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CORSO DI DOTTORATO DI RICERCA IN SCIENZE DELLE PRODUZIONI VEGETALI

CICLO: XXXI

Effects of vineyard management on functional biodiversity

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Padova, 29 September 2018 Giulia Zanettin

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Riassunto

I vigneti italiani sono spesso monocolture su larga scala caratterizzate da un elevato impiego di agrofarmaci e dalla riduzione delle infrastrutture ecologiche. In questi agro-ecosistemi altamente disturbati e semplificati, l'insorgenza di specie non autoctone può verificarsi più facilmente. In questo contesto, la conservazione degli habitat semi-naturali e l'adozione di pratiche di gestione più sostenibili risultano di particolare importanza. Nel presente lavoro, è stata valutata l'influenza delle pratiche di gestione del vigneto e della complessità del paesaggio sulla presenza di tre diverse specie di cicaline. I risultati hanno dimostrato che i fattori investigati possono influenzare le densità di popolazione di tali fitofagi, nonché il loro controllo naturale. Di essi si dovrebbe pertanto tenere considerazione nella pianificazione di strategie di controllo su larga scala. Inoltre, è stato valutato l'effetto sulla presenza sia di fitofagi che artropodi utili di alcune pratiche di manipolazione dell'habitat, come la gestione della vegetazione spontanea presente nell'interfilare e la pratica del sovescio, in quanto tali pratiche possono rivelarsi utili strategie per favorire la presenza di nemici naturali. La presenza di vegetazione non sfalciata ha favorito l'abbondanza di nemici naturali ma anche di alcuni fitofagi, soprattutto in vigneti a conduzione biologica. L'adozione di questa pratica dovrebbe essere attentamente valutata quando la presenza di *Scaphoideus titanus* è accertata nel vigneto, in quanto la presenza di vegetazione non falciata può rendere più difficile il controllo delle popolazioni della cicalina, specialmente nei vigneti biologici. Consentire alle specie vegetali presenti nel miscuglio da sovescio di fiorire per un periodo più prolungato rispetto a quanto tradizionalmente adottato dai viticoltori, può favorire una maggiore presenza e abbondanza di artropodi utili. L'epoca di sfalcio dovrebbe tuttavia essere programmata con precisione per evitare l'eventuale dispersione di fitofagi sulla vite.

Le indagini sulla fenologia di *Erasmoneura vulnerata* suggeriscono che tale specie alloctona può compiere tre generazioni annuali nell'areale in introduzione. Inoltre, la presenza di edifici rurali e di ospiti alternativi in prossimità dei vigneti può favorirne lo svernamento e la dispersione. L'impatto dei nemici naturali sulle popolazioni della cicalina sembra attualmente riguardare unicamente la parassitizzazione delle uova. In questo contesto, l'adozione di un nuovo approccio volto a promuovere l'impatto dei parassitoidi oofagi e dei predatori risulta di fondamentale importanza. A tale scopo, è stata valutata l'efficacia di due predatori generalisti nel controllo della cicalina. I risultati promettenti ottenuti in laboratorio hanno suggerito il rilascio dei predatori anche in un vigneto altamente infestato. Tuttavia, solo in una prova il rilascio dell'antocoride *Orius majusculus* ha significativamente ridotto la densità della cicalina, suggerendo di implementare tecniche di rilascio, densità e tempi al fine di migliorare l'impatto di tale predatore nel controllo del fitofago.

La progettazione di moderni sistemi viticoli dovrebbe integrare pratiche di gestione a minore impatto ambientale con misure di compensazione ecologica atte ad aumentare e migliorare la biodiversità all'interno degli agro-ecosistemi viticoli. Le pratiche di gestione dell'habitat possono contribuire a tale scopo, ma in un contesto estremamente semplificato, la sola presenza di vegetazione temporanea sembrerebbe non bastare a creare un ecosistema più stabile. Poiché l'impiego di pesticidi può favorire esplosioni demografiche di

fitofagi e limitare l'attuazione di strategie di controllo biologico, è necessario individuare strategie più sostenibili al fine di contenere l'impatto dei fitofagi, come osservato per *E. vulnerata* nei vigneti dell'Italia nord-orientale.

Summary

Italian vineyards are large-scale monocultures characterized by high pesticide pressure and removal of ecologically valuable structures. In the resulting highly disturbed and simplified systems, the insurgence of non-native species outbreaks can easier occur. In this framework, the preservation of semi-natural habitats and the adoption of more sustainable vineyard management practices are of particular importance in vineyard agro-ecosystems. In this thesis, the influence of vineyard management and landscape complexity on leafhopper species was investigated. Results shown that investigated factors can affect the leafhopper population densities and their natural control, and therefore they should be considered when pest control strategies are planned on a large scale.

Moreover, some habitat manipulation practices, such as the management of spontaneous groundcover and the use of green manure, could be useful strategies to increase and/or enhance beneficial arthropods by providing fundamental sources for the survival and reproduction of natural enemies. The presence of non-mowed spontaneous grass in vineyards inter-rows favoured the abundance of natural enemies but also of grapevine leafhoppers, especially in organic. In particular, non-mowed vegetation could make harder the control of *Scaphoideus titanus* populations in organic vineyards, and its adoption should be carefully evaluated when the leafhopper occurs in the vineyards. Allowing the green manure to flowering for a prolonged period instead of mowing it early, as traditionally done by the growers, can favour a higher presence and abundance of beneficial arthropods while not influenced phytophagous densities. The timing of mowing must be accurate programmed to avoid the dispersal on leafhopper vectors.

Investigations on the phenology of *E. vulnerata* suggest that the non-native pest can develop three generations per years in the new invaded area, and the presence of rural buildings and alternative hosts at vineyard margins favoured it overwintering and spreading into the vineyards. The impact of natural enemies on pest populations appeared to be limited to egg parasitism by Hymenoptera Mymaridae. A new approach aimed at promoting the impact of egg parasitoids and predators requires to be developed. In this context, the effectiveness of two generalist predators in controlling the leafhopper populations were tested both in laboratory and field conditions. Promising results obtained in laboratory trials suggested to release predators in vineyard, but their release did not give satisfactory results, except in only one trial in which the release of *Orius majusculus* significantly reduced the leafhopper numbers, suggesting to implement release techniques, densities and timing to improve the impact of anthocorids on grape leafhoppers.

The design of modern viticultural systems should integrate management practices with lower environmental impact with ecological compensation measures to increase and enhance biodiversity in the vineyard agro-ecosystems. Habitat management practices can contribute to enhance biodiversity but in extremely simplified context, the only presence of temporary vegetation appears not enough to create a more pest-stable agro-ecosystem. Since the deployment of pesticides can favouring the insurgence of pest outbreaks and limited the successful implementation of biological control, more sustainable biological control

strategies should be implemented to control pest population densities, such as for *E. vulnerata* in vineyards of North-eastern Italy.

Chapter 1

Introduction and aims

Vineyards are perennial crop systems and, for this reason, are considered more stable habitats for a range of species than annual crops. In particular, inter-rows covered by different plant species favour the presence of pollinators (Kehinde and Samways 2014) and invertebrates which provide many ecosystem service, among whom natural biological control is one of the most important for the pest regulation (Shields et al. 2016). In fact, perennial crop systems are theoretically more amenable to conservation biological control than annual crop system since they are subject to lower levels of disturbance (Landis et al. 2000). On the other hand, vineyards are also among the most intensely cultivated agro-ecosystems, typically involving numerous pesticide application, soil tillage operations and high landscape simplification (Nicholls et al. 2008).

Italian grapevine production is often characterized by large-scale monocultures which are recently rapidly expanding. The changes in land use and the intensification of farming systems, through specialization and scale enlargement in order to increase yields and overall efficiency, generally lead to a decrease in biodiversity (Donald et al. 2001) and include high number of agrochemical applications (Tscharntke et al. 2005). The reduction of natural resources in vineyard agro-ecosystems, as consequence of the expansion of vineyard monocultures and the related removal of natural areas, leads to the lack of food resources (such as nectar, pollen, alternate prey) and sheltering, mating and overwintering sites (Corbett and Rosenheim 1996; Landis et al. 2000; van Emden 2002; Ponti et al. 2005; Bianchi et al. 2006; Ricci et al. 2009). In fact, in landscapes dominated by intensive viticulture, the removal of non-crop areas might reduce natural pest control, as natural habitats provide the requisites for a large spectrum of natural enemies, and the spill-over of natural enemies between vineyards and natural habitats is likely to be diminished as the proportion of semi-natural areas decreases (Bianchi et al. 2006; Ricci et al. 2009). Moreover, the deployment of broad-spectrum pesticides drastically simplify arthropod communities, favouring the insurgence of pest populations (Hardin et al. 1995; Johnson and Tabashnik 1999) and limited the successful implementation of biological control (Landis et al. 2000). In these more simplified agro-ecosystems, the abundance and activity of natural enemies has decreased with a consequent reduction in biological control of pests (Corbett and Rosenheim 1996; Symondson et al. 2002; Schmidt et al. 2004; Thorbek and Bilde 2004; Bianchi et al. 2006; Tsitsilas et al. 2006). Therefore, in the resulting highly disturbed and simplified landscapes, biological control and other ecosystem services are declining (Tilman et al. 2001). Those elements of diversity, which are functional to ecosystem services, are of particular importance in agro-ecosystems rather than general diversity (Landis et al. 2000). Grapevine cultivation should attempt to conjugate grape production with environmental quality, through the preservation of semi-natural habitats and the use of ecological infrastructures aimed at increase the biodiversity. The adoption of modern pest control tools in vineyard management strategies can be promoted to conjugate the needs of pest control and biodiversity preservation. For the success of these measures, habitat enrichment should be coupled with grapevine management practices. The design of modern viticultural systems should integrate ecological compensation measures and management practices aimed at biodiversity enhancement. Managed hedgerows can represent a valuable instrument for the compensation of semi-natural habitat losses and biodiversity decrease due to intensive viticulture with a positive effect on pest outbreaks control (Rieux et al. 1999). Groundcover management practices (e.g. native plants use as cover crops) is another viable option to promote the beneficial organisms persistence in agro-ecosystems (Nicholls et al. 2001; Begum et al. 2006). Habitat management is unique in its ability to provide a wide variety of ecosystem services in addition to pest population reduction.

In vineyard agro-ecosystems, the deployment of broad-spectrum pesticides and the reduction of natural resources, which provide fundamental source for a large spectrum of natural enemies, can favour the insurgence of pest populations, also of non-native species. Biological invasions by non-native or alien species are the greatest threats to the ecological and economic safety of the planet. In fact, alien species may have negative impact on humans and/or the environment as they can outcompete native species, alter community structure, disrupt ecosystem functions, and affect cropping systems, leading to increasing costs for eradication and management. Furthermore, alien species can act as vectors for new pathogens resulting in new diseases, reduce the value of land and water for human activities and cause other socio-economic consequences for man (e.g., migrations, cultural changes, etc; Kenis et al. 2009; Europe-aliens.org 2018). Globalization is a complex phenomenon, affecting cultural, social and political aspects with a considerable importance in biological terms. Indeed, the transport of goods and people involves also the movement of harmful organisms, whose establishment is often facilitated by climate change (Roques 2010; Roques et al. 2010; Marini et al. 2011). In recent years, many exotic pests have been introduced accidentally through trade, causing serious damage to the primary sector of many countries. Studying the biology, ecology, natural enemies of the alien species in the new invaded environment is of fundamental importance to set up rationale management strategies in the vineyard. In 2004 the American leafhopper *Erasmoneura vulnerata* (Fitch) (Hemiptera: Cicadellidae) has been recorded for the first time in Europe in vineyards of North-eastern Italy (Duso et al. 2005). Some years following the first record, the leafhopper spread to other regions in Italy and Slovenia (Duso et al. 2008; Seljak 2011) without causing apparent damage in vineyards and therefore has been considered a minor pest of grapevines. In some papers *E. vulnerata* has been reported as a serious pest of grapevines in the USA (Robinson 1926; Beamer 1946), but more recent publications highlighted that this species is rarely dominant in leafhopper communities occurring in American vineyards (e.g., Martinson and Dennehy 1995; Paxton and Thorvilson 1996; Zimmerman et al. 1996). In July of 2016 the first outbreaks of *E. vulnerata* were detected in the Veneto region (Treviso and Vicenza provinces). Symptoms caused by leafhoppers were spread on more than 90% of the canopy including apical leaves and population densities sometimes exceeded densities of 10 nymphs per leaf. Interestingly, infestations were detected both in conventional and organic vineyards. This new scenario suggested a reappraisal of investigations on *E. vulnerata* in order to identify factors affecting the pest outbreaks. Moreover, considering that outbreaks occurred in commercial vineyards despite the use of insecticides, sustainable non-chemical control strategies should be implement to search non-chemical alternatives.

Aims

In this context, my PhD research work aimed to investigate the influence of vineyard management and landscape complexity in order to suggest useful information for the design of modern viticultural systems and implement IPM strategies. Moreover, my work aimed to evaluate the influence of habitat manipulation practices, associated with lower environmental impact management practices, in order to individuate measures for the promotion of functional biodiversity in vineyard agro-ecosystems. Finally, observation on the phenology and colonization pattern of the American leafhopper *E. vulnerata* were carried out in new invaded area to identify factors affecting the pest outbreaks and implement more sustainable non-chemical strategies to controlling its populations.

In particular, the aims of my PhD were focused on:

1) **Investigation on the influence of vineyard management and landscape complexity on the abundance of leafhoppers and the parasitism rate by Mymaridae in vineyards in North-eastern Italy**
   The intensification in grapevine cultivation, and the consequent removal of semi-natural habitats, associated with the use of pesticides in vineyard agro-ecosystems are considered the main causes of the disruption of ecosystems services, such as natural control of grapevine pests. The influence of pest management practices and landscape complexity on leafhoppers should be deeply explored to design modern viticultural systems and implement IPM strategies. Observations were carried out in two consecutive growing season (2016 and 2017) to investigate the effects of vineyard management strategies (conventional vs. organic) and landscape complexity (i.e. the abundance of semi-naturals areas surrounding vineyards) on the populations densities of three leafhoppers species. Moreover, the impact of vineyard management and semi-naturals areas on parasitism rate of Typhlocybinae eggs exerted by egg-parasitoids belonging to the Hymenoptera Mymaridae was evaluated.
2) **Investigation on the influence of inter-rows groundcover vegetation management on arthropods abundance in vineyards of Northeast of Italy**
   Habitat simplification and use of pesticides in vineyard agro-ecosystem drastically simplified arthropod communities, reducing natural pest control. In this context, habitat management practices could be useful strategies to provide fundamental sources for sustaining natural enemies. Field experiments were carried out to investigate the effects of habitat management practices on both pests and beneficial arthropods in vineyards of North-eastern Italy. In particular, three different field experiments were performed to 1) evaluate the influence of the native spontaneous grass of vineyards inter-rows mowing, 2) evaluate the effect of different timing of a commercial green manure mowing, and 3) compare the effect of different green manure mixtures, adopted by farmers to improve the soil characteristics, on arthropods in vineyard agro-ecosystems.

3) **<u>Investigation on the phenology and colonization patterns of the Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) in vineyards of North-eastern Italy</u>**

The American leafhopper *E. vulnerata* was detected for the first time in Europe in 2004 and considered a minor pest of grapevines up to 2016, when first outbreaks were reported in vineyards located in North-eastern Italy. This new scenario suggested a reappraisal of investigations on *E. vulnerata* in order to identify factors affecting the pest outbreaks. Investigations were carried out in 2017 and 2018 in farms located in the Veneto region to shed light on the phenology of *E. vulnerata*. The colonization pattern of adults was studied to shed light on the role of overwintering sites in the pest spread. Moreover, the impact of natural antagonists (in particular that exerted by egg-parasitoids belonging to the Hymenoptera Mymaridae) was evaluated.

4) **<u>Improvement of biological control methods to control *E. vulnerata* populations using of commercial reared generalist predators</u>**

Outbreaks of *E. vulnerata* occurred in commercial vineyards despite the use of insecticides suggesting to search non-chemical alternatives. To improve more sustainable biological control strategies, according to recent rules devoted to the reduction of pesticide use (Directive 2009/128/EU), the effectiveness of commercial reared generalist predators common in Italian agro-ecosystems, such as the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) and the pirate bug *Orius majusculus* (Hemiptera: Anthocoridae), in controlling the leafhopper populations were tested. Preliminary trials were conducted in laboratory; subsequently, predators were release in a highly infested vineyard in 2017 and 2018 growing season.

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Chapter 2

Influence of vineyard management and landscape complexity on the abundance of leafhoppers and the parasitism rate by Mymaridae in vineyards in North-eastern Italy

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Giulia Zanettin collected most of the data, contributed to the analysis and to draft the manuscript.

Abstract

Different leafhopper species can infest Italian vineyards: *Empoasca vitis*, *Zygina rhamni* and *Scaphoideus titanus*. Habitat simplification and use of pesticides have been associated with the reduction of natural control of grapevine pests. In two following years in vineyards of North-eastern Italy, the influence of vineyard management (conventional vs. organic) and landscape complexity (i.e. abundance of semi-naturals areas) on populations densities of leafhopper species and their egg parasitism rate were investigated. Vineyard management affected the abundance of *E. vitis* and *Z. rhamni* on traps, and of *S. titanus* both on suckers and traps. Leafhoppers were more abundant in organic vineyards. Landscape complexity influenced *Z. rhamni* abundance on leaves, which resulted more abundant in vineyards within complex landscapes. Egg parasitism rate was not affected by vineyard management, while resulted higher in vineyards surrounded by higher amount of semi-natural habitats. Results show that vineyard management and landscape complexity can affect the abundance of leafhopper and their natural antagonists, and should be considered when pest control strategies are planned on a large scale.

1. Introduction

Grapevine production can be threatened by various species of leafhoppers (Hemiptera: Cicadellidae). Among these, *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari and *Scaphoideus titanus* Ball are considered the most important in Italian vineyards (e.g., Pavan et al. 1988; Vidano 1964). In particular, *S. titanus* is the key vector of the phytoplasma agent of Flavescence dorée (Schvester et al. 1963; Mori et al. 2002), a serious grapevine yellows disease which has high economic impact since it affects the vigour, the yield of grapevine and the quality of wine (Chuche and Thiéry 2014). Insecticide applications are often mandatory to control *S. titanus* populations in Italy (Decreto Ministeriale n. 32442 del 31/05/2000), while the other leafhoppers are less damaging (Pavan et al. 2000).

The intensification in grapevine cultivation is among the main causes of habitat simplification with the removal of semi-natural habitats. The use of broad-spectrum pesticides and habitat simplification in vineyard agro-ecosystems have been associated with the disruption of ecosystems services such as natural control of grapevine pests (e.g., Duso et al. 2010) . The role of landscape complexity for its impact on the abundance of both pests and natural enemies is often matter of discussion (e.g., Chaplin-Kramer et al. 2011; Veres et al. 2013). Plants present in hedgerows allow the Mymaridae parasitoids to survive even during periods when grape leafhopper eggs are absent on grapevine (Cerutti et al. 1991; Williams et al. 2000). At the same time, pest management practices can exert a differential impact on pests and their natural enemies with unpredictable implications (Rusch et al. 2015). The influence of semi-natural areas and of vineyard management on leafhoppers communities could be deeply explored in order to design modern viticultural systems and implement IPM strategies.

The aim of this study was to investigate the effects of vineyard management strategies (conventional vs. organic) and landscape complexity (i.e. the abundance of semi-naturals areas surrounding vineyards) on the populations densities of three species of leafhoppers and Typhlocybinae egg parasitism rate by Hymenoptera Mymaridae in vineyards in North-eastern Italy.

2. Materials and methods

2.2. Study area

This study was carried out in vineyards located in the Conegliano-Valdobbiadene DOCG area in Northern part of the province of Treviso (Veneto, NE Italy), the most important area for the Prosecco sparkling wine production. This area is characterized by a hilly landscape intensively cultivated with vineyards, and includes 7549 ha of vineyards, while semi-natural areas are restricted to small hedgerows and woodlands.

2.2. Vineyard management and landscape analysis

In the study area, 12 blocks (1.5 x 1.5 km) characterized by similar altitude and exposition were identified. Within each block, at least one conventionally and one organically (according to EU Reg. 889/2008) managed vineyards were selected, for a total of 27 productive vineyards. In all the vineyards the cultivar was Glera. During the two years of investigation insecticide applications were applied against *S. titanus* in late June; in conventional vineyards thiamethoxam based products were used, while pyrethrins were sprayed in organic vineyards. The quantification of landscape complexity was made within a 250 and 500 m radius buffers from the centre of each vineyard. For each buffer, semi-natural habitats, such as woodlands and hedgerows, and vineyard patches were manually digitized from a visual inspection of high-resolution satellite images (Google Earth Pro). In GIS (Quantum GIS 2.6.1, Open Source Geospatial Foundation Project, http://qgis.osgeo.org), the area of each patch was quantified, and the cover percentage of habitats was calculated at the two different landscape scales. Semi-natural areas surrounding vineyards are woodlands and hedgerows similar in plant species composition which mainly included *Carpinus betulus* L., *Castanea sativa* Mill., *Quercus* spp., *Fraxinus ornus* L., *Picea abies* (L.) H. Karst., *Pinus* spp., *Robinia pseudoacacia* L., *Alnus glutinosa* (L.) Gaertn, *Rubus* spp., *Sambucus nigra* L., *Cornus mas* L., *Clematis vitalba* L., *Vitis* spp., *Cornus sanguinea* L. and *Lonicera* spp.

2.3. Sampling methods

The occurrence of grapevine leafhoppers was monitored from June to September in two subsequent years (2016 and 2017). In both growing seasons, the first sampling was performed at the beginning of June, and the following samplings were conducted approximately every four weeks. The occurrence of adults leafhoppers was monitored using yellow sticky traps (24,5 x 40 cm; Serbios s.r.l., Badia Polesine (RO), Italy). Traps had been exposed in the field and monthly changed from June to September. Furthermore, at each sampling data, 25 leaves were collected from each vineyard and observed under a Wild M3C stereomicroscope (10-40 x magnification) in order to assess the abundance of nymphs of *E. vitis* and *Z. rhamni*. The egg parasitism rate of the two leafhoppers (by Hymenoptera Mymaridae) was calculated by dividing the number of parasitoid emergence holes by the sum of the nymph hatching holes and the parasitoid emergence holes. The abundance of *S. titanus* nymphs was assessed by visually sampling the leaves of 25 grapevine suckers in each vineyard in June and July.

2.4. Statistical analysis

A repeated measures linear mixed model with the Proc MIXED of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC) was used to test the effects of vineyard management (conventional vs. organic) and landscape complexity at different spatial scales (250 and 500 m radius) on data collected during the two years of investigation. In each model, year, time, vineyard management and landscape complexity were considered as independent variables and their effect was tested using an F test ($P = 0.05$). In the models, block was considered as random effect. Degrees of freedom were estimated with the Kenward and Roger method. Landscape complexity was considered as continuous variable (from 0 to 100% of semi-natural habitats) and the effect of spatial scale was evaluated by comparing two full models (i.e. as described above) differentiated only by the landscape complexity measured with a 250 m or 500 m radius. Models were compared with Akaike Information Criterion (AICc), which is a measure of relative model fit (Akaike, 2011) and best fitting model was used for inference on all independent variables. The average number of leafhoppers observed on leaves and on traps as wells as the average egg parasitism rate observed in each sampling site were considered as response variables with repeated measures made at different times, i.e. sampling dates and first-order autoregressive were chosen as best fitting covariance structure for correlating different sampling dates. The SLICE option of the LSMEANS statement was used to evaluated with an F test ($P = 0.05$) the effects variation during the experiment. The assumptions of the models were evaluated by inspecting diagnostic plots of model residuals. Prior to the analysis data on leafhoppers density on leaves and traps were log (n+1) transformed while parasitism rate of leafhopper eggs was arcsine-square root transformed. Data on parasitism rate were grouped considering the end of the first and second nymphs generations of *E. vitis* and *Z. rhamni*.

3. Results

The assumption that vineyards were inhabited by *E. vitis*, *Z. rhamni* and *S. titanus* was confirmed.

3.1. *Empoasca vitis*

The abundance of adult catches on traps was differently affected over the two years of investigation (P = 0.042; Table 2.1; Fig. 2.1). In 2016 catches were more abundant in organic than in conventional vineyards (F = 9.48; $DF_{5\text{-}120}$; P < 0.001), in particular from July to September. Differently, no significant differences were observed in adult catches in 2017 (F = 0.94; $DF_{5\text{-}116}$; P = 0.457). Landscape complexity did not influence *E. vitis* catches (Table 2.1). The abundance of nymphs on leaf samples did not result significantly affected by the investigated factors (Table 2.2; Fig. 2.2).

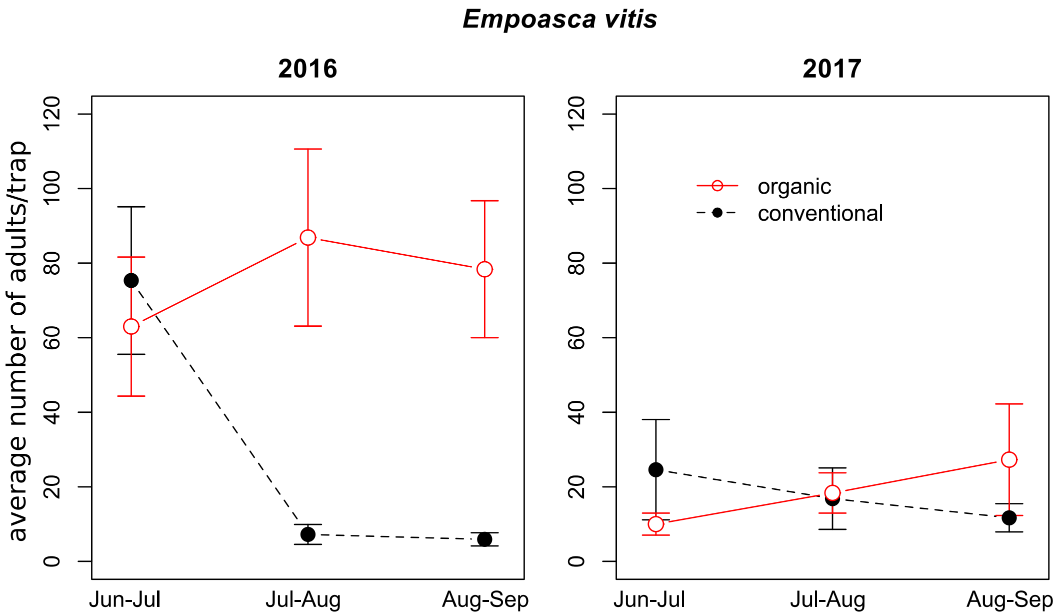

Figure 2.1: Catches of *Empoasca vitis* adults on yellow sticky traps in 2016 and 2017.

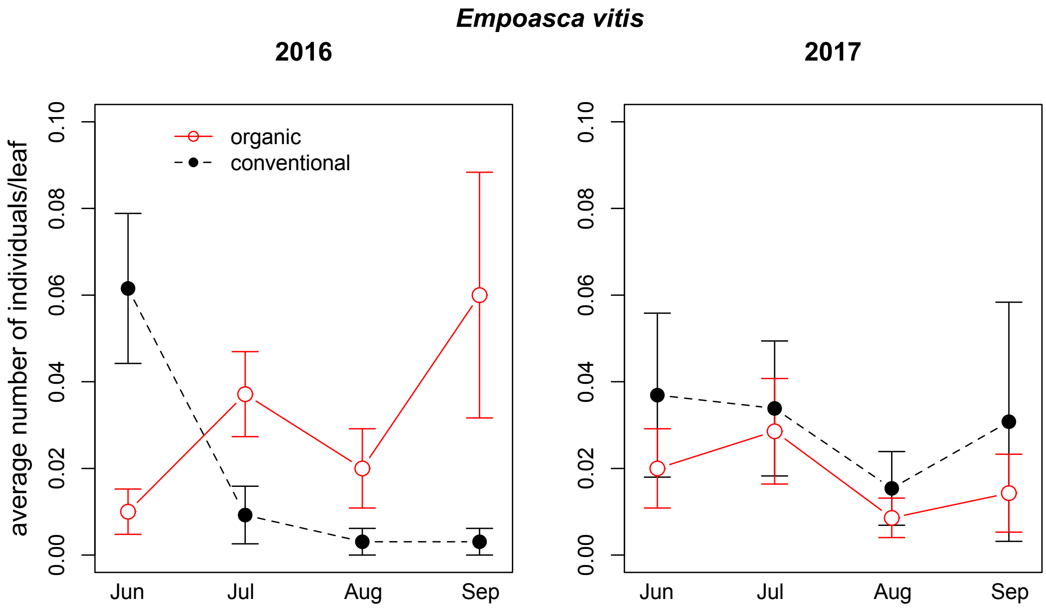

Figure 2.2: Abundance of *Empoasca vitis* nymphs on leaf samples in 2016 and 2017.

3.2. *Zygina rhamni*

On traps, higher *Z. rhamni* numbers were observed in organic than in conventional vineyards (P = 0.005; Table 2.1; Fig. 2.3). Moreover, catches were more abundant in 2017 than in 2016 (P = 0.001) and followed different trends during the growing seasons (P = 0.008; Table 2.1; Fig. 2.3). However, densities of *Z. rhamni* on leaf samples did not result significantly affected by vineyard management practices, time and their interactions (Table 2.2; Fig. 2.4). Differently, they resulted significantly affected by the abundance of semi-natural habitats (P = 0.016 and P = 0.049 for 250 m and 500 m radius, respectively; Table 2.2). In particular, *Z. rhamni* was more abundant in vineyards within complex landscapes and this effect was not influenced by the spatial scale (Fig. 2.5).

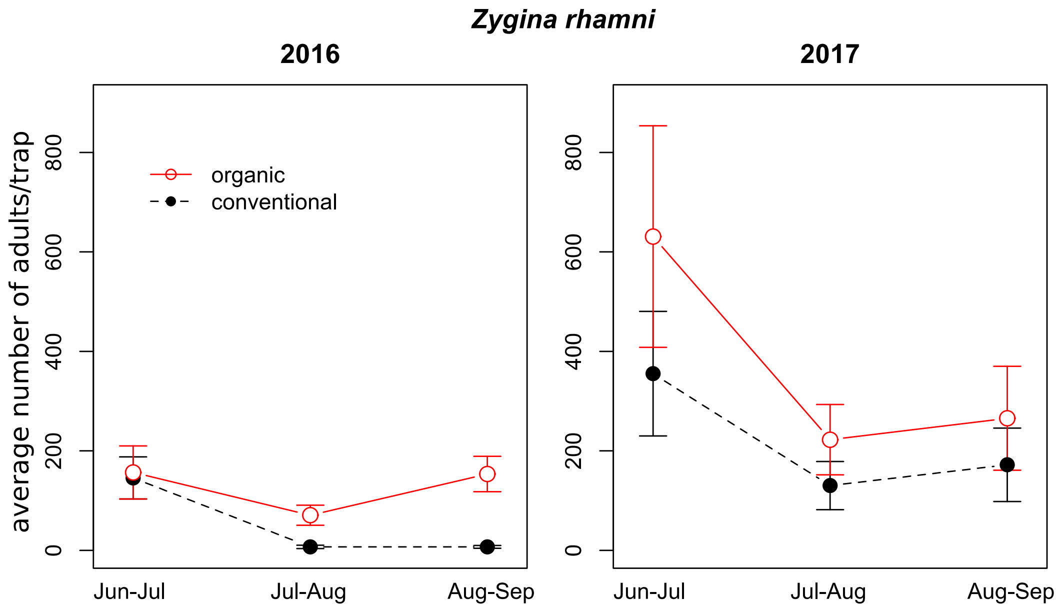

Figure 2.3: Catches of *Zygina rhamni* adults on yellow sticky traps in 2016 and 2017.

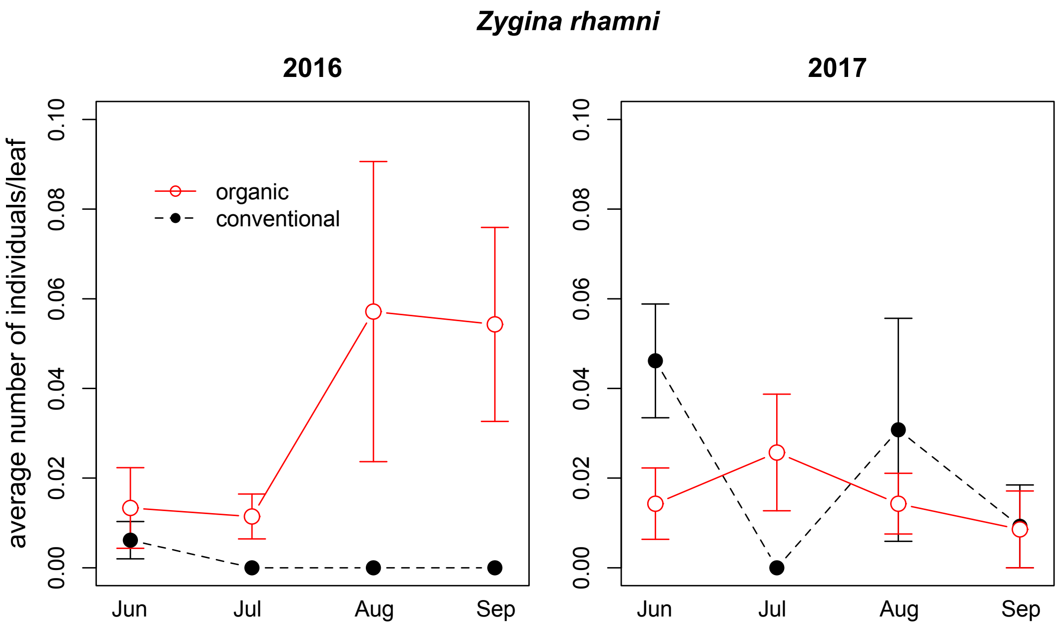

Figure 2.4: Abundance of *Zygina rhamni* nymphs on leaf samples in 2016 and 2017.

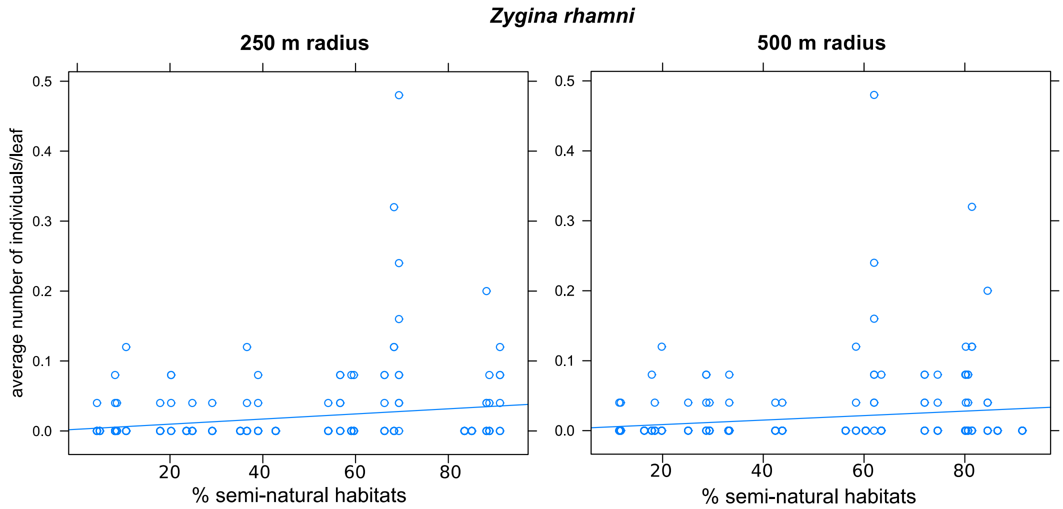

Figure 2.5: Relationship between the percentage of semi-natural habitats and the abundance of *Zygina rhamni* nymphs on leaf samples in a radius of 250 m and 500 m of landscape analysis.

3.3. *Scaphoideus titanus*

The abundance of *S. titanus* adults on traps resulted significantly affected by vineyard management (P = 0.046; Table 2.1). In particular, catches were more abundant in organic than in conventional vineyards (Fig. 2.6). Differently, the abundance of semi-natural habitats did not affect *S.titanus* catches. Densities of *S. titanus* nymphs on suckers were more abundant in organic than in conventional vineyards (P = 0.045; Table 2.2; Fig. 2.7). A higher nymph population density was recorded in 2017 than in 2016 (P = 0.0006) and a significant effect of the interaction “year*vineyard management” was found (P < 0.0001; Table 2.2). *Scaphoideus titanus* nymphs were more abundant in organic vineyards only in 2017 (Fig. 2.7).

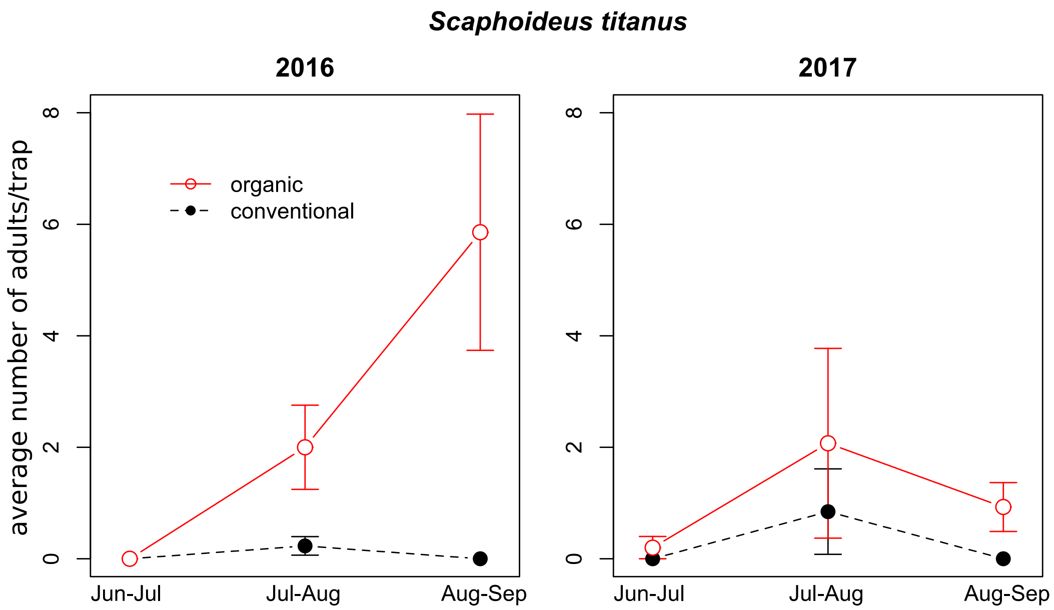

Figure 2.6: Catches of *Scaphoideus titanus* adults on yellow sticky traps in 2016 and 2017.

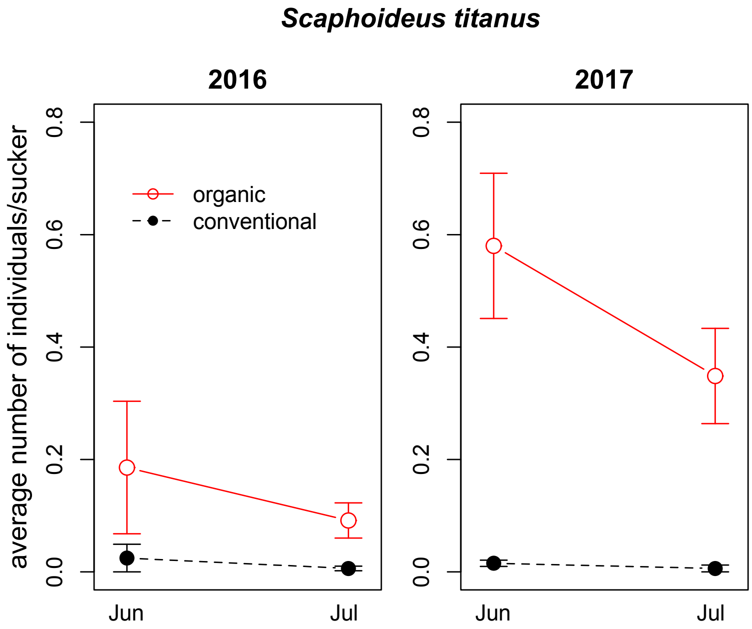

Figure 2.7: Abundance of *Scaphoideus titanus* nymphs on sucker samples in 2016 and 2017.

Table 2.1: Results of the mixed-effect model testing the effect of year, time, vineyard management, and landscape complexity in a radius of 250 m and 500 m from the centre of the vineyard on leafhopper adults caught on yellow sticky traps.

Leafhopper species	Factor or interaction	num DF	den DF	F value	Pr > F		Factor or interaction	num DF	den DF	F value	Pr > F	
	year	1	109	4.24	0.042	*	year	1	111	2.81	0.097	
	time(year)	4	107	2.72	0.033	*	time(year)	4	107	2.96	0.023	*
	management	1	42.5	4.71	0.036	*	management	1	42.6	2.18	0.148	
	year*management	1	109	1.86	0.176		year*management	1	111	2.66	0.106	
	time(year)*management	4	107	2.1	0.085		time(year)*management	4	107	1.82	0.130	
Empoasca vitis	landscape 250 m	1	41.5	0.19	0.663		landscape 500 m	1	42	0.18	0.673	
	landscape 250 m*year	1	107	0.2	0.653		landscape 500 m*year	1	110	0.2	0.654	
	landscape 250 m*time(year)	4	107	1.91	0.114		landscape 500 m*time(year)	4	108	2.36	0.058	
	landscape 250 m*management	1	41.5	0.39	0.535		landscape 500 m*management	1	42	0.01	0.906	
	landscape 250 m*year*management	1	107	0.12	0.733		landscape 500 m*year*management	1	110	0.06	0.801	
	landscape 250	4	107	0.88	0.481		landscape 500	4	108	1.16	0.333	
	year	1	116	11.31	0.001	***	year	1	118	8.61	0.004	**
	time(year)	4	109	3.6	0.009	**	time(year)	4	109	2.91	0.025	*
	management	1	38.7	8.72	0.005	**	management	1	39	4.53	0.040	*
	year*management	1	116	1.25	0.267		year*management	1	118	2.12	0.148	
	time(year)*management	4	109	1.08	0.369		time(year)*management	4	109	0.56	0.693	
Zygina rhamni	landscape 250 m	1	38	0.06	0.809		landscape 500 m	1	38.5	0.08	0.778	
	landscape 250 m*year	1	115	0.07	0.788		landscape 500 m*year	1	117	0.09	0.769	
	landscape 250 m*time(year)	4	109	1.48	0.213		landscape 500 m*time(year)	4	109	2.31	0.063	
	landscape 250 m*management	1	38	0.53	0.471		landscape 500 m*management	1	38.5	0.02	0.884	
	landscape 250 m*year*management	1	115	0.09	0.766		landscape 500 m*year*management	1	117	0.1	0.752	
	landscape 250	4	109	0.28	0.891		landscape 500	4	109	0.7	0.594	
	year	1	108	0.28	0.600		year	1	110	0.75	0.388	
	time(year)	4	110	1.92	0.112		time(year)	4	110	1.85	0.124	
	management	1	50.9	4.18	0.046	*	management	1	50.6	4.05	0.050	*
	year*management	1	108	0.96	0.328		year*management	1	110	1.34	0.250	
	time(year)*management	4	110	1.65	0.167		time(year)*management	4	110	1.95	0.107	
Scaphoideus titanus	landscape 250 m	1	49.6	0	0.994		landscape 500 m	1	49.8	0.01	0.939	
	landscape 250 m*year	1	107	0.28	0.599		landscape 500 m*year	1	109	0	0.958	
	landscape 250 m*time(year)	4	110	0.32	0.862		landscape 500 m*time(year)	4	110	0.45	0.772	
	landscape 250 m*management	1	49.6	0	0.952		landscape 500 m*management	1	49.8	0.16	0.690	
	landscape 250 m*year*management	1	107	0.02	0.892		landscape 500 m*year*management	1	109	0.07	0.795	
	landscape 250	4	110	0.21	0.930		landscape 500	4	110	0.36	0.836	

Average number of individuals of leafhopper was log (n+1) transformed prior the analysis.

Table 2.2: Results of the mixed-effect model testing the effect of year, time, vineyard management, and landscape complexity in a radius of 250 m and 500 m from the centre of the vineyard on leafhopper nymphs on leaves and suckers samplings.

Leafhopper species	Factor or interaction	num DF	den DF	F value	Pr > F		Factor or interaction	num DF	den DF	F value	Pr > F	
	year	1	95.4	0.8	0.373		year	1	94.9	1.36	0.246	
	time(year)	6	164	1.01	0.423		time(year)	6	164	1.52	0.174	
	management	1	58.4	0.44	0.512		management	1	57.8	0.13	0.717	
	year*management	1	95.4	0.69	0.408		year*management	1	94.9	1.03	0.313	
	time(year)*management	6	164	1.55	0.164		time(year)*management	6	164	2.06	0.061	
Empoasca vitis	landscape 250 m	1	58	2.14	0.149		landscape 500 m	1	57.6	1.86	0.178	
	landscape 250 m*year	1	94.9	0.76	0.385		landscape 500 m*year	1	94.6	1.34	0.251	
	landscape 250 m*time(year)	6	164	1.13	0.346		landscape 500 m*time(year)	6	164	1.71	0.122	
	landscape 250 m*management	1	58	0.46	0.500		landscape 500 m*management	1	57.6	0.11	0.740	
	landscape 250 m*year*management	1	94.9	0	0.966		landscape 500 m*year*management	1	94.6	0.09	0.768	
	landscape 250	6	164	0.9	0.500		landscape 500	6	164	1.2	0.307	
	year	1	108	0.01	0.914		year	1	108	0.07	0.797	
	time(year)	6	177	0.72	0.632		time(year)	6	177	1.05	0.393	
	management	1	60.6	0.03	0.855		management	1	59	0.24	0.626	
	year*management	1	108	0.11	0.746		year*management	1	108	0.01	0.931	
	time(year)*management	6	177	0.94	0.468		time(year)*management	6	177	0.74	0.621	
Zygina rhamni	landscape 250 m	1	60.3	6.08	0.017	*	landscape 500 m	1	58.9	4.01	0.050	*
	landscape 250 m*year	1	107	0	0.977		landscape 500 m*year	1	108	0.14	0.704	
	landscape 250 m*time(year)	6	177	1.14	0.343		landscape 500 m*time(year)	6	177	1.45	0.199	
	landscape 250 m*management	1	60.3	0.88	0.352		landscape 500 m*management	1	58.9	0.1	0.755	
	landscape 250 m*year*management	1	107	1.79	0.184		landscape 500 m*year*management	1	108	2.06	0.154	
	landscape 250	6	177	1.41	0.214		landscape 500	6	177	1.05	0.392	
	year	1	85.3	12.56	0.001	***	year	1	77.7	11.42	0.001	***
	time(year)	2	72.4	1.9	0.157		time(year)	2	70.4	1.78	0.175	
	management	1	22.9	4.5	0.045	*	management	1	20.7	4.38	0.049	*
	year*management	1	85.3	12.73	0.001	***	year*management	1	77.7	12.18	0.001	***
	time(year)*management	2	72.4	1.9	0.157		time(year)*management	2	70.4	1.56	0.217	
Scaphoideus titanus	landscape 250 m	1	16.1	0.73	0.404		landscape 500 m	1	12.2	0.05	0.828	
	landscape 250 m*year	1	85.3	2.72	0.103		landscape 500 m*year	1	77.7	3.07	0.084	
	landscape 250 m*time(year)	2	72.4	3.01	0.056		landscape 500 m*time(year)	2	70.4	2.17	0.122	
	landscape 250 m*management	1	22.7	0.9	0.352		landscape 500 m*management	1	20.4	0.16	0.696	
	landscape 250 m*year*management	1	85.3	2.59	0.111		landscape 500 m*year*management	1	77.7	3.32	0.072	
	landscape 250	2	72.4	2.25	0.112		landscape 500	2	70.4	1.52	0.225	

Average number of individuals of leafhopper was log (n+1) transformed prior the analysis.

3.4. Parasitism rate by Mymaridae

The parasitism rate of leafhopper eggs (exerted by Hymenoptera Mymaridae) did not result significantly affected by vineyard management (Table 2.3). Differently, it was affected by the amount of semi-natural habitats (P = 0.0005 and P = 0.0006 for 250 m and 500 m radius, respectively; Table 2.3). A higher egg parasitism rate was observed in vineyards within more complex landscapes than in simplified scenarios (Fig. 2.8).

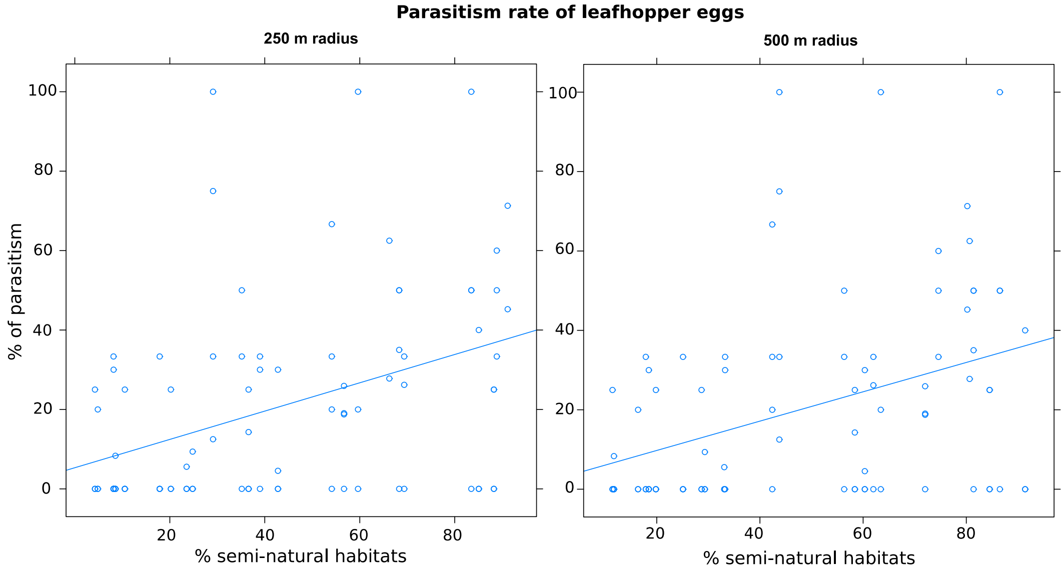

Figure 2.8: Relationship between percentage of semi-natural habitats and parasitism rate of leafhopper eggs in a radius of 250 m and 500 m of landscape analysis.

Table 2.3: Results of the mixed-effect model testing the effect of year, time, vineyard management, and landscape complexity in a radius of 250 m and 500 m from the centre of the vineyard on parasitism rate of leafhopper eggs.

	Factor or interaction	num DF	den DF	F value	Pr > F		Factor or interaction	num DF	den DF	F value	Pr > F	
Parasitism rate of leafhopper eggs	year	1	85.6	0.02	0.877		year	1	86.1	0.03	0.854	
	time(year)	2	51.6	1.44	0.247		time(year)	2	51.9	1.5	0.232	
	management	1	32.5	0.01	0.910		management	1	32.3	0.07	0.796	
	year*management	1	85.6	0.22	0.641		year*management	1	86.1	0.68	0.413	
	time(year)*management	2	51.6	1.5	0.233		time(year)*management	2	51.9	1.34	0.271	
	landscape 250 m	1	33.1	15.14	0.001	***	landscape 500 m	1	32	14.43	0.001	***
	landscape 250 m*year	1	84.5	0.26	0.610		landscape 500 m*year	1	85.5	0.11	0.743	
	landscape 250 m*time(year)	2	53.2	0.8	0.453		landscape 500 m*time(year)	2	52.8	0.65	0.527	
	landscape 250 m*management	1	33.1	2.41	0.130		landscape 500 m*management	1	32	2.18	0.150	
	landscape 250 m*year*management	1	84.5	0.97	0.328		landscape 500 m*year*management	1	85.5	1.5	0.224	
	landscape 250	2	53.2	1.28	0.286		landscape 500	2	52.8	1.51	0.230	

Parasitism rate of leafhopper eggs was arcsine-square root transformed prior the analysis.

4. Discussion

The impact of vineyard management was demonstrated for *E. vitis* and *Z. rhamni* when adult catches were considered. Most of insecticides were applied against *S. titanus* in June-July. The higher abundance of Typhlocybinae adults in organic vineyards could be explained by the use of botanical insecticides (i.e., pyrethrins or spinosyns) characterised by a lower efficacy and persistence compared to the thiamethoxam based insecticides used in conventional viticulture (Mori et al. 2014; Tacoli et al. 2017). The effect of management was not significant on *E. vitis* and *Z. rhamni* nymph populations observed on leaf samples that were lower than those considered as economic thresholds in Italy (Girolami et al. 1989). The low nymph densities observed on leaves contrast to those of adults on traps (within the single species). Leaves were observed in the laboratory under a dissecting microscope to evaluate emergence holes of leafhoppers and their egg-parasitoids. Probably a number of nymphs were missed during these observations because of their reaction to lamp.

Egg parasitism rate of Typhlocybinae by Mymaridae was not affected by vineyard management. Theoretically, parasitism rate should be higher in organic vineyards where the impact of insecticides is considered to be lower compared to that in conventional vineyards. However, the use of botanical insecticides (mainly pyrethrins) or spinosyns on egg-parasitoids can exert detrimental effects on natural antagonists of pests (Biondi et al. 2012).

Vineyard management also affected *S. titanus* abundance both on suckers and on traps. This result may have implications for the spread of the Flavescence Dorée disease in organic vineyards, probably in whole investigated area, that is characterised by a fragmented landscape (Pavan et al. 2012). At this purpose, the number and timing of insecticide applications should be more defined accurately (Mori and Pavan 2014).

The effect of landscape complexity was demonstrated for both *Z. rhamni* nymphs and adults independently on the investigated scale. The presence of wild grapevines and other host plants (i.e., *Rubus* spp. and *Rosa canina* L.) in the semi-natural habitats surrounding vineyards can favour the colonization of *Z. rhamni* in the cultivated areas (Pavan 2000; Mazzoni et al. 2008). Such effects were not observed for *E. vitis* occurring in leaf samples despite the presence of preferred host plants occurred in the surrounding environments (Vidano 1963; Pavan 2000; Viggiani 2003). The presence of natural vegetation around vineyards can favour leafhoppers populations, as observed for *Z. rhamni* in this study, but it can also support their control, as observed by the higher parasitism rate of leafhopper eggs recorded in vineyards surrounded by semi-natural habitats. Semi-natural areas can provide alternative hosts and foods (such as pollen and nectar) to egg-parasitoids as well as refuges and overwintering habitats, with positive effects in the control of pests (Williams and Martinson 2000; Shields et al. 2004; Ponti et al. 2005; Zanolli and Pavan 2011; Thomson and Hoffmann 2013; Gaigher et al. 2015; Smith et al. 2015). Data suggest a relationship between *Z. rhamni* and specific egg-parasitoids. In particular *Anagrus parvus* Soyka *sensu* (Viggiani 2014) is frequently associated with *Z. rhamni* in North-eastern Italy (Zanolli and Pavan 2011; Zanolli et al. 2016).

In conclusion, this study shows that vineyard management and landscape complexity can affect the abundance of leafhopper pests and their natural antagonists. These results can be considered when pest control strategies are planned on a large scale.

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Chapter 3

Influence of vineyard inter-rows groundcover vegetation management on arthropods abundance in vineyards of Northeast of Italy

Manuscript in preparation as: Influence of vineyard inter-rows groundcover vegetation management on arthropods abundance in vineyards of Northeast of Italy

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Giulia Zanettin collected most of the data, performed the statistical analysis and drafted the manuscript.

Abstract

Habitat simplification and use of pesticides in vineyard agro-ecosystem drastically simplified arthropod communities, reducing natural pest control. In this context, habitat management practices could be useful strategies to provide fundamental sources for sustaining natural enemies. In this study the effects of habitat management practices on both pests and beneficial arthropods were evaluated in vineyards of North-eastern Italy, throughout different field experiments: 1) mowing of inter-rows spontaneous grass in both conventional and organic vineyards, 2) different timing of mowing of a green manure mixture, and 3) comparison of different green manure mixtures. In the present study, non-mowed spontaneous grass in vineyards inter-rows favoured the abundance of natural enemies (e.g., predatory mites, reduvids, parasitoid wasps, and spiders), but also of grapevine leafhoppers (*Zygina rhamni* and *Scaphoideus titanus*). Moreover, many arthropods were recorded only or in a higher numbers in organic vineyards. While no differences were observed for leafhopper egg parasitism rate. Different timing of mowing of green manure influenced arthropods abundance: phytophagous mites *Panonychus ulmi* strongly increased in plots without flowering plants, as response of low predatory mite density. Later mowing of green manure favoured beneficial arthropods (e.g., spiders and parasitoids wasps), while not influenced phytophagous densities. Arthropods abundance did not differ among treatments when different green manure mixtures were compared, probably due to low distance adopted in the sampling design. Groundcover management practices, aimed to increase plant biodiversity in vineyards, could be a useful tool to enhance beneficial arthropods, but their adoption should be carefully evaluated when dangerous pest occurs. Semi-natural areas can contribute to create a more pest-stable agro-ecosystem and should be integrated with an appropriated ecological infrastructure surrounding the vineyards.

1. Introduction

In the last decades the intensification in viticulture has been characterized by the expansion of vineyard monoculture with the removal of semi-natural areas that led to the lack of food resources (such as nectar, pollen, alternate prey), sheltering and overwintering sites for beneficial organisms (Corbett and Rosenheim 1996; Landis et al. 2000; van Emden 2002). Moreover, the deployment of broad-spectrum pesticides drastically simplified arthropod communities favouring the resurgence of pests (Hardin et al. 1995; Johnson and Tabashnik 1999) and limited the successful implementation of biological control (Landis et al. 2000). In these simplified agro-ecosystems, the abundance and activity of natural enemies has decreased with a consequent reduction of biological control of pests (Corbett and Rosenheim 1996; Symondson et al. 2002; Schmidt et al. 2004; Thorbek and Bilde 2004; Bianchi et al. 2006; Tsitsilas et al. 2006). In this context, some habitat management practices, such as the management of resident floor vegetation and the use of cover crops, are useful strategies which provide alternative prey and food sources and refuge sites for predators and parasitoids, increasing the diversity and abundance of natural enemies in vineyards (Altieri and Nicholls 2004; Gurr et al. 2004). In fact, conserving and/or increasing plant biodiversity may provide several fundamental resources for beneficial arthropods, contributing to create an appropriate ecological infrastructure within and around vineyards with implications for more pest-stable agro-ecosystems (Altieri and Nicholls 2004; Begum et al. 2004; Gurr et al. 2004; Altieri et al. 2005). In Californian vineyards the cultural practice of spontaneous groundcover grass management resulted in a habitat modification which greatly enhanced the populations and the activity of predators against phytophagous mites (Flaherty 1969). Altieri et al. (2010) suggest a guideline for the implementation of habitat management strategies in vineyards, by assessing the presence of the key natural enemies in the cultivated area and in the surrounding vegetation. After matching the plant resources existing in and around the vineyard with the needs of natural enemies, other plant species can be added to provide shelters and food sources, taking into consideration the biology of those beneficials to favor. It is important to careful select plants to utilize for providing nectar and pollen source, by assessing their influence both on pests and natural enemies. Studies on the impact of different nectar sources on parasitoids survival and fecundity provided important information on which plant species to preserve or artificially introduced into an agro-ecosystem (Wäckers 2004). The use of companion plants has been suggested to promote biological control in agro-ecosystems, in vineyards specifically. In the choice of plants, the availability, the flower attractiveness and its characteristics (e.g., size, shape and color), food accessibility, and the quality in nutritional value must be considered, when choosing flowering plants to attract beneficial arthropods, since they declare which insects will be able to access to flower's pollen and nectar (Wäckers 2004; Altieri et al. 2010). Also the timing of blossom is an important aspect since many natural enemies, which are active only as adults and for a short period during the growing season, need food sources in the early season when prey are scarce (Altieri et al. 2010).

The aims of this study were to evaluate the effects on arthropods, both pests and beneficial ones, of some habitat management practices applied in vineyards of North-eastern Italy. In a first experiment, the influence of the non-mowed native spontaneous grass of vineyards inter-rows was assessed; in a second experiment, the effect of different timing of a commercial green manure mowing was tested; while in a third experiment, was evaluated the influence of different green manure mixtures that farmers use to improve the soil characteristics.

2. Materials and methods

2.1. Influence of non-mowed spontaneous vegetation

2.1.1. Study area

The study was conducted in 2016 and 2017 in the Conegliano-Valdobbiadene DOCG area, in the northern part of Treviso province (Veneto region). For this study, two conventional and two organic (according to EU Reg. 889/2008) managed vineyards were selected. In the second year, three vineyards remained the same while one was changed. In the study area, insecticide applications were mandatory to control *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), the vector of the phytoplasma agent of the Flavescence dorée (Schvester et al. 1963) and in conventional vineyards thiamethoxam based products were the most used, while pyrethrins were mostly sprayed in organic vineyards. During the two years of investigation insecticide applications were applied against *S. titanus* in late June; while, during the sampling period neither insecticide nor acaricide treatments were applied. The studied vineyards were situated in flat conditions at altitude ranging between 93 and 274 m a.s.l. In all vineyards, the cultivated variety was Glera which is the typical cultivar in this area. Spontaneous vegetation was homogeneous among the studied vineyards and characterized by the presence of various plant species; the most common species were: *Amaranthus retroflexus* L., *Chenopodium album* L., *Convolvulus arvensis* L., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Dacus carota* L., *Digitaria* sp., *Hordeum murinum* L., *Plantago lanceolata* L., *Plantago major* L., *Poa pratensis* L., *Ranunculus acris* L., *Rumex acetosa* L., *Setaria* spp., *Silene alba* (Miller) Krause, *Sorghum halepense* (L.) Pers., *Taraxacum officinalis* Weber ex F.H.Wigg., *Trifolium pratensis* L., *Trifolium repens* L., and *Urtica dioica* L.

2.1.2. Sampling design

Experiments were carried out in four vineyards (two conventionally and two organically managed). Two different management strategies of the inter-row groundcover vegetation were compared: non-mowed (NM) and frequently mowed (FM). NM plot consisted in four inter-rows: a mowed inter-row alternated to a subsequent non-mowed inter-row. FM plot consisted in four subsequent inter-rows in which the vegetation was mowed at few centimetres from the ground to not allow flowering. An hammer mulcher was used to cut the inter-row vegetation, while the sub-row weeds were mechanically removed (flat blade). Each treatment was performed in an area of 160 m^2 (four subsequent 20 meters long inter-rows) and replicated twice in each vineyard, for a total of 16 plots (eight conventionally and eight organically managed). The experiment started in mid-July after the insecticide applications against *S. titanus*. At that time groundcover vegetation was managed according to the experimental design. In NM plots mowing was carried out only once as climatic conditions were unfavourable to grasses growth. The first sampling was performed in late July when most of plants were flowering. A total of three samplings were carried out in 2016 and two samplings in 2017, being the last samplings performed two weeks before grape harvest.

2.2. Influence of different timing of a green manure mowing

2.2.1. Study area

To investigate the effect of different timing of a commercial green manure mowing on the presence and the abundance of arthropods, a field experiment was performed in an organic vineyard located at Cessalto (45°42’50.40 N, 12°36.55.44 E, 3 m a.s.l., Treviso province, North-eastern Italy) in 2017. The grapevine variety was Glera, SO4 was used as rootstock, the grapevine training system was Sylvoz and the planting system was 2.70 × 1.20 m corresponding to 3000 vines/ha. The soil was characterized by a medium dough-clayey structure.

2.2.2. Sampling design

Three different management strategies of the vineyard inter-row groundcover were compared:

1) “Standard green manure” (Stand-GM), where vegetation was mowed when most of plants of the mixture were flowering, as traditionally done by the growers;

2) “Green manure with a more prolonged flowering period” (Late-GM), where vegetation was mowed when all the plants of the mixture finished to flower;

3) “Control”, where inter-rows were mowed before plants started to blossom.

Each treatment was performed in an area of 486 m^2 (nine subsequent inter-rows 20 meters long, which whom one third were sown with the green manure mixture), and randomly replicated in four plots. The mixture was sown in October 2016 using a disc seed drill (dose 11g/m^2). The composition of the green manure mixture is reported below (Table. 3.1).

Table 3.1: Composition of the green manure mixture.

Common name	**Scientific name**	**Cultivar**	**Pure seed (%)**
Rye	*Secale cereale*	Conduct	15
Triticale	hybrid of wheat (Triticum) and rye (Secale)	Oxygen	20
Oats	*Avena sativa*	Novella Antonia	15
Vetch	*Vicia sativa*	Mikaela	13
Flax	*Linum usitatissimum*	Sideral	3
White mustard	*Sinapis alba*	Abraham	3
Horseradish	*Brassica rapa* subsp. *campestris*	Carwoodi	4
Kale	*Brassica oleracea*	Malwira	4
Rape	*Brassica napus*	Bonar	3
Blue tansy	*Phacelia tanacetifolia*	Stala	5

The mowing of control plots was performed at the end of March 2017, before plants started to blossom. Brassicaceae species of the mixture started to blossom at the beginning of April 2017 (grapevine sprouting), Poaceae and other families later. The first sampling was performed on May 24, when the majority of plant species present in the green manure plots were blossoming. Stand-GM plots were mowed few days later, while plants continued to blossom in Late-GM plots. The second sampling was done on June 16. Then Late-GM plots were mowed. The last sampling was performed on July 17 when all plots were mowed. During the sampling period neither insecticide nor acaricide treatments were applied.

2.3. Influence of different green manure mixtures

2.3.1. Study area

This experiment was carried out in an organic vineyard located at Carbonera (45°41'4.20 N, 12°17'7.44 E, 30 m a.s.l., Treviso province, North-eastern Italy) in 2018. The variety was Raboso Piave, SO4 was used as rootstock, the wine training system was Sylvoz and the planting system was 2.70 × 1.20 m^2 corresponding to 3000 vines/ha. The soil was characterized by a medium dough structure, with a 70% of skeleton.

2.3.2. Sampling design

Three different mixtures of green manure were compared:

1. "MIX-1": *Avena sativa* L. cv Prevision + commercial mixture composed by buckwheat (KF 83%, RH 99.5%) (30%), *Pisum sativum* cv Arkta (20%), *Vicia sativa* cv Marianna (20%), *Lupinus augustifolium* cv Tango (10%), *Trifolium incarnatum* cv Tardivo (10%), *Trifolium alexandrinum* cv Marmilla (8%) and *Phacelia tanacetifolia* cv Natra (2%);
2. "MIX-2": *Lolium multiflorum* Lam. cv Furore (35%), *Avena sativa* L. cv Teobd40 (15%), *Hordeum vulgare* L. cv Tazio (10%), *Trifolium alexandrinum* cv Erix (20%) and *Vicia sativa* cv Marianna (20%);
3. "MIX-3": Rye (*Secale cereale* L. cv Dukato, 55%) and Vetch (*Vicia villosa* Roth cv Minnie, 45%);
4. "Control", in which the inter-row groundcover was moved before the blossom.

Treatments were replicated four times and each replication consisted of a single inter-row (2.70 m large and 125 m long). Distance between each plot was about 3 m. Seed mixtures were sown at the beginning of November 2017, and started to blossom at the beginning of May 2018. The control plots were mowed on 30th April 2018 while the other plots were not mowed. Leaf samples were collected from May to mid-June 2018 for a total of three sampling dates. During the sampling period neither insecticide nor acaricide treatments were applied.

2.4. Sampling methods

Different techniques were applied to collect arthropods: manual collection of grapevine leaves, beating net and sweeping net samplings. In the experiment devoted to the comparison of different green manure mixtures, only the collection of grapevine leaves was performed. All these sampling techniques were carried out in the central part of each plot. Details of the sampling techniques are reported below.

Leaf sampling

To evaluate the presence of arthropods on grapevine leaves, field samplings were performed during the three experiments. This sampling was focused on the assessment of spider mites (Acari: Tetranychidae), predatory mites (Acari: Phytoseiidae), leafhoppers (Hemiptera: Cicadellidae), mealybugs (Hemiptera: Pseudococcidae) and natural enemies. Twenty-five leaves were randomly collected from vine canopy and immediately transferred to the laboratory where they were observed under a Wild M3C stereomicroscope (10-40 x magnification) to assess the identity and abundance of arthropod species. In the experiment on the influence of non-mowed spontaneous vegetation, the parasitism rate of leafhopper eggs by Hymenoptera Mymaridae was evaluated dividing the number of the parasitoid emerging holes by the sum of the number of nymph emergence holes and parasitoid emergence holes.

Beating net sampling

A beating net (1 x 1 m) was used to collect arthropods from the vine canopy. Each beating net sampling included a total of 4 sub-sample (1 meter of vine canopy row) per each plot. The beating net was positioned between the ground and the vine canopy; then the permanent cordon of the grapevine was shacked for five times and the arthropods that fall on the beating net were quickly collected with an insect aspirator and stored in a plastic tube (50 ml) added with alcohol to prevent predation. Stored material has been identified in the laboratory under a dissecting microscope.

Sweep net sampling

An entomological sweep net was used to collect the arthropods on groundcover vegetation of the vineyard inter-row. The entomological sweep net, with a diameter of 30 cm, was swept for 10 times in the central inter-row of each plot; for NM plots the technique was performed in a non-mowed inter-row. Collected arthropods were removed from the net using an insect aspirator and then put into plastic tube (50 ml) added with alcohol. Later, that material was observed in the laboratory under a dissecting microscope for the identification.

2.5. Statistical analysis

Data on the abundance of main grapevine pests and their natural enemies were analysed using the packages “nlme” (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team 2015) for general mixed-effects models implemented in R 3.0.2 (R Development Core Team 2013). Prior the analysis, average data of collected arthropods were log (n+1) transformed. The parasitism rate of leafhopper eggs was arcsine-square root transformed. The assumptions of the models were evaluated by inspecting diagnostic plots of model residuals.

In the experiment on the influence of non-mowed spontaneous vegetation, a linear mixed-effects model (LME) was used to test the effects of treatment (two different grass mowing strategies), vineyard management strategies (conventional vs. organic) and time on arthropods observed/collected with the three different sampling methods. In each model, treatment, vineyard management and time were considered as categorical fixed factors. Along with the main effects all possible interactions were also tested. To account for the nested design and repeated measures, site identity (n=4) and sampling plot identity (n=16) were included as random factors.

In the experiment on the influence of different timing of green manure mowing, a LME was used to test the effects of treatment (three different groundcover managements) on arthropods observed/collected throughout the experiment with the different sampling methods. In each model, treatment and time were entered as categorical fixed factors. Along with the main effects all possible interactions were also tested. To account for the repeated measures, sampling plot identity (n=12) was included as random factors.

In the experiment on the effect of different green manure mixtures, a LME was used to test the effects of treatment (three different mixtures and the control) on arthropods observed on leaf samples during the experiment. In each model, treatment and time were entered as categorical fixed factors. Along with the main effects all possible interactions were also tested. To account for the repeated measures, sampling plot identity (n=16) was included as random factors.

3. Results

3.1 Influence of non-mowed spontaneous vegetation

Leaf sampling

On leaf samples the presence of predatory mites (Phytoseiidae) and non-specialized mites (Tydeidae) was recorded, while the presence of phytophagous mites (Tetranychidae) was not observed. Regarding the Phytoseiidae, *Kampimodromus aberrans* (Oudemans), *Amblyseius andersoni* (Chant), *Typhlodromus pyri* Scheuten and *Phytoseius finitimus* Ribaga were recorded. In organic vineyards *P. finitimus* was the most abundant species, while in conventional *K. aberrans* and *T. pyri* dominated. Considering this different distribution and their role, predatory mites were analysed together as family group. In both the two years of investigation, grass mowing management influenced predatory mites numbers, which were more abundant in

NM plots than in FM plots (Table 3.2; Figure 3.1). An effect of time was also observed: the density of predatory mites decreased during the time in both years (Table 3.2; Fig. 3.1).

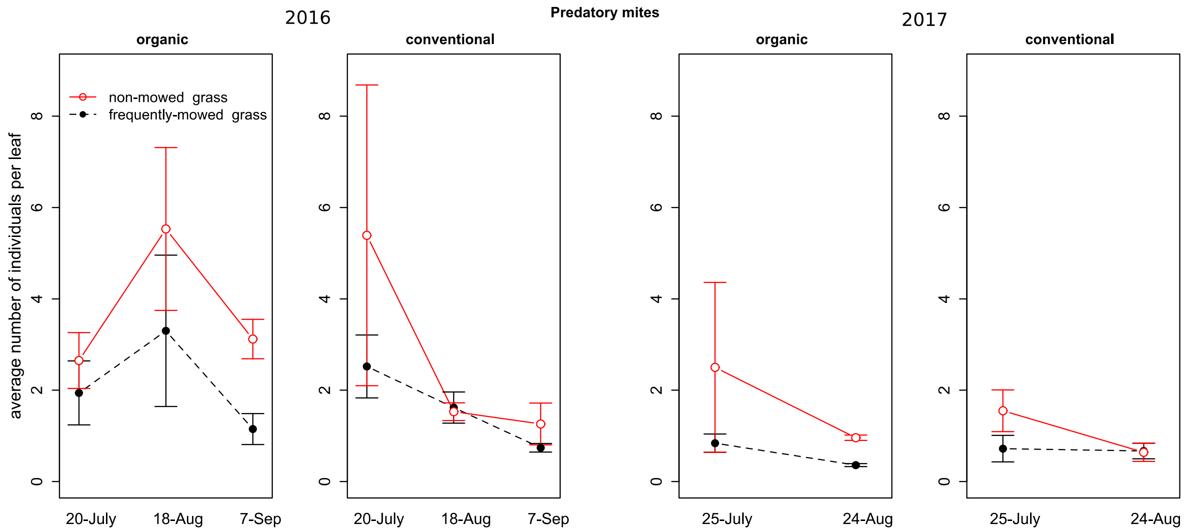

Figure 3.1: Abundance (mean ± std. err.) of predatory mites (Acari: Phytoseiidae) observed on leaf samples during the first experiment in 2016 and 2017.

The abundance of Tydeidae was influenced only by the interaction vineyard management*time, and only in 2016 (Table 3.2); in the first sampling, the Tydeidae were more abundant in conventional than in organic vineyards, and vice versa at the end of August (Fig. 3.2). In 2017 no significant effects were observed (Table 3.2; Fig. 3.2).

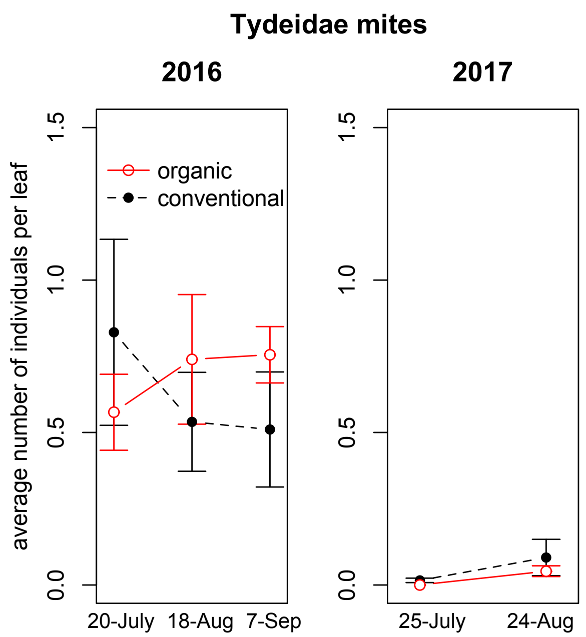

Figure 3.2: Abundance (mean ± std. err.) of Tydeidae (Acari: Phytoseiidae) observed on leaf samples during the first experiment in 2016 and 2017.

Regarding leafhoppers, the presence of *Empoasca vitis* (Göethe) and *Zygina rhamni* Ferrari was recorded on leaves.

In 2016, a significant effect of time and vineyard management*time interaction emerged on *E. vitis*. This leafhopper was more abundant in organic than in conventional vineyards and these differences emerged at the end of the observations (Table 3.2; Fig. 3.3). In the following year, its abundance was significantly affected only by the time (Table 3.2; Fig. 3.3).

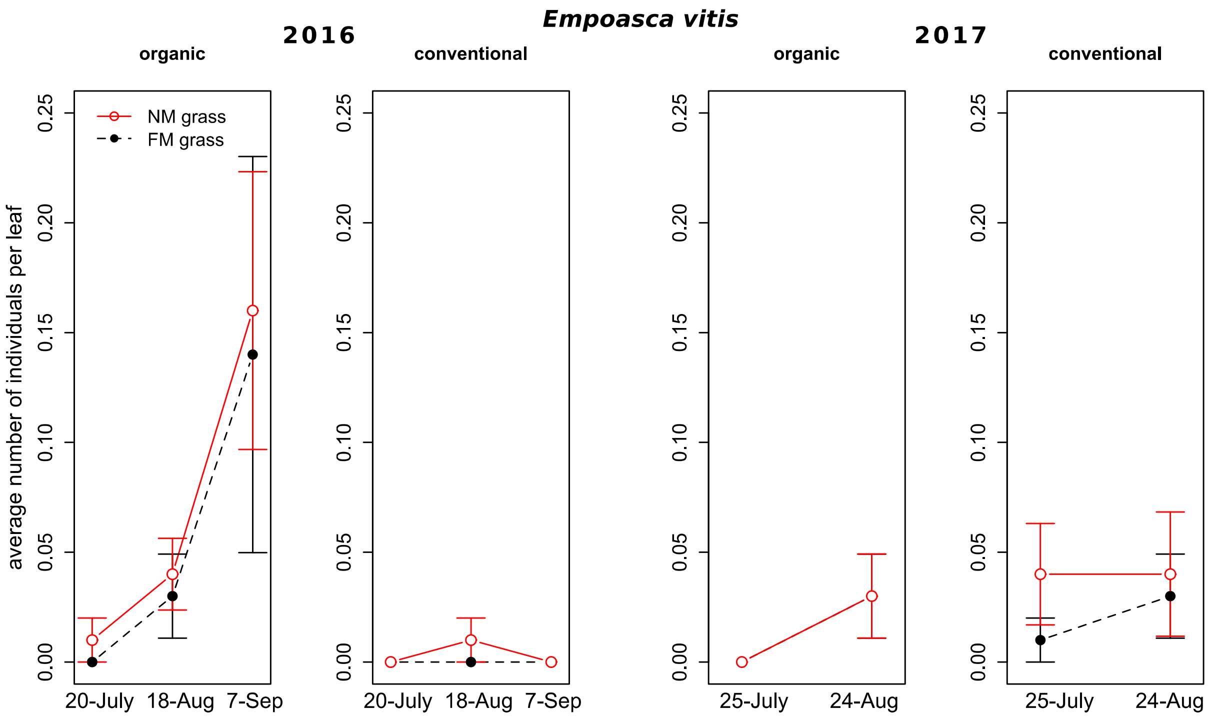

Figure 3.3: Abundance (mean ± std. err.) of *Empoasca vitis* (Hemiptera: Cicadellidae) observed on leaf samples during the first experiment in 2016 and 2017.

The abundance of *Z. rhamni* in 2016 was influenced by grass mowing and grass mowing*management interaction, while no significant effects were observed in 2017 (Table 3.2). In 2016 a higher abundance of *Z. rhamni* was observed in NM plots than in FM plots in organic vineyards, while no effects were observed in conventional vineyards (Table 3.2; Fig. 3.4).

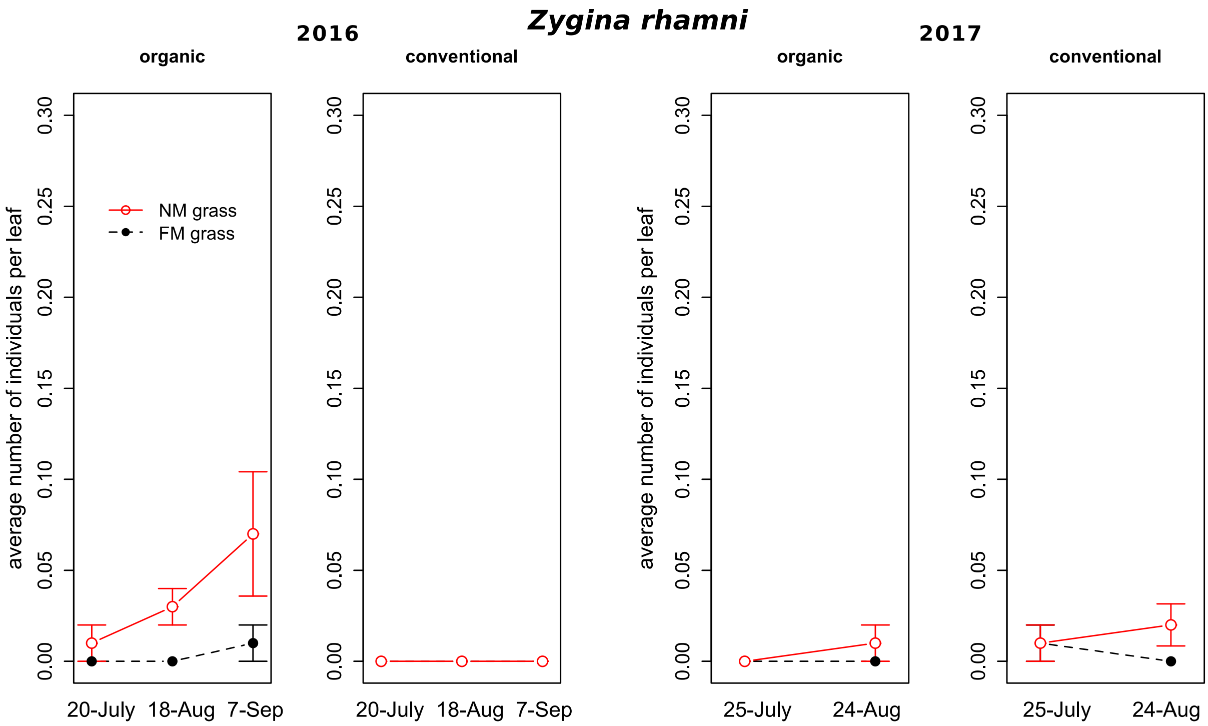

Figure 3.4: Abundance (mean ± std. err.) of *Zygina rhamni* (Hemiptera: Cicadellidae) observed on leaf samples during the first experiment in 2016 and 2017.

In both seasons, the parasitism rate of leafhopper eggs was not affected by the investigated factors and their interactions (Table 3.2).

The abundance of lacewing eggs (Neuroptera: Chrysopidae) did not result influenced by the investigated factors and their interactions (Table 3.2).

Finally, were also recorded the presence of mealybugs, such as *Parthenolecanium corni* (Bouché) (Hemiptera: Coccidae) and *Planoccoccus ficus* (Signoret): their presence was observed especially in organic vineyards. However, they were recorded in very low abundances and therefore not analysed.

Table 3.2: Results of the mixed-effect model testing the effect of treatment, vineyard management, and time on arthropods observed in leaf samples.

	Factor or interaction	2016 num DF	2016 den DF	2016 F value	2016 Pr > F		2017 num DF	2017 den DF	2017 F value	2017 Pr > F	
predatory mites (Acari: Phytoseiidae)	grass mowing	1	10	6.863	0.026	*	1	10	5.359	0.043	*
	management	1	2	0.670	0.499		1	2	0.000	0.987	
	time	2	21	5.461	0.012	*	1	8	6.850	0.031	*
	grass mowing*management	1	10	0.857	0.376		1	10	0.849	0.378	
	grass mowing*time	2	21	0.766	0.477		1	8	2.181	0.178	
	management*time	2	21	2.923	0.076		1	8	0.462	0.516	
	grass mowing*management*time	2	21	1.386	0.272		1	8	0.532	0.487	
non-specialized mites (Acari: Tydeidae)	grass mowing	1	10	0.431	0.526		1	10	0.616	0.451	
	management	1	2	0.099	0.783		1	2	0.102	0.780	
	time	2	21	0.451	0.643		1	8	3.152	0.114	
	grass mowing*management	1	10	1.178	0.303		1	10	0.010	0.924	
	grass mowing*time	2	21	1.317	0.289		1	8	1.679	0.231	
	management*time	2	21	3.612	0.045	*	1	8	0.115	0.743	
	grass mowing*management*time	2	21	0.568	0.575		1	8	0.431	0.530	
Empoasca vitis (Hemiptera: Cicadellidae)	grass mowing	1	10	0.080	0.783		1	10	1.135	0.312	
	management	1	2	12.226	0.073		1	2	0.140	0.744	
	time	2	21	4.617	0.022	*	1	8	6.031	0.040	*
	grass mowing*management	1	10	0.009	0.928		1	10	0.870	0.373	
	grass mowing*time	2	21	0.042	0.959		1	8	0.373	0.558	
	management*time	2	21	5.129	0.015	*	1	8	4.519	0.066	
	grass mowing*management*time	2	21	0.035	0.966		1	8	0.243	0.635	
Zygina rhamni (Hemiptera: Cicadellidae)	grass mowing	1	10	5.933	0.035	*	1	10	1.487	0.251	
	management	1	2	9.204	0.094		1	2	1.023	0.418	
	time	2	21	2.429	0.113		1	8	0.217	0.654	
	grass mowing*management	1	10	6.223	0.032	*	1	10	0.084	0.778	
	grass mowing*time	2	21	1.048	0.368		1	8	2.676	0.141	
	management*time	2	21	2.645	0.095		1	8	0.360	0.565	
	grass mowing*management*time	2	21	1.005	0.383		1	8	0.104	0.756	
Parasistism rate of leafhopper eggs	grass mowing	1	10	0.014	0.907		1	10	0.600	0.457	
	management	1	2	0.154	0.733		1	2	0.491	0.556	
	time	2	18	3.116	0.069		1	7	2.294	0.174	
	grass mowing*management	1	10	0.175	0.685		1	10	1.395	0.265	
	grass mowing*time	2	18	0.052	0.950		1	7	0.258	0.627	
	management*time	2	18	0.806	0.462		1	7	1.737	0.229	
	grass mowing*management*time	2	18	0.479	0.627		1	7	2.806	0.138	
Eggs of lacewing (Neuroptera: Chrysopidae)	grass mowing	1	10	0.296	0.598		1	10	0.928	0.358	
	management	1	2	10.285	0.085		1	2	1.141	0.397	
	time	2	21	1.450	0.257		1	8	1.313	0.285	
	grass mowing*management	1	10	0.505	0.494		1	10	0.036	0.853	
	grass mowing*time	2	21	0.451	0.643		1	8	0	0.997	
	management*time	2	21	0.307	0.739		1	8	0.001	0.979	
	grass mowing*management*time	2	21	0.284	0.755		1	8	0	0.999	

Prior to the analysis, average number of individuals was log (n+1) transformed while parasitism rate of leafhopper eggs was arcsine-square root transformed.

Beating net sampling

The beating net technique allowed to collect additional arthropod species resident on grapevine canopy. During the investigation, the presence of red velvet mites (Acari: Trombididae), earwigs (Dermaptera), stink bugs (Hemiptera: Pentatomidae), leafhoppers, larvae of lacewings (Neuroptera: Chrysopidae), and spiders (Araneae) was recorded in the investigated vineyards. This technique also permitted to collect some individuals of beneficial arthropods such as reduvids (Hemiptera: Reduviidae), minute pirate bugs (Hemiptera: Antocoridae), ground beetles (Coleoptera: Carabidae), coccinellids (Coleoptera: Coccinellidae), and harvestmen (Opiliones); however, they were not considered in the analysis due to their low abundance.

In 2016, red velvet mites were more abundant on vine canopy in NM plots than in FM plots showing a decrease along the time (Table 3.3; Fig. 3.5). In 2017, their presence was not analysed since they were recorded in one vineyard only.

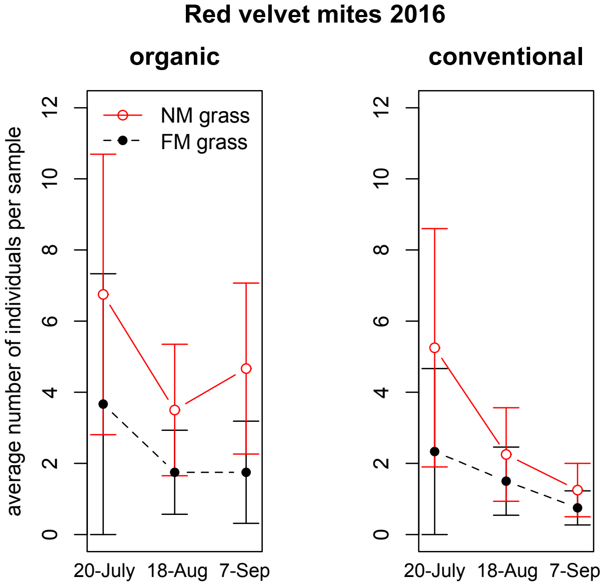

Figure 3.5: Abundance (mean ± std. err.) of red velvet mite (Acari: Trombididae) observed on beating net samples during the first experiment in 2016.

The abundance of earwigs observed in 2016 was influenced by time and the interactions grass mowing*time and vineyard management*time (Table 3.3). Their presence on grape canopy was limited to organic orchards. Here, at the end of the observations, there were higher numbers of earwigs in NM plots as compared to FM plots (Fig. 3.6). In 2017, the presence of earwigs was not influenced by any of the investigated effects (Table 3.3; Fig. 3.6).

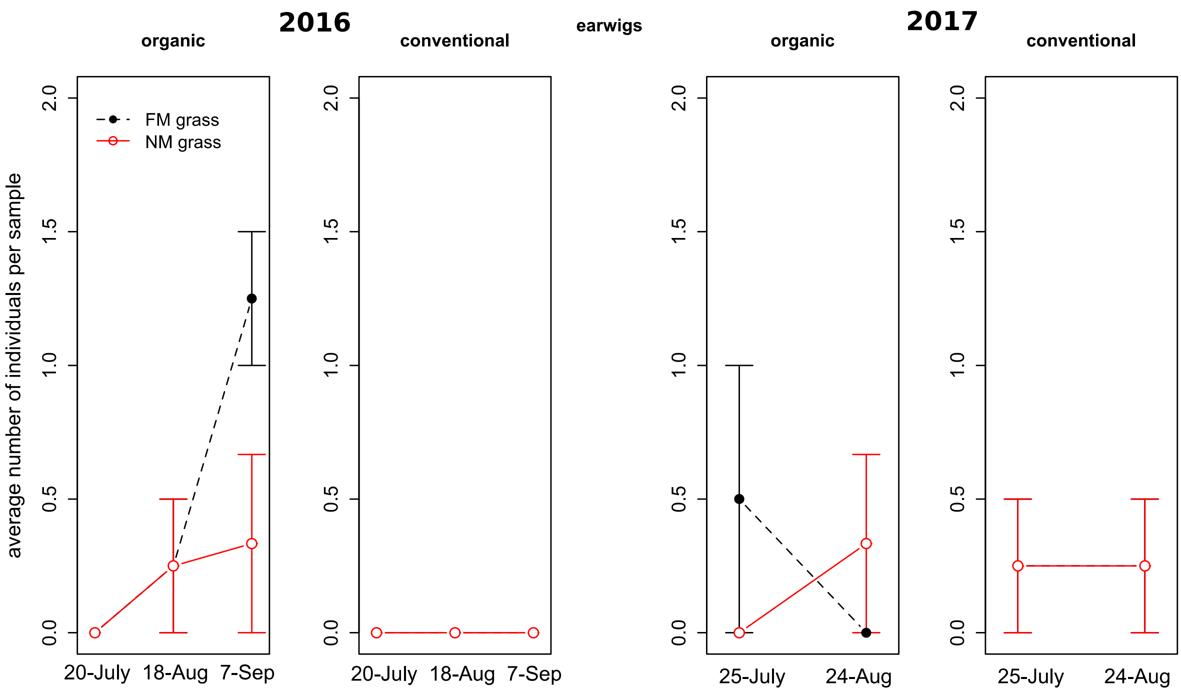

Figure 3.6: Abundance (mean ± std. err.) of earwigs (Dermaptera) observed on beating net samples during the first experiment in 2016 and 2017.

In both years the presence of stink bugs was observed on grapevine canopy, but it did not result influenced by the investigated factors and their interactions (Table 3.3).

In 2016, the abundance of leafhoppers not associated with grapevine was significantly affected by grass mowing*time interaction (Table 3.3); in NM plots an increasing number of leafhoppers was observed on grapevine canopy during the sampling period (Fig. 3.7). Moreover, their abundance increased in organic than in conventional vineyards (Table 3.3; Fig. 3.7). Differently, in 2017 leafhoppers abundance did not result significantly affected by the investigated factors and their interactions (Table 3.3).

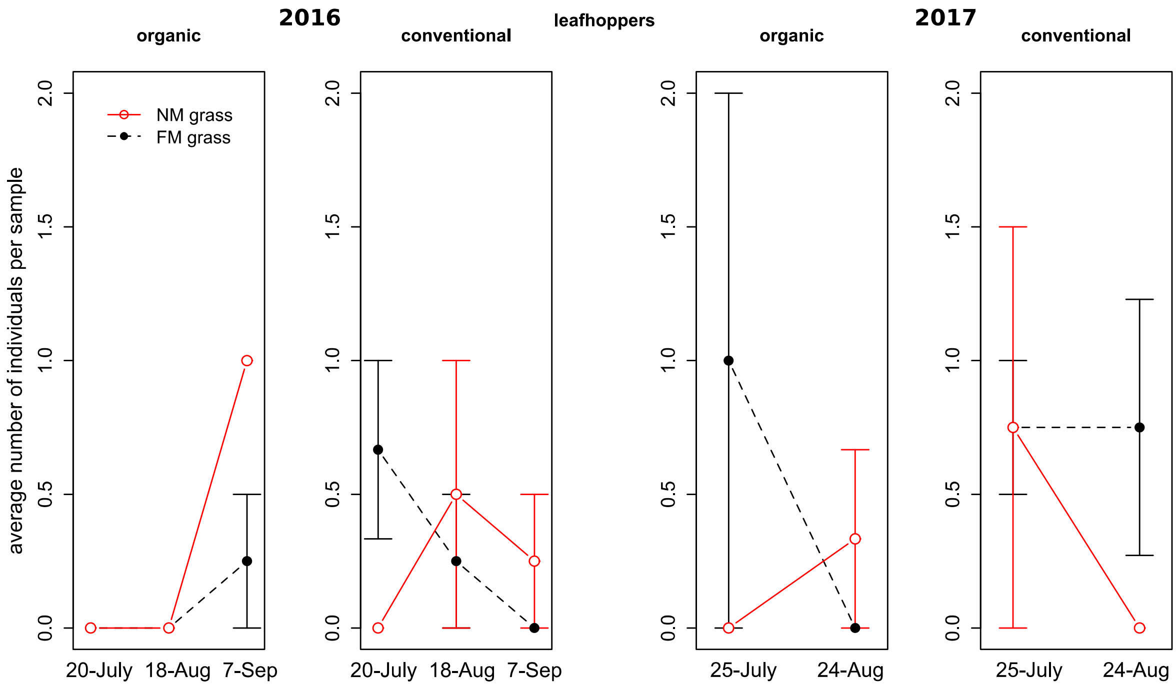

Figure 3.7: Abundance (mean ± std. err.) of leafhoppers (Hemiptera: Cicadellidae) observed on beating net samples during the first experiment in 2016 and 2017.

In 2016, the abundance of lacewing larvae was influenced by time and interaction time*vineyard management: these predators were observed only in organic vineyards and their abundance decreased during the season (Table 3.3; Fig. 3.8). In 2017, their abundance was not significantly affected by the investigated factors (Table 3.3; Fig. 3.8).

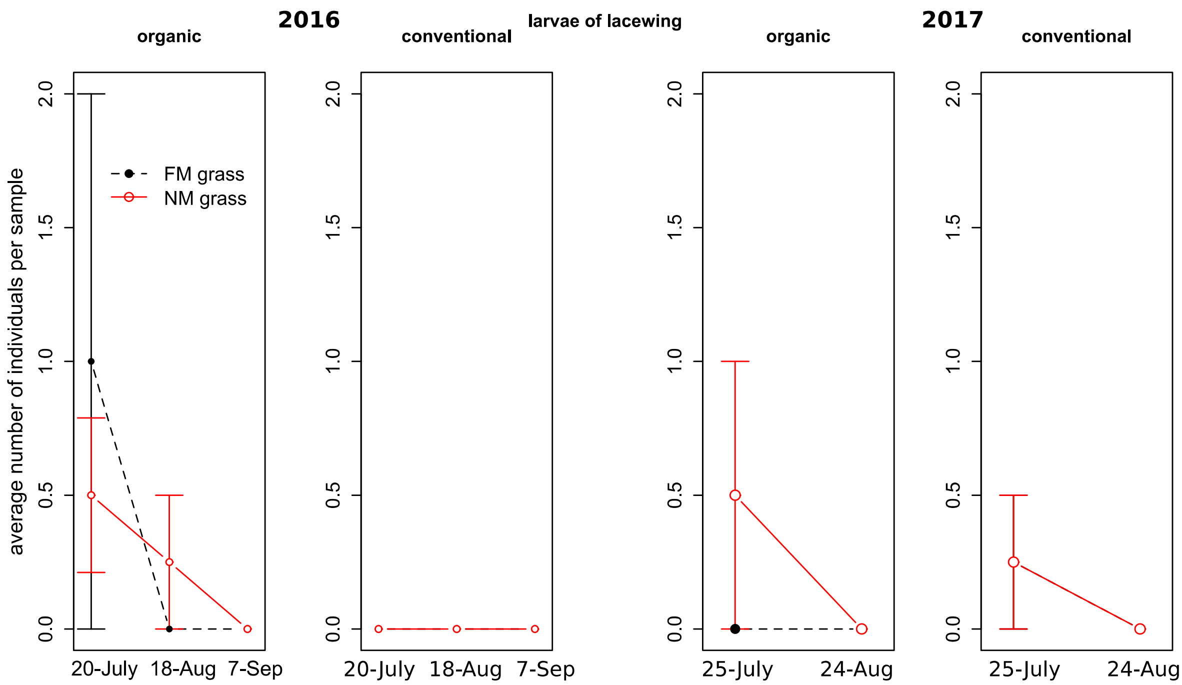

Figure 3.8: Abundance (mean ± std. err.) of lacewing larvae (Neuroptera: Chrysopidae) observed on beating net samples during the first experiment in 2016 and 2017.

In 2016, the abundance of spiders was influenced by vineyard management and by the interactions vineyard management*time and grass mowing*time. Higher spider numbers were reached in organic vineyards as compared to conventional ones (Table 3.3; Fig. 3.9). In the same period spider abundance increased in NM plots but not in FM plots (Table 3.3; Fig. 3.9). In 2017, the presence of spiders was influenced by the interaction time*vineyard management being more abundant in organic than in conventional vineyards, but this effect was higher in the first observation (Table 3.3; Fig. 3.9).

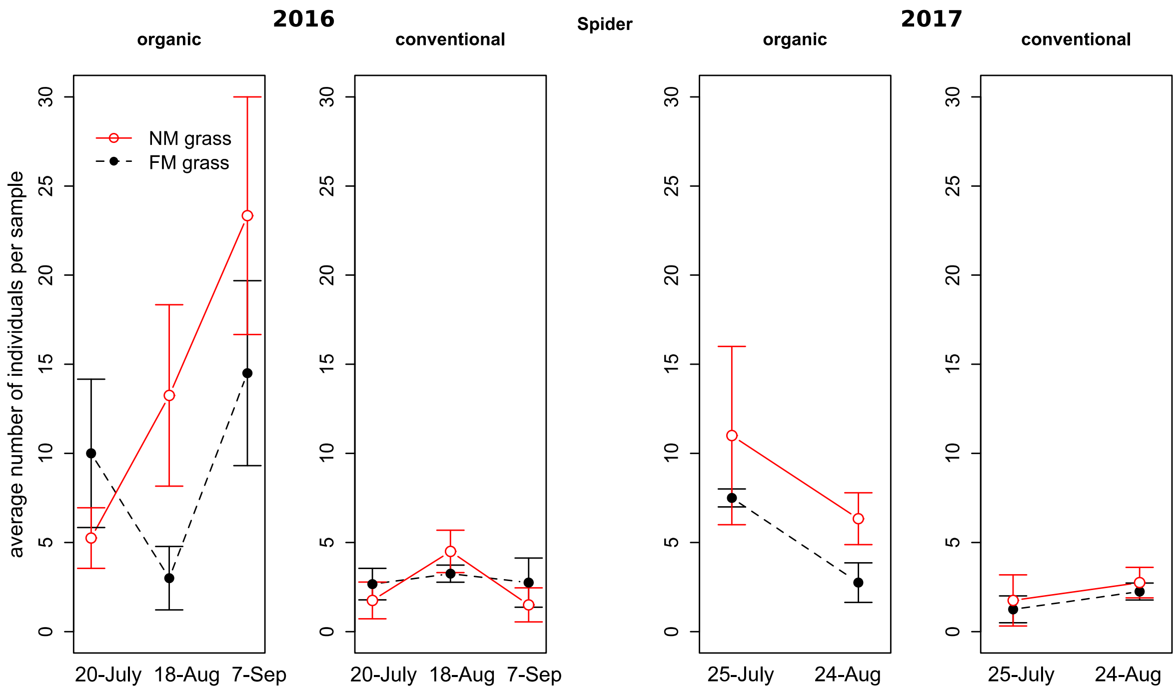

Figure 3.9: Abundance (mean ± std. err.) of spiders (Araneae) observed on beating net samples during the first experiment in 2016 and 2017.

Table 3.3: Results of the mixed-effect models testing the effect of treatment, vineyard management, and time on arthropods collected by beating net sampling.

		2016					2017				
	Factor or interaction	num DF	den DF	F value	Pr > F		num DF	den DF	F value	Pr > F	
Red velvet mites (Acari: Trombididae)	grass mowing	1	10	6.705	0.027	*	not analyzed				
	management	1	2	0.035	0.869						
	time	2	21	5.046	0.016	*					
	grass mowing*management	1	10	0.400	0.541						
	grass mowing*time	2	21	0.053	0.948						
	management*time	2	21	0.213	0.810						
	grass mowing*management*time	2	21	0.180	0.837						
Earwigs (Dermaptera)	grass mowing	1	10	2.728	0.130		1	9	0.002	0.961	
	management	1	2	12.879	0.070		1	2	0.119	0.763	
	time	2	21	9.704	0.001	***	1	8	0.011	0.918	
	grass mowing*management	1	10	2.279	0.162		1	9	0.002	0.967	
	grass mowing*time	2	21	3.521	0.048	*	1	8	0.961	0.356	
	management*time	2	21	9.943	0.001	***	1	8	0.017	0.899	
	grass mowing*management*time	2	21	3.243	0.059		1	8	1.549	0.249	
Stink bugs (Hemiptera: Pentatomidae)	grass mowing	1	10	1.022	0.336		1	9	0.072	0.794	
	management	1	2	0.979	0.427		1	2	0.006	0.944	
	time	2	21	0.941	0.406		1	8	0.161	0.699	
	grass mowing*management	1	10	0.935	0.356		1	9	0.103	0.756	
	grass mowing*time	2	21	0.852	0.441		1	8	4.452	0.068	
	management*time	2	21	0.902	0.421		1	8	0.302	0.598	
	grass mowing*management*time	2	21	0.848	0.443		1	8	0.091	0.771	
Leafhoppers not associated with grapevine (Hemiptera: Cicadellidae)	grass mowing	1	10	0.288	0.603		1	9	1.450	0.259	
	management	1	2	0.275	0.652		1	2	0.879	0.447	
	time	2	21	1.165	0.331		1	8	1.356	0.278	
	grass mowing*management	1	10	1.211	0.297		1	9	0.549	0.478	
	grass mowing*time	2	21	3.788	0.039	*	1	8	0.153	0.706	
	management*time	2	21	5.569	0.012	*	1	8	0.017	0.900	
	grass mowing*management*time	2	21	1.204	0.320		1	8	2.332	0.165	
Larvae of lacewings (Neuroptera: Chrysopidae)	grass mowing	1	10	0.082	0.781		1	9	0.438	0.525	
	management	1	2	0.743	0.479		1	2	0.057	0.833	
	time	2	21	3.934	0.035	*	1	8	3.944	0.082	
	grass mowing*management	1	10	0.052	0.824		1	9	0.549	0.478	
	grass mowing*time	2	21	0.599	0.558		1	8	0.609	0.458	
	management*time	2	21	4.167	0.030	*	1	8	0.001	0.981	
	grass mowing*management*time	2	21	0.630	0.543		1	8	0.964	0.355	
Spiders (Araneae)	grass mowing	1	10	0.236	0.638		1	9	1.246	0.293	
	management	1	2	22.547	0.042	*	1	2	12.007	0.074	
	time	2	21	0.910	0.418		1	8	0.027	0.874	
	grass mowing*management	1	10	2.825	0.124		1	9	1.436	0.261	
	grass mowing*time	2	21	3.564	0.047	*	1	8	0.349	0.571	
	management*time	2	21	5.822	0.010	**	1	8	6.341	0.036	*
	grass mowing*management*time	2	21	0.862	0.437		1	8	0.253	0.628	

Prior to the analysis, average number of individuals was log (n+1) transformed.

Sweep net sampling

Arthropods occurring on vineyard inter-row groundcover were collected by using of sweep net. During the investigation the presence of leafhoppers, nabids (Hemiptera: Nabidae), revudids (Hemiptera: Reduviidae), lacewing larvae, parasitic wasps (Hymenoptera), and spiders was recorded in the investigated vineyards. This technique also permitted to collect some individuals of other beneficials such as minute pirate bugs, ladybirds, hoverflies (Diptera: Syrphidae), predatory thrips (Thysanoptera), and harvestmen (Opiliones); however, they were not considered in the analysis due to their low numbers.

The abundance of leafhoppers not associated with grapevine, in the two years of investigation, was influenced by time showing a significant increment during the sampling period (Table 3.4; Fig. 3.10), and in 2017 also by the interactions grass mowing*vineyard management and vineyard management*time. In the second year of investigation, the leafhoppers were more abundant in FM plots in organic vineyards and less abundant in NM plots in conventional ones (Table 3.4; Fig. 3.10). Moreover, in 2017 the leafhoppers abundance increased during the sampling period in organic vineyards, while remained at constant levels in conventional ones (Table 3.4; Fig. 3.10).

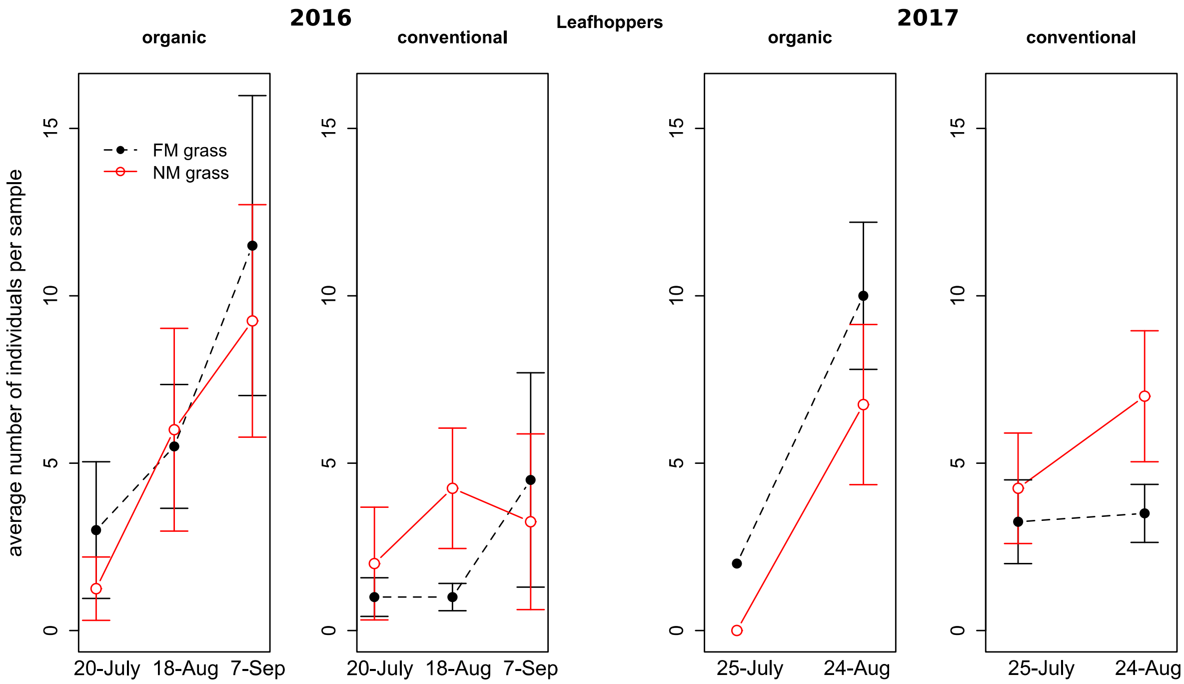

Figure 3.10: Abundance (mean ± std. err.) of leafhoppers (Hemiptera: Cicadellidae) observed on sweep net samples during the first experiment in 2016 and 2017.

Nymphs of *S. titanus* were collected only in 2016. Their abundance was significantly affected by grass mowing, time, grass mowing*time, grass mowing*vineyard management, vineyard management*time, and grass mowing*vineyard management*time (Table 3.4). *Scaphoideus titanus* was recorded only in NM plot in organic vineyards and its abundance increased during the sampling period (Fig. 3.11).

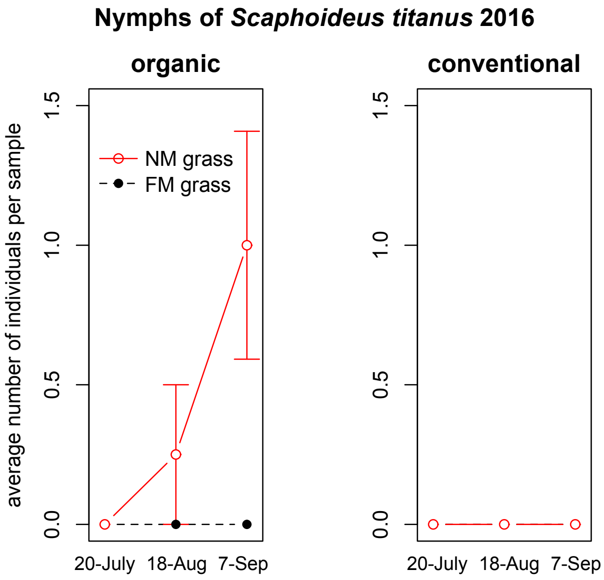

Figure 3.11: Abundance (mean ± std. err.) of *Scaphoideus titanus* (Hemiptera: Cicadellidae) observed on sweep net samples during the first experiment in 2016.

The presence of nabids did not result significantly affected by investigated factors in this study (i.e. grass mowing and vineyard management), but only by the time in 2016 (Table 3.4).

The presence of reduvids was influenced by grass mowing, and in 2016 also by the interaction grass mowing*vineyard management (Table.3.4; Fig. 3.12). A higher number of assassin bugs was observed in NM plots compared to FM plots, but in 2016 this effect emerged only in conventional vineyards (Table 3.4; fig.3.12).

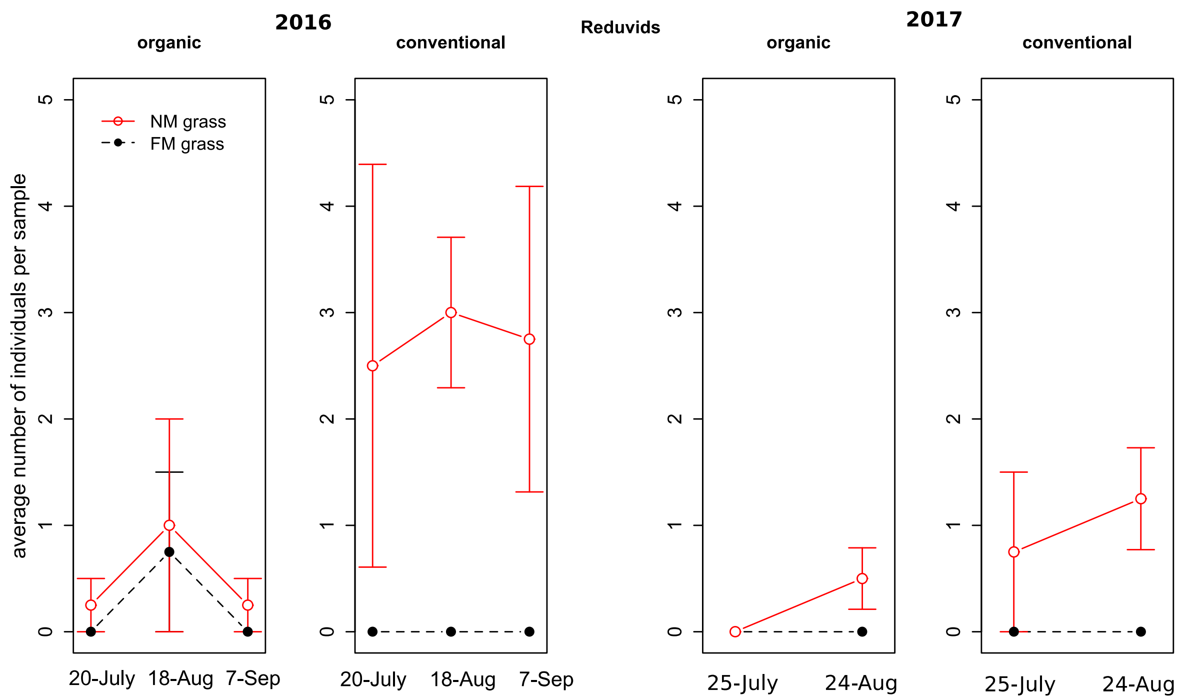

Figure 3.12: Abundance (mean ± std. err.) of reduvids (Hemiptera: Reduviidae) observed on sweep net samples during the first experiment in 2016 and 2017.

The presence of lacewing larvae did not result significantly affected by the investigated factors in this study, but only by the time in 2016 (Table 3.4).

In 2016 the abundance of parasitoid wasps was influenced by the interaction grass mowing*time (Table 3.4): their presence increased only in NM plots (Fig. 3.13). In 2017 the abundance of parasitoid wasps was influenced by grass mowing being higher in NM plots than FM plots (Table 3.4; Fig. 3.13).

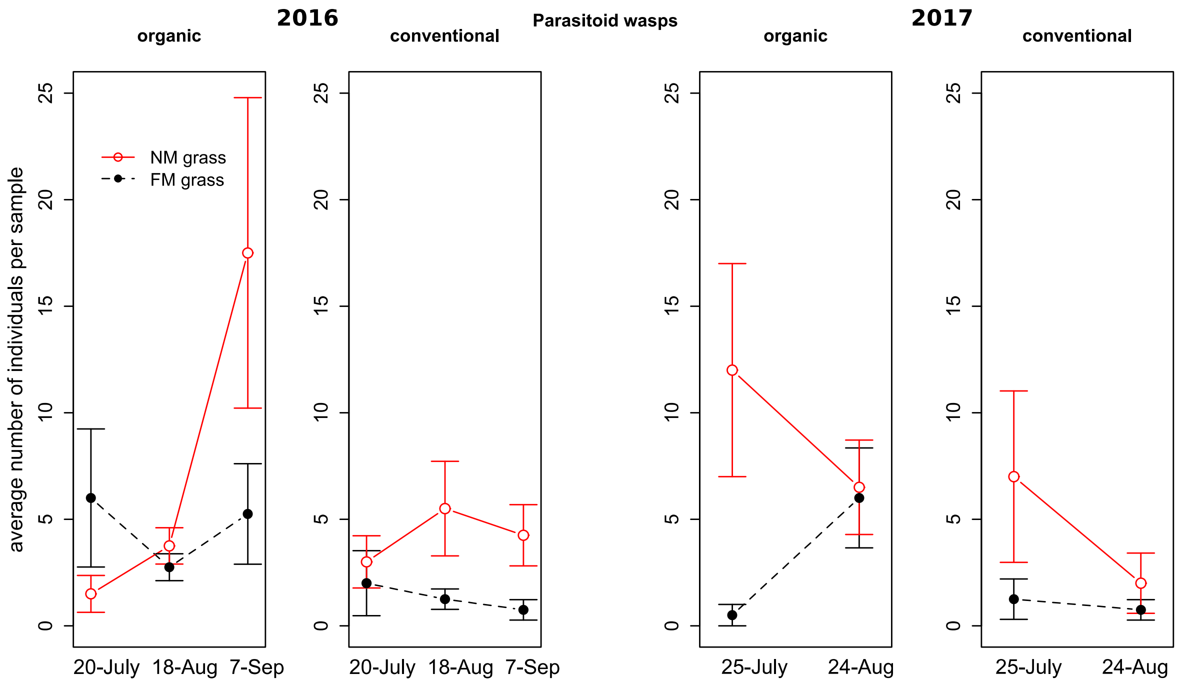

Figure 3.13: Abundance (mean ± std. err.) of parasitoid wasps (Hymenoptera) observed on sweep net samples during the first experiment in 2016 and 2017.

Spiders were more abundant in NM plots than in FM plots (Table 3.4; Fig. 3.14). Additionally, in 2016 their presence increased during the experiment in NM plots, while decreased in FM plots (Table 3.4; Fig. 3.14).

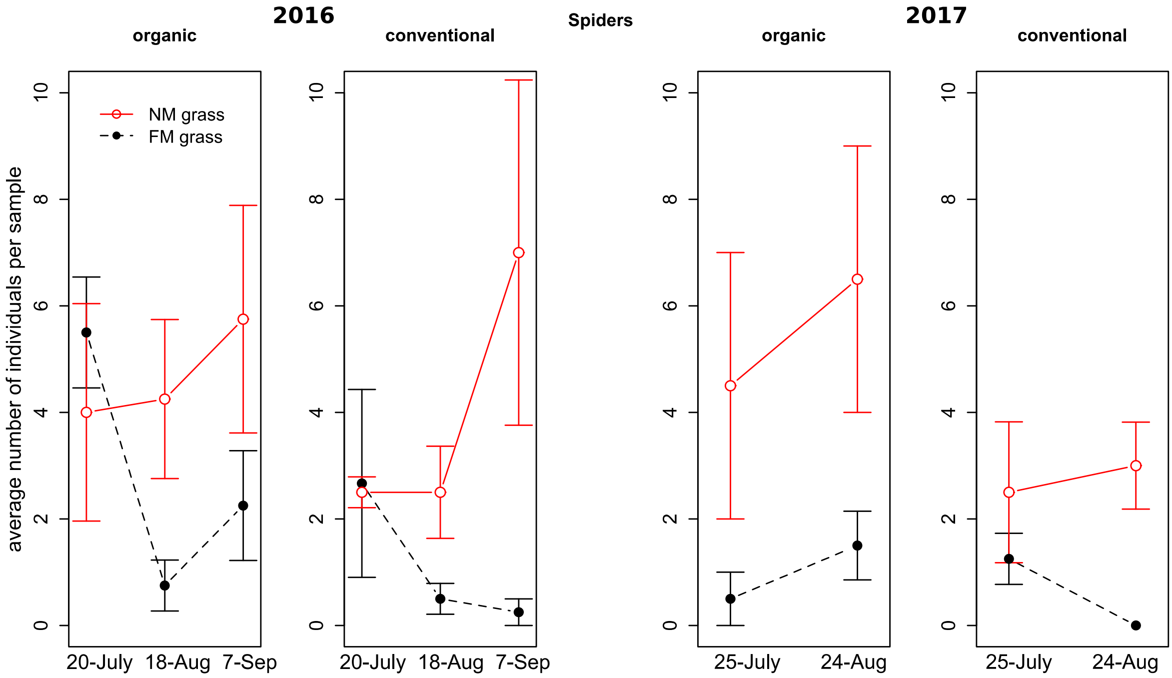

Figure 3.14: Abundance (mean ± std. err.) of spiders (Araneae) observed on sweep net samples during the first experiment in 2016 and 2017.

Table 3.4: Results of the mixed-effect model testing the effect of treatment, vineyard management and time on arthropods on sweep net sampling.

		2016					2017				
	Factor or interaction	**num DF**	**den DF**	**F value**	**Pr > F**		**num DF**	**den DF**	**F value**	**Pr > F**	
Leafhoppers not associated with grapevine (Hemiptera: Cicadellidae)	grass mowing	1	10	0.075	0.790		1	10	0.065	0.805	
	management	1	2	5.583	0.142		1	2	0.161	0.727	
	time	2	23	5.432	0.012	*	1	8	13.109	0.007	**
	grass mowing*management	1	10	1.151	0.309		1	10	5.776	0.037	*
	grass mowing*time	2	23	0.780	0.470		1	8	0.739	0.415	
	management*time	2	23	1.367	0.275		1	8	6.598	0.033	*
	grass mowing*management*time	2	23	0.386	0.684		1	8	0.092	0.770	
Scaphoideus titanus (Hemiptera: Cicadellidae)	grass mowing	1	10	7.321	0.022	*	not analyzed				
	management	1	2	7.647	0.110						
	time	2	23	3.918	0.034	*					
	grass mowing*management	1	10	6.963	0.025	*					
	grass mowing*time	2	23	3.564	0.045	*					
	management*time	2	23	3.752	0.039	*					
	grass mowing*management*time	2	23	3.555	0.045	*					
Nabids (Hemiptera: Nabidae)	grass mowing	1	10	0.072	0.794		1	10	0.312	0.589	
	management	1	2	13.722	0.066		1	2	0.188	0.707	
	time	2	23	3.459	0.049	*	1	8	3.488	0.099	
	grass mowing*management	1	10	0.012	0.916		1	10	0.024	0.880	
	grass mowing*time	2	23	2.916	0.074		1	8	0.286	0.608	
	management*time	2	23	1.893	0.173		1	8	2.681	0.140	
	grass mowing*management*time	2	23	0.753	0.482		1	8	0.038	0.851	
Reduvids (Hemiptera: Reduviidae)	grass mowing	1	10	17.275	0.002	**	1	10	8.398	0.016	*
	management	1	2	6.558	0.125		1	2	1.158	0.394	
	time	2	23	1.144	0.336		1	8	1.621	0.239	
	grass mowing*management	1	10	10.909	0.008	**	1	10	1.158	0.307	
	grass mowing*time	2	23	0.151	0.860		1	8	1.621	0.239	
	management*time	2	23	0.165	0.849		1	8	0.003	0.961	
	grass mowing*management*time	2	23	0.378	0.689		1	8	0.003	0.961	
Larvae of lacewings (Neuroptera: Chrysopidae)	grass mowing	1	10	0.730	0.413		1	10	2.093	0.179	
	management	1	2	0.004	0.957		1	2	0.770	0.473	
	time	2	23	14.774	0.0001	***	1	8	0.000	1.000	
	grass mowing*management	1	10	0.484	0.503		1	10	1.570	0.239	
	grass mowing*time	2	23	0.520	0.601		1	8	0.000	1.000	
	management*time	2	23	0.036	0.965		1	8	0.000	1.000	
	grass mowing*management*time	2	23	0.562	0.578		1	8	0.000	1.000	
Parasitoid wasps (Hymenoptera Terebrantia)	grass mowing	1	10	4.023	0.073		1	10	7.500	0.021	*
	management	1	2	4.205	0.177		1	2	7.322	0.114	
	time	2	23	2.190	0.135		1	8	0.428	0.531	
	grass mowing*management	1	10	2.367	0.155		1	10	0.010	0.923	
	grass mowing*time	2	23	3.421	0.050	*	1	8	5.169	0.053	.
	management*time	2	23	2.931	0.074		1	8	2.531	0.150	
	grass mowing*management*time	2	23	0.594	0.560		1	8	0.863	0.380	
Spiders (Araneae)	grass mowing	1	10	11.773	0.006	**	1	10	11.802	0.006	**
	time	2	23	1.917	0.170		1	2	2.189	0.277	
	management	1	2	1.525	0.342		1	8	0.004	0.950	
	grass mowing*time	2	23	4.860	0.017	*	1	10	0.152	0.705	
	grass mowing*management	1	10	1.425	0.260		1	8	0.805	0.396	
	time*management	2	23	0.086	0.918		1	8	0.776	0.404	
	grass mowing*time*management	2	23	0.883	0.427		1	8	1.660	0.234	

Prior to the analysis average number of individuals was log (n+1) transformed.

3.2 Influence of different timing of a green manure mowing

Leaf sampling

On leaf samples the presence of the phytophagous mite *Panonychus ulmi* (Koch) and of two species of predatory mites (*P. finitimus* and *T. pyri*) was observed. Predatory mites were analysed together as family group. The presence of *P. ulmi* resulted significantly affected by the interaction between treatment and time (Table 3.5). During the sampling period, the abundance of *P. ulmi* strongly increased in the control plots while remained at low levels in both the green manure plots (Fig. 3.15). The presence of predatory mites did not result significantly affected by the different groundcover management but only by the time (Table 3.5; Fig. 3.16).

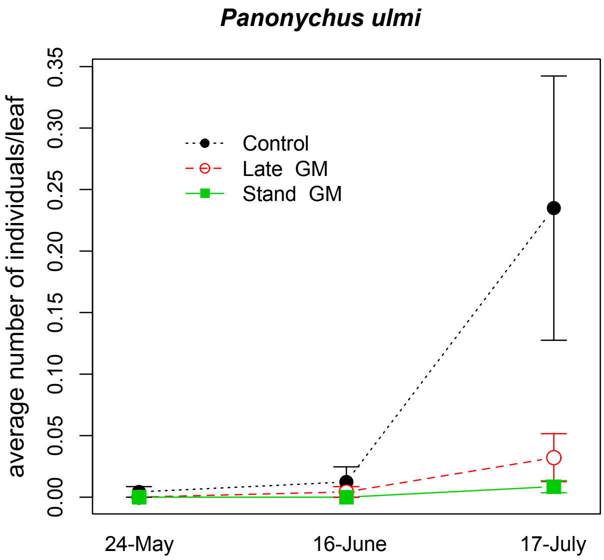

Figure 3.15: Abundance (mean ± std. err.) of *Panonychus ulmi* (Acari: Tetranichidae) observed on leaf samples during the second experiment in 2017.

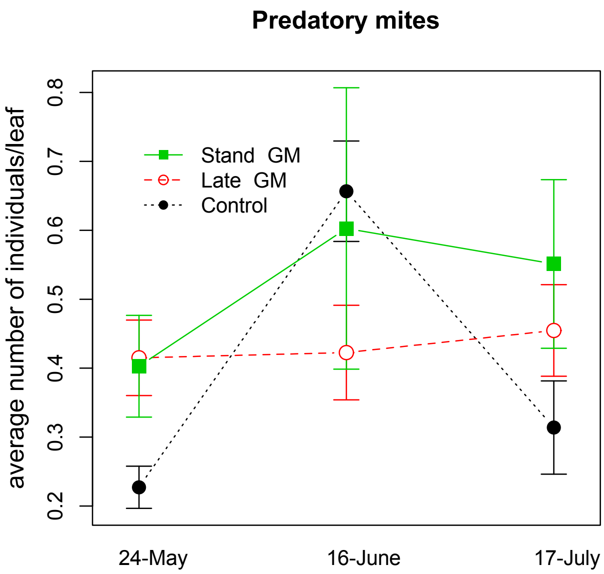

Figure 3.16: Abundance (mean ± std. err.) of predatory mites (Acari: Phytoseiidae) observed on leaf samples during the second experiment in 2017.

Regarding the leafhoppers, the abundance of *E. vitis* and *Z. rhamni* on leaves was significantly affected only by the time, and not by treatment (Table 3.5).

Finally, no effects emerged on the abundance of lacewing eggs, while *P. corni* densities were affected only the time and not by investigated factors (Table 3.5).

Table 3.5: Results of the mixed-effect model testing the effect of treatment and time on arthropods on leaf sampling.

	Factor or interaction	**num DF**	**den DF**	**F value**	**P value**	
Predatory mites (Acari: Phytoseiidae)	treatment	2	9	1,222	0,339	
	time	2	18	3,602	0,048	*
	treatment*time	4	18	1,517	0,239	
Panonychus ulmi (Acari: Tetranychidae)	treatment	2	9	3,594	0,071	
	time	2	18	6,505	0,008	**
	treatment*time	4	18	4,010	0,017	*
Empoasca vitis (Hemiptera: Cicadellidae)	treatment	2	9	0,951	0,422	
	time	2	18	49,937	<0.0001	***
	treatment*time	4	18	0,908	0,480	
Zygina rhamni (Hemiptera: Cicadellidae)	treatment	2	9	0,049	0,952	
	time	2	18	6,203	0,009	**
	treatment*time	4	18	0,885	0,493	
Eggs of lacewing (Neuroptera: Chysopidae)	treatment	2	9	1,619	0,251	
	time	2	18	0,670	0,524	
	treatment*time	4	18	0,569	0,689	
Parthenolecanium corni (Hemiptera: Coccidae)	treatment	2	9	0,545	0,598	
	time	2	18	28,253	<0.0001	***
	treatment*time	4	18	0,915	0,476	

Prior the analysis average number of individuals was log (n+1) transformed.

Beating net sampling

Using the beating net technique various arthropods resident on grapevine canopy were collected. In particular, the presence of leafhoppers, stink bugs, red velvet mites, and spiders were recorded. The technique also permitted to collect other beneficial arthropods such as larvae of lacewing, larvae and adults of ladybirds; however, they were not considered in the analysis due to their low abundance.

The presence of leafhoppers and stink bugs did not result influenced by the investigated factors and their interactions (Table 3.6).

Among beneficial arthropods, the presence of red velvet mites resulted significantly affected only by the time showing, although non-significant, increasing numbers of individuals in Late-GM plots (Table 3.6; Fig. 3.17).

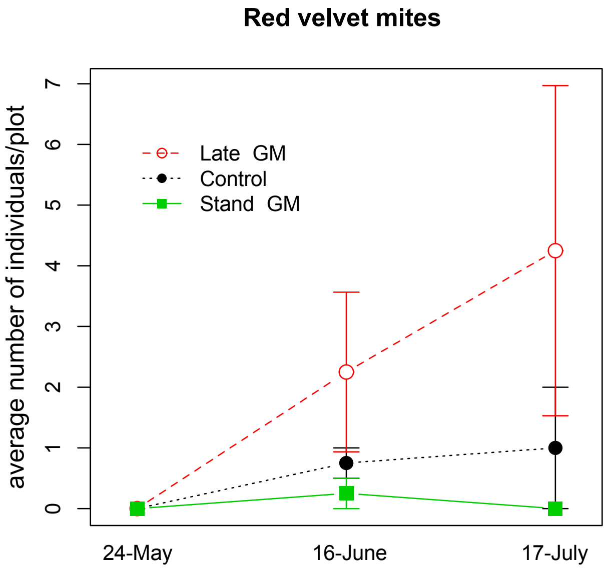

Figure 3.17: Abundance (mean ± std. err.) of red velvet mite (Acari: Trombididae) observed on beating net samples during the second experiment in 2017.

Differently, the presence of adults of ladybirds did not result influenced by the investigated factors and their interactions (Table 3.6).

The management of inter-row groundcover influenced the presence of spiders during the time (Table 3.6); in the last sampling date they were more abundant in Late-GM and in Control plots than in Stand-GM plots (P = 0.026 and P = 0.039 respectively; Fig. 3.18).

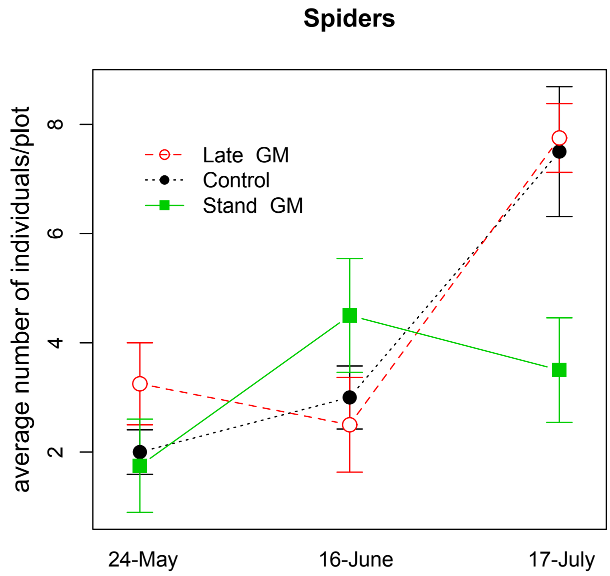

Figure 3.18: Abundance (mean ± std. err.) of spiders (Araneae) observed on beating net samples during the second experiment in 2017.

Table 3.6: Results of the mixed-effect model testing the effect of treatment and time on arthropods on beating net sampling.

	Factor or interaction	num DF	den DF	F value	P value	
Leafhoppers not associated with grapevine (Hemiptera: Cicadellidae)	treatment	2	9	1,789	0,222	
	time	2	18	1,789	0,196	
	treatment*time	4	18	0,963	0,452	
Stink bugs (Hemiptera: Pentatomidae)	treatment	2	9	1,356	0,306	
	time	2	18	1,656	0,219	
	treatment*time	4	18	0,436	0,781	
Red velvet mites (Acari: Trombididae)	treatment	2	9	2,509	0,136	
	time	2	18	5,856	0,011	*
	treatment*time	4	18	1,971	0,142	
Coccinellids (Coleoptera: Coccinellidae)	treatment	2	9	1,000	0,405	
	time	2	18	1,000	0,387	
	treatment*time	4	18	1,000	0,433	
Spiders (Araneae)	treatment	2	9	0,694	0,525	
	time	2	18	12,828	0,0003	***
	treatment*time	4	18	3,436	0,030	*

Prior the analysis average number of individuals was log (n+1) transformed.

Sweep net sampling

The use of the sweep net allowed to collect arthropods present on vineyard inter-row groundcover. During the investigation, the presence of leafhoppers, parasitic wasps, nabids, and spiders were recorded. The technique also permitted to collect some individuals of beneficial arthropods, such as larvae of lacewings, red velvet mites, larvae of coccinellids, larvae and adults of hoverflies, and predatory thrips; however, they were not considered in the analysis due to their low abundance.

The presence of leafhoppers on inter-row vegetation did not result significantly affected by the different strategies of groundcover management, but only by the time (Table 3.7; Fig. 3.19).

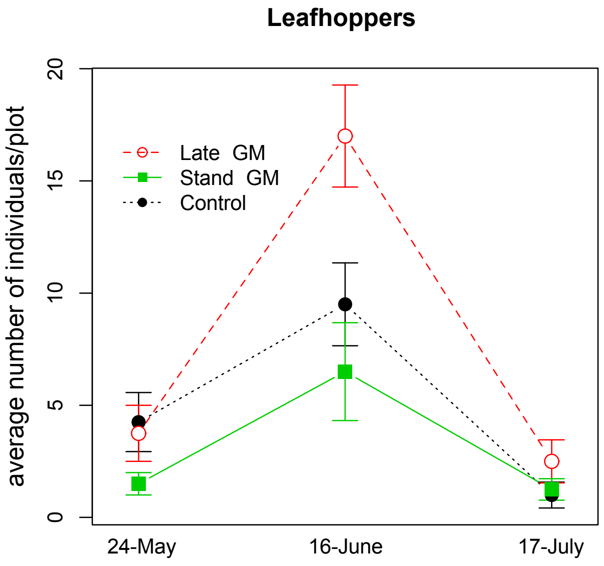

Figure 3.19: Abundance (mean ± std. err.) of leafhoppers (Hemiptera: Cicadellidae) observed on sweep net samples during the second experiment in 2017.

The presence of parasitoid wasps was significantly affected by the strategy of groundcover management (Table 3.7; Fig. 3.24). A higher number of parasitoid wasps was observed in Late-GM plots than in Stand-GM plots (P = 0.013) and in control plots (P = 0.002). Moreover, their presence was affected by the time (Table 3.7), showing a decrease after the withering of the vegetation in the Late-GM plots (Fig. 3.20).

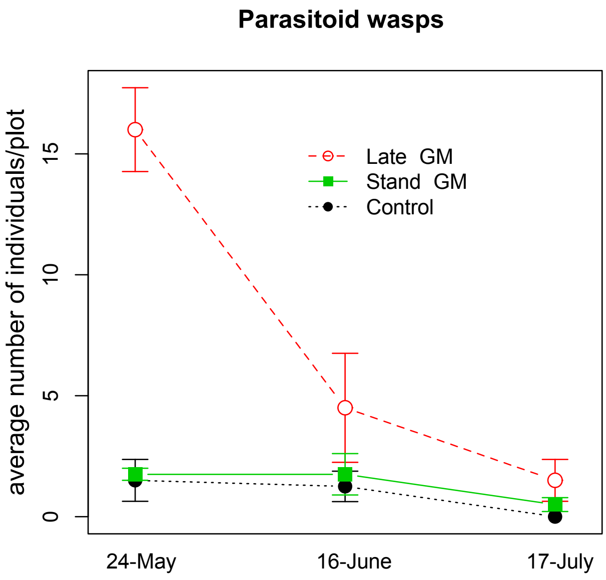

Figure 3.20: Abundance (mean ± std. err.) of parasitoid wasps (Hymenoptera) observed on sweep net samples during the second experiment in 2017.

Differently, no effect of experimental factors was observed on the presence of adults of coccinellids and nabids (Table 3.7).

The presence of spiders was differently affected by the treatment during the sampling period (Table 3.7). They were more abundant in Late-GM plots than in Stand-GM ($P < 0.001$) and Control plots ($P < 0.001$), showing a decrease after the withering of the standing vegetation in the Late-GM plots (Fig. 3.21).

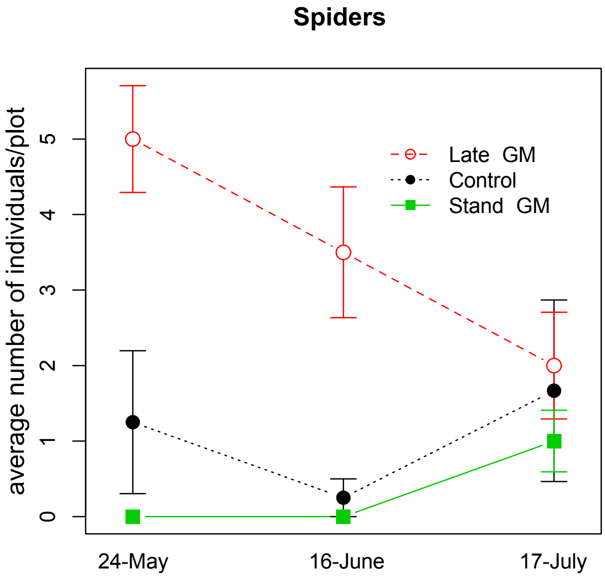

Figure 3.21: Abundance (mean ± std. err.) of spiders (Araneae) observed on sweep net samples during the second experiment in 2017.

Table 3.7: Results of the mixed-effect model testing the effect of treatment, vineyard management practices, and time on arthropods on sweep net sampling.

	Factor or interaction	**num DF**	**den DF**	**F value**	**P value**	
Leafhoppers not associated with grapevine (Hemiptera: Cicadellidae)	treatment	2	9	3,839	0,062	
	time	2	17	32,199	<0.0001	***
	treatment*time	4	17	1,126	0,377	
Parasitoid wasps (Hymenoptera)	treatment	2	9	14,011	0,002	**
	time	2	17	12,532	0,001	***
	treatment*time	4	17	2,626	0,071	
Coccinellids (Coleoptera: Coccinellidae)	treatment	2	9	2,583	0,130	
	time	2	17	2,663	0,099	
	treatment*time	4	17	2,599	0,073	
Nabids (Hemiptera: Nabidae)	treatment	2	9	0,424	0,667	
	time	2	17	1,537	0,243	
	treatment*time	4	17	1,878	0,161	
Spiders (Araneae)	treatment	2	9	12,828	0,002	**
	time	2	17	1,697	0,213	
	treatment*time	4	17	4,139	0,016	*

Prior the analysis average number of individuals was log (n+1) transformed.

3.3 Influence of different green manure mixtures

The presence of *P. ulmi* and of *P. finitimus* was recorded on leaves. Their abundance did not result significantly affected by the presence of green manure (Tab. 3.8).

The presence of *E. vitis*, *Z. rhamni* and *E. vulnerata* was observed in leaf samples. The abundance of these arthropods did not differ among treatments, while the effect of time emerged in the analysis (Tab. 3.8).

During the observation of leaf samples some individuals of predatory thrips, eggs of lacewings, and larvae of ladybirds were observed but they were not considered in the analysis due to their low abundance.

Table 3.8: Results of the mixed-effect model testing the effect of treatment and time on arthropods on leaves samples.

	Factor or interaction	num DF	den DF	F value	P value
Panonychus ulmi (Acari: Tetranychidae)	treatment	3	11	0,809	0,515
	time	2	21	0,126	0,883
	treatment*time	6	21	0,414	0,861
Phytoseius finitimus (Acari: Phytoseiidae)	treatment	3	11	0,536	0,667
	time	2	21	6,232	0,008 **
	treatment*time	6	21	1,494	0,228
Empoasca vitis (Hemiptera: Cicadellidae)	treatment	3	11	0,413	0,747
	time	2	21	11,697	0,0004 ***
	treatment*time	6	21	1,015	0,442
Zygina rhamni (Hemiptera: Cicadellidae)	treatment	3	12	0,497	0,691
	time	2	23	9,558	0,001 ***
	treatment*time	6	23	0,930	0,492
Erasmoneura vulnerata (Hemiptera: Cicadellidae)	treatment	3	11	1,238	0,343
	time	2	21	3,157	0,063
	treatment*time	6	21	0,805	0,578

Prior the analysis average number of individuals was log (n+1) transformed.

4. Discussion

In the first trial the presence of non-mowed vegetation in the vineyard inter-rows influenced the abundance of arthropods on grapevine canopy and/or inside the vineyard agro-ecosystem. On grapevine leaves, the higher number of predatory mites observed in plots with non-mowed vegetation is likely related to the higher amount of pollen provided by flowering plants. Pollen has been shown to serve as alternative food for phytoseiid mites (McMurtry and Croft 1997). Moreover, in the first experiment the higher presence of natural enemies, such as parasitoid wasps, spiders and assassin bugs on non-mowed inter-row vegetation was

related to the presence of standing vegetation, which provides food sources (such as pollen and nectar), refuge zone and alternative prey (e.g., aphids, data not shown) (Altieri and Whitcomb 1980; Landis et al. 2000). A higher presence of spiders was also recorded on canopy of vines close to non-mowed inter-rows; standing vegetation also favoured the presence of spiders by providing habitat and food sources (insects and mites on the vegetation are potential prey), as observed in Californian vineyards (Nicholls et al. 2000; Daane et al. 2018). In fact, weeds are important components of the vineyard agro-ecosystems since they support alternative prey/hosts, pollen or nectar as well as microhabitats that are unavailable in weeded monocultures (Altieri and Whitcomb 1980; Landis et al. 2000). The higher plant biodiversity, which provides several fundamental resources for beneficial arthropods, can contribute to create an appropriate ecological infrastructure within the vineyards resulting in a more pest-stable agro-ecosystems (Altieri and Nicholls 2004; Begum et al. 2004; Gurr et al. 2004; Altieri et al. 2005). Nevertheless, the presence of non-mowed vegetation inside the vineyard can also favour the occurrence of some pests, as reported for *S. titanus* by Trivellone et al. (2013). In the present study, relatively high densities of *Z. rhamni* and *S. titanus* were observed in organic vineyards, in particular in plots with standing vegetation. Regarding *Z. rhamni*, recorded on grapevine leaves, this result is probably influenced by the use of botanical insecticides (e.g. pyrethrins), less effective in controlling leafhoppers populations compared to conventional insecticides (Mori et al. 2014; Tacoli et al. 2017). *S. titanus* was recorded on the NM inter-row vegetation which may offer better micro-climatic conditions, food and refuges. In particular, for *S. titanus*, considering its important role in the spread of the Flavescence Dorée, the presence of standing vegetation could make harder the control of its populations in organic vineyards.
Regarding the parasitism rate of leafhoppers eggs, no differences were observed in the present study, as observed by Nicholls et al. (2000). This could be explained by the different type of plants present on the inter-row. Nicholls et al. (2000) sowed a 30:70 mixture of sunflower (*Helianthus annus* L.) and buckwheat (*Fagopyrum esculentum* Moench), while in this case study no specific plants were used.
A significant effect of vineyard management practices on the abundance of some arthropods (earwigs, larvae of lacewings, and spiders) was observed. Their presence was more abundant in organic than in conventional vineyards or was detected only in the first ones. These results partially agree with those obtained by Caprio et al. (2015) where organic crop systems sustained a higher diversity of carabids and spiders. The meta-analysis conducted by Bengtsson et al. (2005) showed that organic farming often has positive effects on species richness and abundance, and they found that the effects differ between organism groups and landscapes.

The results of the second experiment showed that different timing of mowing of the green manure sown in the vineyard inter-rows can influence the abundance of arthropods on grapevine canopy and/or inside the vineyard agro-ecosystem. The presence of predatory mites on leaves was not significantly affected by green manure but in contrast with spider mite population, more abundant in the control plots. Similar results were obtained by Flaherty (1969), that observed a relatively lower population densities of the phytophagous Pacific mite, *Eotetranychus willamettei* Ewing, on vines where weeds were allowed to grown than on vines in weeded plots. In fact, in plots with spontaneous vegetation the presence of a managed groundcover of *Sorghum halepense* (Poaceae) supported populations of the predatory mite *Metaseiulus occidentalis* (Nesbitt), maintaining the Pacific mite below the economic damage threshold. The grass supported an alternative host for the predatory mite, who colonized adjacent vines sufficiently early to suppress the pest-mite populations. In this experiment, the presence of green manure also favoured the abundance of some beneficial arthropods, such as spiders and parasitoids wasps. They resulted more abundant on green manure strips and, for spiders also on vine canopy adjacent to plots where green manure was late mowed. The presence of the standing vegetation provides habitat and food sources for natural enemies, as observed in the previous trial on the groundcover management and in other studies (Nicholls et al. 2000; Daane et al. 2018). Regarding phytophagous insects, the presence of leafhoppers and stink bugs did not result significantly affected by the different timing of green manure mowing. Therefore results suggest that green manure strips do not promote an increase in pest abundance.

The non-significant results obtained in the third experiment, on the effects of different green manure mixtures on arthropods abundance on leaves, could probably related to the low distance between sampling plots (only 3 m), which cannot impede the aerial dispersal of both pollen of flowering plants (Raynor et al. 1974) and of predatory mites (Tixier et al. 1998). Moreover, also grapevine leafhoppers can actively move among the vines (Decante and Van Helden 2008).

Concluding, alternating mowing in vineyard inter-rows could be a useful practice to increase natural enemies (Altieri and Nicholls 2004; Gurr et al. 2004), but in the investigated area its adoption should be carefully evaluated in organic vineyards where *S. titanus* occurs. Further studies should investigate if the timing of mowing can favour the dispersal of beneficial arthropods from the inter-row vegetation to the grapevine canopy. It has been shown that the mowing of the herbaceous vegetation can force the migration of parasitoids and predators from margins inside the cultivated fields (Perrin 1975) or from cover crops to vine canopy (Nicholls et al. 2000). The timing of mowing must be accurately planned according to the life cycle of natural enemies in order to optimize this practice (Van den Bosch and Telford 1964). However, it should be mentioned that the frequent grass mowing can promote the dispersal of *Hyalesthes obsoletus* Signoret, a vector of phytoplasma associated with Bois noir disease on the grapevine (Mori et al. 2015).
Allowing the green manure to flowering for a prolonged period instead of mowing it early, as traditionally done by the growers, can favour a higher presence and abundance of beneficial arthropods as observed for spiders and parasitoid wasps. It is worth mention that this field experiment was carried out in a very simplified context, where the presence of semi-natural habitats in surrounding area was very low (less than 4% in a radius of 500 m). This implies the low availability of natural enemies inside the vineyard agro-ecosystem observed during the experiment. The presence of green manure can help to increase the plant biodiversity inside the vineyard, and therefore contributed to provide fundamental resources for beneficial arthropods. However, the only presence of temporary vegetation appears not enough to create a more pest-

stable agro-ecosystem; it should be integrated with an appropriated ecological infrastructure surrounding the vineyards (Altieri and Nicholls 2004; Begum et al. 2004; Gurr et al. 2004; Altieri et al. 2005).

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Chapter 4

Outbreaks of the Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) in European vineyards: phenology and colonization patterns

Manuscript in preparation as: Outbreaks of the Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) in European vineyards: phenology and colonization patterns

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Giulia Zanettin contributed in the collection of data and their analysis, and to draft the manuscript.

Abstract

The Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) was detected for the first time in Europe (North-eastern Italy) in 2004. Since then it was recorded in Northern Italy and Slovenia being considered a minor pest of grapevines. In 2016, the first outbreaks of *E. vulnerata* were reported in a number of conventional and organic vineyards located in North-eastern Italy. High population densities and severe leaf symptoms were observed in summer despite the application of pesticides. Investigations were carried out in 2017 and 2018 in a number of farms located in the Veneto region to shed light on the phenology of *E. vulnerata* and these observations strongly suggest that the pest can develop three generations per years. The presence of rural buildings and alternative hosts at vineyard margins favoured *E. vulnerata* overwintering and its spreading into the vineyards. The impact of natural enemies on pest populations appeared to be limited to egg parasitism by Hymenoptera Mymaridae.

1. Introduction

The leafhopper *Erasmoneura vulnerata* (Fitch) (Hemiptera: Cicadellidae) originates from Northern and Central America and it has been considered for longtime as belonging to the genus *Erythroneura* (McAtee 1920; Johnson 1935; Metcalf 1986). In 2006, Dietrich e Dmitriev placed this species in the genus *Erasmoneura* that comprises 11 taxa included *Erasmoneura variabilis* (Beamer), a key pest in Southern California (Wilson et al. 1992; Daane et al. 1996; Dmitriev and Dietrich 2007; Costello 2008; Dmitriev 2017). In the original areas *E. vulnerata* was collected on American and European grapevine species, frequently on *Parthenocissus quinquefolia* (L.) Planch., *Ilex decidua* Walter and *Cercis canadensis* L. (Dmitriev 2017). In some papers *E. vulnerata* has been reported as a serious pest of grapevines in the USA (Robinson 1926; Beamer 1946), but more recent publications highlighted that *E. vulnerata* is rarely dominant in leafhopper communities occurring in American vineyards (e.g., Martinson and Dennehy 1995; Paxton and Thorvilson 1996; Zimmerman et al. 1996).

Erasmoneura vulnerata was recorded for the first time in Europe in 2004 (Castelfranco Veneto, North-eastern Italy) (Duso et al. 2005). Some traits of the *E. vulnerata* behaviour as well as feeding on grapevine leaves were described by Girolami et al. (2006). Field and semi-field studies carried out on pesticide free vines suggested that *E. vulnerata* could develop two to three generations per year (Duso et al. in preparation). Pesticide application was probably the most important factor influencing the population dynamics of *E. vulnerata* in the new invaded areas. In pesticide free plots *E. vulnerata* dominated over *Empoasca vitis* (Göethe) and *Zygina rhamni* Ferrari, while the opposite situation was reported in commercial vineyards where insecticides were commonly applied to control *Scaphoideus titanus* Ball and other pests (Girolami et al. 2000; Duso et al. in preparation).

Some years following the first record in Europe, *E. vulnerata* spread to other regions in Italy and Slovenia (Duso et al. 2008; Seljak 2011) without causing apparent damage in vineyards. In July of 2016 the first outbreaks of *E. vulnerata* were detected in the Veneto region (Treviso and Vicenza provinces). Symptoms caused by leafhoppers were spread on more than 90% of the canopy including apical leaves and population densities sometimes exceeded densities of 10 nymphs per leaf. Interestingly, infestations were detected both in conventional and organic vineyards. The former had been treated mainly with organophosphates, the latter with pyrethrins to control *S. titanus*. This new scenario suggested a reappraisal of investigations on *E. vulnerata* in order to identify factors affecting the pest outbreaks. The phenology of the leafhopper was investigated in 2017 and 2018 in conventional and organic farms comprising a number of *Vitis vinifera* L. varieties. The colonization pattern of adults was studied to shed light on the role of overwintering sites in the pest spread. Moreover, the impact of natural antagonists (in particular that exerted by egg-parasitoids belonging to the Hymenoptera Mymaridae) was evaluated.

2. Materials and methods

2.1. Study sites

In 2017 observations were carried out in two conventional (AC and LC) and two organic (AO and LO) farms located in two close localities in Vicenza province (North-eastern Italy): Lonigo (45°23'1.24''N, 11°23'2.00 E), and Alonte (45°22'0.48''N, 11°25'41.16''E). AC farm comprised three vineyards each made of a single variety (Cabernet Sauvignon, Pinot gris, and Glera). Three vineyards comprising different varieties (Garganega, Chardonnay, and Merlot) were selected in LC farm. AO farm comprised four vineyards (Merlot, Glera, Pinot gris, and Incrocio Manzoni 6013). Two vineyards comprising Merlot and Trebbiano varieties were selected in LO farm. Most of these vineyard owners decided to apply specific insecticides in 2018. Therefore, other farms were selected to study the pest phenology and adult colonization patterns in 2018. *E. vulnerata* phenology was studied in two organic vineyards located at Alonte (AO2, Vicenza province) and Monteforte d'Alpone (MO, Verona province). The first vineyard comprised Trebbiano variety while the second comprised Garganega variety.

2.2. Studies on the phenology of *E. vulnerata*

In 2017 the phenology of *E. vulnerata* was investigated in all farms but the most comprehensive results were obtained in AC and AO farms. In particular, observations were made on Cabernet Sauvignon variety in farm AC and on Merlot, Glera, Pinot gris, and Incrocio Manzoni 6.0.13 varieties in farm AO. Sampling was carried out on insecticide free plots of AC farm while in AO farm pyrethrins were applied in late July to control *S. titanus*. In Veneto region pesticide application is mandatory when *S. titanus* is detected in vineyards. In AC farm leaf sampling was performed every 7-10 days, from May to September. A total of 30 leaves was removed and analysed in the laboratory to assess the abundance of leafhopper species and stages under a Wild M3C stereomicroscope (10-40 x magnification). Motile stages of *E. vulnerata* (and of co-occurring leafhoppers) were subdivided in young nymphs (1^{st}-3^{rd} nymph instars) and aged nymphs (4^{th}-5^{th} nymph instars, adults). Data from leaf samplings were coupled with those on adults captured on four yellow sticky traps placed in the same plots. These sampling procedures were adopted in the remaining farms with some deviation. In particular, 60 leaves were collected from the four varieties of AO farm.

In 2018 the phenology of *E. vulnerata* was investigated in two organic vineyards. In farm AO2 observations were carried out on Trebbiano variety, in farm MO on Garganega variety. Both vineyards were not treated with insecticides. Leaf samplings were performed every 10-15 days, from May to September. A total of 30 leaves were removed and analysed in the laboratory to assess the abundance of leafhopper species and stages under the stereomicroscope. Yellow sticky traps were placed to track adult flights.

2.3. Spatial and temporal distribution of leafhoppers: effects of vineyard margins

Preliminary observations carried out in April before grapevine sprouting revealed the occurrence of many *E. vulnerata* adults on alternative hosts (e.g., *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch) associated to rural buildings, suggesting that leafhoppers overwintered in these sites. The presence of hedgerows contiguous to some vineyards was also considered a factor potentially affecting *E. vulnerata* overwintering (Zimmerman et al. 1996). These observations suggested a role of vineyard margins in the leafhoppers colonization. In 2017, in AC farm four transects, each comprising four yellow sticky traps, were arranged at increasing distance from rural buildings and hedgerows. Four positions were identified for trap placement: A (first row close to rural buildings or hedgerows), B, C, D (at 20, 40, 60 m from A position, respectively). Additional five transects were arranged in AO and LO farms for a total of nine transects. Data will focus on the most common leafhopper species (*E. vulnerata*, *E. vitis* and *Z. rhamni*). Samplings were carried out from May to June. Traps were renewed every 7-10 days and analysed in the laboratory under the stereomicroscope. In 2018, four vineyards different from those considered in 2017 were selected for this study, for a total of four transects. Samplings were carried out from May to July and the experimental design was the same adopted in 2017.

2.4. Natural control

The parasitism rate exerted by Hymenoptera Mymaridae was calculated by dividing the number of parasitoid emergence holes by the sum of the nymph hatching holes and the parasitoid emergence holes, expressed in percentage. A number of emerging cages (Zanolli and Pavan 2011) were used to isolate and identify parasitoid adults.

2.5. Statistical analysis

Data from the experiments aimed at investigating the effect of vineyard margins on the leafhopper colonization were analysed using repeated measures mixed model with the MIXED procedures of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC). Distance from the margin, time of sampling and their interaction were considered as sources of variability in the model and tested using a F test ($\alpha = 0.05$). Transect was considered as random effect term in the model. Pairwise comparison of captures placed at different distances were performed using Tukey's test ($\alpha = 0.05$) on the least-square means. Prior to 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 figures.

3. Results

3.1 The phenology of *Erasmoneura vulnerata*

3.1.1 Phenology in 2017

At the sprouting of 2017 (10th April) high numbers of *E. vulnerata* adults were caught on yellow sticky traps (778-2042 adults per trap in one week in AO and AC farms, respectively; 481-773 adults per trap in LO and LC farms, respectively). Catches of other leafhoppers, in particular *E. vitis* and *Z. rhamni* were much lower (data not shown). In the subsequent weeks *E. vulnerata* catches substantially declined. Frost occurring on 19th April damaged shoots in LC and LO vineyards with effects on canopy formation. The implications of this event on leafhopper phenology suggested to concentrate the observations on AC and AO vineyards. In AC vineyard data were collected on Cabernet Sauvignon insecticide free plots. Young nymphs were detected first in the second half of May and their densities peaked in late May. Aged nymph numbers peaked in early June. The abundance of young nymphs showed two additional peaks in early July and late August followed by those of aged nymphs. Adult catches showed two clear peaks following those of aged nymphs in early July and early August. Data strongly suggest the development of three generations. Densities of other leafhoppers in leaf samples were negligible (data not shown).

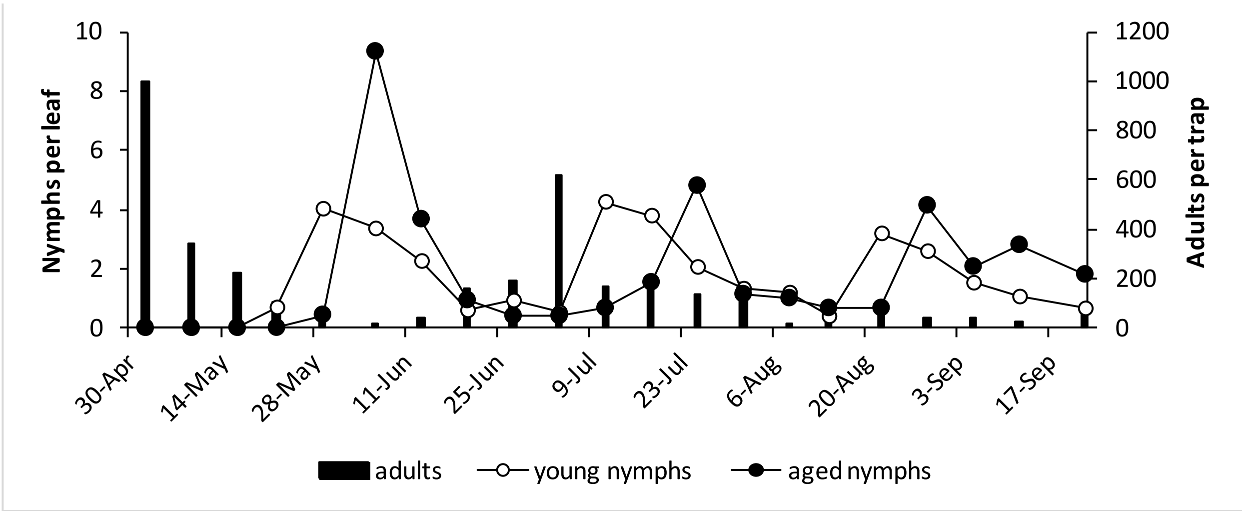

Figure 4.1: The phenology of *Erasmoneura vulnerata* in AC farm (Cabernet Sauvignon, Alonte, 2017).

In AO farm observations were carried out on four different varieties and the phenology of *E. vulnerata* reported in Figure 4.2 combined all these data. Young nymphs were detected from late May and their densities peaked in early June, followed by an increase in aged nymphs and adults. Therefore, the first generation developed in a similar way of AC farm. A new increase in young nymphs in the first half of July was compatible with the start of the second generation. However, in contrast with the situation reported in AC vineyard, aged nymphs reached negligible densities in July which pyrethrins were applied to control *S. titanus*. A slight increase in young nymphs detected in late August-early September could refer to the third generation. *Empoasca vitis* and *Z. rhamni* densities on leaves were negligible (data not shown).

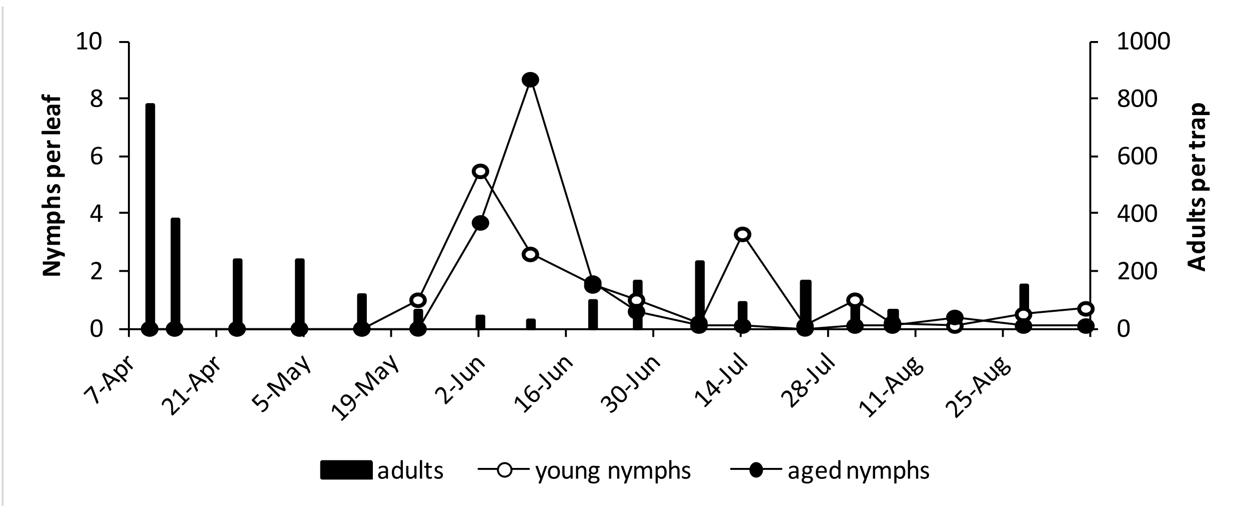

Figure 4.2. The phenology of *Erasmoneura vulnerata* in AO farm (mix of varieties, Alonte, 2017).

3.1.2 Phenology in 2018

In the spring of 2018 catches of *E. vulnerata* adults in vineyards declined from late April-early May to late May. Young nymphs of *E. vulnerata* were detected first in mid-May and their numbers peaked in late May-early June followed by peaks of aged nymphs (Figures 4.3, 4.4). The abundance of young nymphs showed two additional peaks in July and August, confirming the hypothesis of three generations per year. Adult catches gradually increased from July onwards. In most sampling dates catches of *E. vitis* and *Z. rhamni* were lower than those of *E. vulnerata*, and the incidence of this latter on the overall leafhopper catches gradually increased from spring to summer. Therefore, densities of *E. vitis* and *Z. rhamni* nymphs were low throughout the growing season (data not shown).

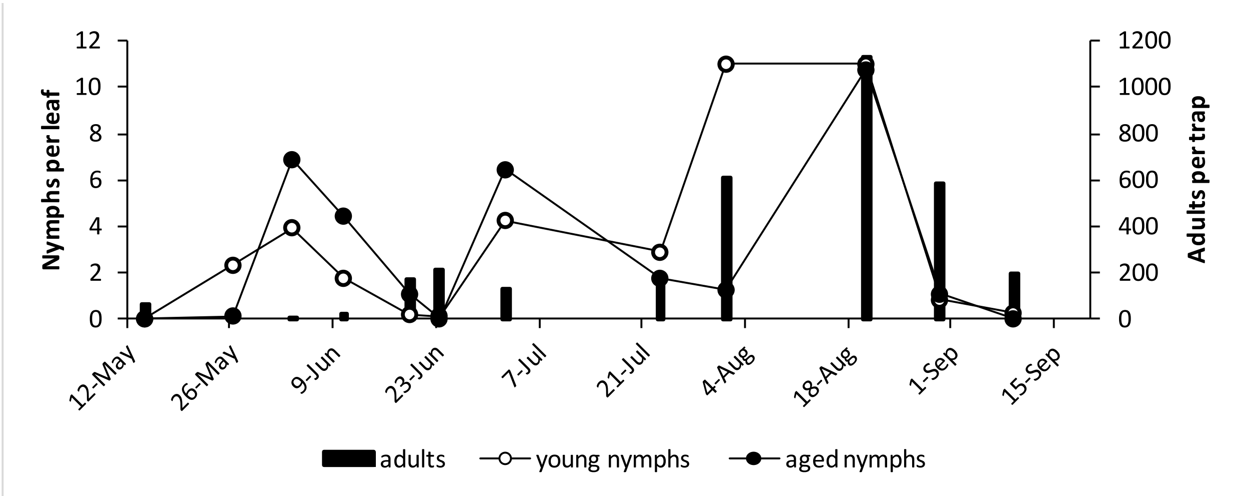

Figure 4.3: The phenology of *Erasmoneura vulnerata* in AO2 farm (Trebbiano, Alonte, 2018).

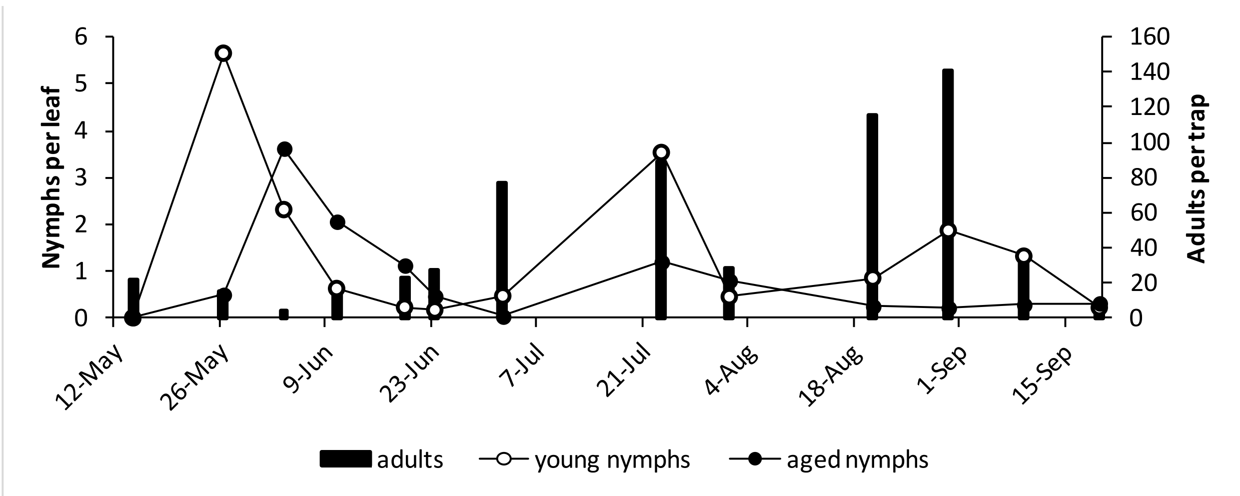

Figure 4.4: The phenology of *Erasmoneura vulnerata* in MO1 farm in 2018 (Garganega, Monteforte d'Alpone, 2018).

3.2 Spatial and temporal dynamics of adult leafhoppers: effects of vineyard margins

3.2.1 Spatial and temporal dynamics in 2017

In 2017 *E. vulnerata* was the most common leafhopper caught on yellow sticky traps (Figures 4.5, 4.6, 4.7). Catches of *E. vulnerata* adults were abundant in early spring when adults left overwintering sites and moved inside the vineyards, then declined ($F = 24.15$; d.f. = 7, 179; $P < 0.0001$; Figure 4.5). Differences among treatments (increasing distances from the margin) were significant ($F = 4.11$; d.f. = 3, 30.6; $P = 0.015$) and the most abundant catches were detected at the vineyard margin (0 m vs. 40 m: $t = 3.01$; d.f.= 30.6; $P = 0.025$; 0 m vs. 60 m: $t = 2.94$; d.f.= 30.7; $P = 0.029$). There were no differences between catches on traps located at 0 m and 20 m ($t = 1.41$; d.f. = 30.6; $P = 0.506$). The remaining comparisons were not significant (20 m vs. 40 m: $t = 1.6$; d.f.= 30.5; $P = 0.392$; 20 m vs. 60 m: $t = 1.54$; d.f.= 30.6; $P = 0.426$; 40 m vs. 60 m: $t = -0.06$; d.f.= 30.6; $P = 0.999$). *Empoasca vitis* and *Zygina rhamni* catches reached the highest levels in June (Figures 4.6 and 4.7). The effect of margins on adult catches was not significant ($F = 1.56$; d.f. = 3, 60; $P = 0.208$; $F = 0.55$; d.f. = 3, 53.2; $P = 0.653$ for *E. vitis* and *Z. rhamni*, respectively).

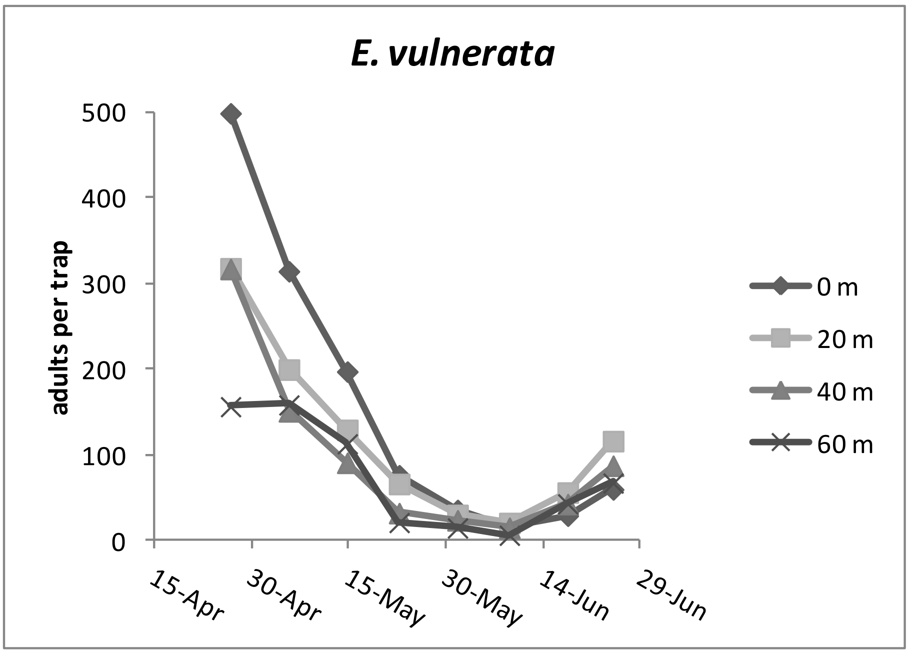

Figure 4.5: Spatial and temporal dynamics of *Erasmoneura vulnerata* at increasing distances from the vineyard margins in 2017.

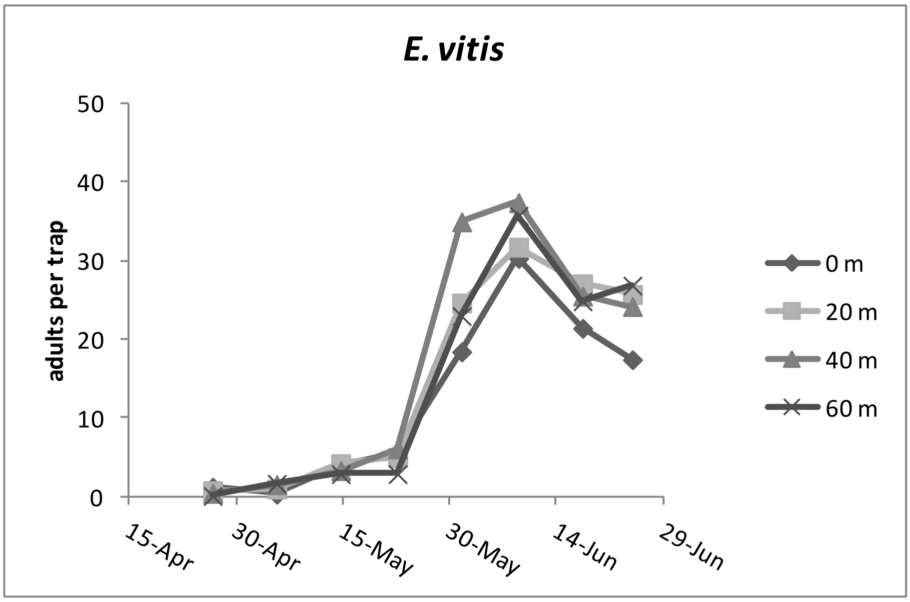

Figure 4.6: Spatial and temporal dynamics of *Empoasca vitis* at increasing distances from the vineyard margins in 2017.

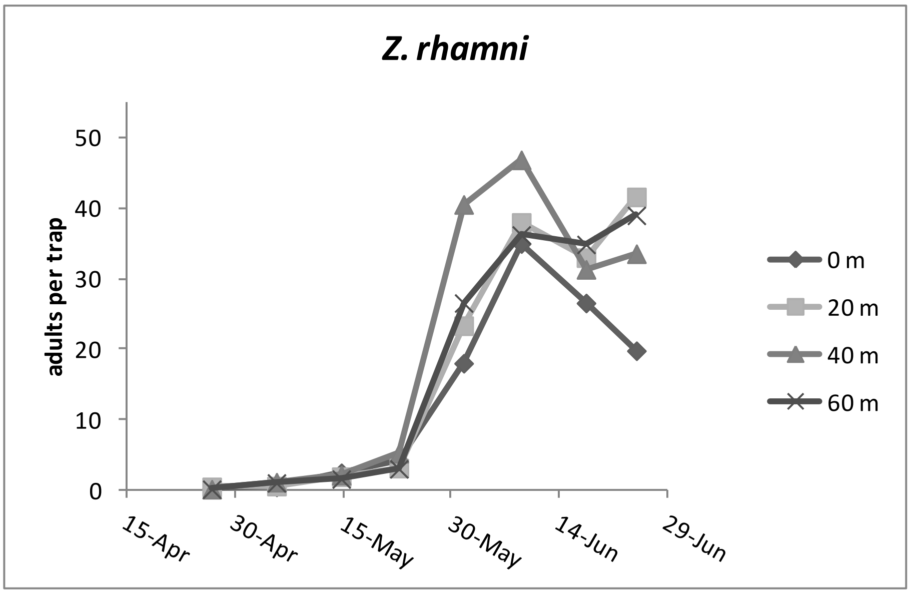

Figure 4.7: Spatial and temporal dynamics of *Zygina rhamni* adults at increasing distances from the vineyard margins in 2017.

3.2.1 Spatial and temporal dynamics in 2018

Catches of *E. vulnerata* adults were more abundant than those of *E. vitis* and *Z. rhamni* in early spring of 2018 (Figure 4.8, 4.9, 4.10). Catches numbers fluctuated over the sampling dates and thus the effect of time was significant ($F = 8.17$; d.f. = 6, 65.4; $P < 0.0001$). Differences among treatments (increasing distances from the margin) were also significant ($F = 5.62$; d.f. = 3, 13; $P = 0.011$) and the most abundant catches were detected at the vineyard margin (0 m vs. 40 m: $t = 3.38$; d.f.= 13; $P = 0.022$; 0 m vs. 60 m: $t = 3.65$; d.f.= 13;

P = 0.013). There were no differences between catches on traps located at 0 m and 20 m (t = 2.82; d.f. = 13; P = 0.061). The remaining comparisons were not significant (20 m vs. 40 m: t = 0.56; d.f.= 13; P = 0.942; 20 m vs. 60 m: t = 0.84; d.f.= 13.1; P = 0.835; 40 m vs. 60 m: t = 0.28; d.f.= 13.1; P = 0.992). *Empoasca vitis* and *Zygina rhamni* catches reached the highest levels in June (Figure 4.9 and 4.10) and the effect of margins on their adults was not significant (F = 0.94; d.f. = 3, 17.1; P = 0.445; F = 1.34; d.f. = 3, 18.2; P = 0.292 for *E. vitis* and *Z. rhamni*, respectively).

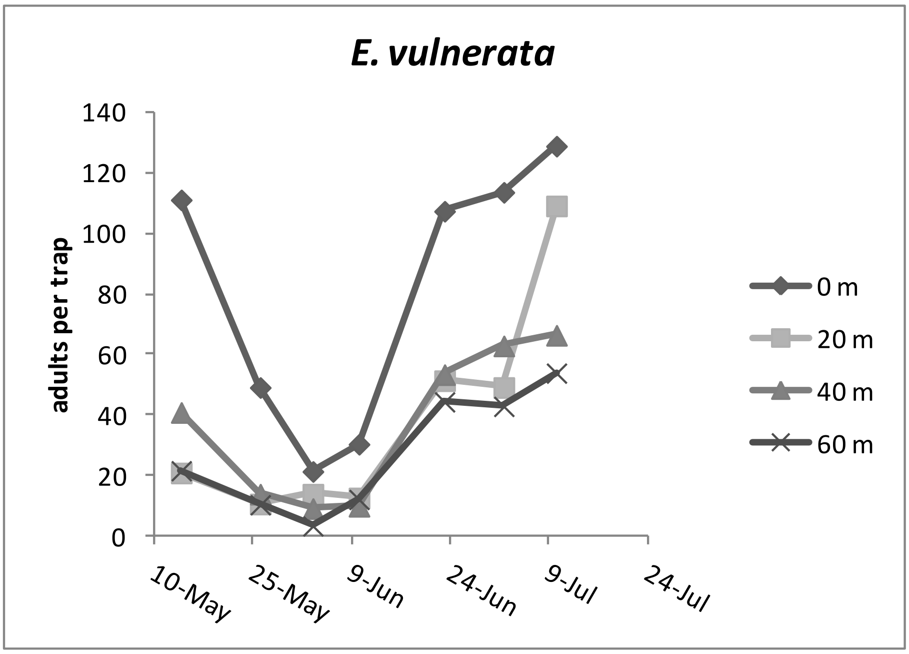

Figure 4.8: Spatial and temporal dynamics of *Erasmoneura vulnerata* at increasing distances from the vineyard margins in 2018.

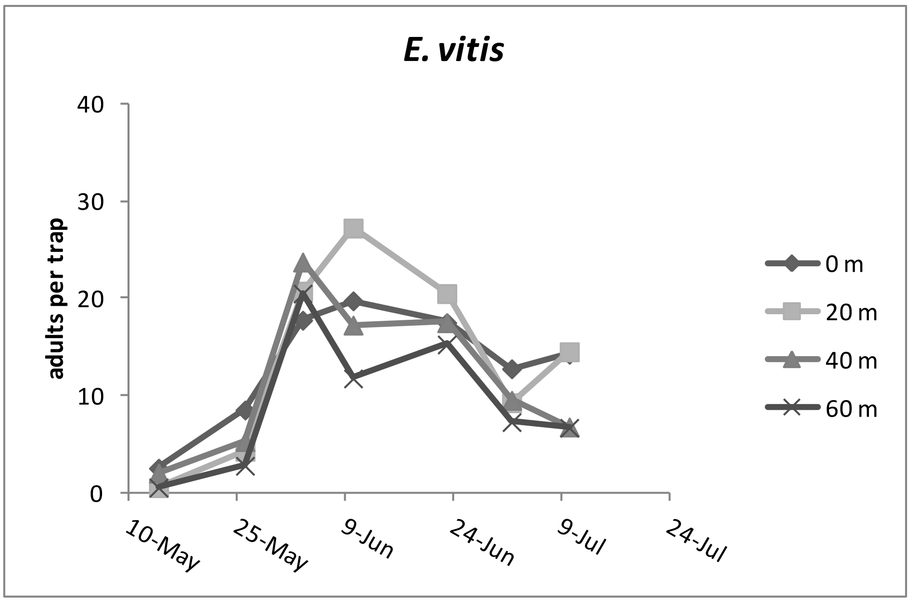

Figure 4.9: Spatial and temporal dynamics of *Empoasca vitis* at increasing distances from the vineyard margins in 2018.

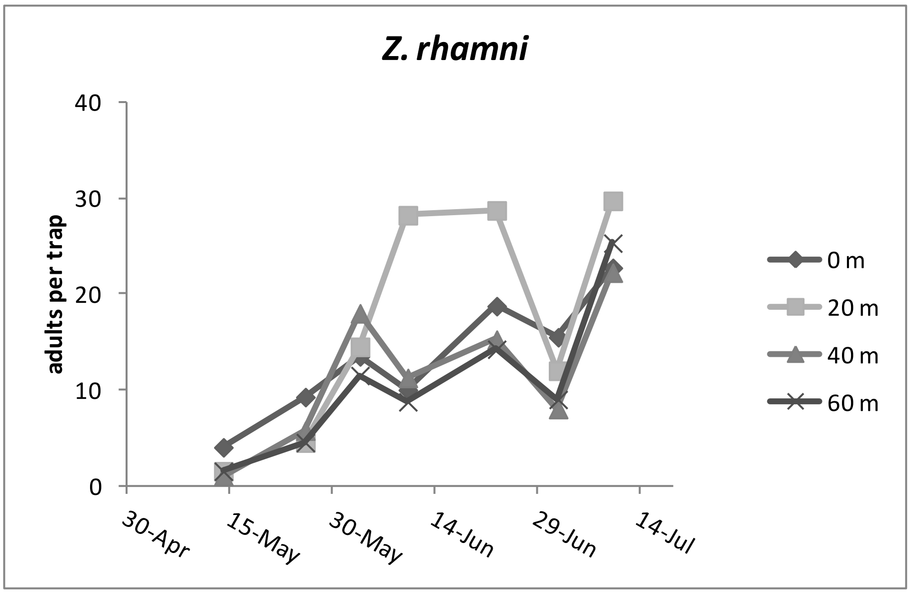

Figure 4.10: Spatial and temporal dynamics of *Zygina rhamni* adults at increasing distances from the vineyard margins in 2018.

3.3. Natural control

The activity of Hymenoptera Mymaridae was observed in all vineyards through the observation of emergence holes left by the parasitoids on leaf veins. Parasitism rates attained low to moderate levels. *Anagrus parvus* Soyka *sensu* (Viggiani 2014) was identified from the material obtained using emergence cages (L. Mazzon and I. Martinez-Sanudo, pers. comm.). This species is frequently associated with *Z. rhamni* in North-eastern Italy (Zanolli and Pavan 2011; Zanolli et al. 2016). Considering the first *E. vulnerata* generation mean, parasitism rates attained 35.6% in AC farm, 27% in AO farm, 48.4% in MO farm, and 28.6% in AO2 farm. In late summer mean parasitism rates increased: 38.8% in AC farm, 34.9% in AO farm, 53.1% in MO farm, and 34.8% in AO2 farm. Considering the limited amount of *E. vitis* and *Z. rhamni* nymphs in leaf samples, most of parasitism detected on them probably involved *E. vulnerata* eggs.

4. Discussion

A recent review (Olivier et al. 2012) considers *E. vulnerata* among the economic important leafhoppers in America and the contribution by Zimmerman et al. (1996) is reported to support this position. In the latter study, made in Colorado, *E. vulnerata* population was mixed with that of *Erythroneura ziczac* Walsh, being the latter more abundant. Leafhopper abundance (in particular *E. ziczac* densities) suggested to apply an insecticide to reduce possible damage and this application affected their population dynamics. On the base of observations made in two subsequent years authors suggested that *E. vulnerata* can complete two generations per year. In order to explain why *E. vulnerata* was less abundant than *E. ziczac* in these vineyards, Zimmerman et al. (1996) suggested three factors: the Colorado environmental conditions, the susceptibility of grape cultivars/hybrids and the behaviour of nymphs that colonize leaf upper surface being more exposed to natural enemies. Some years later, Triapitsyn et al. (2010) carried out a survey in the Colorado sites already investigated by Zimmerman et al. (1996) to shed light on leafhoppers egg parasitoids. Surprisingly *E. vulnerata* was the dominant species among leafhoppers and *E. ziczac* was not detected. Specific publications dealing with the economic impact of *E. vulnerata* in American vineyards are lacking and thus the pest status of this species in native areas is unclear. Outbreaks of *E. vulnerata* in Europe and the lack of detailed data on this species in North-America suggested to investigate factors affecting its potential. Observations on the phenology reported in this study provide new and comprehensive data to support the early hypothesis of the development of three generations per year (Duso et al. 2005). According to Zimmerman et al. (1996) two generations were developed in Colorado but in this case vineyards were treated with insecticides. Overwintering is a crucial phase for *E. vulnerata* vineyard colonization. Zimmerman et al. (1996) detected overwintering adults at the vineyard margins, within evergreen canopy, inside plant structures or under the litter. Results emphasize the importance of rural buildings or hedgerows for the vineyard colonization. A significant edge effect was found in the vineyard colonization by *E. vulnerata* in spring. In this phase vineyards are also colonized by *E. vitis* and *Z. rhamni* but the incidence of these species in the investigated vineyards was limited considering both adult catches on traps and nymph abundance on leaves. It should be mentioned that observations on traps started in late April when probably most of leafhopper adults were already migrated. Nevertheless interspecific competition is strongly suggested by these data collected in June-July that suggest to investigate this phenomenon in semi-field conditions.

Natural control of *E. vulnerata* and *E. ziczac* exerted by egg parasitoids in Colorado appeared to be weak (parasitism rate < 2%) (Zimmerman et al. 1996). A number of limiting factors (nectar availability, predation pressure, climatic conditions, pesticide use) can affect parasitism by Hymenoptera Mymaridae in American vineyards (Segoli 2016). However, parasitism rates calculated in the four vineyards located in North-eastern Italy lasted from 27% to 48.4% in first generation, and from 34.8% to 53.1% in late season. The proportion of *E. vulnerata* on the total leafhopper nymphs was very high in most sampling dates suggesting a role of *A. parvus* as the most prominent antagonist of *E. vulnerata* in the new invaded areas. Parasitism rates did not reach values required to keep *E. vulnerata* populations densities below acceptable levels, but this situation is commonly observed in North-America and Europe. The association of a native parasitoid with an exotic pest has been relatively recent and that this dynamic required to be investigated more in depth. On the other hand, the use of organophosphates in conventional vineyards and pyrethrins in organic vineyards proved to be partially ineffective in controlling *E. vulnerata* (Duso et al. 2017). Therefore, a new approach in pest control should be identified. In this framework the economic impact of *E. vulnerata* on grapevine yield and quality should be urgently investigated in order to define appropriate economic threshold levels and reduce pesticide use. At the same time strategies aimed at promoting the impact of egg parasitoids and predators require to be developed.

Acknowledgements

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Chapter 5

Biological control of the Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) using generalist predators

Manuscript in preparation as: Biological control of the Nearctic leafhopper *Erasmoneura vulnerata* (Fitch) using generalist predators

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Giulia Zanettin conducted laboratory trials, contributed to collect field data and their analysis and to draft the manuscript.

Abstract

Recently, outbreaks of *Erasmoneura vulnerata*, a Nearctic leafhopper recently introduced in Europe, have been reported in North-eastern Italy. Outbreaks occurred despite the use of insecticides suggesting to search non-chemical alternatives. Predation of *E. vulnerata* nymphs by two generalist predators common in Italian agro-ecosystems, i.e. *Chrysoperla carnea* and *Orius majusculus*, was tested in the laboratory. Two prey densities (5 and 10 *E. vulnerata* nymphs) were offered to predators, in particular to *C. carnea* larvae and *O. majusculus* adults. They consumed actively leafhopper nymphs suggesting to release them in vineyards. Three field trials were conducted in a highly infested vineyard in 2017 and 2018 where both predators were released in different plots in comparison with a no-release control. In one of these trials the release of *O. majusculus* reduced significantly leafhopper numbers.

1. Introduction

Erasmoneura vulnerata (Fitch) (Hemiptera: Cicadellidae) is a leafhopper native of Central and Northern America where colonizes grapevines and other hosts (Dmitriev 2017). Little is known on the importance of *E. vulnerata* in American vineyards where populations of this species are often mixed with those of other leafhoppers (Olivier et al. 2012). As an example Zimmerman et al. (1996) analysed the population dynamics of *E. vulnerata* in Colorado vineyards where populations were mixed with those of *Erythroneura ziczac* Walsh being the latter more abundant. Low parasitism rates by egg parasitoids were detected in these vineyards that were treated with insecticides. Authors suggested that *E. vulnerata* nymphs, that colonize frequently leaf upper surfaces, are more exposed to predators than *E. ziczac* nymphs and this phenomenon could affect the balance between the two species. However information on predators potentially involved was not provided. An analysis of the recent literature suggests that *E. vulnerata* plays a minor role within leafhopper communities infesting American vineyards despite its wide geographic distribution.

Erasmoneura vulnerata was intercepted in Italy in 2004, first record for Europe (Duso et al. 2005). Subsequently, this species was widely recorded in North-eastern Italy and Slovenia (Duso et al. 2008; Seljak 2011) but it was detected more in unsprayed than in commercial vineyards. Surprisingly, outbreaks of *E. vulnerata* were observed in July 2016 in a commercial vineyard located in Veneto region (Treviso province) where feeding symptoms were observed on more than 90% of the leaves. In late summer of 2016, additional infested vineyards were recorded in another area of the Veneto region (Vicenza province). Densities exceeding 10 motile forms per leaf were detected with potential implications for grapevine quality and growers disturbance at the harvest. Studies on the phenology and colonization patterns of *E. vulnerata* were carried out in a number of vineyards located in the Veneto region in 2017 and 2018. The impact of egg-parasitoids was moderate resulting ineffective to keep pest densities to acceptable levels (Chapter 4, this thesis). Growers decided to apply insecticides but some of them resulted somewhat ineffective (Duso et al. 2017). Insecticide use is associated with negative effects on the environment and human health, and their misuse can favour pest resistance and pest resurgence. According to recent rules devoted to the reduction of pesticide use (Directive 2009/128/EU) the identification of feasible alternatives to conventional pesticides is a priority in Europe. In this context, the inoculative release of predators could be a useful practice to control leafhopper populations. In Californian vineyards green lacewings (Neuroptera: Chrysopidae) were released to control *Erythroneura variabilis* Beamer and *Erythroneura elegantula* Osborn with some results (Daane et al. 1996; Daane and Yokota 1997). In this study, the effectiveness of two generalist predators, i.e. *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), against *E. vulnerata* was tested. Both species are well known predators of various hemipterans and have been frequently detected in vineyards in North-eastern Italy (Duso and Girolami 1983; Paoletti 1984; Girolami 1987; Bosco and Tavella 2013). In particular, commercially produced predators were tested both in laboratory and in open field conditions.

2. Materials and methods

2.1. Laboratory experiments

A laboratory experiment was carried out to assess the capacity of *C. carnea* and *O. majusculus* to prey upon *E. vulnerata*. Young nymphs of *E. vulnerata* were collected from infested vineyards located in North-eastern Italy (Treviso and Vicenza provinces, Veneto region) and transferred onto grapevine leaf disks inside a Petri dishes (90 mm of diameter, 15 mm of height). Two different prey densities (5 and 10 leafhopper nymphs) were considered in the experiments. Single 3rd instar larvae of *C. carnea* or adults of *O. majusculus* were transferred onto experimental units to evaluate predation capacity. Predators were supplied by Bioplanet (Cesena, Italy), and sent by the producer in plastic bottles, mixed with buckwheat hull to reduce cannibalism. The number of living and dead leafhopper nymphs and of predators were recorded after 24 hours. Three treatments were compared (*C. carnea*, *O. majusculus*, and Control) each having 7 to 10 replicates. Experimental units were maintained in a climatic chamber at 23±2°C and 70-80% of relative humidity with a photoperiod of 16L:8D.

2.2. Field experiments

Considering the results obtained in the laboratory experiments, the effectiveness of the tested predators was assessed in open field conditions. Predators were released in a highly infested vineyard located at Conegliano (North-eastern Italy, 45°52’53.05’’N, 12°17’00.26’’E, 77 m a.s.l.), in 2017 and 2018. The vineyard (cultivar Merlot) was trained with the Guyot system and managed according the Directive 2009/128/EC. No insecticides were applied in plots considered for releases during the experiments. Larvae of *C. carnea* and adults of *O. majusculus* to be released were provided by Bioplanet.

In 2017, three treatments were compared: 1) Release of *C. carnea*; 2) Release of *O. majusculus*; 3) Control. Each treatment comprised four plots (replicates) each having five vines (approximately 20 m^2 of vine canopy). Predators were released on July 25. They were manually distributed on the permanent cordon and the canopy. In particular, about 600 *C. carnea* larvae or 80 *O. majusculus* adults were released on each plot. Samplings were carried out to evaluate leafhopper densities before and after the release, in particular on July 26, 28 and 31. In each plot, 25 leaves were randomly collected and transferred to the laboratory where they were observed under a Wild M3C stereomicroscope (10-40 x magnification) in order to assess the abundance of *E. vulnerata* motile forms and of released predators.

Two experiments were carried out in 2018. In the first one *C. carnea* release was compared with the Control. Each treatment was carried out on eight plots (replicates) comprising five vines. Lacewing larvae were released using biodegradable paper cups (270 x 575 x 360 mm, capacity 60 ml), fixed on the permanent cordon of vines. A mixture of lacewing larvae and buckwheat hull, were placed in each cup, for a total of 20 cups per plot. Two releases of *C. carnea* were performed on 7^{th} June and 3^{rd} July, respectively, using the same number of predators considered in 2017. In the second experiment the effects of *C. carnea* and *O. majusculus* releases (July 21) were compared to the Control, following a design similar to that adopted in the previous year. Each treatment was carried out on four plots comprising five vines. Sampling methods were similar to those used in 2017. Samplings were performed from June 11 to July 6 for the first trial, from July 20 to 11^{th} August for the second trial.

2.3. Statistical analysis

Data from laboratory experiment were analysed using logistic regression with the proc GLIMMIX of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC) considering the binomial distribution of the data. In this analysis the number of surviving nymphs at the end of the experiment over the initial numbers was considered as the dependent variable in the analysis. A χ^2 test ($P = 0.05$) was used to assess the effect of independent variable, that were: predator, prey density and their interactions. A Tukey's test ($P = 0.05$) was used to evaluate differences among treatments for predator and each of the two levels of prey density considered. Data from field experiments were analysed using a repeated measures linear mixed model with the Proc MIXED of SAS®. The model was used to test the effects of predator, time and their interactions on number of *E. vulnerata* motile forms collected during the experiments. The effect of independent variables was tested using a F test ($P = 0.05$). Degrees of freedom were estimated with the Kenward and Roger method. A Tukey's test ($P = 0.05$) to the least square means was applied to evaluate the differences among treatments. The assumptions of the models were evaluated by inspecting diagnostic plots of model residuals and data on leafhoppers density were log (n+1) transformed prior to the analysis.

3. Results

3.1. Laboratory experiment

All predators survived during the experiment. *C. carnea* and *O. majusculus* preyed actively upon *E. vulnerata* nymphs affecting significantly leafhopper survival rates compared to the Control (χ = 57.91, d.f. = 2, 30; P < 0.0001; Fig. 5.1). No differences emerged between predators (Fig 1). Prey density did not affect survival rates (χ = 1.65, d.f. = 1, 30; P = 0.198).

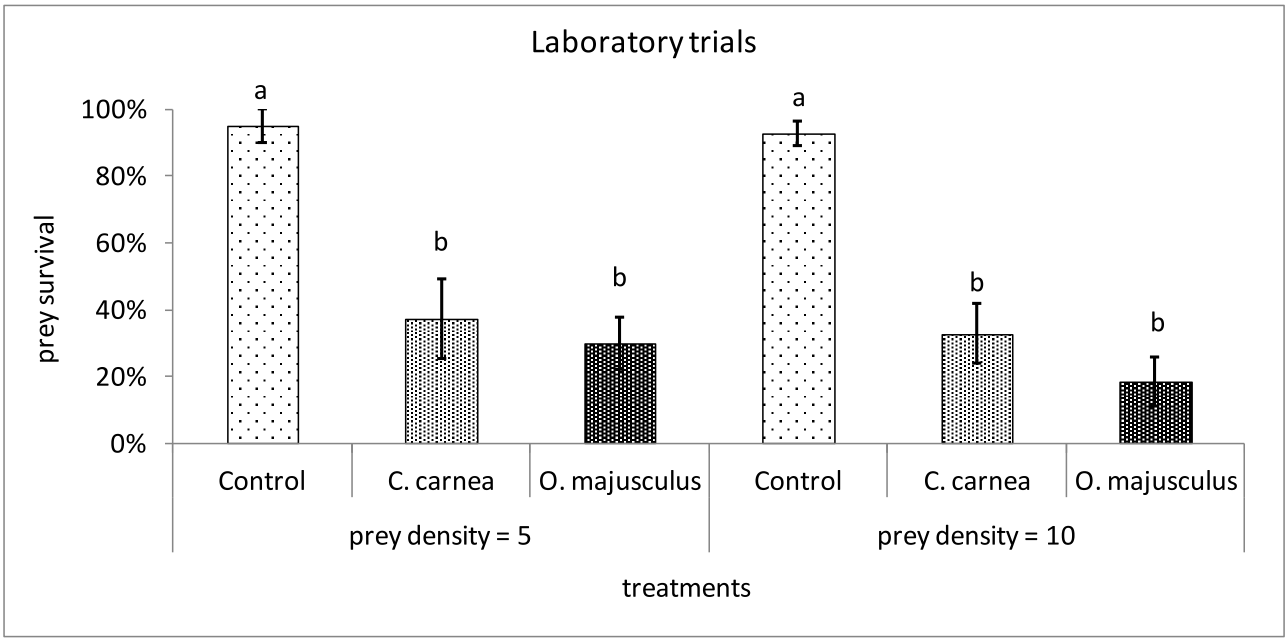

Figure 5.1: Survival rate of *Erasmoneura vulnerata* in treatments (*C. carnea*, *O. majusculus*, and Control) under comparison in the laboratory. Different letters indicate significant differences at Tukey-Kramer test (P = 0.05).

3.2. Field experiments

In 2017 trial, population densities of *E. vulnerata* (most of them were aged nymphs) declined during the experiment carried out in 2017 and thus the effect of time was significant (F = 18.51; d.f. = 2, 18.2; P < 0.0001). Predator releases did not affect significantly leafhopper population densities (F = 0.48: d.f. = 2, 9.8; P = 0.634; Fig. 5.2).

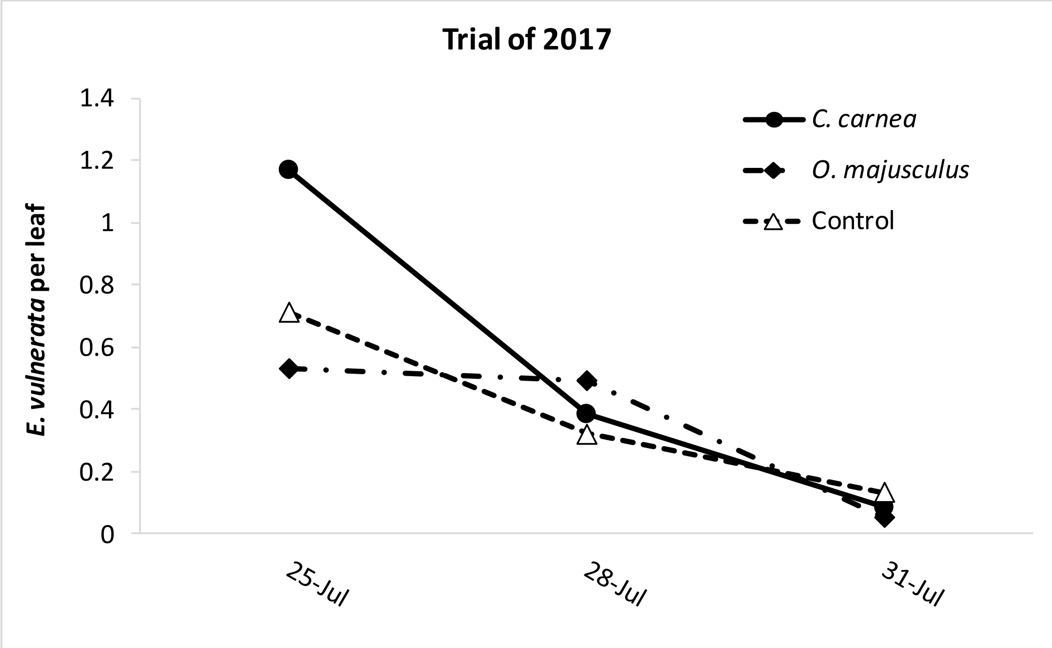

Figure 5.2: *Erasmoneura vulnerata* densities in vineyard plots characterized or not by predator releases in 2017.

In the first trial of 2018, *E. vulnerata* densities fluctuated significantly over the experimental period and the effect of time was significant ($F = 9.27$; d.f. = 3, 37.5; $p < 0.0001$). *Chrysoperla carnea* releases did not reduce significantly leafhopper numbers ($F = 0.17$; d.f. = 1, 22.4; $p = 0.685$; Fig. 5.3).

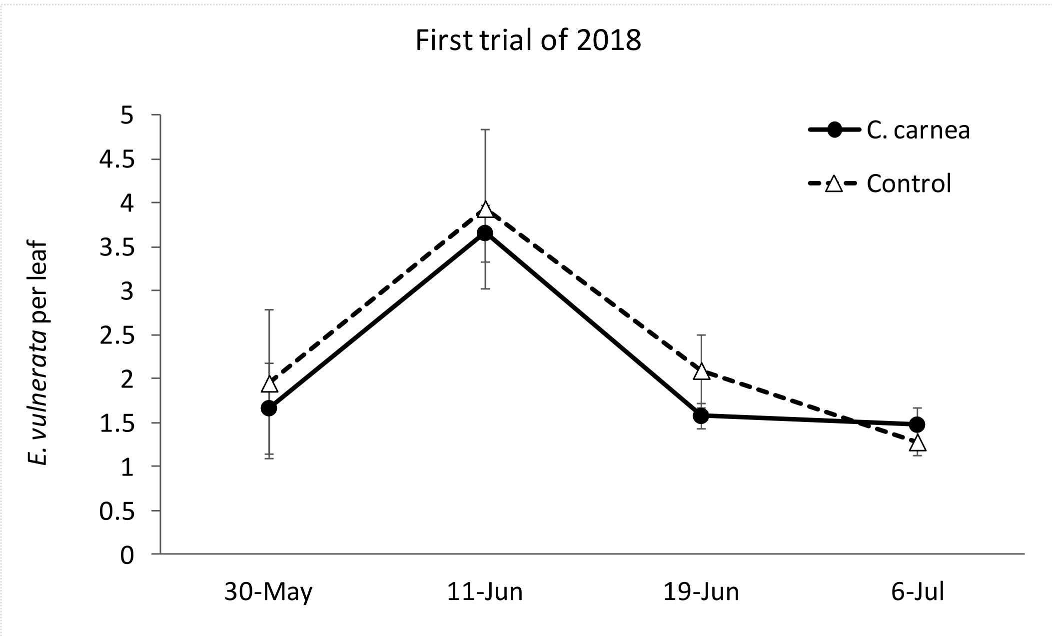

Figure 5.3: *Erasmoneura vulnerata* densities in vineyard plots characterized or not by predator releases in 2018 (first trial).

In the second trial of 2018, *E. vulnerata* densities increased after releases and then declined in all treatments. The effect of time was significant (F = 36.29; d.f. = 3, 27; P < 0.0001). Predator releases significantly affected leafhopper population densities (F = 4.03; d.f. = 2, 13.6; P = 0.036; Fig. 5.4) but with different outcomes between the two predatory species. Leafhopper densities significantly decreased in *O. majusculus* release plots (P = 0.036) compared to the Control plots; in contrast *C. carnea* releases were not associated with a significant reduction of leafhopper numbers (P = 0.349). Nevertheless, there were no differences between *O. majusculus* and *C. carnea* regarding leafhopper densities (P = 0.780).

In all trials, in release plots some individuals of released predators were observed on leaf samples; however, they low densities recorded did not permit their statistical analysis.

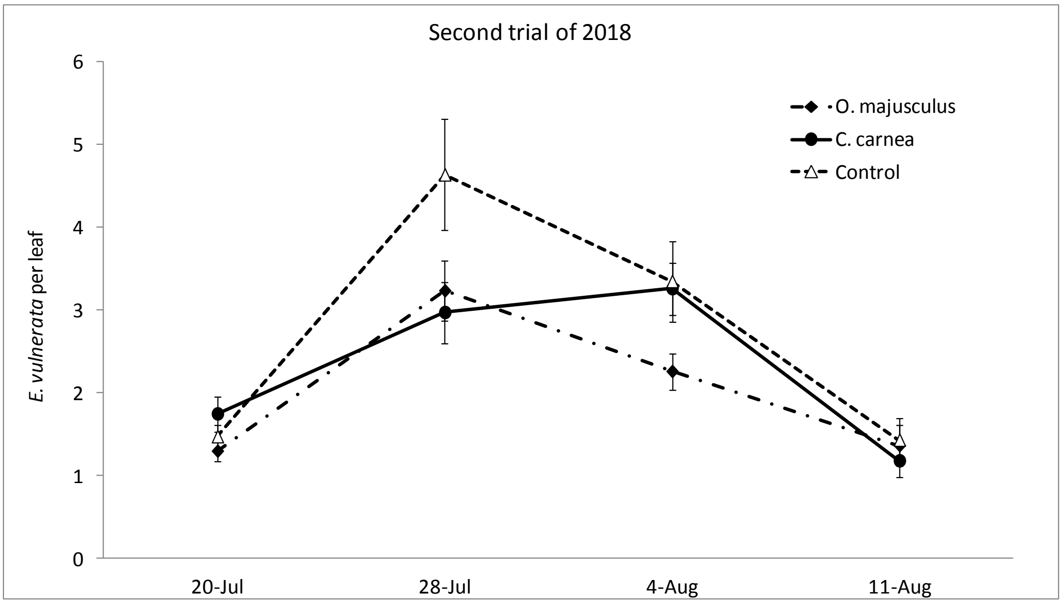

Figure 5.4: *Erasmoneura vulnerata* densities in vineyard plots characterized or not by predator releases in 2018 (second trial).

4. Discussion

Chrysoperla carnea is a high polyphagous predator with about 70 potential prey species within five orders, but mostly of them belong to the Homoptera (e.g. aphids, whiteflies, leafhoppers and mealybugs) (Hagen in Fisher et al. 1999). It has been considered the neuropteran species most used in biocontrol tactics, in particular in augmentation releases especially against aphids and lepidopterans. Few studies have examined predation of leafhoppers by *C. carnea*. Erlandson and Obrycki (2010) compared the predation activity of *C. carnea* upon the leafhopper *Empoasca fabae* Harris with that exhibited by the anthocorid *Orius insidiosus* (Say) and the coccinellid *Coleomegilla maculata* (Degeer). *Chrysoperla carnea* was the most voracious among tested predators and its impact on leafhopper prey appeared to be comparable to that obtained in the present study. Nevertheless, we did not find differences in the capacity of *C. carnea* and *O. majusculus* to reduce *E. vulnerata* survival.

The impact of lacewings on grape leafhoppers have been evaluated in California, where Daane et al. (1996) released *C. carnea* in vineyards infested by *Erythroneura variabilis* Beamer and *E. elegantula* Osborn. In a first trial *C. carnea* larvae were released into cages and leafhopper densities were reduced by 23.5-30.3%. Then *C. carnea* larvae were released on vines following commercial protocols. A significant leafhopper density reduction (33.6 and 31.4% in the first and second generations, respectively) was observed by releasing about 20,000 larvae per hectare. However, unsatisfactory results were obtained using the same approach in other trials. In additional, field trials *C. carnea* eggs were released obtaining a significant leafhopper reduction (9.6% as a mean) in about a half of tested vineyards. Differences in release methods and prey density were claimed as possible factors affecting contrasting results. Experiments showed that leafhopper density reduction was higher releasing lacewing larvae rather than eggs. Authors concluded that prey densities had a significant role in the outcome of these trials: releases did not reduce leafhopper densities below the economic injury thresholds in high pest pressure conditions. Therefore aspects related to release strategies used to augment green lacewings (included *C. carnea*) were evaluated further in the same area (Daane and Yokota 1997). A mixture of lacewing eggs and corn grit placed in paper cups was distributed to every 5th vine in every other row; this system was associated with low egg hatching and larvae dispersal. Egg hatching increased when they were dropped onto the vines from a moving flatbed trailer. In other trials the effect of increasing release rates (from 6,175 to 1,235,000 eggs or larvae per hectare) was compared but prey numbers were not correlated with release densities. Releases were more effective when the nymph generation was in an early stage (before peak). Larval releases confirmed to be more effective than egg releases.

In the present study high numbers of commercially produced lacewing larvae were released, however without decrease in pest pressure. In the 2017 trial predators were released when leafhopper populations were represented mostly by aged nymphs, more difficult to be consumed than young nymphs, and leafhopper densities were naturally declining. Results suggest that timing of release and prey stage were not optimal (Daane and Yokota 1997). In the first 2018 trial, predators were released into cups to reduce losses during release operations. Results were not positive confirming the observations by Daane and Yokota (1997) that adopted a similar experimental approach. Results of *C. carnea* release appeared to be better in the second 2018 trial, but differences with the control were not significant.

Orius majusculus feeds on a variety of arthropod species, such as thrips, spider mites, leafhoppers, aphids, and lepidopteran eggs and young larvae. *Orius majusculus* has been commonly detected in North-eastern Italy preying upon homopterans and spider mites (Duso and Girolami 1983; Paoletti 1984). Predation on grape leafhoppers was seldom observed but ad hoc experiments were not planned. Interactions between *O. majusculus* and leafhoppers have been investigated in Spain. Ardanuy et al. (2016) observed early season increases of *Orius* spp. in maize fields potentially related to the occurrence of leafhoppers, in particular

Zyginidia scutellaris (Herrich-Schäffer). They examined the innate and learned preferences of *O. majusculus* toward volatiles emitted from plants infested by *Z. scutellaris*, *Spodoptera littoralis* Boisduval (a lepidopteran) and *Dalbulus maidis* Delong & Wolcott (another leafhopper). Predators were markedly attracted by volatiles emitted from maize plants infested with *Z. scutellaris* or *S. littoralis*. Feeding by *Z. scutellaris* induces the emission of maize's HIPVs that attract anthocorids into maize fields. In this study, results showed that *O. majusculus* adults consumed actively *E. vulnerata* nymphs in laboratory. Therefore, predators were released in highly infested vineyards distributing them on the grape canopy. Results of 2018 trial were positive while those obtained in 2017 trials were probably affected by release timing as reported for *C. carnea*. Promising results obtained with *O. majusculus* suggest to implement release techniques, densities and timing to improve the impact of anthocorids on grape leafhoppers.

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Conclusions

Italian vineyards are often large-scale monocultures characterized by high pesticide pressure to control pathogens and pests with a widespread removal of ecologically valuable structures. The intensification of farming systems through specialization and scale enlargement in order to increase yields and overall efficiency, generally leads to a decrease in biodiversity, biological control and other ecosystem services (Donald et al. 2001; Tilman et al. 2001; Duso et al. 2010). In this framework, the preservation of semi-natural habitats and the use of ecological infrastructures aimed at increase of biodiversity, which are functional to ecosystem services, are of particular importance in agro-ecosystems (Landis et al. 2000). In this framework, habitat management is unique in its ability to provide a wide variety of ecosystem services in addition to pest population reduction.

In high specialized and more simplified agro-ecosystems, the insurgence of non-native species populations can easier occur, as reported for the American leafhopper *Erasmoneura vulnerata* (Fitch) (Hemiptera: Cicadellidae). In 2016, first outbreaks of the leafhopper were detected in the Veneto region, causing symptoms on more than 90% of the canopy. Since the biological invasions by non-native species may have negative impact on humans and/or the environment, knowlwgde on their biology, ecology, and natural enemies are fundamental to set up rationale management strategies in the vineyard.

In the Chapter 2, the vineyard management strategies affected the population densities of the three leafhoppers: *Empoasca vitis*, *Zygina rhamni*, and *Scaphoideus titanus*. Leafhoppers were more abundant in organic vineyards, where the use of botanical insecticides, characterised by a lower efficacy compared to that of insecticides used in conventional viticulture, poorly control leafhopper populations (Pavan et al. 2012; Mori et al. 2014; Pavan et al. 2014;Tacoli et al. 2017). This result may have implications for the spread of the Flavescence Dorée phytoplasma. At this purpose, the number and timing of insecticide applications should be more defined accurately, especially in organic vineyards (Mori and Pavan 2014).

The influence of landscape complexity was observed on the population of *Z. rhamni*, which resulted more abundant in vineyards within complex scenarios where preferred host plants occur in the semi-natural habitats surrounding vineyards. The higher parasitism rate of eggs of Typhlocybinae species observed in vineyards surrounded by complex landscape confirms the important role of semi-natural areas in providing alternative hosts and foods to egg-parasitoids as well as refuges and overwintering habitats (Van den Bosch and Telford 1964; Williams and Martinson 2000; Ponti et al. 2005; Zanolli and Pavan 2011; Thomson and Hoffmann 2013; Gaigher et al. 2015; Smith et al. 2015).

From the results showed in the Chapter 2, the impact of vineyard management and the influence of landscape complexity both on target pest and their natural antagonists should be considered when pest control strategies are planned on a large scale.

In the Chapter 3, the presence of non-mowed spontaneous grass in vineyards inter-rows favoured the abundance of natural enemies (e.g., predatory mites, assassin bugs, parasitoid wasps, and spiders), but also of grapevine leafhoppers (e.g., *Z. rhamni* and *S. titanus*), especially in organic where botanical insecticides are used. The presence of non-mowed vegetation could probably offer better micro-climatic conditions and refuges and consequent favour the presence of the leafhoppers. Considering the important role of *S. titanus* in the spread of the Flavescence Dorée phytoplasis, the presence of non-mowed vegetation could make harder the control of the vector populations in organic vineyards, and its adoption should be carefully evaluated when the leafhopper occurs in the vineyards.

The different timing of mowing of the green manure sown in the vineyard inter-rows can influence the presence and the abundance of arthropods on grapevine canopy and/or inside the vineyard agro-ecosystem. The phytophagous mites *Panonychus ulmi* strongly increased in plots without flowering plants, as response of low predatory mite density. Moreover, allowing the green manure to flowering for a prolonged period instead of mowing it early, as traditionally done by the growers, can favour a higher presence and abundance of beneficial arthropods (e.g., spiders and parasitoids wasps), while not influenced phytophagous densities (e.g., leafhoppers and stink bugs).

No differences were observed when the effects of different green manure mixtures on arthropods abundance were compared. This is probably related to the low distance between sampling plots, which cannot permit to observing significant effects.

In light of these results (see Chapter 3), some habitat manipulation practices, such as the management of spontaneous groundcover and the use of green manure, could be useful strategies to increase and/or enhance beneficial arthropods. In fact, the presence of vegetation can provide food sources (such as pollen and nectar), refuge zone and alternative prey fundamental for the survival and reproduction of natural enemies (Altieri and Whitcomb 1980; Landis et al. 2000; Nicholls et al. 2000; Poyet et al. 2015; Daane et al. 2018), contributing to create an appropriate ecological infrastructure within the vineyards resulting in a more pest-stable agro-ecosystems (Altieri and Nicholls 2004; Begum et al. 2004; Gurr et al. 2004; Altieri et al. 2005). The timing of mowing must be accurate programmed according to life cycle of the natural enemies in order to better optimize the practice (Van den Bosch and Telford 1964) considering that habitat manipulation can promote the dispersal on leafhopper vectors, such as *S. titanus* and *Hyalesthes obsoletus* Signoret, and therefore its adoption should be carefully evaluated.

Investigations on the phenology of *E. vulnerata*, showed in the Chapter 4, suggest that the pest can develop three generations per years in the new invaded area. The presence of rural buildings and alternative hosts at vineyard margins favoured the leafhopper overwintering and its spreading into the vineyards in spring. The impact of natural enemies on pest populations appeared to be limited to egg parasitism by Hymenoptera Mymaridae and did not reach values required to keep *E. vulnerata* populations densities below acceptable levels. The use of organophosphates in conventional vineyards and pyrethrins in organic vineyards proved to be partially ineffective in controlling *E. vulnerata* (Duso et al. 2017). Therefore, a new approach in pest control should be identified. In this framework the economic impact of *E. vulnerata* on grapevine yield and quality should be urgently investigated in order to define appropriate economic threshold levels and reduce pesticide use. At the same time strategies aimed at promoting the impact of egg parasitoids and predators require to be developed.

To search non-chemical alternatives in controlling the American leafhopper populations, the use of two generalist predators common present in Italian agro-ecosystems, i.e. *Chrysoperla carnea* (Neuroptera) and *Orius majusculus* (Hemiptera) were tested. As showed in the Chapter 5, results on predation of *E. vulnerata* nymphs in the laboratory experiments suggested to release them in a highly infested vineyard. However, in open field conditions the release of predators did not give satisfactory results, except in only one trial in which the release of *O. majusculus* significantly reduced the leafhopper numbers. Promising results obtained in this latter trial suggest to implement release techniques, densities and timing to improve the impact of anthocorids on grape leafhoppers.

In conclusion, grapevine cultivation should attempt to conjugate vine production with environmental quality, through the preservation of semi-natural habitats and the use of ecological infrastructures aimed at increase the biodiversity (Altieri and Nicholls 2004; Begum et al. 2004; Gurr et al. 2004; Altieri et al. 2005). The design of modern viticultural systems should integrate management practices with lower environmental impact with ecological compensation measures to increase and enhance biodiversity in the vineyard agro-ecosystems. Habitat management can contribute to provide a wide variety of ecosystem services, include natural control of pests but, in extremely simplified context, the only presence of temporary vegetation appears not enough to create a more pest-stable agro-ecosystem. Moreover, in highly disturbed and simplified agro-ecosystems the insurgence of non-native pest populations can easier occur, as recently observed for *E. vulnerata* in vineyards of North-eastern Italy. Since the deployment of pesticides can favouring the insurgence of pest outbreaks and limited the successful implementation of biological control (Landis et al. 2000), more sustainable non-chemical strategies should be implement to control pest population densities.

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