nymphs in field conditions

Among products authorized in conventional viticulture, the most effective insecticides, when *S. titanus* was sampled on basal leaves, were two pyrethroids (IRAC Group 3A): etofenprox (96.9%) and deltamethrin (92.2%). The third pyrethroid, i.e., tau-fluvalinate, was less effective (72.5%) than sulfoxaflor (81.2%, IRAC group 4C). The efficacy of acetamiprid (IRAC group 4A) and flupyradifurone (IRAC group 4D) was much lower (49.2% and 41.2%, respectively). When *S. titanus* was sampled on suckers, a higher efficacy was observed for flupyradifurone and acetamiprid (80.6% and 60.8%, respectively).

The mean efficacy levels of insecticides tested in our trials were compared with values reported in the literature, calculated according to Henderson and Tilton. For deltamethrin our mean efficacy value (90.8%) was lower than those reported by Zidaric *et al*. (2013) (100% in a single trial) and Colleluori *et al*. (2020) (98.5% as an average of four trials). Data from the literature on the efficacy of etofenprox (Bosio *et al*., 2004; Lavezzaro *et al*., 2019; Ladurner *et al*., 2020) report values (87% as a mean of trials) similar than those found in the present paper (84.3%). The third pyrethroid in this comparison, tau-fluvalinate, was much less effective in the present study (67.1% as a mean of trials) compared to data reported in the literature (Abbott efficacy of 87.4% according to Colleluori *et al*. (2020)). The most relevant discrepancies between our results and data collected from the literature concerned deltamethrin and tau-fluvalinate and can be associated to different spraying machines used in trials: results from the literature were obtained using backpack sprayers while in the present study using farmer atomizers. Among the remaining insecticides, the most effective resulted sulfoxaflor (mean efficacy of 82.2%). These values are higher than those reported in the literature (60.75% according to Forte *et al*. (2018)). Unfortunately, this active ingredient was associated to potential effects on pollinators and recently its use in open field conditions has been restricted. The mean efficacy of flupyradifurone was lower (60.9%) than that reported in the literature (77.1%) as a mean of some trials (Bosio *et al*., 2004; Forte *et al*., 2018) where it was sprayed using a backpack sprayer. Acetamiprid efficacy was slightly lower that that reported in the literature (55% vs. 61.7% as a mean of trials carried out by Lavezzaro *et al*. (2019)).

In the experimental vineyard where *S. titanus* was sampled also on suckers, the efficacy of pyrethroids slightly decreased compared to that calculated on basal leaves (on average -17%) while that of acetamiprid e flupyradifurone increased (on average +56%). The decrease in efficacy of pyrethroids, acting mainly by contact, could be due to the dilution of the insecticide residues as suckers are rapidly growing shoots (development of new leaves and increase in size of those already present), whereas basal leaves at application timing had already completed their growth. The increase in acetamiprid and flupyradifurone efficacy could be explained by their activity through ingestion as a consequence of a better absorption of insecticides by younger leaves and a greater acropetal translocation in rapidly growing shoots. This hypothesis should be supported by further experiments, and if demonstrated, sucker management and spraying approaches against this pest could be redefined.

The higher efficacy of sulfoxaflor compared to acetamiprid and flupyradifurone (all belonging to IRAC Group 4) could be due to the large use of the latter insecticides and the selection pressure exerted upon *S. titanus* populations. This hypothesis could be supported by literature data that reported a higher efficacy of acetamiprid and flupyradifurone in trials conducted some years ago, before the extensive use of these active ingredients. The lower efficacy of these insecticides on basal leaves than on suckers suggests that the limited coverage of basal leaves determines a low-dosage level that is known to be a factor favouring the selection for resistant strains (Georghiou *et al.*, 1986; Onstad, 2014; Yu, 2008). This hypothesis should be supported by further experiments, but it is recommended to follow the alternance or rotation measures when planning control strategies against *S. titanus* with these insecticides.

The efficacy of organic insecticides against S. titanus nymphs in field conditions

Among organic insecticides, only pyrethrins and kaolin significantly reduced *S. titanus* population densities. The efficacy of pyrethrins (66.6% on basal leaves and 81% on suckers) was higher than that of kaolin (45.5% on basal leaves and 62.9% on suckers). The efficacy of pyrethrins confirms previous trends (Mori *et al.*, 2014; Lavezzaro *et al.*, 2019; Ladurner *et al.*, 2020). Results obtained using kaolin (54.2% of efficacy) stress its potential as a complementary tool against several leafhoppers included *S. titanus* (Gusberti *et al.*, 2008; Tirello *et al.*, 2021). Efficacy levels of pyrethrins increased when the adjuvant Mago was added in the trial with sampling on basal leaves (from 66,6% to 70.8%) and, even if only on average, in the trial with sampling on suckers (from 81.0% to 89.5%). Potassium salts of fatty acids did not significantly reduce nymph populations in both trials (20.3% in that sampling on basal leaves and 37.6% in that sampling on suckers), and efficacy values were lower to those reported in the literature (48.1% as a mean of trials carried out by Tacoli *et al.* (2017) and Forte *et al*. (2018)). *Beauveria bassiana* significantly reduced nymph population densities in the trial in which suckers were sampled (55.0% of efficacy), but not in the trial in which basal leaves were sampled (29.5%); efficacy values are lower than those reported in the literature (60.3% as a mean of trials carried out by Mori *et al.* (2014) and Ladurner *et al.* (2020)) using a backpack sprayer. Contrasting results were obtained with azadirachtin characterised by a low efficacy (7.2%) in the trial in which basal leaves were sampled and a good efficacy (72.6%) in the trial in which suckers were sampled. The efficacy of azadirachtin against *S. titanus* reported in literature for trials conducted under field conditions is moderate (33.4% as a mean of trials carried out by Bottura *et al*. (2003) and Mori *et al*. (2014)). The high efficacy recorded for azidiracthin when sampling was carried out on suckers could be explained by a greater activity for ingestion as a consequence of a better absorption acropetal translocation in rapidly growing shoots. In fact, in a unique sampling carried out on basal leaves, azadirachtin showed a Henderson and Tilton efficacy of 25.6% (data not reported) much lower than that obtained by sampling suckers.

Residual activity of insecticides in semi-field conditions

In the semi-field trials conducted at Padova University, pyrethroids and sulfoxaflor showed a higher residual activity on both nymphs and adults than acetamiprid and flupyradifurone. In contrast, in trials conducted at Verona University all conventional insecticides showed a good residual activity up to seven days from insecticide applications. These differences can be attributed to the different origin of *S. titanus* individuals used in the trials. The insects used in Padova trials came from an organically managed vineyard surrounded by conventionally managed vineyards, whereas those used in Verona trials came from an organic vineyard surrounded mainly by woody vegetation. It can be argued that the organic vineyard used as a source of *S. titanus* for Padova trials had been colonized by leafhopper populations subjected to repeated applications of flupyradifurone and acetamiprid in the last years and thus potentially selected for resistance to these active ingredients. Regarding pyrethroids, the best residual activity was recorded for acrinathrin (> 80% up to 7 days release) and lambda-cyhalothrin (on average of the two trials 86% at 3-day release and 65% at 7-day release). As expected, organic insecticides (i.e., pyrethrins, azadirachtin and *B. bassiana*) showed poor results in terms of residual activity.

Residual activity of insecticides in field conditions

Trials conducted in open field in 2021 and 2022 on nymphs showed only for acrinathrin a remarkable residual activity up to seven days from insecticide application. The efficacy of deltamethrin and etofenprox at three days from insecticide application was good, whereas tau-fluvalinate and the insecticides belonging to IRAC group 4 either showed no residual activity or, as in the case of acetamiprid, this was negligible. Trials conducted on adults confirmed the residual activity of acrinathrin only in the first year, whereas all other active ingredients either showed no efficacy or this was not such as to guarantee sufficient control of *S. titanus* in both years. Among factors affecting these last results we can suggest the role of high temperatures occurring in 2022. The negative correlation between pyrethroid toxicity against insects and high temperatures is well documented (Riskallah, 1984; Brown, 1987; Fabellar and Mochida, 1988; Swain *et al*., 2009; Glunt *et al*., 2018; Agyekum *et al*., 2022). Riskallah *et al*. (1984) demonstrated that permethrin, fenvalerate, deltamethrin, cypermethrin and flucythrinate were more toxic to *Spodoptera littoralis* (Boisd) at 20 °C than at 35 °C. Brown (1987) showed that fenvalerate, flucythrinate and permethrin applied against *Heliothis virescens* (Fabricius) had a lower effectiveness when temperatures increased. Fabellar *et al*. (1988) demonstrated that cypermethrin and deltamethrin had lower LD50 values at 18 °C than at 33 °C against *Nilaparvata lugens* (Stål) and *Nephotettix* sp. Additionally, recent literature (Swain *et al*., 2009; Glunt *et al*., 2019; Agyekum *et al*., 2022) focused on the changes in the insecticide susceptibility to pyrethroids by *Culex* and *Anopheles* spp., highlighting a deep decrease of their effectiveness at high temperatures (above 30 °C). In our trials the daily temperatures measured with the closest weather station (ARPAV, data) to our experimental sites were quite different between the 2021 and 2022 trials, especially when considering the adults. In the 2021 adult trial the temperature ranged from 16.1 °C to 30.8 °C with a mean temperature of 23.2 °C while in 2022 the temperature ranged from 20.0 °C to 35.5 °C with a mean temperature of 27.9 °C. Regarding nymph trials, the temperature in the 2 years where more similar ranging from 18.1 °C to 34.4 °C with a mean temperature of 26.6° C and 16.7 °C and 32.3 °C with a mean temperature of 25° C respectively in 2021 and 2022, and results were more similar. In accordance with these considerations, the higher residual efficacies obtained in the semi-field than in field conditions could be due not only to a better coverage by the insecticide solution but also by the lower temperatures experienced due to the shading net. In fact, leaves exposed to sunlight can have a temperature even 5 degrees higher than shaded ones (Kiaeian *et al*., 2018).

Conclusions

The recent outbreaks of Flavescence dorée are causing extreme concern among winegrowers. They can no longer apply traditional insecticides that proved to be highly effective against *S. titanus* in the past, because of the restrictions by EU authority. The lower impact of available insecticides was claimed as a key factor in recent outbreaks of the vector and the related transmitted phytoplasma disease. This situation suggested to evaluate the effectiveness of available insecticides. Among conventional insecticides, the most effective were acrinathrin, deltamethrin, lambda-cyhalothrin, etofenprox and sulfoxaflor. However, their residual activity seems to be limited and altered by high temperatures occurring in summer. Moreover, most of them belong to IRAC group 3A suggesting that resistance could be a problem in the future. Regarding natural products, pyrethrins were the most effective especially when the adjuvant was added. Kaolin proved to be a complementary tool for *S. titanus* management in organic vineyards. Concerning the other organic products that showed a low efficacy on the investigated development stages (L2-L3) further investigations need to be re-evaluated against newly hatched individuals (L1) trying to delineate a strategy aimed at decreasing leafhopper densities by integrating insecticides belonging to different IRAC groups.

The different efficacy showed on basal leaves and on shoots from insecticides acting mainly by contact than those acting through ingestion suggests canopy or sucker management to concentrate the *S. titanus* individuals on the parts of the canopy most favorable to the insecticide-plant interaction. For pyrethroids and organic insecticides should be necessary suckering and green pruning at least 3-4 days before spraying, while for neonicotinoids the shoots may be present but must in any case be sprayed.

The multiple use of pyrethroids and other non-selective insecticides could originate issues related to secondary pests. Therefore, monitoring insecticide side-effects is crucial to minimize these risks.

The results of the present study provide precise indications on the strategies to be adopted for the containment of the vector of Flavescence dorée phytoplasma. The two cornerstones of this strategy must be an accurate control of nymphs by using effective insecticides and the removal of external sources of infectious *S. titanus* adults. In fact, many insecticides have a good knock-down effect against nymphs and some of them also have a certain level of residual activity. Considering the limited residual activity of most insecticides against adults, the effectiveness of insecticides to control infective individuals colonizing vineyards from the surrounding areas appears negligible and therefore attention must be paid to the eradication of abandoned vineyards and American grapevines growing in wild vegetation.

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Main conclusions

The present PhD thesis evaluated the importance of monitoring techniques and cultural practices to minimize the use of insecticides in the management of grape leafhoppers. Most of the results concerned *Erasmoneura vulnerata* and *Scaphoideus titanus*, but *Empoasca vitis* and *Zygina rhamni* were also considered. Another topic that received special attention was the role of natural vegetation surrounding vineyards. Since it has been considered a potential source of pests and vectors but also of beneficials, relationships between woody vegetation and vineyards require further attention. These aspects have been synthetized in chapter 1.

In chapter 2, the role of woody vegetation in promoting the colonization of vineyards by leafhoppers and their natural enemies was investigated, focusing on the Typhlocybinae. The spring colonization of vineyards by *E. vulnerata* was favored by the proximity of woody vegetation. Considering that *E. vulnerata* adults can cause severe damage at the sprouting, adult colonization should be carefully monitored to prevent such damage. The edge effect was significant for egg parasitoids belonging to the Mymaridae (*Anagrus* atomus). During spring colonization, the populations of *E. vulnerata* and *A*. *atomus* in three out of four case studies were associated. In summer, *E. vulnerata* populations were found to be aggregated in some areas of the vineyards. Associations between *E. vulnerata* and *A. atomus* adults were still found suggesting that egg parasitoids are attracted by infested areas of vineyards. Sentinel plants infested with *E. vulnerata* and *Z. rhamni* eggs were exposed to parasitism. *Anagrus atomus* was attracted by leafhopper eggs confirming that this species is adapting to the American leafhopper. Further research should be conducted on the possibility of increasing the parasitoids' populations through woody vegetation manipulation.

In chapter 3, kaolin proved to be an effective tool against *E. vulnerata* in large scale trials. This result is particularly important for the control of leafhoppers in organically managed vineyards. The use of kaolin was compatible with the activity of egg parasitoids belonging to the Hymenoptera Mymaridae. The impact of kaolin on predatory mites was moderate and not homogenous among the experimental vineyards. Based on the result of this study kaolin appears to be a valuable alternative to insecticides, in particular in organic vineyards.

The effects of leaf removal and insecticide applications on grape leafhoppers and mites were reported in chapter 4. Leafhopper populations (in particular *E. vitis*) were affected by insecticide applications suggesting that active ingredients used in that area are not involved in resistance. Leaf removal reduced leafhopper densities in one out of four case-studies. Insecticides affected predatory mite populations in three out of four case-studies. In one of them phytophagous mites (*Panonychus ulmi*) reached a high population level in insecticide-treated plots. Predatory mite populations were also affected by leaf removal in two out of four case-studies inducing higher *P. ulmi* numbers. Therefore, the effects of leaf removal on grapevine arthropods requires further investigations.

Chapter 5 emphasized the importance of monitoring in controlling *S. titanus*. Moderate to high *S. titanus* populations were detected in a hilly area seriously affected by Flavescence dorée (FD). An accurate monitoring activity coupled with appropriate cultural practices (removal of suckers, canopy management, and rouguing of symptomatic plants) allowed to optimize the use of insecticides against *S. titanus* reducing significantly pest numbers. The spatial distribution of *S. titanus* was also studied showing that pest aggregations declined after the application of insecticides with a correct timing and appropriate active ingredients.

In chapter 6, we observed that the spatial distribution of *S. titanus* was not strictly associated to woody vegetation at any of four agro-ecosystems studied. On the contrary, nymphs and adults were mostly aggregated on the opposite side of groves, while gaps were frequently found close to groves. The patches of *S. titanus* appeared to be influenced by the presence of nearby infested vineyards. The patches of *S. titanus* nymphs during the 2022 growing season reflected those of adults in the previous year. Interestingly, the increase in symptomatic grapevines from 2021 to 2022 appeared to be related to the distribution of adults during the 2021 growing season.

The effectiveness of available insecticides on *S. titanus* was evaluated in chapter 7. Among conventional insecticides, the most effective were deltamethrin and etofenprox. The residual activity of acrinathrin and lambda-cyhalothrin was tested in semi-field conditions showing good results. Among natural products, pyrethrins were the most effective especially when an adjuvant was added; the use of kaolin was promising. However, the residual activity of organic insecticides was very low. The limited residual activity of most insecticides against *S. titanus* adults suggests their low effectiveness to control individuals migrating inside vineyards from infested areas. The control of *S. titanus* nymph populations remains fundamental to prevent a large occurrence of adults in summer.

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