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Working Groups "Pheromones and other semiochemicals in integrated production" & "Integrated Protection of Fruit Crops"

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"Pheromones and other semiochemicals in integrated production"
&
"Integrated Protection of Fruit Crops"

at

Lisbon (Portugal)

20-25 January 2019

"Merging pheromones and other semiochemicals with integrated fruit production: current approaches and applications from research to field implementation in a changing environment"

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Preface

Dear colleagues,

PheroFIP 19, the Joint Meeting of the IOBC-WPRS Working Groups "Pheromones and other semiochemicals in integrated production" and "Integrated Protection of Fruit Crops", has taken place in Lisbon (Portugal), from 20 to 25 January 2019. It was very well organized by the conference chairs Manuela Branco and José Franco (School of Agriculture (ISA), University of Lisbon). The venue was in the Hotel Olissippo Oriente, located close to the Parque das Nações, a renovated area with modern architecture near Tagus river, in the eastern part of Lisbon, which was designed for hosting the last world fair of the 20th century, the Expo 98. It was a great experience to walk during the breaks outside of this outstanding place, which is located not far from the airport, and also close to the city centre.

The meeting was open to all people working on semiochemicals in agriculture and forest systems, as well as to all that work on the protection of fruit crops. The general aim of this meeting was to bring together researchers, students and company representatives dealing with all aspects of pheromones and allelochemicals for the management of pests, and the members of the integrated fruit protection community, i. e. the subgroups "Pome Fruit Arthropods", and "Stone Fruits". Thus, we phrased it "Merging pheromones and other semiochemicals with integrated fruit production: Current approaches and applications from research to field implementation in a changing environment". During five exciting days of exchange of scientific results and ideas, we had around 100 presentations, including a plenary lecture, 7 keynote lectures, and more than 50 scientific talks and 40 posters. Roundabout 140 attendees from 26 countries belonging to universities, research institutes and companies had registered for the meeting. However, we are sorry that some of our US American colleagues had to cancel their participation on short notice due to the federal government shutdown in January in the USA, also affecting the US Department of Agriculture (USDA).

The meeting started with a relaxed welcome reception, followed by five days organized in eight scientific sessions:

- Allelochemical-based pest management tactics
- Pheromone-based pest management tactics
- Managing beneficial insects by semiochemicals
- SIT and biological control of fruit crop pests
- Applied biotremology: a new discipline for pest management. Vibrational signals as semiophysicals
- Invasive pests: a challenge for IPM. Brown marmorated stink bug and other invasive hemipterans
- Invasive pests: a challenge for IPM. Spotted wing drosophila and other invasive dipterans
- New developments in pest monitoring methods

The side program offered an exciting tour to the Alentejo, where we visited Herdade do Esporão, an estate with vineyards and olive groves using organic methods and integrated production, including a tour to the cellars and wineries with wine tasting. Afterwards, we visited the historical city of Évora (UNESCO World Heritage).

We want to thank the local organizers and all attendees for this very interesting meeting, many exciting talks and excellent poster presentations including speed talks, new insights and research ideas and a lot of fun!

Jürgen Gross and Claudio Ioriatti
Convenors

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Allelochemical-based pest management tactics

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Obituary: Heinrich Arn 1937-2019

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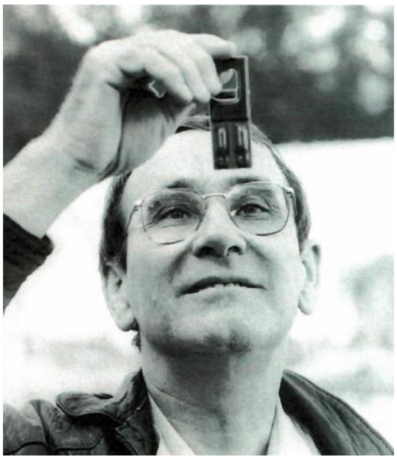

Sadly, in January 2019 Heinrich Arn passed away at the age of 81 at his home in Switzerland. Heinrich was the first convenor of the IOBC Pheromone Working Group in 1975, and he is considered to be one of the most significant pioneers of pheromone research, not only in Europe but worldwide. Heinrich studied chemistry in Bern, after completing his PhD, he pursued a postdoc with the renowned Wendell Roelofs in Geneva, New York between 1968-1970. During this time, he mainly worked on the pheromone identification of tortricid pests. He had chosen a research career in insect sex pheromone because he was inspired by Rachael Carson's famous "Silent Spring", which even today remains a strong driver for people to get away from pesticides, in favour of more sustainable solutions for pests. Together with Wendell, they published several papers on this topic in various journals, including the sex pheromone of the red-banded leafroller in *Nature* in 1968. Heinrich moved back to Switzerland and started work as a research scientist in the Swiss Federal Research Institute (now Agroscope) in 1971. His work resulted in the identification of many lepidopteran sex pheromones, notably the sex pheromone of *Lobesia botrana*. He was the first to develop the coupled gas chromatography/electroantennogram detector (GC/EAD), a very widely-used technique in chemical ecology, and it would be hard to estimate how many important breakthroughs have used this now a standard method in chemical ecology. Later, this technique was modified to GC-sniffing and adopted by food chemistry and fragrance scientists for odour assessment in humans. During his time in Wädenswil Heinrich was technically supported by Stefan Rauscher, a highly skilful research associate who also passed away in 2018. Together with Miklos Toth and Ernst Priesner, Heinrich compiled the known sex pheromones of Lepidoptera into the "Pherolist", which was published online from 1993-2000, and is available in the highly-cited legacy website "The Pherobase". He organized the IOBC Pheromone Working Group meetings well into the early 1990s. Heinrich retired in 1997 from the Swiss Federal Research Institute and moved to SLU, Alnarp, Sweden as a guest professor for three years, and finally retired from science in 2000. He is survived by two children, Christina and Niklas. I feel it was a privilege to work with this passionate and highly effective scientist. The international chemical ecology community is saddened by the loss of this pioneer, but his work lives on.

Photo: Hansueli Trachsel

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Is globulol mediating attack by male pioneers of *Gonipterus platensis* (Col., Curculionidae)?

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Extended Abstract: Weevils of the genus *Gonipterus* (Col., Curculionidae) feed on young eucalyptus leaves, buds and shoots, causing reduced tree growth and mortality. *Gonipterus platensis* is the main coleopteran defoliator of eucalyptus worldwide, being also present in Spain and Portugal, where economic damage occurs particularly at high altitudes. At 700 m above sea level mean defoliation values can reach 75% (Reis et al., 2012). The process of tree selection and colonization by *G. platensis* has not yet been clarified. Although *Gonipterus* spp. feed exclusively on eucalyptus foliage, eucalyptus species preference has been, commonly, reported. Furthermore, the weevils also preferentially select different clones of the same eucalyptus species, even when planted on the same sites (e. g. Paiva and Mateus, 2012).

In an *Eucalyptus globulus* stand, located near Lisbon, Loures municipality (38°49'19"N, 9°12'23"W), a preliminary study was conducted to compare the volatiles emitted by the foliage of trees that were either mechanically damage, damaged by herbivory, or undamaged. Before sampling, trees were thoroughly inspected for signs of the characteristic damage caused by *G. platensis*. Three undamaged trees and three trees showing signs of herbivory – chewed leaves, were selected and one branch was collected from each tree. Three treatments were applied, namely: i) Undamaged leaves, from undamaged branches; ii) Chopped (using scissors) leaves, from undamaged branches; iii) Weevil chewed leaves. For each treatment, three samples consisting of two newly formed leaves were prepared and the volatiles emitted were collected by head space micro-extraction and analysed by gas chromatography mass spectrometry.

Results indicated that some compounds were only detected in damaged leaves and that, in parallel, emissions of most VOCs increased with damage. Surprisingly, globulol was emitted in much lower amounts by herbivory damaged leaves, than by undamaged and mechanically damaged leaves (Figure 1). Additionally, previous studies using GC-MS-EAD showed that globulol was detected by the antennae of both male and female *G. platensis* (Figure 2).

Furthermore, in olfactometer trials, globulol triggered a repellent effect in virgin males. Yet, no response was elicited from either mated males or females (Branco et al. 2018). Since globulol is a common compound present in the emissions of eucalyptus species, two alternative hypothesis might be considered to explain findings: i) *G. platensis* may preferentially select trees with lower globulol levels. This might be due to a reduced capacity to detoxify this compound, known to have antimicrobial properties. Or ii) Inoculation of saliva by the weevils while feeding, might interrupt the cascade mechanism of globulol production. This could signal to weevils that a tree is already under attack by conspecifics. Further research is required.

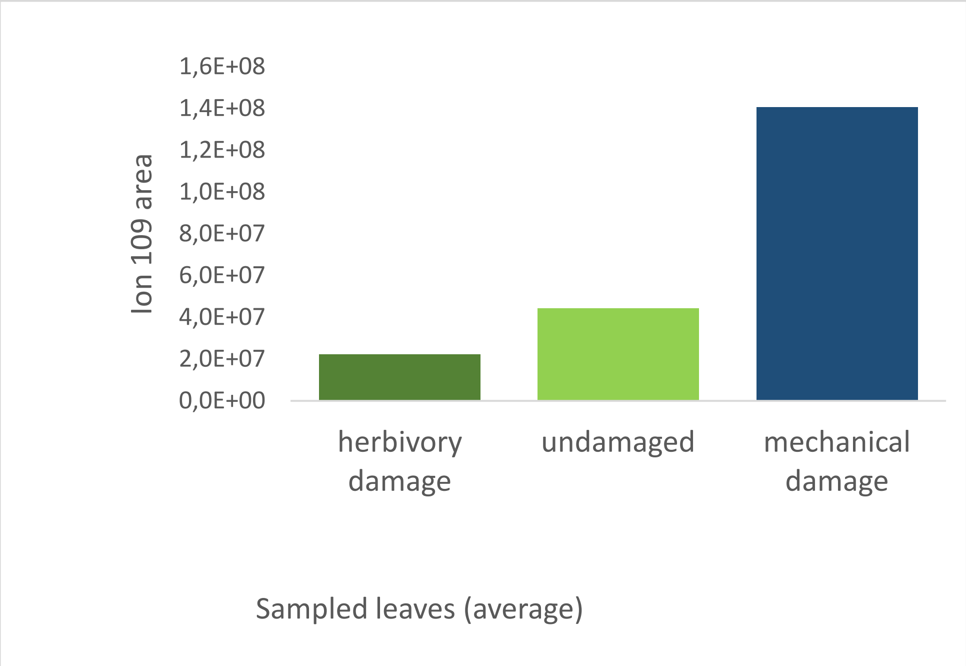

Figure 1. Emission of globulol by *E. globulus* leaves. Bars represent the mean integrated chromatographic areas of ion 109 obtained for each SPME treatment: herbivory damaged, undamaged and mechanical damaged.

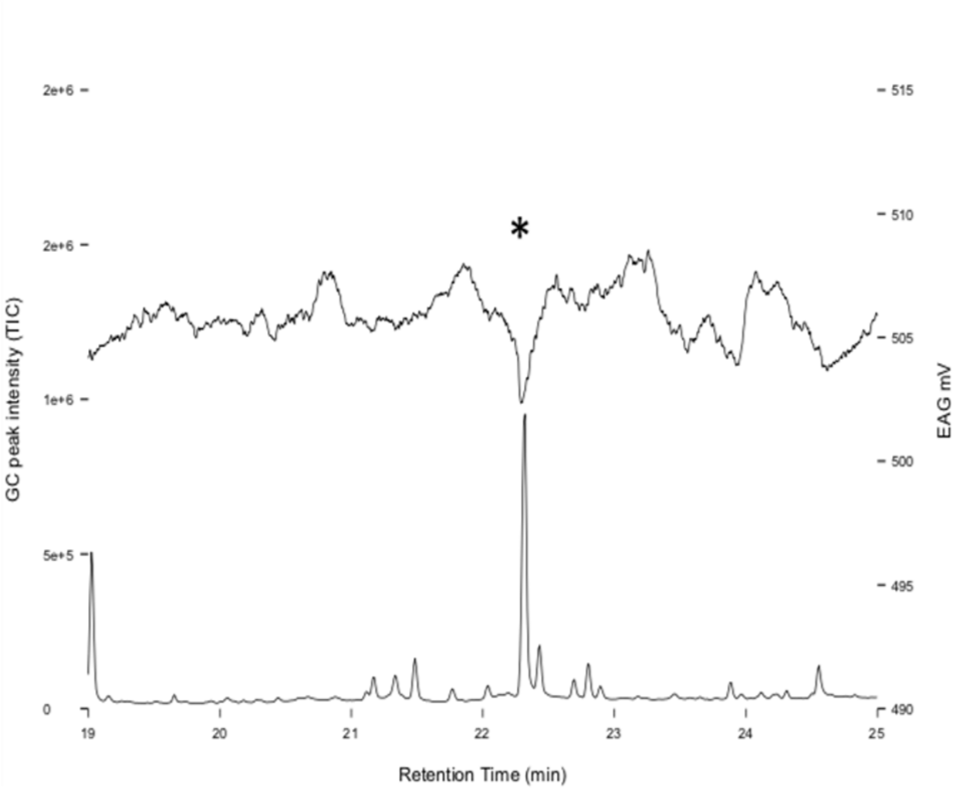

Figure 2. GC-MS chromatographic profile of *E. globulus* (bottom trace) on an INNOWAX column and corresponding EAG recording (top trace) obtained with a female antenna, the peak corresponding to globulol is marked with an *.

Key words: *Eucalyptus globulus*, *Gonipterus platensis*, host selection, globulol

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Investigating the role of leaf color and plant semiochemicals on the behaviour of *Cacopsylla pyri*

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Abstract: Worldwide there are insects that transmit diseases and these are a focus of research. In Europe, fruit trees of the family Rosaceae are seriously affected by phytoplasmas of the apple proliferation group. Phytoplasmas are plant pathogens causing a wide variety of symptoms that can often lead to death of infected plants. Jumping plant lice of the genus *Cacopsylla* are their vectors. It is well known that *Cacopsylla* use chemical cues for orientation and host identification. To overcome the spread of these diseases, especially the transition from infected to healthy plants, several techniques using volatile substances for attracting or repelling vector insects are being studied. In this study, we started to screen possible repellent substances like eugenol to repel *Cacopsylla pyri*, the pear sucker. Secondly, we begun to evaluate the preferences of *C. pyri* for leaf colors from symptomatic and non-symptomatic pear plants, and to determine the relative effect of their spectral reflectance parameters for host finding.

Key words: apple proliferation phytoplasma, push-and-pull, psyllids, application, infochemicals, chemical ecology, kairomones, allomones, pheromones, reflectance, repellents

Introduction

Psyllids play a crucial role in the transmission of phytoplasmas from the apple proliferation group on fruit crops belonging to the Rosaceae (Tedeschi and Alma, 2004), including transmission of *Ca. Liberibacter* (Lopes et al., 2009) on vegetables and Citrus plants. These insects use chemical cues for orientation and host identification (Mayer et al., 2008 a, b; Mann et al., 2012). The psyllid-Phytoplasma and the psyllid-Liberibacter systems have evolved convergently but share many similarities (Gross, 2016).

Former studies indicate that *Candidatus* Phytoplasma pyri, a bacterium causing pear decline disease, is able to overwinter in the body of *C. pyri* (Carraro et al., 2001; Garcia-Chapa et al., 2005) and once infected, to spread the disease over the vegetative period. Considering that the transmission of phytoplasma to plants occurs at the beginning of spring and that the symptoms of infection are more evident at the beginning of autumn, we suspect that at that period, the insects maybe more susceptible to acquiring the bacteria from the plant. We aimed to determine whether the phytoplasma titer in *C. pyri* in mid-autumn and at the end of winter.

In addition the employment of attractive components like β-caryophyllene as a lure for psyllids has been tested (Mayer et al., 2008 a; Eben and Gross, 2013; Gross, 2014). As the semiochemical, produced by infected apple plants, is attractive to both genders of vectoring psyllids, this could be exploited in the development of mass trapping systems for a more sustainable control of these insects in future. In this project, we investigated if (1) leaf color influences the behavior of *Ca. pyri* and (2) if VOCs of infected or healthy trees could enable a mechanism of disease dispersion.

Material and methods

Olfactometer bioassays

Bioassays investigating the vector insect behavior to synthetic compounds were carried out using a dynamic Y-shaped olfactometer. By pumping purified air through two containers, one containing eugenol dissolved in methanol and the other containing just methanol as control, and attaching each outlet with a test arm of the olfactometer, insect preference was tested. The air flow was controlled with a flow meter. The tests were performed with female *C. pyri*. We counted every individual that passed a final mark on one of the test arms within 5 min. T-tests was used to analyze the data.

Spectral measurements

The optical properties of the leaves were measuredusing a USB Ocean Optics FLAME-S-UV-VIS-ES and Xenon Pulse X2 lamp (Ocean Optics) light source. Each leaf was recorded from the adaxial side in the middle vein region. Reflectance properties were measured as the proportion of a diffuse reflectance standard (white standard). The fiber optics probe was mounted inside a matte black plastic tube to exclude ambient light and standardize the distance between each leaf probe at 1 cm. The angle of illumination and reflection was also fixed at 45° to minimize glare. Spectra were calculated at 5 nm intervals from 300 to 700 nm with SpectraSuite software. A black cardboard was used as the background of the leaf to enhance contrast.

Field trials

The field experiments were conducted in the experimental field at Julius Kühn-Institut, Dossenheim, Germany. All samples were stored directly after their collection in a freezer (-20 °C) for counting, sex and species determination. Beating tray samples were taken in October 2018 during the same period so that we could identify symptoms of phytoplasma infection in plants on the field. Symptomatic trees are classified according to the color of the leaves which become abnormally red in autumn and began to drop leaves prematurely. A white funnel cloth tray (50 × 50 cm) was held under a tree limb. Then, 10 limbs were beaten sharply three times each with a pole. Dislodged psyllids fell to the tray and were collected in plastic bags attached to the end of the funnel (Weintraub and Gross, 2013). Both symptomatic and healthy plants were sampled. Seventy-three trees were selected; 33 with symptoms of phytoplasma infection and 40 without (five plants per row).

Eggs and nymphs (Pasqualini et al., 2003) on 10 leaves per tree were also counted. Samples were processed in the laboratory and examined using a binocular dissecting microscope.

Results and discussion

C. pyri females avoided the olfactometer arm containing eugenol. More than 72% of the tested females preferred the control arm ($P < 0.01$). Eugenol is a semiochemical found in a variety of plants including clove buds, cinnamon bark and leaves, tulsi leaves, turmeric, pepper, ginger, oregano and thyme. In addition, several other aromatic herbs including basil, bay, marjoram, mace and nutmeg are also claimed to have significant quantities of eugenol (Raja et al., 2015). Clove essential oil has been widely studied for its insecticidal and repellent activities against many species of pests (Chaieb et al., 2007; Kafle and Shih, 2013; Cortés-Rojas et al., 2014), such as Asian citrus psyllid (Mann et al., 2010). As reported by Tian et al. (2015), clove essential oil treatment reduced the numbers of *C. chinensis* nymphs in a concentration-dependent manner. These results provided us indication that the major components of clove essential oil (eugenol) could be repellent and/or deterrent on female psyllids searching for an appropriate oviposition site. More studies are planned to investigate the potential of those compounds as behavior modifying. The field of insects repellents is moving forward as farmers and consumers demand means of protection from arthropod that are safe to use and environmentally sustainable. Perhaps the most important consideration is improving the longevity of those repellents that are effective but volatile. Hence, we are conducting studies to improve formulations and as well increase volatile longevity.

The spectral measurements showed that non-symptomatic leaves had a higher reflectance (range of 482-585 nm, blue, green and yellow, $P < 0.01$) than symptomatic leaves (range of 585-700 nm, orange and red, $P < 0.01$).

C. pyri was the only psyllid found in the field and the majority were winter-forms (Garcia-Chapa et al., 2005). The mean numbers of eggs laid on symptomatic trees was ten times lower than on non-symptomatic ones ($P < 0.01$). There was no difference in the numbers of nymphs on symptomatic and non-symptomatic trees. In the same period, we could not find differences between the mean number of adults collected on plants with and without phytoplasma symptoms.

The importance of visual stimuli the response to light and trap color was reported for *D. citri* (Hall et al., 2007; Wenninger et al., 2009; Sétamou et al., 2012). Hall et al. (2010) evaluated the preferences of different hues of green and yellow traps. At the peak period of *D. citri* flight, the red and the yellow traps had comparable *D. citri* captures in the lemon grove. The blue and white traps captured fewer *D. citri* adults (Sétamou et al., 2014). Nevertheless, the importance of visual signals for pear psyllids remains unclear. Combining the results of spectral measurements with data from VOCs collection of infected trees will enable us to assess the role of plant volatiles and leaf color in the pear psyllids/pear decline disease system.

These results are part of a pioneer project, which consists the development of new types of repellent formulations and attractive colors for developing efficient mass traps. In that field, nanotechnology can be applied for agrochemicals by using nanoscale carriers; they have controlled release mechanisms which allow the active ingredient to be taken up slowly, thus improving its effectiveness, while reducing the amount applied. Against a background of insect behavior in the field, we want to establish new techniques of vector control for prevention of new phytoplasma infections.

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Interfering host location of *Cacopsylla pruni* with repellent plant volatiles

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Extended Abstract: The phloem-feeding plum psyllid *Cacopsylla pruni* is the only known vector of the phytoplasma '*Candidatus* Phytoplasma prunorum'. This specialized cell wall-less bacterium is restricted to sieve elements in the phloem tissue of *Prunus* ssp. *Ca.* P. prunorum causes European Stone Fruit Yellows (ESFY), one of the most severe diseases in stone fruits. Because infected *Prunus* cultivars yield poorly and die within a few years, ESFY is of high economic interest. *C. pruni* acquires the bacteria during feeding on infected plants. By transmission feeding the pathogens are spread to healthy *Prunus* trees. To date no effective control strategies or cures for phytoplasma diseases are available. To reduce the number of new infections and stem the spread of ESFY innovative control strategies against the vector are required.

C. pruni alternates its host plant twice within one generation (Ossiannilsson 1992). *C. pruni* needs to reproduce on *Prunus* because nymphs are not able to develop on their second host, coniferous trees (Gallinger and Gross, 2018). Therefore, in early spring adults arrive in stone fruit orchards to mate and oviposit on *Prunus* spp. After passing five nymphal stages the young adults (emigrants) migrate, during summer, to firs and other conifers to ensure adequate nutrition for the rest of the year until they return (remigrants) in early spring for reproduction to *Prunus* (Gallinger and Gross, 2018). The long distance alternation between these diverging plant species makes reliable host detection essential.

Insects use secondary plant metabolites such as volatile organic compounds for identification of feeding and oviposition sites. The use of olfactory cues for host location is shown for several psyllid species (Alquézar et al., 2017; Martini et al., 2014; Mas et al., 2014; Mayer et al., 2008 a; 2008 b, 2011; Mayer and Gross, 2007; Soroker et al., 2004). Interestingly a change in preference for volatiles from overwintering and reproduction hosts was found in the migrating psyllid *Cacopsylla melanoneura* (Mayer and Gross, 2007). Additionally the infestation of carrot plants with *Trioza apicalis* can be reduced by the application of sawdust from evergreen overwintering hosts (Nehlin et al., 1994). These findings underlie our hypothesis that *C. pruni* is attracted or repelled by specific volatiles from *Prunus* trees and conifers.

We analyzed and compared the volatile profiles of *Prunus* trees and silver firs to identify species-specific components. In field surveys different infestation levels with *C. pruni* in *Prunus* varieties were found (Carraro et al., 2002; Mergenthaler et al., 2017; Gallinger et al., 2019). Therefore, additionally components that lead to the differentiation of volatile profiles from a high attractive *Prunus* rootstock and a less attractive cultivar were identified (Gallinger et al., 2019).

Typical volatiles of the two Prunus varieties and silver firs were tested for antennal detection of *C. pruni* females and detectable volatiles were investigated for influence on psyllid behavior in Y-shaped olfactometer trials. Most compounds had a repellent effect on *C. pruni* adults. Mixtures of these repellent plant volatiles were incorporated in polypropylene plastic cards as dispensers. Their ability to repel *C. pruni* or mask the odor of an attractive host plant was verified in olfactometer trials (Figure 1). Additionally, these mixtures of potential repellents against *C. pruni* were tested in a first field study. An artificial application of those mixtures could be used for the development of an environmentally safe control strategy.

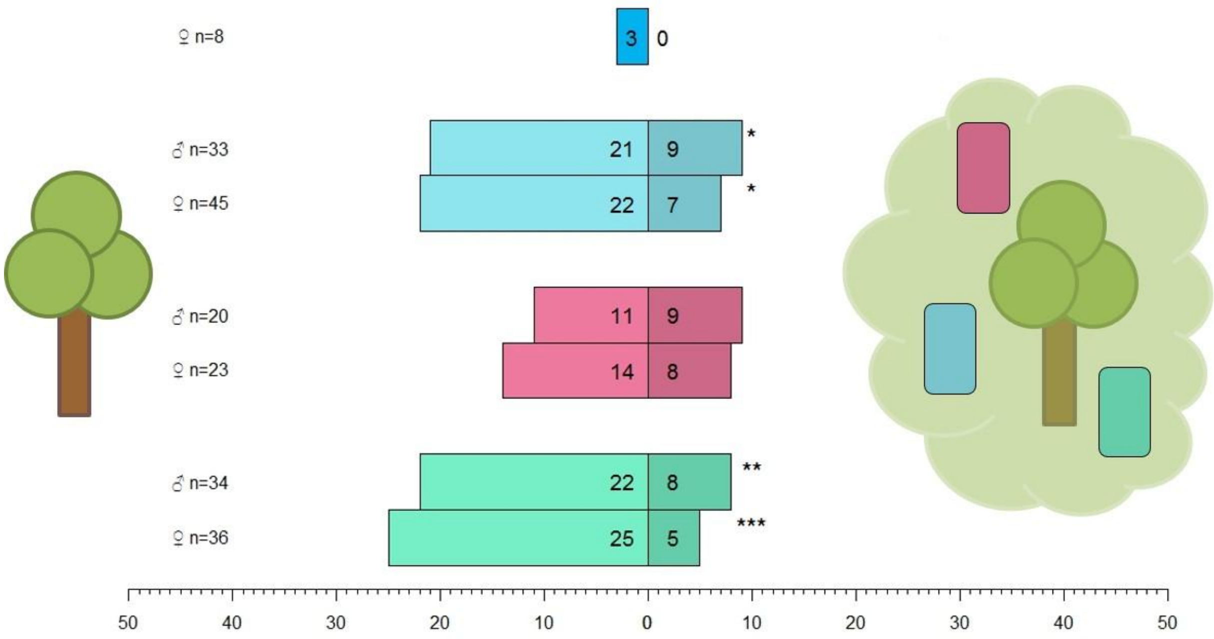

Figure 1. Number of *C. pruni* remigrants which choose the pure odor of a *Prunus* tree (left) over the odor of a *Prunus* tree equipped with different repellent mixtures (green = Mix A – 5%, red = Mix B – 5%, pale blue = Mix C – 5%, mid-blue = Mix C – 20%) incorporated in polypropylene plastic card dispensers (right). Significant differences are marked with asterisk (sign test, *P < 0.05, **P < 0.01, **P < 0.001).

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Attract and Kill for the control of olive fruit fly in Alto Garda, Trentino

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Abstract: Alto Garda Trentino, in northern Italy, is an important economic touristic area characterized by the insubric climate typical of the Lake Garda basin. Good quality oil came from the local olive groves and the olive fruit fly (*Bactrocera oleae* Rossi) represent a serious and potentially devastating threat. The insect outbreaks have increased over the last decade. One objective of the project named "Innovazione e Ricerca per l'Olio Extravergine dell'Alto Garda Trentino" was to evaluate the effectiveness of Attract and Kill products; Eco-Trap (Vioryl), Spintor Fly (Dow AgroSciences) and a device for mass trapping, Flypack (SEDQ). A three year experiment (from 2016 to 2018) was set up in a Casaliva olive orchard located in monte Brione (Riva del Garda, TN). In 2016 at harvest, 49% of olives were infested on the untreated area. Spintor Fly gave the best protection (mean 5% of fruit infested) and Eco-Trap gave intermediate protection (24%). The fruit damage in the untreated gradually lowered in the next two years (42 and 25% respectively) and the tested products (Eco-Trap, Spintor Fly, Flypack) showed similar, good, results (5% or less of fruit infested). The attract and Kill technique has the potential to reduce pollution, residues, resistance, drift and side effects on beneficial arthropods. For good efficacy it should be applied at low infestations and repeated area-wide applications over years for satisfactory pest control. Constant monitoring of the climatic and the insect flight, good agronomic practices and predicting harvest date in case of high infestations remain of fundamental importance for guaranteed crop yields.

Key words: olive fruit fly, Integrated Pest Management, semiochemical-based control

Introduction

The northernmost latitude for olive cultivation in Europe was located in Alto Garda Trentino (northern Italy). The Lake Garda insubric clime and the natural selection of a cold tolerant cultivar, Casaliva, were significant factors to olive growing. The high quality oil was peculiar for some aspects as higher fatty acids content. Olive fruit fly (*Bactrocera oleae* Rossi) represent the most serious pest to olive production. Integrated pest management (IPM) as a control strategy was largely adopted in the past with positive results. However, outbreaks of *B. oleae* have increased in the last ten years (Michelotti, 2017). The technological-scientific progress, the social environmental issues awareness, the use of chemical pesticides and their regulations enforced by the laws were drivers of the pest management (Nestel et al., 2016).

One objective of the project, named "Innovazione e Ricerca per l'Olio Extravergine dell'Alto Garda Trentino", was the knowledge transfer of the reliability of strategies for the management of the olive fruit fly using modern tools.

Material and methods

Semiochemical-based control experiment

A three years experiment (2016-2018) was done to evaluate the effectiveness of different Attract and Kill approaches; mass trapping and “Lure and Kill" tactics. During 2016 and 2017 we compared two “Lure and Kill” bait station products; Eco-Trap (Vioryl), a bag treated with contact insecticide (deltamethrin) containing ammonium bicarbonate and provided with a sexual pheromone (1,7-dioxaspiro[5.5]undecane) dispenser and Spintor Fly (Dow AgroSciences), a sprayable bait protein-specific with ingestion insecticide (spinosad). In addition, in 2017 we also tested a new device for mass trapping, Flypack (SEDQ). This was made of a yellow conic plastic body with four holes containing specific food and sex attractants and a transparent cover treated internally with deltamethrin. In 2018 Flypack and Eco-Trap comparison was repeated.

Study site and design of experiment

The trial was set up in a hilly olive Casaliva orchard (around 70 hectares) located in monte Brione (Riva del Garda, TN). The experimental design, according to European Plant Protection Organization standards (EPPO 2008; 2012 a; 2012 b), consisted of adjacent large plots per treatment (around 3 hectares/plot) subdivided in three subplots size (around 1 hectare each with a net areas of 0.1 hectare or minimum of 20 trees, separated by a minimum of 100 m) at altitudes of 70 to 150 m a.s.l. (Figure 1). The treated plots were rotated each year. Untreated plots, with similar trial conditions, were selected outside the treated area. The experiment began in July each year during the phenological stage of pit hardening (BBCH 75), when the olives were susceptible to egg laying, until harvest.

Eco-Trap and Flypack were hung at a density of 100 and 70 traps per hectare/year respectively. Spintor Fly (a. i. spinosad 0.024%) was applied before the fruit first punctures were found and following the insect infestations (with a maximum of 8 treatments/year) at dosage of 1 l of product diluted in 4 l water/hectare (half of total number of the trees were treated on a small part of foliage with Spintor Fly by backpack sprayer).

Weekly assessments evaluated the fruit infestation in the subplot areas. From 20 trees/subplot we collected a random sample of 5 fruits/tree. The olive total infestation was calculated as sum of olives with eggs, live or dead larvae, pupae, galleries and pupal chambers. All of the Flypack devices were removed from the field soon after the fruit harvest and the flies captured were sexed and counted in the laboratory.

All data collected, were transformed [log(x + 1)] as necessary to meet the assumptions for the Analysis of Variance (ANOVA) followed by Tukey – Kramer test for multiple comparisons (software Statistica STATSOFT, ver.13).

Figure 1. Site location (M. Brione, Riva del Garda, TN) and experimental trial design.

Results

The first year of study, 2016, was characterized by high temperatures, high frequency of rain events and a heavy olive fly infestation naturally occurred in the area. At harvest, 49% of the fruits were infested in the untreated area. Eight applications of Spintor Fly gave the best control (5% fruit damaged). 24% of the fruits were damaged when Eco-Trap was used, similar to untreated.

In the next two years, in the untreated plots, the fruit damage recorded at harvest lowered (42% and 25% in 2017 and 2018, respectively) and all Attract and Kill products tested (Eco-Trap, Spintor Fly and Flypack) showed similar control of *B. oleae* (5% or less of fruit infested) (Table 1).

In 2017 the average number of *B. oleae* captured per Flypack device was 9 (51% females) and no difference was observed in subplots positioned at different altitudes. In 2018 the average number of the *B. oleae* captured was 104 (56% females) with more female captures above 130 m s.l.m. (Table 2).

Table 1. Percent mean values (+/- SD) of olives total infested (eggs + larvae + pupae + exit holes).

Period / Treatment	2016			2017		
	Aug.	Sep.	Oct.	Aug.	Sep.	Oct.
Untreated	11.5 ± 6 bcde**	26.3 ± 9.5 ab	48.5 ± 12.9 a	1.7 ± 2.4 b	17.1 ± 9.2 a	42.0 ± 18.4 a
Eco-trap	7.2 ± 3.9 de	16.3 ± 7.2 abcd	24.1 ± 5.1 abc	0.0 b	0.0 b	5.3 ± 11.2b
Spintor Fly*	3.7 ± 2.1 e	10.8 ± 5.2 bcde	4.9 ± 4.4 e	0.3 ± 0.6 b	0.4 ± 1.3b	1.7 ± 1.2b
Flypack	-	-	-	0.0 b	0.0 b	0.8 ± 1.6b

Period / Treatment	2018		
	Aug.	Sep.	Oct.
Untreated	0 c	16. ± 13.1 ab	24.6 ± 16 a
Eco-trap	0 c	0.6 ± 1.2 c	2.7 ± 3.1 c
Spintor Fly*	-	-	-
Flypack	0 c	2.3 ± 2 c	5.1 ± 3.4 abc

*Number of applications; 8 in 2016, 4 in 2017. **Values followed by different letters were significantly different (Tukey's HSD, $p < 0.05$).

Table 2. Flypack (70 traps/ha); percent mean value of *B. oleae* captured (+/- SD) /device.

Year*	Altitude (m a.s.l.)	Total	Sex ratio	Femals (%)
2017	130-150	9.7 ± 15,6	1.2 ± 1	45.4 ± 25.5
	100-130	7.4 ±6.7	1 ± 1.4	55.5 ± 25.8
	70-100	11.1 ± 12.5	1.1 ± 1.4	49.7 ± 28
	70-150	9.0 ± 11.8	1.2 ± 1.3	50.8 ± 26.4
2018	130-150	73.6 ± 40.1	0.7 ± 0.3a**	61.1 ± 9.3 a
	100-130	97.9 ± 76.2	0.9 ± 0.4b	54.5 ± 11.1b
	70-100	142.5 ± 82.5	1.0 ± 0.4 b	51.8 ± 11.5 b
	70-150	104.4 ± 73.7	0.9 ± 0.4	55.9 ± 11.3

*Period from July to October; **Values followed by different letters were significantly different (Tukey's HSD, $p < 0.05$).

Discussion

Attract and Kill, as an alternative IPM strategy, could help alleviate problems of pesticide pollution, residues, resistance, drift and side effects on beneficial arthropods (Basilios et al., 2002; Bueno and Owen, 2002). From our experience some consideration should be made; the best results were obtained in low *B. oleae* infestations and when repeated over more than one year. Application should be area-wide to guarantee success of the technique if replacing chemical insecticides (El-Sayed et al., 2006).

Spintor Fly provided good protection, and should result in fewer residues on the fruit but it was necessary consider the cost, risk of wash off, low persistence and the impact on no-target arthropods (Gonçalves et al., 2011).

Eco-Trap allowed adequate protection and could combined with the bait spray (e. g. Spintor Fly) or other insecticide products especially during seasons with high infestations near to harvest when fruit are more vulnerable to attack (Lentini et al., 2005; Caleca et al., 2007).

Flypack, when commercially available, could represent a valid alternative to chemical insecticides and bait applications in more urban and touristic areas where treatments are not allowed.

When the risk of immigration of mated female *B. oleae* is low, we could confirm Flypack to be a useful device for spring mass captures of overwintering adults, lowering the future infestation potential (Ragaglini et al., 2007; Marchini et al., 2017; Mucci et al., 2018). Constant *B. oleae* monitoring with local climatic data, good agronomic practices and anticipating the harvest date, still remain fundamental for good control in combination with Attract and Kill methods (Broumas et al., 2002; Ioriatti and Angeli, 2002; Petacchi et al., 2003; Marchi et al., 2015).

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Pheromones and Other Semiochemicals in Integrated Production
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A new bisexual kairomone lure for codling moth

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Abstract: Finally, the promise of pear ester (*E*, *Z*)-2,4-decadienoate as a potent bisexual attractant for codling moth *Cydia pomonella* (L.) has been realized through the creation of powerful new multi-component kairomone blends. This discovery comes nearly 20 years after Dr. Douglas Light revealed the attractiveness of pear ester as the first identified individual kairomone that was effective at rates similar to a sex pheromone and attractive to both sexes of moths (Light et al., 2001). Pear ester used within walnut groves is a powerful attractant, but in pome fruits many factors impacted its effectiveness, such as crop, cultivar, seasonality, crop load, and the presence of fermenting fruit on the orchard floor. Pear ester also performed well when used in combination with the sex pheromone and a 'Combo' lure has been adopted throughout the world to monitor *C. pomonella* in orchards treated with sex pheromones for mating disruption (Knight et al., 2005). A number of studies have developed lure blends including pear ester that have improved its performance (Landolt et al., 2007; Landolt et al., 2014; Jaffe and Landolt, 2018). The use of n-butyl sulfide in combination with acetic acid and pear ester (BSAAPE) has been purported to be a more effective lure than pear ester alone and has recently been tested effectively to mass trap *C. pomonella* in commercial orchards (Jaffe et al., 2018). In a continuing effort to develop new bisexual lures for *C. pomonella* a series of field trials were conducted with delta traps with removable liners and with bucket traps using propylene glycol during 2018. During a serial process of testing new attractant blends a potent multi-component blend was identified. This blend consistently outperformed the use of BSAAPE, by 3-fold. The proportion of female moths caught with the new blend ranged from 60 to 80% during the summer. The new blend was equally effective in apple blocks with low fruit loads and in an orchard with a heavy fruit load with low (early season), moderate (mid-season) and high levels (late season) of fruit injury and even later in the season when the orchard floor was littered with fermenting fruits. The new blend outperformed sex pheromone lures in blocks both untreated or treated with sex pheromone dispensers. The attractiveness of this blend was also tested in combination with sex pheromone lures and male catch was significantly increased and female catch did not decline when the sex pheromone was added.

Key words: apple, kairomones, n-butyl sulfide, acetic acid, pear ester

Introduction

Adult monitoring of codling moth, *Cydia pomonella* (L.), is a key component in implementing effective integrated management programs of this key pest of apple, *Malus domestica* Borkhausen. Development of new kairomone lures allows both moth sexes to be tracked and captures of female moths may benefit several aspects of its' management. Field studies were conducted in 2018 to evaluate new kairomone blends in combination with pear ester (*E,Z*)-2,4-ethyl decadienoate (PE) and acetic acid (AA) for their attraction of male and female codling moth.

Following the identification of a 4-component blend (4-K) consisting of pear ester, (*E,Z*)-2,4-ethyl decadienoate (PE), (*E*)-4,8-dimethyl-1,3,7-nonatriene, pyranoid linalool oxide, and acetic acid (AA) additional studies were conducted to compare moth catches in traps baited with the 4-K lure versus the use of sex pheromone, (*E,E*)-8,10-dodecadien-1-ol (PH) in combination with PE and AA. Separate trials were conducted in orchards either untreated, treated with Cidetrak CM Meso dispensers loaded with PH, or Cidetrak CM-DA Meso dispensers loaded with PH + PE for mating disruption (MD).

Material and methods

(*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT; 99% purity) was obtained from Pherobank (Wageningen, The Netherlands) and loaded into grey halobutyl septa (West Co., Lionville, PA). Prior to loading, septa were extracted three times with dichloromethane (99.9% purity) and air-dried overnight prior to storage in glass jars at -15 °C. DMNT lures were prepared by adding 100 µl of a dilution in dichloromethane to load 10 mg per septum. Similar volumes of dichloromethane were added three times after this initial loading to enhance penetration into the substrate. Lures were stored at -15 °C until used in field studies. Several lures were provided by Trécé Inc. (Adair, OK). A proprietary 2.8 cm^3 resin lure loaded with 10.0 mg decanal (TRE1724), a proprietary 3.2 cm^2 plastic membrane cup lure loaded with 720 mg of acetic acid (TRE3321), and grey halobutyl septa loaded with 3.9 mg of pear ester, (*E,Z*)-2,4-ethyl decadienoate (TRE3460), 3.5 mg of (*E,E*)-8,10-dodecadien-1-ol (Pherocon CM L2, TRE4111), and combinational lures loaded at these rates with both pear ester and sex pheromone (Pherocon CMDA Combo, TRE3461). Linalool oxide pyranoid (99.1% purity, 57.8% *Z*-isomer and 41.3% *E*-isomer) was supplied by Nippon Terpene Chemicals Inc. (Tokyo, Japan), and linalool oxide furanoid (mixture of isomers, > 97% purity) and linalool (97% purity) were purchased from Millipore Sigma (St. Louis, MO). Single or blended linalool and linalool oxides were diluted (10% w/w) in mineral oil and loaded in 1.5 ml Eppendorf microcentrifuge plastic tubes which contained a dental cotton wick to adsorb the solution. These lures were prepared by pipetting either 115 or 230 µl of the oil formulation to create 10 and 20 mg loadings. A 2.0 mm aperture was drilled into the cap of the Eppendorf vials just prior to field application.

A general protocol for field studies was adopted. All studies except one were conducted with the use of orange delta-shaped 28 × 20 × 20 cm traps (Pherocon VI, Trécé Inc.) with liners coated with hot-melt pressure sensitive adhesive (AlphaScents, West Linn, OR). The exception was a single study which was conducted with non-saturating green/white Multipher bucket traps (Great Lakes IPM, Vestaburg, MI) using propylene glycol to retain moths in order to avoid trap liner saturation over a longer trapping period. Treatments were randomized in each orchard with 5 to 10 replicates. Unbaited traps were included as a control treatment in all experiments. However, the mean catch with blank traps was always < 0.2 moths per trap and this treatment was not included in the analyses. Traps were attached to poles and placed in the mid canopy at 3.0-m height, ≥ 30 m apart, and > 20 m from the borders of orchards. Moths were removed, sexed, and counted in the laboratory.

Trials were conducted in three 'Delicious' apple orchards (4 to 8 ha) located south of Wapato, WA (42^o 45' N, 120^o 42'W). Orchards were planted at 720 trees/ha with average tree canopies of 4.0-4.5 m. Orchards were not sprayed with pesticides during 2018. Ten separate trials were conducted between May and August 2018 to evaluate lure blends. Additional studies were conducted to evalaute the msot effective blend against pheromone lures in blocks untreated or treated with either 80 dispensers (Trécé Inc.) ha^{-1} loaded with 900 mg of PH (Pherocon CM Meso-A) or 900 mg PH + 600 mg PE (Pherocon CMDA Meso-A). Four trials were each run in the orchard with no MD and in the orchard with PH dispensers. Two trials were run in the orchard treated with PH + PE dispensers. Trials lasted from 1 to 3 nights to minimize liner saturation and were conducted during August 2018. Traps were placed on different trees within orchards for each trial.

Results and discussion

The addition of decanal to either AA or PE alone significantly increased total and female moth catches. However, the addition of decanal did not improve the attraction of PE + AA. The addition of either the pyranoid (PyrLOX) or furanoid (FurLOX) linalool oxide but not linalool (LOL), increased moth catches with PE but did not increase catches with PE+AA. Similarly, the addition of PyrLOX plus decanal did not improve PE + AA. The addition of (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) to either AA, PE + AA, or PE+AA with decanal did not significantly increase moth catches. However, the addition of PyrLOX to traps with PE + AA and DMNT (4-K lure) significantly increased moth catches compared with PE + AA alone or any of the ternary blends of these volatiles. Females accounted for 60-80% of the total catch with the 4-K lure. The 4-K lure with PyrLOX was a more attractive lure than similar blends that substituted LOL, FurLOX or any of the three binary LOX blends for PyrLOX. The 4-K lure caught nearly 4-fold more total and female moths than the purported attractant N-butyl sulfide when it was used in combination with PE + AA.

Traps baited with 4-K and 4-K + PH lures caught significantly more females than traps baited with PH + PE + AA lures. Traps baited with 4-K + PH lures caught significantly more total moths than traps baited with PH + PE + AA lures in all three orchards. Adding a PH lure to traps with the 4-K lure did not affect female catch, but significantly increased male and total moth catches.

These studies are the first to demonstrate that codling moth can be monitored effectively in apple under MD without the use of sex pheromone lures. The significant increase in female codling moth catch with the 4-K lure suggests that efforts to improve spray timings and action threshold determinations as well as mass trapping can be enhanced with this new lure.

Our results showing that the 4-K lure outperformed the most effective commercial lure for codling moth are remarkable. However, the results must be judged to be tentative until additional studies can more fully evaluate this lure. Behavioral and electrophysiological studies are needed to assess the response of codling moth to purified isomeric preparations. Field studies across the major geographical production areas are necessary to assess the effectiveness of the 4-K lure as significant geographical variation of the attractiveness of PE has been reported. Full seasonal evaluations of the 4-K lure are required across host crops and major cultivars. The performance of the 4-K lure should also be assessed in orchards under various MD technologies, including sprayables, aerosols, and hand-applied dispensers.

Finally, these results suggest that studies should be broadened to include blends of various other volatiles, including key monoterpenes found across host crops, i. e. β-Ocimene, β-Myrcene, limonene, α- and β-pinene, terpineol, and likely volatiles from other chemical groups.

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KLIMAKOM – Insect communication under climate change

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Extended Abstract: Climate change can threaten our food production. Because of changing temperatures and concentrations of greenhouse gases, well-established pest insect control techniques may have to be adapted to the changing requirements to stay effective. In particular temperature, elevated carbon dioxide (CO_2) and ozone concentrations will have significant impacts on the environment (IPCC, 2014) and therefore on the control strategies based on chemical ecology of insects. The aims of the project are the investigation of effects of these fundamental determinants of climate change on the perception and reaction of insects to semiochemicals, in addition to the stability and persistence of volatile organic compounds (VOCs) in the environment. This fundamental research will create the basis for the identification of possible limitations for plant protection methods based on semiochemicals, and to adapt them to a changing environment.

Behavior modifying compounds (pheromones, attractants and repellents) will be evaluated in laboratory, greenhouse and field experiments under different environmental conditions (temperature, elevated CO_2, and O_3 concentrations) examining their effects and efficiency on several insect pest species.

The emphasis is on the mating disruption method, which is widely used in Europe for the biological control of our first model organism *Lobesia botrana* (European grapevine moth) (Figure 1). This includes investigation of the stability of the main component of its sexual pheromone, (7E,9Z)-dodecadienyl acetate (E7,Z9-12Ac) (Roelows et al., 1973) and the release and reception of E7,Z9-12Ac and minor pheromone components by conspecifics. Chemical analysis, including gas chromatography coupled with mass spectrometry, electrophysiological methods, e. g. GC-EAD, behavioral tests in wind tunnels, olfactometers and field cages will be used.

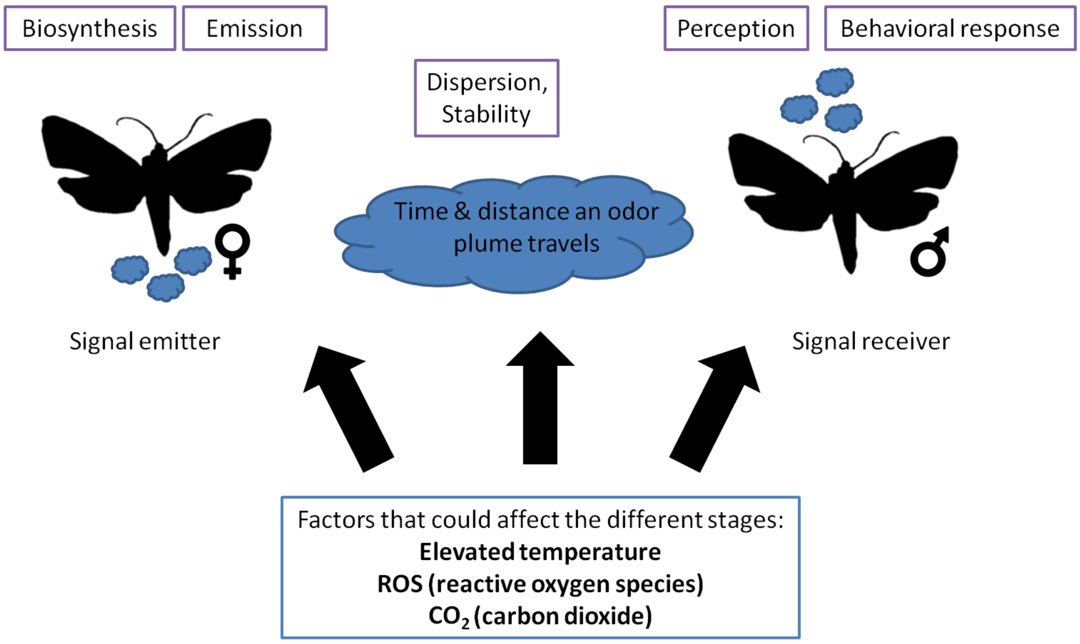

Figure 1. Stages of insect pheromone communication and factors that could affect them.

The second model organism investigated in this project are jumping plant lice of the genus *Cacopsylla* (Hemiptera: Psyllidae), which are well known as vectors of phytoplasma diseases of fruit crops (Jarausch et al., 2018). Psyllids are phloem feeders and both nymphs and adults feed on plant sap. Phytoplasma vector species on apple and stone fruits are univoltine whereas pear psyllids are mostly polyvoltine with overlapping generations (Jarausch et al., 2018). Polyvoltine vector species usually overwinter as adults on or near to their host plants, while univoltine species have an obligate host alternation and migrate in summer (= emigrants) to their overwintering plants (conifers), returning to their respective reproduction host plants in spring (= remigrants) (Mayer et al., 2011; Burckhardt, 1994).

Their migration behavior and their search for appropriate food and oviposition sites are regulated by plant VOCs (Gross, 2016) and non-volatile phloem constituents (Gallinger and Gross, 2018). These differ between plant species and may be influenced by psyllid feeding, phytoplasma infection and further biotic and abiotic factors (Figure 2). The influence of the fundamental determinants of climate change on these multitrophic interactions will be the second emphasis of the study.

The main goal of the project is to improve of plant protection measures. We will focus on two key aspects: 1. Reduction of insecticide spraying in fruit crops and viticulture through non-chemical plant protection measures adapted to future climate change (mating disruption, monitoring, mass trapping). 2. Reduction of new infections of fruit trees with phytoplasma diseases by improved monitoring and selective and targeted control of their vectors.

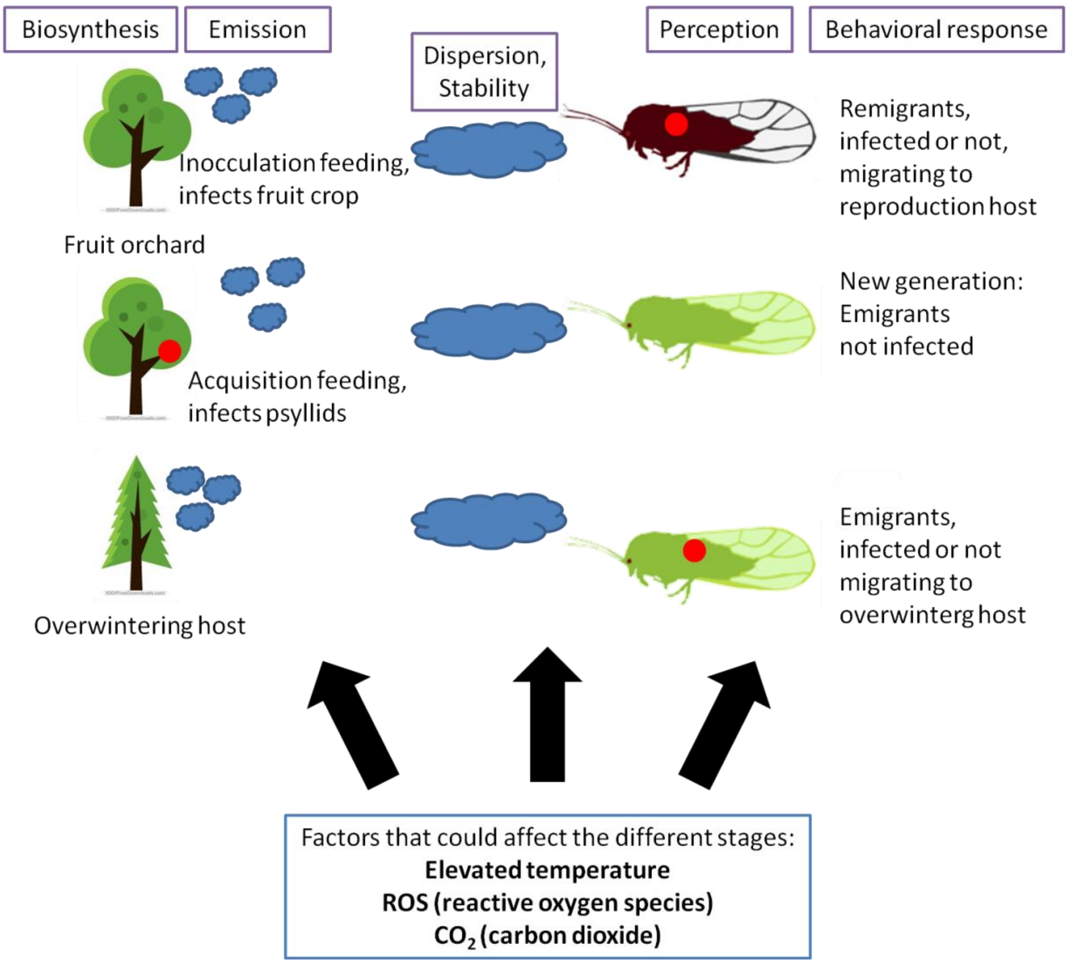

Figure 2. Stages of psyllid interspecific communication during migration between alternate hosts and factors that could affect them.

Here we introduce our new project in which we investigate if greenhouse gases and reactive oxygen species have the potential to alter semiochemically mediated communication within and between insects and their host plants and transmitted pathogens. Our hypothesis is that both pheromone and allelochemical-mediated communication is influenced by the determinants of climate change. The main question posed in this study is: Does the chemical structure of the semiochemicals alter by climate change determinants and/or are both the emitters (insects, plants) and the receivers influenced.

Key words: ozone, carbon dioxide, elevated temperature, chemical communication

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Pheromone-based pest management tactics

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Management of the honeydew moth by mating disruption in vineyard

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Abstract: *Cryptoblabes gnidiella* is a lepidopteran species, which has increased noticeably in the last few years in different crops in the South of Europe, especially in pomegranate, kaki and vineyards. The scarcity of products for its effective control along with the extended use of mating disruption for other coexisting species, such as *Lobesia botrana* in vineyards, makes crucial to develop an alternative based on the use of pheromones for the management of this species. Therefore, the efficacy of mating disruption with the dispenser CRYPTOTEC, at two different doses of dispensers per hectare, was tested in a plot of vineyards in the south of France. The results accomplished so far, show the effectiveness of the dispensers tested, and therefore the mating disruption as a possible strategy in the management of this species.

Key words: Honeydew moth, mating disruption, vineyard

Introduction

The honeydew moth, *Cryptoblabes gnidiella Millière* (Lepidoptera: Pyralidae), is a polyphagous species native to the Mediterranean region which has spread to different areas of Africa, Asia, Europe, New Zealand, Hawaii and South America. It has been mainly related to citrus, pomegranate and grapes, although it has been found in several other crops such as avocado, loquat and kaki. In Southern Europe it has been considered as a secondary pest but in the last years its population and correlated damage has increased considerably in certain crops such as kaki, pomegranate and vineyard.

The efficacy of mating disruption in the control of *C. gnidiella* has been checked in the last 4 years in pomegranate and kaki (Acín, 2018). In vineyard, most of the studies have been focused mainly on the monitoring of this species (Anshelevich et al., 1993; Vidart et al., 2013; Demirel, 2016; Öztürk, 2018), but just a few experiences in mating disruption has been performed, although not concluding enough. Therefore, the efficacy of mating disruption has been evaluated in a plot of vineyard with a high pest pressure, in order to assess the effect of the dispensers tested. This strategy entails a promising tool for the management of this species.

Material and methods

A trial was performed in Baho, in the department of Pyrénées-Orientales in France.

Two plots of 2.3 ha were treated with an experimental mating disruption (MD) dispenser at two different doses, 300 and 400 dispensers per hectare. A third plot of 1.2 ha approximately, without pheromone dispensers, was considered as control. The variety found in all plots was Grenache noir. These plots were chosen due to the high presence of *G. gnidiella* in the last years.

Dispensers were placed before the first flight of adults following a homogeneous distribution. Each dispenser was comprising a blend of Z11-16:Ald and Z13-18:Ald in a ratio 1:1 and a maximum load of 330 mg of pheromone blend.

Efficacy assessment

In order to evaluate the effectiveness of the dispensers, the number of males captured in the monitoring traps and the percentage of damage were recorded.

To monitor the males trapped, four delta traps with the corresponding monitoring dispenser were placed in each plot. These dispensers were replaced every 35-45 days. All monitoring traps were examined at least once a week and males caught in the devices were counted.

Moreover, the efficacy of mating disruption was also measured by the evaluation of damages. For this purpose, the assessment of damage was accomplished before harvest. For this, 200 bunches per plot were searched for damage on 12th September 2018 (BCCH 89). To evaluate the severity of damage, bunches were grouped in classes, according to the following scale:

Class 1 = Less than 10% of damage (very low risk of yield loss)

Class 2 = Damage between 10 and 40% (significance loss of yield)

Class 3 = Damage higher than 40% (total loss of yield).

Results and discussion

The number of males found in the monitoring traps placed in the control plot, was significantly higher than in both plots with mating disruption along the whole trial, as shown in Figure 1, with a total of 1713 captures compared to 1 and 3 in the plots with 400 dispensers/ha and 300 dispensers/ha respectively. Furthermore, an important difference was observed between the captures recorded in the two traps located close to the mating disruption plots and the two situated at a greater distance. An average of 623.5 captures were recorded in the two monitoring traps located further from the two plots in mating disruption and 233 in the traps situated closer, as indicated in Figure 2.

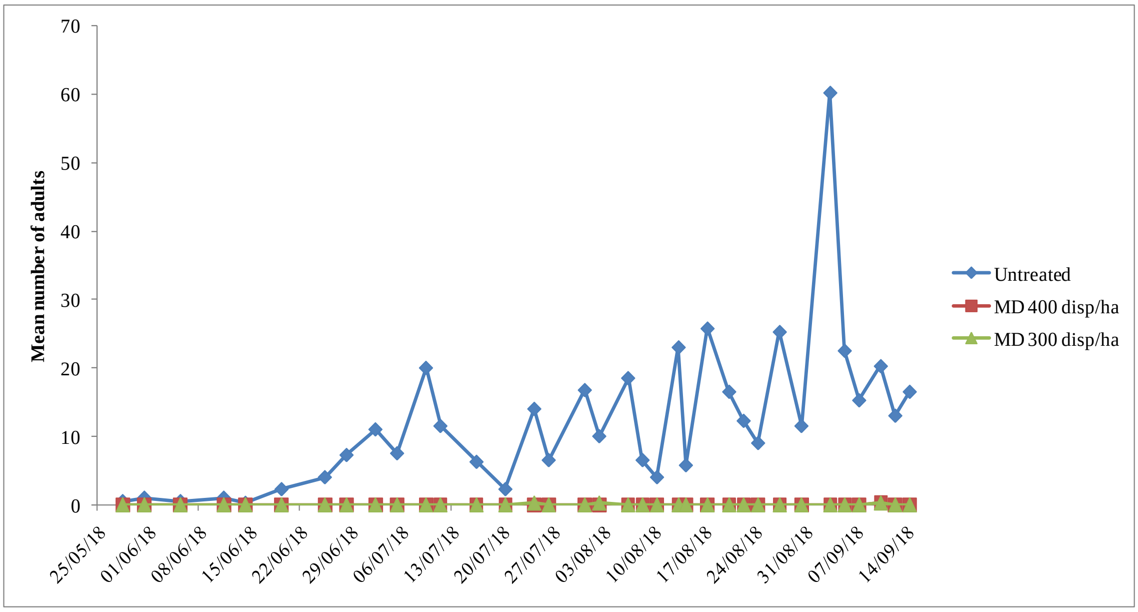

Figure 1. Mean number of *Cryptoblabes gnidiella* males trapped weekly in each plot throughout the trial.

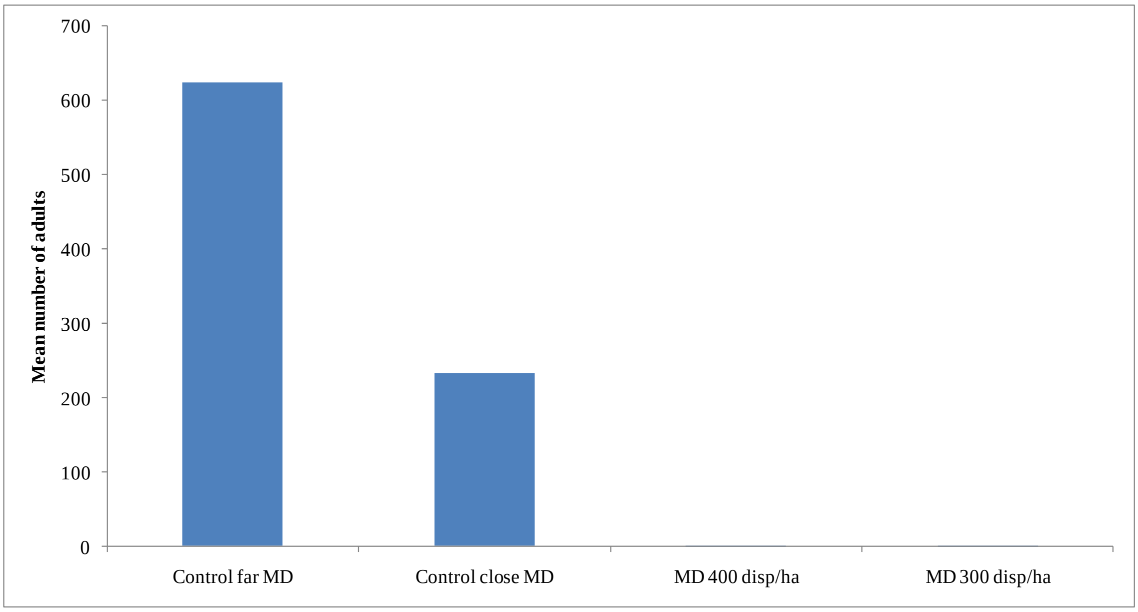

Figure 2. Mean number of *Cryptoblabes gnidiella* males captured in the monitoring traps in each plot.

Regarding the efficacy in terms of damage, significant differences were found between treatments: in the control plot the highest percentage of damages was detected with a total of 17% damaged bunches, in comparison to 4% and 6.5% in mating disruption with 400 and 300 dispensers per hectare respectively, as shown in Figure 3. Considering the severity of damage, there was just significantly differences in bunches of class 1, although in general, the number of damaged bunches in mating disruption was always lower than in the untreated plot. No statistical differences were either observed between both rates of dispensers/ha.

Overall, these results show a good efficacy of mating disruption in the control of *C. gnidiella* in vineyard at the two doses tested.

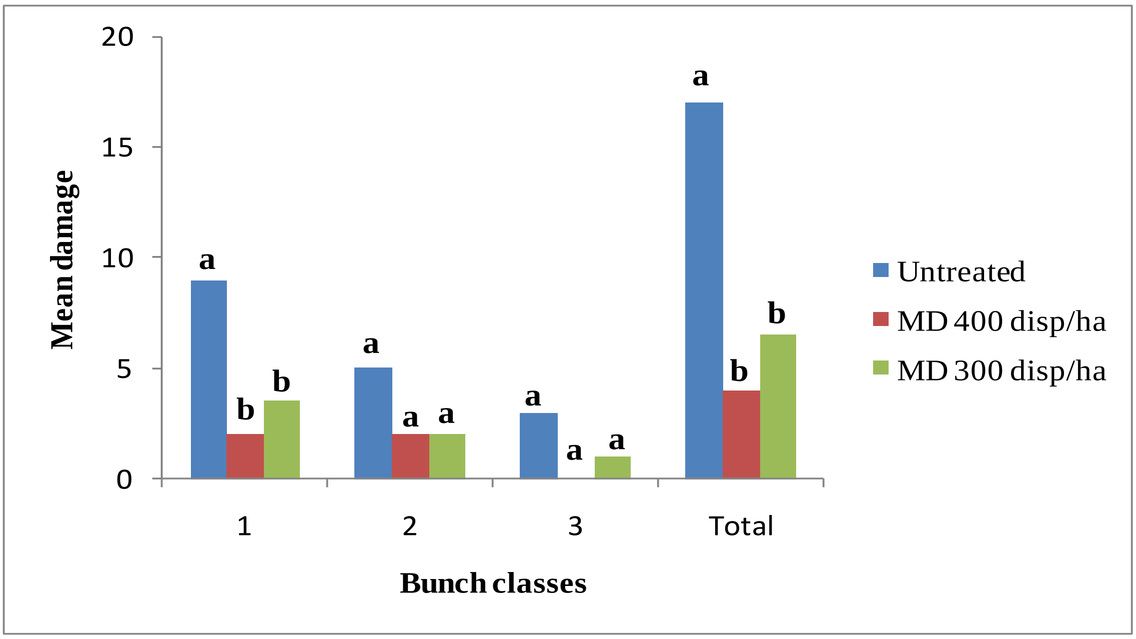

Figure 3. Mean number of damaged bunches for each class. Different letters denote statistical differences between plots (Student-Newman-Keuls test, at 5%).

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The eucalyptus weevil *Gonipterus platensis* (Coleoptera, Curculionidae): new control perspectives based on semiochemicals

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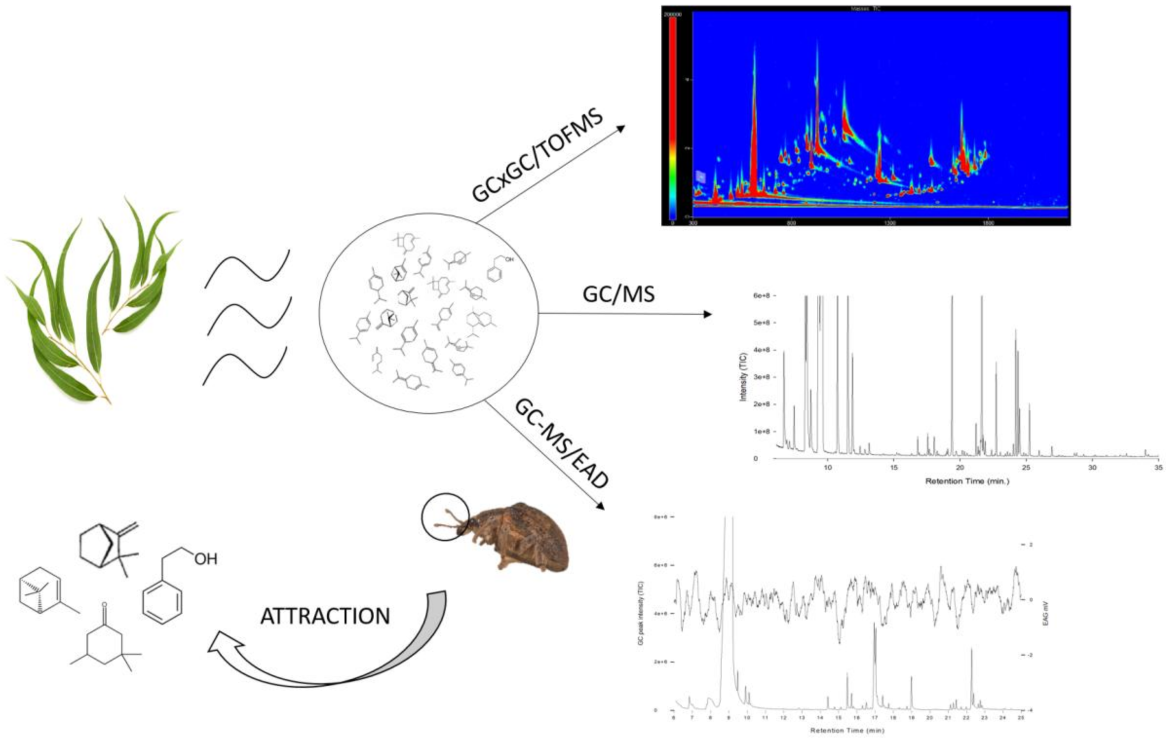
Extended Abstract.: The eucalyptus weevil *Gonipterus platensis*, is a defoliator originating from Oceania, that has spread and invaded large areas where eucalyptus are planted for commercial purposes in Africa, Europe and America (Reis et al., 2012). Attacks of this insect to eucalyptus in several regions often reach values that exceed the economic level of damage, as both larva and adults feed on eucalyptus leaves and young shoots, leading to reduced tree growth and tree mortality. In some climatic regions, the beetle can be controlled by a hymenopteran of the genus *Anaphes*, which is an efficient egg parasitoid (Tooke, 1953). However, in colder and wetter regions, the efficiency of the parasitoid decreases, so that control of the weevil is not achieved (Reis et al., 2012).In Portugal, *G. platensis* reaches pest status in approximately 18.5% of the 812 000 hectares planted with eucalyptus, and has caused a wood loss of about 648.M Euros over the last 20 years (Valente et al., 2018). The use of semiochemicals for integrated pest management (IPM) of *Gonipterus* species is still largely unexplored. In this study we further present a prospect for biotechnological control based on compounds released by its host *Eucalyptus globulus* that might mediate host selection behaviour in this weevil species.

In this study *E. globulus* volatile organic compounds were collected by headspace solid phase microextraction, monolithic material sorption extraction and simultaneous distillation-extraction. Gas chromatography – mass spectrometry/ electroantennographic detection (GC-MS/EAD) was used to determine which compounds triggered antennal responses in *G. platensis*. Further chemical analysis of the extracted compounds was conducted using gas chromatography and mass spectrometry and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *G. platensis* antennal response was elicited by 51 host plant volatiles, and was confirmed for 33 of these compounds using commercial standards. The behavioural response of *G. platensis* to individual compounds was assessed in a two-arm olfactometer with ten parallel walking chambers, coupled to video tracking and data analysis software. The inserts were inserted in the middle of the arm (one in in each individual chamber), where the airflow incoming from both extremities converged. Weevils of known age, sex and mating status were tested. We identified eight compounds with kairomonal activity and two compounds with allomonal property. The behavioural responses were related to the sex and mating condition of the tested insects. Three compounds attracted virgin females or both sexes: camphene, (+)-α-pinene and 2-phenylethanol, and are potential candidates for application in integrated pest management approaches. Furthermore, the two allomones identified may have potential use for developing push-pull strategies (Branco et al., 2018).

In short, sustainable management of *G. platensis* can only be achieved through the adoption of an integrated approach, resorting to an array of techniques: i) Population monitoring based on sampling, coupled with teledetection to assess defoliation rates and inform decision making on treatment applications; ii) Genetic selection of eucalyptus clones showing reduced susceptibility to the weevil; iii) Release/augmentation of biological control agents, in combination with biotechnological strategies; iv) Biotechnological control, based on the use of semiochemicals - both volatile organic compounds (VOCs) released by the host trees as well as pheromones emitted by the weevils, having attractant, deterrent or repellant effects on conspecifics and/or natural enemies. In general, research conducted to develop the proposed tools yielded promising results and is in progress. The strategies envisaged to be implemented in eucalyptus plantations include push–pull techniques, use of trap crops, attraction of natural enemies, weevil repellency and/or oviposition deterrence.

Key words: kairomones, allomones, *Eucalyptus globulus*, *Gonipterus platensis*



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Field effectiveness of pheromone-based mating disruption to control the vine mealybug, *Planococcus ficus* (Hemiptera: Pseudococcidae): results from Italy

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Abstract: In this study, field research aimed at the successful management of VMB was carried out both on table grape and grapevine in various Italian regions. We focused on the potential impact of different amounts of pheromone (respectively 54, 72 and 90 g/ha) per hectare on VMB mating and thus VMB infestation levels. This was achieved by deploying per hectare, respectively, 300, 400 and 500 Isonet® PF reservoir dispensers, each dispenser containing 180 mg of racemic lavandulyl senecioate, and by assessing the effectiveness of the three different rates in reducing VMB infestation levels in comparison to control vineyards. Our findings pointed out that all three tested dispenser rates and thus amounts of pheromone per hectare were effective in reducing the percentage of VMB infested bunches as well as the number of VMB per bunch, with significant differences over the untreated control.

Key words: grapevine; Integrated Pest Management; mating disruption; mealybugs; sex pheromones

Introduction

Pheromone-based mating disruption (MD) has been tested on a wide range of insects of agricultural importance (Miller and Gut, 2015; Ioriatti and Lucchi, 2016). The successful results obtained on a relevant number of pest species boosted its wide-scale employ, with a growing number of commercial products currently available to farmers. Nowadays, the vine mealybug (VMB), *Planococcus ficus* (Hemiptera: Pseudococcidae), is recognized as one of the main insect pests damaging vineyards (Daane et al., 2012). The harmfulness of VMB is due to phloem ingestion, resulting in degeneration of plants, decreased vigour, early defoliation, reduced bunch quality and decreased organoleptic characteristics of the obtained wine. This species produces a large amount of honeydew and wax, which serves as a substrate for sooty molt growing that reduces photosynthetic activity in leaves. Besides, VMB can transmit the grapevine leaf roll-associated virus and increases the risk of ochratoxin A contamination (Douglas and Krüger, 2008; Chiotta et al., 2010; Tsai et al., 2010). A major challenge in vineyard IPM is reducing the amount of broad-spectrum insecticides currently used to manage insect pest populations, with special reference to moths, such as *Lobesia botrana*, and mealybugs (Lucchi and Benelli, 2018).

The chemical ecology of *P. ficus* has been extensively studied, with the identification of its sex pheromone by Hinkens et al. (2001), followed by the description of the site of pheromone emission (Millar et al., 2002). The effective employment of the pheromone main component, lavandulyl senecioate, for MD purposes was reported by Walton et al. (2006), as well as by Cocco et al. (2014). In this scenario, little is still known about the optimization of MD programs against *P. ficus*.

In the present work, field research aimed at the successful management of VMB was recently carried out both on table grape and grapevine in various Italian regions. We focused on the potential impact of different amounts of pheromone (respectively 54, 72 and 90 g/ha) per hectare on VMB mating and thus VMB infestation levels. This was achieved by deploying per hectare respectively 300, 400 and 500 Isonet® PF reservoir dispensers, each dispenser containing 180 mg of racemic lavandulyl senecioate, and by assessing the effectiveness of the three different rates in reducing VMB infestation levels in comparison to control vineyards.

Material and methods

Study site and experimental design

Trials were conducted in 2016 and 2017 on different wine and table grape varieties. In 2016, three trials were carried out in Acate, Ragusa province, Sicily region, Southern Italy. Two trials were performed on early-ripening wine grape varieties, one on Chardonnay and one on Pinot Grigio (expected harvest time: beginning of August), and another trial was carried out on the later-ripening wine grape variety Nero d'Avola (expected harvest time: end of August – beginning of September). In 2017, a total of four trials were conducted. Again, three trials were performed in the province of Ragusa (Sicily region, Southern Italy): one on the early-ripening wine grape variety Chardonnay, one on the later-ripening wine grape variety Nero d'Avola, and one on the table grape variety Italia. A fourth trial was carried out in Colognola ai Colli, Verona province (Veneto region, Northern Italy) on the wine grape variety Garganega. Details on vineyard location and pest history are detailed in Lucchi et al. (2019). The study sites 1 and 4 are the same vineyard block tested over different years; this also applies to sites 3 and 5. A detailed description of each cultivar has been recently provided in Lucchi et al. (2019).

Reservoir dispensers (Isonet® PF, Shin-Etsu Chemical Co. Ltd, Japan) were tested at three different application rates: 300, 400 and 500 dispensers/ha. Isonet® PF consists of two parallel polyethylene tubes, one of which contains an aluminium wire that enables their placement on supports, and the other is filled with 180 mg of racemic lavandulyl senecioate TGAI. The VMB racemic pheromone amount per hectare tested in our MD trials was 54, 72, and 90 g, corresponding to a dispenser density of 300, 400 and 500 Isonet® PF dispensers/ha, respectively.

A randomized block design is not applicable to the large plots required for studies on MD products (European and Mediterranean Plant Protection Organization, 2016) so, each treatment (i.e. Isonet® PF at 300, 400 and 500 dispensers/ha, respectively, and untreated control) was applied to one large plot. There were six sampling units per plot which were distributed in a grid pattern. Each plot was sufficiently large for there to be at least 100 bunches per assessments. Isonet® PF-treated plots were at least 2 ha in size (range: 2.01-4.75 ha). Untreated control plots were not as large (range: 0.12-0.64 ha), but had a comparable pest history and allowed for assessments on the same number of bunches per sampling unit. In all Isonet® PF-treated plots, the dispensers were deployed before the beginning of the migration of VMB nymphs in spring (mid-April in Sicily, end of April in Veneto) (Lucchi et al., 2019).

Male captures and infestation evaluation

Following the method by Walton et al. (2006), *P. ficus* males were monitored during both 2016 and 2017 using red Pherocon Delta IIID sticky traps (Trécé Inc., Adair, OK). Traps were baited with 100 µg of racemic synthetic lavandulyl senecioate from Pherobank B.V. (Wijk bij Duurstede, The Netherlands) in rubber septa lures (Suterra, Inc.). One trap was used per large plot. Trap sticky plates and sex pheromone lures were replaced monthly, and trapped males were counted by direct observation using a stereoscopic microscope (Leica, Germany). Data were analysed as the total number of males caught before harvest.

The MD efficacy of Isonet® PF was evaluated on 100 bunches per sampling unit by determining the percentage of bunches infested by *P. ficus* and the number of VMB/bunch at harvest (BBCH 89). Within each of six sampling units, the number of bunches infested by VMB was counted on 100 randomly selected bunches, giving a total of 600 bunches per plot. Then, the percentage of *P. ficus*-infested bunches was calculated. In addition, the number of VMB per bunch was noted to provide an indication of the damage severity.

Statistical analysis

Mealybug populations in vineyards are typically clumped in their spatial distribution (Geiger and Daane, 2001); in our study the incidence of *P. ficus* infested bunches, and number of VMB per bunch, were not normally distributed. Data transformation, including $ln\ (x + 1)$, was not able to normalize the distribution and homogenize the variance (Shapiro-Wilk test, goodness of fit $P < 0.001$). Thus, non-parametric statistics were used. Differences in male catches, incidence of bunches infested by *P. ficus*, and number of VMB per bunch among treatments (i.e. the tested sex pheromone dispenser Isonet® PF at three different densities per hectare and the untreated control), years, and study site were assessed using Kruskal-Wallis test followed by Steel-Dwass multiple comparison; A *P*-value of 0.05 was selected as threshold to assess significant differences.

Results and discussion

Results pointed out that all three tested dispenser rates and thus amounts of pheromone per hectare were effective in reducing the VMB populations, with significant differences over the untreated control. In detail, our findings show the successful use of MD dispenser Isonet® PF, which led to a significant reduction of VMB infested bunches (%) and number of VMB per bunch, compared with the untreated vineyards, in both wine and table grape varieties. On the other hand, the strong variation in the abundance of male catches between 2016 and 2017 highlighted the poor reliability of male trapping for evaluating MD efficacy compared to VMB direct sampling on grapes. Our data confirm earlier studies highlighting that MD can represent a reliable control method to significantly reduce *P. ficus* populations, particularly after one season characterized by low population density (Walton et al., 2006; Cocco et al., 2014; Mansour et al., 2017). Besides, herein it was observed that there was a lower *P. ficus* infestation in the table grape variety Italia over the other varieties. This may be linked to the differences in historic pesticide strategies in the selected vineyards. Indeed, it is widely known that table grape vineyards have a lower tolerance threshold, thus they are more subjected to insecticide treatments (Cabras and Angioni, 2000; Ravelo-Pérez et al., 2009).

Overall, the results presented here outline a successful MD approach that should be taken into consideration within an IPM program to effectively manage VMB populations. Indeed, if it cannot be used alone as a fully efficient sustainable control option, especially in the case of high mealybug infestations, the use of MD in combination with an effective and safer insecticide, could represent the appropriate way to ensure long-term field efficacy to control vine mealybugs in vineyards (Mansour et al., 2018). Contrary to earlier studies, this research provides evidence for successful MD programs for *P. ficus* control using low pheromone doses per hectare. This can help farmers to reduce costs in terms of both material to be purchased and labour for field application, as well as to adopt area-wide sustainable VMB control strategies for vineyards (Daane et al., 2018).

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The potential of pheromones for controlling *Pseudococcus calceolariae* (Hemiptera: Pseudococcidae) in fruit crops

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Abstract: Mealybugs (Hemiptera: Pseudococcidae) are economically important pests of many fruit crops and the pheromones of several species have been identified. For testing its potential use in the control of *Pseudococcus calceolariae* in fruit crops in Chile, we carried out several field experiments, including the approaches of mating disruption, mass trapping and attract and kill. In mating disruption trials male captures before treatments were similar for control and mating disruption plots, while after mating disruption treatments captures differed significantly. Nevertheless, significant differences in visual counts were not found and populations were very low in all plots. In the mass trapping trial, plots with pheromones had captures between 36 and 40 times that of plots without pheromones, and sticky panels catches were between 13 and 30 times. No significant differences for visual counts were detected. The attract and kill experiment is underway. Pheromones show a great potential as a direct control tactic for *P. calceolariae* in fruit crops.

Key words: mealybugs, mating disruption, mass trapping, attract and kill

Introduction

Mealybugs (Hemiptera: Pseudococcidae) are economically important pests of many fruit crops in several regions of the world, mainly due to their quarantine status for particular markets. The citrophilus mealybug, *Pseudococcus calceolariae* (Maskell), is a pest thought to be native from Australia and now present in over 30 countries covering all continents (Williams, 1985; Daane et al., 2012; García Morales et al., 2016). It is a polyphagous insect present in many plant families (García Morales et al., 2016), and responsible for economic losses in fruit crops such as citrus, avocados, grapes, apples, and berries (Ripa and Larral, 2008; Charles et al., 2010; Daane et al., 2012; Dreistadt, 2012).

The first sex pheromones of mealybug species were identified in the early 1980', but it wasn't until the early 2000's that many new species were added, including that of *P. calceolariae* in 2010 (El-Sayed et al., 2010; Unelius et al., 2011). Recently, we have explored the use of *P. calceolariae* pheromone for its monitoring in fruit crops (Flores et al., 2015). The objective of this work was to evaluate *P. calceolariae* pheromone as a direct control measure in fruit crops through the approaches of mating disruption, mass trapping and attract and kill.

Material and methods

Mating disruption

We have carried out experiments for three seasons in apple orchards and two seasons in citrus orchards. In apples, the first season a low and a high infestation orchards were used. In each orchard six 0.1 ha plots were set: three control (without pheromone) and 3 mating disruption plots. In the second and third season the high infestation apple orchard and a citrus orchard were used. In these two seasons, ten 0.1 ha plots were set in each orchard, with half corresponding to the control treatment and half to the mating disruption treatment. The mating disruption treatment corresponded to 6.2 g of pheromone in a SPLAT formulation, uniformly distributed in the plots. In season one, the mating disruption treatment was set on February, in season two in December and in season three in November. Populations were monitored by pheromones traps and visual inspections to plants, from spring to fall (September to April). Comparisons between control and mating disruptions treatments before and after setting the pheromones in the field were made.

Mass trapping

For this experiment an apple and a blueberry orchard were selected. Eight 10 × 10m plots were established in each orchard and in the 4 corners of each plot a trap and a sticky transparent panel (10 × 8 cm aprox.) hanging from it were set. In half of the plots a pheromone dispenser was added and in the other half a dispenser without pheromone. Traps and panels were replaced monthly (October to April) and brought to the laboratory to count males. Populations in the plants at the centre of the plot were monitored visually. Season long male catches and populations were compared between the control and mass trapping treatment.

Attract and kill

For this experiment three orchards were selected, one table grape, one citrus and one blueberry, and a combined formulation was prepared using SPLAT. The lure contained the pheromones of *P. calceolariae*, *P. viburni* and *P. longispinus*. Treatments were (1) mix of pheromones plus insecticide, (2) mix of pheromones without insecticide and (3) control (SPLAT alone, no pheromones and no insecticides), with three replicates each. The treatment was set on November. Populations are being monitored by pheromones traps and visually from September to the end of the season. Comparisons between the three treatments before and after setting the pheromones in the field will be compared.

Results and discussion

Results of mating disruption trials showed that there was a significant effect of the pheromone treatment in male captures in all orchards and field seasons. Captures before treatments were similar between control and mating disruption plots, while after male captures declined significantly ("trap shutdown") indicating and important disruption in male orientation (Figure 1). Nevertheless, significant differences in visual counts were not found, in part due to the low popultions present in all plots.

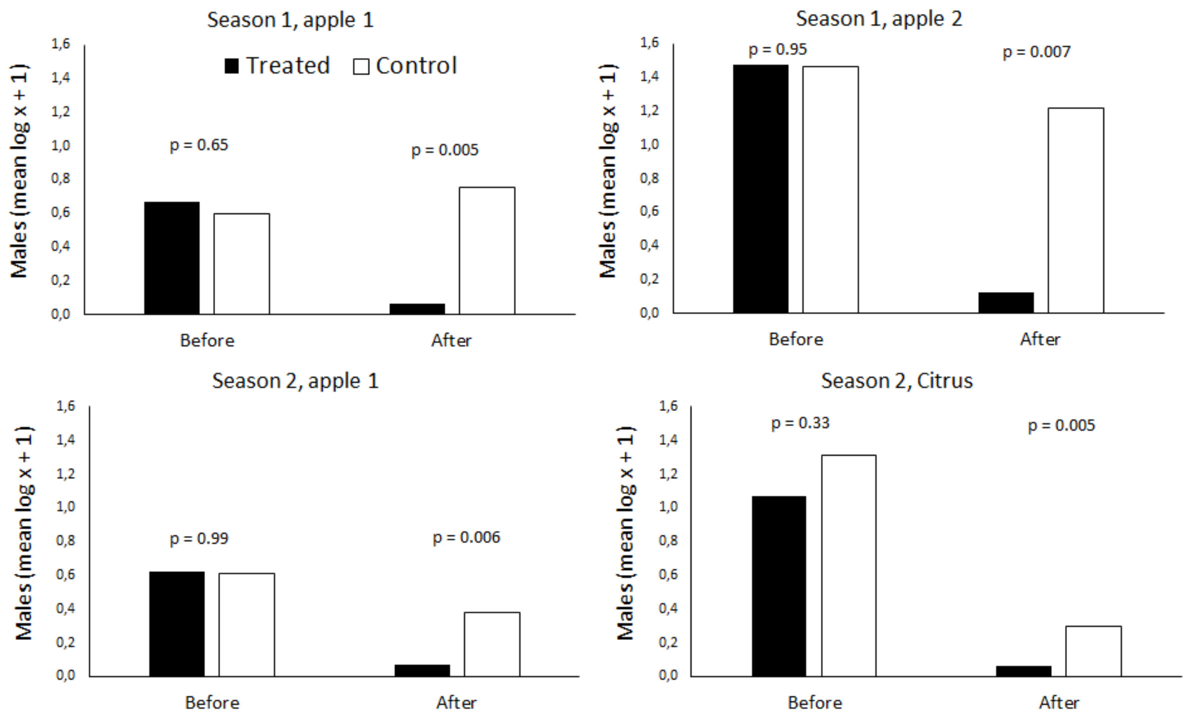

Figure 1. Males captures in pheromone traps in mating disruption plots (Treated) and control plots (Control), before and after applying the pheromones. Different panels represent different orchards and or seasons.

In the case of mass trapping, in plots with pheromones trap captures were between 36 and 40 times that of plots without pheromones, and for the sticky panels it was between 13 and 30 times. Here no significant diffrences for visual counts were detected. The attract and kill experiment captures before treatments were deployed were high, particularly in blueberries (1.6 males per day) and citrus (1.8 males per day). After treatments were deployed, male captures decreased, inlcuding the controls (overall mean 0.1 males per day), and no differences among treatments were found in any of the fruit crops studied. This lower captures probably reflect the species biology, and studies in the future should be carried out earlier in the season.

This study shows the potential use of pheromones as a direct control tactic for *P. calceolariae* in fruit crops. Multiyear trials are necessary to fully evaluate its effects. Its final application as a control measure will depend on an economic assessment of the costs, along with the benefit of lower pesticide residues in the product.

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Incorporating mating disruption into IPM programs for Navel Orangeworm in California almonds

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Extended Abstract: Navel orangeworm (NOW), *Amyelois transitella* (Walker), is the most significant insect pest of more than 525,000 ha of almonds in California. Damage includes direct feeding on kernels and can predispose almonds to infection by *Aspergillus* sp. fungi which produce aflatoxins. Management of NOW requires a combination of integrated pest management (IPM) techniques to keep damage at levels that are commercially acceptable and allow for maximum economic returns to the grower.

For more than a decade IPM programs focusing on sanitation and insecticides timed according to degree-day models did a good job keeping NOW at levels that are generally acceptable industrywide. However, over the past few years the tolerance for damage has decreased due to increased crop values, decreased tolerances for aflatoxins in some export markets, and regional changes in the agricultural ecosystem. Ten years ago California had approximately 300,000 ha of almond orchards that were typically surrounded by non-hosts for NOW, such as grapes or annual vegetable and field crops. Today, almonds are produced on over 525,000 ha, which when added to another 250,000 ha of pistachios and walnuts, means that many orchards are surrounded by other NOW hosts, increasing the need for coordinated areawide efforts against this pest.

Mating disruption is a relatively new technique that provides an opportunity to improve NOW management within IPM programs. It is considered to be a low-risk, sustainable strategy, that can be used in complement with existing programs based on sanitation and traditional insecticides. Currently in California there are four different manufacturers with products that are commercially available to growers. These include two static-release dispenser systems using aerosol-based canisters from Suterra and Pacific Biocontrol, one variable-rate system using aerosol-based canisters where dispensers can be turned on and off remotely from Semios, and a static-release system using passive dispensers from Trécé.

During 2017 we evaluated these four MD systems on 16-ha plots compared to an untreated check. These trials were funded by almond growers through the Almond Board of California. The MD systems were manufactured by four different companies and all released approximately the same amount of pheromone over the course of the season. The systems utilized active or passive dispersion of pheromone, different dispenser densities, and either static or variable rates of pheromone release. All systems were installed around April 1st onto 16-ha plots in three different orchards in Kern County, California. Each orchard had good winter sanitation and was sprayed once or twice with insecticides at hull split. In other words, we evaluated mating disruption as an added component to an existing IPM program, not as a replacement for sanitation or chemical control.

All four MD systems caused greater than 90% reductions in the capture of males in pheromone traps from April through September. Egg captures during the same time period were reduced by 22% where MD was used. Across all orchards the average percentage of NOW-infested kernels was 2.28% for the no-MD checks compared to 1.13 to 1.33% for the four MD products. When all four MD products are averaged, MD reduced NOW damage in the varieties Nonpareil by 35%, Monterey by 51% and Fritz by 55%, with an overall reduction of 46%.

Economic analyses were performed by calculating crop value using assumed per-ha yields of 1,704 kg of nonpareil at $ 5.50/kg and 1,704 kg of pollinizers at $ 4.95/kg base price, plus the addition of quality premiums according Blue Diamond's 2017 Crop Quality Schedule. Where mating disruption was used, grower returns increased by an average of $ 246 per ha. This was comparable to the cost of implementing mating disruption. In other words, adding MD to existing management programs on a 16-ha scale pays for itself. In the process, levels of NOW damage for nuts arriving at the huller were cut in half and risks of aflatoxin were reduced.

During 2017 we also conducted mating disruption demonstration trials to promote sustainable and environmentally-friendly pest management practices that were funded by the California Department of Pesticide Regulation. Six side-by-side comparison orchards were established in the Central Valley (Wasco, Maricopa, Lost Hills, Turlock, Escalon and Ballico). Each trial compared approximately 24 to 40 ha of conventionally-grown almonds to adjacent orchards of similar size where MD was used. Four of the orchards were rectangular, whereas the other two were used to see if MD would work in orchards oriented as triangles.

Across all sites, MD reduced the number of male NOW caught in pheromone traps by 93% from April through September. The average percentage of NOW damage was 2.59% in conventional orchards compared to 1.14% where MD was included. This is a reduction of 55.7%. When only the four rectangular orchards are evaluated, moth captures were reduced by 96% and the average reduction in damage improved to 76.4%. When only considering the two triangular orchards, MD helped reduce male captures, but did not result in a reduction in damage at harvest.

Evaluation of the economic benefits of MD showed that grower returns increased by an average of $ 268 per ha across all sites, and by $ 488 per ha in the four rectangular orchards. Either way you look at it, adding MD paid for itself. Additionally, at one location with relatively low NOW pressure (Wasco), the grower successfully omitted two insecticide sprays in the MD orchard and still had less damage than where the sprays were used. These economic benefits on approximate 40-ha orchards are even larger than the benefits shown previously on 16-ha plots, confirming a long-known fact that the efficacy of MD systems improves as the contiguous acreage under MD increases.

All of our field work in 2017 suggests that incorporating MD for navel orangeworm in almonds in addition to an existing IPM program pays for itself when implemented on at least 16 acres, and generates a positive return on investment when implemented on at least 40 contiguous acres, all while reducing NOW damage and risk of aflatoxins. This statement assumes that the orchard is square to rectangular in shape. If the orchard has a high ratio of edges to middle (such as a triangle or a really long rectangle), growers who want to use MD should pair up with neighboring growers to make larger, more contiguous areas under MD to improve efficacy.

Mating disruption is compatible with, and should be used in conjunction with (not instead of) other historically-proven management techniques: namely winter sanitation, insecticide sprays, and early harvest. Mating disruption is recognized as a sustainable, environmentally-friendly tool for controlling pests that goes hand in hand with industry-led efforts to produce food products for health-conscious consumers around the world.

Key words: integrated pest management, IPM, navel orangeworm, NOW, mating disruption

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Control of oriental fruit moth, *Cydia molesta* Busck and peach twig borer *Anarsia lineatella* Zell. using reduced rate of pheromone dispensers

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Abstract: The main pest on peach in Bulgaria is the oriental fruit moth (OFM), *Cydia molesta* (Busck) (Lepidoptera: Tortricidae). The trials were carried out in the years 2016-2018 in an isolated peach experimental orchard of 1 ha in the Fruit Growing Institute, Plovdiv – Central South Bulgaria. Mating disruption (MD) was tested as an alternative method for controlling OFM and peach twig borer (PTB) *Anarsia lineatella* (Zell.) (Lepidoptera: Gelechiidae) from post-bloom till harvest. The aim of this study was to test the effectiveness of MD in control of OFM and PTB in peach orchards, using CIDETRAK® OFM/PTB MESO dispensers of Trécé Inc., USA, at a rate of 20 dispensers per ha. Monitoring of OFM and PTB flights was carried out using sex pheromone trapping in the years of the study. PHEROCON® VI Delta sticky traps were installed in the trial orchard using a scheme provided by the producer. The damage to shoots was evaluated during the first generation of OFM and PTB on 20 trees, randomly selected within the central area of each block. Correspondingly, fruit damage was recorded on 100 fruits per each selected tree; so, 2000 fruits were inspected for damage from both pests in each block. The level of damaged fruits in the trial plots was compared with that in a reference orchard, located in the vicinity, treated with conventional pesticides. The fruit damage by OFM, in the reference orchard, ranges from 2.9 to 3.7% and by PTB from 1.2 to 1.4% in the successive years. Percentage of damage in the experimental orchard treated with CIDETRAK® OFM/PTB MESO™ was 0.1% by OFM and 0.1% by PTB, which was rather below the economic threshold. The significance of differences in the damage level between the trial and reference orchards was estimated by use of Chi-square tests. CIDETRAK® OFM/PTB MESO™ is effective when used at a dosage of 20 dispensers per ha, applied once during the season, before the first onset of OFM and PTB. The use of CIDETRAK® OFM/PTB MESO™ shows that the number of dispensers used does not affect the effectiveness of mating disruption and the reduced rate of dispensers used will help the growers to decrease labor in the field. Applications of these dispensers can provide an effective control of oriental fruit moth and peach twig borer, with better results than the conventional protection programs employed currently in Bulgaria.

Key words: oriental fruit moth, peach twig borer, *Cydia molesta*, *Anarsia lineatella*, mating disruption, pheromone dispensers, CIDETRAK® OFM/PTB MESO™

Introduction

Chemical pest control in peach and nectarine fruit orchards of Bulgaria has consistented of a broad spectrum of organophosphate and pyrethroid insecticides. Recently their effectiveness has decreased, apparently due to the development of resistance in the pest. However, this is not well documented. Mating disruption technology has been successfully used for control of oriental fruit moth, as reported by Kovanci (2003), Molinari (2007) Lo and Cole (2007) and Kutinkova et al. (2016).

Peach is one of the major fruit crops in Bulgaria. Its main pest is the oriental fruit moth (OFM), *Cydia molesta* (Busck) (Lepidoptera: Tortricidae). The larvae of early OFM generations damage current season shoot tips; then they feed on the developing fruitlets and fruits. Peach twig borer (PTB) *Anarsia lineatella* (Zell.) (Lepidoptera: Gelechiidae) is an Eurasian species that has spread extensively worldwide; its larvae can develop on many plant species from the genera *Cydonia*, *Malus*, *Prunus* and *Pyrus*. In Bulgaria, the main hosts are *Prunus persica* L. (peach) and *Prunus armeniaca* L. (apricot).

The aim of this study was to test the effectiveness of mating disruption method for control of OFM and PTB in peach orchards using CIDETRAK® OFM/PTB MESO™ dispensers developed and manufactured by Trécé Inc., USA. The dispensers are designed to deliver long-lasting performance for the whole season, with remarkably fast application.

Material and methods

Trial orchard

The trials were carried out in the years 2016-2018 in an isolated peach experimental orchard of 1 ha in the Fruit Growing Institute, Plovdiv – Central South Bulgaria. Mating disruption (MD) was tested as an alternative method to the chemical treatments for controlling OFM and PTB from post-bloom till harvest.

CIDETRAK® OFM/PTB MESO™ dispensers were applied at a dosage of 20 dispensers per ha. Only one or two aphicide treatments were applied to the trial area during the years of the study.

Reference orchard

Another 2 ha site served as a reference orchard and it was treated in a conventional way. Five to eight chemical treatments are typically applied during each season to a conventionally treated reference orchard for control of OFM, PTB and other pests. Four to seven of them are usually applied for control of OFM and PTB.

Indices studied

Monitoring of OFM and PTB flights was carried out using sex pheromone trapping in the years of the study. Six PHEROCON® VI Delta sticky traps were installed in the trial orchard using a scheme provided by the producer. The traps were baited with standard OFM L2 – Orfamone and PTB L2 – Anemone and changed every 8 weeks. We used PHEROCON® OFM COMBO A&B DUAL lures, a new product developed by Trécé Inc., USA for the orchards with MD in the trial orchard. The lures were changed every 8 weeks. The traps were installed before OFM and PTB flights started. For comparison, PHEROCON® VI Delta sticky traps and PHEROCON® OFM COMBO A&B DUAL lures were installed in a reference orchard. All pheromone traps were checked twice per week.

Elaboration of data
Damage to shoots was evaluated during the first generation of OFM and PTB on 20 randomly selected trees, within the central area of each orchard. Correspondingly, fruit damage was recorded on 100 fruits per each selected tree. Therefore, 2000 fruits were inspected for damage from both pests in each orchard. The rate of damaged fruits in the trial plots were compared with that in the reference orchard, treated with conventional pesticides only, using the same evaluation techniques. Data on the capture of male moths in the pheromone traps were considered as totals for each date of control and presented in a graphical form. The rate of fruit damage by OFM was expressed as a percentage of damaged fruits. Significance of differences in the damage rate between the trial and reference orchard was estimated by use of Chi-square test.

Results

OFM flight dynamics
The flight of OFM in the reference orchard in 2016-2018 began in the last week of March and second week of April and finished in the second or third week of October (Figure 1). The pest developed 3 generations in 2016 and 2017 and partial 4-th generations in 2018.

The traps installed in the reference orchard caught in total 804 moths in 2016, 508 in 2017 and 1031 in 2018. We used the new PHEROCON® OFM COMBO A&B DUAL lures in 2018, a new product developed by Trécé Inc., USA for the orchards with MD in our trial orchard (Figure 3).

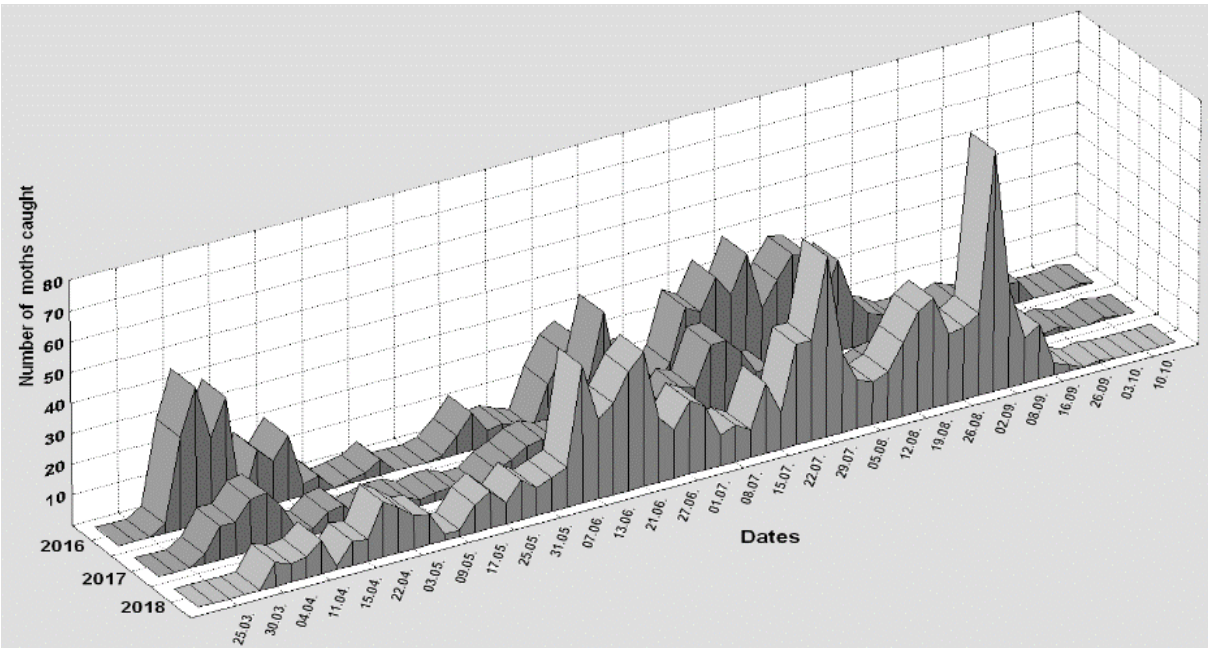

Figure 1. Flight dynamics of OFM in the reference orchard in successive years of study 2016-2018.

PTB flight dynamics

The flight of PTB in the reference orchard, in 2016 -2018 began in the third week of April or in the first week of May and finished from the second week of October untill the last week of October (Figure 2). The traps installed in the reference orchard caught in total 359 moths in 2016, 199 in 2017 and 577 in 2018. The population density of PTB increased season to season. PTB developed 3 generation in all the years of the study.

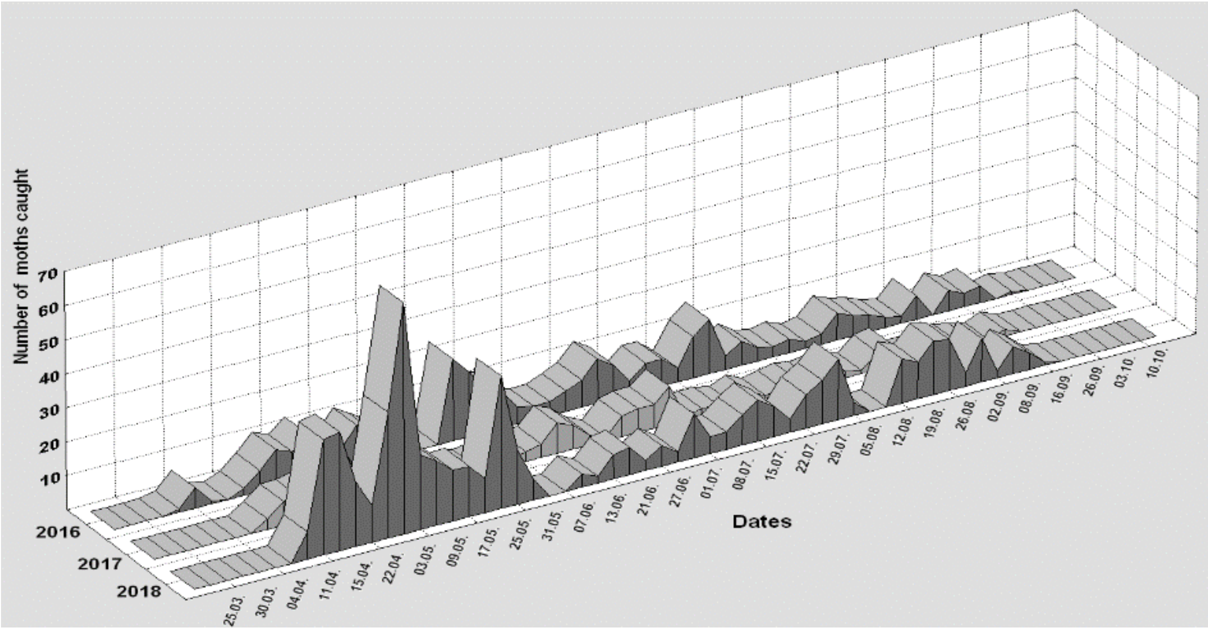

Figure 2. Flight dynamics of PTB in the reference orchard in successive years of study 2016-2018.

***OFM flight dynamics in the trial orchard with new* PHEROCON® OFM COMBO A&B DUAL lures**

We used PHEROCON® OFM COMBO A&B DUAL lures in our trial orchard in 2018, a new product developed by Trécé Inc., USA for orchards with MD. (Figure 3). Monitoring of OFM flight under MD is not possible using the classical sex pheromone baited traps. The new kairomone based lures have been developed to be used under MD. Such tools, have been commercialized and available for *Cydia pomonella* (L.) for several years, but have only recently been commercialized for *C. molesta*. Our results showed that the new lures of Trécé Inc., USA, PHEROCON® OFM COMBO A&B DUAL is significantly more effective than a standard sex pheromone lure to monitor OFM flight under MD, and is very effective in catching female moths.

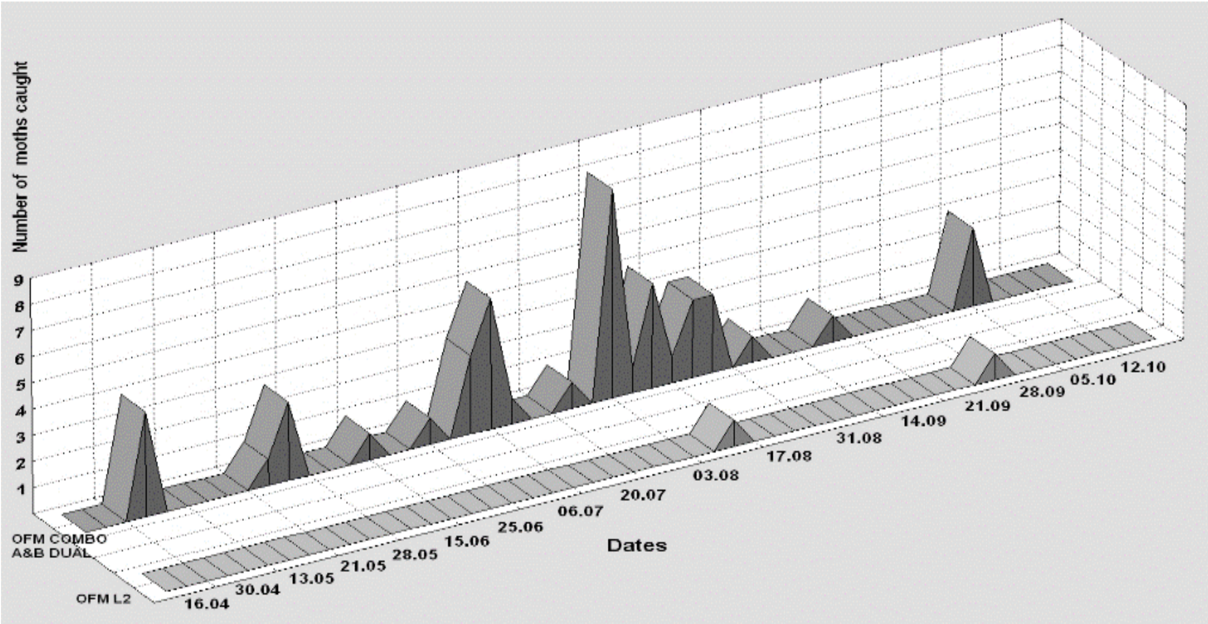

Figure 3. Flight dynamics of OFM in the trial orchard in 2018, where a new lures PHEROCON® OFM COMBO A&B DUAL, were used.

Evaluation of shoot and fruit damage by OFM and PTB during the season

In 2016-2018 in the trial plot, where the CIDETRAK® OFM/PTB MESO™ dispensers was applied, no damages of shoots by OFM were noted (Table 1). Damage rates of shoots were significantly different between the treated plot and the reference (Chi-square test, $p < 0.01$). The fruit damage rate in the trial plot at harvest amounted from 0.0 to 0.1% in all years of the study. Fruit damage in the reference orchard progressed from 2.0% on July 25, 26 up to 2.9-3.7% at harvest of the later ripening cultivars. Damage rates were significantly different between the treated plot and the reference orchard already on July 25, 26 (Chi-square test, $p < 0.01$), and thereafter until harvest (Chi-square tests, $p < 0.001$)

No damages of shoots by PTB in 2016-2018 were noted in the trail plot (Table 2). Damage rates of shoots were significantly different between the treated plot and the reference orchard on June 8, 13 (Chi-square test, $p < 0.01$). The fruit damage by PTB in the trial amounted from 0.0 to 0.1% in all years of the study. Fruit damage in the reference orchard progressed from 0.4-0.5% on July 25, 26 up to 1.2-1.4% at harvest of the later ripening cultivars.

Damage rates were significantly different between the treated plot and the reference orchard already on July 18, 20 (Chi-square test, $p < 0.01$), and thereafter until harvest (Chi-square tests, $p < 0.001$).

Table 1. Evaluation of shoot and fruit damage (%) by *Cydia molesta* in the trial plot and in the conventionally treated orchard in 2016-2018.

Index	Date 2016	Damage (%)		Date 2017	Damage (%)		Date 2018	Damage (%)	
		trial	reference		trial	reference		trial	reference
shoot damage	May 6	0.0	2.6	May 4	0.0	2.1	May 7	0.0	2.3
	May 16	0.0	18.2	May 17	0.0	11.3	May 18	0.0	11.7
	June 13	0.0	19.7	June 8	0.0	16.9	June 11	0.0	17.2
	June 20	0.0	20.3	June 16	0.0	20.1	June 18	0.0	20.2
fruit damage (%)	July 4	0.0	1,0	July 5	0.0	1.1	July 6	0.0	1.3
	July 18	0.0	2.2	July 19	0.0	1.7	July 20	0.0	1.8
	July 26	0.0	3.0	July 25	0.0	2.0	July 26	0.0	2.1
	August 3	0.0	3.1	August 3	0.0	2.1	August 2	0.0	2.2
	August 10	0.0	3.5	August 9	0.0	2.4	August 10	0.0	2.5
	August 22	0.0	3.7	August 23	0.0	2.7	August 24	0.1	2.6
	September 1	0.1	3.7	September 1	0.1	2.9	September 3	0.1	3.1
	at harvest	**0.0-0.1**	**1.0-3.7**	**at harvest**	**0.0-0.1**	**1.1-2.9**	**at harvest**	**0.0-0.1**	**1.3-3.1**

Table 2. Evaluation of shoot and fruit damage (%) by *Anarsia lineatella Zell.* in the trial plot and in the conventionally treated orchard in 2016-2018.

Index	Date 2016	Damage (%)		Date 2017	Damage (%)		Date 2018	Damage (%)	
		trial	reference		trial	reference		trial	reference
shoot damage	May 6	0.0	0.1	May 4	0.0	0.1	May 7	0.0	0.2
	May 16	0.0	0.6	May 17	0.0	0.3	May 18	0.0	0.3
	June 13	0.0	1,1	June 8	0.0	1.9	June 11	0.0	2.0
	June 20	0.0	2.1	June 16	0.0	2.1	June 18	0.0	2.2
fruit damage (%)	July 4	0.0	0.0	July 5	0.0	0.0	July 6	0.0	0.0
	July 18	0.0	0.4	July 19	0.0	0.3	July 20	0.0	0.4
	July 26	0.0	0.5	July 25	0.0	0.4	July 26	0.0	0.5
	August 3	0.0	0.6	August 3	0.0	0.6	August 2	0.0	0.7
	August 10	0.0	0.6	August 9	0.0	0.7	August 10	0.0	0.8
	August 22	0.0	0.9	August 25	0.1	0.8	August- 24	0.1	0.8
	September 3	0.1	1.3	September 1	0.1	1.2	September 3	0.1	1.4
	at harvest	**0.0- 0.1**	**0.0-1.3**	**at harvest**	**0.0-0.1**	**0.0-1.2**	**at harvest**	**0.0-0.1**	**0.0-1.4**

Discussion

Currently, insecticides is the primary method of *C. molesta* control in many countries. Unfortunately, this pest has developed resistance to many of the most useful insecticides, indicating the urgent need for alternative control methods. Resistance of OFM to organophosphate, pyrethroid and carbamate insecticides was detected in Canada (Kanga et al., 1999) and was considered as main cause of failure of conventional plant protection. Apparently, a similar situation may occur in Bulgarian peach orchards. Commercialization and adoption of PTB-MD for *A. lineatella* has been more recent (Pickel et al., 2002; Molinari et al., 2008).

CIDETRAK® OFM/PTB MESO™ dispensers are effective when used at a dosage of 20 dispensers per ha, applied once during the season, before the first onset of OFM and PTB. The use of CIDETRAK® OFM/PTB MESO shows that reducing to 20 dispensers per ha did not affect the effectiveness of mating disruption in comparison with previous works in which we used 80 dispensers per ha (data not shown). The reduced rate of dispensers will help the growers to decrease labor in the field. The new PHEROCON® OFM COMBO A&B DUAL lures of Trécé Inc., USA, is significantly more effective than a standard sex pheromone lures to monitor *C. molesta* flight under MD. This is in line with the reports from Italy in 2019. The availability of new tools such as OFM Combo Dual® lures for both *C. molesta* males and females can improve monitoring of *C. molesta* under MD and help to bring this pest under better control (Preti et al., 2019). The studies are being continued.

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Trials on pheromone mating disruption for reducing population of the Western Corn Rootworm, *Diabrotica virgifera virgifera*, (Coleoptera: Chrysomelidae) in Slovenia

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Abstract: Preliminary results for reducing populations of the Western corn rootworm beetle, *Diabrotoca virgifera virgifera* (Dvv), with sex pheromone in maize fields in Slovenia are reported. Research activities were carried out in years 2015-2018 and were focused on monitoring and mating disruption of adult Dvv. Spermatophores dissected from the females and maize roots injury were checked. Sex pheromone was sprayed with a standard field tractor and a classical field sprayer when the first male adult of Dvv on pheromone traps appeared. According to our preliminary results the mating disruption can be a good control system for reducing the population of Dvv according with the guidelines of Integrated Pest Management.

Key words: *Diabrotica virgifera virgifera*, pheromones, monitoring, mating disruption, spermatophora dissected

Introduction

The Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is an important pest of maize (*Zea mays*), present in North America and also in Europe, whose soil-inhabiting larvae can seriously damage roots of maize and lead to yield losses (Dunbar et. al., 2016). Dvv is present in Slovenia from 2003 and populations are growing up every year because maize is an important host plant which is grown on 40% fields every year. The studies present results for using sex pheromone-mating disruption on two different locations for reducing population of the Dvv.

Material and methods

Monitoring

On two locations in Slovenia, Draža vas by Slovenske Konjice (2015) and Savci by Mala Nedelja (2017), was carried out a monitoring of female Dvv with one pheromone PAL trap Csalomon® on a maize field from June to September. We counted the number of adult males on pheromone traps each week. The pheromone was replaced every month.

Mating disruption (MD)

The MD trials were carried out on the location Draža vas by Slovenske Konjice (2015) and Savci by Mala Nedelja (2017) for reducing the population of Dvv on maize. On each location we have used two treatments; untreated plots and treated plots where synthetic sex pheromone 8-methyl-2-decanol propanoate was used which was applied on volcanic stone zeolite (product CornProtect, Lithos Natural, Austria) in a dose of 4 kg/ha (0.16 g pheromone/ha) with 300 l water/ha. Foliar application was made with a standard tractor and a classical field sprayer when the maize plants were in average 1.2 m of height. On the location Draža vas by Slovenske Konjice in year 2015 we evaluated the biological efficacy of MD by collecting target pest Dvv beetles every seven days from first flight activity (2 July 2015) to the end of flight activity (10 August 2015). In each sampling, a minimum of 100 female and 100 male adults of Dvv were randomly collected in the middle of the untreated plot and treated experimental plot and given in a bottle with 70% alcohol. In the laboratory, the males and females were counted and the rate of the male and female adults was determined. Then the spermatophores were dissected from the females under a stereomicroscope and the spermium content of the spermatophores was examined and the numbers of sterile females was counted in the treatments in each of collecting dates.

Root injury /damage assessment

In 2016 (Draža vas by Slovenske Konjice) and 2018 (Savci by Mala Nedelja) we collected 60 roots (15 roots × 4 replications) on each treatment. Root damage on maize was assessed by node-injury scale.

Results and discussion

In the present experiment, in 2015 on the location Draža vas near Slovenske Konjice we detected reduced mating of males (females were 46% sterile), on the location Savci in year 2017 were 48% females sterile. The field experiments demonstrated that after MD female Dvv layed fewer eggs in soil and we observed in average up to 23% less damage on the roots of maize in year after MD using. In the same year of MD using less damage on maize silks was observed (in average 7 %).

The population of Dvv is very high in Slovenian maize fields therefore the use of continuous rotation is highly recommended for managing Dvv populations. At the same time the combination of safe and environmental friendly crop management practice with the guidelines of Integrated Pest Management using biological control agents (entomopathogenic nematodes) or biotechnology-MD adapted to specific maize production systems in Slovenia is highly recommended.

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Geostatistical approach for spatial distribution analysis of *Lobesia botrana* (Den. & Schiff): (Lepidoptera: Tortricidae) in Douro Demarcated Region (DDR)

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Abstract: The European grapevine moth (EGVM), *Lobesia botrana* (Den. & Schiff.), is the most important vineyard pest in Douro Demarcated Region (DDR) (North of Portugal) where the pest typically develops three generations per year, of which the third is the most damaging one. The mating disruption (MD) technique, an environmental safe control method against this pest was first used in this region in 2000, being increasingly applied since then. However, within this steep slope viticulture region, some major constraints to the use of MD technique were previously identified. The DDR is characterised by a very fragmented landscape with the majority of the vineyards organized in small plots, often bounded by other crops such as olive groves and by unmanaged non-crop habitats. The climatic conditions in DDR allow a high biotic potential for EGMV development. The strong altitude range and rugged orography as well as large number of plots under MD treated are important issues to consider for the successful application of the technique, in order to ensure the homogeneous distribution of the pheromone cloud through the treated area. To overcome some of these constraints, since the end of 2017 a partnership was set up between ADVID, a winegrower organization, the University of Trás-os-Montes and Alto Douro (UTAD) and four wine companies (Real Companhia Velha, Sogevinus Fine Wines, S.A., Dona Matilde Vinhos Lda. e Quinta do Vallado Sociedade Agrícola Lda.) through the project *"CSinDouro – Confusão sexual (CS) contra a traça-da-uva, Lobesia botrana (Den. & Schiff.) em viticultura de montanha: caso particular da Região Demarcada do Douro (RDD)"*. A key objective of this project is to investigate *L. botrana* population spatio-temporal dynamics inside and outside vineyards plots, in order to evaluate the effect of the landscape elements on pest distribution and on the effectiveness of MD. To introduce improvements in the use of MD at the plot level, in DDR conditions, a characterization of spatio-temporal variability, on adult male catches in pheromone traps, was performed through Geostatistical methods (IDW – Inverse Distance Weighting and Kriging), as well as an estimation of EGVM damages, by plot and by farm, in the three pest generations. The achieved results will be used for improving pheromone dispenser location as a way to get a homogeneous distribution of the pheromone cloud, according to landscape characteristics.

Key words: Douro Demarcated Region, geostatistical analysis, *Lobesia botrana*, mating disruption, spatial distribution, vineyard

Introduction

The European grapevine moth (EGVM), *Lobesia botrana* (Den. & Schiff.), is the most worrying vineyard pest in Douro Demarcated Region (DDR), a hilly landscape located in the North of Portugal. The mating disruption (MD) technique, an environmentally safe control method against this pest was first used in this region in 2000, being increasingly applied since then (Carlos et al., 2014).

The EGVM biology and behaviour are largely studied and some of these studies use geostatistical analysis to characterize male population's dynamics and to describe the insect population density in the field. In general, the EGVM male presence is not limited to the vineyards, being also present in olive groves and unmanaged non-crop habitats (Ifoulis and Savopoulou-Soultani, 2006; Sciarretta et al., 2008) which means that landscape composition can easily affect the pest population spatial distribution (Sciarretta and Trematerra, 2014).

Geostatistical techniques were largely used to the relationship between insect population dynamics and the landscape (Beckler et al., 2004; Yawson et al., 2017; Weber et al., 2018) and the practical implications in the pest management (Sciarretta and Trematerra, 2014; Duarte et al., 2015).

This study aimed at investigating the spatial-temporal dynamics of the EGVM populations, both inside and outside of vineyard plots, in order to evaluate the effect of landscape elements on the pest distribution and on the effectiveness of MD, as a basis to improve the use of MD under the DDR conditions. In order to understand the role of spontaneous vegetation and landscape on EGVM population dispersal, we used the geographic information system (GIS) potential to simultaneously analyse various sources of information such as: focus infection location, hotspots position and temporal dynamics.

Material and methods

Study area – vineyards

As experimental areas, five commercial farms, located between the coordinates 1º9'56.32''N, 7º46'13,53''W and 41º12'25,02''N, 7º30'30.39''W within Douro Demarcated Region (DDR), North of Portugal, were used: Quinta de São Luiz, Quinta do Vallado, Quinta Dona Matilde, Quinta do Síbio and Quinta das Carvalhas (Figure 1). The farms are located at both Douro River banks, at different altitudes (ranging from 50 up to 400 m) and spread over two sub regions from DDR: Baixo Corgo and Cima Corgo (Figure 1). In general, these farms are characterised by a very fragmented landscape, where most of the vineyards are organized in small plots, often surrounded by olive groves, woodlots, hedgerows and unmanaged non-crop habitats. These farms account to a total area of 649 ha, and only 46.5% of the area were planted with vines, mainly Portuguese varieties (e. g. Touriga Franca, Touriga Nacional, Tinta Roriz, Sousão, Rufete, etc.). MD technique was applied in 62% of vineyard area (Table 1).

Table 1. Total area and vineyards characterization by wine farm.

Wine Farm	Total area (ha)	Total area of vineyards (ha)	Area of vineyards under MD technique (ha)	Percentage of vineyards under MD technique (%)
Quinta das Carvalhas	315	113	65	88
Quinta do Síbio	34	8	7	87
Quinta Dona Matilde	95	32	16	50
Quinta de São Luiz	130	92	74	80
Quinta do Vallado	75	61	26	43
Total	649	302	188	62

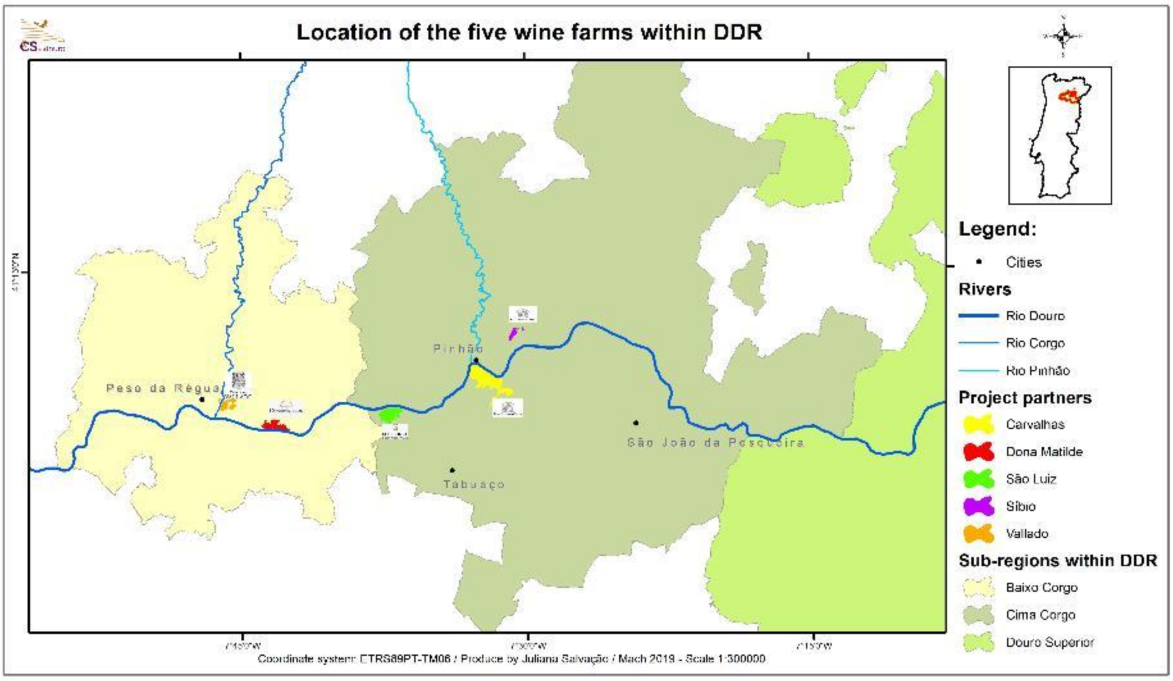

Figure 1. Location of the five wine farms within DDR.

Mating disruption application and data collection methodology

Along 2018, plots were treated using Shin-Etsu Chemical Co. Ltd Isonet-LTT® dispensers loaded with 344 mg of synthetic *L. botrana* female sex pheromone, E7, Z9-12: Ac, at a dose of 400 dispensers/ha. A higher dose than recommend by fabricant (250 dispensers/ha) was used due to several constrains identified under ECOVITIS Project (Carlos et al., 2014), in particular those related with climatic (temperature, humidity, wind) and morphologic conditions (rugged orography). The dispensers were applied during March, before the beginning of adult flight.

In total of 124 sampling points were selected inside and outside of the MD treated area, in order to collect data about EGVM presence. In each sampling point, EGVM males were monitored weekly, from the end of March until the end of October, using 1mg pheromone-baited sticky delta traps. The pheromone dispensers and sticky boards were replaced every

4 weeks. The traps were placed at the height about 1.30 m above ground and positioned within a distance that ranged from 58 m to 298 m, between any two traps. Traps outside of MD plots were placed at least 50 m from the MD plots' border, thus avoiding false hotspots (Trematerra et.al., 2004). Untreated areas consisted in vineyards plots, olive crops and non-crop habitats such as forest and scrubland.

GIS creation and geoestatistical analyses
To develop the geographic database, in a first stage, the wine farms land properties were digitalized by means of a GIS software (ArcGIS 10.x), in a total area of 649 ha, at a scale of 1:2000. Then, landscape was categorized into habitats according to land cover characteristics and the vineyard classified according the cultivars. In the field, sampling point locations were georeferenced using a GPS device. In a second stage, data was embedded in a WebSIG (ArcGIS Online) created for the partnership with the goal of to be used as a database for registering field data, sharing and enable work at real time. For weekly data collection on male catches in traps, a mobile application (Collector for ArcGIS) which enables alphanumeric and photographic data record was used.

A full spatial-temporal distribution and characterization, based on male catches in traps, was performed through geostatistical method Inverse Distance Weighting (IDW) in order to obtain a general frame of the pest presence at a regional level. The IDW is a determinist method, based on a mathematical algorithm, using nearest neighbours to predict values on unsampled locations. It is based upon the distance from the output point; the closer the sampling points are the more influence they have on the estimated value. The geostatistical analysis was performed in ArcGIS 10x., using Spatial Analyst Extension, with Geostatistical Analyst Wizard. In the IDW, a maximum of 8 nearest neighbours were used, as well as a distance power of two and eight sector types for looking at the nearest neighbours. Data collected during the three flight period were processed, based on the catches on untreated plots (control), according to the occurrence in each location, in order to analyse the hotspots position and temporal dynamics per flight.

Results and discussion

EGVM spatial distribution
The IDW interpolation technique was used to produce continuous images for insect population in each wine farm. For each flight, IDW was performed using male cumulative catches, as depicted in Figure 2.

Achieved results showed high differences between the three generations, as well as between the wine farms. In most of the cases, the major EGVM population peak occurs at the 3rd flight, with the exception of São Luiz, were the major peak occurs in the 1rd flight. However, this result could not be very accurate as the control sampling point was located within a vine plot under chemical insecticide application. Along the 2nd and 3rd flights, the pest's spatial distribution changed, and more hotspots were identified; although some hotspots were concentrated inside the vineyard (control point), other occurred in olive grows and non-crop habitats such as forest and scrubland, although they were less evident.

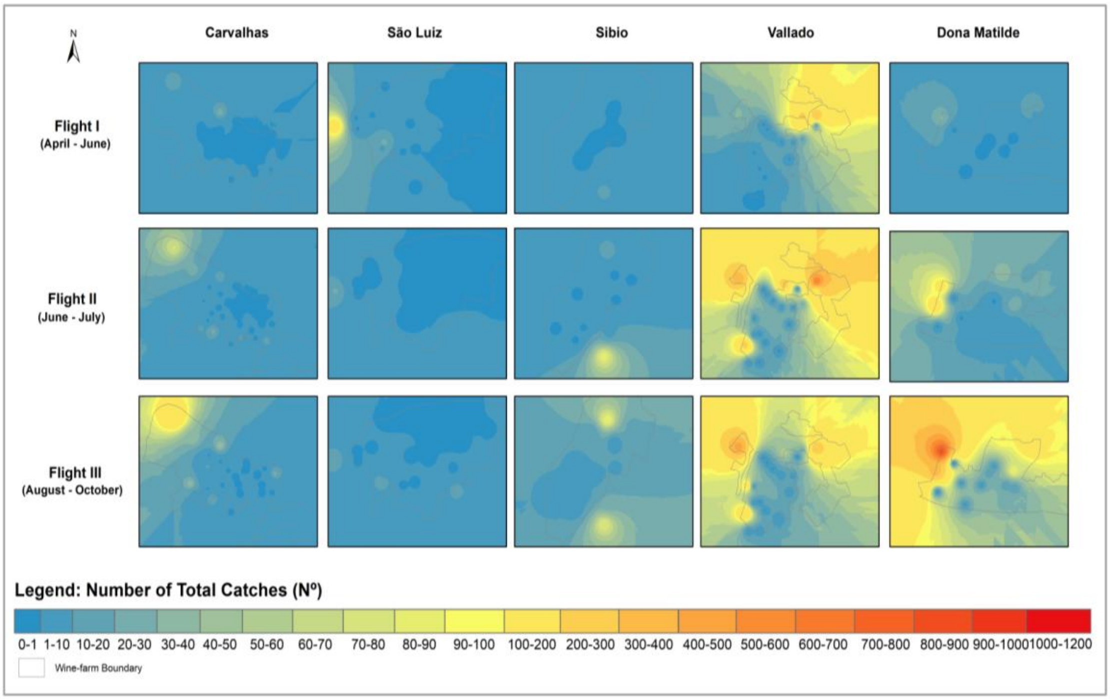

Figure 2. IDW results for the total number of adult males catches per flight in 2018.

Another significant achieved result is related to the presence of EGVM according to vineyards location within DDR. As depicted in Figure 3, two spatial distribution patterns could be established for the studied area, being the presence of EGVM higher for vineyards located in Baixo Corgo than for vineyards located in Cima Corgo (see Figure 1). The pest's pressure was more intense at Vallado (1178 total catches in vineyard control sampling station) and Dona Matilde (977 total catches – control) than at São Luiz (168 total catches), Carvalhas (275 total catches) and Síbio (178 total catches). These two sub-regions within DDR have different climatic characteristics, having Cima Corgo a hotter and driest climate than Baixo Corgo, which strongly influences the biotic potential for EGMV development.

In future works, an integrated analysis on climatic data such as wind speed and wind direction, humidity, temperature, collected in the five wine-farms, will be performed in order to understand about the influence of climate parameters in the results achieved.

These results will be helpful to farmers as they will allow them, in coming years, to know how to reinforce the dispenser's dose and improve pheromone dispenser location to get a homogeneous pheromone cloud distribution, according to terrain morphology and landscape characteristics.

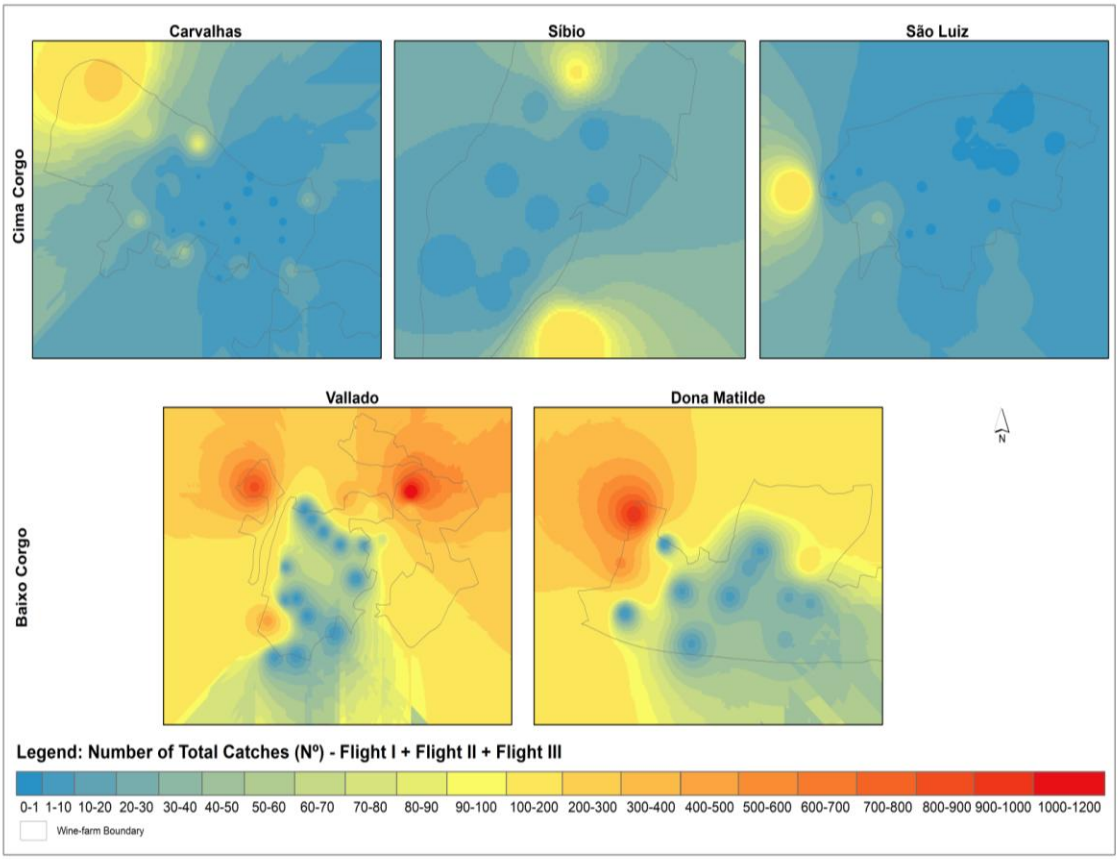

Figure 3. Results of IDW of the number of total adult males catches in 2018.

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Control of plum fruit moth, *Grapholita funebrana* Tr., by ISOMATE® - OFM TT dispensers in plum orchards of Bulgaria

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Abstract: Plum is a traditional fruit crop in Bulgaria. The plum fruit moth, *Grapholita* (syn. *Cydia*) *funebrana* (Tr.), is an important and the most difficult pest to control in plum orchards. The larvae of summer generation feed on fruits and caused damage from early summer till the harvest time. For a long time, pest management in stone fruit orchards in Bulgaria relied on organophosphate and pyrethroid insecticides. Considering environmental concerns the eco-friendly means of control, alternative to chemical insecticides are urgently needed. The ecological approach imposes a wider application of the methods of pest management that decrease or completely eliminate use of chemicals polluting the environment within the integrated fruit production systems. Mating disruption (MD) is a promising solution for control of different pests, among them plum fruit moth. The possibilities for reducing the number of treatments with chemical insecticides against pests in plum orchards of Bulgaria, by use of synthetic sex pheromones have been studied. The trials were carried out in an isolated 2.5-ha private plum orchard in two consecutive years - 2017 and 2018. Catches of male moths in pheromone traps were completely inhibited in the MD block, whereas they were numerous in the reference, conventionally treated orchard during both years of study. Isomate OFM TT dispensers, installed before the first flight of *Grapholita funebrana* males, reduced fruit damage significantly. The percentage of fruits containing plum fruit moth larvae was below the Economic Injury Level (EIL). The positive results obtained in this study indicate that mating disruption for control of plum fruit moth may be an effective alternative to conventional (pesticide) treatments. The studies are being continued.

Key words: IPM, plum fruit moth, *Grapholita funebrana*, mating disruption, pheromone dispensers, Isomate® - OFM TT

Introduction

Plum trees are infested in Bulgaria by a large number of pests. Plum fruit moth *Grapholita* (syn. *Cydia*) *funebrana* (Tr.), is undoubtedly the key pest of plum. The plum fruit moth is an important and the most difficult pest to control in plum orchards. The larvae of summer generation feed in fruits and caused damage from early summer till the harvest time. For a long time, pest management in stone fruit orchards in Bulgaria relied on organophosphate and pyrethroid insecticides. Considering environmental concerns the eco-friendly means of control, alternative to chemical insecticides are urgently needed. The ecological approach imposes a wider application of the methods of pest management that decrease or completely eliminate use of chemicals polluting the environment within the integrated fruit production systems. Mating disruption is a promising solution for control of different pests, among them plum fruit moth. Some successful results of trials in Italy on mating disruption of different tortricid pests, including *Grapholita funebrana*, were reported by (Veronelli and Iodice, 2004). The positive results of the technique mating disruption for control plum fruit moth have been obtained from (Falta et al., 2007; Brouwer and van Doornspeek, 2008; Toffolutti et al., 2008; Kutinkova et al., 2011; Riolo et al., 2010). According to Charmillot et al. (1982), releasing pheromones in all parts of the orchards have been successful but not in all cases. It seems that some isolation from other wooded areas is necessary to control *G. funebrana* with pheromones (Charmillot et al., 1982).

The aim of this study was to test the effectiveness of mating disruption for control of plum fruit moth in plum orchards using Isomate® **-** OFM TT dispensers (Shin-Etsu, Japan).

Material and methods

The trial was carried out in a well-isolated, 2.5-ha commercial orchard near the village Graf Ignatievo, Plovdiv region, South Central Bulgaria, that was established in spring 2012. During two successive years, 2017 and 2018, mating disruption (MD) of *Grapholita funebrana* (Gf) was employed using Isomate® - OFM TT dispensers. In the first week of April, before the start of GF flights, the dispensers were hung in the upper third of tree canopies at the density of 300 pieces per ha. Isomate® - OFM TT are a double dispensers, small red ties contains (Z)-8-dodecyl acetate + (E)-8-dodecyl acetate + (Z)-8-dodecenol.

Monitoring of Gf flight was carried out by sex pheromone trapping in the years of the study. PHEROCON® VI Delta, sticky traps were installed in the trial orchards using a scheme provided by the producer. The traps were baited with standard Gf L2 – Funemone lures and changed every 8 weeks. These products – pheromone traps and baits were developed and are manufactured by Trécé Inc., USA. All pheromone traps were checked twice a week.

For comparison, 2 standard traps were installed in the reference, conventionally treated orchard located in the same region. Seven to eight treatments were applied there during each season, to control Gf and other pests.

During the season, fruit damage was assessed in the trial and reference plot on 1000 fruits, twice a month. At harvest, 1000 fruits were sampled in both orchards, to evaluate the final damage rate. The rate of fruit damage by Gf was expressed as a percentage of damaged fruits. Significance of differences in the damage rate between the trial and reference orchard was estimated by use of Chi-square test

Results and discussion

In the reference orchard the first flight of the overwintering generation in 2017 and 2018 began in the first or second week of April and finished till the second week of September or the first week of October (Figure 1). The traps installed in the reference orchard caught in total 1209 moths in 2017 and 1865 moths in 2018. In the years 2017-2018 the Gf developed three generations.

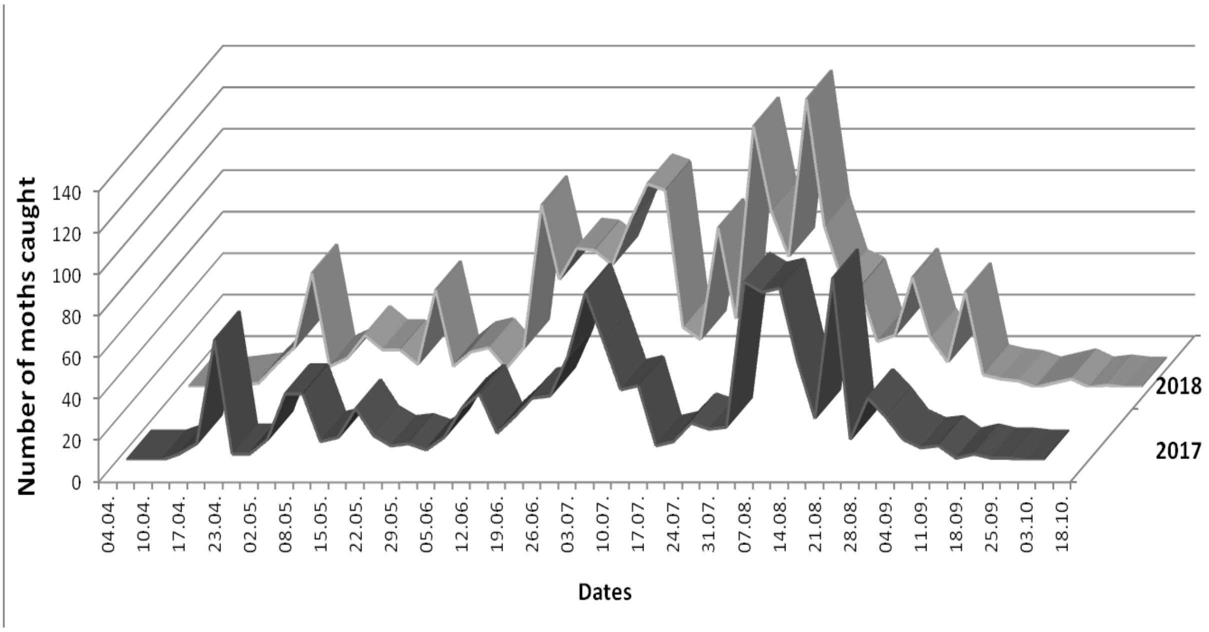

Figure 1. Flight dynamics of GF in the reference orchard in successive years of study 2017-2018.

First signs of fruit damage were noted in the reference orchard at the first week of June in 2017 and 2018. Starting from the middle of June, through August and September, the fruit damage rate increased, reaching finally at harvest 9.2% in 2017, and 7.1% in 2018. In the trial plot only a few damaged fruits were noted at the end of the season; at harvest it was also negligible – 0.5%-0.6% in 2017 and 2018. Damage rates were significantly different between the treated plots and the reference orchard already on 10 July and August 10 in 2017, 13 July and August 15 in 2018, and thereafter until harvest in both years of the study (Chi-square tests, $p < 0.001$) (Table 1).

Table 1. Evaluation of fruit damage (%) by *Grapholita fuberana* Tr. in the trial plot and in the reference in 2016-2017.

2017			2018		
Date	Trial (%)	Reference (%)	Date	Trial (%)	Reference (%)
June 7	0.0	0.1	June 5	0.0	0.1
June 15	0.0	1.3	June 18	0.0	1.1
July 10	0.0	1.9	July 13	0.0	2.1
July 31	0.0	3.2	July 29	0.1	4.7
August 10	0.5	7.9	August 15	0.6	6.8
Before harvest	0.5	9.2	before harvest	0.6	7.1
at harvest	0.5	9.2	at harvest	0.6	7.1

Catches of male moths in the pheromone traps were completely inhibited in the MD block in 2017 and only (single) individuals were caught in 2018, whereas they were numerous in the reference, conventionally treated orchard during both years of our study. Isomate® - OFM TT dispensers, installed before the first flight of *Grapholita funebrana* males, reduced fruit damage significantly. The percentage of fruits containing plum fruit moth larvae, was rather below the Economic Injury Level (EIL) – from 0.5% to 0.6%. In contrast, in the reference, conventionally treated orchard the damage rate reached between 7.1 and 9.2%. The present study shows that the damage caused by Gf larvae in the reference orchard was considerable, in spite of 8 insecticide applications against plum fruit pests. It is suspected that the population of Gf in this orchard had become resistant to some of the insecticides used.

In contrast, mating disruption, by means of Isomate® - OFM TT dispensers, ensures an effective protection from plum fruit moth. In view of increasing concern of consumers in fruit quality and the regulatory restrictions, limiting the use of many insecticides in the EU, this new technology may present a promising alternative to conventional protection programmes in plum orchards of Bulgaria.

The positive results obtained in this study indicate that mating disruption for control of plum fruit moth may be an effective alternative to conventional (pesticide) treatments. This approach to controlling plum fruit moth is in line with the recent EU recommendations that take care of preservation of the natural environment and production of healthy fruits, with no pesticide residues.

Implementation of mating disruption into the commercial plum production in Bulgaria may present an alternative to conventional programmes of protection of plum trees from insect pests with use of chemical pesticides. Thus it would be helpful in preservation of sound environment and in avoiding any risk of pollution of fruit products with pesticides. The studies are being continued.

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Managing beneficial insects by semiochemicals

Pheromones and Other Semiochemicals in Integrated Production and Integrated Protection of Fruit Crops
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Perspectives of multi-species lures for attracting Chrysopidae

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Abstract: Green lacewings (Chrysopidae) are important predators of several soft-bodied insect pests, with preference for Sternorrhyncha, especially aphids. The predatory larvae of *Chrysoperla carnea* species complex have special importance in agroecosystems. Previously a ternary floral bait was developed as a powerful attractant to both sexes of these lacewings, furthermore females were found to lay their eggs in the vicinity of the baits, which is of crucial importance in respect of biological control. Nevertheless, adults of *Chrysoperla* spp. are not predatory, thus it would be beneficial to apply stimuli, which may be attractive to a wider range of green lacewings, including the predatory adults of *Chrysopa* spp.

In order to find potential compounds for development of multi-species lures, field experiments were conducted in Hungary. In this paper we are aiming to give a brief overview of obtained results.

In our field experiments, an aphid sex pheromone compound, nepetalactol, attracted males of *Chrysopa* species, predominantly *C. formosa*, which is frequently present in agroecosystems; however, when nepetalactol was used in combination with the ternary floral bait, the number of attracted *Chrysoperla* spp. adults decreased markedly.

On the other hand, squalene was also found to be attractive to *C. formosa*. Interestingly, this compound was also attractive to males only. When tested in combination with the mentioned ternary floral bait, no significant interaction was found.

Our results indicate that the combination of the ternary floral bait and squalene could be promising for simultaneous attraction of *Chrysoperla* spp. and males of *Chrysopa* spp. Perspectives, challenges and potential practical applications are discussed in this paper.

Key words: chemical ecology, Chrysopidae, semiochemicals, stimulus interaction

Introduction

Green lacewings are important predators of several soft-bodied insect pests, including aphids (Canard 2001). *Chrysoperla* spp. are of special importance in agroecosystems (Pappas et al., 2011).

A ternary floral bait was developed which is a powerful attractant for both sexes of these lacewings (Tóth et al., 2009), furthermore, females were found to lay their eggs in the vicinity of the odour source (Jaastad et al., 2010; Koczor et al., 2015 a; 2017).

Nevertheless, adults of *Chrysoperla* spp. are not predatory, whereas *Chrysopa* spp. are predatory both as larvae and adults (Canard, 2001). Thus, semiochemicals for *Chrysopa* spp. are of special interest, though, from the practical point of view, the potential interaction of these compounds with the ternary floral bait should be taken into consideration as well.

In field experiments conducted in Hungary, semiochemicals involved in the chemical ecology of *Chrysopa* spp. were tested alone and in combination with the ternary floral bait. Hereby we are aiming to give a brief overview of the studies conducted on this subject.

Material and methods

Field experiments were conducted in Hungary from 2008 to 2018 in a mixed orchard near Halásztelek and in Érd-Elvira-major (Pest county, Hungary). In the experiments the CSALOMON® VARL funnel traps were used. Compounds tested in combination with the ternary floral bait included aphid sex pheromone components (Hooper et al., 2002), a plant-originated compound, squalene (Jones et al., 2011) and compounds identified from green lacewings (Aldrich et al., 2009).

Results and discussion

Aphid sex pheromone components

Aphid sex pheromone components attract several green lacewing species including some *Chrysopa* spp. (e. g. Hooper et al., 2002; Aldrich and Zhang, 2016). In field experiments conducted in Hungary, aphid sex pheromone components nepetalactol and nepetalactone attracted males of *Chrysopa* species, predominantly *C. formosa* Brauer, however, these compounds were not attractive to *Chrysoperla* spp. (Koczor et al., 2010; 2015 b). Furthermore, when tested in combination with a ternary floral bait, the number of attracted *Chrysoperla* spp. decreased markedly (Koczor et al., 2010; 2015 b). It was hypothesized to be a potential indirect effect by the *Chrysopa* lacewings attracted, however, as it was found in an early season experiment this effect is due to the compounds themselves (Koczor et al., 2015 b).

Compounds identified from green lacewings

Chrysopa spp. are known for their strong odour emitted upon disturbance, this odour is due to skatole (Aldrich and Zhang, 2016). In field experiments conducted in Hungary, skatole did not attract any green lacewing species (Koczor et al., 2015 b). When tested in combination with a ternary floral bait or nepetalactol baits, the compound did not show any effect on attraction of either *Chrysopa* spp. or *Chrysoperla* spp. (Koczor et al., 2015 b).

On the other hand (*Z*)-4-tridecene has also been identified from several green lacewing species (Zhu et al., 2000; Aldrich and Zhang, 2016; Koczor et al., 2018), and in an experiment in the USA by Zhu et al. (2000) the compound significantly decreased attraction of a Nearctic *Chryosperla* species to 2-phenylethanol. In field experiments conducted in Hungary (*Z*)-4-tridecene significantly decreased attraction of *C. carnea* complex and *C. formosa* to the ternary bait and nepetalactole, respectively (Koczor et al., 2018).

Plant-originated compounds

A plant-originated compound, squalene, was found to attract males of the Nearctic *Chrysopa nigricornis* Burmeister, in field experiments in the USA (Jones et al., 2011). In experiments conducted in Hungary squalene attracted males of *C. formosa*, however, it did not attract *Chrysoperla* spp. (Koczor et al., 2015 c). When tested in combination with the ternary floral bait, the compound did not show any interference (Koczor et al., 2015 c).

Conclusions, perspectives

Our results indicate that the combination of the ternary floral bait and squalene could provide a basis for future development of baits for simultaneous attraction of both sexes of *Chrysoperla* spp. and predatory males of *Chrysopa* spp.

The attraction of only male *Chrysopa* spp. adults to aphid sex pheromone components and squalene is highly interesting, though the ecological background of this phenomenon is currently unknown. Further studies may shed light on this interesting issue of chemical ecology.

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SIT and biological control of fruit crop pests

Pheromones and Other Semiochemicals in Integrated Production and Integrated Protection of Fruit Crops
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Area-wide management of fruit flies using the sterile insect technique

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Abstract: Traditionally, control of fruit flies has been mostly based on the uncoordinated application of insecticides using a localised farm-by-farm approach. This strategy is not effective or sustainable in view of fruit fly movement and is also damaging to the environment. The area-wide approach has its roots in the management of vector-borne diseases and locust pests and has increasingly been used for the management of fruit flies. The aim of a fruit fly area-wide integrated pest management (AW-IPM) strategy is to prevent the targeted population surpassing an economic threshold. AW-IPM targets an entire pest population which requires treating all habitats to avoid leaving infestations from where migrant insects can re-establish in areas of concern. Insect movement, occurring sometimes over long distances, is generally underestimated. Consequently, most conventional pest management is an uncoordinated local action against only segments of a pest population, resulting very often in unsustainable control. In addition, the implementation of control projects requires a phased conditional approach where the next step should only be implemented after the completion of the previous one. Among the control tactics available, the sterile insect technique (SIT) can contribute to the AW-IPM approach in situations where the number of key pests is low, when integrated with other control methods and when technically and economically feasible. Among the methods available for integration, depending on the characteristics of the infested area, could be sanitation, bait sprays, bait stations, biocontrol, and male annihilation technique (MAT).

Key words: area-wide integrated pest management, phased conditional approach, suppression, containment, eradication, prevention

Introduction

Tephritid fruit flies (Diptera: Tephritidae) cause major problems to the production of fruits and vegetables and their trade. The reduction of fruit quality is due to the direct damage caused by the larvae eating the pulp of the fruits. The presence of tephritid fruit flies results in an increase of production costs due the continuous need to control the pest. The conventional use of insecticide application to control fruit flies has a negative impact on the environment and leaves insecticide residues in fruits and vegetables. The environmental impact is being increasingly monitored and is receiving special attention by governments due to more stringent environmental policies. Also, the availability of authorized insecticides is decreasing due to the recommendation by regulatory agencies and new insecticides are often more expensive. Of further importance is the negative impact of insecticides on biocontrol agents resulting in an increase of secondary pests. The pesticide residues alone can disrupt international trade due the restriction on the imports of fruit and vegetable commodities with levels of residues above a certain established level. This is a clear trend in the case of the “low-pesticide residues markets” in the European Union. Other markets are more focused on potentially fruit fly infested commodities being imported, “pest free markets”, and require the exporters to implement phytosanitary measures to reduce fruit infestation in the field and post-harvest phytosanitary treatments to reduce dramatically the risk of pest introduction.

Area-wide integrated pest management (AW-IPM) for fruit flies

Most insect pest control is implemented on a farm-by-farm basis. However, this is very inefficient because insects move, farmers do not coordinate their actions, and only parts of the pest population are suppressed. For example, when a pest attacks various commercial crops, as well as wild and backyard host plants in an area, control actions in farmer fields have only a temporary effect because the pest quickly re-invades from nearby untreated areas and re-infests the commercial crops. Soon the pest problem exists as before. In response, farmers have to apply control measures frequently to protect their crops and this very often is unsustainable.

An AW-IPM approach involves a preventive rather than a reactive strategy whereby all individuals of the pest population are targeted in time and space, resulting in more cost-effective and sustainable pest management (Vreysen et al., 2007).

AW-IPM involves a coordinated effort over larger areas, including not only agricultural, but also natural and other areas where the pest is present. By targeting the sources of re-infestation in the areas surrounding those of agricultural importance, efficient and sustainable pest control is achieved in the entire area and fewer control actions are required. The AW-IPM requires coordination among all stakeholders, long-term commitment, usually multiyear planning, and a centralized organization dedicated exclusively to its implementation.

Furthermore, with lower density of an entire pest population, more selective and less insecticide-reliant management tactics become feasible as part of an AW-IPM approach to address ecological, environmental and resistance concerns. But also, economic and global-trade challenges strongly encourage the collaboration and participation of farmers and all stakeholders in area-wide approaches to insect pest management.

In addition, the AW-IPM should be based on the coverage of the entire area where the target insect population is distributed. Even if the control methods used are not fully effective, the application directed at the entire pest population will result in more sustainable and effective suppression of the pest. In contrast, this is not the case if a fully effective control method is applied, but leaving some areas not covered. The pest will then continue to reproduce in certain parts of the area from where it can repeatedly re-invade the area under effective control, resulting in an increase of the pest population.

Phased conditional approach

The successful implementation of AW-IPM programmes requires adoption of a “phased conditional approach” where progression to the next phase is made conditional upon having finalised all or most of the required activities in the previous phase. This entails (1) collection of necessary baseline data including species present, temporal and spatial dynamics of the target populations over several years, host status, and host sequence; (2) the implementation of a continuous surveillance and control methods that can contribute for the reliability of the baseline data collected; (3) the definition of the AW-IPM programme depending on the objective, the fruit market targeted, the ecological characteristics of the production area, and cost-effective control tactics available to be selected for integration; and (4) the implementation of the programme after completion of steps 1 to 3, including other additional relevant information collected.

Sterile insect technique (SIT)

The SIT is a method of pest control involving area-wide inundative releases of sterile insects to reduce reproduction in a field target population of the same species in an area (Dyck et al., 2005). It is one of the most environmental-friendly methods available, as no toxic material is introduced in the target area, and the released insects cannot get established, as sometimes happens with some other biological control methods.

However, the use of the SIT requires in-depth knowledge of the target pest, as well as efficient mass-rearing, handling, release and monitoring methods. It is best started when the target population is small, at the beginning of the season or after the pest population is being suppressed by using other control methods and has to be applied on an area-wide basis. Besides the technical aspects mentioned, it is management intensive and requires longer term commitment.

The AW-IPM with a SIT component against fruit flies can be implemented using four different strategies, i. e. suppression, containment, eradication, and prevention.

With the markets growing that require lower pesticide residues, the SIT has been increasingly used for fruit fly suppression replacing conventional control. This strategy can also be used to maintain an area of low fruit fly prevalence and can be complemented with additional independent risk mitigation measures (such as a postharvest treatment), as part of a systems approach to reach “pest free markets” (FAO, 2012). This strategy is used against the Mediterranean fruit fly, *Ceratitis capitata* Wied., by the citrus producers in Valencia, Spain, in order to get access to the lucrative USA markets. The low pest prevalence strategy has the advantage of not requiring quarantine checkpoints to prevent reinvasions. It is a continuous pest population suppression and does not preclude eventual pest-free status. It also protects natural enemies, allowing biological control of secondary pests.

Fruit fly containment is used mainly to limit the progress of the fruit flies to the neighbouring pest free area. An example of this strategy is the weekly release of millions of sterile Mediterranean fruit fly males along the Mexico-Guatemala border that has maintained Mexico free of this pest for the past four decades (Enkerlin et al., 2017).

Initially, eradication was the main goal of SIT projects, but currently eradication is mainly used for special situations where it is technically and economically justifiable. Among those situations, the incipient introductions of invasive species in new areas have recently received special attention. For example, a Mediterranean fruit fly outbreak in the Dominican Republic triggered an immediate ban to fruit and vegetable exports to the USA. The Ministry of Agriculture with the support of the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the United States Department of Agriculture (USDA) and the Guatemala-Mexico-USA Moscamed Programme as well as regional agricultural organizations such as Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) and Instituto Interamericano de Cooperación para la Agricultura (IICA) reacted immediately with the implementation of an eradication effort using an AW-IPM approach integrating the SIT. The successful sustainable removal of the Mediterranean fruit fly from the Dominican Republic reopened the fruit and vegetable export markets to the USA valued at that time in US$ 51 million. In these situations, the detection and rapid response to the outbreak is crucial to the successful eradication, irrespective of the control tactics used (FAO, 2016; FAO/IAEA 2017; FAO/IAEA, 2018).

Although the strategy of prevention has been used less extensively, this approach (Mediterranean Fruit Fly Preventive Release Program in California and Florida) has in the last decades prevented the establishment of this pest in the continental USA (Dowell et al., 2000).

Conclusion

(1) AW-IPM is a prerequisite for effective fruit fly control and requires public-private sector collaboration such as participation of the grower organizations, and (2) SIT is management-intensive and should only be applied when integrated with other phytosanitary measures and where technically and economically justifiable.

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Intraguild predation among natural enemies of *Myzus persicae* in peach trees in northeastern Spain

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Extended Abstract: Spain is one of the main European peach producers with almost 30% of the total production. Within Spain, Catalonia, in north-eastern Spain, accounted for 30% of the total production in 2017 (FAOSTAT, 2016; MAPAMA, 2017). The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is the most important aphid species affecting peach trees. Nowadays, its control is reliant on insecticide sprays, but resistance to a wide range of them has been recorded (Slater et al., 2012). This, together with the concerns for human health and environmentally friendly agriculture, calls for the development of alternative tools in order to reduce the use of pesticides. Conservation biological control (CBC) may play an important role in the management of this pest since aphids have a wide range of natural enemies. The inclusion of floral resources near crops may enhance natural enemy populations by providing them with food resources and refuges (Brennan, 2016). Regular field sampling conducted during 2015 and 2016 in peach orchards in the area of Segrià (Catalonia) showed that the most abundant natural enemies were parasitoids and predators, such as *Orius* spp., gall midges and hoverflies (Aparicio et al., 2015). The success of CBC may be affected by the possible interactions between the different natural enemy species present. The interactions are usually studied from the parasitoid point of view, because predators can attack parasitoids, but parasitoids are not able to attack predators (Colfer and Rosenheim, 2001).

In this study, we evaluated with laboratory trials, the potential of the predators in aphid control and the interaction between them and parasitoids. A female *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), a larva of the gall midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) and a larva of the hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) were caged in a microcosm containing a sweet pepper plant infested with *M. persicae*. After three days the number of live aphids was recorded. Our results showed that hoverfly larva was the most effective predator, decreasing aphid population by 93%. The other predators consumed a lower number of aphids. The gall midge reduced aphid populations by 20% and *O. majusculus* by 10%.

It has long been known that the hoverfly larvae feed on parasitized aphids (Meyhöfer and Klug, 2002). However, there is little information regarding the interaction between other predators and aphid parasitoids. Thus, we carried out two experiments to study the food preference of *O. majusculus* and *A. aphidimyza* for unparasitized and parasitized aphids. We performed a no-choice trial with a final count at 24 h and a choice "cafeteria" test in which several counts were performed over 48 h to determine the order in which the prey was consumed by the predators. Our results for *A. aphidimyza* showed that the predator was able to feed on both unparasitized and parasitized aphids, although they consumed more unparasitized aphids and preferred them to parasitized aphids. The same trend was observed in the experiments with *O. majusculus.*

The results obtained in our study suggest that the main natural enemies present in peach orchards in the study area could be valuable potential biological control agents of the green peach aphid. In addition, the interactions among *A. aphidimyza*, *O. majusculus* and the parasitoid wasps may not be detrimental to the biological control of the pest. The provision of food resources, such as flower nectar close to the orchards, may be a useful strategy to enhance the abundance and early presence of parasitoid wasps (Arnó et al., 2012) and *Orius* spp. (Pumariño and Alomar, 2012). *Aphidoletes aphidimyza* improves its fitness when feeding on sugar-rich diets such as nectar. However, morphology of adult predators prevents them from reaching the nectar of some flowers (Aparicio et al., 2018).

Key words: predators, Aphidiinae, green peach aphid, association

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Comparison of parasitism of *Eriosoma lanigerum* by *Aphelinus mali* in IPM and organically managed apple orchards

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Extended Abstract: The woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), is a pest in apple orchards infesting both stems and roots of host trees (Baker, 1915). Heavy infestations with WAA can cause yield and growth reduction of apple trees. WAA weakens the plant by both feeding on roots and inducing root and shoot galls that damage the xylem and interrupt water transportation (Klimstra and Rock, 1985), which can even lead to the death of young trees (Brown and Schmitt, 1990; Brown et al., 1994). The pest status of WAA in Italy has been fluctuating over the years depending on biotic and abiotic factors (Lordan et al., 2015; Angeli, 2015).

The control of the woolly apple aphid by the host-specific solitary parasitoid *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) is cited as one of the most successful examples of classical biological control of insect pests (Mueller et al. 1992; Goossens et al., 2011). DeBach (1964) reported *A. mali* as a successful biological control agent in at least 40 countries, including Italy.

The susceptibility of some apple varieties and dwarfing rootstocks, the simplification of the agroecosystems and the management programs of primary pests and diseases are reported as being potentially responsible for disrupting the biological control of WAA (Angeli and Simoni, 2006; Baldessari et al., 2007; Beers et al., 2010; Beers, 2014; Angeli, 2015). Some recently introduced agronomic techniques, such as single row exclusion netting, also reduce the possibility of colonization of the plant's foliage by predators and parasitoids.

Eriosoma lanigerum becomes active starting from 267 degree-days above the temperature threshold of 5.2 °C (Asante et al., 1991), which generally corresponds to the crop stage of green bud (BBCH 56), whereas *A. mali*, whose lowest developmental threshold is 8.3 °C, needs 255 degree-days to begin its activity (Asante and Danthanarayana, 1992). Consequently, the developmental time of *A. mali* is generally delayed throughout the year compared to its aphid host.

A two-year field experiment (2017-2018) was conducted in organic and integrated pest management (IPM) orchards (Golden Delicious and Fuji varieties) located in plain (180 m) and hilly areas (500-700 m) in the Trentino-South Tyrol region (north-eastern Italy) to determine the efficacy of biological control of WAA populations with *A. mali*. Plant colonization by WAA, flight of *A. mali* and percentage of parasitism of WAA colonies were monitored in the orchards. Particular attention was paid to evaluating the susceptibility of *A. mali* to the common pesticides used in the two crop management systems in relation to plant varieties and altitude of cultivation.

Our results suggest that although *A. mali* reduced WAA populations during summer, but control was insufficient from May until mid-June in both the IPM and organically managed orchards, independent of topography (plain and hilly areas) and apple variety.

The findings are confirmed by Goossens et al. (2011), who reported that parasitism by *A. mali* was insufficient to reduce WWA damage early in the season due to the later emergence from diapause and a slower reproduction of the parasitoid compared to its aphid host. A greater delay of parasitoid development in hilly areas has been observed.

The first peak of flight of *A. mali* in the post-flowering period (6^{th}-20^{th} June), although limited in size, is essential for an exponential flight increase and the corresponding increase of parasitism peaking in summer which occurs generally at the end of July.

Monitoring the number of parasitized aphids (black mummies) and flight activity of *A. mali* in four orchards throughout the season provided information on the developmental stage of the immature parasitoids delayed of about one week on the hills. The phase when *A. mali* larvae develop inside their aphid host is strategic to the timing of pesticide treatments with compounds that pose a risk to *A. mali* adults.

Outbreaks of *E. lanigerum* have often occurred as a result of pesticide applications which decimated biological control agents (McLeod, 1954; Penam and Chapman, 1980). In presence of high infestations of woolly apple aphid, the chemical control of the rosy apple aphid, *Dysaphis plantaginea* with spirotetramat (+ oil) or other aphicides, which remains necessary in IPM, shows a side-effect on WAA that anticipate the high migration waves and the subsequent colonization of growing shoots.

During this two-year study, no differences were observed in the parasitoid-host relationships in the two crop management systems organic and IPM, even though a higher number of WAA was observed in organic orchards. The climatic conditions seem to play a key role in regulating the ability of *A. mali* to control aphid infestations. Investigations are still ongoing, especially into the optimal management of parasitoid populations through simple practices, such as mowing grass at alternate rows.

Key words: monitoring, *Aphelinus mali*, parasitoids, *Eriosoma lanigerum*, wolly apple aphid, IPM, organic

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The sterile insect technique for Mediterranean fruit fly control: a North-Italy pilot project

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Extended Abstract: The Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a pest of East African origin subsequently established in fruit-growing areas of the Mediterranean coast (Balachowski, 1950). It has been described as one of the world's most threatening agricultural pests, attacking more than 300 different host fruits (Liquido et al., 1990; Papadopolus et al., 2001; De Meyer et al., 2002; Malacrida et al., 2007). Historically, it affected fruit crops of southern and central Italian regions, where it infests citrus, apricot, peach, fig, apple and khaki. Recently, due to climate change, fruit damages have been recorded also in the northern regions as Trentino-South Tyrol (Gutierrez and Ponti, 2011).

In Trentino, *C. capitata* was reported for the first time in 1990 when infested apples were found in an orchard close to a supermarket where rotten fruits were thrown away. This first report was followed by others testifying a slow and constant spread of the pest in the region.

At present, the Mediterranean fruit fly is established in scattered areas in the Adige Valley, where it causes economic damage especially on apple, the main fruit crop of the area. To maintain the damage below an economic threshold, specific insecticide treatments with ovo-larvicidal insecticides are necessary in some years. *C. capitata* attacks ripe apple close to harvest and insecticide applications to control the infestation during this period could potentially increase pesticide residues on fruits. To avoid pesticide residue issues, research efforts have focused on the development of environmentally friendly pest management approaches, including mass-trapping, attract and kill and the sterile insect technique (SIT) (Navarro-Llopis et al., 2013; Martinez-Ferrer et al., 2012; Hendrichs et al., 2002). The success of these alternative control measures depends on the density and the spatial distribution of the target pest. Since the population level of *C. capitata* in Trentino is still low and the pest is spread in a defined area bordered by forestry and non-host vegetation, the region seems to be suitable for testing the efficacy of these control strategies.

Here, we report on the preliminary steps made by our research group for implementing SIT as measure to control *C. capitata* in apple orchards.

Apart from the population density, the effectiveness of SIT is associated with the ability of irradiated males to compete with wild males for mating with wild females (Nikolouli et al., 2018). Eggs laid by females that mated with sterile males are not viable and result in no offspring. The competitiveness of released sterile males might be impacted by the insect strain and the rearing method, radiation sterilization, marking, stress during cold storage, shipment to the release site and release procedure (Dyck et al., 2005). In our case, insects belonging to genetic sexing strain *Vienna 8* (obtained from IAEA, Seibersdorf, Austria) have been provided by Bioplanta (Valencia, Spain) by TRAGSA. Even if the International Plant Protection Convention (IPPC, 2005) includes sterile insects produced by biofactories as organic control agents, allowing international exchange and potential use in the crops defence, airfreight and customs clearance are still an issue.

Our first aim was to set up a dynamic procedure for transferring the sterile males from the biofactory to the field. Pupae are sent once a week by airplane to Rome. At the airport, the parcel with the appropriate certification goes through customs clearance and immediately is given to a courier for fast delivery to Fondazione Edmund Mach (FEM) in San Michele all'Adige (Trentino). The time spent by the pupae under hypoxia is critical and should be minimized, otherwise quality parameters could be affected. Once received at FEM, the pupae were divided into batches, two for each release point, kept in a climate chamber under controlled conditions (24 ± 1 °C, RH 70 ± 5%) and supplied with water and food (water, agar 1% and sugar 18%).

We assessed the effect of the stress suffered during shipment on the emergence rate and adult longevity on the insects at arrival (Dyck et al., 2005). Quality control performed included the recording of the percentage of males emerging for each shipment. The emergence rate ranged between 52% and 98%, with an average of 83%. Flight performance was assessed by releasing the insects in three areas of about 10 hectares each along the Adige Valley, which were mainly cultivated with apple orchards. The adult release is performed twice a week from defined points, one per hectare, from the beginning of July until the end of November. Two types of traps were used to evaluate the effectiveness of the SIT strategy and to monitore the distribution of sterile male flies within the released areas: Delta sticky traps (Biogard Delta trap - Biogard, CBC) activated with Trimedlure (ISAGRO) attracting males, and Vaso-trap® with glass jars (by Tap-trap) baited with attractive food (Unipack® Biolure, Suterra) in order to capture both males and females.

The results indicate that the sterile males move more than 300 m in every direction and are evenly distributed in the release areas.

Key words: *Ceratitis capitata*, medfly, sterile insect techniques, Trentino, Italy, apple

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Developing a new biological control strategy for *Cacopsylla* spp. with the novel entomopathogenic fungus *Pandora* sp.

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Extended Abstract: A new fungus species, *Pandora* sp. (Entomophthorales: Entomophthoraceae), was isolated from dead pear psyllid in Denmark in 2016 (Jensen et al., 2018) and cultivated on solid media. In order to assess whether this as yet undescribed species would be able to infect other insects, pathogenicity experiments were performed, with special emphasis on psyllids (Hemiptera: Psyllidae). In the pathogenicity experiments, insects were inoculated with a "conidia shower". For this purpose, mycelia mats were fixed to moist filter paper in the lid of small plastic cups, and a conidial shower was established. This is possible since fungi from Entomophthorales actively discharge their conidia (Hajek et al., 2012). A layer of agar on the bottom of cups increased the relative humidity to above 95% and a filter paper prevented the psyllids from adhering to the agar. The insects were exposed to the shower of the asexual conidia for 24 hours at 20 °C and short-day conditions (L:D 10:14 h). The mortality and post mortal symptoms of the fungal infection were monitored over a period of 10 days after inoculation with *Pandora* sp.

In our experiments, several *Cacopsylla* species, important vectors of phytoplasmas, were successfully infected with this new Pandora sp. The apple psyllid, *Cacopsylla picta* Foerster (Hemiptera: Psyllidae), the vector of the cell wall lacking bacterium '*Candidatus* Phytoplasma mali', the agent of apple proliferation disease, showed significantly increased mortality when inoculated with *Pandora* sp. compared to the control group. Some of the important vectors of phytoplasma diseases in European fruit production, *C. pyri* L. and *C. pyricola* Foerster on pear, and *C. pruni* Scopoli on *Prunus* sp., were also successfully infected and symptoms of the *Pandora* sp. infection on psyllids were observed 24 hours after onset of mortality. Furthermore, the experiment showed that not only the adult stages of *C. pyri* were susceptible to the fungal infection, but also the nymphs of this pear psyllid. Other psyllids were also successfully infected with *Pandora* sp. such as *C. peregrina* Foerster feeding on hawthorn (*Crataegus monogyna* L.). Furthermore, other phloem-feeding hemipteran insects, such as *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) vectoring several plant viruses, showed decreased survival after treatment with this entomopathogenic fungus.

In the research project "PICTA KILL", *Pandora* sp. is being evaluated as a biocontrol agent for psyllid control. In an "Attract- and Kill strategy" (Figure 1) this entomopathogenic fungus should be used as a "kill-component" in combination with specific attractant volatiles. In previous studies it has been shown that uninfected apple psyllids (*C. picta*) were more attracted to phytoplasma-infected apple trees, which emitted higher amounts of the sesquiterpene β-caryophyllene compared to uninfected plants (Mayer et al., 2008 a). In

bioassays, it was demonstrated that the synthetically produced β-caryophyllene was also highly attractive to the new generation of *C. picta* (Mayer et al., 2008 b).

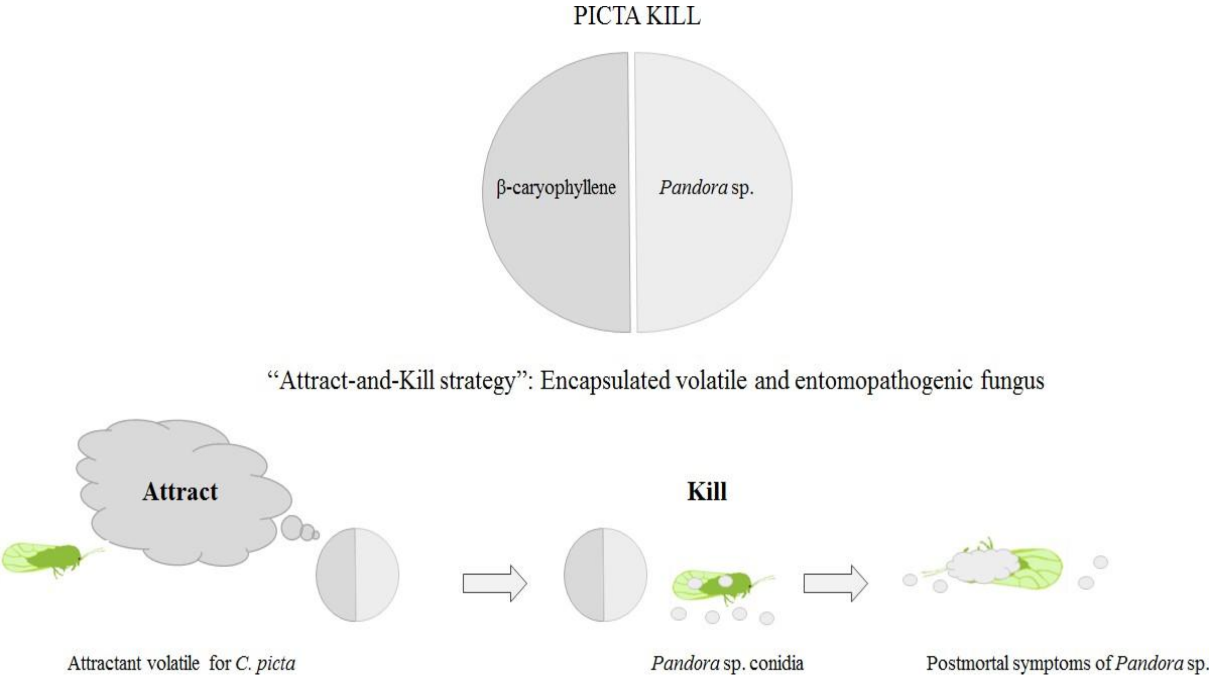

Figure 1. „Attract- and Kill strategy" with encapsulated attractive volatiles and entomopathogenic fungus.

An innovative encapsulated formulation containing the entomopathogenic fungus *Pandora* sp. and volatiles such as β-caryophyllene should increase the specificity of this control approach and decrease the abundance of *C. picta* finally resulting in decreasing numbers of new infestations of apple trees with apple proliferation disease. We are currently designing experiments to elucidate this potential.

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Genetic diversity and initial population size affect the early colonization success of *Mastrus ridens*, a parasitoid used for the control of codling moth

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Extended Abstract: *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae) is one of the main parasitoid species attacking the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in its region of origin (southern Kazakhstan) (Mills, 2005; Retamal et al., 2016). It has been used in several classical biological control programs against the codling moth in North and South America, New Zealand, and Australia and is being considered for release in Europe (Mills, 2005; Devotto et al., 2010; Sandanayaka et al., 2011; Tortosa et al., 2014). *Mastrus ridens* is a specialist gregarious ectoparasitoid that attacks mature codling moth larvae in their cocoons by paralyzing and then laying several eggs on the cuticle of its host. As most hymenopterans, this species has a haplodiploid mode of reproduction, but additionally it displays complementary sex determination (CSD) (Retamal et al., 2016). As a result, populations with low genetic diversity or under inbreeding conditions produce less female progeny and a larger proportion of diploid males (Retamal et al., 2016; Zaviezo et al., 2017). The aim of this study was to examine the effect of initial genetic diversity and founding numbers on *M. ridens* population growth and other associated parameters under laboratory conditions.

For testing the effect of genetic diversity, we established *M. ridens* low diversity (isofemale lines) and high diversity lines (mix of several lines). Then six mated females were placed inside a plastic box (19.5 × 13 × 9 cm) with codling moth larvae and honey. In this way, treatments were established with the same initial insect numbers but different genetic diversity depending on the line used in each box. From then on, additional larvae were added periodically, and at day 21 and 42 a new box was added on top. The two boxes were connected through tubes. Population size was estimated every other day by observing and counting the number of insects through the walls of the box. The experiment at day 70 ended and all individuals present in the boxes were counted, including codling moth larvae and adults. Results were analysed by GLM, and for the apparent growth rate by Exact Wilcoxon test.

For testing the effect of founding numbers, we created more complex arenas made of plastic boxes (23 × 13 × 4.5 cm) with ten internal divisions connected by a single small perforation in each division. In each box, either one unmated female and one male or four unmated females and four males were added. All insects within a box belonged to the same low diversity line, but different lines were used in the boxes. In this way, we varied initial numbers, but the level of genetic diversity was low in all cases. As in the previous experiments, codling moth larvae were added periodically, and also new boxes above the initial ones as time passed. At day 45 the experiment ended and all individuals present in the boxes were counted, including codling moth larvae and adults. Results were analysed by GLM.

Results showed that the effect of genetic diversity on population growth was 17.5% and 11.0% for the whole period in the high and low diversity treatments respectively ($p = 0.047$). High diversity treatments generated 1.6 times the number of individuals than low diversity treatments. These differences were even larger when comparing the number of females produced, which was twice as much for the high diversity treatment ($p = 0.04$). Also the overall sex ratio (proportion of males) was lower in the high diversity treatment (0.51 versus 0.61, $p = 0.02$).

We also found an effect of initial numbers. The most important was the establishment rate (proportion of boxes where production of female offspring occurred), which was 44% in the low population treatment and 83% in the high population treatment. In addition, the total number of individuals produced and total number of female offspring were larger in the high initial number treatment ($p < 0.001$ for both).

This study shows that both genetic diversity and populations numbers affected population growth, female production and sex ratio and thus early colonization success of the experimental laboratory populations. The same effects could be taking place in the field when low numbers are released or laboratory colonies are initiated. This should be considered when carrying out classical or augmentative biological control programs.

Key words: biological control, sex ratio, apple

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Hoverflies as biological control agents of the rosy apple aphid in Mediterranean areas

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Abstract: The aim of this work was to improve biological control of the rosy apple aphid by the promotion of hoverflies. For this reason, we identified which hoverfly species prey on the rosy apple aphid colonies present in the fruit tree growing area of Lleida (Catalonia, NE-Spain), we described the hoverfly species present in this area, and we identified the most attractive flower species that naturally occur in this area to hoverflies. We found three genera of hoverflies (*Episyrphus*, *Eupeodes* and *Sphaerophoria*) predating on rosy apple aphid colonies and six genera (*Sphaerophoria*, *Episyrphus*, *Eupeodes*, *Melanostoma*, *Paragus* and *Xanthogramma*) feeding on flower species. The most visited plants belonged to Compositae and Cruciferae families. Our findings show that hoverflies present in our area have potential for biocontrol of rosy apple aphid populations because the genera found in aphid colonies were the most abundant feeding on the flora that naturally occur in this area.

Key words: hoverfly, Syrphidae, *Dysaphis plantaginea*, ecosystem services, pests control, apple

Introduction

Hoverflies (Diptera: Syrphidae) are an important group of beneficial arthropods due to their double function as providers of ecosystem services such as pollination and biological control of some insect pests. Adult hoverflies need to feed on pollen and nectar for survival and sex maturation, respectively (Gilbert, 1981). This dependence on flower resources has been researched and used in habitat manipulation schemes to enhance agroecosystem services in farmlands (Landis et al., 2000; Wratten et al., 2012). However, most of these studies have been conducted in colder regions and the plant species and their attractiveness may differ in warmer and drier areas such as the Mediterranean. It is precisely in these areas where aphids, like the rosy apple aphid *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae), are major pests of apple (*Malus domestica*) (Rousselin et al., 2017).

In order to improve habitat management measures for aphid biological control, it is necessary to have a better understanding of the aphidophagous hoverflies present in the area and the flower resources they use. The aim of this work was to improve biological control of the rosy apple aphid. For this reason, we described the hoverfly species present in the fruit tree growing area of Lleida (Catalonia, NE-Spain), we identified the most attractive flower species present in this area to hoverflies, and we identified which hoverfly species prey on the rosy apple aphid.

Material and methods

Hoverflies naturally present in the area and their preferred spontaneous flora

During April and May of 2016 and 2017 adult hoverflies visiting the spontaneous flora in different semi-natural habitats in the fruit tree-growing area of Lleida were captured fortnightly with sweep netting in order to identify the species present in the area. The captured individuals were stored in glass vials with ethyl-acetate and the flower species where they were captured were recorded. Once at the laboratory the individuals were identified to genus level.

Hoverflies found in the rosy apple aphid colonies

In order to identify the hoverflies present in rosy apple aphid colonies, eight organic apple orchards were sampled fortnightly from April to May 2018. In each orchard, 12 infested shoots were collected, kept in plastic cups with gauze lids and taken back to the laboratory where they were kept at 20 °C and a photoperiod of 12 h:12 h light:dark. Shoots were left for approximately two weeks to allow the immature stages of the hoverflies to develop. Thereafter, adults were identified to genus level.

Results

A total of 65 individual hoverflies were captured on the spontaneous flora during the sampling period. These belonged to six different genera: *Sphaerophoria* (38.5%), *Episyrphus* (23.1%), *Eupeodes* (15.4%) *Melanostoma* (12.3%), *Paragus* (6.2%), and *Xanthogramma* (4.6%). The captured hoverflies visited 25 different plant species, belonging to 11 families. The most visited families were Compositae (52.3%) and Cruciferae (20.0%) with *Anacyclus clavatus* (29.2%) and *Diplotaxis erucoides* (9.2%) as the most visited plants.

Regarding the hoverflies genera present in the rosy apple aphid colonies, we recorded 67 adults that belonged to *Episyrphus* (59.7%), *Eupeodes* (20.9%), and *Sphaerophoria* (19.4%).

Discussion

This study shows that hoverflies occurring naturally in semi-natural Mediterranean areas have potential for conservation biocontrol of rosy apple aphid populations because of the genera found in the aphid colonies were the most abundant feeding on the spontaneous flora. These findings highlight the importance of spontaneous flora for hoverflies. However, few floral resources are present in the groundcover of orchards because farmers usually mow or shred it. For this reason, efforts should be devoted to manage both weed control and floral promotion in order to increase biological control and, therefore, environmental crop sustainability.

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Applied biotremology:
a new discipline for pest management.
Vibrational signals as semiophysicals

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Biotremology: from basic research to application

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Extended Abstract: The increased awareness that substrate vibrations are an ancient and widespread form of animal communication, and that vibration receptors are ubiquitous in organisms, led to the establishment of biotremology as a new discipline of study of mechanical communication (Hill and Wessel, 2016). Surface-borne mechanical waves provide organisms with information about their environment that is crucial for their survival and reproduction. This information is not limited to intraspecific vibrational communication, but also includes prey detection, predator avoidance and information about habitat quality.

While all insects possess highly sensitive vibration receptors, it is currently estimated that around 200,000 insect species use vibrational communication in a variety of intraspecific interactions (Cocroft and Rodríguez, 2005; Virant-Doberlet and Čokl, 2004). Vibrational signalling is particularly common in Hemiptera, which includes numerous major insect pests, like psyllids, leafhoppers, planthoppers and stink bugs. Reproductive behaviour in psyllids and stink bugs includes both, chemical and vibrational signals. However, in leafhoppers and planthoppers, mate recognition and location of the partner are mediated exclusively by substrate-borne vibrational signals (Čokl and Virant-Doberlet, 2003). During pair formation both sexes emit species- and sex-specific vibrational signals and vibrational interactions include continuous emission of vibrational songs and/or reciprocal exchange of male and female signals in a precisely coordinated duet. Competitive behaviour in males is expressed as emission of disruptive vibrational signals to interfere with ongoing duet and satellite behaviour, where intruding males silently approach the female duetting with another male (Mazzoni et al., 2009).

As a result of a growing realisation of the ubiquitous nature of vibrations in the environment and about the importance of vibrational signals and cues in insect behavioural decisions, the interest to exploit substrate vibrations in pest management also increased in recent years (Čokl and Millar 2009; Mankin, 2012; Polajnar et al., 2015). Every movement of the insect body or its parts induces vibrations in the substrate and such incidental vibrations induced by walking and feeding can be used for monitoring. Detailed knowledge of the biology, ecology and behaviour of the target species is essential in order to exploit or manipulate insect behaviour. Current applications include the use of species-specific vibrational signals emitted in sexual communication for automatic detection (Korinšek et al., 2016) or for playback to attract insects to traps (Mazzoni et al., 2017) and interruption of mating behaviour by playback of natural or synthesized disruptive vibrational signals (Mazzoni et al., 2009). Although vibrational mating disruption is a novel approach (Eriksson et al., 2012), it has been already transferred to the field in the vineyards (Polajnar et al., 2016; Krugner and Gordon, 2018).

Exploitation and manipulation of chemical signals is an integral part of IPM in several important crops and applied biotremology can provide innovative and efficient approaches in pest management of insects in which vibrational signals are an essential part of reproductive behaviour. Besides in depth studies of pest ecology and behaviour, implementation of such approach involves solving several technical challenges that include development of reliable and affordable sensors and playback devices, algorithms for automatic recognition of vibrational signals, as well as optimization of energy consumption, tailored to specific pests and specific field conditions. Taking into account that substrate vibrations are one of the most important and widespread sensory modalities guiding insect behavioural decisions, we believe that with more concentrated effort to solve current technical constraints, exploitation and manipulation of vibrational signals can be successfully included in IPM strategies and provide sustainable solutions in both, open field and greenhouse crop systems.

Key words: biotremology, substrate-borne vibrations, vibrational communication, Hemiptera, IPM

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Exploiting vibrational communication for more efficient trapping of *Halyomorpha halys* (Heteroptera: Pentatomidae)

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Extended Abstract: *Halyomorpha halys*, or the Brown Marmorated Stink Bug (BMSB) is commonly recognized as one of the most notorious invasive insect species. After the population outbreak in some areas of North America, a locally severe population outbreak is also occurring in the north Italy's Po valley (Maistrello et al., 2018), where BMSB has quickly become a key pest in fruit orchards (Maistrello et al., 2017). Its invasion potential is a cause of great concern among farmers and plant protection services in areas with suitable climate worldwide (Kriticos et al., 2017).

Control measures against *H. halys* currently consist of frequent applications of broad-spectrum insecticides, which is adding to environmental pesticide burden and disrupting IPM programs in affected areas (Leskey et al., 2012). Control efforts are hampered by incredible polyphagy of *H. halys*, its high dispersion potential, high reproductive potential and general robustness. Aside from biological control, behavioral manipulation using attraction to male-emitted sexual pheromones is one of the more promising approaches. The male-emitted aggregation pheromone has been identified recently (Khrimian et al., 2014) and is commercially available, although pheromone attraction of *H. halys* has the same shortcoming as in other species of stink bugs: traps' efficiency is more suited for monitoring than mass trapping purpose (Morrison et al., 2015) because attraction to the pheromone source is not precise in this family.

The likely reason for low efficiency of pheromone traps is that communication in stink bugs is bimodal. Pheromones attract males, females, and late-instar nymphs to general vicinity of the emitter (Khrimian et al., 2014), but the final approach during the courtship of stink bugs is mediated by substrate-borne vibrational signals (Virant-Doberlet and Čokl, 2004). Thus, we argue that mechanical vibrations can be used as a "bridge" connecting long-range attraction towards the source of synthetic pheromones and actual elimination.

To explore this possibility, an ongoing research effort was started in 2015, beginning with the basic description of vibration-mediated sexual behaviour of *H. halys*. Courtship involved a stereotypical exchange of signals between a male and a female, which started with the male spontaneously producing vibrational pulses (labeled MS-1), to which receptive females replied. A duet was formed, characterized mainly by sequences of shorter, regularly repeated female signals (FS-2), to which males reacted by searching for the source. Both partners emitted other types of signals during this time as well, ending with copulation (Polajnar et al., 2016).

We then performed a series of laboratory trials in which we reproduced male and female signals using an electromagnetic "shaker". In particular, we tested the potential aggregation function of the long, spontaneously produced MS-1 signals, and the male attraction to the regularly repeated FS-2 signals. We did not observe any effect of MS-1 playback, but FS-2 signals were attractive to adult males on several types and geometries of substrate (both

natural and artificial). We observed a so-called "loitering effect", where the male circled continuously around the exact location where the minishaker was attached to the experimental arena, and never left the area (Mazzoni et al., 2017). Thus, the FS-2 was selected for field trials. A trap prototype was constructed (Biogard®, CBC Europe S.r.l.), implementing a commercial aggregation pheromone dispenser, an electromagnetic "shaker" continuously vibrating the whole trap with the FS-2 sequence, and a circuit emitting electric shocks on top of the standard pyramid panel construction. The males were attracted to the trap by the pheromone and then driven inside cavities in its head, where they were killed by electrocution. Preliminary results show highly male-biased capture rates, thus proving the effect of vibrational playback *per se*.

Further work in the following seasons will focus on improving capture rate, which is crucial if the devices are to be used in mass trapping of *H. halys*. In particular, attractiveness to adult males only needs to be compensated to achieve meaningful reduction of population density.

Key words: biotremology, invasive pest, behavioural manipulation, multimodal trap, IPM

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Invasive pests: a challenge for IPM. Brown marmorated stink bug and other invasive hemipterans

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The invasive *Halyomorpha halys* in Europe: a challenge for integrated fruit production

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Extended Abstract: The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Stal (Heteroptera: Pentatomidae), is a highly polyphagous insect native to eastern Asia, rapidly spreading as a serious pest of global importance of many agricultural crops (Haye and Weber, 2017). It is also a household nuisance, due to the large overwintering aggregations inside man-made structures. It has successfully invaded and established in large areas of North America, in many central, eastern and southern European countries and, most recently, also in Chile (Leskey and Nielsen, 2018). BMSB caused millions of dollars losses in fruit orchards and horticultural crops in the USA, and it rapidly became a key pest of fruit orchards in Northern Italy with severe losses especially on pear (Maistrello et al., 2017). BMSB is also a serious pest of hazelnuts grown in Piedmont and in the countries along the Black Sea (Bosco et al., 2017).

The sneaking behaviour of BMSB makes its worldwide invasion virtually unstoppable. In fact, the propensity to aggregate in narrow, hidden microhabitats (e. g. slots of vehicles, packaging materials, suitcases, clothes etc.) exhibited particularly during overwintering phase, allows these bugs to “hitchhike” unnoticed on practically any type of goods, facilitating human assisted spread all over the world (Maistrello et al., 2018). Interceptions of BMSB are increasingly reported at transitional facilities in many countries, and models highlight the potential for further spread and establishment, threatening horticultural productions of areas with suitable climate in both hemispheres (Kriticos et al., 2017).

BMSB is currently rapidly spreading in European countries causing increasing damage in fruit orchards and other horticultural crops. In northern Italy a BMSB life table calculations performed under outdoor conditions demonstrated a high growth potential for BMSB bivoltine populations with high reproductive rates for both generations ($R0 = 24.04$ and 5.44 respectively) (Costi et al., 2017). A 3-year field survey in the same area showed that the biocontrol potential by native antagonists is presently very limited (Costi et al., 2018).

In the attempt to counteract the fast spread and high damage levels caused by BMSB, farmers intensify treatments with broad-spectrum insecticides, resulting in disruption of the existing Integrated Pest Management (IPM) programs (Leskey et al., 2012; Maistrello et al., 2017), with increasing negative environmental impact. Management of BMSB is particularly challenging. Chemical control proved to be scarcely effective due to the general robustness, as well as to the high polyphagy combined with high mobility of all instars, that results in continuous movements of the bugs between different host plants (Lee and Leskey, 2015). More sustainable approaches to manage BMSB in fruit orchards include the use of exclusion netting systems (Caruso et al., 2017) and behaviour-based strategies such as IPM-CPR (Blaauw et al., 2015). Evaluation of mass rearing of efficient native antagonists for inundative release is also being considered.

Field monitoring of BMSB presently relies on traps baited with aggregation pheromones that showed scarce efficiency and increased damage on the plants surrounding the installation point. The use of substrate-borne vibrational stimuli might favour the development of innovative multi-modal trapping devices as well as behavioural manipulation approaches (Mazzoni et al., 2017).

Key words: brown marmorated stink bug, biological invasions, human assisted spread, fruit orchards, IPM

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Predatory ability of wild generalist predators against eggs and first instar nymphs of *Halyomorpha halys*

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Extended abstract: *Halyomorpha halys* (Heteroptera: Pentatomidae), native to East Asia, is a polyphagous species with more than 300 recognized host plants (Rice et al., 2014). Thanks to its close association with man-made structures during the overwintering period, which facilitates human assisted dispersion (Maistrello et al., 2018), this insect is a fast spreading invasive pest of fruit orchards and many other crops both in the USA. (Leskey and Nielsen, 2018) and in Italy (Maistrello et al., 2017).

Direct damages caused by this insect include malformation, suberisation, discoloration, necrotic areas and watery rot, all of them causing damage that render products unmarketable (Rice et al., 2014). To manage the invasion of this pest, farmers have increased the use of broad spectrum insecticides, resulting in disruption of the most innovative IPM strategies, with serious risks both for the economy and the environment (Maistrello et al., 2017; Leskey et al., 2012). The use of broad spectrum insecticides can also lead to the reduction of populations of beneficial insects, such as pollinators, parasitoids and predators, in consequence altering the natural balance. Therefore, it is necessary to identify more sustainable strategies to control this pest. *H. halys* is an alien species for Italy, thus species specific antagonists are absent in the newly invaded areas. However, native species of generalist predators and parasitoids could eventually use *H. halys* as prey/host, becoming good candidates for the biological control. As matter of fact, generalist predators can be more effective on invasive species than the specialist predators in their introduced range (Chang and Kareiva, 1999).

In this study, the predatory ability of European native generalist insect predators collected from the wild settings was verified on eggs and young instars of *H. halys* using a no choice test. For the study, the following European native species were tested: *Forficula auricularia* (Dermaptera, Forficulidae), *Harmonia axyridis* (Coleoptera, Coccinellidae), *Pholidoptera littoralis* (Orthoptera, Tettigonidae), *Nagusta goedelii* (Rhynchota, Reduviidae), *Rhynocoris iracundus* (Rhynchota, Reduviidae) and two species of the *Himacerus* genus (Rhyncota, Nabidae). These predators were captured in green areas and urban parks of Reggio Emilia (Northern Italy) using tree beating and sweep net techniques.

After a starving period of 24 hours, each predator was individually placed inside a transparent box with a bean plant bearing at least two well developed leaves and the *H. halys* prey item, that consisted of one egg mass or a hatched egg mass (with first instar nymphs) or 5 second instar individuals. *R. iracundus* was also tested with adults of *H. halys*. For each treatment, a control treatment consisted the same set up but without the predator. After 48 hours the survival of the preys specimen and the predators was recorded, and, for the egg masses, emergence was checked during the following 5 days.

From the comparison between the survivors of the treatment group (prey and predator) and the control one (no predators) it emerged that *P. littoralis* is the only species capable of predating on all the instars tested, including eggs. The other predators did not feed on the eggs, although *F. auricularia* damaged them. *N. goedelii* significantly predated the first instars, while *R. iracundus* and nabids of the *Himacerus* genus preyed on second instars. *R. iracundus* significantly predated also on adult individuals of *H. halys*.

This study showed the potential of some European native generalist predators for the biological control of *H. halys*, in particular of *R. iracundus* and *P. littoralis*. The augmentative release of these predators into cultivated areas could negatively affect the survival of *H. halys* in the fields, and especially in combination with augmentative release of native parasitoids, such as *Anastatus bifasciatus* (Hymenoptera, Eupelmidae) (Costi et al., 2018), it might result in increased impact and effectiveness of biological control against this invasive pest.

However further research is necessary both in the laboratory (preference tests) and in the field to verify their effectiveness and the potential impact on the agroecosystem.

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Managing *Halyomorpha halys* by means of exclusion netting: trials 2016-2018

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Extended Abstract: The invasive polyphagous crop pest *Halyomorpha halys*, also known as a brown marmorated stink bug (BMSB), is rapidly spreading in Italy and other European countries. In order to cope with its high harmfulness in fruit orchards, in Emilia-Romagna Region, Northern Italy, farmers have increased the use of broad-spectrum insecticides, resulting in disruption of the most innovative IPM strategies, such as mating disruption (MD) or the use of microbiological products (e. g. CpGV), with serious agro-environmental risks (Maistrello et al., 2017). Several investigations are underway in order to identify more sustainable control strategies against BMSB that include "IPM-CPR" (Blaauw et al., 2014), "attract and kill", trap crop (Morrison III et al., 2015), and exclusion netting. The latest method, designed by the French extension services in 2005 (Severac and Romet, 2007) to control the codling moth, has been tested also in Italy. In both countries, it has been proved that exclusion netting represents one of the most readily available tools for crop protection and an environmentally friendly alternative to pesticides (Alaphilippe et.al., 2016). As a matter of fact, this method is currently applied on several thousand hectares of orchards in both countries (Caruso et al., 2017) and other applications are currently being carried out in different parts of the world (Chouinard et al., 2016). The present study aimed at investigating exclusion nets as a potential strategy to prevent BMSB damage in pear orchards. The experiments performed during 2016-18 included:

1. A laboratory trial was performed to evaluate the effectiveness of different net's mesh size (2.2 × 2.2 mm, 4.0 × 2.5 mm, 5.0 × 1.3 mm, 7.0 × 3.0 mm), against different instars (II, III, IV, V and adults).
2. Field trials, conducted in commercial orchards in the province of Modena, using different types of net as follows:
   a. eight IPM orchards with "anti-hail nets" (mesh size of 7.0 × 3.0 mm) in comparison with 8 uncovered orchards;
   b. eight IPM orchards with "whole orchard-nets" (made of an anti-hail net at the top with mesh size of 7.0 × 3.0 mm, and the net whit mesh size of 4.5 × 2.0 mm on the perimeter), in comparison with 8 uncovered orchards;
   c. eight organic orchards with single-row nets (mesh size of 4.5 × 2.0 mm) in comparison with 8 uncovered orchards.

The results of laboratory trials show that 4.0 × 2.5 and 5.0 × 1.3 mm mesh size net, are able to exclude the most damaging instars, such as adults and older nymphs (IV-V instars). Nets with smaller mesh size (2.2 × 2.2 mm) exclude also third instar nymphs, with no significant efficiency gains. Therefore, the nets 4.0 × 2.5 and 5.0 × 1.3 mm, commonly used

in exclusion netting and already widespread in the field, could be considered as reliable for this type of application. The results of field trials indicated that:

- "anti-hail net" alone reduced damage on pears about 50% compared to un-netted orchards but no differences were detected in the number of applied insecticide treatments (i. e., average of 6.5 treatments/year);
- in "whole orchard-net" damage on pears was reduced about 80% compared to un-netted orchards and insecticide treatments were reduced about 35%;
- in "single-row net" fruit damage was reduced by almost 90% compared to un-netted orchard in organic farms, where no effective insecticides are available/authorized.

These results demonstrated that exclusion netting systems are an efficient and sustainable tool in the framework of IMP and Organic programs for BMSB. However, this species being a very mobile insect (Lee and Leskey, 2015), with five instars of different size able to move in narrow spaces, the efficacy of exclusion netting is not always close to 100%, and some additional insecticide treatments might be necessary. These observed positive results induced the Regional administrations of Northern Italian Regions to contribute financing the installation of exclusion netting with either whole or single-row nets in fruit orchard farms.

Key words: Brown marmorated stink bug, exclusion netting, integrated pest management, pear orchards, invasive insect

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Establishment and current status of *Halyomorpha halys* damaging peaches and olives in the prefecture of Imathia in Northern Greece

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Extended Abstract: *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae), or brown marmorated stink bug (BMSB), is a temperate/subtropical species native to China, Japan, Korea and Taiwan. However, in the last few years, the species was detected in different parts of the world, mainly in North America and most of Europe (Leskey and Nielsen, 2018; Cianferoni, 2018). In northern Europe, particularly, the species has been recorded in the north-east of France in 2012 (Callot and Brua, 2013) and also in Île-de-France (Garrouste et al., 2015, Maurel et al., 2016) and in Corsica (Kriticos et al., 2017). The species was found in 2007 in Liguria, Italy (Maistrello and Dioli, 2014; Dioli et al., 2016) and in 2011 in Athens, Greece (Milonas and Partsinevelos, 2014).

H. halys has a wide host range, including fruit trees, field crops, forest trees as well as wild hosts (Bergman et al., 2016). The BMSB feeding may occur on stems, leaves, shoots although nymphs and adults prefer to feed on developing and mature fruits and seeds of their host plants (Martinson et al., 2015). In this study we present data regarding the appearance and the damage caused by this species for the first time in fruit trees in Northern Greece.

Fieldwork was conducted during 2018 from July to August in two peach fruit orchard plantations located in Nea Nikomidia and Kavasila and one olive tree orchard located in Kavasila in Northern Greece. Each peach orchard, treated as an experimental block, had a parallelogram shape and was separated from other orchards by 10-20 m wide cultivation road. Each block consisted of about 40 peach trees (industrial candy variety Loadel) planted in a regular rectangular 5 × 5 m grid with 10 rows and 10 columns. The tree height was about 2.5-3.5 m, and the trees were about 10+ years old. The olive orchards consisted of about 30 olive trees (halkidiki variety) planted in a 5 × 6 grid with 8 rows and 10 columns. The height of the tress was about 1.5-2.5 m. No insecticides were applied to these orchards and only matting disruption dispensers were used for against *Anarsia lineatella* and *Grapholita molesta*.

One 2-year old branch located about 1-2 m above the ground, where the majority of fruits or olives hang, was used as a sampling unit. In order to have a representative grid of samples in each experimental block every second tree from every second row of each experimental block was used to collect data. Exhaustive counts of sampling units were performed and all fruits on the given branch were inspected for the presence of *H. halys* individuals as well as feeding damages. In total, 132 sampling units were utilized in this study with all fruits or olives inspected.

The presence of *H. halys* individuals (mostly nymphs) was very high in both inspected peach and olive orchards. The feeding damage in most cases was severe and resulted in significant economic losses. The initial damage caused by *H. halys* caused fruit deformation which in most cases lead to the suberifications. In the peach orchards the most severe damage

tended to be located on the periphery of the orchards. The percentage of damage to peaches due to *H. halys* feeding was higher than 50 percent (63 ± 3%). The percentage of damage with at least one feeding entrance to olives was higher than 80 percent (87 ± 6%). However, currently it is unknown whether population of *H. halys* may cause severe damages in other fruit orchards which received pesticides. Nevertheless, since the first record of *H. halys* in 2011 in Athens, at present the pest has occurred regularly in Northern Greece causing considerable damage in screened peaches and olives.

Experience from the USA and Italy has shown that despite being polyphagous, this pest has a preference for peach as the favourite host (Bariselli et al., 2016). However, in this work the damage that occurred on olive trees was significantly higher compared to peach fruits arousing considerable concern. The increasing distribution of this pest, as well as its preference to peach and olives, creates urgent need to develop efficient and sustainable management strategies in the near future.

Key words: marmorated sting bug, sampling, integrated control

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Alternative methods to manage brown marmorated stink bug, *Halyomorpha halys*

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Abstract: As the insecticide treatments remain the most effective tools to manage the brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stäl) in Pennsylvania fruit orchards, we also attempt to develop alternative methods to manage this pest in more sustainable, environmentally responsible way. During the 2018 season we continued to test deltamethrin insecticide treated nets baited with BMSB monitoring lures to monitor BMSB populations around commercial apple orchards. Starting from late July, the treated nets were placed on 2.4 m high shepherd hooks and placed outside of the apple blocks (5-15 m away from the orchard border), at about 50 m distance between each net, however, higher number of nets were placed in the direction of potential influx of BMSB from outside vegetation such as woods. The standard BMSB monitoring sticky traps were used to assess the pest population in the orchards surrounded by the net traps and control blocks. At the peak BMSB activity periods high numbers of BMSB adults and nymphs, as well as native stink bugs, were collected under the net traps. The practical results from our trials are very promising and potentially can be used for the development of alternative BMSB trapping method(s).

Key words: *Halyomorpha halys*, insecticide treated nets, BMSB monitoring, pome fruit, stone fruit

Introduction

The current recommendations to manage brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stäl) continue to rely mostly on the judicious use of insecticide applications. Although, due to continuous improvement in our understanding of BMSB biology and behavior, we have abandoned the initial recommendations of multiple, calendar-based applications, the broad spectrum insecticides still remain the most reliable and economical tool to control BMSB. Utilization of BMSB monitoring traps to better understand the movement of the bugs into orchards and the relative abundance of the pest helped in the decision process "if and when" the treatments are really necessary (Krawczyk et al., 2017).

During last few seasons our research activities concentrated mostly on the development and validation of new approaches in effective BMSB monitoring systems and effective management strategies. The utilization of "ghost traps" and Attract and Kill (A&K) strategies proved very promising in reducing the reliance on pesticides to reduce fruit injury caused by BMSB (Morrison et al., 2018). During the 2016 and 2017 seasons we evaluated commercially available insecticide treated nets (Vestergaard, Lausanne, Switzerland) baited with regular BMSB attractants as a potential tool for use in the A & K program (Kuchar et al., 2018).

Insecticide treated nets placed between the orchard and the source of invading BMSB (e. g., woods) appear to be effective in capturing BMSB adults and nymphs (Krawczyk et al., 2018).

During this project we evaluated and validated the options for the practical use of insecticide treated nets for the monitoring and potential control of brown marmoarted stink bug. The research emphasis was placed on understanding the most effective and economical ways to incorporate insecticide treated nets into practical pest management strategies.

Material and methods

The effectiveness of the "ghost traps" for BMSB monitoring and its potential impact on the pest management was evaluated in four commercial fruit orchards located in southern Pennsylvania. The commercially available long-lasting net containing 0.4% of technical grade deltamethrin (D-Terrence® net, Vestergaard S.A., Lausanne, Switzerland) was placed on 2.4 m ft high two arm metal shepherd hooks. The "ghost traps" were placed outside of the orchard, close to the potential sources of BMSB influx. The number of "ghost traps" per location varied as it was based on the size of the orchard. The smallest experimental orchard (about 0.9 ha) had 5 traps, while the largest block (about 10 ha) was surrounded by12 traps. The traps were spaced at the average of 50-70 m apart. Each "ghost trap" was baited with three commercial BMSB monitoring lures (Pherocon® BMSB Dual Lure, Trece Inc, Adair, OK). The "ghost traps" were placed in the orchards during the first two week of July and observations were conducted until early October. A rectangular piece (0.7 × 0.9 m) of aluminum screen mesh (ScreenGuard, Lowe's Store, 18 × 16 weave) was placed under each trap to monitor the number of dead BMSB adults and nymphs under each trap. Three sticky traps (Pherocon® StinkBug STKY™ Dual Panel, Trécé, Adair, OK) baited with a single BMSB lure, the same as used in the "ghost traps" were placed in each orchard treated with the nets and in a closely localized control block. The numbers of dead BMSB adults and nymphs under each individual "ghost trap" and captured on the sticky traps were recorded weekly.

Additional orchards were used to determine the optimal number of BMSB lures per "ghost trap" and to compare the effectiveness of 'old" nets used during the 2017 season to the effectiveness of brand new, not field exposed nets. For the lure optimization project, multiple "ghost traps" assembled the same way as in the monitoring orchards were placed along an edge of an apple orchard bordering wild woods, at the distance of about 30 m between individual trap. Various numbers of lures: 0, 1, 3, 5 per trap were randomly assigned to an individual trap and each treatment was replicated three times.

The net longevity trial included treatments of a) new, never used D-Terrence net; b) one year old D-Terrence net used during the 2017 season and stored for the winter in the cold storage; c) control net (net identical to the D-Terence net but not treated with insecticide); and d) tall Pyramid trap (AgBio, Inc. Westmister, CO). Additionally, a single trap ("legacy" trap) had a net which was not stored during the winter but was kept outside the whoel year including winter. All nets and traps were baited with three Pherocon BMSB Dual Lures per trap.

Results and discussion

The orchards used for the "ghost net" project experienced various pressure from the BMSB during the 2018 season. The highest numbers of BMSB adults and nymphs were observed at the LB location with the average weekly capture of 43 bugs per trap (Figure 1). The numbers of collected BMSB in the remaining orchards were lower oscillating around 15 BMSB per trap/week. At each location the highest numbers of collected BMSB were observed from Aug. 30 to Sep. 27. The BMSB captures were generally lower (below 10 BMSB/trap/week) in July, early August and in October.

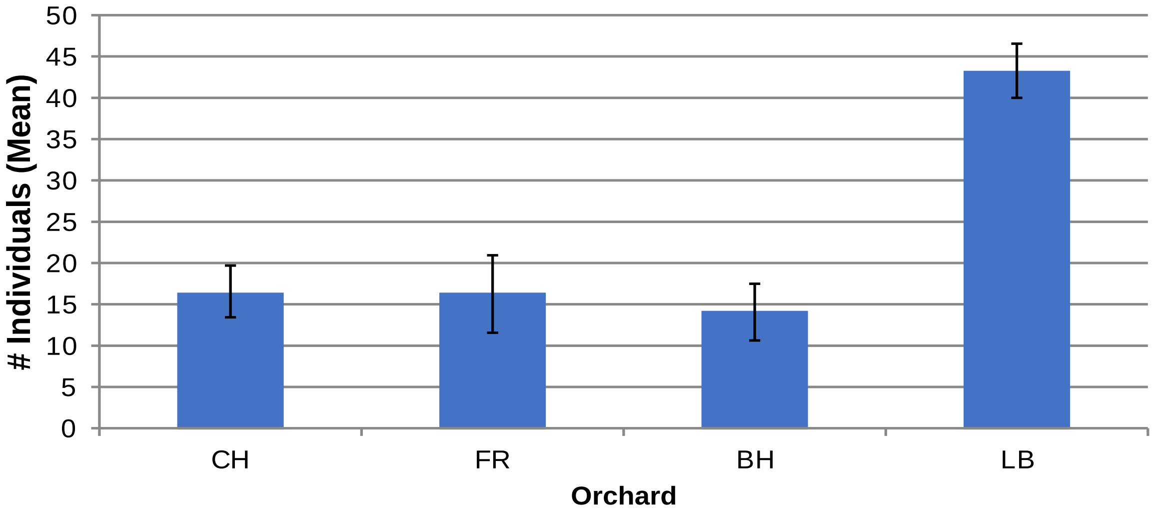

Figure 1. Average weekly captures of BMSB adults and nymphs per “ghost trap” in orchards located in Lancaster and Adams Counties, PA. The numbers of “ghost traps” per orchard: CH had n = 12 traps; FR had n = 5 traps; BH had n = 10 traps and LB had n = 12 traps. PSU FREC 2018.

The number of lures used for baiting insecticide nets significantly influenced the numbers of collected stink bugs (Figure 2). The number of lures placed per single trap was positively correlated with the higher numbers of collected BMSB adults and nymphs. Despite numerical differences, the disparities in the numbers of collected bugs between traps baited with three and five lures were not significant.

The net longevity studies (Figure 3) revealed good effectiveness of nets used for two consecutive seasons (2 Yr net). The Yr 1 net (new nets) and 2 Yr net collected much higher numbers of BMSB adults and nymphs than the 4 ft tall Pyramid traps baited with the same number of lures. A single “legacy” ghost trap kept under outside conditions during previous summer and winter captured similar number of stink bugs as the Pyramid traps. The observed numerically higher captures of BMSB instars in the 2 Yr net traps can possibly be associated with the higher levels of the BMSB aggregation pheromone residues, as some still active residues could be attached to the nets from the exposure to pheromones during the 2017 season. We plan to evaluate the activity of each net age category under laboratory conditions during the 2019 season.

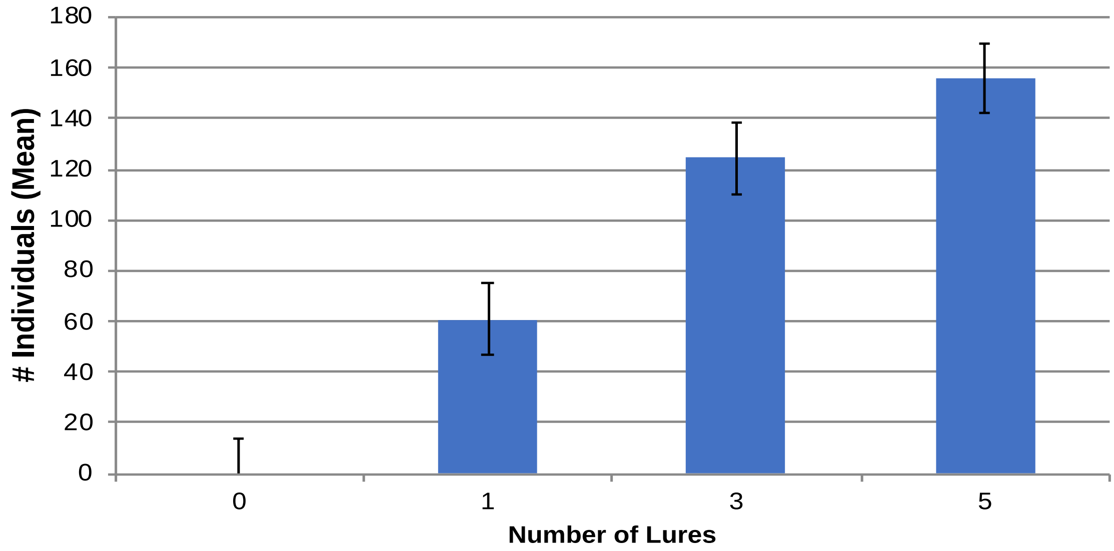

Figure 2. The lure load optimization trial. Average weekly captures of BMSB adults and nymphs per "ghost traps" baited with various number of BMSB monitoring lures per trap. York Spring, PA. PSU FREC 2018.

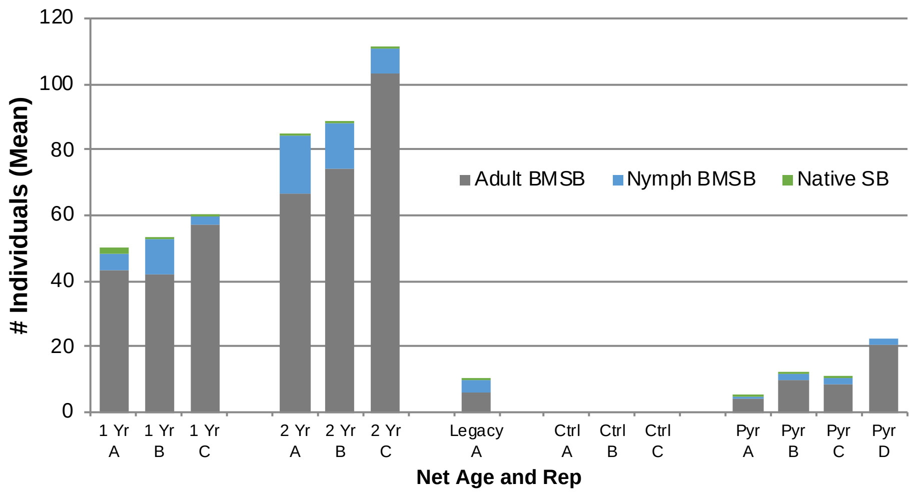

Figure 3. The net longevity trial. Average weekly captures of BMSB adults and nymphs per single "ghost trap". Lancaster, PA. PSU FREC 2019.

The effective use of the long lasting insecticide treated nets in combination with selective, narrow spectrum insecticides for the control of other fruit pests provides great opportunity to re-establish the principles of Integrated Pest Management (IPM) practices and increase the importance of beneficial organisms capable to manage many secondary pests such as scales, aphids or mites threatening orchards under BMSB management practices based on broad-spectrum insecticides.

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Biocontrol of the invasive brown marmorated stink bug *Halyomorpha halys*

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Extended abstract: The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive polyphagous insect pest in the United States, Canada, South America, and Europe, originating from Asia. BMSB has disrupted successful integrated pest management programs (IPM) in fruit orchards, as the most effective control measure for the pest is through the intensive use of broad spectrum insecticides. Use of these insecticides often leads to secondary outbreaks of other orchard pests such as mites, aphids, and scales. An essential step to reestablish these once effective IPM programs is to understand the species composition and potential role of natural enemies in influencing BMSB populations. Since the major outbreak of BMSB in the Mid-Atlantic region of the United States in 2010, several research groups have conducted BMSB surveys to determine BMSB predation and parasitism across agricultural landscapes. These surveys have found native hymenopteran egg parasitoids, including members of the families Scelionidae and Eupelmidae, attacking a low proportion of BMSB eggs, therefore indicating the ability of these native parasitoids to find the eggs of this invasive species. Additionally, BMSB sentinel egg surveys in Maryland during the 2014 season discovered an adventive population of *Trissolcus japonicus* (Ashmead), one of the most successful egg parasitoids of BMSB in its native range of Asia.

The aims of this study were to understand which species of native egg parasitoids attack BMSB, to determine if *T. japonicus* populations are present and growing in and around commercial orchards in Pennsylvania, and to understand which landscape factors play a role in the parasitism of BMSB eggs. BMSB sentinel egg masses were deployed weekly from June through August in 2017 and 2018 at organic and conventionally managed orchards. Egg masses were collected fresh (< 48 hours since laid) from caged BMSB colonies at the Pennsylvania State University Fruit Research and Extension Center (Biglerville, PA), or purchased from an indoor colony (New Jersey Department of Agriculture). BMSB egg masses were deployed on the underside of leaves of known host plants in the orchard, in the surrounding woods, and at the border between them. Eggs were deployed as fresh or frozen, and either left on the leaves of colony host plants or affixed to a white square of card stock paper using double sided sticky tape. Egg masses were deployed for up to 72 hours before collection and placed in a growth chamber for at least six weeks to allow parasitoids to emerge. All emerged parasitoids were identified to genus or species, and rates of parasitism were calculated. Egg masses were dissected after six weeks to determine rates of partially developed parasitoids. In addition to these deployments, egg masses found in the landscape laid by native stink bug species and BMSB were collected to determine natural rates of parasitism. Additional surveys for parasitoids were conducted in three additional locations utilizing yellow sticky cards baited with the BMSB aggregation pheromone. Finally, in 2018,

field sites were monitored for the presence of flowering plants, in order to determine if these food sources for the parasitoids are associated with higher rates of parasitism.

In both years, parasitoid species within the families Scelionidae and Eupelmidae were found to attack BMSB egg masses in the orchard and surrounding landscapes. In 2017, *T. japonicus* populations were detected in two Pennsylvania counties on yellow sticky cards. In 2018, *T. japonicus* populations were detected in three additional counties in Pennsylvania on yellow sticky cards, and also were reared from both BMSB sentinel egg masses and naturally laid BMSB egg masses at the sites where sentinel eggs were deployed. In both years, less than 5% of deployed sentinel egg masses were parasitized. At the organic orchard location, fresh BMSB eggs deployed on leaves were parasitized at a higher rate than fresh and frozen BMSB eggs deployed on index cards, and more parasitoids emerged from egg masses deployed in the wooded sites surrounding the orchard. Wild egg masses (including native stink bug eggs) had a much higher rate of parasitism, with over 55% of wild egg masses being parasitized. Understanding when and where these egg parasitoids are attacking BMSB, and how they find the eggs, will aid in determining the how to conserve these biological control agents.

Key words: brown marmorated stink bug, *Halyomorpha halys*, egg parasitoid, *Trissolcus japonicus*

Acknowledgements

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Habitat and tree species effects on *Trissolcus japonicus* (Hymenoptera: Scelionidae) detections in Virginia, USA

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Abstract: Yellow sticky traps deployed in mid-canopy of host trees of *Halyomorpha halys* (Hemiptera: Pentatomidae) were used to examine the effect of habitat type and tree species on the frequency of *Trissolcus japonicus* (Hymenoptera: Scelionidae) detections in Frederick County, Virginia, USA. Traps were deployed in female tree of heaven growing in three habitat types from May through September, 2018, and also deployed in pairs of tree hosts of along forest edges from June through August, 2018; each pair of trees contained female tree of heaven and hackberry, black locust, black walnut, or cherry. Weekly evaluations revealed significantly more *T. japonicus* captures in hedgerows than at forest edges, and numerically more captures in isolated patches of trees than at the forest edge. *Trissolcus japonicus* was captured in all host species, with no significant differences between any host pair. In 2018, *T. japonicus* captures occurred from 11 May until 9 September, with distinct peaks in early July and early August.

Key words: Parasitoid, biological control, foraging ecology

Introduction

Brown marmorated stink bug (BMSB), *Halyomorpha halys*, continues to pose significant pest issues in the USA (Leskey and Nielsen, 2018). To date, native parasitoids and predators have not adequately regulated its populations (Abram et al., 2017). In Asia, *Trissolcus japonicus* is a key parasitoid of BMSB eggs (Yang et al., 2009; Zhang et al., 2017). Adventive populations of *T. japonicus* detected in the USA since 2014 show indications of geographic range expansion (Talamas et al., 2015; Buffington et al., 2018). Thus, tracking the spread and changes in abundance of *T. japonicus* on a large geographic scale has become a main research priority, requiring the development of efficient sampling methods and information about its foraging ecology. Most BMSB egg masses collected from felled female tree of heaven, *Ailanthus altissima*, were found in the mid- and upper tree canopy, and all masses parasitized by *T. japonicus* were from those canopy locations (Quinn et al., 2019). Yellow sticky traps deployed in the mid-canopy of BMSB host trees were effective for detecting *T. japonicus* (Quinn et al., in press). This sampling approach was used in 2018 to sample *T. japonicus* in Frederick County, Virginia, where the frequency of its detections has increased markedly since 2015.

Material and methods

Habitat effects

Bamboo poles with a hook were used to suspend yellow sticky traps in the mid-canopy of mature female tree of heaven growing in hedgerows, forest edges, and spatially isolated patches (n = 5 per habitat). Traps were replaced weekly from May through September, 2018 and all captured parasitoid specimens of interest were removed *in situ*, identified, and sexed.

Tree species effects

Traps were placed in the mid-canopy of pairs of trees at the forest edge, separated by 10-25 m. Each pair included female tree of heaven plus black locust, black walnut, hackberry, or black cherry (n = 5 per pairing). Traps were replaced weekly from mid-June to mid-September, 2018, and all parasitoid specimens of interest were identified.

Results and discussion

Habitat effects

There was a significant effect of habitat type on *T. japonicus* captures (Kruskal-Wallis and Bonferroni corrected Dunn's test, $\chi^2 = 8.31$, df = 2, $P < 0.02$). Most captures were recorded from hedgerows and isolated patches (Figure 1). Of the 160 parasitoid specimens captured and identified, *T. japonicus* predominated (101), but others known to attack BMSB eggs included *T. euschisti* (29), *T. brochymenae* (10), *T. thyantae* (1), *T. edessae* (1), *Telonomus podisi* (9), and *Telonomus* spp. (9). Captures occurred on most weeks between 11 May and 9 September, with distinct peaks in early July and early August, although males were captured only between June and August (Figure 2). Of the *T. japonicus* captured, 83.2% were female (Figure 2).

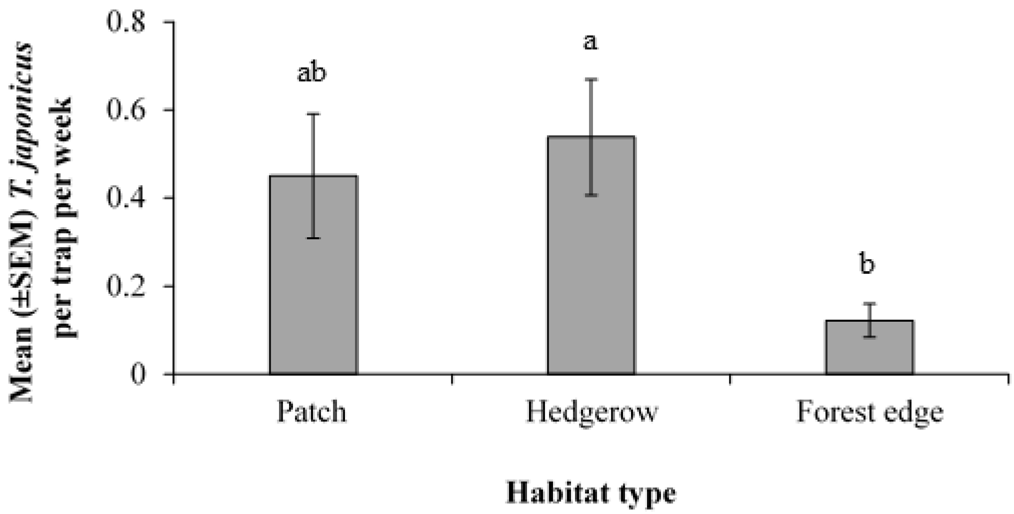

Figure 1. Habitat effects on *T. japonicus* captures in 2018.

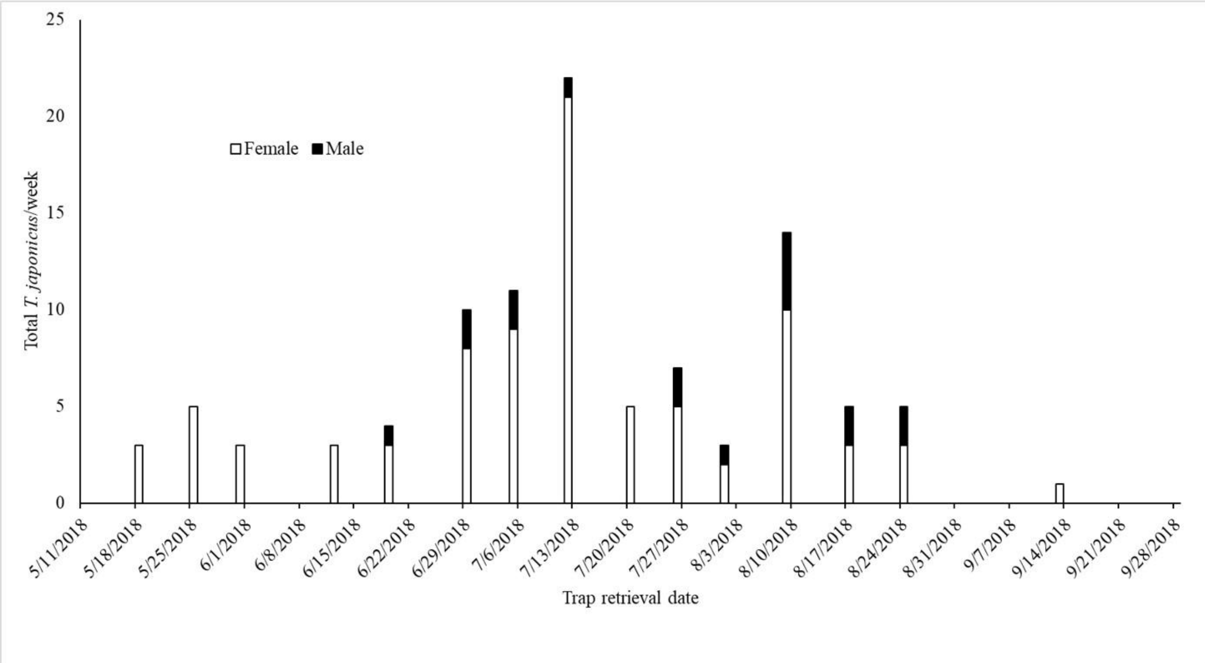

Figure 2. Seasonal captures of *T. japonicus* in 2018.

Tree species effects
There were numerical but not significant differences in captures between species in some pairs ($P \geq 0.09$, Wilcoxon signed-rank test) (Figure 3).

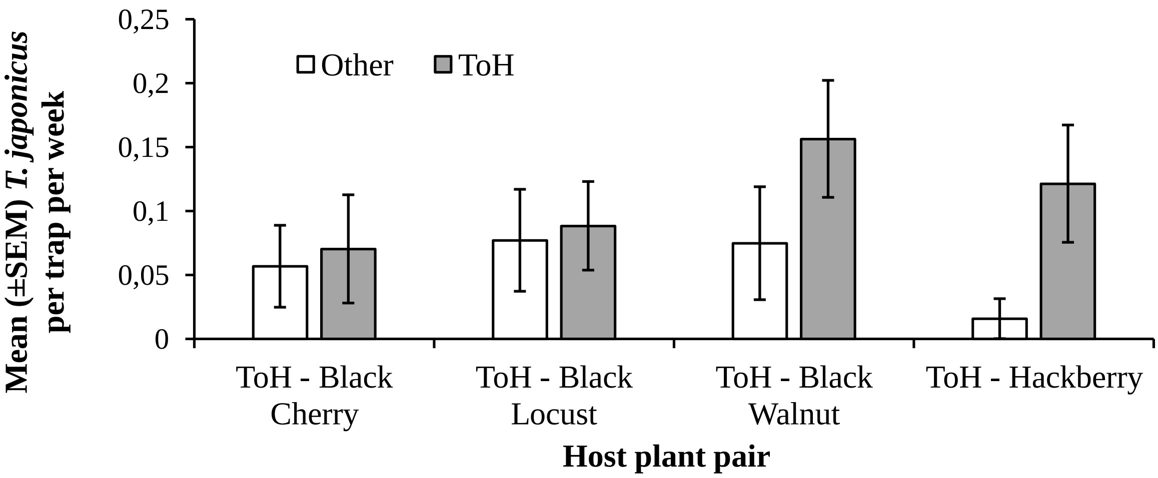

Figure 3. Mean captures of *T. japonicus* per trap per week by host plant in 2018.

Conclusions
These studies have capitalized on a well-established population of *T. japonicus* in Frederick Co., Virginia and used trap-based sampling to generate novel and timely information about aspects of its foraging ecology. Results from studies on the effects of habitat type, tree

species, and its seasonal phenology can inform the placement and deployment timing of traps, to optimize the likelihood of *T. japonicus* detection and therefore the effectiveness and efficiency of efforts to track spatial and temporal changes in its presence and abundance. These results also provide baseline data for assessment of temporal changes in the species composition and relative abundance of *H. halys* parasitoids.

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Pheromone traps for *Halyomorpha halys*: a three-year comparison in pear orchards

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Extended Abstract: The invasive *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) is one of the emerging pests for Italian agriculture. First recorded in Modena (northern Italy) in 2012, it spread progressively in Italy with great rapidity (Maistrello et al., 2018). Since 2014, damage on pear, apple, peach and kiwi attributed to this insect were reported in fruit-growing areas of northern Italy (Bariselli et al., 2016). A survey conducted in 2016 by the Technical Office of Cooperative Fruit Modena Group, on 60 farms managing a total of 400 ha of pear orchards in Modena Province, estimated the economic damage induced by *H. halys* at € 3,072,797 (Maistrello et al., 2017).

H. halys has a high dispersal ability and a marked tendency to move between different host plants (Lee and Leskey, 2015). These ethological characteristics make active monitoring techniques of (frappage, and visual inspections) unsuitable for this pest. These difficulties coupled with the strong concern for the high damaging potential of *H. halys*, led farmers to intensify treatments with broad-spectrum insecticides (Maistrello et al., 2017).

For this reason, the identification of traps able to reliably estimate the presence and the abundance of the pest is a fundamental step to re-establish the principles of integrated pest management, reducing the use of insecticides and guaranteeing at the same time a more effective and rational control of *H. halys* populations.

Traps based on aggregation pheromones are currently the most promising tools for monitoring the presence of *H. halys* and, in the United States, these traps were suggested to support management decision in apple orchards (Short et al., 2017).

In previous trials in the provinces of Modena and Reggio Emilia, the Rescue Pheromone Traps proved to be effective in capturing *H. halys* specimens during the entire production season (Maistrello et al., 2016). Nevertheless, two major critical points were detected, that pose a limitation for their use in commercial fruit orchards. First: a strong variability in catches, probably caused by several external factors such as the attractiveness of surrounding plants, the vigor and the bearing of the plant on which they are installed and the insect population density. Second: an increase of fruit damage on plants located up to 8 meters from the installation point (Vaccari et al., 2018).

The aim of this work, carried out in 2016-2018, was to identify the most reliable system for monitoring *H. halys* in pear orchards, assessing the effectiveness of various trap designs (Rescue Pyramid Trap, AgBio Pyramid Trap, Trécé Double Funnel, Trécé Sticky Sheet) and bait types (Rescue, AgBio, Trécé) available on the Italian market, over the entire production season (beginning of May-second half of October).

Every year the trap comparison was replicated four times, and each replicate was located on the external row of an organic/untreated pear orchard, that was bordered by a hedge or an uncultivated area. Within each replicate, traps were spaced every 30-40 m and their position

was changed randomly every week. The traps were checked weekly, counting *H. halys* adults and nymphs. Data were analyzed by means of analysis of the variance for repeated measures. The effect of the thesis was evaluated by F test ($\alpha = 0.05$) and a t test ($\alpha = 0.05$) was applied for comparison between the thesis.

Preliminary results for 2016-17 show significant differences between trap types and among pheromone lures. Among the commercial combinations (Trap + Bait of the same company) Rescue Trap, proved to be the best devices for monitoring *H. halys* in pear orchards, provided it is correctly installed on the plant, firmly anchoring the base of the trap to the trunk or to a main branch.

Considering only the bait effectiveness, Trécé lures appeared much more effective and reliable than Rescue and AgBio lures.

The combination of Trécé lures with pyramid models (either Rescue or AgBio) provided better reliability of the traps in pear orchards.

Key words: brown marmorated stink bug, aggregation pheromone, invasive species, integrated pest management

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First approach to manage the invasive *Halyomorpha halys* in Italy: a three-year project

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Extended abstract: Soon after the first detection in Italy of the invasive *Halyomorpha halys* (Stål), commonly named Brown Marmorated Stink Bug (BMSB), high losses were reported in Emilia Romagna region on fruit orchards, with up to 60% fruit damaged in integrated crop production and over 90% in organic management (Maistrello et al., 2017). Emilia Romagna is a very intensive fruit production region with over 56,164 hectares of fruit crops and 53,456 hectares of grapevine (ISTAT, 2017) and BMSB created high concern in the whole agricultural sector.

The fast spread and damage levels caused by BMSB forced farmers to increase the numbers of broad-spectrum insecticide treatments, disrupting the Integrated Pest Management (IPM) strategies previously applied on pome fruits, that included mating disruption and use of microbiological products (e. g. CpGV). To face this serious threat, farmers, technicians and researchers of public and private sectors have started in 2016 a three-year project to identify new approaches to manage this pest. This project, called "HALYS", encompasses a series of activities including insights on BMSB biology and monitoring techniques, agro-ecological studies, the biocontrol potential of native antagonists, evaluation on the use of physical barriers and on chemical products authorized in Italy. In particular, the project aimed to identify both prompt effective solutions as well as low impact environmental solutions to control the BMSB infestations.

Since 2016, a yearly based investigation on BMSB survival during overwintering and its biology, based on bugs kept outdoors, the dates and numbers of bugs successfully coming out of overwintering, the beginning of mating of both generations, the emergence of nymphs and adults were periodically communicated to Regional Plant protection Service and to integrated production advisors. This information combined together with the data on egg to adult survival and developmental times are of immediate practical utility for the advisors and farmers, aiding in decision-making, as they are also crucial for the elaboration of forecasting models.

Based on the results achieved so far, none of the chemicals tested in the laboratory showed a satisfying efficacy to kill eggs whereas some effects were recorded on emerging nymphs. The semi-field trials showed a certain persistency with a residual effect at 3-7 days, of chemicals belonging to organo-phosphates, neonicotinoids and pyrethroids, applied at 3 different timings against BMSB nymphs and adults. In open field trials located in the pear

orchards some chemicals caused a certain mortality of BMSB in the short period, with nymphs being more sensitive than adults to sprays. Besides, in agroecological studies an important influence of the surrounding agroecosystem was observed, together with an important impact of overwintering and refuge places of BMSB (e. g., buildings, ornamental and wild trees, hedges) as source of infestation or re-infestation of fruit orchards.

Innovative strategies based on ethology of the insect and optimizing/reducing chemicals application were also evaluated. In particular, the "IPM-CPR (Integrated Pest Management – Crop Perimeter Restructuring)" (Blaauw et al., 2014) was tested on pear orchards for two consecutive years. The strategy that focused on performing insecticide treatments only in the orchards edges, allowed a reduction of 40-50% of insecticidal inputs compared to standard applications on the whole orchard.

Concerning the low impact solutions, exclusion netting either with anti-hail nets associated with border netting or single row nets, proved to be the most effective strategy to prevent BMSB infestation and damage on pear orchards compared to uncovered orchards (Caruso et al., 2017). This technique allows a consistent reduction of chemical sprays and so far it appears to be the only solution applicable in organic orchards to control this pest.

Since BMSB is also present in the vineyards, but it is still not clear the damage that the pest can cause when grapes are destined to winemaking, the project also intended to verify the effects of the massive presence of these bugs during wine making, looking for variations on the quality of the red wine Lambrusco. Results achieved so far indicate no heavy variation on the chemical profile of the wine. Sensorial analysis showed the presence of some anomalous notes not belonging to the typical sensorial scent of Lambrusco, although it does not seem to be attributable to the "bug smell".

In general, the project HALYS gave a first crucial contribution to support farmers and IPM technicians of Emilia Romagna region to manage the BMSB infestations although many aspects remain to be investigated to find sustainable strategies for all the crops affected by this invasive pest.

Key words: Brown Marmorated Stink Bug, integrated control, pome fruits, grapevine

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Agroecosystem impacts on brown marmorated stink bug pheromone trap capture and damage in North Carolina apple orchards

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Extended Abstract: The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive pest of Asian origin that was first detected in the US in the state of Pennsylvania in the mid 1990s. By 2010 it had spread approximately 700 km south to North Carolina, and by 2015 populations were causing economic damage in the central Piedmont and western mountainous regions of the state. It is currently a major pest of apples, peaches, fruiting vegetables, as well as corn and soybeans in these regions of the state. The need for pyrethroid insecticides to control this pest has severely disrupted long-standing apple IPM programs that relied on mating disruption, narrow-spectrum insecticides, and biological control as management tools, and has led to outbreaks of European red mite, woolly apple aphid, and San Jose scale.

Studies were conducted in commercial apple orchards in 2016 and 2017 using pheromone traps (BMSB pheromone + MDT synergist) and damage assessments to determine the phenology and distribution of BMSB populations and damage in apple orchards. Key findings from these studies included; 1) There were no differences in capture of adults in pheromone traps placed in the exterior versus interior of orchards, but nymphal captures were significantly higher in exterior traps; 2) When examined by month (June, July, August and September), the only instance where significant relationships existed between pheromone trap captures and damage was in June; and 3) When damage was assessed along transects from the edge to 50 meters into orchards, damage was significantly higher on the edge row, which was also within 5 m of pheromone traps placed on the exterior of orchards.

These results raise several questions about apple agroecosystem effects on BMSB pheromone trap captures and damage within apple orchards in the Southern Appalachian Mountains of western NC. The lack of significant relationships between pheromone trap captures and damage to apples complicates the interpretation of trap captures and decisions on where to spatially deploy traps in orchards. Also, the lack of differences in captures of bugs in traps placed in the exterior and interior of orchards may suggest agroecosystem factors are affecting the spatial distribution of BMSB trap captures and damage within orchards.

In 2018, studies were conducted to characterize the impact of several agroecosystem effects on BMSB pheromone trap capture and damage in apple orchards, including adjacent habitat, orchard size, cultivar, and pest management practices. The relationship between pheromone trap captures and damage was also assessed. These studies included 25 commercial apple orchards that varied in size and surrounding habitat (e. g., wooded habitats, row crops, pastures, buildings), factors that may affect the spatial and temporal distribution of BMSB in apples. Two parallel sampling transects separated by 50 m were established in each orchard to monitor damage along a gradient from the edge to the center of the orchard; one transect contained pheromone traps at the sampling sites and one did not

have traps. The habitat adjacent to the border sample site was the same for both transects, but varied among orchards. Additional data collected included cultivar, tree height, BMSB insecticide sprays, and other orchard borders. A landscape level multicomponent analysis was conducted to assess the impact of these factors on BMSB trap capture and damage.

While analysis of 2018 data is not yet complete, results showed that the strongest correlation to both BMSB pheromone trap captures and damage to apples was time, with both increasing in successive months from June through September. These results are consistent with the phenology of BMSB in NC, where overwintering adults emerge from late April through May, and first generation adults emerge from late July through August, with peak adult trap captures in mid-September.

Previous studies have shown that non-managed wooded habitats are key reservoirs of reproducing populations of BMSB, and it was hypothesized that they served as key sources of adults infesting apple orchards, and also that BMSB damage to fruit would decline along a gradient from the orchard edge adjacent to a wooded habitat to the center of the orchard. However, there was no significant relationship between pheromone trap capture and adjacent habitat, or between pheromone trap capture and location of trap from the edge of the orchard. The most striking observation was that fruit damage along transects into orchards was dramatically higher in transects containing pheromone traps (40.1% damage) versus no traps (1.0%). Also, there was only a weak correlation between trap capture and distance into the orchard.

These results suggest that BMSB dispersal into apple orchards in the highly diverse habitats of the southern Appalachian Mountains may occur on a scale that is sufficiently large to negate the detection of habitat or edge effects in apple orchards. Apple orchards are relatively small in this region (average size of about 8 ha), and may be less susceptible to nearby habitat effects or dispersal gradients compared to regions with larger orchards. Also, the strong attraction of pheromone lures to BMSB was evident in that high levels of fruit damage were only observed where pheromone traps were located. This bodes well for the use of pheromone lures in attract and kill approaches to managing BMSB.

Key words: Brown marmorated stink bug, apples, pheromone traps, sampling

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Potential new invasive pest species in United States – spotted lanternfly, *Lycorma delicatula*

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Abstract: A new invasive insect species, spotted lanternfly (SLF), *Lycorma delicatula* (White), (Hemiptera: Fulgoridae) was first found in Pennsylvania, USA in September 2014. During the summer 2018 multiple research projects were initiated to understand the available management and monitoring options for this insect. Residual and direct contact insecticide bioassays revealed good efficacy of compounds from various IRAC groups such as neonicotinoids, pyrethroids, carbamates and organophosphates. Nymphs were also affected by products approved for organic production such as natural pyrethrum and mineral oils. Complete mortality of eggs was observed after treatment of overwintering egg masses with chlorpyrifos while partial mortality was observed after treatments with dormant oils, flupyradifurone and dinotefuran. To better understand the movement and behavior of SLF adults and nymphs two available SLF monitoring lures were also tested in field setting inside vineyards, orchards and in woods. Unfortunately, when lures were combined with sticky clear traps or placed with insecticide treated nets none of the tested lure x trap combinations was attractive to nymphs or adults SLF. Additional studies are planned to better understand the potential impact of this new invasive insect species on fruit orchards and vineyards.

Key words: *Lycorma delicatula*, spotted lanternfly, invasive pest, grape pest, monitoring

Introduction

A new invasive insect species, spotted lanternfly (SLF), *Lycorma delicatula* (White), (Hemiptera: Fulgoridae) a potentially economically important insect pest native to China, India, Vietnam and Cambodia was first found in Pennsylvania, USA in September 2014. Within three years since the original detection in very localized area of few towships located in the eastern Berks County, PA, the SLF quarantine zone was expanded in 2017 to over 17 thousands square kilometers, encompasing 13 southeast counties in Pennsylvania and additionally increased during the winter of 2019. The current SLF quarantine area is the home to over 25 percent of Penssylvania orchard and vineyard farms (720 out of 2,686 farms, USDA, NASS, 2013). SLF adults were also recently detected in states of Delaware, New York and New Jersey while eggs and adults were found in Virginia automatically making it a regional and potentially a national pest (Barringer et al., 2015; Parra et al., 2018). This phloem-feeding treehopper insect has a wide range of host plants, with feeding being reported on over 65 species of plants and as such, is projected to become a serious pest of timber, ornamental trees, pome fruit orchards, grapes, stone fruit, and other small fruits such as blueberries. SLF adults were also reported to feed on hops and were observed on several types of vegetables. SLF honeydew (sugary excrement) and sooty mold (fungi growing on honeydew) (Kim et al., 2011) was found to cause economical plant injuries in vineyards in less than two years after the initial detection. SLF spread to more vineyards in 2017, with documented yield reduction in one, and potential, although still undefined long-term effects on health of grapevines. In late August 2017, for the first time, large numbers of SLF were also observed flying into and feeding on the wood and branches of apple trees as well as nectarines and peaches. Direct damage to tree fruit was not observed, but SLF egg masses found on the trees put the trees at risk for heavier and more sustained feeding by SLF nymphs and adults in the spring and summer during the following season.

Material and methods

The initial experiments related to SLF were conducted in the field laboratories, often utilizing grower orchards and buildings located within the SLF quarantine area in south-eastern Pennsylvania, USA. The SLF egg masses, nymphs and adults used in the insecticide trials were collected directly from the infested trees. All SLF monitoring trials were also conducted in and around commercial plantations located within the SLF quarantine area in PA.

Direct ovicidal bioassays

The efficacy of various insecticides against SLF eggs was evaluated either during direct insecticide applications on egg masses collected directly from wild settings during January and February 2018 or by spraying directly SLF egg masses deposited on hosts such as cultivated grapevines (*Vitis* sp.), maple trees (*Acer* sp.) or combination of various wild host trees for a total of 6 separate bioassays. The egg mortalities were assessed in late May - early June after the complete natural hatch of eggs in the control treatment. For the list of tested products please see caption within the Figure 1.

Residual nymphal and adulticidal bioassays

The residual efficacy of various insecticide products available for purchase from retail stores for home owners was evaluated utilizing 3rd and 4th instar SLF nymphs and adults collected directly from wild hosts. The specimens were collected 24-48 hours prior to the bioassays and kept in mesh cages with sunflower, pepper and bean plants serving as the food. Individual sunflower plants were treated with solution of tested insecticides and after air drying at least 20-30 SLF individuals were placed on individual treated plants and enclosed with mesh sleeve. Mortalities were evaluated at 24, 48 and 168 h after the placements of SLF on treated plants. The assortment of tested insecticide active ingredients is listed in Table 1.

SLF monitoring trials

Two commercial lures expected to monitor SLF: PredaLure MS90 (AgBio, Inc, Westminster, CO, USA) and LYCDEL (AlphaScent, Inc. West Linn, OR, USA) were placed at various locations including vineyards, apple and peach orchards and woods next to orchards. The monitoring lures were deployed either on sticky clear traps (Pherocon® StinkBug STKY™ Dual Panel, Trece, Adair, OK, USA) or placed with insecticide treated nets (D-Terrence® net, Vestergaard S.A., Lausanne, Switzerland). The sticky cards were placed at wooden poles at 0.9 m or 1.5 m high. The numbers of collected SLF nymphs or adults were checked weekly and accompanied with a 3 minute visual count of SLF on wild vegetation surrounding each trap.

Table 1. Mortality of SLF nymphs and adults at 24 hours after the exposure to fresh residue of insecticide products available for pest control for homeowners. Means followed by the same letter(s) within each column are not different (ANOVA, LSD *sqrt x* transformation, $p \leq 0.05$).

Active ingredient	Percent mortality at 24 hours after the exposure to dry residue	
	Nymphs	Adults
zeta-cypermethrin	80.0 ab	55.6 cdef
imidacloprid/beta cyfluthrin	100 a	93.3 a
deltamethrin	100 a	88.9 ab
bifenthrin/zeta-cypermethrin	100 a	100 a
carbaryl	98.0 ab	52.2 cdef
taufluvalinate/tebuconazole	95.0 ab	86.7 abc
malathion	100 a	100 a
spinosad	28.0 c	33.4 def
potassium salts of fatty acids	8.0 c	30.0 ef
neem oil (extract)	73.0 b	26.7 f
natural pyrethrum/piperonyl butoxide	88.0 ab	100 a
water (control)	3.0 c	39.0 def

Results and discussion

During the SLF ovicidal bioassays a complete mortality of eggs was observed only after direct treatment of overwintering egg masses with chlorpyrifos (organophosphate) while partial mortality was observed after treatments with dormant oils and dinotefuran (Figure 1). No differences were observed between mortalities exhibited by eggs treated directly on wild hosts or directly on pieces of wood collected from the field and treated in the field laboratory.

Residual and direct contact insecticide bioassays revealed good efficacy of compounds from various IRAC groups such as neonicotinoids (e. g., dinotefuran, imidacloprid), pyrethroids (i. e., bifenthrin, deltamethrin, zeta-cypermenthrin), carbamates (e. g., carbaryl) and organophosphates (e. g., malathion). Nymphs were also affected by products approved for organic production such as natural pyrethrum and mineral oils.

During the SLF lure and trap evaluations none of the lures were attractive to either SLF nymphs or adults. Higher numbers of SLF were observed during the visual observations on surrounding trees than captured by evaluated traps (Figure 2). Also, the placement of traps and the trap height did not influence the numbers of captured of SLF nymphs and adults. Some feeding preferences were observed based on the tress species, although data was highly variable, and no the same patterns were observed at every location.

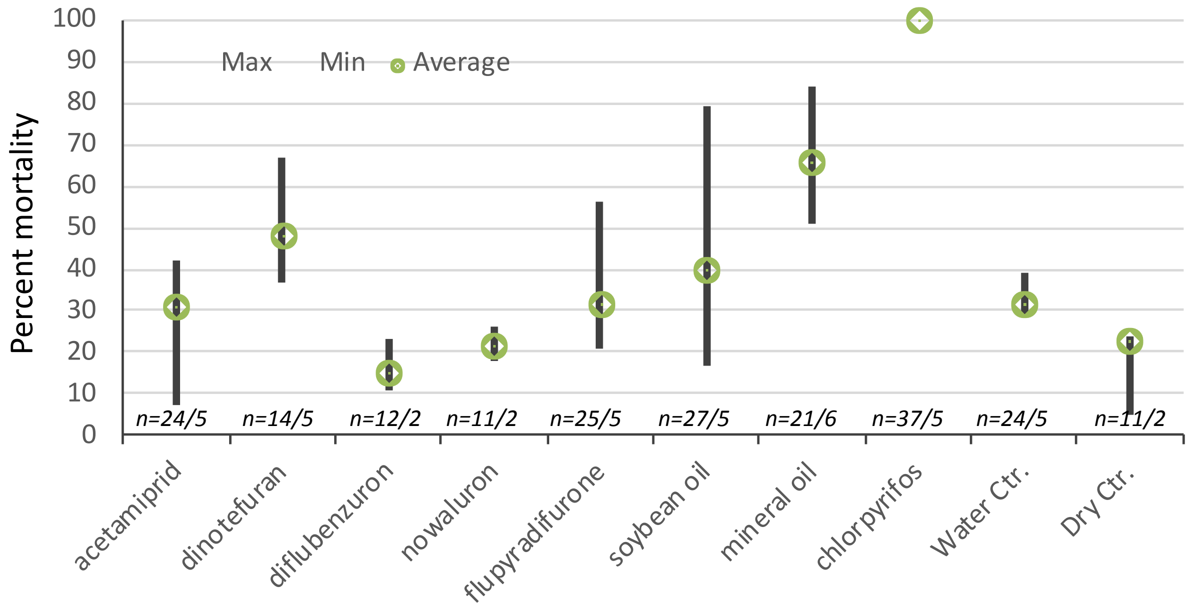

Figure 1. Mortality of SLF egg after direct treatment with various insecticides. Data represents the minimum, maximum and average mortality during multiple bioassays. The number of treated egg masses and number of bioassays with individual compound is presented as “n”.

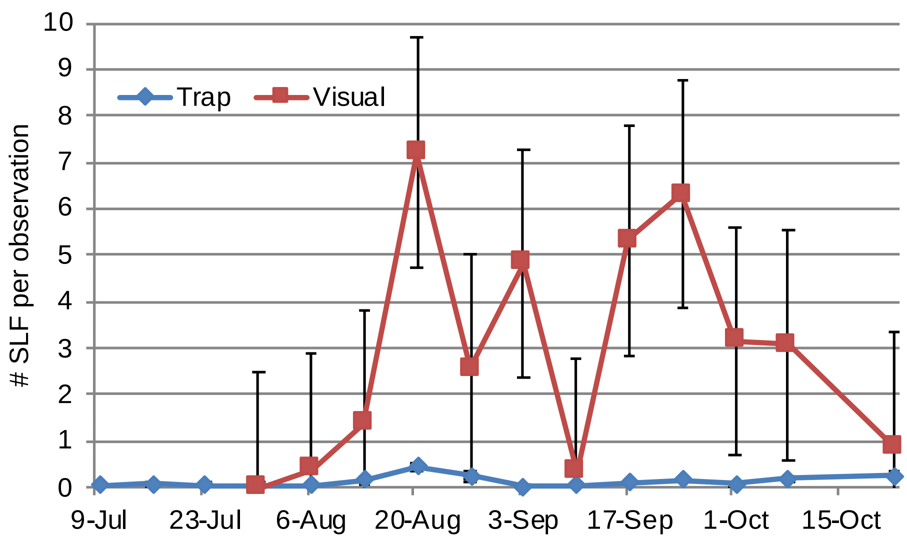

Figure 2. Numbers of SLF nymphs and adults observed utilizing monitoring traps and during weekly visual observations on wild trees surrounding vineyard or orchards. Average data collected from three separate sampling locations with at least 8 traps per location.

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Invasive pests: a challenge for IPM. Spotted wing drosophila and other invasive dipterans

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Comparative study of *Drosophila suzukii* females' behavioral responses to fruit odors of two varieties of *Vitis vinifera*

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Abstract: Spotted wing drosophila *(Drosophila suzukii)* is a key pest of soft fruits. It has been reported as a major invasive pest in Europe and the Americas, causing serious yield loss in soft fruits. Laboratory experiments were carried out to evaluate the behavioral responses of *D. suzukii* females to odors released by fresh and ripe berries of two varieties of table grapes. i. e. *Vitis vinifera, var.* Victoria *and var.* Black magic. The response of *D. suzukii* females to host fruit odors was investigated in two-choice bioassays using Y-tube olfactometer. The berries were tested, separately in an olfactometer, as non-sterilized and sterilized fruits. It was found for the first time that *D. suzukii* female flies were more attracted to non-sterilized fruits than sterilized fruits. Higher attractiveness of fruits was recorded for *var.* Victoria. The results are expected to contribute towards the development of a new concept trap baited with the new lure that can contribute in setting up new environment-friendly control methods such as mass-trapping and attract and kill techniques.

Key words: Spotted wing drosophila, olfactometer, two-choice bioassays, grape varieties

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), is a highly polyphagous pest of fresh fruits. In Europe, it is the only Drosophilid fly with serrated ovipositor, which enables it to oviposit in unwounded fresh fruits, thereby making them unmarketable (Cini et al., 2012). Recently, *D. suzukii* invasion has been reported in Europe and is heavily affecting fruit industry (Rota-Stabelli et al., 2013). Numerous evidences have shown the importance of host plant volatile compounds in oviposition site-selecting behavior by insect herbivores (Verheggen et al., 2008). Recent studies showed that the microbes (yeasts and bacteria) play a vital role in the above process (Becher et al., 2012). At present, monitoring and control tools deployed against *D. suzukii* have been poorly addressed.

The present study aims to evaluate the behavioral responses of *D. suzukii* females to odors released by fresh and ripe berries of grapes, i. e. *Vitis vinifera*, using behavioral bioassays (Y-tube olfactometer), which is expected to contribute in the designing of innovative concepts for plant protection technologies and development of trapping systems for integrated pest management strategies against this pest.

Material and methods

Insect rearing
Drosophila suzukii colony was reared on a standard drosophila diet at 24 ± 1 °C, 65 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Only naive mated females were used in the study. Seven days old adult females were starved for 1 h in 200 ml plastic container on water soaked cotton swab to enhance the flies' response towards odors.

Fruits collection
The fruits collected were two different varieties of table grapes, i. e. *Vitis vinifera var.* Victoria (white grapes) and *var.* Black magic (black grapes). The fruits were freshly picked from Apulia region in Italy at ripe stage followed by storage in PVC (Polyvinylchloride) containers which have no effect on volatiles of fruits after harvesting and were kept at room temperature for 1 h before conducting the test. The fruits were tested non-sterilized and sterilized. Chemical sterilization of fruits was done by dipping fruits in sodium hypochlorite solution (1%) for 1 min, then rinsing with tap water followed by distilled water. Afterwards the berries were air-dried in a laminar flow to avoid any microbial contamination.

Two-choice bioassays
The response of *D. suzukii* females to host fruit odors was investigated in two-choice bioassays using Y-tube olfactometer (stem 29 cm, arm length 22 cm, arm angle 60°, internal diameter 4.5 cm) one arm of Y-tube held 30 g of fresh grape berries which were randomly assigned to avoid positional biases, and the other arm was serving as a control containing 10 ml of D.I water soaked in cotton swab. Each arm was equipped with porous barrier to exclude any possible visual cues. Activated charcoal-filtered and humidified clean air was pumped uniformly through the arms at 20 ml/min. Flies (n = 10) were introduced at once to the stem of Y-tube and were observed for 1 h, after which flies were retrieved, irrespective of the choice. After each test of 10 flies, the olfactometer was washed with distilled water and bakedout in an oven for 1 h at 150 °C. Each variety was tested separately in the olfactometer and each choice test was replicated 10 times. Thus the olfactory responses of 100 flies per variety in comparison to treatment were tested. The experiments were conducted in a laboratory at 23 ± 1 °C, 65 ± 5% relative humidity and 1000 lux light luminance.

Statistical approach
Data were recorded based on the first choice made by a fly. The number of flies in both fruit and control arms is expressed in percentage of the total number of flies making a choice either to fruit or control arm. Flies that did not make a choice were recorded as "no choice". Wilcoxon Signed Rank Test was used to compare the attractiveness of flies towards sterilized and non-sterilized treatments of fruits at $P < 0.05$. Furthermore, for comparison of flies response to control arm and treatments, chi-square test was used (STATISTIX, version 10; Analytical software, Tallahassee, Florida).

Results and discussion

Flies' responses to both varieties of grapes were significantly different. Figure 1 depicts a non-significant response to both varieties in sterilized treatment. However, a difference was recorded among the varieties in non-sterilized treatment. Additionally, the difference within the treatments is evident ($Z = 5.37$, $P = 0.0000$).

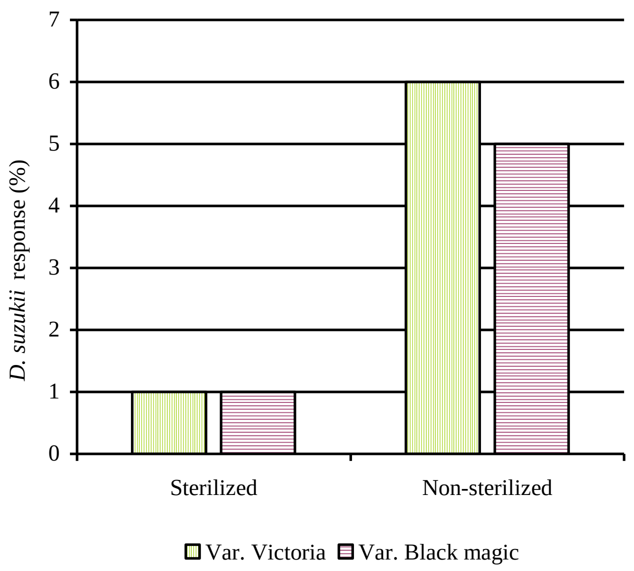

Figure 1. Percentage of *D. suzukii* flies' response to treatment (i. e. sterilized and non-sterilized) in *var*. Victoria and *var*. Black magic in a two-choice bioassay (n = 10).

Likewise, both varieties resulted in significant differences in the flies' responses in non-sterilized treatment: *var*. Victoria (χ^2 = 9.00; d.f. = 1; *P* = 0.0253), *var*. Black magic (χ^2 = 8.00; d.f. = 1; *P* = 0.0047). Figure 2 shows that the flies were more attracted towards non-sterilized than that of control in *var*. Victoria (χ^2 = 9.00; d.f. = 1; *P* = 0.0027).

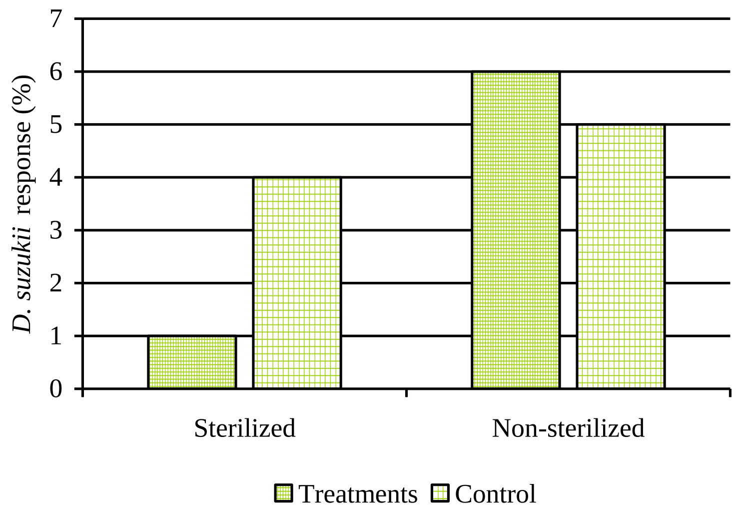

Figure 2. Percentage of *D. suzukii* flies' response to *var*. Victoria, treatments (i. e. sterilized and non-sterilized) and control (n = 10).

Similarly, Figure 3 exhibits that the flies were more attracted towards non-sterilized than that of control in *var*. Black magic (χ^2 = 8.00; d.f. = 1; *P* = 0.0047).

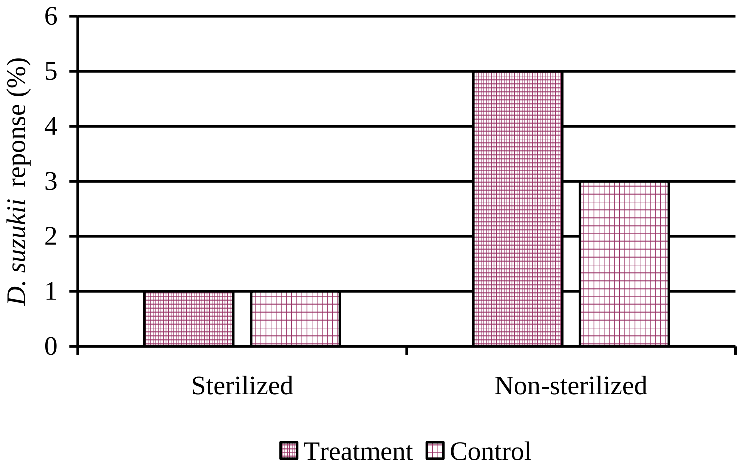

Figure 3. Percentage of *D. suzukii* flies' response to *var*. Black magic, treatments (i. e. sterilized and non-sterilized) and control (n = 10).

The present study reports for the first time the behavioural responses of *D. suzukii* to odors emitted by two varieties of table grapes of agricultural importance in Italy. The obtained data indicate that olfaction has important role in selection of grapes for feeding and oviposition which was confirmed in previous studies (Revadi et al., 2015). The attractiveness of female flies towards grapes varieties in the study reconfirms that *D. suzukii* is capable of infesting a wide range of hosts (Bellamy et al., 2013), and table grapes, i. e. *var*. Victoria is highly susceptible to *D. suzukii* infestation in Apulia region (Baser et al., 2018). From the findings of the experiment it is assumed that *D. suzukii* response was found less due to the spatial variability in host distribution which can affect the bacterial communities associated with the hosts (Corby-Haris et. al., *2007*).

However, flies attraction towards non-sterilized fruits can also be related to microorganisms associated with the fruit surface (Becher et al., 2012). Therefore, further studies are underway to identify microorganisms and volatiles emitted by them that elicit antennal and behavioral activity. Outcomes of these future studies will aid in the development of a selective and efficient synthetic lure. Such synthetic lure may be used as oviposition attractant rather than fermentation bait for effective monitoring and control of this pest.

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Recent records of the Mediterranean fruit fly, *Ceratitis capitata* (Tephritidae, Diptera), in Austria

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Abstract: The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), a polyphagous tephritid, is one of the most important insect pests worldwide. Findings of fruits infested with *C. capitata* in Vienna in 2010 implicated increasing survey activities (especially trap survey) for this fruit fly species and other harmful tephritid species in Vienna and in fruit-producing regions of Austria. Between 2010 and 2018 in total almost 1200 adult *C. capitata* were captured in Austria using different trap-lure combinations. Almost all flies (> 99%) were caught in the city of Vienna, whereas no or only single flies were trapped in other Austrian regions. Molecular sequencing of several individuals revealed that they derived mainly from genetically different origins. However, some of the flies caught at different sampling sites and years in Vienna were clonal. While the origins of the flies caught remain unknown, the repeated findings of individuals of *C. capitata* in Vienna, are very likely linked to recurrent entries of juveniles with infested fruit. However, the findings of genetically clustered individuals in consecutive years support the theory that in certain areas of Vienna these catches might be the result of a possibly temporary establishment.

Key words: *Ceratitis capitata*, medfly, survey, lure, trap, detection, molecular sequencing, ITS

Introduction

Ceratitis capitata (Wiedemann), the Mediterranean fruit fly (medfly), is considered one of the most important pest fruit flies worldwide and can be found on all continents nowadays. This polyphagous species infests different fruits and vegetables, including *Citrus reticulata*, *C. sinensis*, *Mangifera indica* and *Prunus persica* as major host plants (White and Elson-Harris, 1992; EPPO, 2019).

The species has its origin in Africa and is endemic to most sub-Saharan countries, but has spread and became also an important pest in the Mediterranean countries with widespread distribution, e.g. in Italy or Greece (CABI, 2019). A few records of earlier occurrences from Central Europe, where climatic conditions do not favour the establishment of tropical fruit flies, are also available. For instance records for Germany (Baas, 1955; Marr, 1956; Piltz, 1958) or Switzerland (Gast and Müller, 1954), but also detections from Vienna, Austria, (Watzl, 1932; Böhm, 1958; Glaeser, 1979) were recorded in the past. Peach (*Prunus persica*), one of the major medfly host plants is cultivated mainly in east and south-east of Austria and would be available for the development of this pest during the growing season, in addition to different minor host plants (Statistik Austria, 2016).

After records of medfly infested fruits in Vienna from 2010 onwards, survey activities were carried out in the city of Vienna but also in other Austrian provinces in order to detect a potential occurrence of *C. capitata* and other relevant *Ceratitis* species (e. g. *Ceratitis rosa*), which are attracted to the same trap-lure combination.

Survey activities, coordinated by the Austrian Agency for Health and Food Safety in cooperation with regional plant protection services of some Austrian provinces, were established partly within the framework of two Euphresco-Network projects ("FLY DETECT", DOI 10.5281/zenodo.1326227 and "FruitFlyRiskManage").

Material and methods

Sampling sites

The sampling sites for the medfly survey were located in allotments in the city of Vienna (see Figure 1), starting with one sampling site in 2010 (1110-A) and increased to four sites in 2011 and 2012. In 2013, two sites were sampled (traps of two further sites close to market areas were stolen and not replaced). In 2014 and 2015 no specific medfly surveys were carried out. From 2016 onwards survey activities continued and sampling sites were extended to eight of the nine Austrian provinces. Samples were taken in 2016, 2017 and 2018 from 17, 27 and 36 sites, respectively (Table 1).

In Vienna, traps were installed in peach trees or other host plants in allotments and small gardens, but also at Vienna's wholesale market area (1230-Z) in 2011 and 2012, at two fruit and vegetable market areas in 2017 (1150-B and 1160-A), and in 2018 near a biogas plant (1110-B) and a composting plant (1220-C). In the other Austrian regions, traps were installed inside or near commercial orchards or small gardens containing peach trees or other host plants. In addition, traps were installed at Vienna International Airport (point of entry; Lower Austria) in 2018. At sampling sites with host trees, traps were installed in different trees about two metres above the ground in the tree canopy.

Table 1. Number of sampling sites for *Ceratitis capitata* in Austrian regions (2016-2018).

	2016	**2017**	**2018**
Vienna	4	8	14
Lower Austria	3	5	7
Upper Austria	1	1	1
Burgenland	2	3	3
Styria	3	3	5
Carinthia	4	4	4
Salzburg	0	1	0
Tirol	0	2	2

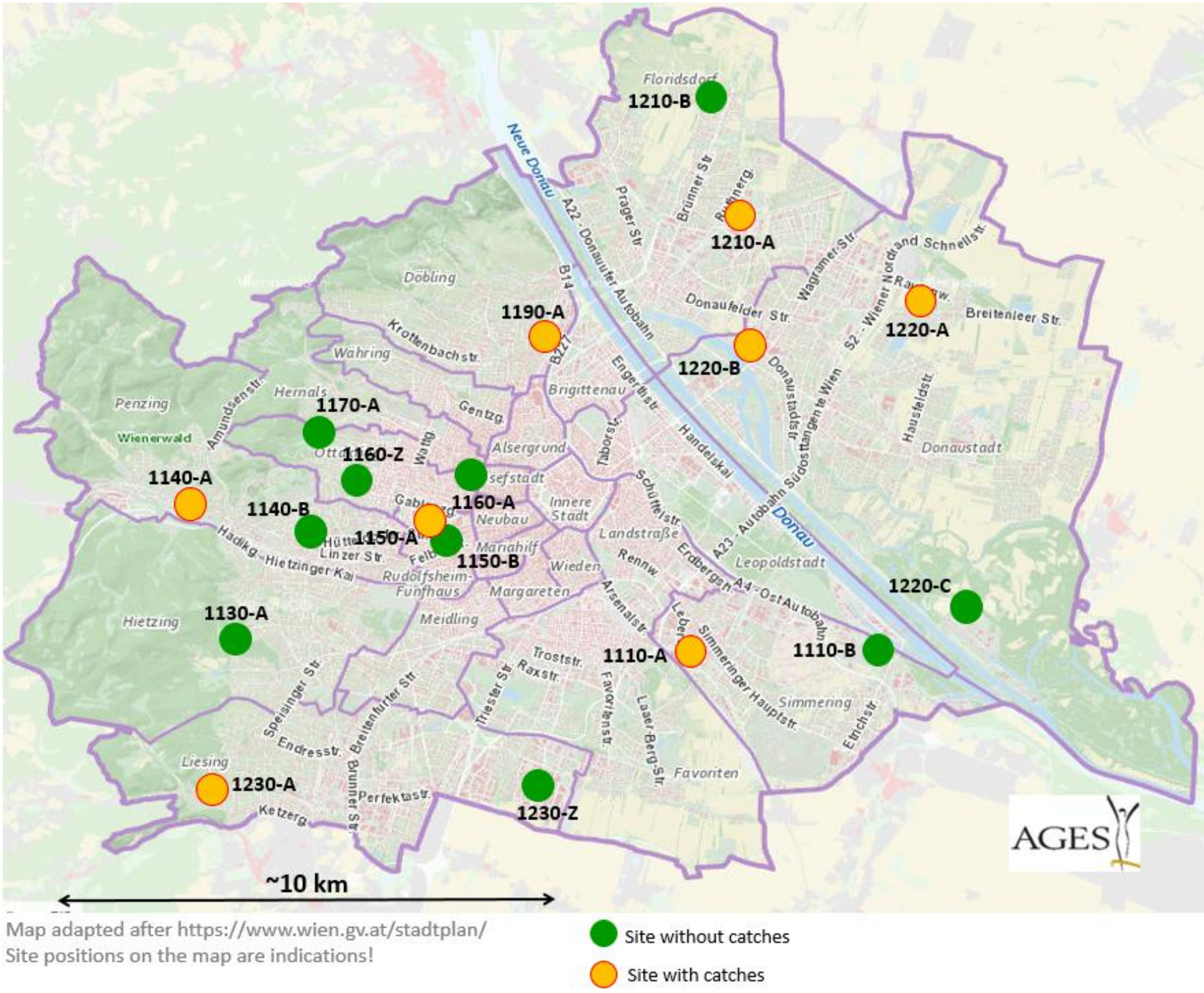

Figure 1. *Ceratitis capitata* sampling sites in Vienna (Austria), 2010-2018, with and without catches.

Traps and lures

In accordance with international recommendations (FAO/IAEA, 2013) a Delta trap was used in 2010, while tephri trap type traps (Maxitrap®, supplier Sociedad Española de Desarrollos Químicos (SEDQ), S.L., ES) were installed in the following years. Traps were charged with a lure attracting male and female flies (mainly containing ammonium acetate) in 2010-2012 (CERATIPROTECT®, supplier SEDQ), while from 2013 onwards plugs containing trimedlure (TML)(supplier Pherobank B.V., NL) were applied to lure male fruit flies. In addition, traps charged with an attractant for female flies (Femilure-45 (prod. code: PH-180-FM45), supplier Russell IPM Ltd, UK) were installed at some sites in 2017 and 2018. Overall one trap per sampling site was installed in 2010-2013. After 2013 mainly two traps per site were installed, except in 2017 at two sites (1150-B and 1160-A, where only one trap per site charged with Femilure-45 were used), and in 2018 at three sites (1210-B and 1220-A and 1220-C, with two traps with TML plus two traps charged with Femilure-45). All lure dispensers were changed several times, in accordance with suppliers' instructions.

Survey period
Between 2010 and 2013 (only Vienna) the survey mainly started in July and finished in September to beginning of October, with the exception of 2011 when traps were installed already by beginning of May. In 2016, the survey period was extended up to a maximum of 5.5 months (VI-XI) in Vienna. In 2017 and 2018 in Vienna, as well as in all other Austrian regions in 2016-2018, the period was approximately from June to October at most of the sites, to cover the season's fruiting periods. Control of trap catches was mainly carried out at two-week intervals.

Identification and molecular analysis
The fruit flies caught were identified morphologically using identification keys and a diagnostic protocol (Carroll et al., 2002; Malumphy, 2007; EPPO, 2011). Morphological identification was confirmed for randomly selected specimens with molecular diagnostic methods.

Sequencing data of ITS 1 PCR (Douglas and Haymer, 2001) amplicons were compared using the software Geneious 10.1.3 (Biomatters Ltd.), and alignment trees were built to identify genetic differences to determine matching origins of the individuals.

Fruit sampling
At three sites with trap catches (1110-A, 1150-A, 1140-A) suspicious fruits were collected from the ground below the trees bearing the traps. Fruits were transferred in sealed boxes to the laboratory (resp. climatic chamber) for development of potential larvae in ideal climatic conditions (temperature: ~ 25 °C, relative humidity: ~ 65%). Sand or tissue was provided below the fruits in order to support pupation of emerging larvae. Adult specimens were identified as described above.

In 2010 (six suspicious peaches (*Prunus persica*) at site 1110-A, week 38), 2015 (one peach, at site 1110-A, week 35) and 2016 (one apricot (*Prunus armeniaca*) at site 1150-A, week 27) few fruits were sampled. In contrast, in 2017 about 50 apricots and about 100 peaches were unsystematically collected from the three sampled sites between mid-July and mid-September. In 2018, samples were taken from two sites (1110-A, 1150-A) between August and October. About 30 peaches (August, from both sites), approximately 250 plums (*Prunus domestica*) (September, 1110-A) and 2 pears (*Pyrus* sp.) (October, 1110-A) were incubated.

Results

Survey activities for *Ceratitis capitata* in Austria between 2010 and 2018 resulted in total captures of 1186 adult fruit fly individuals of this species (see Figure 1 and Table 2; one specimen from a trap for *Bactrocera* sp. is included (1150-A, 2014)). No other *Ceratitis* species was caught. Molecular analyses of selected specimens confirmed the morphological identification.

While at most sites no catches of *C. capitata* were obtained at all, most of the individuals caught (1182 individuals, 99.7%) were recorded at sampling sites in the city of Vienna. No more than two individuals per site were caught at three sites in other regions of Austria (Lower Austria, Burgenland, and Upper Austria; one site per region) in 2016 or 2017. Highest seasonal catches were achieved in August and September, as shown for site 1150-A in Figure 2.

Table 2. *Ceratitis capitata* sampling sites in Vienna (Austria), 2010-2018, with number of individuals caught per site per year (line (-) indicates: no sampling; * indicates: no specific *Ceratitis* survey).

	2010	2011	2012	2013	2014*	2015*	2016	2017	2018
1110-A	1	4	10	29	-	-	219	31	121
1110-B	-	-	-	-	-	-	-	-	0
1130-A	-	-	-	-	-	-	-	-	0
1140-A	-	-	-	-	-	-	-	1	0
1140-B	-	-	-	-	-	-	-	-	0
1150-A	-	9	52	10	1	-	536	82	47
1150-B	-	-	-	-	-	-	-	0	-
1160-A	-	-	-	-	-	-	-	0	-
1160-Z	-	0	-	-	-	-	-	-	-
1170-A	-	-	-	-	-	-	-	-	0
1190-A	-	-	-	-	-	-	-	0	8
1210-A	-	-	5	-	-	-	6	0	0
1210-B	-	-	-	-	-	-	-	-	0
1220-A	-	-	-	-	-	-	3	0	2
1220-B	-	-	-	-	-	-	-	1	2
1220-C	-	-	-	-	-	-	-	-	0
1230-A	-	-	-	-	-	-	-	-	2
1230-Z	-	0	0	-	-	-	-	-	-

Between 2010 and 2012 most of the individuals caught were females (~ 74%), in 2013 only males were caught, and between 2016 and 2018 approximately 95% were males.

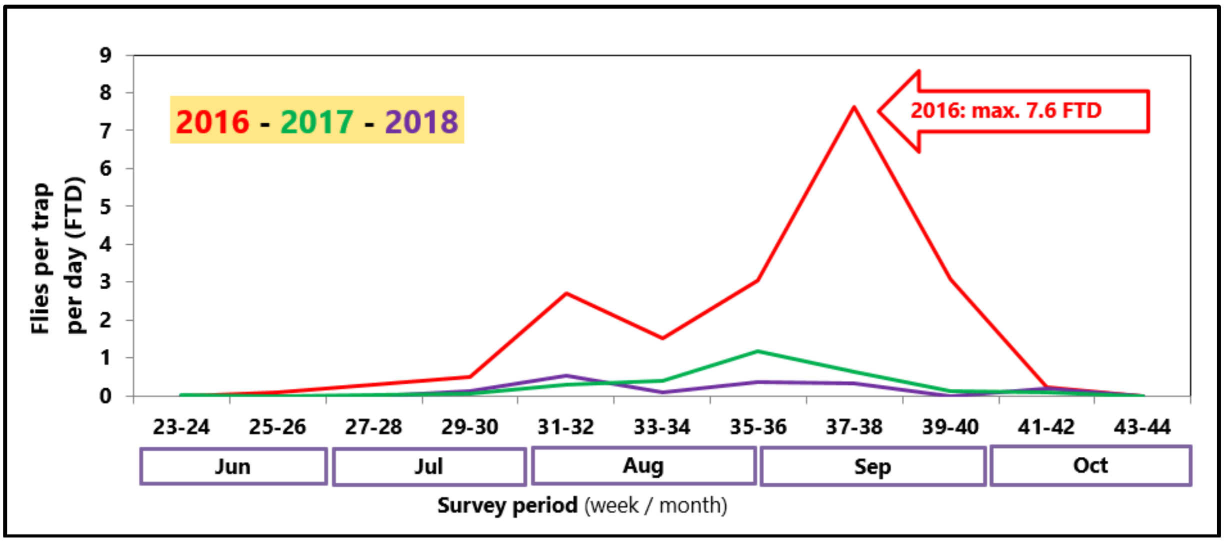

Figure 2. *Ceratitis capitata* – seasonal catches in Vienna (Austria) at site 1150-A, 2016-2018.

Detailed results of Vienna 2010-2013

In 2010, only one female was caught with the Delta trap (1110-A, weeks: 30-33). In the following year (2011) four individuals [3 ♀♀ + 1 (no symbol means: sex unclear, specimen damaged)] were caught at the same site (weeks: 28-40), while nine individuals (1 ♂ + 5 ♀♀ + 3) were caught at site 1150-A (weeks: 29-40); no catches were achieved at the two remaining sites. In 2012, the number of flies caught increased to 67 medflies at three of the four sites [1110-A: 10 individuals (3 ♂♂ + 7 ♀♀), weeks: 29-31, 34-39; 1150-A: 52 individuals (9 ♂♂ + 42 ♀♀ + 1), weeks: 29-40; 1210-A: 5 individuals (3 ♀♀ + 2) weeks: 37-39]. In 2013, totally 39 individuals (only males) were caught at two sites (1110-A: 29 ♂♂, weeks: 27-40; 1150-A: 10 ♂♂, weeks: 32-37).

Detailed results 2016-2018

In 2016, a total of 766 *C. capitata* individuals were caught at six of the 17 sampling sites in Austria. Only two individuals were caught in other Austrian provinces (Lower Austria: 1♀, weeks: 31-32; Burgenland: 1♂, weeks: 39-40), and 764 individuals (720 ♂♂ + 44 ♀♀) were caught at the four sampling sites in Vienna [1110-A: 219 individuals (214 ♂♂ + 5 ♀♀), weeks: 24-40; 1150-A: 536 individuals (497 ♂♂ + 39 ♀♀), weeks: 24-42; 1210-A: 6 ♂♂, weeks: 35-40; 1220-A: 3 ♂♂, weeks: 27-28 & 31-34].

In 2017, 115 individuals of *C. capitata* were caught in Austria at five of the 27 sampling sites with the Maxitrap® + TML combination and two individuals were accidentally caught in yellow panel traps (1150-A, weeks: 41-42; 1220-B, weeks: 37-38) during a survey for *Rhagoletis* sp. (totally 117). Again, only two individuals were caught outside of Vienna at one sampling site (Upper Austria: 2 ♀♀, weeks: 25-26 and 29-30), while in Vienna 115 individuals were recorded [1110-A: 31 individuals (30 ♂♂ + 1♀), weeks: 27-42; 1140-A: 1♂, weeks: 39-40; 1150-A: 81 individuals (80 ♂♂ + 1♀), weeks: 23-42; +2 individuals in yellow panel traps (see above)].

In 2018, 182 individuals of *C. capitata* were caught at six of 36 sampling sites in Austria. All catches were recorded in Vienna (at six of 14 sites), no catches were achieved in other Austrian provinces [1110-A: 121 individuals (115 ♂♂ + 6 ♀♀), weeks: 27-42; 1150-A: 47 individuals (42 ♂♂ + 2 ♀♀ + 3); weeks: 29-37 and 41-42; 1190-A: 8 ♂♂, weeks: 31-32, 37-40; 1220-A: 2 ♀♀, weeks: 37-38; 1220-B: 2 individuals (1 ♂ + 1 ♀); weeks: 37-40; 1230-A: 2 individuals (1 ♂ + 1♀); weeks: 31-32, 39-40]. Three of the total of 12 females were caught in traps charged with other lures: two individuals in traps with lures for *Bactrocera* sp. (methyl eugenol) (1110-A, 1220-B), one in a trap with female lure (Femilure-45) (1220-A).

Results of fruit sampling

From the collected suspicious fruits of the three Viennese sampling sites, *Ceratitis capitata* emerged several times from incubated peaches (totally 31 individuals from up to hundred incubated fruits from 2015 and 2017), apricots (totally 25 individuals from two fruits from 2010 and 2016) and pears (totally 24 individuals from two fruits from 2018) but none from plums. No other fruit fly species was obtained from those fruits.

Results of molecular sequencing

From every sampling site with catches of *C. capitata* 2011-2013 and 2016-2018, at least one specimen was used for molecular sequencing; however, high quality sequences were not obtained for all of those specimens and therefore not all were included in the analyses. Finally 66 sequences, including specimens of two interceptions (Vienna International Airport; origin: Egypt and Uganda), one specimen accidently caught in a trap for *Bactrocera* sp. in 2014

(1150-A) and one specimen reared from an infested peach collected in 2015 (1110-A), were used to build an alignment tree.

While the analyzed specimens were in general from a number of genetically different origins (about four groups, all of those with at least seven closely related but not clonal specimens; and about 20 specimens genetically more distant to others), results showed that approximately one quarter of the sequences were clonal, therefore indicating the same genetic origin. Interestingly samples in the group of clonal specimens derived from four different sampling sites (1110-A, 1150-A, 1190-A, 1230-A) and from six different years (2012, 2013, 2014, 2016, 2017, 2018).

Discussion

The results of our survey activities from the present study, together with the results from our previous findings (Egartner et al., 2017; Egartner et al., 2018) are discussed below.

Ceratitis capitata, the Mediterranean fruit fly, is not native to Austria but has been recorded in Vienna several times at different occasions in the past (Watzl, 1932; Böhm, 1958; Glaeser, 1979). Increasing survey efforts were carried out in Austria between 2010 and 2018, following fruit infestation and single catches in a private garden in Vienna in 2010 (Lethmayer, 2011).

The catches of numerous individuals in Vienna as well as the recorded fruit infestation during this broader survey could be attributed to individuals from different origins. They probably are the outcome of repeated entries from areas with established populations through different pathways (like flight of adult flies or, even more probably, as larval instars in infested fruit from cargo or travellers' luggage) (White and Elson-Harris, 1992; EPPO/CABI, 1996). As host plants were available during summer at the sites with catches, and considering the seasonal trap catches at some sites (see Figure 2), flies caught could also be the offspring of flies that entered the survey area during the trapping period. On the other hand, trap catches could be the result of a possibly temporary established population at some of the sampling sites in Vienna, or of a combination of the various possibilities.

Adult *C. capitata* are able to fly several kilometres (Fletcher, 1989), but according to our results, there is no evidence for an established population in Austrian provinces apart from Vienna. Furthermore, for none of the neighbouring countries north or north-east of Austria are there any published data about established populations (EPPO, 2019). Therefore, natural spread (adult flight) could only be of relevance for catches within Vienna (e. g. at sites with only few catches), as distances to sites with catches in other Austrian regions, are too long.

A more probable pathway for the flies to enter previously not infested areas is long distance transport as larval instars with infested fruits. In international trade, according to EUROPHYT (the European notification system for plant health interceptions), fruit flies are the group of harmful organisms intercepted with fruit and vegetables from non-EU-countries with most interceptions between 2013 and 2017 (EC-DG SANTE, 2018). Furthermore, earlier studies on airline baggage in the United States have revealed that the most commonly infested commodity intercepted by inspectors was fruit and the most commonly intercepted insects were Homoptera and Diptera, which include Tephritidae (Liebhold et al., 2006). However, it should be mentioned that established populations of this pest are also reported from southern European countries (CABI, 2019; EPPO, 2019) and therefore it is likely that infested fruits can enter the survey area without crossing borders of the European Union.

While the origin of the individuals caught in Vienna remains unknown, the given genetic differences of most of the caught and analysed specimens as well as the available information on potential pathways, indicate that most of the catches were flies that arrived in the survey area during the trapping season or are offspring of those individuals. However, for other regions of the world with repeated *C. capitata* catches, this theory of recurring reintroductions was questioned (Papadopoulos et al., 2013). In our case, the repeated findings at the same sites (see Table 2) and the fact that a part of the analysed specimens clustered (over various years), could possibly be interpreted as a (temporary) establishment of this fruit fly species. The hypothesis that a population of *C. capitata* has established itself in Vienna, raises the question of how this species could possibly manage to overwinter, as Austrian winter conditions do not favour the establishment and survival of tropical fruit flies. Even in more southern central European countries like Slovenia, *C. capitata* is only able to establish itself in restricted areas close to the Adriatic, where climatic conditions are more favourable (Seljak, 2012). The potential of *C. capitata* to overwinter in Austria and related hypotheses (Papadopoulos et al., 1996; Papadopoulos et al., 1998) will be addressed in an international collaboration starting in 2019 (H2020-SFS-2018-2020, “In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies ('OFF-Season' FF-IPM).

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Spotted wing drosophila: Extremely meteorosensitive – a base for the development of the Decision Support System "SIMKEF"

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Abstract: The invasive Spotted Wing Drosophila (SWD), *Drosophila suzukii*, is infesting and damaging stone and berry fruits and causing huge economical losses since 5 years in Germany. Population development and infestation level vary between different years, hence regulation measures are difficult to place in an optimal way. To develop a decision support system (DSS "SIMKEF") for control measures, different factors influencing population development, activity and infestation of fruit crops were analysed in a collaboration project for fruit crops (stone fruits and berries) and grapes. The DSS is expected to predict the population dynamics of *D. suzukii* and the actual pest infestation risk for the different crops. For this reason, the interaction of the entire life cycle and activity of *D. suzukii* with the most important meteorological factors, like temperature and relative humidity as well as landscape structures, wood, hedges or fields is described or functionally determined. Laboratory tests, monitoring results and already published data will be combined within the DSS. In this paper first focus is on the influence of cold and freezing temperatures during winter and landscape structure on population development. Second focus is on the influence of temperature and relative humidity as well as ontogenesis of host fruits on oviposition. As a first output of the DSS cherries will be assessed for their susceptibility depending on abiotic and ontogenetic factors.

Key words: *Drosophila suzukii*, Spotted Wing Drosophila, Decision Support System, climate, landscape structure

Introduction

Since the first record in 2011 in Germany, *D. suzukii* has rapidly spread and is found almost all over the country (Vogt et al., 2012; Vogt and Köppler, 2014). Between 2013 and 2018 the pest has shown different infestation levels in the same or different crops in Southern Germany, e. g. 10 up to 100% in cherries, raspberries or blackberries, depending on site, climate or period of time within the season. It is known that long-term survival of *D. suzukii* depends on favorable conditions as temperature and food during the overwintering period (Dalton et al., 2011). Temperature also affects the early season pest pressure (Jung et al., 2018). However, first infestation occurs in early cherries and infestation level can increase or decrease in later cultivars. Also in berries infestation varies throughout the ripening period of several weeks in the same orchard. *D. suzukii* has a temperature-related population development and activity (Tochen et al., 2014; Eben et al., 2017; Evans et al., 2018) and therefore, infestation level also depends on temperature, whereas constant higher temperatures have a negative effect on survival and fecundity (Grumiaux et al., 2019). Relative humidity (RH) also affects population development (Tochen et al., 2015; Eben et al., 2017), particularly with negative effects with humidity levels of 20 and 33% (Tochen et al., 2015). In the field the influence might not be as obvious compared to temperature because of generally higher relative humidity levels in Germany during the reproduction period of SWD (Jung, pers. comm.). According to records of meteorological data in southwest Germany, even in hot and dry summer 2015 as well as in 2018, daily mean values did only reached levels below 55% sporadically. Wong et al. (2018) found no effect of humidity on flight performance in laboratory trials. Beside abiotic factors, habitat structure also influences infestation risk and dispersal of the pest (Jung et al., 2018; Klick et al., 2016; Köppler et al., 2018; Pelton et al., 2016).

The regulation of SWD is difficult because of the broad host range and thus it is a risk for several crops throughout the season. Strongly restricted insecticide registration in Germany combined with a limited number of applications and low efficacy rates add to this. To increase the efficacy of regulation measures in terms of necessity and timing the knowledge of factors influencing the population dynamics and infestation risk is leading to the development of a Decision Support System (DSS “SIMKEF”). In this paper, different exemplary steps to develop the DSS and first results for cherries will be presented. The development of the DSS is still in process.

Material and methods

Materials and methods cannot be described in detail for all different laboratory and field studies as well as data analyses in this chapter. Consequently, only the main aspects for the development of the DSS are mentioned. The developed DSS “SIMKEF” is based on methods described by Rossi et al. (1997) and Racca et al. (2010; 2011 and 2014).

Data input

Temperature and relative humidity: Regional weather data with temperature (2 m above ground, hourly average data) and RH were available from weather stations in fruit and grape growing regions in Germany year-round. Both parameters were also measured with data loggers in those fruit orchards and vineyards, where fruit samples were taken within this project. Additionaly, fruit temperatures exposed to the sun and in shady places were measured to calculate the influence of temperature on different immature stages of *D. suzukii* and thus on the population development during fruit ripening period in the field. Life table studies were made in different temperature and humidity regimes in the laboratory.

Phenology of host fruits: Phenological data of host fruits were taken from databases running by the extension services in Germany as well as from the orchards where field studies were made during the project period. As a scale, the “BBCH-code” (Meier, 2018) was applied: blooming as growth stage 6 “Flowering”, fruit ripening stages of cherries, blackberries raspberries, elderberries, grapes as growth stage 7 “Development of fruit” and growth stage 8 “Maturity of fruit or berries” with subgroups in each case.

D. suzukii activity data: Year-round trap catches [e. g. apple cider vinegar (ACV) and water 1:1, ACV and red wine 2:3, droskidrink = 75% ACV, 25% red vine, 1 teaspoon sucrose/100 ml (Grassi et al., 2014)] provided by the extension services in Germany as well as trap catches in and nearby the orchards where field studies were made during the project period were analysed.

Fruit infestation data: Fruit samples (30 or 50 fruits/sample) were taken in the above mentioned crops in different ripening stages to get information about the initial oviposition and the development of infestation level during fruit ripening in each crop: unripe, half ripe and ripe according to the above mentioned "BBCH-code". Eggs were counted in cherries and grapes directly after sampling and larvae were counted in berries and additionally in cherries. Therefore single fruits were incubated in net-protected boxes for 24 hours at room temperature and put in salt water (NaCL, 10%) for at least 1 hour. Emerged larvae were counted under the microscope for every fruit separately.

Habitat structure: The Digital Basic Landscape Model [Basic DLM] provided general information about habitat structure for Germany in GIS format including details about the spatial distribution of arable land, orchards, vineyards, woodland and hedges (Bundesamt für Kartographie und Geodäsie, 2016). These data were used to analyse the habitat structure nearby the SWD traps.

Data analyses for program module "Risk potential"

This program module was analysed by correlating *D.* suzukii monitoring data (trap catches), habitat structure and weather conditions in different periods during the fruit season as well as during the overwintering period. Data sets were geostatistically evaluated (joined, merged and clipped). Afterwards the influence of the weather conditions and of the amount of woodlands, hedges, e. g. on the quantity of captured flies were statistically analysed by multiple binary logistic regression. In this paper the correlation between the mentioned parameters and winter conditions is shown as an example.

Data analyses for program module "Population dynamics"

For modelling population dynamics, the dependency of SWD development on temperature and humidity was analysed in the laboratory and compared with published data (Tochen et al., 2014; Tochen et al., 2015; Ryan et al., 2016; Kinjo et al., 2014; Kim et al., 2015). The development parameters were modeled as functions of temperature by using the Briére function ($f = p1*x*(x-T_{min})*(T_{max}-x)$^(1/m) (Briére et al., 1999).

Data analyses for program module "Phenology"

Phenological monitoring data were correlated with a calculated developmental rate starting from the Biofix April 1^{st}. The correlation of the data was statistically described by a modified Gomperz function (Racca et al., 2012). To calculate the developmental rate, a function has been developed which was based on the influence of daylight length and temperature.

Development of the DSS („SIMKEF")

In a last step the outputs of the three described program modules were combined to an index (= SIMKEF-index), which discribes the risk of fruit infestation. If the SIMKEF-index reached a statistically defined threshold (specific for different host fruits) a high risk for the first infestation was displayed by the DSS. This threshold has to be validated with field data for different crops and varieties as well as in different regions.

Results and discussion

For the development of the DSS much more data were created and analysed than described in this paper. Hence, only examples are shown and discussed. The development of the DSS and the validation process is still going on.

Program module "Risk potential" – example: overwintering conditions

The analyses showed a significant influence of the longest period of cold days (T_{max} < 8 °C) in winter on the occurrence of *D. suzukii* in May, June and July. Furthermore, there were significant positive influences of the area of hedges on the number of flies in nearby traps. In a first independent validation a correct classification of the predicted risk potential from May to July between 70 and 86 % was reached (Jung *et al*., 2018). Accordingly, this analyses is the base for risk assessment of SWD out of generally available data (GIS data for habitat structure and weather data) before the availability of the first main host fruits in spring. It will also be possible to improve the timing of monitoring and regulation of *D. suzukii* at the beginning of the season.

Program module "Population dynamics" – example: oviposition rate

Life table studies showed a strong correlation between developmental parameters of *D. suzukii* and temperature. Temperatures considerably above 30 °C led to a significant reduction of oviposition. In this study optimum temperature for oviposition was similar to those of a corresponding analysis of literature data (Tochen et al., 2014; Ryan et al., 2016) with 26.2 °C. Studies by Evans et al. (2018) also led to a significant decrease of oviposition at > 28 °C, but fluctuation of temperatures have to be considered with different effects (Eben et al., 2017; Grumiaux et al., 2019). In first laboratory trials RH only indicated a low effect on oviposition within the tested range of 40 to 80% with increasing oviposition rates in higher RH conditions and constant temperatures. Studies by Tochen et al. (2015) resulted in strong negative effects of low humidity of 20 and 33% and significant positive effects on population parameters and activity of SWD in higher RH between 71 and 94%. Predominant average daily field data (e. g. weather station LTZ Augustenberg, upper Rhine valley, Germany) ranged from 40 to 100%. Accordingly, RH is assumed to affect SWD population to a very small extend in the field because of only few events of RH < 40% during the reproductive period of the pest in Germany.

Program module "Phenology" – example: Sweet cherry variety "Schneiders"

Figure 1 is showing the development of cherries depending on time after bud swelling (BBCH 51) until harvest (BBCH 87 to 89) (Meier, 2018) according to temperature and day length for the sweet cherry variety "Schneiders". BBCH 81 (= completed fruit growth, starting of colour change) is assumed to be the starting point for SWD infestation and it was reached if the sum of the daily calculated developmental rate (0 to 1) attained the sum of about 15 to 20 after the Biofix (April 1st).

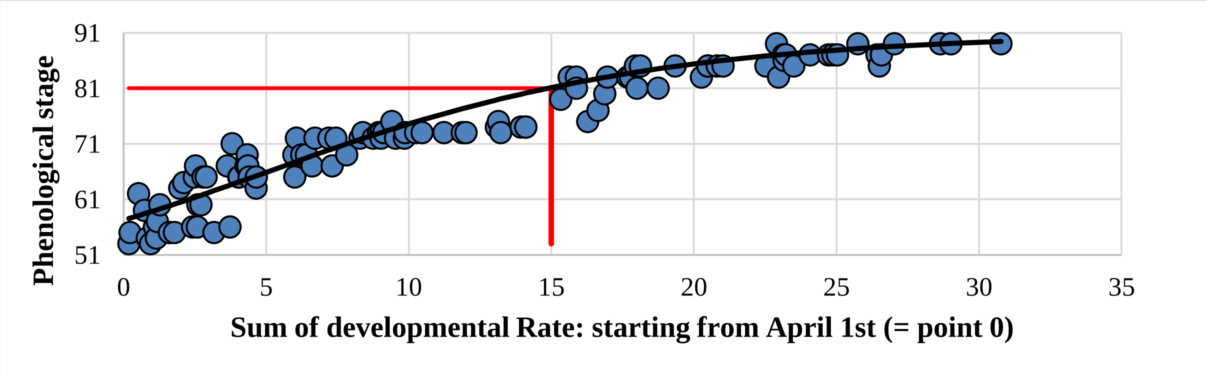

Figure 1. Phenological stages (from BBCH 51 = completed bud growth, still closed, BBCH 81 = completed fruit growth, starting of colour change to BBCH 87-89 = ripe fruits, harvest) and the sum of the developmental rate; the sum of the developmental rate of 15 is correlated to BBCH 81 (red lines) and is assumed to be the starting point for SWD infestation, data from 2005 to 2015, LTZ Augustenberg, Karlsruhe, Germany.

Modelling the ontogenesis of host fruits provides the option to simulate host availability in the adequate stage for oviposition depending on generally available data, like temperature and day length. It can be adapted to different fruit varieties and host fruit species.

Output of the DSS – example: Sweet cherry variety "Schneiders"
The output of the model is a time depending figure under specification of an index (SIMKEF-index) and thereby the threshold for an assumed oviposition risk. For the sweet cherry variety "Schneiders" a threshold of 0.1 was calculated. In 2017 this threshold was reached on May 28th by modeling. In the field 1st oviposition was found on May 29th. Accordingly, calculated model data corresponded with field data in this example. Until now, 20 German and Swiss data sets could be used for model validation for cherries. Thereby the mean observed 1st infestation in the field was found 6 days after exceeding the DSS threshold. Only in two cases the DSS threshold was calculated too late compared to the 1st infestation in the field. The application of the DSS is provided for restricted users on www.isip.de.

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An innovative management approach for spotted wing drosophila (*Drosophila suzukii*) using an environmentally friendly attract and kill formulation

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Abstract: During the last years several data related to the interactions between host plant-associated yeasts and *D. suzukii*, commonly known as the spotted wing drosophila (SWD), were collected and showed that yeast species are attractive to *D. suzukii* in different manners. Moreover, some yeast strains favor larval development, female fecundity and stimulate oviposition. The aim of this work is the identification of a lure with an attractive and phagostimulative component suitable for the development of an attract-and-kill effective formulation. The attractant and feeding stimulant activity of selected yeast cultures towards *D. suzukii* adults were determined in laboratory bioassays and in field trapping tests. Target metabolites content of yeast cultures and yeast culture head space volatiles were determined. The most promising yeast species on an appropriate cultivation substrate were defined.

Key words: *Drosophila suzukii*, yeast, ingestion, attractivity, *H. uvarum*

Introduction

The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura is a polyphagous invasive species in South Tyrol (Italy) which is responsible for major damages to local fruit farming and viticulture.

During the last years several data related to the interactions between host plant-associated yeasts and SWD were collected (Bellutti et al., 2018; Mori et al., 2017; Hamby et al., 2012). Yeast species are attractive to SWD in various ways (Scheidler et al., 2015). Moreover, some yeast strains favor larval development, female fecundity and stimulate oviposition (Bellutti et al., 2018; Spitaler, 2016). These interactions are currently investigated by our research team. The main goal of the study is to develop an effective pest control strategy based on the behavioral manipulation of the insect to specific yeast lures. The expected result is an attract-and-kill formulation, which can attract SWD females and males on target plant leaf surfaces to reduce the load of chemical insecticide residues on the fruits and allow a reduction of the insecticide amount per ha. Headspace volatile compounds, content of metabolites, attractant and phagostimulant activity of selected yeast cultures were determined to define the most appropriate yeast culture for the project purposes.

Material and methods

Yeast cultivation

The following yeasts with the corresponding accession numbers from the NCBI GenBank were used: *Hanseniaspora uvarum* (H.u. 1.21, KP298009), *H. uvarum* (H.u. 2.2, MK567898), *H. uvarum* (H.u. 3.4, MK567905), *Saccharomycopsis vini* (S.v. 1.33, KP289011), *Candida* sp. (C.sp. 3.3, KP298013), *Issatchenkia terricola* (I.t. 2.1, MK567903), *Metschnikowia pulcherrima* (M.p. 3.2, KP298012) and a laboratory strain of *Saccharomyces cerevisiae* (S.c. 288, S288c). The first seven yeasts were isolated from SWD infested fruits at the Research Centre Laimburg (Bellutti et al., 2018).

Yeast solutions were grown in 1 l PDB (24 g/l Difco^{TM} Potato Dextrose Broth, Becton Dickinson) and in 1L YMM (6,8 g/l Yeast Nitrogen Base Without Amino Acids with 20 g/l Glucose, Sigma Aldrich, France) over 30 h at 25 °C and 120 rpm in a 2 l Erlenmeyer flask closed with cotton and aluminum foil. The yeast solutions were stored at -80 °C till use.

CAFE assay

The daily ingestion of different yeast solutions was measured with a modified CAFE assay (Ja et al., 2007). After emerging SWD flies were kept for four days on cornmeal diet ($DSCA_{(a)}$ with dry deactivated yeast) with dry baker's yeast sprinkled over the surface (Bellutti et al., 2018). Before SWD entered the assay, they were kept for 5 h on cotton soaked with water. During the assay single SWD females were kept in 2-ml Eppendorf tubes (Safe-Lock Tubes) with three holes (diameter 1 mm) on the side, at the 1.5-ml mark, for air exchange and one hole at the bottom for capillary insertion. As additional water source and for oviposition a piece of water agar (15 g/l Agar-agar, Merck, Italy) was placed in the lid. The ingestion was measured after 24 h by measuring the difference between zero mark and liquid level. The daily evaporation was measured and subtracted from experimental readings.

All statistical analyses were performed with SPSS. To detect differences between ingestion values a Kruskal-Wallis H test was performed. For pairwise comparison a Dunn-Bonferroni-Test was done.

Trapping trials

Trapping trials with yeasts grown in PDB were performed at the edge of the forest (coordinates: 46.378387, 11.285120) on the 16th of July and 21th of August 2018. 4-ml glass vials were fixed on woody plants at about 1.5 m height and filled with 2 ml bait and 0.4 µl tween 20. The treatments were replicated 10 times at both days. After 24 h the traps were collected and the number of caught SWD flies was counted. Yeast solutions in YMM were not tested in field trapping trial.

Headspace volatile collection and Gas Chromatography-Mass Spectrometry analysis

The headspace volatiles were collected from 8 ml of each of the eight yeast extracts in 20-ml clear glass vials (Sigma-Aldrich, St. Louis, MO) with a DHS syringe (Gerstel Inc., Linthicum, MD). Volatiles were separated on a non-polar HP-5MS column (30 m × 0.25 mm ID, 0.25 µm film thickness, Agilent Technologies) and analyzed with a gas chromatograph (GC 7890A) coupled with a mass spectrometer (MS 5975C Network) (Agilent Technologies, Santa Clara, CA, USA). Significance of quantity differences among strains was measured by ANOVA and posthoc Tukey test.

Analysis of target metabolites

Fermentation broth samples were filtered using hydrophilic SFCA filters (0.2 µm) for the analysis of extracellular metabolites. Carbohydrates and polyols were analysed using pulsed amperometric detection HPAE-PAD (Dionex ICS 5000, Thermo Fisher, US) after dilution of samples with water (1 to 10). Amino acids, vitamins, nucleosides and nucleobases were analysed using liquid chromatography-electrospray ionization-triple quadrupole mass spectrometry UHPLC-QqQ (Dionex UltiMate 3000 UHPLC TSQ Quantiva, Thermo Fisher, US) in multiple reaction monitoring (MRM) mode according to Paglia et al. (2012) with minor modifications. Prior to analysis samples were diluted 1 to 10 with an acetonitrile:methanol:water solution (40:40:20).). The concentration of each metabolite was calculated based on calibration curves of reference standards for each compound and used to generate the heatmaps by the online tool Metaboanalyst (Xia and Wishart et al., 2016).

Results and discussion

CAFE assay

SWD ingested significant different amounts of yeast solutions grown in PDB ($\chi2 = 47.418$, df = 8, $p < 0.001$, $n \geq 17$) and yeast solutions grown in YMM ($\chi2 = 52.406$, df = 8, $p < 0.001$, $n \geq 13$) (Table 1).

Table 1. Average (± SD) ingestion by SWD females of yeast cultures grown in PDB or YMM within 24 h. The control treatment contained the culture media without yeast. Values with the same letter are not significant different ($p \leq 0.05$).

Yeast treatment	**Ingestion (µl) (PDB)**	**Ingestion (µl) (YMM)**
Control	4.12 ± 3.33^{cd}	5.19 ± 2.98^{ab}
S.c. 288	2.43 ± 2.13^{ab}	2.44 ± 2.41^{a}
H.u. 1.21	6.07 ± 3.54^{d}	6.86 ± 2.67^{b}
H.u. 2.2	4.25 ± 3.64^{bcd}	4.82 ± 3.02^{ab}
H.u. 3.4	4.68 ± 3.59^{bcd}	8.13 ± 2.51^{b}
S.v. 1.33	2.97 ± 2.71^{abc}	7.91 ± 1.76^{b}
C.sp. 3.3	1.18 ± 1.41^{a}	2.67 ± 2.80^{a}
I.t. 2.1	3.85 ± 2.41^{abcd}	6.61 ± 2.45^{b}
M.p. 3.2	5.21 ± 3.07^{bcd}	6.83 ± 3.36^{b}

Trapping trial

SWD showed significant differences between the attractiveness to different yeast solutions in the field ($\chi2 = 73.734$, df = 8, $p < 0.001$) (Figure 1). In laboratory trapping trial *H. uvarum* strains and in particular *S. vini* grown in PDB performed better than the other tested yeast species (data not shown).

The results show that attractiveness and ingestion of different yeast species are not always correlated. For an attract-and-kill strategy it is important that the bait is attractive and will be ingested from SWD. This was the case for the three *H. uvarum* strains and *S. vini*.

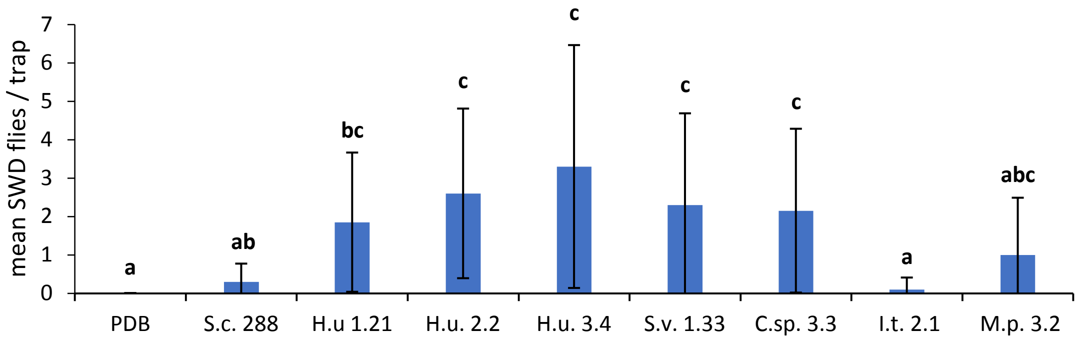

Figure 1. Mean (± SD) number of captured SWD flies per trap (n = 20) within 24 h in a field trial. Means labeled with the same letter are not significant different (Dunn-Bonferroni tests, $p < 0.05$).

Volatiles compounds composition

The volatile compounds of the eight studied yeast strains were identified. In Table 2 the compounds of the yeast cultures grown in PDB and collected with DHS are listed. Volatile compounds detected in YMM yeast culture were few and are not reported.

Table 2. Volatile compounds composition of the eight studied yeast strains. Means (± SD) with the same letter are not significant different ($p \leq 0.05$, n = 5). - = not detected. Values are indicating the compound quantitative averages measured in TIC (Total Ion Current, peak area) by GC-MS analysis, divided by one million.

Compounds	S.c. S288	H.u. 1.21	H.u. 2.2	H.u. 3.4	I.t. 2.1	C.sp. 3.3	S.v. 1.33	M.p. 3.2
ACETATES								
ethyl acetate	-	65.94±27.50^{b}	30.79±16.86^{b}	91.59±24.51^{b}	-	208.45±79.86^{c}	78.62±31.88^{b}	22.71±6.31^{ab}
isoamyl acetate	-	0.96±0.47^{b}	0.37±0.24^{b}	0.81±0.43^{b}	-	23.48±9.55^{a}	-	-
isobutyl acetate	-	-	-	-	-	1.30±0.84	-	-
n-propyl acetate	-	-	-	-	-	1.07±0.93	-	-
ALCOHOLS								
3-methyl-1-butanol	9.61±7.11^{b}	1.27±1.15^{a}	0.54±0.76^{a}	0.41±0.49^{a}	8.25±4.44^{b}	2.81±1.73^{a}	5.41±2.38^{ab}	5.25±2.11^{ab}
ethanol	104.50±100.67^{a}	37.78±18.98^{ab}	30.41±12.69^{ab}	40.54±28.56^{ab}	59.67±30.34^{a}	61.25±33.15^{a}	39.71±6.70^{ab}	130.43±24.75^{c}
isobutanol	1.64±1.45^{a}	-	-	-	3.28±1.53^{b}	-	-	-
BENZENE DERIVATES								
benzaldehyde	0.19±0.07^{a}	-	-	-	0.28±0.17^{b}	-	-	0.37±0.13^{b}
ESTERS								
ethyl hexanoate	0.24±0.11^{a}	-	-	-	0.40±0.22^{b}	-	-	-
ethyl octanoate	0.62±0.46^{a}	-	-	-	1.33±0.43^{b}	-	-	-
ethyl nonanoate	-	-	-	-	0.08±0.08	-	-	-
ethyl-9-decenoate	0.04±0.03	-	-	-	-	-	-	-
ethyl decanoate	0.51±0.52^{a}	-	-	-	0.34±0.11^{a}	-	-	-
MONOTERPENES								
beta-ocimene	-	-	-	-	-	-	0.19±0.06	-

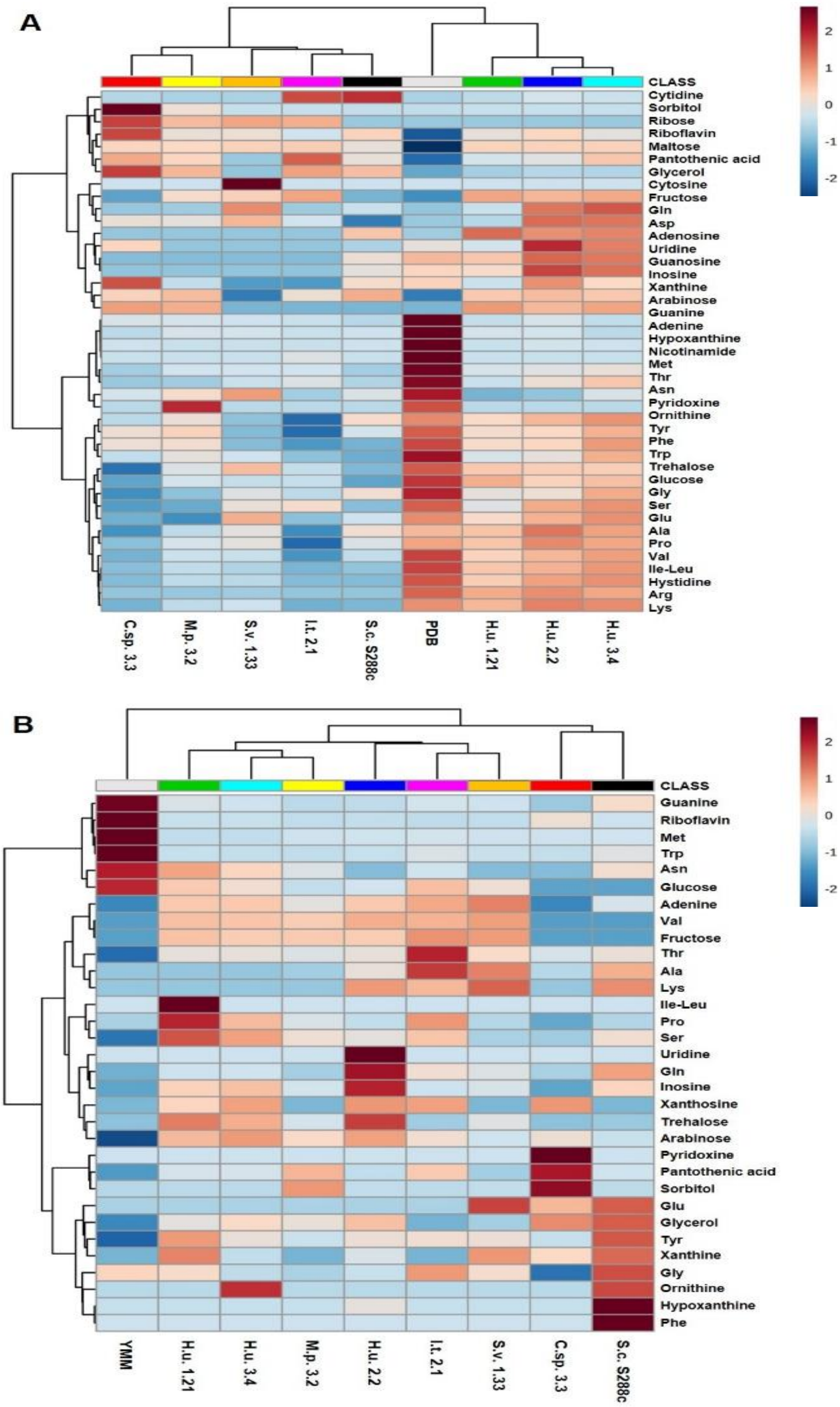

Figure 2. Heatmaps of extracellular metabolites of yeasts grown in PDB (A) and in YMM (B) and the corresponding culture broth. Two separate dendrograms clusters yeasts and medium (*vertical axis*) and metabolites (*horizontal axis*). The length of the lines of the dendrograms are proportional to the distance (correlation). For comparison between yeasts the amount of each metabolite is color-coded with blue to red squares (low to high amount).

Analysis of target metabolites

The target analysis of extracellular metabolites showed important differences in the metabolite content present in the culture broth after 30 h yeast cultivation. Different yeast species consume compounds like carbohydrates and some amino acids and produce other metabolites, like polyols, in different ways.

The concentration of extracellular metabolites found from different yeasts changed depending on the growing medium (Figure 2). Generally, *H. uvarum* strains consume less amino acids and glucose than other yeast species. Almost all available glucose is consumed by *S. cerevisiae* and *Candida* sp., whereas for example trehalose and glutamic acid were higher in all *H. uvarum* strains and in *S. vini* on a nutrient rich medium like PDB.

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New developments in pest monitoring methods

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Herbivory-induced plant volatiles from apple attract *Archips xylosteana* (Lepidoptera: Tortricidae)

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Abstract: Several species of leafrollers (Tortricidae) are important pests of fruit trees and are widely controlled by insecticide treatments by growers. However, due to their negative impact on the ecosystem, human health and the development of resistance, new alternative tools are needed. In our previous studies, we have shown that specific blend of herbivore-induced plant volatiles (HIPVs) released by apple foliage are attractive to conspecific adult males and females of *Pandemis heparana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae) when used together with acetic acid. In this research, we aimed to evaluate the effectiveness of several blends of HIPVs previously characterized in apple trees with and without acetic acid as a binary lure to monitor tortricid moths.

In 2018, a field experiment was carried out in an organically managed apple orchard of South Tyrol (Italy) with the aim to assess several volatile blends as moth attractants. Only few blends, but always in combinations with acetic acid, were able to attract significant numbers of *Archips xylosteana* (Lepidoptera: Tortricidae). The majority of the caught individuals were males. To the best of our knowledge, the results of the present study provide the first non-pheromonal lure for *A. xylosteana*.

Key words: kairomone, monitoring, trapping, integrated pest management, *Malus* x *domestica*

Introduction

Most apple growers use insecticides to manage leafrollers, which have selected resistance and cross-resistance to some classes of chemical compounds (Dunley et al., 2006). In order to delay the development of insecticide resistance, many growers have broadened their management strategies to include the use of microbial and newer conventional insecticides (Sial et al., 2010). Meanwhile, researchers have examined the use of sex pheromones and herbivore-induced plant volatiles (HIPVs) as sustainable alternatives for leafrollers management (Knight, 2010; Knight et al., 2017). Recent studies have shown that acetic acid plays a key role in attracting male and female tortricid leafrollers when used in combination with HIPVs (Basoalto et al., 2017; Giacomuzzi et al., 2017; Knight et al., 2017).

During the last years, populations of the golden variegated moth *Archips xylosteana* L. (Lepidoptera: Tortricidae) increased in Europe (Blaga et al., 2018). The species is monovoltin and widely distributed in Europe, Asia and northern Africa (Blaga et al., 2018). Also it has been detected in North America (Hoebeke et al., 2008) and at present it is a quarantine species in USA. *A. xylosteana* is a polyphagous pest of fruit trees such as apple, pear, plum, and cherry. The larvae may cause significant damage by feeding on new foliage and buds, but also on fruitlets of trees and shrubs of Rosaceae and Fagaceae. Larvae roll leaves to create protected feeding sites. The crucial attack is held by the caterpillars in the first two stages, when the leaves are small and tender unlike other defoliators. A single caterpillar is able to destroy in one day between half a leaf and two future leaves. The most important economic damage is inflicted by the young larvae feeding on the leaves, especially in colder springs, when the leaves develop more slowly (Blaga et al., 2018).

In this study, we evaluated the effectiveness of several blends of HIPVs with and without acetic acid as a binary lure to monitor tortricid moths.

Material and methods

Experimental design: In order to investigate the ecological roles of the HIPVs, few combinations of the characterized volatiles were selected based on our previous experience and the literature. Field experiments were done at the Research Center of Laimburg in 0.5 ha apple Cv. Gala organically managed. The experiment started in June 2018 until 3rd of August. The selected volatiles were loaded together with paraffin oil as an emission retarder inside 1.5 ml Eppendorf vials fitted with dental cotton wicks. Volatiles were released through a 2 mm diameter hole drilled in each vial, placing each lure in white delta traps with sticky inserts. 10 mg of each volatile were loaded together with 100 mg of paraffin oil into the vials, while 500 µl acetic acid were loaded in separated 2 ml vials. The blank samples (treatment n. 1) contained only cotton wicks with paraffin oil. Eight replicates were spaced 6 m apart along tree rows in a completely randomized design. Traps were hung in the tree canopy at a 2-2.5 m height. The number of caught insects was counted weekly and identified.

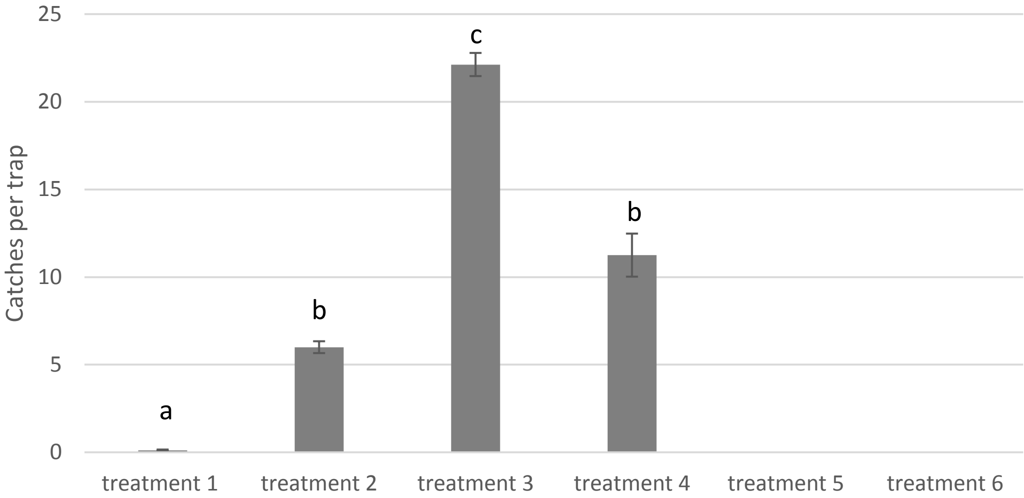
Statistical analysis: Statistical analyses were performed using R software 3.0.213 with package glmmTMB**.** The data were analyzed using generalized linear mixed models (GLMMs). The number of caught insects distributed according to a negative binomial distribution with date used as random effect. Model selection was carried out by a combination of forward and backward selection based on Akaike’s Information Criterion (AIC).

Results and discussion

In this work we report that HIPVs from apple foliage can play an important role to monitor tortricids when combined with acetic acid. This complies with previous studies (Giacomuzzi et al., 2016; Knight et al., 2017). Treatments including acetic acid significantly caught more *A. xylosteana* than treatments without acetic acid (Figure 1, GlmmTMB df = 5, p-values $\leq$ 0.001).

Overall, treatment number 3 (=loaded with paraffin oil, benzaldehyde, methyl salicylate, decanal, nonanal and acetic acid) had the statistically highest catches between all treatments with 22 catches per trap during one-week peak of the flight (Figure. 1). Considering the treatments that contain acetic acid, treatment 3 (GlmmTMB z-value = 4.23, p-value < 0.001) significantly caught more moths than treatment 4 (GlmmTMB z-value = 3.18, p-value = 0.001) and treatment 2 (GlmmTMB z-value = 3.07, p-value = 0.002).

To the best of our knowledge, the results of the present study provide the first specific lure to monitor *A. xylosteana*. Frerot (1983) found that the males of *A. xylosteana* are attracted to blends of (*Z*)- and (*E*)-11-tetradecenyl acetate, two common components of sex pheromones of the subfamily Tortricinae (Ando et al., 1978*). A. xylosteana* may also be attracted to pheromone lures of the oriental fruit moth, *Grapholita molesta*, of the red-banded leafroller, *Argyrotaenia velutinana* and of the oblique banded leafroller, *Choristoneura rosaceana* (Hrdy et al., 1979), however no lures were so far able to attract in high number only *A. xylosteana*.



paraffin oil	x	x	x	-	x	x
benzaldehyde	-	-	x	-	-	x
methyl salicylate	-	x	x	-	x	x
decanal	-	x	x	-	x	x
nonanal	-	x	x	-	x	x
caryophyllene	-	x	-	-	x	-
linalool	-	x	-	-	x	-
acetic acid	-	x	x	x	-	-

Figure 1. Mean (± SE) catches of *Archips xylosteana* (Tortricidae, Lepidoptera) in traps loaded with HIPVs and acetic acid during the peak of the flight.

In our experiments induced plant volatiles were attractive exclusively when combined with acetic acid, further proving the synergizing effect of acetic acid in enhancing lure attractiveness for tortricid moths, as recently found also for *Cydia pomonella* (Kight et al., 2019) and *Grapholita molesta* (Preti et al., 2019). Our results show that a five-component blend consisting of benzaldehyde, methyl salicylate, decanal, nonanal and acetic acid is an attractive for *A. xylosteana*. During 2019 we aim to further confirm the achieved results to optimize the attractive blend for *A. xylosteana* by performing new field experiments.

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Observations on flight activity and voltinism of codling moth *Cydia pomonella* in western central part of Latvia in 2016-2018

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Abstract: Codling moth *Cydia pomonella*, has been considered univoltine in Latvian conditions, with only rare isolated cases of an incomplete second generation. In the light of recent advancements on the topic of climate change, a possible shift towards more than one generation per year has to be considered. Change of voltinism would also require a shift in recommendations for codling moth management. We carried out observations on flight activity of codling moth, using traps baited with lures containing female codling moth sex pheromones. Observations were carried out in one orchard for three consecutive years and three different voltinism patterns were observed ranging from typical univoltine development in 2016 to a pattern strongly suggesting bivoltine development in 2018. Therefore our findings suggest enough developmental plasticity in codling moth to adjust its voltinism to changing meteorological factors. It stays unclear if the second generation is complete and capable for overwintering.

Key words: *Malus x domestica*, monitoring, pheromones, *Cydia pomonella*

Introduction

Codling moth *Cydia pomonella* is a key pest in apple orchards in Latvia, which, if left uncontroled, in favourable conditions can cause more than 50% yield loss (Priedītis, 1996). In the past before widespread use of pheromone baited traps, decision support systems and synthetic pyrethroids, crop losses up to 100% were reported (Ozols, 1973). Nowadays the access to decision support systems allows Latvian farmers to achieve suficient codling moth control via a single neonicotinoid spray at the time of peak egg hatching.

So far codling moth has been considered univoltine with only rare isolated cases of an incomplete second generation described in Latvia (Priedītis, 1996). However, by applying codling moth phenology models to the global climate change predictions, a prognosis was made by Stoeckli et. al. in 2012 that a global shift in codling moth voltinism might take place and the number of generations per vegetation season might increase in temperate climate zones. If the shift to bivoltinism does occour, significant adjustments in codling moth management reccomendations for farmers have to be made to avoid unexpected crop loss. Therefore the aim of this study is to observe changes in codling moth flight pattern over time and predict necessary changes in codling moth management in following years.

Material and methods

Observation site
Observations were carried out in an apple orchard located in western central part of Latvia, town Pūre, 57°01'58.1"N 22°55'03.8"E. Area of the orchard was 1.5 ha. The orchard was managed using principles of integrated pest management. Decisions about codling moth management were made based on pheromone trap catch (local treshold used was in average five to ten males per trap in a week) and prognosis of decision support system RIMpro-Cydia.

Trapping methods
Each year four transparent delta traps with sticky inserts and four green top funnel traps were placed in a lattice pattern in the orchard. The traps were baited with codling moth female mating pheromone lures. All traps and lures were manufactured by Pherobank BV. Codling moth males caught were counted weekly and pheromone lures were replaced every four to five weeks. Periods of observations and number of assessments each year can be seen in Table 1.

Table 1. Exposure timing of traps baited with codling moth sex pheromone lures and number of assessments carried out during exposure time

Year	**Beginning of trap exposure**	**End of trap exposure**	**Number of trap catch assessments**
2016	May 20	August 12	12
2017	May 25	August 17	12
2018	May 10	August 30	16

Obtaining of meteorological data
Meteorological data was obtained from hydrometeorological station Stende, located 28 km North-West from the observation site.

Results and discussion

Codling moth flight activity in 2016
In 2016 codling moth flight activity was reasonably low, and the number of males caught in pheromone lure baited traps never reached the local treshold for justified insecticide use of five to ten males per trap in a week's time (Priedītis, 1999). Figure 1 shows that even though individual males were caught up to August 12, the main flight took place until June 27. The beginning of the flight could not be established with certainty, as a substantial amount of males was registered on the first trap assessment after installing, indicating that the flight had already started before trap installation. The number of males caught during main flight responded to fluctuations in temperature. A brief period of temperatures lower than the long term average from June 5 to June 14 was followed by lower trap catches in the respective assessments, but the number of males caught increased again, when the temperature increased. It matches with the opinions of Ozols (1973), Priedītis (1996) and Alford (1999) who state that flight activity is possible only when temperature exceeds 15 °C. Overall the flight pattern showed typical univoltine development.

Codling moth flight activity in 2017

In 2017 codling moth flight activity was higher than in 2016, while still not exceeding the local treshold for justified insecticide use at any given assesment.The active flight period, as shown in Figure 1, was clearly extended, ranging from June 2 or possibly earlier to August 3, with individual males still getting caught until August 17 or possibly later. According to observations made from 1948 to 1959, usually the flight activity dissipates in the first ten day period of July (Ozols, 1973). Partially the extended flight period in our observations can be attributed to improvements in detection, namely the use of pheromone baited traps instead of ultraviolet light traps, however it is unlikely that first generation males survive until mid August. Therefore our observations suggest that there were at least some larvae that did not enter diapause but pupated around the first or second tertile of July and hatched in August.

From the trap catch data alone it was impossible to tell when the flight of the first generation ends and that of the second begins. In future, to supplement the flight observations, other methods like direct examination for eggs and new larvae should be carried out in August, to distinguish the generations. Overall the flight pattern of codling moth in 2017 showed a possibility of at least a part of the population developing a second generation.

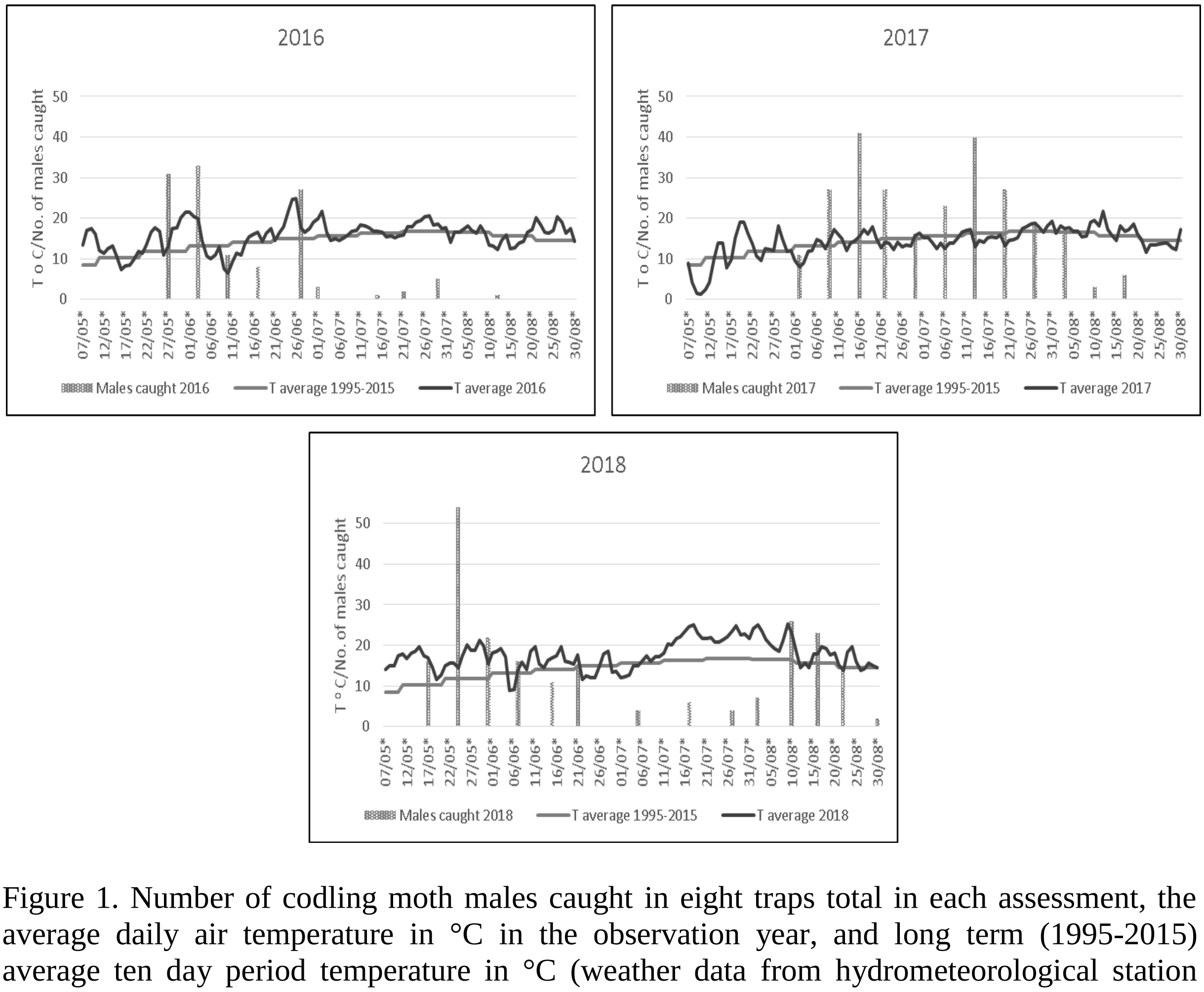

Figure 1. Number of codling moth males caught in eight traps total in each assessment, the average daily air temperature in °C in the observation year, and long term (1995-2015) average ten day period temperature in °C (weather data from hydrometeorological station Stende).

Codling moth flight activity in 2018

The vegetation season of 2018 was distinguished by unusually warm and dry weather conditions. Figure 1 shows that May and July were almost constantly warmer than the long term average. In 2018 the codling moth male trap catches showed two distinct peaks, which can be clearly seen in Figure 1. The first peak took place from May 17 until June 21, reaching its maximum on May 24, when for the only time in three years the local treshold for justified insecticide use was formally reached. The second, slightly lower peak was observed from August 10 until August 22, with only few individual males caught in the period in between the peaks or after them.

This pattern suggests a strong evidence that large part of the population experienced bivoltine development in 2018. However it is unclear if the second generation will successfully develop until maturity, as the development from oviposition to mature larva lasts from 35 to 47 days in Latvian climatological conditions (Priedītis, 1996), and many of the apple varieties grown in this particular orchard are harvested from the end of August to the middle of September, therefore the larvae will have to rely on finishing development in fallen fruit or storage facilities.

Implications for farmers

These observations imply that codling moth shows enough plasticity regarding its phenology to shift from univoltine to bivoltine development depending on meteorological conditions. If the climat change trend towards longer and warmer summers in the temperate zone continues, bivoltine development can become more common. Monitoring of codling moth flight activity should be continued to see wether overwintering is succesful in case of development of a second generation. If that turns out to be the case, adjustments in recommendations of plant protection product applications targeting codling moth should be made. As the development of second generation appears to take place relatively close to harvest, use of conventional insecticides for controling the second generation is going to be problematic. This stresses the importance of approbating and registering novel insecticides with shorter pre-harvest intervals, such as *Bacillus thuringiensis* or *Cydia pomonella* granulosis virus preparations for use in Latvia.

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Improving *Grapholita molesta* monitoring in peach and nectarine orchards under mating disruption by using bisexual lures

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Abstract: Emilia-Romagna is an important peach and nectarine production region in Italy. In this area a primary component of pest management includes the use of sex pheromones for mating disruption (MD) of oriental fruit moth, *Grapholita molesta* Busck (Lepidoptera: Tortricidae). Despite the typical effectiveness of MD to manage *G. molesta*, there have been some sporadic infestations during the last few years. To better investigate this problem, we performed field experiments during 2018 with a new pheromone-kairomone lure, Pherocon® OFM Combo Dual (Trécé Inc., Adair, OK, USA), specifically developed to be used under MD. This binary lure includes the sex pheromone of both *G. molesta* and codling moth, *Cydia pomonella*, plus terpinyl acetate and acetic acid. The experiment was conducted in 13 commercial orchards under MD, comparing moth catches in traps baited either with a standard sex pheromone (Pherocon® OFM L2) or the new lure. Within each orchard five replicates of each lure type were included and studies were conducted over a 6-8 week period using either white or orange delta traps (Pherocon® VI Delta traps) with white sticky liners (Pherocon® VI Delta liners). Our results showed that the traps baited with the new lure caught almost 3 times more moths than traps with the sex pheromone lure, and females comprised 8% of the total catch. We also noted that much fewer non-targets including pollinators were caught in the orange versus the white delta trap. Evidence of foliar and fruit damage from *G. molesta* were found in all orchards, yet the sex pheromone-baited trap caught moths in only 8 of the orchards. Traps baited with the kairomonal lure caught moths in all blocks and adoption and refinement of this new monitoring tool should allow growers to better assess pest pressure. We feel that the development of this new monitoring lure can improve the management of *G. molesta* under MD and facilitate improved pest control for Italian growers.

Key words: *Prunus persica*, Lepidoptera, Tortricidae, delta trap, female moth, kairomones

Introduction

Italy is a leading producer of peaches and nectarines in Europe, with more than 67,000 hectares cultivated in 2017 (FAOSTAT, 2019). Emilia-Romagna is the second most important Italian region, after Campania, with about 11% and 36% of the total peaches and nectarines surface, respectively (IstatAgri, 2019). Though stone fruits are still key crops in Italy, in the last few years, production has decreased due to lower fruit prices, and this change has likely reduced the implementation of intensive and highly-effective management practices. Peach and nectarine orchards in Emilia-Romagna are all managed following the Integrated Fruit Production (IFP), which includes the use of sex pheromones for mating disruption (MD) of oriental fruit moth, *Grapholita molesta* Busck (Lepidoptera: Tortricidae), and the use of some supplemental insecticidal interventions according to the forecasting model of the regional Plant Protection Service.

Unfortunately, since 2016 the occurrence of sporadic infestations of *G. molesta*, both on shoots during the season and on fruits at harvest, has occurred under MD in some production areas of Ravenna and Forlì-Cesena provinces. While these problems are limited, this occurrence and the possibility of an increasing problem is of great concern to the growers.

Sex pheromone baited traps are used to monitor *G. molesta* in orchards not treated with MD to establish timings and the need for insecticide sprays (Emilia-Romagna thresholds: 30 catches/trap/week during the first generation and 10 catches/trap/week for later generations). In orchards treated with MD (> 90% in Emilia-Romagna) the effective use of sex pheromone-baited traps to establish thresholds is limited by the MD treatment, and more commonly, pest occurrence is recorded through labor-intensive visual sampling of the plant canopy to detect larval shoot injury. Development of new lures, which can effectively monitor adult flight under MD, would be a useful and cost-effective tool to monitor *G. molesta*.

The objective of our work reported here was to validate the Pherocon® OFM Combo Dual lure, a new commercial bait of Trécé Inc. (Adair, OK, USA), launched in the Italian market in 2018 by Certis Europe (Saronno, VA, Italy) to monitor *G. molesta* under MD. The new lure for *G. molesta* combines the attractiveness of different sexual and kairomonal volatile compound blends developed recently. It includes two dispensers: one grey septa lure is loaded with a blend of the three components of *G. molesta* sex pheromone and the major component of *C. pomonella* sex pheromone which enhances male catches; and a second membrane lure is loaded with terpinyl acetate and acetic acid which allows traps to catch both moth sexes under MD (Mujica et al., 2018).

Material and methods

Trapping was conducted in Emilia-Romagna in 13 commercial peach and nectarine orchards treated with several types of MD (Table 1) to compare moth catches in traps baited with either the *G. molesta* sex pheromone lure (Pherocon® OFM Combo L2, TRE4102, Trécé Inc.) versus the new kairomonal lure (Pherocon® OFM Combo Dual, TRE3470 + TRE3471, Trécé Inc.). Five replicates of each lure type were randomized within each orchard using Pherocon® VI Delta traps (Trécé Inc.) and white sticky liners. Orange and white traps were used in 6 and 7 orchards, respectively.

Each plot was a minimum of 1.5 hectares and the trap distance was > 25 m from the orchard border and between traps. Trials were performed for a period of 6-8 weeks, between late April and the beginning of September 2018. Traps were checked weekly, liners were replaced, and moths were counted and sexed. Sites were selected among peach and nectarine plots with suspected pest occurrence under MD. Statistical analysis was performed with R software using the Generalized Linear Mixed Models using Template Model Builder (glmm TMB) with a negative binomial distribution ($p < 0.001$).

Table 1. MD type, dispenser density and installation in each trial location of the 13 experiments performed on *Grapholita molesta* monitoring in Emilia-Romagna (Italy) during 2018.

Trial	Location (province)	Cultivar	MD type	N° dispensers per hectare	MD installation
1	Faenza (RA)	Romagna Red	ISOMATE® OFM rosso FLEX	740	March 15th
2		Romagna 3000			
3		Romagna Big			
4		Max 7		700	April 20-25th
5	Roncadello (FC)	August Red	CheckMate OFM®	350	March 31st
6	Bagnacavallo (RA)	Ferclouser	ISOMATE® OFM rosso FLEX	650	End March - beginning April
7		Lami.it			
8	San Tomè (FC)	Red Kyut		700	April 8th
9	Boncellino (RA)	Sweet Lady	RAK® 5+6	700	April 25-30th
10	Villafranca (FC)	Tadibelle	Check Mate Puffer OFM	3.5	March 26th
11	Roncadello (FC)	August Red	CheckMate OFM®	350	March 31st
12	Faenza (RA)	Romagna 3000	ISOMATE® OFM rosso FLEX	740	March 15th
13	Faenza (RA)	Fair Lane	RAK® 5+6	650	March 25-30th

Results and discussion

The new pheromone-kairomone binary lure was significantly more effective than a standard sex pheromone lure among orchards treated with MD (Table 2). Females comprised 8.3% of the total moth catch in traps baited with this new lure, which caught on average 2.7 times more males than the sex pheromone alone. This result is consistent with a previous study, where several new lure blends were compared in the United States, South America and Italy (Mujica et al., 2018). In 8 out of 13 sites treated with MD we also caught male moths in the traps baited only with the sex pheromone. This could be partially explained with the insufficient covering of the MD technique in the selected orchards, however, this aspect needs further investigations. However, larval feeding was recorded (especially on shoots) in all of these blocks, so these non-zero catches in sex pheromone-baited traps is effectively indicating that the pest pressure was very high in these orchards.

The use of different trap color (either orange or white) allowed to consider also the trap and lure selectivity. We noted that the use of orange traps significantly reduced the catch of pollinators, i. e. honeybees and mason bees (Apidae, Megachilidae), which were never caught in orange traps. On the contrary, in the trials conducted with white traps some beneficials were always recorded, regardless the lure baited (data not shown). Previous studies have found that these two trap colors do not differ in the catch of *G. molesta*, but we confirmed a potential effect of trap color on the non-target catch of pollinators (Knight and Miliczky, 2003). The density of beneficials in traps was low, suggesting that selection of trap color is not a major issue for these growers.

Table 2. Average catches of *Grapholita molesta* comparing Pherocon® OFM L2 lure with Pherocon® OFM Combo Dual lure under MD, in 13 peach and nectarine orchards under MD in Emilia-Romagna (Italy) during 2018.

Lures [a]	Mean (SE) moth catch per trap		
	G. molesta males	*G. molesta* females	*G. molesta* total
OFM PH	2.7 ± 1.5 a	0.0 ± 0.0	2.7 ± 1.5 a
OFM/CM PH + TA/AA	7.3 ± 3.0 b	0.7 ± 0.3	8.0 ± 3.2 b
Generalized Linear Mixed Models	Z = -5.693, $P < 0.001$	n.s.	Z = -6.417, $P < 0.001$

The problems with *G. molesta* that some Italian growers using MD have experienced in this region could be due to a number of factors. For example, over the last ten years the peach and nectarine surface has decreased to half in this region, passing from more than 26,500 hectares in 2008 to less than 12,500 hectares in 2018 (IstatAgri, 2019). This drastic contraction has likely affected MD due to increased fragmentation of the MD sites and increased border areas receiving intermediate coverage. Secondly, it could be that because MD is usually set up after flowering, when the first generation of *G. molesta* adults has already started, and this lag period has increased due to recent climate change factors and warmer springs in this region. Thirdly, a possible cause of increased MD control failure could be due to changes in growers' typical spray practices. Growers may have become too reluctant to supplement MD with insecticide sprays and thus allowing pest densities to overwhelm this behavioral control tactic. However, this may being reversed as over the last two years with the arrival of the brown marmorated stink bug, *Halyomorpha halys* Stål (Rhynchota: Pentatomidae), in the region and particularly in the areas where the peach and nectarine production is concentrated more sprays are being applied.

In summary, it is clear that improved monitoring of *G. molesta* under MD using the new binary lure offers enhanced opportunities to track *G. molesta*. Several years of basic field trials will be needed to fully assess its performance and to develop useful thresholds to support management decisions.

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Progress towards identification of a pheromone of the asparagus beetle, *Crioceris asparagi* (Coleoptera; Chrysomelidae)

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Abstract: The asparagus beetle, *Crioceris asparagi* L. (Coleoptera; Chrysomelidae) has become a widespread pest of asparagus, *Asparagus officinalis* (asparagus). There are no known attractants for *C. asparagi* and much of its biology is unknown. Volatiles were collected from individual beetles and a candidate pheromone component was detected in collections from some individuals of the first generation but not the second. This compound elicited an electroantennographic response from *C. asparagi* and the mass spectrum indicated it is a 19-carbon hydrocarbon with four double bonds. Complete structural elucidation and synthesis are in progress.

Key words: asparagus, *Crioceris asparagi*, pheromone, electroantennography, mass spectrometry

Introduction

The asparagus beetle, *Crioceris asparagi* L. (Coleoptera; Chrysomelidae) originated in the Mediterranean region but now occurs wherever asparagus, *Asparagus officinalis*, is grown. Asparagus appears to be the sole source of food with infestations causing severe damage to both the foliage and the commercially valuable spears. Currently, there are no known attractants for *C. asparagi* and much of its biology is still unknown, although a male-produced aggregation pheromone has been identified in the closely-related cereal leaf beetle, *Oulema melanopus* L. (Cossé et al., 2002). An attractive pheromone for *C. asparagi* could be used in population monitoring and possibly control, and contribute towards a greater understanding of its biology.

Material and methods

Insects

Crioceris asparagi beetles were collected as adults and as late larvae or pupae from asparagus fields in Cambridge and Kent. Larvae and pupae were reared through to adults in individual containers in the laboratory at ADAS. In the UK, *C. asparagi* has two generations of adults. Overwintered adults emerge in late spring and mate and lay eggs. Larvae feed on asparagus fronds and spears, pupate in the soil and emerge as adults in late summer and then overwinter as adults. Collections were made from adult beetles of the second generation in June-July 2017 and the overwintered generation in May 2018. First and second summer generations could be separated by timing of adult emergence within the crop. Beetles were sexed by dissection and examination of the genitalia.

Collection and analysis of volatiles

Volatiles were collected from individual beetles on Porapak resin and eluted with dichloromethane. Collections were analysed by gas chromatography (GC) coupled to mass spectrometry (MS) on both polar (DBWax) and non-polar (VF5) columns. Collections were also analysed by GC coupled to electroantennographic (EAG) recording from a beetle antenna with intermittent, "puffed" delivery of the column effluent to the EAG preparation and flame ionisation detection (FID).

Results and discussion

A peak was observed in GC-MS analyses of 18 out of 66 collections from *C. asparagi* beetles of the first, overwintered generation which elicited an EAG response from a beetle antenna (Figure 1), indicating it was a potential pheromone component. This compound was not detected in 30 collections from the second generation, adding further support to it being a component of a sex or aggregation pheromone since only the overwintered beetles mate and those of the second generation overwinter as adults.

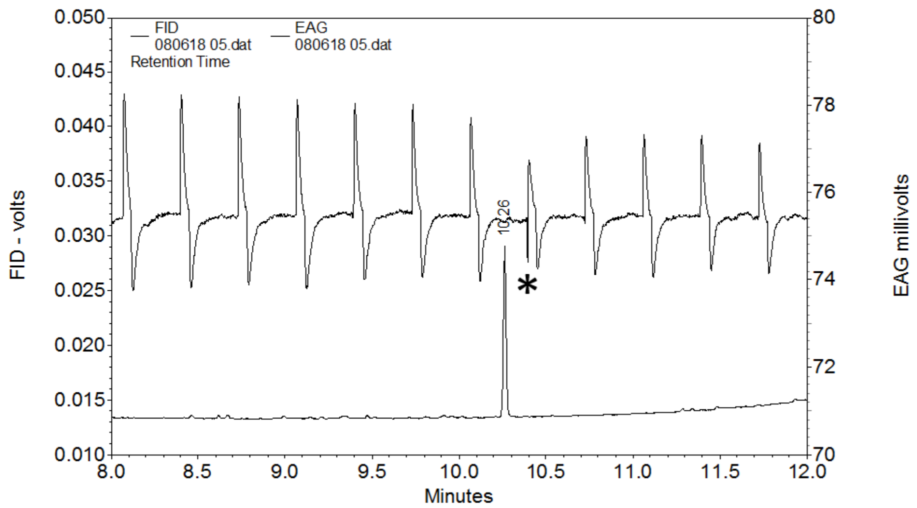

Figure 1. GC-EAG analysis of collection of volatiles from *Crioceris asparagi* (lower trace GC-FID, upper EAG response to intermittent delivery of column effluent; * EAG response).

The sex was determined of most of the beetles that produced this compond in significant quantities. The compound was apparently produced by both sexes (8:7 males:females). This was surprising as pheromones are generally emitted by one sex for any given species.

The mass spectrum had a molecular ion at *m/z* 260 and base peak at *m/z* 79 (Figure 2). The GC retention indices were consistent with it being a hydrocarbon with 19 carbon atoms and four non-conjugated double bonds. Hydrogenation produced nonadecane, confirming that it is a 19-carbon, straight chain hydrocarbon. Authentic (*Z,Z,Z*)-1,3,6,9- and (*Z,Z,Z,Z*)-3,6,9,12-nonadecatetraenes had similar but not identical mass spectra and retention times. This structure is very different from the hydroxyketone identified from *O. melanopus* L. by Cossé et al. (2002), which was not detected in any collections from *C. asparagi* by comparison with an authentic standard. Complete identification and synthesis are in progress.

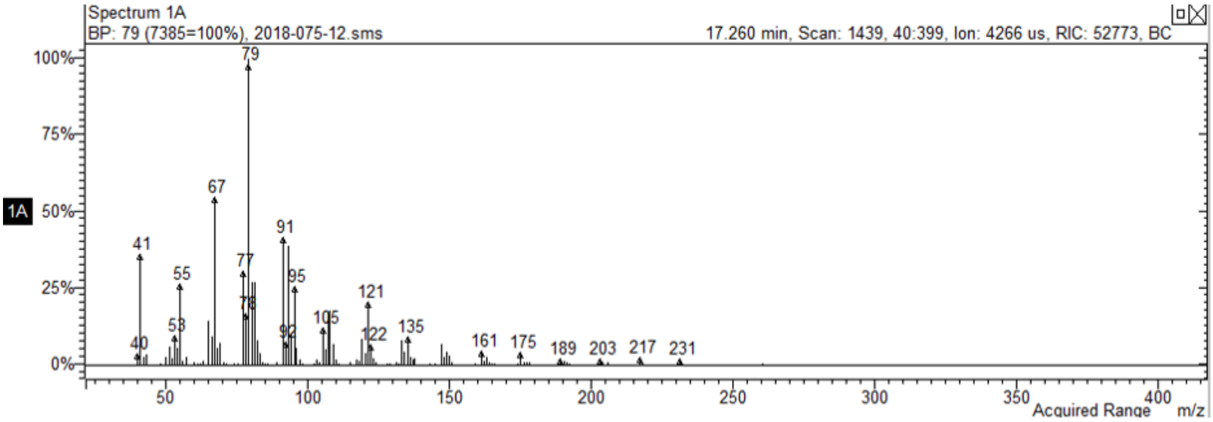

Figure 2. Mass spectrum of the candidate pheromone component from *Crioceris asparagi*.

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The determination of the effectiveness of pheromone traps for the control of Box Tree Moth *Cydalima perspectalis* in Georgia

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Abstract: The Box Tree Moth (BTM) *Cydalima perspectalis* (Walker, 1859) (Lepidoptera; Crambidae) was introduced in 2012 in Georgia and in the next year it began to defoliate *Buxus* spp. in large quantities. Today the situation is quite alarming in Western Georgia, with BTM damaging *Buxus colchica*, which is an endemic species of the Caucasian flora and is threatened now by habitat loss. The larvae feed on leaves and shoots, causing serious damages, defoliating box trees, leading to economic, social and environment problems. During 2017-2018, a WitaTrap® Funnel trap system, with pheromone CYDAWIT® (Witasek, Pflanzenschutz, GmbH, Austria), was installed for the monitoring and control of BTM.

A long term trapping was conducted at two location of boxwood forest in Tsageri-Ambrolauri (South slop of Grate Caucasian mountain range) in West Georgia. In total 450 pheromone traps were set out on the 150 ha at least ten days before the pest was expected to emerge and at the proper height above the ground or in the plant canopy. Three traps per ha were placed, where prevailing winds were carrying the pheromone into the forest area. BTM moths attracted by the pheromone fall into a capture container and cannot fly out anymore. The pheromone traps were emptied and new dispensers were added at two times during the flying period of *C. perspectalis*. The number of captured adults variated from 11 to 176 moths per trap. In total approximately 93000 (2017) and 74000 (2018) *C. perspectalis* were captured in Tsageri-Ambrolauri region during this monitoring period.

Key words: *Cydalima perspectalis*, pheromone trap, *Buxus*, monitoring

Introduction

The Box Tree Moth (BTM) *Cydalima perspectalis* (Walker, 1859) (Lepidoptera; Crambidae) is an insect of Asian origin (Wan et al., 2014; Agius, 2018; EPPO, 2015) that recently invaded most of Europe, Turkey (CABI, 2015; Kenis et al., 2013; Leuthardt, 2013) and the Caucasus, causing serious damage to ornamental box (*Buxus* sp.) shrubs and trees (Matsiakh, 2018; Kenis et al., 2013).

In Georgia BTM was introduced in 2012. During the preparation to the 2014 Winter Olympics in 2012 it was introduced from Italy to Sochi with the planting stock of *Buxus sempervirens*. It then arrived in Georgia and in the next year it began to defoliate *Buxus colchica* in large quantities (Gninenko et al., 2014). *Buxus colchica* is an endemic species of the Caucasian flora. BTM larvae cause damage not only in native habitats of box trees, also in public and private gardens and parks (Matsiakh et al., 2016). The larvae feed on leaves and shoots, causing serious damages, defoliating box trees, leading to economic, social and environment problems (www.tzona.org.ge, 23 April, 2016).

Today the situation is quite alarming in Western Georgia, where *Buxus colchica*, is threatened by habitat loss.

The aim of this research is to test sex pheromone trapping, in order to determine the efficacy of the baits for detecting the male flights of BTM.

Material and methods

Site of investigation

The study of distribution of BTM *C. perspectalis* individuals was conducted in the summer of 2016, in *Buxus colchica* occurring in nature in different regions and locations in Georgia: Imereti, Racha, Tsageri, Samegrelo and Adjara

In every location visual assessments are made in at least 50 individuals of box trees and hedges. Doing this, evaluated plants were distributed in 5 different levels of damage, based on the assessment of the degree of defoliation of box trees by pest larvae, using a 5 point scale (see Table 1).

Table 1. Injury scale of defoliation of box trees by *Cydalima perspectalis* Walker in percent.

#	Defoliation (%)	Damage level	Damage significance
1	0	0	undamaged
2	1-25	1	weak
3	26-50	2	middle
4	51-75	3	strong
5	> 75	4	very strong

Pheromone traps

During 2017-2018, a WitaTrap® Funnel trap system, with pheromone CYDAWIT® (Witasek, Pflanzenschutz, GmbH, Austria), was installed for the monitoring and control of BTM.

A long term trapping was conducted at two locations of boxwood forest in Tsageri-Ambrolauri (South slop of Grate Caucasian mountain range) in West Georgia.In total 450 pheromone traps were set up on the 150 ha, at least ten days before the pest was expected to emerge and at the proper height 2 m above the ground or in the plant canopy. Three traps per ha were placed, where prevailing winds were carrying the pheromone into the forest area.

Results and discussion

This study to establish the degree of damages caused by *C. perspectalis* to *Buxus colchica* was conducted in locations with confirmed presence of this pest in 2016. The results of the research are presented in Table 2.

Table 2. *C. perspectalis* damage level in different locations of Georgia (2016).

#	Region /Area	Defoliation (%)	Damage level	Damage in %	Damage Significance
1.	Imereti	1-25	1	15.7-24.8	weak
2.	Racha	26-50	2	30.5-47.6	middle
3.	Tsageri	51-75	3	65.5-72.3	strong
4.	Samegrelo	51-75	3	52.5-69.5	strong
5.	Adjara	≤ 75	4	75-100	very strong

The results show that *C. perspectalis* is a serious pest on the box trees in the majority of studied localities. The percentages of defoliation ranged from less than 25% to more than 75%. In general it has been observed that the damage level of box tree varied between weak to very strong (15-100%).

During 2017-2018, a funnel trap system, with pheromone traps, was installed for the monitoring and control of BTM in different regions of Georgia. In Figure 1, the areas (in hectares) with native *Buxus* sp. monitored by pheromone traps in different regions of Georgia is represented.

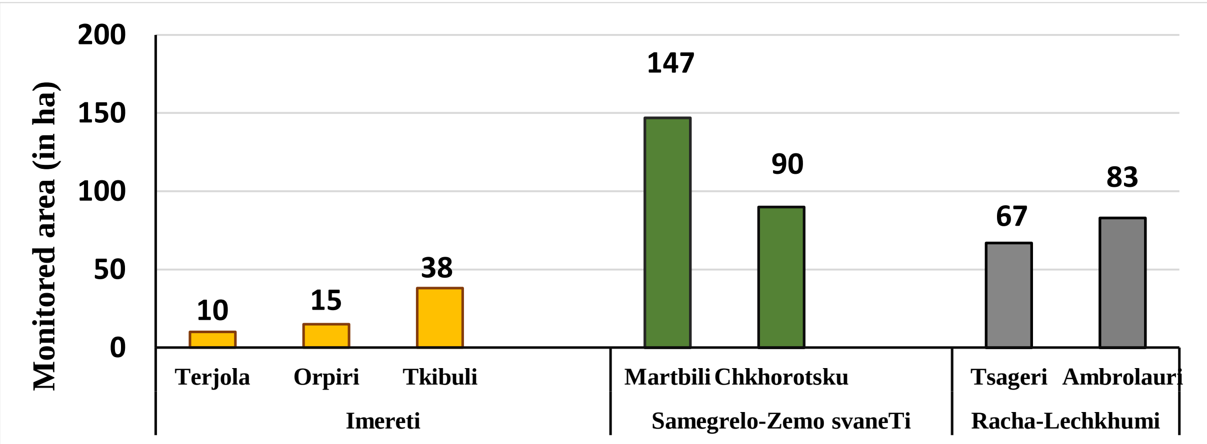

Figure 1. The areas (in ha) in different regions of Georgia with pheromone traps for the monitoring of *C. perspectalis*, 2017-2018.

As described in methods, 450 pheromone traps were installed on 150 ha in Racha-Lechkhumi (Tsageri-Ambrolauri) Region, where we calculated number of baited moth Moth attracted by the pheromone fall into the capture container and cannot fly out anymore (Figure 2).

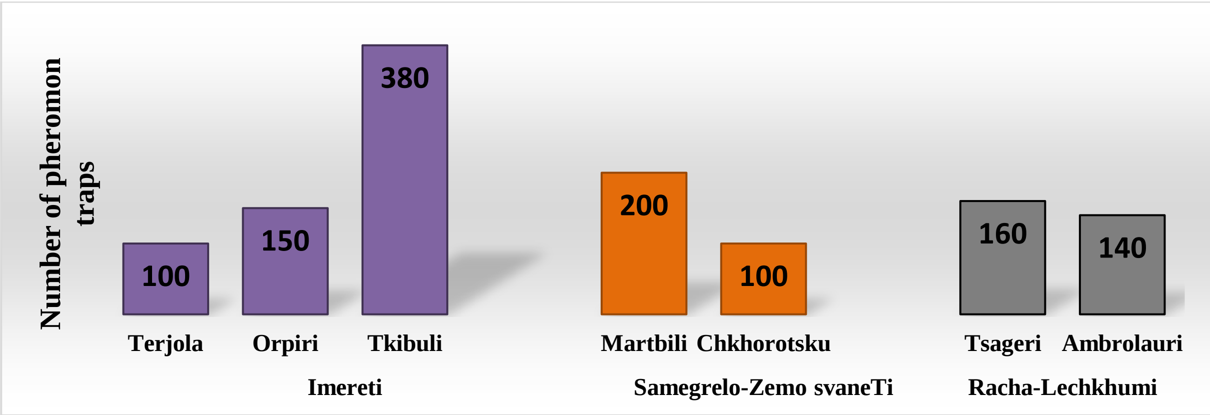

Figure 2. Pheromone traps set up different region of Georgia for monitoring *C.perspectalis*.

The pheromone traps were emptied and new dispensers were added at two times during the flying period of *C. perspectalis*. The number of captured adults varied from 11 to 176 moths in one trap. In total approximately 93000 (2017) and 74000 (2018) of moths were captured in the Tsageri-Ambrolauri (Racha-Lechkhumi region) (Figure 3).

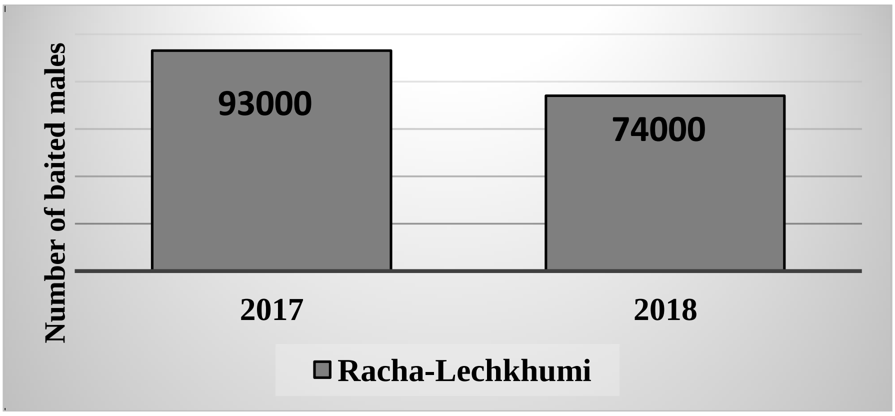

Figure 3. Total number of *C. perspectalis captured* in the Tsageri-Ambrolauri region 2017-2018.

Analyzing the results of study relating to the aim of this research, to use sex pheromone trapping, in order to determine the efficacy of the baits the male flights of BTM, is an effective means for monitoring. Moreover, use pheromone traps for control this insects in the area of South slop of Grate Caucasian mountain range is very important tool, that for its landscape it is difficult to use techniques for insecticide application.

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