UNIVERSITÀ DEGLI STUDI DI UDINE

Research Doctorate in Agricultural Science and Biotechnology
Cycle XXVIII
Director of the School: prof. Mauro Spanghero

PhD THESIS

STUDIES ON ENVIRONMENTAL-FRIENDLY STRATEGIES FOR THE MANAGEMENT OF *LOBESIA BOTRANA* (LEPIDOPTERA: TORTRICIDAE) AND OTHER GRAPEVINE PESTS

PhD STUDENT
Dr. Seyedehfatemeh Kiaeianmoosavi

SUPERVISOR
Prof. Pietro Zandigiacomo

CO-SUPERVISOR
Dr. Francesco Pavan

ACADEMIC YEAR 2015/2016

INDEX

ABSTRACT **1**

CHAPTER I. General introduction **5**

1. Most important grapevine arthropod pests 6

2. Integrated arthropod pest management in vineyards 6

2.1. Biological control 7

2.1.1. Permanent release in vineyards 7

2.1.2. Periodical release 8

2.1.3. Conservation in vineyards 8

2.2. Semiochemical-based control 8

2.3. Resistant plant 9

2.4. Cultural control 9

3. General aim of PhD thesis 10

CHAPTER II. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould

Introduction **13**

1. *Lobesia botrana* biology and damage 13

2. Influence of agronomic practices on grey mould and *Lobesia botrana* 14

3. Aim of this study 15

Materials and methods **16**

1. Influence of bunch-zone leaf removal on *L. botrana* and grey mould 16

2. Spatial distribution of *L. botrana* in relation to sunlight exposure 19

Results **21**
1. Influence of bunch-zone leaf removal *on L. botrana* and grey mould 21
2. Spatial distribution of *L. botrana* in relation to sunlight exposure 23
Discussion and conclusion **28**
1. Influence of bunch-zone leaf removal on *L. botrana* infestation and grey mould 28
2. Spatial distribution of *L. botrana* in relation to sunlight exposure 29
3. Conclusion 30

CHAPTER III. Effects of high temperatures on *Lobesia botrana* egg/larval mortality ……33
Introduction **34**
1. European grapevine moth, *Lobesia botrana* 34
1.1. Description 34
1.2. Biology and damage 36
2. Effects of temperature on *Lobesia botrana* and other tortricids 42
2.1. *Lobesia botrana* 43
2.2. Other carpophagous tortricids 44
3. Influence of sunlight exposure on berry temperature 45
4. Aim of this study 47
Materials and methods **48**
1. Experiments in climatic chamber 48
1.1. *Lobesia botrana* rearing 48
1.2. *Lobesia botrana* eggs exposed to high temperatures 49
1.2.1. Experiment type 1: Exposure of eggs to different constant temperatures 49
1.2.2. Experiment type 2: Exposure of eggs to different temperature cycles 50
1.3. Newly-hatched larvae of *Lobesia botrana* exposed to different temperature cycles 51
1.4. Statistical analysis 52
2. Experiments in the field 53
2.1. Temperatures recorded on berries during *Lobesia botrana* third-flight 53

2.2. *Lobesia botrana* eggs laid on berries exposed or not exposed to sunlight .. 54
Results 56
1. Experiments in climatic chamber 56
1.1. *Lobesia botrana* eggs exposed to high temperatures 56
1.1.1. Experiment type 1: Exposure of eggs to different constant temperatures 56
1.1.2. Experiment type 2: Exposure of different egg stages to different temperature cycles 63
1.2. Newly-hatched larvae of *Lobesia botrana* exposed to different temperature cycles 67
2. Experiments in the field 69
2.1. Temperatures recorded on berries during *Lobesia botrana* third-flight 69
2.1.1. Vineyard A 69
2.1.2. Vineyard B 70
2.2. *Lobesia botrana* eggs laid on berries exposed or not exposed to sunlight .. 73
2.2.1. Number of eggs per cage 73
2.2.2. Percentage of egg hatching 75
2.2.3. Percentage of larval settlement 77
2.2.4. Temperatures recorded and egg/larval mortality 78
2.2.5. Simulated larval infestation in relation to bunch-zone leaf removal79
Discussion and conclusion 81
1. *Lobesia botrana* egg mortality in the laboratory 82
2. *Lobesia botrana* newly-hatched larvae mortality in the laboratory 83
3. Influence of sunlight exposure and row orientation on berry temperature in the field 83
4. *Lobesia botrana* egg distribution in relation to sunlight exposure in the field 84
5. *Lobesia botrana* egg-hatching and larval survival in the field in relation to sunlight exposure 85
6. Conclusion 85

CHAPTER IV. Relation between *Lobesia botrana* and *Botrytis cinerea* …… 87
Introduction..... 88
1. Relation between *Lobesia botrana* and *Botrytis cinerea* damage 88
2. Effects of botrytis on *Lobesia botrana* attractiveness and female's egg laying..... 89
3. Effects of botrytis on *Lobesia botrana* development parameters..... 89
4. Aim of this study 90
Materials and methods..... 92
1. Experiments in the laboratory on *Lobesia botrana* larvae..... 92
1.1. Experiment I. Effect of three basic diets on *Lobesia botrana* larval fitness 93
1.1.1. Diets in comparison 93
1.1.2. Experiment design and sampling..... 94
1.2. Experiment II. Effects of botrytis addition to basic diets on *Lobesia botrana* larval fitness..... 94
1.2.1. Fungi preparation..... 94
1.2.2. Diets in comparison 95
1.2.3. Experiment design and sampling..... 95
1.3. Statistical analysis..... 95
2. Effect of botrytis on *Lobesia botrana* egg laying in the field..... 96
2.1. Experiment design 96
2.2. Sampling and statistical analysis 96
Results..... 97
1. Experiment I. Effect of three basic diets on *Lobesia botrana* larval fitness..... 97
1.1. *Lobesia botrana* larval mortality 97
1.2. Number of *Lobesia botrana* larval-instars..... 97
1.3. Duration of *Lobesia botrana* larval-development..... 97
1.4. Mandible length of *Lobesia botrana* larvae..... 98
1.5. *Lobesia botrana* pupal weight 98
2. Experiment II. Effects of botrytis addition to basic diets on *Lobesia botrana* larval fitness..... 99
2.1. *Lobesia botrana* larval mortality 99
2.2. Number of *Lobesia botrana* larval-instars..... 99
2.3. Duration of *Lobesia botrana* larval-development..... 100

2.4. Mandible length of *Lobesia botrana* larvae 100
2.5. *Lobesia botrana* pupal weight 101
3. Effect of botrytis on *Lobesia botrana* egg-laying in the field 109
Discussion and conclusion 111
1. Comparison between artificial diets and berry diet on *Lobesia botrana* performance 111
2. Effects of botrytis addition to the artificial diets on *Lobesia botrana* performance. 112
3. Influence of botrytis on *Lobesia botrana* egg-laying preference 113
4. Conclusion 114

CHAPTER V. Influence of the vineyard inter-row management on grapevine pests and natural enemies……..…… 117
Introduction..... 118
1. Habitat management in vineyard 118
2. Effect of ground cover on grapevine pests control 119
3. Most common grapevine arthropod pests and their natural enemies..... 120
3.1. *Lobesia botrana* 120
3.2. *Empoasca vitis* 121
3.3. Zygina rhamni 123
3.4. *Drepanothrips reuteri* 124
3.5. *Panonychus ulmi* 125
4. Aim of this study 126
Materials and methods 127
1. Study vineyard 127
2. Treatments in comparison..... 127
3. Weather data and *Lobesia botrana* phenology 129
4. Data collection 129
4.1. Bunch sampling of *Lobesia botrana* and bunch rots 129
4.2. Leaf sampling of leafhopper in the field..... 129
4.3. Checking field-collected leaves in the laboratory..... 130
4.3.1. Leafhopper eggs and *Anagrus* spp. parasitoids..... 130
4.3.2. Phytoseiid mites and Thysanoptera 130

4.4. Beating tray sampling of predators 130
4.5. Yellow sticky traps for sap-sucking pests and natural enemies 131
5. Pruning weight and NDVI index 131
6. Statistical analysis 132
Results 133
1. Weather data 133
2. European grapevine moth, *Lobesia botrana* 134
2.1. Influence of inter-row management 134
2.2. Influence of clone 135
3. Grapevine leafhoppers and *Anagrus* spp. parasitoids 135
3.1. Influence of inter-row management 135
3.1.1. *Empoasca vitis* 135
3.1.2. *Zygina rhamni* 136
3.1.3. Leafhopper eggs and Anagrus spp. parasitoids 136
3.2. Influence of clone 137
3.2.1. *Empoasca vitis* 137
3.2.2. *Zygina rhamni* 137
3.2.3. *Anagrus* spp. parasitoids 137
4. *Drepanothrips reuteri* and *Aeolothrips* sp. predators 138
4.1. Influence of inter-row management 138
4.1.1. *Drepanothrips reuteri* 138
4.1.2. *Aeolothrips* sp. predators 138
4.2. Influence of clone 139
4.2.1. *Drepanothrips reuteri* 139
4.2.2. *Aeolothrips* sp. predators 139
5. Phytoseiid mites 139
5.1. Influence of inter-row management 139
5.2. Influence of clone 140
6. Generalist predators and parasitoids 140
6.1. Spiders 140
6.1.1. Influence of inter-row management 140
6.1.2. Influence of clone 141

6.2. Generalist predatory insects 141
6.2.1. Influence of inter-row management 141
6.2.2. Influence of clone 142
6.3. Parasitoids 142
6.3.1. Influence of inter-row management 142
6.3.2. Influence of clone 143
7. Bunch rots 143
7.1. Influence of inter-row management 143
7.2. Influence of clone 143
8. Plant vigour 144
8.1. Influence of inter-row management 144
8.2. Influence of clone 144
Discussion and conclusion 162
1. Grapevine leafhoppers and *Anagrus* spp. parasitoids 162
2. *Drepanothrips reuteri* and *Aeolothrips* sp. predators 163
3. Phytoseiid mites 164
4. Spiders 165
5. Generalist predatory insects and parasitoids 165
6. *Lobesia botrana* 166
7. Bunch rots 167
8. Plant vigour 167
9. Conclusion 168

AKNOWLEDGEMENTS 197

Scheda informativa 199

ABSTRACT

Chapter I. In the context of IPM strategies, this PhD thesis aims to investigate the possibility to control grapevine pests through environmental-friendly strategies, such as cultural practices and conservation biological control. Moreover, since cultural practices can also influence the spreading of bunch rots due to *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), the relation between the moth and *Botrytis cinerea* Pers. Fr. was also examined.

Chapter II. Bunch-zone leaf removal (BLR) in vineyards was reported as an effective cultural practice to prevent botrytis or *L. botrana* attacks. Field trials were carried out to study the effects of BLR on the two carpophagous generations of the moth and grey mould, simultaneously. BLR applied at the pea-sized berry stage or some days later, during the moths' second-flight, reduced infestation of both carpophagous generations of *L. botrana* and botrytis infection at harvest time. Investigating the effects of BLR on spatial distribution of *L. botrana* larval nests within canopy and eggs on bunches, in relation to sunlight exposure, a lower larval infestation on bunches exposed to sunlight by BLR and more eggs laid on berries exposed to sun than on those facing towards the interior part of the canopy were observed. The female non-avoidance of laying eggs on the sun-exposed side of bunches supports the hypothesis that BLR affects *L. botrana* larval infestation by increasing egg/larval mortality.

Chapter III. Based on high temperatures of berries recorded in vineyards, eggs and larvae of *L. botrana* were exposed to high temperatures in the laboratory. In the field trials effect of different grapevine row-orientation on berry temperature was studied as well as the influence of berry exposure to sunlight on egg distribution, mortality and larval settlement. In the laboratory egg and larval mortality occurred from 40 °C and 37 °C respectively. The berries exposed to sunlight can exceed the air temperature of more than 10 °C, so air temperature higher than 30 °C can determine the berry temperatures the same as those associated with egg/larval mortality in the laboratory. Both the field and laboratory data showed a higher larval-susceptibility to high temperatures comparing to eggs.

Therefore, the cultural practice of BLR reduces *L. botrana* infestation because exposure to sunlight determines high berry temperatures and subsequently egg/larval mortality.

Chapter IV. Based on the frequent coincidence of *L. botrana* and botrytis attacks in vineyards and on the fact that fungus in some experiments favoured female egg-laying, and improved larval performance and female fecundity, some research suggest a mutualistic relationship between the moth and the fungus, and suppose a positive role of fungal sterols in *L. botrana* performance. On the other hand the data reported in literatures about the effect of botrytis on *L. botrana* are not always in agreement with each other. Since some of previous research showed a positive effect of botrytis on *L. botrana* performance adding it to an artificial diet (diet B) with a lower sterol richness than berries, the first step of this research was to compare the performance of larvae fed on three basic diets [i.e., berries (250 mg/100 g sterol content), artificial diets A (160 mg/100 g sterol content) and B (80 mg/100 g sterol content)]. The results showed higher mortality, longer development duration and lower mandible size for larvae fed on diet B in comparison with those on berry diet and diet A. For the second step, dried powder of botrytis was prepared in the laboratory and added to the two artificial diets. Thus, five treatments, i.e. diets A and B with or without botrytis addition, and berries were compared. The addition of botrytis was effective in improving larval performance only for diet B, that is poorer in sterol than both diet A and berry diet. To study the oviposition preference of *L. botrana* female toward botrytis-infected bunches in the field, mated females were released in cages with one healthy and one infected bunch and the number of laid eggs was counted in the laboratory. No preference of females was observed toward infected bunches and infected berries in the field. These data showed that previous research did not definitively prove that botrytis promotes larval performance and females are not stimulated to lay more eggs on botrytis-infected bunches in the field.

Chapter V. In the context of conservation biological control, the influence of two different inter-row management, i.e. tillage (bare soil between rows by cultivation) and mowing (presence of green cover), and two clones of grapevine cv Sauvignon (R3 and R297) on the population of grapevine arthropod pests and their natural enemies were studied in an organic vineyard. Samplings were carried out over two years in the vineyard through grapevine leaf and bunch observations in the field and in the laboratory, beating tray and yellow sticky traps. Most important sap-sucker grapevine pests (i.e., leafhoppers

and thrips) and natural enemies, both specialist and generalist, such as Araneae (spiders), Coleoptera Coccinellidae, Dermaptera, Heteroptera Nabidae, Thysanoptera Aeolothripidae and Acari Phytoseiidae were monitored. Pruning weight and Normalized Difference Vegetation Index were also considered to estimate grapevine vigour. The mowing of inter-rows (vs. tillage) reduced the leafhopper populations (i.e., *Empoasca vitis* and *Zygina rhamni*), as a consequence of the lower plant vigour, and the vine thrips *Drepanothrips reuteri* populations, due to the greater occurrence of their *Aeolothrips* sp. predators. The green cover did not affect *L. botrana* infestation, but the amount of rotten berries contiguous to larval nests may have been reduced since it decreased the botrytis incidence. A role of spiders in pest control could be also supposed, because their population density was favoured by the green covered inter-rows.

Chapter I.

General introduction

1. Most important grapevine arthropod pests

European vineyards are suffered by numerous arthropod pests: some carpophagous, with direct damage, including *Lobesia botrana* (Denis & Schiffermüller) and *Eupoecilia ambiguella* (Hübner) (Lepidoptera: Tortricidae) and many sap feeders, with mostly indirect damage, that belong to leafhoppers, such as *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari and *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), thrips, such as *Drepanothrips reuteri* (Uzel) (Thysanoptera: Thripidae), scales, such as *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and *Parthenolecanium corni* (Bouché) (Hemiptera: Coccidae), and mites, such as the tetranychids *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans) (Acari: Tetranychidae), and the eriophyids *Calepitrimerus vitis* Nalepa and *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae).

Pesticides are usually applied to control these pests, but they become increasingly problematic for technical reasons (many of the most effective insecticides have been recently revoked and there is always a risk that insecticide resistance may evolve), toxicity to humans (the presence of pesticide residues are less and less accepted by the public) and to beneficial arthropods, and environmental pollution due to drift effect.

Nowadays the use of more selective and less-toxic insecticides are being gradually growing in the most agricultural areas of the word and alternative environmental-friendly strategies is developing.

2. Integrated arthropod pest management in vineyards

The reported undesirable effects of pesticides on non-target species, including humans, such as acute and chronic health effects (Hayes and Vaughn, 1977), domestic animal poisonings (Caldwell *et al.*, 1977), effects on wild birds and mammals, fish, bees, beneficial organisms, and small organisms in the soil (Van Steenwyk *et al.*, 1975; Brown, 1978; Bairlein, 1990; Pimentel *et al.*, 1993; Johnson *et al.*, 2010) led a greater focus on Integrated Pest Management (IPM) programs in order to reduce pesticide use.

There are several IPM definitions. However, the use of economic thresholds, multiple management tactics and ecological information are common elements defining IPM.

As alternatives to chemical controls, biological control, semiochemical-based control, resistant plants and cultural control can be adopted in vineyards.

2.1. Biological control

The biological control is a method of controlling pests by using other living organisms. Three types of biological pest control strategies can be considered: permanent release (sometimes called classical biological control), periodical release and conservation. In the first case a single release of natural enemies is made and then these remain naturally without the need for additional releases. In the second case there are periodical releases of natural enemies (one or more times per year). The conservation of natural enemies is probably the most important and readily available biological control practice to growers. Natural enemies are present in all agro-ecosystems, even if expansion of monoculture has reduced their abundance. Increasing the population of present natural enemies by techniques that conserve and promote their presence would lead to decrease pest population in agro-ecosystems. At this purpose, diversification of the vegetation in vineyard habitat, to supply natural enemies with appropriate refuges, microclimates, prey or alternative hosts, and the use of selective pest control products can be adopted.

Application of these three strategies are reported for vineyards.

2.1.1. Permanent release in vineyards

- Release of phytoseiids resistant to pesticides (Duso *et al*., 2006)

Transferring of phytoseiids from vineyards with pesticides resistant populations to others was successfully adopted. Posenato (1994) reported strains of the predator *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) resistant to dithiocarbamates and specific organophosphate (e.g., fenitrotion, methyl-parathion) to control the tetranychid mites.

- Release of *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae)

Releasing of the exotic entomophagous wasp *N. typhlocybae* showed successful establishment and control of the planthopper *Metcalfa pruinosa* Say (Hemiptera: Flatidae)

that had been accidentally introduced from North America (Girolami and Mazzon, 1999; Frilli *et al.*, 2001; Di Bucchianico *et al*., 2004; Angeli *et al*., 2005).

2.1.2. Periodical release

Usage of the Gram-positive bacterium *Bacillus thuringiensis* (Berliner) is a good alternative to chemical control of *L. botrana*. In terms of selectivity, *B. thuringiensis* as a stomach poison has undoubtedly a high ecological value, but there is a limitation due to its short persistence (leaching and photolability) (Pusztai *et al*., 1991; Marchesini *et al*., 2006; Trona *et al*., 2007; Dongiovanni *et al*., 2008).

2.1.3. Conservation in vineyards

Effective habitat management strategies have already been proposed in North America for biological control of *Erythroneura* spp. leafhoppers by *Anagrus* spp. in vineyards (Doutt and Nakata, 1973; Kido *et al*., 1984; Williams, 1984; Williams and Martinson, 2000) and in Europe *A. atomus* was suggested to control *E. vitis* (Cerutti *et al*., 1991; Van Helden and Decante, 2001; Van Helden *et al*., 2003; Boller *et al*., 2004; Ponti *et al*., 2005; Böll *et al*., 2006; Viggiani *et al*., 2006).

2.2. Semiochemical-based control

Semiochemicals are chemicals that convey a signal from one organism to another so as to modify the behaviour of the recipient (also known as behaviour-modifying chemicals). Lepidopteran sex pheromones are one of the most successfully used semiochemicals in pest control. In many commercially developed systems lepidopteran sex pheromones are used, either in monitoring systems or in slow-release formulations to cause mating disruption. It is control method characterized by high selectivity and low environmental impact.

Mating disruption was successfully applied in vineyards against *L. botrana* (Ioriatti *et al*., 2004; Bigot *et al*., 2008; Ioriatti *et al.*, 2012). Five hundred dispensers per hectare (the number of dispensers may vary depending on manufacturer) must be deployed in the vineyards before the onset of the first seasonal *L. botrana* flight. Mating disruption, when applied in optimal conditions, ensures efficacy against *L. botrana* equal to or even higher than insecticide. However, the population density of moth at the time of pheromone

dispenser application must be low. On the other hand, one reason for the low acceptance of this technology by the grape growers could be the high price of the pheromone dispensers.

2.3. Resistant plant

Plant resistance to pest can occur naturally or be produced by plant breeding or techniques of genetic engineering (defence mechanisms of plants are re-created in resistant plant).

Vitis vinifera cultivars are typically grafted onto a grape-phylloxera resistant rootstocks belonging to American grapevine species or their hybrids.

The choice of cultivars less susceptible or tolerant to some pests is the other important issue to consider in vineyards. A different susceptibility of grapevine cultivars was observed for *L. botrana* (Fermaud, 1998; Pavan *et al.,* 2009), *E. vitis* (Pavan and Picotti, 2009; Fornasiero *et al.*, 2016) and *P. ulmi* (Dellei and Szendrey, 1991).

Up to now plant breeding and transgenic plants are not considered in the context of grapevine pest's control.

2.4. Cultural control

Besides biological and other above-mentioned techniques, cultural controls can provide alternative safe strategies to pest management. Cultural practices to control arthropod pests both directly or through host plants modification are promising tools to maintain the pest population under economic injury levels.

Some cultural practices (e.g., irrigation, fertilization, weed control, training system and pruning) can make grapevine less attractive or less favourable to the development of certain pests.

Low nitrogen fertilization, bunch-zone leaf removal and application of growth regulators, that reduce bunch compactness, can decrease *L. botrana* infestations and indirect damage due to the development of bunch rots (Villani *et al*., 1997; Vartholomaiou *et al.*, 2008). There are some reports on better survival of *L. botrana* larvae on the horizontal training system than on the vertical ones (Barbieri *et al.*, 1996), and better on latter than on the head training system (Delrio *et al.*, 1987).

Plants that are not in water-stress are less likely to show *E. vitis* symptoms (Pavan *et al.*, 2000). However, moderate water-stress, applied during egg laying, reduces *E. vitis* infestation (Fornasiero *et al.*, 2012). Since plant vigour is associated with higher population levels of *E. vitis* (Pavan and Pavanetto, 1989; Decante and van Helden, 2001; Decante *et al*., 2009; Pavan and Picotti, 2009), all cultural practices that reduce plant vigour would favour leafhopper control.

Mori *et al.* (2016) reported that nettle control in areas surrounding vineyards reduces vineyard colonization by *Hyalesthes obsoletus* Signoret (Hemiptera: Cixiidae), the vector of phytoplasma that is the causal agent of black wood.

3. General aim of PhD thesis

In the context of IPM strategies, this PhD thesis aims to investigate the possibility to control grapevine pests through environmental-friendly strategies, such as cultural practices and conservation biological control. Moreover, since cultural practices can also influence the spreading of bunch rots due to *L. botrana*, the relation between the moth and *Botrytis cinerea* Pers. Fr. was also studied.

Chapter II.

Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould(*)

(*) This chapter is being printed in Bulletin of Insectology. In this study, I collected the data on "Spatial distribution of *L. botrana* in relation to sunlight exposure". The work was made in cooperation with Dr. Elena Cargnus, Dr. Francesco Pavan, Dr. Giovanni Bigot and Prof. Pietro Zandigiacomo.

Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould

Francesco PAVAN[1], Elena CARGNUS[1], Seyedehfatemeh KIAEIANMOOSAVI[1], Giovanni BIGOT[2], Federico TACOLI[1], Pietro ZANDIGIACOMO[1]

[1] Department of Agricultural, Food, Environmental and Animal Sciences (DI4A), University of Udine, Italy

[2] Studio associato Bigot & Bigot, Cormons (Gorizia), Italy

Abstract

Bunch-zone leaf removal in vineyards has been reported as an effective cultural practice to prevent damage by *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae) or *Botrytis cinerea* Pers. Fr. *L. botrana* larval activity is well known as a factor favouring the spread of *B. cinerea*. In 2007_2014, trials were carried out in a number of vineyards in north-eastern Italy to study the effects of leaf removal on the two carpophagous generations of the moth and on grey mould, simultaneously, and on the spatial distribution in relation to sunlight exposure of *L. botrana* larval nests within the grapevine canopy and eggs on bunches. Bunch-zone leaf removal applied at the pea-sized berry stage or a little later, during the *L. botrana* second-flight, reduced the infestation of both carpophagous generations by about 50% as well as *B. cinerea* infection at harvest time. This latter effect was partially due to moth control. In one of the two vineyards where distribution of larval infestation was studied, bunches not covered by leaves were significantly less infested than those covered. Females confined on bunches facing south and exposed to sunlight preferred to lay eggs on the sun-exposed side of the bunch. The lower *L. botrana* infestation observed both on plots subjected to bunch-zone leaf removal and on bunches facing south could be due to a higher egg/larval mortality caused by the very high temperatures reported for berries exposed to sunlight.

Keywords: European grapevine moth, Botrytis, Green pruning, Cultural control, Egg-laying preference, Sunlight exposure

INTRODUCTION

1. *Lobesia botrana* biology and damage

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is one of the most destructive grapevine pests with economic importance in the Palearctic region and, recently, it has expanded its geographical distribution to the Americas (Ioriatti *et al*., 2012). It is a polivoltine species that performs, depending on the geographical areas and microclimates, from two to four generations per year (Martin-Vertedor *et al*., 2010; Caffarra *et al*., 2012; Pavan *et al*., 2013; Gilioli *et al*., 2016).

The larvae of the second and third generations are carpophagous and can cause yield losses (Roehrich, 1978; Pavan and Sbrissa, 1994; Pavan *et al*., 1998; Moschos, 2006) and favour the spread of bunch rots such as grey mould *Botrytis cinerea* Pers. Fr. (Fermaud and Giboulot, 1992; Roehrich, 1978; Pavan *et al*., 2014a), black aspergilli rot *Aspergillus* Section *Nigri* (Cozzi *et al*., 2009) and sour rot (Bisiach *et al*., 1986).

In areas where *L. botrana* completes three generations per year, some studies reported that both carpophagous generations can favour the spread of grey mould at harvest time (Fermaud and Giboulot, 1992), whereas others showed a greater role of the third generation (Roehrich 1978; Pavan *et al*., 2014a). These latter authors explained their results with the fact that at harvest time the berries bored by the second-generation larvae are mostly shrivelled or fallen, whereas those bored by third-generation larvae are often rotten and still turgid (Pavan and Sbrissa, 1994). Moreover, because the amount of rotten berries non-contiguous to larval nests is not correlated with larval infestation (Pavan *et al*., 2014a),

it can be assumed that the development of grey mould in these berries is independent of larval activity.

2. Influence of agronomic practices on grey mould and *Lobesia botrana*

The spread of grey mould at harvest time is also favoured by plant vigour (Mundy, 2008; Valdés-Gómez *et al*., 2008), leaf density around bunches (Fermaud et *al*., 2001), berry susceptibility and bunch compactness (Vail and Marois, 1991). Some of these factors can be modified by cultural practices (R'Houma *et al*., 1998; Muckensturm and Decoin, 2000; Valdés-Gómez *et al*., 2008; Hed *et al*., 2011). Leaf density around bunches can be managed by manual or mechanical leaf removal, applied from pre-bloom to veraison, with reductions in bunch rots that can even approach 100% (Gubler *et al*., 1987; English *et al*., 1993; Percival *et al*., 1994; Duncan *et al*., 1995; Diago *et al*., 2010; Sivilotti *et al*., 2011).

L. botrana carpophagous generations are usually controlled by pesticides, *Bacillus thuringiensis* Berliner (Ifoulis and Savopoulou-Soultani, 2004) and mating disruption techniques (Ioriatti *et al*., 2011), but grapevine cultivar and cultural practices can contribute to the moth control (Villani *et al*., 1997; Fermaud, 1998; Vartholomaiou *et al*., 2008). In an Italian grape-growing area where *L. botrana* develops only two generations per year, Villani *et al*. (1997) showed that bunch-zone leaf removal applied at the "berries pea-sized" (beginning of *L. botrana* second-flight) or "berries beginning to touch" (about 50% of *L. botrana* second-flight) stages can reduce the second-generation larval infestation by more than 70%. In a Greek grape-growing area where *L. botrana* completes three generations per year (Ifoulis and Savopoulou-Soultani, 2004), Vartholomaiou *et al*. (2008) showed that leaf removal undertaken in June reduced the percentage of infested bunches at harvest time by about 15%. Based on these data, it can not be excluded that the effect of some cultural practices in reducing bunch rots is partially due to *L. botrana* control.

Currently, it is necessary to understand how bunch-zone leaf removal affects the level of *L. botrana* infestation. From the theoretical point of view this practice could reduce egg laying and/or cause a higher egg/larval mortality on sunlight exposed berries. The first hypothesis is based on the supposed preference of *L. botrana* females for bunches placed in a denser canopy, but to our knowledge there is no rigorous demonstration of this

assumption. Moreover, according to this hypothesis, the effect would be evident only if females can choose between plots characterized by different leaf density, unless it is proved that bunch-zone leaf removal reduces female fecundity. The second hypothesis is based on the possibility that eggs and newly-hatched larvae on berries not protected by leaves are more susceptible to meteorological factors (i.e, sunlight, relative humidity and rain). In fact, high temperatures, mostly associated with low relative humidities, cause *L. botrana* egg and larval mortality (Coscollá *et al*., 1986; Rapagnani *et al*., 1988) and berries exposed to sunlight reach higher temperatures than those not exposed (Kliewer and Lider, 1968; Tarara *et al*., 2008). This hypothesis might only be plausible if the females lay eggs on berries independently from the berries previous exposure to sunlight because oviposition occurs after sunset. Regarding this, Zahavi *et al*. (2003) observed that: (i) in a vineyard with north-south oriented rows, the bunches exposed to sunlight in the afternoon (west-facing) were much less infested than non-exposed ones (east-facing); and (ii) in the laboratory, females had a slight preference for laying eggs on bunches collected on the eastern side.

3. Aim of this study

The first aim of this study was to verify the influence of bunch-zone leaf removal on *L. botrana* and grey mould attacks, and to extend it with two additional purposes: (i) to distinguish the direct effect of leaf removal on grey mould spread from the indirect effect associated with *L. botrana* control; and (ii) to evaluate the effect of leaf removal on the two carpophagous generations of *L. botrana*, separately. The second aim was to study the spatial distribution of *L. botrana* in relation to sunlight exposure by comparing in the field: (i) the larval infestation within the grapevine canopy considering row side and leaf coverage; and (ii) the female egg-laying preference for different bunch sides.

MATERIALS AND METHODS

1. Influence of bunch-zone leaf removal on *L. botrana* and grey mould

To study the effect of bunch-zone leaf removal (LR) on *L. botrana* and grey mould, four trials were carried out in north-eastern Italy (Trial 2007, Trial 2008, Trial 2011 and Trial 2013; Tab. 1). In the locality of Trials 2007, 2008 and 2011, *L. botrana* normally has three generations per year, whereas in the locality of Trial 2013 third-generation larvae are detected on bunches only in the warmest years.

Table 1 – Studies carried out during 2007–2014 in four experimental vineyards of north-eastern Italy. LR = Trials on the influence of bunch-zone leaf removal on *Lobesia botrana* and grey mould; LSD = Trials on the spatial distribution of *Lobesia botrana* larval infestation; ESD = Experiment on spatial distribution of *L. botrana* eggs

Trial or Experiment	Locality, district Coordinates Altitude (*)	Cultivar Training system Distances between and along rows	Row orientation
Trial 2007 (LR) **Trial 2008 (LR)**	Cormòns, Gorizia, 45°56'N, 13°27'E 39 m a.s.l.	Chardonnay Guyot 1.5 m and 0.5 m	N30°W
Trial 2011 (LR)	Cormòns, Gorizia 45°57'N, 13°26'E 50 m a.s.l.	Chardonnay Guyot 2.8 m and 1.0 m	N25°W
Trial 2013 (LR) **Trial 2014a (LSD)** **Experiment 2013 (ESD)**	Romans d'Isonzo, Gorizia 45°54'N, 13°27'E 24 m a.s.l.	Chardonnay Guyot 2.7 m and 0.9 m	N65°W
Trial 2014b (LSD)	Buttrio, Udine 46°00'N, 13°20'E 83 m a.s.l.	Chardonnay Guyot 2.5 m and 0.9 m	N80°W

(*) All the vineyards were on the plains, except for Buttrio which was on the south slope of a hill.

Six and two treatments were compared in Trials 2007 and 2008, and in Trials 2011 and 2013, respectively (Tab. 2).

Experimental design was randomized blocks (grapevine rows) with four replicates. The plots within each replicate consisted of at least 16 grapevines. No insecticides were applied other than those used in the trials. In all plots the same fungicides against grapevine downy mildew and grapevine powdery mildew were applied with a trailed air blast sprayer. In Trials 2007 and 2008, to avoid pesticide drift interference, the four blocks (rows) were separated from each other by a border row that was not treated with either the fungicides against grey mould or the insecticides against *L. botrana*.

Table 2 – Treatments considered in the four trials on the influence of bunch-zone leaf removal (LR) on *Lobesia botrana* and grey mould.
BBCH stages (Lorenz *et al.*, 1995): 75, "berries pea-sized, bunches hang"; 79, "majority of berries touching"; 81, "beginning of ripening: berries begin to develop variety-specific colour"; 83, "berries developing colour"; 85, "softening of berries".

Treatments	Trial	Cultural practices, active ingredients (a.i) and products	a.i. per hectare	Number of applications (timing)
Untreated control	All Trials		-	-
LR (leaf removal)	Trial 2007	Manual bunch-zone leaf removal	-	1 (BBCH stage 79)
	Trial 2008	"	-	1 (BBCH stage 75)
	Trial 2011	"	-	1 (BBCH stage 79)
	Trial 2013	Bunch-zone leaf-removal with pneumatic machine Mod. "con 2 testate", Olmi, Castiglione d'Asti (AT), Italy	-	1 (BBCH stage 79)
LR+T2 (leaf removal + insecticide application against *Lobesia botrana* 2nd generation)	Trial 2007	Chlorpyriphos Dursban, DOW Agroscience, 44.5% a.i.	490 mL	1 (7 days after the beginning of hatching)
	Trial 2008	Indoxacarb Steward, Dupont, 30% a.i.	45 g	1 (beginning of egg hatching)
LR+T3 (leaf removal + insecticide application against *Lobesia botrana* 3rd generation)	Trial 2007	Methoxyfenozide Prodigy, Bayer, 22.5% a.i	90 mL	1 (beginning of egg laying)
	Trial 2008	*Bacillus thuringiensis* Berliner DiPel DF, Valent BioSciences Corporation, *B. thuringiensis* subsp. *kurstaki*, strain ABTS-351	1000 g	2 (egg hatching and one week later)
LR+AM (leaf removal+ anti-grey Mould applications)	Trial 2007	Cyprodinil + Fludioxonil Switch, Syngenta, 37.5% and 25% a.i., respectively	300+200 g	3 (BBCH stages 79, 81, 83)
	Trial 2008			3 (BBCH stages 75, 81, 85)
LR+T2+T3+AM	Trial 2007 Trial 2008	See above	See above	See above

Insecticides and anti-grey mould products were distributed with a backpack sprayer [Oleo-Mac Sp-126, Emak S.p.A, Bagnolo in Piano (RE), Italy]. The male flights of *L. botrana* were recorded with pheromone traps (Traptest®, Isagro, Novara, Italy) (Fig. 1). Two traps per vineyard were placed from late April to late September. The traps were checked daily coinciding with the expected beginning of the second and third flights and twice a week up to the end of each flight. Bunch-zone leaf removal consisted of removing all the leaves that covered the bunches. When leaf removal was undertaken in Trials 2007, 2011 and 2013 the *L. botrana* second-flight had started 10 days before, whereas in Trial 2008 five days before.

The second-generation infestation of *L. botrana* was estimated at about 40 days after the beginning of the second flight, whereas the third-generation infestation and bunch rots were estimated at harvest time. In all the trials, 50 bunches per plot (i.e, per replicate of each treatment) were sampled. Bunches were examined directly in the field on 10 grapevines per plot, excluding edge plants, with 4 and 6 bunches collected alternately from each grapevine based on an *a priori* scheme to avoid subjective choice (Pavan *et al*., 1998). The number of larval nests of the second generation was counted without dissecting the bunches. For this generation also the damaged berries were counted except in Trial 2007 because bunches were too compact to accurately count the bored berries without removing them. The number of larval nests of the third generation counted by dissecting the bunches. These nests can be easily recognized from those of the second generation because the damaged berries are still turgid and larvae are normally present. At harvest time, the number of rotten berries was counted, separating those contiguous to *L. botrana* third-generation larval nests (groups of rotten berries in contact with nests) from those that were non-contiguous (groups of rotten berries not in contact with nests).

Count data were square root transformed and submitted to a t-test when the treatments in the comparison were two, or an ANOVA and Tukey's post test when the treatments in the comparison were more than two. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

2. Spatial distribution of *L. botrana* in relation to sunlight exposure

In 2014 the spatial distribution of larval infestation (LSD) in the grapevine canopy was studied in two vineyards with east-west oriented rows (Trials 2014a and 2014b; Tab. 1). Larval infestation on bunches located on north and south sides of rows and subjected or not to leaf removal was compared. Experimental design was randomized blocks (grapevine rows) with four replicates. Within each replicate two plots of 16 grapevines were considered. At the beginning of the moths' second flight (June 12th, BBCH 75 "berries pea-sized, bunches hang") one plot per row was subjected to manual bunch-zone leaf removal on both sides (i.e., north and south facing). Therefore, bunches were classified in four different groups in relation to row side and leaf coverage: (i) located on the north side of rows and covered by leaves; (ii) located on the north side of rows and not covered by leaves; (iii) located on the south side of rows and covered by leaves; (iv) located on the south side of rows and not covered by leaves.

At harvest time the second-generation larval nests were counted because in 2014 the third generation did not develop in the studied grape-growing area. In each plot, with or without leaf removal, all north- and south-facing bunches were sampled up to a total of 50 per row side.

Count data were square root transformed and submitted to a two-way ANOVA with row side and leaf coverage as factors and Tukey's post-test. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

At mid-August 2013 the spatial distribution of eggs laid by *L. botrana* females (ESD) on south-facing bunches was studied in the field (Experiment 2013; Tab. 1). For this purpose, two-day-old *L. botrana* females that had mated and had started to lay eggs in the laboratory were confined at sunset by tulle bags on shoots located on the south side of a grapevine row. On each of 21 shoots with two bunches not covered by leaves, two females were confined for 36 h, in order to allow two egg-laying days. Berries of south-facing bunches were distinguished in two groups: (i) berries facing sun during late morning and afternoon (named "sun-exposed side"); and (ii) berries facing the interior part of the canopy (named "shaded side"). At the end of the experiment the bags were removed and eggs laid on the berries of the sun-exposed and shaded side of bunches separately counted.

To compare the number of eggs laid on berries between the two sides of bunches, data were submitted to the Wilcoxon matched-pairs signed-ranks test. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

RESULTS

1. Influence of bunch-zone leaf removal *on L. botrana* and grey mould

In the four Trials only *B. cinerea* was found among bunch rots.

In Trial 2007, even though leaf removal (LR) had reduced the second-generation infestation of *L. botrana* by 50% on average, the differences were not significant in comparison to the untreated control due to the high variability among replicates (Tab. 3). A significant reduction in infestation was observed with insecticide application (LR+T2). The third generation of *L. botrana* was significantly reduced by LR (50% of efficacy; Tab. 3). A further significant reduction in infestation compared with LR was determined by the insecticide application against the third generation (LR+T3). The total amount of rotten berries by grey mould was significantly reduced in the two treatments with fungicide applications (LR+AM and LR+T2+T3+AM) and by the insecticide application against the third generation (LR+T3) (Tab. 3). The rotten berries contiguous to larval nests were significantly reduced only when insecticides and/or fungicides against grey mould were added to LR, even if the insecticide against the second generation (LR+T2) did not significantly differ from LR. The rotten berries non-contiguous to *L. botrana* larval nests were significantly reduced only in the two treatments with fungicide applications (LR+AM and LR+T2+T3+AM). In this year there were four days of rainfall during the six days before sampling on August 14th (data from http://www.osmer.fvg.it/OSMER).

In Trial 2008, LR significantly reduced the second-generation infestation of *L. botrana* (56% of efficacy; Tab. 3). A further significant reduction in infestation was determined by the addition of the insecticide application (LR+T2). The third-generation

infestation of *L. botrana* was significantly reduced by LR (59% of efficacy; Tab. 3). Only the addition of all pesticides together (LR+T2+T3+AM) significantly increased moth control compared to LR. *B. thuringiensis* against the third generation (LR+T3) did not significantly improve the control of this generation and was tendentially less effective than indoxacarb against the second generation (LR+T2), since only this latter treatment did not differ from LR+T2+T3+AM. The total number of rotten berries and the number of rotten berries contiguous to *L. botrana* larval nests were significantly reduced by LR (about 75% of efficacy) and only the addition of all pesticides together (LR+T2+T3+AM) resulted in a further significant reduction in infected berries (Tab. 3). The number of non-contiguous rotten berries was significantly reduced by LR (84% of efficacy) but there was no further significant reduction following fungicide and insecticide applications. Thus, the efficacy of leaf removal was higher for non-contiguous than contiguous berries. The number of contiguous rotten berries was reduced by leaf removal in higher proportion than the number of third-generation larval nests (75% vs 59%), due to a lower number of contiguous rotten berries per larval nest (2.3 in the untreated control and 1.7 in LR). In this year, the global radiation, to which the UV radiation is positively correlated, was very high and rains were absent during the eight days before the sampling carried out on September 2nd (data from http://www.osmer.fvg.it/OSMER).

In Trial 2011, LR significantly reduced the second-generation infestation of *L. botrana*, even though a high variability among replicates was observed (74% of efficacy; Tab. 3). At harvest time, neither *L. botrana* larval nests of the third generation nor grey mould were recorded.

In Trial 2013, LR significantly reduced both the second- and third-generation infestation of *L. botrana* (33% and 63% of efficacy, respectively; Tab. 3). The berries bored by third-generation larvae were not rotten. The amount of rotten berries non-contiguous to larval nests was not significant different between treatments.

The control of the second generation by bunch-zone leaf removal was effective both when leaf removal was carried out 10 days after the beginning of the *L. botrana* second-flight (i.e., Trials 2007, 2011 and 2013) and when it was carried out 5 days after the beginning of the flight (i.e., Trial 2008). In Trials 2007, 2011 and 2013 females had already laid many eggs, but larval hatching had not yet started, whereas in Trial 2008 females had just started to lay eggs (data not reported).

2. Spatial distribution of *L. botrana* in relation to sunlight exposure

In Trial 2014a, there was no difference in the second-generation infestation between the north- and south-facing bunches ($F_{1,12}$ = 2.96, P = 0.11), whereas in Trial 2014b the south-facing bunches were significantly more infested than the north-facing bunches ($F_{1,12}$ = 10.87, P = 0.006) (Fig. 2). In Trial 2014a, there was no difference in the infestation between the covered and not-covered bunches ($F_{1,12}$ = 1.56, P = 0.24), whereas in Trial 2014b the covered bunches were significantly more infested than the not-covered bunches ($F_{1,12}$ = 24.72, P = 0.0003) (Fig. 2). Only in Trial 2014b the interaction between the two factors was significant ($F_{1,12}$ = 13.43, P = 0.003) since the higher infestation recorded in the south-facing bunches was due exclusively to those covered by leaves (27.0 and 7.7 larval nests on 100 bunches in covered and not covered groups, respectively).

In Experiment 2013, *L. botrana* females laid more eggs on the sun-exposed side than on the shaded side of the south-facing bunches not covered by leaves (Fig. 3).

Table 3 – Number per 100 bunches ± standard deviation of *Lobesia botrana* larval nests (l.n.), *L. botrana* damaged berries (d.b.), *Botrytis cinerea* rotten berries at harvest time (r.b.) observed in the treatments in the four trials. Different small letters among treatments indicate significant differences at 0.05 (Tukey post-test or t-test). 2nd = second generation; 3rd = third generation; tot. = total; con. = contiguous to larval nests; non con. = non contiguous to larval nests. LR = bunch-zone leaf removal at "berry pea-sized" or "majority of berries touching" stages; T2 and T3 = one insecticide application against the second and the third generations of *L. botrana*, respectively; AM = three fungicide applications against grey mould.

	LR	LR+T2	LR+T3	LR+AM	LR+T2+T3+ AM	Untreated control	ANOVA or t-test
Trial 2007							
l.n.-2nd	27.5±13.3 ab	5.5±4.4 a	–	17.5±8.7 ab	7.0±2.6 a	61.0±47.3 b	$F_{4,15}$=6.837 P=0.0024
l.n.-3rd	81.5±20.7 c	64.5±19.8 c	25.5±13.7 ab	61.0±30.6 bc	16.0±5.2 a	162.5±38.9 d	$F_{5,18}$=22.656 P<0.0001
r.b.-tot.	403.0±152.0 bc	367.0±133.6 bc	225.0±57.4 ab	136.0±80.2 a	80.0±40.8 a	579.5±227.9 c	$F_{5,18}$=11.341 P<0.0001
r.b.-con	155.0±96.3 cd	86.0±47.5 bc	22.5±15.9 ab	29.0±16.8 ab	7.5±10.0 a	286.0±100.4 d	$F_{5,18}$=17.487 P<0.0001
r.b.-non con.	248.0±99.6 b	281.0±100.8 b	202.5±56.2 ab	107.0±83.8 a	72.5±45.1 a	293.5±130.3 b	$F_{5,18}$=5.226 P=0.0039
Trial 2008							
l.n.-2nd	92.3±28.0 b	2.5±3.0 a	–	72.5±27.92 b	5.5±9.71 a	209.0±60.1 c	$F_{4,15}$=26.860 P<0.0001
d.b.-2nd	333.8±139.5 b	7.5±9.0 a	–	257.5±126.2 b	16.5±27.9 a	804.0±260.25 c	$F_{4,15}$=35.380 P<0.0001
l.n.-3rd	20.5±5.3 b	12.5±9.6 ab	13.0±9.9 b	19.0±8.4 b	1.5±1.9 a	50.0±6.9 c	$F_{5,18}$=13.612 P<0.0001
r.b.-tot.	55.5±21.4 b	29.5±9.2 ab	46.5±23.1 b	26.0±13.6 ab	6.0±6.7 a	243.5±106.3 c	$F_{5,18}$=13.451 P<0.0001
r.b.-con	35.5±12.8 b	15.5±13.4 b	18.5±15.2 b	23.0±12.9 b	0.0±0.0 a	116.5±36.5 c	$F_{5,18}$=20.043 P<0.0001
r.b.-non con.	20.0±12.0 a	14.0±10.2 a	28.0±17.5 a	3.0±2.6 a	6.0±6.7 a	127.0±72.5 b	$F_{5,18}$=13.450 P<0.0001
Trial 2011							
l.n.-2nd	7.0±3.8 a					27.0±18.7 b	t_6=2.463 P=0.049
d.b.-2nd	15.0±8.4 a					79.0±47.9 b	t_6=3.301 P=0.016
Trial 2013							
l.n.-2nd	48.0±5.7 a					71.5±8.9 b	t_6=4.543 P=0.0039
d.b.-2nd	117.0±22.3 a					189.0±24.9 b	t_6=4.207 P=0.0060
l.n.-3rd	27.0±15.9 a					73.0±31.2 b	t_6=2.951, P=0.0026
d.b.-3rd	48.0±31.9 a					146.0±61.9 b	t_6=3.062, P=0.0022
r.b.–non con.	117.3±112.9 a					241.8±207.9 a	t_6=1.152, P=0.29

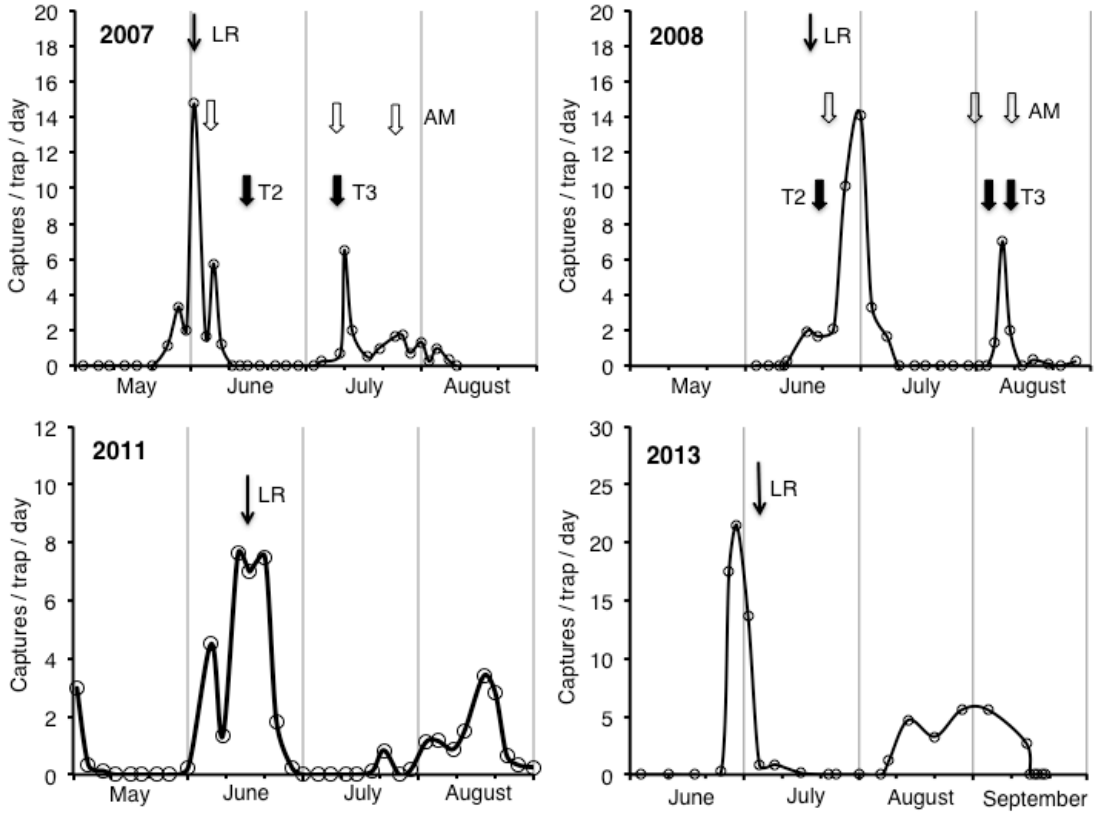


Figure 1 – Second and third flights of *Lobesia botrana* males recorded in the four trials of table 2. The timings of bunch-zone leaf removal (LR), fungicides against grey mould (AM) and insecticides against moths (T2 = second generation, T3 = third generation) are indicated.

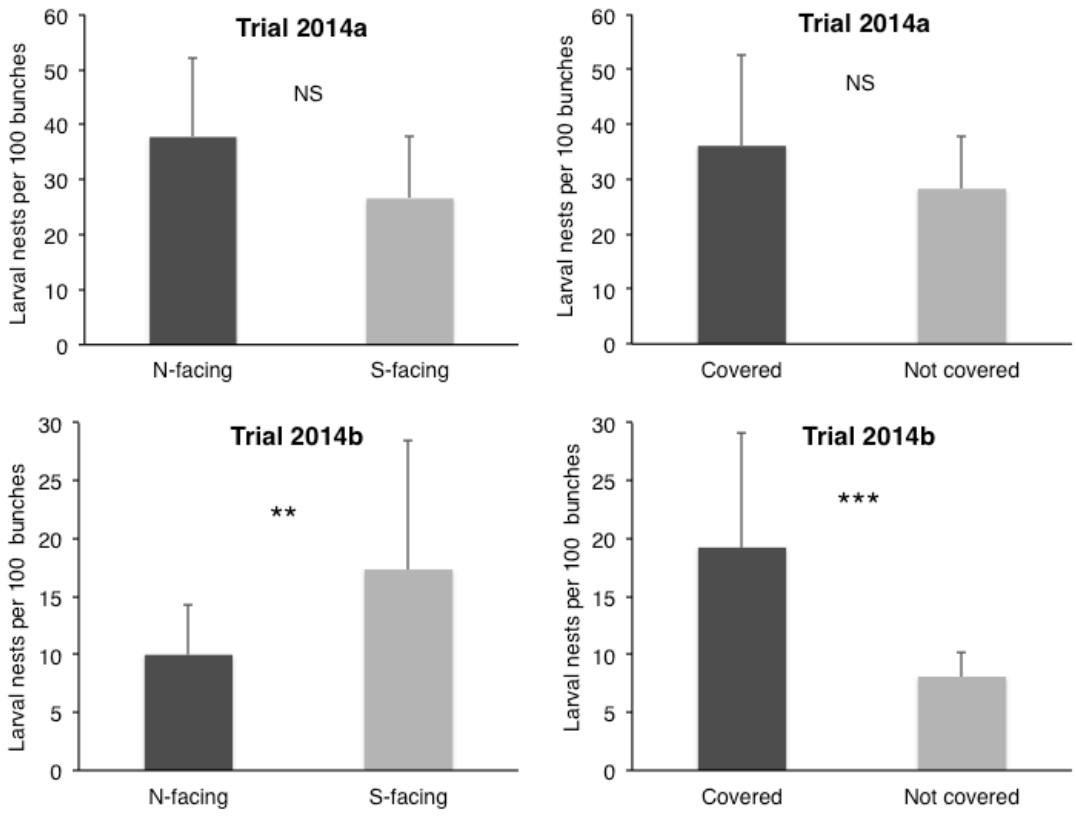


Figure 2 – Second-generation infestation of *Lobesia botrana* (mean+standard deviation) observed in the two parallel trials conducted in 2014 on bunches located on the north- or south-facing sides of the rows and covered or not covered by leaves (i.e. exposed to sunlight by leaf removal). NS, **, *** indicate respectively not significant differences, significant differences at 0.01 level and significant differences at 0.001 at two-ways ANOVA.

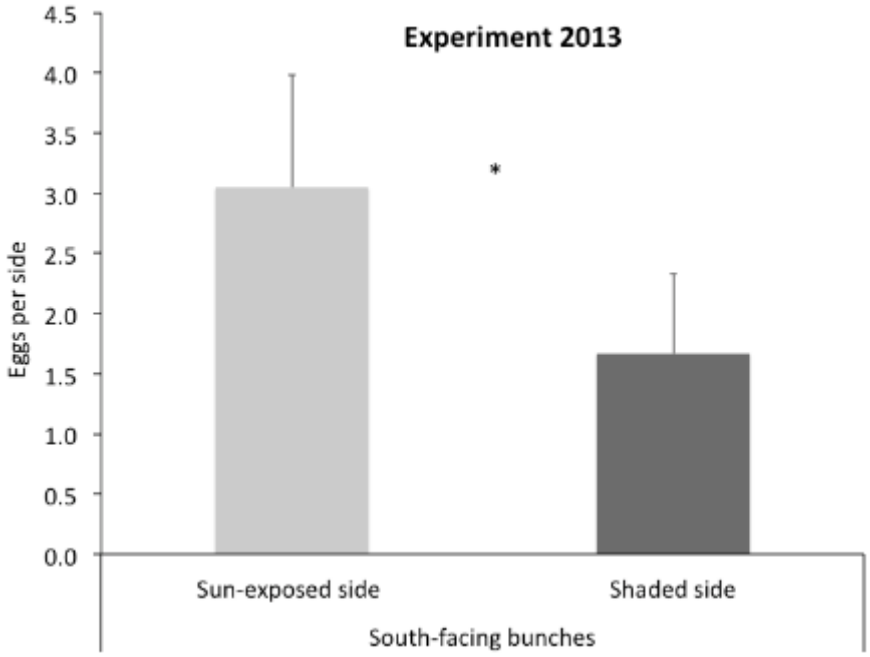


Figure 3 – Partitioning of eggs (mean + SE) laid by *Lobesia botrana* females between the two sides of south-facing bunches. Females were confined by tulle bags on 21 shoots with two bunches not covered by leaves. A significant difference was reported at the 0.05 level according to the Wilcoxon matched-pairs signed-ranks test (W = 106, No = 20, P = 0.0484).

DISCUSSION AND CONCLUSION

1. Influence of bunch-zone leaf removal on *L. botrana* infestation and grey mould

Bunch-zone leaf removal carried out within ten days of the beginning of the *L. botrana* second-flight, before the expected beginning of egg hatching, reduced larval infestation of the second generation by about 50%, confirming the data reported in Villani *et al*. (1997) that had previously studied the effect of leaf removal specifically on this moth generation. Our study demonstrated for the first time that the positive effect of the leaf-removal, performed to control the second generation, persisted even into the third generation, whose eggs are laid about a month after the green-pruning practice. This result is of practical relevance because the third generation causes higher yield losses (Pavan and Sbrissa, 1994) and favours more grey mould than the second generation (Pavan *et al*., 2014a).

In Trial 2008, the control of the third generation of *L. botrana* was tentendially better guaranteed by the application with indoxacarb against the second generation than by the specific application with *B. thuringiensis*. The lack of efficacy of *B. thuringiensis* could be explained by the bunch compactness, which does not ensure thorough coverage of berries by the product sprayed, and by the very high UV radiation, that is known to inactivate *B. thuringiensis* toxin (Ignoffo and Garcia, 1978). The efficacy of Indoxacarb could be explained by its high persistence that was able to partially control the third generation (Pavan *et al*., 2014b), even though it was applied against the second generation.

During this study high grey mould levels were observed at harvest time both in Trials 2007 and 2008, but the incidence of grey mould was higher in 2007 than in 2008 in

accordance with rainfall conditions recorded in the two years. Contrary to expectations, only in 2008 leaf removal significantly reduced the incidence of *B. cinerea*. It could be due to high rainfall recorded in 2007 and hence to the very high number of hours of wetness, which nullified the benefit of leaf removal. The reduction in rotten berries involved both those non-contiguous to *L. botrana* larval nests (not associated with moth activity) and those contiguous to larval nests (associated with moth activity). Leaf removal had a greater effect on non-contiguous rotten berries, but in any case the reduction in contiguous rotten berries was more than proportional to the reduction in the number of larval nests.

2. Spatial distribution of *L. botrana* in relation to sunlight exposure

In Israel, Zahavi *et al*. (2003) showed that females of *L. botrana* prefer to lay eggs on bunches that are less exposed to sunlight and they hypothesized that this is due to different characteristics of the exposed berries. Our data showed that on south-facing bunches females preferred to lay eggs on the side of bunch that had been exposed to sunlight in the hours before. Therefore, the females that laid eggs after sunset did not avoid berries that had been previously exposed to sunlight but even showed a slight preference for them. The fact that *L. botrana* females prefer to lay on the berries of the ancestral host plant *Daphne gnidium* L., that are not naturally covered by leaves, than on grape berries, indirectly confirms that exposed fruits are not avoided for oviposition (Maher and Thiery, 2006). Higher temperatures in Israel than in northern Italy could explain differences in egg-laying preference. However, the data of Zahavi *et al*. (2003) showed that females did not completely avoid sun-exposed bunches, but that they simply laid 20-25% fewer eggs on these. In any case, these differences in oviposition were not sufficient to explain the three times lower levels of infestation observed in the field on the sun-exposed bunches. Other factors must be considered to explain such differences, and egg/larval mortality is one of these.

In the vineyard with east-west oriented rows located on the south slope of a hill, the infestation in south-facing bunches (i.e., sun-exposed row-side) was lower in bunches not covered than in those covered by leaves. These results could be explained by a lower level of egg laying or by a higher level of egg/larval mortality on sun-exposed bunches.

However, considering that females did not avoid laying eggs on the sun-exposed side of bunches and that the shaded side of these bunches is not directly exposed to sunlight as well as the bunches covered by leaves, the egg/larval mortality hypothesis seems to be more plausible. In this regard, egg susceptibility to higher temperature has been demonstrated (Götz, 1941; Coscollá *et al*., 1986). In the laboratory, Coscollá *et al*. (1986) showed that the critical temperatures are above 40 °C and that the incidence of mortality increases with low relative humidity and exposure time. The same authors suggested that in the field the eggs directly exposed to sunlight could have a higher temperature than the air. In this regard, many studies have shown that berries exposed to sunlight have a higher temperature, ranging up to 10 °C or more above air temperature (Kliewer and Lider, 1968; Millar, 1972; Smart and Sinclair, 1976; Pieri and Fermaud, 2005). Therefore, berries can exceed the critical temperature (i.e, 40 °C), even when the air temperature is lower than this value. A negative effect of high constant temperatures even on larvae was shown (Rapagnani *et al*., 1988), but these results are not directly applicable to field conditions where the temperatures are variable over the day. The role of high temperatures associated with low relative humidity in egg/larval mortality could explain why in the hilly vineyard, differently from the flatland vineyard, the bunches exposed to sunlight were significantly less infested than those covered by leaves.

3. Conclusion

The data collected in this study allowed us to add two important knowledge on the role of bunch-zone leaf removal on *L. botrana* and grey mould control, as they showed that: (i) part of the grey-mould reduction is mediated by moth control; and (ii) leaf removal, carried out during the second flight of *L. botrana,* reduced not only the second- but also the third-generation of the moth.

These results suggest that leaf removal affects *L. botrana* larval infestation by increasing egg/larval mortality. Indeed, female non-avoidance of laying eggs on the sun-exposed side of bunches reject the hypothesis that leaf removal affects female fecundity as a consequence of greater difficulty in finding suitable sites for egg laying. However, more

research is necessary to determine with certainty whether egg/larval mortality hypothesis is true.

Bunch-zone leaf removal is an advisable cultural practice in an Integrated Pest Management context because it also allows a better bunch coverage by insecticides and anti grey-mould applications. Because bunch-zone leaf removal influences yield and must quality, and can be associated with sunburn (e.g., Verdenal *et al*., 2013), the choice to adopt this practice must be made while taking into account all the possible positive and negative effects.

Chapter III.

Effects of high temperatures on *Lobesia botrana* egg/larval mortality (*)

(*) In this study, I collected all the laboratory data and most of the field data. The work was made in cooperation with Dr. Elena Cargnus, Dr. Francesco Pavan and Prof. Pietro Zandigiacomo.

INTRODUCTION

1. European grapevine moth, *Lobesia botrana*

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is one of the most destructive grapevine pests with economically importance in the Palearctic region (i.e., Europe, Asia and Africa). Recently, it has expanded its geographical distribution and was found in Chile in 2008, California in 2009, and Argentina in 2010 (Ioriatti *et al*., 2012). *L. botrana* larvae cause direct damage to grapevines by feeding on flowers and berries, and some indirect damages due to the developing of bunch rots.

1.1. Description

L. botrana is a holometabolic insect with four developmental stages: adult, egg, larva and pupa (Figs. 1, 2, 3, 4). Adult males are smaller and have a tighter abdomen than adult females, and if they are being harassed, they move with quicker movements.

Eggs are lenticular, flattened (0.6 × 0.7 mm) (Fig. 2). They pass through three stages: "white", "red-eyes" (two red dots are visible for transparency) and "black head" (black head capsule visible for transparency). Eggs are laid singly, and more rarely in small group of two or three.

Larvae complete their development in five, sometimes six, instars (Pavan *et al*., 2010, 2013) (Fig. 3). The first instar is 0.9-1 mm in length. Larva colour evolves from a creamy white with a black head-capsule upon emergence to a darker yellowish brown or green maroon with a brown head-capsule in the subsequent instars; reflecting the colours of its gut contents and equipped with hair. Reaching maturity, larvae (8-10 mm in length)

assume a blue-violet colour, cease feeding, and, when found a suitable site form a fusiform cocoon silk, without trapping debris, inside which metamorphosis takes place. Pupae, first greenish and then dark brown, are characterized by 8 hooked bristles at apex (Fig. 4).

Figure 1 – *Lobesia botrana* adult. Leaden-grey bar in the middle of the forewings (A. Villani).

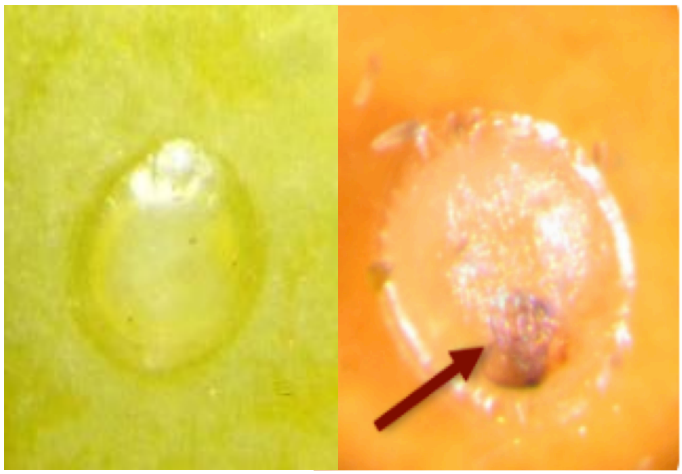


Figure 2 – *Lobesia botrana* eggs. Red-eye egg on berry (left, F. Pavan) and black head egg observed under dissection microscope (right).

Figure 3 – Mature larva of *Lobesia botrana* on inflorescence of grape (F. Pavan).

Figure 4 – Overwintering pupa of *Lobesia botrana* removed from its silken cocoon under grapevine bark (F. Pavan).

1.2. Biology and damage

L. botrana is an extremely polyphagous species (Bovey, 1966; Moleas, 1988; Coscollá, 1997; Stavridis and Savopoulou-Soultani, 1998). The spurge flax *Daphne gnidium* is hypothesized to be its original host plant (Bovey, 1966; Lucchi and Santini, 2011), but *Vitis vinifera* could be considered currently as its most important host plant. However, there are also some observations on even better development of *L. botrana* larvae on *Olea europaea* than on *V. vinifera* (Stavridis and Savopoulou-Soultani, 1998).

L. botrana is a polivoltin species that performs, depending on the geographical areas and microclimates, from two to four generations per year (under optimal conditions

they have been registered a fifth incomplete) (Roehrich and Boller, 1991; Coscollá, 1997; Ioriatti *et al.*, 2012).

This tortricid moth overwinters from September-October until the following spring as a diapausing pupa which is induced in the second half of July in northern latitudes of Italy (Coscollá, 1997).

In northern Italy, adult emergence from overwintering pupae usually begins in the second half of April and has a peak between late April and mid May (Zangheri *et al*., 1987).

The females of overwintering generation (i.e., adults of the first flight) begin to lay eggs from one to three days after mating on inflorescences, preferentially on smooth surfaces (Galet, 1982), usually singly, but sometimes in groups of two or three, and continue to oviposit throughout their life (Torres-Vila *et al.*, 1997; Maher and Thiéry, 2004).

The egg incubation period is 7-11 days, depending on the temperature, and the newly hatched larvae feed on stamens and ovary of a flower bud (anthophagous generation). Subsequently larvae attack the neighbour flower buds, join them together in groups of 6-8 with silky threads, forming the so-called "glomerule" (Fig. 5). After blooming, larvae start to feed on just-set berries. Larval development is completed in about four to five weeks, depending on weather conditions, during which larvae develop normally through four moults, so five instars. The larva of fifth instar, reaching maturity, builds a white silky cocoon inside the “glomerule” or in other sites, where it pupates. The duration of metamorphosis varies depending on the temperature (8 days at 26° C and 17 days at 18° C).

L. botrana first-generation larvae, that feed on flowers and small berries, do not cause serious damage (Bovey, 1966; Galet, 1982; Coscollá, 1997). Clusters are able to withstand attacks of 1-4 glomerules by one or two larvae, or loss of 30 flowers without yield losses (Roehrich and Schmid, 1979). *L. botrana* first-generation can show distinct preferences for some grapevine cultivars (Geisler, 1959; Galet, 1982). The larval population levels observed in each cultivar was positively correlated with inflorescence earliness and negatively correlated with inflorescence hairiness (Pavan *et al*., 2009).

Figure 5 – *Lobesia botrana* "glomerule" formed by first-generation larvae (left, F. Pavan), and damage of larvae (right, F. Pavan).

In Italy, the second flight of adults usually begins in the second half of June (Zangheri *et al*., 1987). Females lay the second-generation eggs on the surface of unripe green berries. After 5-9 days, depending on the temperature, the newly hatched larvae emerge from the eggs and enter inside of an unripe green berry, digging a penetration hole at the insertion of the pedicel or at the point of contact between two berries (thigmotropism) (Fig. 6). Larvae then move to neighbouring berries in the same bunch using silk threads to join berries together. Damage can include two to six berries per larva, depending on the grape cultivar (Pavan and Girolami, 1986; Delrio *et al.* 1987; Pavan *et al.*, 1987; Coscollá, 1997). Larval development usually completes before harvest time. Mostly from veraison, the feeding activity of the larvae promotes the development of grey mould (*Botrytis cinerea*), mainly in the saprophytic form on dead tissues, which leads to even greater injury than that caused by the insect itself (Fig. 7). The bored and rotten berries show different levels of dehydration and may even fall (Pavan *et al.*, 1993) (Fig. 8). Some berries are able to heal wounds without rotting. Near harvest time, the berries rotten due to larval activity can spread the infection to contiguous not-bored berries. The direct and indirect damage caused by the larvae of this *L. botrana* generation are affected by both the grapevine phenological stage, in which larval development occurs, and the cultivar harvest-time. In

fact, it is higher when larvae develop on berries next to harvest time and for the early-harvested cultivars, because in both cases the berries are in a phonological stage more susceptible to rots (Pavan *et al*., 1993; Pavan and Sbrissa, 1994).

Figure 6 – *Lobesia botrana*, larvae of the second generation. A hole of larval penetration with sawdust (top left, F. Pavan), a visible subsurface tunnel dug by a larva (top right, F. Pavan) and a larval penetration at the point of contact between two contiguous berries (down, F. Pavan).

Figure 7 – Berries eroded by *Lobesia botrana* larvae of the second generation infected also by *Botrytis cinerea* (F. Pavan).

Figure 8 – Rots developed from berries eroded by the *Lobesia botrana* larvae of the second generation (F. Pavan).

The larval stage lasts 19 to 25 days, depending on temperature. In areas with warmer climate, the larvae complete their development from the last ten days of July and pupate preferably within the folds of the leaves or inside of the bunches. Newly emerged adults within a few days give place to a third generation. In more northern regions, where the moth has only two generations per year, the pupae are formed from the second week of August and enter diapause under a suitable shelter.

The females of the third flight lay eggs on ripe or ripening berries. The incubation period of the eggs is 5-7 days. The larvae, unlike those of the previous generation, feed on the surface of berries without penetrating inside (Fig. 9). Larval development lasts 22-28 days, depending on the temperature, and the larvae leave bunches to form diapausing pupae in winter shelters that are mainly in crevices and cracks of the trunk and cordons.

L. botrana third generation larvae (second carpophagous generation) cause higher damage than the previous generation, because they feed on berries near to maturity. In fact, in this phonological stage the berries are very susceptible to rots, such as *Botrytis*, *Penicillium*, *Aspergillus*, and *Alternaria* (Dalla Montà *et al.*, 2007), so the indirect damage is favoured (Fig. 10). The weight losses caused by the larvae of this generation are highly variable and may depend on the cultivar, in particular the berry size, and the weather

conditions. The longer the damaged bunches remain in the field, the more the larvae and the pathogenic agents of rottenness have berries available, so the harvest time for is the other important factor (Coscollá, 1997).

Figure 9 – Bunches with surface damage caused by *Lobesia botrana* larvae of the third generation (F. Pavan).

Figure 10 – Rot developed from the *Lobesia botrana* nest of the third generation larvae (the arrow indicates the larva) (F. Pavan).

For carpophagous generations a higher cultivar susceptibility was associated with a higher bunch compactness (Pavan and Girolami, 1986; Pavan *et al.*, 1993; Fermaud, 1998; Snjezana, 2004; Sharon *et al*., 2009) and kairomones present on berry surface (Maher and Thiéry, 2004). In north-eastern Italy, the late-harvested cultivars (e.g. Raboso veronese) are the more heavily infested by larvae of third generation (Zangheri *et al.,* 1987). The

development of rots associated with the moth is due to the second generation, in the grape-growing areas where the moth completes only two generations per year, and mostly to the third generation, where full three generations per year exist (Pavan *et al.*, 2014). The development of rot *Aspergillus* is also associated with the presence of ochratoxin in bunches (Cozzi *et al.*, 2006).

Phenology of the *L. botrana* second-generation differed between areas where the moth has always three generations and more northern areas, where the moth is typically bi-voltine. In fact, it was pointed out that the timing of larval development is longer in growing areas where the third generation is negligible compared to warmer areas, where the third generation is important and damaging (Pavan *et al.*, 2006, 2013). The slower development is characterized by six larval instars and is normally associated with the production of overwintering pupae, although in some cases it is observed a delayed third flight (late August-early September) that determines abundant oviposition only on late-maturing cultivars and on bunches from the second flowering. The fastest development is characterized by five larval instars and is associated with earlier third flight (from late July-early August) that determines abundant oviposition also on early maturing cultivars.

2. Effects of temperature on *Lobesia botrana* and other tortricids

Temperature could be considered as a key factor regulating growth in poikilothermic organisms especially insects due to the fact that many metabolic reactions are temperature-dependent (Nijhout, 1994). On the other hand, thermal death mechanism caused by high temperatures is believed to be due to an increase of both respiration and metabolism up to a critical thermal limit and to other destroying effects on nervous and endocrine systems, and cell wall (Neven, 2000). Therefore, species that are not able to survive a broad range of temperature conditions will not also be able to persist during frequent extreme temperature events brought about by global climate change (Bürgi and Mills, 2012).

2.1. *Lobesia botrana*

European grapevine moth *L. botrana*, which is specifically considered in this study, was also the subject of some investigations about the effects of temperature on its different life aspects.

Coscollá *et al*. (1986) studied the mortality of *L. botrana* eggs under different relative humidity (RH) and temperature conditions and the results showed that at 40 °C the egg mortality increases in comparison with control (30% vs 15%) just when they have been submitted to RH lower than 20% (10-15%) for 6 h, whereas at 43 °C the egg mortality was very high (62%) in comparison with control (4%) at 20% RH for 5 h and 30 min. On the other hand, submitting the eggs to constant temperature for almost all their embryonic developmental period showed 100% of eggs mortality even with lower temperature and higher relative humidity (Briere and Pracros, 1998) in comparison with those reported in Coscollà *et al*. (1986) (32 °C vs 43 °C and 65% RH vs 20% RH). Similar study was conducted by Rapagnani *et al.* (1988) demonstrating that eggs and larval mortality increased from constant 30 °C and was 100% at constant 35 °C, whereas the pupal mortality increased from constant 33 °C and was 100% at constant 35 °C.

Irigaray *et al.* (2006) studied the effects of one non-constant (average 24 °C) and six constant temperatures (15, 18, 21, 24, 27, 30 °C) on head capsules size of all larval instars of *L. botrana*. Head capsules were larger at 15-21 °C than other temperatures. At non-constant temperature (average 24 °C), the head capsules were larger than at constant 24 °C.

Fecundity of *L. botrana* is also affected by temperature and it is found to be optimal at 20–27 °C (Gabel, 1981).

While high temperatures showed several negative effects on *L. botrana*, it was found that increments of temperature up to 30 °C will decrease larval developmental time, but at 34 °C first instar larvae developed significantly longer than at 22–32 °C (Briere and Pracros, 1998).

Rain and wind are other abiotic factors that can influence the activity and survival of *L. botrana* also interacting with temperature. Although they play a lesser role than do temperature and relative humidity, long period of heavy rain and wind could hinder adult movement, mating and oviposition, resulting in low density of larval population (Bovey, 1966).

2.2. Other carpophagous tortricids

Fifth instar of *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) was found by several authors, as the most heat-resistance stage (Yokoyama *et al.*, 1991; Neven and Mitcham, 1996; Feng *et al.*, 2004, Wang *et al*. 2004). Mortality of 100% of this larval stage, as reported by Yokoyama *et al*. (1991), was obtained through exposure to 45 °C for more than 55 min, while based on Neven and Mitcham (1996) the exact time required for 100% mortality of *C. pomonella* fifth instar at 45 and 47 °C was 124 and 72 min, respectively. 50 °C for 10 min in water bath also showed the same results (Feng *et al.*, 2004). Neven (2005) found two controlled conditions in chamber, 45 °C for 45 min and 47 °C for 25 min under a 1% oxygen, 15% carbon dioxide, -2 °C dew point environment, sufficient to control all life stage of codling moth in sweet cherries while saving the fruit marketing quality. Fecundity of *C. pomonella* is also affected by temperature to which larvae were exposed during their development. As it was found by Yokoyama *et al.* (1991), exposing fourth and fifth larval instars of *C. pomonella* to 44 or 45 °C for 20 min caused females to lay fewer eggs. White (1981) found that transferring from 26 to 33 °C at various time during larval development caused the sterility of most adult males. On the other hand, Howell and Schmidt (2002) observed that constant temperatures as low as 25 °C increased mortality on eggs and larvae of *C. pomonella*, but in the field conditions the codling moth tolerated temperatures well above this thermal death point. In their opinion every adverse effect such as sterility and prolonged emergence are all due to the rearing at constant temperatures. They found no high temperature threshold for this pest in its natural setting in Washington. When this pest was reared under simulated field condition even with peak of 41.8 °C, no increment of egg, larvae or pupal mortality was observed.

The first instar of the oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) was found to be the most susceptible instar to high temperature (100% mortality at 42 °C for 25 min and 43 °C for 20 min) (Yokoyama and Miller, 1987). Liang *et al.* (2014) studied *G. molesta*-adult response to a single hot event (38˚C for 4 h) exposure and no immediate mortality was reported for adults although females' survival slightly declined, but hatching eggs were significantly lower when both or only one gender was heated. If females were directly exposed to thermic stress exhibited longer lifespan and oviposition period, while if males mated with them were exposed, a delay in onset of oviposition was observed.

Whiting and Hoy (1998) found that in the light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) the mortality increases to the increase of fruit-surface temperature from 20 to 40 °C and the tolerance to high temperature increases from first to fifth larval instar. Also Bürgi and Mills (2012), comparing in the laboratory the effect of air temperature ranging from 32 °C to 40.4 °C on all life stages, showed that the tolerance to high temperature increases from first to fifth larval instars. Alderson *et al*. (1998) studied effects of heating rate as quarantine treatment on the mortality of *E. postvittana* fifth instar. Lethal time for 99% mortality of fifth instar larvae was longer when temperature increases gradually from 20 to 43 °C than when the larvae were directly exposed at 43 °C.

3. Influence of sunlight exposure on berry temperature

The effects of sunlight on development and composition of grape berries could be considered as a key ingredient to the grapevine production quality (Kliewer and Lider, 1968; Crippen and Morrison, 1986; Dokoozlian and Kliewer, 1996; Bergqvist *et al*., 2001; Weiss *et al.*, 2003). These authors also agree that berry quality improves due to fruit exposure to sunlight. Solar radiation directly heats bunches and affects ripening rates. Sugar and acid content are functions of accumulated temperatures and temperature range (Mullins *et al*., 1992). Canopy manipulations, trellis design and row orientation can lead to provide daily and seasonal solar radiation regimes for better products (Smart, 1985; Pieri, 2010). Grifoni *et al*. (2008) studied effects of row orientation on the distribution of light and shade on plants and the penetration of the different components of solar radiation into the plant canopy and they found noticeable influence of vines row orientation on received global PAR (photosynthetically active radiation) by both sides of rows (shaded and full sunlight exposed).

It is known that sunlight exposure increases linearly the temperature of berries (Smart and Sinclair, 1976; Bergqvist *et al*., 2001) and the greatest impact of light on fruit development happens during initial stages of berry growth (Dokoozlian and Kliewer, 1996).

Bergqvist *et al*. (2001) measured berry temperature on east-west oriented rows for full exposure, moderate exposure and shaded situations. Fully-exposed berries were found to be 7 °C warmer than air temperature and about 10 °C of the shaded ones at mid-day. In their survey the temperature of fully exposed and shaded bunches on south part of the rows was significantly higher of 3-4 °C compared to those on north part. On the south part of canopy, fully and moderately exposed bunches received mostly direct sunlight in the afternoon while on the north part of canopy they received indirect or diffuse light during same period.

Also Millar (1972) measured exposed or shaded berry temperatures and compared it with air temperature. Exposed bunches had higher temperature of 1.4 to 7.3 °C than air temperature while the temperature of shaded ones was 0.5 to 4.4 °C lower than that of air.

Jogaiah *et al*. (2012) compared the temperatures of berry skin in relation to row orientation and sunlight exposure. They found that the temperature of shaded bunches was only little higher than air temperature whereas that of sun-exposed bunches, both to south and west sides, was 5.6–8.4 °C higher than air temperatures.

However, the exposed bunch would have different thermal conditions in its different parts. As it was mentioned in Kliewer and Lider (1968), according to the position of berries in the bunch and whether or not they are exposed to direct sunlight, their temperature would be different (with a maximum difference of 6.3 °C). Even a single berry with direct sun light exposure, could be considered as three parts respect to its temperature (front, middle and rear) (Kliewer and Lider, 1968).

Smart and Sinclair (1976) measured skin temperature of green berries up to 12 °C above air temperature in the field with 3 °C of gradient across exposed berries. They found berries of loose bunches with lower temperatures than those on tight bunches.

The sun-exposed west-facing bunches could be 10 °C above air temperature with defoliation (Pieri and Fermaud, 2005).

4. Aim of this study

Leaf removal is a widely used practice in lots of grape-growing areas. For most growers the main ideas behind it are to fight diseases pressure and influence grape quality. It is also known that bunch-zone leaf removal influences pests' population in vineyards such as leafhoppers (Stapleton *et al*., 1990) and *L. botrana* (Villani *et al*., 1999; Vartholomaiou *et al.*, 2008; Chapter II, Pavan *et al*., in press).

The specific objective of this study was to understand why bunch-zone leaf removal reduces *L. botrana* larval infestation (see Chapter II). How above reported, the sun-exposed bunches could be 10 °C or more above air temperature with defoliation and adverse effects of high temperature on tortricids in general and *L. botrana* in particular are well known. The results reported in Chapter II of this PhD thesis suggest that bunch-zone leaf removal affects *L. botrana* larval infestation by increasing egg/larval mortality. Indeed, female's non-avoidance of laying eggs on the sun-exposed side of bunches suggests a negation of the hypothesis that leaf removal affects female fecundity as a consequence of greater difficulty in finding suitable sites for egg laying. If bunch-zone leaf removal reduced infestation only as a result of female preference for laying eggs on leaf covered bunches, the absence of choice would make this cultural practice useless.

At this purpose it was studied the effect of:

1. Temperature on eggs in the laboratory;
2. Temperature on larvae in the laboratory;
3. Different grapevine row-orientation on berry temperature;
4. Berry exposure to sunlight on egg distribution in the field;
5. Berry exposure to sunlight on egg mortality in the field;
6. Berry exposure to sunlight on larval settlement in the field.

MATERIALS AND METHODS

1. Experiments in climatic chamber

Different experiments were conducted in climatic chamber (Sanyo Versatile Environmental Test Chamber, Sanyo Corp, Japan) to evaluate the effect of high temperatures on eggs and larvae of *L. botrana*.

1.1. *Lobesia botrana* rearing

Mass rearing of *L. botrana* was conducted in climatic room at 24±1 °C temperature, 70±5% relative humidity and at 16:8 (D:L) photoperiod.

The rearing was originated from larvae collected in May 2013 in a Pinot Gris vineyard located in north-eastern Italy (locality Corona di Mariano del Friuli, Gorizia district, 45°55'30"N, 13°29'44"E, 40 m a.s.l.). In order to prevent inbreeding effects, in May 2014 new larvae of *L. botrana* were collected in a Pinot Gris vineyard located in north-eastern Italy (locality Spessa di Cividale, Udine district, 46°2'37"N, 13°26'23"E, 112 m a.s.l.) and introduced into previous rearing.

L. botrana larvae collected in the field were individually reared in cylindrical polystyrene boxes (diameter 5 cm, high 1.8 cm) in order to eliminate parasitized or infected larvae. Adults obtained by larvae were put in cylindrical tubes lined with plastic bags (15 × 25 cm) where they were fed through soaked cotton-wool with 10% saccharose solution (Fig. 11). Mated females laid eggs on the internal surface of plastic bag. After converting adults in a new bag, the old one was cut in broad strips that were put in rectangle polystyrene boxes (length 9 cm, width 6 cm, high 1.8 cm), where larvae, after egg hatching,

were fed on artificial diet (Rapignani *et al.*, 1990). The pupated larvae were moved in another clean rectangle polystyrene boxes until adult emergence.

Figure 11 - Cylindrical tube lined with plastic bags with *Lobesia botrana* adults and saccharose soaked cotton wool inside.

1.2. *Lobesia botrana* eggs exposed to high temperatures

Experiments on the influence of high temperatures on *L. botrana* eggs were conducted in two climatic chambers (Sanyo Versatile Environmental Test Chamber, Sanyo Corp, Japan) programmed at different temperatures, 60±5% relative humidity and at 16:8 (D:L) photoperiod. One chamber was used as control. The relative humidity was based on that usually recorded in the field conditions.

1.2.1. Experiment type 1: Exposure of eggs to different constant temperatures

In order to determine the susceptibility of different egg stages of *L. botrana* to high temperatures, eggs of *L. botrana* belonging to different stages (i.e., white, red eyes and

black head) were exposed to different constant high temperatures for 3 or 6 hour and for one or two exposure periods (Tab. 1). The eggs laid on plastic bag were controlled under dissection microscope and eggs of each stage were marked. The marked eggs belonging to each stage were put under desired thermal condition. Each experiment was consisted of matching control group kept at constant 24 °C for the same period. Eggs were checked daily to record changes during the experiments (death, going to the next stage or hatch). For most temperature conditions 2–4 independent replications were conducted. Each replicate was normally consisting of 50 eggs.

Table 1 – Experiments carried out on the effects of exposure to high constant temperatures on *Lobesia botrana* eggs.

Temperature	Exposure time	Egg stages (*)	Number of replicates (N° eggs per stage in each replicate)
37 °C	6 h	W, R	1 (50)
	6 h vs 6 h + 6 h**	W, R, B	3 (50, 50, 50)
40 °C	6 h	W, R, B	4 (55, 50, 20, 50)
	3 h vs 3 h + 3 h***	W, R, B	3 (50, 50, 50)
	6 h vs 6 h + 6 h**	W, R	3 (50, 50, 50)
43 °C	6h	W, R	2 (50, 50)

* W = white egg, R = red-eyes eggs, B = black-head eggs.
** After the first 6 h of exposure time, egg groups were placed at 24 °C for 18 h and after the second 6 h, they were transferred to the 24 °C until the end of experiment.
*** After the first 3 h of exposure, egg groups were placed at 24 °C for 21 h and after the second 3 h, they were transferred to the 24 °C until the end of experiment.

1.2.2. Experiment type 2: Exposure of eggs to different temperature cycles

Based on air and berry temperature recorded in early August in a vineyard of north-eastern Italy, two 24-h temperature cycles were considered for this experiment (Fig. 12). In order to eliminate a possible effect of heated plastic strips, the mortality of the three egg stages of *L. botrana* were examined also on green glass marbles (used as artificial berries) and real berries (berries of table grape from organic agriculture). To obtain laying eggs on artificial and real berries, they were confined with mated females for two days.

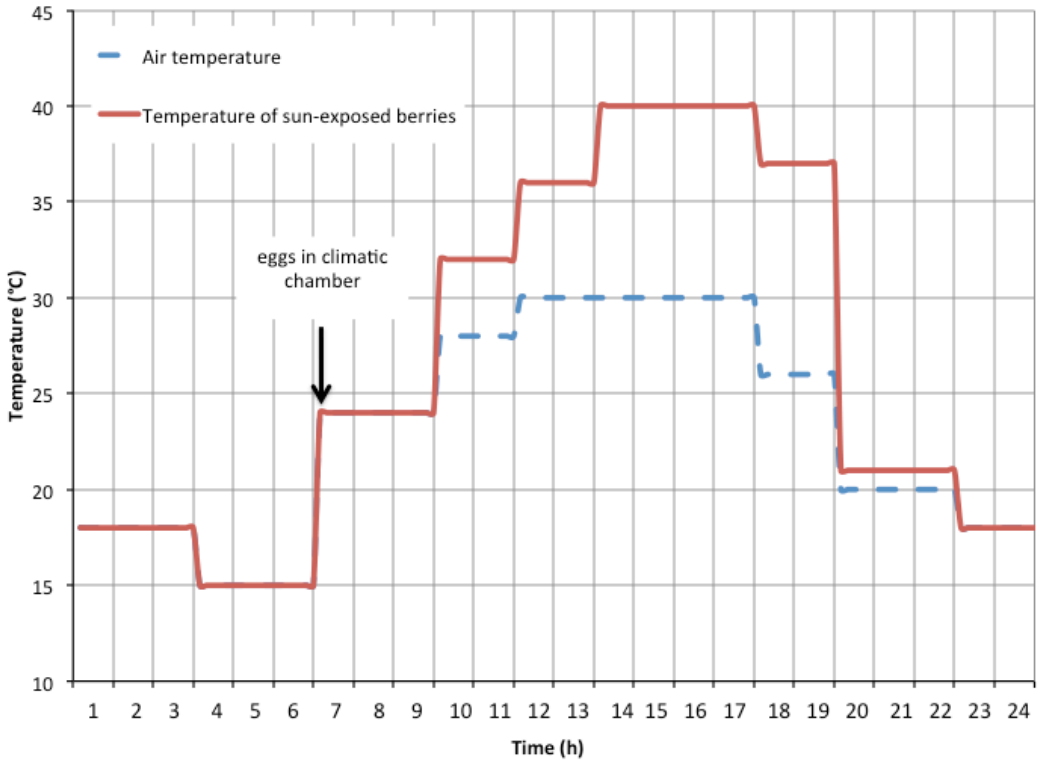


Figure 12 – Two 24-h temperature cycles to which the eggs of *Lobesia botrana* were exposed based on temperatures recorded in early August in a vineyard of north-eastern Italy. Sun-exposed berries belonged to bunches not-covered by leaves and facing south-west orientation.

1.3. Newly-hatched larvae of *Lobesia botrana* exposed to different temperature cycles

The experiments were conducted in the two climatic chambers used for egg experiments. The two climatic chambers were set with two different 24-h temperature cycles based on temperatures of air (peak of 30 °C) and on sun-exposed south-west facing berries (peaks of 37 °C or 40 °C) (Fig. 13). At 7th h of the temperature cycles, when the temperature was the same of the rearing room (24 °C), newly-hatched larvae, from the eggs that were laid at control condition (24 °C), and small bunches (consisted of 3-4 berries) from organic agriculture, included in separate polystyrene boxes, were placed in both experimental chambers. As it is shown in figure 13 the temperature then gradually increased to reach a peak of 30 °C for control cycle and peaks of 37 or 40 °C for high-temperature cycles. At the beginning of high temperature period (14th h of temperature cycles) larvae were put on berries in both chambers. Peaks of high temperatures were kept

for 4 hours and then the temperature gradually decreased. After 24-h, when the temperature reached again 24 °C, the berries were removed from climatic chambers and checked under stereomicroscopy to record the number of settled larvae.

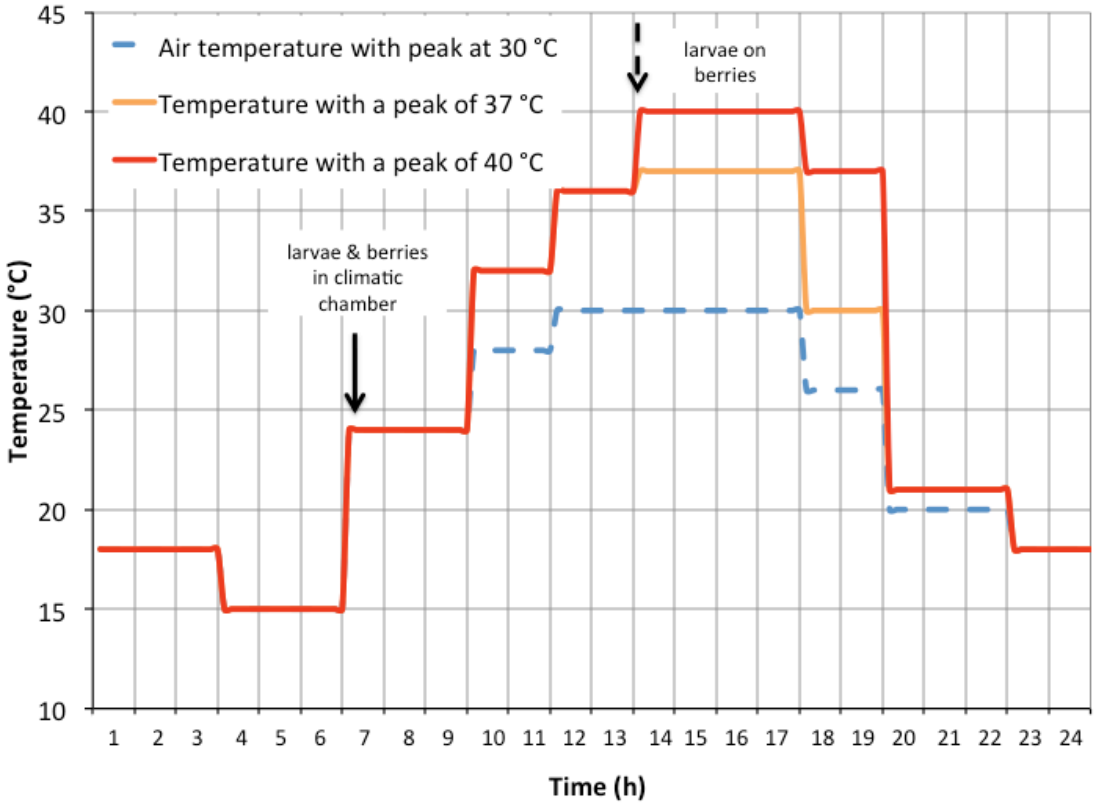


Figure 13 – Three 24-h temperature cycles to which the larvae of *Lobesia botrana* were exposed. The cycle with peak of 30 °C simulated the air temperatures, whereas the two cycles with peaks of 37 or 40 °C simulated the temperatures of sun-exposed berries belonged to bunches not-covered by leaves and facing south-west orientation.

1.4. Statistical analysis

To compare the percentage of egg or larval mortality in experiment involving one treatment in addition to control, χ^2 test (one replication) and Chochram Q test (more than one independent replication) were used. To compare the percentage of egg or larval mortality in experiments involving more replications and more than one treatments in addition to control, one-way ANOVA was used after arcsine transformation of data. To compare Abbott efficacies in different experiments, two- or three-way ANOVA was used after arcsine transformation of data.

2. Experiments in the field

Experiments on the effects of sunlight exposure on both berry temperature and egg/larval mortality were conducted.

2.1. Temperatures recorded on berries during *Lobesia botrana* third-flight

In two vineyards (A and B) of north-eastern Italy the temperatures of berries were measured in August 2013 and 2015 respectively, in coincidence with egg-laying and egg-hatching of the third generation of *L. botrana*.

The vineyard A (locality Romans d'Isonzo, Gorizia district, 45°54'24"N, 13°27'9''E, 26 m a.s.l., cv. Chardonnay) has a rectangular area (230 × 21 m) with 8 rows N65°W oriented. The vines, trained to Guyot system, were planted at the distance between and within rows of 2.7 and 0.9 m, respectively. At 16 August 2013, on the south face of grapevines of an inner row of the vineyard the temperatures of upper surface of leaves, exposed or non-exposed to sunlight, berries of bunches, exposed or non-exposed to sunlight, were measured. The data were hourly recorded from 10:00 to 17:00 Central European Time (CET, UTC+1). At 10:00 direct sunlight hit the first south-facing bunches and at 18:00 the south-facing bunches were no longer hit by the direct sunlight.

The vineyard B (locality Domanins, Pordenone district, 46°0'31''N, 12°48'58''E, 58 m a.s.l., cv. Sauvignon) has an annular area (inner and outer circumference diameters of 38 m and 76 m), with grapevine displaced in 56 ray-like rows, each 38 m long. The vines, trained to Guyot system, were planted at a within-row distance of 1 m and at an inter-row distance variable from 2.5 m to 6.5 m, respectively from the inner to the outer vineyard circumferences. At 8 August 2015 on bunches not covered by leaves, the temperatures of berries facing 8 different spatial orientations, corresponding to the two sides of 4 differently oriented rows (i.e., NS, EW, N45°E, N45°W), were hourly measured from 9:00 to 18:00 CET. At 9:00 direct sunlight hit the first east-facing bunches and at 19:00 none south-facing bunches were anymore hit by direct sunlight.

In vineyard A weather station located near the vineyard was used for temperature data (OSMER web site). In vineyard B air temperature was recorded using iButton® computer microchip temperature loggers (DS1922L Thermochron®, Maxim Integrated

Products, Inc.) interfaced to Thermodata viewer package (Thermodata Pty. Ltd.). Two microchips were placed into the canopy in shaded position at 180 cm from ground level.

Temperature on leaves and berries was measured using a portable handheld noncontact infrared thermometer Raynger® ST Raytek™ (Spectrum® Tecnologies, Inc.). At each sampling time and for each sunlight exposure (vineyard A) or spatial orientation (vineyard B), the temperature of 5 leaves (only vineyard A) and 5 bunches was measured. The five bunches per vineyard chosen to record temperature were previously marked on five different grapevines. At each sampling time the temperature measurements started 5 minutes before reaching hour interval and lasted not more than ten minutes. In vineyard B, at each sampling time the information if bunches were hit or not by sunlight was also annotated.

To compare the average temperatures on berries facing the different cardinal or inter-cardinal directions ANOVA and Tukey's range test were used. To study the relation between average temperature and insolation hours or temperature peak of berries a correlation analysis was used.

2.2. *Lobesia botrana* eggs laid on berries exposed or not exposed to sunlight

An experiment was carried out in July 2015 on Pinot Gris vineyard located in north-eastern Italy (locality Udine, Udine district, 46°01'51"N, 13°13'29"E, 87 m a.s.l. N35°W row orientation and vines trained by Guyot system) to assess the influence of berry high temperatures, due to sunlight exposure, on *L. botrana* eggs and larvae. The experiment was carried out in three different periods (i.e., 7–14, 10–17 and 17–24 July). The 1st day of each period, ten vines were marked, excluding the border plants of the vineyard. On each vine a shoot with two south-west facing bunches was marked. One bunch was well exposed to the sunlight and the other was covered by leaves so to be never hit by sun rays. Each couple of bunches was checked for absence of *L. botrana* eggs. Then, the shoot with the two bunches was placed into a tulle cage (diameter 15 cm × length 25 cm). The berries of sunlight exposed bunches were divided in two groups: (i) berries facing sun during late morning and afternoon (hereafter named "sunny side") and (ii) berries facing the interior part of the canopy (hereafter named "shaded side").

Two-days old *L. botrana* females that had mated and had started to lay eggs in the laboratory were transferred to the field. Four females were released inside each cage. The

3rd day of each period the cages were removed and for the sunlight exposed bunches the sunny- and shaded-side berries were distinguished and marked. The 7th day of each period, bunches were collected and transferred to the laboratory to be checked under dissection microscope. Number of eggs (hatched and dead) laid on each bunch, distinguishing sunny from shaded side for sunlight-exposed bunch, were counted. For the second and third periods, on each bunch also the number of larvae that had built their nest (i.e., larval settlement) was recorded.

During two days of the experiment (14 and 22 July), temperatures of berries were measured in the warmest hours. The measurements were done using a portable handheld noncontact infrared thermometer Raynger® ST Raytek™ (Spectrum® Technologies, Inc.). During the three experimental-periods the air temperature was measured using an iButton® computer microchip temperature logger (DS1922L Thermochron®, Maxim Integrated Products, Inc.) interfaced to Thermodata viewer package (Thermodata Pty. Ltd.). Two microchips were placed into the shaded canopy zone at 180 cm from ground level. Climatic data from a weather station (Sant'Osvaldo, Udine, OSMER) located 500 m from the vineyard were also used (http://www.osmer.fvg.it/OSMER).

To compare the number of eggs in sunny and shaded sides of sunlight-exposed bunch and that on both-sides of exposed and covered bunches, a t-test was used. To compare the percentage of dead eggs on total laid eggs and larval settlement on newly-hatched larvae, Cochram Q test was used.

RESULTS

1. Experiments in climatic chamber

1.1. *Lobesia botrana* eggs exposed to high temperatures

1.1.1. Experiment type 1: Exposure of eggs to different constant temperatures

Almost all eggs exposed to 43 °C for one exposure period of 6 h died (Fig. 14). The differences were highly significant in comparison with the control (24 °C) both at white (Cochran Q = 13.34, P = 0.0003) and red-eyes stages (Cochran Q = 12.01, P = 0.0005).

For eggs exposed to 40 °C for one exposure period of 6 h, the average mortality ranged from 46.8% to 75.4% in relation to the different stages (Fig. 15). Statistically differences were observed in comparison with control (24 °C) for all egg developmental stages (white, Cochran Q = 9.67, P = 0.0019; red-eyes, Cochran Q = 13.04, P = 0.0003; black-head, Cochran Q = 12.82, P = 0.0003). The Abbott mortality was not significantly different among the three egg stages ($F_{2,9} = 2.65$, P = 0.12).

For eggs exposed to 37 °C for one exposure period of 6 h, the mortality was less than 10% and no significant effect was detected either for white ($\chi^2 = 0.097$; P = 0.76) or for red-eyes stages ($\chi^2 = 0.041$; P = 0.84) in comparison with control (24 °C) (Fig. 16).

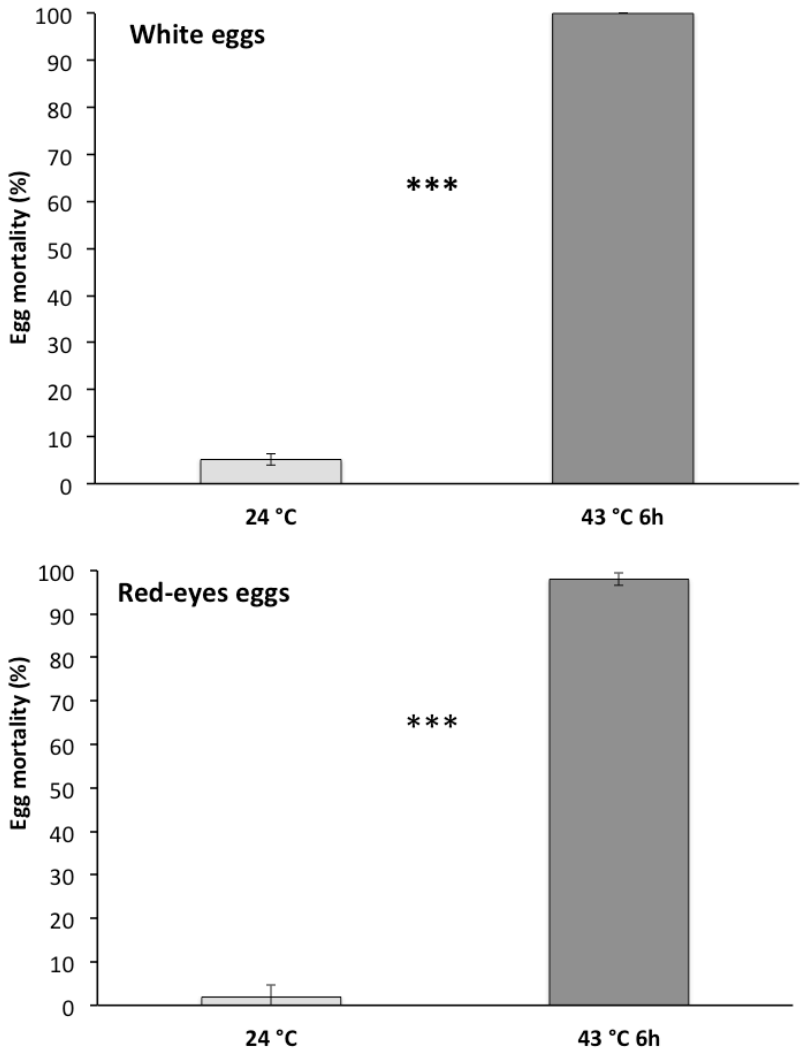


Figure 14 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on plastic bags and exposed to two different temperature regimes at two different egg stages. *** = significant differences at Cochran Q test <0.001.

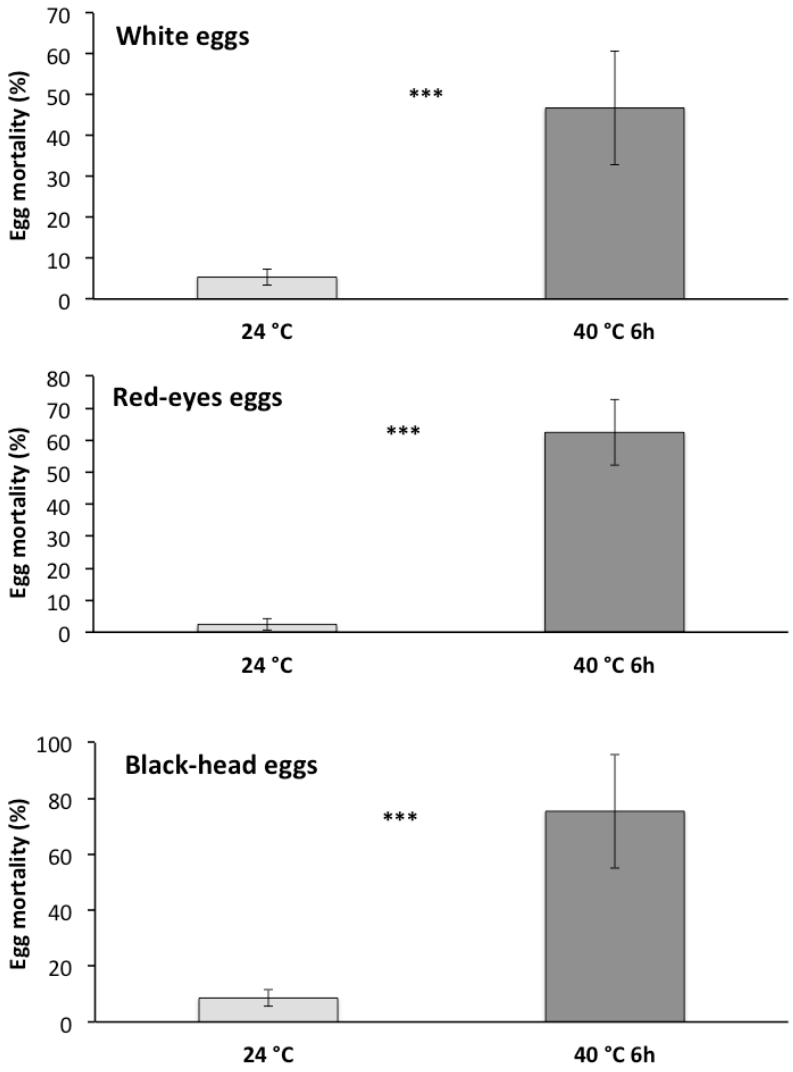


Figure 15 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on plastic bags and exposed to two different temperature regimes at three different egg stages. *** = significant differences at Cochran Q test < 0.001.

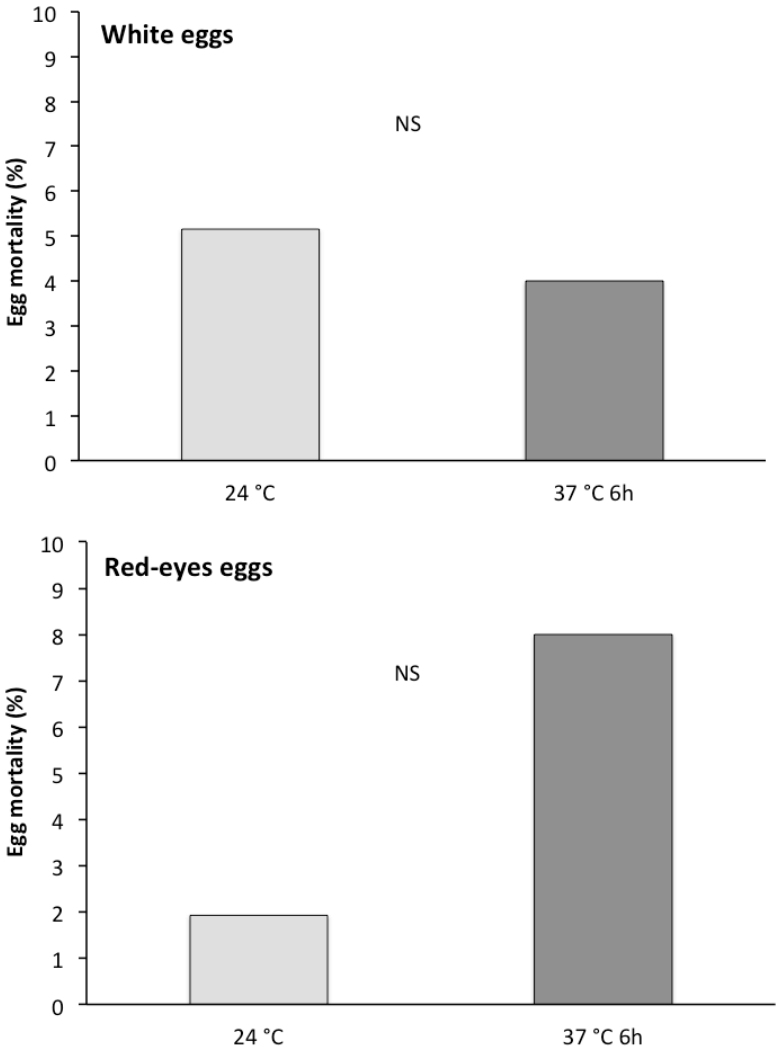


Figure 16 – Mortality of *Lobesia botrana* eggs laid on plastic bags and exposed to two different temperature regimes at two different egg stages. NS = not significant differences at χ^2 <0.05.

Concerning exposure to 40 °C for one or two exposure periods of 3 h, white eggs did not show a significant increase of mortality in comparison with the control (24 °C) ($F_{2,6}$ = 2.6, P = 0.18) (Fig. 17). Red-eyes eggs exposed to 40 °C for one or two exposure periods of 3 h had a significant higher mortality than control (24 °C) ($F_{2,6}$ = 148.2, P < 0.0001) and two exposure periods significantly increased the mortality in comparison with one. Black-head eggs exposed to 40 °C for one or two exposure periods of 3 h had a significant higher mortality than control (24 °C) ($F_{2,6}$ = 39.0, P < 0.0004) and no significant difference between one and two exposure periods was observed. Considering the eggs exposed to one exposure period of 3 h at 40 °C, the Abbott mortality was significantly higher for red-eyes and black-head eggs than for white eggs ($F_{2,6}$ = 19.16, P = 0.0025). Considering the eggs

exposed to two exposure periods of 3 h at 40 °C, the Abbott mortality was significantly higher for black-head eggs than for white eggs ($F_{2,6} = 8.36$, $P = 0.018$) with red-eyes eggs not different from the other two egg stages.

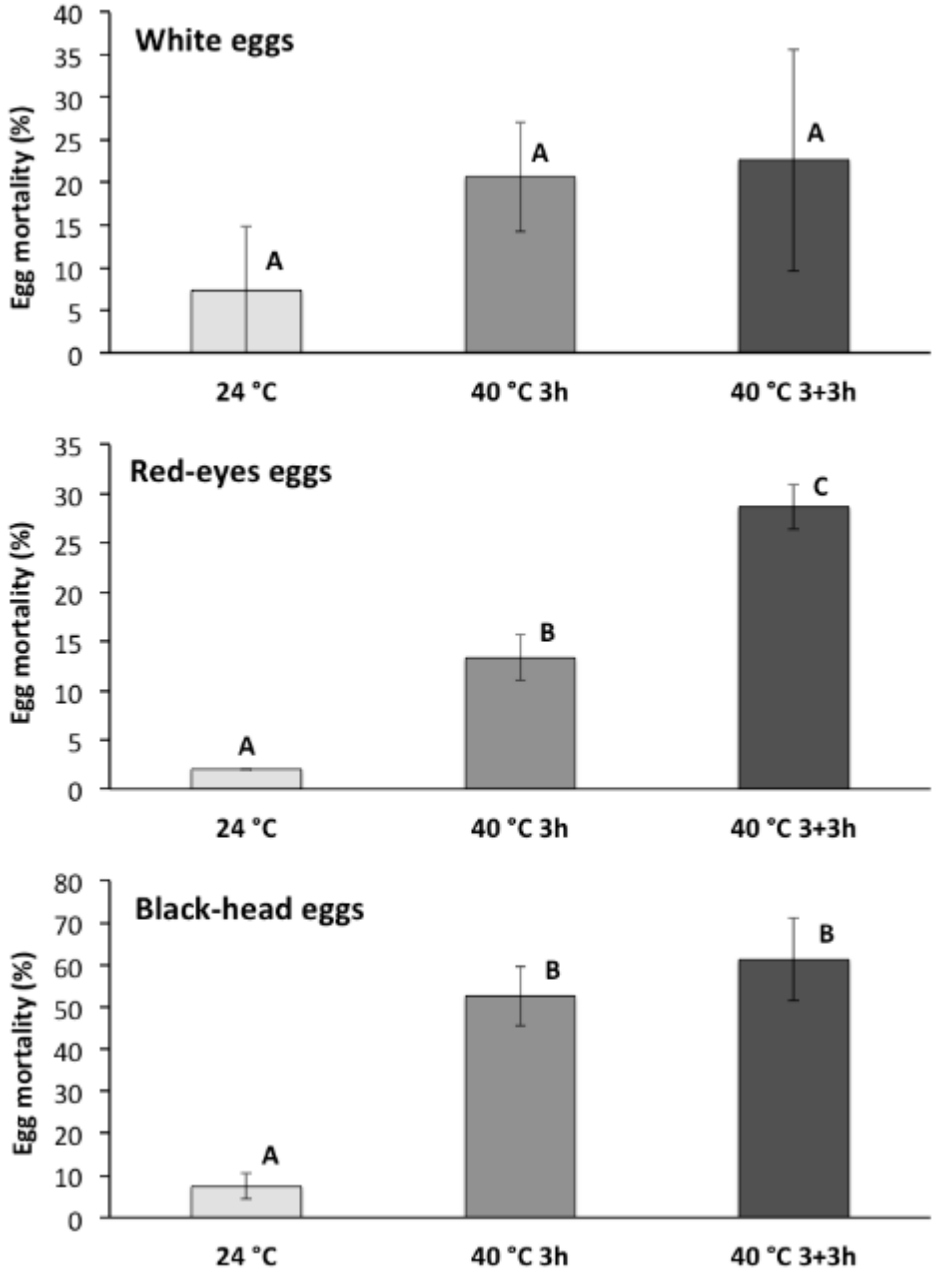


Figure 17 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on plastic bags and exposed to three different temperature regimes at three different egg stages. Different capital letters indicate significant differences at Tukey's test < 0.01.

Exposure to 40 °C for one or two exposure periods of 6 h significantly increased the mortality of white eggs and red-eyes in comparison with corresponding control (24 °C) (for white eggs, $F_{2,6} = 29.0$, $P = 0.004$; for red-eyes eggs, $F_{2,6} = 92.3$, $P = 0.0004$) (Fig. 18). For both white and red-eyes eggs, two exposure periods significantly increased the mortality in comparison with one. Considering the eggs exposed for one or two exposure periods of 6 h at 40 °C the Abbott mortality was significantly higher for red-eyes eggs than for white eggs (one exposure period, $t_4 = 9.27$, $P = 0.0008$; two exposure periods, $t_4 = 2.92$, $P = 0.043$).

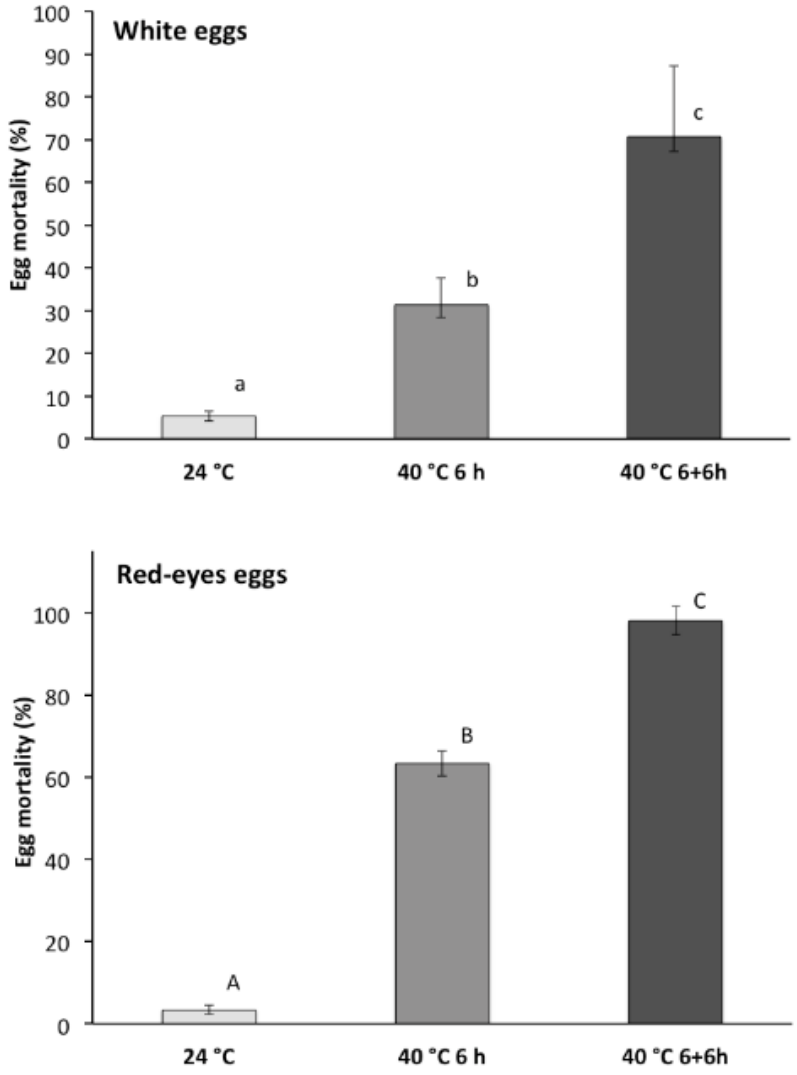


Figure 18 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on plastic bags and exposed to three different temperature regimes at two different egg stages. Different small and capital letters indicate significant differences at Tukey's test < 0.05 and < 0.01, respectively.

Considering together the last two experiments at 40 °C (Fig. 17 and 18), the Abbot mortality was significantly higher for red-eyes eggs in comparison with white eggs, for 6 h of exposure in comparison with 3 h of exposure and for two exposure periods in comparison with one (Tab. 2). All two-way interactions were significant, whereas the three-way interaction was not.

Table 2 – Comparison of Abbott mortality in *Lobesia botrana* eggs laid on plastic bags and exposed to 40 °C for 3 or 6 h and for one or two exposition periods.

Source of variation	F	P	mean±standard deviation
A. Egg stage	$F_{1,16}$= 36.41	1.08×10^{-7}	
- white			28.7±23.5
- red eyes			48.7±33.7
B. Exposure hours	$F_{1,16}$= 125.3	2.52×10^{-16}	
- 3 h			17.0±26.8
- 6 h			60.4±12.5
C. Exposure periods	$F_{1,16}$= 37.18	8.43×10^{-8}	
- one			28.7±22.1
- two			48.7±34.7
A×B	$F_{1,16}$= 25.86	3.87×10^{-6}	
A×C	$F_{1,16}$= 5.71	0.02	
B×C	$F_{1,16}$= 18.78	5.67×10^{-5}	
A×B×C	$F_{1,16}$= 1	0.32	

Exposure to 37 °C for one or two exposure periods of 6 h did not significantly increase the mortality of white, red-eyes and black-head eggs in comparison with corresponding control (24 °C) (for white eggs, $F_{2,6} = 0.2$, $P = 0.81$; for red-eyes eggs, $F_{2,6} = 1.6$, $P = 0.25$; for black-head eggs, $F_{2,6} = 3.6$, $P = 0.09$) (Fig. 19).

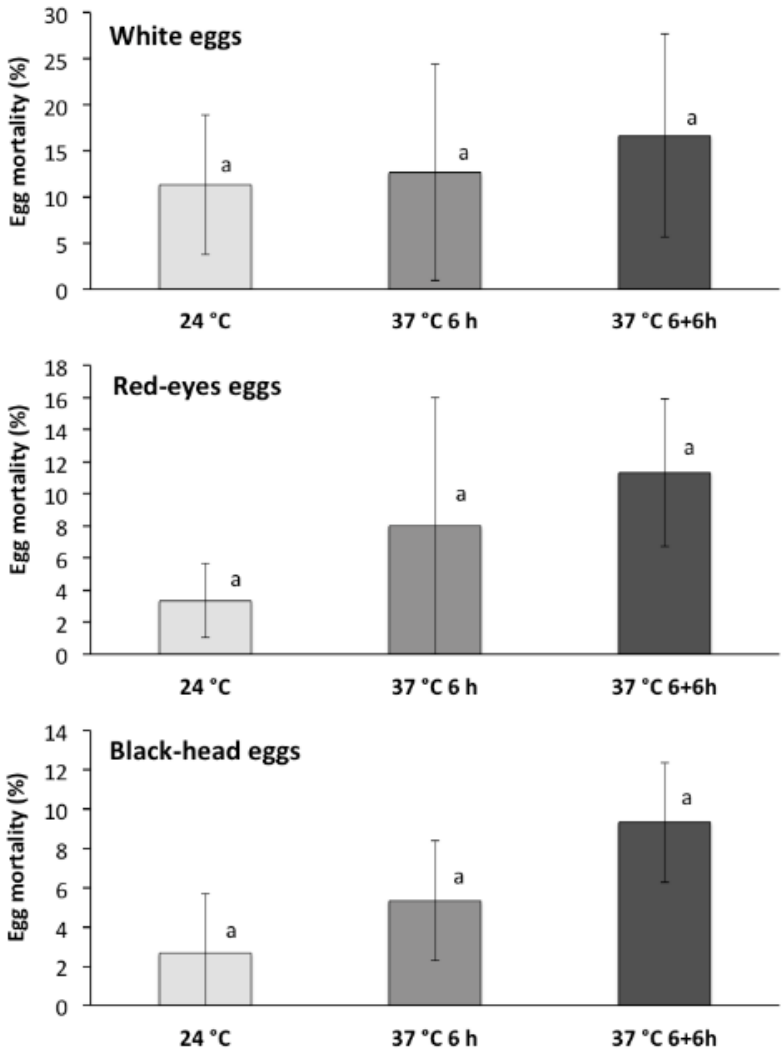


Figure 19 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on plastic bags and exposed to three different temperature regimes at three different egg stages. Different small letters indicate significant differences at Tukey's test < 0.05.

1.1.2. Experiment type 2: Exposure of different egg stages to different temperature cycles

Exposure to a 24-h temperature cycle characterized by a peak of 40 °C for 4 h showed a significant higher mortality than control characterized by a peak of 30 °C for 6 h, for all egg-laying substrates and egg stages (Fig. 20, 21 and 22).

Considering together the three experiments, the Abbot mortality was significantly higher for white eggs in comparison with other egg stages and for red-eyes eggs in comparison with black-head eggs (Tab. 3). The egg-laying substrate did not influence the egg mortality. A significant interaction between egg stage and substrate was observed, probably due a higher mortality of white eggs on berry control (see Fig. 20).

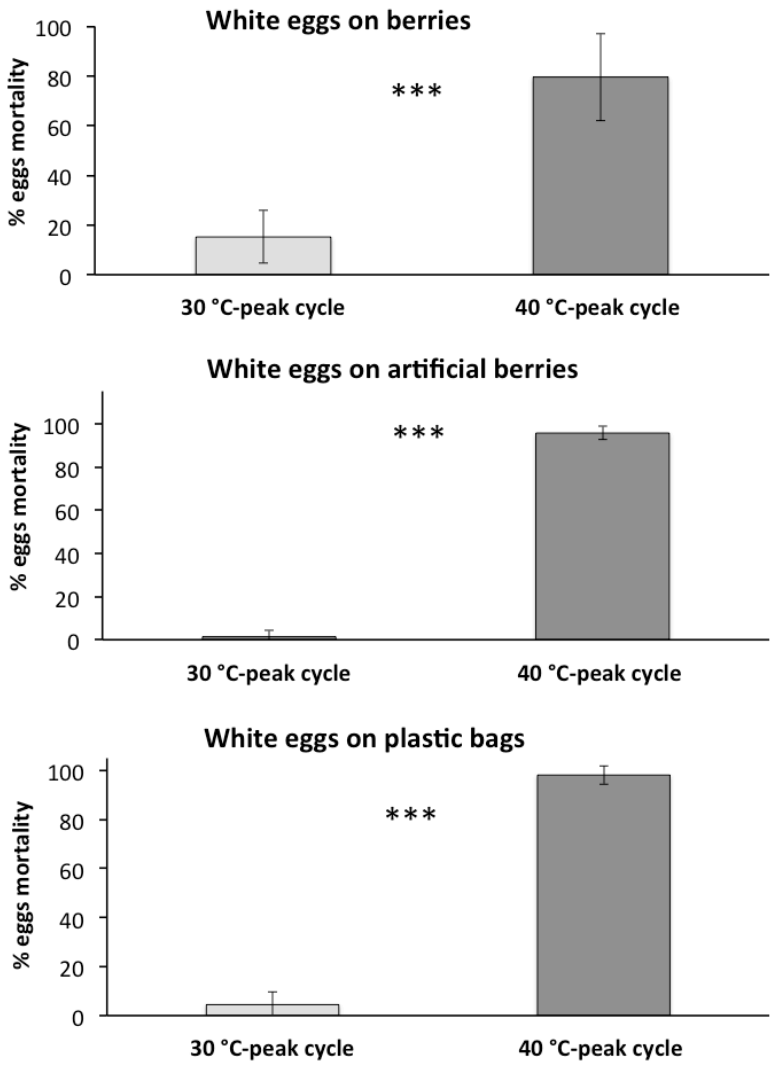


Figure 20 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on three different substrates and exposed at white stage to two different 24-h cycles of temperatures characterized respectively by a peak of 6 h at 30 °C (air cycle) and a peak of 4 h at 40 °C (berry cycle). *** = significant differences at Cochran Q test < 0.001 (for berries, Cochram Q = 11.2, P = 0.0008; for artificial berries, Cochram Q = 13.84, P = 0.0002; for plastic bag, Cochram Q = 18.20, P < 0.0001).

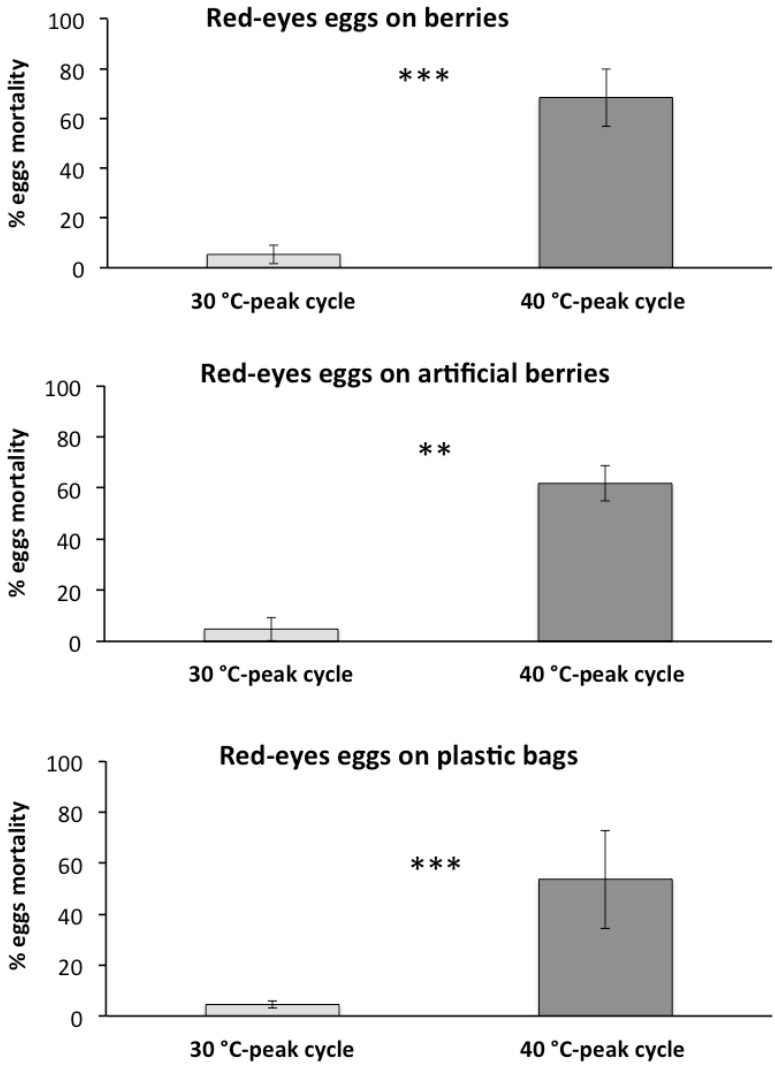


Figure 21 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on three different substrates and exposed at red-eyes stage to two different 24-h cycles of temperatures characterized respectively by a peak of 6 h at 30 °C (air cycle) and a peak of 4 h at 40 °C (berry cycle). ** and *** = significant differences at Cochran Q test < 0.01 and < 0.001 respectively (for berries, Cochram Q = 10.45, P = 0.0012; for artificial berries, Cochram Q = 9.50, P = 0.002; for plastic bag, Cochram Q = 11.37, P = 0.0008).

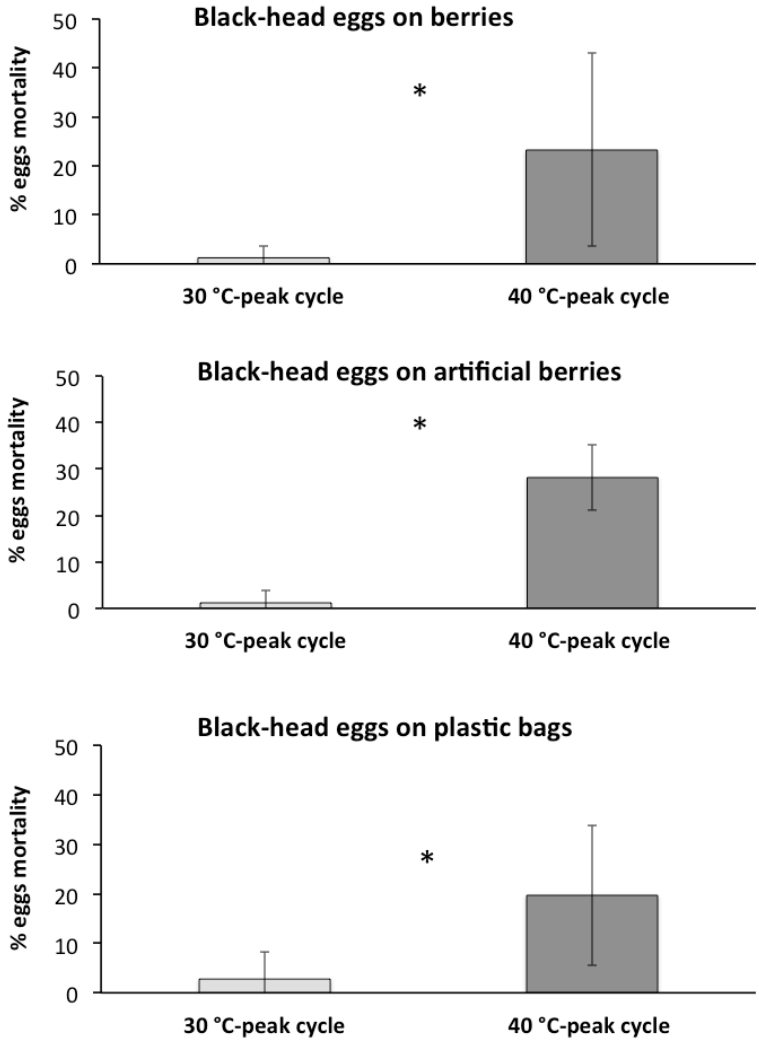


Figure 22 – Mortality of *Lobesia botrana* eggs laid (mean±standard deviation) on three different substrates and exposed at black-egg stage to two different 24-h cycles of temperatures characterized respectively by a peak of 6 h at 30 °C (air cycle) and a peak of 4 h at 40 °C (berry cycle). * = significant differences at Cochran Q test < 0.05 (for berries, Cochram Q = 5.33, P = 0.0021; for artificial berries, Cochram Q = 4.25, P = 0.039; for plastic bag, Cochram Q = 5.06, P < 0.0024).

Table 3 – Three-way ANOVA analysis on Abbott mortality of *Lobesia botrana* eggs recorded in the three experiments with different variable temperatures. Different small letters on the column indicate significant differences at 0.05 level (Tukey's test).

Source of variation	F	P	Mean±standar deviation
A. Egg stage	$F_{2,27} = 68.57$	2.6×10^{-11}	
- white			90.5±14.1 c
- red eyes			59.3±14.4 b
- black head			23.3±13.4 a
B. Substrate	$F_{2,27} = 0.69$	0.51	
- berries			55.7±29.1 a
- artificial berries			60.8±29.5 a
- plastic bag			56.5±36.34 a
A×B	$F_{4,27} = 2.73$	0.050	

1.2. Newly-hatched larvae of *Lobesia botrana* exposed to different temperature cycles

Newly-hatched larvae exposed to 24-h temperature cycles including 4 h at high temperatures (37 or 40 °C) showed a significant decrease of settlement in comparison with larvae exposed to a 24-h temperature cycle with a peak of 30 °C for 6 h (for "37 °C cycle", Cochram Q = 3.88, P = 0.049; for "40 °C cycle", Cochram Q = 6.33, P = 0.012) (Fig. 23).

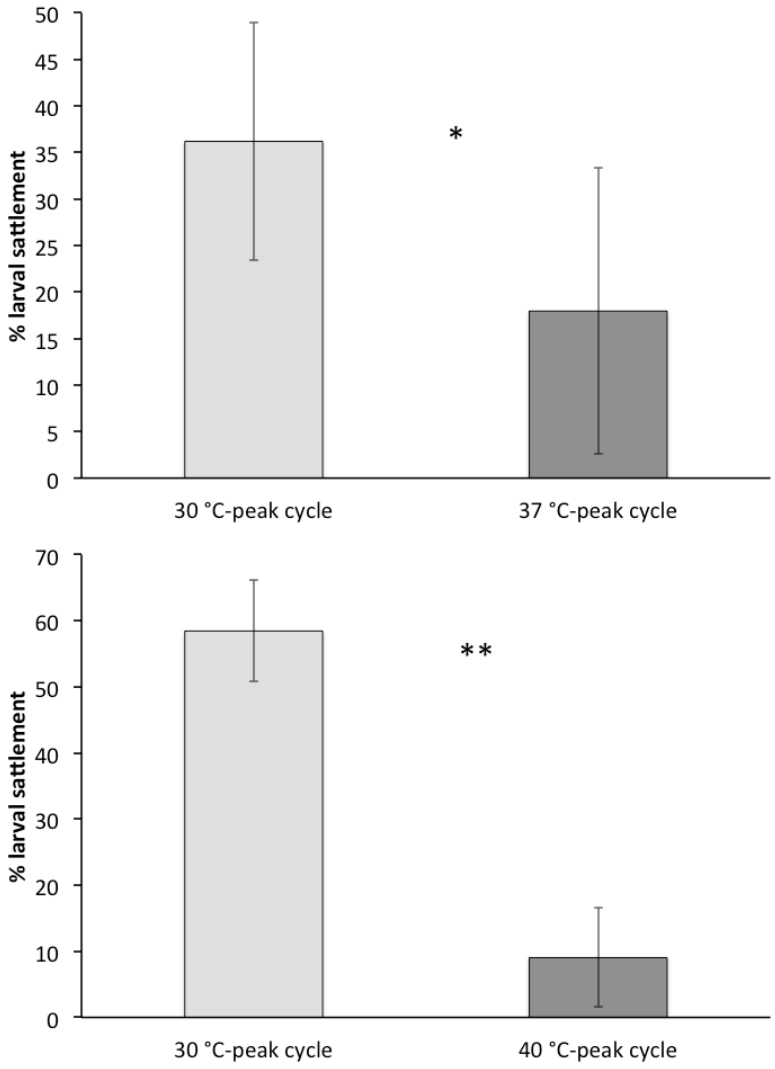


Figure 23 – Percentage of newly-hatched larvae (mean±standard deviation) of *Lobesia botrana* settled on grapevines berries after exposure in climatic chamber to a 24-h temperature cycle simulating air temperature (peak of 30 °C for 6 h) or 24-h temperature cycle simulating sunlight-exposed berry temperature (peak of 37 °C or 40 °C for 4 h). * and ** = significant differences at Cochran Q test < 0.05 and < 0.01 respectively.

2. Experiments in the field

2.1. Temperatures recorded on berries during *Lobesia botrana* third-flight

2.1.1. Vineyard A

During measurement period, the air temperatures were on average 29.5 °C and ranged from 27.1 °C (17:00) to 30.7 °C (14:00) (Fig. 24).

The average temperature of leaves non-exposed to sunlight were 28.5 °C and ranged from 27.0 °C (17:00) to 29.5 °C (14:00). They were lower than air temperatures from 10:00 to 16:00 with maximum differences at 11:00 e 12:00.

The temperatures of leaves exposed to sunlight were on average 32.7 °C and ranged from 30.4 °C (11:00) to 34.7 °C (16:00). They were always higher than air temperatures with differences progressively increasing from 11:00 (0.94 °C) to 16:00 (5.1 °C).

The average temperature of berries non-exposed to sunlight was 31.1 °C and ranged from 29.1 °C (10:00) to 32.8 °C (15:00). They were always higher than air temperature with a difference trend increasing from 12:00 (0.49 °C) to 17:00 (3.14 °C).

The temperatures of berries exposed to sunlight were on average 37.4 °C and ranged from 32.0 °C (10:00) to 40.6 °C (15:00). They were higher than 36 °C from 11:00 to 17:00 and always above air temperature with a difference trend increasing from 10:00 (3.6 °C) to 15:00–17:00 (10.1–10.3 °C).

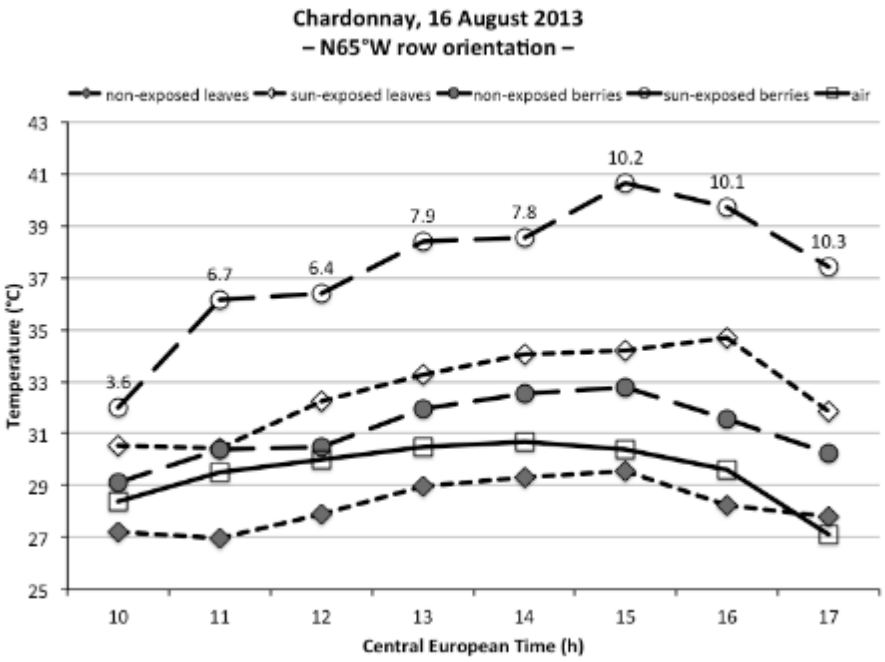


Figure 24 – Temperatures recorded in vineyard A from 10:00 to 17:00 CET on leaves and berries, both sun-exposed and not sun-exposed. Also the average air temperature in the different CET hours is reported. Labels on "sun-exposed berries" temperatures indicate the difference between these temperatures and air temperatures in the different CET hours.

The leaves and berries exposed to sunlight always showed higher temperatures than those non-exposed (leaves: 28.3±0.96 for non-exposed and 32.7±1.7 for exposed, t_{14} = 6.53, P < 0.0001; berries: 31.1±1.28 for non-exposed and 37.4±2.7 for exposed, t_{14} = 5.99, P < 0.0001) (Fig. 24). The minimum and maximum differences in temperature for both leaves and berries were recorded at 10:00 (3.32 °C and 2.88 °C) and 16:00 (6.44 °C and 8.14 °C), respectively, with almost always increasing trend between 10:00 and 16:00.

During the sampling period both non-exposed and sunlight exposed berries showed higher average temperatures of the corresponding leaves, showing a lesser ability of thermoregulation (non-exposed: 31.1±1.28 for berries and 28.3±0.96 for leaves, t_{14} = 5.12, P = 0.0002; exposed: 37.4±2.7 for berries and 32.7±1.7 for leaves, t_7 = 4.27, P = 0.0008).

2.1.2. Vineyard B

During the measurement period, the average air temperature was 34.1 °C and varied from 30.6 °C (9:00) to 35.6 °C (15:00-16:00) (Fig. 25).

In the NS oriented row the berries of east-facing bunches had temperatures similar to air temperature (on average only 0.9 °C higher), even if at 9:00 and 10:00 the

temperatures were of 5.6 °C and 6.9 °C higher than air temperature, respectively. The berries of west-facing bunches had temperatures higher than air temperature (on average 3.2 °C higher) with differences higher than 5 °C from 15:00 to 18:00 (peak 11.6 °C).

In the EW oriented row the berries of north-facing bunches had temperatures similar to air temperature (on average only 0.1 °C higher). The berries of south-facing bunches showed higher temperature than air temperature (on average 3.1 °C higher) with differences higher than 7 °C from 11:00 to 13:00.

In the N45°E oriented row the berries of north-west facing bunches had temperatures a little higher than air temperature (on average only 0.1 °C higher) due to a small increase in temperature from 15:00 to 18:00 (max differences at 18:00 with 4.5 °C). The berries of south-east facing bunches showed higher average temperature than air temperature (5.7 °C higher) and in particular the temperature was higher from 9:00 to 12:00 with differences ranging from 4.7 °C to 9.3 °C (7.2 °C on average).

In the N45°W oriented row the berries of north-east facing bunches had temperatures a little higher than air temperature on average (1.1 °C higher) and continuously from 11:00 to 15:00, although these berries were never directly exposed to sun rays in this interval. The berries of south-west facing bunches showed on average a very higher temperature than air temperature (7.1 °C) and always more than 5° C higher from 11:00 to 18:00 reaching a max difference of 12.1 °C (8.5 °C on average).

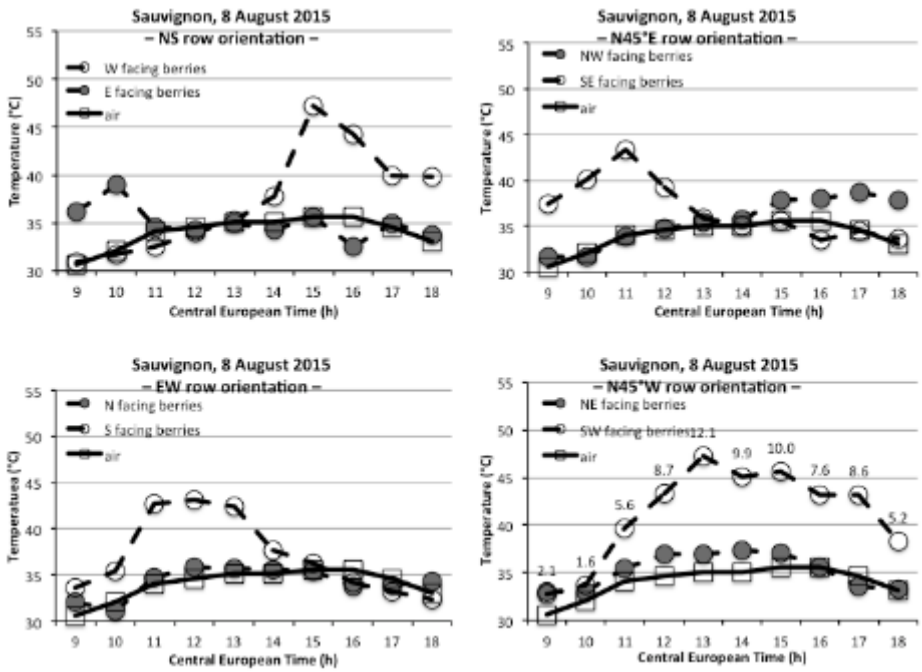


Figure 25 – Temperatures recorded in vineyard B from 9:00 to 18:00 CET on berries of bunches not covered by leaves placed on both sides of differently oriented rows. Also the average air temperature in the different CET hours is reported. Labels on “SW facing berries” temperatures indicate the difference between these temperatures and air temperatures in the different CET hours.

The berry average temperatures of bunches not covered by leaves were the highest for the south-west facing berries, intermediate for those facing south-east, south and west, and slightly higher or equal to air average temperature for those of other orientations (Tab. 4). The differences in average berry temperatures between the different exposures showed a good correlation with insolation hours ($P = 0.0102$) and temperature peak ($P = 0.0055$) of berries. The berries average temperatures were significantly higher for west in comparison with east facing barriers and for south-west in comparison with both south and south-east facing berries showing the direct sun rays exposure in the afternoon is more favourable to high temperatures of berries than that in the late morning. The berries of the NS oriented row had more hours with temperatures above 37 °C than the EW oriented row, although this latter berries faced south.

Table 4 – Temperatures and insolation hours recorded in vineyard B on berries facing different cardinal or inter-cardinal directions. Different small letters on the column indicate significant differences at 0.05 level (Tukey's test).

Row orientation	Berries facing	Sampling period with direct sunlight on berries (number of sampling in the period)	Berry temperatures (average °C ± standard deviation)*	Berry temperature peak (°C)	Number of sampling times with berries above 37 °C
NS	E	9:00–11:00 (3)	35.0±0.30 ab	39.0	1
	W	13:00–18.00 (6)	37.33±0.87 c	47.2	5
EW	N	18:00 (1)	34.18±0.26 a	35.8	0
	S	10:00–15:00 (6)	37.12±0.70 c	43.2	4
N45°E	NW	14:00–18:00 (5)	35.54±0.46 b	38.6	4
	SE	9:00–13:00 (5)	36.85±0.43 c	43.4	4
N45°W	NE	(0)	35.21±0.26 ab	37.4	0
	SW	11:00–18:00 (8)	41.2±0.94 d	47.2	8
Air temperature			34.1	35.6	0

* ANOVA $F_{7,32} = 69.79$; $P < 0.0001$

2.2. *Lobesia botrana* eggs laid on berries exposed or not exposed to sunlight

2.2.1. Number of eggs per cage

On exposed bunches, *L. botrana* females laid more eggs on sunny side than on shaded side, but the differences were significant only for the period 10–17 July (7–14 July, $t_9 = 2.19$, $P = 0.056$; 10–17 July, $t_8 = 3.20$, $P = 0.013$; 17–24 July, $t_3 = 1.09$, $P = 0.36$) (Fig. 26).

L. botrana females laid a not significant different number of eggs in the exposed and covered bunches (7–14 July, $t_9 = 1.12$, $P = 0.29$; 10–17 July, $t_8 = 0.80$, $P = 0.45$; 17–24 July, $t_4 = 1.28$, $P = 0.27$) (Fig. 26).

Comparing sunny side of exposed bunches with all other positions (i.e., shaded side of exposed bunches and both sides of covered bunches), females laid more eggs on shaded positions, but the differences were significant only for the period 7–14 July (7–14 July, $t_9 = 2.68$, $P = 0.025$; 10–17 July, $t_8 = 1.87$, $P = 0.10$; 17–24 July, $t_4 = 2.28$, $P = 0.09$) (Fig. 27). Assuming that the surface of sunny-exposed berries are about a quarter of the total berry surface, the weighted ratio of eggs laid on sun-exposed berries to shaded berries was about

1.5, 1.9, 1.0 in the three periods, respectively. It suggests that not only females did not avoid lay eggs on sun-exposed berries, but that these berries are sometime preferred.

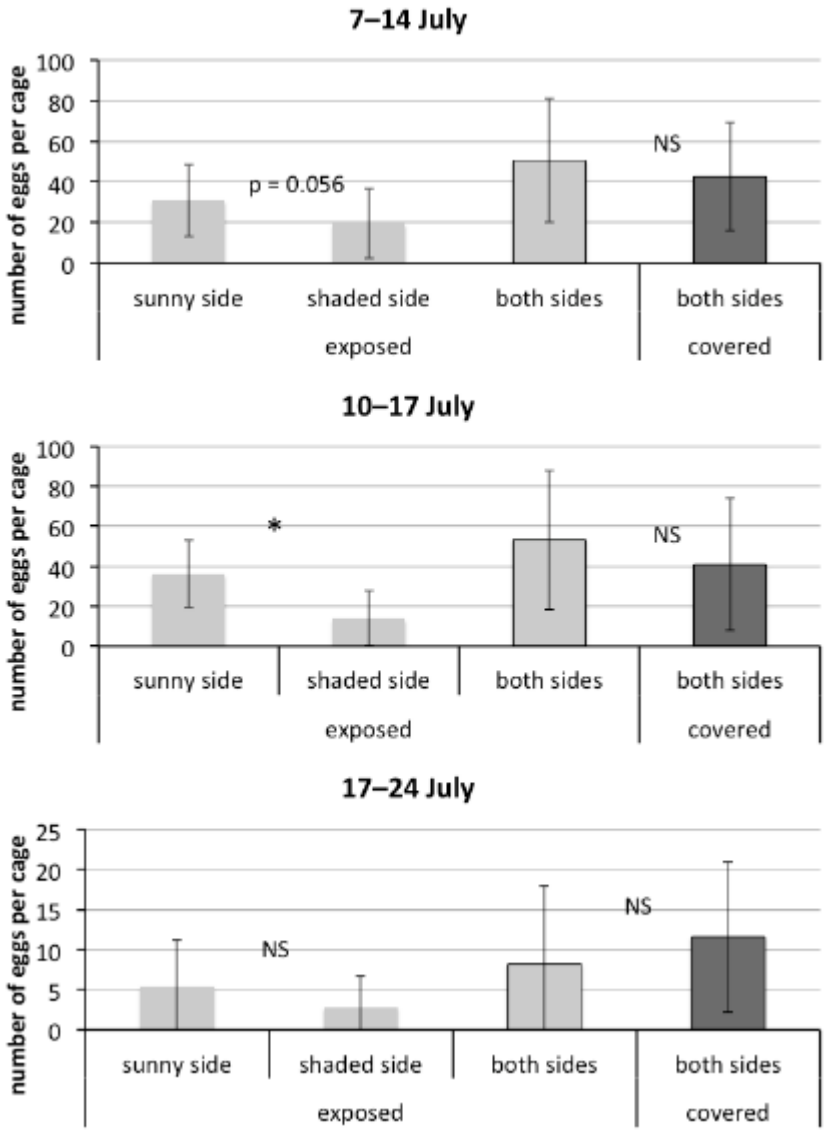


Figure 26 – Number of eggs laid (mean±standard deviation) in the field (vineyard cv. Pinot Gris, locality Udine) by *Lobesia botrana* females on the berries of exposed and covered bunches (i.e., without or with leaf coverage). For exposed bunches the number of eggs laid on sunny and shaded sides was also separately reported. NS and * indicate differences statistically not significant and significant < 0.05, respectively, at a t-test.

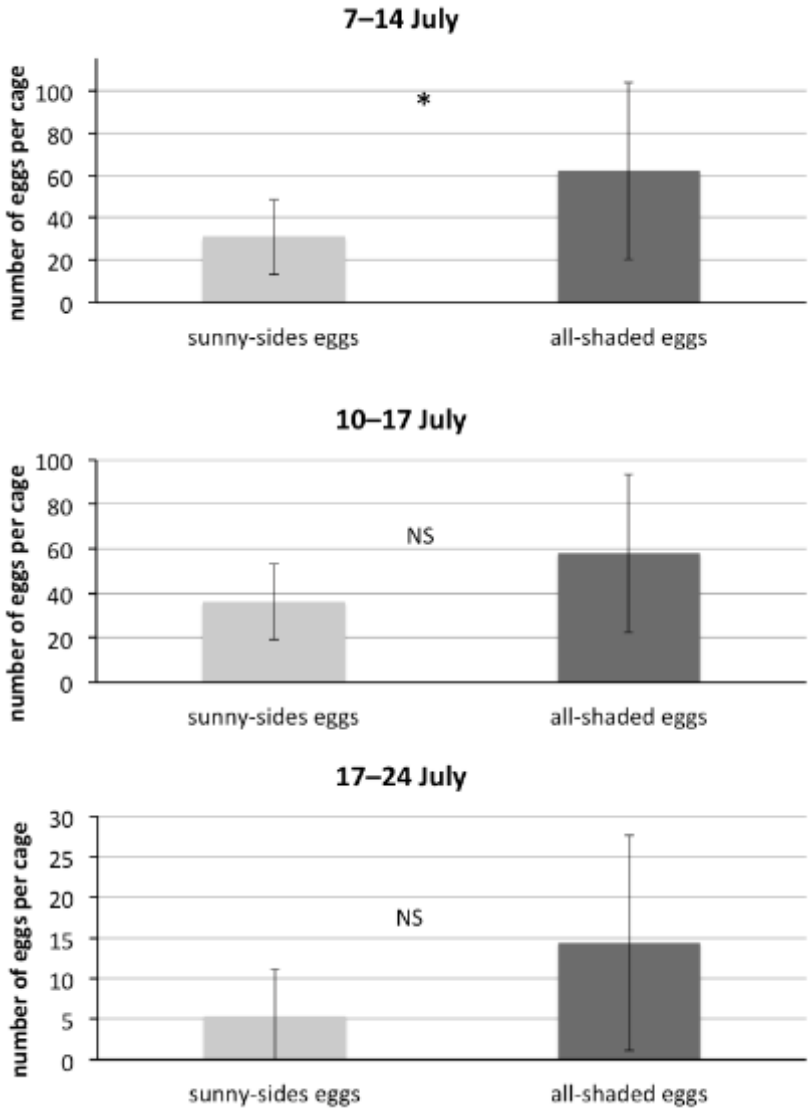


Figure 27 – Number of eggs laid (mean±standard deviation) in the field by *Lobesia botrana* females on sunny-exposed berries (i.e., berries of sunny side of exposed bunches) in comparison with all the shaded berries (i.e., sum of berries of covered bunches and berries of shaded side of exposed bunches). NS and * indicate differences statistically not significant and significant < 0.05, respectively, at a t-test.

2.2.2. Percentage of egg hatching

On exposed bunches, the percentage of *L. botrana* egg hatching was always higher on shaded side but the differences were not significant (7–14 July, Cochram Q = 2.75, P = 0.09; 10-17 July, Cochram Q = 2.12, P = 0.14; 17–24 July, not possible statistical analysis due to lack of eggs in shaded side in one cage) (Fig. 28).

The percentage of egg hatching was always higher on covered bunches than on exposed bunches with significant differences in two of the three periods 7–14 and 17–24

July (7–14 July, Cochram Q = 5.4, P = 0.02; 10-17 July, Cochram Q = 1.08, P = 0.31; 17–24 July, Cochram Q = 6.42, P = 0.008) (Fig. 28).

Comparing sunny side of exposed bunches with all other positions, the percentage of egg hatching was always higher in shaded positions with significant differences in two of the three periods (7–14 July, Cochram Q = 4.45, P = 0.035; 10-17 July, Cochram Q = 2.39, P = 0.12; 17–24 July, Cochram Q = 6.38, P = 0.011) (Fig. 29).

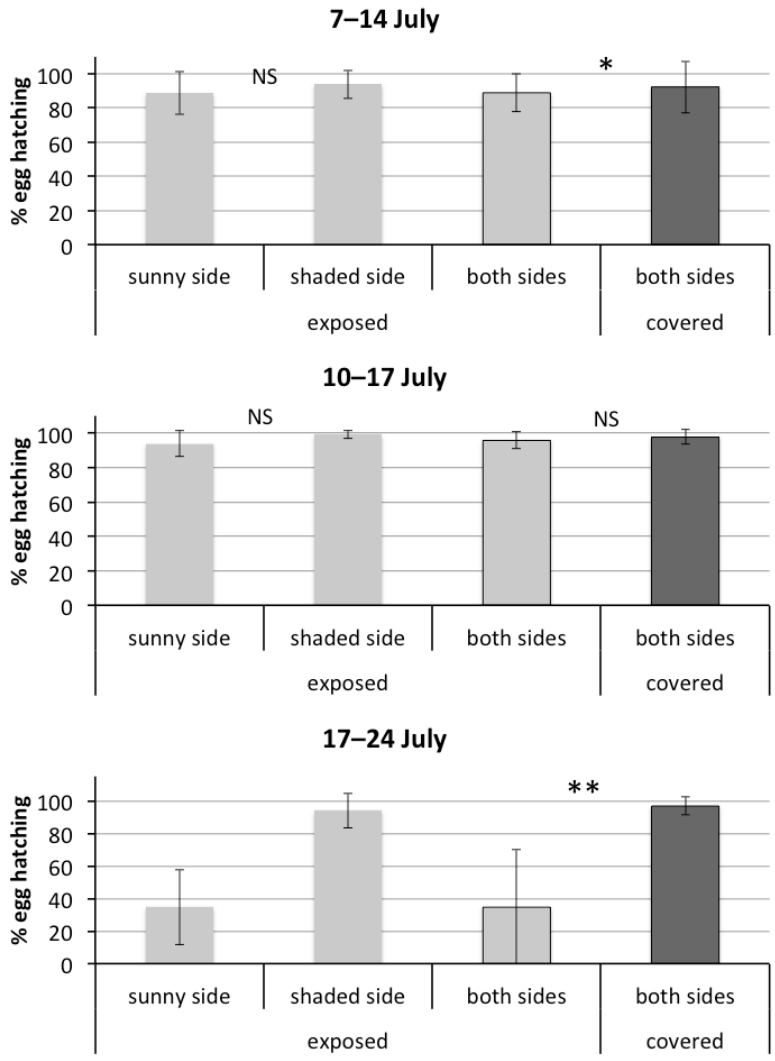


Figure 28 – Percentage of *Lobesia botrana* eggs hatched (mean±standard deviation) in the field on all the berries of exposed and covered bunches (i.e., without or with leaf coverage). For exposed bunches the % egg hatching on sunny and shaded sides was also separately reported. In the period 17–24 July the percentage on shaded side and sunny side were not statistically compared because in shaded side of a cage there were not eggs laid. NS, *, ** indicate differences statistically not significant and significant < 0.05, < 0.01, respectively, at Cochram Q test.

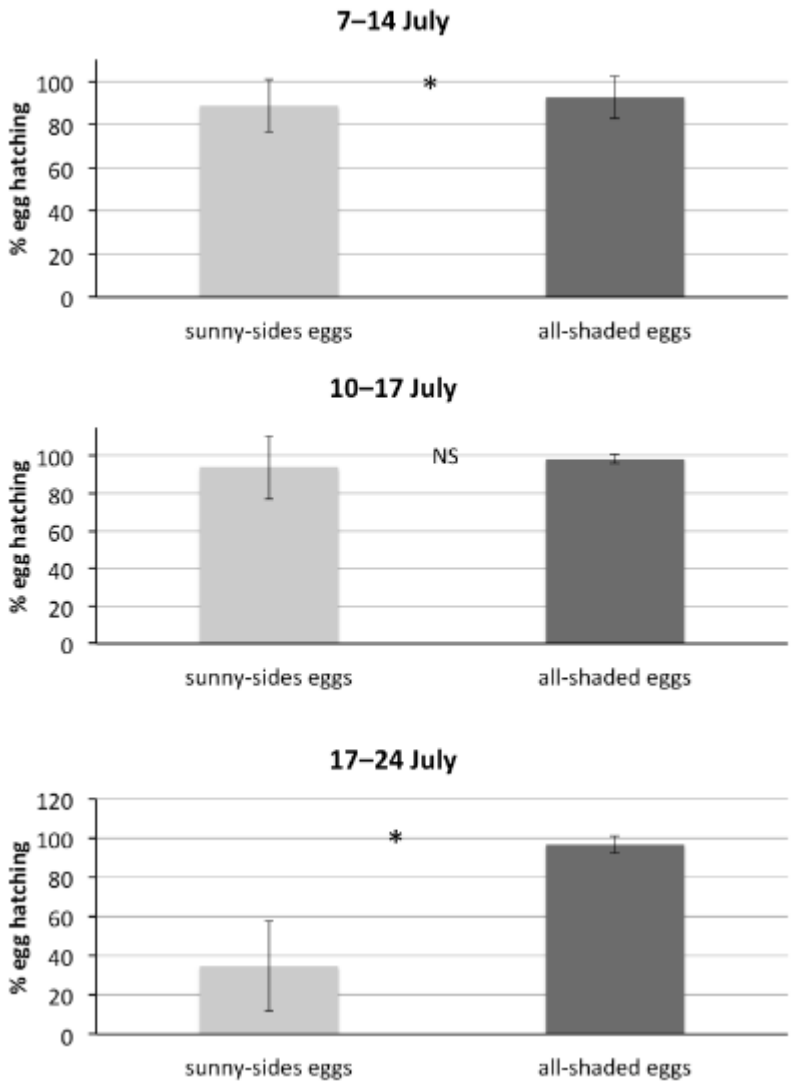


Figure 29 – Percentage of *Lobesia botrana* eggs hatched (mean±standard deviation) in the field on sunny-exposed berries (i.e., berries of sunny side of exposed bunches) in comparison with all shaded berries (i.e., sum of berries of covered bunches and berries of shaded side of exposed bunches). NS and * indicate differences statistically not significant and significant < 0.05, respectively, at Cochram Q test.

2.2.3. Percentage of larval settlement

The percentage of larval settlement, based on newly-hatched larvae, was always lower on exposed than on covered bunches with significant differences in one of the two periods in which the larval settlement was recorded (10-17 July, Cochram Q = 8.64, P = 0.0033; 17–24 July, Cochram Q = 2.26, P = 0.13) (Fig. 30).

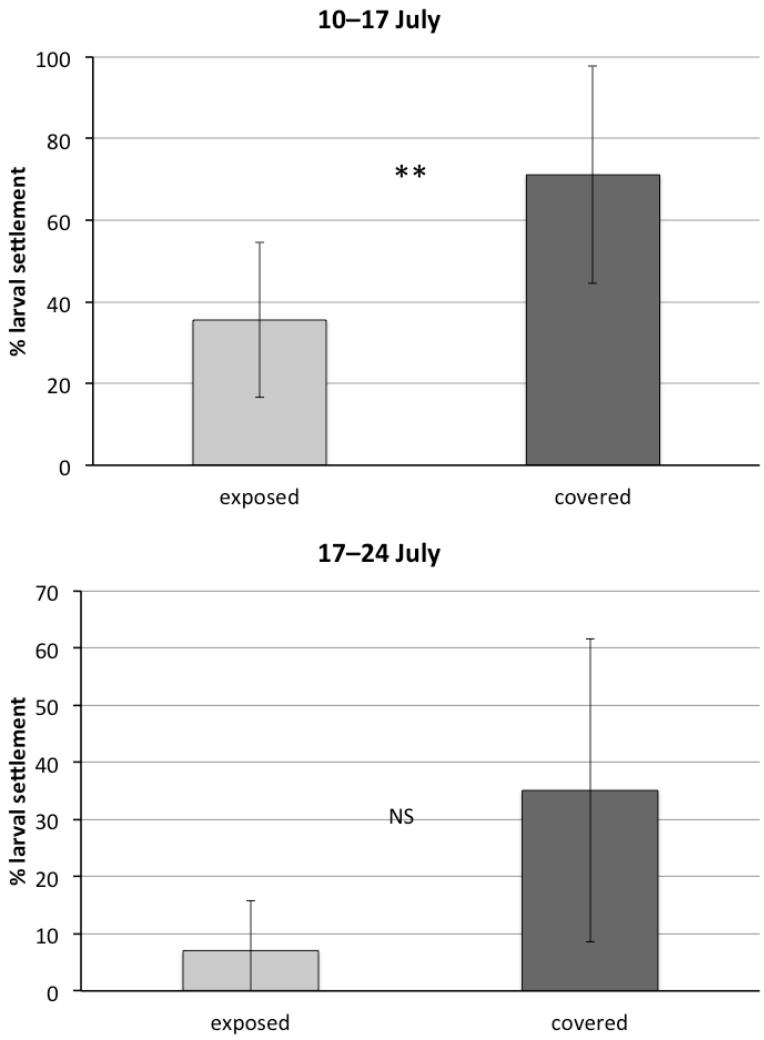


Figure 30 – Percentage of *Lobesia botrana* newly-hatched larvae (mean±standard deviation) that settled in the field on the berries of exposed and covered bunches (i.e., without or with leaf coverage). NS, ** indicate differences statistically not significant and significant < 0.01, respectively, at Cochram Q test.

2.2.4. Temperatures recorded and egg/larval mortality

Increasing average air temperatures were observed in the field from the first to the third periods, with differences of about 1 °C from the first to the second period and about 3 °C between the last two periods (Tab. 5). The highest air temperatures recorded in the third period were associated with lower both number of cages with eggs (10, 9, 4 in the three periods, respectively) and eggs per cage (93, 93, 25 in the three periods, respectively). Considering total eggs, the highest air temperatures recorded in the third period determined also a higher total egg mortality (8, 4, 23% in the three periods, respectively) and larval mortality (55, 68% in the last two periods, respectively). Considering only the eggs laid on

exposed bunches, the negative effect of the highest air temperatures recorded in the third period was even more evident on both egg mortality (11, 5, 51% in the three periods, respectively) and larval mortality (65, 84% in the last two periods, respectively).

Temperature measurements of sun-exposed berries on 14 July, in coincidence with expected egg-hatching of the first period, showed peaks of 37 °C at 12:00 (CET) and 43 °C at 15:00 with difference from air temperatures of about 4 and 8 °C, respectively. Temperature measurements of sun-exposed berries on 22 July, in coincidence with expected egg-hatching of the third period, showed peaks of 50 °C at 12:00 and 48 °C at 15:00 with difference from air temperatures of about 12 and 9 °C, respectively.

Table 5 – Air temperatures recorded during the phenological stages of *Lobesia botrana* in three experiments (periods) carried out in the Pinot Gris vineyard located at Udine.

Experimental period and *Lobesia botrana* phenology	**Average temperatures (°C) recorded during egg-laying and -hatching**	
	Daily average	**Daily max**
Period 7–14 July		
- egg-laying days (8–10)	24.4	29.0
- hatching-larvae days (12–14) *	26.1	32.5
Period 10–17 July		
- egg-laying days (11–13)	25.3	31.8
- hatching-larvae days (15–17)	27.8	34.2
Period 17–24 July		
- egg-laying days (18–20)	29.3	35.7
- hatching-larvae days (22–24)	30.3	37.2

* 5 days were supposed for egg-hatching

2.2.5. Simulated larval infestation in relation to bunch-zone leaf removal

Considering that by bunch-zone leaf removal bunches are exposed to sunlight and basing on egg distribution among covered and sunlight exposed berries and percentage of larval settlement in the field, the simulated reduction of larval infestation ranged from 28% to 78% in relation to the considered period (i.e., 10–17 or 17–24 July) and two

hypothesized levels of sunlight exposure (i.e., 100% or 50%) ensuring by leaf removal (Tab. 6).

Table 6 – Simulation of *Lobesia botrana* larval infestation based on the field data of periods 10–17 and 17–24 July assuming bunch-zone leaf removal. Three situations were hypothesized: all bunches are covered by leaves and larval settlement was that expected according to the observed settlement in covered bunches; all bunches are sun-exposed (without leaf coverage) and larval settlement was that expected according to the observed settlement in sun-exposed bunches; 50% bunches are exposed and larval settlement was that expected according to spatial distribution of eggs between covered by leaves and sun-exposed bunches and the corresponding larval settlement.

Period	Laid eggs			% larval settlement calculated on laid eggs		Expected alive larvae in three hypothesized situations			% expected reduction of infestation levels	
	Total	Covered	Exposed	Covered	Exposed	100% bunches covered	100% bunches exposed	50% bunches exposed	100% bunches exposed	50% bunches exposed
10–17 July	835	358	477	71.1	35.7	594	298	425	49.8	28.4
17–24 July	98	58	40	35.1	7.6	34	7	23	78.3	32.0

DISCUSSION AND CONCLUSION

Previous research had demonstrated that bunch-zone leaf removal carried out within ten days of the beginning of the *L. botrana* second-flight reduces both second and third generations of the moth (Chapter II; Pavan *et al*., in press).

This study, based on both laboratory and field data, is a large contribution to know the mechanisms by which defoliation reduces moth larval infestations. From the theoretical point of view exposure of bunches to sunlight by bunch-zone leaf removal could (1) reduce the number of laid eggs and/or (2) cause a higher mortality in eggs and/or newly-hatched larvae.

The first hypothesis is true if the following two conditions are satisfied: (i) *L. botrana* females prefer lay eggs on berries of bunches not exposed to sunlight as previously reported in Zahavi *et al.* (2003), and (ii) the exclusive availability of bunches exposed to sunlight determines a lower fecundity of females.

The second hypothesis is more logically acceptable than the first if the eggs and newly-hatched larvae not protected by leaves are more susceptible to meteorological factors (i.e. sunlight, relative humidity and rain). In fact, high temperatures, mostly associated with low relative humidity, cause *L. botrana* egg mortality (Coscollá *et al*., 1986). However, temperatures that cause egg mortality in the laboratory are very higher than those of air temperature in the field conditions and they can be reached only by the berries exposed to sunlight (Kliewer and Lider, 1968; Spayd *et al.*, 2002; Tarara *et al.*, 2008). This second hypothesis is more likely to be true if females prefer lay eggs on berries exposed to sunlight as previously reported in Pavan *et al*. (in press; see Chapter II).

1. *Lobesia botrana* egg mortality in the laboratory

Considering the fact that under field conditions the berries exposed to the sun can reach temperatures (Smart and Sinclair, 1976; Bergqvist *et al*., 2001) that have been proved in the laboratory to cause high egg mortality (Coscollá *et al.*, 1986), and females prefer to lay their eggs on the side of bunches that had been exposed to sunlight in the hours before (see Chapter II; Pavan *et al*., in press), the present study has brought new elements in favour of the egg mortality hypothesis. In particular, it was showed that in the laboratory at 60% of relative humidity and constant 43 °C per 6 h the mortality was 100% and it decreased at about 50% at constant 40 °C per 6 h, whereas at constant 37 °C per 6 h the egg mortality did not differ in comparison with constant 24 °C. The mortality significantly increased when the eggs were exposed to constant high temperatures for two consecutive exposure-periods and it was decreased when the exposure time was reduced from 6 h to 3 h and it was higher for red-eyes eggs (about 50%) than for white eggs (about 30%). These data showed a higher sensitivity of eggs in comparison to Coscollá *et al.* (1986). In particular, at constant 40 °C per 6 h the mortality was significantly higher than at constant 24 °C, even if the relative humidity was higher than 20% (i.e., 60%) and at constant 43 °C per 6 h the mortality was 100% while in Coscollá *et al.* (1986) was about 60%. The egg mortality not only increases with exposure time, according to Coscollá *et al.* (1986), but also with the number of consecutive exposure-periods.

When the eggs were exposed to a 24-h temperature cycle characterized by a peak of 40 °C for 4 h, to simulate the field conditions, it was observed an average egg mortality similar to that observed on eggs exposed to constant 40 °C, but the mortality decreased from white eggs (about 90%), to red-eyes eggs (about 60%), to black-head eggs (about 25%) showing that the eggs sensitivity decreases with development. Probably when the eggs are gradually subjected to high temperatures, the susceptibility of different egg stages changes.

On artificial egg-laying substrates (i.e., plastic bag or green glass marbles), the effect of high temperatures on egg mortality was not different from that observed on berries, showing that the results obtained using an artificial substrate can be extrapolated to eggs laid on berries.

2. *Lobesia botrana* newly-hatched larvae mortality in the laboratory

As it was reviewed in Zalucki *et al*., (2002), the first instar larvae of Lepidoptera was considered as critical life stage, with high mortality. Weather condition or microclimate could be one of the most effective factor due to quite long period of 'erratic stage' of first larval instar of *L. botrana* before settlement. Literature data on *L. botrana* report only the effect of constant temperature on larval survival. In particular, Rapagnani *et al.* (1988) showed that at constant 35 °C the larval mortality was 100%.

In this study newly-hatched larvae were exposed to 24-h temperature cycles and the larval settlement on berries was significantly reduced under a 24-h temperature cycle with a peak of 37 °C for 4 h (peak of 6 h at 30 °C in the control). The differences comparing with control increased when the larvae were exposed to the 24-h temperature cycle with the peak of 40 °C for 4 h. In any case at 37°C, larval survival was significantly affected with 4 h of exposure, whereas egg survival was not significantly affected by one or two cycles of 6 h. Therefore newly-hatched larvae showed a higher sensitivity than eggs.

3. Influence of sunlight exposure and row orientation on berry temperature in the field

Berries exposed to sunlight, in the same field conditions that had showed the efficacy of bunch-zone leaf removal in reducing *L. botrana* infestation, reached temperatures very higher than air temperature (even more than 10 °C higher) during the hours that sun rays directly hit them. This confirms the possibility of sunlight exposed berries to reach high temperatures for some hours that was proved to cause egg/larval mortality in the laboratory condition.

Among the different row orientations, N45°W orientation determines the highest number of hours with temperature near to egg/larval-mortality limits followed by EW and NS orientations. The higher temperatures of bunches recorded in N45°W orientation (i.e., bunches south-west exposed) than in EW orientation (i.e., bunches south exposed) could be

due to the fact that when the sun is in the west position hits bunches in a more perpendicular way than when it is in the south position.

4. *Lobesia botrana* egg distribution in relation to sunlight exposure in the field

In this study the females did not show significant preference for egg laying when they could choose between sun-exposed and covered south-facing bunches, even if the two out of three experiments, those with highest oviposition levels, showed a tendentially higher number of eggs on sun-exposed bunches. Therefore, the females that laid eggs after sunset did not avoid bunches that had been previously exposed to sunlight. In a previous laboratory study, Zahavi *et al.* (2003) showed a slight preference by females to lay eggs in the laboratory on bunches that in the field had not been exposed to sunlight but also in this study females did not completely avoid sun-exposed bunches.

In the south sun-exposed bunches a female preference for laying eggs on sunny side was observed. This result is in agreement with the results reported in Chapter II (Pavan *et al*., in press) and confirms that the females lay more eggs on the side of the bunch that during the day had been directly exposed to sunlight. However, it does not allow to say that females are attracted by the berries that had been directly exposed to the sun, because there is not a significant preference for sun-exposed bunches in comparison with covered bunches. The preference for sunny side may also be a light effect being the part of bunch more illuminated by twilight before and moonlight after in agreement with the fact that in the laboratory *L. botrana* females show a positive phototropism.

Considering female egg-laying preference, the preference-performance correlations would be the term that several authors discuss about. In the majority of ovipositing insects, gravid females determine where their offspring will live and feed. Therefore, there could be a close match between the site chosen by females to oviposite and the situation where their offspring performance would be maximum (Nylin and Janz, 1993; Barker and Maczka, 1996), on the other hand this positive correlation could not always be found (Nylin and Janz, 1993; Underwood, 1994). However, Scheirs *et al*. (2000) reported that herbivorous insects usually seem to make poor choices for their offspring developments. Considering

the results of this chapter and those presented in the Chapter II (Pavan *et al.*, in press), high egg/larval mortality on sun exposed berries and female slight preference toward sun-exposed bunches/berries would indicate a negative correlation of preference-performance.

5. *Lobesia botrana* egg-hatching and larval survival in the field in relation to sunlight exposure

In the field, the sunlight exposure can determine a reduction in the percentage of egg hatching, but the differences were important only when the air temperature reached peaks of temperature higher than 35 °C and the berries higher than 45 °C.

The negative effect of berry high temperatures appeared more evident on the larval settlement. These field data were in agreement with the laboratory data that had showed a higher sensibility of larvae than eggs to high temperatures.

The simulation of larval infestation, in consequence of the bunch-zone leaf removal and the increased egg/larval mortality in the bunches exposed to sunlight, indicates that the high temperatures reached by the berries exposed to the sun are able to explain the observed reduction in larval infestation in the plots of vineyards submitted to leaf removal.

6. Conclusion

The previously exposure or not of bunches to sunlight did not influence the egg-laying preference by *L. botrana* females that oviposit after sun set.

Berries belonging to bunches, not covered by leaves and west or south exposed to sun rays, reach temperatures very higher than air (even more than 10 °C).

These temperatures both in laboratory and in the field were associated with high eggs and especially newly-hatched larvae mortalities.

Therefore, the cultural practice of bunch-zone leaf removal reduces *L. botrana* infestation because it favours berry exposure to sunlight, high berry temperatures and subsequently egg/larval mortality.

Chapter IV.

Relation between *Lobesia botrana* and *Botrytis cinerea* (*)

(*) In this study, I collected all the laboratory data and most of the field data. The work was made in cooperation with Dr. Elena Cargnus, Dr. Francesco Pavan and Prof. Pietro Zandigiacomo.

INTRODUCTION

1. Relation between *Lobesia botrana* and *Botrytis cinerea* damage

Bunch rot caused by *Botrytis cinerea* Pers. Fr. is a serious disease of grapevines that can cause heavy losses near the harvest time. Carpophagous generations of the European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), were associated with a higher incidence of this fungus (Roehrich, 1978; Galet, 1982; Savopoulou-Soultani and Tzanakakis, 1988; Pavan *et al.*, 2014).

Yield losses are caused by first-generation of *L. botrana* through attacking flowers and by second and third generations because larvae feed on berries and favour *B. cinerea* spread (Fermaud and Giboulot, 1992).

Fermaud and Le Menn (1989, 1992) demonstrated the potential role of *L. botrana* larvae as vector of *B. cinerea*, because larvae can carry the conidia both on tegument and in their gut. A natural contamination of second-generation larvae by *B. cinerea* in a range of 35 to 95% was observed. Therefore, *L. botrana* can favour the spread of *B. cinerea*, not only supporting fungus penetration into the berries through the larval holes, but also because larvae actively transport the conidia.

2. Effects of botrytis on *Lobesia botrana* attractiveness and female's egg laying

Various chemical cues mediate the insect gravid females to identify the oviposition site. Chemicals that attract females may be originated from host plant or microbial assemblages on it (Dicke, 1996; Kellner, 2008).

The frequent coincidence of *L. botrana* and *B. cinerea* in vineyards raised so many studies on the possible attractiveness of this fungus for the females to oviposit.

Mondy *et al.* (1998a) studied in the laboratory *L. botrana* female egg-laying preference by considering a two-choice test: two uninfected grape bunches (choice I) or one uninfected and one infected with the fungus (choice II). When both grape bunches were uninfected, an equal quantity of eggs on each bunch was counted. Conversely, the females showed a highly significant oviposition preference for the infected bunches compared to the control. On opposite, Tasin *et al.* (2011) found repellent effect of infected berries to egg-laying females. Moreover, Tasin *et al.* (2012) found no difference in the orientation response of *L. botrana* towards healthy compared to *B. cinerea* infected bunches after 1-3 days by infection, and a repellent effect after 7-9 days by infection. Masante-Roca *et al.* (2007) did not find an oriented flight preference by *L. botrana* towards berries infected by *B. cinerea.*

3. Effects of botrytis on *Lobesia botrana* development parameters

As it was mentioned before, damage of *L. botrana* to ripe and nearly ripe berries is often accompanied by infection of the bunch by the grey mould fungus *B. cinerea* (Savopoulou-Soultani and Tzanakakis, 1988). It is known that *B. cinerea* modify sugar content and organic acids of berries by developing on them (Hong *et al.*, 2012). On the other hand, quantity and quality of food play an important role in the biology of Lepidoptera, affecting aspects like development rate, variation in the number of instars, pupal weight, fecundity and mortality (Cisneros and Barnes 1974; Pashley *et al.*, 1995). Therefore, there are several studies on possible improvement of *L. botrana* fitness due to

feeding on infected berries (Savopoulou-Soultani and Tzanakakis, 1988; Fermaud and Le Menn, 1989, 1992; Mondy *et al.*, 1998a,b; Mondy and Corio-Costet, 2000, 2004; Tasin *et al.*, 2011).

Mondy *et al.* (1998b) investigated attraction of first-instar larvae of *L. botrana* in the presence of *B. cinerea*. Their results showed significant preference of larvae to the synthetic media or grape berries infected with *B. cinerea* to uninfected berries. Mondy *et al.* (1998a) tried to study specifically the mutualism relationship between these two organisms, so they compared growth rate, mortality, fecundity and oviposition potential of *L. botrana* in the presence or absence of botrytis mycelium in the artificial diet or infected and uninfected berries in greenhouse. In both vivo and vitro experiments developmental duration was shorter when *L. botrana* was in contact with botrytis, but in vivo experiments the shorter developmental time was associated with tendentially lower pupal weight. Moreover, females from larvae that fed on artificial diet with botrytis addition showed a higher fecundity. In field experiments carried out during pre- and post-diapause period, Mondy and Corio-Costet (2004) showed that botrytis determined a higher larval survival and a faster larval development in the diapausing larvae and a higher fecundity in females after wintering diapause. However, Savopoulou-Soultani and Tzanakakis (1988) did not find significant differences neither for developmental duration and pupal weight between infected and uninfected berries, but only a higher fecundity by females. On the other hand, fungus had not showed always beneficial effect for *L. botrana* since Tasin *et al.* (2011) observed that male pupal weight from larvae reared on diet supplemented with *B. cinerea* was lower compared with control.

Mondy and Corio-Costet (2000) suggested that the positive effect of botrytis on *L. botrana* development is due to the fact that botrytis supplies fungus sterols that could be useful for moulting hormone biosynthesis by *L. botrana* larvae.

4. Aim of this study

Insects lack the capacity to synthetize sterol in their body so they must acquire it from their diet (Hobson, 1935). In Mondy and Corio-Costet (2000) botrytis was considered as a good source of sterol for *L. botrana* lead to a better performance of larvae and

suggesting a mutualism relation between these two organisms. However, other studies reported negative or no effects of botrytis on *L. botrana* performance. It appears that the data reported in literatures about the effect of botrytis on *L. botrana* are not always in agreement.

It is to consider that under field conditions berry's nutritional amount (such as sterols) allows the normal development of *L. botrana* larvae, therefore in the laboratory using a diet with the comparable amount of sterols to the berries is important. In particular, to determine the real effect of adding botrytis to the artificial diet on the *L. botrana* development, the nutritional value of basic diet (i.e., phytosterol richness) should be comparable to that of berries. Mondy *et al*. (1998a,b) demonstrated a positive effect of botrytis addition on *L. botrana* performance by using a diet with approximately 80 mg/100 g [[1]] sterol content. As Ruggiero *et al.* (2013) reported that the sterol content of berry diet is about 250 mg/100 g, the diet used in Mondy *et al*. (1998a,b) seems to be poor in terms of sterols (almost three times less than berry).

On the basis of this consideration, first aim of the present study was to compare in the laboratory the performance of *L. botrana* larvae fed on berries, on the diet reported in Mondy *et al*. (1998a,b) and on the diet reported in Rapagnani *et al.* (1990) with about 160 mg/100 g [[2]] sterol content. After comparing the performance of the larvae fed on the two artificial diets with those fed on berries, the second aim of this study was to compare both artificial diets with or without botrytis addition and healthy berries.

The other aspect with contrast results in different studies was the female's egg laying preference toward botrytis infected bunches. Two different research groups in the laboratory [i.e., Mondy *et al.* (1998a) and Tasin *et al.* (2011, 2012)] had shown opposite results, i.e. preference or repellence from infected berries. As no field study was carried out on botrytis influence on the oviposition, the third aim was to test the oviposition preference of *L. botrana* females comparing healthy and naturally botrytis-infected bunches in the field.

[[1]] In Mondy's diet the only important source of sterols is cholesterol, which represents the 0.08% of diet.

[[2]] In Rapagnani's diet there are two important sources of sterols: cholesterol, that represents the 0.12% of diet, and wheat germ, that represents the 9% of diet and has a content of phytosterols of 400 mg/100 g (Phillips *et al.*, 2005).

MATERIALS AND METHODS

1. Experiments in the laboratory on *Lobesia botrana* larvae

The experiments were conducted in climatic chamber (Sanyo Versatile Environmental Test Chamber, Sanyo Corp, Japan) with 70±5 % relative humidity and 16:8 (D:L) photoperiod. The temperature was programed according to different experiments from 24±0.5 to 25±0.5 °C.

The rearing originated from larvae collected in May 2013 in a Pinot Gris vineyard located in north-eastern Italy (locality Corona di Mariano del Friuli, Gorizia district, 45°55'30"N, 13°29'44"E, 40 m a.s.l.). In order to prevent inbreeding effects, in May 2014 new larvae of *L. botrana* were collected in a Pinot Gris vineyard located in north-eastern Italy (locality Spessa di Cividale, Udine district, 46°2'37"N, 13°26'23"E, 112 m a.s.l.) and introduced into previous rearing.

L. botrana larvae collected in the field were individually reared in cylindrical polystyrene boxes (diameter 5 cm, high 1.8 cm) in order to eliminate parasitized or infected larvae. Adults obtained by larvae were put in cylindrical tubes lined with plastic bags (15 × 25 cm) where they were fed through soaked cotton-wool with 10% saccharose solution (Fig. 11 in Materials and methods of Chapter III). Mated females laid eggs on the internal surface of plastic bag. After converting adults in a new bag, the old one was cut in broad strips that were put in rectangle polystyrene boxes (length 9 cm, width 6 cm, high 1.8 cm), where larvae, after egg hatching, were fed on artificial diet (Rapignani *et al.*, 1990). The pupated larvae were moved in another clean rectangle polystyrene boxes until adult emergence.

1.1. Experiment I. Effect of three basic diets on *Lobesia botrana* larval fitness

1.1.1. Diets in comparison

In 2014 three basic diets were compared:

- Berry diet. Thompson seedless berries from organic agriculture without sign of fungal infection were used. Before starting of the Experiment I, berries were dipped in 90% ethyl alcohol for one second to avoid possible fungal infection;
- Diet A (Tab. 1);
- Diet B (Tab. 1).

Table 1 – Ingredients of artificial diets for *Lobesia botrana* larvae used in the Experiments I and II.

Ingredients	Diet A (based on Rapagnani *et al.*,1990)	Diet B (based on Mondy *et al.*, 1998a)
Casein	4.29%	4.6%
Sucrose	3.62%	2.3%
Cellulose	-	4%
Wheat germ	9.08%	-
Lucerne meal	2.44%	-
Wesson salt	0.064%	1.3%
Agar	2.44%	3.3%
Oil	0.24% (sunflower)	0.1% (maize)
Cholesterol	0.12%	0.08%
Vitamin source	1.86% (yeast extract) 1.95% (ascorbic acid)	2.6% (vanderzant vitamin mixture)
Anti-bacteria and anti-fungi	0.195% (sorbic acid) 0.24% (acetic acid) 0.122% (3,4-dimethoxy-5-hydroxybenzoic acid methyl ester)	0.195% (sorbic acid) 0.24% (acetic acid) 0.122% (3,4-dimethoxy-5-hydroxybenzoic acid methyl ester)
Water	to 100%	to 100%

1.1.2. Experiment design and sampling

Newly hatched larvae were placed individually in polystyrene boxes (length 9 cm, width 6 cm, high 1.8 cm) on the two artificial diets or berries, until pupation. Thirty boxes were used for each diet (90 in total). Boxes contaminated with micro-organisms were discarded.

Total developmental duration (from newly-hatched larva to pupa), mortality, number of larval instars were recorded for each individual. To count the number of larval instars, each box was checked daily to observe the shedding of the head capsule and moulting to the next instar. Head capsule of each ecdysis were collected and mounted individually on slides in Berlese's liquid. For each head capsule mounted on slides the left mandible was measured to a precision of 1.25 μm using a calibrated ocular micrometre under an optical microscope at 400× magnification (Zeiss Axioplan). At pupa stage the sex of each individual was distinguished, following the characters reported in Galet (1982), and pupal weight was measured.

During the Experiment I the temperature was on average 24±0.5 °C.

1.2. Experiment II. Effects of botrytis addition to basic diets on *Lobesia botrana* larval fitness

1.2.1. Fungi preparation

Botrytis cinerea isolated from a vineyard in 2014 was used for Experiment II. Fungi was grown in Petri dishes on a potato dextrose agar (PDA) and kept at 25 °C for 3 days. They spent other 3 days at 25 °C and UV condition and then, about 10 days at 25 °C and normal light for sporulation. The surface of sporulated fungi in Petri dishes was washed with sterile water. The conidial suspension was centrifuged and adjusted for 1.06×10^6 conidia/mL. To have mycelia of botrytis, liquid medium was prepared with malt dextrose and inoculated with 2 mL of conidial suspension. After two weeks of incubation at 25 °C, obtained mycelium was freeze dried to be added to artificial diets.

1.2.2. Diets in comparison

In 2015 five diets (3 basic and 2 with botrytis addition) were compared:

- Berry diet (as reported in section 1.1.1.);
- Diet A (Tab. 1);
- Diet A with botrytis (4% freeze-dried powder of *Botrytis cinerea* mycelium);
- Diet B (Tab. 1);
- Diet B with botrytis (4% freeze-dried powder of *Botrytis cinerea* mycelium).

1.2.3. Experiment design and sampling

As reported for the Experiment I, newly hatched larvae were placed individually in test boxes on mentioned diets and berry diet until pupation. Forty individuals were used in each diet (200 in total). During daily check of boxes in the berry trials, those infected with fungus were replaced with healthy berries.

As reported for the Experiment I, total developmental duration (from newly-hatched larva to pupa), mortality and number of larval instars were recorded, and mandible size of each larval instar and pupal weight were measured. As above reported, the pupa sex was distinguished.

During the Experiment II the temperature was on average 25±0.5 °C.

1.3. Statistical analysis

To compare the percentage data Ryan's test was performed.

To compare the developmental duration and the mandible length, the data were previously checked for normality assumption and then, if so, ANOVA and Tukey's post-test were used, whereas, in negative case, Kruskal-Wallis test and Dunn's Multiple Comparison test were used.

2. Effect of botrytis on *Lobesia botrana* egg laying in the field

2.1. Experiment design

In 2015, a field experiment was carried out in an organic vineyard of north-eastern Italy (locality Fratta of Romans d'Isonzo, Gorizia district, 45°53'43''N, 13°27'28''E, 26 m a.s.l., cv. Chardonnay). Ten shoots with two bunches, one naturally infected by *B. cinerea* and one healthy, were chosen. Each couple of bunches was put in the same condition, considering the sun-light exposure, and inserted inside a tulle cage. Five 48-h-old mated females were released into each cage. After three days the tulle cages were removed.

2.2. Sampling and statistical analysis

Coinciding with cage removal, bunches were collected and the number of laid eggs on each bunch was counted in the laboratory. For infected bunches the eggs laid on infected berries and on those in contact with them were distinguished from eggs laid on healthy berries. Also the number of berries of each bunch was counted so to refer the average number of laid eggs not only to the bunch but also to the berry.

To compare the number of eggs per bunch or berry a paired t-test was used.

RESULTS

1. Experiment I. Effect of three basic diets on *Lobesia botrana* larval fitness

1.1. *Lobesia botrana* larval mortality

The proportion of larvae that complete their development from newly-hatched larva to pupa was influenced by the three basic diets. The mortality during larval development was significantly higher for diet B in comparison with diet A, while berry diet showed an intermediate value (Fig. 1, upper chart). The number of larvae that completed their development was 22 out of 27 for berry diet, 29 out of 30 for diet A and 18 out of 30 for diet B.

1.2. Number of *Lobesia botrana* larval-instars

The number of larval instars to develop from newly-hatched larva to pupa varied as a function of diet. All larvae fed on berry diet and diet A completed their development in five instars, whereas a significant proportion of larvae fed on diet B needed a further instar (i.e., 6^{th} instar) to pupate (Fig. 2, upper chart).

1.3. Duration of *Lobesia botrana* larval-development

The larval developmental duration was significantly different among the three basic diets (ANOVA, $F_{2,66} = 61.88$, $P < 0.0001$) (Fig. 3, upper chart). In particular, larvae fed on

diet B, both those developed in five and six instars, required a significant higher number of days to complete their development in comparison with larvae fed on other two diets.

The differences were the same when males and female were separately considered (Tab. 2).

1.4. Mandible length of *Lobesia botrana* larvae

The average length of the larval mandibles was similar for the three basic diets in the first two instars and it began to be significantly different from the third one (Kruskal-Wallis test: 1st instar, KW = 5.90, P = 0.12; 2nd instar, KW = 1.9, P = 0.59; 3rd instar, KW = 12.1, P = 0.007; 4th instar, KW = 44.6, P < 0.0001; 5th instar, KW = 48.7, P < 0.0001) (Fig. 4, upper chart).

The fourth and fifth instars of larvae that completed their development through five instars on diet B had shorter mandibles than larvae fed on berry diet and diet A. Individuals that passed through six instars on diet B had shorter mandibles than those fed on berry diet from the third instar.

On diet B, the fifth instar of individuals that developed through six instars had shorter mandibles than those developed through five instars. When the mandible length of the fifth instar was less than 0.195 mm, the larvae went through one more moult.

No significant differences were observed between mandible size of larvae fed on berry diet and diet A.

The mandible length of the last larval-instar of larvae that passed through six instars on diet B, differently from those developed in five instars on the same diet, was not significantly different from individuals on berry diet and diet A (Fig. 4, lower chart). Therefore, the larvae feeding on diet B required a further instar (i.e., the sixth one) to reach the same size of larvae feeding on berry diet and diet A.

1.5. *Lobesia botrana* pupal weight

The pupal weight was significantly different among the three basic diets (Kruskal-Wallis test, KW = 19.06, P < 0.0001) (Fig. 5, upper chart). In particular, pupae reared on diet A were heavier than those reared on diet B and berry diet. The differences were the same when males and female were separately considered (Tab. 2).

Table 2 – Duration of *Lobesia botrana* larval development and pupal weight (males, females and total) in the three diets in comparison in Experiment I.

Diets	N°	Duration larval development (days)	Pupal weight (g)
Males			
Berry	12	25.0±4.7 A	8.1±2.1 A
Diet A	17	22.3±2.1 A	10.02±1.1 B
Diet B	12	35.2±7.6 B	7.6±1.2 A
Females			
Berry	10	26.3±2.6 A	10.9±2.1 a
Diet A	12	23.6±2.1 A	13.8±2.7 b
Diet B	6	39.3±5.0 B	10.2±2.0 a
Total			
Berry	22	25.6±3.9 A	9.4±2.5 A
Diet A	29	22.9±2.1 A	11.6±2.6 B
Diet B	18	36.6±6.5 B	9.2±1.8 A

2. Experiment II. Effects of botrytis addition to basic diets on *Lobesia botrana* larval fitness

2.1. *Lobesia botrana* larval mortality

The proportion of larvae that completed their development from newly-hatched larva to pupa differed among diets (Fig. 1, lower chart). The mortality during larval development was significantly higher for diet B than for diet A, in agreement with the Experiment I. With the addition of botrytis, the larval mortality in diet B did not differ any more from diet A. The mortality recorded on berry diet showed an intermediate value between the two artificial diets in agreement with Experiment I. The number of larvae that completed their development was 34 out of 40 for berry diet, 40 out of 40 for diet A, 30 out of 40 for diet B, 39 out of 40 for diet A + botrytis and 36 out of 40 for diet B + botrytis.

2.2. Number of *Lobesia botrana* larval-instars

All larvae fed on berry diet and diet A completed their development in five instars, whereas a significant proportion of larvae fed on diet B developed in six instars (Fig. 2,

lower chart). This result was in agreement with that obtained in Experiment I even if the proportion of larvae developed in six instars was lower. Adding botrytis to diet B, no further instar was needed to pupate.

2.3. Duration of *Lobesia botrana* larval-development

The larval developmental duration was significantly different among the diets (Kruskal-Wallis test, KW = 102.3, P < 0,0001) (Fig. 3, lower chart). The larvae fed on diet A developed faster than those fed both on diet B, independently from the instar group (i.e., larvae developed in five or six instars), and berry diet. This result was in agreement with Experiment I for diet B but not for berry diet. Larvae developed in six instars on diet B needed significantly more days to reach pupal stage in comparison with larvae developed in five instar on the same diet and also in comparison with those fed on berry diet. Adding botrytis to diet B, no more significant difference in larval developmental duration with diet A was observed.

Considering separately the males and females, the results were statistically in agreement with those referred to the total (Tab. 3).

2.4. Mandible length of *Lobesia botrana* larvae

The average length of the larval mandibles began to be significantly different among the three basic diets from the second instar (Kruskal-Wallis test: 1^{st} instar, KW = 4.13, P = 0.53; 2^{nd} instar, KW = 25.7, P = 0.0001; 3^{rd} instar, KW = 16.2, P = 0.006; 4^{th} instar, KW = 39.4, P < 0.0001; 5^{th} instar, KW = 58.3, P < 0.0001) (Fig. 6, upper chart).

The fifth instar of larvae that complete development in five instars on diet B had shorter mandibles than those of the same instar fed on berry diet and diet A. In the Experiment I the differences were already occurred in fourth instar. Individuals that developed in six instars on diet B had significantly shorter mandibles than those fed on berry diet in the second, fourth and five instars and those fed on diet A from the third instar. These data are substantially in agreement with those of the Experiment I.

In agreement with the Experiment I, individuals developed through six instars on diet B had shorter mandibles than those developed in five instars on diet B in the fourth and fifth instars.

Unlike the Experiment I, significant differences were observed between mandibles size of the larvae fed on berry diet and diet A from the fourth instar. However, the larvae fed on berry diet in the second instar had the highest mandible length and only progressively their performance were reduced in comparison with larvae fed on artificial diets.

The mandible length of the last-larval instar of individuals developed in six instars on diet B, unlike those developed in five instar on the same diet, was not significantly different from individuals fed on berry diet and diet A (Fig. 6, lower chart). Therefore, in agreement with the Experiment I, the larvae developed on diet B required a further instar (i.e. the sixth one) to reach the same size of larvae developed on berry diet and diet A.

No significant difference was observed in mandibles length of larvae fed on diet A with botrytis and without botrytis addition. While diet B with botrytis addition presented larvae with significantly longer mandibles length in the fourth and fifth instars in comparison with those fed on diet B without botrytis (Fig. 6, upper chart).

2.5. *Lobesia botrana* pupal weight

The pupal weight was significantly different among the three basic diets (Kruskal-Wallis test, $KW = 28.6$, $P < 0.0001$) (Fig. 5, lower chart). In particular, in agreement with the Experiment I, the weight of pupae reared on diet A was higher than those reared on diet B. Adding botrytis to diet B, pupal weight increased significantly and no more differences were observed in comparison with diet A, whereas the addition of botrytis to diet A did not increase the pupal weight.

The differences were similar when males and female were separately considered (Tab. 3).

Table 3 – Duration of *Lobesia botrana* larval development and pupal weight (males, females and total) in the three diets in comparison in the Experiment II.

Diets	N°	Duration larval development (days)	Pupal weight (g)
Males			
Berry	18	22.5±3.6 B	7.9±1.4 A
Diet A	23	17.9±1.6 A	9.5±1.3 B
Diet B	16	22.6±2.6 B	7.8±1.0 A
Diet A + Botrytis	15	17.1±1.6 A	10.5±1.8 B
Diet B + Botrytis	16	16.9±1.9 A	9.3±1.2 B
Females			
Berry	16	23.1±1.7 B	12.5±1.4 AB
Diet A	17	18.8±2.0 A	14.7±2.8 C
Diet B	14	25.9±4.0 B	11.7±1.0 A
Diet A + Botrytis	24	18.2±1.1 A	14.7±1.7 C
Diet B + Botrytis	20	18.7±2.3 A	13.9±2.6 BC
Total			
Berry	34	22.8±2.9 B	10.1±2.7 AB
Diet A	40	18.3±1.8 A	11.7±3.3 ABC
Diet B	30	24.1±3.7 B	9.6±2.2 A
Diet A + Botrytis	39	17.8±1.4 A	13.1±2.7 C
Diet B + Botrytis	36	17.9±2.3 A	11.9±3.1 BC

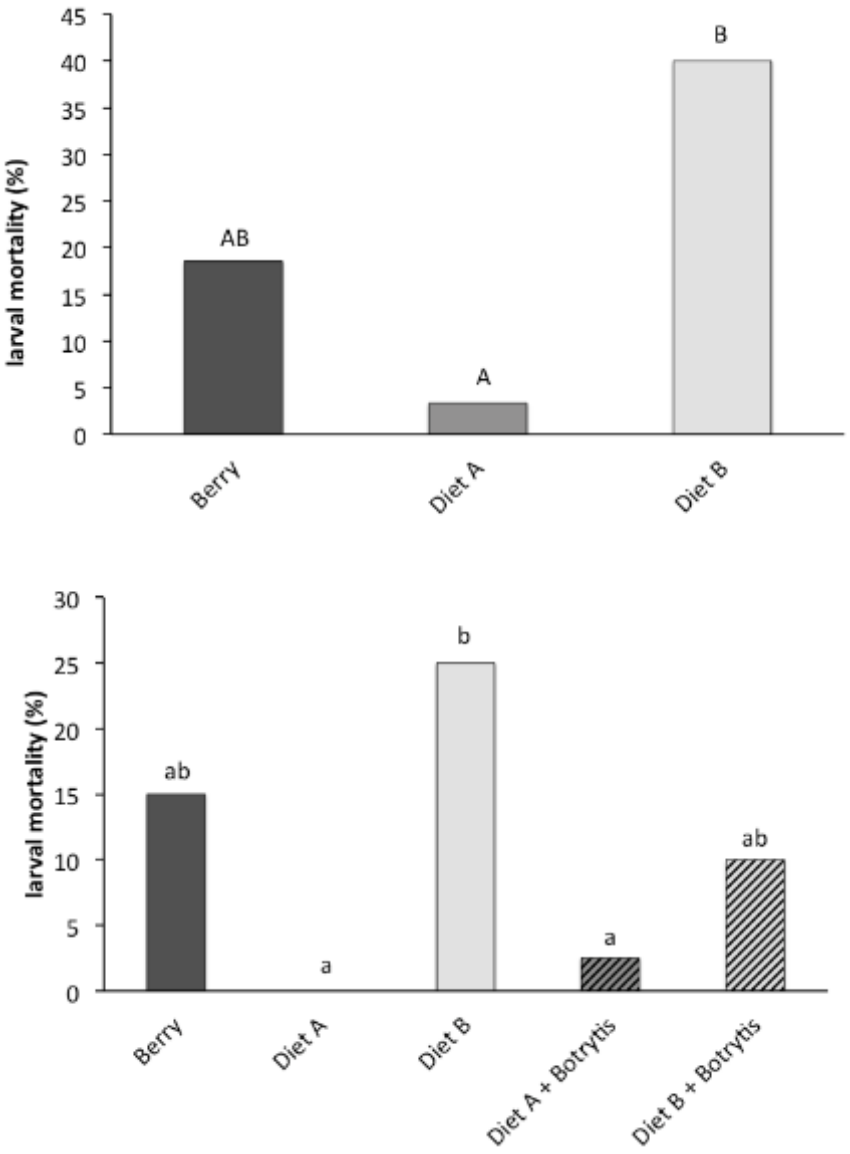


Figure 1 – Percentage of *Lobesia botrana* larvae that died during their development in the two Experiments I (upper chart) and II (lower chart), and in the different diets. Different small and capital letters indicate statistical differences at Ryan's test < 0.05 and < 0.01.

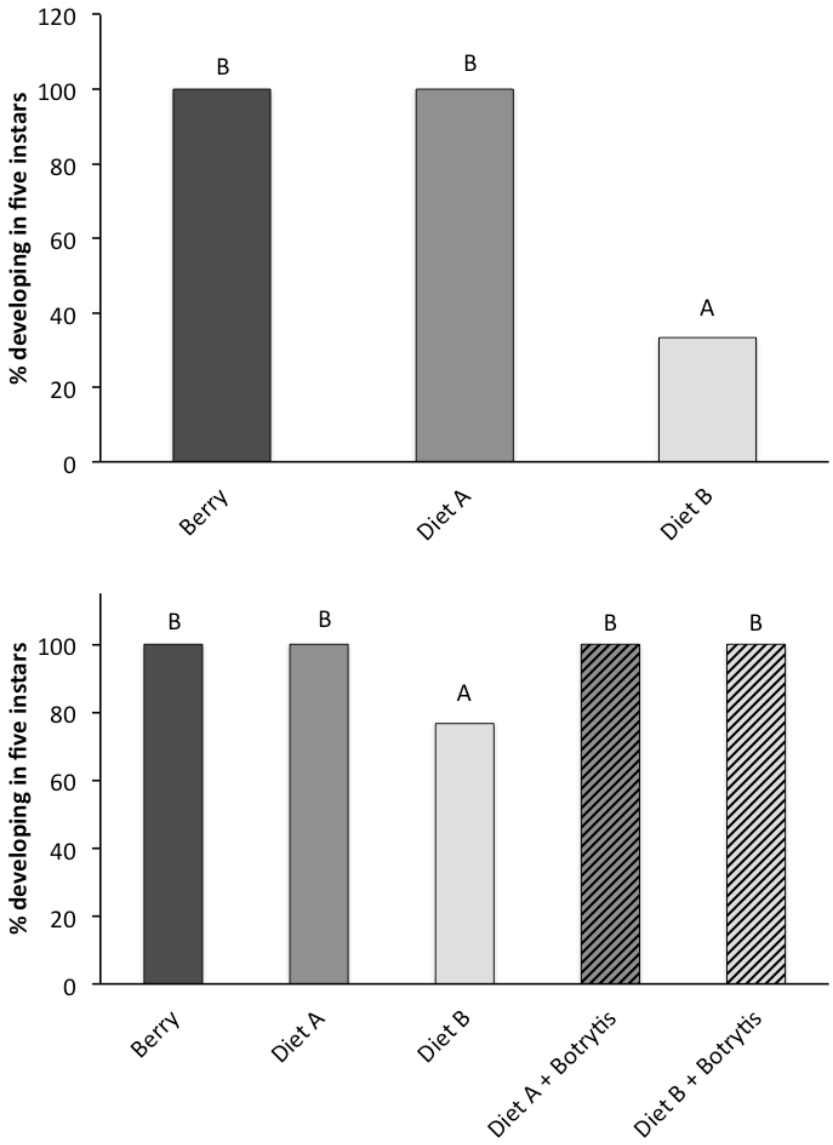


Figure 2 – Percentage of *Lobesia botrana* larvae that complete their development in five instars in the Experiments I (upper chart) and II (lower chart), and in the different diets. Different capital letters indicate statistical differences at Ryan's test < 0.01.

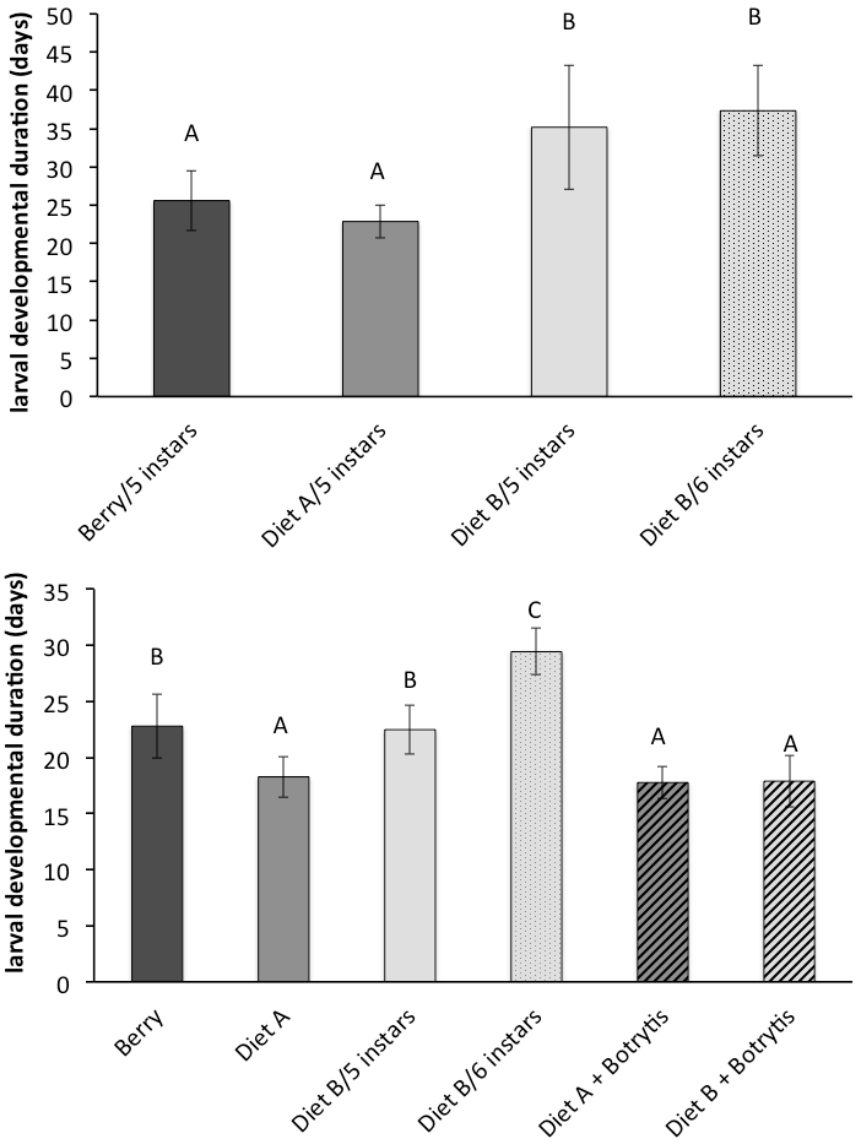


Figure 3 – Developmental duration of *Lobesia botrana* larvae (mean±standard deviation) recorded in the Experiments I (upper chart) and II (lower chart) for larvae fed on different diets. For diet B the larvae developed in 5 and 6 instars were distinguished. Different capital letters indicate statistical differences at Tukey's test < 0.01.

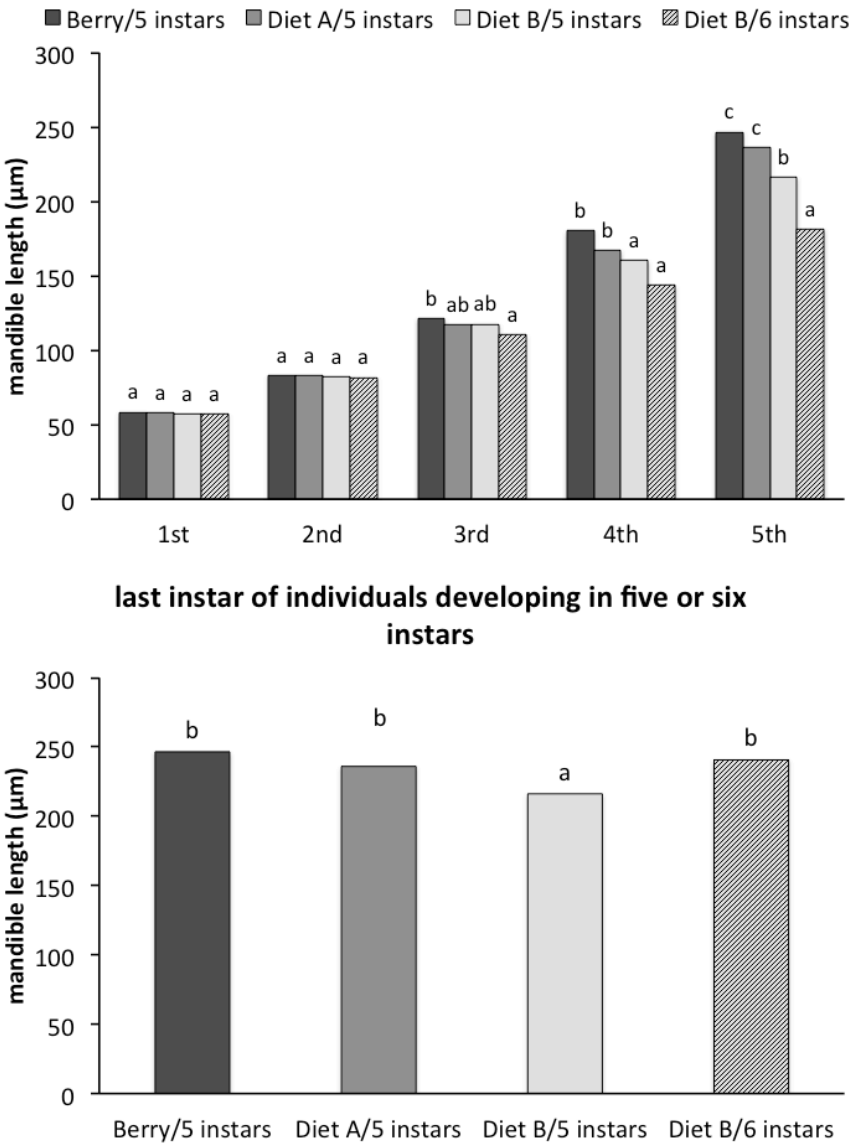


Figure 4 – Mandible length of *Lobesia botrana* larval-instars recorded in the Experiment I for larvae fed on three basic diets. For diet B the larvae developed in 5 and 6 instars were distinguished. In upper chart the larvae of the first five instars were compared. In the lower chart the last instar was compared for larvae developed both in five and six instars. Different small letters indicate statistical differences at Dunn's multiple comparison test < 0.05.

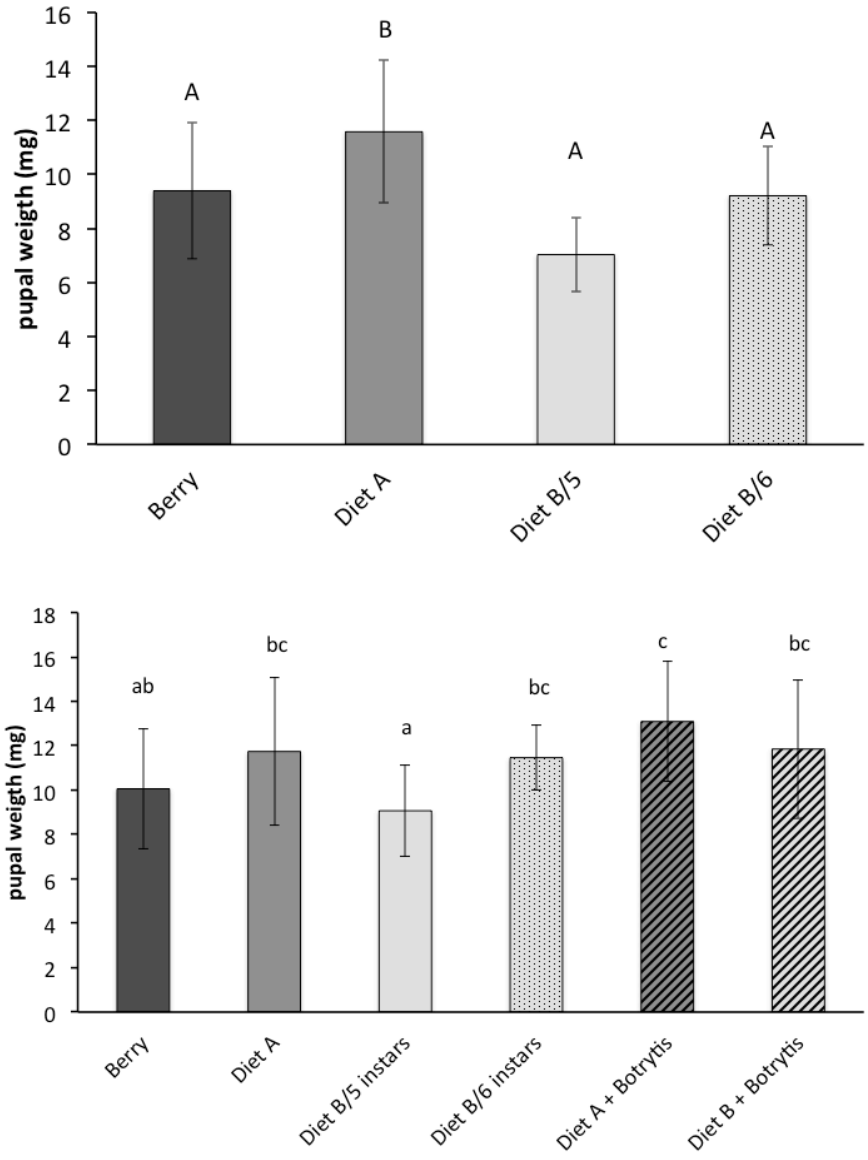


Figure 5 – Pupal weight of *Lobesia botrana* larvae (mean±standard deviation) recorded in the Experiments I (upper chart) and II (lower chart) for larvae fed on different diets. Different small and capital letters indicate statistical differences at Dunn's multiple comparison test < 0.01.

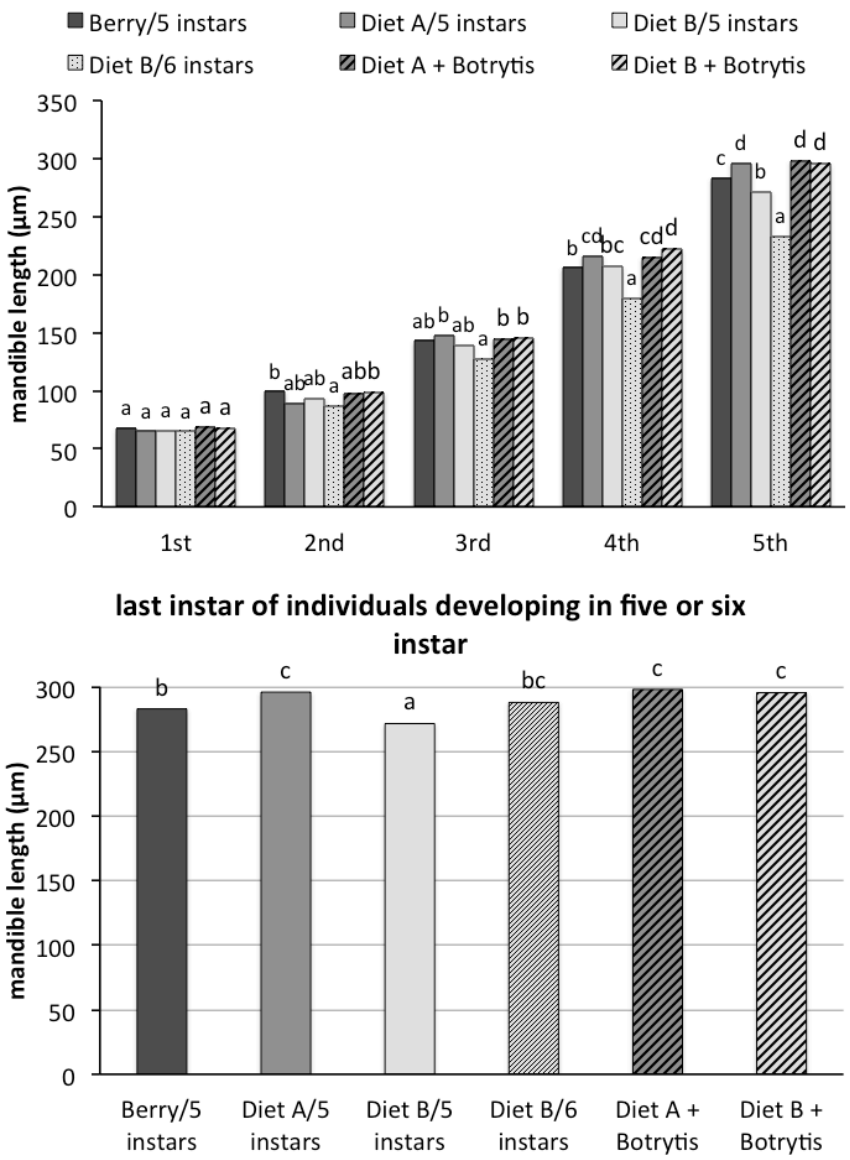


Figure 6 –Mandible length of *Lobesia botrana* larval-instars recorded in the Experiment II for larvae fed on five different diets. For diet B the larvae developed in 5 and 6 instars were distinguished. In upper chart the larvae of the first five instars were compared. In the lower chart the last instar was compared for larvae developed both in five and six instars. Different small letters indicate statistical differences at Dunn's multiple comparison test < 0.05.

3. Effect of botrytis on *Lobesia botrana* egg-laying in the field

In the two-choices field experiment *L. botrana* females did not show any significant egg-laying preference between healthy and botrytis infected bunches, considering both the number of eggs per bunch (paired t-test, $t_9 = 1.61$, P = 0,14) and the number of eggs per berry (paired t-test, $t_9 = 1.50$, P = 0.17) (Fig. 7).

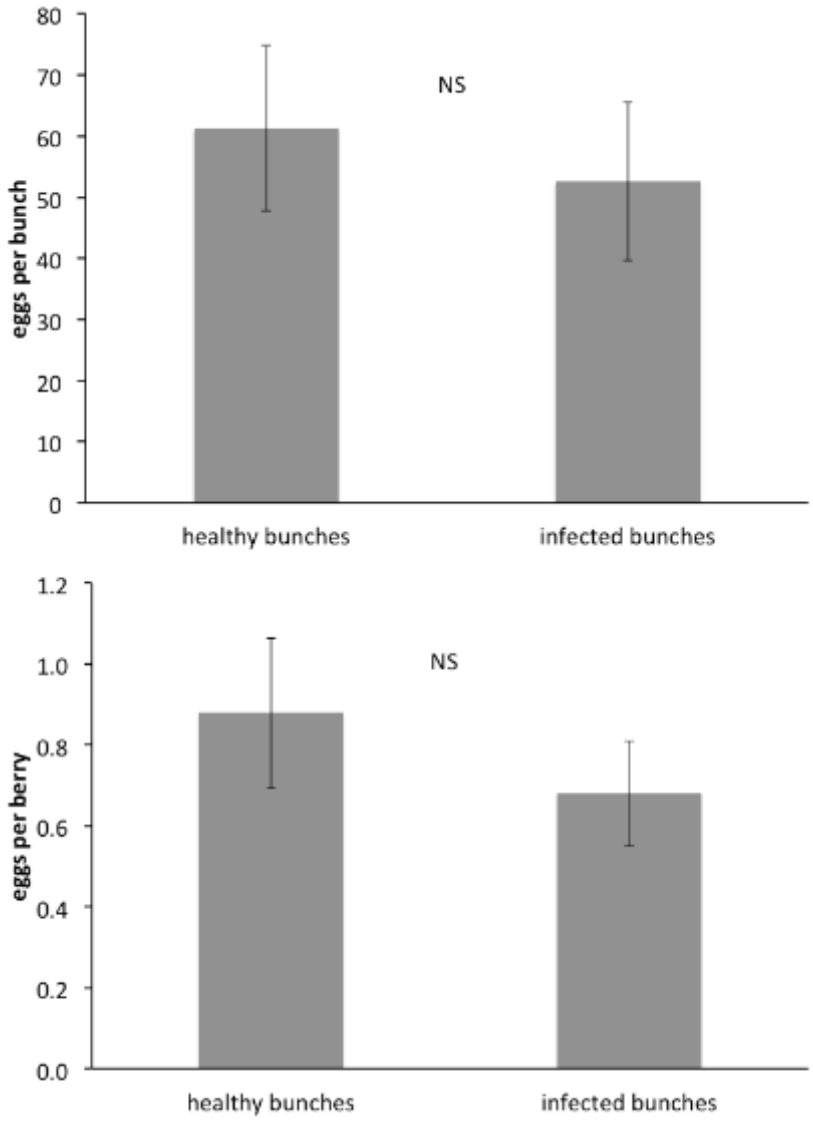


Figure 7 – *Lobesia botrana* eggs laid (mean±standard error) on healthy or infected bunches in a two-choices experiment conducted in the field. In the upper and lower charts, the number of eggs per bunch and berries were considered, respectively. NS = difference not significant at a paired t-test.

Considering the infected bunches, *L. botrana* females did not show any significant egg-laying preference between botrytis infected berries, or healthy berries in contact with infected ones, and healthy berries (paired t-test, $t_9 = 0.39$, $P = 0.71$) (Fig. 8).

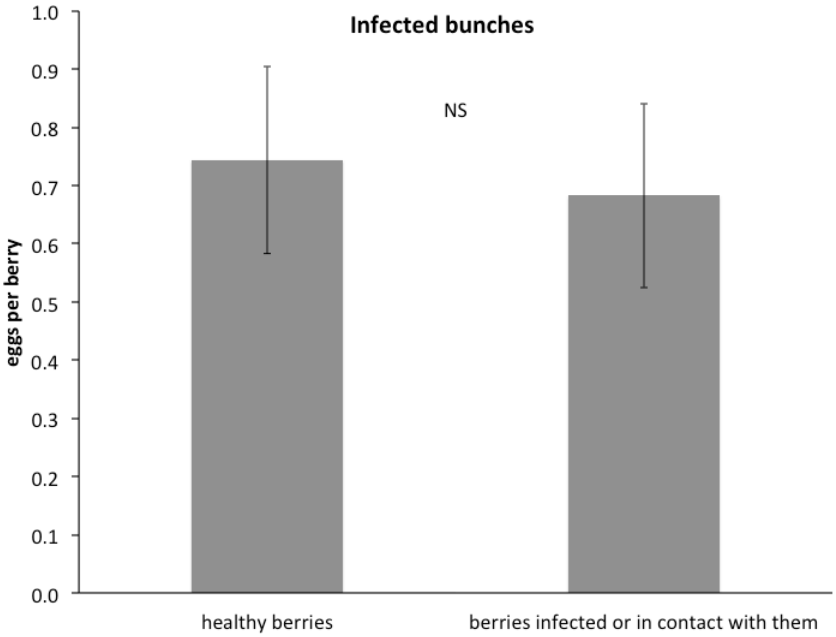


Figure 8 – *Lobesia botrana* eggs laid (mean±standard error) on healthy berries and sum of eggs laid on infected berries and healthy ones in contact with them. NS = difference not significant at a paired t-test.

DISCUSSION AND CONCLUSION

1. Comparison between artificial diets and berry diet on *Lobesia botrana* performance

The quantity and quality of food have a direct influence on essential aspects of the insects biology (Hagley and Barber, 1992; Pashley *et al*., 1995; Bentancourt *et al*., 2003) and as it was mentioned before to know the exact influence of one supplement addition in insects diet on their performance, it is necessary to have a basic diet with comparable nutritional value as natural diet in the field condition. The natural diet of an insect, berries in the case of *L. botrana*, can be assumed generally to be the best for their natural development (Vans Driesche and Bellows, 1996).

In the Experiment I the quality of the diet A (Rapagnani *et al*., 1990) was comparable to that of the berry diet regarding to the larval developmental duration and the mandible size of the larvae, and even better in terms of pupal weight; but in the Experiment II, the quality of the berry diet was showed to be lower than that of diet A in the same parameters (larval developmental duration and mandible size). However, the results of larval mandible size showed predominance of larvae fed on the berry diet at the beginning of growth (second instar), then progressively their performance were reduced in comparison with larvae fed on artificial diets. This is probably due to the fact that, in coincidence with the replacement of berries contaminated by fungi, the larvae were disturbed and forced to build a new larval nest. Costs and benefits of shelter-building behaviours have been studied in several species of Lepidoptera (Lill *et al*., 2007). Costs of nest construction include time lost from feeding, plus energy investment in silk

(Berenbaum *et al*., 1993). So, it does not seem reasonable to attribute the smaller size and the longer developmental duration of the larvae fed on berry diet to lower quality of berry diet in comparison with the artificial diet.

In both the experiments the quality of diet B (Mondy *et al*., 1998a) was lower than that of both berry diet and diet A for all the parameters considered (survival, larval development duration, larval mandible size and pupal weight). Some larvae fed on diet B even needed a further instar to complete their development.

The most important difference between the artificial diets respect to the berry diet was about sterol contents [i.e., 250 mg/100 g in berry (Ruggiero *et al*., 2013), 160 mg/100 g in diet A (120 g from cholesterol and 40 g from wheat germ), 80 mg/100 g in diet B (all from cholesterol)]. Therefore, the amount of sterols in the diet A was more similar to the berry diet in comparison with diet B.

2. Effects of botrytis addition to the artificial diets on *Lobesia botrana* performance

In agreement with sterol-lack hypothesis, the addition of botrytis, an important source of sterols (Mondy and Corio-Costet, 2000), increased the performance of *L. botrana* larvae developed in diet B (very poor in sterols in comparison to the berry diet) but not in diet A (sterol contents more similar to the berry). Therefore, an artificial diet with a nutritional value lower than berry is not appropriate to demonstrate that botrytis enhances larval performance.

When botrytis was added to the berry diet in the laboratory the performance of larvae was not significantly improved, but an increase of female's fecundity was associated with botrytis addition (Savopoulou-Soultani and Tzanakakis, 1988).

In two experiments with bunches on grapevines (Mondy *et al*., 1998a; Mondy and Corio-Costet, 2004) an improvement of larval performance was recorded. In the first study a shorter larval development duration was observed on bunches artificially infected with botrytis, but it was associated with a tendential reduction of pupal weight and no increase of fecundity was observed. In the second study, larval performance and female's fecundity were higher in botrytis infected bunches, but non-infected bunches was obtained applying a

fungicide against botrytis and negative effects on larval survival and development of this product can not be excluded.

In conclusion, the only result without doubt was that of Savopoulou-Soultani and Tzanakakis (1988) that in the laboratory observed an increase of fecundity of females due to the presence of botrytis during their larval development, even if the larval size and the development duration were not significantly influenced.

3. Influence of botrytis on *Lobesia botrana* egg-laying preference

The egg-laying preference of *L. botrana* females toward botrytis infected bunches in the field was the other aspect of this relationship investigated in this study. Considering female egg-laying preference, the preference-performance correlations would be the term that several authors discuss about. In the majority of ovipositing insects, gravid females determine where their offspring will live and feed. Therefore, there could be a close match between the site chosen by females to oviposite and the situation where their offspring performance would be maximum (Nylin and Janz, 1993; Barker and Maczka, 1996), on the other hand this positive correlation could not always be found (Nylin and Janz, 1993; Underwood, 1994).

The healthy berry's composition is different either from those infected by botrytis or the healthy berries in infected bunch (Hong *et al*., 2012) and also their volatile emitting. One of the immune response of plant to the botrytis infection in still healthy berries is to increase levels of the amino acids required for synthesis of cell wall, in order to defeat against the increased threat of cell wall degradation as a result of botrytis infection (Hong *et al*., 2012). So an increase in attraction and oviposition of herbivorous lepidopteran females toward pathogenic fungal volatiles in some studies (Cossé *et al*., 1994; Cardoza *et al*., 2003) could be due to their positive influences in the nutritional content of plant. The presence of ready degraded source of food for the offspring in this case, supported the positive correlation of preference-performance.

About the relationship between *L. botrana* and botrytis the data reported in literature are not consistent. Mondy *et al*. (1998a, 1998b) observed a preference for laying eggs on infected bunches associated with a higher performance on infected berries. On the other

hand, Tasin *et al.* (2012) found the avoidance of *L. botrana* females to land and oviposit on botrytis-infected berries. This was reported to be as a result of the odour modification of infected berries that is known to occur during fungi phytopathogenic infection process (Rohlfs and Churchill, 2011). While adding botrytis to the *L. botrana* larval diet showed no significant increment in larval performance (Tasin *at al*., 2011). Therefore, also considering that Masante-Roca *et al*. (2007) did not show an oviposition preference for botrytis by females, the positive correlation of preference-performance hypothesis appears to be somewhat controversial. Moreover, it is important to highlight that all these studies were carried out in the laboratory.

In our study, conducted in the field, mated females showed no egg-laying preference toward infected bunch and no significant differences were found among the number of eggs laid on healthy berries in healthy bunch and infected berries in infected bunch and healthy berries in infected bunch.

4. Conclusion

Based on critical analysis of literature and our data, the existence of a positive correlation of preference-performance seems hard to sustain.

In fact, about oviposition preference of females for botrytis-infected bunches, our field data suggest that females are indifferent to the presence of botrytis, in agreement with the laboratory data of Masante-Roca *et al*. (2007). At least, based on the tendentially higher number of egg laid on healthy bunches (statistical probability equal to 0.14-0.17), we can suppose that the presence of botrytis exerts a slight deterrent effect, in agreement with laboratory data of Tasin *et al*. (2012). However, our data exclude with certainty that in the field the females are stimulated to lay more eggs on botrytis-infected bunches.

Regarding larval performance our data showed that previous research based on addition of botrytis to artificial diet do not prove definitively that botrytis promotes larval performance. However, the increase of female's fecundity due to larval feeding on botrytis was demonstrated by Savopoulou-Soultani and Tzanakakis (1988). In our opinion, this fact is valid even if it is not associated with a female preference to lay eggs on botrytis-infected bunches. We can suppose that the larvae during their development can feed on infected

berries, even if the botrytis is not already present on berries where females lay eggs. In fact, the tunnels bored by larvae in the berries can be infected by fungus spores dispersed by air currents within vineyards.

Chapter V.

Influence of the vineyard inter-row management on grapevine pests and natural enemies (*)

(*) In this study I have collaborated to the collection of all field data and the laboratory activities. The work was made in cooperation with Dr. Elena Cargnus, Dr. Francesco Pavan and Prof. Pietro Zandigiacomo.

INTRODUCTION

1. Habitat management in vineyard

In the context of Integrated Pest Management (IPM) cultural practices have to be considered different from pesticide applications (Isaacs *at al*., 2012). Pests' control strategies, based on habitat management and cultural control, enhance the environmental quality by reducing the amount of chemical pollutants in soil, water and air. Habitat management in vineyards could impact the arthropod pest abundance both directly, by affecting its dispersal, mortality or reproduction, and indirectly, by affecting its natural enemies (predators, parasitoids, entomopathogens) (Symondson *et al*., 2002; Nicholas *et al*., 2005; Mills *et al*., 2012; Walton *et al*., 2012). Plant diversity, both hedgerows and green cover, could increase beneficial species diversity, providing to natural enemies nutritional resources (nectar as a source of carbohydrates and pollen as a source of proteins), alternative prey or hosts, overwintering sites and shelter from adverse conditions (reviewed by Altieri and Letourneau, 1982; Risch *et al*., 1983; Andow, 1991; Landis *et al*., 2000; Veres *et al*., 2011). In addition, a reduced use of agricultural machines for pesticide applications, tillage and frequent/complete inter-row mowing make the soil microbiological activity more efficient.

2. Effect of ground cover on grapevine pests control

In perennial tree cultures, such as vineyards and orchards, diverse strategies of ground cover management were studied in order to characterize the arthropod communities, especially the abundance and activity of beneficials.

The importance of herbaceous vegetation in vineyards (both native grasses and cover crops) to obtain an increased number of natural enemies and greater control of pest populations, such as spider mites, thrips and leafhoppers are well known (Altieri and Schmidt, 1985; Settle *et al*., 1986; Nicholls *et al*., 2000; Hanna *et al*., 2003; Costello and Daane, 1998, 1999; Daane and Costello, 1998; Begum *et al*., 2006). In particular, the inter-row ground cover can enhance pest suppression by increasing survival, reproduction and density of beneficials since in this habitat they can find alternative preys or hosts and also alternative foods because many of them are omnivorous species, either at a specific stage of development or for a lifetime. Therefore, the flowering plants, as source of extra-floral nectar, floral nectar, honeydew or pollen, allow to maintain predators and parasitoids inside vineyards through all their life stages (Altieri, 1999; Landis *et al*., 2000; Begum *et al*., 2006; Jonsson *et al*., 2010). On the contrary, the tillage of inter-rows, applied mainly to eliminate the competition for water and nutrients between vines roots and grasses, alters habitat and reduces food availability for natural enemies and, then, can favour grapevine pests (Sharley *et al*., 2008).

It was evidenced that ground cover can enhance the richness of natural enemies was found from several studies in which the comparison among the following types of inter-row management in vineyards was carried out: a) bare soil, b) resident grass cover and c) cover crop [types a, b and c, in Costello and Daane (1998) and Campos *et al*. (2006); a and b, in Serra *et al*. (2006); Sharley *et al*. (2008) and Nunes *et al*. (2015); a and b, in Berndt *et al*., (2006); a and c, in Daane and Costello (1998), Costello and Daane (2003) and Altieri *et al*. (2005); different c-types, in English-Loeb *et al*. (2003) and Begum *et al*. (2006)].

3. Most common grapevine arthropod pests and their natural enemies

In the northern Italy vineyard ecosystems, the most widespread pests are the grapevine moth *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), the leafhoppers *Empoasca vitis* (Göthe) and *Zygina rhamni* Ferrari (Hemiptera: Cicadellidae), the vine thrips *Drepanothrips reuteri* (Uzel) (Thysanoptera: Thripidae), the tetranychid mites *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans) (Acari: Tetranychidae), and the eriophyid mites *Calepitrimerus vitis* Nalepa and *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae). These pests have many natural enemies. Some are specialist, as egg parasitoids *Anagrus* spp. (Hymenoptera: Mymaridae) for leafhoppers and predator *Aeolothrips* spp. (Thysanoptera: Aeolothripidae) for thrips, others are generalist, as Heteroptera [e.g., *Nabis* sp. (Nabidae), *Orius* sp. (Antochoridae), Neuroptera [(e.g., *Chrysoperla carnea* (Stephensen) (Chrysopidae)], Coleoptera Coccinellidae, Diptera Syrphidae and spiders (Araneae)] and moreover others are specialized generalist, as phytoseiid mites (Acari: Phytoseiidae) for tetranychid and eriophyid mites. Other Hymenoptera parasitoids than *Anagrus* spp. can be specialist or generalist. Therefore, the vineyard inter-row management could affect the density and diversity of these beneficial taxa.

3.1. *Lobesia botrana*

Biology, damage and abiotic control factors are reported in Chapter III.

Many natural enemies are biotic control factors of *L. botrana* and they were the subject of several studies especially since 1960's (Levi, 1873; Deseo, 1980; Deseo *et al.*, 1981; Marchesini and Dalla Montà, 1994, 1998). Among these predators appear to have less important role in the control of *L. botrana* than do parasitoids. An important predatory activity against the pupae is performed by mites *Allothrombium fuliginosum* (Hermann) (Acari: Trombidiidae) (Zangheri *et al.*, 1987), whereas many insects are predators of *L. botrana* larvae (Marchesini and Dalla Montà, 1994, 1998).

Among parasitoids of larvae and pupae, the most important action is performed by Hymenoptera, Ichneumonoidea (Ichneumonidae and Braconidae) and Chalcidoidea (Eulophidae and Pteromalidae). Diptera Tachinidae are other parasitoids presented in

vineyards (Marchesini and Dalla Montà, 1994, 1998; Bagnoli and Lucchi, 2006). Hymenoptera Trichogrammatidae (*Trichogramma* spp.) play an important role in the parasitization of the *L. botrana* eggs.

Fungi can infect a large percentage of overwintering pupae. The bacteria *Bacillus thuringiensis* Berliner var. *kurstaki* and var. *aizawai* are effectively and extensively used against *L. botrana*, both in conventional and organic vineyards (Deseo *et al.*, 1981; Ifoulis and Savopoulou-Soultani, 2004; Shahini *et al.*, 2010).

Chemical control of *L. botrana* eggs and larvae could be considered as the most widely used control method, due to low cost and high efficiency. As an alternative to neurotoxic organophosphates (e.g., chlorpyriphos and methyl chlorpyriphos) new neurotoxic insecticides (i.e., oxadiazines, spinosyns, avermectins and anthranilic diamides), compounds accelerating moulting and chitin synthesis inhibitors were developed. The organophosphates are neurotoxic insecticides characterized by larvicidal activity and some of them can also be employed with curative criterium, i.e. when the larvae of the second generation are already penetrated inside the berries (Pavan and Duso, 1986). More recent neurotoxic insecticides (e.g., indoxacarb, chlorantraniliprole and emamectin benzoate) exhibit good larvicidal activity and showed a certain ovicidal activity (Bassi *et al.*, 2007; Ioriatti *et al.*, 2009). Chitin synthesis inhibitors (e.g., lufenuron) and moulting accelerator compounds (MAC, e.g., methoxyfenozide) show both larvicidal and ovicidal activities (Charmillot *et al.*, 2006).

3.2. *Empoasca vitis*

The leafhopper *Empoasca vitis* (Göthe) (Hemiptera: Cicadellidae) is a serious pest of grapevines in Europe (Vidano, 1963; Baggiolini *et al*., 1968; Cerutti *et al*., 1988; Baillod *et al.,* 1990; Pavan *et al*., 2000). *E. vitis* is a polyphagous and polivoltine leafhopper that overwinters as adults on evergreen plants and completes from 1 to 4 generations per year in European vineyards (Schvester *et al*., 1962; Vidano, 1963; Baggiolini *et al*., 1968; Cerutti *et al*., 1988; Pavan *et al*., 1988). *Actinidia chinensis* (kiwi) and *Vitis vinifera* (grapevine) are the main host among the cultivated plants.

This leafhopper is a phloem feeder. Symptoms of *E. vitis* are different among cultivars, with leaf margins typically turning reddish in red cultivars and yellowish in white cultivars (Vidano, 1963; Carle and Moutous, 1965). Infestation can also cause the leaf

lamina to partially dry out. Symptoms are associated with a reduction in photosynthesis, mesophyll conductance and transpiration rate (Candolfi *et al.*, 1993). Feeding activity can cause yield losses and sugar content reduction (Moutous and Fos, 1971; Baillod *et al.*, 1993; Pavan *et al.*, 2000) (Fig. 1).

Figure 1 – *Empoasca vitis*. Adult (left, F. Pavan), nymph (top right, F. Pavan), and leaf yellowing caused by feeding on white grape cultivar (down right, F. Pavan).

In north-eastern Italy, where the leafhopper has three generations per year, the second one is usually the most dangerous (Pavan *et al.*, 2000).

Some cultivars (e.g., Carménère and Sauvignon Blanc) were usually more infested than others (e.g., Cabernet Sauvignon and Pinot Gris) (Pavan *et al*., 2009; Fornasiero *et al*., 2016). The sensitivity varied among cultivars, i.e. some of them showed more symptoms than expected on the basis of infestation levels (e.g., Carménère and Merlot), in contrast with others (e.g., Rhine Riesling and Chardonnay). Leafhopper populations are increased by plant vigour (Decante and van Helden, 2001; Decante *et al*., 2009; Pavan and Picotti, 2009) and reduced by water stress (Costello, 2008; Fornasiero *et al*., 2012).

The egg parasitoid *Anagrus atomus* (L.) (Hymenoptera: Mymaridae) is the main natural enemy for *E. vitis* (Cerutti *et al.*, 1991, Pavan and Picotti, 2009; Zanolli and Pavan 2011, 2013). This parasitoid overwinters in leafhopper eggs on roses and bramble and completed one generation in these eggs before attacking eggs of *E. vitis* in vineyards so it is recommended that *A. atomus* should be favoured by surrounding vineyards with plants carrying leafhopper eggs (Cerutti *et al*., 1991).

On the other hand, the activity of natural enemies of *E. vitis* is not always enough to maintain this pest under economic thresholds. Chemical control is necessary only when

population level exceeds one nymph per leaf for most sensitive cultivars (e.g., Carménère and Sauvignon Blanc) or two nymphs per leaf for less sensitive cultivars (e.g., Chardonnay and Pinot Gris) (Pavan *et al*., 2000). Pyrethroids (e.g., etofenprox), neonicotinoids (e.g., thiamethoxam) or growth regulators (e.g., buprofezin) can be used.

3.3. Zygina rhamni

Zygina rhamni Ferrari (Hemiptera: Cicadellidae) is a mesophyll-feeding leafhopper that infests grapevine. It is a oligophagous species that overwinters as eggs and adults on bramble, *Rubus* gr. *fruticosus*, and dog rose, *Rosa canina*, on which can complete a spring generation before moving towards grapevines where completed three generations per year (Vidano, 1963; Pavan, 2001; Mazzoni *et al.*, 2008).

Symptoms caused by *Z. rhamni* on leaves are almost sudden appearance of chlorotic pale green, yellowish-white or silver spots due to it's feeding on leaf mesophyll cells. It causes a progressive reduction in the amount of chlorophyll at the leaf surface and eventual leaf fall (Fig. 2) (Vidano, 1963). The most extensive part of damage is caused by nymphs, however there is no report on *Z. rhamni* potential for causing economic damage. In recent years, severe infestations have been reported in several vine-growing areas in central and southern Italy (Viggiani *et al.*, 2003; Mazzoni *et al.*, 2004). Control strategies and natural enemies reported for *Z. rhamni* are the same reported for *E. vitis*.

Figure 2 – *Zygina rhamni*. Adult (left, F. Pavan), nymph (top right, F. Pavan) and discolored spots caused by feeding (down right, F. Pavan).

3.4. *Drepanothrips reuteri*

The vine thrips *Drepanothrips reuteri* Uzel (Thysanoptera: Thripidae) is considered as an important pest of *V. vinifera* and is capable of causing economic damage in the vineyards of northern Italy (Girolami *et al.*, 1989). The host plants of the vine thrips include: *V. vinifera*, *Quercus* sp., *Betula* sp., *Corylus* sp. and *Salix* sp. (Marullo, 2004). The pest is recorded widely across Europe, California, Illinois and Chile. Regarding the number of generations, in California six (Bailey, 1942) and in Hungary four (Jenser *et al.*, 2010) overlapping generations were recorded.

Figure 3 – *Drepanothrips reuteri*. Short internodes and deformed leaves due to a summer attack (top, F. Pavan), adult (down left, V. Girolami) and necrotic patches on leaf (down right, F. Pavan).

The vine thrips can cause damage in the spring, immediately after sprouting, and in the summer from July (Girolami *et al*., 1989). In case of heavy damage the shoot remains short (short internodes), the leaves curl up towards their surface and edges get brown and dry out (Fig. 3). The leaves splits up along the veins, later long holes develop on the leaves. On fully developed leaves, necrotic patches of reddish brown colour and of 1-2 mm size will be formed. Feeding activity on berries at fruit setting can determine the presence of necrotic areas and deformation. The presence of natural enemies in vineyards belonging to thrips, such as *Haplothrips* spp. and *Aeolothrips* spp., and phytoseiid mites plays a role in keeping *D. reuteri* populations below the thresholds (2-4 individuals per leaf in the spring

and a dozen on top leaves in summer). If their activity was shown insufficient, insecticides of microbial origin, such as spinosad and abamectin, can be used.

3.5. *Panonychus ulmi*

The red mite *Panonychus ulmi* (Koch) (Acari: Tetranychidae) is a polyphagous species that is considered as the most important spider mite in European vineyards. Many trees and shrubs, especially apple (Hardman *et al.*, 1985), pear and peach are other hosts of this pest. It is widespread in temperate regions and less damaging in hot areas. *P. ulmi* overwinters as groups of red onion shape eggs, usually laid in the vicinity of buds and at the insertion of 1-year old wood on 2-year old one. In spring, from April to May, eggs hatch and juvenile forms cause the first damage by feeding on leaves. *P. ulmi* feeds on the mesophyll cells causing leaf discoloration (leaf bronzing) and a decrease in CO_2 exchange rates, followed in the heavier cases by an early leaf fallen (Rilling and Düring, 1990) (Fig. 4). At the beginning of the growing season, the spider mites prefer basal leaves and later, leaves located at the middle of shoots (Candolfi *et al.*, 1992). The range of 18 to 41 days depending on temperature was reported as their mean generation time (Ramsdell and Jubb, 1979; Herbert, 1981). Therefore, it may have 6-9 generations, partly overlapping, per year.

Figure 4 – *Panonychus ulmi*. Damage to sprouting (left, F. Pavan), adult female and red onion shape eggs (top right, V. Girolami), and leaf bronzing caused in summer by feeding (down right, F. Pavan).

Predatory phytoseiid mites are the most important factor controlling Tetranychidae. These species are common in the vineyards of northern Italy: *Kampimodromus aberrans* (Oudemans), *Amblyseius andersoni* (Chant), *Thyplodromus pyri* Scheuten (Duso, 1992; Duso and Pasqualetto, 1993; Duso *et al*., 2003). Eriophyid mites (Acari: Eriophyidae), pollens and fungi play an important role in integrated mite management as an alternate food source for predatory mites. This is a critical component in the system, since it allows predators to maintain themselves during periods of low spider mite density.

4. Aim of this study

The aim of this study was to compare the influence of two different strategies of vineyard ground cover management, i.e. tillage and alternate mowing of inter-rows, on the occurrence of grapevine pests and composition and abundance of specialist and generalist natural enemies.

MATERIALS AND METHODS

1. Study vineyard

The study was carried out during two consecutive years (i.e., 2013 and 2014) in an organic vineyard of north-eastern Italy (locality Cormons, Gorizia district, 45°56' 56"N, 13°27'11"E, 46 m a.s.l., cv. Sauvignon). The vineyard was of about 0.52 hectares in size and planted in 1980 with N48°E oriented rows. The vines were spaced 1.7 m within rows and 2.7 m between rows and grown with Guyot training system (Fig. 5). The vines of nine adjacent rows were of clone Sauvignon R3 and those of seven adjacent rows of clone Sauvignon 297. The soil is of sedimentary origin, with abundant skeleton and medium texture. Prior to the study every second inter-row was tilled.

2. Treatments in comparison

From early spring 2013 two ground cover management treatments were compared:

- Inter-row mowing (hereafter mowing) characterized by presence of permanent resident vegetation, or green cover, between rows;
- Inter-row tillage (hereafter tillage) characterized by maintenance of bare soil between rows by cultivation.

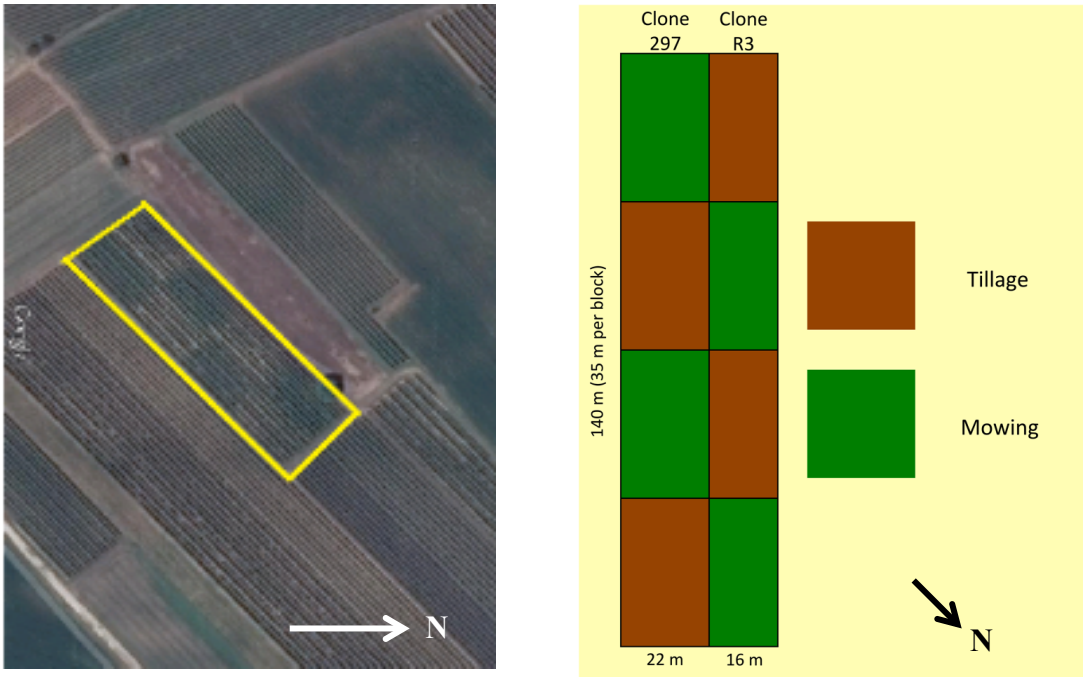


Fig. 5 – The study vineyard (from Google Earth) and treatments in comparison.

Four replicates per treatment, arranged in a systematic block design with two blocks, were considered (Fig. 5). One block, consisted of nine rows of Sauvignon clone R3 (140 × 22 m) and had 4 plots, two per treatment, of about 750 m^2. The other block consisted of seven rows of Sauvignon clone 297 (140 × 16 m) (Fig. 1) and had 4 plots of about 550 m^2.

In the mowing treatment every second inter-row was mowed at one time. In order to have a flowering vegetation at least in one side of the rows, in one of two adjacent inter-rows the mowing was carried out when in the other inter-row the plants began flowering.

Therefore, permanent nectar and pollen productions as food supply for natural enemies were achieved throughout all the season. On contrary, in the tillage treatment all the inter-rows were ploughed before plants flowering to avoid nectar and pollen production. In the two treatments, mowing and tillage were performed two times per year (6 May and 1 July 2013; 5 May and 5 July 2014), using a shredding machine and a tractor-mounted tiller, respectively.

No insecticide and only sulphur- and copper-based fungicides were applied on grapevines.

3. Weather data and *Lobesia botrana* phenology

A weather station (Gradisca d'Isonzo, Gorizia, OSMER) located at about 6 km from the trial site were used for climate data collection (http://www.osmer.fvg.it/OSMER). Male flight phenology of *L. botrana* and the grapevine phenology were provided by the Consorzio Tutela Vini DOC Friuli Isonzo.

4. Data collection

Different sampling methods (i.e., bunch sampling, leaf sampling, beating tray, yellow sticky traps) were done during the growing season from May to September in both study years (2013-14) for grapevine pests and beneficial arthropods. At harvest time also data on bunch rots were collected. Samplings were done in the two central rows of each plot, excluding the five plants next to the plot border.

4.1. Bunch sampling of *Lobesia botrana* and bunch rots

The second generation of *L. botrana* larval infestation was estimated at about 40 days after the beginning of the second flight, whereas third-generation infestation was estimated at harvest time (2 August and 4 September 2013; 1 August and 9 September 2014). At each sampling fifty bunches per plot were examined on 10 different plants. Four and six bunches were chosen alternately from each grapevine based on an *a priori* scheme to avoid subjective choice (Pavan *et al*., 1998). On each bunch, larval nests (each nest consisted of a group of berries with feeding and webbing damages caused by a single larva) and, only for the second generation, berries with feeding damages were counted.

At harvest time, on the same bunches sampled for *L. botrana*, percentages of berries with grey mould, sour rot and black aspergilli rot were also estimated.

4.2. Leaf sampling of leafhopper in the field

In the mid canopy, on 100 leaves per plot, chosen in the middle portion of shoots, leafhopper nymphs of *E. vitis* and *Z. rhamni* were counted every two weeks (26 June–28

August 2013; 20 May–25 August 2014). The morphological and behavioural features considered to distinguish the nymphs of these two species in the field are reported in Pavan *et al*. (1992).

4.3. Checking field-collected leaves in the laboratory

4.3.1. Leafhopper eggs and Anagrus *spp. parasitoids*

In the mid canopy, 10 and 20 leaves per plot were collected in the middle portion of shoots on 12 August 2013 and 9 September 2014, respectively. Leaf samples, each closed into plastic bag, were placed in portable fridge and taken to the laboratory. Under a dissection microscope, the emergence holes of leafhopper nymphs and those of *Anagrus* spp. adults (parasitized eggs) on veins were counted (Picotti and Pavan, 1993). The total number of leafhopper eggs laid was calculated by adding the leafhopper and parasitoid holes. The percentage of eggs parasitized by *Anagrus* spp. was calculated by the ratio between parasitized eggs and total eggs. The obtained data are the sum of *E. vitis* and *Z. rhamni* eggs since it is not possible to know if hatching holes or parasitoid emergence holes are associated with the first or the second leafhopper species.

4.3.2. Phytoseiid mites and Thysanoptera

In each plot a sample of ten leaves from the middle portion of shoots (in both study years) and 10 young leaves from the apical portion of shoots (2014 only) were periodically collected (monthly, 9 May–28 August 2013; every two weeks, 20 May–25 August 2014) and taken to the laboratory as above reported. On each leaf under a dissection microscope motile forms of phytoseiid mites and Thysanoptera were counted and slide mounted. They were identified to species or genus level under an optical microscope at 400× magnification (Zeiss Axioplan) on the basis of morphological features (Mound *et al*., 1976; Miedema, 1987; Karg, 1991; Tixier *et al*., 2013).

4.4. Beating tray sampling of predators

In each plot per date (every two weeks, 9 May–28 August 2013; 20 May–25 August 2014) predators were collected placing a beating tray (74 × 45 cm) under the canopy of 10 plants (5 and 5 on two adjacent rows) and shaking the cane of each plant for 5 times.

Specimens fallen in the beating tray, and belonging to Araneae (spiders), Coleoptera Coccinellidae, Dermaptera and Heteroptera Nabidae, were collected by aspirators and preserved in 70% ethyl alcohol.

In the laboratory under a dissection microscope specimens were counted and identified at different taxa levels.

4.5. Yellow sticky traps for sap-sucking pests and natural enemies

Yellow sticky traps [trap size 24 × 12 × 0.2 cm, smeared with glue (Temo-O-Cid®, Kollant SpA, Italy)] were used to capture the most important sap-suckers grapevine pests (i.e., Cicadomorpha and Thysanoptera) and natural enemies, both specialist and generalist. Two traps per plot were placed in the mid canopy, but not covered by leaves. Traps were hung on a grapevine trellis wire at about 1.5 m from the ground level. Every two weeks the traps were replaced (9 May–27 August 2013, 6 May–21 August 2014).

Within the arthropods captured by traps the taxa taken into account were: i) the leafhoppers *E. vitis* and *Z. rhamni*; ii) the vine thrips *Drepanothrips reuteri*; iii) the predators such as Araneae (spiders), *Aeolothrips* spp., Coleoptera Coccinellidae, Diptera Syrphidae and Neuroptera Chrysopidae; and iv) the parasitoids belonging to Hymenoptera Ichneumonoidea (Braconidae and Ichneumonidae) and Hymenoptera Chalcidoidea. In the last taxon Hymenoptera Mymaridae of *Anagrus* sp. was distinguished from the others. The arthropod specimens, both those collected by beating tray and those captured on traps, were identified to different taxonomic level on the basis of their morphological features (Le Quesne, 1965; Tamanini, 1988; Viggiani, 1994; Roberts, 1995; Bellmann, 2011).

5. Pruning weight and NDVI index

Pruning weight and Normalized Difference Vegetation Index (NDVI) were considered as an estimate of the plant vigour.

Samplings about pruning weight were carried out on 13 January 2014 and 18 March 2015. One-year old canes of five plants per plot were weighted (kg per plant) with a digital dynamometer.

The NDVI was calculated by processing the data provided by optical sensors which measure the reflectance of different wavelengths from vegetation (Rouse *et al.,* 1974).

6. Statistical analysis

To compare data on arthropods recorded in the two treatments during the growing season a paired t-test, after root square transformation of data, was used. To compare data on arthropods in each sampling date, a two-way ANOVA, after logarithmic (x+1) transformation of data, was performed considering two factors (i.e., inter-row management and clone) and their interaction. To compare the percentage of parasitized leafhopper-eggs in the two treatments the Fisher's exact solution test was used.

For statistical analysis the software Graph Pad InStat 3.0 for Macintosh was used.

RESULTS

1. Weather data

The spring 2013 was characterized by high rainfall and average temperatures almost always below 20 °C (Fig. 6). The period from mid-June to mid-August was marked by lack of rain and average temperatures almost always above 25 °C, with peaks next to 30 °C. Only in the second half of August it started to rain again and average temperatures were kept below 25 °C up to harvest time (4 September).

The spring 2014 was characterized by a below-average rainfall and average temperatures quite always below 20 °C until early June when they quickly increased up to a peak of 27 °C. From mid-June to harvest time (9 September) rains were very frequent and average temperatures rarely exceeded 25 °C and in the last period they fell below 20 °C.

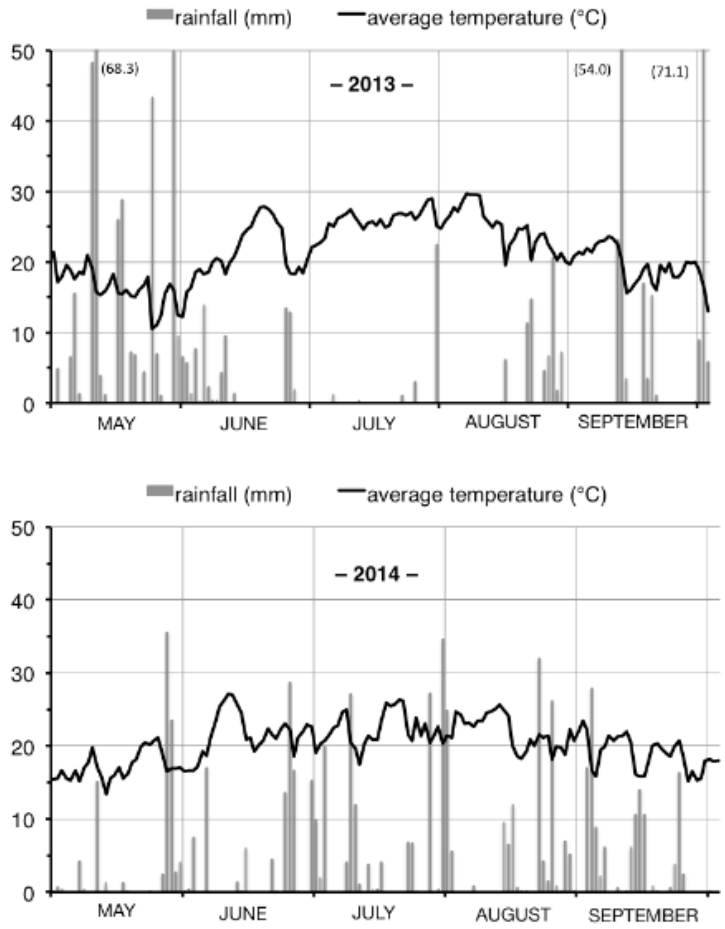


Figure 6 – Weather data recorded in 2014 and 2015 in a weather station near the study vineyard.

2. European grapevine moth, *Lobesia botrana*

2.1. Influence of inter-row management

Both in 2013 and 2014 the second-generation larval nests and damaged berries were not significantly different between the two treatments (larval nests 2013, $F_{1,4} = 0.11$, $P = 0.76$; damaged berries 2013, $F_{1,4} = 0.08$, $P = 0.79$; larval nests 2014, $F_{1,4} = 0.12$, $P = 0.75$; damaged berries 2014, $F_{1,4} = 0.13$, $P = 0.73$) (Fig. 7).

Both in 2013 and 2014 the third-generation larval nests were not significantly different between the two treatments (2013, $F_{1,4} = 0.11$, $P = 0.76$; 2014, $F_{1,4} = 2.69$, $P = 0.18$) (Fig. 8).

2.2. Influence of clone

In 2013 the second-generation infestation was higher in the clone 297 than in the clone R3, but the differences were significant only for damaged berries (larval nests, $F_{1,4}$ = 5.1, P = 0.09; damaged berries, $F_{1,4}$ = 8.04, P = 0.05). In 2014 larval nests and damaged berries were not significantly different between the two clones (larval nests, $F_{1,4}$ = 0.47, P = 0.53; damaged berries, $F_{1,4}$ = 0.31, P = 0.61) (Fig. 9).

In 2013 the third-generation larval nests were not significantly different between the two clones (2013, $F_{1,4}$ = 0.88, P = 0.40), whereas in 2014 the infestation was higher in the clone R3 than in the clone 297 (2014, $F_{1,4}$ = 14.21, P = 0.02) (Fig. 10).

3. Grapevine leafhoppers and *Anagrus* spp. parasitoids

3.1. Influence of inter-row management

3.1.1. Empoasca vitis

In 2013 *E. vitis* infestation level was very low with a max of 5 nymphs on 100 leaves in the tillage treatment at mid-July in coincidence with the second generation (Fig. 11). No significant differences in nymph population were observed between the two treatments in comparison (paired t-test, t_4 = 0.82, P = 0.46). Captures of *E. vitis* adults by yellow sticky traps were significantly higher in tillage treatment only in mid-July (paired t-test, t_8 = 1.45, P = 0.19; 16 July, $F_{1,4}$ = 11.0, P = 0.03) (Fig. 12).

In 2014 the *E. vitis* infestation level reached a max of about 1 nymph per leaf in the tillage treatment in late August in coincidence with the third generation (Fig. 11). Nymph population was significantly higher in the tillage treatment than in the mowing one from early August (paired t-test, t_7 = 1.74, P = 0.13; 1 August, $F_{1,4}$ = 9.0, P = 0.04; 12 August, $F_{1,4}$ = 20.1, P = 0.01; 25 August, $F_{1,4}$ = 9.8, P = 0.04). Captures of *E. vitis* adults were significantly higher in the tillage treatment than in the mowing one considering both the whole sampling period (paired t-test, t_8 = 3.23, P = 0.012) and the sampling dates from early July (1 July, $F_{1,4}$ = 52.3, P = 0.002; 15 July, $F_{1,4}$ = 18.3, P = 0.01; 29 July, $F_{1,4}$ = 44.5, P = 0.003; 12 August, $F_{1,4}$ = 56.7, P = 0.002; 25 August, $F_{1,4}$ = 16.5, P = 0.015) (Fig. 12).

3.1.2. **Zygina rhamni**

In both years *Z. rhamni* infestation level was always lower than 10 nymphs on 100 leaves (Fig. 13). The nymph populations were higher in the tillage treatment than in the mowing one, but the differences were not significant (2013, paired t-test, $t_4 = 1.87$, $P = 0.13$; 2014, paired t-test, $t_5 = 1.22$, $P = 0.28$).

The captures of *Z. rhamni* adults by yellow sticky traps were higher in the tillage treatment than in the mowing one referred to both the whole sampling period (2013, paired t-test, $t_8 = 2.16$, $P = 0.06$; 2014, paired t-test, $t_8 = 4.89$, $P = 0.001$) and single sampling dates (2013, 13 August, $F_{1,4} = 10.3$, $P = 0.03$; 2014, 1 July, $F_{1,4} = 18.2$, $P = 0.01$; 15 July, $F_{1,4} = 14.0$, $P = 0.02$; 29 July, $F_{1,4} = 8.7$, $P = 0.04$) (Fig. 14).

3.1.3. Leafhopper eggs and **Anagrus** *spp. parasitoids*

In both years the captures of *Anagrus* spp. adults by yellow sticky traps were significantly higher in the tillage treatment, where higher leafhopper infestations were observed, than in the mowing treatment with significant differences referred to both the whole sampling period (2013, paired t-test, $t_8 = 2.58$, $P = 0.03$; 2014, paired t-test, $t_8 = 6.56$, $P = 0.0002$) and single sampling dates (2013, 30 July, $F_{1,4} = 15.3$, $P = 0.02$; 2014, 3 June, $F_{1,4} = 11.8$, $P = 0.03$; 17 June, $F_{1,4} = 9.6$, $P = 0.04$; 1 July, $F_{1,4} = 62.4$, $P = 0.001$; 12 August, $F_{1,4} = 44.9$, $P = 0.003$; 25 August, $F_{1,4} = 9.7$, $P = 0.04$) (Fig. 15).

In 2014, the number of total and hatched leafhopper eggs, recorded on leaves at harvest time, was significantly higher in the tillage treatment than in the mowing one (total eggs, $t_6 = 4.37$, $P = 0.005$; hatched eggs, $t_6 = 4.02$, $P = 0.007$), whereas in 2013 no differences in egg numbers were observed (total eggs, $t_6 = 0.25$, $P = 0.81$; hatched eggs, $t_6 = 0.33$, $P = 0.75$) (Fig. 16). The percentage of parasitized eggs was only tendentially higher in the tillage treatment than in the mowing one (2013, Fisher's Exact Test, $P = 0.65$; 2014, Fisher's Exact Test, $P = 0.36$).

3.2. Influence of clone

3.2.1. Empoasca vitis

In 2013 there were significant differences between the two clones neither for nymphs on leaves (paired t-test, $t_4 = 1.83$, $P = 0.14$) nor for adult captures by yellow sticky traps (paired t-test, $t_8 = 1.47$, $P = 0.18$) (Fig. 17 and 18).

Also in 2014 there were no significant differences referred to whole sampling period (for nymphs, paired t-test, $t_7 = 0.44$, $P = 0.67$; for adult captures, paired t-test, $t_8 = 0.95$, $P = 0.37$), but in single sampling dates the clone R3 was more infested than the clone 297 (for nymphs, 2 July, $F_{1,4} = 8.64$, $P = 0.04$; 12 August, $F_{1,4} = 45.1$, $P = 0{,}002$; for adult captures, 29 July, $F_{1,4} = 13.1$, $P = 0.02$; 12 August, $F_{1,4} = 24.6$, $P = 0.01$) (Fig. 17 and 18).

3.2.2. Zygina rhamni

In 2013, referring to the whole sampling period, there were significant differences between the two clones neither for nymphs on leaves (paired t-test, $t_4 = 2.24$, $P = 0.09$) nor for adult captures by yellow sticky traps (paired t-test, $t_8 = 1.54$, $P = 0.16$) (Fig. 19 and 20). Only in mid-August adult captures were significantly higher in the clone 297 than in clone R3 (13 August, $F_{1,4} = 10.1$, $P = 0.03$).

Also in 2014, referring to the whole sampling period, there were significant differences between the two clones neither for nymphs (paired t-test, $t_5 = 0.52$, $P = 0.63$) nor for adult captures (paired t-test, $t_8 = 0.88$, $P = 0.40$) (Fig. 19 and 20). Only in early July nymphs were significantly higher in the clone 297 than in clone R3 (2 July, $F_{1,4} = 22.5$, $P = 0.009$).

3.2.3. Anagrus *spp. parasitoids*

Both in 2013 and 2014, referring to the whole sampling period, there were no significant differences between the two clones for captures of *Anagrus* spp. adults (2013, paired t-test, $t_8 = 1.61$, $P = 0.15$; 2014, paired t-test, $t_8 = 0.13$, $P = 0.90$) (Fig. 21). Only in early June 2013 significantly higher captures were observed in the clone 297 than in the clone R3 (4 June, $F_{1,4} = 47.7$, $P = 0.002$).

4. *Drepanothrips reuteri* and *Aeolothrips* sp. predators

4.1. Influence of inter-row management

4.1.1. Drepanothrips reuteri

In 2013 the captures of *D. reuteri* adults on yellow sticky traps were not significantly different between the two treatments referred to the whole sampling period (paired t-test, $t_8 = 1.11$, $P = 0.30$), but in July at the peak of population the adult captures were significantly higher in the tillage treatment than in the mowing one (2 July, $F_{1,4} = 13.6$, $P = 0.02$; 16 July, $F_{1,4} = 11.3$, $P = 0.03$) (Fig. 22).

In 2014 the captures were significantly higher in the tillage treatment than in the mowing one referred to both the whole sampling period (paired t-test, $t_8 = 3.78$, $P = 0.005$) and the capture peak (1 July, $F_{1,4} = 7.91$, $P = 0.05$) (Fig. 22). The juvenile-stage population on leaves confirmed the significant differences observed for adults at the peak (15 July, $F_{1,4} = 15.5$, $P = 0.02$), but not in referred to the whole sampling period (paired t-test, $t_{14} = 0.55$, $P = 0.59$) (Fig. 23).

4.1.2. Aeolothrips *sp. predators*

In 2013 *Aeolothrips* sp. adult captures on yellow sticky traps were not significantly different between the two treatments in referred to both whole sampling period (paired t-test, $t_8 = 1.15$, $P = 0.28$) and single sampling dates (Fig. 24). However, in two consecutive dates the captures were tendentially higher in the mowing treatment, where lower *D. reuteri* population were observed, than in the tillage one (30 July, $F_{1,4} = 3.91$, $P = 0.12$; 13 August, $F_{1,4} = 4.34$, $P = 0.11$).

In 2014 *Aeolothrips* sp. captures were higher in the mowing treatment, where lower *D. reuteri* population were observed, than in the tillage one referred to both whole sampling period (paired t-test, $t_8 = 3.51$, $P = 0.01$) and single sampling dates (20 May, $F_{1,4} = 28.7$, $P = 0.006$; 15 July, $F_{1,4} = 20.6$, $P = 0.01$; 29 July, $F_{1,4} = 7.24$, $P = 0.05$) (Fig. 24).

4.2. Influence of clone

4.2.1. Drepanothrips reuteri

Both in 2013 and 2014 the captures of *D. reuteri* adults on yellow sticky traps were higher in the clone 297 than in the clone R3, with significant differences only in referred to single sampling dates for 2013 (whole sampling period, paired t-test, $t_8 = 1.09$, $P = 0.31$; 2 July, $F_{1,4} = 37.0$, $P = 0.004$; 16 July, $F_{1,4} = 7.8$, $P = 0.05$) and whole sampling period for 2014 (paired t-test, $t_8 = 2.62$, $P = 0.03$) (Fig. 25). In 2014 the juvenile stages confirmed the higher susceptibility of the clone 297 only referred to the peak sampling (whole sampling period, paired t-test, $t_{14} = 0.14$, $P = 0.89$; 15 July, $F_{1,4} = 18.4$, $P = 0.01$) (Fig. 26).

4.2.2. Aeolothrips *sp. predators*

In 2013 *Aeolothrips* sp. adult captures were higher in the clone 297, where higher *D. reuteri* population were observed, than in the clone R3 with significant differences only referred to single sampling dates (whole sampling period, paired t-test, $t_8 = 1.62$, $P = 0.14$; 2 July, $F_{1,4} = 63.6$, $P = 0.001$; 30 July, $F_{1,4} = 31.0$, $P = 0.005$) (Fig. 27).

Also in 2014 *Aeolothrips* sp. adult captures were higher in the clone 297, where higher *D. reuteri* population were observed, than in the clone R3 with significant differences referred to both whole sampling period (paired t-test, $t_8 = 3.83$, $P = 0.005$) and single sampling dates (20 May, $F_{1,4} = 9.9$, $P = 0.04$; 17 June, $F_{1,4} = 7.46$, $P = 0.05$; 15 July, $F_{1,4} = 17.5$, $P = 0.01$; 29 July, $F_{1,4} = 10.7$, $P = 0.03$) (Fig. 27).

5. Phytoseiid mites

5.1. Influence of inter-row management

The phytoseiid mite species recorded over the sampling period were *Kampimodromus aberrans* (Oudemans) (95% in 2013 and 98% in 2014) and *Amblyseius andersoni* Chant (5% in 2013 and 2% in 2014).

In 2013 no significant differences were observed in phytoseiid mite populations on leaves between the two treatments in comparison (paired t-test, $t_5 = 1.20$, $P = 0.35$), even if

a higher density of this predator mite was observed in the mowing treatment than in the tillage one from early June to early July (Fig. 28).

In 2014, the phytoseiid density was always higher in the mowing treatment than in the tillage one from mid May to mid July with significant differences in two sampling dates (17 June, $F_{1,4} = 8.89$, $P = 0.04$; 15 July, $F_{1,4} = 63.8$, $P = 0.001$), but not referred to the whole sampling period (paired t-test, $t_7 = 1.63$, $P = 0.15$) (Fig. 28).

5.2. Influence of clone

In 2013 the amount of phytoseiid mites was higher in the clone 297 than in the clone R3 with differences close to the significance level referred to the whole sampling period (paired t-test, $t_5 = 1.81$, $P = 0.13$) (Fig. 28).

In 2014 not only the 2013 trend was not confirmed (paired t-test, $t_7 = 0.28$, $P = 0.79$), but in a single sampling date the phytoseiid density was higher in the clone R3 than in the clone 297 (15 July, $F_{1,4} = 272.9$, $P = 0.001$) (Fig. 29).

6. Generalist predators and parasitoids

6.1. Spiders

6.1.1. Influence of inter-row management

In both years of study the number of spider specimens collected by beating tray were higher than that of insects (respectively 89% and 11% in 2013; 84% and 16% in 2014). In 2013 the most abundant spider families were Oxyopidae (66%), Thomisidae (11%) and Araneidae (4%), whereas the rest of spiders belonged to other nine families (19%). In 2014 the most spider families were Oxyopidae (61%), Araneidae (15%) and Thomisidae (9%), whereas other specimens belonged to other 10 families (15%).

In 2013 the amount of spiders collected on canopy by beating tray was not different between the two treatments ($t_6 = 0.69$, $P = 0.51$), whereas in 2014 a significant higher number of spiders were collected in the mowing treatment than in the tillage one referred to both the whole sampling period (paired t-test, $t_7 = 2.49$, $P = 0.04$) and single sampling dates

(15 July, $F_{1,4} = 11.9$, P = 0.03; 01 August, $F_{1,4} = 12.4$, P = 0.02; 25 August, $F_{1,4} = 21.9$, P = 0.01) (Fig. 30).

The spider specimens captured on yellow sticky traps were not separated to family level due to difficulties with the examination of their diagnostic characters. In both years no differences were observed in the captures of spiders by yellow sticky traps (2013, paired t-test, $t_8 = 1.29$, P = 0.23; 2014, paired t-test, $t_8 = 1.24$, P = 0.25) (Fig. 31), even if in most sampling dates the captures was higher in the mowing treatment than in the tillage one.

In 2014 the captures over time increased using beating try and decreased using yellow sticky traps.

6.1.2. Influence of clone

Both in 2013 and 2014 the amount of spiders collected by beating tray was higher in the clone 297 than in the clone R3 but the differences were significant only in 2014 (2013, paired t-test, $t_6 = 1.51$, P = 0.18; 2014, paired t-test, $t_7 = 2.52$, P = 0.04) (Fig. 32).

In 2013, but not in 2014, the number of spiders captured by yellow sticky traps was higher in the clone R3 than in the clone 297 (2013, paired t-test, $t_8 = 2.41$, P = 0.04; 30 July 2013, $F_{1,4} = 26.6$, P = 0.001; 2014, $t_8 = 0.53$, P = 0.61) (Fig. 33).

6.2. Generalist predatory insects

6.2.1. Influence of inter-row management

Both in 2013 and 2014 the amount of predatory insects captured on canopy by beating tray was not significantly different between the two treatments (Nabidae 2013, paired t-test, $t_6 = 1.76$, P = 0.13; Coccinellidae 2013, paired t-test, $t_6 = 1.05$, P = 0.34; Dermaptera 2013, paired t-test, $t_6 = 1.09$, P = 0.32; Nabidae 2014, paired t-test, $t_5 = 1.63$, P = 0.16; Coccinellidae 2014, paired t-test, $t_6 = 0.87$, P = 0.42; Dermaptera 2014, paired t-test, $t_7 = 0.78$, P = 0.46) (Fig. 34).

Both in 2013 and 2014 the amount of predatory insects captured by yellow sticky traps was not significantly different between the two treatments (Coccinellidae 2013, paired t-test, $t_8 = 1.29$, P = 0.23; Chrysophidae 2013, paired t-test, $t_8 = 0.69$, P = 0.51; Syrphidae 2013, paired t-test, $t_8 = 1.53$, P = 0.16; Coccinellidae 2014, paired t-test, $t_8 = 0.56$, P = 0.59; Chrysophidae 2014, paired t-test, $t_8 = 0.79$, P = 0.45; Syrphidae 2014, paired t-test, $t_8 = 0.69$, P = 0.51) (Fig. 35, 36 and 37). Only the captures of Syrphidae in May 2013 were

significantly higher in the tillage treatment than in the mowing one (21 May, $F_{1,4} = 12.4$, P = 0.02).

6.2.2. Influence of clone

Both in 2013 and 2014 the amount of Coccinellidae and Nabidae captured with beating tray was not significantly different between the two clones in comparison (Coccinellidae 2013, paired t-test, $t_6 = 2.35$, P = 0.06; Nabidae 2013, paired t-test, $t_6 = 0.45$, P = 0.67; Coccinellidae 2014, paired t-test, $t_6 = 0.76$, P = 0.48; Nabidae 2014, paired t-test, $t_5 = 0.47$, P = 0.66 (Fig. 38). The amount of Dermaptera was significantly higher in clone R3 than clone 297 in 2014 (paired t-test, $t_7 = 3.85$, P = 0.006) but not in 2013 (paired t-test, $t_6 = 2.12$, P = 0.09).

Both in 2013 and 2014 the amount of Coccinellidae and Chrysopidae captured with yellow sticky traps was not significantly different between the two clones in comparison (Coccinellidae 2013, paired t-test, $t_8 = 1.70$, P = 0.13; Chrysophidae 2013, paired t-test, $t_8 = 0.0$, P = 0.0; Coccinellidae 2014, paired t-test, $t_8 = 1.60$, P = 0.15; Chrysophidae 2013, paired t-test, $t_8 = 0.67$, P = 0.52) (Fig. 39 and 40). The amount of Syrphidae was significantly higher in the clone 297 than in the clone R3 in 2013 (paired t-test, $t_8 = 2.41$, P = 0.04) but not in 2014 (paired t-test, $t_8 = 0.90$, P = 0.39) (Fig. 41).

6.3. Parasitoids

6.3.1. Influence of inter-row management

In 2013 the captures of parasitoids, both Ichneumonoidea and Chalcidoidea, by yellow sticky traps were significantly higher in the mowing treatment than in the tillage one (Ichneumonoidea, paired t-test, $t_8 = 2.59$, P = 0.03; Chalcidoidea excl. *Anagrus*, paired t-test, $t_8 = 2.29$, P = 0.05) (Fig. 42 and 43).

In 2014 these differences were not confirmed (Ichneumonoidea, paired t-test, $t_8 = 0.41$, P = 0.69; Chalcidoidea excl. *Anagrus*, paired t-test, $t_8 = 0.73$, P = 0.48) (Fig. 42 and 43).

6.3.2. Influence of clone

Both in 2013 and 2014 the captures of Ichneumonoidea by yellow sticky traps were not significantly different between the two clones (2013, paired t-test, $t_8 = 0.90$, $P = 0.39$; 2014, paired t-test, $t_8 = 1.45$, $P = 0.18$) (Fig. 44).

In 2013 the captures of Chalcidoidea by yellow sticky traps were significantly higher in the clone R3 than in the clone 297 (paired t-test, $t_8 = 2.29$, $P = 0.05$), but this difference was not confirmed in 2014 (paired t-test, $t_8 = 0.81$, $P = 0.44$) (Fig. 45).

7. Bunch rots

7.1. Influence of inter-row management

Both in 2013 and 2014 the percentage of berries infected by grey mould was significantly higher in the tillage treatment than in the mowing one (2013, $F_{1,4} = 9.96$, $P = 0.03$; 2014, $F_{1,4} = 56.3$, $P = 0.002$) (Fig. 46).

In 2013, but not in 2014, the incidence of sour rot was significantly higher in the tillage treatment than in the mowing one (2013, $F_{1,4} = 9.96$, $P = 0.03$; 2014, $F_{1,4} = 1.18$, $P = 0.34$) (Fig. 47).

In 2013, it was present also black aspergilli rot without significant differences between the two treatments ($F_{1,4} = 0.08$, $P = 0.93$) (Fig. 50).

7.2. Influence of clone

In 2013, but not in 2014, the percentage of berries infected by grey mould was significantly higher in the clone 297 than in the clone R3 (2013, $F_{1,4} = 24.4$, $P = 0.01$; 2014, $F_{1,4} = 0.06$, $P = 0.81$) (Fig. 48).

Both in 2013 and 2014 the percentage of berries infected by sour rot was not significantly different between the two clones, even if in 2014 the differences were close to the significance with a higher incidence in the clone 297 than in the clone R3 (2013, $F_{1,4} = 0.11$, $P = 0.75$; 2014, $F_{1,4} = 6.16$, $P = 0.07$) (Fig. 49).

In 2013, black aspergilli rot was significantly more spread in the clone 297 than in the clone R3 ($F_{1,4} = 12.85$, $P = 0.02$) (Fig. 50).

8. Plant vigour

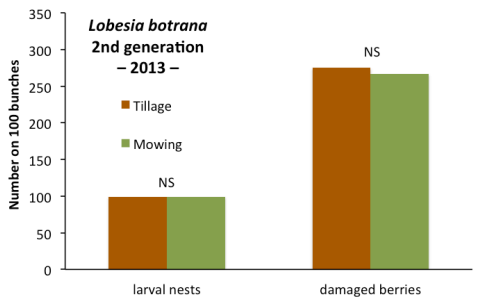
8.1. Influence of inter-row management

Both in 2014 and 2015 pruning weight of canes was higher in the tillage treatment than in the mowing one, but the differences were not significant, even if close to be significant in the second year (2014, $F_{1,4} = 0.17$, $P = 0.70$; 2015, $F_{1,4} = 5.98$, $P = 0.07$) (Fig. 51).

Both in 2014 and 2015 the index of vigour NDVI was significantly higher in the tillage treatment than in the mowing treatment (harvest 2013, $F_{1,4} = 54.6$, $P = 0.002$; July 2014, $F_{1,4} = 56.01$, $P = 0.002$; harvest 2014, $F_{1,4} = 12.02$, $P = 0.03$) (Fig. 52).

8.2. Influence of clone

No significant influence of clone was observed for both pruning weight (2014, $F_{1,4} = 4.38$, $P = 0.10$; 2015, $F_{1,4} = 1.39$, $P = 0.14$) and NDVI (harvest 2013, $F_{1,4} = 0.25$, $P = 0.64$; July 2014, $F_{1,4} = 3.61$, $P = 0.13$; harvest 2014, $F_{1,4} = 3.0$, $P = 0.16$), even if both parameters of plant vigour were tendentially higher for the clone the 297 than for the clone R3 (Fig. 51 and 52).



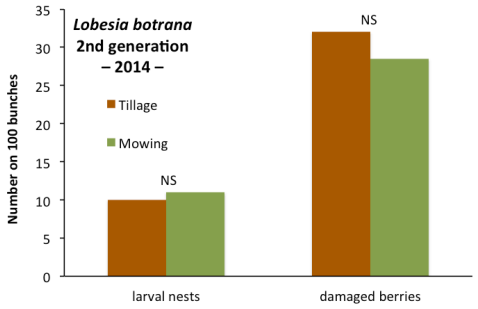
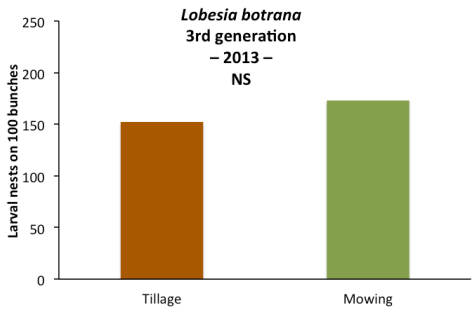


Figure 7 – Larval nests and damaged berries associated with the second generation of *Lobesia botrana* recorded in 2013 and 2014 in the two treatments in comparison. NS = not significant differences at ANOVA test.



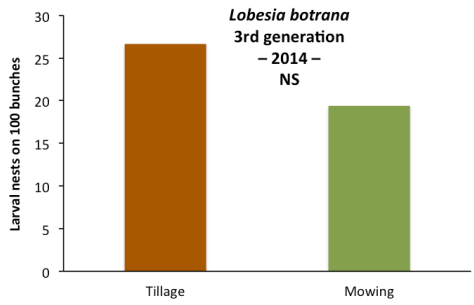
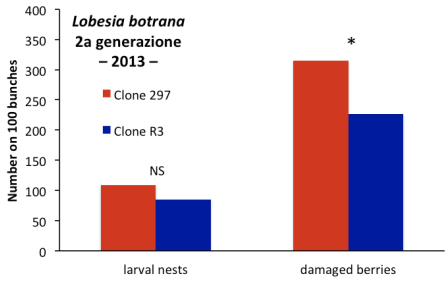


Figure 8 – Larval nests associated with the third generation of *Lobesia botrana* recorded in 2013 and 2014 in the two treatments in comparison. NS = not significant differences at ANOVA test.



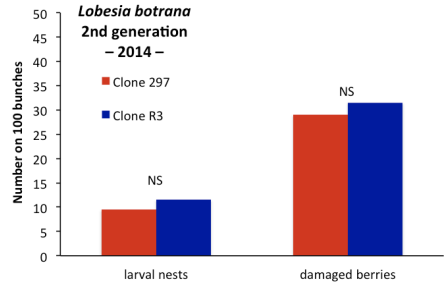
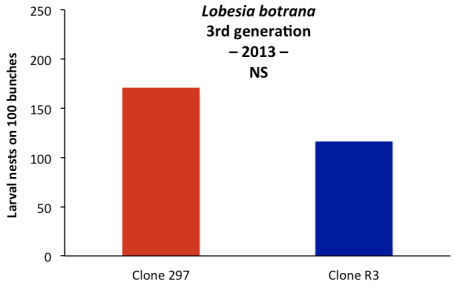


Figure 9 – Larval nests and damaged berries associated with the second generation of *Lobesia botrana* recorded in 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant 0.05 differences at ANOVA test



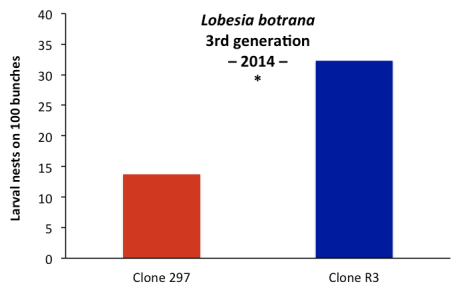
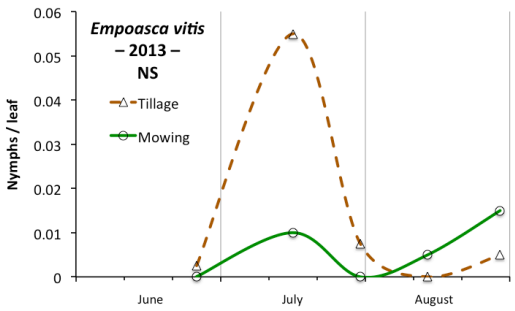


Figure 10 – Larval nests associated with the third generation of *Lobesia botrana* recorded in 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant 0.05 differences at ANOVA test.



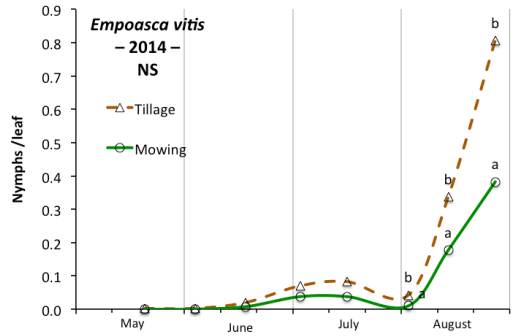
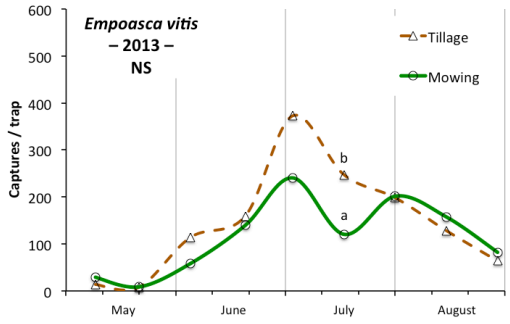


Figure 11 – *Empoasca vitis* nymphs recorded over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.



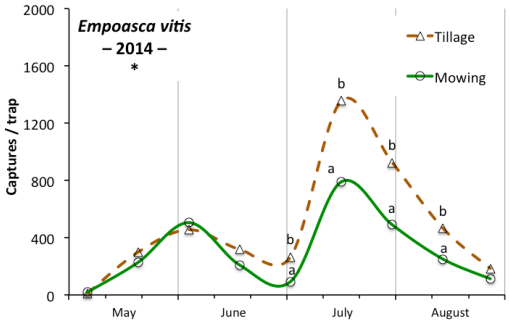


Figure 12 – *Empoasca vitis* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS and * = not significant and significant differences < 0.05 between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

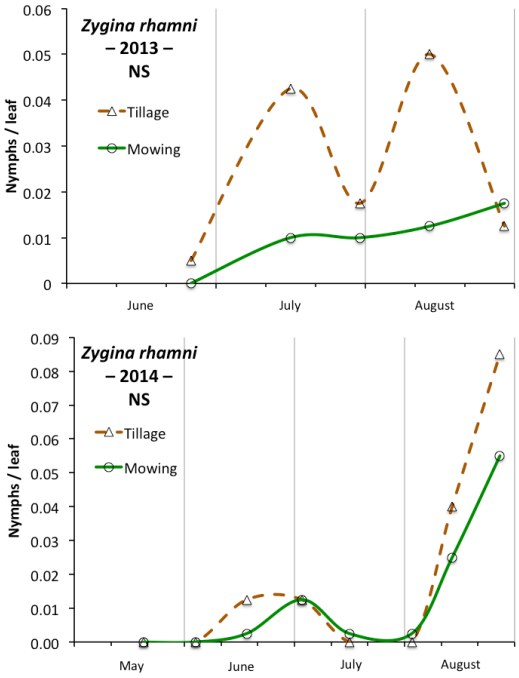


Figure 13 – *Zygina rhamni* nymphs recorded over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatment at a paired t-test.

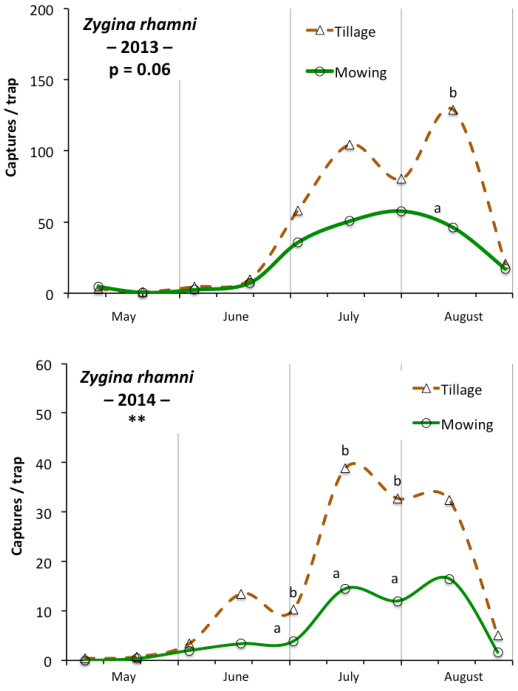


Figure 14 – *Zygina rhamni* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. ** = significant differences between treatments < 0.01 at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

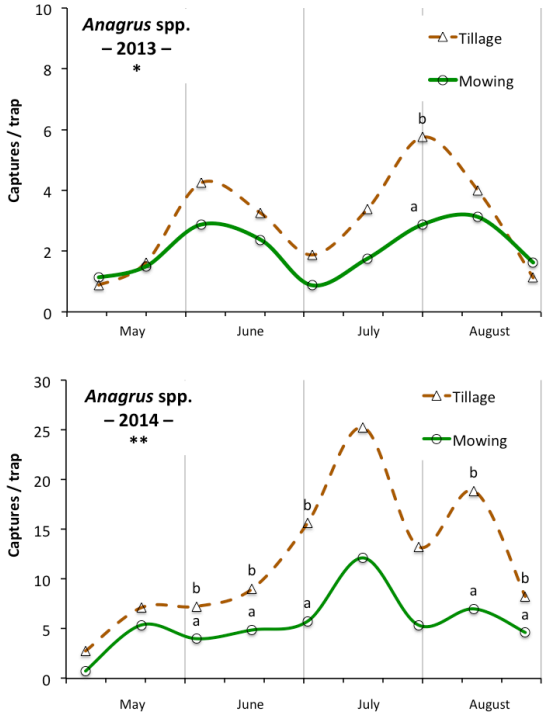


Figure 15 – *Anagrus* spp. adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. * and ** = significant differences between treatments < 0.05 and < 0.01 at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

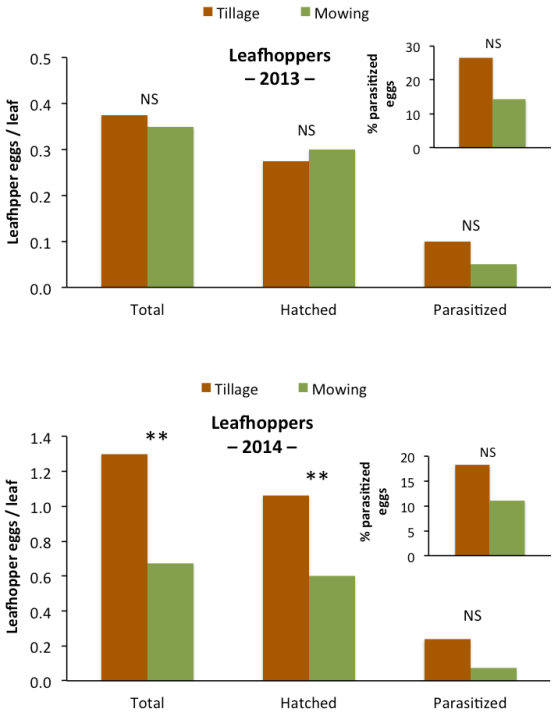
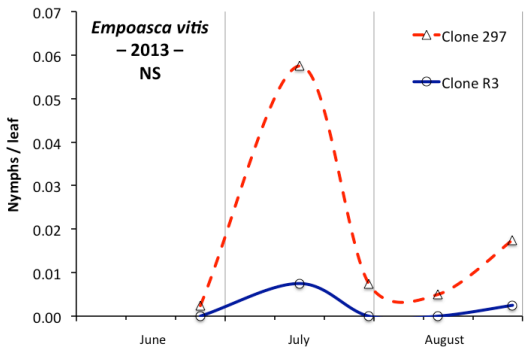
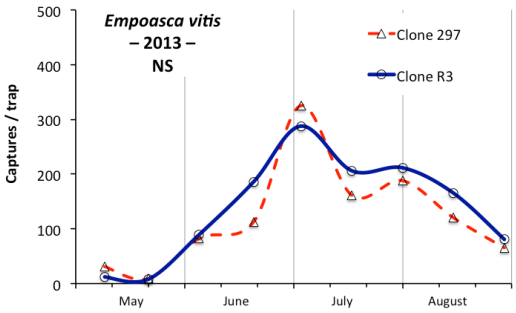
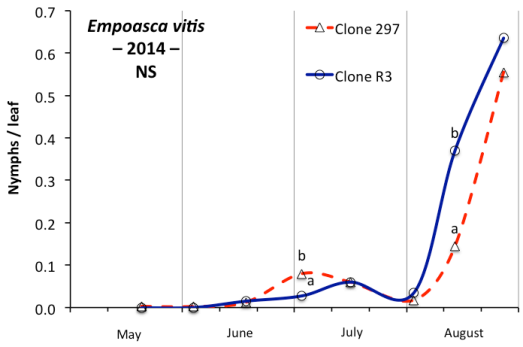


Figure 16 – Eggs (total, hatched and parasitized) of *Empoasca vitis* and *Zygina rhamni* observed on leaves in 2013 and 2014. The percentage of parasitized eggs is also reported. NS and ** = not significant and significant differences < 0.01 at t-test (eggs / leaf) or Fisher's Exact test (percentage of parasitized eggs).







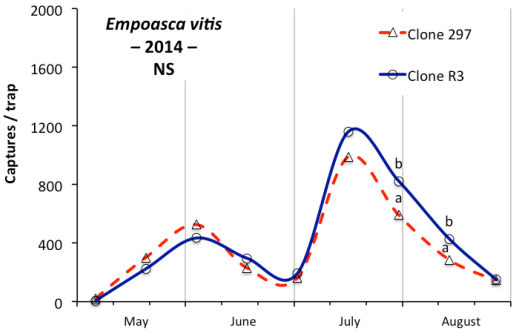


Figure 17 – *Empoasca vitis* nymphs recorded over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

Figure 18 – *Empoasca vitis* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

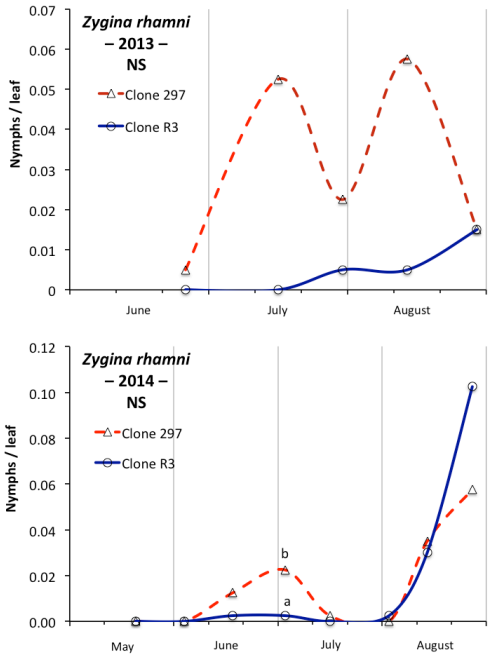


Figure 19 – *Zygina rhamni* nymphs recorded over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

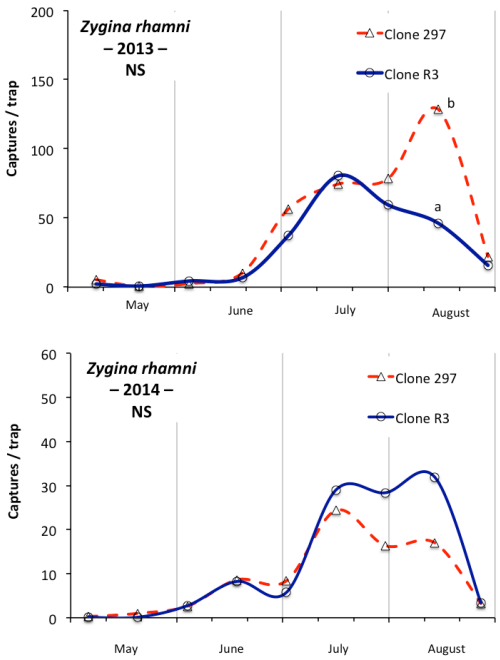


Figure 20 – *Zygina rhamni* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

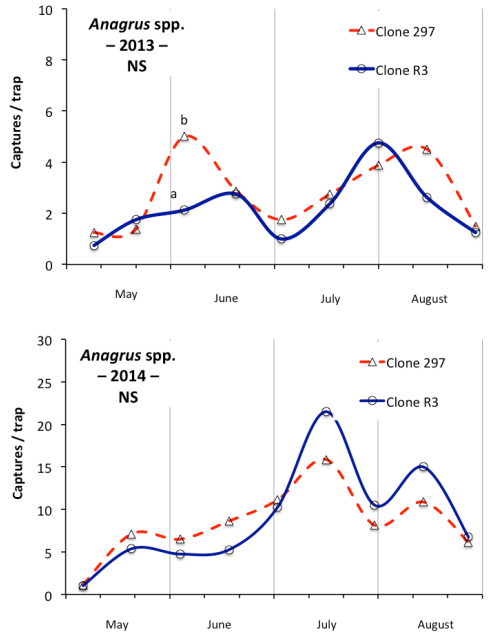


Figure 21 – *Anagrus* spp. adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

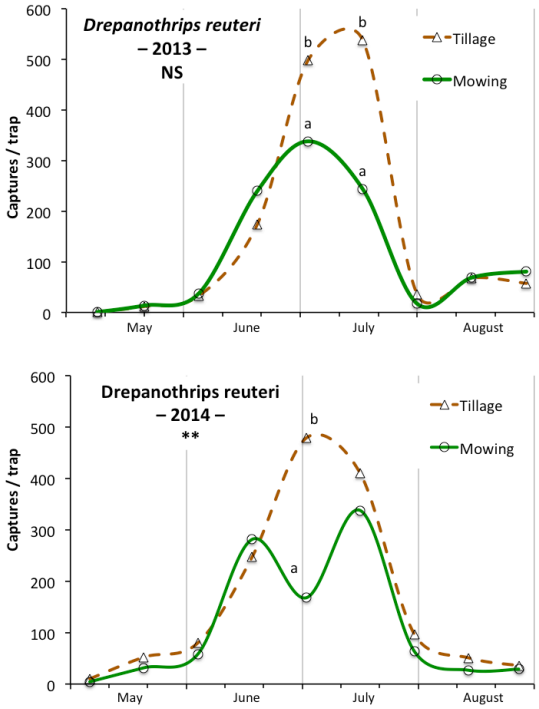


Figure 22 – *Drepanothrips reuteri* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS and ** = not significant and significant < 0.01 differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

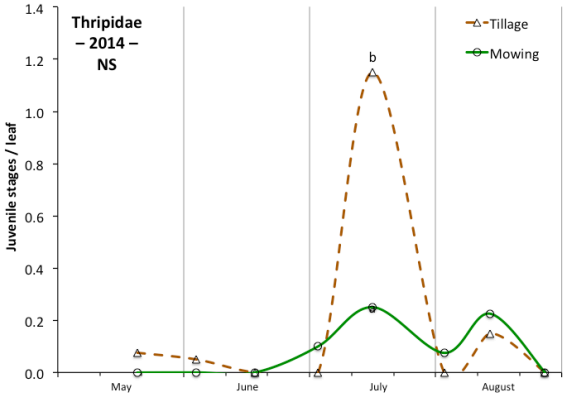


Figure 23 – Juvenile stages of thrips recorded over vegetative season 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

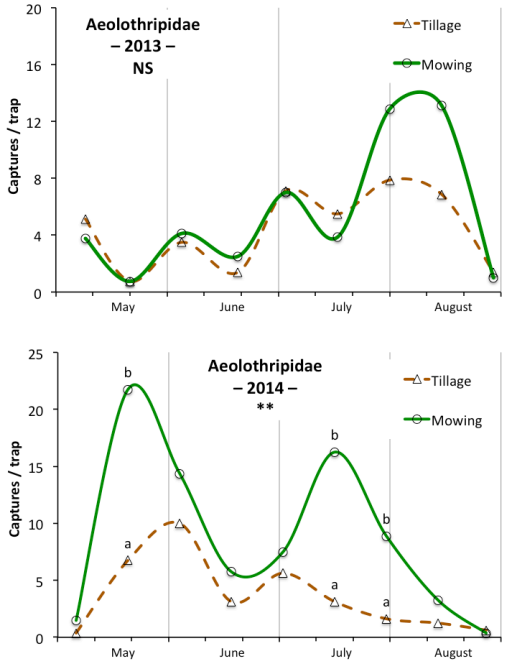


Figure 24 – Aeolothripidae predator adults of genus *Aeolothrips* captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS and ** = not significant and significant < 0.01 differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

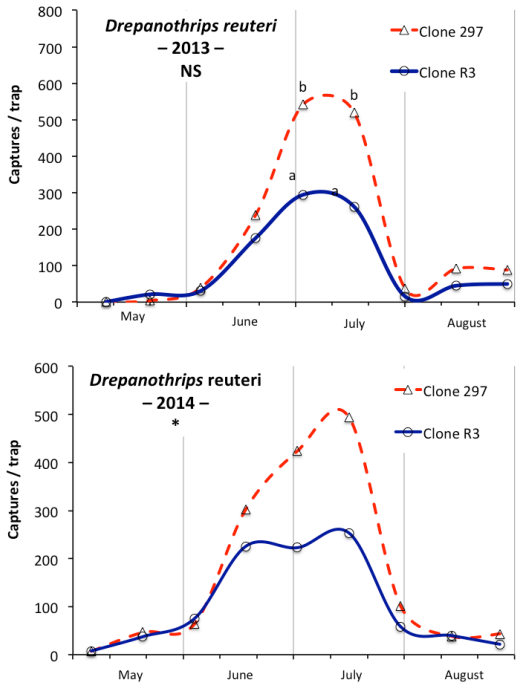


Figure 25 – *Drepanothrips reuteri* adults captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant < 0.05 differences between treatments at a paired t-test.

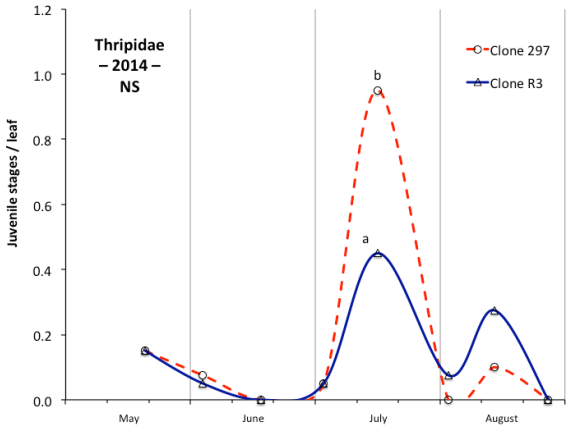


Figure 26 – Juvenile stages of thrips recorded over vegetative season 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

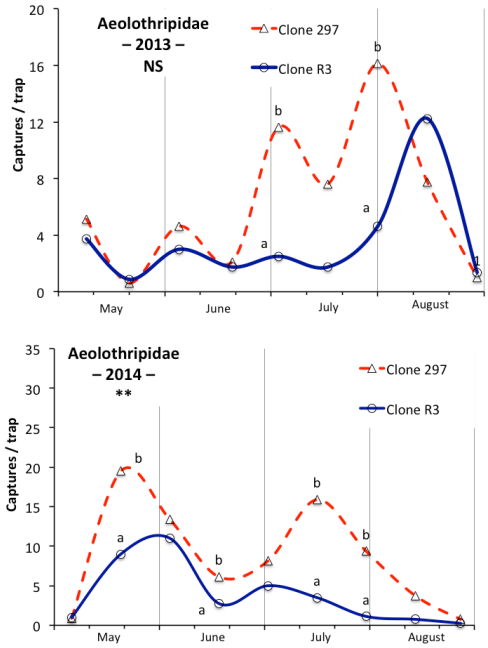
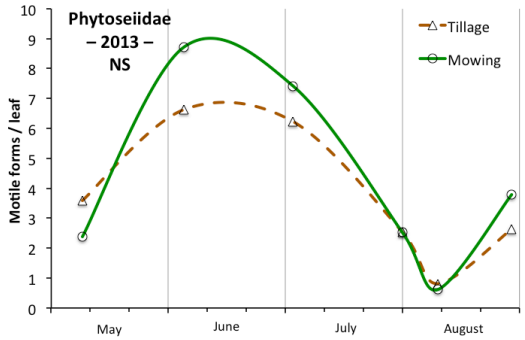


Figure 27 – Aeolothripidae predator adults of the genus *Aeolothrips* captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS and ** = not significant and significant < 0.01 differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.



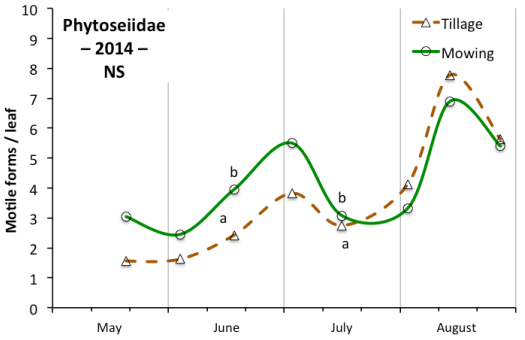
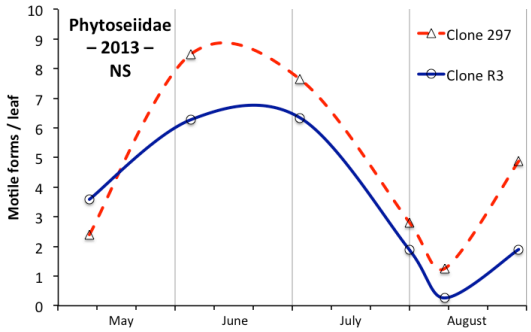


Figure 28 – Motile forms of Phytoseiidae recorded on leaves over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.



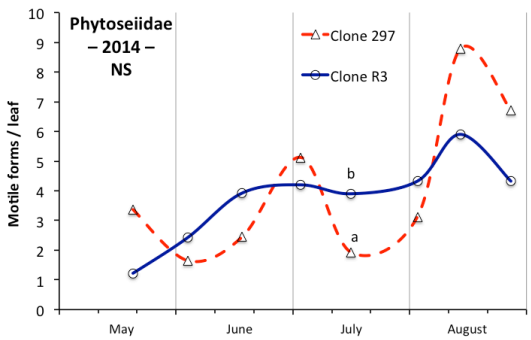


Figure 29 – Motile forms of Phytoseiidae recorded on leaves over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

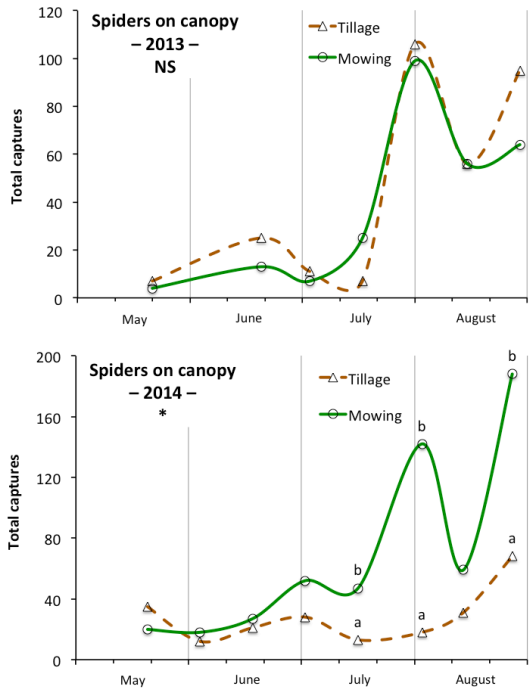


Figure 30 – Spiders captured on canopy by beating tray over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS and * = not significant and significant differences < 0.05 between treatments at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05.

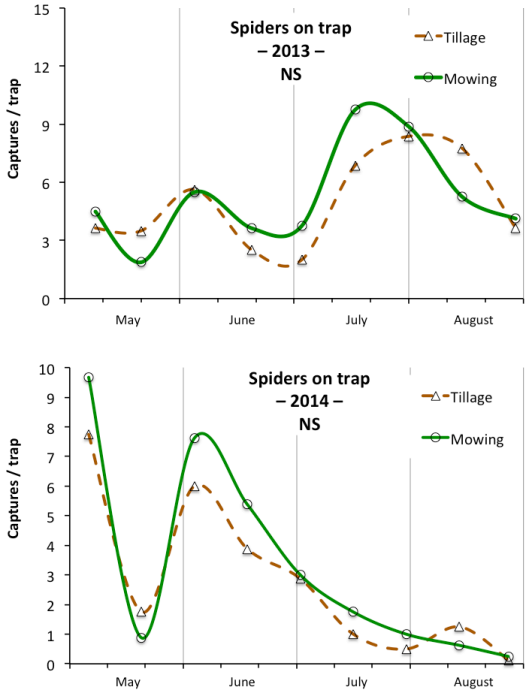
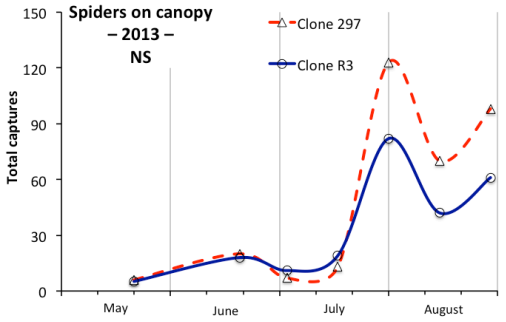


Figure 31 – Spiders captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test.



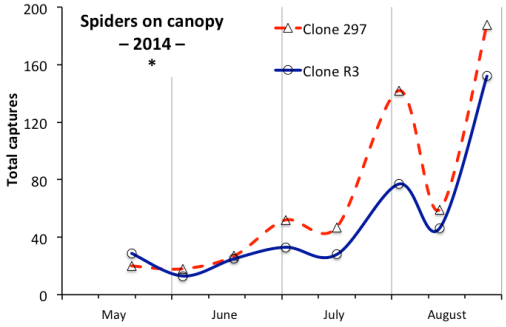
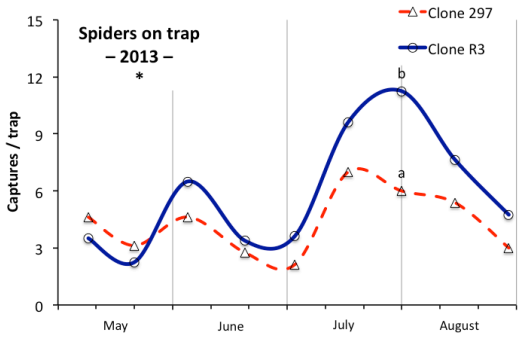


Figure 32 – Spiders captured on canopy by beating tray over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between clones at a paired t-test. Different small letters in the same date indicate significant differences at Tukey's test < 0.05



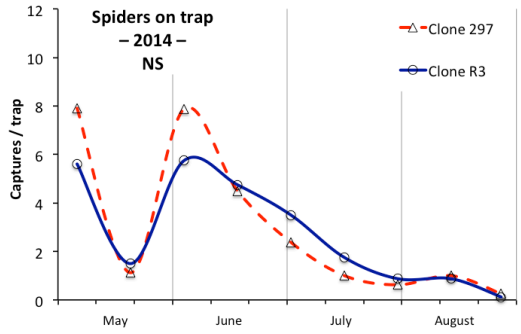


Figure 33 – Spiders captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant < 0.05 differences between treatments at a paired t-test.

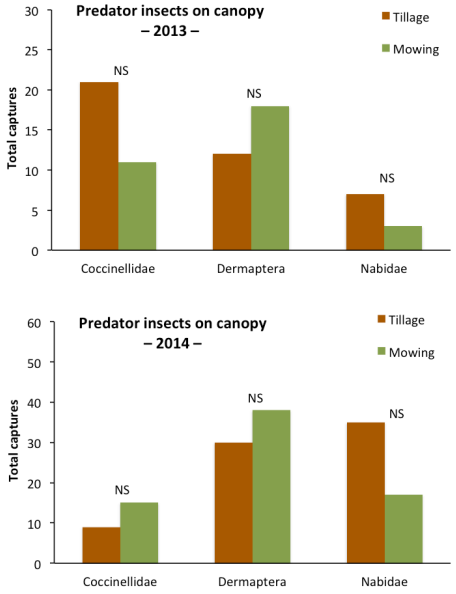


Figure 34 – Predatory insects captured on canopy by beating tray over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test.

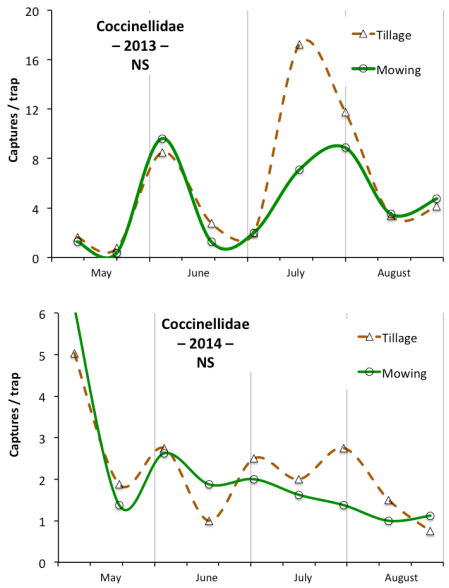


Figure 35 – Coccinellidae captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test.

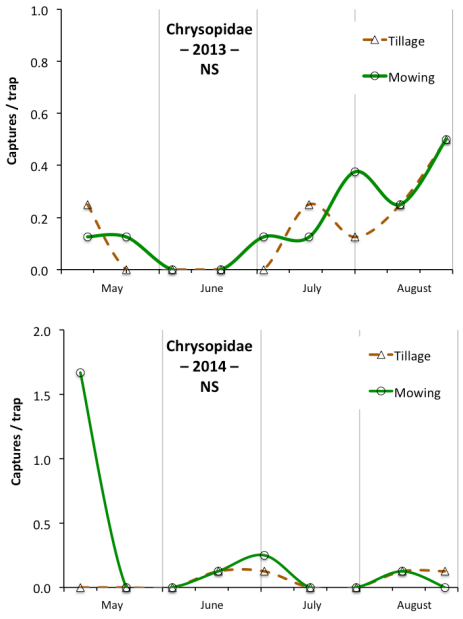


Figure 36 – Chrysopidae captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test.

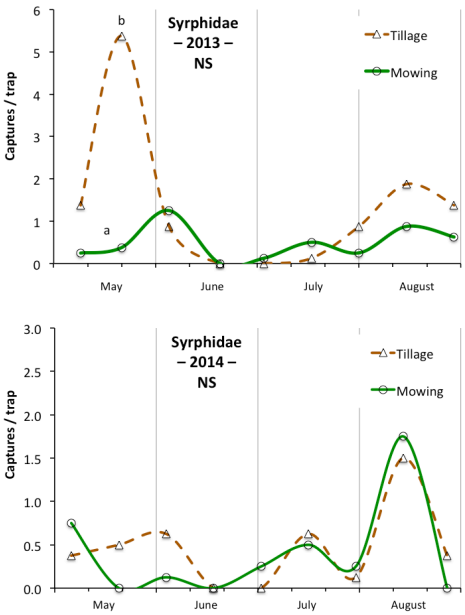


Figure 37 – Syrphidae captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two treatments in comparison. NS = not significant differences between treatments at a paired t-test.

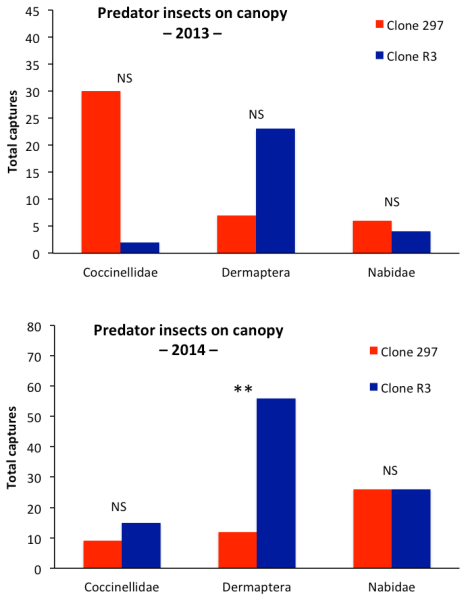


Figure 38 – Predatory insects captured on canopy by beating tray over vegetative seasons 2013 and 2014 in the two clones in comparison. NS and ** = not significant and significant < 0.01 differences between clones at a paired t-test.

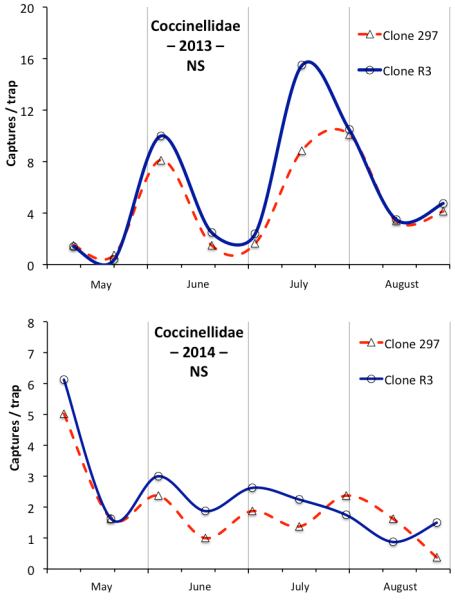


Figure 39 – Coccinellidae captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between clones at a paired t-test.

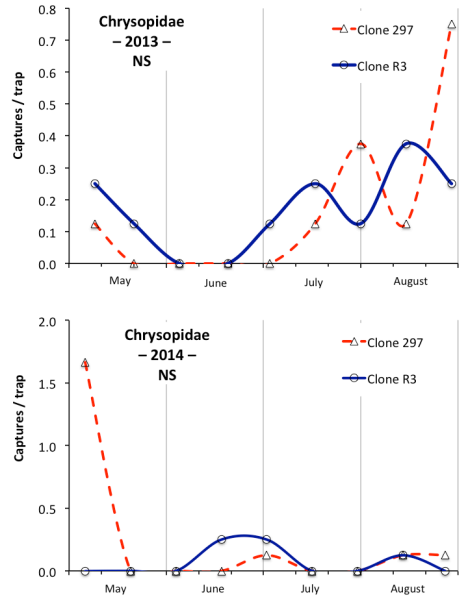


Figure 40 – Chrysopidae captures by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS = not significant differences between clones at a paired t-test.

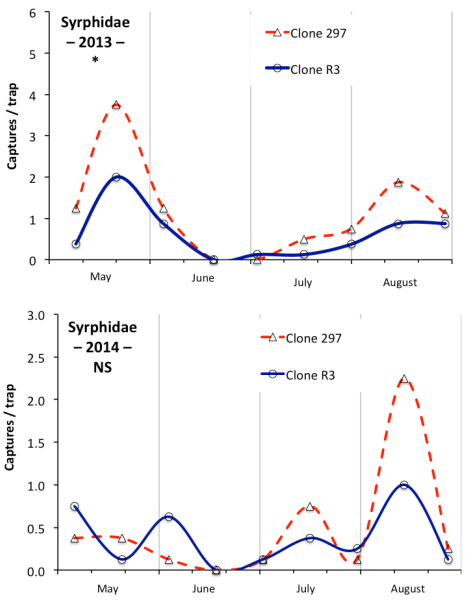


Figure 41 – Syrphidae captured by yellow sticky traps over vegetative seasons 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant < 0.05 differences between clones at a paired t-test.

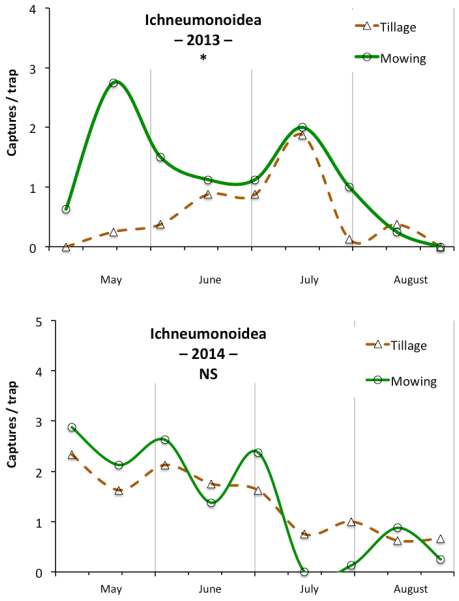


Figure 42 – Ichneumonoidea captured by yellow sticky traps over 2013 and 2014 in the two treatments in comparison. NS and * = not significant and significant < 0.05 differences between treatments at a paired t-test.

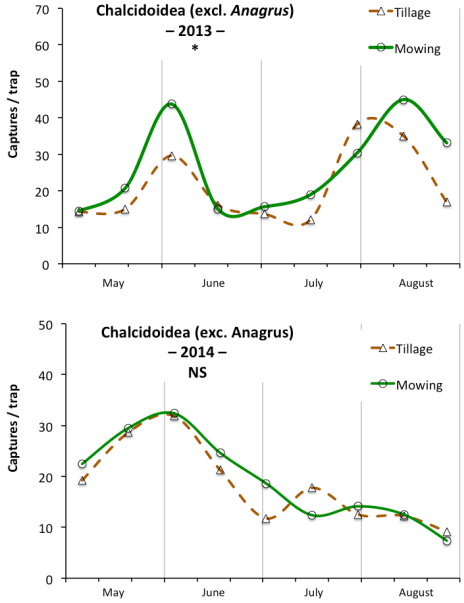


Figure 43 – Chalcidoidea captured by yellow sticky traps over 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant < 0.05 differences between treatments at a paired t-test.

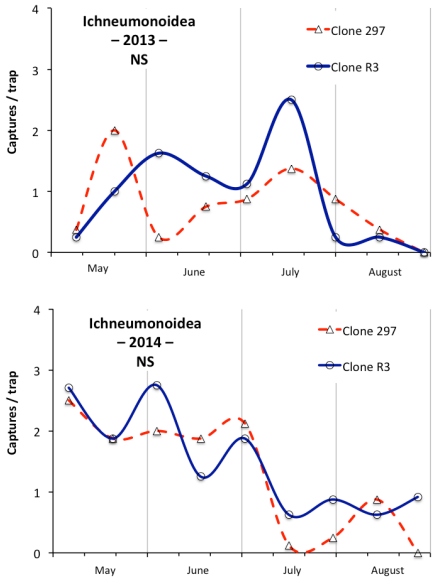


Figure 44 – Ichenumonoidea captured by yellow sticky traps over 2013 and 2014 in the two clones in comparison. NS and ** = not significant and significant < 0.01 differences between clones at a paired t-test.

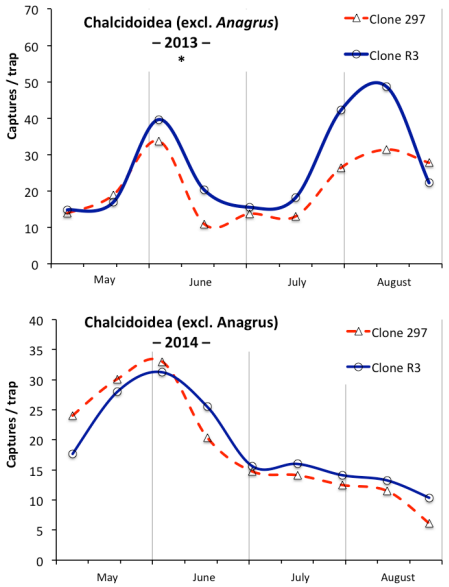


Figure 45 – Chalcidoidea captured by yellow sticky traps over 2013 and 2014 in the two clones in comparison. NS and * = not significant and significant < 0.05 differences between clones at a paired t-test.

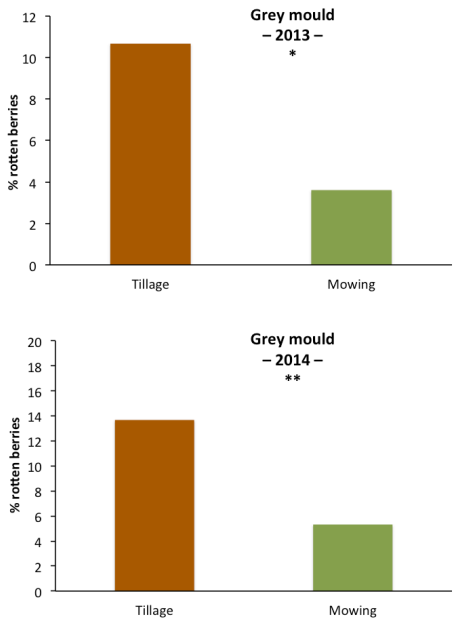


Figure 46 – Grey mould recorded at harvest time in 2013 and 2014 in the two treatments in comparison. * and ** = significant 0.05 and 0.01 differences at ANOVA test.

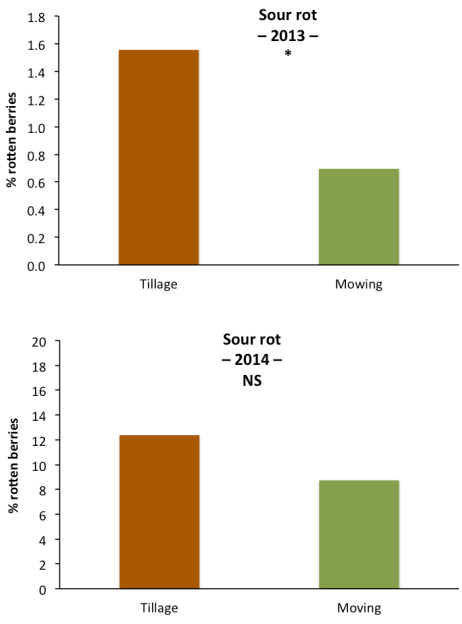


Figure 47 – Sour rot recorded at harvest time in 2013 and 2014 in the two treatments in comparison. NS and * = not significant and significant 0.05 differences at ANOVA test.

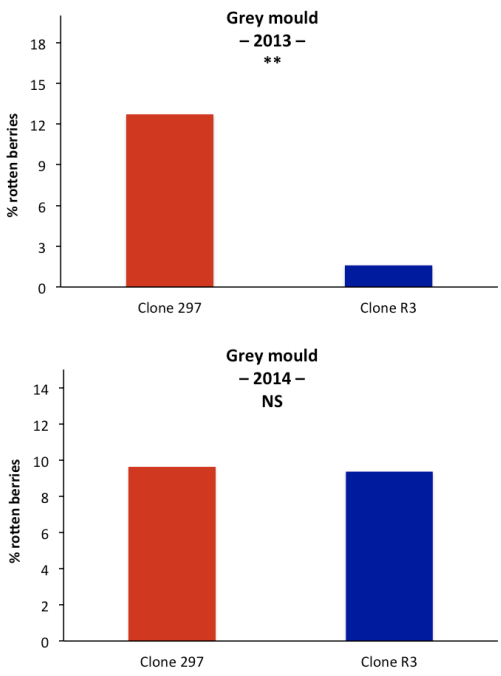


Figure 48 – Grey mould rot recorded at harvest time in 2013 and 2014 in the two clones in comparison. NS and ** = not significant and significant 0.01 differences at ANOVA test.

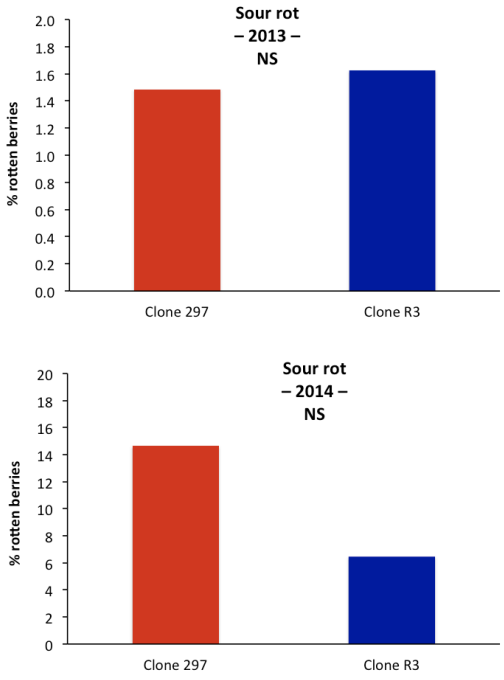


Figure 49 – Sour rot recorded at harvest time in 2013 and 2014 in the two clones in comparison. NS = not significant differences at ANOVA test.

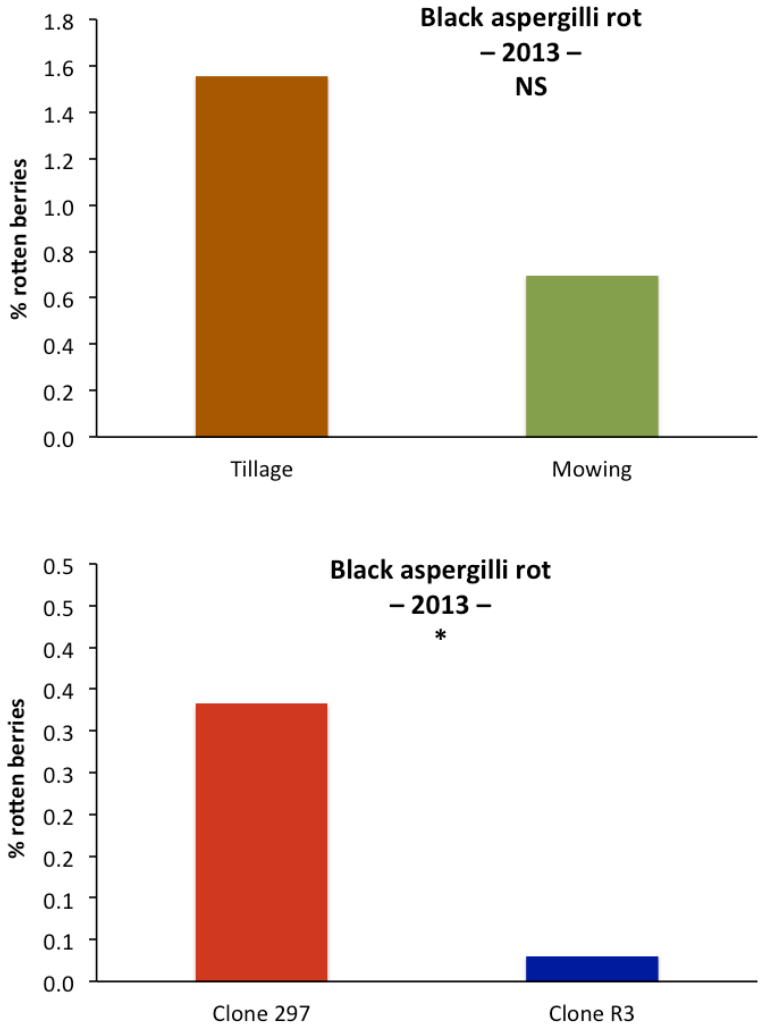


Figure 50 – Black aspergilli rot recorded at harvest time in 2013 and 2014 in the two treatments and clones in comparison. NS and * = not significant and significant 0.05 differences at ANOVA test.

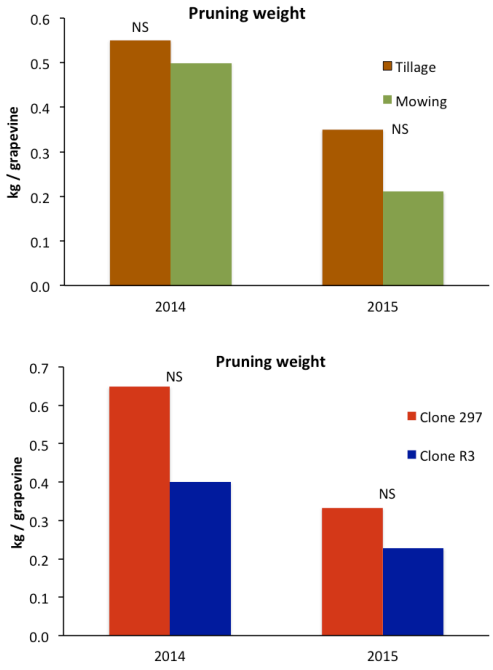


Figure 51 – Pruning weight recorded during the rest season 2013-2014 (2014) and 2014-2015 (2015) in the two treatments and clones in comparison. NS = not significant differences at ANOVA test.

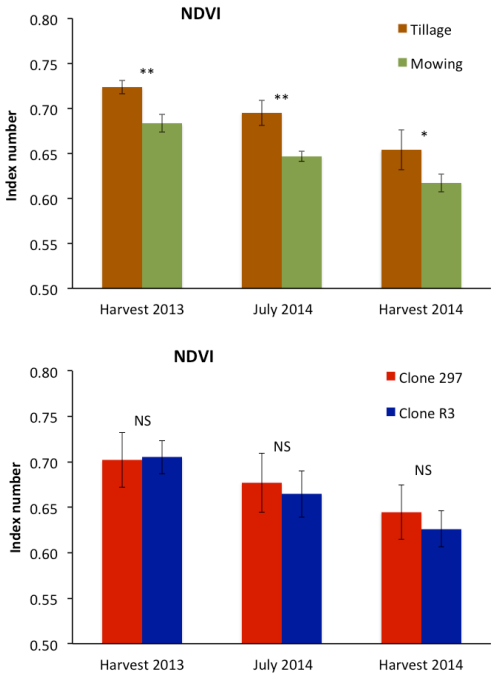


Figure 52 – NDVI recorded in 2013 and 2014 vegetative seasons in the two treatments and clones in comparison. NS, *, ** = not significant, significant < 0.05 and significant 0.01 differences at ANOVA test.

DISCUSSION AND CONCLUSION

1. Grapevine leafhoppers and *Anagrus* spp. parasitoids

In both study years (i.e., 2013 and 2014), *E. vitis* and *Z. rhamni* infestations were higher in the tillage treatment than in the mowing one considering both nymphs sampled on leaves and adults captured by yellow sticky traps.

The higher leafhoppers density in tillage treatment cannot be explained by a different activity of the eggs parasitoid *Anagrus* spp., because they were more abundant in the tillage treatment than in the mowing one and a tendentially lower percentage of leafhopper parasitized eggs in the mowing treatment was observed. Therefore, it seems that these mymarid parasitoids were not favoured by the availability of herbaceous flowering plants. Similar results were obtained in other studies on *Anagrus epos* Girault, a parasitoid of North American leafhoppers in vineyards (Nicholls *et al*., 2000).

A lower leafhopper infestation in the mowing treatment might be related to the reduced vine vigour as well as to the higher abundance of spiders on canopy, as already reported for American leafhoppers (Nicholls *et al*., 2000; Altieri *et al*., 2005; Wilson *et al*., 2015). In particular, in Californian vineyards a higher spider density, favoured by ground cover, reduced *Erythroneura* spp. abundance (Costello and Daane, 2003). According to vigour hypothesis, in the tillage treatment, with bare soil in the inter-rows, both (i) a higher vine-vegetation vigour (see, NDVI parameter) and (ii) a higher number of leafhopper eggs on leaves (significant differences only in 2014), were observed in comparison to the mowing treatment, with permanent herbaceous plants cover, indicating that the leafhoppers prefer to lay eggs on more vigorous vines. These results are in agreement with higher infestation levels observed on more vigorous cultivars, plants and shoots (Vidano *et al*.,

1988; Pavan and Pavanetto, 1989; Decante *et al*., 2009; Pavan and Picotti, 2009). According to spider hypothesis, in 2014 a higher abundance of spiders were observed in the mowing treatment than in the tillage treatment and the leafhopper reduction effect associated with the presence of permanent herbaceous vegetation cover was higher for nymphs (52% of reduction based on cumulated nymphs-day) than for hatched eggs (44% of reduction). Therefore, a higher predation of leafhopper nymphs by spiders can be supposed. Serra *et al*. (2006), that studied the effect of tilled and ground cover inter-rows in a Sardinian vineyard (Italy) on grapevine pests, showed that populations of the leafhopper *Jacobiasca lybica* (Bergevin & Zanon) were not influenced by the ground cover.

In 2013, in comparison with 2014, the third generations of both leafhopper species was very scarce. Because in the summer 2013 two months were without rainfall, a negative effect of vine reduced water supply on leafhopper population level can be supposed in agreement with Fornasiero *et al*. (2012). The decrease of leafhopper populations could be due to reduced egg laying but also to egg and nymph difficulty of survival, as reported for other grapevine leafhoppers (Daane and Williams, 2003; Costello, 2008).

2. *Drepanothrips reuteri* and *Aeolothrips* sp. predators

In both study years, the infestations of *D. reuteri* were higher in the tillage treatment than in the mowing one. The lower population density of thrips pest in this latter treatment appeared linked to a higher abundance of its natural enemies belonging to *Aeolothrips* sp. Higher populations of these predators in the mowing treatment from May can be due to a higher availability of pollen in herbaceous flowering vegetation that represent an important alternative food in the diet of these insects (Trdan *et al*., 2005).

In both study years, the Sauvignon clone 297 was more infested than clone R3. In this case the differences can be explained by a tendentially higher vigour of grapevines in the clone 297.

3. Phytoseiid mites

In both years from May to the end of July the phytoseiid mite populations were higher in the mowing treatment than in the tillage one, whereas from the late summer to the harvest the populations of this predators appeared similar in the two treatments.

For the first sampling period (May-June) the abundant phytoseiid population in the mowing treatment can be related to the increased pollen production from the flowering plants of the inter-rows. A relationship between abundance of pollen and many predatory mites was also highlighted by many studies. For example, the herbaceous vegetation inside vineyards favours the phytoseiid mite persistence because they can develop and reproduce by feeding on pollen (Boller and Frey, 1990; Duso and Camporese, 1991; Schausberger, 1992; Lozzia and Rigamonti, 1998; Duso *et al*., 1997, 2002, 2004; Girolami *et al*., 2000; Madinelli et al, 2002; Pozzebon *et al*., 2005, 2015). The amount of windborne pollen (mainly Poaceae) on grapevine canopy increases through reducing the frequency of inter-row mowing (Girolami *et al*., 2000). In addition, the presence and the amount of pollen is closely related to the occurrence of predatory mite populations as well as the occurrence of tydeiid mites (Acari: Tydeidae), an alternative prey of phytoseiid mites (Camporese and Duso, 1995).

The annulled differences in the predator population density observed in from mid-July to the end of August, in both study years, could be related to factors different than inter-row management. In fact, in 2013 the population rapidly decreased in both treatment from August as consequence of two-month absence of rainfall that caused strong plant water stress and, in turn, lack of pollen production from grasses resulting in adverse environment conditions for these predators. In 2014 the similar phytoseiid population density in the two treatments from half July could be related to the downy mildew infection on grapevine leaves observed in both treatments. Since spores of the fungus are an alternative food for the predatory mites of the family Phytoseiidae (Duso *et al*., 2003; Pozzebon and Duso, 2008), the benefits of higher pollen availability in the mowing treatment become less evident.

4. Spiders

The spider captures on canopy were very higher by beating tray than by yellow sticky traps. In both study years, the spider captures by beating tray showed a trend characterized by a heavy increase from mid-July, whereas no typical trend was observed for spider captures by yellow sticky traps. The increase of captures by beating tray in the second part of the season could be due to the occurrence of juvenile specimens developed from spider taxa that overwinter as adults and then lay eggs in the spring (Marc *et al*., 1999).

In the first year the different management of soil did not influence significantly the spider population density for both sampling methods, whereas in the next year these predators were more abundant in the mowing treatment for the beating tray sampling. This result could be explained by the spider biology characterized by a single generation per year, therefore favourable green cover effects can be only evidenced from the second year of the inter-row management establishment. The results are in agreement with literature data in which the importance of ground plants in vineyards as source of spider preys through whole season and the increase of populations through next years are reported (Costello and Daane, 1998).

In this study, it was found that the spiders are the most abundant predators collected by the beating tray on grapevine canopy, especially in the second year of study. This result is in agreement with several other studies that reported spiders as the most common resident generalist predators in green covered vineyards (Marc *et al*., 1999; Costello and Daane, 1999; Altieri *et al*., 2005; Bolduc, 2005; Isaia *et al*., 2006; Keresztes *et al*., 2012).

In both years, in particular from mid-summer, the spider populations were most abundant in the more vigorous Sauvignon clone 297 than in the Sauvignon clone R3.

5. Generalist predatory insects and parasitoids

Generalist predatory insects (Coccinellidae, Chrysopidae, Dermaptera, Nabidae) recorded on the grapevine canopy (by beating tray and yellow sticky traps) were not affected by the type of inter-row management since, in both years of study, no significant

differences were observed between the tillage and the mowing treatments either for the arthropods collected by beating tray or for those captured by yellow sticky traps.

Hymenoptera parasitoids of the superfamily Ichneumonoidea (Braconidae and Ichneumonidae) were not affected by the type of inter-row management in both years of study. The Hymenoptera parasitoids belonging to the superfamily Chalcidoidea (excl. *Anagrus* spp.) were more abundant in the mowing treatment in 2013, but not in 2014. Differences could be due to the flowering plant species that occurred in the ground cover as they provide alternative foods (i.e., alternative hosts, nectar, pollen), as well as better microclimatic conditions. In fact, it is well known the importance of the presence of flowering plants as source for the Hymenopteran parasitoids sugar consumption in the vineyard (Berndt *et al*., 2006; Irvin *et al*., 2014). In particular, the Compositae and Umbelliferae families, small and relatively open flowers are especially useful (Altieri *et al*., 2005). Regarding the results on Chalcidoidea obtained in this study in 2013, also in Australian vineyards it was found that Trichogrammatidae on the canopy decreased in abundance after tillage (Sharley *et al*., 2008).

6. *Lobesia botrana*

In both years of study, the *L. botrana* infestation was not influenced by the type of inter-row management adopted in the studied vineyard.

A minor infestations in the mowing treatment could be associated with a greater presence of natural enemies or a lower vigour of grapevines. The absence of differences showed that the increase of generalist predators, such as spiders, and hymenopteran parasitoids is not effective in reducing a carpophagous insect that are not usually submitted to an efficiently density-dependent biological control. Also the higher vigour of vines in the tillage treatment did not seem to be favourable to the European grapevine moth. However, in the study of Serra *et al*. (2006) the attacks of *L. botrana* were always higher in the tilled plots, which showed a higher percentage of damaged bunches at harvest. For these authors the differences could be explained by the fact that grapevines of the cover crop plots had less vigorous sprouts that produced smaller and less clustered bunches, less preferred by the moth. Regarding the spider activity on *L. botrana,* these results are in agreement with the

data reported in Addante *et al*. (2003, 2008). In these studies the usefulness of spider activity in limiting the *L. botrana* adults was found, but not that of the larvae, mainly because predators have poor possibilities of reaching the larvae protected inside or among berries.

7. Bunch rots

The permanent green cover of the inter-row allowed a reduced incidence of grey mould in both study years, especially in the second one. A higher grey mould incidence observed in the tillage treatment probably was related to the increased vines vigour, as highlighted by the index NDVI, and the increased size of the berries and bunch compactness (data not reported).

The sour rot incidence was significantly influenced by the type of inter-row management in the 2013, but only tendentially in the next year of study. As for the grey mould, the permanent green cover reduced the incidence of this disease. Differences between the two types of inter-row management were less evident for sour rot than for grey mould. In fact, the incidence of sour rot is influenced primarily by weather conditions, while the botrytis is very influenced by plant vigour and bunch compactness, too (Bisiach *et al*., 1986; Gay Eynard and Morando, 2003; Cravero and Rabino, 2005; Kast and Rupp, 2009).

A higher incidence of grey mould was observed in the clone 297 than in clone R3, probably because the first was tentendially more vigorous than the second.

8. Plant vigour

The two studied types of inter-row management affected the vine vigour because the vines of the tillage treatment showed a higher vigour than those of the mowing one.

The vigour differences could be explained by the evidence that the soil tillage allows to obtain a greater vegetative vine growing due to the absence of water and nutrients

competition with herbaceous plants of the inter-rows (Maigre and Murisier, 1992; Wopert *et al*., 1993; Rives, 2000; Gay *et al*., 2004).

These differences were better evidenced by the NDVI than by the weight pruning data scored, because this last parameter is difficult to significantly modify in only two years by the starting of the two different inter-row managements. In any case in the second year the differences were higher than in the first one.

9. Conclusion

In the vineyard studied during two years the results shown the influence of different inter-row management on the occurrence of grapevine pests and natural enemies.

The populations of some grapevine pests were lower and the abundance of their natural enemies was higher in the inter-row mowing treatment, characterized by the presence of permanent resident vegetation (i.e. green cover) between rows, than in the inter-row tillage treatment, characterized by maintenance of bare soil between rows by cultivation.

It was shown that the green cover of inter-rows allow the decrease of the grapevine vigour and consequently the leafhopper populations.

The populations of vine thrips was reduced in the green covered inter-rows, due to the greater occurrence of their *Aeolothrips* sp. predators.

An important role of spiders, which presence was favoured by the green covered inter-rows, for the pest control could be supposed.

The green cover did not affect *L. botrana* infestation, but the amount of rotten berries contiguous to larval nests may have been reduced since it decreased botrytis incidence.

Chapter VI.

References

Addante R., Moleas T., Ranieri. (2003) Preliminary investigations on the interaction between spiders (Araneae) and grapevine moth (*Lobesia botrana* (Denis et Schiffermüller)) populations in Apulian vineyards. *OILB/SROP Bulletin*, **26**, 8, 111-115.

Addante R., Di Gioia S., Calculli C., Pollice A. (2008) The impact of spiders (Aranae) on *Lobesia botrana* (Denis & Schiffermüller) population density. *IOBC/WPRS Bulletin*, **36**, 221-231.

Alderson S.L., Waddell B.C., Ryan a N. (1998) Effects of heating rate on the mortality of lightbrown apple moth. In O'Callaghan M. (ed), pp. 199–203, *51st New Zealand Plant Protection Conference*, 12 August 1998, Hamilton, New Zealand.

Altieri M.A. (1999) The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems and Environment*, **74**, 1/3, 19-31.

Altieri M.A., Letourneau D.K. (1982) Vegetation management and biological control in agroecosystems. *Crop Protection*, **1**, 4, 505-430.

Altieri M.A., Schmidt L.L. (1985) Cover crop manipulation in northern California apple orchards and vineyards: Effects on arthropod communities. *Biological Agriculture and Horticulture*, **3**, 1, 1-24.

Altieri M.A., Ponti L., Nicholls C.I. (2005) Manipulating vineyard biodiversity for improved insect pest management: case studies from northern California. *International Journal of Biodiversity Science and Management*, **1**, 4, 191-203.

Andow D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561-586.

Angeli G., Maines R., Fanti M., Forti D., Sofia M., Baldessari M., Tomasi C., Sandri O., Delaiti L., Ioriatti C., Girolami V., Cross J., Ioriatti C. (2005) Biological control of *Metcalfa pruinosa* with *Neodrynus typhlocybae*: establishment and diffusion of the parasitoid in Trentino Alto Adige (Italy). *OILB/SROP Bulletin*, **28**, 7, 271-274.

Baggiolini M., Canevascini V., Tencalla Y., Caccia R., Sobrio G., Cavalli S. (1968) La cicadella verte *Empoasca flavescens* F. (Homoptera: Typhlocybidae), agent d'altérations foliaires sur vigne. *Recherche Agronomique en Suisse*, **7**, 43-69.

Bagnoli B., Lucchi A. (2006) Parasitoids of *Lobesia botrana* (Den. & Schiff.) in Tuscany. *IOBC/WPRS Bulletin*, **29**, 11, 139-142.

Bailey S.F. (1942) The grape or vine Thrips, *Drepanothrips reuteri*. *Journal of Economical Entomology*, **35**, 382–386.

Baillod M., Jermini M., Schmid A. (1990) Essais de nuisibilité de la cicadelle verte, *Empoasca vitis* Goethe sur le cépage Merlot au Tessin et le cépage Pinot noir en Valais. *IOBC/WPRS Bulletin*, **13**, 158-161.

Baillod M., Jermini M., Antonin P., Linder C., Mittaz C., Carrera E., Udry V., Schmid A. (1993) Stratéegies de lutte contre la cicadelle verte de la vigne, *Empoasca vitis* (Göthe). Efficacité des insecticides et problématique liée à la nuisibilité. *Revue Suisse de Viticulture Arboriculture Horticulture*, **25**, 133-141.

Bairlein F. (1990) Estimating density and reproductive parameters for terrestrial field testing with birds. In Somerville L., Walker C. H. (eds), pp. 113-128, *Pesticide Effects On Terrestrial Wildlife*, Taylor and Francis, London.

Barbieri R., Cavallini G., Pollini A. (1996) Le tignole della vite: strategie ed esperienze di lotta. *L'Informatore Agrario*, **52**, 14, 75-79.

Barker A.M., Maczka C.J.M. (1996) The relationships between host selection and subsequent larval performance in three free-living graminivorous sawflies. *Ecological Entomology*, **21**, 4, 317-327.

Bassi A., Alber R., Wiles J.A., Rison J.L., Frost N.M., Marmor F.W., Marcon P.C. (2007) Chlorantraniliprole: a novel anthranilic diamide insecticide. pp. 52-59, *XVI International Plant Protection Congress*, 15-18 October 2007, Glasgow, Scotland, UK.

Begum M., Gurr G.M., Wratten S.D., Hedberg P.R., Nicol H.I. (2006) Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology*, **43**, 3, 547-554.

Bellmann H. (2011) Guida ai ragni d'Europa. Muzzio editore, Roma, 427 pp.

Bentancourt C.M., Scatoni I.B., Gonzalez A., Franco J. (2003) Effects of larval diet on the development and reproduction of *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera: Tortricidae). *Neotropical Entomology*, **32**, 4, 551-557.

Berenbaum M.R., Green E.S., Zangerl A.R. (1993) Web costs and web defense in the parsnip webworm (Lepidoptera: Oecophoridae). *Environmental Entomology*, **22**, 4, 791-795.

Bergqvist J., Dokoozlian N., Ebisuda N. (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *American Journal of Enology and Viticulture*, **52**, 1, 1–7.

Berndt L., Wratten S., Frampton C., Hoddle M. S. (2000) The use of buckwheat flowers to enhance efficiency of leafroller parasitoids in a New Zealand vineyard. pp. 121-123, *California Conference on Biological Control II, The Historic Mission In Riverside*, 11-12 July 2000, California, USA.

Berndt L.A., Wratten S.D., Scarratt S.L. (2006) The influence of floral resource subsidies on parasitism rates of leafrollers (Lepidoptera: Tortricidae) in New Zealand vineyards. *Biological Control*, **37**, 1, 50-55.

Bigot G., Ostan M., Sandra M., Pavan F., Zandigiacomo P. (2008) Lotta contro la tignoletta della vite con il metodo della confusione sessuale in Friuli Venezia Giulia. *Atti Giornate Fitopatologiche*, **1**, 203-210.

Bisiach M., Minervini G., Zerbetto F. (1986) Possible integrated control of grapevine sour rot. *Vitis*, **25**, 118-128.

Bolduc E., Buddle C.M., Bostanian N.J., Vincent C. (2005) Ground-dwelling spider fauna (Araneae) of two vineyards in Southern Quebec. *Environmental Entomology*, **34**, 3, 635-645.

Böll S., Schwappach P., Herrmann J.V. (2006) Planting dog roses – an efficient method to promote mymarid populations in vineyards? *IOBC/WPRS Bulletin*, **29**, 11, 175-181.

Boller E.F., Frey B. (1990) Bluhende Rebberge in der Ostschwiez. I. Zur Bedeutung des Pollens für die Raubmilben. *Schwiez Zeitschrift für Obst-und Wienbau*, **126**, 401-405.

Boller E.F., Häni F., Phoeling H.M. (2004) Ecological infrastructure: Ideabook on functional biodiversity at the farm level. Temperate Zones Of Europe, 1st edn. IOBC/OILB, Switzerland, 212 pp.

Briere J.F., Pracros P. (1998) Comparison of temperature-dependent growth models with the development of *Lobesia botrana* (Lepidoptera: Tortricidae). *Environmental Entomology*, **27**, 1, 94–101.

Brown A.W.A. (1978) Ecology of pesticides. *John Wiley & Sons,* New York, 525 pp.

Bürgi L.P., Mills N.J. (2012) Ecologically relevant measures of the physiological tolerance of light brown apple moth, *Epiphyas postvittana*, to high temperature extremes. *Journal of Insect Physiology*, **58**, 9, 1184–1191.

Caffarra A., Rinaldi M., Eccel E., Rossi V., Pertot I. (2012) Modelling the impact of climate change on the interaction between grapevine and its pests and pathogens: European grapevine moth and powdery mildew. *Agriculture, Ecosystems & Environment*, **148**, 89-101.

Caldwell S.T., Keil J.E., Sandifer S.H. (1977) Survey of animal pesticides poisoning in South Carolina, 1976. *Veterinary and Human Toxicology*, **19**, 3, 166-168.

Camporese P, Duso C. (1995) Life history and life table parameters of the predatory mite *Typhlodromus talbii. Entomologia Experimentalis et Applicata*, **77**, 2, 149-157.

Campos L., Franco J.C., Monteiro A., Lopez, C. (2006) Influência do enrelvamento na abundância de artródodes associados a uma vinha da Estremadura, *Ciência e Técnica Vitivinícola*, **21**, 1, 33-46.

Candolfi M.P., Boller R.E., Wermelinger B. (1992) Spatio-temporal distribution of *Panonychus ulmi* Koch (Acari: Tetranychidae) on Guyot-trained grapevines. *Journal of Applied Entomology*, **114**, 244-250.

Candolfi M.P., Jermini M., Carrera E., Candolfi-Vasconcelos M.C. (1993) Grapevine leaf exchange, plant growth, yield, fruit quality and carbohydrate reserves influenced by the grape leafhopper, *Empoasca vitis*. *Entomologia Experimentalis et Applicata*, **69**, 3, 289-296.

Cardoza Y.J., Teal P.E.A., Tumlinson J.H. (2003) Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behavior by *Cotesia marginiventris*. *Environmental Entomology*, **32**, 5, 970-976.

Carle P., Moutous G. (1965) Observations sur le mode de nutrition sur vigne de quatre espèces de cicadelles. *Annales des Épiphyties*, **16**, 4, 333-354.

Cerutti F. Baumgartner J., De Lucchi V. (1988) Ricerche sull'ecosistema "vigneto" nel Ticino: I. Campionamento delle popolazioni di *Empoasca vitis* Göethe (Homoptera, Cicadellidae, Typhlocybinae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, **61**, 29-41.

Cerutti F., Baumgärtner J., Delucchi V. (1991) The dynamics of grape leafhopper *Empoasca vitis* Göethe populations in southern Switzerland and the implications for habitat management. *Biocontrol Science and Technology*, **1**, 3, 177-194.

Charmillot P.J., Pasquier D. (2002) Grape tortricids: population dynamics during 1995 to 2001. *Revue Suisse de Viticulture, Arboriculture et Horticulture*, **34**, 1, 31-32

Charmillot P.J., Pasquier D., Salamin C., Briand F. (2006) Efficacité larvicide et ovicide sur les vers de la grappe *Lobesia botrana* et *Eupoecilia ambiguella* de différents insecticides appliqués par trempage des grappes. *Revue Suisse de Viticulture Arboriculture Horticulture*, **38**, 5, 286-295.

Cisneros F.H., Barnes M.M. (1974) Contribution to the biological and ecological characterization of apple and walnut host races of Codling moth, *Laspeyresia pomonella* (L.): Moth longevity and oviposition capacity. *Environmental Entomology*, **3**, 3, 402-406.

Coscollá R. (1997) La polilla del racimo de la vid (*Lobesia botrana* Den. y Schiff.) Generalitat Valenciana, Conselleria de Agricoltura, Pesca y Alimentación, Valencia, Spain, 613 pp.

Coscollá R., Sánchez J., Beltrán V. (1986) Estudio preliminar sobre mortalidad de huevos de *Lobesia botrana* Den. y Schiff. por efecto de altas temperaturas y bajas humedades relativas en laboratorio. *Boletín de Sanidad Vegetal, Plagas*, **12**, 3–7.

Cossé A.A., Endris J.J., Millar J.G., Baker T.C. (1994) Identification of volatile compounds from fungus-infected date fruit that stimulate upwind flight in female *Ectomyelois ceratoniae*. *Entomologia Experimentalis et Applicata*, **72**, 3, 233-238.

Costello M.J. (2008) Regulated deficit irrigation and density of *Erythroneura* spp. (Hemiptera: Cicadellidae) on grape. *Journal of Economic Entomology*, **101**, 1287-1294.

Costello M.J., Daane K.M. (1998) Influence of ground cover on spider populations in a table grape vineyard. *Ecological Entomology*, **23**, 1, 33-40.

Costello M.J., Daane K.M. (1999) Abundance of spider and insect predators on grapes in central California. *The Journal of Arachnology*, **27**, 531-538.

Costello M.J., Daane K.M. (2003) Spider and leafhopper (*Erythroneura* spp.) response to vineyard ground cover. *Environmental Entomology*, **32**, 5, 1085-1098.

Cozzi G., Pascale M., Perrone G., Visconti A., Logrieco A. (2006) Effect of *Lobesia botrana* damages on black aspergilli rot and ochratoxin A content in grapes. *International Journal of Food Microbiology*, **111**, Supplement 1, S88-S92.

Cozzi G., Haidukowski H., Perrone G., Visconti A., Logrieco A. (2009) Influence of *Lobesia botrana* field control on black aspergilli rot and ochratoxin A contamination in grapes. *Journal of Food Protection*, **72**, 4, 894-897.

Cravero S., Rabino M. (2005) Sfogliatura e botrite, marciume acido ed altre alterazioni del grappolo. *L'Informatore Agrario*, **61**, 21, 43-44.

Crippen D.D., Morrison J.C. (1986) The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. *American Journal of Enology and Viticulture*, **37**, 4, 243–247.

Daane K.M., Costello M.J. (1998) Can cover crops reduce leafhopper abundance in vineyards? *California Agriculture*, **52**, 2, 27-33.

Daane K.M., Williams L.E. (2003) Manipulating vineyard irrigation amounts to reduce insect pest damage. *Ecological Applications*, **13**, 6, 1650-1666.

Dalla Montà L., Marchesini E., Pavan F. (2007) Relazione fra tignole della vite e attacchi di *Botrytis cinerea*. *Informatore Fitopatologico*, **57**, 4, 28-35.

Decante D., Van Helden M. (2001) Intraplot distribution of leafhoppers damaging Bordelais vineyards. *IOBC/WPRS Bulletin*, **24**, 7, 203-210.

Decante D., Van Leeuwen C.,Van Helden M. (2009) Influence of plot characteristics and surrounding vegetation on the intra-plot spatial distribution of *Empoasca vitis*. *Agricultural and Forest Entomology*, **11**, 4, 377-388.

Dellei A., Szendrey L. (1991) The mite infestation of different grapevine varieties in the vineyards of Heves County. *Növényvédelem*, **27**, 2, 55-61. [in Hungarian]

Delrio G., Luciano P., Prota R. (1987) Researches on grape-vine moths in Sardinia. In Cavalloro R. (ed), pp. 57-67, *Proceeding of a meeting of the EC Expert' Group on Integrated Pest Control in Viticulture,* 26-28 September 1985, Portoferraio, Italy.

Deseo K.V. (1980) Due fitofagi di secondaria importanza nei vigneti emiliani: *Euzophera bigella* Zell. ed *Ephestia parasitella* ssp. *Unicolorella staud*. (Lepidoptera: Pyralidae). *Informatore Fitopatologico*, **30**, 6, 7-9.

Deseo K.V., Marani F., Brunelli A., Bertaccini A. (1981) Observations on the biology and diseases of *Lobesia botrana* Den. and Schiff. (Lepidoptera: Tortricidae) in Central-North Italy. *Acta Phytopathologica Academiae Scientiarum Hungaricae*, **16**, 3/4, 405-431.

Diago M.P., Vilanova M., Tardaguila J. (2010) Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* (L.) Tempranillo wine. *American Journal of Enology and Viticulture*, **61**, 3, 382-391.

Di Bucchianico N., Girolami V., Mazzocchetti A., Mazzon L. (2004) Introduzione in Abruzzo di *Neodryinus typhlocybae* (Ashmead) per la lotta biologica contro *Metcalfa pruinosa* (Say). *Atti Giornate Fitopatologiche*, **1**, 123-130.

Dicke M. (1996) The role of microorganisms in tri-trophic interactions in systems consisting of plants, herbivores, and carnivores. In Colwell RR, Simidu U, Ohwada K (eds), pp. 71-84, *Microbial Diversity in Time and Space*. Plenum press, New York, USA.

Dokoozlian N.K., Kliewer W.M. (1996) Influence of Light on Grape Berry Growth and Composition Varies during Fruit Development. *Journal of the American Society for Horticultural Science*, **121**, 5, 869–874.

Dongiovanni C., Giampaolo C., DI Carolo M., Natale P., Venerito P. (2008). Valutazione dell'efficacia di differenti modalità di applicazione di *Bacillus thuringiensis* nei confronti della tignoletta della vite in Puglia. *Atti Giornate Fitopatologiche,* **1**, 199-202.

Doutt R.L., Nakata J. (1973) The *Rubus* leafhopper and its egg parasitoid: An endemic biotic system useful in grape pest management. *Environmental Entomology,* **2**, 3, 381-386.

Duncan R. A., Stapleton J. J., Leavitt G.M. (1995) Population dynamics of *epiphytic mycoflora* and occurrence of bunch rots of wine grapes as influenced by leaf. *Plant Pathology*, **44**, 6, 956-965.

Duso C. (1992) Role of predatory mites *Amblyseius aberrans* (Oudemans), *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) in vineyards. III. Influence of variety characteristics on the success of *A. aberrans* and *T. pyri*. *Journal of Applied Entomology*, **114**, 1-5, 455-462.

Duso C., Camporese P. (1991) Developmental times and ovoposition rates of predatory mites *Typhlodormus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) reared on different foods. *Experimental and Applied Acarology*, **13**, 2, 117-128.

Duso C., Pasqualetto C. (1993) Factors affecting the potential of phytoseiid mites (Acari: Phytoseiidae) as biocontrol agents in north-Italian vineyards. *Experimental and Applied Acarology*, **17**, 4, 241-258.

Duso C., Malagnini V., Paganelli A. (1997) Indagini preliminari sul rapporto tra polline e *Kampimodromus aberrans* (Acari: Phytoseiidae) su *Vitis vinifera* L. *Allionia*, **3**, 229-239.

Duso C., Malagnini V., Paganelli A., Aldegheri L., Bottini M. (2002) Phytoseiid mites - pollen relationships: observation in a vineyard and the surrounding vegetation. In Bernini F., Nannelli R., Nuzzaci G., de Lillo E. (eds), pp. 375-389, *Proceedings of the IV Symphosium of the European association of acarologists on Acarid phylogeny and evolution: adaptation in mites and tiks*, Kluwer Academic Publishers.

Duso C., Malagnini V., Drago A., Pozzebon A., Galbero G., Castagnoli M., De Lillo E. (2003) The colonization of phytoseiid mites (Acari: Phytoseiidae) in a vineyard and the surrounding hedgerows. *OILB/SROP Bulletin*, **26**, 4, 37-42.

Duso C., Pozzebon A., Capuzzo C., Bisol P.M., Otto S. (2003) Grape downy mildew spread and mite seasonal abundance in vineyards: evidence for the predatory mites *Amblyseius andersoni* and *Typhlodromus pyri*. *Biological Control*, **27**, 3, 229-241.

Duso C., Malagnini V., Paganelli A., Aldeghieri L., Bottini M., Otto S. (2004) Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. *BioControl*, **49**, 4, 397-415.

Duso C., Pozzebon A., Malagnini V. (2006) Augmentative releases of beneficials in vineyards: factors affecting predatory mite (Acari: Phytoseiidae) persistence in the long-term period. *OILB/SROP Bulletin*, **29**, 11, 215-219.

English J. T., Kaps M. L., Moore J. F., Hill J., Nakova M. (1993) Leaf removal for control of Botrytis bunch rot of wine grapes in the midwestern United States. *Plant Disease*, **77**, 12, 1224-1227.

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**, 2, 173-181.

Fermaud M. (1998). Cultivar susceptibility of grape berry clusters to larvae of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, **91**, 4, 974-980.

Fermaud M., Giboulot A. (1992) Influence of *Lobesia botrana* larvae on field severity of Botrytis rot of grape berries. *Plant Disease*, **76**, 4, 404-409.

Fermaud M., Le Menn R. (1989) Association of *Botrytis cinerea* with grape berry moth larvae. *Phytopathology*, **79**, 6, 651-656.

Fermaud M., Le Menn R. (1992) Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae. *Phytopathology*, **82**, 12, 1393-1398.

Fermaud M., Liminana J. M., Froidefond G., Pieri P. (2001) Grape cluster microclimate and architecture affect severity of *Botrytis* rot of ripening berries. *IOBC/WPRS Bulletin*, **24**, 7, 7-9.

Feng X., Hansen J.D., Biasi B., Tang J., Mitcham E.J. (2004) Use of hot water treatment to control codling moths in harvested California "Bing" sweet cherries. *Postharvest Biology and Technology*, **31**, 1, 41–49.

Filip I. (1986) Breeding zones of the grape moth (*Lobesia botrana* Den & Schiff) in Romania. *Probleme de Protecţia Plantelor*, **14**, 1, 25-30.

Fornasiero D., Duso C., Pozzebon A., Tomasi D., Gaiotti F., Pavan F. (2012) Effects of irrigation on the seasonal abundance of *Empoasca vitis* in North-Italian vineyards. *Journal of Economic Entomology*, **105**, 1, 176–185.

Fornasiero D., Pavan F., Pozzebon A., Picotti P., Duso C. (2016) Relative infestation level and sensitivity of grapevine cultivars to the leafhopper *Empoasca vitis* (Hemiptera: Cicadellidae). *Journal of Economic Entomology*, **109**, 1, 416-425.

Frilli F., Villani A., Zandigiacomo P. (2001) *Neodryinus typhlocybae* (Ashmead) antagonista di *Metcalfa pruinosa* (Say). Risultati di liberazioni pluriennali effettuate in Friuli-Venezia. *Entomologica*, **35**, 27-38.

Gabel B. (1981) Über den Einflufi der Temperatur auf die Entwicklung und Vermehrung des Bekreuzten Traubenwicklers, *Lobesia botrana* Den. et Schiff. (Lepid., Tortricidae). *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz*, **54**, 6, 83-87.

Galet P. (1982) Les maladies et les parasites de la vigne. Tome II. Les parasites animaux. *Imprimerie du Paysan du midi*, Montpellier, 1004 pp.

Gay G., Morando A. (2003) La gestione agronomica del vigneto: influenza di alcune scelte sugli attacchi di *Botrytis cinerea*. *Informatore Fitopatologico*, **53**, 4, 9-16.

Gay G., Bovio M., Minati J.L., Morando A., Novello V., Ambrosoli R. (2004) La gestion du sol par rapport au système de conduite dans un vignoble en forte pente. *Journal International des Sciences de la Vigne et du Vin*, **38**, 1, 71-74.

Geisler G. (1959) Untersuchungen zur Resistenzzüchtung gegen "Heuwurm"-Befall bei Reben. *Vitis*, **2**, 2, 84-100.

Gilioli G., Pasquali S., Marchesini E. (2016) A modelling framework for pest population dynamics and management: An application to the grape berry moth. *Ecological modelling*, **320**, 348-357.

Girolami V., Mazzon L. (1999) Controllo di Metcalfa pruinosa ad opera di Neodryinus typhlocybae. *L'Informatore Agrario*, **55**, 19, 87-91.

Girolami V., Duso C., Refatti E., Osler R. (1989) Lotta integrata in viticoltura. Malattie della vite. IRIPA, Mestre, 100 pp.

Girolami V., Borella E., Di Bernardo A., Malagnini V. (2000) Influenza positiva sui fitoseidi della fioritura del cotico erboso. *L'Informatore Agrario*, **56**, 21, 71-73.

Götz B. (1941) Laboratoriumsuntersuchungen über den Einfluß von konstanten und variierenden Temperaturen, relativer Luftfeuchtigkeit und Licht auf die Embryonalentwicklung von *Polychrosis botrana*. *Anzeiger für Schädlingskunde*, **17**, 7, 73-83, 86-96, 125-129.

Grifoni D., Carreras G., Zipoli G., Sabatini F., Dalla Marta A., Orlandini S. (2008) Row orientation effect on UV-B, UV-A and PAR solar irradiation components in vineyards at Tuscany, Italy. *International journal of biometeorology*, **52**, 8, 755-763.

Gubler W. D., Marios J. J., Bledsoe A. M., Bettiga L. J. (1987) Control of Botrytis bunch rot of grape with canopy management. *Plant Disease*, **71**, 7, 599-601.

Hagley E.A.C., Barber D.R. (1992) Effect of food sources on the longevity and fecundity of *Pholetesor ornigis* (Weed) (Hymenoptera: Braconidae). *Canadian Entomologist*, **124**, 2, 341-346.

Hanna R., Zalom F.G., Roltsch W.J. (2003) Relative impact of spider predation and cover crop on population dynamics of *Erythroneura variabilis* in a raisin grape vineyard. *Entomologia Experimentalis et Applicata*, **107**, 3, 177-191.

Hardman J.M., Herbert H.J., Sanford K.H., Hamilton D. (1985) Effect of populations of the European red mite, *Panonychus ulmi*, on the apple variety Red Delicious in Nova Scotia. *The Canadian Entomologist*, **117**, 10, 1257-1265.

Hayes W.J., Vaughn W.K. (1977) Mortality from pesticides in the United States in 1973 and 1974. *Toxicology and Applied Pharmacology*, **42**, 2, 235-252.

Hed B., Ngugi H.K., Travis J. W. (2011) Use of gibberellic acid for management of bunch rot on Chardonnay and Vignoles grape. *Plant Disease*, **95**, 3, 269-278.

Herbert H.J. (1981) Biology, life tables, and intrinsic rate of increase of the European red mite, *Panonychus ulmi* (Acarina:Tetranychidae). *The Canadian Entomologist*, **113**, 1, 65-71.

Hobson R.P. (1935) On a fat-soluble growth factor required by blow-fly larvae. II. Identity of the growth factor with cholesterol. *Biochemical Journal*, **29**, 9, 2023-2026.

Hong Y., Martinez A., Liger-Belair G., Jeandet P., Nuzillard J., Cilindre C. (2012) Metabolomics reveals simultaneous influences of plant defence system and fungal growth in *Botrytis cinerea* infected *Vitis vinifera* cv. Chardonnay berries. *Journal of Experimental Botany*, **63**, 16, 695-709.

Howell J.F., Schmidt R.S. (2002) Codling moth (Lepidoptera: Tortricidae): development at constant and at orchard temperatures. *Journal of Agricultural and Urban Entomology*, **19**, 1, 15–23.

Ifoulis A.A., 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, 340-343.

Ignoffo C. M., Garcia, C. (1978) UV-photoinactivation of cells and spores of *Bacillus thuringiensis* and effects of peroxidase on inactivation. *Environmental Entomology*, **7**, 2, 270-272.

Ioriatti C., Bagnoli B., Lucchi A., Veronelli V. (2004). Vine moths control by mating disruption in Italy: results and future prospects. *Redia*, **87**, 117-128.

Ioriatti C., Anfora G., Angeli G., Mazzoni V., Trona F. (2009) Effects of chlorantraniliprole on eggs and larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). *Pest Managemant Science*, **65**, 6, 717-722.

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.

Ioriatti C., Lucchi A., Varela L. (2012) Grape berry moths in Western European vineyards and their recent movement into the New World. In Bostanian N.J., Vincent C., Isaacs R. (eds), pp. 339-359, *Arthropod Management in Vineyards: Pests, Approaches, and Future Directions*, Springer, London.

Irigaray F.J., Moreno F., Pérez-Moreno I., Marco V. (2006) Larval growth and the effect of temperature on head capsule size in *Lobesia botrana* (Lepidoptera: Tortricidae). *Environmental Entomology*, **35**, 2, 189–193.

Irvin, N. A., Pinckard T.R., Perring T.M., Hoddle M.S. (2014) Evaluating the potential of buckwheat and cahaba vetch as nectar producing cover crops for enhancing biological control of *Homalodisca vitripennis* in California vineyards. *Biological Control*, **76**, 10-18.

Isaacs R., Vincent C., Bostanian N.J. (2012) Vineyard IPM in a changing world: Adapting to new pests, tactics, and challenges. In Bostanian N. J., Vincent C., Isaacs R. (eds.), *Arthropod management in vineyards: pest, approaches, and future directions*. Springer Science+Business Media B.V., Dordrecht, The Netherlands, 505 pp.

Isaia M., Bona F., Badino G. (2006) Influence of landscape diversity and agricultural practices on spider assemblage in Italian vineyards of Langa Astigiana (Northwest Italy). *Environmental Entomology*, **35**, 2, 297-307.

Jenser G., Szita É., Szénàsi Á., Vörös G., Tóth M. (2010) Monitoring the population of vine thrips (*Drepanothrips reuteri* Uzel) (Thysanoptera: Thripidae) by using fluorescent yellow sticky traps. *Acta Phytopathologica et Entomologica Hungarica,* **45**, 2, 329-335.

Jogaiah S., Striegler K.R., Bergmeier E., Harris J. (2012) Influence of cluster exposure to sun on fruit composition of 'Norton' grapes (*Vitis estivalis* Michx) in Missouri. *International Journal of Fruit Science*, **12**, 4, 410-426.

Johnson R.M., Ellis M.D., Mullin C.A., Frazier M. (2010) Review. Pesticides and honey bee toxicity – USA. *Apidologie*, **41**, 3, 312-331.

Jonsson M., Wratten S.D., Landis D., Tompkins J.M.L., Cullen R. (2010) Habitat manipulation to mitigate the impacts of invasive arthropod pests. *Biological Invasions*, **12**, 9, 2933-2945.

Karg W. (1991) Die Raubmilbenarten der Phytoseiidae Berlese (Acarina) Mitteleuropas sowie angrenzender Gebiete. *Zoologische Jahrbuecher Abteilung fuer Systematik Oekologie und Geographie der Tiere*, **118**, 1, 1-64.

Kast W.K., Rupp D. (2009) Auswirkungen klimatischer Veränderungen auf die Phänologie der Rebe und die Bedingungen wahrend der Traubenreife. *Mitteilungen Klosterneuburg,* **59**, 3-7.

Kellner R.L. (2008) The role of microorganisms for eggs and progeny, In Hilker M., Meiners T. (eds), pp. 149-167, *Chemoecology of Insect Eggs and Egg Deposition*, Oxford, UK: Blackwell Publishing Ltd.

Keresztes B., Mikulás J., Markó V. (2012) Effects of different management systems on the ground surface spider (Araneae) communities in a vineyard near Kecskemet. *Növényvédelem*, **48**, 5, 203-213. [in Hungarian]

Kido H., Flaherty D.L., Bosch D., Valero K.A. (1984) French prune trees as overwintering sites for the grape leafhopper egg parasite. *American Journal of Enology and Viticulture*, **35**, 3, 156-160.

Kliewer W.M., Lider L.A. (1968) Influence of cluster exposure to the sun on the composition of Thompson seedless fruit. *American Journal of Enology and Viticulture*, **19**, 3, 175-184.

Kührt U., Samietz J., Dorn S. (2005) Thermoregulation behaviour in codling moth larvae. *Physiological Entomology*, **30**, 54-61.

Landis D.A., Wratten S.D., Gurr G.M. (2000) Habitat management to conserve natural enemies of arthropod pest in agriculture. *Annual Review of Entomology*, **45**, 175-201.

Le Quesne W.J. (1965) Handbooks for the identification of British insects. Vol. II, Part 2(a): Hemiptera Cicadomorpha (excluding Deltocephalinae and Typhlocybinae). Royal Entomological Society of London, Hampton, GB, 64 pp.

Levi A. (1873) Intorno ad alcuni parassiti della tignola dell'uva. *Bollettino della Associazione Agraria Friulana*, II, **(seri I)**, 214-226.

Liang L.N., Zhang W., Ma G., Hoffmann A.A., Ma C.S. (2014) A single hot event stimulates adult performance but reduces egg survival in the oriental fruit moth, *Grapholitha molesta. Plos One*, **9**, 12, e116339.

Lill J.T., Marquis R.J., Walker M.A., Peterson L. (2007) Ecological consequences of shelter sharing by leaf-tying caterpillars. *Entomologia Experimentalis et Applicata*, **124**, 1, 45-53.

Lorenz D. H., Eichhorn K. W., Bleiholder H., Klose R., Meier U., Weber E. (1995) Growth stages of the grapevine: phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. vinifera). Codes and descriptions according to the extended BBCH scale. *Australian Journal of Grape and Wine Research*, **1**, 2, 100-103.

Lucchi A., Santini L. (2011) Life history of *Lobesia botrana* on *Daphne gnidium* in a Natural Park of Tuscany. *IOBC/WPRS Bulletin*, **67**, 197-202.

Madinelli S., Mori N., Girolami V. (2002) Importanza del polline del cotico erboso per la presenza di fitoseidi. *L'Informatore Agrario*, **58**, 15, 125-127.

Maher N., Thiéry D. (2004) A bioassay to evaluate the activity of chemical stimuli from grape berries on the oviposition of *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research*, **94**, 1, 27-33.

Maher N., Thiery D. (2006) *Daphne gnidium*, a possible native host plant of the European grapevine moth *Lobesia botrana*, stimulates its oviposition. Is a host shift relevant? *Chemoecology*, **16**, 3, 135-144.

Maigre D., Murisier F. (1992) Comparaison de techniques d'entretien des sol viticoles dans trois sites pédoclimatiques différents de Suisse romande. *Revue Suisse de Viticulture, d'Arboriculture et d'Horticulture*, **24**, 3, 173-177.

Marc P., Canard A., Ysnel F. (1999) Spiders (Araneae) useful for pest limitation and bioindication. *Agriculture, Ecosystems and Environmental*, **74**, 1-3, 229-273.

Marchesini E., Dalla Montà L. (1994) Observations on natural enemies of *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) in Venetian vineyards. *Bollettino di Zoologia Agraria e di Bachicoltura*, **26**, 2, 201-230.

Marchesini E., Dalla Montà L. (1998) I nemici naturali della tignoletta dell'uva nei vigneti del Veneto. *Informatore Fitopatologico*, **48**, 9, 3-10.

Marchesini E., Ruggiero P., Posenato G. (2006). Efficacia di *Bacillus thuringensis* subsp. *aizawai* nei contronti della tignoletta della vite (*Lobesia botrana*). *Atti Giornate Fitopatologiche*, **1**, 105-110.

Martin-Vertedor D., Ferrero-Garcia J. J., Torres-Vila L. M. (2010) Global warming affects phenology and voltinism of *Lobesia botrana* in Spain. *Agricultural and Forest Entomology*, **12**, 2, 169-176.

Marullo R., (2004) Host-plant range and relationships in the Italian thrips fauna. *Acta Phytopathologica et Entomologica Hungarica*, **39**, 1-3, 243-254.

Masante-Roca I., Anton S., Delbac L., Dufour M.C., Gadenne C. (2007) Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex, and mating status. *Entomologia Experimentalis et Applicata*, **122**, 3, 239-245.

Mazzoni V., Lucchi A., Santini L. (2004) Indagine faunistica sugli Auchenorrinchi di vigneti liguri e toscani (Rhynchota: Homoptera). *Frustula Entomologica* (2002) n.s. **25**, 181-194.

Mazzoni V., Anfora G., Ioriatti C., Lucchi A. (2008) Role of winter host plants in vineyard colonization and phenology of *Zygina rhamni* (Hemiptera: Cicadellidae: Typhlocybinae). *Annals of the Entomological Society of America*, **101**,6, 1003-1009.

Miedema E. (1987) Survey of Phytoseiids mites (Acari: Phytoseidae) in orchards and surrounding vegetation of northwestern Europe, especially in The Netherland. Keys description and figures. *Netherlands Journal of Plant Pathology*, **93**, suppl. 2, 64 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 N. J., Vincent C., Isaacs R. (eds.) *Arthropod management in vineyards: pest, approaches, and future directions*. Springer Science+Business Media B.V., Dordrecht, The Netherlands, 505 pp.

Millar A. A. (1972) Thermal regime of grapevines. *American Journal of Enology and Viticulture*, **23**, 4, 173-176.

Mills N.J., Daane K.M. (2005) Biological and cultural controls, Non pesticide alternatives can suppress crop pests. *California Agriculture*, **59**, 1, 23-28.

Moleas T. (1988) *Lobesia botrana* Den. et Schiff. (Tortricidae: Lepidoptera), un potenziale pericolo per l'actinidia (*Actinidia chinensis* Planchon). *Informatore Fitopatologico*, **38**, 12, 71-73.

Mondy N., Corio-Costet M.F. (2000) The response of the grape berry moth (*Lobesia botrana*) to a dietary phytopathogenic fungus (*Botrytis cinerea*): the significance of fungus sterols. *Journal of Insect Physiology*, **46**, 12, 1557-1564.

Mondy N., Corio-Costet M.F. (2004) Feeding insects with a phytopathogenic fungus influences their diapause and population dynamics. *Ecological Entomology*, **29**, 6, 711-717.

Mondy N., Charrier B., Feramud M., Pracros P., Corio-Costet M. (1998a) Mutualism between a phytopathogenic fungus (*Botrytis cinerea*) and a vineyard pest (*Lobesia botrana*). Positive effects on insect development and oviposition behaviour. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie,* **321**, 8, 665-671.

Mondy N., Pracros P., Fermaud M., Corio-Costet M.F. (1998b) Olfactory and gustatory behaviour by larvae of *Lobesia botrana* in response to *Botrytis cinerea*. *Entomologia Experimentalis et Applicata*, **88**, 1, 1-7.

Mori N., Pozzebon A., Duso C., Reggiani N., Pavan F. (2016) Vineyard colonization by *Hyalesthes obsoletus* (Hemiptera: Cixiidae) induced by stinging nettle cut along surrounding ditches. *Journal of Economic Entomology*, **109**, 1, 49-58.

Moschos T. (2006) Yield loss quantification and economic injury level estimation for the carpophagous generations of the European grapevine moth *Lobesia botrana* Den. et Schiff. (Lepidoptera: Tortricidae). *International Journal of Pest Management*, **52**, 2, 141-147.

Mound L.A., Morison G.D., Pitkin B.R., Palmer J.M. (1976) Thysanoptera. In Wotson A. (ed), **1**, pp. 1-79, *Handbooks for the identification of British insects*. Royal Entomological Society of London.

Moutous G., Fos A. (1971) Essais de lutte chimique contre la Cicadelle de la Vigne *Empoasca flavescens* Fabr. Résultats 1970. *Revue de Zoologie Agricole et de Pathologie Végétale*, **70**, 2, 48-56.

Muckensturm N., Decoin M. (2000). La pourriture grise de la vigne. Pourquoi la lutte échoue parfois, et comment la faire réussir. *Phytoma*, **525**, 50-52.

Mullins M., Bouquet A., Williams L. (1992) Biology of the grapevine. Cambridge, Cambridge University Press, 252 pp.

Mundy D. C. (2008) A review of the direct and indirect effects of nitrogen on botrytis bunch rot in wine grapes. *New Zealand Plant Protection*, **61**, 306-310.

Neven L.G. (2000) Physiological responses of insects to heat. *Postharvest Biology and Technology*, **21**, 1, 103–111.

Neven L.G. (2005) Combined heat and controlled atmosphere quarantine treatments for control of codling moth in sweet cherries. *Journal of Economic Entomology*, **98**, 3, 709-715.

Neven L.G., Mitcham J. (1996) CATTS (Controlled Atmosphere/Temperature Treatment System): A novel tool for the development of quarantine treatments. *American Entomologist*, **42**, 1, 56-59.

Nicholls C.I., Parrella M.P., Altieri M.A. (2000) Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agricultural and Forest Entomology*, **2**, 2, 107-113.

Nijhout H.F. (1994) Insect Hormones. Princeton, Princeton University Press, 267 pp.

Nunes C., Teixeira B., Carlos C., Gonçalves F., Martins M., Crespi A., Sousa S., Torres L., Amaro da Costa C. (2015) Biodiversidade do solo em vinhas com e sem enrelvamento. *Revista de Ciências Agrárias*, ***38***, 2, 248-257.

Nylin S., Janz N. (1993) Oviposition preference and larval performance in *Polygonia calbum* (Lepidoptera: Nymphalidae): the choice between bad and worse. *Ecological Entomology*, **18**, 4, 394-398.

Pashley D.P., Hardy T.N., Hammond A.M. (1995) Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, **88**, 6, 748-755.

Pavan F. (2001) The role of *Rubus* bushes in the life cycle of two typhlocybinae infesting European vineyards. *Redia*, **83**, 47-60.

Pavan F., Duso C. (1986) Il controllo delle tignole della vite (*Lobesia botrana* Den. e Schiff.; *Eupoecilia ambiguella* Hb.). 1: Confronto sull' efficacia di insetticidi diversi. *Rivista di Viticoltura e di Enologia*, **39**, 4, 148-157.

Pavan F., Girolami V. (1986) Lotta guidata alle tignole della vite su uve da vino nell'Italia nord-orientale. *L'Informatore Agrario*, **42**, 30, 35-41.

Pavan F., Pavanetto E. (1989) Seasonal abundance of Typhlocybinae at different leaf position of vines. In Cavalloro R. (ed), pp. 135-141, *Proceeding of a meeting of the EC Expert' Group on Influence of environmental factors on the control of grape pests, diseases & weeds*, Thessaloniki, Greece, 6-8 October 1987, A.A. Balkema, Rotterdam.

Pavan F., Picotti P. (2009) Influence of grapevine cultivars on the leafhopper *Empoasca vitis* and its egg parasitoids. *BioControl,* **54**, 1, 55-63.

Pavan F., Sbrissa F. (1994) Dannosità delle tignole della vite *Lobesia botrana* ed *Eupoecilia ambiguella*, su cultivar a maturazione tardiva nell'Italia nord-orientale. *Frustula Entomologica*, **17**, 43-53.

Pavan F., Sacilotto G., Girolami V. (1987) Damage evolution, larval sampling and treatment period for grape moths. In Cavalloro R. (ed), pp. 39-49, *Proceeding of a meeting of the EC Expert' Group on Integrated Pest Control in Viticulture,* 26-28 September 1985, Portoferraio, Italy. Published by A. A. Balkema, Rotterdam, The Netherlands.

Pavan F., Pavanetto E., Duso C., Girolami V. (1988) Population dynamics of *Empoasca vitis* (Goethe) and *Zygina rhamni* (Ferr.) on vines in northern Italy. In Vidano C., Arzone A. (eds), pp. 517-524, *Proceedings of the 6th Auchenorryncha Meeting*, 7-11 September 1987, Torino, Italy.

Pavan F., Picotti P., Girolami V. (1992) Strategie per il controllo di *Empoasca vitis* Göthe su vite. *L'Informatore Agrario*, **48**, 24, 65-72.

Pavan F., Girolomi V., Cecchini A., Turbian E. (1993) Evolution of damage of grape berry moths, *Lobesia botrana* (Den. and Schiff.) and *Eupoecilia ambiguella* (Hb.), in north-eastern Italy and chemical control. *Redia*, **76**, 2, 417-431.

Pavan F., Girolami V., Sacilotto G. (1998) Second generation of grape berry moths, *Lobesia botrana* (Den. & Schiff.) (Lep., Tortricidae) and *Eupoecilia ambiguella* (Hb.) (Lep., Cochylidae): spatial and frequency distributions of larvae, weight loss and economic injury level. *Journal of Applied Entomology*, **122**, 7, 361-368.

Pavan F., Stefanelli G., Villani A., Gasparinetti P., Colussi G., Mucignat D., Del Cont Bernard D., Mutton P. (2000) Danni da *Emapoasca vitis* (Göthe) (Homoptera: Cicadellidae) in vigneti dell'Italia nordorientale e soglie d'intervento. *Frustula Entomologica*, n.s. **21**, 109-124.

Pavan F., Zandigiacomo P., Dalla Montà L. (2006) Influence of the grape-growing area on the phenology of *Lobesia botrana* second generation. *Bulletin of Insectology*, **59**, 2, 105-109.

Pavan F., Stefanelli G., Cargnus E., Villani A. (2009) Assessing the influence of inflorescence traits on the susceptibility of grape to vine moths. *Journal of Applied Entomology*, **133**, 5, 394-401.

Pavan F., Floreani C., Barro P., Zandigiacomo P., Dalla Montà L. (2010) Influence of generation and photoperiod on larval development of *Lobesia botrana* (Lepidoptera: Tortricidae). *Environmental Entomology*, **39**, 5, 1652-1658.

Pavan F., Floreani C., Barro P., Zandigiacomo P., Dalla Montà L. (2013) Occurrence of two different development patterns in *Lobesia botrana* (Lepidoptera: Tortricidae) larvae during the second generation. *Agricultural and Forest Entomology*, **15**, 4, 398-406.

Pavan F., Bigot G., Cargnus E., Zandigiacomo P. (2014a) Influence of the carpophagous generations of the European grapevine moth *Lobesia botrana* on grape bunch rots. *Phytoparasitica*, **42**, 1, 61-69.

Pavan F., Cargnus E., Bigot G., Zandigiacomo P. (2014b) Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies. *Bulletin of Insectology*, **67**, 2, 273-280.

Pavan F., Cargnus E., Kiaeian Moosavi F., Bigot G., Tacoli F., Zandigiacomo P. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould. *Bulletin of Insectology* (in press).

Percival D.C., Fisher K.H., Sullivan J.A. (1994) Use of fruit zone leaf removal with *Vitis vinifera* L. cv Riesling grapevines. II. Effects on fruit composition, yield, and occurrence of bunch rot (*Botrytis cinerea* Pers.). *American Journal of Enology and Viticulture*, **45**, 2, 133-139.

Phillips K.M., Ruggio D.M., Ashraf-Khorassani M. (2005) Phytosterol composition of nuts and seeds commonly consumed in the United States. *Journal of Agricultural and Food Chemistry*, **53**, 24, 9436-9445.

Picotti P., Pavan F. (1993) Studi su *Anagrus atomus* (Linnaeus) (Hymenoptera, Mymaridae) parassitoide oofago di *Empoasca vitis* (Göthe) (Homoptera, Cicadellidae) su vite. 1. Dinamica di popolazione in assenza di trattamenti insetticidi. *Bollettino del Laboratorio di Entomologia agraria "Filippo Silvestri"*, **48**, 105-115.

Pieri P. (2010) Modelling radiative balance in a row-crop canopy: Row-soil surface net radiation partition. *Ecological Modelling*, **221**, 5, 791-801.

Pieri P., Fermaud M. (2005) Effects of defoliation on temperature and wetness of grapevine berries. *Acta Horticulturae*, **689**, 109-116.

Pimentel D., McLaughlin L., Zepp A., Lakitan B., Kraus T., Kleinman P., Vancini F., Roach W.J., Graap E., Keeton W.S., Selig G. (1993) Environmental and economic effects of reducing pesticide use in agriculture. *Agriculture, Ecosystems & Environment*, **46**, 1-4, 273-288.

Ponti L., Ricci C., Veronesi F., Torricelli R. (2005) Natural hedges as an element of functional biodiversity in agroecosystems: the case of Central Italy vineyard. *Bulletin of Insectology*, **58**, 1, 19-23.

Posenato G. (1994) Popolazioni di *Amblyseius aberrans* (Oud.) resistenti ad esteri fosforici e ditiocarbammati. *L'Informatore Agrario*, **50**, 24, 41-43.

Pozzebon A., Duso C. (2008) Grape downy mildew *Plasmopara viticola*, an alternative food for generalist predatory mites occurring in vineyards. *Biological Control*, **45**, 3, 441-449.

Pozzebon A., Duso C., Malagnini V. (2005) Predatory mite population dynamics in vineyards: the role of alternative foods. *In 2ème Colloque International sur les Acariens des Cultures*, 24-25 October 2005. Montpellier, France.

Pozzebon A., Loeb G.M., Duso C. (2015) Role of supplemental foods and habitat structural complexity in persistence and coexistence of generalist predatory mites. *Scientific Reports*, **5**, 14997.

Pusztai M., Fast P., Gringorten L., Kaplan H., Lessard T., Carey P.R. (1991) The mechanism of sunlight-mediated inactivation of *Bacillus thuringiensis* crystals. *Biochemistry Journal*, **273**, 1, 43-47.

Ramsdell D.R., Jubb G.L. (1979) Seasonal life history of European red mite on concord grapes in Erie country, Pennsylvania. *Environmental Entomology*, **8**, 6, 1018-1020.

Rapagnani M.R., Caffarelli V., Barlattani M. (1988) *Lobesia botrana* Schiff.: studio in laboratorio del ciclo di sviluppo in funzione della temperatura, pp. 973–980, *Atti del XV Congresso Nazionale Italiano di Entomologia,* 13-17 giugno 1988, L'Aquila, Italy.

Rapagnani M.R., Caffarelli V., Barlattani M., Minelli F. (1990) Descrizione di un allevamento, in laboratorio, della tignoletta dell'uva. *Bollettino dell'Istituto di Entomologia "Guido Grandi" della Università degli Studi di Bologna*, **44**, 57-64.

R'Houma A., Chérif M., Boubaker A. (1998) Effect of nitrogen fertilization, green pruning and fungicide treatments on Botrytis bunch rot of grapes. *Journal of Plant Pathology*, **80**, 2, 115-124.

Risch S., Andow D., Altieri M.A. (1983) Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. *Environmental Entomology*, **12**, 3, 625-629.

Rilling G., Düring H. (1990) Structure and function of grapevine leaves (*Vitis vinifera* L.) as affected by the European red mite (*Panonychus ulmi*). *Vitis*, **29**, 1, 27-42.

Rives M. (2000) Vigour, pruning, cropping in the grapevine (*Vitis vinifera* L.). I. A literature review. *Agronomie, EDP Sciences,* **20**, 1, 79-91.

Roberts M.J. (1995) Spiders of Britain and northern Europe. Harper Collins Publishers, London, 383 pp.

Roehrich R. (1978) Recherches sur la nuisibilité de *Eupoecilia ambiguella* Hb et *Lobesia botrana* Den et Schiff. *Phytoma*, **32,** 191, 106-124.

Roehrich R., Boller E. (1991) Tortricids in vineyards. In Van der Geest L.P.S., Evenhuis H.H. (eds), pp. 507–514, *Tortricids Pests, their Biology, Natural Enemies and Control.* Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Roehrich R., Schmid A. (1979) Lutte intégrée en viticulture. Tordeuses de la grappe: évaluation du risque, determination des periodes d'intervention et recherche de méthodes de lutte biologique. In Ross K., Berger H. (eds), pp. 245-254, *Proceeding in International Symposium of IOBC/WPRS on Integrated Control in Agriculture and Forestry,* 8-12 October 1979*,* Vienna, Austria.

Rohlfs M., Churchill A.C.L. (2011) Fungal secondary metabolites as modulators of interactions with insects and other arthropods. *Fungal Genetics and Biology*, **48**, 1, 23-34.

Rouse J.W., Haas R.H., Schell J.A., Deering D.W. (1974) Monitoring vegetation systems in the Great Plains with ERTS. In Fraden S.C., Marcanti E.P., Becker M.A. (eds.), pp. 309-317, *Third ERTS-1 Symposium*, 10–14 December 1973, NASA SP-351, Washington D.C. NASA.

Ruggiero A., Vitalini S., Burlini N., Bernasconi S., Iriti M. (2013) Phytosterols in grapes and wine, and effects of agrochemicals on their levels. *Food Chemistry*, **141**, 4, 3473-3479.

Savopoulou-Soultani M., Tzanakakis M.E. (1988) Development of *Lobesia botrana* (Lepidoptera: Tortricidae) on grapes and apples infected with the fungus *Botrytis cinerea*. *Environmental Entomology*, **17**, 1, 1-6.

Savopoulou-Soultani M., Nikolaou N., Milonas P.G. (1999) Influence of maturity stage of grape berries on the development of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae. *Journal of Economic Entomology*, **92**, 3, 551-556.

Schausberger P. (1992) Vergleichende Untersuchungen über den Einfluss unterschiedlicher Nahrung auf die Praimaginalentwichlung und die Reproduktion von *Amblyseius aberrans* Oud. Und *Amblyseius finlandicus* Oud. (Acarina: Phytoseiidae). *Journal of Applied Entomology*, **113**, 476-486.

Scheirs J., De Bruyn L., Verhagen R. (2000) Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **267**, 1457, 2065–2069.

Schvester D., Moutous G., Bonfils J., Carle P., (1962) Étude biologique des cicadelles de la vigne dans le sud-ovest de la France. *Annales des Epiphyties*, **13**, 3, 205-237.

Serra G., Lentini A., Verdinelli M., Delrio G. (2006) Effects of cover crop management on grape pests in a Mediterranean environment. *OILB/SROP Bulletin*, **29**, 11, 209-214.

Settle W.H., Wilson L.T., Flaherty D.L., English-Loeb G.M. (1986) The variegated leafhopper, an increasing pest of grapes. *California Agriculture*, **40**, 7-8, 30-32.

Shahini S., Kullaj E., Çakalli A., Çakalli M., Lazarevska S., Pfeiffer D.G., Gumeni F. (2010) Population dynamics and biological control of European grapevine moth (*Lobesia botrana*: Lepidoptera: Tortricidae) in Albania using different strains of *Bacillus thuringiensis*. *International Journal of Pest Management*, **56**, 3, 281-286.

Sharley D.J., Hoffmann A.A., Thomson L.J. (2008) The effects of soil tillage on beneficial invertebrates within the vineyard. *Agricultural and Forest Entomology*, **10**, 3, 233-243.

Sharon R., Zahavi T., Soroker V., Harari A.R. (2009) The effect of grape vine cultivars on *Lobesia botrana* (Lepidotpera: Tortricidae) population levels. *Journal of Pest Science*, **82**, 2, 187–193.

Sivilotti P., Malossini G., Stocco M., Colussi G., Bregant F., Gallas A., Miletti R., Fabbro A. (2011) Tecniche di defogliazione della vite e marciumi del grappolo. *L'Informatore Agrario*, **67**, 22, 67-71.

Smart R.E. (1985) Principles of grapevine canopy microclimate manipulation with implications for yield and quality. Review. *American Journal of Enology and Viticulture*, **36**, 3, 230–239.

Smart R.E., Sinclair T.R. (1976) Solar heating of grape berries and other spherical fruits. *Agricultural Meteorology*, **17**, 4, 241–259.

Snjezana H. (2004). Susceptibility of some grapevine cultivars in area of vineyards of Podgorica on the attack of European grape berry moth - *Lobesia botrana* Den & Schiff, Lepidoptera, Trotricidae. *Acta Horticulture*, **652**, 355-358.

Spayd S.E., Tarara J.M., Mee D.L., Ferguson J.C. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*, **53**, 3, 171–182.

Stapleton J.J., Barnett W.W., Marois J.J. Gubler W.D. (1990) Leaf removal for pest management in wine grapes. *California Agriculture*, **44**, 5, 15–17.

Stavridis D.G., Savopoulou-Soultani M. (1998) Larval performance on and oviposition preference for known and potential hosts by *Lobesia botrana* (Lepidoptera: Tortricidae). *European Journal of Entomology*, **95**, 1, 55-63.

Symondson W.O.C., Sunderland K.D., Greenstone M.H. (2002) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561-594.

Tamanini L. (1988) Tabelle per la determinazione dei più comuni Eterotteri italiani. Heteroptera. *Memorie della Societa Entomologica Italiana, Genova*, **67**, 2, 359-471.

Tasin M., Betta E., Carlin S., Gasperi F., Mattivi F., Pertot I. (2011) Volatiles that encode host-plant quality in the grapevine moth. *Phytochemistry*, **72**, 16, 1999-2005.

Tasin M., Knudsen G.K., Pertot I. (2012) Smelling a diseased host: grapevine moth responses to healthy and fungus-infected grapes. *Animal Behaviour*, **83**, 2, 555-562.

Tarara J.M., Lee J., Spayd S.E., Scagel C.F. (2008) Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture*, **59**, 3, 235–247.

Thiéry (2005) Vers de la grappe. Les connaître pour s'en protéger. Vigne et Vin Publications Internationales, Bordeaux, France, 60 pp.

Tixier M.-S., Baldassar A., Duso C., Kreiter S. (2013) Phytoseiidae in European grape (*Vitis vinifera* L.): bio-ecological aspects and keys to species (Acari: Mesostigmata). *Zootaxa*, **3721**, 2, 101-142.

Torres-Vila L.M. (1996) Effect of preimaginal development temperature on biotic potential of grape berry moth, *Lobesia botrana* (Denis & Schiffermüller, (1775)) (Lepidoptera: Tortricidae). *SHILAP, Sociedad Hispano-Luso-Americana de Lepidopterología*, **24**, 94, 197-206.

Torres-Vila L.M., Schmitz V., Stockel J. (1992) Efecto de la evolución fenológica de la variedad de vid en la instalación y supervivencia en primera generación de las orugas de la polilla del racimo (*Lobesia botrana* Den. and Schiff.; Lepidoptera: Tortricidae). *Boletín de Sanidad Vegetal, Plagas*, **18**, 4, 755-764.

Torres-Vila L.M., Oustry L., Schmitz V., Roehrich R., Stockel J. (1993) Action of relative humidity and temperature fluctuation on non-diapausing pupae of the grape vine moth *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae). *Boletín de Sanidad Vegetal, Plagas*, **19**, 1, 79-93.

Torres-Vila L.M., Stockel J., Roehrich R., Rodriguez-Molina M.C. (1997) The relation between dispersal and survival of *Lobesia botrana* larvae and their density in vine inflorescences. *Entomologia Experimentalis et Applicata*, **84**, 2, 109-114.

Trdan S., Andjus L., Raspudić E., Kač M. (2005) Distribution of *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae) and its potential prey Thysanoptera species on different cultivated host plants. *Journal of Pest Science*, **78**, 4, 217-226.

Trona F., Marchesini E., Baldessari M., Sofia M., Angeli G. (2007) *Bacillus* si conferma efficace contro la tignoletta della vite. *L'Informatore Agrario*, **63**, 23, 74-77.

Tzanakakis M.E., Savopoulou-Soultani M., Oustapassidis C.S., Verras S.C., Hatziemmanuel H. (1988) Induction of dormancy in *Lobesia botrana* by long day and high temperature conditions. *Entomologia Hellenica*, **6**, 7-10.

Underwood D.L.A. (1994) Intraspecific variability in host plant quality and ovipositionaI preferences in *Eucheira socialis* (Lepidoptera: Pieridae). *Ecological Entomology*, **19**, 3, 245–256.

Vail M. E., Marois J. J. (1991) Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. *Phytopathology*, **81**, 2, 188-191.

Valdés-Gómez H., Fermaud M., Roudet J., Calonnec A., Gary C. (2008) Grey mould incidence is reduced on grapevines with lower vegetative and reproductive growth. *Crop Protection*, **27**, 8, 1174-1186.

Van Driesche R., Bellows T.S. (1996) *Biological Control*. Chapman and Hall, New York. 539 pp.

Van Helden M., Decante D. (2001) The possibilities for conserva- tion biocontrol as a management strategy against *Empoasca vitis*. *IOBC/WPRS Bulletin*, **24**, 7, 291-297.

Van Helden M., Decante D., Papura D. (2003) Possibilities for conservation biological control against grape pests in the Bordeaux region. *IOBC/WPRS Bulletin*, **26**, 4, 191-196.

Van Steenwyk R.A., Toscano N.C., Ballmer G.R., Kido K., Reynolds H.T. (1975) Increases of *Heliothis spp*. in cotton under various insecticide treatment regimes. *Environmental Entomology*, **4**, 6, 993-996.

Vartholomaiou A.N., Navrozidis E.I., Payne C.C., Salpiggidis G.A. (2008) Agronomic techniques to control *Lobesia botrana*. *Phytoparasitica*, **36**, 3, 264-271.

Verdenal T., Zufferey V., Spring J.-L., Viret O. (2013) Conséquences physiologiques de effeuillage de la vigne – Revue de littérature. *Revue Suisse de Viticulture, Arboriculture, Horticolture*, **45**, 3, 148-155.

Veres A., Petit S., Conord C., Lavigne C. (2011) Does landscape composition affect pest abundance and their control by natural enemies? A review. *Agriculture, Ecosystems and Environment*, **166**, 110-117.

Vidano, C. (1963) Alterazioni provocate da insetti in *Vitis osservate*, sperimentate e comparate. *Annali Facoltà Scienze Agrarie. Univiveristà di Torino*, **1**, 514-644.

Vidano C., Arnò C., Alma A., (1988) On the *Empoasca vitis* intervention threshold on vine (Rynchota, Auchenorryncha). In Vidano C. Arzone A. (eds.), pp. 525-537, *Proceedings of the 6th Auchenorryncha Meeting,* 7–11 September 1987, CNR-IPRA, Turin, Italy.

Viggiani G. (1994) Lotta biologica e integrate nella difesa fitosanitaria. Vol. **1**. Lotta biologica. Liguori Editore, Napoli, 517 pp.

Viggiani G., Jesu R., Sasso R. (2003) Cicaline della vite e loro ooparassitoidi in vigneti del Sud Italia. *Bollettino Laboratorio Entomologia Agraria "Filippo-Silvestri"*, **59**, 3-31.

Viggiani G., Di Luca A., Matteucig G. (2006) The egg parasitoids of the genus *Anagrus* (Hymenoptera: Mymaridae) as functional biodiversity of the vineyard agroecosystem. *IOBC/WPRS Bulletin*, **29**, 6, 157-160.

Villani A., Stefanelli G., Pavan F. (1997) Influenza della potatura verde sulla seconda generazione delle tignole dell'uva. *Notiziario ERSA*, **10**, 4, 12-15.

Walton V.M., Daane K.M., Addison P. (2012) Biological control of arthropods and its application in vineyards. In Bostanian N. J., Vincent C., Isaacs R. (eds.) *Arthropod management in vineyards: pest, approaches, and future directions*, 505 pp., Springer Science+Business Media B.V., Dordrecht, The Netherlands.

Wang S., Yin X., Tang J., Hansen J.D. (2004) Thermal resistance of different life stages of codling moth (Lepidoptera: Tortricidae). *Journal of Stored Products Research*, **40**, 5, 565-574.

Weiss S.B., Luth D.C., Guerra B. (2003) Potential solar radiation in a Vertical Shoot Positioned (VSP) trellis at 38° N latitude. *Practical Winery & Vineyard*, May/June, 16-23.

White L.D. (1981) Survival and reproduction of codling moths exposed to 33° during larval and pupal development. *Entomologia Experimentalis et Applicata*, **29**, 1, 98-102.

Whiting D., Hoy L. (1998) Effect of temperature establishment time on the mortality of *Epiphyas postvittana* (Lepidoptera: Tortricidae) larvae exposed to a high-temperature controlled atmosphere. *Journal of Economic Entomology*, **91**, 1, 287-292.

Williams D.W. (1984) Ecology of the blackberry-leafhopper-parasite system and its relevance to California grape agroecosystems. *Hilgardia*, **52**, 4, 1-32.

Williams L., Martinson T.E. (2000) Colonization of New York vineyards by *Anagrus* spp. (Hymenoptera: Mymaridae): Overwintering biology, within-vineyard distribution of wasps, and parasitism of grape leafhopper, *Erythroneura* spp. eggs. *Biological Control*, **18**, 2, 136-146.

Wilson H., Miles A.F., Daane K.M., Altieri M.A. (2015) Landscape diversity and crop vigor influence biological control of the Western grape leafhopper (*E. elegantula* Osborn) in Vineyards. *PLoS ONE***, 10**, 11, e0141752.

Wolpert J.A., Phillips P.A., Striegler R K., McKenry M.V., Foott J.H. (1993). Berber orchardgrass tested as cover crop in a commercial vineyard. *California Agriculture*, **47**, 5, 23-25.

Yokoyama V.Y., Miller G.T. (1987) High temperature for control of oriental fruit moth (Lepidoptera: Tortricidae) in stone fruits. *Journal of Economic Entomology*, **80**, 3, 641-645.

Yokoyama V.Y., Miller G.T., Dowell R. V. (1991) Response of Codling moth (Lepidoptera: Tortricidae) to high temperature, a potential quarantine treatment for exported commodities. *Journal of Economic Entomology*, **84**, 2, 528-531.

Zahavi T., Ally H., Thiery D. (2003) Can we expect *Lobesia botrana* to distribute its eggs partly using differential exposure of bunches to light ? *Integrated Protection and Production in Viticulture*, **26**, 8, 151-154.

Zalucki M.P., Clarke A.R., Malcolm S.B. (2002) Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology*, **47**, 361–393.

Zangheri S., Dalla Montà L., Duso C. (1987) Observations on biology and control of grape moths in Venetia. In Cavalloro R. (ed), pp. 27-37, *Proceedings of a Meeting of the EC Experts' Group on Integrated pest control in viticulture*, 26-28 September 1985, Portoferraio, Italy.

Zanolli P., Pavan F. (2011) Autumnal emergence of *Anagrus* wasps, egg parasitoids of *Empoasca vitis*, from grapevine leaves and their migration towards brambles. *Agricultural and Forest Entomology*, **13**, 4, 423-433.

Zanolli P., Pavan F. (2013) Occurrence of different development time patterns induced by photoperiod in *Anagrus atomus* (Hymenoptera: Mymaridae), an egg parasitoid of *Empoasca vitis* (Homoptera: Cicadellidae). *Physiological Entomology*, **38,** 4, 269-278.

AKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my advisor Professor **Pietro Zandigiacomo** and Co-advisor Professor **Francesco Pavan**. I am very grateful for their patience, motivation, enthusiasm, and immense knowledge, taken together, make them great mentors. Along with my advisors I had the opportunity of working with Doctor **Elena Cargnus**. She has been supportive since the days I began studying in the University of Udine, not only by providing a research assistantship over almost three years, but also academically and emotionally through the rough road to finish this thesis.

I owe a great deal of appreciation and gratitude to the people with whom I worked out of university, most of the results described in this thesis would not have been obtained without a kind collaboration of the Company Perleuve S.r.l. (locality Cormòns, Gorizia district; Dr. Giovanni Bigot, Dr. Alessandro Freccero, Dr. Carlos Alberto Lujan, Dr. Davide Musetti, Michele Stecchina) and of the owners of the farms Bigot Federico (locality Cormòns, Gorizia district), Danieli Marina (locality Buttrio, Udine district), Puiatti Tenimenti Angelini S.R.L. (locality Farra di Isonzo, Gorizia district), I Magredi di M. Tombacco & C. S.a.s. (locality Domanins, Pordenone district), A. Servadei of Udine University (locality San Osvaldo, Udine district; Dr. Moreno Greatti).

Other past and present group members that I have had the pleasure to work with or alongside of are Filippo Michele Buian for technical support in laboratory activities and Chiara Floreani for collaboration in laboratory samplings.

The members of pathology group deserve my sincere expression of thanks for helping me to carry out part of my research work on *Botrytis cinera*. I would like to thank Serena Moruzzi for isolation process and especially Emanuela Torelli; I owe gratitude to her who willingly devoted so much time in giving guidance to me. She made significant contributions to the isolation of Botrytis and leading the way in the preparation of dried powder mycelium of this fungus.

I would also like to thank my reading committee members: Dr. Nicola Mori and Dr. Bruno Bagnoli; for their time, interest, and helpful comments.

In addition, I would like to thank my office mate: Laura Fourtunato. She was a wonderful friend ever since we began to share an office. And I also thank Federico Tacoli, my other great office mate. I would also like to extend huge, warm thanks to some special

individuals that caused me to have my beloved husband with me during this period and also helped us in so many ways, Prof. Daniele Goi, Prof. Maria De Nobili and Prof. Marco Contin, some of the greatest Italian people I have ever met.

My time at University of Udine was made enjoyable in large part due to the many friends and groups that became a part of my life. Thanks to Philippo, I would never forget about the many happy moments with entomology group in coffee break every morning at 10 O'clock.

Special thanks to my family. I have an amazing family, unique in many ways, and the stereotype of a perfect *family* in many others. Words can not express how grateful I am to my father Seyed Eisa Kiaeian Moosavi and my mother Masoumeh Manouchehri for all of the sacrifices that they've made on my behalf. Their prayer for me was what sustained me thus far. I would also like to thank my brothers, Amir Hossein and Kasra for their love and companionship throughout my life and especially to my older brother Amir Hossein who suggested me studying in Udine and caused me to have such a wonderful experience and his darling wife Atena Beykaii; they kindly hosted me for my first months of staying in Udine.

Above all, my special acknowledgement goes to of many years, **Ali Khabaz**. My best friend and beloved husband. His unconditional support has been essential all these years. This thesis is the result of him who were always there with his valuable suggestions and almost unbelievable support when I really needed. It doesn't seem sufficient but I thank him with appreciation and love for his encouragement, care, understanding and creating a pleasant atmosphere for me. I doubt that I will ever be able to convey my appreciation fully, but I owe him this PhD thesis.

I finish with **Iran**, where the most basic source of my life energy resides.

Scheda informativa
Dottorato di Ricerca in Scienze e Biotecnologie Agrarie.
Università di Udine

Nome e cognome: Seyedehfatemeh Kiaeianmoosavi
Data di Nascita: 25/03/1985
Laurea in: Agricultural Engineering-Entomology
Conseguimento della Laurea: 07/02/2012, Sari Agricultural Sciences and Natural Resources University, Iran
Ciclo di dottorato: XXVIII
Data conseguimento titolo: 07/04/2016
Supervisore: Prof. Pietro Zandigiacomo

Elenco delle pubblicazioni fatte nel corso del dottorato
(specificare se published, in press, accepted, submitted, *in litteris*)

- Pavan F., Cargnus E., Kiaeian Moosavi F., Bigot G., Tacoli F., Zandigiacomo P. (2016) Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould. Bulletin of Insectology (in press).
- Kiaeian Moosavi S.F., Tacoli F., Pavan F., Zandigiacomo P. (2015) Environmentally friendly control of arthropods pests in vineyards. Poster presented at PhD EXPO 2015, University of Udine, 18 June 2015.

Elenco dei convegni cui ha partecipato
(con le informazioni su: ente organizzatore, titolo, sede, data e l'informazione se il candidato ha presentato papers o posters)

- Bilancio fitosanitario viticolo 2014 nel Triveneto, Meeting held in Susegana, (Treviso, Italy), 7 November 2014.
- 4th Annual Meeting of the European PhD Network in "Insect Science", held in Paluzza (Udine, Italy), 19-22 September 2013.

Elenco dei corsi, seminari, conferenze a cui ha partecipato
(in particolare quelli organizzate dal collegio di dottorato, ma non solo, cui ha partecipato. Anche in questo caso con i dettagli necessari: relatore, titolo, data ecc.)

- Presented by Dr. Markus Schwarzländer, Imaging subcellular physiology in living plants, 17 June 2015.
- Presented by Prof. Christina Grozinger, Bee health: from genes to landscapes, 12 June 2015.
- Presented by Dr. Wolfgang Schmidt, Translational fitness of messenger RNAs: Impact of environmental signals, 25 February 2015.
- Presented by Carlo Vascotto, Emanuele De Paoli and Michele Morgante, Next generation sequencing and qPCR workshop, 8-12 December 2014
- Presented by Prof. Ulas Cinar, Genomics of meat quality study and expression of QTLs, 25 September 2014.
- Presented by prof. Angelika Mustroph, The role of ATP- and Ppi- dependent phosphofructokinase in plants, 22 May 2104.
- Presented by prof. Angelika Mustroph, The response of plant to Oxygen deficiency stress, 21 May 2014.