

Universidade de Trás-os-Montes e Alto Douro

**Towards a sustainable control of arthropod pests in Douro  
Demarcated Region vineyards with emphasis on the grape berry moth,  
*Lobesia botrana* (Denis & Schifermüller)**

Tese de Doutoramento em Ciências Agronómicas e Florestais

Cristina da Conceição Ribeiro Carlos

Orientadores

Professora Doutora Laura Monteiro Torres  
Professor Doutor Antonio Maria Luís Crespi



Vila Real, 2017



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## Abstract

The aim of the present thesis was to contribute to the development of an environmentally sustainable pest management strategy for controlling the main arthropod pests in Douro Demarcated Region (DDR) vineyards, with emphasis on the grape berry moth, *Lobesia botrana*, a key-pest of the crop in this region. Due to the impact of *L. botrana* in the quality of the wines produced in DDR, the increasing regulation of pesticides in Europe and the increasing interest for sustainable production approaches, we intend to contribute for the development of an effective control strategy against this pest, based on conservation biological control and mating disruption technique with the support of degree-day models to better timing sampling or control operations and ultimately to improve Integrated Pest Management tactics.

With the overall goal mentioned, the following aspects were studied: a) evaluation of the impact of non-crop habitats (NCH) adjacent to vineyards as well as ground cover vegetation of terraced vineyards, on the overall biodiversity of arthropods, including natural enemies (predators and parasitoids) and main vineyard pests; b) survey of *L. botrana* parasitoids carried over a nine-year period; c) development of a degree-day ( $DD^\circ$ ) models to predict the occurrence of the main phases of the flight curve of *L. botrana*; two starting points for degree-day accumulation were tested, a biological event (the first male catch) and a calendar date (January 1<sup>st</sup>) and d) evaluation of the effectiveness of using mating disruption technique (MD) against *L. botrana* in DDR, as well as identification of main constraints to its use in the region.

The results obtained showed the importance of maintaining both NCH on the neighborhood of vineyards and vegetation of slopes and horizontal alleys to enhance functional biodiversity of this agroecosystem. It was found that they could act as potential ecological infrastructures in the increase of populations of natural enemies of vineyard's pests, namely predators (spiders and coccinelids) and parasitoids.

Although closely related groups displayed different responses to land use and habitat preferences, the abundance of omnivores and predators was, in general, higher in NCH than in vineyards. Preserving NCH near the vineyards, as well as promoting local vegetation, enhances the presence of ground-dwelling arthropods.

It was found that the complex of *L. botrana* parasitoids, as well as the potential role played by each species to control the pest, are greatly variable in space and time, with the rates

of parasitism being substantially higher in the pest 1<sup>st</sup> generation compared to that of the other two generations. The most promising candidate to enhance conservation biological control of *L. botrana* in DDR vineyards is *Elachertus* sp. (Hym: Eulophidae). This is because, on one hand, this larval ectoparasitoid has a wide geographical distribution in the studied region and on the other hand, in most favorable conditions, the percentage of parasitism can reach 60%, mainly in the 1<sup>st</sup> generation of the pest. *Campoplex capitator* and *Brachymeria tibialis* appears to have a complementary role on the parasitism of the first and second generations of *L. botrana*, respectively. Moreover, it was found that the parasitism rate was related with ground cover management and chemical treatments. Results suggest that, for enhancing conservation biological control of *L. botrana* in DDR, a high abundance and diversity of vegetation (ground covers) inside or at the edge of vineyards plots should be enhanced and a selection of pesticide with minimal risks to parasitoid's activity should be performed.

Nonlinear models based on Boltzmann regression equations were developed and predicted with reasonable accuracy the flight phenology of *L. botrana*, although the one that use first catches as starting point for accumulation was more accurate on predicting the second and third flights of the insect. Even though the use of a biofix, here considered to be a biological event, seems to improve the model accuracy, the use of a fixed calendar date (January 1<sup>st</sup>) should be preferred, from the practical point of view and considering large scale application of an IPM strategy.

In general MD was more effective in years of low pest population density, when applied in large areas, with more points of release per hectare, and after consecutive seasons. Some major constraints to the use of this technique in DDR could be identified, namely: the high biotic potential of *L. botrana*; the climate conditions, particularly the high summer temperatures; the effect of the winds on the distribution of the pheromone on the hill; the impact of slope; the fragmentation of many vineyards and the size of the treated area.

**Keywords:** biodiversity, conservation biological control, ecological infrastructures, integrated pest management, mating disruption

## Dissemination

### Scientific publications

#### International peer-reviewed papers

- Carlos C, Gonçalves F, Torres L. The use of a degree-day model to forecast flight activity of the grape berry moth, *Lobesia botrana*, in Douro Demarcated Region (Northeast of Portugal). Crop Protection (accepted after revision)
- Carlos C, Gonçalves F, Sousa S, Salvação J, Sharma L, Soares R, Manso J, Nóbrega M, Lopes A, Soares S, Aranha J, Villemant C, Marques G, Torres L (2013) Environmentally safe strategies to control the European Grapevine Moth, *Lobesia botrana* (Den. & Schiff.) in the Douro Demarcated Region. Ciência e Técnica Vitivinícola: 28: 1006-1011

#### Internacional reviewed papers

- Carlos C, Gonçalves F, Sousa S, Nóbrega M, Manso J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D, Val MC, Franco JC, Thistlewood H, Torres L (2014) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. IOBC/WPRS Bull, 105: 93-102
- Carlos C, Afonso S, Crespi A, Aranha J, Thistlewood H, Torres L (2012) Biodiversity of plants and arthropods in key ecological structures of vineyards of the Alto Douro region. IOBC/WPRS Bull, 75: 51-55
- Carlos C, Val MC, Marques G, Torres L (2011) New approaches for management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal. Am J Enol Vitic, 62 (3): 389A-389A
- Carlos C, Alves F, Torres L (2010) Eight years of practical experience with mating disruption to control grape berry moth, *Lobesia botrana*, in Porto Wine Region. IOBC/WPRS Bull: 54: 405-409
- Carlos C, Costa JR, Tão CB, Alves F, Torres LM (2006) Parasitismo associado à traça da uva, *Lobesia botrana* (Den. & Schiff.) na Região Demarcada do Douro. Bol San Veg Plagas, 32: 355-362
- Carlos C, Costa J, Gaspar C, Domingos J, Alves F, Torres L (2004) Mating disruption to control the grape vine moth, *Lobesia botrana* (Den. & Schiff.) in a Porto Wine Region vineyard: a three-year study. IOBC/WPRS Bull: 28 (7): 283-287

#### Poster presentations in international conferences

- Carlos C, Gonçalves F, Val MC., Sousa S, Nóbrega M, Manso J, Soares R, Martinho, A, Soares S, Torres L (2015) Mating disruption against *Lobesia botrana* Den. & Schiff) using Isonet Ltt dispensers in the Douro Wine Region (Portugal). Proceedings Conference IPM innovation in Europe, 14<sup>th</sup>-16<sup>th</sup> Jan 2015, Poznan, Poland: 52
- Carlos C, Meireles S, Val MC, Alves F, Crespi A, Torres L (2011) Enhancing functional Biodiversity in Douro Wine Region vineyards. 34th OIV - World Congress of Vine and Wine. 20th -27th June 2011, Porto. Abstract ID: 468

#### Oral presentations in international conferences

- Goula M., Gonçalves F, Carlos C, Torres L (2016) Heteroptera from vineyards and adjacent vegetation. Proceedings 17th Congresso Ibérico de Entomologia. Sociedade Portuguesa de Entomologia (SPEN), Laboratório Nacional de Engenharia Civil (LNEC), Asociación española de Entomología (AeE). 5th-8th Sept. 2016. Centro de Congressos do Laboratório de Engenharia Civil, Lisboa
- Carlos C, Gonçalves F, Sousa S, Salvação J, Sharma L, Soares R, Manso J, Nóbrega M, Lopes A, Soares S, Aranha J, Villemant C, Marques G, Torres L (2013) Environmentally safe strategies to control the European Grapevine Moth, *Lobesia botrana* (Den. & Schiff.) in the Douro Demarcated

Region. 18th International Symposium GiESCO, Faculty of Sciences of the University of Porto. July 7th - 11th 2013

- Carlos C, Gonçalves F, Sousa S, Nóbrega M, Manso J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D, Val MC, Franco JC, Thistlewood H, Torres L (2013) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. IOBC-WPRS Meeting Group "Integrated Protection and Production in Viticulture". Ascona, Switzerland, 13th -17th October. 2013
- Carlos C, Val MC, Marques G, Torres L (2011) New approaches for management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal. 62th ASEV National Conference. June 20–24, 2011. Monterey, California USA, 62
- Carlos C, Val C, Rataux A, Aranha J, Crespí A, Marques G, Torres L (2011) Does the landscape of Douro Wine Region affect the complex of antagonists of the grape berry moth? Proceedings 18th IaleUK Conference "Landscape ecology and ecosystem services", Wolverhampton University, Telford Campus, England. 6th-8th Sept. 2011. 71
- Carlos, C., Alves, F. & Torres, L. (2008) Eight years of practical experience with mating disruption to control grape berry moth, *Lobesia botrana*, in Porto Wine Region. VII International Conference on Integrated Fruit Production, Avignon, France, 27-30 October: 118-119
- Carlos C, Alves F, Torres L (2010) Constrains to the application of mating disruption against *Lobesia botrana* in Douro Wine Region. In 3<sup>rd</sup> International Congress of mountain and Steep slope viticulture, Castiglione di Sicilia. Sicily, Italy. 12th – 14th May 2010. 103-110
- Carlos CR, Costa JR, Tão CB, Alves F, Torres LM (2005) Parasitismo associado à traça-da-uva, *Lobesia botrana* (Den. & Schiff.) na Região Demarcada do Douro. Proceedings IV Congreso Nacional de Entomología Aplicada. X Jornadas Científicas de la SEEA. I Jornadas Portuguesas de Entomologia Aplicada, Escola Superior Agrária de Bragança, 17th-21th October. 2005, Bragança

#### **Poster presentations in national conferences**

- Carlos C, Sousa S, Nave A, Gonçalves F, Fernandes R, Crespí A, Torres L (2012) Artrópodes associados à flora da Região Demarcada do Douro. Semana da Biodiversidade – ADVID. Quinta do Tedo, Tabuaço, 28 May 2012
- Fernandes R, Carlos C, Crespí A, Torres L (2011) Artrópodes associados à vegetação espontânea das vinhas da região demarcada do Douro. 9º Encontro Nacional de Protecção Integrada, Escola Superior Agrária de Viseu, 17-18 Nov. 2011
- Carlos C, Crespí A, Torres L (2011) A gestão da flora da vinha da Região Demarcada do Douro no incremento dos serviços facultados pelo ecossistema. Conference "Gestão e Conservação de Habitats e Flora Associada". Sociedade Portuguesa de Botânica, ALFA - Associação Lusitana de Fitossociologia. Escola Superior Agrária de Coimbra, 25 March 2011

#### **Oral presentations in national conferences**

- Carlos C, Gonçalves F, Val MC, Sousa S, Nóbrega M, Manso J, Soares R, Martinho A, Soares S, Torres L (2014) A confusão sexual da traça-da-uva na Região Demarcada do Douro com recurso a difusores Isonet-LTT. Proceedings 1st Symposium SCAP Novos Desafios na protecção das plantas / 7th Congress Sociedade Portuguesa de fitopatologia. 20th-21th November. 2014, Auditório INIAV, Quinta do Marquês, Oeiras: 36
- Carlos C, Gonçalves F, Sousa S, Salvação J, Nóbrega M, Manso J, Soares R, Lopes A, Franco JC, Thistlewood H, Aranha J, Torres (2013) Resultados da aplicação do novo difusor de feromona ISONET-LTT em duas explorações com historial de ataque de traça-da-uva na RDD. Workshop "Maximização dos serviços do ecossistema vinha", Ecovitis project. Vila Real, 13th-14th November
- Carlos C (2012) Biodiversidade de plantas e artrópodes associados à vinha da RDD. Forum INFOWINE "Thinking "out of the Bottle". VINIDEAS. 31 May 2012, Vila Real

- Carlos C (2012) Biodiversidade funcional em viticultura no Douro. Workshop “Vitivinicultura e Biodiversidade, Boas práticas agrícolas e Valor acrescentado”. QUERCUS. Sec. Estado Agricultura, Lisboa, 20th November 2012
- Carlos C (2012) Biodiversidade de plantas e artrópodes associados à vinha da RDD. Semana da Biodiversidade – ADVID. Quinta do Tedo, Tabuaço, 28 de May 2012
- Carlos C (2011) Biodiversidade funcional em viticultura. Conference “Biodiversidade em viticultura – Um potencial de diferenciação no mercado?”. Auditório do INRB IP / INIA, Dois Portos, 15 December 2010
- Carlos C, Val M C, Meireles S, Crespi A, Torres L (2010) Biodiversidade e protecção biológica de conservação contra pragas da vinha, na Região Demarcada do Douro. 12th Encontro Nacional da Sociedade Portuguesa de Ecologia “Serviços dos Ecossistemas. Desafios e ameaças num mundo em mudança”. Porto, 18th-20th October

### **Technical documents**

- Gonçalves F, Carlos C, Crespo L, Torres L (2014) O campo no seu bolso nº 2. Amigos desconhecidos do agricultor - aracnídeos, insectos e centopeias. In Torres, L (coord.). Edibio Edições, Lda, 118pp. ISBN 978-972-99697-4-4
- Gonçalves F, Carlos C, Torres L (2013) Inimigos naturais das pragas da vinha: insectos e aracnídeos. Quem são e onde estão? Associação para o Desenvolvimento da Viticultura Duriense, 81pp. ISBN 978-989-98368-2-2
- Gonçalves F, Carlos C, Torres L (coord) (2013) Fauna associada à vinha da Região Demarcada do Douro. Associação para o Desenvolvimento da Viticultura Duriense, 57. ISBN 978-989-98368 -3-9
- Carlos C (2012) Technical Notes 4 - Mating Disruption of the grapevine moth in the Douro Region. ADVID Technical notes. 12 pp. ISBN: 978-989-95481-5-2
- Carlos C (2012) Technical Notes 1 - The grapevine moth. ADVID Technical notes. 12 pp. ISBN: 978-989-95481-4-5

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# Chapter 1

## General Introduction





## 1.1 General introduction

The grapevine moth, *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae) is among the most economically important insect pests in Europe and has recently been found in vineyards in Chile, California and Argentina (Varela et al. 2010). It is a multivoltine species with two to five annual generations (Ioriatti et al. 2011). The larvae of the first-generation feed on flower bunches while the next generations feed on ripening and ripe berries. Fungi, especially grey mold *Botrytis cinerea*, develop rapidly on the damaged grapes, causing entire clusters to rot (Tasin et al. 2005).

At the Douro Demarcated Region (DDR), an important winegrowing area (43,670 hectares) located in the Northeast of Portugal, where “Port” D.O.C. wine and other remarkably high-quality table wines are produced, this pest is feared by growers mostly for its impact on quality, while damages are highly variable amongst years, ranging from 0 to 90% of infested clusters at harvest (Carlos et al. 2014). As a result, it has received considerable attention by researchers attempting to develop effective control strategies against it.

Prediction of *L. botrana* flight activity during the growing season has been considered critical, to improve IPM tactics through better timing of sampling or control operations. The need to ensure effective and sound strategies to control this important pest requires the development of tools that can help to predict its development for initiating sampling programs or timing insecticide sprays, to increase their efficacy, and so reducing their number, as well as its environmental impact. Traditionally, the control of *L. botrana* relies primarily on the use of insect growth regulators (IGRs) or pyrethroid insecticides, once or twice a year, against the second and/or the third generation. This latest is particularly difficult to control, since larvae quickly penetrate ripening fruit. Because economic injury to grapes occurs when neonates feed on grape clusters, control measures applied, in particular, against the third generation, primarily target *L. botrana* eggs. Therefore, precise timing for spraying with ovicides (e.g. IGR's), before hatching eggs, is particularly important to maximize their efficacy and avoid damages.

The increased regulation of pesticides in Europe, the concerns of the public about the environmental impacts of viticulture and the consumers demand of residue-free products has led to an increase, in the last decade, of environmentally safe pest management strategies, with emphasis on conservation biological control and mating disruption (MD) technique.

According to the Centre for the Research, Study, Protection, Co-ordination and Advancement of Mountain Viticulture (CERVIM), the DDR is the largest and most heterogeneous viticulture region of the world. Despite the intensification of the last thirty years, given the need to reduce operating costs and mitigate the harshness of the labor, the most representative and well-preserved part (about 10% of the total area), the 'Alto Douro Wine Region' (ADV), was classified in 2001 as UNESCO World Heritage Site, for its unique character as an 'evolved continuing cultural landscape'. A significant part of this area is still occupied by non-crop habitats (NCH) such as scrublands (20.4%) and woodlands (13.2%). In the area covered by vineyards (42.9%), a significant part is occupied by grassy/shrubby slopes and/or by dry stone walls (Andresen and Rebelo 2013). While most vineyards in the world are nowadays typically extreme monocultures, with little remaining native vegetation and a suite of introduced weeds, whose provision of ecosystem services is, as a result, at a low level, the DDR offer strong potential from this standpoint, due to the occurrence of a significant area of non-crop habitats.

The occurrence of non-crop habitats has several advantages from the biodiversity point of view, in particular, in conservation biological control, by providing important resources for natural enemies, such as refugia, overwintering habitat, nectar, pollen and alternate hosts or prey, supporting natural enemy populations in nearby crop fields, which can lead to increased levels of biological control of pests. These habitats have been reported to act as biodiversity reservoirs for plants, insects, birds and mammals (Bianchi et al. 2006).

According to Böller et al. (2004), a high potential for a species rich and natural green cover has been found in sloping vineyards, with small-scale terraces. The plant community found in terraced vineyards contains several perennial plant species of value in fostering beneficial predators and parasitoids and therefore these banks serve as an internal ecological infrastructure of the vineyard and face the grapevine at very short distance.

The characteristic landscape of DDR represents however some constraint for the successful application of pheromone based mating disruption (MD) technique, an innovative method of protection used against *L. botrana*, registered in Portugal since 2002 and applied in DDR since 2000. Nowadays, area in DDR under MD is estimated to be only 350 ha (0.8% of DDR vineyard area), due the difficulties on the adaptation of this method of protection to DDR conditions.

The proposed PhD thesis aims at contributing to the development of an environmentally sustainable pest management strategy to control main arthropod pests in Douro Demarcated Region vineyards, with emphasis on the grape berry moth, *L. botrana*.

The thesis is structured into six chapters, and each of them correspond to papers that have been submitted to international scientific journals with referees. These chapters focus on specific issues that are considered important to achieve the final goal of the thesis, which is to support the DDR wine production sector with useful tools for increasing its sustainability at both economic and environmental levels.

In addition to the introduction and conclusion, the chapters and the corresponding objectives are:

- **Second chapter**, to evaluate the diversity of arthropods, as well as the impact, on their communities, of neighboring non-crop habitat and vegetation of terraced vineyards;

- **Third chapter**, to identify the diversity of soil arthropods and evaluate the impact of neighborhood non-crop habitats as well as soil ground cover, on their community;

- **Fourth chapter**, to survey *L. botrana* parasitoids and evaluate their relative importance, according to the generation, and landscape / vineyard management determinants;

- **Fifth chapter**, to investigate the flight activity of *L. botrana*, by analyzing data on male catches in sex pheromone traps recorded over a 20-year period, and develop degree-day models for predicting flights occurrence;

- **Sixth chapter**, to investigate the effectiveness of the use of mating disruption against *L. botrana*, using wired pheromone dispensers and identify the main constraints to its successful application.

## 1.2 References

- Andresen T, Rebelo J (2013) Assessment of the State of Conservation of the Property Alto Douro Wine Region - Evolutive and Living Cultural Landscape - Assessment Report. Porto: CIBIO UP/UTAD
- Bianchi FJJA, Booij CJH, Tscharntke T (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proc R Soc Lond B Biol. Sci, 273, 1715–1727

- Böller EF, Häni F, Poehling HM (eds.) (2004) Ecological infrastructures: Ideabook on functional biodiversity at the farm level. Temperate zones of Europe. Swiss Centre for Agricultural Extension and Rural Development, Switzerland, 212 pp
- Carlos C, Gonçalves F, Sousa S, Nóbrega M, Manso J, Salvação J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D, Val M, Franco JC, Aranha J, Thistlewood H, Torres L (2014) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. IOBC-WPRS Bull 105: 93-102
- Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A (2011) Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). J Econ Entomol 104(4): 1125-1137
- Tasin M, Anfora G, Ioriatti C, Carlin S, de Cristofaro A, Schmidt S, Bengtsson M, Versini G, Witzgall P (2005) Antennal and behavioral responses of grapevine moth *Lobesia botrana* females to volatiles from grapevine. J Chem Ecol 31(1): 77-87
- Varela LG, Smith RJ, Cooper ML, Hoenisch RW (2010) European grapevine moth, *Lobesia botrana*, in Napa Valley vineyards. Practical Winery & Vineyard. March/April:1–5



## Chapter 2

### **Can woodlands, scrublands and vegetation of terraced vineyards in Douro Demarcated Region enhance functional biodiversity of arthropods?**



Part of the content of this chapter was presented/ published in:

Carlos C, Afonso S, Crespí A, Aranha J, Thistlewood H, Torres L (2012) Biodiversity of plants and arthropods in key ecological structures of vineyards of the Alto Douro region. IOBC-WPRS Bulletin, 75: 51-55. ISBN: 978-92-9067-252-4

Carlos C, Val C, Rataux A, Aranha J, Crespí A, Marques G, Torres L (2011) Does the landscape of Douro Wine Region affect the complex of antagonists of the grape berry moth? Proceedings 18th IaleUK Conference “Landscape ecology and ecosystem services”, Wolverhampton University, Telford Campus, England. 6th-8th Sept. 2011. 71



## **Abstract**

A study was held in Douro Demarcated Region (Northeast of Portugal) vineyards with the aim of evaluating the diversity of arthropods, as well as the impact, on their communities, of neighboring non-crop habitats (NHC) and vegetation of terraced vineyards. Arthropods were sampled three times during 2010 in three wine farms using a D-Vacuum machine (D-VAc) and yellow sticky traps, both in NCH, and inside vineyards (vine canopies, slopes or horizontal alleys), at three distances from the edge (5, 25 and 50 m). A total of 6,923 arthropods was caught with the D-Vac, mainly from Hemiptera (39.7%), Hymenoptera (21.7%), Araneae (12.9%) and Coleoptera (9.0%). Concerning to yellow sticky traps, 29,255 individuals were caught, mainly from Hemiptera (68.3%), Hymenoptera (24.2%) and Coleoptera (5.8%).

Although a higher abundance and richness of several beneficial groups was found in NCH (i.e. Coccinellidae, Araneae and parasitoids), the positive impact of NHC on vineyards located on their proximity was only found for the Coccinellidae. On the other hand, vegetation present in slopes or on horizontal alleys have benefited particularly Araneae and parasitoids, although in the case of Myrmecidae it was found that their abundance was also related with the abundance of Cicadellidae, their main host.

Our results highlight the importance of maintaining NCH on the neighborhood of vineyards, to enhance functional biodiversity of this agroecosystem. Also, important from this point of view, seems to be the slopes, that despite being a cultivated habitat, are subjected to a low intensity management. These findings emphasize the importance of maintaining both areas, NCH and slopes with vegetation, in such vineyards, for supporting functional diversity and assist in conservation biological control strategies.

**Keywords:** Predators, Parasitoids, ecosystem services, non-crop habitats, functional agrobiodiversity



## 2.1 Introduction

The 'Douro Demarcated Region' (DDR), where “Port” D.O.C. wine and others remarkably high-quality table wines are produced, covers an area of approximately 250,000 ha, of which 43,600 ha are planted with vines. It is characterized by many deep valleys and steep slopes along the Douro River and its tributaries, with schists soils, cold winters, hot summers and low rainfall. Despite the intensification practiced during the last thirty years, given the need to reduce operating costs and mitigate the harshness of the labor, the most representative and well-preserved part (about 10% of the total area), the “Alto Douro Vinhateiro” (ADV), was classified in 2001 as UNESCO World Heritage Site, for its unique character as an 'evolved continuing cultural landscape'. A significant part of this area is still occupied by non-crop habitats, specially by scrublands (20.4%) and woodlands (13.2%). Moreover, in the area covered by terraced vineyards (42.9%), a significant part is occupied by grassy/shrubby slopes and/or dry-stone walls (Andresen and Rebelo 2013).

The simplification of cultivated landscapes is particularly acute in wine grape regions as the geographic branding wine further encourages regional land use conversion from natural habitats to high-value grape production. The loss of both agrobiodiversity and natural habitats that surround agroecosystems can lead to the loss of multiple ecosystem services, including biological control (Miles et al. 2012), that was estimated worldwide, in 1997, in approximately US\$410 billion per year (Costanza et al. 1997). Thus, populations of arthropod pests may proliferate in vineyards, due to lack of habitat and/or food resources required for enhancing the impact of their natural enemies.

Because of the increased regulation of pesticides in Europe and the concerns about the environmental impacts of viticulture, research about ecological-based pest management strategies, has attracted increasing interest in recent decades in particular about those aimed at promoting conservation biological control of pests (e.g. Thomson and Hoffmann, 2009 and references therein). However, and while it is known that the improvement of wild plant diversity in vineyards may sustain higher landscape biodiversity, providing refuge and food source for several vertebrates and arthropods, including those that are beneficial for pest control (Sanguankee and León, 2011), to our knowledge little research has been done on how vineyard management, as well as adjacent semi-natural habitats, affects different arthropod's

communities. In particular, few studies have been conducted about the significance, from this point of view, of terraced vineyard.

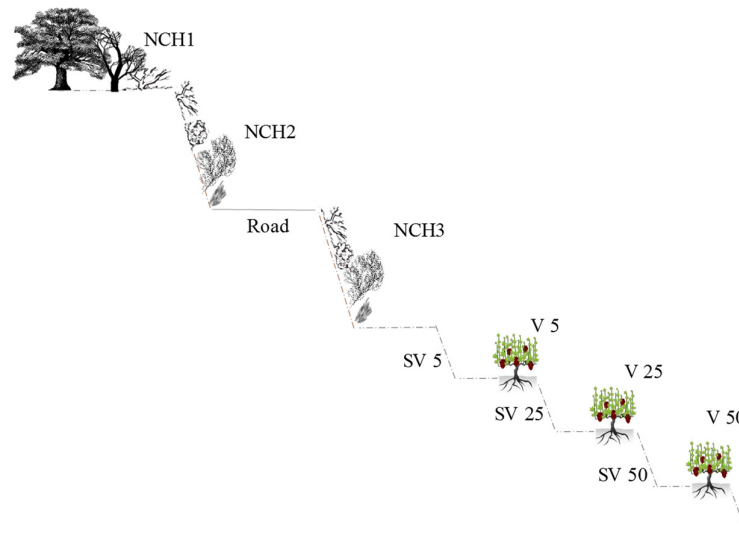
Considering the need of preserving non-crop habitats existing in DDR, and the increasing interest of local farmers for conservation biological control strategies, this study aimed at evaluating the impact of NCH adjacent to vineyards, as well as of the vegetation of terraced vineyards on the overall biodiversity of arthropods, particularly on natural enemies (predators and parasitoids) and on main pest of DDR vineyards ecosystem. Therefore, we hypothesize that the presence of NCH and vegetation of terraced vineyards have a positive impact on arthropods diversity, including on natural enemies of pests in the context of increasing functional biodiversity of the agroecosystem.

## **2.2 Material and methods**

### **2.2.1 Study sites**

The study was carried out in 2010 in three terraced vineyard agroecosystems, located in the DDR. They were chosen because they had, in their neighborhood, woodland and/or scrubland lots, thereafter designated non-crop habitats (NCH). The corresponding agroecosystem (vineyard of *c.v. Touriga Nacional* plus neighborhood non-crop habitats) will be thereafter referred as sites A, B and C, located respectively at quinta das Carvalhas (S. João Pesqueira county), quinta de S. Luiz (Tabuaço county) and Quinta do Seixo (Tabuaço county).

At site A (41°10'47''N, 7°32'09''W) the vineyard was 8-years old, was set on one row terraces and North East faced. It was bordered by a Mediterranean woodland of the type *Rusco aculeati Querceto suberis viburnetosum tini* (NCH1); moreover, there were also a slope (NCH2) mainly covered with shrubs and herbaceous species and a second slope (NCH3) mainly covered by shrubs, herbaceous vegetation and some dispersed trees (Fig. 2.1).



**Figure 2.1** General characteristics of site A agroecosystem (NCH- Non-crop habitats; SV-Slope vegetation; V- Vineyard).

At site B (41°09'08''N, 7°37'04''W) the vineyard was 11-years old, was set on one row terraces and West faced. It was bordered by NCH characterized by the combination of a Mediterranean scrubland of the type *Erico arboreae arbutetum unedonis viburnetosum tini* and by an herbaceous border just located near the vineyard edge. Inside vineyard, some dispersed shrubs were left on slopes.

At site C (41°10'15''N, 7°33'05''W) the vineyard was 10-years old, was set on two row terraces and East faced. It was bordered by NCH which formerly was a cropped area (vineyard and olive production until 1980), being occupied mainly by scrubland dominated by *Rubus ulmifolius*, combined with an herbaceous cover.

All vineyards were conducted under Integrated Production Guidelines. Weeds under vines were controlled through one application of herbicide at the end of winter (glyphosate plus oxyfluorfen). The vegetation of slopes and ground cover between vines was mowed twice mechanically, first in March and later in June/July.

Landscape composition around each site was calculated within a GIS framework, with a 200-m radius, resulting in different proportions of land use categories. The elements woodland / forest, scrubland / shrubby slopes, riparian gallery, water elements and orchards and vegetable gardens, when conducted extensively, were considered as part of ecological infrastructures (EI).

### 2.2.2 *Flora and arthropods assessments*

Plant and arthropod communities were assessed in both non-cropped and vineyards habitats in three replicate sampling stations, each separated by a minimum of 50 m. In vineyards, assessments of *arthropods* were done in vine canopies, as well as in the ground cover of the slopes (sites A and B) or in the horizontal alleys (site C), at 5, 25 and 50 m from NCH. Vineyards *flora* was assessed in the ground cover of the slopes (sites A and B) or in the horizontal alleys (site C), at the same distances.

The number of sampling stations assessed by site varied between 21, at sites B and C (3 in NCH plus 18 in vineyards habitats) and 27, at site A (9 in NCH plus 18 in vineyards habitats). This means that, in total, 69 sampling points were assessed on the three sites. Each habitat assessed will be designated: non-crop habitat (NCH), slope vegetation (SV), ground cover vegetation (GC) and vines (V).

Vegetation was assessed twice, in late May and in early September, using the phytostuctural method (Crespí et al. 2005). However, as assessments of arthropods were done during summer period, only the later was considered for further analysis. In each sampling station and site, all vascular plant species were inventoried in a surface of 4 m<sup>2</sup>. Moreover, the percentage of herbaceous cover, fresh cover, and total cover (fresh plus dry) was recorded.

Arthropods were accessed through two sampling methods: by suction and with yellow sticky traps. According to Yi et al. (2012), the combination of different methods is highly recommended for comprehensively sampling of larger taxa, where different species often vary strongly in their behavior and ecological niche.

For *suction sampling*, a Dietrick Vacuum insect net (D-Vac) machine (Rincon-Vitova Insectaries, Inc., Ventura, CA, US, model 122) was used during one minute along a transect of 20 m; the procedure was repeated at each sampling station. At sites A and B, as the access to slopes was easier, suction sampling was performed on slopes; at site C, since vineyards were installed in two rows, suction was performed only on vegetation present in the horizontal alley. Arthropods caught were conserved in identified recipients containing diethyl ether and conducted to laboratory for identification. Samplings were repeated three times from July to September (July 28<sup>th</sup>, August 26<sup>th</sup> and September 28<sup>th</sup>).

*Yellow sticky traps* consisted in a 20.0 cm x 24.5 cm plastic rectangle with sticky surface, which were vertically hanged in trees, shrubs or vineyards, according the sampling point, 1.30



m above the ground, during a week period. This procedure was performed three times, between June and August (June 24<sup>th</sup>, July 21<sup>st</sup> and August 26<sup>th</sup>).

Under a stereoscopic microscope, all collected arthropods were sorted to the morphospecies level and identified at the taxonomic level of order or family level, based on literature (Triplehorn and Johnson 2005). Main morphospecies caught were later identified by taxonomists. Each taxon was further classified into functional trophic groups (predators, parasitoids, herbivores, omnivores, detritivores, or pollen/nectar feeders). Because spiders are one of the most abundant predators in vineyards (Costello and Daane 1999) and due to their close relationship to their habitats, those collected by suction sampling were studied in great depth since individuals caught in yellow sticky traps were too damaged to allow their identification. Thus, Araneae was classified in eight guilds, following Cardoso et al. (2011) based on their foraging strategy (type of web and hunting method), prey range (stenophagous or euryphagous), vertical stratification (ground or foliage) and circadian activity (diurnal or nocturnal) in: (1) sensing web weavers, (2) sheet web weavers, (3) space web weavers, (4) orb web weavers; (5) specialists; (6) ambush, (7) ground hunters, and (8) other hunters.

### ***2.2.3 Data analysis***

To compare differences in vegetation cover among habitats within each site, the richness of plants and the percentage of herbaceous cover, fresh cover and total cover (fresh plus dry), was analyzed by a nonparametric Kruskal–Wallis test, followed by the multiple comparison mean ranks by Fisher's LSD, as described by Marôco (2011).

Abundance (N) and richness (S) of arthropods found in each habitat were calculated. In a first step, Generalized Linear Models (GzLM) were used to test for differences in abundance and richness of arthropods between farms, sampling dates and habitats. To uniform data, for this analysis, woodland slope (NCH2) and road slope (NCH3) habitats from site A were not included. Due to the geographical differences and different habitats accessed in each site, the analysis of the impact of non-crop habitats on the abundance and richness of arthropods was performed individually in each farm.

Separated models were applied to each sampling methods (suction and yellow sticky traps) for total arthropods, main functional groups (predators, parasitoids and herbivores), for each functional group, main orders, families or species. Dependent variables were analyzed

fitting a Normal, Poisson or Negative Binomial error, according with their distribution. In some cases, data was log-transformed to achieve normality. Pairwise comparison of estimated marginal means was used to estimate significant differences between modalities and a least significant difference test was used to separate them. Data of arthropods found in vineyard canopy at the harvest time was also analyzed through GzLM.

Spearman rank correlations were used to determine the significance of relationship between abundance and richness of arthropods (both trophic groups and taxonomic taxa) and the vegetation parameters (percentage of herbaceous cover, total cover and richness). As vegetation data was related to one sampling performed in September, for this analysis, total data of arthropods collected were pooled for each sampling point. All correlations were carried out as pairwise two-side tests.

To investigate to which extent Araneae move from NCH to the interior of vineyards, similarity between modalities in that assemblages collected by suction was analyzed. For each habitat, data collected by sampling point and sampling date were pooled to form one unique sample per habitat and farm. Similarity was calculated in Estimate S (version 9.1.0) (Colwell 2013) and using Chao Jaccard abundance-based estimator, an index that reduce bias, due to sample size and include the effect of unseen shared species (Chao et al. 2005). Then, a Multidimensional scaling (MDS) was performed using Proxscal algorithm. To perform MDS, a preliminary 22-dimensional analysis was run and then repeated using three dimensions, as suggested by the analysis of the scree plot and the plots transformed proximities vs distances from preliminary analysis (Marôco 2011). The goodness of fit was evaluated through STRESS-I and DAF (Dispersion Accounted For) using the reference values defined by Marôco (2011). DAF is equivalent to  $R^2$  and is the amount of variance which is explained by the three dimensions. The models, correlations and MDS were fitted using IBM SPSS version 20.0 (Armonk, NY: IBM Corp.). Significance was reported at the level of  $p < 0.05$ . Means and standard errors are shown in text, tables and/or figures.

## 2.3 Results

### 2.3.1 Flora

At site A, 59 species were identified belonging to 29 families. The most frequent families were: Asteraceae (32.3%), Rosaceae (6.5%), Ericaceae (5.9%) and Poaceae (5.9%). At site B, 36 plant species were identified, belonging to 24 families. The more frequent families were: Asteraceae (39.5%), Apiaceae (13.2%) and Fabaceae (9.3%). At site C, 24 species were identified, belonging to 14 families. The most frequent families were Asteraceae (45.1%), Poaceae (17.6%) and Apiaceae (14.7%) (Table 2.1).

The only site in which significant differences were found between vegetation parameters was site A, where vegetation richness was significantly higher in NCH than in vineyard slopes ( $\chi^2_{\text{KW}} = 20.32$ ,  $p=0.009$  for richness of plants). The same trend was found, generally, for the percentage of herbaceous cover ( $\chi^2_{\text{KW}} = 16.91$ ,  $p=0.031$ ), for the percentage of fresh cover ( $\chi^2_{\text{KW}} = 18.96$ ;  $p=0.015$  and for the percentage of total cover ( $\chi^2_{\text{KW}} = 20.75$ ;  $p=0.008$ ) (S 2 – Table 2.1).

**Table 2.1** Plant species most frequently found in each studied site and habitat (average percentage of frequency at each sampling station, n = 3).

Site	Habitat	Species most frequently observed
A	NCH1	<i>Arbutus unedo</i> , <i>Ruscus aculeatus</i> (1.0); <i>Asplenium onopteris</i> , <i>Crataegus monogyna</i> , <i>Erica arborea</i> , <i>Quercus x coutinhoi</i> , <i>Hedera hibernica</i> , <i>Rubus ulmifolius</i> , <i>Teucrium scorodonia</i> (0.7)
	NCH2	<i>Cistus salvifolius</i> , <i>Erica arborea</i> (1.0); <i>Andryala integrifolia</i> , <i>Arbutus unedo</i> , <i>Cytisus striatus</i> , <i>Rubus ulmifolius</i> (0.7)
	NCH3	<i>Cytisus striatus</i> , <i>Eschscholzia californica</i> (1.0); <i>Cistus salvifolius</i> , <i>Holcus lanatus</i> (0.7)
	SV 5	<i>Hedera hibernica</i> , <i>Pistacia terebinthus</i> , <i>Sedum album</i> , <i>Umbilicus rupestris</i> (0.5)
	V 5	<i>Hypochoeris radicata</i> , <i>Polygonum aviculare</i> (1.0); <i>Dittrichia viscosa</i> (0.7)
	SV 25	<i>Galium mollugo</i> , <i>Ortega hispanica</i> (0.7)
	V 25	<i>Chondrilla juncea</i> , <i>Conyza sumatrensis</i> , <i>Hypochoeris radicata</i> (0.7)
	SV 50	<i>Dittrichia graveolens</i> (1.0); <i>Conyza arvensis</i> , <i>Conyza sp.</i> , <i>Hypochoeris radicata</i> , <i>Polygonum aviculare</i> (0.7)
B	V 50	<i>Andryala integrifolia</i> , <i>Conyza arvensis</i> , <i>Hypochoeris radicata</i> , <i>Dittrichia graveolens</i> (1.0)
	NCH	<i>Rubus ulmifolius</i> (1.0); <i>Arbutus unedo</i> , <i>B. dioica</i> , <i>M. ciliata</i> , <i>Phillyrea angustifolia</i> , <i>Quercus rotundifolia</i> , <i>Ruscus aculeatus</i> (0.7)
	V 5	<i>Chondrilla juncea</i> , <i>Conyza sumatrensis</i> , <i>Cynodon dactylon</i> , <i>Daucus carota</i> (0.7)
	SV 5	<i>Daucus carota</i> (1.0); <i>Andryala integrifolia</i> , <i>Lactuca virosa</i> (0.7)
	V 25	<i>Daucus carota</i> (1.0); <i>Conyza sumatrensis</i> , <i>Hypochoeris radicata</i> , <i>Lactuca virosa</i> (0.7)
	SV 25	<i>Andryala integrifolia</i> , <i>Bituminaria bituminosa</i> (1.0); <i>Daucus carota</i> (0.7)
	V 50	<i>Conyza sumatrensis</i> (1.0); <i>Lactuca virosa</i> (0.7)
	SV 50	<i>Andryala integrifolia</i> , <i>Bituminaria bituminosa</i> (1.0); <i>Chondrilla juncea</i> , <i>Daucus carota</i> (0.7)
C	NCH	<i>Ailanthus altissima</i> , <i>Rubus ulmifolius</i> , <i>Conyza sumatrensis</i> , <i>Daucus carota</i> , <i>Salix atrocinerea</i> , <i>Vitis vinifera</i> (0.7)
	V 5	<i>Conyza sumatrensis</i> (1.0); <i>Conyza bonariensis</i> , <i>Digitaria sanguinalis</i> , <i>Hypochoeris radicata</i> , <i>Lactuca virosa</i> (0.7)
	GC 5	<i>Daucus carota</i> (1.0); <i>Andryala integrifolia</i> , <i>Conyza sumatrensis</i> , <i>Digitaria sanguinalis</i> , <i>Lactuca virosa</i> (0.7)
	V 25	<i>Conyza sumatrensis</i> , <i>Daucus carota</i> (1.0); <i>Conyza bonariensis</i> , <i>Lactuca virosa</i> (0.7)
	GC 25	<i>Daucus carota</i> (1.0); <i>Andryala integrifolia</i> , <i>Bituminaria bituminosa</i> , <i>Conyza sp.</i> , <i>Digitaria sanguinalis</i> , <i>Hypochoeris radicata</i> (0.7)
	V 5	<i>Conyza bonariensis</i> , <i>Conyza sumatrensis</i> , <i>Daucus carota</i> , <i>Digitaria sanguinalis</i> (0.7)
	GC 50	<i>Bituminaria bituminosa</i> , <i>Daucus carota</i> (0.7)

Habitats: NCH- Non-crop habitat; V- vineyard; SV- slope vegetation; GC-Ground cover in the horizontal alley. 5, 25 and 50 indicates the distances (m) from the NCH

### 2.3.2 Arthropods

#### Suction sampling

Across all sites, 6,923 arthropods were collected. These were represented by Insecta (83.7%), Arachnida (16.3%) and Malacostraca (0.01%). The main taxon caught were, by decreasing order of abundance: Hemiptera (39.7%), Hymenoptera (21.7%), Araneae (12.9%), Coleoptera (9.0%), Diptera (8.3%), Acari (3.4%) and Thysanoptera (3.0%). Hemiptera were

grouped into 22 families, from which the most abundant were Cicadellidae (35.9%), Aleyrodidae (30.5%) and Lygaeidae (8.6%). Hymenoptera, included 25 families from which the most abundant were Platygasteridae (25.5%), Formicidae (17.2%), Mymaridae (14.8%) and Eulophidae (12.2%). Parasitic Hymenoptera were mainly represented by Platygasteridae, Mymaridae, Eulophidae and Encyrtidae, which totalized altogether close to 74.0% of the parasitic Hymenoptera assemblage.

Araneae were grouped into 12 families, from which the most abundant were, by decreasing order: Dictynidae (16.0%), Salticidae (15.7%), Thomisidae (13.5%), Oxyopidae (11.9%) Araneidae (10.5%), Theridiidae (6.2%) and Philodromidae (3.0%). Ecological guilds of Araneae were by decreasing order of abundance: other hunters (Salticidae, Philodromidae, Miturgidae, Linyphiidae and Oxyopidae) (30.2%), space web weavers (Dictynidae and Theridiidae) (27.2%), ambush hunters (Thomisidae) (12.9%), orb web weavers (Uloboridae and Araneidae) (8.6%), ground hunters (Gnaphosidae) (1.6%), and sheet web weavers (Pisauridae) (1.4%).

Coleoptera were grouped into 18 families, being the most abundant: Coccinellidae (43.2%) in which *Scymnus* sp. dominated (87.4% of the total assemblage of this family), Chrysomelidae (19.5%), and Curculionidae (18.0%). Although in a lower number, Anthicidae (*Anthicus* sp.), Carabidae (*Dromius* (*Dromius*) *meridionalis* Dejean), Cleridae (*Dasytes* sp.) and Malachiidae were caught, representing altogether less than 6% of the Coleoptera.

According their trophic status, arthropod assemblage was dominated by herbivorous (46.7%), followed by predators (18.1%), parasitoids (17.6%), detritivores (4.8%), omnivorous (3.9%) and pollen/nectar feeders (0.2%). The most abundant group of natural enemies found were parasitic Hymenoptera (17.5% of arthropods assemblage), Araneae (12.9%) and Coccinellidae (3.9%), both predators.

Although suction sampling method caught a lower number of arthropods than sticky traps, the specimens caught were in better conditions for further classification, allowing to calculate their richness. For this reason, most of the analysis on the impact of habitats was done in data collected by suction sampling and the catches from sticky traps were only considered on the study of the dynamic of Coccinellidae and *Empoasca vitis* (Goethe).

### *Yellow sticky traps*

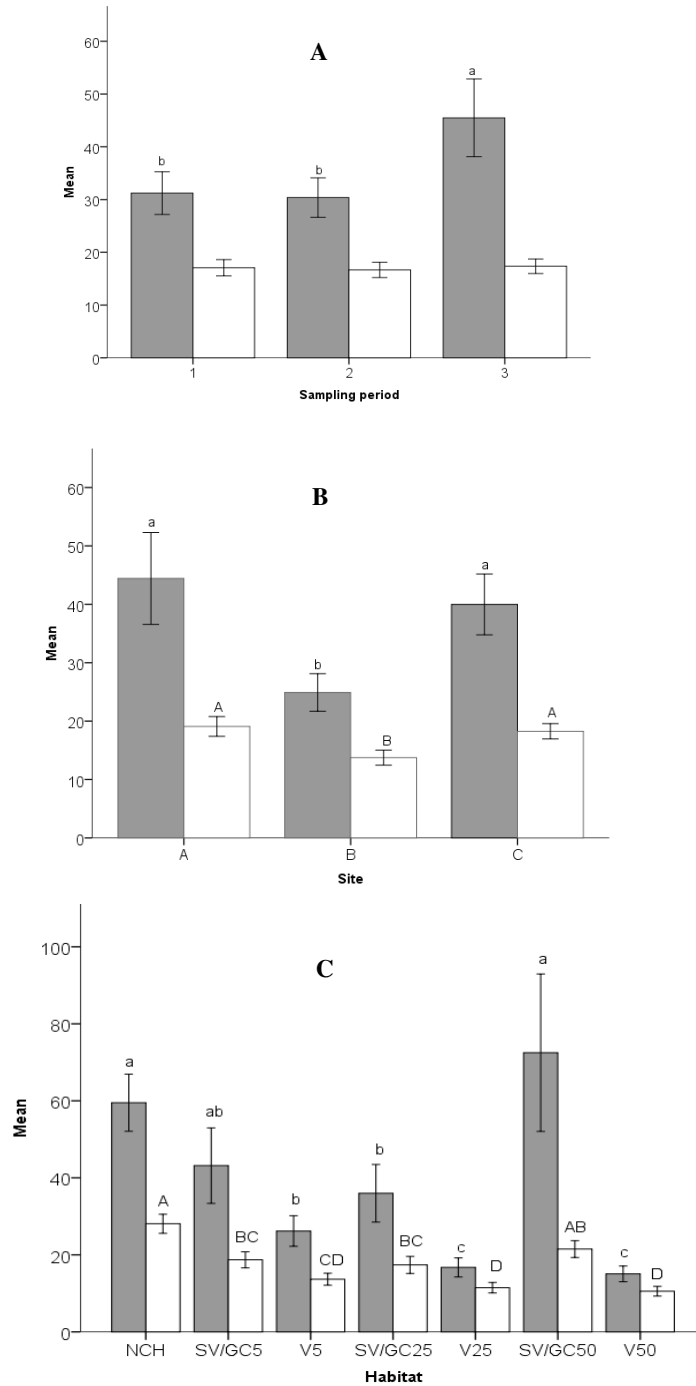
A total of 29,255 individuals was collected, mostly belonging to Insecta (99.4%). The assemblage was dominated by Hemiptera (68.3%), followed by Hymenoptera (24.2%) and by Coleoptera (5.8%). Araneae, Syrphidae, Chrysopidae, Trichoptera and Lepidoptera were caught in very low numbers, representing altogether less than 2.0% of the assemblage. Hemiptera were mainly represented by Cicadellidae (98.3% of the total) from which 26 species were identified. The most abundant were *E. vitis* (88.2% of the Cicadellidae) and *Neotalitrus fenestratus* (Herrich-Schäffer) (7.1%). Several other Hemipteran families were caught, belonging to Delphacidae, Issidae, Aphrophoridae, Cixiidae (including *Hyalesthes obsoletus* Signoret), and Dytiscidae (including Dictyophara (Dictyophara) europaea (Linnaeus, 1767)). Most Hymenoptera caught belonged to Chalcidoidea (89.4%), and were too damaged to allow further identification. Within Coleoptera, the most abundant families were Coccinellidae (59.8%), Carabidae (18.8%), Malachidae (5.8%) and Staphylinidae (2.3%). According to their trophic status, arthropod assemblage was dominated by herbivorous (68.3%) and by parasitoids (22.7%).

### **2.3.3 Effect of sampling period, site and habitat on arthropods assemblage**

Arthropod's abundance was significantly higher in the third sampling period (September 28<sup>th</sup>), than at any other of the two sampling dates (i.e. July 28<sup>th</sup> and August 26<sup>th</sup>) (Wald  $\chi^2 = 9.33$ ,  $p = 0.009$ ); however, richness did not differ significantly between these dates (Wald  $\chi^2 = 4.91$ ,  $p = 0.086$ ) (Figure 2.2A).

Arthropods were significantly more abundant at sites A and C, compared to site B (Wald  $\chi^2 = 11.77$ ,  $p = 0.003$ ), a pattern also found for richness (Wald  $\chi^2 = 12.89$ ,  $p = 0.002$ ) (Figure 2.2B).

Concerning the assessed habitats both, arthropods abundance (Wald  $\chi^2 = 98.99$ ,  $p < 0.001$ ) and richness (Wald  $\chi^2 = 79.07$ ,  $p < 0.001$ ) were statistically higher in NCH and in vegetation of slopes /horizontal alleys located at 50 m from NCH (SV50/ GC50) than in vine canopies located at higher distances (V25 and V50). However, none of these metrics differed significantly from the obtained either on slopes or in ground cover sampling stations located at 5 m from NCH (SV5/ GC5) (Figure 2.2C).



**Figure 2.2** Mean ( $\pm$ S.E.) of abundance (grey bars) and richness (white bars) of arthropods collected by suction sampling, in each sampling period (A), site (B) and habitat (C). Statistical significance was based on marginal estimated means for  $p < 0.05$ . Legend: NCH – woodland and/or scrubland lots; SV – slope vegetation; GC 5, 25 and 50 – ground cover vegetation at, respectively, 5, 25 and 50 m from NCH; V5, 25 and 50 – vine canopy at respectively, 5, 25 and 50 m from NCH

### 2.3.4 Effect of the distance from the NCH and the vegetation, on arthropod's abundance and richness

#### *Herbivores*

The main herbivores caught belonged to Hemiptera, which were mainly represented by Cicadellidae, particularly *E. vitis* but also *N. fenestratus*.

At site A, the abundance of Cicadellidae, and particularly, of the green leafhopper, *E. vitis*, was, higher in vine canopies located at the higher distance from NCH (V50), than in NCH or than in vine canopies located at 25 m from NCH (Wald  $\chi^2=22.583$ ,  $p<0.001$ ) (S2 – Table 2.7). However, richness did not differ significantly between habitats (Wald  $\chi^2=5.333$ ,  $p=0.255$ ). In the same site, abundance of Cicadellidae, as a whole, ( $R_{ho}=0.55$ ,  $p<0.05$ ) and *E. vitis* ( $R_{ho}=0.58$ ,  $p<0.05$ ), were positively correlated with the percentage of herbaceous cover. At site B, the richness of Cicadellidae was positively correlated with the richness of cover ( $R_{ho}=0.52$ ,  $p<0.05$ ) (S2 – Table 2.3).

#### *Parasitoids*

At site A, the abundance of parasitoids was higher in NCH2 and in vineyard slopes located at 50 m from NCH (SV 50) than in the vine canopies located at 25 m (V25) or 50 m (V50) from NCH area (Wald  $\chi^2 = 31.345$ ,  $p<0.001$ ); however, their abundance in slopes located at 50 m from NCH (SV50) did not differ from that observed in all NCH (NCH 1, 2 and 3) nor slopes located at 25 m from NCH (Wald  $\chi^2 = 31.35$ ,  $p<0.001$ ). Particularly, the abundance of Eulophidae was significantly higher in NCH2 than in any habitat located in vineyard, with exception of slopes located at 25 m from NCH (Wald  $\chi^2=27.981$ ,  $p<0.001$ ). The richness of parasitoids was higher in NCH3, than in vine canopies located at 50 m from NCH area (Wald  $\chi^2 = 24.729$ ,  $p=0.002$ ) (S2 – Table 2.4).

The abundance and richness of parasitoids caught in site A was positively correlated with the percentage of ground cover ( $R_{ho}=0.39$ ,  $p<0.05$ , for abundance, and  $R_{ho}=0.48$ ,  $p<0.05$  for richness). Concerning to site B, no differences were found either in the abundance or in the richness of parasitoids, between any habitat assessed (Wald  $\chi^2 = 5.541$ ,  $p=0.477$  for abundance; Wald  $\chi^2 = 7.280$ ,  $p=0.296$  for richness). However, their richness was positively correlated with percentage of total cover ( $R_{ho}=0.49$ ,  $p<0.05$ ) (S2 – Table 2.3).



At site C, the abundance of parasitoids was significantly higher in ground covers located at 50 m from NCH (GC 50) than in vine canopies located at the same distance (V50) (Wald  $\chi^2 = 37.10$ ,  $p < 0.001$ ); however, their abundance did not differ between ground covers located at 25 m from NCH (GC 25) or NCH itself. Particularly, the abundance of Eulophidae was significantly higher in NCH than in any habitats located in their proximity; however, it did not differ significantly from vine canopies or ground covers located at 50 m from NCH (Wald  $\chi^2 = 23.497$ ,  $p = 0.001$  for site C). Richness of parasitoids was significantly higher in NCH than in any other habitat assessed, with exception of ground covers located at 50 m from NCH (GC50) (Wald  $\chi^2 = 41.043$ ,  $p < 0.001$ ) (S2 – Table 2.6).

At site A, Mymaridae were more abundant in the vegetation of slopes at the distance of 25 m from NCH (SV 25) than at any other sampled habitat, except for slopes in the distance of 50 m from NCH (SV50) (Wald  $\chi^2 = 26.198$ ,  $p < 0.001$ ) (S2 – Table 2.4). At site B, no significant differences were found between habitats. At site C, a significantly higher abundance was found in ground covers located at 50 m from NCH (GC50) than in NCH (Wald  $\chi^2 = 37.464$ ,  $p < 0.001$ ) (S2 – Table 2.6).

### *Predators*

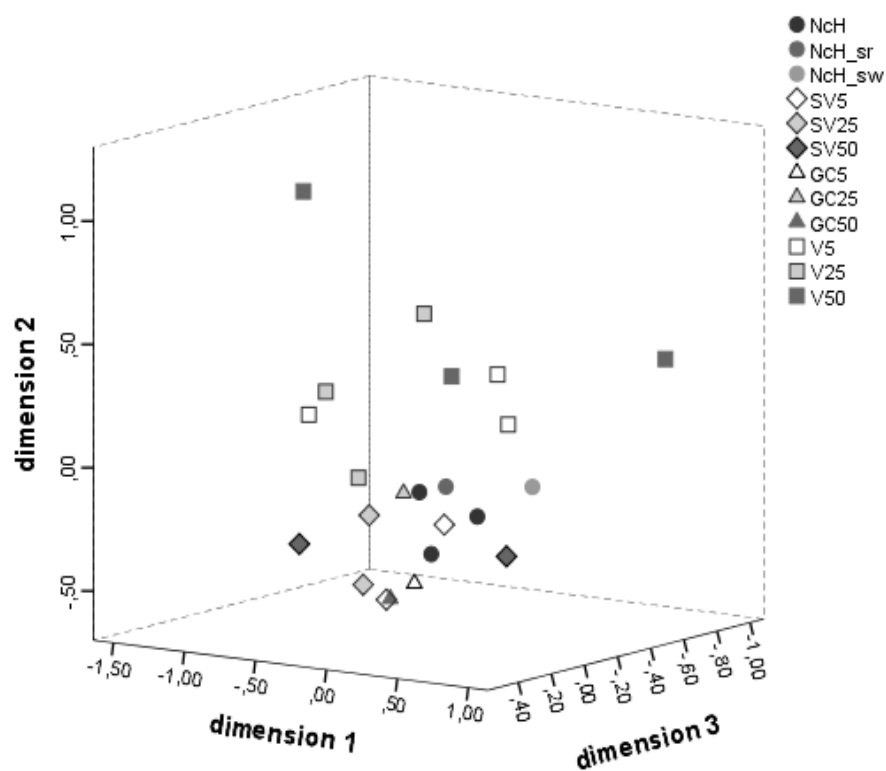
The main predators caught in our study were Araneae and Coccinellidae. The abundance of Coccinellidae at site A was significantly higher both in the shrubby/weedy margin of NCH2 and in vine canopies located near this margin (V5), than either in vine canopies located at higher distances from NCH area (V 25 and V 50) or in NCH1 (Wald  $\chi^2 = 27.364$ ,  $p < 0.001$ ) (S2 – Table 2.7). No significant differences were found concerning their richness (Wald  $\chi^2 = 9.103$ ,  $p = 0.059$ ). At site B, the abundance of Coccinellidae was significantly higher in NCH and in vine canopies located near NCH (V5), than in vine canopies located at higher distance of NCH (V 25 and V 50) (Wald  $\chi^2 = 52.433$ ,  $p < 0.001$ ). Richness was higher in both NCH and vine canopies located at lower distance from the edge (V5) than in vine canopies located at higher distances (V25 or V50) (Wald  $\chi^2 = 30.286$ ,  $p < 0.001$ ) (S2 – Table 2.8). At this site, the abundance of Coccinellidae was positively correlated with both the percentage of cover ( $R_{ho} = 0.71$ ,  $p < 0.05$ ) and the richness of vegetation cover ( $R_{ho} = 0.70$ ,  $p < 0.05$ ) (S2 – Table 2.3).

At site C, no differences could be found on either the abundance or the richness of Coccinellidae in any habitat assessed (S2 – Table 2.9) but their richness was positively correlated with the richness of vegetation cover ( $R_{ho} = 0.61$ ,  $p < 0.05$ ) (S2 – Table 2.3).

The abundance of Araneae was higher in NCH than in vine canopies located at 50 m from NCH (V50), in all sites; however, no significant differences were found between NCH and slopes / ground cover sampling stations located at the same distance (SV50 and GC50) (Wald  $\chi^2=42.781$ ,  $p<0.001$ , for site A; Wald  $\chi^2=32.589$ ,  $p<0.001$ , for site B; Wald  $\chi^2=17.475$ ,  $p=0.008$  for site C) (S2 – Tables 2.4, 2.5 and 2.6). In both sites A and B, Araneae richness was higher in NCH than in all vine canopies, but did not differ from vegetation of slopes (Wald  $\chi^2=34.438$ ,  $p=0.001$  for site A; Wald  $\chi^2=34.991$ ,  $p=0.001$  for site B) (S2 - Tables 2.4 and 2.5). At site B, both abundance and richness of Araneae were positively correlated with the percentage of ground cover ( $R_{ho}=0.75$ ;  $p<0.01$ , for abundance; ( $R_{ho}=0.71$ ;  $p<0.01$ , for richness) (S2 – Table 2.3).

#### *Similarity between communities of Araneae*

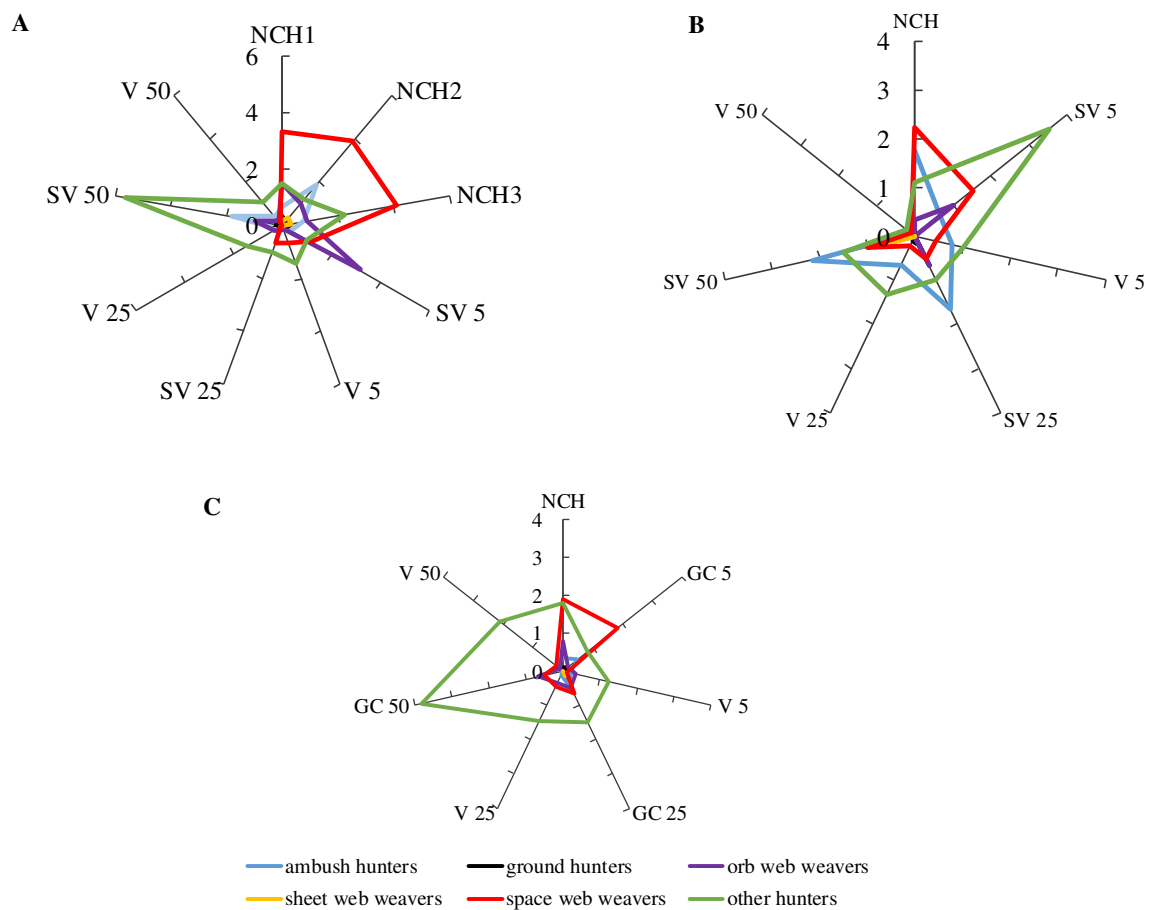
The ordination analysis using Chao-Jaccard similarity index on data of Araneae caught in all sites and habitats (Fig. 2.3), showed that there is similarity in spider's community among the different habitats ((STRESS-I = 0.168 (fair) and D.A.F = 0.972 (very good)). Thus, the communities found on non-crop habitats and on the vegetation of slopes and/or ground cover were, in general, more similar between each other, than with those found in the vineyard, which suggest a limited spillover from communities found in NCH or cover vegetation to vine canopy.



**Figure 2.3** Multidimensional scaling ordination based on Chao-Jaccard similarity index between spider communities collected by suction in the different farms and habitats (STRESS-I = 0.168 (fair); D.A.F = 0.972 (very good)) (NCH – woodland and/or scrubland lots; SV – slope vegetation; GC 5, 25 and 50 – ground cover vegetation at, respectively, 5, 25 and 50 m from NCH; V5, 25 and 50 – vine canopy at, respectively, 5, 25 and 50 m from NCH)

### *Analysis of functional guilds of Araneae*

The other hunter's guild (which include Salticidae, Phylodromidae, Miturgidae, Linyphiidae and Oxyopidae) were more abundant in vines and in slopes (at sites A and B) or in ground covers (at site C) than in NCH sampling stations, while the guild space web weavers (Dictynidae and Theridiidae) were found to be more associated with NCH (woodland, scrubland or sloppy habitats) (Fig. 2.4).



**Figure 2.4** Mean abundance of each functional guild of Araneae in each habitat and site. (NCH – woodland and/or scrubland lots; SV – slope vegetation; GC 5, 25 and 50 – ground cover vegetation at, respectively, 5, 25 and 50 m from NCH; V5, 25 and 50 – vine canopy at, respectively, 5, 25 and 50 m from NCH)

## **2.4 Discussion**

### **2.4.1. Flora**

With exception of site A, in which we found a higher richness and percentage of cover in NCH than in slopes of vineyards, the differences found among sampling stations were not significant. According to Barberi (2010), some of the plant families identified in each site (e.g. Apiaceae, Asteraceae and Fabaceae) could play an important ecological role, by nurturing a complex of beneficial arthropods.

### **2.4.2 Effect of sampling period, site and habitat on arthropods**

#### *Sampling period*

The higher abundance of arthropods found in the third sampling period (September 28<sup>th</sup>) compared to the other two, may have been related with a combined effect of climate conditions and composition and quality of ground cover, especially because, between the first and the second sampling dates (i.e. July-August), a significant amount of vegetation became dried, due to the high temperatures and lack of rainfall registered. According to Frampton and Dorne (2007), drought has been found to negatively affect the abundance of arthropods on farmland through changes in vegetation structural complexity. Also, as reviewed by Pérez-Bote and Romero (2012), strong seasonality is a feature of most ecosystems, particularly in Mediterranean habitats, where the seasonal fluctuations of temperature and rainfall create marked pulses of productivity and animal activity.

#### *Site*

The higher abundance and richness found in sites A and C, compared to site B, were probably related with the different landscape composition around each site. The proportion of ecological infrastructures (EI) surrounding the vineyard plot represented up to 33.9% of the total surface categorized at site A, 24.4% at site B and 11.8% at site C (data not showed). Site A contained a higher percentage of EI around the vineyard plot. Although site B contained a higher percentage of EI than site C, the existence of a riparian gallery near this last may have influenced favorably in its abundance and richness. This positive influence of riparian galleries

on abundance and diversity of arthropods was reported by Smith et al. (2015) and Landis et al. (2000).

### *Habitat*

The high abundance and richness of arthropods found in NCH and vegetation of slopes (sites A and B) and/or ground cover of the horizontal alley (site C) is discussed in more detail in the next point.

### **2.4.3 Effect of distance from NCH and vegetation on arthropod's abundance and richness**

#### *Herbivores*

Positive relations were found between vegetation cover and herbivore metrics. On the other hand, a high abundance of Cicadellidae was found, in site A, in vine canopies located at 50 m from NCH than in vine canopies located at 25 m from NCH. Although NCH have been reported as having alternative host plants for the polyphagous *E. vitis* (Bosco et al. 1996; Cerutti et al. 1991), Decante and van Helden (2006) found that surrounding landscape play a major role in its population dynamics. According to Bosco et al. (1997) and Cerutti et al. (1991), the proximity of a mixed forest could increase the presence of natural enemies inside the plot and thus decrease overall populations of the phytophage. Also, predators, whose abundance was found to be higher in NCH, may have limited the population of *E. vitis* in the border of the vineyards at site A. This result is also in line with the reported by Nicholls et al. (2001), who found that, in Californian vineyards, leafhopper exhibited density gradients with a tendency to reach higher numbers in the centers of the vineyards.

#### *Parasitoids*

A high abundance and richness of parasitoids as a whole, and of Eulophidae in particular, were found in NCH, and in vegetation of slopes/horizontal alleys located at higher distances from NCH (25 and 50 m); moreover, positive relations were found between parasitoid's metrics and vegetation cover. According to Nicholls et al. (2001), the abundance and diversity of entomophagous insects within a field depends on either the plant species composition of the surrounding vegetation, or the spatial extent of its influence on natural enemy abundance, which in turn is determined by the distance to which natural enemies disperse into the crop. In our

study, although the abundance and richness of parasitoids were high in NCH, we did not find that the proximity of these habitats resulted in a higher presence of them in vineyards located nearby, contrary to the findings of Thomson and Hoffmann (2009), who found that wooded vegetation adjacent to vineyards enhanced the abundance of hymenopteran parasitoids, including Eulophidae. Instead, we found that the abundance of overall parasitoids, and Eulophidae, was higher on NCH and on the vegetation of the slopes / ground covers inside vineyards, at higher distance from NCH.

The abundance of Mymaridae in vegetation of slopes/horizontal alleys located at 25 m from NCH point to their relationship with leafhoppers, their main hosts. This result is in line with Nicholls et al. (2001), who found that the abundance of these parasitoids followed the patterns of leafhoppers. Thomson and Hoffmann (2009) also found a negative impact of NCH on the abundance of Mymaridae.

Moreover, Cerutti et al. (1991) found that *Anagrus atomus* (Linnaeus) appears to overwinter in leafhopper eggs, mainly on roses and blackberries. This can, in part, explain the higher abundance and richness of mymarids found in site C, where a high presence of blackberries (*Rubus sp.*) and *E. vitis* was reported. The riparian gallery found close to the vineyard canopy located at the higher distance from the NCH (V50) could also have impacted their abundance. According to Smith et al. (2015), an increase in the abundance of several wasp families is found with riparian vegetation.

### *Predators*

Coccinellidae abundance and richness in vineyards were found to benefit from the proximity of NCH, as well as soil cover with vegetation, which is in line with the reported by Nicholls et al. (2001), Thomson and Hoffman (2013) and Smith et al. (2015). Woltz et al. (2012) observed that the abundance of Coccinellidae is more influenced by landscape characteristics (adjacent crop type) than by enhanced local resources (vegetation characteristics), although Burgio et al. (2004) reported that *Crataegus monogyna* (Rosaceae), *Salix sp.* (Salicaceae), *Daucus carota* (Apiaceae) and *Conyza canadiensis* (Asteraceae) are searched by Coccinellidae, referring that trees and shrubs species can provide shelter for adult ladybirds, mainly in late summer, when many crops are harvested. Results also point to a positive impact of both NCH and vegetation of slopes and/or horizontal alleys of terraced vineyards on Araneae abundance,

which is in line with the findings of previous studies. Thus, Costello and Daane (2003) found that spider density was higher in ground cover modalities. Caprio et al. (2015) also found that overall community of spiders (both abundance and richness) were positively correlated with grassland area, forest patch area, heterogeneous landscape, and negatively correlated with vineyard area, and that abundance of spiders significantly increased with scrubland area. Košulič and Hula (2013) reported that vineyard terraces can act as an important refuge for rare steppe species of spiders in the unified intensive agricultural landscape and that suitable conditions of vineyard terraces (steep slopes with exposed substrate, low vegetation structure, sparse vegetation coverage) enable many rare endangered species to occur. Franin et al. (2016) also found that weedy strips in field margins contained a high number of predators (spiders and ladybugs) referring that spiders also benefited from the presence of dry vegetation. However, and contrary to the reported by Hogg and Daane (2010), who found that abundance and diversity of spiders were significantly higher at the vineyard edge than in higher distance from woodland, we could not find a clear impact positive of NCH in vineyards located in their proximity. In our study both abundance and richness of Araneae were positively affected by vegetation present in the slopes and/or the horizontal alleys. Hence, these findings suggest, that in DDR terraced vineyards, both areas could act as an internal ecological infrastructure inside vineyards, although a limited spillover seemed to occur from communities found in either NCH or cover vegetation to vine canopy.

In the analysis of Araneae results, it is important to note that, as stated by Marc et al. (1999), they do not constitute a homogeneous functional group and on the contrary exhibit significant behavioral diversity in relation to their different predation strategies, their dispersal modes and their great ability to resist adverse ecological conditions.

In our study, the other hunter's guild was more abundant in vine's canopy and in slopes or ground covers than in NCH sampling stations. Araneae from this guild hunt freely or ambush and keep in the foliage and in the ground (Huang et al. 2014). Oberg et al. (2007) found that Linyphiidae family (which belong to the other hunters' guild), are particularly impacted by the surrounding landscape, while Hogg and Daane (2010) found that they dominated spider composition on aerial traps but were not abundant on the vines.

On the contrary, space web weaver's guild was found to be more associated with NCH. This is probably because they have specific spatial needs for web building and prey catching,



and an open and relatively simpler vegetation structure may limit them. So, web-building spiders are particularly dependent on vegetation to provide suitable web-attachment sites, since their webs are frequently three-dimensional and require multiple attachment sites, as reviewed by Rypstra and Carte (1995).

## **2.5 Conclusions**

Compared to many other wine-producing regions of the world, DDR still has relatively large amounts of semi-natural habitat fragments in the cultivated landscape, with emphasis on NCH and local vegetation of slopes, which were found in our study to have a positive impact on abundance and diversity of several groups of natural enemies of pests.

According to Bianchi et al. (2006), the diversity and density of these natural enemies, in agricultural landscapes may decline with increasing distance from NCH, and the average distance between NCH and fields may affect the timing of field colonization. Other studies have demonstrated a higher abundance and diversity of natural enemies in vineyards adjoining non-crop habitats (Gaigher and Samways 2010; Hogg and Daane 2010; Thomson and Hoffmann 2009; Thomson and Hoffmann 2010) and a gradually decreasing in their diversity with increasing distance from natural habitats.

In our study, although a higher abundance and richness of several beneficial groups was found in NCH (i.e. Coccinellidae, Araneae and parasitoids), the positive impact of NCH on vineyards located on their proximity was only found for the Coccinellidae. On the other hand, vegetation present in slopes or on horizontal alleys have benefited particularly Araneae and parasitoids, although in the case of Mymaridae it was found that their abundance was also related with the abundance of Cicadellidae, their main host.

Araneae were the predators more abundant in the studied sites, although a reduced spillover was found between vegetation cover and vine canopy, possibly since they found more suitable conditions (moderate climate and no pesticide application) in the vegetation than in vine canopy. Even so, they may play a role in conservation biological control of phytophagous, particularly those who spend part of their life in the soil, which should be investigated.

The diversity and abundance of parasitoids, in particular as regards to Eulophidae, were positively affected by both NCH and ground cover vegetation. However, in the case of Mymaridae, which are known as the main parasitoids of Cicadellidae, their abundance was

found to be related mainly with that of their host. Since it was found that the abundance of Cicadellidae also benefited from ground cover vegetation it is important to investigate habitat management strategies aimed at enhancing the impact of Mymaridae on the populations of the pest. From this point of view, it is considered interesting to evaluate the impact of the riparian corridors on the populations of the parasitoid.

As a whole, our results indicate that, in terraced vineyards, the local vegetation present in slopes / ground covers can act as valuable ecological infrastructure, whose judicious use increase the functional biodiversity of the farm, namely by potentially increasing the density of wild populations of natural enemies to enhance their impact on pests.

Böller et al. (2004), referring to the high potential for functional biodiversity of small scale terraced vineyards with a high botanical diversity of a green cover, states that by mowing the banks in alternating fashion and with lowest possible frequency, the flora can be converted into a plant community similar to that in meadows with low management intensity which contains several perennial plant species of value in fostering beneficial parasitoids and therefore this banks serve as an internal ecological infrastructure of the vineyard and face the grapevine at very short distance.

The enhancement of the impact of natural enemies on pests may provide an economic benefit by potentially reducing the need for chemical applications to control pests, as well as increasing the economic and environmental sustainability of the wine industry.

## **2.6 References**

- Andresen T, Rebelo J (2013) Assessment of the state of conservation of the property Alto Douro Wine Region - Evolutive and Living Cultural Landscape - Assessment Report. Porto: CIBIO UP/UTAD
- Barberi P, Burgio G, Dinelli G (2010) Functional biodiversity in the agricultural landscape: relationships between weeds and arthropod fauna. *Weed Research* 50, 388–401
- Bianchi FJJA, Booij CJH, Tschamntke T (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proc R Soc Lond B Biol Sci* 273: 1715-1727

- Böller EF, Häni F, Poehling H-M (eds.) (2004) Ecological infrastructures: Ideabook on functional biodiversity at the farm level. Temperate zones of Europe. Swiss Centre for Agricultural Extension and Rural Development, Switzerland, 212 pp
- Bosco D, Alma A, Bonelli S, Arzone A (1996) Phenology and within vineyard distribution of *Empoasca vitis* Goethe adults (Cicadellidae Typlocybinae). Redia LXXIX (1), 1–9
- Bosco D, Alma A, Arzone A (1997) Studies on population dynamics and spatial distribution of leafhoppers in vineyards (Homoptera: Cicadellidae). Ann. Appl. Biol. 130, 1–11
- Burgio G, Ferrari R, Pozzati M, Boriani L (2004) The role of ecological compensation areas on predator populations: an analysis on biodiversity and phenology of Coccinellidae (Coleoptera) on non-crop plants within hedgerows in Northern Italy. Bull Insect 57 (1): 1-10
- Caprio E, Nervo B, Isaia M, Allegro G, Rolando A (2015) Organic versus conventional systems in viticulture. Comparative effects on spiders and carabids in vineyards and adjacent forests. Agric Syst 136: 61-69
- Cardoso P, Pekár S, Jocqué R, Coddington JA (2011) Global Patterns of Guild Composition and Functional Diversity of Spiders. PLoS ONE 6(6): e21710. doi:10.1371/journal.pone.0021710
- Cerutti F, Baumgartner J, Delucchi V (1991) The dynamic of grape leafhopper *Empoasca vitis* Goethe populations in southern Switzerland and the implications for habitat management. Biocontrol Sci Technol 1: 177-194
- Chao A, Chazdon RL, Colwell RK, Shen T.J (2005) A new statistical approach for assessing compositional similarity based on incidence and abundance data. Ecology Letters 8:148-159
- Colwell RK (2013) EstimateS: Statistical estimation of species richness and shared species from samples, Version 9.1.0. <http://viceroy.eeb.uconn.edu/estimates/>
- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Suttonkk P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. Nature 387:253-60
- Costello MJ, Daane KM (1999) Abundance of spiders and insect predators on grapes in central California. J Arachnol 27: 531-538

- Costello MJ, Daane KM (2003) Spider and leafhopper (*Erythroneura* spp.) response to vineyard ground cover. *Envir Entomol* 32(5):1085-1098
- Crespí AL, Bernardos S, Castro A, Fernandes CP, Amich F (2005) Phytostructural characterization of several vegetation types in northern Portugal. II. The structural expressivity and the resistance of the vegetation. *Plant Biosyst* 139: 387-389
- Decante D, van Helden M (2006) Population ecology of *Empoasca vitis* (Goethe) and *Scaphoideus titanus* (Ball) in Bordeaux vineyards: influence of migration and landscape. *Crop Protection* 25 (7): 696-704
- Franin K, Barić B, Kuštera G (2016) The role of ecological infrastructure on beneficial arthropods in vineyards. *Span J Agric Res* 14 (1), e0303. doi: 10.5424/sjar/2016141-7371
- Frampton GK, Dorne JL (2007) The effects on terrestrial invertebrates of reducing pesticide inputs in arable crop edges: a meta-analysis. *J Appl Ecol* 44: 362-373
- Gaigher R, Samways MJ (2010) Surface active arthropods in organic vineyards, integrated vineyards and natural habitat in the Cape Floristic Region. *J Insect Conserv* 14: 595-605
- Hogg BN, Daane KM (2010) The role of dispersal from natural habitat in determining spider abundance and diversity in California vineyards. *Agric Ecosyst Environ* 135(4): 260-267
- Huang P-S, Lin H-C, Lin C-P, Tso I-M (2014) The effect of thinning on ground spider diversity and microenvironmental factors of a subtropical spruce plantation forest in East Asia. *Eur J Forest Res* 133 (5): 919-930
- Košulić O, Hula V (2013) Rare and remarkable spiders (Araneae) from vineyard terraces in Pálava region (South Moravia, Czech Republic). *Acta Univ Agric Silviculturae Mendelianae Brun* 61: 663-676
- Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu Rev Entomol* 45: 175-201
- Marc P, Canard A, Ysnel F (1999) Spiders (Araneae) useful for pest limitation and bioindication. *Agric Ecosyst Environ* 74: 229-273
- Marôco J (2011) *Análise estatística com o SPSS Statistics*. ReportNumber – *Análise e Gestão de informação*, 5ª edição, 990 pp
- Miles A, Wilson H, Altieri M, Nicholls C (2012) Habitat Diversity at the Field and Landscape Level: Conservation Biological Control Research in California Viticulture. In Bostanian

- NJ Vincent C, Isaacs R (eds.) *Arthropod Management in Vineyards: Pests, Approaches, and Future Directions*, Springer, New York pp159-189
- Nicholls CI, Parella M, Altieri MA (2001) The effects of a vegetational corridor on the abundance and dispersal of insect biodiversity within a northern California organic vineyard. *Landscape Ecol.* 133: 133-146
- Öberg S, Ekbom B, Bommarco R (2007) Influence of habitat type and surrounding landscape on spider diversity in Swedish agroecosystems. *Agriculture, Ecosystems and Environment* 122: 211–219
- Pérez-Bote JL, Romero A (2012) Epigeic soil arthropod abundance under different agricultural land uses. *Span J Agric Res* 10(1): 55-61
- Rypstra AL, Carter PE (1995) The web-spider community of soybean agroecosystems in Southern Ohio. *J. Arachnol* 23: 135–144
- Sanguankee PP, León RG (2011) Weed management practices determine plant and arthropod diversity and seed predation in vineyards. *Weed Res* 51: 404-412
- Smith IM, Hoffmann AA, Thomson LJ (2015) Coccinellid abundance in shelterbelts is affected more by adjacent crop type and aphid abundance than vegetation characteristics. *Biol. Control* 87: 47-55
- Thomson LJ, Hoffmann AA (2009) Vegetation increases the abundance of natural enemies in vineyards. *Biological Control* 49: 259-269
- Thomson LJ, Hoffmann AA (2010) Natural enemy responses and pest control: importance of local vegetation. *Biological Control* 52: 160-166
- Thomson LJ, Hoffmann AA (2013) Spatial scale of benefits from adjacent woody vegetation on natural enemies within vineyards. *Biol. Control* 64: 57-65
- Triplehorn CA, Johnson NF (2005) Borror and DeLong's *Introduction to the Study of Insects*. Belmont, Thomson Brooks/Cole, 7th ed., 864 pp
- Woltz JM, Isaacs R, Landis DA (2012) Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agric Ecosyst Environ* 152: 40–49
- Yi Z, Jinchao F, Dayuan X, Weiguo S, Axmacher JC (2012) A comparison of terrestrial arthropod sampling methods. *Journal of Resources and Ecology* 3(2): 174- 182

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## Supplement 2

**S2 - Table 2.1** Richness (mean  $\pm$  S.E) of species and percentage of different type of soil cover (mean  $\pm$  S.E) found in each habitat

Site / Habitat	Richness		% herbaceous cover		% of fresh cover		% of total cover (fresh + dry)	
<b>A</b> NCH1	9.00 $\pm$ 2.00	a	8.33 $\pm$ 3.33	ab	80.00 $\pm$ 0.00	a	83.33 $\pm$ 1.67	a
NCH2	9.67 $\pm$ 1.67	a	5.00 $\pm$ 0.00	b	26.67 $\pm$ 6.01	ab	61.67 $\pm$ 4.41	b
NCH3	7.33 $\pm$ 0.67	ab	5.00 $\pm$ 0.00	b	16.00 $\pm$ 4.93	abc	60.0 $\pm$ 10.00	b
SV 5	1.33 $\pm$ 0.67	d	0.00 $\pm$ 0.00	c	6.67 $\pm$ 4.41	d	6.67 $\pm$ 4.41	c
V 5	8.00 $\pm$ 1.00	a	8.33 $\pm$ 1.67	ab	8.33 $\pm$ 1.67	cd	31.67 $\pm$ 4.41	c
SV 25	4.33 $\pm$ 0.33	cd	5.00 $\pm$ 0.00	b	5.00 $\pm$ 0.00	d	51.67 $\pm$ 12.02	b
V 25	5.00 $\pm$ 0.58	bc	6.67 $\pm$ 1.67	a	6.67 $\pm$ 16.7	d	56.67 $\pm$ 11.67	b
SV 50	4.00 $\pm$ 0.00	cd	5.00 $\pm$ 0.00	b	5.00 $\pm$ 0.00	d	30.00 $\pm$ 0.00	c
V 50	7.33 $\pm$ 1.20	ab	10.00 $\pm$ 2.89	a	10.00 $\pm$ 2.89	bcd	56.67 $\pm$ 12.02	b
$\chi^2_{kw}$	20.32		16.91		18.96		20.75	
$p$	0.009		0.031		0.015		0.008	
<b>B</b> NCH	8.67 $\pm$ 1.86		5.00 $\pm$ 0.00		55.00 $\pm$ 25.17		83.33 $\pm$ 4.41	
SV 5	3.33 $\pm$ 0.33		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		53.33 $\pm$ 6.01	
V 5	4.67 $\pm$ 0.33		6.67 $\pm$ 1.67		6.67 $\pm$ 1.67		36.67 $\pm$ 8.82	
SV 25	5.00 $\pm$ 1.53		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		38.33 $\pm$ 12.02	
V 25	5.00 $\pm$ 0.58		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		23.33 $\pm$ 3.33	
SV 50	5.00 $\pm$ 1.00		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		43.33 $\pm$ 16.67	
V 50	3.00 $\pm$ 1.15		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		30.00 $\pm$ 7.64	
$\chi^2_{kw}$	9.606		6.00		10.099		11.287	
$p$	0.152		0.423		0.121		0.080	
<b>C</b> NCH	5.67 $\pm$ 1.67		3.33 $\pm$ 1.67		58.33 $\pm$ 26.82		66.67 $\pm$ 18.56	
SV 5	5.33 $\pm$ 0.88		15.00 $\pm$ 10.00		15.00 $\pm$ 10.00		43.33 $\pm$ 13.64	
V 5	5.33 $\pm$ 0.88		6.67 $\pm$ 1.67		6.67 $\pm$ 1.67		26.67 $\pm$ 12.02	
SV 25	6.67 $\pm$ 1.20		10.00 $\pm$ 2.89		16.67 $\pm$ 7.26		41.67 $\pm$ 6.01	
V 25	4.67 $\pm$ 0.33		5.00 $\pm$ 0.00		5.00 $\pm$ 0.00		18.33 $\pm$ 1.67	
SV 50	2.67 $\pm$ 1.33		8.33 $\pm$ 6.01		11.67 $\pm$ 9.28		25.00 $\pm$ 12.58	
V 50	4.33 $\pm$ 1.67		11.00 $\pm$ 4.58		11.0 $\pm$ 4.6		20.00 $\pm$ 5.00	
$\chi^2_{kw}$	6.173		5.372		5.069		9.096	
$p$	0.404		0.497		0.535		0.168	

Statistical significance was based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.2** Average ( $\pm$  SE) abundance and richness of arthropods, per sampling period, site and common habitats assessed

	Treatment	Abundance		Richness	
Sampling period	1	31.22 $\pm$ 4.06	b	17.08 $\pm$ 1.55	
	2	30.38 $\pm$ 3.73	b	16.67 $\pm$ 1.45	
	3	45.48 $\pm$ 7.37	a	17.37 $\pm$ 1.38	
Wald $\chi^2$		9.33		4.91	
<i>p</i>		0.009		0.086	
Site	A	44.43 $\pm$ 7.86	a	19.09 $\pm$ 1.69	a
	B	24.91 $\pm$ 3.21	b	13.74 $\pm$ 1.28	b
	C	39.98 $\pm$ 5.22	a	18.26 $\pm$ 1.31	a
Wald $\chi^2$		11.77		12.89	
<i>p</i>		0.003		0.002	
Habitat	NCH	59.52 $\pm$ 7.41	a	28.07 $\pm$ 2.48	a
	SV/GC 5	43.17 $\pm$ 9.81	ab	18.72 $\pm$ 2.09	bc
	V5	26.19 $\pm$ 3.96	b	13.67 $\pm$ 1.66	cd
	SV/GC 25	36.00 $\pm$ 7.48	b	17.39 $\pm$ 2.21	bc
	V25	16.74 $\pm$ 2.47	c	11.48 $\pm$ 1.36	d
	SV/GC 50	72.50 $\pm$ 20.44	a	21.50 $\pm$ 2.18	ab
	V50	15.07 $\pm$ 2.04	c	10.56 $\pm$ 1.27	d
Wald $\chi^2$		98.99		79.07	
<i>p</i>		<0.001		<0.001	

Statistical significance based on marginal estimated means for  $p < 0.05$



**S 2 - Table 2.3** Significant Spearman correlation ( $R_{ho}$ ) between abundance and richness of arthropods captured by suction and yellow sticky traps and vegetation parameters (% of cover, richness and % of herbaceous cover)

	% Total cover (fresh + dry)			Richness			% herbaceous cover
	A	B	C	A	B	C	A
<b>Abundance</b>							
Total (a)	-	0.79**	0.44*	-	0.50*	-	-
Herbivores (a)	-	0.71**	0.52*	-	-	-	-
Cicadellidae (b)	-	-	-	-	-	-	0.55*
<i>E. vitis</i> (b)	-	-	-	-	-	-	0.58*
Parasitoids (a)	0.39*	-	-	-	-	-	-
Predators (a)	-	0.72**	-	-	0.48*	-	-
Spiders (a)	-	0.75**	-	-	-	-	-
Coccinellidae (b)	-	0.707*	-	-	0.70*	-	-
Syrphidae (b)		-	-	0.67**	-	-	-
<b>Richness</b>							
Total (a)	0.45*	0.73**	-	-	-	-	-
Herbivores (a)	0.43*	0.63**	0.49*	-	-	-	-
Cicadellidae (b)	-	-	-	-	0.52*	-	-
Parasitoids (a)	0.48*	0.49*	-	-	-	-	-
Predators (a)	-	0.78**	-	-	-	-	-
Spiders (a)	-	0.71**	-	-	-	-	-
Coccinellidae (b)	-	-	-	-	-	0.61*	-

Correlations are significant at the 0.05 (\*), 0.01 (\*\*); (a) – arthropods collected by suction (n = 27 for site 1. n = 21 for site 2 and n = 21 for site 3); (b) - arthropods collected with sticky traps (n = 15 for site 1. n = 12 for site 2 and n = 12 for site 3)

**S2 - Table 2.4** Abundance and richness (mean  $\pm$  SE) of arthropods collected by suction sampling in each habitat in site A and results of generalized linear model (GzLM) analysis

Abundance						
	Herbivores	Parasitoids	Eulophidae	Mymaridae	Predators	Spiders
NCH1	29.3 $\pm$ 3.1 abc	7.7 $\pm$ 2.4 abcde	0.7 $\pm$ 0.3 bc	0.0 $\pm$ 0.0 b	10.2 $\pm$ 1.7 abc	9.3 $\pm$ 1.7 ab
NCH2	26.4 $\pm$ 7.2 abc	14.8 $\pm$ 5.2 a	4.3 $\pm$ 1.6 a	0.2 $\pm$ 0.1 b	14.2 $\pm$ 4.1 abc	11.4 $\pm$ 3.8 a
NCH3	21.1 $\pm$ 6.0 bc	7.7 $\pm$ 2.4 abc	1.6 $\pm$ 0.6 ab	0.2 $\pm$ 0.1 b	13.0 $\pm$ 2.4 abc	11.6 $\pm$ 2.5 a
SV 5	12.5 $\pm$ 3.1 bcd	3.5 $\pm$ 0.5 cde	1.2 $\pm$ 0.4 bc	0.2 $\pm$ 0.2 b	9.0 $\pm$ 3.8 abcd	8.5 $\pm$ 2.9 abc
V 5	18.7 $\pm$ 5.9 bcd	5.4 $\pm$ 1.2 bcde	0.9 $\pm$ 0.4 bc	0.2 $\pm$ 0.2 b	8.7 $\pm$ 1.8 ac	3.0 $\pm$ 0.7 bce
SV 25	18.3 $\pm$ 8.9 bcd	8.5 $\pm$ 2.9 abcd	1.5 $\pm$ 0.7 ab	3.8 $\pm$ 2.7 a	2.8 $\pm$ 1.1 d	2.7 $\pm$ 1.2 cd
V 25	9.3 $\pm$ 4.0 cd	3.2 $\pm$ 0.8 de	0.3 $\pm$ 0.2 c	0.2 $\pm$ 0.1 b	4.1 $\pm$ 1.5 cd	2.8 $\pm$ 0.8 cd
SV 50	90.5 $\pm$ 44.2 a	11.3 $\pm$ 4.8 ab	0.3 $\pm$ 0.2 bc	1.2 $\pm$ 0.5 ab	12.3 $\pm$ 4.9 abc	11.2 $\pm$ 4.5 a
V 50	7.2 $\pm$ 1.6 d	3.4 $\pm$ 0.6 e	0.6 $\pm$ 0.2 bc	0.3 $\pm$ 0.3 b	7.2 $\pm$ 2.8 abcd	1.7 $\pm$ 0.7 de
<sup>(1)</sup> Wald $\chi^2$	(NB) 38.447	(NB) 31.345	(NB) 27.981	(NB) 26.198	(NB) 20.001	(NB) 42.781
<i>p</i>	<0.001	<0.001	<0.001	<0.001	0.010	<0.001
Richness						
NCH1	8.8 $\pm$ 0.7 ab	5.2 $\pm$ 1.5 ac	-	-	6.8 $\pm$ 1.1 ac	6.2 $\pm$ 1.1 ab
NCH2	11.9 $\pm$ 1.7 ab	9.0 $\pm$ 2.3 ac	-	-	7.6 $\pm$ 1.8 ab	6.0 $\pm$ 1.5 ab
NCH3	7.8 $\pm$ 1.0 bc	5.3 $\pm$ 1.4 ab	-	-	7.7 $\pm$ 1.1 a	6.2 $\pm$ 1.1 a
SV 5	5.8 $\pm$ 1.9 bc	3.0 $\pm$ 0.6 bc	-	-	4.0 $\pm$ 1.2 cd	3.5 $\pm$ 1.2 bcd
V 5	6.6 $\pm$ 1.6 bc	4.3 $\pm$ 0.8 bc	-	-	4.6 $\pm$ 1.1 bcd	2.6 $\pm$ 0.6 cd
SV 25	6.7 $\pm$ 1.9 bc	4.3 $\pm$ 0.8 bc	-	-	2.3 $\pm$ 0.8 d	2.2 $\pm$ 0.9 cd
V 25	4.6 $\pm$ 1.7 c	2.9 $\pm$ 0.7 bc	-	-	2.9 $\pm$ 0.9 d	2.1 $\pm$ 0.6 cd
SV 50	8.2 $\pm$ 1.1 ac	4.8 $\pm$ 0.8 ac	-	-	5.7 $\pm$ 1.7 ad	4.5 $\pm$ 1.3 ac
V 50	4.9 $\pm$ 1.0 c	2.1 $\pm$ 0.5 c	-	-	2.9 $\pm$ 1.0 d	1.3 $\pm$ 0.5 d
<sup>(1)</sup> Wald $\chi^2$	(N) 24.666	(NB) 24.729	-	-	(N) 24.585	(N) 34.438
<i>p</i>	0.002	0.002	-	-	0.002	<0.001

(1) – under brackets are presented the distribution and link function: N – normal identity; NB – negative binomial with log link; statistical significance based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.5** Abundance and richness (mean  $\pm$  SE) of arthropods collected by suction sampling in each habitat in site B and results of generalized linear model (GzLM) analysis

Abundance						
	Herbivores	Parasitoids	Eulophidae	Mymaridae	Predators	Spiders
NCH	22.9 $\pm$ 6.3 a	3.3 $\pm$ 0.6	0.6 $\pm$ 0.3	0.1 $\pm$ 0.1	8.7 $\pm$ 1.5 ab	6.4 $\pm$ 1.6 a
SV 5	11.5 $\pm$ 5.4 abc	5.8 $\pm$ 1.9	1.3 $\pm$ 0.7	0.7 $\pm$ 0.3	8.5 $\pm$ 2.4 ab	8.0 $\pm$ 2.1 a
V 5	9.4 $\pm$ 4.4 ab	4.3 $\pm$ 1.0	1.0 $\pm$ 0.3	0.2 $\pm$ 0.1	10.8 $\pm$ 2.9 a	2.2 $\pm$ 0.8 b
SV 25	13.0 $\pm$ 6.2 ab	6.3 $\pm$ 4.2	0.7 $\pm$ 0.5	1.0 $\pm$ 0.8	5.8 $\pm$ 2.2 ab	5.3 $\pm$ 2.0 ab
V 25	6.4 $\pm$ 2.4 bc	3.1 $\pm$ 1.1	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	4.6 $\pm$ 1.2 b	2.6 $\pm$ 0.7 b
SV 50	8.8 $\pm$ 3.0 abc	6.0 $\pm$ 3.2	0.5 $\pm$ 0.2	0.7 $\pm$ 0.5	7.7 $\pm$ 2.1 ab	6.5 $\pm$ 1.6 ab
V 50	2.8 $\pm$ 0.7 c	2.4 $\pm$ 1.4	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	1.2 $\pm$ 0.5 c	0.4 $\pm$ 0.2 c
<sup>(1)</sup> Wald $\chi^2$	(NB) 21.708	(NB) 5.541	(NB) 8.365	(NB) 6.506	(NB) 28.211	(NB) 32.589
<i>p</i>	0.001	0.477	0.213	0.260	<0.001	<0.001
Richness						
NCH	6.4 $\pm$ 0.8 a	3.0 $\pm$ 0.5	-	-	6.0 $\pm$ 1.1 a	4.4 $\pm$ 1.0 a
SV 5	6.7 $\pm$ 2.4 a	4.0 $\pm$ 1.0	-	-	4.8 $\pm$ 0.9 ab	4.3 $\pm$ 0.8 a
V 5	4.9 $\pm$ 1.6 ab	3.7 $\pm$ 0.8	-	-	3.3 $\pm$ 0.7 b	1.6 $\pm$ 0.6 bc
SV 25	5.2 $\pm$ 1.3 a	3.5 $\pm$ 1.6	-	-	3.5 $\pm$ 1.2 ab	3.2 $\pm$ 1.0 ab
V 25	4.2 $\pm$ 1.2 ab	2.3 $\pm$ 0.8	-	-	3.1 $\pm$ 0.5 b	2.0 $\pm$ 0.4 bc
SV 50	5.8 $\pm$ 1.5 a	3.3 $\pm$ 1.4	-	-	5.3 $\pm$ 1.2 ab	4.7 $\pm$ 1.1 a
V 50	2.2 $\pm$ 0.5 b	1.4 $\pm$ 0.5	-	-	0.9 $\pm$ 0.3 c	0.4 $\pm$ 0.2 c
<sup>(1)</sup> Wald $\chi^2$	(N*) 13.703	(NB) 7.280	-	-	(NB) 27.988	(N) 34.991
<i>p</i>	0.033	0.296	-	-	<0.001	<0.001

(1) under brackets are presented the distribution and link function: N – normal identity; NB – negative binomial with log link; \* - data transformed in log (x+1); statistical significance based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.6** Abundance and richness (mean  $\pm$  SE) of arthropods collected by suction sampling in each habitat in site C and results of generalized linear model (GzLM) analysis

Abundance						
	Herbivores	Parasitoids	Eulophidae	Mymaridae	Predators	Spiders
NCH	27.3 $\pm$ 5.5 a	15.2 $\pm$ 3.5 abd	2.6 $\pm$ 0.5 a	1.4 $\pm$ 0.7 bc	7.6 $\pm$ 1.2	5.8 $\pm$ 0.9 a
GC 5	45.0 $\pm$ 5.4 a	8.2 $\pm$ 1.9 bde	0.2 $\pm$ 0.2 c	4.2 $\pm$ 2.5 ab	4.2 $\pm$ 2.4	4.2 $\pm$ 2.1 ab
V 5	6.6 $\pm$ 1.3 b	3.8 $\pm$ 1.0 e	0.7 $\pm$ 0.2 bc	0.3 $\pm$ 0.2 c	3.1 $\pm$ 0.8	2.0 $\pm$ 0.44 b
GC 25	24.2 $\pm$ 8.8 a	10.7 $\pm$ 1.9 acd	0.8 $\pm$ 0.3 bc	4.8 $\pm$ 0.9 ab	3.8 $\pm$ 1.2	3.8 $\pm$ 1.2 ab
V 25	4.3 $\pm$ 0.8 b	4.0 $\pm$ 0.8 e	0.3 $\pm$ 0.2 c	1.6 $\pm$ 0.5 bc	4.3 $\pm$ 0.7	2.7 $\pm$ 0.5 b
GC 50	33.5 $\pm$ 10.4 a	21.5 $\pm$ 8.7 a	1.5 $\pm$ 0.8 ab	11.7 $\pm$ 5.5 a	6.2 $\pm$ 2.4	5.8 $\pm$ 2.3 a
V 50	5.1 $\pm$ 0.8 b	5.2 $\pm$ 1.4 ce	1.4 $\pm$ 0.8 ab	1.0 $\pm$ 0.4 bc	4.6 $\pm$ 0.7	2.8 $\pm$ 0.4 b
<sup>(1)</sup> Wald $\chi^2$	(NB) 76.695	(NB) 37.104	(NB) 23.497	(NB) 37.464	(NB) 11.553	(N) 17.475
<i>p</i>	<0.001	<0.001	0.001	<0.001	0.073	0.008
Richness						
NCH	8.2 $\pm$ 1.1 a	9.1 $\pm$ 1.5 a	-	-	4.8 $\pm$ 0.7 a	3.7 $\pm$ 0.4
GC 5	7.3 $\pm$ 2.4 a	3.8 $\pm$ 1.0 cd	-	-	2.5 $\pm$ 0.9 b	2.5 $\pm$ 0.8
V 5	3.9 $\pm$ 0.6 b	2.3 $\pm$ 0.5 d	-	-	2.4 $\pm$ 0.5 b	1.9 $\pm$ 0.4
GC 25	7.2 $\pm$ 1.6 a	5.2 $\pm$ 0.5 bc	-	-	3.2 $\pm$ 0.9 b	3.2 $\pm$ 0.9
V 25	3.6 $\pm$ 0.6 b	3.2 $\pm$ 0.5 cd	-	-	3.1 $\pm$ 0.5 b	2.3 $\pm$ 0.5
GC 50	8.8 $\pm$ 0.7 a	7.2 $\pm$ 1.2 ab	-	-	2.5 $\pm$ 0.6 b	2.2 $\pm$ 0.5
V 50	3.7 $\pm$ 0.6 b	4.3 $\pm$ 1.1 cd	-	-	3.4 $\pm$ 0.4 b	2.4 $\pm$ 0.3
<sup>(1)</sup> Wald $\chi^2$	(N*) 41.311	(N) 41.043	-	-	(N) 14.279	(P) 7.092
<i>p</i>	<0.001	<0.001	-	-	0.027	0.312

(1) – under brackets are presented the distribution and link function: N – normal identity; NB – negative binomial with log link; \* - data transformed in log (x+1); statistical significance based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.7** Abundance and richness (mean  $\pm$  SE) of arthropods collected in yellow sticky traps at each habitat in site A and results of generalized linear model (GzLM) analysis

Abundance								
	Cicadellidae	<i>Empoasca vitis</i>	Parasitoids	Predators	Coccinellidae	Araneae	Neuroptera	Syrphidae
NCH1	65.4 $\pm$ 12.7 bc	48.8 $\pm$ 11.7 bd	46.7 $\pm$ 13.1	11.0 $\pm$ 2.4 bc	4.3 $\pm$ 1.2 b	2.0 $\pm$ 0.6	0.4 $\pm$ 0.2	1.7 $\pm$ 0.8
NCH2	37.3 $\pm$ 6.6 c	23.1 $\pm$ 7.4 cd	49.3 $\pm$ 13.9	23.1 $\pm$ 6.7 a	18.0 $\pm$ 5.7 a	0.8 $\pm$ 0.3	0.4 $\pm$ 0.3	1.3 $\pm$ 0.9
V5	85.6 $\pm$ 8.4 ab	76.6 $\pm$ 8.4 ab	43.0 $\pm$ 10.3	18.2 $\pm$ 3.2 ab	11.2 $\pm$ 2.9 a	0.8 $\pm$ 0.3	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2
V 25	76.2 $\pm$ 16.3 bc	63.3 $\pm$ 12.0 bc	37.4 $\pm$ 9.5	8.3 $\pm$ 1.5 c	4.8 $\pm$ 1.2 b	0.9 $\pm$ 0.3	0.8 $\pm$ 0.3	0.2 $\pm$ 0.2
V 50	137.1 $\pm$ 42.0 a	118.4 $\pm$ 38.3 a	44.3 $\pm$ 8.1	8.0 $\pm$ 1.1 c	3.2 $\pm$ 0.9 b	1.7 $\pm$ 0.3	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0
<sup>(1)</sup> Wald $\chi^2$	(NB) 22.583	(N) 15.007	(N) 0.712	(N) 15.527	(NB) 27.364	(P) 9.088	(P) 2.460	(NB) 5.847
<i>p</i>	<0.001	0.005	0.950	0.004	<0.001	0.059	0.652	0.119
Richness								
NCH1	5.0 $\pm$ 0.5	-	-	-	2.4 $\pm$ 0.4	-	-	-
NCH2	4.4 $\pm$ 0.5	-	-	-	3.8 $\pm$ 0.5	-	-	-
V5	4.8 $\pm$ 0.5	-	-	-	2.8 $\pm$ 0.5	-	-	-
V 25	3.7 $\pm$ 0.4	-	-	-	2.6 $\pm$ 0.4	-	-	-
V 50	4.6 $\pm$ 0.4	-	-	-	2.0 $\pm$ 0.4	-	-	-
<sup>(1)</sup> Wald $\chi^2$	(N) 5.333	-	-	-	(N) 9.103	-	-	-
<i>p</i>	0.255	-	-	-	0.059	-	-	-

(1) – under brackets are presented the distribution and link function: N – normal identity; NB – negative binomial with log link; statistical significance based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.8** Abundance and richness (mean  $\pm$  SE) of arthropods collected in yellow sticky traps at each habitat modalities in site B and results of generalized linear model (GzLM) analysis

Abundance								
	Cicadellidae	<i>Empoasca vitis</i>	Parasitoids	Predators	Coccinellidae	Araneae	Neuroptera	Syrphidae
NCH	90.2 $\pm$ 16.4	75.1 $\pm$ 13.8	98.8 $\pm$ 18.0 a	57.0 $\pm$ 25.7 a	15.8 $\pm$ 3.2 a	1.7 $\pm$ 0.7 ab	2.1 $\pm$ 0.9	4.4 $\pm$ 1.8
V 5	117.6 $\pm$ 20.5	84.6 $\pm$ 10.8	64.7 $\pm$ 10.6 b	20.4 $\pm$ 2.2 b	7.1 $\pm$ 1.3 b	3.0 $\pm$ 0.6 a	3.1 $\pm$ 1.0	2.3 $\pm$ 0.8
V 25	117.1 $\pm$ 28.4	82.2 $\pm$ 26.1	36.2 $\pm$ 4.5 b	9.1 $\pm$ 1.2 c	2.8 $\pm$ 0.8 c	1.6 $\pm$ 0.5 ab	1.3 $\pm$ 0.7	1.3 $\pm$ 0.7
V 50	143.1 $\pm$ 40.9	106.3 $\pm$ 33.5	43.2 $\pm$ 13.9 b	6.3 $\pm$ 1.1 c	1.6 $\pm$ 0.5 c	0.7 $\pm$ 0.2 b	0.8 $\pm$ 0.3	0.8 $\pm$ 0.5
<sup>(1)</sup> Wald $\chi^2$	(N) 1.989	(N) 1.160	(N) 16.412	(NB) 69.272	(NB) 52.433	(NB) 10.452	(NB) 5.861	(NB) 5.607
<i>p</i>	0.575	0.763	0.001	<0.001	<0.001	0.015	0.119	0.132
Richness								
NCH	5.4 $\pm$ 0.6	-	-	-	3.1 $\pm$ 0.3 a	-	-	-
V 5	4.3 $\pm$ 0.4	-	-	-	2.7 $\pm$ 0.3 a	-	-	-
V 25	4.9 $\pm$ 0.5	-	-	-	1.6 $\pm$ 0.2 b	-	-	-
V 50	4.7 $\pm$ 0.7	-	-	-	1.1 $\pm$ 0.4 b	-	-	-
<sup>(1)</sup> Wald $\chi^2$	(N) 2.327	-	-	-	(N) 30.286	-	-	-
<i>p</i>	0.507	-	-	-	<0.001	-	-	-

(1) – under brackets are presented the distribution and link function: N – normal identity; NB – negative binomial with log link; statistical significance based on marginal estimated means for  $p < 0.05$

**S2 - Table 2.9** Abundance and richness (mean  $\pm$  SE) of arthropods collected in yellow sticky traps at each habitat modalities in site C and results of generalized linear model (GzLM) analysis

Abundance								
	Cicadellidae	<i>Empoasca vitis</i>	Parasitoids	Predators	Coccinellidae	Araneae	Neuroptera	Syrphidae
NCH	255.1 $\pm$ 40.0	235.9 $\pm$ 37.3	71.7 $\pm$ 13.2	21.9 $\pm$ 3.4	13.4 $\pm$ 2.3	1.6 $\pm$ 0.5	0.3 $\pm$ 0.2	0.4 $\pm$ 0.3
V 5	243.8 $\pm$ 69.2	228.3 $\pm$ 66.3	47.9 $\pm$ 12.0	11.9 $\pm$ 4.9	9.7 $\pm$ 4.9	1.0 $\pm$ 0.4	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2
V 25	440.0 $\pm$ 123.1	425.7 $\pm$ 120.2	71.4 $\pm$ 19.8	17.1 $\pm$ 4.5	12.3 $\pm$ 4.7	1.6 $\pm$ 0.5	0.6 $\pm$ 0.3	0.4 $\pm$ 0.2
V 50	373.2 $\pm$ 115.4	358.4 $\pm$ 112.6	83.4 $\pm$ 19.3	14.0 $\pm$ 2.3	8.2 $\pm$ 1.9	1.2 $\pm$ 0.3	0.6 $\pm$ 0.2	0.0 $\pm$ 0.0
Wald $\chi^2$	(N) 3.484	(N) 3.809	(N) 2.770	(N) 4.203	(N) 1.397	(P) 1.481	(P) 2.668	(P) 0.181
<i>p</i>	0.323	0.283	0.428	0.240	0.706	0.687	0.446	0.914
Richness								
NCH	3.9 $\pm$ 0.6	-	-		3.6 $\pm$ 0.4	-	-	-
V 5	4.2 $\pm$ 0.4	-	-		3.3 $\pm$ 0.7	-	-	-
V 25	3.7 $\pm$ 0.4	-	-		2.8 $\pm$ 0.4	-	-	-
V 50	3.1 $\pm$ 0.4	-	-		3.2 $\pm$ 0.5	-	-	-
Wald $\chi^2$	(N) 3.573	-	-		(N) 1.311	-	-	-
<i>p</i>	0.311	-	-		0.727	-	-	-

Statistical significance based on marginal estimated means for  $p < 0.05$





## **Chapter 3**

### **Functional biodiversity of soil arthropods associated with terraced vineyards in Douro Demarcated Region**



Part of the content of this chapter was presented/ published in:

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## Abstract

We investigated the impact of neighborhood non-crop habitats (NCH) and vegetation in Douro Demarcated Region (DDR) terraced vineyards on ground-dwelling arthropod communities. Three agroecosystems were sampled, using pitfall traps located either in NCH as inside vineyards, at three distances (5, 25 and 50 m from NCH). Vegetation cover and species richness were evaluated around each trap. Omnivores (Formicidae) and detritivores (mainly Collembola) were the dominating groups, accounting for 42.0% and 33.7% of the sample, respectively. Predators, mainly dominated by Scydmaenidae (Coleoptera) and Araneae, accounted for 19.4% of the sample. Although closely related groups displayed different responses to land use and habitat preferences, the abundance of omnivores and predators was, in general, higher in NCH than in vineyards. In Formicidae, the generalist/ opportunist group dominated, being highly represented by *Pheidole pallidula*; this group occurred mainly in NCH and was positively correlated with the percentage of fresh vegetation cover. In Araneae, sheet web weavers were the most abundant group, being positively correlated with percentage of total vegetation cover and richness of plants. This study indicates that preserving NCH close to the vineyards, as well as promoting local vegetation, enhances the presence of ground-dwelling arthropods.

**Keywords:** non-crop habitats, vegetation cover, functional groups, biodiversity



### 3.1 Introduction

During the second half of the twentieth century, the agriculture suffered a strong expansion and intensification, with high pesticide application and intensive land-use practices that has caused biodiversity losses and has profoundly changed the functioning of agroecosystems (Kleijn et al. 2009; Geiger et al. 2010). Growing calls for agriculture landscapes to be managed as “multifunctional” systems, creates new mandates, as well as opportunities, to maintain and enhance ecosystem services, as part of productive agroecosystems (Garbach et al. 2014). To preserve agriculture for future generations, production systems that conserve and improve soil quality (Doran and Zeiss 2000), health and resilience must be developed, including the use of ground cover, reduced tillage and herbicide application and promoting landscape heterogeneity (Lehman et al. 2015).

While vineyards are among the most intensive forms of agriculture, often resulting in simplified landscapes (Nascimbene et al. 2013), in areas of Douro Demarcated Region (DDR), such as the 'Alto Douro Wine Region', classified as UNESCO World Heritage Site, the characteristics of landscape offers great opportunity from the standpoint of biodiversity, by including a significant area of non-crop habitats (e.g. woodland remnants, grassy slopes, or terraces with natural vegetation and dry-stone walls) (Andresen et al. 2004). This landscape deserves also high conservation priority because of ecological, historical and economic importance (high quality wine production). Moreover, as DDR is one of the world biggest region of hillside vineyards, the use of ground covers for soil protection against erosion is common.

Arthropods are the most diverse group of organisms, with over one million described species (May 1988) and many groups are important drivers of ecosystem functions such as nutrient cycling, pest control, pollination, maintenance of soil structure (Petchey and Gaston 2002; Tscharrntke et al. 2005). Many studies have highlighted the positive effects of good management practices on ground-dwelling arthropods diversity in vineyards (Isaia et al. 2006; Thomson & Hoffmann 2007; Bruggisser et al. 2010; Addison et al. 2013), as well as of the existence of non-crop habitats in the margins of vineyard plots (Bolduc et al. 2005; Gaigher and Samways 2010; Thomson et al. 2009). However, responses across taxa haven't been consistent, with different organisms responding in different ways. Most of studies have focused on the overall biodiversity of vineyards, and less attention has been addressed to the effect of habitat management strategies in different functional groups (Thomson and Hoffmann 2007; Trivellone et al.

2012; Caprio et al. 2015). This is of major interest, since species with variable ecological requirements may respond differently to different habitat management systems. The classification into functional groups allows to evaluate responses to the land-use gradient using broad functional characteristics easily observed, without requiring detailed knowledge of natural history or species designation. According to Buschke and Seaman (2011), functional feeding groups can be used to obtain the same ecological information as family level data, with much less sampling effort and taxonomic expertise. However, few authors have conducted studies under artificial vineyard terraced ecosystems (Košulič et al. 2014), yielding results only for spiders.

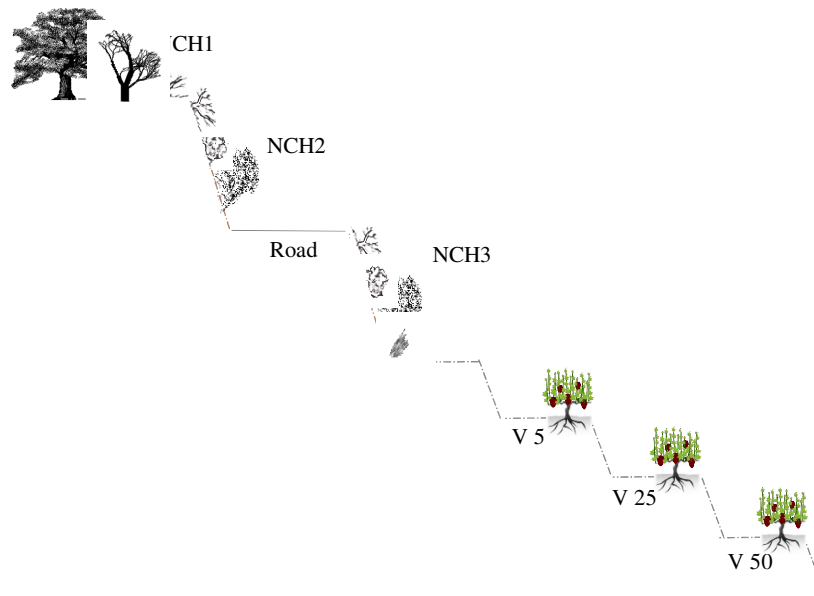
In the present study, it was intended to: a) gain knowledge about the biodiversity of ground-dwelling arthropods in terraced vineyards of DDR; b) analyze the impact of the presence of non-crop habitats (NCH) near terraced vineyards on ground-dwelling arthropods and particularly on specific functional groups; c) analyze the impact of vegetation on such groups. The ultimate goal, is to show the advantages of preserving these ecological infrastructures in the proximity of terraced vineyards and maintaining soil vegetation cover in the vineyards, in order to enhance the agroecosystem functional biodiversity.

## **3.2 Materials and Methods**

### **3.2.1 Study sites**

The study was carried out from July to October 2010 in three terraced vineyard agroecosystems, located in the DDR. Separated by more than 3 km, the three sites were chosen according the presence, in their neighborhood, of woodland and/or scrubland lots, thereafter designated non-crop habitats (NCH). The corresponding agroecosystem (cv. *Touriga Nacional* plus neighborhood non-crop habitats) will be thereafter referred as sites A, B and C, located respectively at quinta das Carvalhas (S. João Pesqueira county), quinta de S. Luiz (Tabuaço county) and Quinta do Seixo (Tabuaço county).

At site A (41°10'47''N, 7°32'09''W) the vineyard (8-year-old) was set on one row terraces and North East faced. It was bordered by a Mediterranean woodland of the type *Rusco aculeati Querceto suberis viburnetosum tini* (NCH1); moreover, there were a slope (NCH2) mainly covered with shrubs and herbaceous species and a second slope (NCH3) mainly covered by shrubs, herbaceous vegetation and some dispersed trees (Fig. 3.1).



**Figure 3.1** Experimental design followed at site A.

Habitats: NCH- Non-crop habitats; V: Vineyard.

At site B (41°09'08''N, 7°37'04''W) the vineyard (11-year-old) was set on one row terraces and West faced. It was bordered by NCH characterized by the combination of a Mediterranean scrubland of the type *Erico arboreae arbutetum unedonis viburnetosum tini* and by an herbaceous border just located near the vineyard edge. Inside vineyard surface, some dispersed shrubs were left on slopes.

At site C (41°10'15''N, 7°33'05''W) the vineyard (10-year-old) was set on two row terraces, and East faced. It was bordered by NCH which formerly was a cropped area (vineyard and olive production until 1980), being occupied mainly by scrubland dominated by *Rubus ulmifolius*, combined with an herbaceous cover.

All vineyards were conducted under Integrated Production Principles. Vegetation under vines were controlled through one application of herbicide at the end of winter (glyphosate and oxyfluorfen). The vegetation of slopes and ground cover between vines was mowed twice mechanically, first in March and later in June/July.

### 3.2.2 Data collection

Plant and arthropod communities were assessed in both non-cropped and vineyards habitats in three replicate sampling stations, each separated by a minimum of 50 m. At site A, a total of 18 sampling stations were assessed (9 in NCH plus 9 in vineyards habitats). In the other sites (B and C), the samplings were performed in 12 sampling stations (3 in NCH plus 9 in vineyards habitats). This means that, in total, 42 sampling points were assessed on the three sites.

#### *Arthropods*

Ground-dwelling arthropods were sampled three times, in July 16<sup>th</sup>, August 19<sup>th</sup>, and October 22<sup>nd</sup> using *pitfall traps*, a method that enable rapid and efficient collection of data amenable to statistical analysis (Topping and Sunderland 1992). This method is one of the most used since it works when the observer is absent, it is very simple and inexpensive, as reviewed by Zaller et al. (2015). The traps consisted in plastic containers of 10 cm deep by 7 cm in diameter, half filled with 25% of ethylene glycol, buried in the ground up to the rim. They were installed either in NCH, as inside vineyards replicated sampling points, at 5, 25 and 50 m from NCH. After 24 hours (Pérez-Bote and Romero 2012; Cárdenas et al. 2015), traps were collected and arthropods were stored in 70% ethanol, until identification.

Based on literature (reviewed by Triplehorn and Johnson 2005), arthropods were initially identified at the taxonomic level of order or family, and sorted into morpho-species, being later identified by taxonomists. Each taxon was classified into trophic groups (predators, parasitoids, herbivores, omnivores, detritivores, and pollen/nectar feeders).

Araneae were also classified on the base of their foraging strategy (type of web and hunting method), in the following groups: (a) sensing web weavers, (b) sheet web weavers, (c) space web weavers, (d) orb web weavers; (e) specialists; (f) ambush, (g) ground hunters, and (h) other hunters (Cardoso et al., 2011). Formicidae were classified into functional groups, according to Roig and Espadaler (2010) as: (a) generalists and/or opportunistic (GO); (b) cold-climate specialists and/or shade habitats (SpCold); (c) hot climate specialists and/or open habitats (SpWarm), (d) and cryptic (Cr).



## *Flora*

Vegetation was assessed twice, in late May and in early September 2010, in a 4 m<sup>2</sup> surface around each pitfall trap, using the phytostructural method (Crespí et al. 2005), which estimates plant-community vegetation structure and floristic composition. However, as assessments of arthropods were done during summer period, only the later was considered for further analysis. The vegetation coverage was estimated, through the percentage of fresh cover and the percentage of total cover (fresh plus dry). The number of plant species (richness) was also recorded.

### **3.2.3 Statistical analysis**

Differences in the richness of plants and in the percentage of vegetation cover (both fresh cover and total cover (fresh plus dry)) among sites or among habitats, within each site, were analyzed by a nonparametric Kruskal–Wallis test, followed by the multiple comparison mean ranks by Fisher’s LSD, as described by Marôco (2011). Data are presented as the median and interquartile range. The abundance and richness of arthropods, were calculated by pooling data from all site together and in each site separately, due to local geographical differences found.

Abundance implicitly means the number of arthropods caught in pitfall traps and is interpreted as an estimation of the ‘activity density’ of the captured species (Melbourne, 1999). The abundance and richness of arthropods were analyzed using generalized linear models (GzLM), assuming a Poisson distribution with log link function. Site, time and habitat were used as independent variables (fixed effects). The analysis started fitting the full model that included all independent variables, followed by stepwise procedure to remove non-significant variables (Crawley 2007). The significance of independent variables was obtained using a Wald test. Tukey’s pairwise comparison test, were used to estimate significant differences between groups in each independent variable. As significant effects were found between sites, separated models were also performed for each site, using time and habitats as independent variables. Analyses were performed with R (version 3.2.2) (R Development Core Team 2015), using “*MASS*” package (Venables and Ripley 2002). Data are presented as mean ± standard error.

Under the “*vegan*” package facilities R (Oksanen et al. 2016), non-metric multidimensional scaling (NMDS) was used to examine the similarities of ant

communities among the studied sites, identified by site and habitat. Prior to ordination, we removed individuals observed in only one location, to facilitate solution convergence in these non-linear methods. For a good and interpretable result, we employed NMDS fixing a 2-dimensional solution and using all the available dissimilarities indices in “vegdist” function, choosing the convergent solution with lowest stress value, under 0.1.

The results of NMDS ordination allows to interpret axis by evaluating the position of each sample. The similarity with other samples is determined by its distance to other samples in the analysis. Shorter distances represent higher homogeneity in community composition. The influence of each environmental variable (vegetation parameters) was verified by the function “envfit”, which uses the  $R^2$  value and several randomizations (1000 permutations) to define the significance of these variables. To associate community composition with vegetation parameters, we used the “envfit” function to evaluate the associations between NMDS site scores, as multivariate response variables, and sites environmental values related with vegetation parameters (total cover, fresh cover and richness of cover). The  $R^2$  values are presented, as the measure of these associations. The significance level for all analyses was 0.05. The MDS plot was produced using the R package ggplot2 (Wickham 2009).

The resulted NMDS ordination allowed to separate functional groups of ants, according to habitats and vegetation parameters. The ultimate 2-dimensional NMDS solution was found with *Euclidean* dissimilarity and had an associated stress of 0.095, indicating a solution with useful interpretative ability.

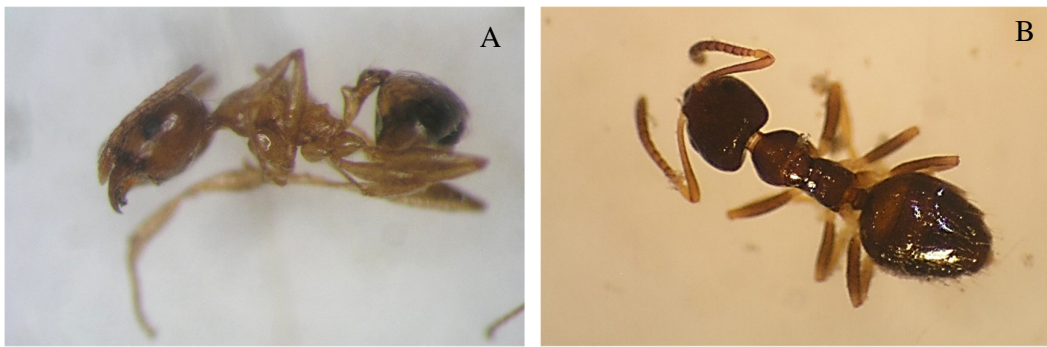
### 3.3 Results

#### 3.3.1 Arthropods

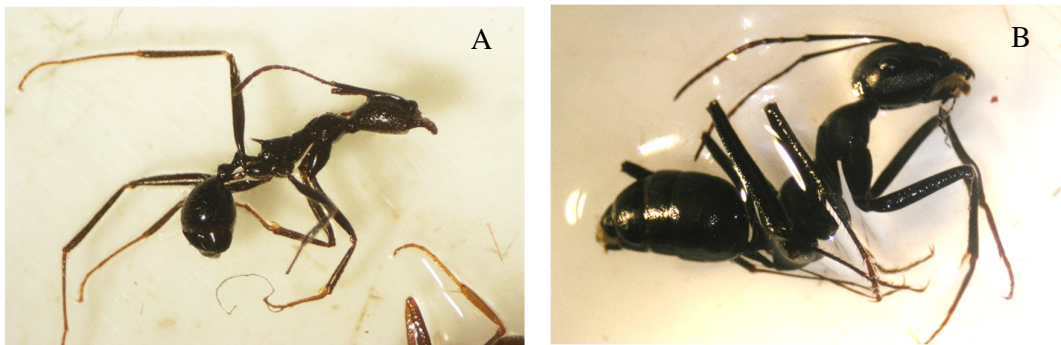
A total of 2,825 arthropods was captured in the sampling stations assessed. The assemblage was dominated by Hymenoptera (42.7%), Collembola (30.8%), Coleoptera (10.9%), Acari (6.3%) and Araneae (3.9%). The numerically dominant groups were: omnivores (42.0% of the sample), detritivores (33.7%) and predators (19.4%). Herbivorous, parasitoids and pollen/nectar feeders represented altogether 2% of the total assemblage (Table 3.1).

Among **omnivores**, Formicidae was the most abundant family (1,186 individuals), being represented by three sub-families: Myrmicinae (75.9%), Formicinae (20.3%) and Dolichoderinae (3.8%). These belonged to 11 genera and 11 species, of which the most

abundant was *Pheidole pallidula* (Nylander 1849) (Fig. 3.2A), followed by *Plagiolepis pygmaea* (Latreille 1798) (Fig. 3.2B) and *Aphaenogaster gibbosa* (Latreille 1798) (S3-Table 3.1). Also, two Iberian endemism were identified, i.e. *Aphaenogaster iberica* Emery 1908 (Fig. 3.3A) (7.3% of the total), and *Cataglyphis hispanica* (Emery 1906) (Fig. 3.3B) (0.4%). Most specimens caught belonged to the generalist/opportunistic (GO) group (76.3%) which were followed by Cryptic (Cr) (9.7%), hot climate specialists and/or open habitats (SpWarm) (7.3%) and cold-climate specialists and/or shade habitats (SpCold) (5.0%) (S3-Table 3.2).



**Figure 3.2** Specimens of *Pheidole pallidula* (A) and *Plagiolepis pygmaea* (B)



**Figure 3.3** Specimens of *Aphaenogaster iberica* (A) and *Cataglyphis hispanica* (B)

**Table 3.1** Abundance (N), relative percentage (%) and richness (S) of arthropod morpho-species found in each functional trophic group and abundance (N) of each group in the studied sites (A, B and C)

Functional trophic group	N	%	S	N (A)	N (B)	N (C)
Omnivorous	1187	42,0	18	461	416	310
Detritivores	951	33,7	21	269	543	139
Predators	547	19,4	62	195	254	98
Herbivorous	38	1,3	24	13	16	9
Parasitoids	18	0,6	12	8	5	5
Pollen / nectar	1	0,04	1	0	1	0
Others	83	2,9	26	45	26	12
	2825	100	164	991	1261	573

**Detritivores** belonged mainly to Collembola (91.6%), and in lower number to Psocoptera (3.9%) and Acari (1.9%). Coleoptera, Oligotomidae and Isopoda represented, altogether, less than 3% of this group.

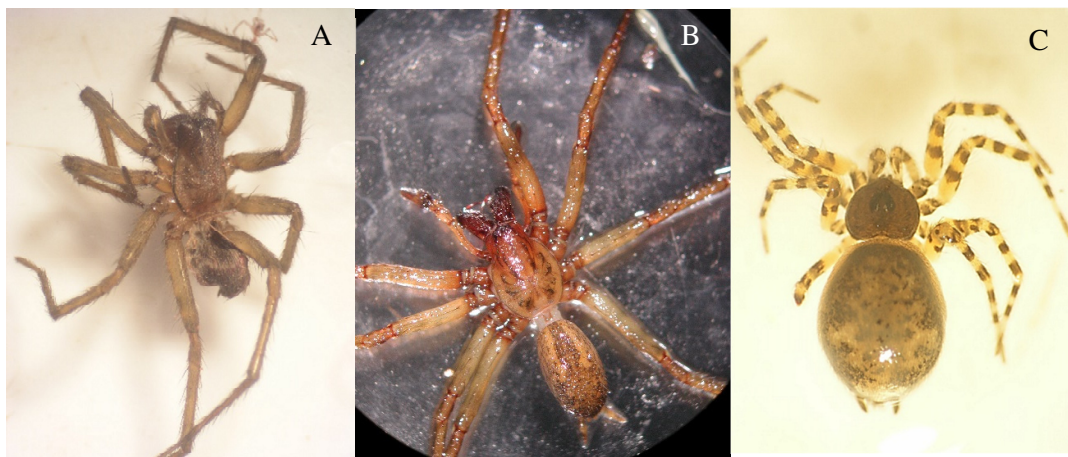
**Predators** belonged to eight taxa being Coleoptera (52.7%), Acari (23.8%), Araneae (20.3%), Opiliones (1.5%) and Chilopoda (1.1%) the most representatives. Coleoptera was dominated by Scydmaenidae (73.7%) (Fig. 3.4) and Anthicidae (18.3%). Carabidae and Staphylinidae only represented, respectively, 7.3% and 0.7% of the group (S3- Table 3.3).



**Figure 3.4** Specimen of *Palaeostigus palpalis* (Coleoptera: Scydmaenidae)

Predatory mites (Acari) were mainly represented by Erythraeidae (61.5%) and Anystidae (30.8%). Araneae included 13 families, being Agelenidae (21.6%), Gnaphosidae (18.9%) and Zodaridae (9.0%) the most representatives. Twenty-three

genera and 19 species were identified (S3- Table 3.4). *Malthonica lusitanica* Simon, 1898 (Agelenidae) was the most abundant species (10.8% of total Araneae). Three Iberian endemisms, namely *Eratigena bucculenta* (L. Koch, 1868) (Fig. 3.5A), *Eratigena feminea* Simon, 1870 (Fig. 3.5B) (Agelinidae) and *Oecobius machadoi* Wunderlich, 1995 (Fig. 3.5C) (Oecobiidae) were identified. When separated into functional groups, sheet web weavers were the dominant group, with 28.8% of individuals, followed by ground hunters (21.6%), other hunters (18%), space web hunters (18%) specialists (9%), and ambush hunters and sensing web weavers both representing 0.9% (S3- Table 3.5).



**Figure 3.5** Specimens of *Eratigena bucculenta* (A), *Eratigena feminea* (B) and *Oecobius machadoi* (C)

### 3.3.2 Flora

Altogether, 74 plant species, belonging to 39 families were identified. At site A, the percentage of fresh cover was higher in NCH, when compared with NCH3 or any of vineyard habitats ( $\chi^2_{\text{KW}}=12.91$ ,  $p=0.024$ ) (S3-Figure 3.1). When sites were analyzed together, the same trend was found, either for the percentage of fresh cover ( $\chi^2_{\text{KW}}=13.46$ ,  $p=0.004$ ) as for the percentage of total cover (fresh plus dry) ( $\chi^2_{\text{KW}}=15.12$ ,  $p=0.002$ ), which were higher in NCH than in vineyard habitats (S3-Figures 3.1 and 3.2). Richness didn't significantly differ among habitats or sites (S3- Figure 3.3). In general, the families most common were Asteraceae, Poaceae and Rosaceae (for more detailed information about species and families, in each habitat and site, see S3- Tables 3.6 and 3.7).

### 3.3.3 Effect of sampling date, site and habitat on arthropods

The abundance of total arthropods differed significantly between sampling dates (Wald $\chi^2=45.9$ ,  $p<0.001$ ) (Table 3.2; Fig. 3.6A), sites (Wald $\chi^2=283.6$ ,  $p<0.001$ ) (Fig. 3.7A) and habitats (Wald $\chi^2=44.2$ ,  $p<0.001$ ) (Fig. 3.8A). Richness differed between sampling dates (Wald $\chi^2=34.2$ ,  $p<0.001$ ) (Fig. 3.6B) and sites (Wald $\chi^2=9.8$ ,  $p<0.01$ ) (Fig. 3.7B). Both abundance and richness were higher in July 16<sup>th</sup> than in either August 19<sup>th</sup> or October 22<sup>nd</sup> (Figs. 3.6A and 3.6B).

**Table 3.2** Abundance (mean  $\pm$  SE) of arthropods caught, according to sampling date

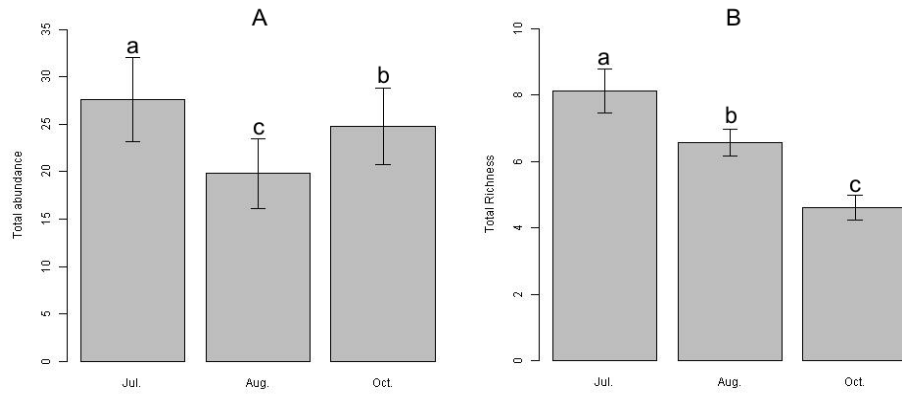
Sampling date	Total arthropods	Detritivores	Omnivores	Predators
W1- July 16 <sup>th</sup>	26.5 $\pm$ 3.9 a	2.2 $\pm$ 0.8 c	19.0 $\pm$ 2.9 a	3.9 $\pm$ 0.5
W2- August 19 <sup>th</sup>	19.5 $\pm$ 3.2 c	3.7 $\pm$ 1.0 b	9.1 $\pm$ 1.2 b	5.0 $\pm$ 2.6
W3- October 22 <sup>nd</sup>	23.2 $\pm$ 3.5 b	16.9 $\pm$ 3.4 a	1.5 $\pm$ 1.0 c	4.4 $\pm$ 1.1
Wald $\chi^2$	45.9	638.1	605.9	
$p$	<0.001	<0.001	<0.001	n.s
df	2	2	2	

Values with the same letter aren't significantly different from each other ( $p<0.05$ )

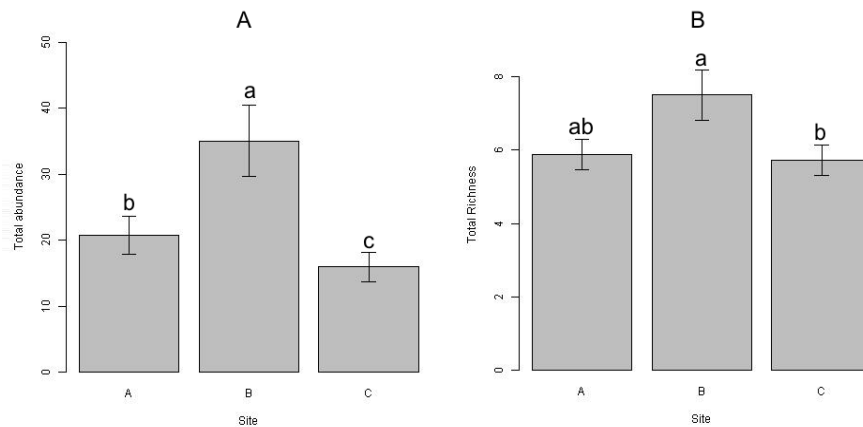
When considering the trophic groups, the abundance of omnivores was significantly higher in July and lower in October, while an inverse pattern of abundance was found for detritivores, which was higher in October. For predators, no differences were found between sampling dates (Table 3.2).

Considering the sites, the abundance of arthropods was higher in site B, compared to the other two sites (Fig. 3.7A). However, richness was significantly higher in site B than in site C, but didn't differed between site A and both sites B and C (Fig. 3.7B).

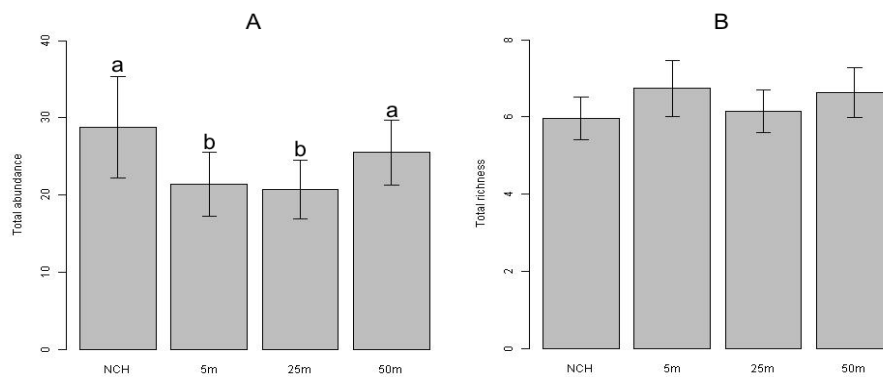
Considering the habitat, arthropods were more abundant in NCH, and in vineyards at 50 m, than in shorter distances (5 and 25 m) (Fig. 3.4A). Richness didn't differ between habitats (Fig. 3.8B).



**Figure 3.6** Abundance (A) and richness (B) (mean  $\pm$  S.E) of arthropods found in each sampling date; values with the same letter aren't significantly different from each other ( $p < 0.05$ ).



**Figure 3.7** Abundance (A) and richness (B) (mean  $\pm$  S.E) of arthropods found in each site. Values with the same letter aren't significantly different from each other ( $p < 0.05$ ).



**Figure 3.8** Abundance (A) and richness (B) (mean  $\pm$  S.E) of arthropods found in each habitat. NCH - Non-crop habitat; 5 m, 25 m and 50 m – distances from NCH, inside the vineyards; Values with the same letter aren't significantly different from each other ( $p < 0.05$ ).

### 3.3.4 Effect of habitat and vegetation on arthropods

#### Total arthropods

At site A, the abundance of arthropods was significantly higher in NCH1 than in any other habitat, whereas an opposite pattern was observed at site C, where abundance was higher in the distance of 50 m from the NCH, when compared with the other habitats. At site B, the abundance of arthropods didn't differ between habitats (Table 3.3).

**Table 3.3** Abundance (mean  $\pm$  S.E) of total arthropods, predators, detritivores and omnivores among sites and in all sites, together

Site	Habitat	Total arthropods	Omnivores	Detritivores	Predators
<b>A</b>	NCH 1	34.8 $\pm$ 6.9 a	11.8 $\pm$ 5.1 a	5.7 $\pm$ 1.4 b	12.5 $\pm$ 6.3 a
	NCH 2	20.1 $\pm$ 3.8 bc	11.9 $\pm$ 4.8 a	2.9 $\pm$ 1.2 bc	4.3 $\pm$ 1.7 b
	NCH 3	14.0 $\pm$ 2.9 d	6.0 $\pm$ 2.9 c	5.7 $\pm$ 1.7 b	1.7 $\pm$ 0.5 c
	V 5	14.7 $\pm$ 2.4 cd	7.6 $\pm$ 2.5 bc	2.7 $\pm$ 1.6 c	3.6 $\pm$ 0.6 bc
	V 25	14.4 $\pm$ 2.2 d	7.2 $\pm$ 2.1 bc	4.2 $\pm$ 1.9 bc	2.0 $\pm$ 0.4 bc
	V 50	23.7 $\pm$ 7.6 b	10.7 $\pm$ 4.3 ab	10.7 $\pm$ 7.9 a	1.8 $\pm$ 0.7 c
	Wald $\chi^2$	106.4	44.2	68.2	102.1
	<i>p</i>	<0.001	<0.001	<0.001	<0.001
	df	5	5	5	5
<b>B</b>	NCH	38.7 $\pm$ 15.1	16.9 $\pm$ 8.0 a	5.4 $\pm$ 2.7 b	14.7 $\pm$ 12.2 a
	V 5	37.7 $\pm$ 2.9	14.7 $\pm$ 2.5 a	15.9 $\pm$ 1.9 a	5.7 $\pm$ 0.6 b
	V 25	32.0 $\pm$ 10.2	8.9 $\pm$ 5.8 b	19.1 $\pm$ 9.7 a	3.9 $\pm$ 1.3 b
	V 50	31.8 $\pm$ 8.9	5.8 $\pm$ 2.4 b	19.9 $\pm$ 10.4 a	4.0 $\pm$ 1.4 b
	Wald $\chi^2$		64.2	95.5	88.3
	<i>p</i>	n.s.	<0.001	<0.001	<0.001
	df		3	3	3
<b>C</b>	NCH	14.8 $\pm$ 6.5 b	10.3 $\pm$ 7.0 a	1.2 $\pm$ 0.7 c	2.3 $\pm$ 0.5
	V 5	12.0 $\pm$ 2.3 b	5.3 $\pm$ 2.0 b	3.2 $\pm$ 1.8 b	3.1 $\pm$ 0.7
	V 25	15.8 $\pm$ 2.5 b	8.2 $\pm$ 2.5 ab	4.6 $\pm$ 2.2 ab	2.4 $\pm$ 0.6
	V 50	21.1 $\pm$ 5.1 a	10.6 $\pm$ 3.3 a	6.4 $\pm$ 3.8 a	3.0 $\pm$ 0.7
	Wald $\chi^2$	24.1	19.8	37.2	
	<i>p</i>	<0.001	<0.001	<0.001	n.s.
	df	3	3	3	
<b>All sites</b>	NCH	28.1 $\pm$ 6.6 a	13.2 $\pm$ 4.1 a	3.9 $\pm$ 1.2 d	9.5 $\pm$ 4.8 a
	V 5	21.4 $\pm$ 4.1 b	9.2 $\pm$ 2.8 b	7.3 $\pm$ 2.1 c	4.1 $\pm$ 0.6 b
	V 25	20.7 $\pm$ 3.8 b	8.1 $\pm$ 2.1 b	9.3 $\pm$ 3.5 b	2.8 $\pm$ 0.5 c
	V 50	25.5 $\pm$ 4.2 a	9.0 $\pm$ 2.0 b	12.3 $\pm$ 4.5 a	2.9 $\pm$ 0.6 bc
	Wald $\chi^2$	44.2	60.7	143.74	136.7
	<i>p</i>	<0.001	<0.001	<0.001	<0.001
	df	3	3	3	3

NCH - Non-crop habitat, V 5 m, V 25 m and V 50 m - Distances from NCH; Values with the same letter aren't significantly different from each other ( $p < 0.05$ )



The richness of arthropods wasn't different between habitats, in any site, but a positive correlation ( $r=0.61$ ;  $p<0.05$ ) (Table 3.4) was found between this parameter and richness of vegetation, at site C.

**Table 3.4** Significant Pearson's correlations found between groups of arthropods and richness and/or percentage of cover (fresh and or fresh + dry)

Groups of arthropods	R	% Fresh cover				% Total cover (fresh + dry)		Richness flora		
		T	A	B	C	T	B	T	B	C
Total arthropods	S +									0.61*
Detritivores	N -							0.33*		
Predators	N +		0.70**						0.61*	
Omnivores	S -	0.34*	0.62**			0.42**		0.46**		
Ants (SpCold)	N -					0.38*				
"	S -					0.34*		0.45*		
Ants (Cr)	S -	0.39*				0.35*	0.66*	0.32*		
Ants (G/O)	N +			0.70*						
"	S -	0.36*	0.63**		0.61*					
Spiders (SWW)	N +					0.33*		0.33*		
"	S +	0.35*				0.35*				
Spiders (Sp)	N -					0.40*				
"	S -					0.40*				

N - Abundance; S - Richness; R - Pearson correlation (- negative; +-positive); Correlations are significant at  $p<0.05$  (\*) and  $p<0.01$  (\*\*). T- data of all sites together; A: Site A; B: Site B; C: Site C; SpCold (cold-climate specialists and/or shade habitats); Cr (cryptic); GO (generalists and/or opportunistic); Sp (specialists); SWW (sheet web weavers)

### *Omnivores*

Considering all sites together, abundance of omnivores was higher in NCH, compared to vineyard habitats (Wald $\chi^2=60.7$ ,  $p<0.001$ ). However, in each site, their distribution showed different trends (Table 3.3).

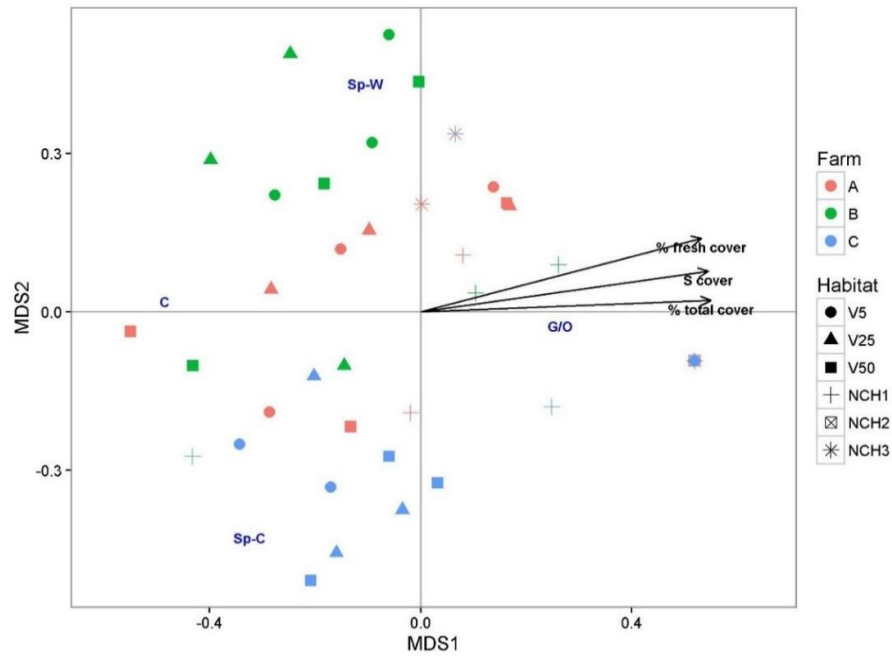
Considering Formicidae, the dominant omnivores, at sites A and B, the group of **generalists/opportunists** (G/O), were significantly more abundant in woodland (NCH1) and scrubland (NCH), respectively, than in vineyard habitats (Table 3.5); at site B, their abundance was positively correlated with the percentage of fresh cover ( $r= 0.70$ ,  $p<0.05$ ) (Table 3.4). However, their richness was negatively correlated with the percentage of fresh cover on site A ( $r=0.63$ ,  $p<0.01$ ), site C ( $r=0.61$ ,  $p<0.05$ ) or on all sites analyzed together ( $r=0.36$ ,  $p<0.05$ ) (Table 3.4).

**Table 3.5** Abundance (mean  $\pm$  S.E) of each functional group of Formicidae among sites and in all sites, together

Site	Habitat	Cr		G/O		SpCold		SpWarm	
<b>A</b>	NCH 1	0.3 $\pm$ 0.2	b	11.2 $\pm$ 4.8	a	0.2 $\pm$ 0.2		0.2 $\pm$ 0.2	
	NCH 2	0.0 $\pm$ 0.0	ab	11.7 $\pm$ 4.7	b	0.0 $\pm$ 0.0		0.0 $\pm$ 0.0	
	NCH 3	0.1 $\pm$ 0.1	b	5.3 $\pm$ 2.5	cd	0.0 $\pm$ 0.0		0.6 $\pm$ 0.4	
	V 5	1.6 $\pm$ 1.0	b	5.4 $\pm$ 1.9	d	0.2 $\pm$ 0.2		0.3 $\pm$ 0.2	
	V 25	1.1 $\pm$ 0.8	b	5.3 $\pm$ 1.4	d	0.0 $\pm$ 0.0		0.3 $\pm$ 0.2	
	V 50	5.0 $\pm$ 4.4	a	5.1 $\pm$ 2.0	d	0.2 $\pm$ 0.1		0.2 $\pm$ 0.2	
	Wald $\chi^2$	107.5		88.0					
	<i>p</i>	<0.001		<0.001		n.s		n.s	
	df	5		5					
<b>B</b>	NCH	0.0 $\pm$ 0.0		16.2 $\pm$ 7.6	a	0.1 $\pm$ 0.1		0.6 $\pm$ 0.3	b
	V 5	1.3 $\pm$ 0.4		8.2 $\pm$ 1.9	b	0.1 $\pm$ 0.0		5.0 $\pm$ 0.3	a
	V 25	1.3 $\pm$ 0.8		5.3 $\pm$ 4.7	b	0.6 $\pm$ 0.6		1.2 $\pm$ 0.6	b
	V 50	0.9 $\pm$ 0.6		2.0 $\pm$ 1.1	c	0.7 $\pm$ 0.7		1.1 $\pm$ 0.4	b
	Wald $\chi^2$			125.6				49.1	
	<i>p</i>	n.s		3		n.s		3	
	df			<0.001				<0.001	
<b>C</b>	NCH	0.4 $\pm$ 0.4		9.4 $\pm$ 7.0	a	0.3 $\pm$ 0.2	b	0.1 $\pm$ 0.1	
	V 5	0.3 $\pm$ 0.2		4.3 $\pm$ 1.8	b	0.7 $\pm$ 0.4	b	0.0 $\pm$ 0.0	
	V 25	0.2 $\pm$ 0.1		6.4 $\pm$ 2.3	ab	1.3 $\pm$ 0.3	ab	0.1 $\pm$ 0.1	
	V 50	0.1 $\pm$ 0.1		8.2 $\pm$ 3.0	a	2.2 $\pm$ 0.7	a	0.0 $\pm$ 0.0	
	Wald $\chi^2$			19.7		16.7			
	<i>p</i>	n.s		<0.001		<0.001		n.s	
	df			3		3			
<b>All</b>	NCH	0.3 $\pm$ 0.2	c	12.4 $\pm$ 3.9	a	0.2 $\pm$ 0.1	b	0.3 $\pm$ 0.1	b
	V 5	1.1 $\pm$ 0.5	b	6.0 $\pm$ 1.9	b	0.3 $\pm$ 0.2	b	1.8 $\pm$ 0.8	a
	V 25	0.9 $\pm$ 0.4	bc	5.7 $\pm$ 1.7	b	0.6 $\pm$ 0.2	ab	0.6 $\pm$ 0.2	b
	V 50	2.0 $\pm$ 1.5	a	5.1 $\pm$ 1.3	b	1.0 $\pm$ 0.4	a	0.4 $\pm$ 0.2	b
	Wald $\chi^2$	35.5		107.4		20.4		43.4	
	<i>p</i>	<0.001		<0.001		<0.001		<0.001	
	df	3		3		3		3	

NCH - Non-crop habitat, V 5 m, V 25 m and V 50 m - Distances from NCH; Cr - Cryptic, G/O - generalists/opportunists, SpCold - cold-climate specialists and/or shade habitats; SpWarm- hot climate specialists and/or open habitats. Values with the same letter are not significantly different from each other at the indicated level ( $p < 0.05$ )

NMDS analysis also revealed that G/O, seems to be more associated with non-crop habitats and positively influenced by vegetation, particularly by fresh cover (Fig. 3.9).



**Figure 3.9** Non-metric Multidimensional scaling ordination showing distinct ant community composition between habitats including relationships with vegetation parameters (% total cover; % fresh cover and Richness (S) of cover). The two NMDS axis were significantly correlated with total percentage of cover ( $r^2=0.293$ ,  $p=0.001$ ), percentage of fresh cover ( $r^2=0.167$ ,  $p=0.028$ ) and richness of flora ( $r^2=0.228$ ,  $p=0.004$ ), meaning that these variables explained 29.3%, 16.7% and 22.8% of the variation on Formicidae given by the two axes. GO: generalists and/or opportunists; C - cryptic; SpW - hot climate specialists and/or open habitats; SpC - cold-climate specialists and/or shade habitats

**Cryptics (Cr)** were significantly more abundant in the traps located in the vineyards at the distance of 50 m from NCH (Table 3.5), when sites were analyzed together, and at site A. However, their richness was negatively correlated with percentage of fresh cover ( $r=0.39$ ,  $p<0.05$ ), total cover ( $r=0.35$ ,  $p<0.05$ ), and richness of flora ( $r=0.32$ ,  $p<0.05$ ), when all sites were analyzed together, and with percentage of total cover at site B ( $r=0.66$ ,  $p<0.01$ ) (Table 3.4). These results were confirmed by NMDS analysis which revealed that Cr were more associated with vineyard's habitats, and negatively affected by vegetation (Fig. 3.9).

**Cold climate's specialists (SpCold)** were significantly more abundant in vineyards located at the distance of 50 m from NCH, when all sites were analyzed together (Table 3.5); significant correlations with vegetation parameters were only found when all sites were analyzed together. Thus, their abundance and richness were negatively correlated

with percentage of total cover ( $r=0.38$ ,  $p<0.05$ , for abundance) (Table 3.4). **Hot climate specialists** (SpWarm) were significantly more abundant in vineyards located at the distance of 5 m from NCH, when all sites were analyzed together and at site B (Table 3.5).

NMDS analysis also revealed that specialist's ants (SpCold, SpWarm) were more associated with vineyard's habitats (Fig. 3.9).

#### *Detritivores*

Within habitats, detritivore's abundance was consistently higher in vineyards located at the distance of 50 m from NCH, than in NCH (Table 3.3), although richness didn't differ between habitats.

#### *Predators*

In sites A and B, and when considering all sites together, predators were more abundant in NCH than in vineyard habitats located at 50 m from NCH (Table 3.3). However, the richness of predators didn't differ between habitats.

Although spiders were caught in a very low number, possibly due to the short sampling period (only 24 h), not allowing to analyze the impact of habitat on their abundance using GzLM, results suggest an influence of local vegetation on their distribution, considering correlations found between some functional guilds and vegetation parameters (Table 3.4). Abundance of sheet web weavers was positively, although weakly correlated with the percentage of total cover ( $r= 0.33$ ,  $p<0.05$ ) and with richness of plants ( $r= 0.33$ ,  $p<0.05$ ) while their richness was positively correlated with the percentage of total cover ( $r= 0.35$ ;  $p<0.05$ ). On the contrary, the abundance and richness of specialist's (Zodariidae family) seems to have been negatively affected by the total percentage of cover (Table 3.4).

### **3.4 Discussion**

Although the results obtained in the present study should be regarded with caution since they correspond to a particular period of the year, only 1 year and have been obtained with a single trap by location and period, they point to the importance of the presence of non-crop habitats near to vineyards in the increment of ground-dwelling arthropods abundance, in particular, of the predator group. Thus, local vegetation seems

to have played a major role in the abundance and richness increment of ants and spiders' communities, although this effect differed between functional groups (some related taxa displayed markedly different responses to land use and habitat preferences).

#### ***3.4.1 Effect of sampling date***

In this study, a clear effect of the sampling date on the abundance and richness of ground-dwelling arthropods assemblage was found. This could have been related to a combined effect of climate conditions, composition and complexity of vegetation of the ground cover. Thus, as reported by Frampton and Dorne (2007), drought has been found to negatively affect the abundance of arthropods on farmland through changes in vegetation structural complexity. On the other hand, Knapp and Řezáč (2015) found a substantial intra-annual variation in arthropods spatial distribution, and point out that such variation could be driven by substantial changes in microclimate and food sources experienced by arthropods within arable fields, during a season. In fact, strong seasonality is a feature of most ecosystems, particularly in Mediterranean habitats, where the seasonal fluctuations of temperature and rainfall create marked pulses of productivity and animal activity (reviewed by Pérez-Bote and Romero, 2012). The seasonal and annual variability of soil invertebrate assemblages has potentially important implications on community dynamics in the study systems, since the changes in species composition may affect their interactions and food web dynamics over time.

#### ***3.4.2 Effect of habitat and vegetation***

According to Gaigher and Samways (2010) findings, a high complexity of vegetation contributes to a high diversity of arthropods (predators, detritivores and phytophagous feeding guilds), and natural habitats sustain a high overall arthropod diversity. These authors report positive correlations between the abundance and richness of arthropods and both the percentage of vegetation cover and the richness of vegetation in vineyards, and states also that management activities inside vineyards affects negatively arthropod's abundance and diversity. The effect of general decline in arthropod abundance and richness, with increasing land-use and management intensity, was also reported by Pérez-Bote and Romero (2012). In the present study, the richness of ground-dwelling arthropods assemblage wasn't significantly affected by the increasing

distance from NCH, which could indicate that the intensity of management practices inside vineyards didn't had such negative impact, and that vegetation found inside vineyard habitats might have played an important role on such result. It should be noted that the management practices in DDR vineyards do not include, in a regular basis, tillage operations, that Sharley et al. (2008) have reported as having negative impact on ants.

Also, it was found that the response to the habitat differed between functional groups of arthropods, which could in part be because different plant associations, with different structure, result in a wide variety of microhabitats. However, as reported by Gaigher and Samways (2010), the vegetation structure can also differentially affect the susceptibility of different species to pitfall trapping, and so discretion should be used when comparing habitats with different ground cover.

The fact that total arthropods assemblage distributed differently in each site, suggest that it was more influenced by local parameters, than by the proximity of the non-cropped areas. The different results found between different groups of Formicidae suggest a high impact of local scale factors such as soil structure, as reported by Peck et al. (1998) and/or ground cover vegetation. In short, the abundance of generalists/opportunist's Formicidae seemed to have been influenced mainly by both the proximity of NCH and by fresh vegetation, although vegetation parameters affected negatively their richness, which can be due to a dominance of some species in relation to other, as reported by Bestelmeyer and Wiens (1996). On the other hand, cryptics and specialists of cold climates ants seems to have preferred vineyard habitats, being however influenced negatively by vegetation parameters. More than 90% of captures of the Iberian endemic cryptic species *A. iberica*, were obtained inside vineyards (unpublished data), which highlights the added value of this agroecosystem for this species.

Our results suggest that vegetation can impact positively the abundance of G/O Formicidae, which is in line with Holec et al. (2006), who found that pitfall trapping reveal significantly higher activity of *Lasius niger*, a generalist foraging Formicidae, in tall and dense vegetation stands, than in low and sparse vegetation. These authors also report that nesting and foraging may differ between ant's microclimatic requirements and the formation of vegetation mosaics may be important to change in their populations during succession, particularly in summer period. According to Bestelmeyer and Wiens (1996), cryptic ants forage mainly in the soil and leaf litter, although they may be dominant above the litter, in interactions with epigeic species. It is possible that, in DDR

case, terraces found within vineyards may have an impact on the distribution of ants among vineyards, as they are indeed islands of shrubby and herbaceous vegetation within vineyards, offering resources as well as protection against the high summer temperatures and therefore, the higher abundance of some functional groups, such as for example cryptics, could have been due to its impact.

Because ants are ubiquitous in agroecosystems, easily sampled, taxonomically well known, and, as demonstrated in the present study, often correlated with vegetation and habitats, they can probably be useful to develop terrestrial bio-indicators in DDR vineyards.

Detritivore's abundance (mainly collembolan) was consistently higher in vineyard habitats located at 50 m from NCH, than in NCH itself, although richness wasn't significantly affected by the habitat. This is in line with Sandler *et al.* (2014), who reported that no-tillage management practices with very low or null movements can result in an increase of collembolan populations. It is also possible that the presence of predators, including spiders, in NCH have affected negatively their populations, because, according to Lawrence and Wise (2000) findings, spider predation may reduce Collembola densities enough to lower rates of litter disappearance on the forest floor.

Predators were found to be more abundant in NCH, compared to vineyard habitats located at longer distances, pointing out the importance of the presence of these structures near vineyards. Several authors have reported an impact of non-crop habitats in the presence of ground-dwelling predators, particularly on Araneae (Sackett *et al.* 2009; Thomson and Hoffmann 2009; Cardenas *et al.* 2015; Knapp and Řezáč 2015), Staphylinidae (Thomson and Hoffmann 2009) and Carabidae (Nash *et al.* 2008). As demonstrated by Knapp and Řezáč (2015), even small non-crop habitat islands can substantially enhance biodiversity of agricultural landscape, but some larger non-crop habitats are necessary to maximize arthropod diversity. These authors found that NCH influenced the richness of spiders (positively) and carabids (negatively). The contrasting patterns can be due to possible effect of intra-guild predation (killing and eating species that use similar resources), being potential competitors (Polis and McCormick 1987). Yet, according to the same authors, the local environmental conditions (tree cover, shrub cover, grass cover, litter depth) is in general more influential than the area per se.

In the present study, web-spider and specialist-spider communities responded differently to plant community, which agrees with Rypstra and Carte (1995), Caprio *et*

al. (2015) and Cardenas et al. (2015). According to the reviewed by Rypstra and Carte (1995), web-building spiders are particularly dependent on vegetation because it provides to them suitable web-attachment sites. Isaia et al. (2006) and Öberg et al. (2007) also found that heterogeneity of landscape impacted differently several groups of ground-dwelling spiders. However, Košulič et al. (2014) suggest that, in terraced vineyards, the presence of large patches of uniform grasslands around vineyard terraces is more important for spiders than the presence of different habitats, like woodlands, ruderals and other agriculture ecosystems. Košulič and Hula (2013) reported that vineyard terraces can act as an important refuge for rare steppe species of spiders in the unified intensive agricultural landscape, explaining that suitable conditions of vineyard terraces (steep slopes with exposed substrate, low vegetation structure, sparse vegetation coverage) enable many rare endangered species to occur. Franin et al. (2016) found that weedy strips in field margins contained a high number of spiders, and that this group also benefit from the presence of dry vegetation. In our study, the three Iberian endemisms *O. machadoi* (sensing web weaver), *E. bucculenta* and *E. feminea* (sheet web weavers) were caught only in vineyard habitats, which highlights the added value of terraced vineyards for their conservation.

It was found that richness of overall arthropods, as well as of functional groups found in vineyards, weren't significantly different from those found on NCH, which can indicate that: a) management practices performed hasn't a significant impact on diversity of arthropods, and b) vegetation existing in vineyard's can represent ecological advantages from the functional biodiversity point of view, since it offer food resources as well as shelter in particular for ants and spiders, providing resilience to DDR vineyard agroecosystem. Particularly, in one row terraced vineyards system, the existence of local vegetation on slopes related with reduced mowing frequency, can represent an added value to the enhancement of functional biodiversity in vineyards. This is in line with Nascimbene et al. (2013) findings, who predict that in the Conegliano-Valdobbiadene DOCG area, the maintenance of high plant diversity is mainly related to the management intensity applied to vineyards on steep slopes, where low mowing frequency is recommended.

The results of this study indicate that viticulture landscapes in DDR terraced vineyards have considerable potential to support a range of ground-dwelling arthropod groups, in result of the relatively low intensity management, in particular, in one row



terraces, and of the presence of remnants of local vegetation. Therefore, the maintenance and sympathetic management of remnant vegetation in the border of vineyards plots and of ground cover in vineyards should be encouraged for enhancing functional biodiversity.

These measures are likely to reduce management costs and to yield positive effects on the diversity of several taxonomic groups, including organisms that benefit ecosystem services such as biological control of phytophagous species.

### 3.5 References

- Addison P, Baauw AH, Groenewald GA (2013) An initial investigation of the effects of mulch layers on soil-dwelling arthropod assemblages in vineyards. *South African Journal of Enology and Viticulture* 34 (2), 266-271
- Andresen T, Bianchi de Aguiar F, Curado MJ (2004) The Alto Douro Wine Region greenway. *Landscape and Urban Planning* 68, 289-303
- Bestelmeyer BT, Wiens JA (1996) The effects of land use on the structure of ground-foraging ant communities in the argentine Chaco. *Ecological Applications* 6 (4), 1225-1240
- Bolduc E, Buddle CM, Bostanian NJ, Vincent C (2005) Ground-dwelling spider fauna (Araneae) of two vineyards in Southern Quebec. *Environmental Entomology* 34(3), 635-645
- Bruggisser OT, Schmidt-Entling MH, Bacher S (2010) Effects of vineyard management on biodiversity at three trophic levels. *Biological Conservation* 143(6), 1521-1528
- Buschke FT, Seaman MT (2011) Functional Feeding Groups as a Taxonomic Surrogate for a Grassland Arthropod Assemblage. *African Invertebrates*, 52(1):217-228. <https://doi.org/10.5733/afin.052.0112>
- Caprio E, Nervo B, Isaia M, Allegro G, Rolando A (2015) Organic versus conventional systems in viticulture: Comparative effects on spiders and carabids in vineyards and adjacent forests. *Agricultural Systems* 136, 61–69
- Cárdenas M, Pascual F, Campos M, Pekár S (2015) The spider assemblage of olive groves under three management systems. *Environmental Entomology* 44(3), 509-518
- Cardoso P, Pekár S, Jocqué R, Coddington JA (2011) Global patterns of guild composition and functional diversity of spiders. *PLoS ONE* 6 (6), e21710. doi:10.1371/journal.pone.0021710

- Crawley MJ (2007) The R book. John Wiley and Sons, Ltd, Chichester, 950 pp
- Crespí AL, Bernardos S, Castro A, Fernandes CP, Amich F (2005) Phytostructural characterization of several vegetation types in northern Portugal. II. The structural expressivity and the resistance of the vegetation. *Plant Biosystems* 139 (3), 387 – 398
- Doran JW, Zeiss MR (2000) Soil health and sustainability: managing the biotic component of soil quality. *Appl Soil Ecol* 15: 3–11
- Frampton GK, Dorne JL (2007) The effects on terrestrial invertebrates of reducing pesticide inputs in arable crop edges: a meta-analysis. *Journal of Applied Ecology* 44, 362–373
- Franin K, Baric B, Kustera G (2016) The role of ecological infrastructure on beneficial arthropods in vineyards. *Spanish Journal of Agricultural Research* 14(1), e0303. doi:[http://dx.doi.org/ 10.5424/sjar/2016141-7371](http://dx.doi.org/10.5424/sjar/2016141-7371)
- Gaigher R, Samways MJ (2010) Surface-active arthropods in organic vineyards, integrated vineyards and natural habitat in the Cape Floristic Region. *Journal of Insect Conservation* 14, 595–605
- Garbach K, Milder JC, Montenegro M, Karp DS, DeClerck FAJ (2014) Biodiversity and ecosystem services in agroecosystems. *Encyclopedia Agricultural Food Systems* 2: 21–40. <https://doi.org/10.1016/B978-0-444-52512-3.00013-9>
- Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, Ceryngier P, Liira J, Tscharnkte T, Winqvist C, Eggers S, Bommarco R, Pärt T, Bretagnolle V, Plantegenest M, Clement LW, Dennis C, Palmer C, Oñate JJ, Guerrero I, Hawro V, Aavik T, Thies C, Flohre A, Hänke S, Fischer C, Goedhart PW, Inchausti P (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11, 97–105
- Holec M, Frouz J, Pokorný R (2006) The influence of different vegetation patches on the spatial distribution of nest and the epigeic activity of ants (*Lasius niger*) on a spoil bank after brown coal mining (Czech Republic). *European Journal of Soil Biology* 42, 158–165
- Isaia M, Bona F, Badino G (2006) Influence of landscape diversity and agricultural practices on spider assemblage in Italian vineyards of Langa Astigiana (NW-Italy). *Environmental Entomology* 35, 297-307
- Kleijn D, Kohler F, Báldi A, Batáry P, Concepción ED, Clough Y, Díaz M, Gabriel D, Holzschuh A, Knop E, Kovács A, Marshall EJP, Tscharnkte T, Verhulst J (2009) On

- the relationship between farmland biodiversity and land use intensity in Europe. *Proceedings of the Royal Society of London B* 276, 903–909
- Knapp M, Řezáč M (2015) Even the smallest non-crop habitat islands could be beneficial: distribution of carabid beetles and spiders in agricultural landscape. *PLoS ONE* 10(4), e0123052. doi:10.1371/journal.pone.0123052
- Košulič O, Hula V (2013) Rare and remarkable spiders (Araneae) from vineyard terraces in Pálava region (South Moravia, Czech Republic). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 61, 663–676
- Košulič O, Michalko R, Hula V (2014) Recent artificial vineyard terraces as a refuge for rare and endangered spiders in a modern agricultural landscape. *Ecological Engineering* 68, 137–154
- Lawrence KL, Wise DH (2000) Spider predation on forest-floor collembolan and evidence for indirect effects on decomposition. *Pedobiologia* 44, 33–39
- Lehman RM, Acosta-Martinez V, Buyer JS, Cambardella SA, Collins HP, Ducey TF, Halvorson JJ, Jin VL, Johnson JMF, Kremer RJ, Lundgren JG, Manter DK, Maul JE, Smith JL, Stott DE (2015) Soil biology for resilient, healthy soil. *J Soil Water Conserv* 70 (1): 12A–18A
- Marôco J (2011) *Análise estatística com o SPSS Statistics*. ReportNumber – *Análise e Gestão de informação*, 5ª edição, 990 pp
- May RM (1988) How many species are there on Earth? *Science* 241, 1441–1449
- Melbourne BA (1999) Bias in the effect of habitat structure on pitfall traps: An experimental evaluation. *Australian Journal of Ecology* 24, 228–239
- Nascimbene J, Marini L, Ivan D, Zottini M (2013) Management intensity and topography determined plant diversity in vineyards. *PLoS ONE* 8(10), e76167. doi:10.1371/journal.pone.0076167
- Nash MA, Thomson LJ, Hoffmann AA (2008) Effect of remnant vegetation, pesticides, and farm management on abundance of the beneficial predator *Notonomus gravis* (Chaudoir) (Coleoptera: Carabidae). *Biological Control* 46, 83–93
- Öberg S, Ekbom B, Bommarco R (2007) Influence of habitat type and surrounding landscape on spider diversity in Swedish agroecosystems. *Agriculture Ecosystems & Environment* 122, 211–219
- Oksanen J, Blanchett FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHM, Wagner H (2016) *Vegan: community. Ecology Package*.

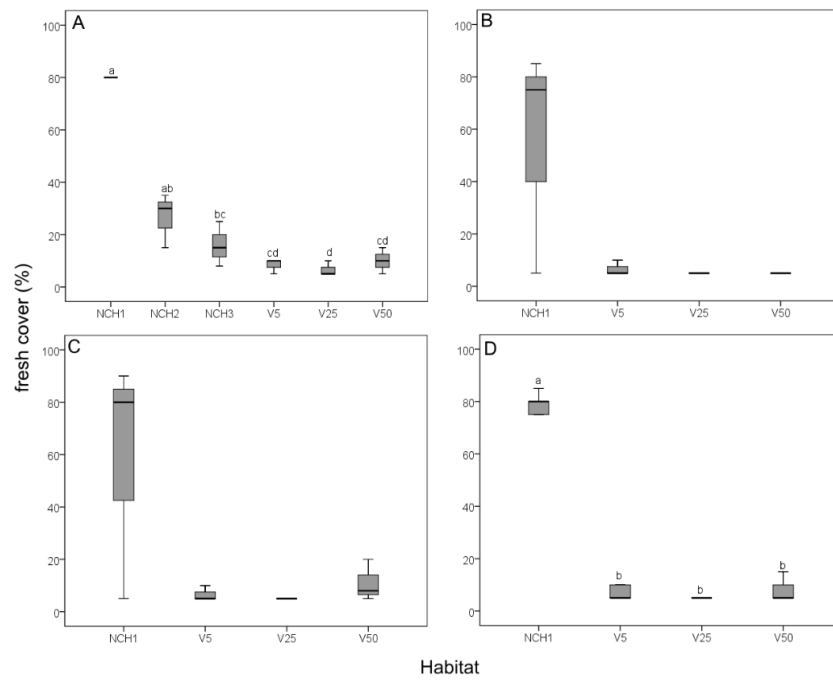
- R Package 2.4-1. [WWW document] URL <http://CRAN.R-project.org/package=vegan> [Accessed on 9 September 2016]
- Peck SL, McQuaid B, Campbell CL (1998) Using ant species (Hymenoptera: Formicidae) as a biological indicator of agroecosystem condition. *Environmental Entomology* 27, 1102 – 1110
- Pérez-Bote JL, Romero A (2012) Epigeic soil arthropod abundance under different agricultural land uses. *Spanish Journal of Agricultural Research* 10(1), 55–61
- Petchey OL, Gaston, K.J. (2002) Functional diversity (FD), species richness and community composition. *Ecology Letters* 5, 402–411
- Polis GA, McCormick SJ (1987) Intraguild predation and competition among desert scorpions. *Ecology* 68, 332-343
- R Development Core Team (2015) R: A Language and Environment for Statistical Computing. Vienna, Austria: the R Foundation for Statistical Computing. [WWW document] URL <http://www.R-project.org/> [Accessed on 1 August 2016]
- Roig X, Espadaler X (2010) Proposal of functional groups of ants for the Iberian Peninsula and Balearic Islands and their use as bioindicators. *Iberomyrmex* 2, 28–29
- Rypstra AL, Carter PE (1995) The web-spider community of soybean agroecosystems in Sothern Ohio. *Journal of Arachnology* 23, 135–144
- Sackett TE, Buddle CM, Vincent C (2009) Dynamics of spider colonization of apple orchards from adjacent deciduous forest. *Agriculture Ecosystems & Environment* 129, 144-148
- Sandler RV, Falco LB, Di Ciocco CA, Castro-Huerta R, Coviella CE (2014) The degree of change of collembolan community structure related to anthropic soil disturbance. *PeerJ PrePrints* 2, e721v1: <https://doi.org/10.7287/peerj.preprints.721v1>
- Sharley DJ, Hoffmann AA, Thomson LJ (2008) The effects of soil tillage on beneficial invertebrates within the vineyard. *Agriculture and Forest Entomology* 10, 233-243
- Thomson LJ, Hoffmann AA (2007) Effects of ground cover (straw and compost) on the abundance of natural enemies and soil macro invertebrates in vineyards. *Agriculture and Forest Entomology* 9, 173 – 179
- Thomson LJ, Hoffmann AA (2009) Vegetation increases the abundance of natural enemies in vineyards. *Biological Control* 49, 259–269

- Topping CJ, Sunderland KD (1992) Limitations to the use of pitfall traps in ecological studies exemplified by a study of spiders in a field of winter wheat. *Journal of Applied Ecology* 29:485–491
- Triplehorn CA, Johnson NF (2005) Borror and DeLong's Introduction to the Study of Insects. Belmont. Thomson Brooks/Cole. 7th ed, 864 pp
- Trivellone V, Paltrinieri LP, Jermini M, Moretti M (2012) Management pressure drives leafhopper communities in vineyards in Southern Switzerland. *Insect Conservation and Diversity* 5, 75–85. doi:10.1111/j.1752-4598.2011.00151.x
- Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* 8: 857–874
- Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*. 4th edition, Springer, New York
- Wickham H (2009) “ggplot2: elegant graphics for data analysis”. New York: Springer
- Zaller JG, Kerschbaumer G, Rizzoli R, Tiefenbacher A, Gruber E, Schedl H (2015): Monitoring arthropods in protected grasslands: comparing pitfall trapping, quadrat sampling and video monitoring. *Web Ecology*, 15:15-23

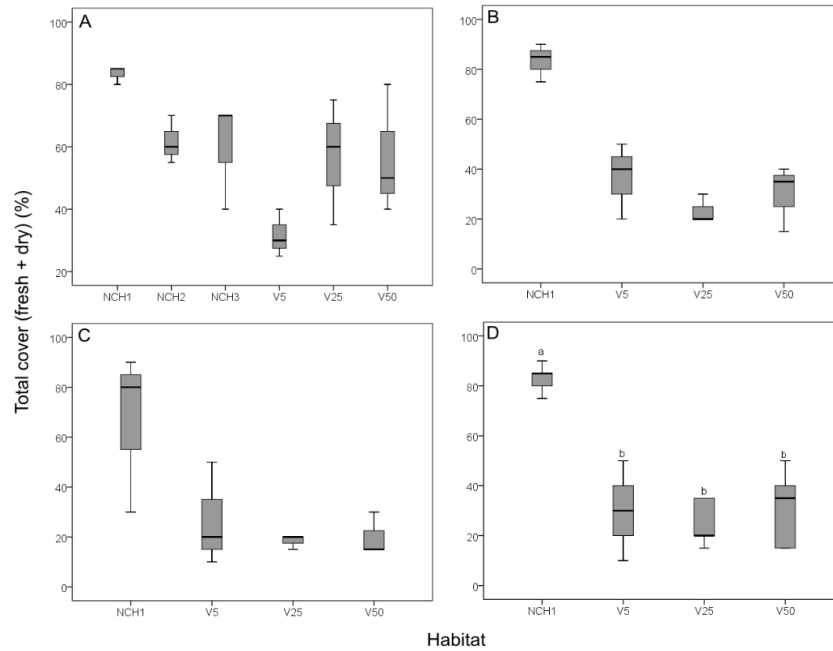
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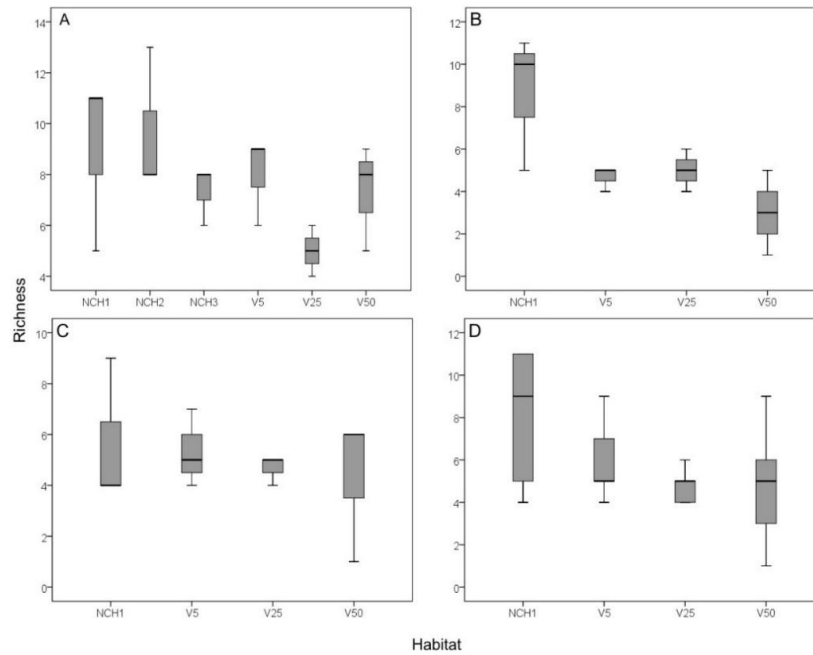
### Supplement 3



**S3- Fig. 3.1** Percentage of fresh cover (median  $\pm$  interquartiles) found in each site (A- site A; B- Site B; C- site C; D-all sites together) and habitat; values with the same letter are not significantly different from each other ( $p < 0.05$ ).



**S3 - Fig 3.2** Percentage of total cover (median  $\pm$  interquartiles) found in each site (A- site A; B- Site B; C- site C; D-all sites together) and habitat; values with the same letter are not significantly different from each other ( $p < 0.05$ ).



**S3- Fig 3.3** Richness (median  $\pm$  interquartiles) of plants found in each site (A- site A; B- Site B; C- site C; D-all sites together) and habitat; values with the same letter are not significantly different from each other ( $p < 0.05$ ).

**S3 -Table 3.1** Taxonomic (specie and sub-family), functional group, abundance (N) and relative percentage (%) of Formicidae caught during the study period

Species	Sub-family	Functional group	N	%
<i>Pheidole pallidula</i> (Nylander 1849)	Myrmicinae	G/O	624	52,6
<i>Plagiolepis pygmaea</i> (Latreille 1798)	Formicinae	G/O	117	9.9
<i>Aphaenogaster gibbosa</i> (Latreille 1798)	Myrmicinae	Cr	113	9.5
<i>Aphaenogaster iberica</i> Emery 1908 *	Myrmicinae	G/O	86	7.3
<i>Iberoformica subrufa</i> Roger 1859	Formicinae	SpCold	57	4.8
<i>Tapinoma</i> sp.	Dolichoderinae	G/O	45	3.8
<i>Messor barbarus</i> (Linnaeus 1767)	Myrmicinae	SpWarm	42	3.5
<i>Crematogaster auberti</i> Emery 1869	Myrmicinae	G/O	33	2.8
<i>Camponotus cruentatus</i> (Latreille 1802)	Formicinae	SpWarm	30	2.5
Formicinae sp	Formicinae	-	20	1.7
<i>Camponotus pilicornis</i> (Roger 1859)	Formicinae	SpWarm	7	0.6
<i>Cataglyphis hispanica</i> (Emery 1906)*	Formicinae	SpWarm	5	0.4
<i>Lasius</i> sp.	Formicinae	SpCold	2	0.2
<i>Camponotus piceus</i> (Leach 1825)	Formicinae	SpWarm	2	0.2
<i>Aphaenogaster</i> sp	Myrmicinae	Cr	1	0.1
<i>Solenopsis</i> sp.	Myrmicinae	Cr	1	0.1
<i>Cataglyphis</i> sp.	Formicinae	SpWarm	1	0.1
			1186	100

GO - generalists and/or opportunists; Cr - cryptic; SpWarm - hot climate specialists and/or open habitats; SpCold - cold-climate specialists and/or shade habitats; \* - Iberian endemism (according to Janicki *et al.*, 2016); - damaged specimens not identified

## References

Janicki, J., Narula, N., Ziegler, M., Guénard, B. & Economo, E.P. (2016) Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: The design and implementation of antmaps.org. *Ecological Informatics* **32**, 185-193



**S3- Table 3.2** Abundance (N), richness (S) and relative percentage (%) of functional group of Formicidae found in each site and in all sites

Functional group		A	B	C	Total
G/O	N	363	286	256	905
	%	78.9	68.8	82.6	76.3
	S	5	5	5	5
Cr	N	73	32	10	115
	%	15.9	7.7	3.2	9.7
	S	3	1	1	3
SpWarm	N	14	71	2	87
	%	3.0	17.1	0.6	7.3
	S	2	6	1	7
SpCold	N	5	13	41	59
	%	1.1	3.1	13.2	5.0
	S	2	2	1	2
Others*	N	5	14	1	20
	%	1.1	3.4	0.3	1.7
	S	1	1	1	1
		460	416	310	1186

GO - generalists and/or opportunists; Cr - cryptic; SpWarm - hot climate specialists and/or open habitats; SpCold - cold-climate specialists and/or shade habitats; \* - other ants which were damaged and were identified only until the subfamily level, being difficult to assess the functional group

**S3-Table 3.3** Taxonomic (specie and sub-family), abundance (N) and relative percentage (%) of predators belonging to Coleoptera caught during the study period

Specie	Family	N	%
<i>Palaeostigus palpalis</i> (Latreille 1804)	Scydmaenidae	213	73.7
<i>Anthicus</i> sp.	Anthicidae	53	18.3
<i>Nebria (Nebria) brevicollis</i> (Fabricius 1792)	Carabidae	13	4.5
<i>Microlestes</i> sp.	Carabidae	4	1.4
<i>Harpalus (Pseudoophonus) rufipes</i> (De Geer, 1774)	Carabidae	1	0.3
<i>Penetretus rufipennis</i> (Dejean 1828)	Carabidae	1	0.3
<i>Olisthopus</i> sp.	Carabidae	1	0.3
Carabidae sp.	Carabidae	1	0.3
<i>Anotylus</i> sp.	Staphylinidae	1	0.3
<i>Micropeplus staphylinoides</i> Marsham 1802	Staphylinidae	1	0.3
		289	100

**S3-Table 3.4** Species of Araneae identified in all sites, during the study period

Specie	Family	N	%
<i>Malthonica lusitanica</i> Simon, 1898	Agelenidae	12	10.8
<i>Zodarion</i> sp.	Zodariidae	9	8.1
<i>Hahnina nava</i> (Blackwall, 1841)	Hahniidae	7	6.3
<i>Euophrys rufibarbis</i> (Simon, 1868)	Salticidae	6	5.4
<i>Scytodes velutina</i> Heineken & Lowe, 1832	Scytodidae	6	5.4
<i>Callilepis concolor</i> Simon, 1914	Gnaphosidae	5	4.5
<i>Nomisia</i> sp.	Gnaphosidae	5	4.5
<i>Malthonica</i> sp.	Agelenidae	5	4.5
<i>Gnaphosidae</i> sp.	Gnaphosidae	4	3.6
<i>Linyphiidae</i> sp.	Linyphiidae	4	3.6
<i>Tegenaria</i> sp.	Agelenidae	4	3.6
<i>Episinus</i> sp.	Theridiidae	4	3.6
<i>Lycosidae</i> sp.	Lycosidae	3	2.7
<i>Zelotes semirufus</i> (L. Koch, 1882)	Gnaphosidae	2	1.8
<i>Gnaphosa alacris</i> Simon, 1878	Gnaphosidae	2	1.8
<i>Eratigena feminea</i> Simon, 1870 *	Agelenidae	2	1.8
<i>Theridiidae</i> sp.	Theridiidae	2	1.8
<i>Xysticus bufo</i> (Dufour, 1820)	Thomisidae	1	0.9
<i>Leptodrassex simoni</i> (Dalmás, 1919)	Gnaphosidae	1	0.9
<i>Callilepis</i> sp.	Gnaphosidae	1	0.9
<i>Micaria</i> sp.	Gnaphosidae	1	0.9
<i>Prinerigone vagans</i> (Audouin, 1826)	Linyphiidae	1	0.9
<i>Typhochrestus bogarti</i>	Linyphiidae	1	0.9
<i>Neaetha</i> sp.	Salticidae	1	0.9
<i>Aelurillus luctuosus</i>	Salticidae	1	0.9
<i>Oecobius machadoi</i> Wunderlich, 1995 *	Oecobiidae	1	0.9
<i>Eratigena bucculenta</i> (L. Koch, 1868) *	Agelenidae	1	0.9
<i>Hahnina</i> sp.	Hahniidae	1	0.9
<i>Marilynia bicolor</i> Bosmans, 1990	Dictynidae	1	0.9
<i>Euryopsis episinoides</i> (Walckenaer, 1847)	Theridiidae	1	0.9
<i>Steatoda incomposita</i> (Denis, 1957)	Theridiidae	1	0.9
<i>Titanoeca</i> sp.	Titanoecidae	1	0.9
<i>Zodarion styliiferum</i> (Simon, 1870)	Zodariidae	1	0.9

\* - Iberian endemism (according to Cardoso and Morano 2010)

## References

Cardoso, P. and Morano, E. (2010) The Iberian spider checklist (Araneae). *Zootaxa* **2495**, 1–52

**S3-Table 3.5** Abundance (N), richness (S) and relative percentage (%) of functional groups of Araneae found in sites A, B and C and in all sites together (Total)

Functional group		A	B	C	Total
Sheet web weavers (SWW)	N	15	11	6	32
	%	34.9	25.0	25.0	28.8
	S	4	4	5	7
Ground hunters (GH)	N	7	13	4	24
	%	16.3	29.5	16.7	21.6
	S	4	9	3	9
Other hunters (OH)	N	12	5	3	20
	%	27.9	11.4	12.5	18.0
	S	4	3	1	7
Space web weavers (SpWW)	N	3	6	1	10
	%	7.0	13.6	4.2	9.0
	S	2	5	1	6
Specialists (Sps)	N	2	3	5	10
	%	4.7	6.8	20.8	9.0
	S	1	2	1	2
Ambush hunters (AH)	N	1	0	0	1
	%	2.3	0.0	0.0	0.9
	S	1	0	0	1
Sensing web weavers (SeWW)	N	0	0	1	1
	%	0.0	0.0	4.2	0.9
	S	0	0	1	1
Others	N	3	6	4	13
	%	7.0	13.6	16.7	11.7
Total		40	38	20	111

SWW - Agelenidae and Hahniidae; GH - Gnaphosidae and Lycosidae; OH - Linyphiidae, Salticidae and Scytodidae; SpWW - Dictynidae, Theridiidae and Titanoecidae; Sps – Zodariidae; AH – Thomisidae; SeWW - Oecobiidae

**S3-Table 3.6** Species of plants most frequently observed with average frequency under brackets (n= sampling stations), total number (N) of species and families identified and families with higher number of species identified in each habitat and site (in bold, the most frequent species in each habitat)

Site/Hab.	Species most frequently observed in sampling stations	N species	N families	Families with higher number of species identified (%)
A	<b><i>Arbutus unedo</i></b> , <b><i>Ruscus aculeatus</i></b> (1.0), <i>Asplenium onopteris</i> , <i>Crataegus monogyna</i> , <i>Erica arborea</i> , <i>Quercus x coutinhoi</i> , <i>Hedera hibernica</i> , <i>Rubus ulmifolius</i> , <i>Teucrium scorodonia</i> (0.66)	12	8	Poaceae (25.0); Asparagaceae (16.7); Ericaceae (16.7); Rosaceae (16.7)
NCH 1				
NCH 2	<b><i>Cistus salvifolius</i></b> , <b><i>Erica arborea</i></b> (1.0), <i>Andryala integrifolia</i> , <i>Arbutus unedo</i> , <i>Cytisus striatus</i> , <i>Rubus ulmifolius</i> (0.66)	21	13	Asteraceae (19.0)
NCH 3	<b><i>Cytisus striatus</i></b> , <b><i>Eschscholzia californica</i></b> (1.0), <i>Cistus salvifolius</i> , <i>Holcus lanatus</i> (0.66)	16	12	Apiaceae, Asteraceae, Fagaceae, Rosaceae (12.5)
V 5	<b><i>Hypochoeris radicata</i></b> , <b><i>Polygonum aviculare</i></b> (1.0), <i>Dittrichia viscosa</i> (0.66)	19	9	Asteraceae (31.6)
V25	<b><i>Chondrilla juncea</i></b> , <b><i>Conyza sumatrensis</i></b> , <b><i>Hypochoeris radicata</i></b> (0.66)	12	7	Asteraceae (31.6)
V50	<b><i>Andryala integrifolia</i></b> , <b><i>Convolvulus arvensis</i></b> , <b><i>Hypochoeris radicata</i></b> , <i>Dittrichia graveolens</i> (1.0)	15	8	Asteraceae (31.6)
B	<b><i>Rubus ulmifolius</i></b> (1.0), <i>Arbutus unedo</i> , <i>Bryonia dioica</i> , <i>Melica ciliata</i> sbsp <i>magnolia</i> , <i>Phillyrea angustifolia</i> , <i>Quercus rotundifolia</i> , <i>Ruscus aculeatus</i> (0.66)	18	17	Ericaceae (11.1)
NCH				
V 5	<b><i>Chondrilla juncea</i></b> , <b><i>Conyza sumatrensis</i></b> , <b><i>Cynodon dactylon</i></b> , <b><i>Daucus carota</i></b> (0.66)	10	8	Asteraceae (30.0)
V25	<b><i>Daucus carota</i></b> (1.0), <i>Conyza sumatrensis</i> , <i>Hypochoeris radicata</i> , <i>Lactuca virosa</i> (0.66)	10	5	Asteraceae (60.0)
V50	<b><i>Conyza sumatrensis</i></b> (1.0), <i>Lactuca virosa</i> (0.66)	6	3	Asteraceae (66.7)
C	<b><i>Ailanthus altissima</i></b> , <b><i>Rubus ulmifolius</i></b> , <b><i>Conyza sumatrensis</i></b> , <b><i>Daucus carota</i></b> , <b><i>Salix atrocinerea</i></b> , <b><i>Vitis vinifera</i></b> (0.66)	11	8	Asteraceae (27.3); Rosaceae (18.2)
NCH				
V 5	<b><i>Conyza sumatrensis</i></b> (1.0), <i>Conyza bonariensis</i> , <i>Digitaria sanguinalis</i> , <i>Hypochoeris radicata</i> , <i>Lactuca virosa</i> (0.66)	10	4	Asteraceae (60.0)
V25	<b><i>Conyza sumatrensis</i></b> , <b><i>Daucus carota</i></b> (1.0), <i>Conyza bonariensis</i> , <i>Lactuca virosa</i> (0.66)	8	4	Asteraceae (37.5); Poaceae (37.5)
V50	<b><i>Conyza bonariensis</i></b> , <b><i>Conyza sumatrensis</i></b> , <b><i>Daucus carota</i></b> , <b><i>Digitaria sanguinalis</i></b> (0.66)	9	4	Poaceae (44.4); Asteraceae (33.3);

**S3-Table 3.7** Total frequency of observations (F), relative percentage of frequencies (%) and richness (S) of plant species found, per family, in all sites

Family	F	%	S
Anacardiaceae	5	1.9	2
Apiaceae	5	1.9	2
Araliaceae	16	6.1	2
Asparagaceae	2	0.8	1
Aspleniaceae	6	2.3	2
Asteraceae	2	0.8	1
Boraginaceae	86	32.7	10
Caryophyllaceae	1	0.4	1
Celastraceae	2	0.8	1
Chenopodiaceae	4	1.5	2
Cistaceae	1	0.4	1
Cucurbitaceae	1	0.4	1
Cuscutaceae	1	0.4	1
Dioscoreaceae	4	1.5	2
Ericaceae	1	0.4	1
Fabaceae	4	1.5	2
Fagaceae	23	8.7	8
Guttiferaceae	10	3.8	3
Labiatae	1	0.4	1
Lamiaceae	1	0.4	1
Olacaceae	18	6.8	5
Papaveraceae	3	1.1	2
Phytolaccaceae	3	1.1	1
Poaceae	1	0.4	1
Polypodiaceae	2	0.8	2
Primulaceae	2	0.8	1
Rosaceae	1	0.4	1
Rubiaceae	1	0.4	1
Salicaceae	1	0.4	1
Santalaceae	1	0.4	1
Scrophulariaceae	1	0.4	1
Smarandaceae	1	0.4	1
Solanaceae	1	0.4	1
Thymelaeaceae	2	0.8	1
Urticaceae	263	100	74
Vitaceae			

## Chapter 4

### **Towards the development of a conservation biological control strategy against *Lobesia botrana* in Douro Demarcated Region (Portugal)**



Part of the content of this chapter was presented/ published in:

- Carlos C, Gonçalves F, Sousa S, Salvação J, Sharma L, Soares R, Manso J, Nóbrega M, Lopes A, Soares S, Aranha J, Villemant C, Marques G, Torres L (2013) Environmentally safe strategies to control the European Grapevine Moth, *Lobesia botrana* (Den. & Schiff.) in the Douro Demarcated Region. *Ciência e Técnica Vitivinícola*: 28: 1006-1011.
- Carlos C, Val MC, Marques G, Torres L (2011) New approaches for management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal. *Am J Enol Vitic*, 62 (3): 389A-389A
- Carlos C, Val C, Rataux A, Aranha J, Crespí A, Marques G, Torres L (2011) Does the landscape of Douro Wine Region affect the complex of antagonists of the grape berry moth? Proceedings 18th IaleUK Conference “Landscape ecology and ecosystem services”, Wolverhampton University, Telford Campus, England. 6th-8th Sept. 2011. 71
- Carlos C, Costa JR, Tão CB, Alves F, Torres LM (2006) Parasitismo associado à traça da uva, *Lobesia botrana* (Den. & Schiff.) na Região Demarcada do Douro. *Bol San Veg Plagas*, 32: 355-362





## Abstract

Because of the increased regulation of pesticides in Europe and the concerns about environmental impacts of viticulture, research about conservation biological control (CBC) of pests has grown in the last two decades. However, little attention has been addressed to main determinants of parasitism of *Lobesia botrana*, a major pest in Southern Europe vineyards. The unique character of Douro Demarcated Region (DDR) landscape and the management practiced on vineyards agroecosystem offers scope for the use of CBC. This study aimed at: (i) identifying parasitoids associated with each generation of *L. botrana*, and evaluating their relative abundance in DDR, and (ii) evaluating the effect of neighboring non-crop habitats and of management practices (chemical treatments and soil cover) on populations of parasitoid's species.

A total of 3,226 larvae/pupa of *L. botrana* were collected, out of which 485 parasitoids emerged. Sixteen different taxa were recorded, the majority belonging to Hymenoptera namely, *Elachertus* sp., *Elasmus* cf. *bistrigatus*, *Elasmus* cf. *steffani*, *Elasmus* sp., *Baryscapus* sp., an Eulophinae not identified (Eulophidae), *Campoplex capitator*, *Itoplectis maculator*, a Cryptinae not identified (Ichneumonidae), *Brachymeria tibialis*, *Hockeria* sp. (Chalcididae), *Dibrachys cavus* (Pteromalidae), *Ascogaster quadridentata* (Braconidae), *Goniozus gallicola* and *Goniozus claripennis* (Bethylidae). From Diptera, *Eurystaea scutellaris* (Tachinidae) was recorded. *Elachertus* sp. had a wide geographical distribution, being responsible for the highest rates of parasitism (up to 62%) recorded in *L. botrana*'s first generation. *C. capitator* and *B. tibialis* were found to have a complementary role, in the first and second generations of the pest, respectively. Moreover, it was found that the parasitism rate was related with ground cover management and chemical treatments.

**Key-words:** *Elachertus* sp., *Eurystaea scutellaris*, ecological infrastructures, landscape, ground cover, chemical treatments



## 4.1 Introduction

The grapevine moth, *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae) is among the most economically important insect pests in Europe and has recently been found in vineyards in Chile, California and Argentina (Varela et al. 2010).

The damage of *L. botrana* is mainly caused by larval feeding on grape clusters, which renders them susceptible to the infection of pathogenic fungi like the grey mold disease, *Botrytis cinerea* Persoon: Fries (Leotiales: Sclerotiniaceae) (Roehrich and Boller 1991) or black mold *Aspergillus* spp. (Thiéry 2008), making this moth the most economically important pest of grapevine in the wine-growing areas, worldwide (Ioriatti et al. 2011).

Traditionally, the control of the pest relies on the use of insect growth regulators (IGRs), once or twice a year, against the second and/or the third generation (Carlos et al. 2010). Increased regulation of pesticides in Europe, concerns of public about the environmental impacts of viticulture and consumers demand of residue-free products, has led to the increase in the last decade of the use of environmentally safe pest management strategies, with emphasis on conservation biological control.

Conservation biological control is distinct from classical biological control in that it involves making better use of agent species that are already present in a region rather than releasing new species (Gurr and You 2016). This approach is a combination of protecting biological control agents and providing resources so that they can be more effective (Eilenberg 2006). Therefore, it includes passive conservation by the avoidance of actions which disfavor the natural enemies, for example by limited and selective use of pesticides, and active conservation. The latter could be the use of habitat management strategies aimed to provide key ecological resources to natural enemies or to have direct effects on pest's independent of natural enemies (Poveda et al. 2008; Letourneau et al. 2011; Lu et al. 2014).

As stated by Jonsson et al. (2008), in addition to being able to contribute to safer and more effective biological control practices, conservation biological control has several other advantages, which include the following: (1) be based on concepts which are easy for growers to understand; (2) be a practice which individual growers can adopt;

(3) usually undergo a change of landscape and therefore can be used to support ‘green’ marketing strategies.

To implement a successful conservation biological control program, it is essential to have a good knowledge of the identity of the natural enemies that are present in the pest’s environment, as well as of the influence of environmental variation in natural enemy’s community structure and diversity (Thacker 2002). Parasitoids are important natural enemies of many crop pests and may act as keystone species in ecosystems (LaSalle 1993). Those associated with *L. botrana* have been studied in various European countries, and one hundred or so papers have been published on the subject (Loni et al. 2016), which showed that the species, as well as their abundance and diversity, varied as a function of geographical location. Yet, to date, the list of parasitoids is still incomplete. Thus, Loni et al. (2016) found recently for the first time, associated with *L. botrana*, the species *Bracon admotus* Papp, *Habrobracon concolorans* (Marshall) and *H. pillerianae* Fisher, obtained from larvae living on *Daphne gnidium* Linnaeus.

Despite evidence of the importance of a biological control based on larval parasitoids as one possible future strategy against the pest, surprisingly the development of such programs has received very little attention and suffers from a lack of studies (Moreau et al. 2010, Thiéry et al. 2011). Also, and while neighboring natural landscapes have long been regarded as probably having an important role in natural enemies’ population level (Genini 2000), to our knowledge, there is no studies about their role in enhancing conservation biological control of *L. botrana* by its complex of parasitoids.

The Douro Demarcated Region (DDR) with a winegrowing area of 43,670 hectares is one of the largest and most heterogeneous viticulture region of the world (ICOMOS 2001). Despite the intensification of the last thirty years, given the need to reduce operating costs and mitigate the harshness of the labor, a significant part of DDR area is still occupied by non-crop habitats, in particular, by scrublands and woodlands. In the area covered by vineyards, a significant part is occupied by grassy/shrubby slopes and/or by dry stone walls (Andresen and Rebelo 2013), which are important from the standpoint of biodiversity. Moreover, as DDR is one of the world biggest region of hillside vineyards, the use of soil cover vegetation against erosion is common. Potentially, these are infrastructures in the sense of Böller et al. (2004), whose ecological qualities in the provision of ecosystem services such as conservation biological control, must be assessed

on the landscape scale, because as already shown, local impact of natural enemies is strongly affected by vegetation patterns at landscape scale, not only local scale (Perovic et al. 2010; Paredes et al. 2015). Also, while a key constraint to the use of landscape-scale approaches to be actively exploited in conservation biological control is, according to Gurr and You (2016), the fact that agricultural landscapes are often intensively sprayed with insecticides that can strongly impact natural enemies, this is not the case in DDR, because these non-crop habitats are not treated.

Because, as stated by Böller et al. (2004), the ecological quality of the agroecosystem vineyard is determined by the quality of the green cover and of the availability of neighboring ecological infrastructures, DDR vineyards may be considered an agroecosystem with a high ecological potential, interesting as a case study of the tendency toward a more biodiversity friendly management that reflects a general change in the attitude of wine consumers and producers (see Viers et al. 2013).

From the information presented above, it is clear that, to implement a successful conservation biological control program against *L. botrana*, more information is needed on the potential importance of their parasitoids in relation with the generation of the pest, and the landscape / vineyard management determinants. Therefore, the aim of the present study was to answer to the following questions: (1) which species of parasitoids occur naturally in DDR vineyards associated to each of the three generations of *L. botrana* and what is the corresponding abundance? and (2) is the parasitism of *L. botrana* associated with chemical treatments, green cover vegetation and/or neighboring ecological infrastructures?

## **4.2 Material and methods**

The study was carried over a 9-year period (2002 to 2015), in several commercial vineyards of DDR, with variable characteristics (S4 - Table 4.1).

### ***4.2.1 Management practices***

Most vineyards were managed under Integrated Production (IP) principles, receiving chemical applications for the control of the main diseases (powdery, downy mildew, *Botrytis* rot) and pests (*L. botrana*). All pesticides were applied at commercial

doses. From the information on spraying regime obtained from growers, indices were calculated to quantify relative management intensity in relation to pesticide use, following the methodology proposed by Thomson and Hoffmann (2006). To quantify pesticide applications during the study period, each product used per site was assigned with an environmental risk level (“chemical impact”), according to the biocide coding system available on Oliveira et al. (2014), based on its potential impact on hymenopteran parasitoids. Risk levels were weighted as it follows: low risk = 1, medium risk = 2, medium–high risk = 3. Weights of products per site were summed to obtain a relative measure of the magnitude of pesticide (S4-Table 4.3). The vineyards were also characterized, according to soil management (bare soil or soil cover vegetation) (S4 - Table 4.1).

#### **4.2.2 Parasitism of *Lobesia botrana***

Samples of larvae / pupae of *L. botrana* were collected from vineyards, at the end of each of its three generations, by examining random samples of bunches. The estimation of damages of *L. botrana* was calculated in each sampling point. In the laboratory, they were placed individually in special breeding containers into controlled conditions (22°C; RH: 65±10%, photoperiod 16:8 (L:D)); according to the generation, the larvae were fed with natural substrate (i.e., parts of inflorescences or grapes) until pupation. Pupae were checked daily until adult of *L. botrana* or parasitoid emergence. Under a stereoscopic microscope, all parasitoids were sorted into morphospecies, preserved in 70% ethanol and later identified. The Tachinidae species were identified with the key of Martinez (2011) and Hymenoptera with that provided by Villemant and Delvare (2011) for the main common species of vineyard tortricid parasitoids. Identification of poorly represented species were done using specific keys (Tryapitsyn 1988; Graham 1995).

#### **4.2.3 Landscape analysis**

Landscape composition around each sampling point was calculated within a GIS framework (ArcGIS® 9.3, Esri), with a radii of 50 and 100 m, overlaying aerial photographs of Bing Maps (ESRI 2016). The radii were chosen according to literature (Lavandero et al. 2005; Scarratt et al. 2008; Thomson and Hoffmann 2013). Seven

measures of landscape composition were calculated in addition to the area covered by vineyards: (1) woodland / forest; (2) scrubland / shrubby slopes; (3) riparian gallery; (4) water elements; (5) orchards and vegetable gardens; (6) roads and (7) houses and other buildings. The elements woodland / forest / scrubland / shrubby slopes, riparian gallery, water elements and orchards and vegetable gardens, when conducted extensively, were considered as part of neighboring ecological infrastructures (EI). Based on this information, four landscape variables were obtained (percentage of vineyards, percentage of EI, Shannon-Wiener and Evenness indexes), at each radii, within each point assessed (S4 – Table 4.2).

#### 4.2.4 Data analyses

Abundance (N) and richness (S) of species were calculated to provide information on parasitoid diversity in each location. Parasitism was calculated as the percentage of parasitism, according to the following formula:

$$Parasitism (\%) = (number\ of\ Lb \frac{parasitized}{total}) * 100$$

For this analysis, only samples with more than 10 individuals of *L. botrana* were considered.

The damages of *L. botrana* were expressed through the number of nests per 100 bunches assessed, for the first generation, and through the percentage of clusters attacked, for the second and third generation.

To evaluate the impact of landscape, as well as of management practices (soil cover vegetation and chemical treatments) on percentage of parasitism, statistical analyzes were performed. To account for variability in the response variables assessed in this study, we choose model selection procedure as an alternative to traditional hypothesis tested (Canham and Uriarte 2006; Johnson and Omland 2004). We selected generalized linear mixed models (GzLMM) for the analyses, as data on study plots were nested farms within several years of the study. Pearson's correlations were previously used, to determine which landscape variable were more related with parasitism rates, and the percentage of EI at the radii of 100 m was selected. Thus, this landscape variable, as well as the variables soil cover vegetation and chemical impact of treatments were used as fixed factors, while

the year and the variety were used as random factors. We performed a set of models by combining the random and fixed variables. After this process, we got a set of 20 alternative models, from which we selected the most parsimonious, using the Aikake Information Criteria (AIC). Models with a difference in  $AIC > 2$  indicate that the worse model has virtually no support and can be omitted from further consideration. A binomial error structure was selected. The  $R^2$  of the best model was used as a measure of goodness-of-fit. All analyses were taken with the function contained in the package “lme4” written for the R environment (Bates et al. 2013; R 2012).

## 4.3 Results

### 4.3.1 Parasitism of *Lobesia botrana*

A total of 3,226 individuals of *L. botrana* (larvae/pupa) were collected, from which 485 parasitoids were obtained. Part of this assemblage (27) was damaged and could not be identified. Of the remaining 458 individuals, sixteen different taxa were recorded, from which ten were identified at species level, the majority belonging to Hymenoptera. The most abundant parasitoids were *Elachertus* sp. (Eulophidae) (Fig. 4.1), *Campoplex capitator* Aubert (Ichneumonidae) (Fig. 4.2) and *Brachymeria tibialis* (Walker) (Chalcididae) (Fig. 4.3), which represented 62.5%, 12.6% and 12.0% of the total assemblage of parasitoids emerged, respectively (Table 4.1).

Other parasitoids were identified in a smaller number: *Dibrachys cavus* (Walker), (Pteromalidae) (Fig. 4.4); *Ascogaster quadridentata* Wesmael (Braconidae) (Fig. 4.5); *Elasmus cf bistrigatus* Graham, *Elasmus cf steffani* Graham (Eulophidae) (Fig. 4.6); *Itoplectis maculator* (Fabricius) (Ichneumonidae); *Goniozus gallicola* (Kieffer), *Goniozus claripennis* Förster (Bethyridae) and *Eurystaea scutellaris* (Robineau-Desvoidy) (Tachinidae) (Table 4.1).

Individuals from *Baryscapus* sp. and *Elasmus* sp. (Eulophidae) and *Hockeria* sp. (Chalcididae) were also reported. Moreover, two morphospecies were only identified at the sub-family level (one Eulophinae, from Eulophidae and one Cryptinae, from Ichneumonidae). The Eulophidae dominated the assemblage of parasitoids collected, followed by Ichneumonidae and Chalcididae (Table 4.1).



**Table 4.1** Abundance (N) and relative percentage (%) of *Lobesia botrana* parasitoids identified in Douro Demarcated Region, during the studied period (2002-2015), and parasitized generation

Species	Family	N	%	Generation of Lb
<i>Elachertus</i> sp.	Eulophidae	303	62.5	1; 2; 3
<i>Campoplex capitator</i>	Ichneumonidae	61	12.6	1; 2
<i>Brachymeria tibialis</i>	Chalcididae	58	12.0	1; 2
<i>Dibrachys cavus</i>	Pteromalidae	10	2.1	2
<i>Ascogaster quadridentata</i>	Braconidae	9	1.9	1
<i>Elasmus</i> cf <i>bistrigatus</i>	Eulophidae	3	0.6	1; 2
<i>Eurystaea scutellaris</i>	Tachinidae	3	0.6	1; 2
<i>Gonyozus claripennis</i>	Bethylidae	2	0.4	1; 3
Cryptinae sp.	Ichneumonidae	2	0.4	2
<i>Elasmus</i> cf <i>steffani</i>	Eulophidae	1	0.2	3
<i>Itoplectis maculator</i>	Ichneumonidae	1	0.2	1
<i>Baryscapus</i> sp.	Eulophidae	1	0.2	2
Eulophinae sp.	Eulophidae	1	0.2	1
<i>Elasmus</i> sp.	Eulophidae	1	0.2	2
<i>Hockeria</i> sp.	Chalcididae	1	0.2	2
<i>Gonyozus gallicola</i>	Bethylidae	1	0.2	2



**Figure 4.1** *Elachertus* sp.



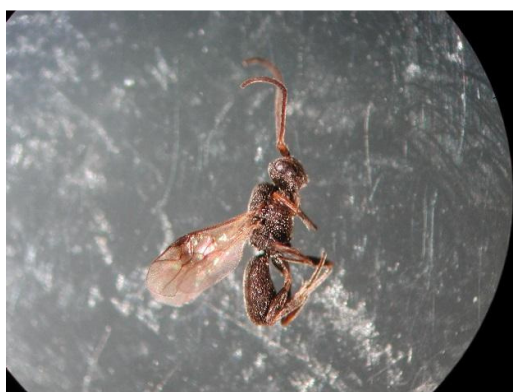
**Figure 4.2** *Campoplex capitator*



**Figure 4.3** *Brachymeria tibialis*



**Figure 4.4** *Dibrachys cavus*



**Figure 4.5** *Ascogaster quadridentata*



**Figure 4.6** *Elasmus* cf. *steffani*

The average rates of *L. botrana* parasitism decreased sharply from the first to the third generation. Thus, they ranged from 0.0 to 61.5% in the first generation, from 0.0 to 36.8% in the second generation and from 0.0 to 12.1% in the third generation (Table 4.2; S4 – Table 4.4).

**Table 4.2** Parasitism rates (mean  $\pm$  SE) found per generation of *Lobesia botrana*, in Douro Demarcated Region during the studied period (2002-2015)

Generation of <i>Lb</i>	Variation of rates of parasitism	Average parasitism ( $\pm$ se)	Number of sampling points assessed
1 <sup>st</sup>	0.0-61.5	15.6 $\pm$ 3.4	32
2 <sup>nd</sup>	0.0-36.8	8.9 $\pm$ 2.7	18
3 <sup>rd</sup>	0.0-12.1	2.0 $\pm$ 1.4	10

The higher rates of parasitism were found in *L. botrana* first generation, mostly due to *Elachertus* sp. (up to 61.5%) and *C. capitator* (up to 47%) (Table 4.3).

**Table 4.3** Variation of the parasitism rates (%) caused in *Lobesia botrana* by each of the five-main species of parasitoids identified in Douro Demarcated Region during the studied period (2002-2015)

Generation	<i>Elachertus</i> sp.	<i>Campoplex capitator</i>	<i>Brachymeria tibialis</i>	<i>Dibrachys cavus</i>	<i>Ascogaster quadridentata</i>
1 <sup>st</sup>	0.0-61.5	0.0-46.9	0.0-2.8	0.0	0.0-7.1
2 <sup>nd</sup>	0.0-6.8	1.4-8.3	0.0-32.8	0.0-4.7	0.0
3 <sup>rd</sup>	0.0-8.3	0.0	0.0	0.0	0.0

*Elachertus* sp. is an ectoparasitoid associated mainly with young larvae; it was the only parasitoid obtained in the three generations of the pest, although 91.4% of the individuals were reared from the first generation (Table 4.4); *C. capitator* is a larval endoparasitoid of which 80.3% individuals were also obtained from first generation, while the rest emerged from the second generation (Table 4.4).

**Table 4.4** Relative abundance (N) and percentage (%) of the main parasitoid species of each generation of *Lobesia botrana* in Douro Demarcated Region, during the studied period (2002-2015). Only samples with more than 10 larvae/pupae of *L. botrana* were included in the analysis

Generation	<i>Elachertus</i> sp.		<i>Campoplex capitator</i>		<i>Brachymeria tibialis</i>		<i>Dibrachys cavus</i>		<i>Ascogaster quadridentata</i>	
	N	%	N	%	N	%	N	%	N	%
1 <sup>st</sup>	277	91.4	49	80.3	7	12.1	0	0.0	9	100.0
2 <sup>nd</sup>	19	6.3	12	19.7	51	87.9	9	100.0	0	0.0
3 <sup>rd</sup>	7	2.3	0	0.0	0	0.0	0	0.0	0	0.0
	303	100.0	61	100	58	100	9	100.0	9	100.0

The third higher rate of parasitism, i.e. up to 32.8%, was found in *L. botrana* second generation due to *B. tibialis* (Table 4.3); this is a solitary pupal endoparasitoid, of which 87.9% of the individuals were reared from *L. botrana* second generation, while the remaining emerged from the first generation (Table 4.4). *A. quadridentata*, a solitary egg-larval endoparasitoid, was reared only from the first generation, while *D. cavus*, which develops as gregarious larvo-pupal parasitoid, was only obtained from the second generation of *L. botrana* (Table 4.4).

The levels of infestation of grapes by *L. botrana* were higher in both the first and second generations, when compared with the third generation (Table 4.5).

**Table 4.5** Analysis of level of infestation by *L. botrana* per generation found in vineyards, during the study period (2002-2015).

Generation	Months	Variation on level of damages assessed	Mean $\pm$ SE	Number of observations
1 <sup>st</sup>	May-Jun	1.0-187.0*	31.4 $\pm$ 4.5*	68
2 <sup>nd</sup>	Jun-July	0.0-75.0**	31.3 $\pm$ 4.5**	24
3 <sup>rd</sup>	Aug-Sept	1.0-67.0**	15.5 $\pm$ 2.5**	44

\* Number of nests / 100 bunches); \*\* % clusters attacked (viable eggs or berries damaged)

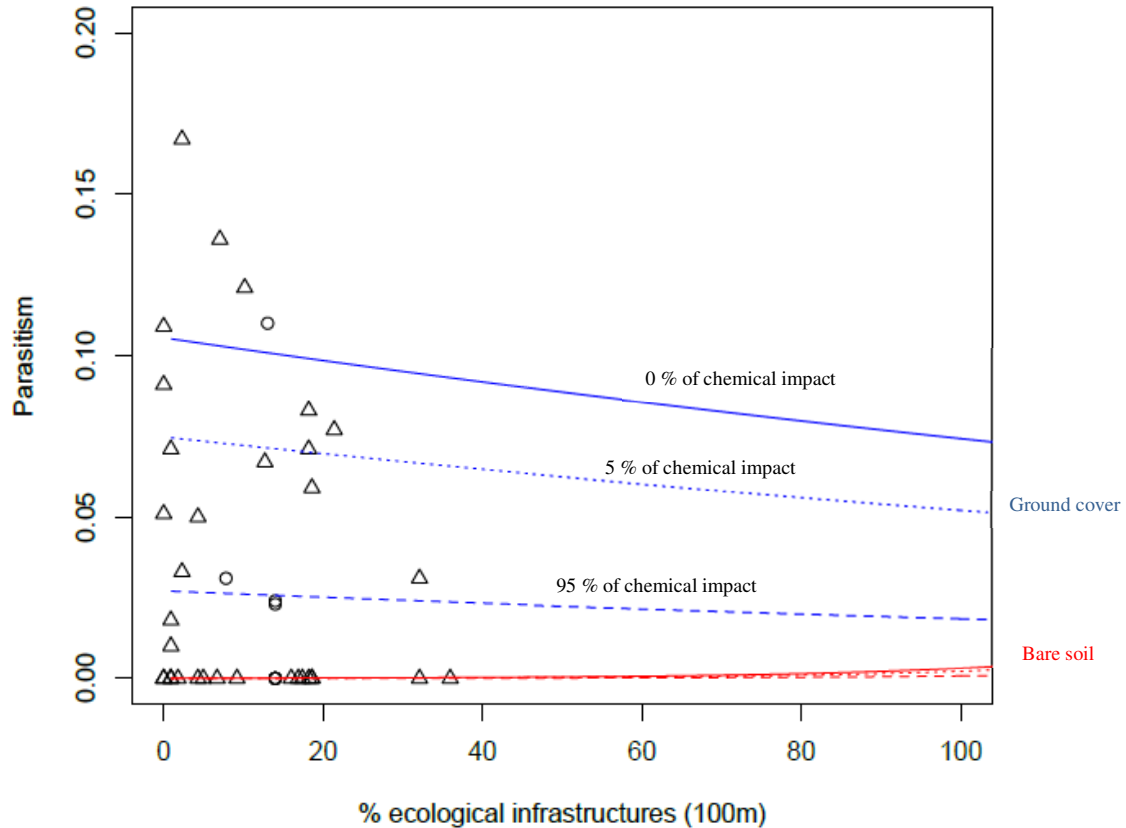
### 4.3.2 Main determinants of parasitism

From the comparison between alternative models tested, the best model (lower AIC) was obtained by combining the interaction between type of soil management (ground cover/bare soil) and the proportion of EI at 100 m, plus the chemical impact of treatments (type of soil management X proportion of EI (100) + chemical impact). Fixed factors of best model explain 11.7% of variability, while fixed and random effects explain 61.0%. (Table 4.6).

**Table 4.6** Comparison of alternative models (using AIC) for *Lobesia botrana* parasitism rates tested in the study. The best model (lowest AIC) is indicated in bold type. For brevity, only the most parsimonious (lowest AIC) of all possible models are reported. R<sup>2</sup> refer to the best model.

Fixed effects		Random effects	AIC
Basic Models	General response	No	460,890
	Proportion of EI (100)	No	290,757
	Chemical impact	No	453,889
	Proportion of EI (100) + Chemical impact	No	292,648
	General response	yes	249,845
	Proportion of EI (100)	yes	243,471
	Chemical impact	yes	250,929
	Proportion of EI (100) + Chemical Impact	yes	245,676
Soil management models	General response	No	443,726
	Proportion of EI (100)	No	294,648
	Chemical impact	No	438,598
	Proportion of EI (100) + Chemical impact	No	281,654
	Type of soil management X Proportion of EI (100)	No	294,756
	Type of soil management X Proportion of EI (100) + Chemical impact	No	296,647
	General response	yes	229,079
	Proportion of EI (100)	yes	229,528
	Chemical impact	yes	230,151
	Proportion of EI (100) + Chemical impact	yes	229,165
	Type of soil management X Proportion of EI (100)	yes	216,416
	<b>Type of soil management X Proportion of EI (100) + Chemical impact</b>	<b>yes</b>	<b>211,559</b>
R <sup>2</sup> <sub>c</sub> (fixed effects)			<b>0,117</b>
R <sup>2</sup> <sub>m</sub> (fixed + random effects)			<b>0,609</b>
EI – ecological infrastructures			

The best model resulted in a graphical representation, which illustrates the relation between rate of parasitism and the proportion of ecological infrastructures (EI) at 100 m radii, the soil management and the chemical impact of treatments (Figure 4.7).



**Figure 4.7** Evolution of parasitism rates, according to the proportion of ecological infrastructures at 100 m. The three lines in blue, located above, represents the relation obtained in vineyards with ground cover (solid line - predictions of the model with 0% of the chemical impact (score=0); dotted line- predictions of the model with 5% of the chemical impact measured on the experiment; dashed line- predictions of the model with 95% of the chemical impact measured on the experiment). The three lines in red located below represents the same relations, but in vineyards with bare soil.

Concerning the effect of soil management, the rate of parasitism seems to have increased substantially more in vineyards with ground cover (in blue on Figure 4.7) than in those with bare soil (in red on Figure 4.7), in which parasitism was almost inexistent. However, in vineyards with ground cover, a higher percentage of ecological infrastructures (EI) seem to have had a negative impact on parasitism of *L. botrana*. On

the other hand, on vineyards with bare soils, the higher percentage of ecological infrastructures resulted only in a slightly increase of parasitism rates.

When comparing the impact of chemical pesticides applied, results indicated that those with higher chemical impact (95% of the score) resulted in a substantial decrease in rates of parasitism. So, the higher rates of parasitism were found in vineyards with ground cover, under the lower rate of chemical impact (0%), which point to a positive impact of ground cover on parasitism and a negative impact on it of chemical treatments.

## 4.4 Discussion

### 4.4.1 Parasitoids diversity in DDR

The results from this study indicates that the cohort of parasitoids associated with *L. botrana* in DDR vineyards is quite vast and rather different from the obtained in other European countries, although also variable in space and time (Villemant et al. 2011; Bagnoli and Lucchi 2006). In this study, the key parasitoid found was *Elachertus* sp., being the complex of parasitoid more related with the found in Italian vineyards (Bagnoli and Lucchi 2006), with exception of *B. tibialis*. *C. capitator* is widespread in most European vine-growing areas and is one of the species considered as having the higher potential of parasitism in most published studies (e.g. Coscollá 1980; Moreau et al. 2010; Thiéry et al. 2001; Thiéry and Xuéreb 2003; Thiéry and Xuéreb 2004; Xuéreb and Thiéry 2006). In Venetian vineyards, Marchesini and Dallá Montà (1994) reported, besides *C. capitator* and *Dribachys affinis* Masi, two other Ichneumonidae (i.e. *Dicaelotus inflexus* Thomson and *Tranosemella prerogator* (Linnaeus)). Moreover, in studies conducted in Swiss and French vineyards, Moreau et al. (2010) found that the most abundant species, with wider geographical distribution, was the Ichneumonidae *Exochus notatus* Holmgren, while in Valencian vineyards, Coscollá (1998), reported the higher presence of the Pteromalidae *D. cavus* and *D. affinis*, a finding also supported by data obtained by Perez Moreno et al. (2000), in vineyards from Rioja.

In an extensive review recently published on the parasitoids of the families Tachinidae and Braconidae associated to the larvae of *L. botrana* in Italy, Scaramozzino et al. (2017) report 21 species of Braconidae, from which eight were identified only at generic level, and only two species of Tachinidae i.e. *Actia pilipennis* (Fallen) and

*Phytomyptera nigrina* (Meigen). The species *E. scutellaris*, which record in Italy was considered by Scaramozzino et al. (2017) as doubtful, in the present study was obtained in the first and second generations of the moth, although with a parasitism rate of only 0.6%.

In Portugal, the published studies about *L. botrana* parasitoids are scarce. Thus, in the northwest of the country, Ribeiro et al. (2001) have noticed the presence of *D. affinis* in the moth overwintering generation, while Carlos et al. (2006) and Carlos et al. (2011), in a previous study carried in DDR have identified *Elachertus affinis*, *Brachymeria* sp., *C. capitator*, *D. cavus*, *Elasmus* sp., *A. quadridentata* and *G. gallicola*.

*Elachertus* Spinola, 1811 (Hymenoptera: Eulophidae) are primary parasitoids of a variety of lepidopteran larvae, and several of their host species are economically important (Schauff 1985). In Europe, 40 species are described and, to date, *E. affinis* is reported only from French mainland, Hungary, Italian mainland and North Africa (Mitroiu 2013) and Spain (Villemant et al. 2011). According to Villemant et al. (2011), the literature about this species is scarce. Also, while many *Elachertus* species are gregarious ectoparasitoids (Boucek and Askew 1968; Schauff 1985), in DDR only one specimen was obtained from each *L. botrana* larva parasitized.

#### **4.4.2 Determinants of parasitism**

The remarkable decrease found in parasitism rates of the second and third generations of *L. botrana* compared to the first, also reported by Bagnoli and Lucchi (2006) for Tuscany (Italy) and by Akbarzadeh (2012), for Orumieh (Iran), can be expected in regions whose hot dry summers causes drought of the soil ground vegetation. This is the case of DDR, where rainfall during the season April-September is only between 189 and 326 mm, depending on the location (Jones and Alves 2012). On such conditions, in late spring/early summer, the green cover turns into a dried mulching cover, reducing dramatically the availability of pollen and nectar resources, with an expected reduction in parasitoid's survival. This is consistent with Segoli and Rosenheim (2013), who stated that *Anagrus* wasps, a small parasitoid of leafhoppers, seem to be highly limited by sugar resources in the field, latter in the season.



On the one hand, the duration of larval development of *L. botrana* is longer in the first generation compared to the other two, especially since it takes place during a period of the year when temperatures are lower. This leads to the insect being exposed to parasitoids over a longer period (Thiéry and Moreau 2005). The comparatively lower rate of parasitism observed in the third generation compared to the previous two may also be related to the reduced host density, as shown by Xuéreb and Thiéry (2006) for *C. capitator*. Thus, the high temperatures that usually occurs in July and August in DDR, may cause mortality of eggs and larva of *L. botrana* third generation, especially on vineyards more exposed to dry conditions. According to Moosavi et al. (2017), temperatures of 40°C reached by berries exposed to sunlight can cause high egg and larval mortality.

In this study, an increment in *L. botrana* parasitism rates was found in vineyards managed with ground cover vegetation, compared to those with bare soil, in which parasitism was almost non-existent. However, ecological infrastructures seem to have caused the decrease of parasitism rates in soils with ground cover and to slightly increased these rates in bare soils.

The importance of non-crop vegetation or soil cover vegetation in agricultural landscapes, for providing resources to parasitoids, such as refugia, overwintering habitat, nectar, pollen and alternate hosts or prey, have been reported by several authors (Bianchi et al. 2006; Gaigher et al. 2015; Smith et al. 2015; Thomson and Hoffmann 2009; Silva et al. 2010; Thomson et al. 2010). The availability of floral vegetation in these landscapes can provide adult parasitoids with sugar resources that are required for energy and physiological maintenance thereby potentially enhancing their efficiency as biological control agents (Heimpel and Jervis 2005). Also, it is known that the potential of floral resources to enhance parasitoid success depends on their ability to move easily between flower and pest patches (Lavandero et al. 2005; Lee et al. 2006). Moreover, differences have been found in the response of parasitoids to spatial scale, attributable to foraging and dispersion distance, which, in turn have been related to parasitoid size (Van Nouhuys and Hanski 2002). Due to its small size, *Elachertus* sp., which was by far the most abundant parasitoid found in the present study, may forage over modest distances and hence may have been highly dependent on the ground cover vegetation within vineyards. This is in line with what is reported in the literature for *Anagrus* parasitoids that parasitize

the eggs of leafhopper pests (genus *Erythroneura*) in vineyards (English-Loeb et al. 2003; Segoli and Rosenheim 2013). Accordingly, attempts to enhance biological control in vineyards by planting prune trees that provide overwintering refuge for *Anagrus* have had a limited success, as the abundance of the parasitoid declined gradually with increasing distance from the refuge (Corbett and Rosenheim 1996). The exposed could also, in part, explain the reduced impact of ecological infrastructures in *L. botrana* parasitism rates found in bare soils, in the present study.

The negative impact of the ecological infrastructures on parasitism showed by the model, could be explained, at least in part, by the existence of intraguild interactions, as discussed in several works (Rosenheim et al. 1995; Brodeur and Rosenheim 2000; Muller and Brodeur 2002; Straub et al. 2008; Traugott et al. 2012). In fact, ecological infrastructures along with soil ground cover was found to promote the abundance of predators, in DDR vineyards (Carlos, unpublished data), which, in the present study could hypothetically have disrupted the biological control delivered by parasitoids, through the predation of both parasitoid adults and parasitized immatures of *L. botrana*.

The higher rates of parasitism found in vineyards managed under the lower rate of chemical impact (0%) was expectable since it is known that chemicals can cause a wide range of unintentional effects on parasitoids (reviewed by Thomson and Hoffmann 2006). As shown by Thomson et al. (2000), parasitism can be high in vineyards with low chemical use and particularly low sulphur inputs. Sulphur, that is commonly used in DDR vineyards, have also been shown to be highly toxic to *Anagrus* (Jepsen et al. 2007). In the present study, several chemical treatments could have impacted negatively parasitoid populations (S1- Table 3), namely sulphur, mancozeb and indoxacarb, with medium risk of toxicity (2) and imidacloprid or deltamethrin, with a high risk of toxicity (3) (Oliveira et al. 2014). Moreover, phosalone that was used in 2002 and later discontinued, is reported in the *IOBC Pesticide Side Effect Database* as having high toxicity to parasitoids (IOBC-WPRS 2016).

#### **4.5 Conclusions**

A complex of sixteen different taxa of parasitoids of *L. botrana* was identified in the DDR sampled vineyards, the majority belonging to Hymenoptera. The cohort of

parasitoids and the potential role played by each species in the control of the pest showed a great variability in space and time, with rates of parasitism being substantially higher in its first generation, compared to any of the two other generations. This can be linked to different biotic and abiotic factors. However, the higher presence of vegetation cover that occurs in the first generation, which results in a higher availability of floral resources, was found to increase parasitism rates and thus it is expected to enhance the success of parasitoids on achieving a more effective biological control of *L. botrana*.

The most promising candidate to boost conservation biological control of the pest in DDR vineyards was found to be the eulophidae *Elachertus* sp. This is because, on one hand, this larval ectoparasitoid has a wide geographical distribution in the studied region and, on the other hand, in most favorable conditions, the percentage of parasitism caused can reach over than 50%, even when the host population is low. To our knowledge, this is the first study which report *Elachertus* sp. as a key parasitoid of *L. botrana*.

As a whole, the results of the present study suggest potential for conservation biological control of *L. botrana* first generation and, to a lesser extent, second generation in DDR, if soil cover, including a high proportion of native perennial herbs, is encouraged. The use of plants native to a region in habitat management to support natural enemy populations has several advantages, as reported by Isaacs et al. (2009) and Landis et al. (2012). Thus, because they are locally adapted, they require, in general, little management after establishment and can persist within the agroecosystem for decades. The maintenance of such habitats into the vineyard, beyond biocontrol, can provide other benefits such as conserving wildlife, protecting water quality and reducing erosion and runoff (references in Tillman et al. 2012). On the other hand, the use of native plants contributes to their conservation, which is especially important in Mediterranean Basin, since this is one of the world's richest places in terms of plant diversity (Cuttelod et al. 2008), being considered a hyper-hot candidate for conservation support (Myers et al. 2000).

The choice of chemicals with low toxicity to parasitoids is also a critical point to contribute to the preservation and maintenance of natural enemies in the vineyard and should be carefully considered.

The unexpected negative interaction effect found in the present study between the percentage of the ecological infrastructures and the ground cover of vineyards, in the parasitism rates, should be further investigated.

In conclusion, the results of the present study indicate that the control of *L. botrana* to be effective in DDR, such as probably in other Mediterranean continental climate, should integrate conservation biological control with other sustainable strategies such as mating disruption technique (Carlos et al. 2014) and the application of microbiological control agents (e.g. Ifoulis and Savopoulou-Soultani 2004).

#### 4.6 References

- Akbarzadeh SG (2012) Larval Parasitoids of *Lobesia botrana* (Denis and Schiffermüller, 1775) (Lepidoptera: Tortricidae) in Orumieh Vineyards. J. Agr. Sci. Tech. 14: 267-274
- Andresen T, Rebelo J (2013) Assessment of the State of Conservation of the Property Alto Douro Wine Region - Evolutive and Living Cultural Landscape - Assessment Report. Porto: CIBIO UP/UTAD
- Bagnoli B, Lucchi A (2006) Parasitoids of *Lobesia botrana* (Den. & Schiff.) in Tuscany. IOBC/wprs Bull 29 (11): 139-142
- Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5
- Bianchi FJJA, Booij CJH, Tscharntke T (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proc R Soc Lond B Biol Sci 273: 1715-1727
- Böller EF, Häni F, Poehling HM (eds.) (2004) Ecological infrastructures: Ideabook on functional biodiversity at the farm level. Temperate zones of Europe. Swiss Centre for Agricultural Extension and Rural Development, Switzerland, 212 pp
- Boucek Z, Askew RR (1968) Hym. Chalcidoidea. Palearctic Eulophidae (excl. Tetrastichinae). *Index of Entomophagous Insects* 260 pp (Eds Delucchi V, Remaudière G.) Le François, Paris

- Brodeur J, Rosenheim JA (2000) Intraguild interactions in aphid parasitoids. *Entomol. Exp. Appl.* 2000; 97: 93–108
- Canham CD, Uriarte M (2006) Analysis of neighborhood dynamics of forest ecosystems using likelihood methods and modeling. *Ecol Appl* 16: 62-73
- Carlos C, Costa JR, Tão CB, Alves F, Torres LM (2006) Parasitismo associado à traça da uva, *Lobesia botrana* (Dennis & Schiffermüller) na Região Demarcada do Douro. *Bol San Veg Plagas* 32: 355-362
- Carlos C, Alves F, Torres L (2010) Constraints to the application of mating disruption against *Lobesia botrana* in Douro Wine Region. In 3rd International Congress of mountain and Steep slope viticulture, Castiglione di Sicilia. Sicily, Italy. 12th – 14th May 2010. 103-110
- Carlos C, Val MC, Marques G, Torres L (2011) New approaches for management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal. *Am J Enol Vitic*, 62 (3): 389A-389A
- Carlos C, Gonçalves F, Sousa S, Nóbrega M, Manso J, Salvação J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D., Val M.C., Franco JC, Aranha J, Thistlewood H. & Torres L (2014) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. *IOBC-WPRS Bulletin*, 105: 93-102
- Corbett A, Rosenheim JÁ (1996) Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape. *Ecol. Entomol.* 21, 155–164
- Coscollá R (1998) Polillas del racimo (*Lobesia botrana* Den. y Schiff.) In: Barrios GS, Coscollá RR, Lucas AE, Perez JJO, Perez JLM, Toledo JP (eds.). *Los parásitos de la vid*, MAPA-Mundi Prensa, Madrid, pp 29-42
- Coscollá R (1980) Aproximación al estudio del parasitismo natural sobre *Lobesia botrana* Den. y Schiff. en las comarcas vitícolasValencianas. *Bol Serv Plagas* 6: 5-15
- Cuttelod A, García N, Abdul Malak D, Temple H and Katariya V (2008) The Mediterranean: a biodiversity hotspot under threat. In: J.-C. Vié, C. Hilton-Taylor and S.N. Stuart (eds). *The 2008 Review of The IUCN Red List of Threatened Species*. IUCN Gland, Switzerland

- Eilenberg J (2006) Concepts and visions of biological control. In: J. Eilenberg and H.M.T. Hokkanen (eds.), *An Ecological and Societal Approach to Biological Control*, 1–11. Springer
- English-Loeb G, Rhainds M, Martinson T, Ugine T (2003) Influence of flowering cover crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. *Agricultural and Forest Entomology* 5: 173–181
- ESRI, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo and the GIC User community (World imagery Bing Map consulted in August 2016)
- Gaigher R, Pryke JS, Samways MJ (2015) High parasitoid diversity in remnant natural vegetation, but limited spillover into the agricultural matrix in South African vineyard ecosystems. *Biol Conserv* 186: 69-74
- Genini M (2000). Antagonistes de la cicadelle verte et des vers de la grappe dans le vignoble valaisan et les milieux environnants. *Rev. Suisse Viti. Arbo. Hort.* 32: 153-160.
- Graham MWR (1995) European *Elasmus* (Hymenoptera, Chalcidoidea, Elasmidae) with a key and descriptions of five new species. *Entomol. Month. Mag.* 131: 1–23.
- Gurr GM and You M (2016) Conservation Biological Control of Pests in the Molecular Era: New Opportunities to Address Old Constraints. *Front. Plant Sci.* 6:1255. doi: 10.3389/fpls.2015.01255
- Heimpel GE, Jervis MA (2005) Does floral nectar improve biological control by parasitoids? In: Wäckers F, van Rijn PCJ, Bruin J (eds). *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. Cambridge: University Press. pp. 267–304
- ICOMOS (2001). Alto Douro, Portugal n° 1046. Advisory Body Evaluation. Available on [whc.unesco.org/en/list/1046/documents/](http://whc.unesco.org/en/list/1046/documents/)
- Ifoulis AA, Savopoulou-Soultani M (2004). Biological Control of *Lobesia botrana* (Lepidoptera: Tortricidae) Larvae by Using Different Formulations of *Bacillus thuringiensis* in 11 Vine Cultivars Under Field Conditions. *Journal of Economic Entomology* 97(2):340-3. DOI: 10.1603/0022-0493-97.2.340

- Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A (2011) Chemical Ecology and Management of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology* 104(4): 1125–1137. <https://doi.org/10.1603/EC10443>
- Bostanian et al. (eds.), *Arthropod Management in Vineyards: Pests, Approaches, 339 and Future Directions*, DOI 10.1007/978-94-007-4032-7\_14: 339-359
- IOBC-WPRS (2016) IOBC Pesticide Side Effect Database Available on [https://www.iobc-wprs.org/ip\\_ipm/IOBC\\_Pesticide\\_Side\\_Effect\\_Database.html](https://www.iobc-wprs.org/ip_ipm/IOBC_Pesticide_Side_Effect_Database.html)
- Isaacs R, Tuell J, Fiedler A, Gardiner M, Landis D (2009) Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Front Ecol Environ* 7(4):196–203. doi:10.1890/080035
- Jepsen SJ, Rosenheim JA, Bench ME (2007) The effect of sulfur on biological control of the grape leafhopper, *Erythroneura elegantula*, by the egg parasitoid *Anagrus erythroneurae*. *Biocontrol* 52, 721–732
- Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends Ecol Evol* 19: 101-108
- Jones GV, Alves F (2012) Impact of climate change on wine production: a global overview and regional assessment in the Douro Valley of Portugal. *Int J Global Warming* 4(3/4): 383-406. doi: <http://dx.doi.org/10.1504/IJGW.2012.049448>
- Jonsson M, Wratten SD, Landis DA, Gurr GM. (2008) Recent advances in conservation biological control of arthropods by arthropods. *Biological Control* 45: 172–175
- Landis DA, Gardiner MM, Tompkins J (2012) Using native plant species to diversify agriculture. In: Gurr GM, Wratten SD, Snyder WE, Read DMY (eds) *Biodiversity and insect pests: key issues for sustainable management*. Wiley-Blackwell, Oxford, pp 276–308
- LaSalle J (1993) Parasitic Hymenoptera, biological control and biodiversity. – In: LaSalle, J. and Gauld, I. D. (eds), *Hymenoptera and biodiversity*. C.A.B.I, pp. 197–215
- Lavandero B, Wratten SD, Shishehbor P, Worner S (2005) Enhancing the effectiveness of *Diadegma semiclausum* (Helen): quantifying movement after use of nectar in the field. *Biol Control* 34: 152-158
- Lee JC, Andow DA, Heimpel GE (2006) Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. *Ecol. Entomol.* 31, 470–480

- Letourneau DK, Armbrrecht I, Rivera BS, Lerma JM, Carmona EJ, Daza MC, Escobar S, Galindo V, Gutiérrez C, López SD, Mejía JL, Rangel AMA, Rangel JH, Rivera L, Saavedra CA, Torres AM, Trujillo AR (2011) Does plant diversity benefit agroecosystems? A synthetic review. *Ecol. Appl.* 21, 9–21. doi:10.1890/09-2026.1
- Loni A, Samartsev KG, Scaramozzino PL, Belokobylskij SA and Lucchi A. (2016) Braconinae parasitoids (Hymenoptera, Braconidae) emerged from larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) feeding on *Daphne gnidium* L. *ZooKeys* 587: 125-150
- Lu ZX, Zhu PY, Gurr GM, Zheng XS, Read DMY, Heong KL, Yang YJ, Xu HX (2014) Mechanisms for flowering plants to benefit arthropod natural enemies of insect pests: Prospects for enhanced use in agriculture. *Insect Sci.* 21, 1–12. doi: 10.1111/1744-7917.12000
- Marchesini E, Dalla Montá L (1994) Observations on natural enemies of *Lobesia botrana* (Den. & Schiff.) (Lepidoptera Tortricidae) in Venetian vineyards. *Boll Zool Agr Bachic Ser* 26: 201-230
- Martinez M (2011) Clé d'identification des familles, genres et/ou espèces de diptères auxiliaires, parasitoïdes ou prédateurs des principaux insectes nuisibles à la vigne. In : Sentenac (ed.) *La faune auxiliaire des Vignobles de France*. France Agricole, Paris : 119-140
- Mitroiu MD (2013) Fauna Europaea: Hymenoptera: Chalcidoidea. Fauna Europaea version 2.6, <http://www.fauna-eu.org>
- Moreau J, Villemant C, Benrey B, Thiéry D (2010) Species diversity of larval parasitoids of the European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae): The influence of region and cultivar. *Biol Control* 54: 300–306
- Moosavi FK, Cargnus E, Pavan F, Zandigiacomo, P (2017) Mortality of Eggs and Newly Hatched Larvae of *Lobesia botrana* (Lepidoptera: Tortricidae) exposed to high temperatures in the laboratory. *Environ Entomol.* 2017, 1-8. doi: 10.1093/ee/nvx035
- Muller CB, Brodeur J (2002) Intraguild predation in biological control and conservation biology. *Biological Control* 25: 216–223
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403 (6772): 853–858



- Oliveira B, Barata A, Prates A, Mendes F, Bento F, Gaspar L, Cavaco M (2014) Protecção integrada das culturas. Vol. III- Efeitos secundários dos produtos. DGAV, Lisboa Available at <http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?actualmenu=4318089&generico=4317470&cboui=4317470>. Accessed 15 August 2016
- Paredes D, Cayuela L, Gurr GM, Campos M (2015) Is ground cover vegetation an effective biological control enhancement strategy against olive pests? PLoS ONE 10:e0117265.doi:10.1371/journal.pone.0117265
- Perez Moreno I, Sáenz de Cabezón FJ, Marco V (2000) Evaluación del parasitismo natural sobre crisálidas hibernantes de polilla del racimo (*Lobesia botrana* Den. Y Schiff.) en viñedos de La Rioja. Bol San Veg Plagas 26: 715-722
- Perovic DJ, Gurr GM, Raman A, Nicol HI (2010) Effect of landscape composition and arrangement on biological control agents in a simplified agricultural system: a cost-distance approach. Biol. Control 52, 263–270. doi: 10.1016/j.biocontrol.2009.09.014
- Poveda K, Gomez MI, Martinez E (2008) Diversification practices: their effect on pest regulation and production. Rev. Colom.Entomol. 34, 131–144
- R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at [www.R-project.org](http://www.R-project.org)
- Ribeiro JJA, Martins F, Mendonça TR, Lavadinho AMP (2001) Natural parasitism of *Lobesia botrana* during the hibernation period in the Region of Vinhos Verdes. IOBC/wprs Bull 24 (7): 117-120
- Roehrich R, Boller E (1991) Tortricids in vineyards. In: Van der Gesst, L.P.S., Evenhuis, H.H. (Eds.), Tortricid Pests, their Biology Natural Enemies and Control. Elsevier, Amsterdam, pp. 507–514
- Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA (1995) Intraguild predation among biological-control agents: theory and evidence. Biological Control 5, 303–335
- Scaramozzino PL, Loni A, Lucchi A (2017) A review of insect parasitoids associated with *Lobesia botrana* (Denis & Schiffermüller, 1775) in Italy. 1. Diptera Tachinidae and Hymenoptera Braconidae (Lepidoptera, Tortricidae). ZooKeys 647: 67–100. <https://doi.org/10.3897/zookeys.647.11098>

- Segoli M, Rosenheim JA (2013) Spatial and temporal variation in sugar availability for insect parasitoids in agricultural fields and consequences for reproductive success. *Biological Control* 67: 163–169
- Scarratt SL, Wratten SD, Shishehbor P (2008) Measuring parasitoid movement from floral resources in a vineyard. *Biol Control* 46(2): 107–113
- Schauff ME (1985) Taxonomic study of the Nearctic species of *Elachertus* Spinola (Hymenoptera: Eulophidae). *Proceeding of Entomological Society of Washington*, 87
- Silva EB, Franco JC, Vasconcelos T, Branco M (2010) Effect of ground cover vegetation on the abundance and diversity of beneficial arthropods in citrus orchards. *Bull Entomol Res* 100 (04), 489-499
- Smith I, Hoffmann AA, Thomson LJ (2015) Ground cover and floral resources in shelterbelts increase the abundance of beneficial Hymenopteran families. *Agric For Entomol* 17: 120-128
- Straub CS, Finke DL, Snyder WE (2008) Are the conservation of natural enemy biodiversity and biological control compatible goals? *Biological Control* 45: 225–237
- Thacker JRM (2002) *An Introduction to Arthropod Pest Control*. Cambridge University Press, Cambridge
- Thiéry D, Xuéreb A, Villemant C, Sentenac G, Delbac L, Kuntzman P (2001) Les parasites larvaires de tordeuses de vignobles: aperçu de quelques espèces présentes dans 3 régions viticoles françaises. *IOBC/wprs Bull* 24 (7): 135–142
- Thiéry D, Xuéreb A (2003) Relative abundance of several larval parasitoids of *Lobesia botrana* on different varieties of grapes. *IOBC/wprs Bull* 26 (8), 147–150
- Thiéry D, Xuéreb A (2004) Vers une lutte biologique contre eudémis, *Lobesia botrana*? *Mondaviati Bordeaux – 1-2 Décembre 2004*: 47-52
- Thiéry D, Moreau J (2005) Relative performance of European grapevine moth (*Lobesia botrana*) on grapes and other hosts. *Oecologia* 143, 548–557
- Thiéry D (2008) *Les tordeuses nuisibles à la vigne*. Féret Publication, Bordeaux, France.
- Thiéry D, Delbac L, Villemant C, Moreau J (2011). Control of grape berry moth larvae using parasitoids: should it be developed? *Integrated protection and production in viticulture IOBC/wprs Bull* 67, 189-196

- Thomson LJ, Glenn DC, Hoffmann AA (2000) The effects of sulfur on *Trichogramma* egg parasitoids in vineyards: measuring toxic effects and establishing release windows. *Australian Journal of Experimental Agriculture* 40, 1165–1171
- Thomson LJ, Hoffmann AA (2006) Field validation of laboratory-derived IOBC toxicity ratings for natural enemies in commercial vineyards. *Biol Control* 39: 507-515
- Thomson LJ, Hoffmann AA (2009) Vegetation increases the abundance of natural enemies in vineyards. *Biol Control* 49: 259-269
- Thomson LJ, Hoffmann AA (2010) Natural enemy responses and pest control: importance of local vegetation. *Biol Control* 52: 160-166
- Thomson LJ, Hoffmann AA (2013) Spatial scale of benefits from adjacent woody vegetation on natural enemies within vineyards. *Biol Control* 64: 57-65
- Tillman PG, Smith HA, Holland JM (2012) Cover crops and related methods for enhancing agricultural biodiversity and conservation biocontrol: Successful case studies. In: Gurr GM, Wratten SD, Snyder WE, Read DMY (Eds), *Biodiversity and Insect Pests: Key Issues for Sustainable Management*. New York, NY: John Wiley & Sons, pp. 309–327
- Traugott M, Bell JR, Raso L, Sint D, Symondson WO (2012) (Epub 2011 Oct 10). Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation. *Bulletin of Entomological Research* 102(2): 239-47
- Tryapitsyn VA (1988) Family Bethyidae (Bethyids) In Tryapitsyn VA (ed) *Keys to the insects of the European part of the USSR*. Vol. 3. Part 2. Translated from *Opredelitel nasekomykh Evropeiskoi chasti SSSR*. EJ Brill, Leiden: 3-19
- Van Nouhuys S, Hanski I (2002) Colonization rates and distances of a host butterfly and two specific parasitoids in a fragmented landscape. *Journal of Animal Ecology* 71, 639–650
- Varela LG, Smith RJ, Cooper ML, Hoenisch RW (2010) European grapevine moth, *Lobesia botrana*, in Napa Valley vineyards. *Practical Winery & Vineyard*. March/April: 1–5
- Viers JH, Williams JN, Nicholas KA, Barbosa O, Kotzé I, Spence L, Webb LB, Adina Merenlender A and Reynolds M. (2013) Vinecology: pairing wine with nature. *Conservation Letters* 6: 5 September/October (2013) 287–299
- Villemant C, Delvare G, Martinez M, Sentenac G, Kuntzmann P (2011) *Parasitoïdes de*

- tordeuses. In : Sentenac (ed.) *La faune auxiliaire des Vignobles de France*. France Agricole, Paris : 119-140
- Villemant C, Delvare G (2011) Clé simplifiée d'identification des espèces d'Hyménoptères parasitoïdes des Tortricidae de la vigne. In : Sentenac (ed.) *La faune auxiliaire des Vignobles de France*. France Agricole, Paris: 292-311
- Xuéreb A, Thiéry D (2006) Does natural larval parasitism of *Lobesia botrana* (Lepidoptera: Tortricidae) vary between years, generation, density of the host and vine cultivar? Bull Entomol Res 96, 105-110

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## Supplement 4

**S4- Table 4.1** Characteristics of vineyards assessed

Sampling points	Wine farm	Years	Generations of Lb assessed	Latitude (degree, min, sec) N	Longitude (degree, min, sec) W	Variety	Vineyard system	Management of soil	Production program	Management of soil on slope	Altitude (m)
1	A	2011-12	1, 2	41° 12'32"	7° 26' 7"	T. Nacional	V	GC	IP	-	130
2	A	2011-13	1, 2, 3	41° 12'21"	7° 25' 47"	T. Franca	V	GC	IP	-	136
3	A	2011	1	41°12'31"	7°26'13"	T. Franca	V	GC	IP	-	120
4	A	2012	3	41°12'33"	7°26'12"	T. Amarela	V	GC	IP	-	120
5	A	2012	3	41°12'28"	7°25'49"	T. Franca	V	GC	IP	-	128
6	B	2002	1	41° 11'30"	7° 31' 39"	T. Franca	P2	GC	IP	H	196
7	C	2011	1	41° 10'29"	7° 31' 53"	several	T	H	IP	-	367
8	C	2011-12	1, 3	41° 11'10"	7° 32' 38"	several	T	GC	IP	GC	159
9	C	2011-12	1, 2, 3	41° 10'35"	7° 31' 33"	several	T	H	IP	-	181
10	C	2012-13	3	41° 10'44"	7° 31' 58"	T. Nacional	P1	E	IP	E	204
11	C	2013	2	41° 10'18"	7° 31' 09"	T. Franca	V	E	IP	-	389
12	C	2013	2, 3	41° 10'04"	7° 30' 55"	T. Franca	V	E	IP	-	293
13	C	2011-13	1, 3	41° 11'07"	7° 32' 29"	several	T	E	IP	-	154
14	D	2011-12	1, 2, 3	41° 08'44"	7° 22' 54"	Chardonnay	V	GC	IP	-	539
15	E	2005	1	41° 08'60"	7° 43' 44"	T. Franca	P2	GC	IP	H	186
16	F	2011-13	1, 2, 3	41° 15'03"	7° 29' 09"	Malvasia fina	FV	GC	IP	-	575
17	F	2011-12	1, 2	41° 15'34"	7° 28' 36"	Arinto	FV	GC	IP	-	612
18	F	2011-12	1, 2, 3	41° 15'06"	7° 28' 36"	Encruzado	FV	GC	IP	-	589
19	F	2013	3 <sup>a</sup>	41° 15'56"	7° 28' 51"	Boal	FV	GC	IP	-	629
20	F	2011, 2012	1, 2, 3	41°15'20"	7°28'33"	Moscatel galego	FV	GC	IP	-	600
21	G	2002, 2004, 2007, 2011-12	1, 2	41° 09'14"	7°37'20"	T. Franca	P2	GC	IP	H	211
22	G	2009, 2011, 213	1, 2, 3	41° 09'15"	7°37'52"	T. Franca	P2	T	Conv.	H	234
23	G	2011	1, 3	41° 09'09"	7°37'04"	T. Nacional	P1	GC	IP	GC	205

24	G	2011	1	41° 09'14"	7°37'43"	T. Nacional	P2	GC	IP	H	242
25	G	2011-12; 2015	1	41° 09'24"	7°37'26"	T. Franca, T. Barroca	P2	GC	IP	H	114
26	G	2011, 2015	1	41° 09'17"	7°37'38"	T. Franca	V	GC	IP	-	183
27	G	2015	1	41° 09'5"	7°37'25"	T. Nacional	P2	GC	IP	H	300
28	G	2011, 2015	1	41° 09'1"	7°37'17"	Viosinho	P2	GC	IP	H	290
29	G	2011-12	1	41° 9'27"	7°37'7"	several	T	H	IP	H	111
30	G	2011-12, 2015	1, 3	41° 09'23"	7°36'53"	T. Nacional	P1	GC	IP	H	224
31	H	2005	2	41° 10'25"	7° 33' 06"	T. Franca	V	GC	IP	-	88
32	H	2011	2	41°10'3"	7°33'2.62"	T. Franca	V	GC	IP	-	157
33	H	2011	2, 3	41°10'14"	7°33'5"	T. Nacional	P2	GC	IP	GC	126
34	H	2011	2	41° 09'49"	7° 33' 16"	T. Franca	P2	GC	IP	H	277
35	H	2011	2, 3	41° 09'58"	7° 33' 21"	T. Franca	P2	GC	IP	H	240
36	H	2011	3	41° 09'57"	7° 33' 35"	T. Franca	P2	GC	IP	H	191
37	I	2005, 2011	1	41° 09'56"	7° 33' 16"	T. Franca	P2	GC	IP	H	125
38	I	2011	1, 2	41° 09'28"	7° 46' 06"	T. Franca	P2	GC	IP	H	132
39	I	2011	1 <sup>a</sup>	41° 09'42"	7° 46' 03"	T. Franca	FV	GC	IP	GC	71
40	J	2009, 2011	1, 2, 3	41° 11'12"	7° 30' 42"	T. Franca	P2	GC	IP	H	121
41	J	2011	1 <sup>a</sup>	41° 10'25"	7° 30' 58"	T. Franca	P2	GC	IP	H	305
42	J	2015	1	41° 10'42"	7° 29' 59"	T. Franca	P2	GC	IP	H	205
43	L	2012	3	41° 10'7"	7° 49' 49"	several	P2	GC	IP	H	326

Organization of vineyards on land: P1: earth-banked terraces of one row; P2: earth-banked terraces of two rows; V: vertical vineyards; T: traditional vineyards, installed with no organization of land; FV- Flat vineyards. Management of soil: GC- ground cover; H- herbicide; T: tilled; production system: IP: Integrated production; Conv: conventional.

**S4-Table 4.2** Landscape analysis of the sampling points

Wine farm/ Sampling point	Buffer 50 m				Buffer 100 m			
	% Vines	% EI	H	E	% Vines	% EI	H	E
A1	100,00	0,00	0,00	0,00	91,70	0,00	0,41	0,41
A2	80,50	6,50	0,89	0,45	68,70	16,90	1,45	0,56
B6	97,40	0,00	0,17	0,17	89,40	3,36	0,61	0,31
C7	84,00	3,30	0,75	0,47	78,30	7,82	1,87	0,72
C8	71,70	17,60	1,24	0,53	38,10	54,09	1,92	0,83
C9	93,40	0,00	0,35	0,35	78,80	13,04	1,52	0,59
C10	87,40	0,00	0,55	0,55	89,30	1,80	0,56	0,36
C11	76,70	8,00	1,08	0,54	45,30	21,40	2,06	0,73
C12	57,70	21,40	1,57	0,78	46,70	32,10	1,97	0,85
C13	78,80	9,30	0,99	0,50	40,90	35,95	2,31	0,89
D14	85,00	0,00	0,61	0,61	86,10	2,30	0,67	0,42
E15	87,20	3,90	0,73	0,37	76,40	13,60	1,70	0,66
F16	100,00	0,00	0,00	0,00	91,20	0,90	0,48	0,24
F17	79,10	8,30	1,17	0,59	71,40	18,60	1,63	0,82
F18	100,00	0,00	0,00	0,00	83,30	0,00	0,81	0,51
F19	86,20	0,00	0,62	0,39	49,80	9,20	1,69	0,60
G21	87,80	3,30	0,64	0,40	80,40	6,70	0,90	0,57
G22	91,40	7,00	0,54	0,27	74,60	14,00	1,39	0,54
G23	89,30	6,80	1,17	0,45	74,70	18,20	1,29	0,50
G24	83,70	9,20	0,80	0,51	72,80	17,40	1,64	0,82
H31	54,10	26,40	2,02	0,72	26,90	49,70	2,50	0,83
H34	89,40	0,00	0,49	0,49	81,50	7,05	0,89	0,45
H35	81,00	11,00	0,95	0,48	75,80	10,17	1,47	0,57
H36	85,80	0,00	0,59	0,59	74,70	4,97	1,18	0,51
I37	90,40	0,00	0,46	0,46	62,30	16,00	1,65	0,71
I38	96,80	0,60	0,23	0,14	87,20	4,30	0,79	0,34
I39	81,70	12,40	0,85	0,54	59,10	18,20	2,53	0,80
J40	93,80	0,00	0,34	0,34	95,00	0,00	0,29	0,29
J41	77,10	5,50	1,01	0,51	54,30	12,70	1,83	0,79
J42	98,50	0,00	0,12	0,12	85,40	9,70	0,83	0,42

H'- Shannon index for landscape; E- Eveness index for landscape; % vines- % surface occupied by vineyards; % EI- % surface occupied by EI)

**S4-Table 4.3** Number of treatments performed in each vineyard, score calculated according to Thomson and Hoffmann (2006), and reference to the active ingredients applied with medium or high toxicity reported on literature (risk 2-medium; risk 3- high)

Wine farm/ Sampling point	Year	Sampling date	Treatments performed	Chemical impact *	Active ingredients used with medium or high toxicity reported
A1	2011	23/05	3	7	Sulphur (2),
A2	2013	25/06	5	10	Sulphur (2)
B6	2002	13/05	3	8	Sulphur (2); phosalone (3)
B6	2002	30/07	4	10	Sulphur (2); phosalone (3)
C7	2011	20/05	4	9	Sulphur (2)
C8	2011	20/05	4	9	Sulphur (2)
C8	2012	06/06	4	7	Sulphur (2)
C9	2011	20/05	4	9	Sulphur (2)
C9	2011	21/07	6	15	Sulphur (2); indoxacarb (2)
C9	2012	06/06	4	7	Sulphur (2)
C10	2012	12/09	6	9	Sulphur (2)
C11	2013	24/07	4	8	Sulphur (2)
C12	2013	24/07	4	8	Sulphur (2)
C12	2013	19/09	5	11	Sulphur (2)
C13	2013	19/09	5	11	Sulphur (2)
D14	2011	31/05	3	7	Sulphur (2)
D14	2011	01/08	5	11	Sulphur (2)
E15	2005	12/05	3	6	Sulphur (2)
F16	2011	30/05	2	5	Sulphur (2)
F16	2011	02/08	5	11	Sulphur (2)
F16	2012	02/10	6	11	Sulphur (2)
F16	2013	17/06	2	4	Sulphur (2)
F16	2013	31/07	4	8	Sulphur (2)
F17	2011	30/05	2	5	Sulphur (2)
F17	2011	02/08	5	12	Sulphur (2)
F17	2012	22/06	3	7	Sulphur (2)
F18	2011	30/05	2	5	Sulphur (2)
F18	2011	02/08	5	12	Sulphur (2)



F18	2012	22/06	3	7	Sulphur (2)
F19	2013	03/10	5	9	Sulphur (2)
G21	2002	13/05	2	4	Sulphur (2)
G21	2002	30/07	5	15	Sulphur (2); mancozeb (2)
G21	2004	04/06	3	7	Sulphur (2)
G21	2007	14/05	2	5	Sulphur (2)
G21	2011	19/05	3	7	Sulphur (2)
G22	2009	26/05	3	9	Sulphur (2); deltamethrin (3)
G22	2011	19/05	2	6	Sulphur (2)
G22	2011	19/07	6	20	Sulphur (2); deltamethrin (3); indoxacarb (2)
G22	2011	13/09	6	20	Sulphur (2); deltamethrin (3); indoxacarb (2)
G22	2013	31/05	3	7	Sulphur (2); indoxacarb (2)
G22	2013	24/07	5	11	Sulphur (2); indoxacarb (2)
G22	2013	25/09	5	11	Sulphur (2); indoxacarb (2)
G23	2011	19/05	3	7	Sulphur (2)
G23	2011	13/09	3	7	Sulphur (2)
G24	2011	19/05	3	7	Sulphur (2)
H31	2005	20/07	5	15	Sulphur (2); mancozeb (2)
H34	2011	25/07	5	12	Sulphur (2); mancozeb (2)
H35	2011	25/07	5	12	Sulphur (2); mancozeb (2)
H35	2011	20/09	6	13	Sulphur (2); mancozeb (2)
H36	2011	20/09	6	13	Sulphur (2); mancozeb (2)
I37	2005	11/05	2	4	Sulphur (2)
I37	2011	18/05	2	4	-
I38	2011	18/05	2	6	Sulphur (2)
I38	2011	20/07	6	21	Sulphur (2); mancozeb (2); imidacloprid (3)
I39	2011	18/05	2	4	-
J40	2009	22/05	3	6	Sulphur (2)
J40	2011	24/05	3	6	Sulphur (2)
J40	2011	21/07	7	15	Sulphur (2); indoxacarb (2)
J40	2011	08/09	7	15	Sulphur (2); indoxacarb (2)
J41	2011	24/05	3	6	Sulphur (2)
J42	2015	25/05	2	5	Sulphur (2)

**S4-Table 4.4** Parasitism rates and parasitoid taxa identified in each sampling point, by generation and year

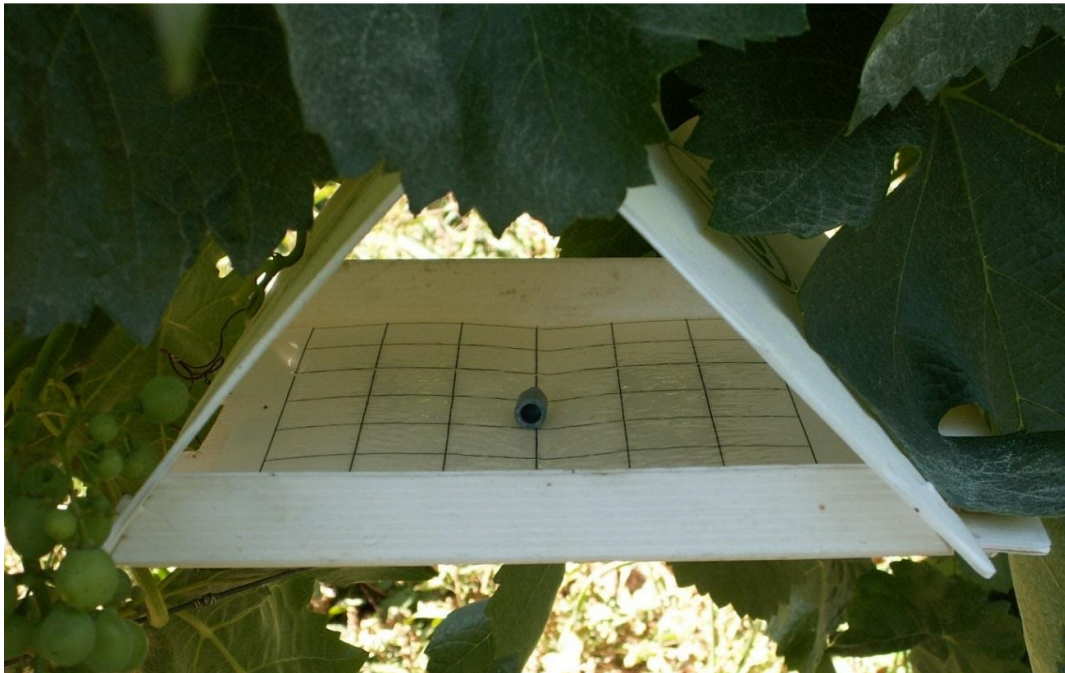
Sampling points	Wine farm	Year	Number of larva/pupa collected	Generation Lb	% Parasit	S	<i>Campoplex capitator</i>	<i>Itoplectis maculator</i>	Cryptinae	<i>Elachertus</i> sp.	<i>Baryscapus</i> sp.	<i>Eulophinae</i>	<i>Elasmus bistrigatus</i>	<i>Elasmus</i> sp	<i>Brachymeria tibialis</i>	<i>Hockeria</i> sp.	<i>Ascogaster quadridentata</i>	<i>Dybrachys cavius</i>	<i>Gonyozus gallicola</i>	<i>Gonyozus claripennis</i>	<i>Eurystoea scutellaris</i>	Not identified	N
1	A	2011	13	1	<b>61.5</b>	1				8													8
2	A	2013	10	2	0	0																	0
6	B	2002	109	1	10.1	4	6			1					3		1						11
6	B	2002	125	2	<b>36.8</b>	4				1					41			3	1				46
7	C	2011	98	1	3.1	1				3													3
8	C	2011	80	1	<b>37.5</b>	1				30													30
8	C	2012	237	1	<b>54.4</b>	5	4	1		122		1	1										129
9	C	2011	25	1	40	1				10													10
9	C	2011	73	2	11	3	1			5					2								8
9	C	2012	42	1	<b>45.2</b>	2				18					1								19
11	C	2013	13	2	7.7	1																1	1
12	C	2013	32	2	3.1	1																1	1
12	C	2013	19	3	0	0																	0
13	C	2013	26	3	0	0																	0
14	D	2011	48	1	16.7	2				7											1		8
14	D	2011	30	2	3.3	1				1													1
15	E	2005	155	1	5.2	3	2			4												2	8
16	F	2011	99	1	1	1											1						1
16	F	2011	55	2	1.8	1															1		1
16	F	2012	21	3	0	0																	0
16	F	2013	14	1	7.1	1	1																1
16	F	2013	14	2	0	0																	0
17	F	2011	68	1	5.9	2	1										3						4
17	F	2011	22	2	0	0																	0
17	F	2012	12	1	0	0																	0
18	F	2011	58	1	0	0																	0
18	F	2011	39	2	5.1	1				2													2
18	F	2012	11	1	9.1	1				1													1

19	F	2013	13	3	0	0																0	
21	G	2002	158	1	12.7	2	11	9														20	
21	G	2002	103	2	6.8	3	4				1	2										7	
21	G	2004	99	1	45.5	4	1	35									1			8		45	
21	G	2007	32	1	46.9	1	15															15	
21	G	2011	13	1	0	0																0	
22	G	2009	37	1	10.8	3	1						2							1		4	
22	G	2011	217	1	2.3	2	1	4														5	
22	G	2011	41	2	2.4	1		1														1	
22	G	2011	14	3	0	0																0	
22	G	2013	28	1	0	0																0	
22	G	2013	21	2	0	0																0	
22	G	2013	14	3	0	0																0	
23	G	2011	14	1	7.1	1		1														1	
23	G	2011	24	3	8.3	1		2														2	
24	G	2011	34	1	0	0																0	
31	H	2005	89	2	24.7	9	5	2	3			1	1	1		1			1	7		22	
34	H	2011	44	2	13.6	3			3				1		2							6	
35	H	2011	12	2	33.3	2	1						3									4	
35	H	2011	33	3	12.1	3			1								1			2		4	
36	H	2011	10	3	0	0																0	
37	I	2005	98	1	2	1	2															2	
37	I	2011	13	1	0	0																0	
38	I	2011	20	1	5	1			1													1	
38	I	2011	23	2	0	0																0	
39	I	2011	20	1	0	0																0	
40	J	2009	38	1	2.6	1								1								1	
40	J	2011	22	1	40.9	2			7											2		9	
40	J	2011	64	2	10.9	4			2				1		3					1		7	
40	J	2011	12	3	0	0																0	
41	J	2011	15	1	6.7	1	1															1	
42	J	2015	14	1	21.4	2			2						1							3	
Total			2927		15.4	16	57	1	2	283	1	1	2	1	55	1	9	9	1	2	3	25	453
							12.6	0.2	0.4	62.5	0.2	0.2	0.4	0.2	12.1	0.2	2.0	2.0	0.2	0.4	0.7	5.5	100



## Chapter 5

### **The use of a degree-day model to forecast flight activity of the grape berry moth, *Lobesia botrana*, in Douro Demarcated Region (Northeast of Portugal)**



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## Abstract

The European grape berry moth, *Lobesia botrana*, is among the most economically important insect pests in Europe and has recently been found in vineyards in Chile, California and Argentina. Predicting the insect's flight phenology during the growing season is critical to improve IPM tactics through better timing of sampling or control operations. The aim of this study was to characterize the flight phenology of *L. botrana* in Douro Demarcated Region (DDR) as well as to develop degree-day (°DD) models for predicting main pest flights, based on data of male captures in sex pheromone traps and temperature data, both recorded over a 20-year period. Nonlinear models based on Boltzmann regression equations were developed using the percentage of accumulated male catches and °DD accumulation, considering two starting points for this accumulation, a biological event (the first male catch) and a calendar date (January 1<sup>st</sup>), both using as lower and upper thresholds 7.3°C and 33°C, respectively. Both models predicted, with reasonable accuracy, the flight phenology of *L. botrana*, although the one that use first catches as starting point for accumulation was more accurate on predicting the second and third flights of the insect. Although the use of a biofix seems to improve model's accuracy, the use of a fixed calendar date (January 1<sup>st</sup>) should be preferred, from the practical point of view and considering large scale application on an IPM strategy. The results obtained could be useful in timing *L. botrana* control measures, especially biorational pesticides application that require accurate information on insect phenology to be effective.

**Keywords:** European grapevine moth . Degree-day models . Pest management . Pheromone traps





## 5.1 Introduction

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is an important vineyard-pest in the European and Mediterranean areas (Ioriatti et al. 2011) and recently was found in Chile, California and Argentina (Gonzales 2010; Varela et al. 2010). This is a multivoltine species which, according to Ioriatti et al. (2011), can develop two to five annual generations. The larvae of first generation feed on bud clusters while the next generations feed on ripening and ripe berries. Fungi, especially grey mold, *Botrytis cinerea*, develop rapidly on the damaged grapes, causing entire clusters to rot (Fermaud and Giboulot 1992).

The Douro Demarcated Region (DDR) is an important winegrowing area (43,670 hectares) located in the Northeast of Portugal, where “Port wine” D.O.P (“Denominação de Origem Protegida”) is produced. In this region, damages caused by the pest are highly variable amongst years, ranging from 0 to 90% of infested clusters at harvest (Carlos et al. 2014). Thus, the need to ensure effective and sound strategies to control this important pest requires the development of tools to support sampling programs and/or timing insecticide sprays, to increase their efficacy, and so reducing their number, as well as environmental impact.

Traditionally, the control of *L. botrana* in DDR relies primarily on the use of insect growth regulators (IGRs), once or twice a year, against the second and/or the third generation. This later is particularly difficult to control, since larvae quickly penetrate ripening fruit. As economic damages to grapes occurs when neonates feed on grape clusters, control measures applied, mainly against the third generation, should primarily target *L. botrana* eggs. Therefore, precise timing of sprays with ovicides (e.g. IGR's), before the eggs hatch, is particularly important to maximize their efficacy and therefore avoid damages.

Several researchers have proposed predictive models for the development of *L. botrana*, both in the laboratory and in the field, based on the relationship between the temperature and the developmental rate of the insect (Baumgärtner and Baronio 1988; Briere and Pracros 1998; Cravedi and Mazzoni 1994; Del Tío et al. 2001; Gabel and Mocko 1984; Gallardo et al. 2009; Heit et al. 2015; Milonas et al. 2001; Savopoulou-Soultani et al. 1996). Other authors have studied the relationship between *L. botrana*

pheromone trap catches and degree-days (DD°) accumulations, using phenological models, to determine the best time for spraying (Del Tio et al. 2001; Gallardo et al. 2009; Heit et al. 2015; Milonas et al. 2001; Ortega-Lopez et al. 2014). This last approach typically comprises one to several regression models. In the simpler case, cumulative counts or proportions of seasonal counts, are related with cumulative DD and predicted distribution is compared with the observed one, to measure the accuracy of the model (Hardman 2012). Physiological models have been also developed (Amo-Salas et al. 2011; Gilioli et al. 2016; Gutierrez et al. 2012; Moravie et al. 2006; Ortega-Lopez et al. 2014; Schmidt et al. 2003), including both abiotic variables (e.g. relative humidity, photoperiod), and biotic variables (e.g. overwintering population density, mortality, fecundity, larval diet) that can have impact on the development of *L. botrana*. However, despite their realistic approach on predicting the development of the pest, and their usefulness, especially when included on Decision Support Systems programs, from the practical point of view and because of lack of physiological data, the DD models has the advantages of being simpler to develop and easier to use by growers, after validated locally, because the timing of the emergence of *L. botrana* adults varies according to sites, climate and years (Gallardo et al. 2009).

The starting point for °DD accumulation is one of the main cause of uncertainty in °DD models. In the previous models, °DD accumulations started on a calendar date (e.g. January 1<sup>st</sup> used by Del Tío et al. (2001) and Lozzia and Vita (1987), March 1<sup>st</sup> used by Gallardo et al. (2009) and Milonas et al. (2001) and March 5<sup>th</sup> used by Gabel and Mocko (1984)). However, as the rate of insect development depends on temperature, forecasts should be able to be improved by starting DD° accumulations from some definite biological event. Accordingly, Riedl et al. (1976), and Jones et al. (2013) found that in codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the model accuracy could be improved by using the first moth capture to synchronize the model to field populations. Thus, this biological event to start computing °DD has been used till recently (Jones et al. 2013; Blomefield and Giliomee 2014; Joshi et al. 2016). To our knowledge, no studies have investigated the possibility of use it to start °DD accumulation for *L. botrana*.

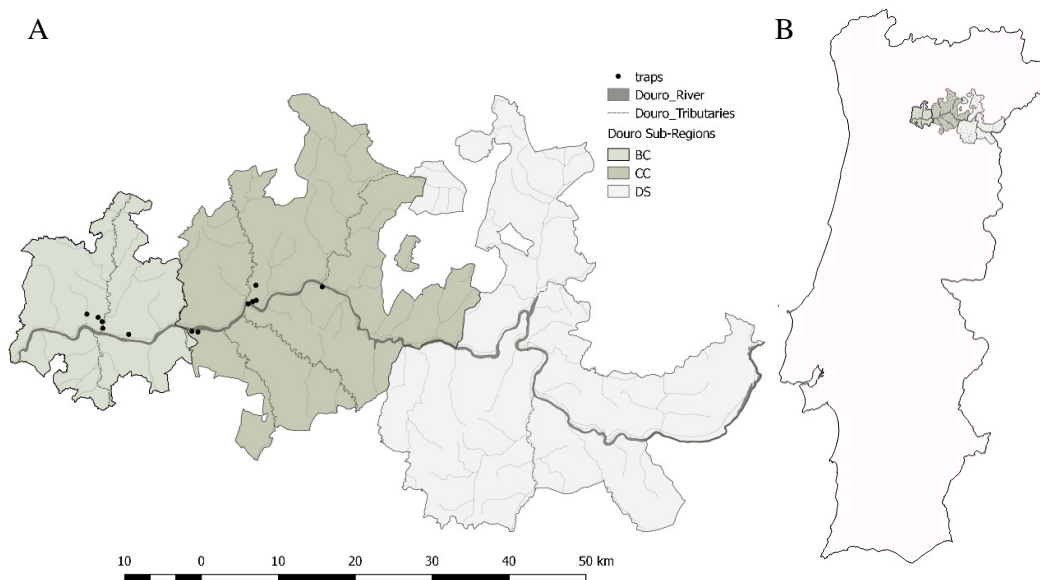
The present study aimed at describing the flight phenology of *L. botrana* in DDR, in relation to degree-day accumulations, as well as developing DD° models, as key-tools to improve monitoring and forecasting of the pest in the studied region, using two

different starting points for temperature accumulation: a calendar date (1<sup>st</sup> January) and a biological event, i.e. biofix (first captures on traps). Specifically, through the analysis of pheromone trap catch data collected over a 20-year period in relation with degree-day accumulations, it was intended to (1) describe the beginning and the peak of each flight of *L. botrana*; (2) develop nonlinear models considering two different starting points for °DD accumulation; 3) and validate such models in DDR conditions. Such information may be important when optimizing *L. botrana* control measures within an integrated pest management (IPM) approach.

## 5.2 Material and methods

### 5.2.1 Study area

The DDR consists of three sub-regions: Baixo Corgo (BC), Cima Corgo (CC), and Douro Superior (DS) (Fig. 5.1A). The western most part of the region is approximately 70 km from the coast and the eastern most areas border with Spain (Fig. 5.1B).



**Figure 5.1** A) Detailed location of the trapping system at the DDR (BC-Baixo Corgo, CC- Cima Corgo, DS- Douro Superior, sub-regions). B) Location of the DDR in Portugal (made by C. Carlos through Quantum GIS Development Team, 2014). Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>.

The climate of DDR is characterized by a strong inter-annual consistency of total insolation, temperature, and potential evapotranspiration and significant inter-annual variation in precipitation. From the analysis of the WorldClim database for the 1950-2000-time period (Hijmans et al. 2005), it was found that, during the active growth stages of grapevines (April to September), the average rainfall varies between 189 and 326 mm, while it is only 50 to 85 mm during the ripening stage (from July to September). The low precipitation along with significant temperature and radiation availability give rise to situations of intense summer plant-soil water stress, particularly in the Cima Corgo and Douro Superior sub-regions. Growing season temperatures (April–October) in the region for the period 1950–2000 averaged 17.8°C, over the entire region, but ranged from a low of 12.1°C, in the upper elevations in the Baixo Corgo, to 19.7°C, in the warmest areas in the Douro Superior. Overall, the region is 65% an Intermediate climate, 24% a Warm climate and nearly 10% a ‘Hot’ climate type (Jones and Alves 2012). For the 1931-1960 climate normal time period, it was found that the growing season variation range, in average maximum temperatures, was nearly 8°C, from 22.4 to 30.3°C. During August, which was the warmest month of summer, maximum values reached 37.0°C. For these reasons, viticulture in the DDR is carried out over a considerable area of the land in moderate to very severe conditions (Jones 2012).

The present study was conducted during the period of 1989 to 2016 in nine wine farms located in Baixo Corgo and Cima Corgo sub-regions (Figure 5.1A). The studied vineyards were grown either on terraces or on vertical rows and located at variable altitude (92 -222 m a.s.l) (Table 5.1). Also, they were conducted under IPM guidelines, being sprayed 1 or 2 times (mainly with IGR’s) against grape berry moth, each time economic threshold level (1-10 % clusters damaged) was surpassed in the second and/or third generation. The soil was maintained with natural ground cover between rows, controlled with herbicide in the rows.

**Table 5.1** General description of the study sites as well as wine varieties

Winefarm (sub-region)	Latitude and longitude coordinates (°, min., sec.)	Wine varieties	Altitude (m)	Number and description of years assessed
Cedro (BC)	41°10'27.51" N 7°47'33.12" W	Tinta Amarela, Tinta Barroca	92	2 (1989, 1991)
Vale Vinhas (BC)	41°10' 4.54" N 7°46'36.29" W	Tinta Amarela, Tinta Barroca	222	1 (1990)
D. Matilde (BC)	41° 8'58.98" N 7°43'43.81" W	Touriga Franca	196	4 (2000, 2008, 2013, 2014*)
Vallado (BC)	41° 9'32.49" N 7°45'59.05" W	Touriga Franca, Syrah, Tinta Amarela	163	11 (2004-2016*)
Pacheca (BC)	41° 8'35.87"N 7°48'48.94"W	Touriga Franca	102	1 (2016*)
S. Luiz (CC)	41° 9'13.30" N 7°37'20.05" W	Touriga Franca	220	11 (2000-2015*)
Bomfim (CC)	41°11'30.62" N 7°31'39.98" W	Touriga Franca	113-196	4 (2002-2003, 2014*-2015*)
Aciprestes (CC)	41°12'21.29" N 7°25'45.67" W	Touriga Franca	139	2 (2013-2014*)
Carvalhas (CC)	41°11'10.53" N 7°32'39.94" W	Touriga Franca	159	2 (2013-2014*)

BC, Baixo Corgo; CC, Cima Corgo / \* data used for validation of models

### 5.2.2 Data collection

The flight activity of *L. botrana* males was monitored, in each vineyard, using one pheromone Delta trap (AgriSense BCS Ltd.) with sticky floors baited with 1 mg synthetic sex pheromone (E7,Z9-12:Ac). Traps were hung 1.0-1.3 m above ground and checked weekly. Sticky floors were replaced and pheromone lures changed monthly. Traps were installed in early March and maintained until the end of September/ middle of October. Temperature data was obtained from several meteorological stations located either in farms or at its proximity (< 5 km).

Males emerging from overwintering pupae constituted the flight of the overwintering generation, here designated as first flight. The beginning and the peak of each flight were determined, given their importance for the management of the pest. The

beginning of the first flight was that of the first capture in early spring. The peak of captures was that of the maximum adult catches in each generation. Bimodal peaks at short time intervals were considered from individuals of the same generation. According to Magalhães (2006), the peak of *L. botrana* oviposition in DDR region occurs at about the same time as the peak of male catches in pheromone traps, which in turn coincides with the period when 50% of the individuals of the flight are captured.

### **5.2.3 Models development**

Degree-days (°DD) were computed using the UC IPM Web degree-day calculator developed by the University of California (<http://www.ipm.ucdavis.edu/WEATHER/ddretrieve.html>), using a single sine wave function (Allen, 1976). An upper intermediate cutoff was selected to slow down °DD accumulation and avoid overestimation of heat units, when temperatures rise above the upper threshold.

The lower and upper development thresholds were defined as 7.3°C and 33°C, respectively (Savopoulou-Soultani et al. 1996; Brière and Pacros 1998). DD were calculated, for each flight and each sampling station, using two events as starting point, a calendar date (January 1<sup>st</sup>) and a biological event (first male capture on traps).

Boltzmann sigmoidal regression equations were determined for each flight, based on the relationship between the percentage of accumulated adult catches in pheromone traps and °DD accumulation, as described by Kumral et al. (2005) for the olive moth, *Prays oleae* (Bernard). The percentage of accumulated catches in pheromone traps was used as dependent variable and °DD accumulation as independent variable. For this analysis, the fourth flight was not considered due to the reduced number of data and because in many cases it was only possible to identify its beginning. Moreover, as this flight coincided with grape harvesting, the risk of losses was low. For more robustness, for each flight period, data from all years (1989-2013) and sites were pooled and analyzed together. Boltzmann equations were calculated through the following equation:

$$Y = Bottom + \frac{(Top - Bottom)}{1 + e^{\left[\frac{(V50-x)}{slope}\right]}}$$

where Y, is the cumulative percentage of adult catches,  $x$  is the degree-day accumulated, V50 is the degree-day accumulated to obtain 50% of catches and the slope describes the steepness of the curve.

Cumulative percentage of adult catches varies from Bottom (lower value) to Top (upper value). Analysis were performed by using IBM SPSS v19. Constraints were incorporated to Bottom ( $\geq 0$ ) and Top ( $\leq 100$ ) parameters. Standard errors of estimated parameters were calculated by bootstrapping. The coefficient of determination ( $R^2$ ) was used to indicate the proportionate amount of variation in the cumulative percentage explained by the DD° accumulations in the nonlinear regression model.

#### **5.2.4 Models validation/ accuracy**

To determine model accuracy, data collected on the three-year's period of 2014-2016, were used. For each flight and year DD° accumulations were introduced in the Boltzmann equations and predicted cumulative percentage of catches were assessed. Then, linear regressions were performed, to compare the predicted values by Boltzmann equations and the observed values of the cumulative percentage of adult catches.

In linear regressions, observed values were used as dependent variables (y-axis) and predicted values as independent variables (x-axis) (Piñeiro et al. 2008). The relationship was evaluated by calculating the coefficient of determination ( $R^2$  values), which measure the proportion of the variance in observed values that is explained by the predicted ones.

Moreover, to assess the statistical significance of regression parameters, it was tested the significance of slope = 1 and intercept = 0 parameters, respectively (Piñeiro et al. 2008), considering that the slope and intercept describe the consistency and the model bias, respectively (Mesple et al. 1996; Smith and Rose 1995). Significance was reported at the level of  $p < 0.05$ . According to Piñeiro et al. (2008), three situations could occur: a) if slope  $\neq 1$ , then model predictions have no consistency with observed values; b) if slope = 1 but intercept  $\neq 0$ , then the model is biased; c) if slope = 1 and intercept = 0, then disagreement between model predictions and observed data is due entirely to the unexplained variance. Moreover, and as advised by Piñeiro et al. (2008), the Root Mean

Square Deviation (RMSD), which represents the mean deviation of predicted values with respect to the observed ones, was also calculated. RMSD is presented in the same units as the model variable under evaluation and is calculated through the following equation:

$$RMSD = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (pi - oi)^2}$$

where  $p$  is the predicted value and  $o$  is the observed value.

### 5.3 Results

#### 5.3.1 *Lobesia botrana* flight phenology

Data of captures of *L. botrana* males, collected over the 20 years' studied period (observed values) showed that, on average, the first flight occurred by mid-March, the second by mid-June, the third by the end of July and the fourth by the beginning of September (Table 2).

By using the first male capture (i.e. biofix) as starting point for °DD accumulation, it was found that, on average, first flight peaked at  $193.7 \pm 3.2$  °DD; second flight began at  $753.6 \pm 1.6$  °DD and peaked at  $982.6 \pm 3.5$  °DD; third flight began at  $1513.0 \pm 2.5$  °DD and peaked at  $1808.2 \pm 4.0$  °DD. The beginning of a fourth flight was found to occur at  $2253.4 \pm 7.1$  °DD (Table 2). On the other hand, when January 1<sup>st</sup> was used as starting point for °DD accumulation, it was found that, on average, first flight began at  $282.8 \pm 2.2$  °DD and peaked at  $470.0 \pm 3.6$  °DD; second flight began at  $1034.9 \pm 2.0$  °DD and peaked at  $1257.5 \pm 3.2$  °DD; third flight began at  $1798.7 \pm 2.8$  °DD and peaked at  $2090.7 \pm 4.1$  °DD. The beginning of a fourth flight was found to happen at  $2539.8 \pm 6.8$  °DD (Table 5.2).

The mean °DD needed to complete each generation was found to be  $745,3 \pm 1.9$ ,  $764.4 \pm 2.8$  and  $762.0 \pm 6.2$ , for the first, the second and the third generations, respectively.



**Table 5.2** Observed values (means  $\pm$  SE) for the occurrence of first catches of *L. botrana* males of each flight (in Julian-days (JD) and degree-days ( $^{\circ}$ DD)) and peak of catches (in  $^{\circ}$ DD) by using first male catches (1<sup>st</sup> catch) and January 1<sup>st</sup> (1 jan) as a starting point for DD $^{\circ}$  accumulation. DDR (1989-1991, 2000-2013)

Flight	N	First catches			Peak of catches	
		JD	$^{\circ}$ DD (1 <sup>st</sup> catch)	$^{\circ}$ DD (1 jan)	$^{\circ}$ DD (1 <sup>st</sup> catch)	$^{\circ}$ DD (1 jan)
First	23	81.3 $\pm$ 0.3	-	282.8 $\pm$ 2.2	193.7 $\pm$ 3.2	470.0 $\pm$ 3.6
Second	37	162.1 $\pm$ 0.2	753.6 $\pm$ 1.6	1034.9 $\pm$ 2.0	982.6 $\pm$ 3.5	1257.5 $\pm$ 3.2
Third	36	210.5 $\pm$ 0.2	1513.0 $\pm$ 2.5	1798.7 $\pm$ 2.8	1808.2 $\pm$ 4.0	2090.7 $\pm$ 4.1
Fourth	17	253.1 $\pm$ 0.4	2253.4 $\pm$ 7.1	2539.8 $\pm$ 6.8	-	-

N- Number of samples (years x sites)

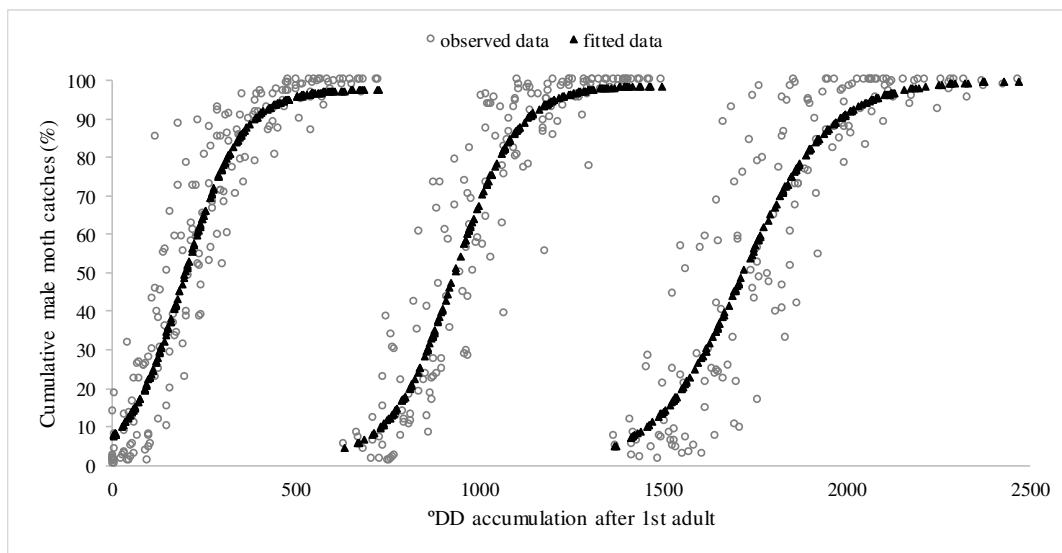
### 5.3.2 Nonlinear models developed

The coefficients of determination obtained (Table 5.3 and Table 5.4) suggests that trap catches accumulation and degree-day are highly related, using both events for starting  $^{\circ}$ DD accumulation, but particularly in the first flight, using first male catches ( $R^2= 0.89$ ) and in the second flight, using January 1<sup>st</sup> ( $R^2= 0.89$ ). On the third flight, coefficients of determination were lower but similar using both starting points for  $^{\circ}$ DD accumulation ( $R^2=0.80$ , with first male catches;  $R^2= 0.81$ , with January 1<sup>st</sup>).

**Table 5.3** Boltzmann regression equations showing the relation between DD $^{\circ}$  accumulation ( $x$ ) and cumulative percentage of *L. botrana* adult male catches ( $Y$ ) across all sites and years, using first male catches as biofix. DDR (1989-1991, 2000-2013)

	First flight	Second flight	Third flight
Regression equation	$Y = \frac{97.73}{1 + e^{\frac{(197.3-x)}{197.28}}}$	$Y = 1.69 + \frac{97.04}{1 + e^{\frac{(934.8-x)}{84.5}}}$	$Y = \frac{100.00}{1 + e^{\frac{(1719.9-x)}{119.4}}}$
df	193	167	160
V50	197.28	934.77	1719.85
CI (95%)	183.01 – 211.56	907.18 - 962.36	1679.16 – 1760.55
$R^2$	0.89	0.87	0.80

$R^2$  - Coefficient of determination; d.f. - degree of freedom; V50 - degree-day accumulated to obtain 50% of catches; CI - Confidence intervals for V50

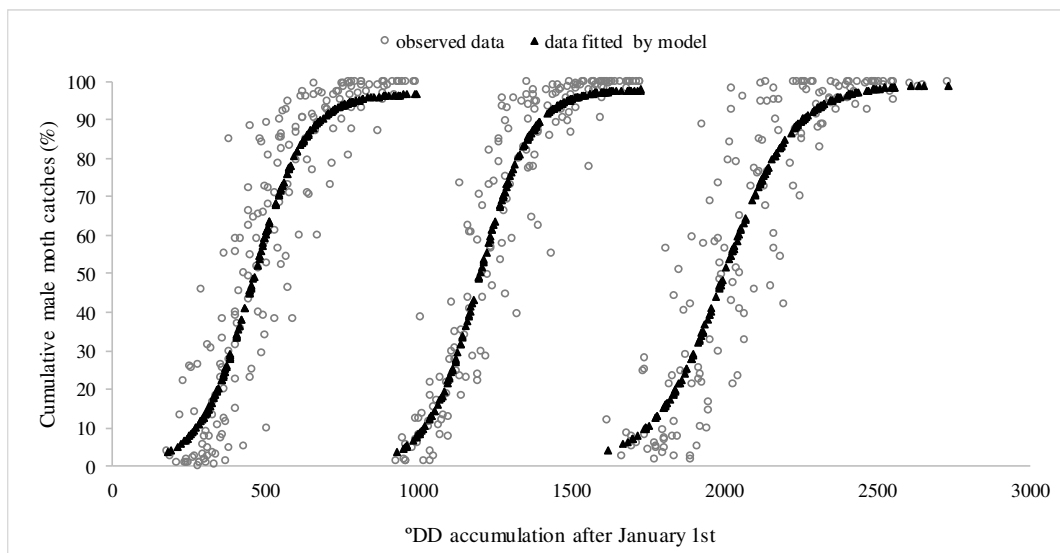


**Figure 5.2** Cumulative male trap catches of *L. botrana* for the first, second and third flights vs. DD°, using first male catches as biofix. DDR (1989-1991, 2000-2013)

**Table 5.4** Boltzmann regression equations showing the relation between DD° accumulation ( $x$ ) and cumulative percentage of *L. botrana* adult male catches ( $Y$ ) across all sites and years, using January 1<sup>st</sup> as starting point for DD° accumulation. DDR (1989-1991, 2000-2013)

	First flight	Second flight	Third flight
Regression equation	$Y = \frac{97.07}{1 + e^{\frac{(460.8-x)}{85.7}}}$	$Y = \frac{98.19}{1 + e^{\frac{(1200.8-x)}{83.4}}}$	$Y = 1.0 + \frac{98.40}{1 + e^{\frac{(2002.0-x)}{111.9}}}$
df	193	167	160
V50	460.8	1200.81	2001.99
CI (95%)	438.65 – 482.94	1181.21 – 1220.42	1964.81-2039.18
$R^2$	0.84	0.89	0.81

$R^2$  - Coefficient of determination; d.f. - degree of freedom; V50 - degree-day accumulated to obtain 50% of catches; CI - Confidence intervals for V50



**Fig. 5.3** Cumulative male trap catches of *L. botrana* for the first, second and third flights vs. DD°, using January 1<sup>st</sup> as starting point for DD° accumulation. DDR (1989-1991, 2000-2013)

### 5.3.3 Models validation/ accuracy

The validation of models through linear regressions during the period of 2014-2016 showed that predicted values for cumulative catches of *L. botrana* could explain, with reasonable accuracy, the variability of observed cumulative catches (Table 5.5). However, in the third flight, the models developed seems to result more biased ( $a \neq 0$ ) and/or not so consistent with observed values ( $b \neq 1$ ), particularly on predicting flight activity of *L. botrana* in 2016, as models developed using first catches (i.e. biofix) as starting point for DD° accumulation (Table 5.5).

**Table 5.5** Parameters obtained on linear regression between observed values of cumulative catches of *L. botrana* males in the pheromone traps obtained in the period 2014-2016 and predictive values obtained by the models, using 1<sup>st</sup> catches and January 1<sup>st</sup>, as starting points for °DD accumulation (SP-DD°)

SP-DD°	year	1 <sup>st</sup> flight							2 <sup>nd</sup> flight							3 <sup>rd</sup> flight						
		n	R <sup>2</sup>	F	p	b ± SE <sup>a</sup>	a ± SE <sup>b</sup>	RMSD <sup>c</sup>	n	R <sup>2</sup>	F	p	b ± SE <sup>a</sup>	a ± SE <sup>b</sup>	RMSD <sup>c</sup>	n	R <sup>2</sup>	F	p	b ± SE <sup>a</sup>	a ± SE <sup>b</sup>	RMSD <sup>c</sup>
1 <sup>st</sup> catch	2014	19	0.86	107.1	***	0.93 ± 0.09	10.86 ± 5.79	14.97	30	0.85	163.02	***	0.91 ± 0.07	9.23 ± 5.17	13.594	25	0.81	100.34	***	1.03 ± 0.10	-6.95 ± 7.68	15.91
	2015	18	0.95	323.1	***	1.11 ± 0.06	-7.83 ± 4.52	8.33	14	0.94	181.15	***	1.03 ± 0.08	1.03 ± 5.45	9.613	14	0.72	31.12	***	0.90 ± 0.16	-15.01 ± 13.43	27.99
	2016	20	0.96	387.0	***	1.07 ± 0.05	2.64 ± 3.61	10.36	13	0.91	100.12	***	1.14 ± 0.12	<b>-19.23 ± 8.35</b>	16.930	12	0.96	274.16	***	1.20 ± 0.07	-11.5 ± 5.12	9.06
	total	57	0.90	554.5	***	1.01 ± 0.04	3.79 ± 2.92	11.59	57	0.86	335.65	***	0.99 ± 0.05	0.97 ± 3.91	13.613	51	0.76	151.9	***	0.99 ± 0.08	-7.22 ± 6.08	18.93
1 <sup>st</sup> Jan	2014	19	0.94	275.0	***	1.10 ± 0.07	-7.34 ± 4.61	9.05	30	0.89	233.05	***	0.98 ± 0.06	-0.69 ± 4.93	11.16	25	0.75	70.19	***	1.04 ± 0.12	-11.58 ± 9.63	19.62
	2015	18	0.93	221.9	***	1.08 ± 0.07	2.88 ± 4.80	11.99	14	0.92	129.76	***	0.96 ± 0.08	<b>12.31 ± 5.57</b>	14.64	14	0.76	37.45	***	0.83 ± 0.14	-5.17 ± 10.79	24.12
	2016	20	0.96	456.48	***	<b>1.34 ± 0.06</b>	<b>-27.96 ± 4.62</b>	12.25	13	0.82	50.47	***	1.24 ± 0.17	<b>-35.53 ± 13.74</b>	25.80	12	0.98	515.3	***	<b>1.29 ± 0.06</b>	<b>-25.85 ± 4.32</b>	10.93
	total	57	0.91	576.75	**	<b>1.14 ± 0.05</b>	<b>-8.13 ± 3.31</b>	11.19	57	0.80	221.99	***	0.97 ± 0.07	-0.70 ± 4.87	16.43	51	0.78	171.33	***	1.02 ± 0.08	-11.72 ± 6.05	19.40

n- Number of observations; R<sup>2</sup> –Coefficient of determination; F- F-test; p - significance (\*\*\* p<0.001; \*\*p<0.01)

a- the values of slope (b) marked in bold means that b≠1 and the model predictions have no consistency with observed values (for details see Material and methods section)

b- The values of intersect (a) marked in bold means that a≠0 and the model is biased

c- Root Mean Square Deviation

## 5.4 Discussion and conclusion

Average dates (JD) and degree-days ( $^{\circ}\text{DD}$ ) corresponding to main flight events (beginning and peak of catches in each flight) of *L. botrana* were obtained for the first time in DDR, using data collected over a 20-year's period. Such information is useful for DDR growers, because gives a first approach of *L. botrana* flight behavior in this region. The average dates of the beginning and peak of the three-main flight are strategic data for scheduling risk assessments.

The comparison of our results with those of previous studies is difficult, because several authors have proposed different starting points for accumulation, and different lower thresholds to model the development of *L. botrana*.

The analysis of the obtained data supports the existence of a fourth flight of *L. botrana*, in September, a finding that is in line with the recorded in Ribera del Duero (Spain) (Amos-Salas 2011) and Veneto (Italy) (Marchesini and Dalla Montà 2004) and could be attributable to global warming (Martín-Vertedor et al. 2010).

An aspect in the development of  $^{\circ}\text{DD}$  models can be the choice of starting point for  $^{\circ}\text{DD}$  accumulation. For *L. botrana*, the models previously developed started it in an arbitrary date (e.g. January 1<sup>st</sup> or March 1<sup>st</sup>), which does not reflect any definite biological event. In our study, both models (i.e. using as started point for  $^{\circ}\text{DD}$  accumulation either biological event or fixed calendar data) showed a good capability in predicting adult accumulated catches, particularly in the first and second flights.

From the years used for model's validation, 2016 show a lower accuracy on predicting adult's accumulated catches. This could have been due to the atypical evolution of climate conditions of this year. According to ADVID (2016), climate conditions on 2016 in DDR were characterized by a strong variability on temperatures and precipitation. During spring, temperatures were significantly lower than average and precipitation was significantly higher (more than the double) than average. These conditions may have affected traps efficiency to attract adults, since trap catches are negatively affected by adverse weather conditions, especially by the occurrence of rain events. During summer, temperatures in DDR were significantly higher than average, without precipitation for more than 3 months. These climate conditions may have induced a summer diapause of the pest; Tzanakakis et al. (1988) observed under laboratory conditions, that, dormancy

occurred under a long day photoperiod length, when eggs were submitted to high temperatures (30°C), indicating the possibility of a diapause mediating dormancy. Within field context, it is known that many invertebrates enter in diapause or quiescent phases in response to unfavorable summer conditions, particularly in arid environments; however, the factors that are involved are poorly understood (Sgrò et al. 2016).

The lower accuracy of both models on predicting the third flight may have been due to several other aspects such as: a) the possibility of generations overlapping; thus, according Roidakis and Karandinos (2001) and Deseo et al. (1981), short day-lengths (<13h) during egg and larval stages affects strongly pupal diapause. In DDR latitudes, such critical day-length occur after September 4<sup>th</sup> (<http://www.sci.fi/~benefon/sol.html>). If larvae of third generation hatch and develop during early August, under a photoperiod higher than 13h, they might be able to reach adult stage, originating a fourth flight, and a fourth generation might develop from middle September on; b) the insecticide treatments, especially those carried against the third generation of the pest, that could have influenced the flight dynamic; c) the effect of grape varieties, which was reported by several authors (e.g. Aguiar et al. 2003; Moreau et al. 2006b; Thiery et al. 2014) as having an important impact in *L. botrana* development and fecundity. Moreover, according to Thiery et al. (2014) the size and shape of the grape bunches in the different varieties, as well as other characteristics of the larval micro-habitat, may influence *L. botrana* development time. These authors suggest that the difference of compactness of clusters among varieties, could infer differences in sunlight exposure and temperature having possibly an impact on the insect development; d) changes in the larval diet quality, linked to the ripeness of grapes which was previously reported as impacting the development rate of *L. botrana*; thus, according to Savopoulou-Soultani et al. (1999), larval development is faster on mid-mature berries than on mature berries, depending on varieties. Pavan et al. (2013) also reported that, in some warmer areas, the third flight develop from late July / early August and that this occurrence is associated with a shorter development time of the second-generation larvae that was independent from temperatures. This occurrence is referred because of either a reduction in instar development times or a lower number of larval instars. Moreau et al. (2006a) shown that larvae performed differently when fed on diets containing different parts of berries. They concluded that seeds are less suitable than the pulp + skin or the entire berry for almost all life history traits of *L. botrana* (extending

the duration of development, causing higher mortality, and lower mating success as well as fecundity. According to the same authors these differences in suitability between different parts of berries may be explained by either difference in their nutritive quality or the presence of secondary compounds, e.g., tannins that reduce protein digestibility or act as feeding deterrents.

It was found that the model that used a Biofix (first catches) for starting °DD accumulation was more accurate (less biased and/or more consistent) in predicting flight activity of *L. botrana*, probably due to the higher synchronization of the model to field populations. From a purely academic perspective, the use of a biofix seems to be a biologically sensible way to improve model accuracy. However, as stated by Jones et al (2008) the use of “no-biofix” models simplify management (no need to install and check traps before beginning of first flight) and eliminates mistakes associated with poor trap catch, particularly in low-pressure situations and where mating disruption reduces trap efficiency. Moreover, according to Jones et al. (2013), the use of a biological event can be a source of error for large-scale implementation due to weekly trap check intervals (instead of daily). In fact, since that traps will be deployed for supervised pest management rather than research purposes, consultants in DDR rarely will check them more than once a week during the critical period around the day in which the occurrence of the first adult is expected, which will increase the error in the calculation of °DD.

Thus, our study is in line with the reported by Jones et al. (2008), who concluded that although the use of a biofix might still be useful in some research studies, as a biologically sensible way to improve model accuracy, the use of models developed using a fixed calendar date (January 1<sup>st</sup>) should be considered on a IPM strategy, since it is much easier to use and eliminates a key point of confusion among IPM consultants, especially when considering its large-scale application through winegrower's organizations.

The obtained information will be useful to support growers in the determination of the best moment to estimate the risk associated with *L. botrana* and, on the other hand, to time treatments against the pest, mainly if account is taken of the growing interest for biorational insecticides, where precise timing of treatments is important.

The proposed nonlinear models and, particularly the one that uses a calendar date (January 1<sup>st</sup>) for the starting °DD accumulation, might be useful to provide *L. botrana*

forecasts in a large-scale management strategy, given the practical limitations on the ability to correctly monitor the pest activity in the field. Their application can therefore improve the effectiveness of IPM in DDR area, considering its lack of complexity, and that the required tools (e.g. sex-pheromone traps and weather stations) are already being used. Monitoring of grapevine pests, their natural enemies, and diseases during the phenological development of the vine is locally being carried out by IPM teams, such as the Association for the Development of Viticulture in the Douro Region (ADVID), which co-operate with the winegrowers and the agricultural administrations to determine the best moments to carry phytosanitary treatments. Therefore, the results obtained would not replace common monitoring programs or using economic thresholds, before deciding to apply insecticides. In practice, they specify the time to start careful vineyard monitoring, from which growers can then make better control decisions in a timely manner. However, field evaluation and possible reevaluation of the model parameters should be done. The effect of global warming on the life history of *L. botrana* should be carefully evaluated. Its effect on the advance of the insect phenology throughout the last two decades was already demonstrated in vine-growing areas of south-western Spain (Martín-Vertedor et al. 2010). An assessment of future climate conditions in DDR show ensemble mean temperature increases for February-March from 9.0°C in the year 2000 up to 12.0°C by the end of the 21st century (+3°C), whereas for May it increases from 14.8°C up to 18.8°C (+4°C) (Santos et al. 2013). On the other hand, as stated by Reineke and Thiery (2016), grapevine harvest dates might advance as well, limiting putative damage due to an earlier appearance of late-season generations or an increase number of *L. botrana* generations.

In conclusion, the proposed two models, which were the first developed in DDR to predict the flight activity of *L. botrana*, could be a useful tool towards a more sustainable use of pesticides against a key pest of commercial European vines, thus complying with EU Directive 128/2009.



## 5.5 References

- ADVID (2016) Boletim ano vitícola 2016 – Balanço final. Association for the Development of Viticulture in the Douro Region, Peso da Régua, 17 pp
- Aguiar A, Carlos C, Bastos MMSM, Mexia A (2003) Ataques de traça da uva *Lobesia botrana* (Den. & Schiff.) em diferentes castas das regiões dos Vinhos Verdes e Douro. Actas do 6º Encontro Nacional de Protecção Integrada. Escola Superior Agrária de Castelo Branco. 14-16 May 2003: 78-82
- Allen JC 1976. A modified sine wave method for calculating degree days. Environ. Entomol. 5:388-396
- Amo-Salas M, Ortega-López V, Harman R, Alonso-González A (2011) A new model for predicting the flight activity of *Lobesia botrana* (Lepidoptera: Tortricidae). Crop Prot 30 (6):1586-1593. doi:10.1016/j.cropro.2011.09.003
- Baumgärtner J, Baronio P (1988) Modello fenologico di volo del *Lobesia botrana* Den. et Schiff (Lep. Tortricidae) relativo alla situazione ambientale della Emilia-Romagna. Boll Inst Entomol Univ Bologna 43: 157-170
- Blomefield TL, Giliomee JH (2014) Validation of the phenology model for the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), in South African pome fruit orchards. African Entomology 22 (1), 30-48 (2014)
- Brière JF, Pracros P (1998) Comparison of temperature-dependent growth models with the development of *Lobesia botrana* (Lepidoptera: Tortricidae). Environ Entomol 27: 94-101. doi: <http://dx.doi.org/10.1093/ee/27.1.94>
- Carlos C, Gonçalves F, Sousa S, Nóbrega M, Manso J, Salvação J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D, Val M, Franco JC, Aranha J, Thistlewood H, Torres L (2014) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. IOBC-WPRS Bulletin 105: 93-102
- Cravedi P, Mazzoni E (1994) Verification of the relation between degree-days and pheromone trap catches of *Lobesia botrana* (Den. & Schiff.) (Lepidoptera Tortricidae). Redia 77: 109-122
- Del Tío R, Martinez JL, Ocete ME (2001) Study of the relationship between sex pheromone trap catches of *Lobesia botrana* (Den. and Schiff) (Lep., Tortricidae) and

- the accumulation of degree-days in Sherry vineyards (SW of Spain). J Appl Entomol 125: 9–14. doi: 10.1111/j.1439-0418.2001.00507.x
- Deseo KV, Marani F, Brunelli A, Bertaccini, A (1981) Observations on the biology and diseases of *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) in Central-North Italy. Acta Phytopathol Hun 1: 405-431
- Fermaud M, Giboulot A (1992) Influence of *Lobesia botrana* larvae on field severity of *Botrytis* rot of grape berries. Plant Disease, 76, 404–409
- Gabel B, Mocko V (1984) Forecasting the cyclical timing of the grape vine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). Acta Entomol Bohemoslovaca 81: 1-14
- Gallardo A, Ocete R, Lopez MA, Maistrello L, Ortega F, Semedo A, Soria FJ (2009) Forecasting the flight activity of *Lobesia botrana* (Denis and Schifferrmüller) (Lepidoptera, Tortricidae) in Southwestern Spain. J App Entomol 133: 626-632. doi: 10.1111/j.1439-0418.2009.01417.x
- Gilioli G, Pasquali S, Marchesini E (2016) A modelling framework for pest population dynamics and management: An application to the grape berry moth. Ecol Model 320: 348–357. doi:10.1016/j.ecolmodel.2015.10.018
- Gonzales M (2010) *Lobesia botrana*: polilla de la uva. Rev Enol 2:2–5
- Gutierrez A P, Ponti L, Cooper ML, Gilioli G, Baumgartner J, Duso C (2012) Prospective analysis of the invasive potential of the European grapevine moth, *Lobesia botrana* (Den. and Schiff.) in California. Agric For Entomol 14: 225-238. doi: 10.1111/j.1461-9563.2011.00566.x
- Hardman JM (2012) Modeling arthropods to support IPM in vineyards. In: Bostanian NJ, Vincent C, Isaacs R (Eds) Arthropod Management in Vineyards: Pests, Approaches, and Future Directions. Springer, Dordrecht, pp 37–52
- Heit G, Sione W, Cortese P (2015) Three years analysis of *Lobesia botrana* (Lepidoptera: Tortricidae) flight activity in a quarantined area. J. Crop Prot 2015(4):605-615
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, and Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25(15):1965-1978
- Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A (2011) Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). J Econ Entomol 104(4): 1125-1137. doi: <http://dx.doi.org/10.1603/EC10443>

- Jones GV (2012) A Climate Assessment for the Douro Wine Region: An Examination of the Past, Present, and Future Climate Conditions for Wine Production. Association for the Development of Viticulture in the Douro Region, Peso da Régua. 93 pp
- Jones GV, Alves F (2012) Impact of climate change on wine production: a global overview and regional assessment in the Douro Valley of Portugal. *Int J Global Warming* 4(3/4): 383-406. doi: <http://dx.doi.org/10.1504/IJGW.2012.049448>
- Jones VP, Doerr M, Brunner JF (2008) Is biofix necessary for predicting codling moth (Lepidoptera: Tortricidae) emergence in Washington state apple orchards? *J. Econ. Entomol.* 101,1651–1657. doi:10.1093/jee/101.5. 1651
- Jones VP, Hilton R, Brunner JF, Bentley WJ, Alston DG, Barrett B, Van Steenwyk RA, Hull LA, Walgenbach JF, Coates W, Smith TJ (2013) Predicting the emergence of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), on a degree-day scale in North America. *Pest Management Science* 69:1393-1398
- Kumral NA, Kovanci B, Akbudak B (2005) Pheromone trap catches of the olive moth, *Prays oleae* (Bern.) (Lep., Plutellidae) in relation to olive phenology and degree-day models. *J Appl Entomol* 129: 375-381. doi: 10.1111/j.1439-0418.2005.00985.x
- Lozzia GC, Vita G (1987) Preliminary notes on application of a predictive model for *Eupoecilia ambiguella* (Hbn.) and *Lobesia botrana* (Den. & Schiff.) flight in Lombardia (Italy) in relation to temperature. Proceedings of Joint Expert's Meeting "Influence of environmental factors on the control of grape pest, diseases and weeds", Thessaloniki, 6 – 8 October 1987: 35 – 47
- Magalhães P (2006) Estudo da estimativa do risco e dos níveis económicos de ataque de *Lobesia botrana* (Dennis & Schiffermuller) na casta Touriga Franca na Região do Douro. Graduate Thesis in Agronomic engineering. Faculdade de Ciências da Universidade do Porto
- Marchesini E, Dalla Montà L (2004) Nel Veneto quattro generazioni di tignoletta della vite. *L'Informatore Agrario* 60(4): 75-78
- Martín-Vertedor D, Ferrero-García JJ, Torres-Vila LM (2010) Global warming affects phenology and voltinism of *Lobesia botrana* in Spain. *Agr Forest Entomol* 12: 169 – 176. doi: 10.1111/j.1461-9563.2009.00465.x
- Mesple F, Troussellier M, Casellas C, Legendre P (1996) Evaluation of simple statistical criteria to qualify a simulation. *Ecol Model* 88: 9–18

- Milonas P, SavopoulouSoultani M, Stavridis D (2001) Day-degree models for predicting the generation time and flight activity of local populations of *Lobesia botrana* (Denis & Schifferrmüller) (Lepidoptera: Tortricidae) in Greece. J Appl Entomol 125: 515-518. doi: 10.1046/j.1439-0418.2001.00594.x
- Moravie M, Davison A, Pasquier D, Charmillot P (2006) Bayesian forecasting of grape moth emergence. Ecol Model 197: 478-489. doi: 10.1016/j.ecolmodel.2006.03.030
- Moreau J, Arruego X, Benrey B, Thiery D (2006a) Differences in nutritional quality of parts of *Vitis vinifera* berries affect fitness of the European grapevine. Entomol Exp Appl 119:93–99. doi:10.1111/j.1570-7458.2006.00390
- Moreau J, Benrey B, Thiéry D (2006b) Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). Bull Entomol Res 96: 205-212. doi: 10.1079/BER2005417
- Ortega-Lopez V, Amo-Salas M, Ortiz-Barredo A, Diez-Navajas AM (2014) Male flight phenology of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae) in different wine-growing regions in Spain. Bull Entomol Res 104: 566-575. doi: 10.1017/S0007485314000339
- Pavan F, Floreani C, Barro P, Zandigiacomo P, Dalla Monta L (2013) Occurrence of two different development patterns in *Lobesia botrana* (Lepidoptera: Tortricidae) larvae during the second generation. Agric For Entomol 14: 398-406. doi: 10.1111/afe.12027
- Piñeiro G, Perelman S, Guerschman JP, Paruelo JM (2008) How to evaluate models: Observed vs. predicted or predicted vs. observed? Ecol Model 216: 316–322. doi:10.1016/j.ecolmodel.2008.05.006
- Reineke A, Thiéry D (2016) Grapevine insect pests and their natural enemies in the age of global warming. J. Pest Sci 89: 313-328 doi:10.1007/s10340-016-0761-8
- Riedl H, Croft BA Howitt AJ (1976) Forecasting codling moth phenology based on pheromone trap catches and physiological-time models. Can. Entomol. 108: 449-460.
- Roditakis NE, Karandinos MG (2001) Effects of photoperiod and temperature on pupal diapause induction of grape berry moth *Lobesia botrana*. Physiol Entomol 26: 329-340. doi/10.1046/j.0307-6962.2001.00253.x
- Santos JA, Grätsch SD, Karremann MK, Jones GV, Pinto JG (2013) Ensemble projections for wine production in the Douro Valley of Portugal. Climatic Change 117: 211–225 doi: 10.1007/s10584-012-0538-x

- Savopoulou-Soultani M, Milonas PG, Skoulakis GE (1996) Development and life-fertility tables for *Lobesia botrana* (Lepidoptera: Tortricidae) at constant temperatures. *Recent Res Dev Entomol* 1: 73-81
- Savopoulou-Soultani M, Nikolaou N, Milonas PG (1999) Influence of maturity stage of the grape berries on the development of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae. *J Econ Entomol* 92: 551-556. doi: <http://dx.doi.org/10.1093/jee/92.3.551>
- Schmidt K, Hoppmann D, Holst H, Berkelmann-Lohnertz B (2003) Identifying weather-related covariates controlling grape berry moth dynamics. *OEPP/EPPO Bull.* 33:517–524.
- Sgrò CM, Terblanche JS, Hoffmann AA (2016) What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* 61:433–51
- Smith EP, Rose KA (1995) Model goodness-of-fit analysis using regression and related techniques. *Ecol Model* 77: 49–64
- Thiéry D, Monceau K, Moreau J (2014) Different emergence phenology of European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae) on six varieties of grapes. *Bull Entomol Res* 104 (3): 277-287. doi: 10.1017/S000748531300031X
- Tzanakakis ME, Savopoulou-Soultani M, Oustapassidis CS, Verras SC, Hatziemmanuel H (1988) Induction of dormancy in *Lobesia botrana* by long day and high temperature conditions. *Entomol Hellenica* 6: 7-10
- Varela LG, Smith RJ, Cooper ML, Hoenisch RW (2010) European grapevine moth, *Lobesia botrana*, in Napa Valley vineyards. *Practical Winery & Vineyard*. March/April:1–5

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## Chapter 6

### Does mating disruption of *Lobesia botrana* (Den. & Schiff) works under Douro Demarcated Region conditions?



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- Carlos C**, Gonçalves F, Sousa S, Nóbrega M, Manso J, Salvação J, Costa J, Gaspar C, Domingos J, Silva L, Fernandes D., Val M.C., Franco JC, Aranha J, Thistlewood H. & Torres L (2014) Success of mating disruption against the European grapevine moth, *Lobesia botrana* (Den. & Schiff): a whole farm case-study in the Douro Wine Region. IOBC-WPRS Bulletin, 105: 93-102
- Carlos C**, Gonçalves F, Sousa S, Salvação J, Sharma L, Soares R, Manso J, Nóbrega M, Lopes A, Soares S, Aranha J, Villemant C, Marques G, Torres L (2013) Environmentally safe strategies to control the European Grapevine Moth, *Lobesia botrana* (Den. & Schiff.) in the Douro Demarcated Region. Ciência e Técnica Vitivinícola: 1006-1011
- Carlos C**, Val MC, Marques G, Torres L (2011) New approaches for management of European Grapevine Moth, *Lobesia botrana*, in the Douro Wine Region, Portugal. Am J Enol Vitic, 62 (3): 389A-389A





## **Abstract**

The grapevine moth, *Lobesia botrana* (Den & Schiff.) is the most important pest in the Douro Demarcated Region (Portugal) and has typically three generations per year, of which the third is the most damaging to grapes. The damage is highly variable amongst years, ranging from 0 to 90% of infested clusters at harvest. The use of mating disruption (MD), an environmentally friendly method to control this pest, is widely recommended in IPM strategies. However, some major constraints to its implementation have been identified in our region: high biotic potential of the moth; high summer temperatures; the steep terrain in many vineyards. Most importantly, the size of the treated area is an important factor for success of the method, which works best in large and contiguous areas. However, the landscape of the Douro Region is very fragmented. Most of the vineyards are small, often bounded by other crops such as olive groves, and by unmanaged natural or abandoned crop habitats, where alternative plant hosts of *L. botrana* (e.g. *Daphne gnidium* L.) are common. Our objective was to investigate the effectiveness of MD against *L. botrana* within a series of contiguous habitats managed within the “Quinta de São Luiz”, as a case-study. It also served as a demonstration project and to highlight the importance of applying MD on an area-wide scale. The technique was first applied within the farm in 2001 on 4 ha, and expanded to the present 90 ha. MD was shown to be most effective for control of *L. botrana* after consecutive seasons of application, when large areas were treated, and in years of low pest population density.

**Key words:** mating disruption, *Lobesia botrana*, vineyard, pheromone, area-wide



## 6.1 Introduction

The grapevine moth, *Lobesia botrana* (Den. and Schiff.) is a key pest of commercial vineyards, mainly in Southern Europe, causing damages to grapes, directly through the feeding activity of larvae and indirectly by promoting *botrytis* to bunches (Fermaud and Le Menn 1989). As a result, it has received considerable attention by researchers attempting to develop effective control strategies against it. Public demand of residue-free products also augmented the interest for environmentally safe pest management such as mating disruption (MD) technique, that is currently being implemented on 140,000 ha in Europe (Ioratti et al. 2011).

Although this technique has been successfully applied in many European countries namely in Germany (Kast 2001), Switzerland (Charmillot and Pasquier 2001) and northern Italy (Varner et al. 2001), in southern regions, such as Italy (Nucifora et al. 1996; Bagnoli et al. 2001), Greece (Moschos et al. 2004) and Cyprus (Vassiliou 2009) several constraints to the successful application of this technique have been identified.

In Portugal, where MD is registered for *L. botrana* since 2002 (Isonet-L®, from Shin-Etsu Chemical Co.), the area of vineyard under the technique was approximately 800 ha, in 2005 (Eira 2006) and presently is estimated to be around 1,500 ha, from which 350 ha in Douro Demarcated Region (DDR). In this region, MD has been applied since 2000 and since then researchers, technicians and growers are collaborating to identify the main constraints to its successful application. Some of those constraints are related with the biology of the pest, namely with its long life cycle and its high biotic potential, others constraints are related with natural conditions of this region, such as the orography (high steepness), and the fragmentation, size and shape of the plots, finally others are related with climate conditions, such as the wind pattern and the high summer temperatures that, in some years, leads to the exhaustion of the pheromone in the dispensers by the end of July, just when *L. botrana* begin the third generation, which is the most damaging (Carlos et al. 2010).

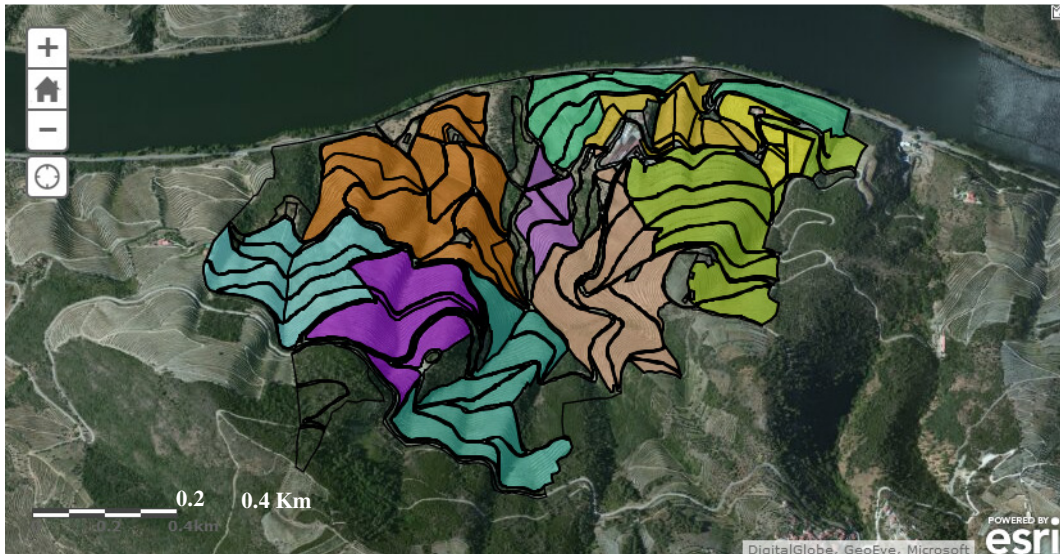
The objective of this work was to investigate the effectiveness of MD against *L. botrana* in a commercial wine farm located in the DDR, during the period 2001-2013. The obtained results are critically discussed and constraints are analyzed, as a basis for the successful adaptation of this technique in DDR. These results also intend to highlight the

importance of applying MD on an area-wide scale through a demonstration project (EcoVitis).

## 6.2 Material and methods

### 6.2.1 Study sites

The experiments were carried out from 2001 to 2013 in a commercial wine-farm (Quinta de S. Luiz, from Sogevinus Fine Wines, SA company) located at 41°08'46.29''N, 7°37'48.90''W in the Cima Corgo sub region of the DDR, on the left side of the Douro river. Grapevine, mainly constituted by Portuguese varieties (cv. *Touriga Franca*, *Touriga Nacional*, *Tinta Roriz*) were grown on slopes ranging from 30-50% (in either small terraces or vertical planted rows) and conducted in Royat training system. The soil was maintained with natural ground cover between rows and with herbicide on the row. The farm with approximately 132 ha, is composed by several land use elements, namely, vineyards under IPM program (69%), forest (11%), roads (10%), scrubland (4%), olives (3%), and urban elements (1.4%). These several land use elements are all dispersed on plots of variable size and form, and located at different altitudes (80-400 m) as shown on Figure 6.1.



**Figure 6.1** S. Luiz farm (Source: ESRI Arcgis online). The colored polygons represent area under MD; each division of the scale represent 0.1 km.

### 6.2.2 Type and number of dispensers

Shin-Etsu Chemical Co. Ltd Isonet® dispensers, loaded with the synthetic female sex pheromone, E7,Z9-12:Ac (dispensers with 172 mg of pheromone in 2000-2010, and with 300 mg in 2010-2013) were used (Table 6.1).

**Table 6.1** General information about the surfaces and the type and number of dispensers applied in S. Luiz farm during the present study

Year	Surface (ha)	Type of dispenser	Dose / ha	Dates of deployment of dispensers
2001	3.0	Isonet-L	650	12-Mar
2002	25.0	Isonet -L	560	19-Mar
2003	15.0	Isonet -L	600	21-Mar
2004	16.2	Isonet -L	560	12-Mar
2009*	82.1*	Isonet -L	610*	17-Mar *
2010	4.0	Isonet -Ltt	473	26-Mar
“	82.1	Isonet -L	370	20-Jul
2011	90.0	Isonet -Ltt	442	7 - 9 Jul
2012	90.0	Isonet -Ltt	478	26-Mar
2013	90.0	Isonet -Ltt	460	27-Mar

\* Reinforcement of dispensers in part of the area (34.13 ha with 516 dispensers/ha on July 23)

Between 2001 and 2004, the treated area ranged from 3.0 to 25.0 ha (Table 6.1). Isonet-L dispensers were applied mainly during March, before or shortly after the beginning of the first flight period at a density of 560-650 ha<sup>-1</sup>, depending on the experimental site (surface, steepness, winds), with a reinforce of 10-20% in the 15-20 m of the border and in the top of the hills.

From 2005 to 2008, MD was not used and the control of *L. botrana* was done by spraying with either *Bacillus thurigiensis* or insect growth regulators (flufenoxuron or fenoxicarb). In 2009, dispensers (Isonet-L) were applied in 82.1 ha (almost all the total surface of vineyards of this farm). To reinforce the dispensers of some specific plots, identified in previous years as having higher pressure of the pest, a second deployment of dispensers was done in a total of 34.13 ha, just before the beginning of the third flight period at the end of July (Table 6.1).

In 2010, Shin-Etsu provided dispensers loaded with an higher amount of pheromone (300 mg of E7,Z9-12:Ac), i.e. Isonet – Ltt, to test its efficacy in an experimental area of

4.0 ha, that were applied in a rate of 473 dispensers / ha (which is above the recommended rate of 400 dispensers / ha). On the rest of the area, Isonet-L dispensers were applied only in July, to evaluate the possibility to control effectively the 3<sup>rd</sup> generation, by avoiding the early exhaustion of the pheromone in the dispensers. The same strategy was conducted in 2011 on the whole surface of S. Luiz, this time using only Isonet-Ltt dispensers. Since then, Isonet-Ltt has been applied during March, before the beginning of the first flight period at a density of 460-480 ha<sup>-1</sup> (Table 6.1).

### ***6.2.3 Evolution of pheromone in dispensers***

The active ingredient in the dispensers was calculated by gravimetric analysis, an alternative and reliable method to evaluate performance of dispensers, as indicated by Caruso et al. (2012). These authors found that there is a statistically significant relationship between the percentage of active ingredient content obtained by gas chromatography and weighing ISONET dispensers. To verify the release over time by weighing, a balance with 0.0001g accuracy was used to weight samples of 10 dispensers collected monthly from March to September in 2002 (for Isonet-L) and from April to November in 2013 (for Isonet-Ltt). In 2013 the dispensers were collected at 80 and 400 m of altitude, being deployed on the field specifically to measure the evolution of the pheromone.

The average active ingredient in the dispensers was calculated in each data and analyzed considering the monthly mean temperatures registered on an automatic weather station (ADCON Telemetry A723) located at S. Luiz farm.

### ***6.2.4 Evaluation of mating disruption efficacy***

MD efficacy was evaluated by comparing either pheromone-baited trap catches and grape infestation, between treated and untreated plots, at several “inspection stations” which varied from three in 2001 to 20 in 2013. The untreated plot (control) was located on a vineyard distant over than 50 m from the plots under MD.

Captures were monitored through a network of between 3 and 22 pheromone delta traps (AgriSense BCS Ltd.) baited with a 1 mg E7,Z9-12:Ac red rubber septum, installed in the centre of each plot. In some years, they were also installed in the border of the plot.

Traps were checked weekly and captured moths were counted and removed. Percentage of male disorientation was calculated for each weekly trapping interval.

Grape infestation was assessed by inspecting samples of 50 to 100 randomly inflorescences or grapes, according to the season, during each of the three generations of the insect. In addition, a sample of 50 to 100 grapes was collected at harvest and dissected to look for larvae. In the first generation, the level of infestation was expressed as the number of nests per 100 inflorescences, while in the second and in the third generations it was expressed by the percentage of grapes with at least one berry infested. Each time the economic threshold level was surpassed (i.e., 200 nests / 100 clusters for the 1<sup>st</sup> generation, 5% for the second and third generations), a treatment was applied with either some IGR or *B. thuringiensis*, mainly in the second and the third generations. According to our experience (Carlos, unpublished data), the infestation varies according to altitude, being normally higher in the lower altitudes. Thus, data of infestation were analysed as the infestation in the top, in the middle and in the bottom of the farm.

## **6.3 Results and discussion**

### ***6.3.1 Evolution of pheromones in the dispensers***

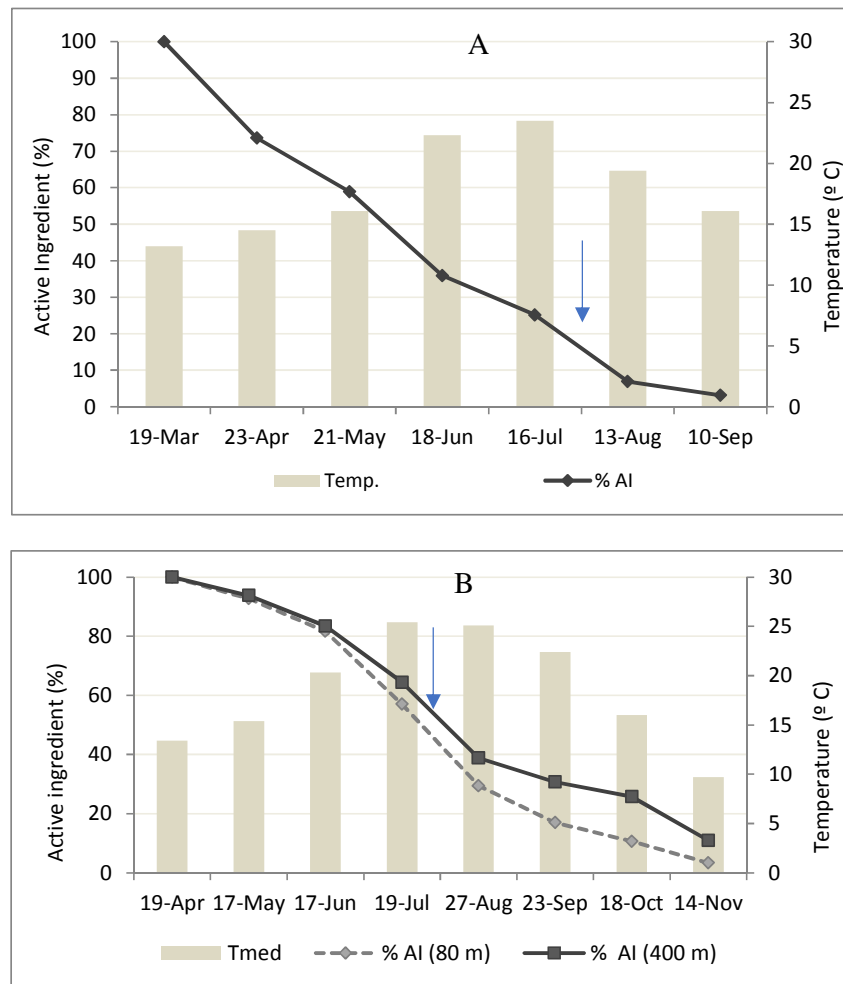
In 2002, a loss of more than 25% of active ingredient on the dispensers was found in April (Figure 6.2), only one month after its deployment in the field.

In the studied farm, it is normal to have wind speed higher than 1.5 m/s during the period from April to August (based on ADVID weather data station, located at S. Luiz farm). The reduced canopy structure of the grapevines, at this time, could have amplified the wind impact on the releasing rate of the pheromone on the dispensers.

On the period of June-August, with the high mean temperatures registered, more than 93% of active ingredient was already lost. These results are in accordance with those reported by Moschos et al. (2004) in Greece and by Vassiliou (2009) in Cyprus, who found that if the temperatures are high during the preceding period, little pheromone is left in the dispensers during the crucial period of the third generation, that, in DDR, occurs in mid-August.

In 2013, the highest loss of pheromone of Isonet-Ltt dispensers happened on July and August, coinciding with the period of higher temperatures. At the time of the

beginning of the third flight, the remaining of pheromone in the dispensers was around 50%, at 400 m of altitude, and 40%, at 80 m of altitude. These results, indicate that the rate of release of pheromone is higher in the lower altitudes, next to the river, and demonstrate the better performance of Isonet-Ltt dispensers compared to Isonet-L in DDR, by allowing a higher dose of pheromone in the dispenser at the beginning of the third generation.



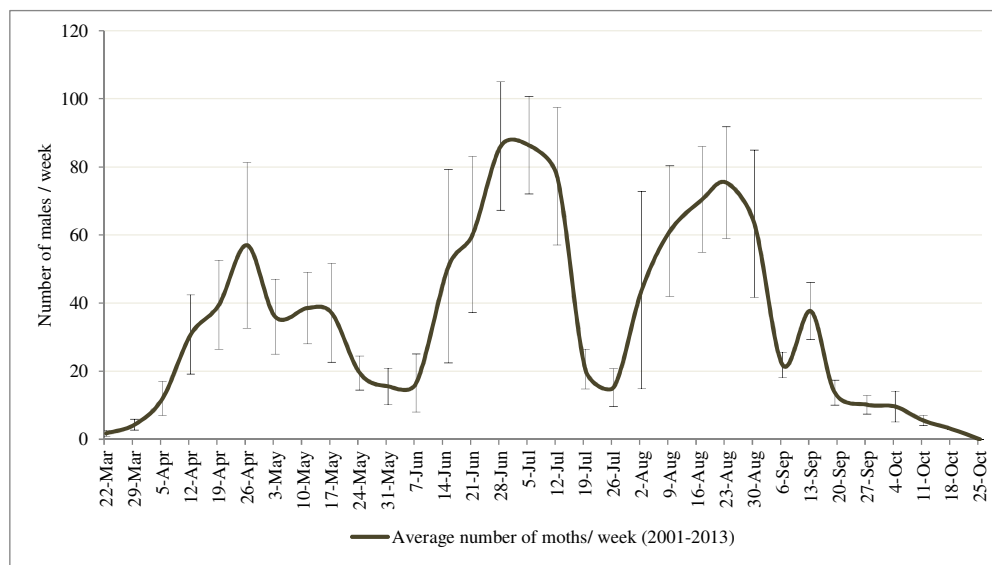
**Figure 6.2** Evolution of the % of active ingredient on dispensers and monthly mean temperatures in 2002 (A) and 2013 (B). Arrows indicate the usual period for the beginning of the 3<sup>rd</sup> generation in DDR.



### 6.3.2 Mating disruption efficacy

#### Trap catches

The average number of moths captured per week on the period 2001-2013 revealed a long flight period occurred between the middle of March till middle October (around 30 weeks). Usually *L. botrana* have three complete flight curves but, in some years, a fourth flight can happen on September (Figure 6.3).



**Figure 6.3** Flight curve of *L. botrana* based on the number (mean  $\pm$  standard error) of male moths captured per week on control plots, during the period 2001-2013.

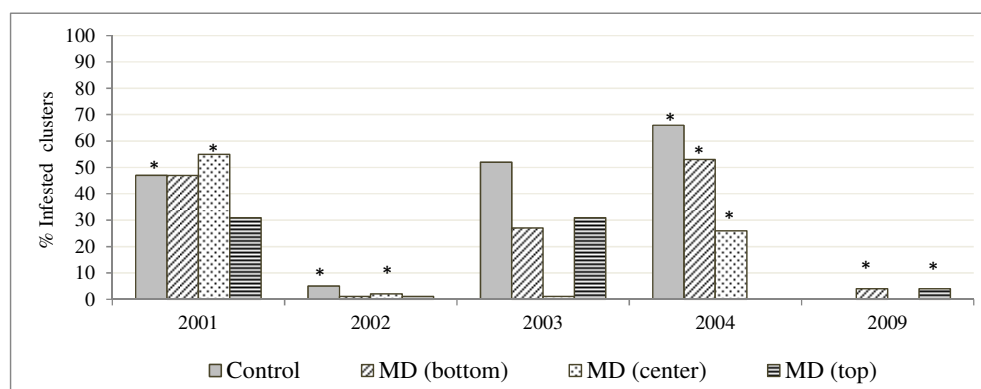
In comparison with the control plot, the captures in the traps located in the MD plots were reduced by 95-100% (Table 6.2), which suggests that treatment with pheromone almost prevented completely the male from locating the sources of synthetic sex pheromone.

**Table 6.2** Number of *L. botrana* on control plots and % of reduction of catches on MD plots

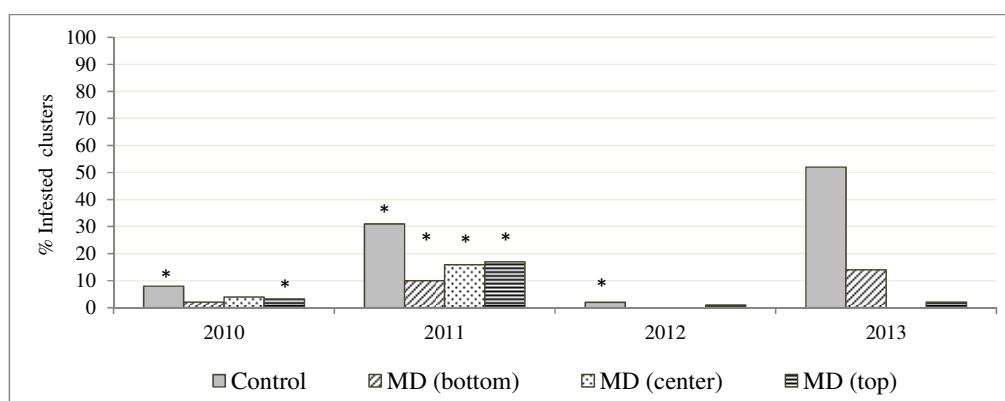
Year	2001	2002	2003	2004	2009	2010	2011	2012	2013
Nb <i>L. botrana</i> (control plots)	1134	719	457	1585	1291	636	951	1069	887
% reduction (MD plots)	99.8- 100.0	98.1- 100.0	99.3	99.7- 100.0	98.7- 99.9	95.3- 99.8	95.5- 99.6	98.5- 100.0	99.2- 100.0

## Infestation

The infestation recorded at the harvest on the control and MD plots was highly variable among years (Figures 6.4 and 6.5).



**Figure 6.4** Infested clusters (expressed as % of grapes with at least one berry infested) at different locations of S. Luiz at harvest on the period 2001-2009 (ISONET-L). \* Sprayed with insecticide in 2<sup>nd</sup> or 3<sup>rd</sup> generations



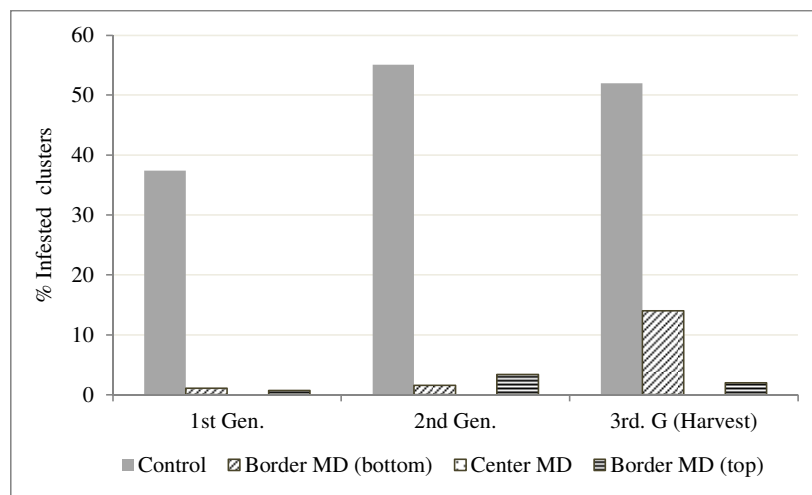
**Figure 6.5** Infested clusters (expressed as % of grapes with at least one berry infested) at different locations of S. Luiz at harvest on the period 2010-2013 (ISONET-Ltt). \* Sprayed with insecticide in 2<sup>nd</sup> or 3<sup>rd</sup> generations

On the period of 2001-2004, when MD was applied to smaller areas, the infestation was not reduced to a level where MD could be used alone (especially on the bottom and

on the top of the farm) and insecticides had to be applied, mostly on the beginning of the 3<sup>rd</sup> generation. According to Stockel and Chichignoud (1994), the MD area should have a minimal of 10.0 hectares, to avoid the migration of mated females from the neighboured vineyards into the MD treated vineyards. On the conditions of DDR, and in particular of S. Luiz farm, it looks that the minimal area under MD should be higher.

When the area under MD was enlarged to the whole farm, a significant reduction of infestation was observed. Also, in 2009 the second application of dispensers in July, in addition with the insecticide treatment, improved the efficacy of MD against the pest. On the other hand, the deployment of the dispensers only in July (in 2010 and 2011), could have contributed for the reduction of the pest infestation found in 2012 (Figure 6.4). However, the better results obtained since 2011 were very probably due to the use of the Isonet-Ltt dispensers, loaded with higher dose of pheromone.

On 2013, for the first year, the infestation on MD plots was significantly reduced to a level where MD could be used alone, especially on the center of the surface treated (Figure 6.6). These results agree with the reported in the literature (Anfora et al. 2005, Charmillot et al. 1995), suggesting, on this case, that the dispensers applied in previous years improved the efficacy of this technique, and a reduction of dispensers can be envisaged.



**Figure 6.6** Infestation of clusters in each generation of *L. botrana*, at S. Luiz in 2013

In both 2013 and 2004 an increase on infestation was found at harvest, at the bottom of the treated surface which, having in mind the results of the loss of pheromone in the dispensers at the two studied altitudes, suggest a poor performance of MD in S. Luiz lower altitudes.

As stated by Ioratti et al. (2005), little research has been conducted to evaluate how the basic technique of installing MD should be modified for specific agronomic conditions of vineyards installed in steep slopes and with rows planted in different cardinal directions in relation to wind direction. For example, in North Italy, particularly in Trentino and Tuscany conditions, an uneven dispenser distribution of 70% dosage in the 30% upper side of vineyard slope surface with the remaining 30% evenly applied in the rest of the surface has been advised, recommended (Ioratti et al. 2005). Considering our results, it seems that, in Douro conditions, this strategy cannot be applied.

In DDR, the wind pattern on the valley seems to remove the pheromone from both the bottom and the top of the hill. This can be explained by the occurrence of local winds known as “valley and mountain breezes” created by differences of temperatures between the land and the water surfaces along the all day. This effect usually results on the circulation of air from the top to the bottom of the hill, during the night and the morning, and on its circulation from the bottom to the top of the hill on the afternoon (Manso, J. pers. com.). It is possible that this trend in circulation of breezes can be modified along the season, and years, considering the differences of temperatures of land and water surfaces and should be analyzed more deeply.

The fact that the wind circulates up at the hill on the afternoon, and that *L. botrana* have a crepuscular behavior, could have contributed to the poor results obtained at lower altitudes.

## 6.4 References

Bagnoli B, Cosci F, Santini L, Lucchi A (2001) La lutte par confusion sexuelle contre *Lobesia botrana* en Toscane: les conditions locales affectent-elles l'efficacité de la méthode?. IOBC wprs Bulletin 24 (7): 85-86

- Carlos C, Alves F, Torres, L (2010) Constrains to the application of mating disruption against *Lobesia botrana* in Douro Wine Region. In CERVIM, Sicilia, 11-14 May 2010 (Cd-Rom)
- Caruso S, Franceschelli F, Iodice A, Ardizzoni M (2012) Release rate of Shin-Etsu products: comparison of GC and weighing Emilia-Romagna region (North Italy) official data with Shin-Etsu internal data (2010–2011) IOBC/WPRS Working Group "Pheromones and other semio-chemicals in integrated production", 1-5 October 2012, Bursa, Turkey
- Charmillot PJ, Pasquier D (2001) Technique de confusion, lutte classique et dynamique des populations des vers de la grappe. IOBC wprs Bulletin 24 (7): 87-90
- Fermaud M, Le Menn R (1989) Association of *Botrytis cinerea* with grape berry moth larvae, Phytopathology, 79: 651-656
- Ioriatti C, Bagnoli B, Lucchi A, Veronelli, V (2005) Vine moths control by mating disruption in Italy: results and future prospects. Redia, 87:117-128
- Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A (2011) Chemical Ecology and Management of *Lobesia botrana* (Lepidoptera: Tortricidae). Journal of Economic Entomology, 104 (4):1125-1137
- Kast WK (2001) Twelve years of practical experience using mating disruption against *Eupoecilia ambiguella* and *Lobesia botrana* in vineyards of the Wuerttemberg region, Germany. IOBC wprs Bulletin 24 (2): 71-73
- Moschos T, Souliotis C, Broumas T & Kapothanassi V (2004) Control of the European Grapevine Moth *Lobesia botrana* in Greece by the mating disruption technique: a three-year survey. Phytoparasitica 32 (1): 83-96
- Nucifora A, Buonocore E, Colombo A, Boncoraglio P, Campo G, Nucifora M T (1996) Il metodo della confusione sessuale nella lotta contro *Lobesia botrana* (Den. et Schiff.) (Lepidoptera, Tortricidae) su uva "Italia" in vigneti della Sicilia orientale (I contributo). Inform. Fitop. 1: 56-61
- Varner M, Lucin R, Mattedi L, Forno F (2001) Experience with mating disruption technique to control grape berry moth, *Lobesia botrana*, in Trentino. IOBC wprs Bulletin 24 (2): 81-88

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## **Chapter 7**

### **General discussion and conclusions**







## 7.1 General discussion and conclusions

In this last chapter, it was intended to give an overview of the main results obtained in each chapter, as well as to point out future perspectives of applied research on the areas developed.

The proposed PhD thesis aims to contribute to the development of an environmentally sustainable pest management strategy to control main arthropod pests in DDR vineyards, with emphasis on the grape berry moth, *L. botrana*, to provide the wine production sector with useful tools for increasing its sustainability at both economic and environmental levels.

It is known that occurrence of non-crop habitats (NCH) (e.g. woodland, scrubland, weedy or shrubby margins) has several advantages from the biodiversity point of view, in particular in conservation biological control, by providing important resources to natural enemies of crop pests, such as refugia, overwintering habitat, nectar, pollen and alternate hosts or prey, that support populations of these beneficial near crop fields, and can lead to increased levels of biological control. Even though a significant part of the DDR is occupied by these non-crop habitats, there was a lack of knowledge about their impact on functional biodiversity, including conservation biological of pests, in the vineyard. To achieve such goal, a study was done on the impact of non-crop habitats adjacent to vineyards, as well as ground cover vegetation of terraced vineyards, on overall biodiversity of arthropods, including natural enemies (predators and parasitoids) and main crop pests. Moreover, a survey of *L. botrana* parasitoids was carried out over a nine-year period, and their relative importance was evaluated, according to the generation, and landscape / vineyard management determinants.

Because prediction of flight activity of *L. botrana* during the growing season is critical to improve Integrated Pest Management tactics against the pest, through better timing of sampling or control operations, this flight activity was studied by analyzing data on male catches in sex pheromone traps recorded over a 20-year period, and degree-day models for predicting flights occurrence were developed.

Lastly, and as mating disruption is an environmentally friendly method very promising to control *L. botrana*, its effectiveness in DDR was investigated using different

wired pheromone dispensers and main constraints of DDR conditions for its successful application were studied.

The results obtained on chapter 2 and 3 points to the high value of the non-crop vegetation found in the vineyard agroecosystem and showed the different relations established with several guilds of arthropods. A positive impact was found of NCH and vegetation on main predators (Araneae and Coccinellidae), parasitoids, and omnivores (Formicidae), although the results suggest a differentiated response from functional groups to habitat and vegetation, which should be considered in conservation management. Two Iberian endemisms of ants, *Aphaenogaster iberica* and *Cataglyphis hispanica* and three Iberian endemisms of spiders, *Eratigena bucculenta*, *Eratigena feminea* and *Oecobius machadoi*, were identified, which highlight the high conservation value of Douro vineyard ecosystem. As a whole, the results showed the importance of maintaining NCH on the neighborhood of vineyards, due to the positive impact found on arthropod biodiversity of this agroecosystem. Despite of vineyards slopes are a cropped habitat, the lower management performed on such areas can result in a high diversity of arthropods inside vineyards.

In chapter 4, sixteen different taxa were recorded, the majority belonging to Hymenoptera namely, *Elachertus* sp., *Elasmus* cf *bistrigatus*, *Elasmus* cf *steffani*, *Elasmus* sp., *Baryscapus* sp., an Eulophinae not identified (Eulophidae), *Campoplex capitator*, *Itoplectis maculator*, a Cryptinae not identified (Ichneumonidae), *Brachymeria tibialis*, *Hockeria* sp. (Chalcididae), *Dibrachys cavus* (Pteromalidae), *Ascogaster quadridentata* (Braconidae), *Goniozus gallicola* and *Goniozus claripennis* (Bethyidae). From Diptera, *Eurystaea scutellaris* (Tachinidae) was reported. It was found that, the complex of parasitoids of *L. botrana* and the potential role played by each species in the natural control of the pest showed a great variability in space and time, being rates of parasitism substantially higher in the 1<sup>st</sup> generation of *L. botrana*, compared to the other two generations. The most promising candidate to enhance conservation biological control of *L. botrana* in DDR vineyards is *Elachertus* sp. (Eulophidae) because, on one hand this larval ectoparasitoid has a wide geographical distribution in the studied region and on the other hand, in most favorable conditions, the percentage of parasitism caused can reach 60%, mainly in the 1<sup>st</sup> generation of the pest. *C. capitator* and *B. tibialis* appears to have a complementary role, on the parasitism of the first and second generation of the

pest respectively, depending the parasitism rates caused, on the location of vineyards. These results suggest that, for enhancing conservation biological control of *L. botrana* in DDR, a high abundance and diversity of vegetation (ground covers) inside or at the edge of vineyards plots should be enhanced and the selection of pesticide with minimal risks to parasitoids activity should be performed.

In chapter 5, average dates (JD) and degree-days ( $^{\circ}\text{DD}$ ) corresponding to main flight events (beginning and peak of catches in each flight) of *L. botrana* were obtained for the first time in DDR, using data collected over a 20-year's period. Such information is useful for DDR growers, because gives a first approach of *L. botrana* flight behavior in this region and should aid in timing damage assessments. Nonlinear models based on Boltzmann regression equations were developed, using the percentage of accumulated male catches and  $^{\circ}\text{DD}$  accumulation and considering two starting points for this accumulation, a biological event (the first male catch) and a calendar date (January 1<sup>st</sup>), both using as lower and upper thresholds 7.3°C and 33°C, respectively. Both models predicted, with reasonable accuracy, the flight phenology of *L. botrana*. Even though the use of a biofix seems to improve model's accuracy, the use of a fixed calendar date (January 1<sup>st</sup>) should be preferred, from the practical point of view and considering large scale application on an IPM strategy. The results obtained could be useful in timing *L. botrana* control measures, especially biorational pesticides application that require accurate information on insect phenology to be effective.

Finally, in chapter 6, it is shown that the application of the tested dispenser over the years allowed the identification of some major constraints to the use of the technique in DDR, namely: the high biotic potential of the moth; the climate conditions, particularly the high summer temperatures; the effect of the winds on the distribution of the pheromone on the hill; the impact of slope; the fragmentation of many vineyards and the size of the treated area. It was also found that in general, MD was most effective in years of low pest population density, when applied in large areas, with more points of release per hectare, and after consecutive seasons.

## 7.2 Future perspectives

With the development of the present thesis, several questions that should be analyzed in future investigation are reported and recommendations to improve the results obtained are presented.

In chapter 2 and 3, the impact of habitats and native vegetation on functional biodiversity of arthropods was studied. Further work is needed to identify the main relations existing between species of native plants mostly found in DDR vineyard ecosystem and beneficial arthropods, to enhance the presence of plants in which beneficials are found in higher abundance in vineyards, to assist in conservation biological control of pests.

On chapter 2 and 4, the possible impact of riparian galleries on the biodiversity of arthropods in vineyards located nearby, particularly on parasitoids, is hypothesized, as reported in literature. Considering the climate conditions of DDR during the summer period, which impact on the communities of native plant species, as well as on the availability of floral resources and consequently, on the abundance of parasitoids, and since several vineyards in DDR are bordered by riparian galleries, investigation should be conducted to evaluate the impact of such ecological infrastructure on the presence of several groups of beneficial arthropods.

Considering that, as shown in chapter 3, a higher presence of some groups of arthropods (e.g. collembolans) were found inside the vineyard, although several authors have reported a negative impact of management practices on their abundance, it is suggested that, in future studies, further analysis should be conducted to understand the impact of soil characteristics, as well as of management practices performed in vineyards on the abundance of the reported arthropods.

The phenological models developed in chapter 5 should be tested in the future by wine grower's associations, to evaluate their performance and accuracy on predicting flying activity of *L. botrana*, and assist in IPM decision making thus allowing to reduce pesticide application, as well as pest damage.

Finally, in chapter 6, several recommendations are given to improve the effectiveness of the use of mating disruption against *L. botrana*, in DDR conditions. Winegrowers of this region are encouraged to apply this environmental safe control

method in larger areas, to increase area wide effect, as it was proven to be the most effective way to maximize its effectiveness in steep slope viticulture regions.