UNIVERSITÀ DEGLI STUDI DI TORINO

SCUOLA DI DOTTORATO IN SCIENZE
DELLA NATURA E TECNOLOGIE INNOVATIVE

DOTTORATO IN
SCIENZE AGRARIE, FORESTALI ED AGROALIMENTARI

CICLO: XXX

Innovative pest control strategies in IPM orchards

Valentina Candian

Docente guida:	**Coordinatore del Ciclo:**
Prof. Rosemarie Tedeschi	**Prof. Aldo Ferrero**

ANNI
2014-2015/2015-2016/2016-2017

Contents

1 PREFACE

Integrated pest management (IPM) has become fundamental in European plant protection as regards food safety and environmental protection. When pest damage becomes economically harmful, farmers generally use insecticides to protect the crops. Frequent insecticide treatments, however, increase hazards for workers, consumers and environment. Moreover, a consistent use of insecticides often induces resistance in treated insect populations (Ben-Yakir *et al*., 2014).

In the last years, the implementation of the new Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market and the directive 2009/128/EC regarding the sustainable use of pesticides have drastically changed the European plant protection management. Therefore, many chemicals have disappeared from the European market, launching new challenges for the control of pathogenic insects and fungi. Moreover, hazardous effects of agrochemicals on both humans and the environment, the increasing problems related to the occurrence of resistant pests as well as the introduction of exotic pests require the implementation of new methods for the integrated pest management.

In the last few years, physical exclusion strategies proved to be effective for the control of key and emerging pests (Castellano *et al*., 2008; Chouinard *et al*., 2016). At the same time, the use of entomopathogenic fungi offers an attractive alternative to the use of chemical pesticides (Lydia *et al*., 2017; Wang and Wang, 2017).

2 OBJECTIVES

The objectives of this research were to evaluate two innovative pest control strategies for IPM orchards. Experimental trials were carried out in Piedmont (NW Italy) in order to assess the efficacy of different kinds of net (colours and meshes) in containing key and emerging insect pests in fruit orchards. Any possible effects of the net on orchard arthropod communities, with a special regard to the predators, as well as on the fruit quality, were considered.

Moreover, biological and genomic interactions between the entomopathogenic fungi *Isaria fumosorosea* and the ‘*Candidatus* Liberibacter asiaticus’ psyllid vector *Diaphorina citri* Kuwayama were analysed. The *D. citri* mortality time course infection after treatments with *I. fumosorosea* at different concentrations and with different inoculation strategies was evaluated. In addition, pathogen effector genes from *I. fumosorosea* at early time points of infection were identified.

3 EXCLUSION NETS AGAINST KEY AND EMERGING PESTS IN PIEDMONT ORCHARDS

3.1 INTRODUCTION

Orchards are among the most complex ecosystems in agriculture because of their perennial nature. Nevertheless, until a few years ago, crop protection programs were mainly focused on synthetic pesticides to prevent or limit pest damage (FAO, 2009). The application of several pesticides is required to ensure both the sustainability of the perennial tree structures and the visual quality of the fruits, which is a key point for commercialization. For instance, over 35 treatments per year are applied to French apple orchards, including a range of 7–15 insecticides specifically targeted towards the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae: Olethreutinae) (Butault *et al*., 2010). In Italy, 6–7 treatments are necessary to contain *C. pomonella* in apple orchards (Pavarino and Vittone, 2014).

Alternative control methods reduce environmental impact compared with insecticide treatments but often target a single key pest. Specific control methods have been developed mostly focusing on microbiological insecticides (Arthurs *et al*., 2007) and on mating disruption using synthetic sex pheromones for tortrix moths (Witzgall *et al.,* 2010). In the last years, both methods are widely implemented, but insecticides are still needed to supplement these strategies under high pest densities (Butault *et al*., 2010). Moreover, *C. pomonella* was the first pest in which field resistance to an entomopathogenic virus was detected (Asser-Kaiser *et* *al*., 2007). In this scenario, insect exclusion nets may represent a promising efficient tool to protect crops even from more than one pest at the same time.

Exclusion nets have been used in agriculture since the middle of the 20th century (Scarascia-Mugnozza *et al.*, 2011; Merrill, 1967), and more commonly since the 1990s, when they became widespread as a protection tool against whiteflies in greenhouses (Berlinger *et al.*, 2002). Various types of net coverings are widely used in various countries around the world to provide protection from birds, frugivorous bats, hail, wind, frost and sunburn damage (Lloyd *et al*., 2005). Moreover, exclusion nets also allow a significant reduction in the number of insecticides applications needed to protect the crop from all pests. These nets are almost exclusively made of clear high density polyethylene (HDPE) and have an average lifespan of six (Sauphanor *et al*., 2009) to ten (Rigden, 2008) years under field conditions.

The main mode of action of nets is to act as a barrier to deny access to the crop. Despite their high sustainability (Alaphilippe *et al*., 2016) and stable efficacy under variable conditions, they seldom have been considered cost-effective until the last three decades when, in many parts of the world, the exclusion nets have progressively found wider applications (Tasin *et al*., 2008). The characteristics and effectiveness of exclusion systems adapted for fruit tree protection have been studied in relation to many key pests of pome and stone fruits. These systems can be also classified as either *complete* (single row strategy) or *incomplete* (single-plot exclusion-net) exclusion systems (Chouinard *et al*, 2016). In the *incomplete* exclusion, the soil is not excluded from the system allowing several key pest species (e.g. plum curculio, tephritid flies, European apple sawfly) to complete their life cycle and remain inside the enclosed area. In *complete* exclusion however, the soil is excluded from the enclosed zone.

3.1.1 Principal applications of the exclusion nets in orchard crops

The effectiveness of the net in excluding the codling moth in apple orchards has long been known (Tasin *et al*., 2008). Exclusion nets have been used successfully in France against *C. pomonella* since the early 2000s and they were tested in an experimental apple orchard in southern Quebec from 2012 to 2016 to see their applicability in North American conditions (Chouinard *et al*., 2017). The Alt'Carpo [a French designation meaning "codling moth arrest"] system is the first and one of the most widely used commercial exclusion systems for pome fruit in the world. It is estimated that this exclusion system is applied on about 2000 ha in Southern France (mainly on apples) and 350 ha in Italy (mainly on pears) (Alaphilippe *et al*., 2016). Codling moth exclusion systems are known to protect crops also from birds, mirids (Alaphilippe *et al*., 2016) and *Zeuzera pyrina* (L.) (Lepidoptera: Cossidae) (Sauphanor *et al*., 2009). In pear orchards, where the Alt'Carpo system is applied, *Cacopsylla pyricola* Foerster (Hemiptera: Psyllidae) re-infestations are prevented when exclusion nets are installed right after an initial insecticide application (Romet *et al*., 2010).

The efficacy of the exclusion nets in containing *C. pomonella* populations in apple orchards and how the net affects its biology have been more investigated compared to other pests. The impact of the exclusion nets against the codling moth is not only confined to being an actual physical barrier against the movement of the moths in-out from the orchard but they also interfere with the pest biology. Under simulated conditions, anti-hail nets retarded adult development up to five days in trees covered with nets than in trees without them. This negative effect on the development is probably due to lower temperatures under the net (Kuhrt *et al*., 2006).

Disrupting effects of the net were also observed during the mating. A decreased number of males able to locate calling females or a synthetic source of sex pheromone were observed by Tasin *et al*. (2008) under net coverage. Moreover, the net significantly reduces the flight in the higher part of the canopy. It is known that males and females fly to the upper part of the canopy for mating and mated females may then re-distribute themselves in the canopy in order to lay eggs evenly. The percentage of mated tethered females located in the upper part of the canopy is significantly reduced when the net is present. The interference of the net on the mating process is not only related to the approaching phase, but also to some aspects of courtship and mating (Tasin *et al*., 2008). The net indeed interferes with the flight of the male moths inside the orchard during their approach towards the females reducing mating success and may also interferes by causing a visual disturbance to the searching males (Tasin *et al*., 2008; Sauphanor *et al*., 2012).

With the increasing number of cherry orchards covered to prevent fruit cracking by rain, exclusion nets become a viable and cost-effective control method for the European cherry fruit fly, *Rhagoletis cerasi* (L.)

(Diptera: Tephritidae), a major pest of European cherry crops (Daniel and Grunder, 2012). Nets with 1.3×1.3 mm mesh are effective against this pest as long as nets are installed prior to egg-laying and removed right before the harvest time (Brand *et al*., 2013; Höhn *et al*., 2012). In high pest pressure orchards, the single row strategy allows to reduce the infestation by up to 98% (Brand *et al*., 2013).

Moreover, thanks to their mechanical action, exclusion nets could be a ready tool in case new exotic pest introduction. Nets have been proved to be useful for the control of the exotic pest *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in cherry, cranberry, raspberry and blueberry crops in France, Canada and North America (Charlot *et al*., 2014; Cormier *et al*., 2015; Rogers *et al*., 2016). Exclusion nets are also currently being investigated as a potential solution to the devastating problems caused by the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in the United States (Marshall and Beers, 2016).

3.1.2 Exclusion nets and their impact on secondary pests and diseases

Despite exclusion nets are primarily designed to exclude a single pest species, they have some effects on the entomofauna of fruit trees. Those effects are more obvious in the case of single row strategy. Whilst the effectiveness of the exclusion nets against some key pests is well known, the secondary effects of the exclusion nets on other pests and beneficial insects have not yet been thoroughly assessed.

Secondary effects on the development of secondary pests such as *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae), *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) and Tingidae (Hemiptera) in apple orchards were observed by Alaphilippe *et al*. (2016). In Italian organic pear orchards, nets favor the development of *Leucoptera malifoliella* (Costa) (Lepidoptera: Lyonetiidae), a minor pest that can readily pass through the meshes and develop within enclosed environments (Sévérac and Siegwart, 2013).

Mites also appear to be indirectly affected by exclusion nets. Sévérac and Siegwart (2013) reported that ‘Pink Lady’ apples grown without nets needed two more acaricide applications than apples grown under Alt’Carpo nets applied with the single row strategy. Probably the absence of insecticides in the netted plot could have benefited mite predators (Sauphanor *et al*., 2009).

Different researches showed contrasting results on the efficacy of the exclusion nets in containing aphid populations. Dib *et al*. (2010) proved the efficiency of the net for the control of *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in France apple orchards while in other researches nets have been shown to induce population flare-ups for some species such as *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) (Alaphilippe *et al*., 2016). Moreover contrasting effects were observed for other species such as *Aphis pomi* De Geer (Hemiptera: Aphididae) and *D. plantaginea*, with occasional flare-ups under nets (Chouinard *et al*., 2016). Probably an inconsistent effect on their natural enemies could be the reason (Chouinard *et al*., 2016). Direct and indirect behavioral effects on small size pests (e.g. mites) can be induced by the biotic and abiotic modifications of the environment caused by the nets (Chouinard *et al*., 2016). For example, regarding biotic modifications, the avoidance of broad-spectrum pesticides can allow the development of indigenous predatory mites that are already in the system. On the contrary, the impact of abiotic factors (relative humidity, temperature, rainfalls) can be modified and, in turn, can modify pests development and fecundity inside the nets (Chouinard *et al*., 2016).

Other indirect effects include plant pathogenic fungi and postharvest diseases. In apple orchards, a slight reduction in the occurrence and intensity of apple scab, *Venturia inaequalis* (Cooke) G. Wint on susceptible cultivars such as ‘Pink Lady’ and ‘Gala’ were observed, but the reason for this effect was not determined (Sévérac and Siegwart, 2013). A lower incidence of postharvest diseases recorded on fruits grown under exclusion nets may be due to the fruit protections from climatic, parasitic and non-parasitic agents that are responsible for creating entry ports for various diseases (Sévérac and Siegwart, 2013).

3.1.3 Photoselective nets

Recently, photoselective nets have been developed with the aim of improving plant production thanks to their optical properties in addition to their physical protective action. The photoselective netting technology was developed during the past years by a joint R&D effort of the Volcani Center (ARO) along with Polysack Plastics Industries in Israel. This technology introduced specific spectral filtration and scattering features into the netting materials. The photoselective netting approach was initially targeted towards specifically stimulating desired physiological plant responses, which are regulated by light, and which determine the productivity and product quality. They are produced in different textures, colours and for different crops and climates.

In literature, photoselective red, yellow, blue, green nets are often indicate as “colored photoselective nets” while grey, white and pearl nets are called “neutral photoselective nets” (Shahak, 2011). Depending on the thread pigmentation and knitting design with different fibers and density to create specific shade indices (Castellano, 2008), photoselective nets provide different mixtures of natural, unmodified light and scattered, spectrally modified light (Shahak *et al.*, 2004a; Rajapakse and Shahak, 2007). Light quality modification in terms of light transmittance and scattering by different nets is reported in Table 1.

Table 1. Light quality modification by photoselective nets (UV: ultra violet; B: blue; G: green; Y: yellow; R: red; FR: far red). Source: Shahak, 2008.

Net	**Absorption**	**Transmittance**	**Scattering**
Blue	UV + Y + R + FR	B + G	++
Red	UV + B + G	R + FR	++
Yellow	UV + B	G + Y + R + FR	++
White	UV	B + G + Y + R + FR	++
Pearl	UV	B + G+ Y + R + FR	+++
Grey	all (+IR)	–	+
Black	all	–	–

Photoselective nets were initially tested in ornamental crops which are traditionally grown under black shade nets with particular attention first to the foliage and then to the cut-flowers and pot-plants. The vegetative growth rate and vigor is stimulated by red and yellow nets while dwarfing is increase by the blue net. The grey net specifically enhances branching and bushiness and also reduces leaf size and variegation in some species (Oren-Shamir *et al*., 2001; Shahak, 2008). Photoselective nets are able to influence length and density of flowering stems (longer and thicker under yellow and red net; shorter under blue net), time of flowering (shorter under red net) and the number of flower per branch (pearl net) in some cultivars (Shahak, 2011).

Positive results obtained on ornamental plants had encouraged new researches on vegetables and deciduous fruit crops. The production of leafy crops greatly depends on their growth rate. In field condition, red and pearl nets allow a significantly increase production of lettuce and basil compared to blue or black nets. Pepper productivity is increased under photoselective net with highest yields under red net (Shahak, 2008) while pearl and yellow nets significantly maintain a better fruit quality after storage and shelf life simulations (Shahak, 2011) probably cause their significant reduction in *Alternaria* spp. population (Ilić and Fallik, 2017). Red net significantly reduced post-harvest fruit weight loss (Fallik *et al*., 2009; Goren *et al*., 2010). Moreover, red and pearl nets improve tomato fruit quality (Ilić *et al*., 2012 and 2015).

On fruit trees, photoselective nets that reduce the 15-30% of shading factors are generally used. Positive effects on flowering, fruit-set, fruit size, colour and internal quality, in addition to non-specific reduction of water stress, superficial damage, and sunburn are observed on plants grown under these nets (Shahak *et al*., 2004a and b; Rajapakse and Shahak, 2007; Shahak *et al*., 2008). Positive effects are observed on apples and peaches (Shahak *et al*., 2004b), pears and table grapes (Shahak *et al*., 2008), kiwifruit (Basile *et al*., 2008).

3.1.4 Use of photoselective nets against pests

In the last years, the response of insects to light has long been investigated (Johansen *et al*., 2011). Different species have light photoreceptors for different region (i.e. yellow, blue, red, UV) and with different peak sensitivity. For example, aphids and whiteflies have light receptors in the ultraviolet (UV) region with peak sensitivity at 330–340 nm and in the green-yellow region with peak sensitivity at 520–530 nm (Doring and Chittka, 2007) while thrips have light receptors in the yellow region (540–570 nm), the blue region (440–450 nm) and the UV region (350–360 nm) (Vernon and Gillespie, 1990).

The response of insects to light is strongly affected by the intensity of radiation, the shape and contrast of the radiation source and the physiological state of the insect. High light intensity often inhibits the expected behavioral response to an attractive color. For instance the preference of aphids towards yellow instead of green may be explained by the higher reflectance of yellow in the green spectral domain (Prokopy *et al*., 1983). Indeed, when aphids are exposed to monochromatic lights of the same intensity, they preferred green over yellow (Hardie, 1989).

Greenhouse films or screens containing UV absorbing additives were previously found to provide better pest protection than standard cladding materials (Antignus and Ben-Yakir, 2004; Kumar and Poehling, 2006). Moreover, the horticultural studies on photoselective nets showed the positive potential of these nets in containing pests even though the holes of these nets are large enough to allow free passage of aphids, whiteflies and thrips (Shahak *et al*., 2009). The photoselective nets contain pigments known to attract whiteflies and thrips (i.e. yellow and blue colour) but optical disruption caused by the reflected light may interfere with distant host finding by the pests. This is because it is expected that when pests are near or on the plants, other sensory, such as humidity gradient and plant odors, can substitute the optical investigation (Ben-Yakir *et al*., 2012b). This aspect has been studied in the last few years on aphids, whiteflies and thrips in relation to their direct damage by feeding on crops and their capacity to transmit viral pathogens (Ben-Yakir *et al*., 2012b).

Several studies have shown that, in choice experiments, insects prefer to move to environments with a high intensity of UV light (Diaz and Fereres, 2007) and are repelled by a high intensity reflected UV light (Summers *et al*., 2004) making photoselective nets a useful additional tool for crop protection. Mostly pearl and yellow photoselective nets are able to significantly reduce pest populations. The incidences of aphids and whiteflies populations under pearl and yellow nets in bell peppers and tomatoes, as well as the consequent transmission of viral diseases, is significantly reduces compared to coverage with black and red nets (Shahak *et al*., 2009). The mechanisms by which yellow and pearl nets provide protection against aphids and whiteflies are still not entirely clear.

Pest protection by the pearl net is related to repellency due to its light reflective capacity, which is 2-5 fold higher compared to red and black nets. On the other hand, the yellow net induce aphids and whiteflies to land, feed and settle on the net surface because of its attractive colour.

After the pest try to probe and feed on the net, they usually fly away in what is calling a ‘rejection flight’ (Kring, 1972) reducing in this way the efficacy of the virus transmission. Photoselective nets thus do not provide full pest control, but they can be incorporated into IPM strategies taking advantage of their optical repelling or arresting proprieties to reduce the use of insecticides (Ben-Yakir *et al*., 2012a, 2012b; Ilić and Fallik, 2017).

Even though the effect of photoselective nets is mainly investigated on pests, observations of their impact on beneficial insects have been much less detailed. Researches on parasitoids are only marginal while they are almost totally absent on predators. Reports on crops protected with photoselective nets indicate species-specific responses in predators and in parasitoids. *Aphidius colemani* Viereck (Hymenoptera: Braconidae), and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) did not show interruption of their host location ability under photoselective net (Chyzik *et al*., 2003) while a negative effect on the host location ability of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae) was recorded under photoselective net (Chyzik *et al*., 2003; Chiel *et al*., 2006).

Legarrea *et al*. (2012) studied the impact of UV-absorbing nets on the visual cues of two important predator species, *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) showing how each species can react in a very different way to the presence of the exclusion net. Indeed, the anthocorid was caught in higher numbers in traps placed under regular nets, whereas the mites preferably chose environments in which the UV radiation was attenuated.

In the frame of the LIFE+ SU.SA.FRUIT project (Low pesticide IPM in sustainable and safe fruit production - LIFE13 ENV/HR/000580), experimental trials were carried out in Piedmont (NW Italy) in order to assess the efficacy of different kinds of net in containing key and emerging insect pests in fruit orchards. Experimental trials were carried out in a three-year period. During the first year, prototypes with different types of net were set up in order to compare different colours and meshes. According to the results obtained in the first year, in terms of effects on the entomological and pathological aspects and on fruit quality, semi-field trials were carried out in the following two years using the most promising net. Populations of key pests such as *C. pomonella* and *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae: Olethreutinae), as well as of new exotic pests, such as *D. suzukii* and *H. halys* were monitored. Moreover, any possible effects of the net on orchard arthropod communities, with a special regard to predators, as well as on the fruit quality, were considered.

3.2 MATERIALS AND METHODS

3.2.1 Selection of the most promising net

3.2.1.1 Experimental sites and design

In 2015, different kinds of net were tested using prototypes in an apple (cv. Baigent Brookfield®) and in a peach orchard (cv. Royal Glory®) located in the province of Cuneo (Table 2). A specific anti-drosophilid net (mesh 0.9×1.0 mm) [Artes Politecnica Srl, Schio (VI), Italy] and three anti-hail photoselective nets (mesh 2.4×4.8 mm) [AGRITENAX, now AGRINTECH S.r.l., Eboli (SA), Italy] of different colours (yellow, pearl, red) were tested (Figure 1).

Table 2. Description of the experimental orchards.

Site	Position	A.s.l. (m)	Crop	Cultivar	Area (ha)	Orchard age (YR)
Magliano Alfieri (CN)	44°45'29.3"N 8°04'58.7"E	138	Peach	Royal Glory®	0.6	3
Cervignasco (CN)	44°41'35.7"N 7°30'47.0"E	280	Apple	Baigent Brookfield®	3.9	13

The trials were arranged in a randomized complete block design with three replicates for each of the following treatments: 1) trees netted with anti-drosophilid net (D); 2) trees netted with yellow anti-hail net (Y); 3) trees netted with pearl anti-hail net (P); 4) trees netted with red anti-hail net (R); 5) un-netted control trees (C). In each orchard, 15 plots of 3 neighbouring trees on the row were selected. In the apple orchard, the trees in the three replicates of D, Y, P, and R were isolated with the net that was set up hooking its upper side to the anti-hail net support and fixing the lower side to the ground with metal pegs. In the peach orchard, nets were deployed on wooden crosses and the lower sides were fixed to the ground with metal pegs (Figure 1). In both the orchards, the cages were installed at the beginning of May (May 5th in the peach orchard, May 8th in the apple orchard) at the end of flowering and they were removed at the end of the harvest time.

Figure 1. Prototypes setting-up in the peach orchard.

During the experimental trials, no insecticide treatments were applied from the setting-up of the nets until the end of the harvest time in each D, Y, P, R and C repetition. The first objective of the research was to evaluate the physical exclusion due to the net, but further trees without net coverage and treated with insecticides (I) were also monitored in order to compare the effectiveness of the nets and of chemical insecticide treatments in both orchards. Treatment I was considered for the evaluation of the impact of the net on beneficial insects. Treatment I was tested in both orchards by means of a knock-down treatment while surveys with yellow sticky traps were carried out in I only in the apple orchard. The routine pest control inputs applied on I trees is reported in Table 3. In both the orchards, the mating disruption against Tortricidae was not applied. Fungicides treatments were done in the same way outside and inside the nets (directly through the net coverage) following the routine pest control schedule of the growers.

Table 3. Insecticidal treatments applied on I (trees without net and with insecticide treatments) from the net setting-up until the harvest time.

Orchard	Cultivar	Active ingredient	Trade name	Target	Quantity/ha	No. of treatments	Date
1	Royal Glory®	Chlorpyrifos	Alise’ 75WG	*Grapholita molesta* (Busck)	0.04 kg	1	May 9th
		Thiacloprid	Calypso	*Grapholita molesta* (Busck)	0.19 L	1	June 4th
		Saccharopolispora spinosa (toxins)	Laser	*Grapholita molesta* (Busck); *Anarsia lineatella* Zeller	0.2 L	1	June 16th
2	Baigent® Brookfield	Chlorpyrifos	Terial 75 WG	Tortricidae	0.76 kg	1	May 28th
		Chlorpyrifos	Terial 75 WG	Tortricidae	0.76 kg	1	July 3rd
		Etofenprox	Trebon Star ECC	Tortricidae	0.75 L	1	August 10th

3.2.1.2 Monitoring of *Cydia pomonella*, *Grapholita molesta* and *Anarsia lineatella*

To evaluate the presence and abundance of Tortricidae and other moths, inside (D, Y, P, R) and outside (C) the exclusion nets, traps with sex pheromones [CSALOMON® Budapest (BU), Hungary] were used in both the orchards. In the peach orchard, a pheromone trap for *G. molesta* and another for *A. lineatella* were used in each repetition, while in the apple orchard a trap for *C. pomonella* and another for *G. molesta* were used in each repetition for monitoring the pest populations. In the apple orchard, the feeding attractant CSALOMON® “BISEX” [CSALOMON® Budapest (BU), Hungary] made with a pear ester (Tóth *et al*., 2014) was also applied in each *C. pomonella* pheromone trap in order to collect also female specimens. Traps were placed at 2.50 m from the ground due to the flying characteristics of these pests (Tasin *et al.*, 2008). Sex pheromones and feeding attractants were replaced every four weeks to consistently ensure their effectiveness. Every 10 days, catches were counted and pests were transferred into tubes for the species determination. Even though pheromone traps and feeding attractants are specific, they could also capture individuals not belonging to the target species so a determination of the species by morphological analysis of the aedeagus was necessary. The last abdominal segment of the captured specimens was dissected and boiled for few seconds in potash (15%). The abdominal segment was subsequently washed in water and dissected in glycerol for the genitalia extraction and the species determination by comparison with dichotomous keys (Gilligan and Epstein, 2014).

3.2.1.3 Monitoring of *Drosophila suzukii*

Although at the moment *D. suzukii* is not a key pest in Piedmont fruit orchards, the pest populations were monitored during the trial. Therefore, a trap with *Droskidrink* (74.5% apple vinegar, 25% red wine and sugar) [Az Agr. Prantil Elisabetta, Prio di Vervo - Val di Non (TN)], a feeding attractive, was used inside each cage (D, Y, P, R) and in each control (C). A transparent plastic bottle was filled with 250 mL of *Droskidrink* and a drop of soap was added with surfactant function. The bottle was closed and four symmetrical holes were applied in the upper part of the bottle in order to allow the insect entrance. For each repetition, a trap was installed at 1.50 m from the ground. Every 10 days, the material caught by each trap was collected and stored in 70% alcohol for subsequent determination and *D.* suzukii counts while the attractive solution was replaced with new *Droskidrink*. The prominent serrated ovipositor of the female and the presence of a dark spot on the leading edge near the tip of each wing as well as the presence of three to six bristles on the first pair of leg for the male were observed for the species determination following Vlach, 2010.

3.2.1.4 Monitoring of *Halyomorpha halys*

A RESCUE!® Stink Bug Trap activate with the RESCUE!® Stink Bug Attractant [Sterling International, Inc. Spokane (WA), USA] was applied in the middle of each orchard to evaluate the presence of *H. halys*. From the net installation until the end of the trial, traps were checked every 10 days and the lure was changed every seven weeks accordingly to manufacturer’s instructions. The specimens collected into the traps during each survey were identified and counted. Moreover, every 10 days, in each repetition inside (D, Y, P, R) and outside (C) the net, five branches from each tree were beated to assess the presence and the abundance of the pest during the growing season.

3.2.1.5 Damage on fruits

To evaluate the effectiveness of the different kinds of net, the damage on fruits caused by *C. pomonella*, *G. molesta* and *H. halys* was evaluated all along the trials and at the harvest time. Since the net setting-up, 30 fruits per each covered (D, Y, P, R) and un-covered (C) replicate were visually inspected every 10 days to evaluate the damage caused by *G. molesta* and *H. halys* in the peach orchard and the damage caused by *C. pomonella* and *H. halys* in the apple orchard during the growing season. Overall, the whole production in the peach orchard, and 330 fruits in the apple orchard were checked in each replicate. In particular, the damage caused by *G. molesta* was evaluated considering that one larva visits 3 shoots before damaging a fruit (Zangheri *et al*., 1999). For this reason, 30 shoots (10 shoots from each tree) in each D, Y, P, R and C replicate were also checked every 10 days. The total damage caused by *G. molesta* in each repetition was finally evaluated with the following mathematical formula:

$$\frac{[(\%\ damaged\ shoots/3) + \%\ damaged\ fruits]}{2}$$

Due to the very low production, at the harvest time, the whole production (at least 40 fruits on average for Y, P, R, C and 24 fruits in D) of the peach orchard (harvest day: July 8th) was evaluated for fruit damages. In the apple orchard, three picking dates occurred (August 21st and 28th; September 3rd) and 100, 300 and 100 apples were respectively harvested in each covered (D, Y, P, R) and un-covered (C) cage. Damage caused by *G. molesta* and *H. halys* in the peach orchard and damage caused by *C. pomonella* and *H. halys* in the apple orchard were evaluated.

3.2.1.6 Monitoring of other pests and beneficial insects

During the trial, every 10 days from the net setting-up until the harvest time, 30 shoots (10 shoots from each tree) in each D, Y, P, R and C replicate were checked to evaluate the presence and the abundance of aphids.

The abundance of beneficial insects (before, after and all along the trial) was evaluated thanks to chromotropic sticky traps and knock-down treatments. A Glutor YELLOW (25×20 cm) [BIOGARD® Division, Cesena (FC), IT] chromotropic sticky trap was used in each covered (D, Y, P, R) and un-covered (C) replicate. Only in the apple orchard, a yellow sticky trap was also hanged on three trees without net but treated with insecticides (I) to compare the net efficacy against chemical treatments. Traps were changed every 10 days from the net setting-up until the end of the harvest time. The collected specimens were examined and sorted in the following clusters: 1) *total catches*, 2) *predators*, 3) *pests*.

Before the net setting-up and after the end of the harvest time a knock-down treatment with the pyrethroid deltamethrin (Decis® Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL^{-1}) was applied. Before the net setting-up, the knock-down treatment was applied on three trees arranged along the diagonal in both the orchards. After the harvest time it was applied on one tree per each Y; P; R, D, C and I replicate. After 3 hours and a final beating of the canopy, all the arthropods killed by the knock-down treatment were collected on a nylon tarpaulin (3×2 m) lying under the canopy of the trees, and then put into plastic tubes (50 mL) with 70% alcohol until the determination. In order to assess the arthropod fauna abundance depending on the treatment and, in particular, the possible effect of the nets on the predators, the collected specimens were examined and sorted in the following clusters: 1) *total catches*, 2) *predators*. For the final knock-down treatment the cluster *H. halys* was added to previous groups.

3.2.1.7 Monitoring of climatic conditions

From the net setting-up until the harvest time, temperature and relative humidity were monitored at one-hour intervals in both the orchards. A data logger Hobo® H08-004-02 or a Hobo® H8 Pro Series [Onset Computer Corporation, Bourne, (MA) USA] was installed in one of the three D, Y, P, R and C replicates. The data were downloaded and analyzed using BoxCar Pro software v.3.7.2.

Moreover, only in the apple orchard, the photosynthetically active radiation (PAR) of covered and un-covered trees were also evaluated in one Y; P; R, D and C replicate. A Data Logger WatchDog 1000 Series Micro Station [Spectrum Technologies, Aurora (IL) USA] was located on the central tree in each replicate. The PAR at three different heights (high, middle and basal) was recorded at one-hour intervals from the net setting-up until the harvest time.

3.2.1.8 Fruit quality

At the harvest time, the fruit quality of the covered (D, Y, P, R) and un-covered (C) trees were evaluated. Colour, firmness, total sugar and the starch content [only on apple] were evaluated. In each orchard, 180 fruits per treatment were analysed for the colour and 135 fruits per treatment were checked for firmness, total sugar and starch content.

The colour was measured on the external part of the fruit using a portable colour analyser [Chroma Meter, model CR-400, Minolta, Langenhagen, Germany] equipped with a measuring head of 8 mm-diameter area. The CIELAB scale defined by the Commission International de L'Eclairage was used to describe the colour with the L* a* b* space coordinates. The colour parameters were expressed with a colour index (Martínez-Las Heras *et al*., 2016).

The firmness was measured using a manual standard penetrometer [52200 Fruit penetrometer, Turoni, Forlì, Italy] (diameter of the probe 8 mm) with a kg scale. For each fruit, a slice of skin was removed using a cutter, and the probe was pushed into the flesh tissue to a depth of 9 mm.

For the total soluble solid, fruits were squeezed and the juice was distributed into a plastic tube. After centrifugation, the subnatant was measured with a digital refractometer [PAL series, ATAGO CO, LTD, Tokyo, Japan].

The quantity of starch was obtained by means of the Lugol test. Iodine, present in the Lugol solution, reacts with starch and shows a characteristic blue-violet colour. The result was expressed by means of an index value that is related to the starch degradation degree. A scale of 1–10 is used for apples (Sansavini and Ranalli, 2012); the first degree in this scale indicates no degradation (maximum quantity of starch), while the tenth indicates that almost all the starch has been degraded.

3.2.1.9 Data analysis

The statistical analyses were performed using SPSS v23.0 and v24.0 (SPSS Inc., Chicago, IL, USA) and outcomes were considered significant at $P<0.05$.

The mean percentage of catches was calculated for each category [Peach orchard: *G. molesta*; *D. suzukii*. Apple orchard: the total sum of *G. molesta*, *C. pomonella*, *Synanthedon myopaeformis* (Borkhausen) (Lepidoptera: Sesiidae); *D. suzukii*] considering the total number of specimens caught in each N and C replicates on the total number of catches recorded in each orchard. In the peach orchard, the mean percentage on shoots and fruits damaged by *G. molesta* per treatment was considered. The number of arthropods collected with yellow sticky traps and with the final knock-down treatment were used for the statistical analysis.

The data collected with the entomological survey, as well as the results obtained with the PAR and the quality of fruit at the harvest time were checked for homogeneity of variance (Levene test) and normality (Shapiro-Wilk test), and compared using a one-way ANOVA; in the case of significant differences, the means were separated by Tukey’s test. In case of percentages data, values were arcsine square root transformed before the analysis. If the assumptions of ANOVA were not met, the data were analysed using the Kruskal-Wallis test, and the means were pairwise compared using the Mann-Whitney U test.

3.2.2 Semi-field trials

3.2.2.1 Experimental sites and design

Semi-field trials were carried out in 2016 and 2017 in two nectarine orchards (cv. Amiga* and Fire Top®) and two apple orchards (cv. Baigent Brookfield® and Galaval*), located in the province of Cuneo (NW Italy) (Table 4). All the orchards were equipped with an anti-hail net system with a green net in nectarine orchards and a grey net in apple orchards.

The trials were arranged in a randomized complete block design with three replicates for each of the following treatments: 1) netted trees (N); 2) un-netted control trees (C); 3) trees without net but treated with insecticides (I). In each orchard, nine plots (three for each treatment) of neighbouring trees on the row (16 trees in nectarine orchards, 20 trees in apple orchards) were selected. In the three replicates of N, the plots were further covered by the pearl anti-hail photoselective net Tenax Iridium (mesh 2.4×4.8 mm) [AGRITENAX, now AGRINTECH S.r.l., Eboli (SA), Italy]. The nets were set up hooking their upper side to the anti-hail net support and fixing the lower side to the ground with metal pegs (Figure 2).

Table 4. Description of the experimental orchards for semi-field trials in 2016 and 2017.

Orchard	**Site**	**Position**	**A.s.l. (m)**	**Species**	**Cultivar**	**Area (ha)**	**Orchard age (YR)**
1	Savigliano (CN)	44°37'19.5"N 7°37'32.6"E	321	*Prunus persica* (L.) Batsch	Amiga*	0.6	13
2	Savigliano (CN)	44°37'20.8"N 7°37'31.6"E	321	*Prunus persica* (L.) Batsch	Fire Top®	0.6	13
3	Cervignasco (CN)	44°41'35.7"N 7°30'47.0"E	280	*Malus domestica* Borkh.	Baigent Brookfield®	3.9	13
4	Revello (CN)	44°39'51.1"N 7°24'33.5"E	351	*Malus domestica* Borkh.	Galaval*	1.1	3

Figure 2. Setting-up of the pearl photoselective anti-hail net in the nectarine orchard.

The exclusion nets were placed at the petal fall and removed at the end of the harvest time. Immediately after the closing of the nets, a knock-down treatment with the pyrethroid deltamethrin (Decis® Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL^{-1}) was performed to eliminate pest populations. Then, during the experimental trials, no further insecticide treatments were applied in the three plots of N and C. In the remaining three replicates of I, the trees received routine pest control inputs both in 2016 and 2017 as reported in Table 5 and Table 6.

The treatment I was only used to compare the effectiveness of the net to the effectiveness of insecticide treatments with reference to fruit damage at the harvest time and to insect abundance assessed through the final knock-down treatment. These parameters were chosen due to the low number of catches of *H. halys* by pheromone traps in spite of high levels of fruit damage observed in the study area.

Table 5. Insecticidal treatments applied on I (trees without net and with insecticide treatments) from the net setting-up until the harvest time in 2016.

Orchard	**Cultivar**	**Active ingredient**	**Trade name**	**Target**	**No. of treatments**	**Date**
1	Amiga*	Chlorpyrifos methyl	Reldan	*G. molesta*	1	July 1st
		Etofenprox	Trebon up	*G. molesta*	1	July 18th
2	Fire Top®	Chlorpyrifos methyl	Reldan	*G. molesta*	1	July 1st
		Etofenprox	Trebon up	*G. molesta*	1	July 18th
3	Baigent Brookfield®	Chlorpyrifos	Terial 75 WG	*C. pomonella*	2	June 20th, July 3rd
		Chlorpyrifos methyl	Reldan	*C. pomonella*	1	July 29th
		Etofenprox	Trebon Star ECC	*C. pomonella*	1	August 10th
4	Galaval*	Chlorpyrifos methyl	Runner M	Tortricidae	2	July 5th and 20th
		Etofenprox	Trebon up	Tortricidae	1	August 16th

Table 6. Insecticidal treatments applied on I (trees without net and with insecticide treatments) from the net setting-up until the harvest time in 2017

Orchard	**Cultivar**	**Active ingredient**	**Trade name**	**Target**	**No. of treatments**	**Date**
1	Amiga*	Phosmet	Spada	*G. molesta*	1	May 12th
		Chlorpyrifos methyl	Pirenex	*G. molesta*	2▲	May 25th, June 5th
		Etofenprox	Trebon UP	*G. molesta*	2	June 15th, July 13rd
		Chlorpyrifos methyl	Runner LO	*G. molesta*	1	July 1st
		Deltamethrin	Decis	*H. halys*	1	July 29th
2	Fire Top®	Phosmet	Spada	*G. molesta*	1	May 12th
		Chlorpyrifos methyl	Pirenex	*G. molesta*	2▲	May 25th, June 5th
		Etofenprox	Trebon UP	*G. molesta*	2	June 15th, July 13rd
		Chlorpyrifos methyl	Runner LO	*G. molesta*	1	July 1st
		Deltamethrin	Decis	*H. halys*	1	July 29th

▲ Insecticide treatment applied to alternate row.

Table 6. (Continue).

Orchard	Cultivar	Active ingredient	Trade name	Target	No. of treatments	Date
3	Baigent Brookfield®	Abamectin	Zoro 1,9 EV	*Aculus schlechtendali* (Nalepa)	1	May 15th
		Chlorpyrifos	Terial 75 WG	*Synanthedon myopaeformis* (Borkhausen)	1	May 25th
		Methoxyfenozide	Intrepid	Tortricidae	1	June 7th
		Phosmet	Spada	*C. pomonella*	2	June 17th, July 19th
		Etofenprox	Trebon UP	*C. pomonella*	1	July 1st
		Chlorpyrifos methyl	Reldan Lo	*H. halys*	1	July 15th
4	Galaval*	Metossifenozide	Prodigy	*C. pomonella*	2	May 25th
		Chlorpyrifos methyl	Runner Lo	*C. pomonella*	2	June 30th, July 14th
		Fosmet	Spada 50 WG	*Anthonomus pomorum* (L.)	1	July 14th
		Etofenprox	Trebon UP	*C. pomonella*	1	August 8th

▲ Insecticide treatment applied to alternate row.

3.2.2.2 Monitoring of *Cydia pomonella*, *Grapholita molesta* and *Anarsia lineatella*

The protocol applied during 2015 was followed also in the years 2016 and 2017. A pheromone trap for *G. molesta* and another for *A. lineatella* in the nectarine orchards, as well as a trap for *C. pomonella* and another for *C. molesta* in apple orchards were used in each N and C repetitions. For *C. pomonella* the feeding attractant was also used with the pheromone trap located in N and C repetition in apple orchards. The collected specimens were determined by comparison with dichotomous keys (Gilligan and Epstein, 2014).

3.2.2.3 Monitoring of *Drosophila suzukii*

In order to evaluate the population of this pest in the semi-field trials, the same protocol followed in 2015 was applied. In each orchard, a trap filled with *Droskidrink* was used in each N and C repetition. Every 10 days, traps were checked and collected specimens were observed under a stereomicroscope and determined following Vlach, 2010.

3.2.2.4 Monitoring of *Halyomorpha halys*

To evaluate the presence and abundance of this pest inside and outside the exclusion nets, a DEAD-INN™ Stink Bug Traps [AgBio, Westminster, (CO) USA] (high 121.92 cm), baited with the Xtra Combo lure provided with the trap, was placed in a N replicate and in a C replicate in each orchard in both years. Only in the cv. Amiga* traps for *H. halys* were not used in the last year of the trials. The lure was composed by the aggregation pheromones produced by the males of *H.*

halys (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R, 6S, 7R, 10S)-10,11-epoxy-1-bisabolen-3-ol and by the aggregation pheromone of *Plautia stali* Scott (Heteroptera: Pentatomidae) [methyl-(E,E,Z)-2,4,6-decatrienoate]. From the net installation until the end of the trials, traps were checked every 10 days and the lure was changed every four weeks accordingly to manufacturer’s instructions. The specimens collected into the traps during each survey were identified and counted. Moreover, every 10 days, in each N and C repetition, five branches of three randomly selected trees were beated to assess the presence and the abundance of the pest during the growing season.

3.2.2.5 Damage on fruits

Since the net setting-up, 30 fruits per replicate in the treatments N and C (10 fruits per plant on three randomly selected trees) were visually inspected every 10 days to evaluate the damage caused by *G. molesta*, *C. pomonella* and *H. halys* during the growing season. Overall, 180 nectarines and 270 apples were checked in each replicate in 2016 while 240 nectarines and 300 apples were visually inspected in 2017.

At the harvest time, nectarines and apples were sampled from each replicate of the treatments N, C and I, and analyzed for damage caused by tortrix moths and *H. halys*. The fruits were picked in different dates following the growers’ indications. Nectarines were always harvested in two picking dates while in apple orchards three picking dates occurred in 2016 and two dates in 2017 (Table 7).

Table 7. Picking dates occurred in each orchard in 2016 and 2017

Cultivar	**2016**	**2017**
Amiga*	July 26th, August 2nd	July 24th and 31st
Fire Top®	August 2nd and 8th	July 24th and 31st
Baigent Brookfield®	August 23rd and 29th, September 6th	August 16th and 23rd
Galaval*	August 23rd and 30th, September 6th	August 17th and 25th

At the harvest time, 240 fruits per repetition were sampled in each picking date in both nectarine and apple orchards. Only in the apple orchards in 2016, a third picking date occurred and 30 fruits were collected in each repetition. Overall, 480 nectarines and 510 apples (480 in 2017) were picked in each treatment (N, C and I) per year, with a total of 8640 fruits in each nectarine orchard and 8910 fruits in each apple orchard were harvested in the two years. The number of fruits damaged by *G. molesta*, *C. pomonella* and *H. halys* was recorded. The damage caused by *H. halys* was identified according to Acebes-Doria *et al.* (2016): nectarines and apples were considered damaged if punctures, dimples, areas with superficial discoloration with or without depressions and areas with necrotic tissue after slicing the fruits were observed. In addition, on nectarines the presence of gummosis and fruit deformations was also evaluated.

3.2.2.6 Monitoring of other pests and beneficial insects

During the trial, every 10 days from the net setting-up until the harvest time, 30 shoots (10 shoots from each tree) were checked to evaluate the presence and the abundance of aphids in each N and C replicate. To evaluate the arthropod fauna in the semi-field trials in both the years, yellow sticky traps and knock-down treatments were used following the same protocols applied during the prototype trials. A Glutor YELLOW chromotropic sticky trap was used in each N and C replicate. The collected specimens were examined under a stereomicroscope for the identification, counted and sorted according to the external morphology in the cluster *predators*.

Moreover, at the end of the harvest time of each year, the knock-down treatment with the pyrethroid deltamethrin was applied on one tree per repetition in the treatments N, C, I in each orchard. The collected specimens were examined and sorted in the following clusters: 1) *total catches*, 2) *predators*, 3) *H. halys*.

3.2.2.7 Monitoring of climatic conditions

In each orchard, a data logger Hobo® Pro v2 (U23-002) [Onset Computer Corporation, Bourne, (MA) USA] was installed in one N and one C replicate. From the net setting-up until the harvest time, temperature and relative humidity were hourly monitored; data were downloaded and analyzed using the HOBOware Pro software v3.7.5.

Moreover, the PAR was evaluated in one C and one N replicate in each apple orchard in 2016 and in each nectarine orchards in 2017. Data Loggers WatchDog 1000 Series Micro Station [Spectrum Technologies, Aurora (IL) USA] were used and the PAR was recorded once a hour from the net setting-up until the harvest time. Each data Logger was provided with a protective box and three sensors. In each replicate, sensors were arranged at three different heights: high-position (3.00 m from the ground), middle-position (1.70 m from the ground) and basal-position (0.50 m from the ground). Moreover, only in N, a further sensor was arranged at middle height outside the net.

3.2.2.8 Fruit quality

The colour index, the firmness, the total soluble solid and nutraceutical parameters (total anthocyanins and total polyphenols) were analysed at the harvest time to evaluate any possible effects of the nets on the fruit quality in N, C and I. In each orchard and for each treatment, 180 fruits were analysed for colour, firmness and the total soluble solid following the protocols used in the prototype trials. Only in 2017 and only for the apple orchards, 60 fruits (instead of 180) were analysed for the nutraceutical parameters.

The total anthocyanin and the total phenol were analyzed separately on the skin and on the fruit pulp for the apples, while the tissues were mixed for the nectarines. Every sample came from 10 fruits randomly selected per treatment and orchard for each fruit species (4 fruits for each replicates per treatment and apple orchards in 2017). Both analyses were performed starting from an extract. The nectarine and apple extract was obtained using 10 g of fruit added to 25 mL of extraction solution (500 mL of methanol, 23.8 mL of de-ionized water and 1.4 mL of 37% hydrochloric acid). After 1 h in the dark at room temperature, the samples were thoroughly homogenized for 1 min with an ULTRA TURRAX [IKA, Staufen, Germany], and centrifuged at 3,019 g for 15 min. The supernatant obtained by centrifugation was collected, transferred into glass test tubes, and stored at -20°C until analysis. The total anthocyanin content was quantified according to the pH differential method of Cheng and Breen (1991). Anthocyanins were estimated by the difference in absorbance at 510 and 700 nm in a buffer at pH 1.0 and pH 4.5. The results were expressed as mg of cyanidin-3-glucoside (C3G) equivalents per 100 g of fresh weight (FW). The total phenolic content was measured using Folin–Ciocalteu reagent with gallic acid as a standard at 765 nm following the method of Slinkard and Singleton (1977). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of FW.

3.2.2.9 Data analysis

The statistical analyses were performed using SPSS v24.0 [SPSS Inc., Chicago, IL, USA] and outcomes were considered significant at $P<0.05$. The mean percentage of catches was calculated for each category [Peach orchard: *G. molesta*; *D. suzukii*; total sum of Drosophilidae. Apple orchard: the total sum of *G. molesta*, *C. pomonella*, *S. myopaeformis*; *D. suzukii*; total sum of Drosophilidae] considering the total number of specimens caught in each N and C replicates on the total number of catches recorded in each orchard and compared using a t-test for two independent samples. The number of arthropods collected with yellow sticky traps was compared using a t-test for two independent samples. The numbers of fruits damaged by *G. molesta* and *H. halys* at the harvest time were compared using a generalized linear mixed model (GLMM; random effect: plot; fixed effects: treatment, block, picking date) with a binary distribution and logit link and Bonferroni correction. Block and picking date effects were used in order to assess if pests were more concentrated on the borders or in the middle of the orchards and to evaluate any variation of the damage intensity during the harvest period.

Moreover, any possible interactions between the treatment and the picking date effects were investigated. The number of arthropods collected by the knock-down treatment and the data on quality and nutraceutical parameters of fruit at the harvest time were checked for homogeneity of variance (Levene test) and normality (Shapiro-Wilk test), and compared using a one-way ANOVA; in the case of significant differences, the means were separated by Tukey’s test. If the assumptions of ANOVA were not met, the data were compared using Kruskal-Wallis test, and the means were separated using a Mann-Whitney U test.

3.3 RESULTS

3.3.1 SELECTION OF THE MOST PROMISING NET

The results obtained during the prototype trials are reported below, broken down by crop.

3.3.1.1 Peach orchards

3.3.1.1.1 Monitoring of and damage caused by *Grapholita molesta* and *Anarsia lineatella*

In the peach orchard, abundant catches of *G. molesta* were recorded all along the trial while *A. lineatella* was never trapped. The critical threshold for chemical treatments against *G. molesta* is, as set by the Piedmont Regional rules (Regione Piemonte, 2015), at 10 males catched per trap per week starting from the second flight. In the investigated area, *G. molesta* performs four-five generations per year. During the trial, the end of the first generation and the overlap between the second and the third generation were recorded. The critical threshold was never reached under the pearl net and the anti-drosophilid net while under yellow and red net was exceeded during the ten days previous the harvest time (Figure 3). Only 2 specimens were caught in D in June while in C, pests were trapped with peaks up to 3 times higher than the critical threshold.

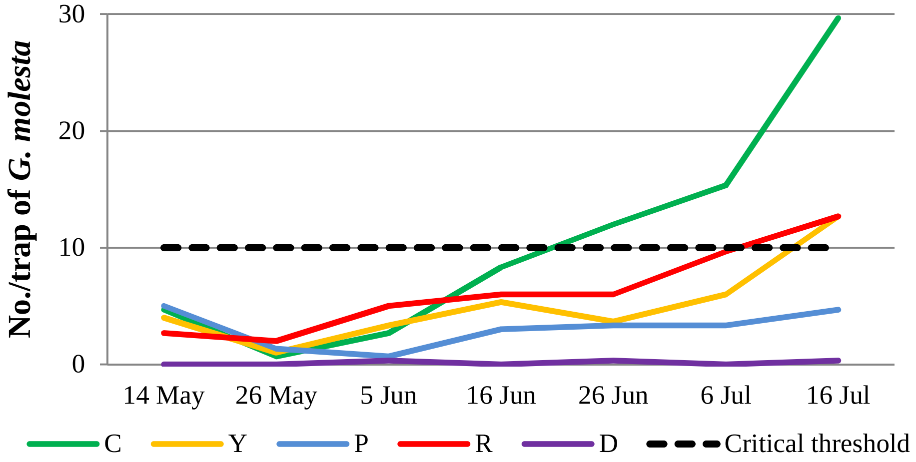


Figure 3. Mean number of catches of *G. molesta* in pheromone traps all along the growing season in treatments C (un-netted control trees), Y (yellow net), P (pearl net), R (red net), D (anti-drosophilid net).

The analysis of the mean percentage of *G. molesta* catches per trap on the total catches in the orchard shows significant differences between treatments (one-way ANOVA: df=4, 10; F=28.29; P=0.000). The anti-drosophilid net proved to be the most effective barrier for the protection of the crop followed by the pearl and yellow photoselective anti-hail nets (Figure 4).

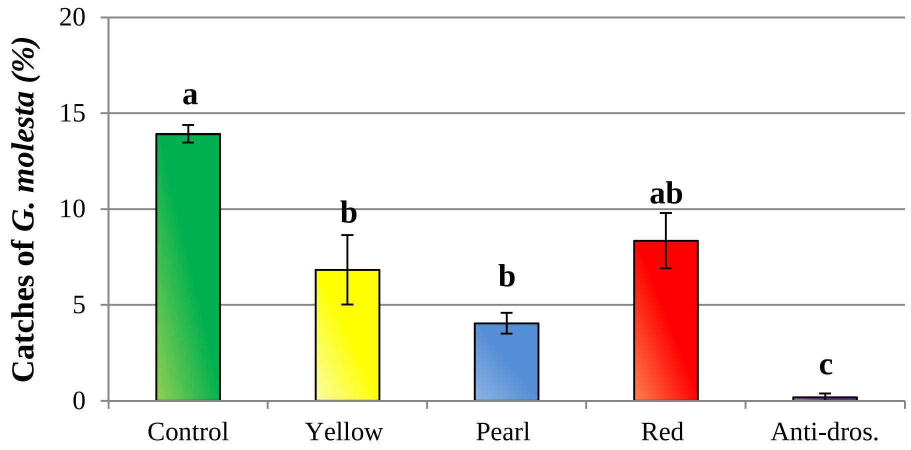


Figure 4. Mean percentage of *G. molesta* catches per trap on the total catches in the peach orchard. Bars with a letter in common are not significantly different (Tukey's test, P<0.05).

The damage caused by *G. molesta* during the growing season on shoots and fruits were evaluated considering that one larva visits 3 shoots before damaging a fruit (Zangheri *et al.*, 1999). Statistical differences were recorded between the treatments (one-way ANOVA: df=4, 60; F=4.54; P=0.003) (Figure 5). The anti-drosophilid net was confirmed to be the most effective barrier. No statistical differences as regards the damage on the fruits at the harvest time between the treatments were recorded.

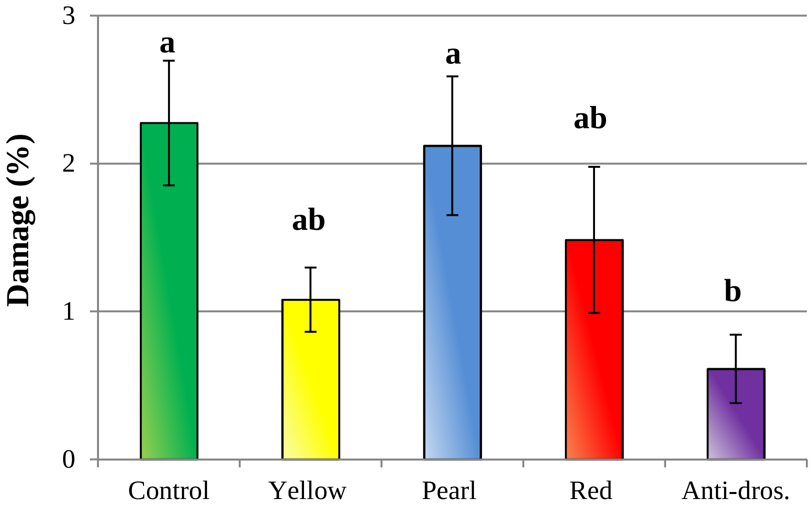


Figure 5. Mean percentage of shoots and fruits damaged by *G. molesta* on the total of shoots and fruits damaged in the orchard. Bars with a letter in common are not significantly different (Tukey’s test, P<0.05).

3.3.1.1.2 Monitoring of *Drosophila suzukii*

During the prototype trials, only few specimens of *D. suzukii* were collected with the traps (Table 8). Only three specimens were caught under the anti-drosofilid net in the peach orchard. In order to evaluate the effectiveness of the four nets in containg *D. suzukii* populations, all the specimens belonging to the family Drosophilidae were considered during the statistical analysis. Generally, catches of Drosophilidae under the anti-drosophilid net were recorded close to the harvest time, when the net was often opened and closed to check the fruit degree of ripeness, and at the harvest time.

Table 8. Total catches of *D. suzukii* and Drosophilidae collected per each treatment in peach orchard.

Treatment	**Total *D. suzukii* (no. of specimens)**	**Total Drosophilidae (no. of specimens)**
Control	40	864
Yellow photoselective net	14	573
Pearl photoselective net	12	699
Red photoselective net	17	548
Anti-drosophilid net	3	68

Statistical differences of the mean percentage of Drosophilidae caught were recorded between the treatments (one-way ANOVA: df=4, 10; F=10.19; P=0.001). The anti-drosophilid net proved to be the most effective barrier for the protection of the crop followed by the three photoselective anti-hail nets (Figure 6).

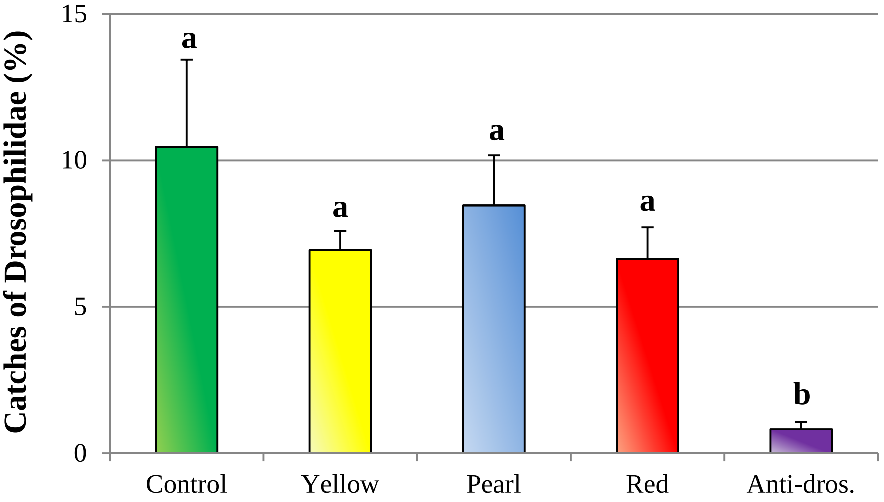


Figure 6. Mean percentage of Drosophilidae catches per trap on the total catches in the peach orchard. Bars with a letter in common are not significantly different (Tukey's test, P<0.05).

3.3.1.1.3 Monitoring of and damage caused by *Halyomorpha halys*

Halyomorpha halys was never collected during the trial neither with pheromone traps nor beating the tree branches. Moreover, fruits damaged by *H. halys* were never observed all along the trial and at the harvest time.

3.3.1.1.4 Monitoring of other pests and beneficial insects

Shoot visual inspections, yellow sticky traps and a final knock-down treatment were used to assess the impact of the nets on arthropod fauna abundance. Aphids were never observed in all the peach orchard.

All the specimens belonging to Miridae (Heteroptera); Chrysopidae (Neuroptera); *Diabrotica virgifera* subsp. *virgifera* LeConte (Chrysomelidae) and Coccinellidae (Coleoptera); Syrphidae (Diptera) trapped with the yellow sticky traps were considered in the cluster *total catches*. Their amounts were not significantly different between the treatments but a higher number of specimens were collected in C and Y (Table 9). Chrysopidae, Coccinellidae and Syrphidae were grouped in *predators*. No statistical differences were recorded in these clusters but the highest number of catches was recorded in C. In particular, 17 Coccinellidae were collected in C, 8 in Y, 1 in P and no specimens were collected in R and D. *Lygus rugulipennis* Poppius (Heteroptera: Miridae) and *D. virgifera* subsp. *virgifera* were grouped in the cluster *pests* even though these pests are not worrisome for this crop. Statistical differences between the treatments were not recorded for *pests*.

Table 9. Insects collected every ten days with yellow sticky traps (mean±SE). No significant differences were found by ANOVA (total catches) and by Kruskal-Wallis test (predators, pests).

Treatment	Total catches	Predators	Pests
Control	15.33±4.84	9.67± 3.28	5.67±1.76
Yellow photoselective net	12.00±5.51	3.33±2.03	8.67±4.81
Pearl photoselective net	8.00±4.25	1.33±0.82	6.67±4.53
Red photoselective net	5.33±4.33	0.67±0.33	4.67±4.18
Anti-drosophilid net	0.33±0.33	0.00±0.00	0.33±0.33

In the knock-down treatment applied before the net setting-up, specimens belonging to Araneae (Aranaeidae), Acarina, Heteroptera (Anthocoridae and Pentatomidae), Homoptera (Delphacidae and Aphidoidea), Coleoptera (Carabidae, Staphylinidae, Coccinellidae, Chrysomelidae, Nitidulidae, Bostrichidae, Curculionidae, Cantharidae and Tenebrionidae), Diptera (Micropezidae and Sciaridae), Mecoptera (Panorpidae), Hymenoptera (Apoidea, Formicidae, Symphyta, Calcidoidea and Braconidae) were collected. An average of 31 specimens of which five predators were sampled under each treated tree.

After the harvest time, the knock-down treatment allowed the collections of specimens belonging to Araneae (Aranaeidae), Acarina, Dermaptera (Forficulidae), Thysanoptera (Thripidae), Heteroptera (Anthocoridae, Nabidae, Miridae, Lygeidae and Pentatomidae), Homoptera (Cicadellidae), Neuroptera (Chrysopidae), Coleoptera (Carabidae, Staphylinidae, Coccinellidae, Chrysomelidae, Nitidulidae and Curculionidae), Diptera (Drosophilidae) that were grouped in the cluster *total catches*. Even though statistical differences between the treatments were not found, a lower number of specimens were collected in D and in

I (Table 10). The highest captures were recorded in P and C. In the cluster *predators Allothrombium fuliginosum* Hermann (Acarina), Anthocoridae and Nabidae, Chrysopidae, Staphylinidae and Coccinellidae were grouped. Statistical differences were not found in *predators* but the highest captures were recorded in P, mostly represented by Coccinellidae. *Halyomorpha halys* was never collected with the knock-down treatment.

Table 10. Insects collected with the knock-down treatment applied after the harvest time (mean±SE). No significant differences were found by Kruskal-Wallis test.

Treatment	**Total catches**	**Predators**
Control	36.33±25.3	5.00±0.6
Yellow photoselective net	14.00±3.00	4.00±0.6
Pearl photoselective net	45.33±17.7	30.67±14.4
Red photoselective net	15.67±4.2	4.67±0.9
Anti-drosophilid net	7.00±2.6	1.67±0.9
Insecticidal treatments	6.67±0.3	2.33±0.3

3.3.1.1.5 Monitoring of climatic conditions

During the prototype trials, temperature and relative humidity were also monitored. No significant temperature deviations were recorded between covered and control trees, but, in general, higher temperatures were recorded under the net (Figure 7). In particular, temperatures similar to the ones of the control were detected under the pearl net while under the anti-drodsophilid net and the red net temperatures of one and a half mean degree higher were recorded.

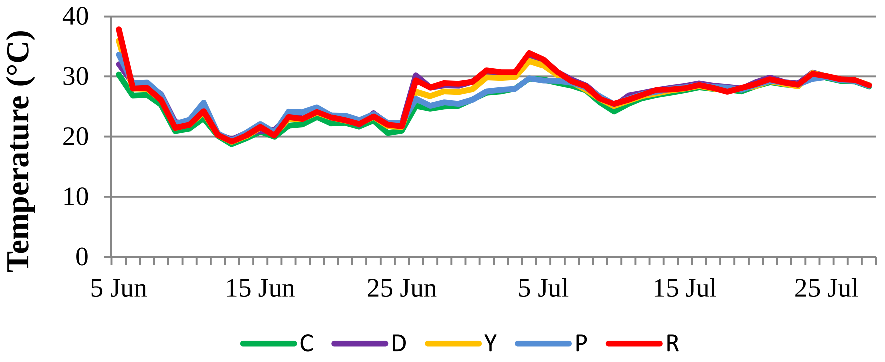


Figure 7. Daily mean temperature recorded inside and outside nets in the peach orchard in treatments C (un-netted control trees), Y (yellow net), P (pearl net), R (red net), D (anti-drosophilid net).

During the trial, higher humidity values were recorded under the nets (Figure 8) but values similar to the control were detected under the pearl one. Unfortunately, under the anti-drosophilid net it was not possible to record the humidity during all the trail. Therefore, it was not possible to accurately compare the humidity trend with the other treatments. The strong decrease of the relative humidity in the last two weeks of June is probably due to a strong wind. This decrease was not so sharp under the anti-drosophilid net probably due to its dense mesh.

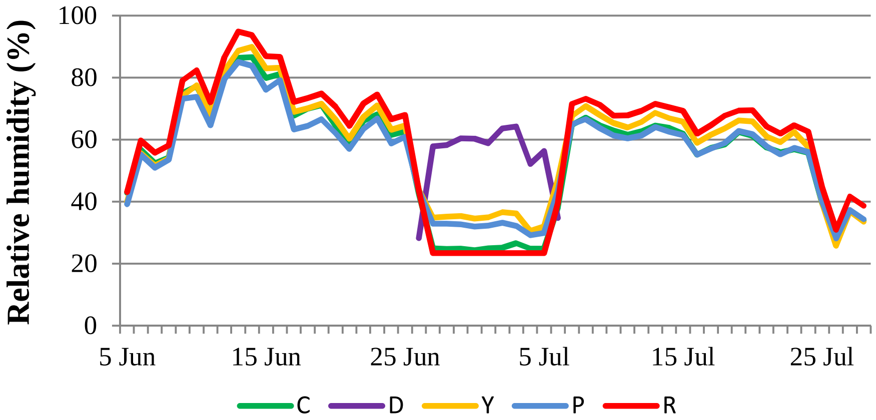


Figure 8. Daily mean relative humidity detected inside and outside nets in the peach orchard in treatments C (un-netted control trees), Y (yellow net), P (pearl net), R (red net), D (anti-drosophilid net).

3.3.1.1.6 Fruit quality

The peach orchard was hit by a strong frost which has significantly reduced the production; for this reason the results obtained from the qualitative analysis are not reliable.

3.3.1.2 Apple orchard

3.3.1.2.1 Monitoring of and damage caused by *Cydia pomonella* and *Grapholita molesta*

In the apple orchard, low catches of Tortricidae were recorded all along the trial. In particular, some specimens were collected with the traps only during the second decad of August. In total, only one *C. pomonella* in C and one in R were trapped while two *G. molesta* were collected in C, one in P and one in Y. No damage to the fruits caused by these pests were observed all along the trail and damaged fruits by *G. molesta* were never observed in all the repetitions at the harvest time. *Cydia pomonella* population never reached worrisome levels, therefore damages were never observed in C, P, Y and D while only three damaged fruits were picked at the harvest time in R.

3.3.1.2.2 Monitoring of *Drosophila suzukii*

In the apple orchard, *D. suzukii* was never collected under anti-drosophilid net while, among the photoselective nets, the lowest captures were obtained with the pearl net (Table 11).

Table 11. Total catches of *D. suzukii* and Drosophilidae collected per each treatment in the apple orchard.

Treatment	**Total *D. suzukii* (no. of specimens)**	**Total Drosophilidae (no. of specimens)**
Control	130	1598
Yellow photoselective net	41	1153
Pearl photoselective net	18	1700
Red photoselective net	66	1338
Anti-drosophilid net	0	100

Given the low number of catches of *D.* suzukii, in order to evaluate the effectiveness of the four nets in contain the pest populations, all the specimens belonging to the family Drosophilidae were considered during the statistical analysis. Comparing the mean percentage of Drosophilidae caught with the attractive traps, statistical differences were found between the treatments (one-way ANOVA: df=4, 15; F=13.20; P=0.001). The anti-drosophilid net proved to be the most effective barrier for the protection of the crop (Figure 9). The highest catches were recorded under the pearl net.

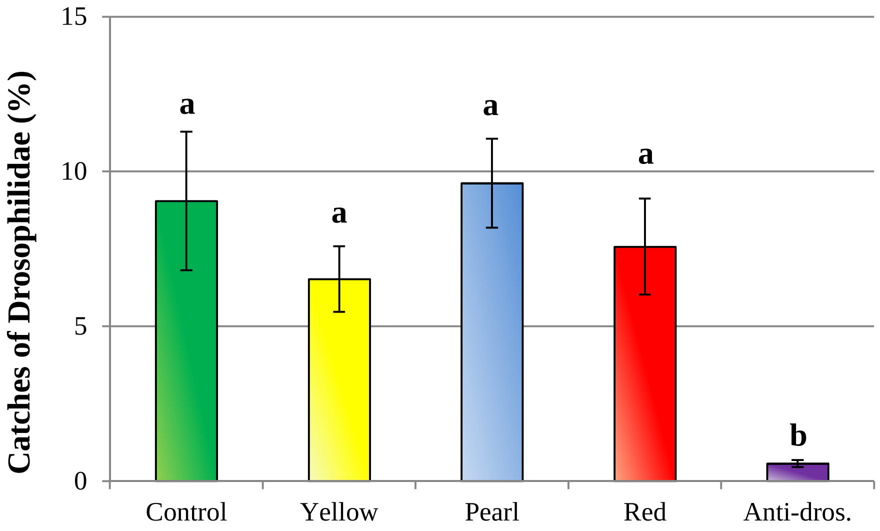


Figure 9. Mean percentage of Drosophilidae catches per trap on the total catches in the apple orchard. Bars with a letter in common are not significantly different (Tukey's test, P<0.05).

3.3.1.2.3 Monitoring of and damage caused by *Halyomorpha halys*

Halyomorpha halys was never collected during the trial neither with pheromone traps nor beating the tree branches. Grower's personal communications highlighted how some specimens were observed in the field in October. Fruits damaged by *H. halys* were never observed all along the trial and at the harvest time.

3.3.1.2.4 Monitoring of other pests and beneficial insects

Shoot visual inspections, yellow sticky traps and a final knock-down treatment were used to assess the impact of the nets on orchards arthropod fauna. In the apple orchard, few specimens of *A. pomi* and *D. plantaginea* were observed every 10 days during the shoot visual inspections. Out of 90 shoots totally observed per treatment, 9 infested shoots were recorded in Y; 7 in P; 3 in R, C and D. No significant differences between the treatments were recorded but in Y a greater number of infested shoots with more than two aphids in each shoot was observed. In particular, 6 colonies with more than ten aphids each and three colonies with a number of specimens between 2 and ten each were observed. In D, only 2 aphids and one single colony with more than ten specimens were recorded in only one visual inspection of the trial. In P; R and C few occasional single aphids and colony with two to ten aphids were observed. Two colonies with more than ten aphids were observed in P while colonies with more than 10 aphids were never recorded in R and C.

Although pheromone traps are commonly used to specifically monitor pest populations, during the trial, several specimens of *S. myopaeformis* were observed in the *C. pomonella* trap. This aspecific capture is due to the feeding attractant. Therefore, in order to evaluate the efficacy of the nets, all the *C. pomonella*, *G. molesta* and *S. myopaeformis* caught with the traps were considered. In particular, 2 adults of *C. pomonella*, 4 specimens of *G. molesta* and 41 *S. myopaeformis* were collected in all the orchard. A total of 25 specimens were caught in C, 8 in Y, 4 in P, 10 in R while no specimens were collected in D. Significant differences between the treatments were observed (one-way ANOVA: df=4, 15; F=14.01; P=0.000) with a lower number of catches recorded under net compared to the control (Figure 10).

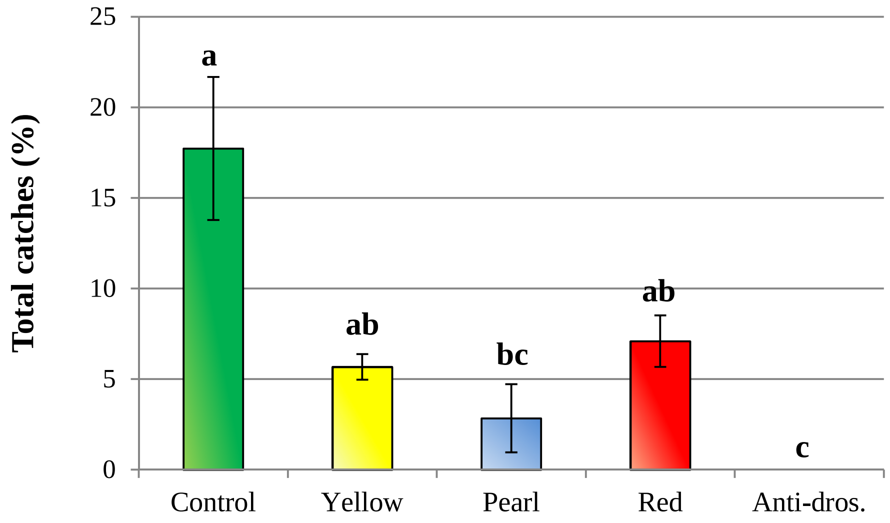


Figure 10. Mean percentage of total catches per trap on the total catches in the apple orchard. Bars with a letter in common are not significantly different (Tukey's test, P<0.05).

The anti-drosophilid net proved to be the most effective barrier for the containment of these pests. No significant differences were recorded between the three photoselective nets, but lower catches were detected under the pearl net.

In order to assess the impact of nets on the orchard arthropod fauna, its abundance, with particular attention to beneficial insects, was assessed in each repetition by yellow sticky traps all along the trial and with a knock-down treatment before the net setting-up and after the harvest time. All the specimens belonging to *Lygus rugulipennis*, Lygeidae, Chrysopidae, *D. virgifera* subsp. *virgifera*, Coccinellidae and Syrphidae trapped with the yellow sticky traps were considered in the cluster *total catches*. Their amounts were significantly different between the treatments (Kruskal-Wallis test: df=5, χ^2=15.88, P=0.007) with a higher number of specimens collected in C followed by I and the three photoselective nets (Table 12). Moreover, Chrysopidae, Coccinellidae and Syrphidae specimens were grouped in *predators*. For this cluster, statistical differences were recorded between the treatments (Kruskal-Wallis test: df=5, χ^2=14.10, P=0.015) (Table 12). In particular, hoverfly were mostly collected in C and in I (96 and 39 specimens totally collected, respectively). Only one hoverfly was caught during the first decad of the trial in D, probably because the specimens was locked inside during the net setting-up. After that, hoverflies were never sampled under the anti-drosophilid net because the mesh is too dense but they were collected in Y, P and R (7, 3, 3 specimens were caught respectively) because the mesh of anti-hail nets allowed the entry of hoverflies of small size. Chrysopidae where collected only in C and I while Coccinellidae where mostly trapped in C, followed by I and the three photoselective nets and were never collected in D.

In the cluster *pests* specimens belonging to the species *L. rugulipennis* and to the family Lygeidae as well *D. virgifera* subsp. *virgifera* specimens were grouped even though these pests are not worrisome for this crop. Statistical differences were recorded between the treatments (one-way ANOVA: df=5, 12; F=10.99, P=0.000) with the highest captures (mainly *L. rugulipennis* and Lygeidae) in C and P replicates (Table 12). *Diabrotica virgifera* subsp. *virgifera* was never trapped under net.

Table 12. Insects collected every ten days with yellow sticky traps (mean±SE). In column, means followed by different letters are significantly different (total catches, predators: Mann-Whitney U-test, P≤0.05; pests: Tukey's test, P<0.05).

Treatment	**Total catches**	**Predators**	**Pests**
Control	45.67±6.17 **a**	39.33± 5.92 **a**	6.33±0.33 **a**
Yellow photoselective net	5.67±0.33 **c**	4.00±1.54 **c**	1.67±0.88 **bc**
Pearl photoselective net	6.33±1.33 **c**	2.00±1.00 **cd**	4.33±1.20 **ab**
Red photoselective net	3.33±0.88 **c**	2.33±1.20 **cd**	1.00±0.58 **bc**
Anti-drosophilid net	0.33±0.33 **d**	0.33±0.33 **d**	0.00±0.00 **c**
Insecticidal treatments	18.67±2.19 **b**	16.67±1.77 **b**	2.00±0.58 **bc**

In the knock-down treatment applied before the net setting-up, specimens belonging to Acarina, Homoptera (Delphacidae and Aphidoidea), Coleoptera (Staphylinidae, Coccinellidae, Chrysomelidae, Curculionidae, Scarabaeidae and Tenebrionidae), Diptera (Micropezidae and Sciaridae), Lepidoptera (Geometridae), Hymenoptera (Apoidea, Formicidae and Calcidoidea) were collected. An average of 45 specimens of which 4 predators were sampled under each treated trees.

After the harvest time with the knock-down treatment, specimens belonging to Collembola, Acarina, Dermaptera (Forficulidae), Thysanoptera (Thripidae), Heteroptera (Anthocoridae, Nabidae, Miridae, Lygeidae and Pentatomidae), Homoptera (Cicadellidae and Aphidoidea), Neuroptera (Chrysopidae), Coleoptera (Carabidae, Staphylinidae, Elateridae, Coccinellidae, Cerambicidae, Chrysomelidae, Nitidulidae, Silvanidae, Curculionidae and Tenebrionidae), Diptera (Drosophilidae) and Hymenoptera were collected and grouped in the cluster *total catches*. Their amounts were significantly different between the treatments (Kruskal-Wallis test: df=5, χ^2=14.99, P=0.010) with a lower number of specimens collected on the un-netted trees (C and I replicates) (Table 13). The highest captures were recorded under Y and R nets.

In the cluster *predators A. fuliginosum*, Forficulidae, Chrysopidae, Anthocoridae, Nabidae, Miridae, Staphylinidae and Coccinellidae were grouped. Statistical differences between the treatments were recorded in *predators* (Kruskal-Wallis test: df=5, χ^2=14.02, P=0.015) with the highest captures in Y followed by R, P and D. In particular, specimens belonging to Acarina, Anthocoridae and Coccinellidae were the most represented with 28, 51 and 62 specimens respectively caught in the all orchard. During the knock-down treatment, 41 aphids were totally collected in the orchard. In particular 16 aphids were collected in P, 14 in Y, 7 in R, 4 in D and no catches were recorded in C and I. *Halyomorpha halys* was never collected under the net coverage, but 5 specimens were caught in C and one in I.

Table 13. Insects collected with the final knock-down treatment (mean±SE). In column, means followed by different letters are significantly different (total catches, predator: Mann-Whitney U-test, P≤0.05).

Treatment	Total catches		Predators	
Control	12.33±4.33	**d**	2.67±0.67	**c**
Yellow photoselective net	174.67±13.92	**a**	94.67±36.56	**a**
Pearl photoselective net	71.00±16.09	**bc**	31.33±20.85	**ab**
Red photoselective net	172.67±76.23	**ab**	90.67±67.27	**ab**
Anti-drosophilid net	38.33±10.90	**c**	15.67±5.67	**b**
Insecticidal treatments	11.00±49.42	**d**	4.00±0.58	**c**

3.3.1.2.5 Monitoring of climatic conditions

As in the peach orchard, temperature and relative humidity were recorded during the trials. Unfortunately, the Data Loggers used provided only partial temperature recordings and unreliable relative humidity data. Despite the partial registrations, no significant temperature deviations were recorded between covered and un-covered trees as in the peach orchard (Figure 11).

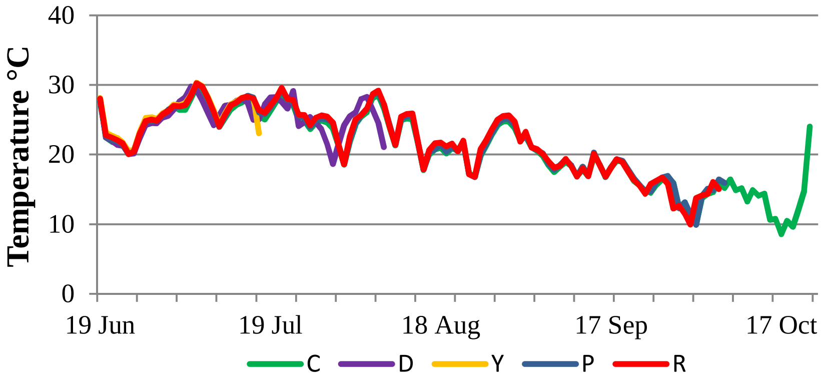


Figure 11. Daily mean temperature recorded inside and outside nets in the apple orchard in treatments C (un-netted control trees), Y (yellow net), P (pearl net), R (red net), D (anti-drosophilid net).

During the trial, the PAR under the different nets was also evaluated at three different heights setting at 100 the lightness recorded in the control replicate. Statistical differences between the treatments were recorded in all the heights (high-position one-way ANOVA: df=4, 10; F=39.95, P=0.000; middle-position one-way ANOVA: df=4, 10; F=50.97, P=0.000; basal-position Kruskal-Wallis test: df=4, χ^2=12.97, P=0.011). The minor shading was observed under pearl net at all heights (Figure 12).

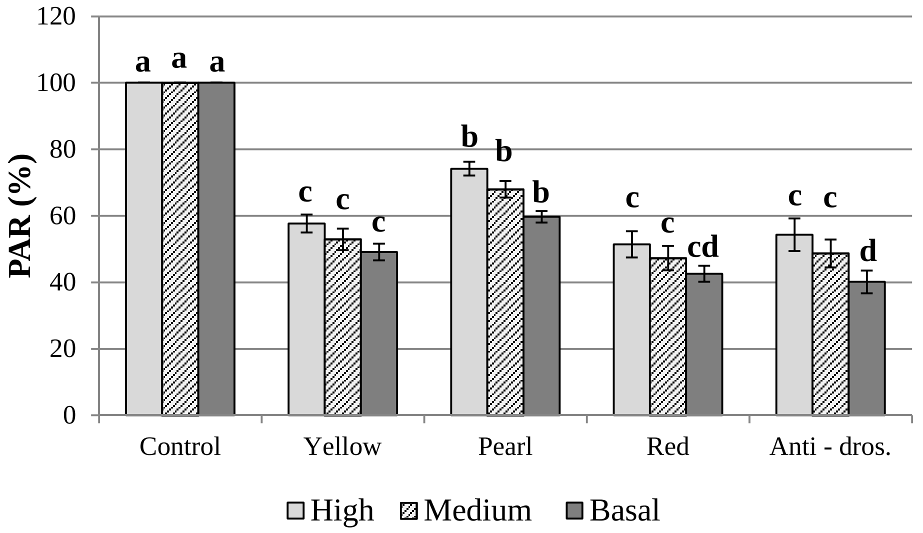


Figure 12. Luminous intensity measured on plant covered with different kind of net in the apple orchard at different heights setting at 100 the external lightness (control). Bars with a letter in common are not significantly different (high and middle-positions: Tukey's test, $P<0.05$; basal-position: Mann-Whitney U-test, $P\leq0.05$).

3.3.1.2.6 Fruit quality

The evolution of the colour index, firmness, total solid soluble and the starch content during the prototype trials is reported in Table 14. Statistical differences were recorded for the colour index (Kruskal-Wallis test: df=4, $\chi^2=12.66$, P=0.013) with more coloured fruits observed under the pearl net. Statistical differences between the treatments were also found for the firmness (one-way ANOVA: df=4, 670; F=6.87, P=0.000) and for the total solid soluble (Kruskal-Wallis test: df=4, $\chi^2=26.62$, P=0.000) but were not recorded for the starch content. The highest firmness was recorded under the pearl net while fruits with a major content in sugar were harvested outside nets followed by the pearl, red and the anti-drosophilid net (Table 14).

Table 14. Colour index, firmness, total solid soluble and starch content (mean±SE) in apples harvested under different nets. In column, means followed by different letters are significantly different (colour index and total solid soluble: Mann-Whitney U-test, P<0.05; firmness: Tukey's test, P<0.05).

Treatment	**Colour index**	**Firmness (g cm^{-2})**	**Total solid soluble (°Brix)**	**Starch (Lugol test)**
Control	28.32±1.12 **a**	6.71±0.05 **b**	12.57±0.06 **a**	8.97±0.06
Yellow net	25.71±0.81 **b**	6.73±0.05 **b**	12.11±0.06 **d**	9.07±0.04
Pearl net	30.61±2.70 **ab**	6.96±0.04 **a**	12.40±0.06 **b**	8.98±0.05
Red net	26.59±0.93 **b**	6.72±0.04 **b**	12.37±0.06 **bc**	9.01±0.07
Anti-drosophilid net	27.34±0.95 **ab**	6.68±0.05 **b**	12.35±0.08 **bc**	8.83±0.06

3.3.1.3 FINAL FEEDBACK

According to the results obtained, a final feedback of the nets was proposed to identify the most promising anti-insect net for the Piedmontese (NW Italy) orchards (Table 15). Scores were calculated separately for the protection ensured by different kinds of net towards different pests and for the net impact on the abundance of predators in the peach and in the apple orchard. The outcome for each category (*protection* and *predators*) was expressed in scores based on the result obtained by the statistical analyses.

In the peach orchard, the final feedback for the category *protection* was calculated taking into account the mean percentage of *G. molesta* and Drosophilidae catches on the total number of catches in the orchard, the mean percentage on shoots and fruits damaged by *G. molesta* during the growing season and the mean percentage of fruits damaged by *G. molesta* at the harvest time per treatment on the total number of damaged fruits at harvest time. In the apple orchard, the mean percentage of the sum of tortrix moths and *S. myopaeformis* catches, and Drosophilidae catches on the total number of catches in the orchard and the mean number of aphids per treatment were considered. In each orchard, three scores were assigned to the different categories taken into account: pest catches (1=0-5.9%, 2=6-10.9%, 3=11-15.9%) damage on shoots and fruits during the growing season (1=0-1.5%, 2=1.6-3%, 3=3.1-4.5%) damage on fruits at harvest time (1=0-1.5%, 2=1.6-3%, 3=3.1-4.5%). The final feedback rating for the category *protection* was assessed, per each treatment, considering the total sum of scores obtained for pest catches, damage during the growing season and at harvest time.

In both the orchards, the final feedback for the category *predators* was calculated considering the mean number of predators caught with yellow sticky traps and with the final knock-down treatment. Three scores were assigned both for the captures obtained with yellow sticky traps (1=0-5.9, 2=6-10.9, 3=11-15.9) and for the predators collected with the final knock-down treatment. In this case, considering the different amount of catches observed in the different orchards, two different scores were assigned for catches recorded in the peach orchard (1=0-5.9, 2=6-10.9, 3=11-15.9) and in the apple orchard (1=0-30.9, 2=31-60.9, 3=61-95.9). The final feedback rating for the category *predators* was assessed, per each treatment, considering the total sum of scores obtained for insects caught with yellow sticky traps and with the final knock-down treatment.

In both the orchards the anti-drosophilid net proved to be the most effective barrier for the protection of the crops followed by the three photoselective anti-hail nets. Among the photoselective nets, the best results were obtained with the pearl net in the peach orchard both for the category *protection* and *predators* while in the apple orchard the pearl net was the best net for the category *protection.*

Table 15. Final feedback for the evaluation of the best anti-insect net in Piedmontese orchards. To a greater number of + corresponds an higher level of the crop protection and a major abundance of predators.

Treatment	**Peach orchard**		**Apple orchard**	
	Protection	***Predators***	***Protection***	***Predators***
Control	+	+++	+	+++
Yellow net	+++	+	++	+++
Pearl net	+++	+++	+++	++
Red net	+++	+	++	+++
Anti-drosophilid net	++++	+	++++	+

In order to choose the net to be used in the following trials (realization of semi-field trials), a compromise has been achieved considering also pathological aspects and fruit quality evaluated as well in the frame of the LIFE+ SU.SA.FRUIT project. Significant differences between the treatments were not recorded in both the orchards. However, lower incidence of *Monilia fructicola* (Winter) Honey was observed under the pearl net in the peach orchard. In the apple orchard, rots determined by *Botrytis* spp., *Alternaria* spp. and *Penicillium* spp. were less frequent under the different nets especially under the pearl net (Davide Spadaro, DISAFA, personal communication).

In the peach orchard, the results obtained from the qualitative analysis were not representative due to the low number of fruits harvested per plant, while in the apple orchard the pearl net positively influenced the color index and the firmness of fruits. During the light analysis evaluations, the lowest shading was recorded under the pearl net while the highest shading was detected under the red net. Overall, the best results were obtained with the pearl net and for this reason this net was used for the semi-filed trials.

3.3.2 SEMI-FIELD TRIALS

Below are reported the results obtained during the semi-field trials broken down for nectarine orchards and apple orchards.

3.3.2.1 Nectarine orchards

3.3.2.1.1 Monitoring of and damage caused by *Grapholita molesta* and *Anarsia lineatella*

In the nectarine orchards, *G. molesta* and *A. lineatella* were collected in both the years with pheromone traps. *Anarsia lineatella* was never collected in N while few specimens were collected in C in both the years. In 2016, only one specimen was collected in Amiga* and in Fire Top® while four specimens were sampled in Fire Top® in 2017.

During the two years of the trial, *G. molesta* was collected both in N and in C with highest catches recorded in C. Statistical differences between the treatments were observed in 2016 (Amiga* t-test: df=4, t=5.47, P=0.005; Fire top® t-test: df=4, t=5.13, P=0.028) and in 2017 (Amiga* t-test: df=4, t=7.76; P=0.001; Fire Top® t-test: df=4, t=5.31, P=0.006) in both the orchards as shown in Table 16.

Table 16. Mean percentage of *G. molesta* (mean±SE) catches per trap on the total catches in the nectarine orchards in 2016 and 2017. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	***Grapholita molesta***	
		2016	**2017**
Amiga*	N	6.0±2.5 **b**	6.5±1.5 **b**
	C	27.3±2.0 **a**	28.8±1.9 **a**
Fire Top®	N	6.1±2.4 **b**	6.1±0.7 **b**
	C	27.2±1.3 **a**	27.2±4.7 **a**

Treatment: N = netted trees, C = un-netted control trees.

In 2016, peaks of catches were reached on June 20th and July 19th while in 2017 peaks were observed on June 15th and July 17th. The critical threshold (Regione Piemonte, 2016 and 2017) was never reached in Amiga* both in C and N while in Fire Top®, the threshold was exceeded only once in C during 2016 (Figure 13).

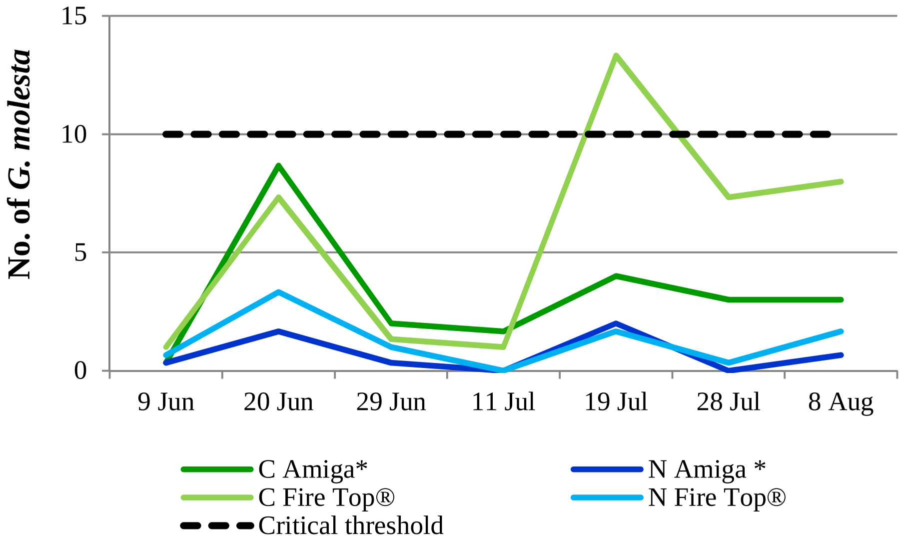


Figure 13. Mean number of *G. molesta* trapped all along the 2016 growing season in treatments C (un-netted control trees) and N (netted trees).

In 2017, both in Amiga* and in Fire Top® (Figure 14) the critical threshold was exceeded three times in C. In N, the threshold was exceeded only once in N on July 17th in Amiga* (28 mean specimens/trap) while in Fire Top® the threshold was exceeded twice on June 15th (10 mean specimens/trap) and July 17th (26 mean specimens/trap) (Figure 14).

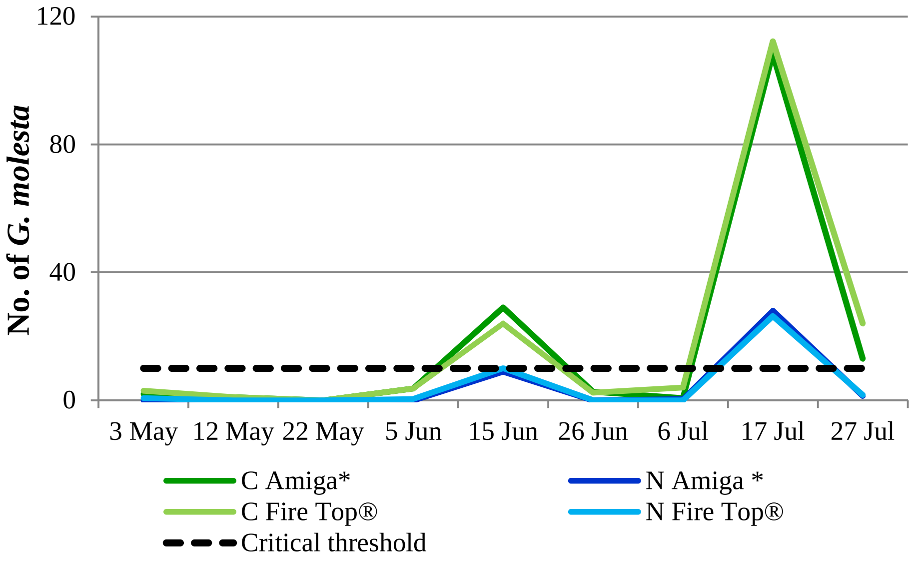


Figure 14. Mean number of *G. molesta* trapped all along the 2017 growing season in treatments C (un-netted control trees) and N (netted trees).

Since the net setting-up until the harvest time, shoots and fruits were visually inspected for damage caused by *G. molesta* in the treatments N and C. Damage on shoots caused by *G. molesta* during the growing season were recorded only in 2016 with mild damages observed in both the cultivar. In Amiga*, two damaged shoots on June 9th and one on July 19th were observed in C. No damage was recorded in N. In Fire Top®, two damaged shoots in C and three in N were observed on June 9th while only one damaged shoot was recorded on June 20th in C.

No fruits damaged by *G. molesta* were observed all along the trial in 2016 and 2017 with the visual inspection but damaged fruits were recorded at the harvest time in N, C and I. During the first year in Amiga* the 0.42% damaged fruits were observed in N, 4.20% in C and 2.12% in I, while in Fire Top® no damaged fruits were recorded in N, the 0.14% in C and 0.13% in I. Statistical differences between the treatments were not found but a lower damage or the absence of damage was recorded in N. No significant differences were observed for the block and picking date effects as no interactions between the treatments and the picking dates were recorded in any orchard.

In 2017, significant differences between the treatments were observed both in Amiga* (GLMM: df=2, 10; F=28.85, P=0.000) and in Fire Top® (GLMM: df=2, 10; F=12.72, P=0.000), with a significantly lower damage in N (Table 17). Statistical differences between the picking dates were also found both in Amiga*(GLMM: df=1, 10; F=65.88, P=0.024) and in Fire Top® (GLMM: df= 1, 10; F=7.74, P=0.009), with a higher damage in the first picking date (Table 17). No interactions between the treatments and the picking dates were recorded in any orchard. Moreover, the GLMM was used to analyze the block effect in order to assess if the pest was more concentrated on the borders or in the middle of the orchards. Significant differences were recorded only in the cv. Amiga* in 2016 (GLMM: df=2, 10; F=9.58, P=0.005) with a higher damage from the edge bordering alfalfa unto the orchard center.

Table 17. Percentages (mean±SE) of nectarines damaged by *G. molesta* assessed in each picking date and in total on fruits sampled at harvest time in 2017 (no.=240 fruits per repetition in the first and second dates). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P<0.05).

Cultivar	**Treatment**	**1st picking date**	**2nd picking date**	**Total***
Amiga*	N	1.4±0.3	0.2±0.2	0.6±0.3 **c**
	C	17.4±1.1	4.9±0.9	9.4±0.9 **a**
	I	6.7±0.7	1.7±0.6	3.4±0.6 **b**
	Total	5.7±0.5 **a**	1.3±0.4 **b**	
Fire Top®	N	0.5±0.4	0.4±0.2	0.4±0.2 **b**
	C	10.1±1.8	3.7±0.8	6.2±0.9 **a**
	I	9.6±1.8	1.7±0.5	4.1±0.7 **a**
	Total	3.8±1.1 **a**	1.3±0.3 **b**	

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

* Total mean percentage of damaged fruits (1st + 2nd picking date) per treatment.

3.3.2.1.2 Monitoring of *Drosophila suzukii*

During the semi-field trials, abundant catches of *D. suzukii* were recorded in both the cultivar on the un-netted control trees (C). The first specimens were collected in mild-June in 2016 and early in May in 2017. During the first year of the trial, 182 *D. suzukii* were totally collected in C and 21 in N in the cultivar Amiga* while in Fire Top® 387 and 39 specimens were respectively trapped in C and N. Statistical differences between the treatments were recorded both in Amiga* (t-test: df=4, t=16.96, P=0.000) and in Fire top® (t-test: df=4, t=4.24, P=0.013) (Table 18).

In 2017, catches were slightly lower than the previous year with 64 specimens collected in C and 21 in N in Amiga* and 101 specimens trapped in C and 36 N in Fire Top®. Statistical differences between the treatments were observed both in Amiga*(t-test: df=4, t=3.50, P=0.025) and in Fire Top® (t-test: df=4, t=3.50, P=0.025) (Table 18).

Table 18. Mean percentage of *D. suzukii* and total Drosophilidae (mean±SE) catches per trap on the total catches in the nectarine orchards. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	***Drosophila suzukii***		**Total Drosophilidae**	
		2016	**2017**	**2016**	**2017**
Amiga*	N	3.4±0.5 **b**	8.2±2.7 **b**	13.0±1.5 **b**	11.6±0.8
	C	20.9±1.7 **a**	25.1±2.1 **a**	20.3±0.9 **a**	21.8±1.0
Fire Top®	N	3.1±1.8 **b**	8.8±1.8 **b**	9.1±1.8 **b**	13.2±0.3
	C	30.3±5.9 **a**	24.6±4.6 **a**	24.2±1.6 **a**	20.2±2.5

Treatment: N = netted trees, C = un-netted control trees.

In 2016, peaks of Drosophilidae catches were reached from mid-July until the harvest time in both the cultivars. In Amiga* 1,014 specimens belonging to Drosophilidae were collected in C and 651 in N while in Fire Top® 1,064 Drosophilidae were trapped in C and 602 in N. Statistical differences were observed both in Amiga*(t-test: df=4, t=3.77, P=0.020) and in Fire Top® (t-test: df=4, t=5.00, P=0.038) (Table 18).

In 2017, peaks of Drosophilidae catches were recorded from June to July 17th in Amiga* while in Fire Top® one more peak was observed on June 26th. During the second year of the trial, the number of Drosophilidae catches was higher than in the previous year with 2,139 specimens totally collected in C and 1,139 in N in Amiga* while in Fire Top® 1,982 Drosophilidae were trapped in C and 1,294 in N. Even though statistical differences between the treatments were not recorded, a lower number of Drosophilidae were collected in N in both the cultivars.

3.3.2.1.3 Monitoring of and damage caused by *Halyomorpha halys*

Halyomorpha halys was never collected with the beating of the branches in both the years of the trial except for one adult collected on the un-netted control trees (C) in Fire Top® on June 15th in 2017.

The pest was detected by traps in the surveyed sites only during the first year of the trial, but its population density was variable among the sites and along the season (Figure 15 and 16). In both the nectarine orchards, it was never collected in traps in N, whereas it was caught in traps in C but in low amounts and close to the harvest time. In Amiga*, 1 nymph and 4 adults were collected in early July and early August, respectively, while in Fire Top® 3 nymphs, and 5 nymphs and 1 adult were caught in late July and early August, respectively. In all the orchards *H. halys* was observed walking rapidly on net surface and reaching the trees through the openings on the top of the anti-hail net system. Moreover, in all the orchards, the trees in the repetitions of N and of C were the pheromone trap was placed (i.e., one of three repetitions per treatment and orchard) showed the highest fruits damage rate in the respective treatment.

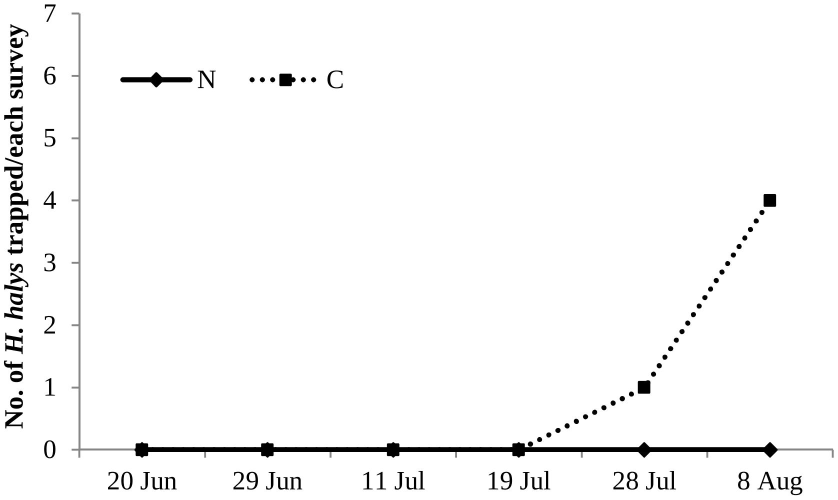


Figure 15. Total number of catches of *H. halys* in pheromone traps in the cultivar Amiga* in treatments N (netted trees) and C (un-netted control trees) in 2016.

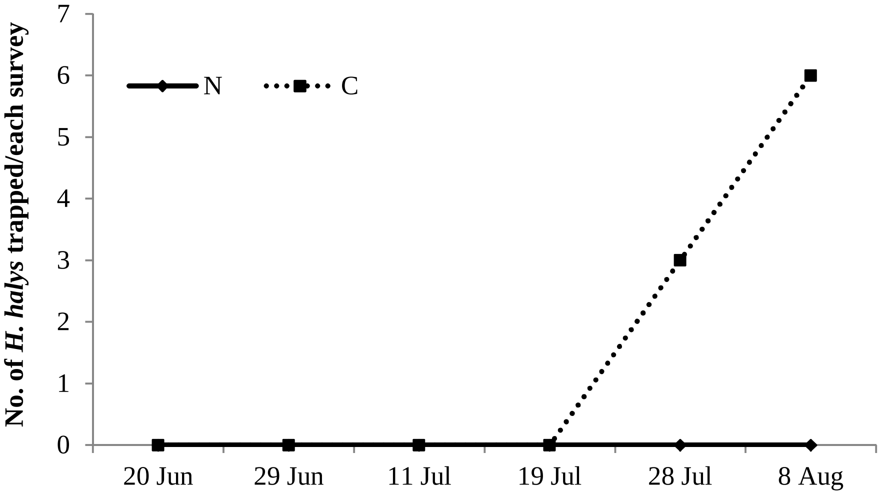


Figure 16. Total number of catches of *H. halys* in pheromone traps in the cultivar Fire Top® in treatments N (netted trees) and C (un-netted control trees) in 2016.

In 2016, during the growing season, a low number of damaged fruits was recorded by visual inspection in all the orchards. Both in Amiga* and in Fire Top®, no damaged fruits were observed in N, while in C, damaged fruits were observed starting from late June. Out of 540 checked fruits in C, in each nectarine orchard, only 23 damaged fruits were recorded in Amiga* (2.1%) and 18 in Fire Top® (1.7%). In 2017, out of 240 checked fruits, no damaged fruits were observed in N while 6 damaged fruits (0.83%) were recorded in C in Amiga*. In Fire Top®, 6 damaged fruits were observed in N (0.83%) and 46 (6.39%) in C.

The damage on fruits observed in each orchard at the harvest time in 2016 is reported in Table 19. By statistical analysis with the GLMM, in the nectarine orchards significant differences between the picking dates were not found, while significant differences between the treatments were observed both in Amiga* (GLMM: df=2, 10; F=65.88, P=0.024) and in Fire Top® (GLMM: df=2, 10; F=7.74, P=0.009), with a significantly lower damage in N. No interactions between the treatments and the picking dates were recorded in any orchard. Moreover, the GLMM was used to analyze the block effect in order to assess if *H. halys* was more concentrated on the borders or in the middle of the orchards. Significant differences for the block effect were recorded only in Amiga* (GLMM: df=2, 10; F=5.57, P=0.024) with a higher damage from the edge bordering alfalfa until the orchard center.

Table 19. Percentages (mean±SE) of nectarines damaged by *H. halys* assessed in each picking date and in total on fruits sampled at harvest time in 2016 (no.=240 fruits per repetition in the first and second dates). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P<0.05).

Cultivar	**Treatment**	**1st picking date**	**2nd picking date**	**Total***
Amiga*	N	4.4±1.9	5.6±1.3	4.9±1.2 **c**
	C	45.6±4.7	52.6±2.9	49.1±2.8 **a**
	I	22.9±4.0	19.9±2.2	21.4±2.2 **b**
	Total	18.4±2.7	20.2±1.7	
Fire Top®	N	8.3±4.7	5.6±8.4	11.5±4.5 **b**
	C	46.6±8.6	60.1±13.7	53.4±8.3 **a**
	I	32.0±7.7	37.8±13.7	34.8±7.7 **ab**
	Total	25.1±4.9	35.6±7.9	

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.
* Total mean percentage of damaged fruits (1st + 2nd picking date) per treatment.

During the harvest time in 2017, the number of fruits damaged by *H. halys* was higher compared with the previous year as reported in Table 20. Statistical differences between the treatments were observed in Amiga* (GLMM: df=2, 10; F=32.96, P=0.000) and in Fire Top® (GLMM: df=2, 10; F=50.87, P=0.000), with a significantly lower damage in N. No differences between the blocks neither interactions between the treatments and the picking dates were recorded in any orchard while significant differences between the picking dates were recorded only in Fire Top® (GLMM: df=1, 10; F=41.89, P=0.000) with a higher damage recorded at the first picking date.

Table 20. Percentages (mean±SE) of nectarines damaged by *H. halys* assessed in each picking date and in total on fruits sampled at harvest time in 2017 (no.=240 fruits per repetition in the first and second dates). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P<0.05).

Cultivar	**Treatment**	**1st picking date**	**2nd picking date**	**Total***
Amiga*	N	12,6±4,8	9,6±3,1	11,0±2,8 **c**
	C	69,7±7,0	65,0±5,1	67,4±4,4 **a**
	I	59,9±7,6	38,6±5,3	49,2±4,8 **b**
	Total	44,2±5,2	33,2±3,5	
Fire Top®	N	51,1±3,4	27,2±4,6	38,5±3,2 **c**
	C	90,0±2,0	77,0±4,5	84,6±2,2 **a**
	I	74,2±2,9	45,5±5,2	60,8±3,1 **b**
	Total	75,0±1,9 **a**	50,4±3,3 **b**	

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

* Total mean percentage of damaged fruits (1st + 2nd picking date) per treatment.

3.3.2.1.4 Monitoring of other pests and beneficial insects

During the semi-field trials, no aphids were observed all along the growing season in both the orchards. Specimens belonging to Anthocoridae, Hemerobiidae, Chrysopidae, Staphylinidae, Coccinellidae and Syrphidae collected with yellow sticky traps were grouped in *predators*. Significant differences between the treatment were observed both in 2016 (Amiga* t-test: df=4, t=12.12, P=0.000; Fire Top® t-test: df=4, t=13.99, P=0.000) and in 2017 (Amiga* t-test: df=4, t=23.29, P=0.000; Fire Top® t-test: df=4, t=5.99, P=0.004) (Table 21) with higher captures in C.

Table 21. Total specimens (mean±SE) caught with the yellow sticky traps per treatment in the nectarine orchards in 2016 and 2017. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	**Predators**	
		2016	**2017**
Amiga*	N	1.67±1.20 **b**	7.67±2.03 **b**
	C	41.00±3.01 **a**	74.00±2.00 **a**
Fire Top®	N	1.00±0.58 **b**	1.33±0.33 **b**
	C	25.67±1.67 **a**	58.67±9.56 **a**

Treatment: N = netted trees, C = un-netted control trees.

The final knock down treatment was not applied in 2017 following growers' need to carry out technical operations into the field. In 2016, in the cluster *total catches*, all the specimens killed by the knock-down treatment were considered. Specimens belonging to Araneae (Aranaeidae), Acarina, Psocoptera, Thysanoptera (Thripidae), Heteroptera (Anthocoridae, Nabidae, Tingidae, Coreidae, Lygeidae and Pentatomidae), Homoptera (Cicadellidae and Aphidoidea), Neuroptera (Hemerobiidae and Chrysopidae), Coleoptera (Staphylinidae, Coccinellidae, Chrysomelidae and Curculionidae), Diptera (Syrphidae and Drosophilidae), Lepidoptera and Hymenoptera were collected. Statistical differences between the treatments were not observed in both the orchards but in Fire Top® a lower number of specimens was collected in treatment I (Table 22). Moreover, Aranaeidae, *A. fuliginosum*, Anthocoridae, Nabidae, Hemerobiidae, Chrysopidae, Staphylinidae, Coccinellidae and Syrphidae were grouped in *predators*. Numbers of predators were significantly different between the treatments in 2016 in Amiga* (Kruskal-Wallis test: df=2, χ^2=6.76, P=0.034) with higher numbers in C (Table 22). Finally, lower numbers of *H. halys* were generally collected in N, even if significant differences were found only in Fire Top® (one-way ANOVA: df=2, 6; F=9.37, P=0.014) (Table 22).

Table 22. Insects collected during the final knock-down treatment (mean±SE) in 2016. In column, means followed by different letters are significantly different (Amiga*: Mann-Whitney *U*-test, P<0.05; Fire Top®: Tukey's test, P<0.05).

Date	**Cultivar**	**Treatments**	**Total catches**	**Predators**	***H. halys***
August 16th	Amiga*	N	23.00±4.00	3.00±0.00 **b**	0.00±0.00
		C	112.33±16.68	11.67±4.25 **a**	19.33±11.26
		I	23.33±0.67	2.00±0.58 **b**	8.00±3.21
August 9th	Fire Top®	N	354.33±304.36	8.67±3.18	6.67±6.17 **b**
		C	155.67±37.82	15.33±6.44	35.33±3.53 **a**
		I	52.67±6.64	6.67±1.20	9.33±5.46 **b**

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

3.3.2.1.5 Monitoring of climatic conditions

During the second year of the semi-field trials, temperature and relative humidity trends were almost identical outside and inside net. In Amiga*, slightly higher values of relative humidity were recorded outside net while slightly higher values of the mean daily temperature were recorded under the net coverage (Figure 17).

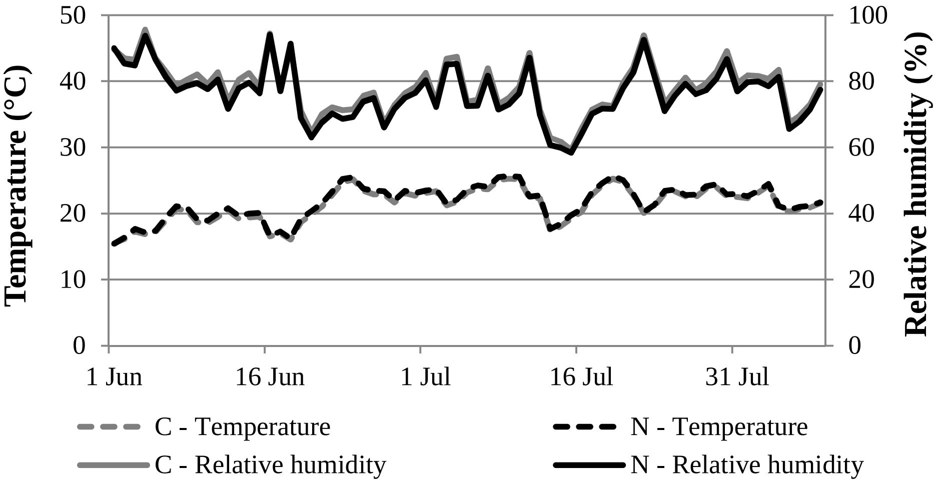


Figure 17. Daily mean temperature and daily mean relative humidity recorded in Amiga* in treatments N (netted trees) and C (un-netted control trees) in 2016.

In Fire Top®, slightly higher values of relative humidity were recorded under net while slightly higher values of the mean daily temperature were recorded outside net (Figure 18).

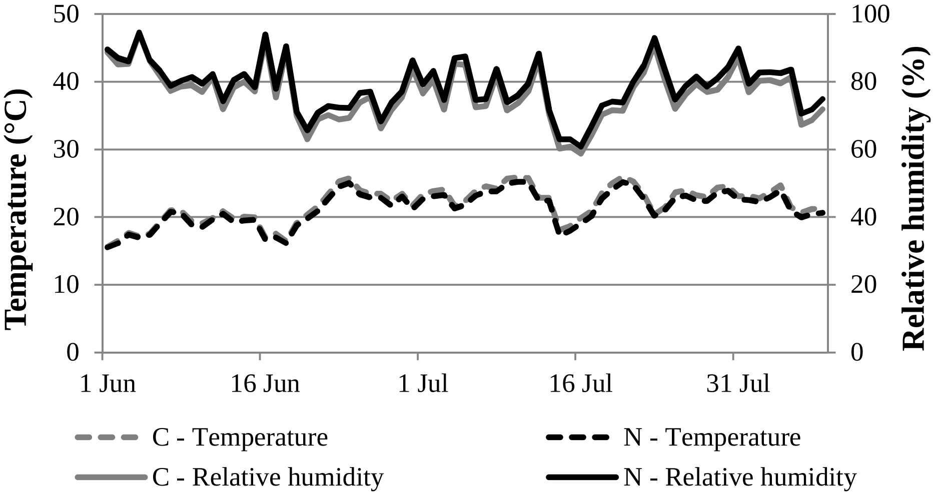


Figure 18. Daily mean temperature and daily mean relative humidity recorded in Fire Top® in treatments N (netted trees) and C (un-netted control trees) in 2016.

In the second year of the semi-field trials, temperature trend was almost identical outside and inside net while slightly higher values of relative humidity were recorded outside net in both the cultivars (Figures 19 and 20).

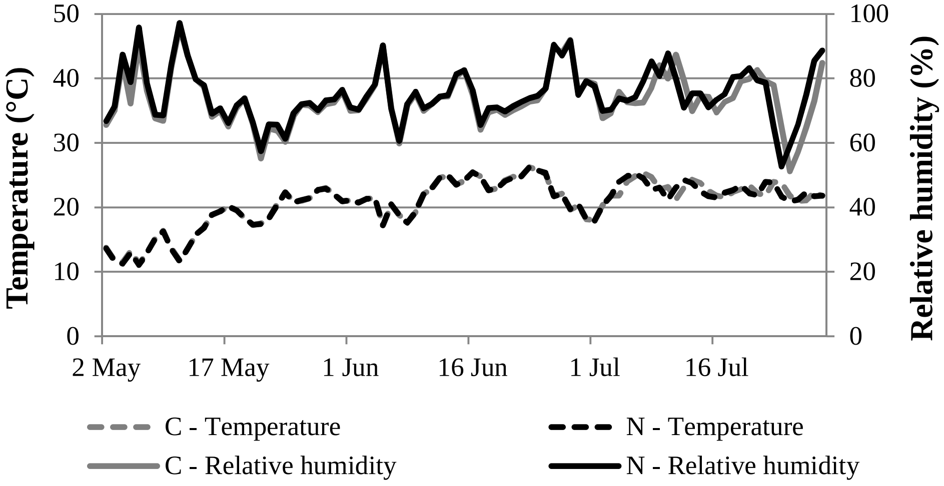


Figure 19. Daily mean temperature and daily mean relative humidity in Amiga* in treatments N (netted trees) and C (un-netted control trees) in 2017.

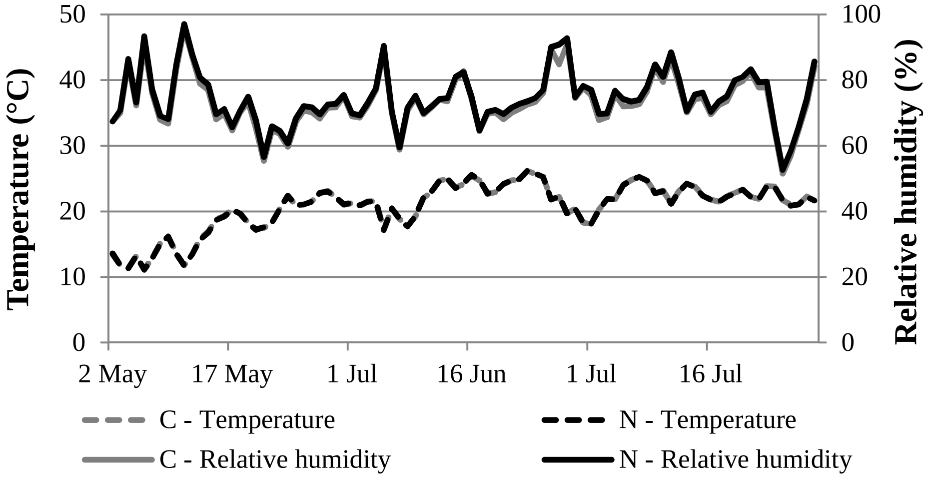


Figure 20. Daily mean temperature and daily mean relative humidity in Fire Top® in treatments N (netted trees) and C (un-netted control trees) in 2017.

During the first year of the semi-field trials, the PAR was recorded in both the nectarine orchards. Since the orchards were neighbouring, the PAR in C was evaluated only in Amiga*. In the high part of the canopy, higher values were recorded in N in Fire Top® and in C in Amiga* (Figure 21a). In the middle height part of the canopy, similar trends were recorded inside and outside net in both the cultivars. Moreover, the further sensor outside the net at middle height allowed the recording of the light reflected from the net (Figure 21b). In the basal part of the canopy, higher PAR was recorded under photoselective net in both orchards (Figure 21c).

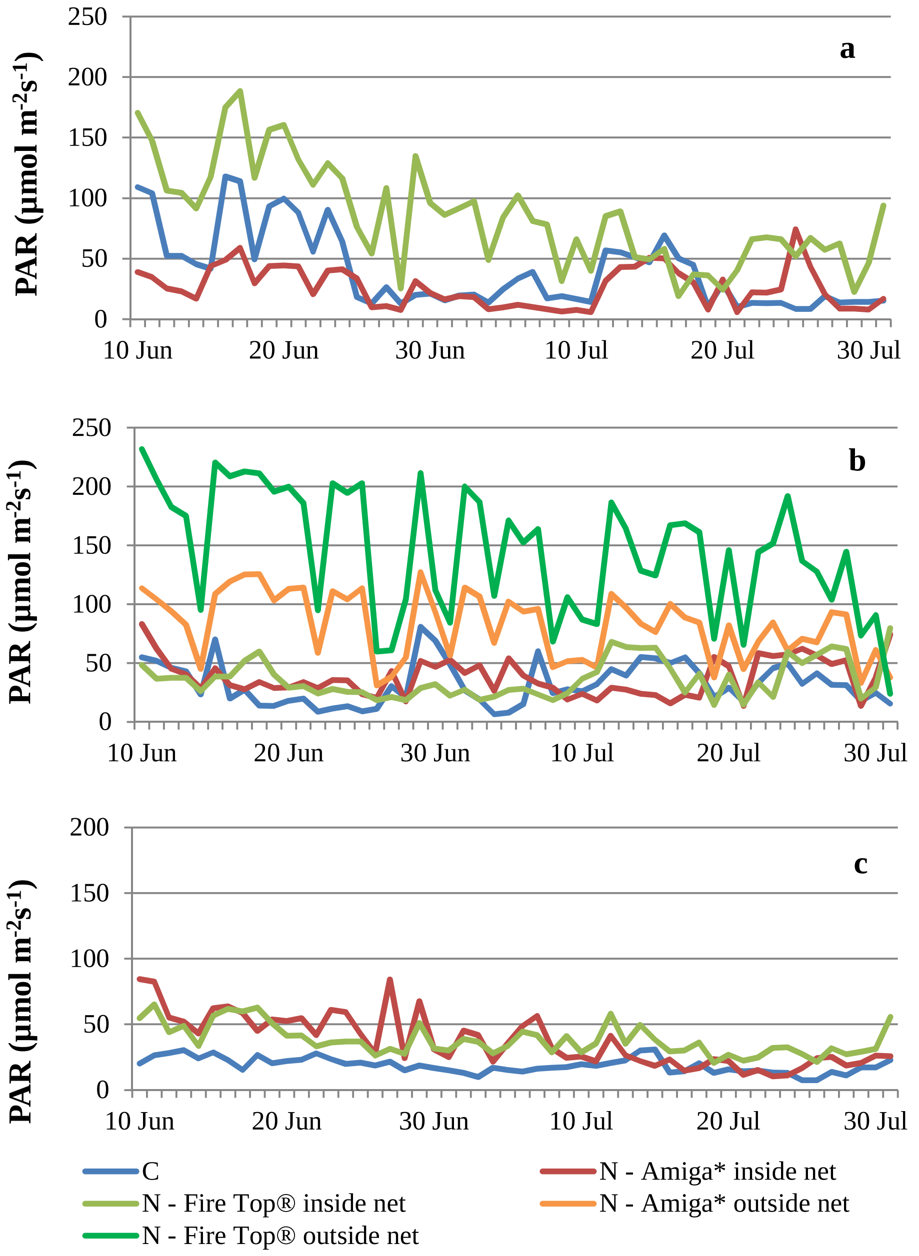


Figure 21. Daily mean PAR recorded in the high (a), middle (b) and basal (c) part of the canopy in Amiga* and in Fire Top® in treatments C (un-netted control trees) and N (netted trees) in 2017.

3.3.2.1.6 Fruit quality

For the nectarines, no statistical differences between the treatments were observed for all the quality analyses performed for Amiga* and Fire Top® in 2016 (Table 23). A lower colour index parameter was recorded in Amiga* for all the treatments compared to Fire Top®. Regarding the total polyphenol and anthocyanin tested on the whole fruit, no statistical differences were observed between the treatments. In Fire Top®, a higher concentration of total anthocyanins was measured for all the treatments compared to Amiga* (Table 23).

In Amiga*, during the second year of the semi-field trials, statistical differences between the treatments were observed only for the total solid soluble (one-way ANOVA: df= 2, 227; F=4.58, P=0.011) (Table 24). In Fire Top®, statistical differences between the treatments were recorded for the colour index (one-way ANOVA: df=2, 227; F=11.09, P=0.000) and the total solid soluble (Kruskal-Wallis test: df=2, χ^2=31.28, P=0.000) (Table 24). Regarding the total polyphenol and anthocyanin tested on the whole fruit, no statistical differences were observed between the treatments. However, a higher concentration of total polyphenols was observed in N (Table 24).

Table 23. Colour index, firmness, total solid soluble, total polyphenols and total anthocyanins (mean±SE) of the two picking dates for nectarine orchards in 2016. No significant differences were found by ANOVA.

Cultivar	**Treatment**	**Colour index**	**Firmness ($g\ cm^{-2}$)**	**Tot. solid soluble (°Brix)**	**Tot. polyphenols ($mg_{GAE}\ 100_g^{-1}$)**	**Tot. anthocyanins ($mg_{C3G}\ 100_g^{-1}$)**
Amiga*	N	33.11±2.41	5.45±0.11	8.62±0.09	34.23±3.49	8.74±0.30
	C	35.03±2.56	5.63±0.08	8.88±0.08	33.52±1.80	7.54±1.55
	I	39.42±2.87	5.83±0.09	8.57±0.08	38.98±3.76	8.74±12.17
Fire Top®	N	49.12±1.91	4.23±0.11	8.15±0.07	38.85±3.12	17.49±1.47
	C	48.05±1.58	4.49±0.06	8.41±0.06	43.73±3.69	20.93±3.29
	I	50.59±2.46	4.20±0.07	8.24±0.11	44.30±3.98	11.60±2.49

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

Table 24. Colour index, firmness, total solid soluble, total polyphenols and total anthocyanins (mean±SE) of the two picking dates for nectarine orchards in 2017. In column, means followed by different letters are significantly different (Amiga* total solid soluble and Fire Top® colour index: Tukey's test, $P<0.05$; Fire Top® total solid soluble: Mann-Whitney *U*-test, $P<0.05$).

Cultivar	**Treatment**	**Colour index**	**Firmness ($g\ cm^{-2}$)**	**Tot. solid soluble (°Brix)**	**Tot. polyphenols ($mg_{GAE}\ 100_g^{-1}$)**	**Tot. anthocyanins ($mg_{C3G}\ 100_g^{-1}$)**
Amiga*	N	30.75±1.04	4.90±0.12	8.19±0.08 **b**	33.80±2.43	7.43±2.02
	C	30.76±1.22	4.87±0.12	8.57±0.09 **a**	31.09±1.90	12.25±1.81
	I	27.83±1.14	5.18±0.14	8.41±0.11 **ab**	29.02±2.33	9.45±2.23
Fire Top®	N	36.68±1.92 **b**	4.24±0.08	8.30±0.08 **c**	65.43±6.92	9.74±1.98
	C	44.92±1.61 **a**	4.21±0.08	8.71±0.08 **b**	51.51±3.71	10.95±2.29
	I	38.00±1.58 **b**	4.08±0.06	8.89±0.10 **a**	47.92±1.97	13.28±2.61

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

3.3.2.2 Apple orchards

3.3.2.2.1 Monitoring of and damage caused by *Cydia pomonella* and *Grapholita molesta*

The presence and the abundance of tortrix moths were monitored all along the trials in each N and C replicate. During the trial, low catches of Tortricidae were recorded with the pheromone traps in both the apple orchards. In Baigent Brookfield®, *C. pomonella* was never trapped in both the years. In 2016, 13 *G. molesta* were recorded in C and one in N while 11 *G. molesta* were collected in C and two in N in 2017. In Galaval*, only one *C. pomonella* and two *G. molesta* were recorded in C in 2016. During the second year of the semi-field trial, *G. molesta* was never trapped in this orchard and only 2 *C. pomonella* were collected in C. Damage on fruits caused by Tortricidae were never observed in both the apple orchards all along the two years of the trial but fruits damaged by *C. pomonella* at the harvest time were recorded in Galaval* in 2017. In particular, 4 damaged fruits were collected in N and 6 in C and in I.

3.3.2.2.2 Monitoring of *Drosophila suzukii*

Population of *D. suzukii* and other Drosophilidae were monitored in each N and C replicate with a feeding attractive. Catches of *D. suzukii* were recorded in both the cultivar on the un-netted control trees (C). The first specimens were trapped in June in 2016 and in May in 2017. In particular, abundant catches were recorded in 2016 when 631 *D. suzukii* were totally collected in C and 118 in N in Baigent Brookfield® while in Galaval* 394 and 117 specimens were trapped in C and N, respectively.

Significant differences between the treatments were recorded both in Baigent Brookfield® (t-test: df=4, t=7.07, P=0.002) and in Galaval* (t-test: df=4, t=0.56, P=0.025) with a lower number of catches collected in N (Table 25).

In 2017, catches well below than the previous year were recorded. In Baigent Brookfield®, 111 specimens were totally collected in C and 21 in N while 147 specimens were trapped in C and 50 N in Galaval*. Statistical differences between the treatments were observed both in Baigent Brookfield® (t-test: df=4, t=4.47, P=0.011) and in Galaval* (t-test: df=4, t=6.36, P=0.003) with a lower number of catches recorded in N (Table 25).

Table 25. Mean percentage of *D. suzukii* and total Drosophilidae (mean±SE) catches per trap on the total catches in the apple orchards. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	***Drosophila suzukii***		**Total Drosophilidae**	
		2016	**2017**	**2016**	**2017**
Baigent Brookfield®	N	5.3±2.0 **b**	5.3±1.9 **b**	10.1±2.9	14.3±1.4
	C	28.1±2.2 **a**	28.8±4.3 **a**	23.3±4	19.1±1.3
Galaval*	N	7.6±0.9 **b**	8.5±1.8 **b**	10.6±0.7 **b**	14.5±0.9
	C	25.7±4.1 **a**	24.9±2.8 **a**	22.7±2.5 **a**	18.9±1.3

Treatment: N = netted trees, C = un-netted control trees.

The total amount of specimens belonging to Drosophilidae trapped in the orchards was also taking into account. In 2016, 3,406 specimens belonging to Drosophilidae were collected in C and 1,473 in N in Baigent Brookfield® while Galaval* 2,095 Drosophilidae were trapped in C and 978 in N. Statistical differences were observed only in Galaval* (t-test:

df=4, t=2.83, P=0.047) with a lower number of catches collected in N (Table 25).

In 2017, catches were slightly higher than in the previous year. No significant differences between the treatments were recorded but a lower number of specimens was trapped in N. In Baigent Brookfield®, 2,398 specimens were totally collected in C and in 1,795 N while Galaval* 4,174 Drosophilidae were trapped in C and 3,207 in N.

3.3.2.2.3 Monitoring of and damage caused by *Halyomorpha halys*

Halyomorpha halys was never collected with the beating of the branches in both 2016 and 2017. The pest was detected by traps in all surveyed sites, but its population density was variable among the sites and along the years. In 2016, catches were higher than in nectarine orchards but later in the season when the trials in the nectarine orchards were already ended. In N, a few specimens were caught after the end of the harvest time only in Baigent Brookfield®, on which 10 nymphs and 1 nymph were collected in early and late September, respectively. In C, in Baigent Brookfield®, 496 nymphs and 5 adults were overall caught from mid-August, with a peak of 309 specimens in the second half of September (Figure 22). In Galaval*, 10 nymphs and 37 adults were caught at the end of October (Figure 23).

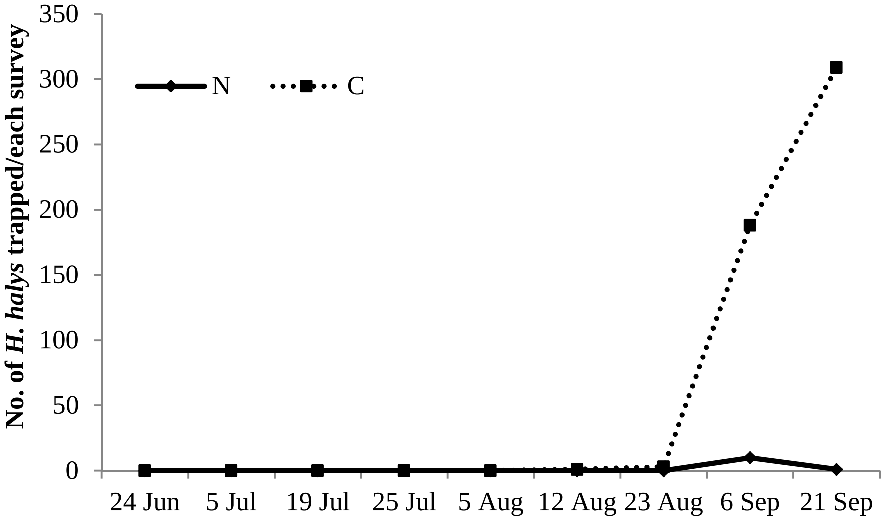


Figure 22. Total number of catches of *H. halys* in pheromone traps in the cultivar Baigent Brookfield® in treatments N (netted trees) and C (un-netted control trees) in 2016.

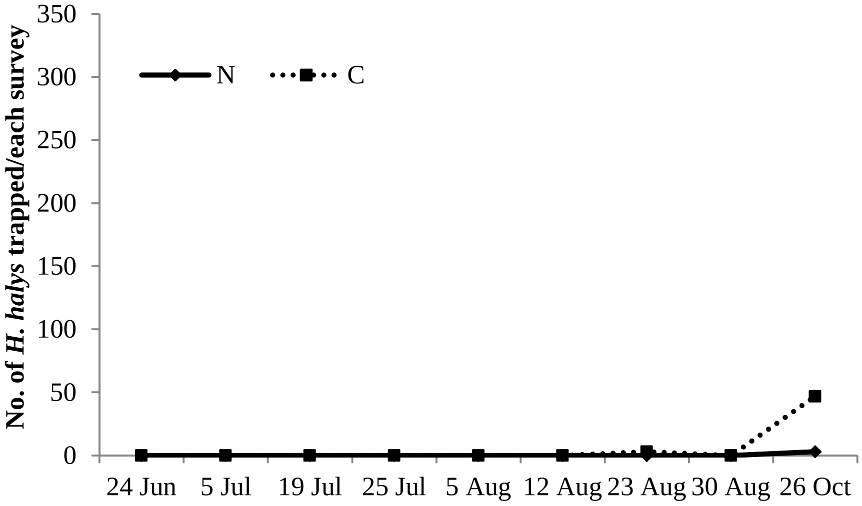


Figure 23. Total number of catches of *H. halys* in pheromone traps in the cultivar Galaval* in treatments N (netted trees) and C (un-netted control trees) in 2016.

In 2017, *H. halys* was detected in both the orchards from the early growing season until the harvest time with peaks of catches close to the fruit ripening. The pest was never trapped in N in Galaval* while in Baigent Brookfield® only 2 nymphs were collected. In C, 42 nymphs and 23 adults were overall caught in Baigent Brookfield® (Figure 24) while 180 nymphs and 18 adults were totally trapped in Galaval* (Figure 25).

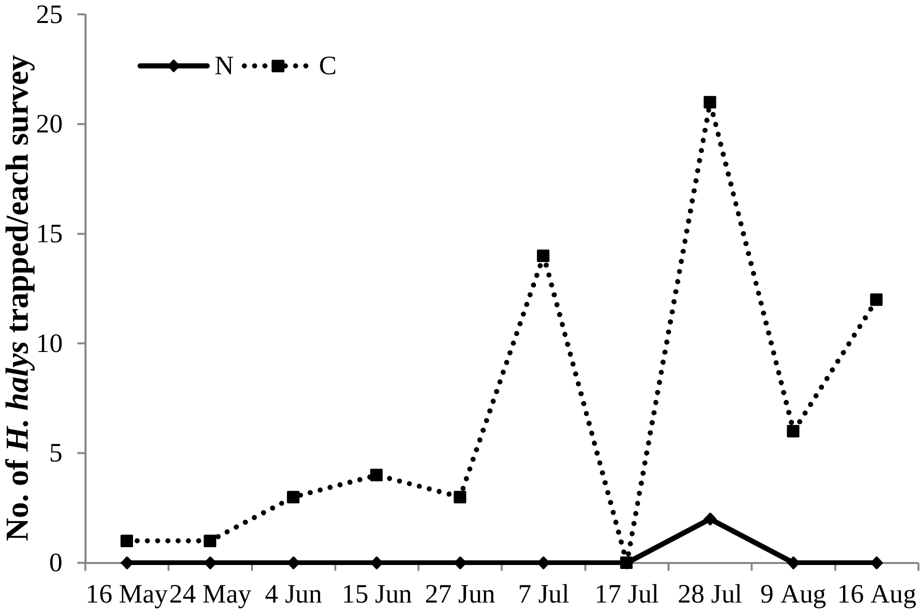


Figure 24. Total number of catches of *H. halys* in pheromone traps in the cultivar Baigent Brookfield® in treatments N (netted trees) and C (un-netted control trees) in 2017.

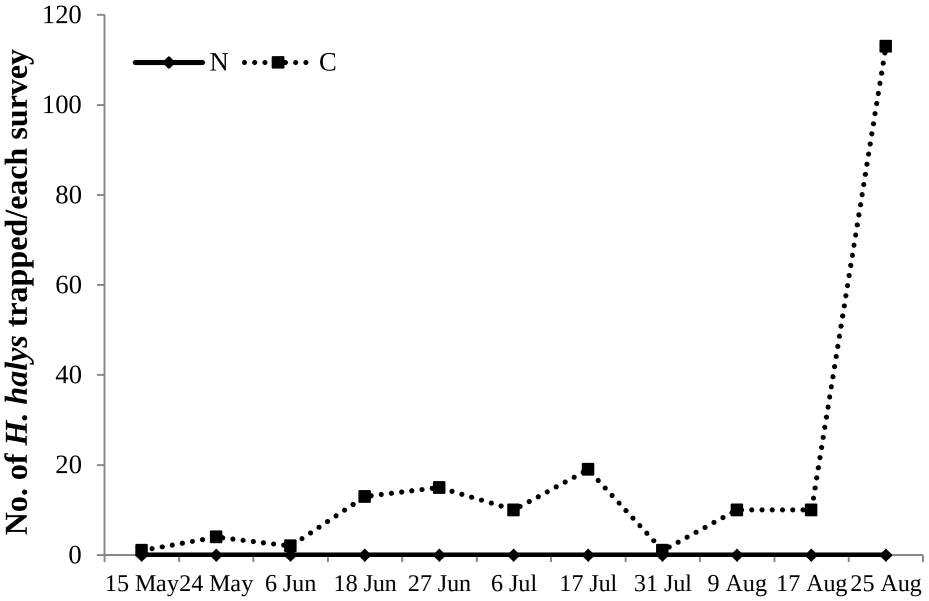


Figure 25. Total number of catches of *H. halys* in pheromone traps in the cultivar Galaval* in treatments N (netted trees) and C (un-netted control trees) in 2017.

Such as in the nectarine orchards, *H. halys* was observed walking rapidly on net surface and reaching the trees through the openings on the top of the anti-hail net system. The highest damage rate was observed in the repetitions of N and of C where the pheromone trap was placed (i.e., one of three repetitions per treatment and orchard). In 2016, during the growing season, a low number of damaged fruits was recorded in both the apple orchards where damaged fruits were observed only in the first week of August. Out of 540 checked fruits in each apple orchard, in Galaval* no damaged fruits were found in N and only one damaged fruit was recorded in C. In Baigent Brookfield®, 3 damaged fruits (0.4%) were recorded in N and 4 (0.5%) in C. In the growing season 2017, a higher number of damaged fruits was recorded in both the apple orchards compared with the first year of the semi-field trial. In Baigent

Brookfield®, out of 900 checked fruits per treatment, 25 damaged fruits (2.78%) were found in N and 21 damaged fruits (2.33%) were recorded in C. In Galaval*, 12 damaged fruits (1.21%) were recorded in N and 17 (1.72%) in C, out of 990 checked fruits per treatment.

The damage on fruits observed in each apple orchard at the harvest time is reported in Table 26 for the year 2016. By statistical analysis with the GLMM, in the apple orchards, significant differences between the treatments were not observed, while differences between the picking dates were recorded only in Galaval* (GLMM: df=2, 16; F=5.93, P=0.012) with a significantly lower damage in the first picking date. No interactions between the treatments and the picking dates were recorded in any orchard. Moreover, such as for the nectarine orchards, the block effect was analyzed in order to assess if *H. halys* was more concentrated on the borders or in the middle of the orchards. No differences for the block effect were recorded.

Table 26. Percentages (mean±SE) of apples damaged by *H. halys* assessed in each picking date and in total on fruits sampled at harvest time in 2016 (no.=240 fruits per repetition in the first and second dates, no.=30 fruits per repetition in the third date). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P<0.05).

Cultivar	**Treatment**	**1st picking date**	**2nd picking date**	**3rd picking date**	**Total***
Baigent Brookfield®	N	6.2±2.2	4.6±1.2	6.9±3.9	5.8±1.4
	C	7.7±2.4	6.0±1.5	7.6±3.8	7.1±1.6
	I	3.1±1.5	5.3±1.5	10.3±5.1	5.5±1.4
	Total	5.3±1.2	5.3±0.9	8.1±2.5	
Galaval*	N	2.4±1.7	7.6±1.8	4.4±2.2	4.3±1.3
	C	3.4±2.0	17.0±2.5	11.7±3.2	9.0±1.9
	I	2.9±1.8	8.9±1.9	14.8±3.6	7.4±1.7
	Total	2.9±1.1 **b**	10.6±1.3 **a**	9.2±1.9 **a**	

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

* Total mean percentage of damaged fruits (1st + 2nd picking date) per treatment.

In 2017, damaged fruits at the harvest time were observed in both the orchards with highest damage recorded in Galaval* (Table 27). Significant differences between the treatments were observed in Baigent Brookfield® (GLMM: df=2, 10; F=9.12, P=0.006) and in Galaval* (GLMM: df= 2, 10; F=9.46, P=0.005) as reported in Table 27. Statistical differences between the picking dates were observed in Baigent Brookfield® (GLMM: df=1, 10; F=5.02, P=0.049) and in Galaval* (GLMM: df=1, 10; F=6.44, P=0.029) with a significantly lower damage in the second picking date in Baigent Brookfield® and in the first picking date in Galaval*. No interactions between the treatments and the picking dates were recorded in any orchard. Significant differences for the block effect were recorded only in Baigent Brookfield® (GLMM: df=2, 10; F=10.75, P=0.003) with a higher concentration on the borders.

Table 27. Percentages (mean±SE) of apples damaged by *H. halys* assessed in each picking date and in total on fruits sampled at harvest time in 2017 (no.=240 fruits per repetition in the first and second dates). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P<0.05).

Cultivar	**Treatment**	**1st picking date**	**2nd picking date**	**Total***
Baigent Brookfield®	N	2.7±1.6	1.4±0.8	1.9±0.8 **b**
	C	12.5±4.1	4.7±1.6	7.7±2.1 **a**
	I	2.3±1.6	0.6±0.5	1.2±0.7 **b**
	Total	4.3±1.6 **a**	1.6±0.6 **b**	
Galaval*	N	4.0±0.8	9.8±3.7	6.3±1.4 **b**
	C	13.2±1.4	23.3±5.3	17.7±2.3 **a**
	I	16.3±1.5	16.5±4.6	16.4±2.4 **a**
	Total	9.7±0.8 **b**	15.8±2.7 **a**	

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

* Total mean percentage of damaged fruits (1st + 2nd picking date) per treatment.

3.3.2.2.4 Monitoring of other pests and beneficial insects

In the apple orchards, during the shoots visual inspections in each N and C replicate, *A. pomi* and *E. lanigerum* were observed in 2016 while only *A. pomi* was observed in 2017. In Baigent Brookfield®, 5 infested shoots in C and 20 in N were recorded in 2016 while in the following year 107 infested shoots in C and 56 in N were observed. In Galaval*, a lower number of infested shoots was always detected in N with 4 and 23 infested shoots in 2016 and 2017, respectively against 38 and 81 shoots recorded in C. Significant differences were found only in Galaval* both in 2016 (t-test: df=4, t=2.91, P=0.044) and in 2017 (t-test: df=4, t=5.47, P=0.005).

During the semi-field trials, low catches of Tortricidae were recorded but high infestations of *S. myopaeformis* were observed in all the orchards especially in 2017. In Baigent Brookfield®, 38 specimens were collected in C and 2 in N in 2016 while 111 specimens were trapped in C and 18 in N during the following year. In Galaval*, 4 *S. myopaeformis* were recorded in N in both the years while 71 and 108 specimens were trapped in C in 2016 and in 2017, respectively. In order to evaluate the efficacy of the net, all the *C. pomonella, G. molesta* and *S. myopaeformis* caught with the pheromone traps were considered during the statistical analysis. Statistical differences between the treatments were observed in 2016 both in Baigent Brookfield® (t-test: df=4, t=-7.22, P=0.002) and in Galaval* (t-test: df=4, t=-6.52, P=0.003) and only in Galaval* in 2017 (t-test: df=4, t=-5.55, P=0.005) with a lower number of catches recorded under net (Table 28). Even though in Baigent Brookfield® in 2017 statistical differences were not recorded, a lower number of specimens were collected under the net.

Table 28. Mean percentages (±SE) of total catches per trap on the total catches in the apple orchards in 2016 and 2017. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	**Total catches**	
		2016	**2017**
Baigent Brookfield®	N	1.85±1.07 **b**	4.7±0.8
	C	31.48±3.21 **a**	28.6±11.1
Galaval*	N	1.71±1.71 **b**	1.2±0.3 **b**
	C	31.62±2.26 **a**	32.1±8.0 **a**

Treatment: N = netted trees, C = un-netted control trees.

In order to assess the impact of nets on the orchard arthropod fauna abundance, yellow sticky traps (in N and C) and knock-down treatments (in N, C and I) were used in both the orchards. Specimens belonging to Araneae (Aranaeidae), Heteroptera (Anthocoridae) [only in Baigent Brookfield® in 2016], Neuroptera (Hemerobiidae [except in Galaval* in 2017] and Chrysopidae), Coleoptera (Staphylinidae and Coccinellidae); Diptera (Syrphidae) collected all along the trial with the yellow sticky traps were totally grouped in *predators* and statistically analyzed. Higher catches were always obtained on the un-netted control trees (C) but statistical differences were recorded only in Galaval* in 2016 (t-test: df=2, t=6.99, P=0.002) and in Baigent Brookfield® in 2017(t-test: df=4, t=4.63, P=0.010) (Table 29).

Table 29. Total specimens (mean±SE) of predators caught with the yellow sticky traps per treatment in the apple orchards in 2016 and 2017. Means followed by different letters are significantly different (t-test, P<0.05).

Cultivar	**Treatment**	**Predators**	
		2016	**2017**
Baigent Brookfield®	N	74.67±27.51	2.33±0.88 **b**
	C	137.00±9.45	15.33±2.66 **a**
Galaval*	N	7.00±1.00 **b**	0.66±0.66
	C	67.33±8.56 **a**	7.00±3.05

Treatment: N = netted trees, C = un-netted control trees.

In the final knock-down treatment, all the specimens killed by the treatment were considered in the cluster *total catches*. Specimens belonging to Araneae (Aranaeidae), Acarina, Dermaptera (Forficulidae), Psocoptera, Thysanoptera (Thripidae), Heteroptera (Anthocoridae, Nabidae, Tingidae, Coreidae, Lygeidae and Pentatomidae), Homoptera (Cicadellidae and Aphidoidea), Neuroptera (Hemerobiidae and

Chrysopidae), Coleoptera (Staphylinidae, Coccinellidae, Chrysomelidae and Curculionidae), Diptera (Syrphidae and Drosophilidae), Lepidoptera and Hymenoptera were collected. Their amounts were significantly different between the treatments only in Galaval* in 2016 with a lower number of specimens collected in treatment I (one-way ANOVA: df=2, 6; F=5.18, P=0.049) (Table 30). Moreover, Aranaeidae, *A. fuliginosum*, Forficulidae, Anthocoridae, Nabidae, Hemerobiidae, Chrysopidae, Staphylinidae, Coccinellidae and Syrphidae were grouped in *predators*. Given the severity of the presence of *H. halys* in the last years in Piedmont, this pest was considered individually. Statistical differences between the treatments were not found for the cluster *predators* and for *H. halys* (Table 30). In 2016, lower numbers of *H. halys* were generally collected in N while in 2017 in Galaval*, 8 first age nymphs were collected in N.

Table 30. Number of insects collected during the final knock-down treatment (mean±SE). In column, means followed by different letters are significantly different (Tukey’s test, P<0.05).

Cultivar	**Date**	**Treatments**	**Total catches**	**Predators**	***H. halys***
Baigent Brookfield®	20 September 2016	N	199.00±162.56	42.00±30.50	0.33±0.33
		C	57.00±2.89	25.00±3.61	2.67±2.67
		I	50.33±9.96	30.33±8.45	0.33±0.33
Baigent Brookfield®	13 October 2017	N	26.00±2.52	9.67±1.76	0.00±0.00
		C	69.00±21.73	10.67±3.67	0.00±0.00
		I	38.67±12.41	10.00±1.15	0.33±0.33
Galaval*	19 October 2016	N	36.33±3.48 **ab**	23.67±4.10	0.33±0.33
		C	40.33±5.78 **a**	28.00±7.37	1.33±1.33
		I	22.67±2.03 **b**	12.67±1.45	0.00±0.00
Galaval*	3 October 2017	N	43.67±10.17	19.67±5.04	2.67±2.67
		C	41.67±3.53	17.67±3.93	0.00±0.00
		I	26.00±13.58	9.33±3.38	2.67±0.33

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides.

3.3.2.2.5 Monitoring of climatic conditions

During the semi-field trials, climatic conditions were evaluated in both the apple orchards only in 2016 while in 2017 they were checked only in Galaval*. As in the nectarine orchards, temperature and relative humidity trends were almost identical outside and inside net. In Baigent Brookfield®, slightly higher values of temperature and relative humidity were recorded outside net (Figure 26).

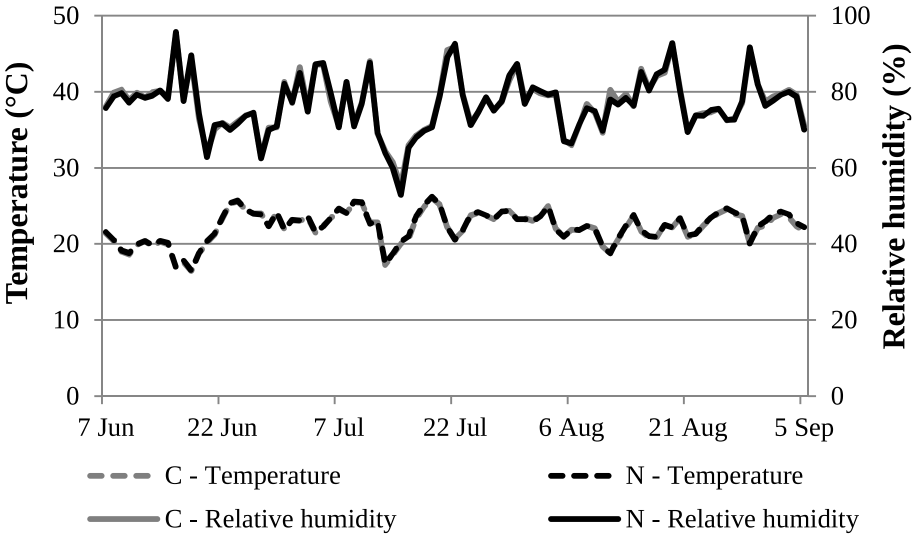


Figure 26. Daily mean temperature and daily mean relative humidity recorded in Baigent Brookfield® in treatments N (netted trees) and C (un-netted control trees) in 2016.

In Galaval*, slightly higher values of relative humidity were recorded inside the net while slightly higher values of the mean daily temperature were recorded outside the net coverage in both years (Figures 27 and 28).

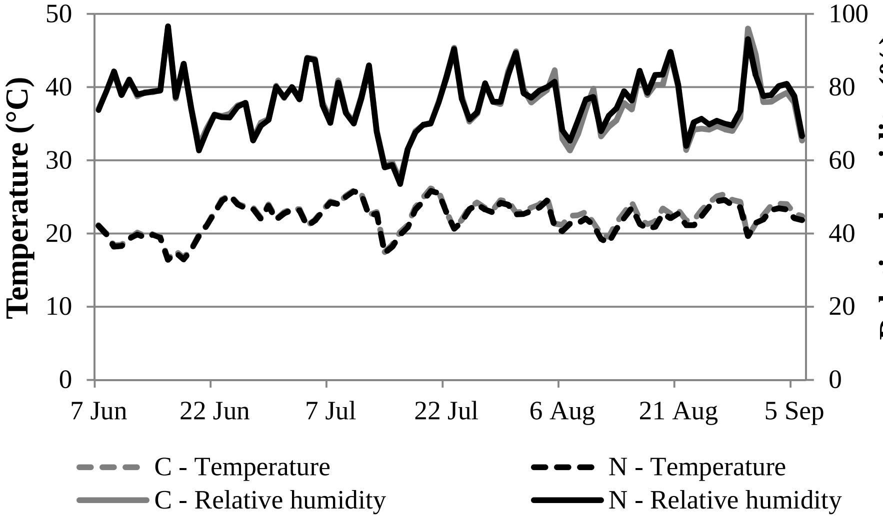


Figure 27. Daily mean temperature and daily mean relative humidity recorded in Galaval* in treatments N (netted trees) and C (un-netted control trees) in 2016

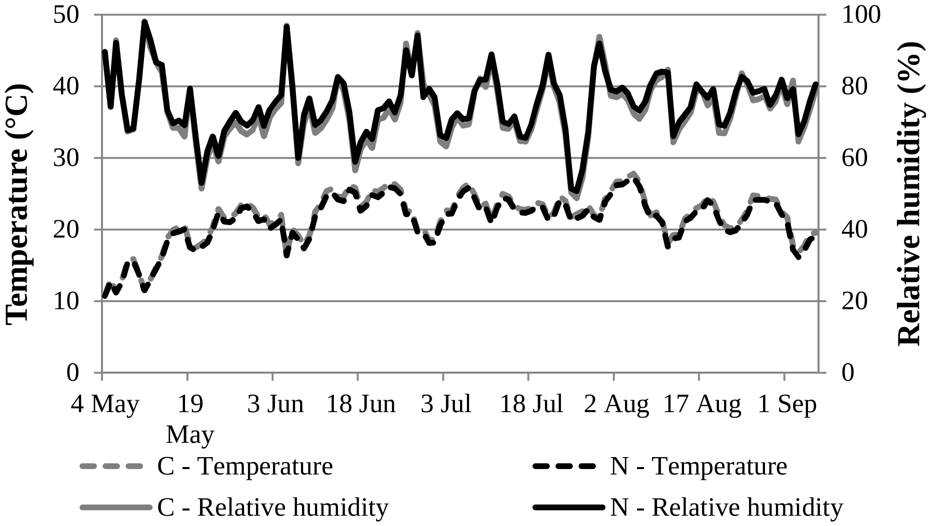


Figure 28. Daily mean temperature and daily mean relative humidity recorded in Galaval* in treatments N (netted trees) and C (un-netted control trees) in 2017.

During the first year of the semi-field trials, the PAR was recorded in both the apple orchards. In Baigent Brookfield®, higher values of PAR were generally recorded in July and then drastically dropped in August. Lower PAR values were observed in the high part of the canopy under the photoselective net in July while from August the trend was almost identical outside and inside the net but slightly higher values were recorded in C (Figure 29a). However, higher values were observed under the photoselective net for all the trial in the middle height of the canopy (Figure 29b) and from the second decade until the end of July in the basal part (Figure 29c). In August, more consistent values were observed in N compared to C in the basal part of the canopy. Moreover, a sensor outside the net was arranged in the N replicate at middle height. In N, the sensor placed outside, but close to the photoselective net, recorded the part of light reflected from the net (Figure 29b).

In Galaval*, higher values of PAR were generally recorded in July and at the beginning of August. In the high part of the canopy, lower PAR values were observed under the photoselective net until the end of August, then similar trends were recorded in C and in N (Figure 30a). Higher values were observed in C in the middle part of the canopy until mid-August, then similar trends were recoded (Figure 30b). As already observed in Baigent Brookfield®, highest PAR values were always recorded with the sensor arranged in N but outside the net coverage that recorded the part of light reflected from the net (Figure 30b). In August, more consistent values were observed in N compared to C in the basal part of the canopy (Figure 30c).

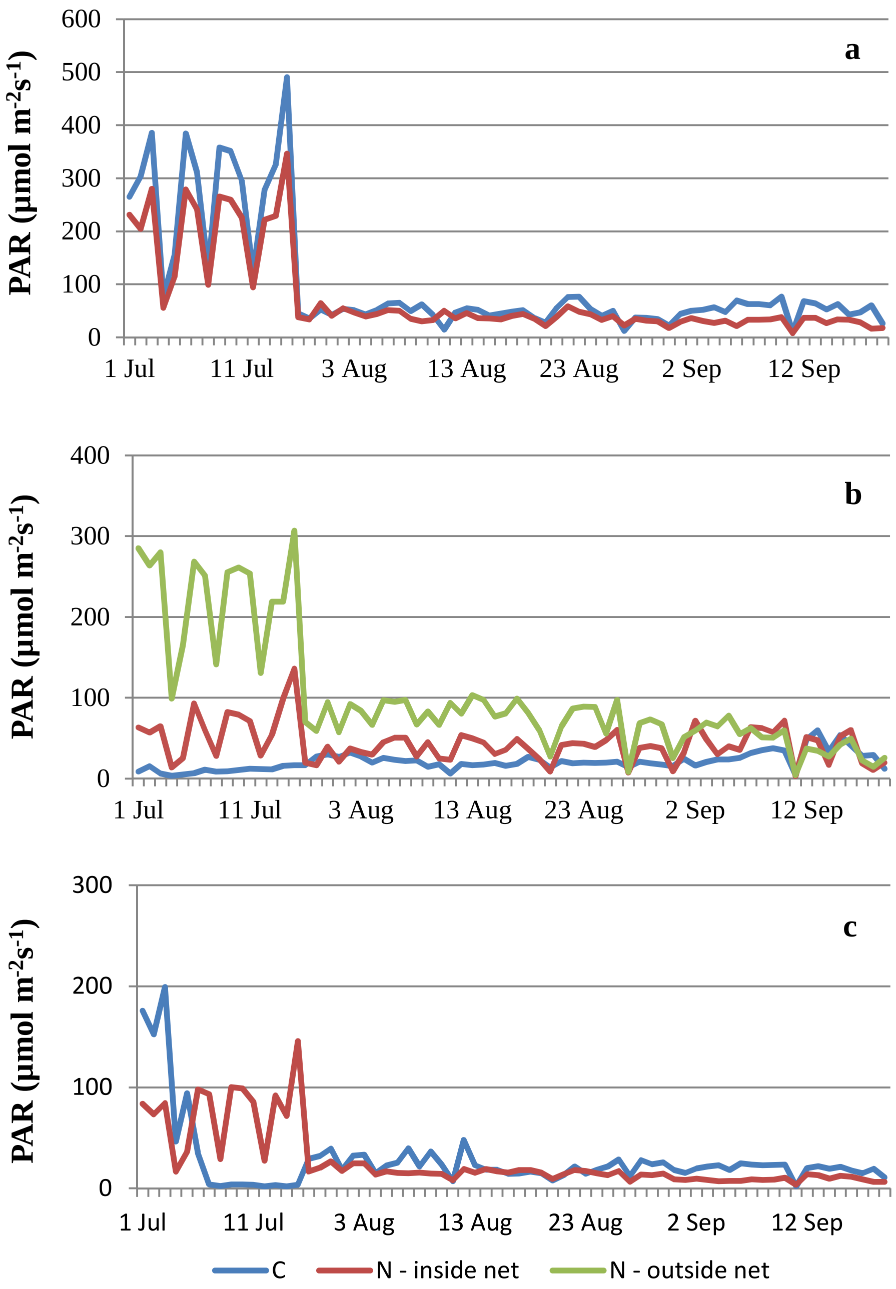


Figure 29. Daily mean PAR recorded in the high (a), middle (b) and basal (c) part of the canopy in Baigent Brookfield® in treatments C (un-netted control trees) and N (netted trees) in 2016.

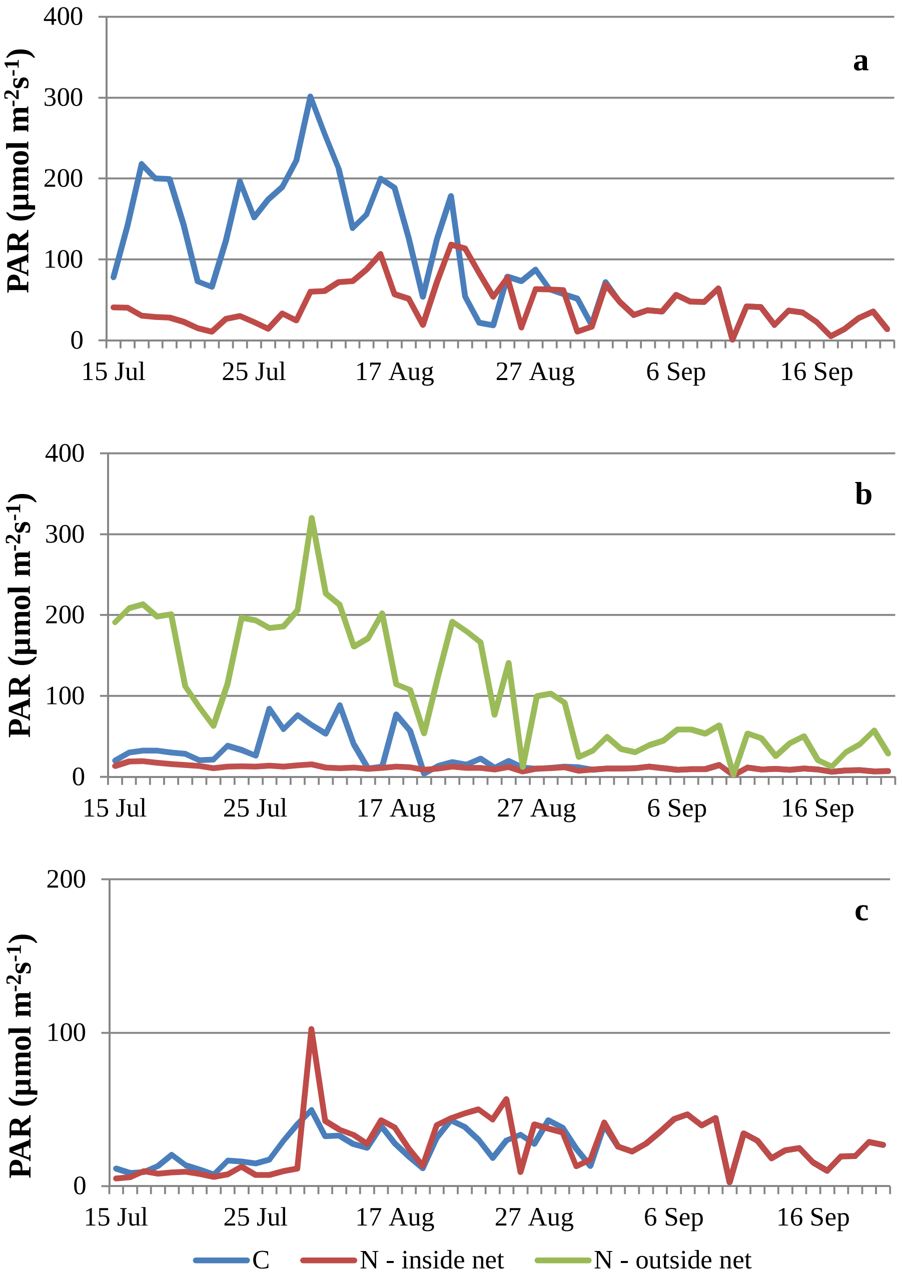


Figure 30. Daily mean PAR recorded in the high (a), middle (b) and basal (c) part of the canopy in Galaval* in treatments C (un-netted control trees) and N (netted trees) in 2016.

3.3.2.2.6 Fruit quality

No statistical differences between the treatments were observed in both the cultivars for the colour index, the firmness and the total solid soluble in 2016 (Table 31).

Table 31. Colour index, firmness and total solid soluble (mean±SE) of the two picking dates in 2016 for apple orchards. No significant differences were found by ANOVA.

Cultivar	**Treatment**	**Colour index**	**Firmness ($g\ cm^{-2}$)**	**Total solid soluble (°Brix)**
Baigent Brookfield®	N	39.09±0.66	7.45±0.06	13.85±0.09
	C	40.01±0.93	7.58±0.06	13.45±0.09
	I	49.79±1.00	7.51±0.07	14.00±0.08
Galaval*	N	44.46±1.15	7.99±0.08	12.89±0.09
	C	50.06±0.96	7.72±0.09	13.59±0.08
	I	54.25±1.26	8.15±0.40	13.03±0.09

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides

In 2016, significant differences between the treatments were observed for the total phenols in the peel (one-way ANOVA: df=2, 51; F=3.51, P=0.037) in Baigent Brookfield®, and in the pulp in Galaval* (one-way ANOVA: df=2, 51; F=3.32, P=0.044), with higher values in N and C, respectively (Table 32). Moreover, in Baigent Brookfield®, statistical differences between the treatments were recorded for the total anthocyanins in the pulp (one-way ANOVA: df=2, 51; F=9.50, P=0.010) with higher values in C (Table 32).

Table 32. Total polyphenols and total anthocyanins (mean±SE) of the two picking dates in 2016 for apple orchards. In column, means followed by different letters are significantly different (Tukey's test, P<0.05).

Cultivar	**Treatment**	**Total polyphenols (mg_{GAE} $100g^{-1}$)**		**Total anthocyanins (mg_{C3G} $100g^{-1}$)**	
		Pulp	**Peel**	**Pulp**	**Peel**
Baigent Brookfield®	N	0.00±0.00	61.72±2.22 **a**	1.15±0.16	32.96±0.80 **b**
	C	0.00±0.00	44.91±0.48 **b**	1.17±0.33	58.02±2.96 **a**
	I	0.00±0.00	39.14±2.74 **b**	2.10±0.44	32.84±1.63 **b**
Galaval*	N	13.99±4.29 **a**	28.51±2.99	2.71±41.8	19.28±1.71
	C	9.16±2.48 **ab**	36.98±3.23	5.94±1.83	17.47±1.70
	I	0.00±0.00 **b**	25.11±2.22	1.56±0.43	18.09±1.68

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides

During the second year of the semi-field trials, statistical differences between the treatments were observed in Baigent Brookfield® for the firmness (one-way ANOVA: df=2, 177; F=10.55, P=0.000) while in Galaval* significant differences were recorded for the total solid soluble (one-way ANOVA: df=2, 177; F=17.51, P=0.000) with lower values observed in N (Table 33).

In 2017, no significant differences between the treatments were observed in both the cultivars for the total polyphenols and the total anthocyanins. In general, a low total anthocyanins content was observed both in Baigent Brookfield® and Galaval* in all the treatments while higer values of total phenols in the peel were recorded in N in both the cultivars (Table 34).

Table 33. Colour index, firmness and total solid soluble (mean±SE) of the two picking dates in 2017 for apple orchards. In column, means followed by different letters are significantly different (Tukey's test, P<0.05).

Cultivar	**Treatment**	**Colour index**	**Firmness ($g\ cm^{-2}$)**	**Total solid soluble (°Brix)**
Baigent Brookfield®	N	38.77±1.08	8.00±0.08 **b**	11.99±0.10
	C	41.11±1.10	8.55±0.09 **a**	12.34±0.14
	I	40.23±1.15	8.39±0.09 **a**	12.29±0.11
Galaval*	N	60.23±1.94	8.82±0.09	11.60±0.13 **b**
	C	60.29±2.01	9.08±0.11	12.52±0.15 **a**
	I	55.74±1.63	8.98±0.12	12.63±0.13 **a**

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides

Table 34. Total polyphenols and total anthocyanins (mean±SE) of the two picking dates in 2017 for apple orchards. No significant differences were found by ANOVA (Baigent Brookfield®: total polyphenols in the pulp and in the peel, total anthocyanins in the peel; Galaval*: total polyphenols in the pulp and in the peel, total anthocyanins in the pulp) and by Kruskal-Wallis test. (Baigent Brookfield®: total anthocyanins in the pulp; Galaval* total anthocyanins in the peel).

Cultivar	**Treatment**	**Total polyphenols ($mg_{GAE}\ 100g^{-1}$)**		**Total anthocyanins ($mg_{C3G}\ 100g^{-1}$)**	
		Pulp	**Peel**	**Pulp**	**Peel**
Baigent Brookfield®	N	28.13±4.36	344.93±12.34	6.29±9.87	0.00±0.00
	C	19.75±3.42	283.95±51.40	0.00±0.00	0.00±0.00
	I	29.74±2.21	392.66±13.78	0.00±0.00	0.00±0.00
Galaval*	N	17.02±2.05	92.26±5.84	0.24±0.17	0.00±0.00
	C	9.20±1.35	72.50±9.84	0.24±0.17	12.30±8.39
	I	9.57±2.57	46.22±6.31	1.29±1.29	18.93±18.3

Treatment: N = netted trees, C = un-netted control trees, I = trees without net but treated with insecticides

3.4 DISCUSSION

The increasing attention for the food safety, the environmental protection and the increasing problems related to the occurrence of resistant pests as well as the introduction of exotic pests require the implementation of new methods for the integrated pest management. In this context, innovative efficient and sustainable control strategies are required in order to preserve the orchard production and high fruit quality.

During the first year of the trials, nets with different colours and meshes were used to identify the most promising anti-insect net for the Piedmont (NW Italy) orchards. The presence of pests was significantly reduced under the nets even though, in some case, a high damage was detected under net. In particular, a high damage on shoots was observed under pearl net but it was due to the contact between the net and the plant vegetation that allowed the oviposition by the pest. No statistical differences as regards the damage on the fruits at the harvest time between the treatments were observed due to the small number of fruits harvested in the peach orchard and to the very low tortrix moth population in the apple orchard that was not such as to cause high damage at the production.

The anti-drosophilid net proved to be the most effective barrier for the protection of the crops. However, this net is very heavy and it requires a more robust supporting structure than the one usually used for the simple anti-hail protection in order to avoid a “sail effect” caused by the wind. Moreover, considering that in apple and peach orchards the usually cultivation practices would required frequent openings of the exclusion net and that *D. suzukii* is not yet a worrisome pest (at least in the investigated area), the choice has fallen upon the photoselective anti-hail nets. Furthermore, taking into account also the fruit quality and the incidence of pathogenic fungi (data retrieved as part of the LIFE+ SU.SA.FRUIT project), the best overall results were obtained with the pearl net.

Interesting observations on the impact that the net colour and its mechanical action may have on other pests and on beneficial arthropods were recorded. It has been reported that, using the photoselective nets, the optical disruption caused by the reflected light may interfere with distant host finding by the pests. The light inside the net contains less UV light and therefore becomes “invisible” to the pests or higher levels of reflected/scattered sunlight could deter the pests landing (Ben-Yakir *et al*., 2008). Aphids seem not to be influenced by the photoselective net while whitefly and thrips capacity to reach the target plants is reduced by photoselective nets. It appears that these pests remain on their preferred colored nets for a long time (‘rejection flight’), and as a result they are less likely to infest the plants underneath (Ben-Yakir *et al*., 2008). Photoselective nets may have the same influence on beneficial insects.

During prototype trials, yellow and red nets showed to have a greater insect attractiveness not only under the net. In particular, an increase of the beneficial insects was observed under the yellow net as already shown by Shahak *et al*. (2009) but also on the external net surface. On the contrary, the red net seemed to be more attractive for tortrix moths. The influence of the exclusion nets on aphid populations is not yet clear but it seems to be a species-specific response (Dib *et al*. 2010; Chouinard *et al*., 2016). Few specimens of *A. pomi* and *D. plantaginea* were observed in the apple orchard during the trials, but no strong infestations have ever been detected under the nets.

The net is thin enough to keep out the target pests, but at the same time large enough to allow most beneficial insects to pass through. Indeed, abundant catches were obtained with yellow sticky traps under the photoselective nets as well as, at the end of the harvest time, for the cluster *predators*, with the knock-down treatment. Moreover, the color of the photoselective nets does not seem to have influenced the abundance of *total catches* and *predators*.

In insects, temperature plays an important role on the growth rate and the pre-adult development (Honek and Kocourek, 1990, Tochen *et al*., 2014). In the prototype trials, temperature and relative humidity differences inside and outside the nets were slight, therefore they did not influence pest development. More differences between the netted and the un-netted control trees as well as among the nets were observed for the PAR. The pearl net mostly enhance the scattering in the UV indeed was the only net under which PAR values similar to the un-netted control trees were achieved. Even though the anti-drosophilid net is not a photoselective net, similar PAR values were recorded under this net compared to yellow and red photoselective net. It is well known that PAR directly influence fruit quality (Shahak *et al*., 2004a and 2004b) and nutraceutical compounds (Zoratti *et al*., 2015). Accordingly, in the prototype trials, fruits harvested under pearl net were the once with the highest quality. Considering the entomological and pathological aspects as well as the fruit quality results, the pearl net resulted the most promising net in Piedmont orchards and was used for the semi-field trials.

The results obtained during the semi-field trials with the pearl photoselective net confirmed its effectiveness in the protection of the orchards against key pests and *H. halys*. Furthermore, the net proved not to negatively affect the arthropod fauna present in the orchards as well as the quality of fruit production. In parallel, the net has an impact on Drosophilidae populations and secondary pests (aphids and *S. myopaeformis*).

Even if the effectiveness of the exclusion nets against *G. molesta* and *S. myopaeformis* has not been so long investigated, it is reasonable to suppose that their impact on these pests is always due to the physical barrier proprieties of the net and on its interference on the pests biology. The anti-hail photoselective pearl net proved to be effective in reducing the pest populations and their damage on fruits. The net was able to contain tortrix moths and *S. myopaeformis* populations. The moth catches with the pheromone traps were very low under nets and significantly more abundant outside nets. As a consequence, in the nectarine orchards, under the exclusion nets a damage 8-fold lower than the one observed in I (i.e. trees without net but treated with insecticides) was recorded.

Even though *D. suzukii* does not represent a key pest in nectarine and apple orchards, the efficacy of the net against this pest was evaluated. Different researches proved that only nets with a mesh thinner than 1 mm^2 are effective in excluding this pest (Kawase *et al.*, 2008; Cormier *et* *al*. 2015). Indeed, during the selection of the most promising net, the anti-drosophilid net proved to be the best net in containing the pest population as also demonstrated by Firlej *et al*. (2014) in experimental tests conducted in blueberry in Canada and by Rogers *et al*. (2016) on raspberry. However, in the semi-field trials, where only the pearl net (2.4×4.8 mm) was used, satisfactory results in reducing *D. suzukii* populations, and generally the Drosophilidae abundance, were recorded under the nets, both in nectarine and apple orchards. This is probably due more to the optical proprieties of the photoselective net than to its mechanical activity.

In Italy, a great concern for fruit crops certainly increases with the introduction of the new exotic pest *H. halys*. The initial absence of reliable monitoring tools and the low effectiveness of chemical control have led *H. halys* becoming a serious pest in fruit crops in northern Italy, where it has found favourable conditions for its establishment. In particular, in Piedmont, *H. halys* has a great potential in reaching high infestation levels. The orchards chosen for this study are located in an area in which the presence of *H. halys* was already reported. During the prototype trials, the pest was never detected but it was observed in bordering crops or later in the season. In the orchards chosen for the semi-field trials, the presence of *H. halys* considerably increased in 2016 and even more in 2017 although in an uneven pattern. Therefore, particular attention was given to this insect during the trials.

Halyomorpha halys was recorded in all the experimental orchards in 2016 and only in apple orchards in 2017, but its population density was very variable depending on the season and on the crop. In the four orchards in 2016, *H. halys* was never trapped at the beginning of the growing season but only close to harvest time while in 2017 the pest was recorded only in the apple orchards earlier in the growing season and with higher catches. The net always avoided the entry of the adults while only two nymphs were collected under net demonstrating the ability of the net to effectively block the entrance of the pest. Short *et al.* (2017) showed how insecticide applications at a cumulative threshold of 10 adults of *H. halys* collected in a pheromone trap in apple orchard were effective at reducing fruit injury. This threshold was exceeded only from August in 2016 while in 2017 it was exceeded from the end of June.

Overall, comparing the results obtained with the different monitoring techniques and the fruit damage at the harvest time, it is evident how the catches by pheromone traps did not always reflect the real abundance of the pest in the field, probably due to various reasons. It was already highlighted by AgBio (http://www.agbio-inc.com/) that overwintered adults emerging in the spring do not respond to the lure (Morrison *et al*., 2015). Probably, upon emergence from overwintering sites, being in a dispersal phase searching for food sources, *H. halys* is more attracted by kairomones emitted by plants compared to the lures used in traps. On the contrary, consistently higher captures were recorded in apple orchards in late summer, a period in which decreasing day length and temperature trigger *H. halys* an aggregation behaviour before moving to overwintering sites (Lee *et al*., 2013). Moreover, studies on genetic diversity of Italian populations revealed the presence of various *H. halys* haplotypes in Piedmont (Cesari *et al*., 2018), which could have a different response (less attractiveness) to the lure, for example at the beginning of the season.

The uneven *H. halys* population density in the orchards as assessed by damage rate at the harvest time is also due to a different attractiveness of the various crops. Despite its high polyphagy, *H. halys* can be considered a fruit specialist, seeking and moving among trees that differentially bear fruit in space and time (Martinson *et al*., 2015). It is evident that *H. halys* firstly moves to other temporary hosts, which may be used as a water source (Lee *et al*., 2013), before colonizing crops. As a consequence, the first damaged fruits were observed close to the beginning of the harvest time in all the orchards. Then, among the different crops, *H. halys* has a preference for peaches as a favourite host. This behaviour seems to be due to the fact that peach is the only crop able to support the development of the pest from the end of May until the harvest time (Blaauw *et al*., 2016).

Besides the different number of specimens captured inside and outside the net by the traps, the best evidence for the effectiveness of the nets against *H. halys* comes from the assessment of damaged fruits in the treatments in comparison. Indeed, under the exclusion nets, a damage 4-fold lower and 2-fold lower than the one observed in insecticide treated trees was recorded in nectarine and apple orchards, respectively. In particular, in nectarine orchards the number of damaged fruits was always significantly lower inside than outside nets even when trees were regularly treated with insecticides by the growers (I). Only in Fire Top® in 2017, a higher damage (up to 38.5%) compared with the one recorded in the previous year, was observed under the net. This was probably due to various reasons: a higher pressure of the pest in the field not recorded with the pheromone traps and the contact of the net with the vegetation, due to the great vegetative growth rate of the cultivar, may have influenced the mechanical barrier action of the net. Specific insecticidal treatments against *H. halys* were applied only in 2017 when few products were registered for the first time against this pest in Italy. However, the pyrethroid-like (etofenprox) and the insecticide classes of organophosphates (chlorpyriphos and chlorphyriphos methyl) have been shown to be effective (Leskey *et al*., 2014; Blaauw *et al*., 2015, 2016). In Piedmont (NW Italy), the current European, National and Regional directives place severe restrictions on the use of chemical products for crop protection, limiting the number of the allowed treatments in order to reduce residues in food. Blaauw *et al*. (2015, 2016) agree that the short residual activity of many compounds makes necessary to repeat the treatments every 7-10 days, but this, beyond being not always applicable in our region, would nullify the integrated pest management principles now largely adopted in fruit orchards. This study showed that the exclusion nets can be more effective than chemical treatments in containing *H. halys* damage; thus, considering also the phytosanitary directives, the net coverage can be a great-value alternative for the management of *H. halys*.

In apple orchards, the differences between the treatments are less marked than in the peach orchards, probably due to the low pest density before the harvest time. The lower number of injured apples compared to nectarines could also be due to a lower level of expression of the damage. It is proved that *H. halys* feeding on apples during the last 1-2 weeks before the harvest time may not produce symptoms; however, apples showing no surface injury at harvest time may develop both surface and internal injuries following a period of post-harvest cold storage (Bergh *et al.*, 2016). It should also be taken into account that these trials were carried out on early ripening apple cultivars, and that probably late ripening apple cultivars may be subjected to a higher pressure by this pest.

Statistical differences for the block effect were recorded only in Amiga* in 2017 and in Baigent Brookfield® in 2017, but in general the damage was higher on netted and un-netted trees closer to the edges, and mainly when the orchard borders on other peach orchards, soybean or alfalfa fields. In particular, in Galaval*, the damage was higher on netted and un-netted apples close to the edge bordering a peach orchard, while the nectarines were more damaged in the edge bordering soybean or alfalfa. Leskey *et al.* (2012) already found that *H. halys* is a perimeter-driven threat. In their research, injury was usually significantly greater at the exterior of orchard relative to the interior, suggesting an adult emigration from overwintering sites in the early season and from wood lots or cultivated hosts later in the season.

The influence of the exclusion nets on aphid populations is not yet clear and contrasting results were obtained by different authors monitoring different aphid species (Dib *et al.*, 2010; Chouinard *et al.*, 2016). In nectarine orchards, aphids were never observed during the semi-field trials while in apple orchards colonies of aphids occurred. Generally, a low number of infested shoots was observed under net except in Baigent

Brookfield® in 2016 where, according with Chouinard *et al*. (2016), colonies were mainly composed by *E. lanigerum*. In complete exclusion systems, Marliac *et al*. (2013) reported side-effects of codling moth exclusion netting on Miridae, Anthocoridae, Syrphidae and Coccinellidae, natural predators of the rosy apple aphid *D. plantaginea*. Similarly Dib *et al*. (2010) and Romet *et al*. (2010) reported a lower abundance of Syrphidae and Coccinellidae under apple netted plots than in un-covered ones. The possible effect of the nets on the arthropod fauna, in particular predators, was evaluated all along the trials with yellow sticky traps and with a final knock-down treatment after the harvest time. According with Dib *et al*. (2010) and Romet *et al*. (2010), a significant lower number of predators was collected under net in all the orchards during the growing seasons. Generally, the net mesh was large enough to allow tiny beneficial insects to pass through [mainly *Stetorus* spp. (Coccinellidae), Anthocoridae and Staphylinidae]. Larger size insects such as Hemerobiidae, Chrysopidae and Syrphidae were collected only in the first weeks after the net setting up probably following the hatching of eggs laid on the plants covered with the net before the net installation.

Different results were obtained with the final knock-down treatments showing how the presence of the net did not negatively influence the abundance of the total catches and predators, although the net coverage caused a reduction in *H. halys* population. Only in Amiga*, the number of *predators* was significantly lower under the net. These contrasting results are probably mainly due to different aspects such as the timing of the survey (i.e. yellow sticky traps were used during the growing season;

the knock-down treatment was applied after the harvest time) and the optical characteristics of the net. Indeed, studies on the abundance of *Psyttalia concolor* (Szèpl.) (Hymenoptera, Braconidae) and *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae) populations under photoselective nets showed how these insects are not very attracted to yellow sticky traps (Velázquez *et al*., 2012). That may suggest that the disruption of visual behaviours caused by photoselective nets, could interfere with this monitoring technique. Anyway, the pearl net used in the current research is thin enough to keep out the most worrisome pests, but at the same time large enough to allow most beneficial insects to pass through.

The mesh size is a very critical issue not only for the exclusion effectiveness, but also for the consequences on the pest biology and the microclimate occurring under the net. Changes to the orchard microclimate are significantly greater where nets with small mesh size are used. Kuhrt *et al*. (2006) showed how a reduction of less than 1°C in air temperature within the canopy caused by some net coverages is negligible for the development of codling moth. During the semi-field trials, temperature and relative humidity trends were almost identical outside and inside net without particular consequences on pests biology due to the net. Despite the expectations, the pearl net does not seem to enhance the development of pathogens (Davide Spadaro, DISAFA; personal communication). Actually, apples coming from the trials and subjected to cold storage after the harvest time revealed interesting preliminary results on the effect of the nets on physiological disorders such as bitter pit. For both cultivars, and in particular for Galaval*, the nets reduced the incidence of bitter pit (Candian *et al.*, 2018) as already observed by do Amarante *et al.* (2011) supposing that a lower leaf transpiration under the nets might increase the xylem transport of calcium to the fruits in detriment to the shoots, therefore reducing bitter pit. Moreover, no differences between the treatments were found as regards the incidence of apple scab and brown rot (Candian *et al.*, 2018).

Significant differences between the treatments emerged for the PAR. Even though photosensitive nets cause a shading of about 25-30% of the light in the PAR region, they transform the direct light into diffused light promoting the penetration of light into the foliage, increasing the PAR and therefore the photosynthetic efficiency, thus influencing the quality of the fruits (Shahak *et al.*, 2004a; Rajapakse and Shahak 2007; Shahak *et al.*, 2009; Basile *et al.*, 2012; Bravetti *et al.*, 2012). In particular, the pearl net is the one that mostly enhanced the scattering in the UV.

During the trials, a fluctuating PAR trend was observed outside the net while an almost constant luminous volume was created under the net. Therefore, the photoselective net allows to maintain a luminous volume over the season and in all the tree canopy. Crops grown under pearl photoselective nets allow the plant to perceive a 15-20% more light compared to other nets when grown in absence or low presence of light (Shahak, 2014). Apples grown under higher PAR values have major nutraceutical content in comparison to others in which the light is significantly lower (Baiamonte *et al.*, 2016). Overcolour of peaches and nectarines is strongly influenced by the availability of light (Lewallen and Marini, 2003). Moreover, the interaction of solar radiation with photoselective nets and the net braking action on the winds exert a significant influence on the microclimatic conditions present in the orchard that influence fruit quality (Shahak *et al.*, 2004a and 2004b).

During the semi-field trials, fruit quality was not negatively influenced by the net coverage; actually, in some cases, the pearl photoselective net was able to enhance the nutraceutical properties. A greater source of total polyphenol compounds in the peel of the Baigent Brookfield® apples grown under net (N) compared to the un-netted treatments (C and I) may have therapeutic value (Scalbert *et al.*, 2005; Almeida *et al.*, 2008; Mileo and Miccadei, 2015; Zhang and Tsao, 2016).

In terms of costs, in all the cases in which an anti-hail net system is already present in the orchard, a single-plot exclusion-net system is more feasible, entailing a 2,300 €/ha cost increase depr eciable in 15 years. This strategy allows to save approximately 280 € ha^{-1} per year compared with chemical control against *C. pomonella* in areas with a high moth pressure (Pavarino and Vittone, 2014). All the more reasons, this saving will be even higher considering the cost of chemical control against *H. halys*. In the semi-field trials, the exclusion nets allowed to perform 7 insecticide treatments less than in I in each orchard.

Naturally, it is necessary to ensure the uniformity of the closure of the anti-hail net on the top to prevent any entrance of the pest. As a consequence, an easy opening system and a sufficient space for the entry and the manoeuvre of the machineries should be provided. By contrast, in orchards without an anti-hail net coverage the single row strategy could be economically more advantageous. However, in this case, farming operations such as pruning and harvesting will be harder, whereas fungicide treatments will be easier because of their application through the net.

3.5 CONCLUSION

In Piedmont (NW Italy), the anti-hail photoselective pearl net proved to be a good exclusion system that can prevent more than one pest species at a time allowing the reduction of costs associated with insecticide use. The use of the exclusion nets opens up new opportunities as a “ready to use” tool against other worrisome emerging pests, such as the highly polyphagous *Popillia japonica* Newman (Coleoptera: Rutelidae) recently reported in northern Italy (Pavesi, 2014). Moreover, positive effects of photoselective nets on the fruit quality were observed as already reported by several authors (Shahak *et al*., 2004; Retamales *et al*., 2008; Basile *et al*., 2012). Although in areas of landscape value, exclusion nets may have a strong visual impact, in highly specialized fruit-growing areas, already equipped with anti-hail systems and subjected to a high pressure of the phytophagous, they can be a great resource and an “environment-friendly” strategy.

4 USE OF ENTOMOPATHOGENIC FUNGI TO STUDY FUNGUS-INSECT INTERACTION, THE CASE OF *ISARIA FUMOSOROSEA-DIAPHORINA CITRI* IN CITRUS

4.1 INTRODUCTION

4.1.1 Entomopathogenic fungi as an alternative to chemical pesticides

Entomopathogens are important natural enemies of many insect and mite species and as such have been recognized as providing an important ecosystem service. Indeed, entomopathogenic fungi have been widely investigated as biological control agents of pest insects in attempts to improve the sustainability of crop protection (Roy *et al*., 2010). Although this potential was first explored from the late nineteenth century onwards, interest in microbial control declined with the introduction of synthetic chemical insecticides in the 1940s and 1950s (Charnley, 1991). Compared with chemical insecticides, microbial control offers a number of advantages.

Environmental benefits include increased safety for humans, reduced contamination of food, soil and groundwater. Moreover, the high selectivity of microbial control agents results in increased biodiversity and activity of the beneficial arthropods. Further advantages include limited development of host resistance and the compatibility with other biocontrol agents. However, high selectivity may result in the need for additional control measures if more than one major pest is present and the limited persistence can also become an issue for successful control (Lacey *et al*., 2001; Lydia *et al*., 2017).

Despite there being an estimated 700 species of entomopathogenic fungi in approximately 90 genera (Roberts and Humber, 1981), most of the commercially produced fungi are species of *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* thanks to their relatively easy mass production. Entomopathogenic fungi infect their insect hosts by penetrating through the cuticle or through body openings. Thanks to this mode of action, they are capable of infecting non-feeding stages such as eggs and pupae and sap-feeding hemipteran species (Payne *et al*., 1988). They have evolved specialized mechanisms for the enzymatic degradation of the integument and for overcoming insect defense compounds. The relationships by which different fungal species obtain energy from their insect hosts include biotrophy (nutrition derived only from living cells, which ceases once the cell has died), necrotrophy (killing and utilization of dead tissues), and hemibiotrophy (initially biotrophic and then becoming necrotrophic) (Vega *et al*., 2009).

Studies describe that insects killed by entomopathogenic fungi often take longer to die than if treated with chemical pesticides but the damage to crops is decreased during the disease incubation period. Indeed, researches on pathogen-induced and host-mediated behavioral changes demonstrates the range of altered behaviors exhibited by invertebrates including behaviorally induced fever, elevation seeking, reduced or increased activity, reduced response to semiochemicals and changes in reproductive behavior (Roy *et al*., 2006).

The virulence of entomopathogenic fungi involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity. Moreover, entomopathogenic fungi have life cycles that are synchronized with insect host stages and environmental conditions (Shahid *et al*., 2012). Entomopathogenic fungi produce extra-cellular lipases, chitinases and proteases which help them to overcome the insect cuticle as the first barrier of infection. Once inside the insect, the fungus develops as a yeast-like form and produces secondary metabolites that inhibit the insect immune system, modify the insect behavior, or act as post-mortem antibiotics against competing microorganisms like other fungi or bacteria. Hundreds of small molecules with insecticidal activity and probably other biological functions such as destruxins, beauvericin, oosporein, bassianolide, cordycepin, and beauverolides have been identified from entomopathogenic fungi.

After reaching the host hemocoel, the fungal cells modify their wall structure in response to hemocyte recognition, encapsulation and melanizations (Holder and Keyhani, 2005; Lewis *et al*., 2009; Wanchoo *et al*., 2009). Moreover, entomopathogenic fungi secrete effector proteins and secondary metabolites to evade the host immunity by counteracting host receptors (Wang and Wang, 2017). It has been described that entomopathogenic fungal genomes such as *Beauveria bassiana* (Bbas) (Xiao *et al*., 2012), *Metarhizium anisopliae* (Manis) (Hu *et al*., 2014) and *Isaria fumosorosea* Wize (Shang *et al*., 2016), encode for a repertoire of extracellular (apoplastic) with pathogenicity-related effector proteins such chitin-binding proteases, cell wall degrading enzymes and others small-secreted proteins associated that function in gene-for-gene relationship in fungus-insect interactions and thereby contribute to fungal virulence (Shang *et al*., 2016; Wang and Wang, 2017).

4.1.2 Pathogenicity-related effector proteins from entomopathogenic fungi

Secreted proteins and in particular the effectors, are key factors involved in pathogen-host interactions. Fungal effector is use to define any secreted molecule that modulates the interaction between the fungus and its host (Lo Presti *et al*., 2015) (Figure 1).

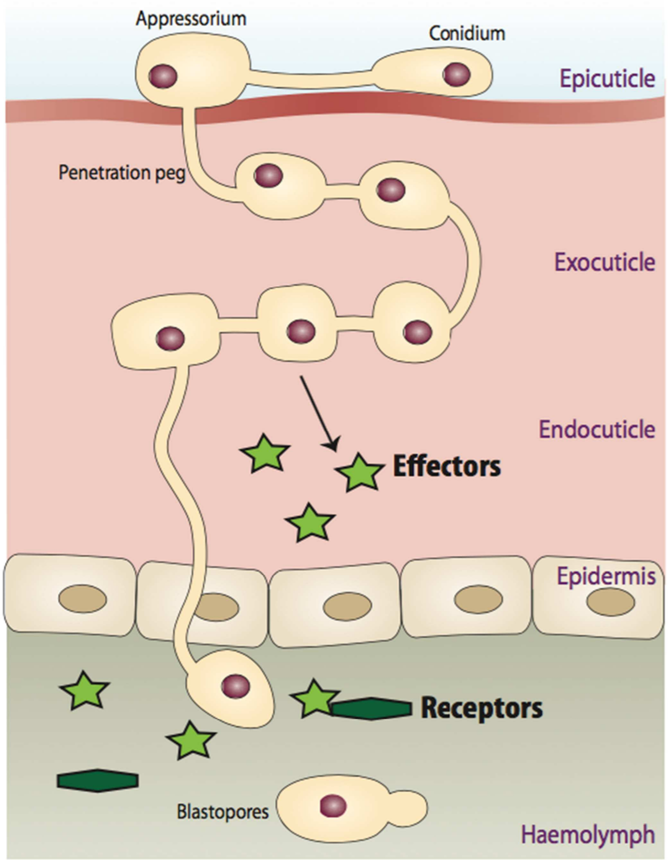


Figure 1. Infection process by fungal entomopathogens. The *in vivo* development cycle of entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae*, involves sequential steps. First, conidia (spores) adhere to the host cuticle, then the conidia germinate and the germ tube and appressorium (penetration structure) are produced. The cuticle is penetrated by a combination of mechanical pressure and the action of cuticle- degrading enzymes. The fungus grows by vegetative growth in the host haemocoel and external conidia are produced upon the death of the host. The host cuticle is the first line of defence against infection and has a central role in determining fungal specificity. If the fungus breaches the cuticle, successful infection can only result if the fungus can overcome the innate immune response of the insect. Insects respond in both a cellular and humoral manner to fungal infection, with immune activation occurring as early as the point of cuticle degradation during the penetration step. Fungal effectors proteins are secreted in the endocuticle and haemolymph (green starts). Fungal effectors are recognized by receptors in the host in the haemolymph. Fungi have two main strategies for overcoming host defence responses; development of cryptic growth forms that are effectively masked from the insect defence responses, and production of immunomodulating substances that suppress the host defence system.
Modified by Thomas and Read, 2007.

All fungi that colonize hosts are recognized by the immune system and elicit host defenses. These initial defense responses are triggered by invariant molecular patterns exposed by the microbe, referred to as pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs). In fungi, the cell-wall component chitin functions as such a PAMP. After fungi contact, chitin oligomers are released from the fungal cell wall through plant chitinases. PAMPs are recognized through membrane localized pattern recognition receptors (PRRs), which trigger a first line of defense reactions called PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). Moreover, PRR signaling can also be triggered by host-derived damage-associated molecular patterns (DAMPs).

The activation of PRR signaling results in rapid responses that include the accumulation different molecules (reactive oxygen intermediates; activation of ion channels; activation of specific defense-related mitogen) collectively leading to an accumulation of antimicrobial compounds. For example, in plant cells, after PRR activation, changes in hormone biosynthesis occur and cell-walls are reinforced by callose deposition (Lo Presti *et al.*, 2015).

Usually PTI is considered to be effective against non-adapted indeed, to establish a compatible interaction leading to proliferation, fungi must avoid eliciting PTI or either cope with or suppress it. For this purpose, the fungus must inactivate toxic metabolites or secrete effectors, which may be either toxic secondary metabolites or proteins that kill the host. Alternatively, effectors can be secreted proteins that shield the fungus, suppress the host immune response, or manipulate host cell physiology.

The identification and functional elucidation of effectors in plant pathogens help establish the gene-for-gene relationships in pathogen-host interactions (Stergiopoulos and de Wit, 2009). Protein effectors are most often secreted via the conventional endoplasmic reticulum and Golgi apparatus route. For this reason, they must contain an N-terminal secretion signal (Lo Presti *et al*., 2015). Effector candidates can thus be identified bioinformatically by the presence of this signal. In general, effectors are expressed only after contact with the host. Their expression profile is tightly tuned to the different infection stages and may be affected by the cell type and/or organ being infected (Okmen and Doehlemann, 2014).

Nowadays, the gene-for-gene relationships in pathogen-host relations has not been as well determined in animal-pathogen interactions but is known in plant-pathogen and in some insect-plant interactions (Hogenhout *et al*., 2009; Kamoun, 2007; Bos *et al*., 2010).

Fungal effectors usually evolve quickly: they are highly divergent or even originate at a species-specific level (Wang and Wang, 2017). However, common features have been found for these effector proteins, which are usually Small Secreted Cysteine-rich Proteins (SSCPs) (less than 300 amino acids in length) (Stergiopoulos and de Wit, 2009). On the basis of this algorithm, a plethora of SSCP-type effectors (345 proteins on average) were identified in genome of entomopathogenic fungi (Shang *et al*., 2016), suggesting the presence of a model of interaction similar to that of plant pathogens.

4.1.3 Application of entomopathogenic fungi in orchard

Although entomopathogenic fungi are well known as biological control agent on herbaceous crops; less applications, fragmented information and mostly laboratory experiment are recorded on orchard crops. On cherry tree, the entomopathogenic fungi effectiveness was evaluated on *Ceratitis capitata* (Wiedemann), *Rhagoletis indifferens* Curran and *R. cerasi* Wiesmann (1933) (Diptera: Tephritidae). *Metarhizium anisopliae* and *B. bassiana* demonstrate their effectiveness on *C. capitata* (Mochi *et al*., 2006; Castillo *et al*., 2000; Dimbi *et al*., 2003) while *R. indifferens* is susceptible only to *M. anisopliae* (Yee and Lacey, 2005). Moreover, effects of six fungus isolates on the mortality of different life stages of *R. cerasi* were recently assessed in laboratory conditions. *Beauveria bassiana* and *I. fumosorosea* caused 90–100% mortality and had the strongest influence on fecundity (Daniel and Wyss, 2009).

For the first time, on peach and almond trees in Tunisia, *B. bassiana* and *Metacordyceps liangshanensis* (Ascomycota: Hypocreales, Clavicipitaceae) were reported infecting *Pterochloroides persicae* (Cholodkovsky, 1899) (Hemiptera, Aphididae) populations (Mdellel *et al*., 2015). The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is a major pest of stone and pome fruits in North America shown to be susceptible to *Beauveria* spp. and *M. anisopliae* in laboratory experiments (Alston *et al*., 2005).

On citrus crops, *Hirsutella thompsonii* Fisher has received considerable attention for the containment of the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (McCoy and Couch, 1982). Moreover, *B. bassiana* has shown promise for control of *P. oleivora* and other citrus mites

(Alves *et al*., 2005; Shi and Feng, 2006) and for the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Poprawski *et al*., 1999). *Beauveria bassiana* strain GAr 17 B3 and *M. anisopliae* strain FCM Ar 23 B3 have been identified as effective control agents of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) wandering fifth instars under laboratory conditions (Coombes *et al*., 2016).

4.1.4 The Asian citrus psyllid *Diaphorina citri* and its interaction with *Isaria fumosorosea*

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), has become the most important pest that affects citrus production. This pest is responsible for transmission of three species of alpha-proteobacterium: '*Candidatus* Liberibacter asiaticus' (CLas), '*Ca*. L. africanus' and '*Ca*. L. americanus' (Murray and Schleifer, 1994), the causal agent of citrus greening or huanglongbing (HLB), one of the most destructive diseases of citrus in the United States and world (Garnier *et al*., 2000; Bove 2006; Brlansky and Rogers 2007; Gottwald, 2007; Gottwald *et al*., 2007; Gottwald, 2010; Ammar el *et al*., 2013; D'Alessandro *et al*. 2013; Orduño-Cruz *et al*., 2015). HLB outbreak has high economic costs to worldwide citrus industry, and currently there is not cure for the disease (Hodges and Spreen, 2012).

Diaphorina citri, similar to other hemipterans, has a stylet bundle consisting on a pair of outer mandibular and inner maxillary stylets, piercing, sucking mouthparts (Garzo *et al*., 2012). The psyllids insert their piercing-sucking mouthparts into the plant vascular system (phloem) to feed, thus transmitting the pathogen (Andrade and Hunter, 2017). The ACP is confined to Rutaceae, occurring on wild hosts as well as on citrus crops, especially lemons [*C. limon* (L.) Burm. f.] and limes [*C. aurantiifolia* (Christm.) Swingle]. The preferred host is *Murraya paniculata* (L.) Jack, a rutaceous plant often used for hedges. The pest is widely spread in all the continents except in Europe and its distribution is wider than HLB distribution since it occurs in Afghanistan, Bangladesh, Brazil, Hong Kong, Japan, Macau, Myanmar, Singapore and Sri Lanka where the bacterium has not been recorded (EPPO Global Database). *Diaphorina citri* was identified in many countries Central and North America in the 1990s.

In Florida, it was first reported in 1998 and has since spread throughout the country (Knapp, 1998). HLB disease management tactics relied exclusively on applications of insecticides to control the psyllid and imposed intensive insecticide application for *D. citri* and frequent eradication of diseased citrus plants (Gottwald, 2007; Hall and Gottwald, 2011). Eradication was finally declared infeasible since 2006 after the hurricane seasons in 2004 and 2005 had aided the spread the psyllids throughout all citrus producing areas in Florida (Alvarez *et al*., 2016).

The use of insecticides still represents the most widely used method for the control of *D. citri* in countries such as Brazil, USA and Mexico. It is well know that using chemical insecticides as the main control strategy is not sustainable, and has known negative side effects such as environmental pollution, generating genetic resistance in insect pests, encouragement of secondary pests and reduction of natural enemy populations (Doutt and Smith, 1971; Qureshi and Stansly, 2009). Although insecticides are considered effective at reducing psyllid populations, research indicates that even intensive insecticide programs are ineffective at preventing the spread of HLB. Moreover, the excessive reliance on few insecticides has also resulted in reduced susceptibility of *D. citri* towards neonicotinoids, pyrethroids, organophosphates and carbamates (Avery *et al*., 2013).

One of the most promising alternatives to pesticides to control arthropod pests is the use of biological control using entomopathogenic fungi that can provide an alternative strategy and environment friendly alternatives to chemical insecticides for pest control (Orduño-Cruz *et al*., 2015; Pappas *et al*., 2017). Entomopathogenic fungi play an important role in the regulation of insect populations in nature as well as representative species have been developed as promising environmentally friendly microinsecticides (Wang and Wang, 2017). Nowadays, it is widely accepted the used of biological control entomopathogenic agents in addition to chemical control, in the form of commercial biopesticides for the management of ACP in the field (Hall *et al*., 2012).

For example, several studies (Meyer *et al*., 2008; Orduño-Cruz *et al*., 2015; Patt *et al*., 2015) reported the efficacy of *I. fumosorosea*, which has been isolated from over 40 species of arthropod representing 10 orders (Hussein *et al*., 2016; Dymarska *et al*., 2017), and other different entomological fungal species against *D. citri in vivo* bioassays under controlled conditions (Hunter *et al*., 2011; Avery *et al*., 2013; Galindo-Velasco *et al*., 2015; Patt e*t al*., 2015) and field production systems (Kumar *et al*., 2017). These studies represent a first step towards evaluating the potential use of *I. fumosorosea* in an IPM program to suppress ACP populations not only in the greenhouse but also in the field under natural disease challenge conditions in an HLB endemic region.

4.2 MATERIALS AND METHODS

Trials were carried out at the University of Florida in the Indian River Research and Education Center (IRREC) in Fort Pierce to: 1) evaluate *D. citri* mortality after treatments with *I. fumosorosea* at different concentrations; 2) identify pathogen effector genes from the entomopathogenic fungi *I. fumosorosea* genome involved during the interaction with the psyllids at early days post inoculation (dpi).

4.2.1 Source of insects

Asian citrus psyllid *Diaphorina citri* specimens, infected with '*Candidatus* Liberibacter asiaticus' (CLas), were provided from the Insectary Lab at the USDA-ARS laboratory colony established during early 2000 at the U.S. Horticultural Research Laboratory in Fort Pierce, Florida (FL). Originally collected from *Citrus*, the psyllids have been continuously reared on orange jasmine, *Murraya paniculata* (L.) Jack, isolated in Plexiglas (0.6×0.6×0.6 m) or BugDorm-2® cages [MegaView Science Education Services Co., Ltd, Taichung, Taiwan]. Mass rearings were carried out in environmental chambers under the following controlled conditions: 20±28°C, 40±80% RH and under a photoperiod of 14:10 (L:D). The original colony has not had field collected psyllids added since establishment.

4.2.2 Citrus leaves

Certificated HLB-free sweet oranges, *Citrus sinensis* (L.) Osbeck cv. 'Valencia', were grown inside BugDorm-4® cages (47.5×47.5×93 cm)

[MegaView Science Education Services Co., Ltd, Taichung, Taiwan] to avoid the possible infection by infected *D. citri* (psyllids carrying '*Ca.* Liberibacter asiaticus' bacteria). Plants were regularly pruned every 3-4 weeks in order to ensure the production of young leaves used during the trials.

4.2.3 Preparation of the conidial suspensions

The commercial product PFR-97 20% WDG [Certis USA; Columbia, USA], a formulation with blastopores of *I. fumosorosea* (originally called Apopka 97 strain), was used to isolate the entomopathogenic fungi in potato dextrose agar PDA plates. Conidia are the asexual stage produced from the blastospores germination in PDA plates. The PFR-97 was grown on PDA plates at 25°C for 17 days. After this incubation time, conidial suspensions of the isolate were prepared collecting the conidia from the mycelia grown in the PDA plates using a microbiological loop. The harvested conidial was suspended in 5 mL of a 0.03% Tween 80 solution diluted in sterile distilled water, and placed in a 50 mL sterile centrifuge tube. Suspension was filtered into a new 50 mL centrifuge tube through a sterile cloth to remove any remaining fungal structures (mycelia) from the PDA plates. The conidia concentration was estimated using a haemocytometer and then adjusted accordingly to obtain two conidial suspensions with final concentrations of 10^5 and 10^7 spores mL^{-1}.

4.2.4 Infection of psyllids using a single drop inoculation of a spore solution of *I. fumosorosea*

Mixed-sex adults of *D. citri* were inoculated with 10^5 (If^^{5}) and 10^7 spores mL^{-1} (If^^{7}) suspensions of the *I. fumosorosea* isolate while the control group (K) was treated only with 0.03% Tween 80. To each specimen, 0.4 µL of the suspension was topically applied between the head and the thorax. Psyllids were organized into groups of 10 specimens for each treatment (If^^{5}, If^^{7}, K). Each group of psyllids was added to a citrus leaf (Valencia cultivar) used as food source and placed into a Magenta™ box (7.7×7.7×9.7 cm) [Sigma-Aldrich® now Merck KGaA, Darmstadt, Germany]. Two holes closed by cotton balls were applied to the Magenta™ box and the box was closed in the upper part with a fine mesh net to allowed the air circulations. The trials were carried out in two separated climatic chambers (one for the control and one for the treatments with the fungi) but with the same environmental conditions (T 25±2 °C, RH 80%, 16:8 h L:D photoperiod) to avoid control contaminations. In total, 1,260 psyllids (420 psyllids for each treatment) were used. The experiment was carried out using a completely randomized design with 14 replicates and it was repeated on three different occasions (repetitions). The mortality was evaluated every 24 hours for 10 days post inoculation (dpi).

4.2.5 Infection of psyllids using a spray inoculation of a spore solution of *I. fumosorosea*

Mixed-sex adults of *D. citri* were inoculated with 10^7 mL^{-1} (If^^{7}) suspension of the *I. fumosorosea* isolate which is comparable to field rates for other fungal biopesticide products (Puterka, 1999) or only with 0.03% Tween 80 for the control group (K) by spraying the spores Nalgene® aerosol sprayer [Nalge Nunc International, Rochester, NY] directly onto the adult psyllids for a couple of seconds. Psyllids were organized into 3 groups with 20 specimens for each treatment (If^^{7}, K). Each group of psyllids was added to a citrus leaf (Valencia cultivar) used as food source and placed into a centrifuge tube of 50mL [Thermo Scientific, Waltham, Ma, USA] filled with 5 mL of potato dextrose agar to support the detached citrus leave and closed in the upper part with a fine mesh net. The mortality was evaluated every 24 hours for 6 days post inoculation (dpi).

4.2.6 Identification of *I. fumosorosea* effectors genes candidates for qPCR analysis

To identify pathogen effector genes from *I. fumosorosea* involved during the interaction with the hot (CLas positive) psyllids, new trials were carried out for the collection of alive psyllid specimens spray inoculated with fungal spores. Psyllids were inoculated with 10^{7} mL^{-1} (If^^{7}) suspension of the *I. fumosorosea* isolate or only with 0.03% Tween 80 for the control group (K). Five 50 mL centrifuge tubes with 20 psyllids for each treatment were transferred on detached young citrus leaves after the treatment. One psyllid per tube was collected after 1, 24, 48, 72, 96 hours post inoculation (hpi) for each time course. In total, 30 alive psyllids were taken for each treatment. Alive psyllids were collected and immediately stored into tubes with 100 µL of RNAlater™ Stabilization Solution [Invitrogen™, Carlsbad, CA, USA] and stored at -20°C until use. The trials were carried out in climatic chambers with the environmental conditions (T 25±2°C, 16:8 h L:D photoperiod).

Reported sequences from *I. fumosorosea* ARSEF 2679 strain (NCBI bioproject accession number PRJNA342686) by Shang *et al*. (2016) were used to investigate genes encoding for pathogenicity related small secreted proteins. Out of the 10,061 proteins of *I. fumosorosea*, 345 encoded for small secreted proteins (Shang *et al*., 2016). Out of the 345 proteins, 7 proteins with the highest percentage of cysteines were selected as candidate effector genes to test the expression during fungus-insect interaction in citrus (Figure 2 and Table 1).

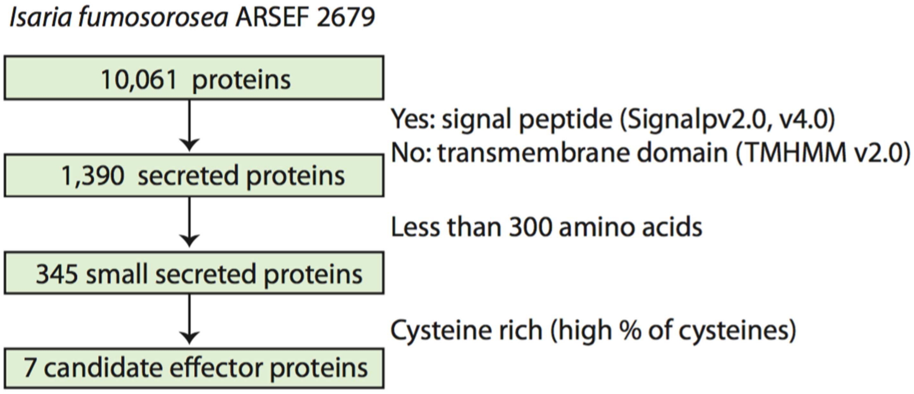


Figure 2. Pipeline for the identification of *I. fumosorosea* candidate effectors for qPCR analyses.

Table 1. List of *I. fumosorosea* effector genes selected for qPCR analyses in this study. Gene IDs correspond to the reference genome strain ARSEF2679.

Gene ID	**Genbank ID**	**Description**	**Signal peptide length (aa) SignalP v2.0**	**Protein length (aa)**	**No. of cysteines**	**% of cysteines**
ISF_09453	XP_018699736.1	Spider potassium channel inhibitory toxin	18	80	10	13
ISF_00119	XP_018708176.1	Hypothetical protein	21	84	10	12
ISF_05991	XP_018703093.1	Hypothetical protein	18	100	10	10
ISF_06731	XP_018702375.1	Hypothetical protein	21	103	10	10
ISF_07009	XP_018702258.1	Hypothetical protein	21	128	12	9
ISF_09687	XP_018699537.1	Hypothetical protein	31	284	4	1
ISF_09081	XP_018700102.1	Extracellular membrane protein, CFEM domain (IPR008427)	24	184	5	3

4.2.6.1 RNA extraction and qPCR analysis from psyllids infected with *Isaria fumosorosea*

All collected alive psyllids were used for total RNA extraction using the Direct-zol RNA Miniprep plus kit [Zymo Research, Irvine, CA, USA] and treated with 500 µL of TRI Reagent® according to the manufacturer's protocol. Total RNA concentration and purity were determined from the ratio of absorbance readings at 260 and 280 nm, using a Nanodrop Lite Spectrophotometer [Thermo Scientific, Waltham, Ma, USA]. First-strand cDNA was synthesized using the Super Script™ IV First-Strand cDNA Synthesis Reaction [Invitrogen™, Carlsbad, CA, USA] following the manufacturer’s instructions. Primers listed in Table 2 were used in a 15 µL reaction with 7.5 µL of 2 × Fast SYBR™ Green Master Mix reagent and 2 µL of cDNA template. Quantitative real time qPCRs were performed in a StepOnePlus qPCR machine using StepOnePlus ‘Fast Protocol’. Reactions for each time point of infection were performed in triplicates. To normalize the qPCR data for *I. fumosorosea* genes during infection on psyllids, we used the reported specific primers ISFBetaTb2F and ISFBetaTbR designed to amplify *I. fumosorosea* beta tubulin endogenous gene (Table 2) (Song *et al*., 2012).

Table 2. List of primers used in this study to amplify *I. fumosorosea* effector genes via qPCR.

Primer name	Primer sequence 5'->3'	Length (bp)	Reference
ISF09453F	GTCCTCACCGCCCTCATG	18	This study
ISF09453R	CAGACCATGGGAGGCACG	18	
ISF00119F	GACTCTGCTCCTCACCACC	19	This study
ISF00119R	GTTGCACTTCAGACCACCG	19	
ISF05991F	CAACACCAACCTCGAAGCC	19	This study
ISF05991R	CGTCGTTACCGCAGTACCT	19	
ISF06731F	CCCATGGGTGTCGAAGTCA	19	This study
ISF06731R	GGTACGCAGATGGACTTGC	19	
ISF07009F	GTTGACCCCGGTACTGTCC	19	This study
ISF07009R	AAGAAGTACTCGGGCCGTC	19	
ISF09687F	GAGTTGAGTATTGCCGCCG	19	This study
ISF09687R	TTCGAGCATGGCCTTCTGT	19	
ISF09081CtF	TAAGAGGAAGCAGGCTGGG	19	This study
ISF09081CtR	GACCATGTCCTCGTAGCGA	19	
ISFBetaTb2F	CGCCGTCCTCGTCGATCTTGAG	22	Song *et al.*, 2012
ISFBetaTb2R	GCACCCTCAGTGTAGTGACCCTTG	24	

4.2.7 Statistical analyses

Mortality data obtained with the single drop inoculation were analyzed using SAS. The cumulative mortality percentage was arcsine transformed and analysed by a general linear model (GLM) performed and executed on a PRO_WIN 6.1 platform [SAS 2002-2012; SAS Institute Inc., Cary, NC, USA]. Outcomes were considered significant at $P<0.05$ and the means were separated by Tukey’s test in case of significant differences.

The statistical analyses for the mortality data obtained with the spray treatments were performed using SPSS v24.0 [SPSS Inc., Chicago, IL, USA] and outcomes were considered significant at $P<0.05$. The cumulative mortality percentage was arcsine transformed and compared using a generalized linear model (GLM) with a normal distribution and identity link and Bonferroni correction was applied.

Gene expression qPCR data was initially processed with StepOne software version 2.3 and the exported results were analyzed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) for relative quantification of the gene expression of the seven evaluated *I. fumosorosea* effectors (Tables 1 and 2).

4.3 RESULTS

4.3.1 Infection of psyllids using a single drop inoculation of a spore solution of *I. fumosorosea*

A mortality range between 64-95% for the treatment *If*^5 and between 52-90% for the treatment *If*^7 was recorded. Significant differences between the treatments were recorded at different day post inoculation (dpi 3: df=2, 55; F=1.85, P=0.016; dpi 6: df=2, 55 F=2.60, P=0.006; dpi 10: df=2, 55; F=1.63, P=0.043) with higher values rates obtained with the less concentrated solution (Figure 3).

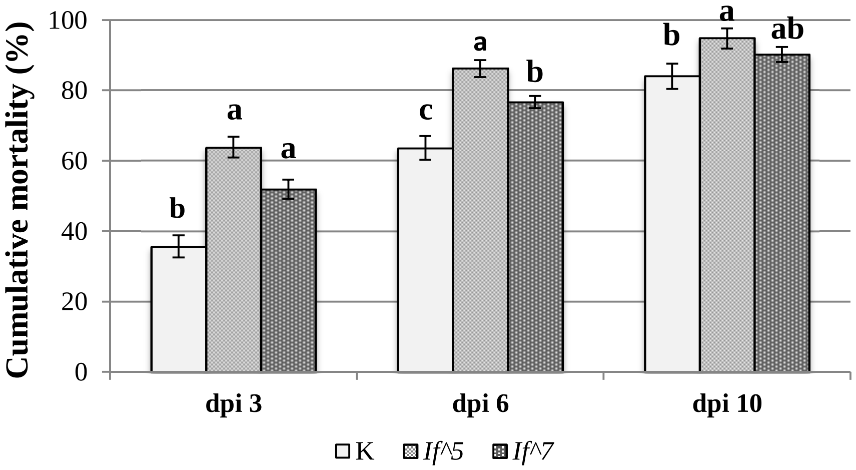


Figure 3. Mean of the cumulative mortality of *D. citri* in each treatment at different days post inoculation (dpi). Bars with a letter in common are not significantly different (Tukey's test, P<0.05).

In *If*^5 and *If*^7 treatments, dead specimens were observed to form a sporulating cadaver cemented in a feeding position to the leaf surface by hyphae growing from their tarsi. Moreover, in the days preceding the death, dying individuals but without the hyphae presence on the body shown a reduction of the feeding behaviour and the almost the whole body paralysis.

4.3.2 Infection of psyllids using a spray inoculation of a spore solution of *I. fumosorosea*

During the trials performed spraying the psyllids, a very low mortality in the control was recorded with only 2 cumulative dead specimens totally observed after 6 dpi (Figure 4). In *If*$\wedge^{7}$, dead psyllids were observed starting from the 2 dpi to reach the 90% of cumulative mortality after 6 dpi (Figure 4). Statistical differences between the treatments were observed starting from the third day post inoculation until the end of the trial (dpi 3: df=1, F=96.00, P=0.000; dpi 4: df=1, F=37.50, P=0.000; dpi 5: df=1, F=234.38, P=0.000; dpi 6: df=1, F=384.00, P=0.000). Similar to the single drop inoculation approach, sporulating cadavers and paralyzed specimens were observed in the psyllid samples collected from spray inoculation.

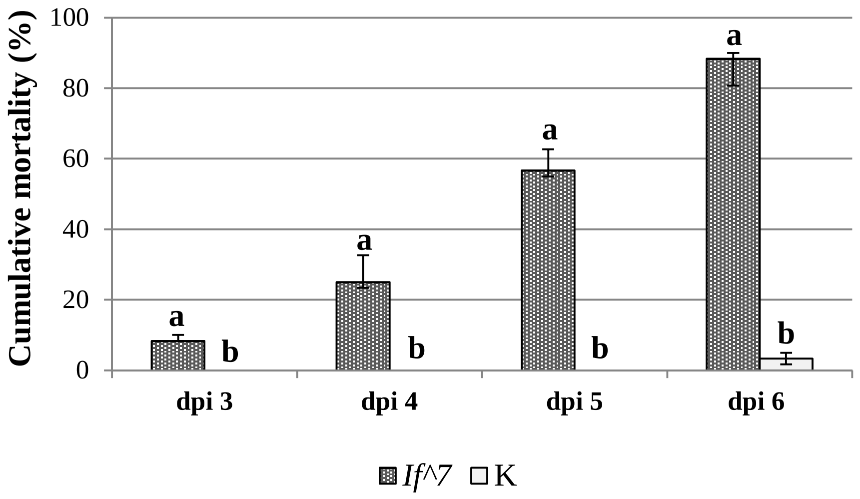


Figure 4. Mean of the cumulative mortality of *D. citri* treated by spray inoculation at different days post inoculation (dpi). Different letters indicate statistical differences (Tukey's test, P<0.05).

4.3.3 Identification of *I. fumosorosea* candidate effector genes

Initially, qPCR analyses from psyllid samples collected from single drop inoculation were performed but, due to the limited amount of total RNA (low yield) no sufficient cDNA was obtained for downstream gene expression analyses. Therefore, a spray inoculation approach was tested and implemented with a group of psyllids instead of single psyllids.

4.3.3.1 Relative expression of *I. fumosorosea* effector genes via qPCR

Relative expression results via qPCR showed the up regulation of five genes from *I. fumosorosea*. The two most highly up-regulated at early time points of infection (from 1h to 48h) with a concentration of 10^7

spores mL^{-1} were ISF_06731 (NCBI Reference Sequence: XP_XP_018702375) and ISF_09081 (NCBI Reference Sequence: XP_018700102) (Figure 5). These two highly expressed genes ISF_06731 and ISF_09081 were originally described as hypothetical proteins in the annotation of ARSEF 2679 reference genome strain. A blast search against NCBI and pfam search were performed and shown that ISF_09081 contained a conserved fungus-specific CFEM (Conserved Extracellular Fungal Membrane, IPR008427) domain which potential roles in pathogenicity (Table 1) (Kim *et al*., 2016).

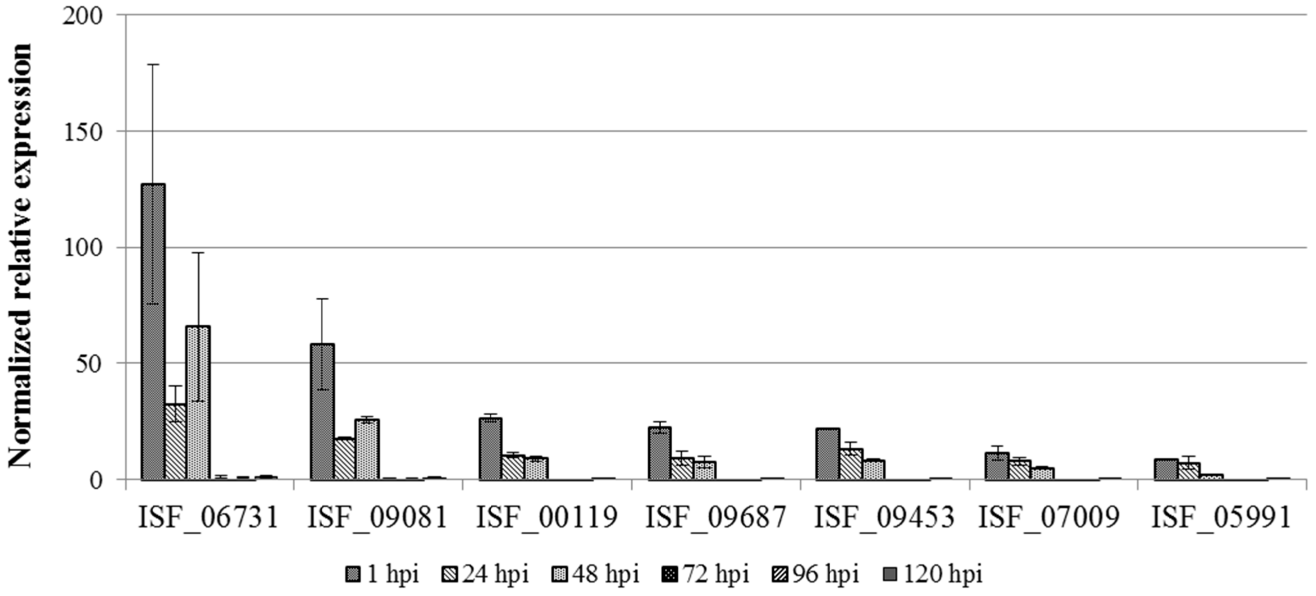
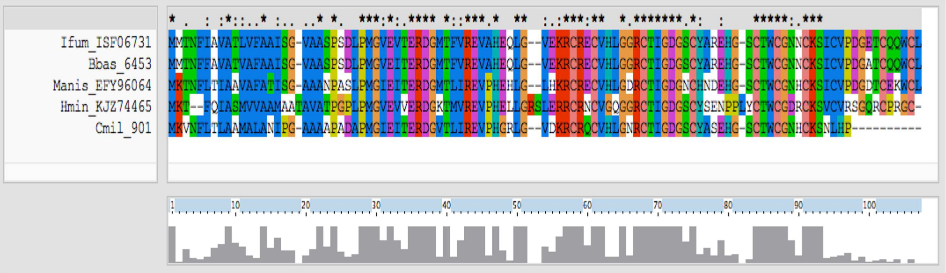


Figure 5. Gene expression of seven *I. fumosorosea* effector genes via qPCR. hpi = hours post inoculation.

Fungal effectors can be conserved across different species or species specific. Protein sequences of two *I. fumosorosea* effector genes which we found to be up-regulated during early time points of infection ISF_06731 is conserved in other entomopathogenic fungal pathogens including *B. bassiana*, *M. anisopliae*, *H. minnesotensis*, *Lecanicillium lecanii* (Llec) and *Cordyceps militaris* (Cmil) (Figure 6). ISF_06731 specific biological function is unknown but the conserved sequence may suggest a conserved activation in gene expression during infection in other insect pests.



```
               *  .   :  :*::..*  :..  ..* *.   ***:*:.**** *::***.*  **   :.:***:**  *.*******.*:   :     *****:.***
Ifum_ISF06731  MMTNFIAVATLVFAAISG-VAASPSDLPMGVEVTERDGMTFVREVAHEQLG--VEKRCRECVHLGGRCTIGDGSCYAREHG-SCTWCGNNCKSICVPDGETCQQWCL
    Bbas_6453  MMTNFFAVATVAFAAISG-VAASPSDLPMGVEITERDGMTFVREVAHEQLG--VEKRCRECVHLGGRCTIGDGSCYAREHG-SCTWCGNNCKSICVPDGATCQQWCL
Manis_EFY96064 MKTNFLTIAAVAFATISG-AAANPASLPMGIEITERDGMTLIREVPHEHLG--LHKRCRECVHLGDRCTIGDGNCHNDEHG-SCTWCGNHCKSICVPDGDTCEKWCL
Hmin_KJZ74465  MKT--FQIASMVVAAMAATAVATPGPLPMGVEVVERDGKTMVREVPHELLGRSLERRCRNCVGQGGRCTIGDGSCYSENPPLYCTWCGDRCKSVCVRSGQRCPRGC-
     Cmil_901  MKVNFLTLAAMALANIPG-AAAAPADAPMGIEITERDGVTLIREVPHGRLG--VDKRCRQCVHLGNRCTIGDGSCYASEHG-SCTWCGNHCKSNLHP----------
```

Figure 6. Protein alignment of *I. fumosorosea* effector candidate ISF_06731 showing conserved amino acid sequences of entomopathogenic fungi *B. bassiana*, *M. anisopliae*, *Hirsutella minnesotensis* (Hmin), *L. lecanii* and *C. militaris*.

4.4 DISCUSSION

The wide use of synthetic chemical pesticides together with increase insecticide resistance, environmental impact and regulations, raised the research for alternative control strategies. Researches on entomopathogenic fungi indicate important advancement on the use of fungi for insect biocontrol, in particular in production, formulation, and application of fungal entomopathogens.

Under laboratory conditions, *I. fumosorosea* (PFR97 strain) conidia suspension showed to be effective against *D. citri* with the single drop inoculation as well as with the spray treatment. The blastospore formulation of this strain is known to cause significantly higher mortality of the citrus pest than conidia within the first 2 days. This is probably due to a faster germination rates of the blastospores, but 7 dpi, psyllids mortality reached 100% under both blastospores and conidial treatments compared to the untreated control (Avery *et al*., 2011). In the trial with the single drop inoculation, statistical differences between the treatments and the control were recorded at 3 and 6 dpi. The highest concentration of the conidial formulation was found to be less effective probably due to the excessive concentration of the solution which led to the defensive response of the psyllid pest through tissue necrosis causing a slower infection rate.

The mortality rate obtained with the spray inoculation was performed using the conidial suspensions at the final concentration of 10^7 mL^{-1}. It must to be taken into account that during the spray treatment the concentration of the solution is reduced and it can be considered comparable to the 10^5 mL^{-1} concentration (Pasco Avery, IRREC, personal communication). Indeed, the mortality rate observed at 6 dpi is comparable with the one recorded with the single drop inoculation in the $If\wedge^{5}$ treatment. Lower mortality rates in the first days post inoculation (1, 2 and 3 dpi) were recorded with the spray treatment. It is well know that temperature, relative humidity and water activity influence the entomopathogenic fungi germination (Luz and Fargues, 1997). The trial with the spray inoculation was carried out in climate chambers without the humidity control. Probably the humidity inside these chambers was lower than the ones used during the single drop inoculation trials allowed a slower germination of the conidia.

After the entomopathogenic fungi treatment, dead specimens showed changes in the behavior feeding, paralyses and sporulating cadavers. As already observed by Meyer *et al*. (2008) and Avery *et al*. (2009), specimens treated with *I. fumosorosea* strain formed sporulating cadavers in a feeding position to the leaf surface by hyphae growing from their tarsi. Moreover, Avery *et al*. (2011) showed that adult psyllids infected by *I. fumosorosea* produce less honeydew than healthy psyllids suggesting that they may feed less, which could potentially reduce the spread of the bacteria '*Ca*. Liberibacter asiaticus' and therefore reduce the damage produced by huanglongbing disease in citrus. Indeed, several authors have indicated that entomopathogenic fungal spores produce bioactive metabolites and cuticle degrading enzymes which may cause avoidance and antifeedant behavior in insects (Meyling and Pell, 2006; Quesada-Moragaet *et al*., 2006; Ali *et al*., 2010; Baverstock *et al*., 2010). *Beauveria bassiana* and *M. anisopliae* have been shown to produce secondary metabolites within insect hosts with effects ranging from paralysis to immunosuppression (Hajek and St. Leger, 1994; Kershaw *et al*., 1999). There are limited information on metabolites and toxins produced by *Isaria* species complex. The first record was the isolation of beauvericin from *I. fumosorosea* (Bernardini *et al*., 1975) moreover, dipicolinc acid (Roberts, 1981) and two beauverolides L and La (Jegorov *et al*., 1994) were identified from *I. fumosorosea* known to have insecticidal activity (Roberts, 1981; Asaff *et al*., 2005).

Although entomopathogenic fungi are well known as biological control agent of insects, the mechanisms underlying their molecular pathogenesis are rather limitedly understood. In particular, the functional activity of the effector repertoire has not been characterized in the interaction between fungi and insect. Few genome sequences are available from insect pathogenic fungi. *Metarhizium robertsii* J.F. Bisch., Rehner & Humbe was first sequenced, (Gao *et al*., 2011), followed by *Cordyceps militaris* (L.) (Zheng *et al*., 2011), *B. bassiana* (Xiao *et al*., 2012) and *Ophiocordyceps* spp (Wichadakul *et al*., 2015). Only more recently functional characterization of protein involved in fungal infection, virulence factors and effector-like proteins to evade host immunity have been investigated (Shang *et al*., 2016; Cen *et al*., 2017).

Fungal plant pathogens effector genes are induced during infection while there are still no visible necrotic lesions and/or visible damage in the host plant tissues. Since there are not gene expression studies during fungal-insect interaction for other known entomopathogenic fungi, the hypothesis behind the research was that *I. fumosorosea* effector genes are up-regulated during early time points of infection of the host, in this case insect psyllids. This is the first study which reports entomopathogenic fungal effector genes that are up-regulated during infection *in vivo*. At least two genes (ISF_06731 and ISF_09081) originally described as hypothetical proteins from *I. fumosorosea* ARSEF 2679 reference genome strain shown this distinct pattern of expression. The annotation of hypothetical protein is assigned to a gene that encodes for a protein but the corresponding translation product has not been functionally characterized yet or showed not sequence similarity/protein domain hits to other characterized proteins from databases like Pfam, Interproscan, Swissprot. In this study, information on the gene expression patterns and potential roles for these two effectors from *I. fumosorosea* were gained. In addition, ISF_06731 effector gene sequence showed conservation with other well-known insect pathogenic fungi such as *B. bassiana* and *M. anisopliae* suggesting potential conservation of insect host targets. ISF_09081 effector mature protein sequence contains a fungus-specific CFEM domain (Conserved Extracellular Fungal Membrane, IPR008427) with potential roles in pathogenicity.

4.5 CONCLUSION

Isaria fumosorosea can be efficiently used to control *D. citri* under laboratory conditions as already proved by other researches (Orduño-Cruz *et al*., 2015; Kumar *et al*., 2017). *Isaria fumosorosea* offers an alternative biocontrol strategy in the field to contribute in the integrated pest management of psyllids infected with '*Ca*. Liberibacter asiaticus' bacteria and to mitigate the losses in citrus groves affected by HLB. Genomes from entomopathogenic fungi encode for effector genes which can be identified from their conserved sequence features like carrying secretion signals and protein length. Some of these effector genes are induced during infection and are potentially required for the establishment of the pathogen and successful colonization of the host insect. This is the first study which reports two entomopathogenic fungal effector genes that are up-regulated during infection *in vivo*. Further studies to decipher their biological function are required, but these results contribute to the understanding of the mechanism regulating the infection of entomopathogenic fungi and will help in the design of novel management strategies against *D. citri*. For instance, it will be possible to select more virulent strains and to identify new target sites for the implementation of innovative control strategies such as the application of the RNA interference (RNAi) technology.

5 REFERENCES

ACEBES-DORIA A. L., LESKEY T. C., BERGH J.C., 2016. Injury to apples and peaches at harvest from feeding by *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs early and late in the season. *Crop Protection*, 89: 58–65.

ALAPHILIPPE A., CAPOWIEZ Y., SEVERAC G., SIMON S., SAUDREAU M., CARUSO S., VERGNANI S., 2016. Codling moth exclusion netting: an overview of French and Italian experiences. *IOBC/WPRS Bulletin*, 112: 31–35.

ALI S., HUANG Z., REN S., 2010. Production of cuticle degrading enzymes by *Isaria fumosorosea* and their evaluation as a biocontrol agent against diamondback moth. *Journal of Pest Science*, 83: 361–370.

ALMEIDA I.F., VALENTÃO P., ANDRADE P.B., SEABRA R.M., PEREIRA T.M., AMARAL M.H., COSTA P.C., BAHIA M.F., 2008. *In vivo* skin irritation potential of a *Castanea sativa* (chestnut) leaf extract, a putative natural antioxidant for topical application. *Basic & Clinical Pharmacology & Toxicology*, 103: 461–467.

ALSTON D.G., RANGEL D.E.N., LACEY L.A., GOLEZ H.G., KIM J.J., ROBERTS D.W., 2005. Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar* (Coleoptera: Curculionidae) larvae. *Biological Control*, l35: 163–71.

ALVAREZ S., ROHRIG E., SOLÍS D., THOMAS M.H., 2016. Citrus greening disease (Huanglongbing) in Florida: economic impact, management and the potential for biological control. *Agricultural Research*, 5: 109–118.

ALVES S.B., TAMAI M.A., ROSSI L.S., CASTIGLIONI E., 2005. *Beauveria bassiana* pathogenicity to the citrus rust mite *Phyllocoptruta oleivora*. *Experimental and Applied Acarology*, 37: 117–122.

AMMAR EL-D., ALESSANDRO R., SHATTERS R.G.J.R., HALL D.G., 2013. Behavioral, ultrastructural and chemical studies on the honeydew and waxy secretions by nymphs and adults of the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae). *PLoS One*, 8: e64938.

ANDRADE E.C., HUNTER W.B., 2017. RNAi feeding bioassay: development of a non-transgenic approach to control Asian citrus psyllid and other hemipterans. *Entomologia Experimentalis et Applicata*, 162: 389–396.

ARTHURS S.P., LACEY L.A., MILICZKY E.R., 2007. Evaluation of the codling moth granulovirus and spinosad for codling moth control and impact on non-target species in pear orchards. *Biological Control*, 41: 99–109.

ANTIGNUS Y., BEN-YAKIR D., 2004. Ultraviolet-absorbing barriers, an efficient integrated pest management tool to protect greenhouses from insects and virus diseases. In: *Insect Pest Management - Field and Protected Crops*, Horowitz A.R. and Ishaaya I. (eds.), Springer Publishers, Berlin: pp. 319–335.

ASAFF A., CERDA-GARCIA-ROJAS C., DE LA TORRE M., 2005. Isolation of dipicolinic acid as an insecticidal toxin from *Paecilomyces fumosoroseus*. *Applied Microbiology and Biotechnology*, 68: 542–547.

ASSER-KAISER S., FRITSCH E., UNDORF-SPAHN K., KIENZLE J., EBERLE K.E. GUND N.A., REINEKE A., ZEBITZ C.P.W., HECKEL D.G., HUBER J., JEHLE J.A., 2007. Rapid emergence of baculovirus resistance in codling moth due to dominant sex-linked inheritance. *Science*, 317: 1916–1918.

AVERY P.B., HUNTER W.B., HALL D.G., JACKSON M.A., POWELL C.A., ROGERS M.E., 2009. *Diaphorina citri* (Hemiptera: Psyllidae) infection and dissemination of the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) under laboratory conditions. *Florida Entomologist*, 92: 608–618.

AVERY P.B., WEKESA V.W., HUNTER W.B., HALL D.G., MCKENZIE C.L., OSBORNE L.S., POWELL C.A., ROGERS M.E., 2011. Effects of the fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on reduced feeding and mortality of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Biocontrol Science and Technology*, 21: 1065–1078.

AVERY P.B., PICK D.A., ARISTIZÁBAL L.F., KERRIGAN J., POWELL C.A., ROGERS M.E., ARTHURS S.P., 2013. Compatibility of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) blastospores with agricultural chemicals used for management of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). *Insects*, 4: 694–711.

BAIAMONTE I., RAFFO A., NARDO N., MONETA E., PEPARAIO M., D'ALOISE A., KELDERER M., CASERA C., PAOLETTI F., 2016. Effect of the use of anti-hail nets on codling moth (*Cydia pomonella*) and organoleptic quality of apple (cv. Braeburn) grown in Alto Adige

Region (Northern Italy). *Journal of the Science of Food and Agriculture*, 96: 2025–2032.

BASILE B., ROMANO R., GIACCONE M., BARLOTTI E., COLONNA V., CIRILLO C., SHAHAK Y., FORLANI M., 2008. Use of photo-selective nets for hail protection of kiwifruit vines in Southern Italy. *Acta horticulturae*, 770: 185–192.

BASILE B., GIACCONE M., CIRILLO C., RITIENI A., GRAZIANI G., SHAHAK Y., FORLANI M., 2012. Photo-selective hail nets affect fruit size and quality in Hayward kiwifruit. *Scientia Horticulturae*, 141: 91–97.

BAVERSTOCK J., ROY H.E., PELL J.K., 2010. Entomopathogenic fungi and insect behavior: from unsuspecting hosts to targeted vectors. *BioControl*, 55: 89–102.

BEN-YAKIR D., HADAR M.D., OFFIR Y., CHEN M., TREGERMAN M., 2008. Protecting crops from pests using OptiNet® and ChromatiNet® shading nets. *Acta horticulturae*, 770: 205–212.

BEN-YAKIR D., ANTIGNUS Y., OFFIR Y., SHAHAK Y., 2012a. Colored shading nets impede insect invasion and decrease the incidences of insect-transmitted viral diseases in vegetable crops. *Entomologia Experimentalis et Applicata*, 144: 249–257.

BEN-YAKIR D., ANTIGNUS Y., OFFIR Y., SHAHAK Y., 2012b. Optical manipulations: an advance approach for reducing sucking insect pests. Ishaaya I. *et al*. (eds.), *Advanced Technologies for Managing Insect Pests*, pp. 249–267.

BEN-YAKIR D., ANTIGNUS Y., OFFIR Y., SHAHAK Y., 2014. Photoselective nets and screens can reduce insect pests and diseases in agricultural crops. *Acta Horticulturae*, 1015: 95–102.

BERGH C., ACEBES-DORIA A., LESKEY T., MORRISON R., SHORT B., KRAWCZYK G., WALGENBACH J., AGNELLO A., JENTSCH P., HAMILTON G., NIELSEN A., BLAAUW B., WALTON V., WIMAN N., HEDSTROM C., SHEARER P., BEERS B., 2016. Integrated pest management for brown marmorated stink bug in orchard crops. *Brown Marmorated Stink Bug SCRI CAP Orchard Crop Commodity Team and Northeastern IPM Center*. http://www.stopbmsb.org/where-is-bmsb/cropby-crop/orchard-crops/.

BERLINGER M.J., TAYLOR R.A.J., LEBIUSH-MORDECHAI S., SHALHEVET S., SPHARIM I., 2002. Efficiency of insect exclusion screens for preventing whitefly transmission of tomato yellow leaf curl virus of tomatoes in Israel. *Bulletin of Entomological Research*, 92: 367–373.

BERNARDINI M., CARILLI A., PACIONI G., SANTURBANO B., 1975. Isolation of Beauvericin from *Paecilomyces fumosoroseus*. *Phytochemistry*, 14: 1865.

BLAAUW B.R., POLK D., NIELSEN A.L., 2015. IPM-CPR for peaches: incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach: behaviorally-based methods to manage *H. halys* and key pests in peach. *Pest Management Science*, 71: 1513–1522.

BLAAUW B.R., JONES V.P., NIELSEN A.L., 2016. Utilizing immunomarking techniques to track *Halyomorpha halys* (Hemiptera: Pentatomidae) movement and distribution within a peach orchard. *PeerJ*, 4: e1997.

BOVE J.M., 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, 88: 7–37.

BRAND G., HÖHN H., KUSKE S., SAMIETZ J., 2013. Management of European cherry fruit fly (*Rhagoletis cerasi*) with exclusion netting: first results. *IOBC/WPRS Bulletin*, 91: 401–404.

BRAVETTI M., BELLEGGIA A., NERI D., 2012. Reti fotoselettive in frutteto: aumentano fotosintesi e qualità. *L'informatore agrario*, 42: 55–58.

BRLANSKY R.H., ROGERS M.E., 2007. Citrus huanglongbing: understanding the vector-pathogen interaction for disease management. Online, *APSnet Features*. http://www.apsnet.org/publications/apsnetfeatures/Pages/Huanglongbing.aspx.

BOS J.I.B., PRINCE D., PITINO M., MAFFEI M.E., WIN J., HOGENHOUT S.A., 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics*, 6: e1001216.

BUTAULT J.P., DEDRYVER C.A., GARY C., GUICHARD L., JACQUET F., MEYNARD J.M. NICOT P., PITRAT M., REAU R., SAUPHANOR B., SAVINI I., VOLAY T., 2010. Ecophyto R&D. Quelles voies pour réduire l'usage des pesticides? *Synthèse du rapport d'étude*, INRA Editeur (France), p.90.

CANDIAN V., PANSA M.G., BRIANO R., PEANO C, TEDESCHI R., TAVELLA L., 2018. Exclusion nets: a promising tool to prevent *Halyomorpha halys* from damaging nectarines and apples in NW Italy. *Bulletin of Insectology*, 71: 21–30.

CASTELLANO S., SCARASCIA MUGNOZZA G., RUSSO G., BRIASSOULIS D., MISTRIOTIS A., HEMMING S., WAAIJENBERG D., 2008. Plastic nets in agriculture: a general review of types and applications. *Applied Engineering in Agriculture*, 24: 799–808.

CASTILLO M.A., MOYA P., HERNÁNDEZA E., PRIMO-YÚFERAB E., 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biological Control*, 19: 274–282.

CEN K., LI B., LU Y., ZHANG S., WANG C., 2017. Divergent LysM effectors contribute to the virulence of *Beauveria bassiana* by evasion of insect immune defenses. *PLoS Pathogens*, 13: e1006604.

CESARI M., MAISTRELLO L., PIEMONTESE L., BONINI R., DIOLI P., LEE W., PARK C.G., PARTSINEVELOS G.K., REBECCHI L., GUIDETTI R., 2018. Genetic diversity of the brown marmorated stink bug *Halyomorpha halys* in the invaded territories of Europe and its patterns of diffusion in Italy. *Biological Invasions*, 20: 1073.

CHARLOT G., WEYDERT C., MILLAN M., BRACHET M.L., WARLOP F., 2014. Nets and covers to protect cherry trees from rain and insects, pp. 222–227. In: *Proceedings of Ecofruit. 16th International Conference on Organic-Fruit Growing*, Hohenheim, 17-19 February 2014. Fördergemeinschaft Ökologischer Obstbau e.V., Weinsberg, Germany.

CHARNLEY A., 1991. Microbial pathogens and insect pest control. *Letters in Applied Microbiology*, 12: 149–157.

CHENG G.W., BREEN P.J., 1991. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society for Horticultural Science*, 116: 865–869.

CHIEL E., MESSIKA Y., STEINBERG S., ANTIGNUS Y., 2006. The effect of UV-absorbing plastic sheet on the attraction and host location ability of three parasitoids: *Aphidius colemani*, *Diglyphus isaea* and *Eretmocerus mundus*. *BioControl*, 51: 65–78.

CHOUINARD G., FIRLEJ A., CORMIER D., 2016. Going beyond sprays and killing agents: exclusion, sterilization and disruption for insect pest control in pome and stone fruit orchards. *Scientia Horticulturae*, 208: 13–27.

CHOUINARD G, VEILLEUX J., PELLETIER F., LAROSE M., PHILION V., CORMIER D., 2017. Impact of exclusion netting row covers on arthropod presence and crop damage to ‘Honeycrisp’ apple trees in North America: a five-year study. *Crop Protection*, 98: 248–254.

CHYZIK R., DOBRININ S., ANTIGNUS Y., 2003. Effect of a uv-deficient environment on the biology and flight activity of *Myzus persicae* and its hymenopterous parasite *Aphidius matricariae*. *Phytoparasitica*, 31: 467–477.

COOMBES C.A., HILL M.P., MOORE S.D., DAMES J.F., 2016. Entomopathogenic fungi as control agents of *Thaumatotibia leucotreta* in citrus orchards: field efficacy and persistence. *BioControl*, 61: 729–739.

CORMIER D., VEILLEUX J., FIRLEJ A., 2015. Exclusion net to control spotted wing *Drosophila* in blueberry fields. *IOBC/WPRS Bulletin*, 109: 181–184.

D'ALESSANDRO C.P., JONES L.R., HUMBER R.A., LÓPEZ LASTRA C.C., SOSA GOMEZ D.R., 2013. Characterization and phylogeny of *Isaria* spp. strains (Ascomycota: Hypocreales) using ITS1-5.8S-ITS2 and elongation factor 1-alpha sequences. *Journal of Basic Microbiology*, 53: 1–11.

DANIEL C., WYSS E., 2009. Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. *Journal of Applied Entomology*, 133: 473–483.

DANIEL C., GRUNDER J., 2012. Integrated management of European cherry fruit fly *Rhagoletis cerasi* (L.): situation in Switzerland and Europe. *Insects*, 3: 956–988.

DIB H., SAUPHANOR B., CAPOWIEZ Y., 2010. Effect of codling moth exclusion nets on the rosy apple aphid, *Dysaphis plantaginea*, and its control by natural enemies. *Crop Protection*, 29: 1502–1513.

DIMBI S., MANIANIA N.K., LUX S.A., EKESI S., MUEKE J.K., 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. fasciventris Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). *Mycopathologia*, 156: 375–382.

DO AMARANTE C.V.T., STEFFENS C.A., ARGENTA L.C., 2011. Yield and fruit quality of ‘Gala’ and ‘Fuji’ apple trees protected by white anti-hail net. *Scientia Horticulturae*, 129: 79–85.

DORING T.F., CHITTKA L., 2007. Visual ecology of aphids - a critical review on the role of colours in host finding. *Arthropod-Plant Interactions*, 1: 3–16.

DOUTT R.L., SMITH R.F., 1971. The pesticide syndrome – diagnosis and suggested prophylaxis. In: C.B. Huffaker (Editor), *Biological Control*. Plenum Press, New York, pp. 3–14.

DYMARSKA M., GRZESZCZUK J., URBANIAK M., JANECZKO T., PLASKOWSKA E., STEPIEN L., KOSTRZEWA-SUSLOW E., 2017. Glycosylation of 6-methylflavone by the strain *Isaria fumosorosea* KCH J2. *PLoS One*, 12: e0184885.

EPPO Global Database. https://gd.eppo.int/taxon/DIAACI (Accessed: 8th February 2018).

FALLIK E., ALAKALI-TUVIA S., PARSELAN Y., AHARON Z., OFFIR Y., MATAN E., YEHEZKEL H., RATNER K., ZUR N., SHAHAK Y., 2009. Can colored shade nets maintain sweet pepper quality during storage and marketing? *Acta Horticulturae,* 830: 37-43.

FAO (Food and Agriculture Organization of the United Nations), 2009. Increasing crop production sustainability: the perspective of biological process. *FAO*, Rome.

FIRLEJ A., VANOOSTHUYSE F., VEILLEUX J., CORMIER D., 2014. Évaluation de filets d’exclusion contre la drosophile à ailes tachetées en bleutiére au Québec. *Rapport d'étape réalisé dans la cadre du programme Prime-Vert, sous-volet 11.1 – Appui à la Stratégie phytosanitaire québecoise en agriculture*. pp 8.

GALINDO-VELASCO E., LEZAMA-GUTIERREZ R., CRUZ-VAZQUEZ C., PESCADOR-RUBIO A., ANGEL-SAHAGUN C.A., OJEDA-CHI M.M., RODRIGUEZ-VIVAS R.I., CONTRERAS-LARA D., 2015. Efficacy of entomopathogenic fungi (Ascomycetes: Hypocreales) against adult *Haematobia irritans* (Diptera: Muscidae) under stable conditions in the Mexican dry tropics. *Veterinary Parasitology,* 209: 173–178.

GAO Q., JIN K., YING S.H, ZHANG Y., XIAO G., SHANG Y., DUAN Z., HU X., XIE X.Q., ZHOU G., PENG G., LUO Z., HUANG W., WANG B., FANG W., WANG S., ZHONG Y., MA L.J., ST LEGER R.J., ZHAO G.P., PEI Y., FENG M.G., XIA Y., WANG C., 2011. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genetics,* 7: e1001264.

GARNIER M., JAGOUEIX-EVEILLARD S., CRONJE P.R., LE ROUX H.F., BOVE J.M., 2000. Genomic characterization of a liberibacter present in an ornamental rutaceous tree, *Calodendrum capense,* in the Western Cape Province of South Africa. Proposal of '*Candidatus* Liberibacter africanus subsp. capensis'. *International Journal of Systematic and Evolutionary Microbiology*, 50: 2119–2125.

GARZO E., BONANI J.P., LOPES J.R.S., FERERES A., 2012. Morphological description of the mouthparts of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Arthropod Structure & Development*, 41: 79–86.

GILLIGAN T.M., EPSTEIN M.E., 2014. Tortricids of agricultural importance, interactive keys developed in lucid 3.5. Last updated August 2014. http://idtools.org/id/leps/tortai/Cydia_pomonella.htm.

GOREN A., ALKALAI-TUVIA S., PERZELAN Y., AHARON Z., FALLIK E., SHAHAK Y., 2010. The effect of colored shade nets on sweet bell pepper quality after prolonged storage and shelf life. *Acta Horticulturae*, 927: 565–570.

GOTTWALD T.R., 2007. Citrus canker and citrus Huanglongbing, two exotic bacterial diseases threatening the citrus industries of the western hemisphere. *Outlooks on Pest Management*, 18: 274–279.

GOTTWALD T.R., 2010. Current epidemiological understanding of citrus Huanglongbing. *Annual Review of Phytopathology*, 48: 119–139.

GOTTWALD T.R., DA GRAÇA J.V., BASSANEZI R.B., 2007. Citrus huanglongbing: the pathogen and its impact. Online, *Plant Health Progress*.

HALL D.G., GOTTWALD T.R., 2011. Pest management practices aimed at curtailing citrus Huanglongbing disease. *Outlooks on Pest Management*, 22: 189–192.

HALL D.G., HENTZ M.G., MEYER J.M., KRISS A.B., GOTTWALD T.R., BOUCIA D.G., 2012. Observations on the entomopathogenic fungus *Hirsutella citriformis* attacking adult *Diaphorina citri* (Hemiptera: Psyllidae) in a managed citrus grove. *BioControl*, 57: 663–675.

HAJEK A.E., ST LEGER R.J., 1994. Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology,* 39: 293–322.

HARDIE J., 1989. Spectral specificity for targeted flight in the black bean aphid, *Aphis fabae*. *Journal of Insect Physiology,* 35: 619–626.

HODGES A.W., SPREEN T.H., 2012. Economic Impacts of Citrus Greening (HLB) in Florida, 2006/07-2010/11. *Electronic Data Information Source* (EDIS) FE903. Gainseville, FL, University of Florida.

HOGENHOUT S.A., VAN DER HOORN R.A., TERAUCHI R., KAMOUN S., 2009. Emerging concepts in effector biology of plant-associated organisms. *Molecular Plant-Microbe Interactions,* 22: 115–122.

HÖHN H., WALDER R., MÜHLENZ I., SAMIETZ J., LINDER C., 2012. Alternatives au diméthoate pour lutter contre la mouche de la cerise. *Revue suisse de Viticulture, Arboriculture, Horticulture,* 44: 152–158.

HOLDER D.J., KEYHANI N.O., 2005. Adhesion of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* to substrata. *Applied and Environmental Microbiology*, 71: 5260–5266.

HONEK A., KOCOUREK F., 1990. Temperature and development time in insects: a general relationship between thermal constants. *Zoologische Jahrbucher: Abteilung fur Systematik, Okologie und Geographie der Tiere*, 117: 401–439.

HU X., XIAO G., ZHENG P., SHANG Y., SU Y., ZHANG X., LIU X., ZHAN S., ST. LEGER R.J., WANG C., 2014. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, 111: 16796–16801.

HUNTER W.B., AVERY P.B., PICK D., POWELL C.A., 2011. Broad spectrum potential of *Isaria fumosorosea* against insect pests of citrus. *Florida Entomologist*, 94: 1051–1054.

HUSSEIN H.M., SKOKOVA HABUSTOVA O., PUZA V., ZEMEK R., 2016. Laboratory evaluation of *Isaria fumosorosea* CCM 8367 and *Steinernema feltiae* Ustinov against immature stages of the Colorado potato beetle. *PLoS One*, 11: e0152399.

JEGOROV A., SEDMERA P., MATHA V., SIMEK P., ZAHRADNÍČKOVÁ H., LANDA Z., EYAL J., 1994. Beauverolides L and La from *Beauveria tenella* and *Paecilomyces fumosoroseus. Phytochemistry*, 37: 1301–1303.

JOHANSEN N.S., VANNINEN I., PINTO D.M., NISSINEN A.I., SHIPP L., 2011. In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. *Annals of Applied Biology*, 159: 1–27.

JONES J.D., DANGL J.L., 2006. The plant immune system. *Nature*, 444: 323–329.

KAMOUN S., 2007. Groovy times: filamentous pathogen effectors revealed. *Current Opinion in Plant Biology*, 10: 358–365.

KAWASE S., UCHINO K., YASUDA M., MOTOORI S., 2008. Netting control of cherry drosophila *Drosophila suzukii* injurious to blueberry. *Bulletin of the Chiba Prefect Agriculture Research Center*, 7: 9–15.

KERSHAW M.J., MOORHOUSE E.R., BATEMAN R., REYNOLDS S.E., CHARNLEY A.K., 1999. The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insect. *Journal of Invertebrate Pathology*, 74: 213–223.

KIM K.-T., JEON J., CHOI J., CHEONG K., SONG H., CHOI G., LEE, Y.-H., 2016. Kingdom-wide analysis of fungal small secreted proteins (SSPs) reveals their potential role in host association. *Frontiers in Plant Science*, 7: 186.

KNAPP J.L., HALBERT S., LEE R., HOY M., CLARK R., KESINGER M., 1998. The Asian citrus psyllid and citrus greening disease. *Citrus Industry Magazine*, 79: 28–29.

KRING J.B., 1972. Flight behavior of aphids. *Annual Review of Entomology*, 17: 461–492.

KUHRT U., SAMIETZ J., HÖHN H., DORN S., 2006. Modelling the phenology of codling moth: Influence of habitat and thermoregulation. *Agriculture, Ecosystems and Environment*, 117: 29–38.

KUMAR P., POEHLING H.M., 2006. UV-blocking plastic films and nets influence vectors and virus transmission on greenhouse tomatoes in the humid Tropics. *Environmental Entomology*, 35: 1069–1082.

KUMAR V., AVERY P.B., AHMED J., CAVE R.D., MCKENZIE C.L., OSBORNE L.S., 2017. Compatibility and efficacy of *Isaria fumosorosea* with horticultural oils for mitigation of the Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Liviidae). *Insects*, 8: pii: E119.

ILIĆ Z.S., MILENKOVIĆ L., STANOJEVIĆ L., CVETKOVIĆ D., FALLIK E., 2012. Effects of the modification of light intensity by color shade nets on yield and quality of tomato fruits. *Scientia Horticulturae*, 139: 90–95.

ILIĆ S.Z., MILENKOVIĆ L., ŠUNIĆ L., FALLIK E., 2015. Effect of coloured shade-nets on plant leaf parameters and tomato fruit quality. *Journal of the Science of Food and Agriculture*, 95: 2660–2667.

ILIĆ S.Z., FALLIK E., 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: a review. *Environmental and Experimental Botany*, 139: 79–90.

LACEY L.A., FRUTOS R., KAYA H.K., VAIL P., 2001. Insect pathogens as biological control agents: Do they have a future? *Biological Control*, 21: 230–248.

LEE D.H., SHORT B.D., JOSEPH S. V., BERGH J.C., LESKEY T.C., 2013. Review of the biology, ecology and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan and the Republic of Korea. *Environmental Entomology*, 42: 627–641.

LEGARREA S., WEINTRAUB P.G., PLAZA M., VIÑUELA E., FERERES A., 2012. Dispersal of aphids, whiteflies and their natural enemies under photoselective nets. *BioControl*, 57: 523–532.

LESKEY T.C., SHORT B.D., BUTLER B.R., WRIGHT S.E., 2012. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. *Psyche*, 2012: 535062.

LESKEY T.C., SHORT B.D., LEE D.H., 2014. Efficacy of insecticide residues on adult *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. *Pest Management Science*, 70: 1097–1104.

LEWALLEN K.S., MARINI R.P., 2003. Relationship between flesh firmness and ground color in peach as influenced by light and canopy position. *Journal of the American Society for Horticultural Science*, 128:163–170.

LEWIS M. W., ROBALINO I.V., KEYHANI N.O., 2009. Uptake of the fluorescent probe FM4-64 by hyphae and haemolymph-derived in vivo hyphal bodies of the entomopathogenic fungus *Beauveria bassiana*. *Microbiology*, 155: 3110–3120.

LIVAK K.J., SCHMITTGEN T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, 25: 402–408.

LLOYD A., HAMACEK E., GEORGE A., NISSEN R., WAITE G., 2005. Evaluation of exclusion netting for insect pest control and fruit quality enhancement in tree crops. *Acta Horticulturae*, 694: 253–258.

LO PRESTI L., LANVER D., SCHWEIZER G, TANAKA S., LIANG L., TOLLOT M., ZUCCARO A., REISSMANN S., KAHMANN R., 2015. Fungal effectors and plant susceptibility. *Annual Review of Plant Biology*, 66: 513–545.

LUZ C., FARGUES J., 1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. *Mycopathologia*, 138: 117–125.

LYDIA C.H., SUNIL V., RENUKA K., SANDHYA V., BENTUR J.S., 2017. Entomopathogenic fungi as potential bio-control agents and mode of action against insect pests. *Agriculture Update*, 12 (TECHSEAR-1): 277–283.

MARLIAC G., SIMON S., FLEURY A., ALAPHILIPPE A., DIB H., CAPOWIEZ Y., 2013. Contrasting effects of codling moth exclusion netting on the natural control of the rosy apple aphid. *IOBC/WPRS Bulletin*, 91: 81–85.

MARTÍNEZ-LAS HERAS R., AMIGO-SÁNCHEZ J.C., HEREDIA A., CASTELLÓ M.L., ANDRÉS A., 2016. Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage. *CyTA - Journal of Food*, 14: 333–339.

MARTINSON H.M., VENUGOPAL P.D., BERGMANN E.J., SHREWSBURY P.M., RAUPP M.J., 2015. Fruit availability influences the seasonal abundance of invasive stink bugs in ornamental tree nurseries. *Journal of Pest Science*, 88: 461–468.

MARSHALL A., BEERS E., 2016. Efficacy and non-target effects of net enclosures in apple orchards. In: *Proceedings of 90th Annual Orchard Pest and Disease Management Conference*, 13–15 January 2016, Portland, OR, p. 34.

MCCOY C.W., COUCH T.L., 1982. Microbial control of the citrus rust mite with the mycoarcaricide. *Florida Entomologist*, 65: 116–126.

MDELLEL L., GUESMI J., KAMEL M.B.H., 2015. Identification of two entomopathogenic fungi naturally infecting *Pterochloroides persicae* (Cholodkovsky 1899) (Hemiptera, Aphididae) in peach orchards in Tunisia. *European Journal of Environmental Sciences*, 5: 158–160.

MERRILL H.A., 1967. Control of nuisance pests in suburbia. In: *Proceedings of the 3rd Vertebrate Pest Conference*, March 1967, San Francisco, California, USA, pp. 7–9.

MEYLING N.V., PELL J.K., 2006. Detection and avoidance of an entomopathogenic fungus by a generalist insect predator. *Ecological Entomology*, 31: 162–171.

MEYER J.M., HOY M.A., BOUCIAS D.G., 2008. Isolation and characterization of an *Isaria fumosorosea* isolate infecting the Asian citrus psyllid in Florida. *Journal of Invertebrate Pathology*, 99: 96–102.

MILEO A.M., MICCADEI S., 2015. Polyphenols as modulator of oxidative stress in cancer disease: new therapeutic strategies. *Oxidative Medicine and Cellular Longevity*, 2016, 1–17.

MOCHI D.A., MONTEIRO A.C., DE BORTOLI S.A., DÓRIA H.O.S., BARBOSA J.C., 2006. Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. *Neotropical Entomology*, 35: 382–389.

MORRISON W.R., LEE D.H., SHORT B.D., KHRIMIAN A., LESKEY T.C., 2016. Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive *Halyomorpha halys* in apple orchards. *Journal of Pest Science*, 89: 81–96.

MURRAY R.G.E., SCHLEIFER K.H., 1994. Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. *International journal of systematic bacteriology*, 44: 174–176.

OKMEN B., DOEHLEMANN G., 2014. Inside plant: biotrophic strategies to modulate host immunity and metabolism. *Current Opinion in Plant Biology*, 20: 19–25.

ORDUÑO-CRUZ N., GUZMAN-FRANCO A.W., RODRIGUEZ-LEYVA E., ALATORRE-ROSAS R., GONZALEZ-HERNANDEZ H., MORA-AGUILERA G., 2015. In vivo selection of entomopahtogenic fungal isolates for control of *Diaphorina citri* (Hemiptera: Liviidae). *Biological Control*, 90: 1–5.

OREN-SHAMIR M., GUSSAKOVSKY E.E., SHPIEGEL E., NISSIM-LEVI A., RATNER K., OVADIA R., GILLER Y.E., SHAHAK Y., 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum*. *Journal of Horticultural Science and Biotechnology*, 76: 353–361.

PAPPAS M.L., BROEKGAARDEN C., BROUFAS G.D., KANT M.R., MESSELINK G.J., STEPPUHN A., WACKERS F., VAN DAM N.M., 2017. Induced plant defences in biological control of arthropod pests: a double-edged sword. *Pest Management Science*, 73: 1780–1788.

PATT J., CHOW A., MEIKLE W.G., GRACIA C., JACKSON M.A., FLORES D., SETAMOU M., DUNLAP C.A., AVERY P.B., HUNTER W.B., ADAMCZYK J.J., 2015. Efficacy of an autodisseminator of an entomopathogenic fungus, *Isaria fumosorosea*, to supress Asian citrus psyllid, *Diaphorina citri*, under greenhouse conditions. *Biological Control*, 88: 37–45.

PAVARINO A., VITTONE G., 2014. Protezione dalla carpocapsa: strategia a confronto. *Rivista di Frutticoltura e Ortofloricoltura*, 4: 53–54.

PAVESI M., 2014. *Popillia japonica* specie aliena invasiva segnalata in Lombardia. *Informatore Agrario*, 70: 53–55.

PAYNE C.C., JONES M.H.K., HARPAZ I., AKINGBOHUNGBE A.E., PATERSON R.R.M., AHMED S.M.K.H., 1988. Pathogens for the control of insects: where next? [and discussion]. *Philosophical Transactions of the Royal Society of London Series B*, 31: 225–248.

PROKOPY R.J., COLLIER R.H., FINCH S., 1983. Leaf color used by cabbage root flies to distinguish among host plants. *Science*, 221: 190–192.

POPRAWSKI T.J., PARKER P.E., TSAI J.H., 1999. Laboratory and field evaluation of hyphomycete insect pathogenic fungi for control of brown citrus aphid (Homoptera: Aphididae). *Environmental Entomology*, 28: 315–321.

PUTERKA G.J., 1999. Fungal pathogens for arthropod pest control in orchard systems: mycoinsecticidal approach for pear psylla control. *BioControl*, 44: 183–210.

QUESADA-MORAGA E., CARRASCO-DÍAZ J.-A., SANTIAGO-ÁLVAREZ C., 2006. Insecticidal and antifeedant activities of proteins secreted by entomopathogenic fungi against *Spodoptera littoralis* (Lep., Noctuidae). *Journal of Applied Entomology*, 130: 442–452.

QURESHI J.A., STANSLY P.A., 2009. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. *Biological Control*, 50: 129–136.

RAJAPAKSE N.C., SHAHAK Y., 2007. Light quality manipulation by horticulture industry. In: Whitelam G. and Halliday K. (eds.), *Light and Plant Development.* Blackwell Publishing, UK, pp: 290–312.

REGIONE PIEMONTE, 2015. Norme Tecniche di produzione Integrata. http://www.bdfagro.it/bdf-archivi/disciplinari/piemonte/dpi_piemonte_2015.pdf (Accessed: 4th June 2015).

REGIONE PIEMONTE, 2016. Norme Tecniche di produzione Integrata. http://www.regione.piemonte.it/agri/area_tecnico_scientifica/settore_fitosanitario/dwd/pan/NormeTecnichePiemonte2016.pdf (Accessed: 25th May 2016).

REGIONE PIEMONTE, 2017. Norme Tecniche di produzione Integrata. https://www.reterurale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/15591 (Accessed: 17th July 2017).

RETAMALES J.B., MONECINO J.M., LOBOS G.A., ROJAS L.A., 2008. Colored shading increase yields and profitability of highbush blueberries. *Acta Horticulturae*, 770: 193–198.

RIGDEN P., 2008. To net or not to net. Queensland Department of Primary Industries and Fisheries, Queensland, p. 64.

ROBERTS D.W., 1981. Toxins of entomopathogenic fungi. In: *Microbial control of pests and plant diseases* 1970-1980. HD Burges (Ed.), Academic press, ISBN 0121433609, London and New York, pp: 441–464.

ROBERTS D.W., HUMBER R.A., 1981. Entomogenous fungi. In: *Biology of Conidial Fungi*. Cole G.T., Kendrick B. (Eds),. Academic Press, New York, pp: 201–236.

ROGERS M.A., BURKNESS E.C., HUTCHISON W.D., 2016. Evaluation of high tunnels for management of *Drosophila suzukii* in fall-bearing red raspberries: potential for reducing insecticide use. *Journal of Pest Science*, 89: 815–821.

ROMET L., SEVERAC G., WARLOP F., 2010. Overview of ALT’CARPO concept and its development in France, in: Ecofruit. Proceedings of 14th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, 22–24 Febuary 2010 Hohenheim, Germany, Fördergemeinschaft, (Ed.) Ökologischer Obstbau, E.V. (Ed.), Weinsberg, Germany, pp. 176–182.

ROY H.E., STEINKRAUS D., EILENBERG J., HAJEK A., PELL J.K., 2006. Bizzare interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology*, 51: 331–357.

ROY H.E., EOIN L.B., CHANDELR D., GOETTEL M.S., PELL J.K., WAJNBERG E., VEGA F.E., 2010. Deep space and hidden depths: understanding the evolution and ecology of fungal entomopathogens. *BioControl*, 55: 1–6.

SANSAVINI S., RANALLI P., 2012. Indici di maturazione, qualità e perdite post-raccolta. *Manuale di ortofrutticoltura edagricole*, pp: 169–172.

SAUPHANOR B., DIRWIMMER C., BOUTIN S., CHAUSSABEL A.L., DUPONT N., FAURIEL J., GALLIA V., LAMBERT N., NAVARRO E., PARISI L., PLENET D., RICAUD V., SAGNES J.-L., SAUVAITRE D., SIMON S., SPEICH P., ZAVAGLI F., 2009. Tome IV: analyse comparative de différents systèmes en arboriculture fruitière, volet I. Ecophyto R&D: vers des systèmes de culture économes en produits phytosanitaires. In: *Rapport of Expertise Collective Inra.* Inra Rennes, France.

SAUPHANOR, B., SEVERAC, G., MAUGIN, S., TOUBON, J.F., APOWIEZ Y., 2012. Exclusion netting may alter reproduction of the codling moth (*Cydia pomonella*) and prevent associated fruit damage to apple orchards. *Entomologia Experimentalis et Applicata*, 145: 134–142.

SCALBERT A., MANACH C., MORAND C., RÉMÉSY C., JIMÉNEZ L., 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45: 287–306.

SCARASCIA-MUGNOZZA G., SICA C., RUSSO G., 2011. Plastic materials in European agriculture: actual use and perspectives. *Journal of Agriculture Engineering*, 3: 15–28.

SÉVÉRAC G., SIEGWART M., 2013. Protection Alt'Carpo, nouvelles études sur trois ans. *Phytoma*, 668: 33–37.

SHAHAK Y., 2008. Photo-selective netting for improved performance of horticultural crops: a review of ornamental and vegetable studies in Israel. *Acta Horticulturae*, 770: 161–168.

SHAHAK Y., 2011. Photoselective netting: the concept, research and implementation in various crops. In: Proceedings of International CIPA Conference 2012 on Plasticulture for a Green Planet. Ed.: Sadka. A. *Acta Horticulturae*, 1015: 155–162.

SHAHAK Y., 2014. Photoselective netting: an overview of the concept, R&D and practical implementation in agriculture. *Acta Horticulturae*, 1015: 155–162.

SHAHAK Y., GUSSAKOVSKY E.E., GAL E., GANELEVIN R., 2004a. ColorNets: Crop protection and light-quality manipulation in one technology. *Acta Horticulturae*, 659: 143–151.

SHAHAK Y., GUSSAKOVSKY E.E., COHEN Y., LURIE S., STERN R., KFIR S., NAOR A., ATZMON I., DORON I., GREENBLAT-AVRON Y., 2004b. ColorNets: a new approach for light manipulation in fruit trees. *Acta Horticulturae*, 636: 609–616.

SHAHAK Y., RATNER K., GILLER Y.E., ZUR N., OR E., GUSSAKOVSKY E.E., STERN R., SARIG P., RABAN E., HARCAVI E., DORON I., GREENBLAT-AVRON Y., 2008. Improving solar energy utilization, productivity and fruit quality in orchards and vineyards by photoselective netting. *Acta Horticulturae*, 772: 65–72.

SHAHAK Y., RATNER K., ZUR N., OFFIR Y., MATAN E., YEHEZKEL H., MESSIKA Y., POSALSKI I., BEN-YAKIR D., 2009. Photoselective netting: an emerging approach in protected agriculture. *Acta Horticulturae*, 807: 79–84.

SHAHID A.A., RAO A.Q., BAKHSH A., HUSNAIN T., 2012. Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Archives of Biological Sciences. Archives of Biological Sciences*, 64: 21–42.

SHANG Y., XIAO G., ZHENG P., CEN K., ZHAN S., WANG C., 2016. Divergent and Convergent Evolution of Fungal Pathogenicity. *Genome Biology and Evolution*, 8: 1374–1387.

SHI W.B., FENG M.G., 2006. Field efficacy of application of *Beauveria bassiana* formulation and low rate pyridaben for sustainable control of citrus red mite *Panonychus citri* (Acari: Tetranychidae) in orchards. *Biological Control*, 39: 210–217.

SHORT B.D., KHRIMIAN A., LESKEY T.C., 2017. Pheromone-based decision support tools for management of *Halyomorpha halys* in apple orchards: development of a trap-based treatment threshold. *Journal of Pest Science*, 90: 1191–1204.

SLINKARD K., SINGLETON V.L., 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28: 49–55.

SONG T.T., YING S.H., FENG M.G., 2012. High resistance of *Isaria fumosorosea* to carbendazim arises from the overexpression of an ATP-binding cassette transporter (ifT1) rather than tubulin mutation. *Journal of Applied Microbiology*, 112: 175–84.

STERGIOPOULOS I., DE WIT P.J.G.M., 2009. Fungal effector proteins. *Annual Review of Phytopathology*, 47: 233–63.

SUMMERS C.G., MITCHELL J.P., STAPLETON J.J., 2004. Management of aphid-borne viruses and *Bemisia argentifolii* (Homoptera: Aleyrodidae) in zucchini squash by using UV reflective plastic and wheat straw mulches. *Environmental Entomology*, 33: 1447–1457.

TASIN M., DEMARIA D., RYNE C., CESANO A., GALLIANO A., ANFORA G., IORIATTI C., ALMA A., 2008. Effect of anti-hail nets on C*ydia pomonella* behavior in apple orchards. *Entomologia Experimentalis et Applicata*, 129: 32–36.

THOMAS M.B., READ A.F., 2007. Can fungal biopesticides control malaria? *Nature Reviews Microbiology*, 5: 377–383.

TOCHEN S., DALTON D.T., WIMAN N.G., HAMM C., SHEARER P.W., WALTON V.M., 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, 43:501–510.

TÓTH M., JÓSVAI J., HÁRI K., PÉNZES B., VUITY Z.S., HOLB I., SZARUKÁN I., KECSKÉS Z.S., DORGÁN-ZSUGA I., KOCZOR S., VOIGT E., 2014. Pear ester based lures for the codling moth *Cydia pomonella* L. — A summary of research effort in Hungary. *Acta Phytopathologica et Entomologica Hungarica*, 49: 37–47.

VLACH J., 2010. Identify *Drosophila suzukii.* Oregon Department of Agricolture. http://www.liebegg.ch/upload/cms/user/150730_Identifikation2.pdf (Accessed: 8th June 2015).

VEGA F.E., GOETTEL M.S., BLACKWELL M., CHANDLER D., JACKSON M.A, KELLER S., KOIKE M., MANIANIA N.K., MONZÓN A., OWNLEY B.H., PELL J.K., RANGEL D.E.N., ROY H.E., 2009. Fungal entomopathogens: new insights on their ecology. *Fungal ecology*, 2: 149–159.

VELÁZQUEZ E., LEGARREA S., MORALES I., FERERES CASTIEL A., AMOR PARRILLA F., FERNÁNDEZ M.M., RODRIGUEZ D., VIÑUELA SANDOVAL E., 2012. Flying capacity of *Psyttalia concolor* and *Chrysoperla carnea* under a UV-absorbing net (Bionet®) in presence and absence of crop. *IOBC/WPRS Bulletin,* 79: 113–119.

VERNON R.S., GILLESPIE D.R, 1990. Spectral responsiveness of *Frankliniella occidentalis* (Thysanoptera, Thripidae) determined by trap catches in greenhouses. *Environmental Entomology*, 19: 1229–1241.

WANCHOO A., LEWIS M.W., KEYHANI N.O., 2009. Lectin mapping reveals stage-specific display of surface carbohydrates in in vitro and haemolymph-derived cells of the entomopathogenic fungus *Beauveria bassiana*. *Microbiology*, 155: 3121–3133.

WANG C., WANG S., 2017. Insect Pathogenic Fungi: Genomics, Molecular Interactions, and Genetic Improvements. *Annual Review of Entomology*, 62: 73–90.

WICHADAKUL D., KOBMOO N., INGSRISWANG S., TANGPHATSORNRUANG S., CHANTASINGH D., LUANGSA-ARD J.J., EURWILAICHITR L., 2015. Insights from the genome of *Ophiocordyceps polyrhachis-furcata* to pathogenicity and host specificity in insect fungi. *BMC Genomics*, 16: 881.

WITZGALL P., KIRSCH P., CORK A., 2010. Sex pheromones and their impact on pest management. *Journal of Chemical Ecology*, 36: 80–100.

YEE W.L., LACEY L.A., 2005. Mortality of different life stages of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Entomological Science*, 40: 167–177.

XIAO G., YING S.H., ZHENG P., WANG Z.L., ZHANG S., XIE X.Q., SHANG Y., ST LEGER R.J., ZHAO G.P., WANG C., FENG M.G., 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Scientific Reports*, 2: 483.

ZANGHERI S., BRIOLLINI G., CRAVEDI P., DUSO C., MOLINARI F., PASQUALINI E., 1999. *Tortricidae e Cochylidae.* Edizioni L'informatore Agrario. *Lepidotteri dei fruttiferi e della vite*, pp. 60–103.

ZHANG H., TSAO R., 2016. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science*, 8: 33–42.

ZHENG P., XIA Y.L., XIAO G.H., XIONG C.H, HU X., ZHANG S.W., ZHENG H.J., HUANG Y., ZHOU Y., WANG S.Y., ZHAO G.P., LIU X.Z., ST LEGER R.J., WANG C.S., 2011. Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Genome Biology*, 12: R116.

ZORATTI L., JAAKOLA L., HÄGGMAN H., GIONGO L., 2015. Modification of sunlight radiation through colored photo-selective nets affects anthocyanin profile in Vaccinium spp. berries. Xu C, ed. *PLoS ONE*, 10: e0135935.

6 ACKNOWLEDGEMENTS

I would like to thank all the people who helped me during my Ph.D.

Dr. Marco Giuseppe Pansa, Dr. Karin Santoro, Dr. Rossella Briano, Luca Rivoira, Enrico Tomatis for their support in experimental activities. The growers Silvano Ezio Bertorello, Giuseppe Borretta, Enzo Farinasso and Andrea Ingaramo for their hospitality. The EU LIFE13 ENV/HR/000580 project “Low pesticide IPM in sustainable and safe fruit production” that supported this work.

Dr. David Hall and Ms. Kathy Moulton from the United States Horticultural Research Laboratory in Fort Pierce, Florida that supplied ‘*Candidatus* Liberibacter asiaticus’ (CLas) infected *Diaphorina citri* psyllids. Dr. Pasco Avery from the Indian River Research and Education Center at the University of Florida, Fort Pierce, Florida that provided *I. fumosorosea* strain PFR97 and suggestions. Dr. Wayne Hunter from the United States Horticultural Research Laboratory in Fort Pierce, Florida for the Internship supervision. Dr. Marco Pitino and Dr. Shi Qingchun for their support during the qPCR analyses.

A special thank goes to Prof. Liliana Maria Cano who gave me the opportunity to work in her laboratory during my period abroad and to Prof. Rosemarie Tedeschi for her careful supervision and precious support.

7 APPENDIX

During the Ph.D. period the following paper was published:

CANDIAN V., PANSA M.G., BRIANO R., PEANO C., TEDESCHI R., TAVELLA L., 2018. Exclusion nets: a promising tool to prevent *Halyomorpha halys* from damaging nectarines and apples in NW Italy. *Bulletin of Insectology*, 71: 21–30.

Bulletin of Insectology ***71*** *(1): 21-30, 2018*
ISSN 1721-8861

Exclusion nets: a promising tool to prevent *Halyomorpha halys* from damaging nectarines and apples in NW Italy

Valentina Candian, Marco G. Pansa, Rossella Briano, Cristiana Peano, Rosemarie Tedeschi, Luciana Tavella
Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Italy

Abstract

Halyomorpha halys (Stal) (Heteroptera Pentatomidae) is becoming one of the most worrisome pests for many fruit crops worldwide causing serious fruit damage and thus heavy economic losses. Insecticide treatments are not so effective in containing this pest, and they should be repeated every 7-10 days. Therefore, exclusion nets represent one of the most readily available tools for crop protection and an environmental friendly alternative to pesticides. In this study, the use of exclusion nets was investigated in semi-field conditions as a potential strategy to protect nectarine and apple orchards from *H. halys* in NW Italy. The presence and abundance of the pest inside and outside the exclusion nets, as well as the damage on fruits all along the trials and at the harvest time were evaluated. Moreover, the possible effects of the net on the arthropod fauna (mainly predators) and on fruit quality and nutraceutical parameters were considered. This study showed that the exclusion nets are a very promising and sustainable tool for the management of *H. halys*. In particular, the exclusion nets reduced damage on peaches by 45% compared to unnetted and untreated trees, and on apples by 20% compared to the chemical control treatments. Moreover, the pearl anti-hail photoselective net used in our trials proved not to negatively affect the arthropod fauna present in the orchards as well as the quality of fruit production.

Key words: photoselective nets, brown marmorated stink bug, fruit orchards, fruit damage, quality and nutraceutical parameters, arthropod fauna.

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stal) (Heteroptera Pentatomidae), is a pest native to eastern Asia that, in recent years, has become invasive in North America and Europe. In particular, in Europe it has been recorded in Switzerland (2004), Liechtenstein (2007), Greece (2011), France, Germany, Italy (2012), Hungary (2013), Romania and Serbia (2015), and it continues to spread further east in Abkhazia, Georgia and Russia (Kriticos *et al*., 2017). In Italy, the first detection of *H. halys* occurred in Emilia Romagna in 2012 (Cesari *et al*., 2015), and then in Piedmont in 2013 (Pansa *et al*., 2013).

According to the studies on haplotypes, the populations recorded in these two Italian regions came from different areas (Cesari *et al*., 2017). Since then the pest has progressively spread in the Po Valley, and at present, it is reported nearly everywhere in North Italy, while it was occasionally detected in Central and South Italy (Bariselli *et al*., 2016). Following its first detection, *H. halys* has become a serious pest on many fruit crops. Initially, the most damaged crops were pear in Emilia Romagna and nectarine in Piedmont (Pansa *et al*., 2013; Cesari *et al*., 2015). However, in 2015-2016, the reports on damage caused by *H. halys* have increased in Piedmont involving other crops such as Asian pear, apple, hazelnut, corn and some vegetables (Pizzinat and Vittone, 2015; Rancati *et al*., 2017; Bosco *et al*., 2017).

The economic consequences of *H. halys* establishment are devastating. In 2010, high densities of this stink bug caused as much as 100% crop loss in some apple and peach orchards in the Eastern USA (Leskey *et al*., 2012) while in 2011, nearly 100% of the sweet corn was damaged in Maryland (Kuhar *et al*., 2012). At the moment, only local and fragmented information is available on the economic injury level of *H. halys* in different crops. In sweet corn, as low as one *H. halys* per ear is capable of causing great levels of kernel injury (Cissel *et al*., 2015) while for soybean the economic threshold of 5 bugs per 15 sweeps was confirmed (Aigner *at al*., 2016). In apple orchards, Short *et al*. (2017) showed how insecticide applications at a cumulative threshold of 10 adults of *H. halys* collected in a pheromone trap were effective at reducing fruit injury. Moreover, in this scenario, chemical control is particularly difficult due to the high mobility and polyphagy of *H. halys*. The lethal activity of insecticides depends on the bug generation, being the overwintered adults more susceptible than those ones of the summer generations (Leskey *et al*., 2014). Pyrethroids and neonicotinoids are effective in containing *H. halys* but the short residual activity of many compounds makes necessary to repeat the treatments every 7-10 days (Blaauw *et al*., 2015; 2016). The frequent pesticide applications have increased the cost of chemical treatments and have reduced the capacity of natural enemies to contain other pests making this management practice neither economically nor environmentally sustainable (Blaauw *et al*., 2016).

Exclusion nets represent one of the most readily available tools for crop protection and an environmental friendly alternative to pesticides (Castellano *et al*., 2008; Chouinard *et al*., 2016). In the recent years, the exclusion nets have progressively found wider application. Their effectiveness in excluding *Cydia pomonella* (L.) (Lepidoptera Tortricidae) in apple orchards has long been known (Tasin *et al*., 2008; Pasqualini *et al*., 2013). Moreover, exclusion nets proved to be useful for the control of aphids in apple orchards (Dib *et al*., 2010) and *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae) in cherry, raspberry and blueberry crops (Charlot *et al*., 2014; Cormier *et al*., 2015; Rogers *et al*., 2016).

Recently, coloured and photoselective anti-hail nets have been developed with the aim of improving plant production thanks to their optical properties in addition to their physical protective action. It is known that the coloured (e.g. blue, green, yellow, red) and neutral (e.g. white, grey, pearl) photoselective nets modify the spectral composition of solar light transmitted and reflected as well as transform the direct light into diffused light (Shahak *et al*., 2004; Basile *et al*., 2012; Ben-Yakir *et al*., 2012). For these reasons, photoselective nets are able to influence the fruit quality and nutraceutical compounds (Basile *et al*., 2012), and to decrease the infestation levels of aphids and whiteflies on tomato and pepper compared with black nets (Ben-Yakir *et al*., 2012).

This research was carried out in the frame of a project aimed at implementing IPM in the Croatian and Italian fruit production, reducing the use of pesticides, both in the field and in post-harvest (LIFE13 ENV/HR/000580). In this context, different types of net were previously compared in apple and peach orchards through prototypes with different colour and mesh (Candian *et al*., 2016). According to the results obtained with these prototypes, semi-field trials were set up in commercial orchards to test the effectiveness of a pearl photoselective anti-hail net in preventing *H. halys* from colonizing plants and damaging nectarines and apples in NW Italy. The effectiveness of the exclusion net was assessed by monitoring the pest populations and by evaluating the damage on fruits both during the growing season and at the harvest time. Moreover, any possible effects of the net on orchard arthropod communities, with a special regard to the predators, as well as on fruit quality were considered.

Materials and methods

Experimental sites

Semi-field trials were carried out in two nectarine orchards (cv. Amiga* and Fire Top®) and two apple orchards (cv. Baigent Brookfield® and Galaval*), located in the province of Cuneo (NW Italy) in 2016 (table 1). All the orchards were equipped with a structure for anti-hail net system.

Experimental design

The trials were arranged in a randomized complete block design with three replicates for each of the following treatments: 1) netted trees (N); 2) unnetted control trees (C); 3) trees without net but treated by insecticides (I). During the experimental trials, no insecticide treatments were applied in the three plots of N and C. In the remaining three replicates of I, the trees received routine pest control inputs as reported in table 2.

In each orchard, nine plots of neighbouring trees on the row, each consisting of 16 nectarine trees or 20 apple trees, were selected. In the three replicates of N, the trees were isolated by the pearl anti-hail photoselective net Tenax Iridium (mesh: 2.4 × 4.8 mm) [AGRITENAX, now AGRINTECH S.r.l., Eboli (SA), Italy]. The nets were set up hooking their upper side to the anti-hail net support and fixing the lower side to the ground with metal pegs. The exclusion nets were placed at the petal fall and removed at the end of the harvest time. Immediately after the closing of the nets, a knock-down treatment with the pyrethroid deltamethrin (Decis® Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL^{-1}) was performed to avoid any presence of the pest.

Monitoring of *H. halys*

To evaluate the presence and abundance of the pest inside and outside the exclusion nets, two DEAD-INN™ Stink Bug Traps (AgBio, Westminster, CO, USA) (high 121.92 cm), baited with the Xtra Combo lure provided with the trap, were placed one in a N replicate and one in a C replicate in each orchard. The lure was composed by the aggregation pheromones produced by the males of *H. halys* (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol

Table 1. Locations and characteristics of the sites where field surveys were carried out in 2016.

Orchard	Site	Position	Species	Cultivar	Area (ha)	Orchard age
1	Savigliano	44°37'19.5"N 7°37'32.6"E 321 m a.s.l.	*Prunus persica* (L.) Batsch	Amiga*	0.6	13
2	Savigliano	44°37'20.8"N 7°37'31.6"E 321 m a.s.l.	*Prunus persica* (L.) Batsch	Fire Top®	0.6	13
3	Cervignasco	44°41'35.7"N 7°30'47.0"E 280 m a.s.l.	*Malus domestica* Borkh.	Baigent Brookfield®	3.9	13
4	Revello	44°39'51.1"N 7°24'33.5"E 351 m a.s.l.	*Malus domestica* Borkh.	Galaval*	1.1	3

Table 2. Insecticidal treatments applied on I (i.e., trees without net and with insecticide treatments) from the net setting-up until the harvest time.

Orchard	Cultivar	Active ingredient	Trade name	Target	Quantity/ha	N° of treatments	Date
1	Amiga*	Chlorpyrifos methyl	Reldan	*Grapholita molesta* (Busck)	2.92 L	1	1st July
		Etofenprox	Trebon up	*Grapholita molesta* (Busck)	0.73 L	1	18th July
2	Fire Top®	Chlorpyrifos methyl	Reldan	*Grapholita molesta* (Busck)	2.92 L	1	1st July
		Etofenprox	Trebon up	*Grapholita molesta* (Busck)	0.73 L	1	18th July
3	Baigent Brookfield®	Chlorpyrifos	Terial 75 WG	Tortricidae	0.76 kg	2	20th June, 3rd July
		Chlorpyrifos methyl	Reldan	Tortricidae	3 L	1	29th July
		Etofenprox	Trebon Star ECC	Tortricidae	0.75 L	1	10th August
4	Galaval*	Chlorpyrifos methyl	Runner M	Tortricidae	3.23; 2.45 L	2	5th and 20th July
		Etofenprox	Trebon up	Tortricidae	0.57 L	1	16th August

and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and by the aggregation pheromone of *Plautia stali* Scott [methyl-(E,E,Z)-2,4,6-decatrienoate]. From the net installation until the end of the trials, traps were checked every 10 days and the lure was changed every four weeks accordingly to manufacturer's instructions. The specimens collected into the traps during each survey were identified and counted.

Damage on fruits all along the trials and at the harvest time

Since the net setting-up, 30 fruits per replicate in the treatments N and C (10 fruits per plant on three randomly selected trees) were visually inspected every 10 days to evaluate the damage caused by *H. halys* during the growing season. Overall, 180 nectarines and 270 apples were checked in each replicate.

At the harvest time, nectarines and apples were sampled from trees of each replicate in the treatments N, C and I, and analyzed for damage caused by *H. halys*. The fruits were picked in different dates following the growers' indication. Nectarines were harvested in two picking dates (Amiga*: July 26 and August 2; Fire Top®: August 2 and 8), while in apple orchards three picking dates occurred (Baigent Brookfield®: August 23 and 29, September 6; Galaval*: August 23 and 30, September 6). In each of the first two picking dates, 240 fruits per repetition were sampled in both nectarine and apple orchards, while in the third picking date 30 fruits per repetition were collected only in the apple orchards. Overall, 1440 nectarines and 1530 apples were picked in each treatment (N, C and I), with a total of 4320 fruits in each nectarine orchard and 4590 fruits in each apple orchard. The damage was identified according to Acebes-Doria *et al.* (2016): nectarines and apples were considered damaged if punctures, dimples, areas with superficial discoloration with or without depressions and areas with necrotic tissue after slicing the fruits were observed. In addition, on nectarines the presence of gummosis and fruit deformations was also evaluated. Similar symptoms could be caused also by native stink bugs which however have never been reported as noxious to peaches and apples in the study area.

Final knock-down treatment

At the end of the harvest time, to evaluate the arthropod fauna in the orchard, a knock down treatment with the pyrethroid deltamethrin (Decis® Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL^{-1}) was applied on one tree per repetition in the treatments N, C, I in each orchard. Before the treatment, a nylon tarpaulin (3 × 2 m) was lain under the canopy of the treated trees to make the arthropod collection easier. After 3 h, the canopy was beaten, and all the killed arthropods were collected on the nylon tarpaulin, and transferred to the laboratory into plastic tubes (50 mL) with 70% alcohol. In order to assess the arthropod fauna abundance depending on the treatment and, in particular, the possible effect of the net on the predators, the collected specimens were examined and sorted in the following clusters: 1) 'total catches', 2) 'predators', and 3) *H. halys*.

Quality and nutraceutical analysis

The following quality parameters were examined: the colour index, the firmness and the total soluble solid, while the following nutraceutical parameters were evaluated: the total anthocyanins and the total polyphenols.

The colour and the firmness were analyzed on 30 fruits per treatment and orchard for each fruit species. The colour was measured on the external part of the fruit using a portable colour analyser (Chroma Meter, model CR-400, Minolta, Langenhagen, Germany) equipped with a measuring head of 8 mm-diameter area.

The CIELAB scale defined by the Commission Internationale de L'Eclairage was used to describe the colour with the L* a* b* space coordinates. The colour parameters were expressed with a colour index (Martínez-Las Heras *et al*., 2016). The firmness was measured using a manual standard penetrometer (52200 Fruit penetrometer, Turoni, Forlì, Italy) (diameter of the probe 8 mm) with a kg scale. For each fruit, a slice of skin was removed using a cutter, and the probe was pushed into the flesh tissue to a depth of 9 mm. For the total soluble solid, 15 fruits were squeezed, the juice was distributed into a plastic tube and after centrifugation the subnatant was measured with a digital refractometer (PAL series, ATAGO CO, LTD, Tokyo, Japan).

The total anthocyanin and the total phenol were analyzed separately on the skin and on the fruit pulp for the apples, while the tissues were mixed for the nectarines. Every sample came from 10 fruits randomly selected per treatment and orchard for each fruit species. Both analyses were performed starting from an extract. The nectarine and apple extract was obtained using 10 g of fruit added to 25 mL of extraction solution (500 mL of methanol, 23.8 mL of de-ionized water and 1.4 mL of 37% hydrochloric acid). After 1 h in the dark at room temperature, the samples were thoroughly homogenized for 1 min with an ULTRA TURRAX (IKA, Staufen, Germany), and centrifuged at 3,019 g for 15 min. The supernatant obtained by centrifugation was collected, transferred into glass test tubes, and stored at −20 °C until analysis. The total anthocyanin content was quantified according to the pH differential method of Cheng and Breen (1991). Anthocyanins were estimated by the difference in absorbance at 510 and 700 nm in a buffer at pH 1.0 and pH 4.5. The results were expressed as mg of cyanidin-3-glucoside ($_{C3G}$) equivalents per 100 g of fresh weight (FW). The total phenolic content was measured using Folin-Ciocalteu reagent with gallic acid as a standard at 765 nm following the method of Slinkard and Singleton (1977). The results were expressed as mg of gallic acid equivalents ($_{GAE}$) per 100 g of FW.

Data analysis

The statistical analyses were performed using SPSS v23.0 (SPSS Inc., Chicago, IL, USA) and outcomes were considered significant at $P < 0.05$. The numbers of damaged fruits per treatment and orchard at the harvest time were compared using a generalized linear mixed model (GLMM; random effect: plot; fixed effects: treatment, block, picking date) with a binary distribution and logit link, and Bonferroni correction was applied. The data on arthropods collected by the knock-down treatment and the data on quality and nutraceutical parameters of fruit at harvest were checked for homogeneity of variance (Levene test) and normality (Shapiro-Wilk test), and compared using a one-way ANOVA; in the case of significant differences, the means were separated by Tukey's test. If the assumptions of ANOVA were not met, the data were analyzed using the Kruskal Wallis test, and the means were pairwise compared using the Mann-Whitney U test.

Results

Monitoring of *H. halys*

H. halys was detected by traps in all surveyed sites, but its population density was variable among the sites and along the season (figure 1). In both the nectarine orchards, it was never collected in traps in N, whereas it was caught in traps in C but in low amounts and close to

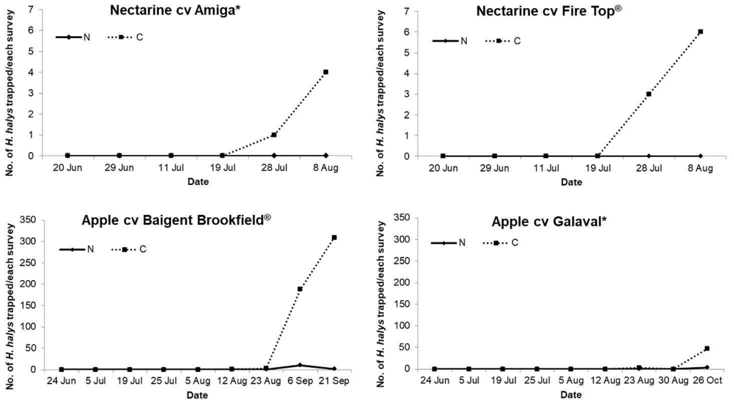


Figure 1. Total number of catches of *H. halys* in pheromone traps in the cultivar Amiga*, Fire Top®, Baigent Brookfield® and Galaval* in treatments N (netted trees) and C (unnetted control trees).

the harvest time. In Amiga*, 1 nymph and 4 adults were collected in early July and early August, respectively, while in Fire Top®, 3 nymphs, and 5 nymphs and 1 adult were caught in late July and early August, respectively. In apple orchards, the catches were higher than in nectarine orchards but later in the season when the trials in the nectarine orchards were already ended. In N, a few specimens were caught after the end of the harvest time only in Baigent Brookfield®, on which 10 nymphs and 1 nymph were collected in early and late September, respectively. In C, in Baigent Brookfield®, 496 nymphs and 5 adults were overall caught from mid-August, with a peak of 309 specimens in the second half of September. In Galaval*, 10 nymphs and 37 adults were caught at the end of October.

In all the orchards *H. halys* was observed to walk rapidly on net surface and reach the trees through the openings on the top of the anti-hail net system. Moreover, in all the orchards, the trees in the repetitions of N and of C with the pheromone trap (i.e., one of three repetitions per treatment and orchard) showed the highest damage rate in the respective treatment.

Damage on fruits during the growing season and at the harvest time

During the growing season, a low number of damaged fruits was recorded by visual inspection in all the orchards. Both in Amiga* and in Fire Top®, no damaged fruits were observed in N, while in C, damaged fruits were observed starting from late June. Out of 540 checked fruits in C in each orchard, only 23 damaged fruits were recorded in Amiga* (2.1%) and 18 in Fire Top® (1.7%). In both the apple orchards, damaged fruits were observed only in the first week of August. In Galaval*, no damaged fruits were found in N and only one damaged fruit was recorded in C. In Baigent Brookfield®, 3 damaged fruits (0.4%) were recorded in N and 4 (0.5%) in C.

The damage on fruits observed in each orchard at the harvest time is reported in table 3. By statistical analysis with the GLMM, in the nectarine orchards significant differences between the picking dates were not found, while significant differences between the treatments were observed both in Amiga* (F = 65.878, P = 0.024) and in Fire Top® (F = 7.735, P = 0.009), with a significantly lower damage in N. In the apple orchards, significant differences between the treatments were not observed, while differences between the picking dates were recorded only in Galaval* (F = 5.933, P = 0.012) with a significantly lower damage in the first picking date. No interactions between the treatments and the picking dates were recorded in any orchard. Moreover, the GLMM was used to analyze the block effect in order to assess if *H. halys* was more concentrated on the borders or in the middle of the orchards. Significant differences for the block effect were recorded only in Amiga* (F = 5.570, P = 0.024) with a higher concentration on the borders.

Final knock-down treatment

In the cluster 'total catches', all the specimens killed by the knock-down treatment were considered. Specimens belonging to Aranaeidae (Araneae); Acarina; Forficulidae (Dermaptera) [only in apple orchards]; Psocoptera; Thripidae (Thysanoptera); Anthocoridae, Nabidae, Tingidae, Coreidae, Lygeidae and Pentatomidae (Heteroptera); Cicadellidae and Aphidoidea (Homoptera); Hemerobiidae and Chrysopidae (Neuroptera); Staphylinidae, Coccinellidae, Chrysomelidae and Curculionidae (Coleoptera); Syrphidae and Drosophilidae (Diptera); Lepidoptera; and Hymenoptera were collected. Their amounts were significantly different be-

Table 3. Percentages (mean ± SE) of damaged fruits assessed in each picking date and in total on fruits sampled at harvest time (no. = 240 fruits per repetition in the first and second dates, no. = 30 fruits per repetition in the third date). In column for treatments and in row for picking dates, means followed by different letters are significantly different by the GLMM analysis (Bonferroni correction, P < 0.05).

Cultivar	Treatment	1st picking date	2nd picking date	3rd picking date	Total
Amiga*	N	4.4 ± 1.9	5.6 ± 1.3		4.9 ± 1.2 **c**
	C	45.6 ± 4.7	52.6 ± 2.9		49.1 ± 2.8 **a**
	I	22.9 ± 4.0	19.9 ± 2.2		21.4 ± 2.2 **b**
	Total	18.4 ± 2.7	20.2 ± 1.7		
Fire Top®	N	8.3 ± 4.7	5.6 ± 8.4		11.5 ± 4.5 **b**
	C	46.6 ± 8.6	60.1 ± 13.7		53.4 ± 8.3 **a**
	I	32.0 ± 7.7	37.8 ± 13.7		34.8 ± 7.7 **ab**
	Total	25.1 ± 4.9	35.6 ± 7.9		
Baigent Brookfield®	N	6.2 ± 2.2	4.6 ± 1.2	6.9 ± 3.9	5.8 ± 1.4
	C	7.7 ± 2.4	6.0 ± 1.5	7.6 ± 3.8	7.1 ± 1.6
	I	3.1 ± 1.5	5.3 ± 1.5	10.3 ± 5.1	5.5 ± 1.4
	Total	5.3 ± 1.2	5.3 ± 0.9	8.1 ± 2.5	
Galaval*	N	2.4 ± 1.7	7.6 ± 1.8	4.4 ± 2.2	4.3 ± 1.3
	C	3.4 ± 2.0	17.0 ± 2.5	11.7 ± 3.2	9.0 ± 1.9
	I	2.9 ± 1.8	8.9 ± 1.9	14.8 ± 3.6	7.4 ± 1.7
	Total	2.9 ± 1.1 **b**	10.6 ± 1.3 **a**	9.2 ± 1.9 **a**	

Treatment: N = netted trees, C = unnetted control trees, I = trees without net but treated by insecticides.

tween the treatments only in Galaval* with a lower number of specimens collected in treatment I (P = 0.049) (table 4). In the nectarine orchards, Aranaeidae (Araneae), *Allothrombium fuliginosum* (Hermann) (Acarina), Anthocoridae and Nabidae (Hemiptera), Hemerobiidae and Chrysopidae (Neuroptera), Staphylinidae and Coccinellidae (Coleoptera), Syrphidae (Diptera) were grouped in 'predators'. In the apple orchards, 'predators' included also Forficulidae (Dermaptera). Numbers of predators were significantly different between the treatments in Amiga* (P = 0.034), on which higher numbers were collected in C (table 4). Finally, lower numbers of *H. halys* were generally collected in N, even if significant differences were found only in Fire Top® (P = 0.014) (table 4).

Quality and nutraceutical analysis

For the nectarines, no statistical differences between the treatments were observed for all the quality analyses performed for Amiga* and Fire Top® (table 5). A lower colour index parameter was obtained in Amiga* for all three treatments compared to Fire Top®. Regarding the total polyphenol and anthocyanin tested on the whole fruit, no statistical differences were observed between the treatments (Amiga*: P = 0.48; P = 0.50; Fire Top®: P = 0.08; P = 0.07). In Fire Top®, a higher concentration of total anthocyanins was measured for all three treatments compared to Amiga* (table 5).

For the apples, no statistical differences between the treatments were observed in both the cultivars for the quality parameters (table 6). In Baigent Brookfield® apple fruits the non-significant differences were confirmed for the total phenols in the pulp (P = 0.44) and for the total anthocyanins in the pulp (P = 0.27). In Galaval*, stastistical differences were not recorded for the total phenol in the peel (P = 0.56) and for the total anthocyanin in the pulp and in the peel (P = 0.09 and P = 0.31, respectively). Significant differences between the treatments were observed for the total phenols in the peel (P = 0.04) in Baigent Brookfield®, and in the pulp in Galaval* (P = 0.04), with higher values in N and C, respectively. Moreover, in Baigent Brookfield®, statistical differences between the treatments were recorded for the total anthocyanins in the pulp (P = 0.01) with higher values in C.

Discussion and conclusions

The initial absence of reliable monitoring tools and the low effectiveness of chemical control have led *H. halys* becoming a serious pest in fruit crops in North Italy, where it has found favourable conditions for its establishment. In particular, in Piedmont (NW Italy), *H. halys* has a great potential in reaching high infestation levels, since about 40% of the overwintered adults proved to

Table 4. Insects collected after the knock-down treatment (mean ± SE). In column, means followed by different letters are significantly different (Amiga*: Mann-Whitney *U*-test, P < 0.05; Fire Top®, Galaval*: Tukey test, P < 0.05).

Date	Cultivar	Treatments	Total catches	Predators	*H. halys*
16 August	Amiga*	N	23.00 ± 4.00	3.00 ± 0.00 **b**	0.00 ± 0.00
		C	112.33 ± 16.68	11.67 ± 4.25 **a**	19.33 ± 11.26
		I	23.33 ± 0.67	2.00 ± 0.58 **b**	8.00 ± 3.21
09 August	Fire Top®	N	354.33 ± 304.36	8.67 ± 3.18	6.67 ± 6.17 **b**
		C	155.67 ± 37.82	15.33 ± 6.44	35.33 ± 3.53 **a**
		I	52.67 ± 6.64	6.67 ± 1.20	9.33 ± 5.46 **b**
20 September	Baigent Brookfield®	N	199.00 ± 162.56	42.00 ± 30.50	0.33 ± 0.33
		C	57.00 ± 2.89	25.00 ± 3.61	2.67 ± 2.67
		I	50.33 ± 9.96	30.33 ± 8.45	0.33 ± 0.33
19 October	Galaval*	N	36.33 ± 3.48 **ab**	23.67 ± 4.10	0.33 ± 0.33
		C	40.33 ± 5.78 **a**	28.00 ± 7.37	1.33 ± 1.33
		I	22.67 ± 2.03 **b**	12.67 ± 1.45	0.00 ± 0.00

Treatment: N = netted trees, C = unnetted control trees, I = trees without net but treated by insecticides.

Table 5. Colour index, firmness, total solid soluble, total polyphenols and total anthocyanins (mean ± SE) of the two picking dates for nectarine orchards. No significant differences were found by ANOVA.

Cultivar	Treatment	Colour index	Firmness (g cm^{-2})	Tot. solid soluble (°Brix)	Tot. polyphenols (mg$_{GAE}$ 100g^{-1})	Tot. anthocyanins (mg$_{C3G}$ 100g^{-1})
Amiga*	N	33.11 ± 2.41	5.45 ± 0.11	8.62 ± 0.09	34.23 ± 3.49	8.74 ± 0.30
	C	35.03 ± 2.56	5.63 ± 0.08	8.88 ± 0.08	33.52 ± 1.80	7.54 ± 1.55
	I	39.42 ± 2.87	5.83 ± 0.09	8.57 ± 0.08	38.98 ± 3.76	8.74 ± 12.17
Fire Top®	N	49.12 ± 1.91	4.23 ± 0.11	8.15 ± 0.07	38.85 ± 3.12	17.49 ± 1.47
	C	48.05 ± 1.58	4.49 ± 0.06	8.41 ± 0.06	43.73 ± 3.69	20.93 ± 3.29
	I	50.59 ± 2.46	4.20 ± 0.07	8.24 ± 0.11	44.30 ± 3.98	11.60 ± 2.49

Treatment: N = netted trees, C = unnetted control trees, I = trees without net but treated by insecticides.

Table 6. Colour index, firmness, total solid soluble, total polyphenols and total anthocyanins (mean ± SE) of the two picking dates for apple orchards. In column, means followed by different letters are significantly different (Tukey's test, $P < 0.05$).

Cultivar	Treatment	Colour index	Firmness (g cm^{-2})	Total solid soluble (°Brix)	Total polyphenols (mg_{GAE} $100g^{-1}$)		Total anthocyanins (mg_{C3G} $100g^{-1}$)	
					Pulp	Peel	Pulp	Peel
Baigent Brookfield®	N	39.09 ± 0.66	7.45 ± 0.06	13.85 ± 0.09	0.00 ± 0.00	61.72 ± 2.22 **a**	1.15 ± 0.16	32.96 ± 0.80 **b**
	C	40.01 ± 0.93	7.58 ± 0.06	13.45 ± 0.09	0.00 ± 0.00	44.91 ± 0.48 **b**	1.17 ± 0.33	58.02 ± 2.96 **a**
	I	49.79 ± 1.00	7.51 ± 0.07	14.00 ± 0.08	0.00 ± 0.00	39.14 ± 2.74 **b**	2.10 ± 0.44	32.84 ± 1.63 **b**
Galaval*	N	44.46 ± 1.15	7.99 ± 0.08	12.89 ± 0.09	13.99 ± 4.29 **a**	28.51 ± 2.99	2.71 ± 41.8	19.28 ± 1.71
	C	50.06 ± 0.96	7.72 ± 0.09	13.59 ± 0.08	9.16 ± 2.48 **ab**	36.98 ± 3.23	5.94 ± 1.83	17.47 ±1.70
	I	54.25 ± 1.26	8.15 ± 0.40	13.03 ± 0.09	0.00 ± 0.00 **b**	25.11 ± 2.22	1.56 ± 0.43	18.09 ± 1.68

Treatment: N = netted trees, C = unnetted control trees, I = trees without net but treated by insecticides.

survive thanks to the progressive exit from the shelters as a strategy, occurring for a long period between early March and mid-June (authors' observation). In this context, innovative efficient and sustainable control strategies are required in order to preserve high quality fruit productions. Our results prove the effectiveness of the exclusion nets in the protection of the orchards against this pest: the catches with the pheromone traps were none at all or very low under nets, and more abundant outside nets.

The orchards chosen for our study are located in an area in which the presence of *H. halys* was already reported in the previous year, but considerably increased in 2016 although in an uneven pattern. In fact, in our trials, *H. halys* was recorded in all the experimental orchards, but its population density was very variable depending on the season and on the crop. In all these orchards, *H. halys* was never trapped at the beginning of the growing season but only close to the harvest time and, overall, catches by pheromone traps did not always reflect the real abundance of the pest in the field, probably due to various reasons. It was already highlighted by AgBio (http://www.agbio-inc.com/) that overwintered adults emerging in the spring do not respond to the lure. Probably, upon emergence from overwintering sites, being in a dispersal phase searching for food sources, *H. halys* is more attracted by kairomones emitted by plants compared to the lures used in traps. On the contrary, consistently higher captures were recorded in apple orchards in late summer, a period in which decreasing day length and temperature trigger *H. halys* an aggregation behaviour before moving to overwintering sites (Lee *et al*., 2013). Moreover, studies on genetic diversity of Italian populations revealed the presence of various *H. halys* haplotypes in Piedmont (Cesari *et al*., 2017), which could have a different (less efficient) response to the lure, for example at the beginning of the season.

The uneven *H. halys* population density in the orchards as assessed by damage rate at the harvest is also due to a diverse attractiveness of the different crops. Despite its high polyphagy, *H. halys* can be considered a fruit specialist, seeking and moving among trees that differentially bear fruit in space and time (Martinson *et al*., 2015). It is evident that *H. halys* firstly moves to other temporary hosts, which may be used as a water source (Lee *et al*., 2013), before colonizing crops. As a consequence, the first damaged fruits were observed close to the beginning of the harvest time in all the orchards. Then, among the different crops, *H. halys* has a preference for peaches as a favourite host. This behaviour seems to be due to the fact that peach is the only fruit crop able to support the development of the pest from the end of May until the harvest (Blaauw *et al*., 2016).

Besides the different number of specimens captured inside and outside the net by the traps, the best evidence for the effectiveness of the nets against *H. halys* comes from the assessment of damaged fruits in the three treatments in comparison. In particular, in nectarine orchards the number of damaged fruits was always significantly lower inside than outside nets even when trees were regularly treated with insecticides by the growers (I). As shown in table 2, there are no specific insecticidal treatments applied against *H. halys* because, at the moment, there are few products registered against this pest in Italy. However, the insecticide classes of pyrethroids (etofenprox) and organophosphates (chlorpyriphos and chlorphyriphos methyl) have been shown to be effective (Leskey *et al*., 2014; Blaauw *et al*., 2015; 2016). In Piedmont, the current European, National and Regional directives place severe restrictions on the use of chemical products for crop protection limiting the number of the allowed treatments in order to reduce residues in food. Blaauw *et al*. (2015; 2016) agree that the short residual activity of many compounds makes necessary to repeat the treatments every 7-10 days, but this, beyond being not always applicable in our region, would nullify the integrated pest management now largely adopted in fruit orchards. Our studies showed that the exclusion nets are more effective than chemical treatments in containing *H. halys* damage; thus, considering also the phytosanitary directives, the net coverage can be a great-value alternative for the management of *H. halys*. In apple orchards, the differences between the treatments, more evident in the last picking date, were never significant, probably due to the low pest density before harvest. The lower number of injured apples compared to nectarines could also be due to a lower level of expression of the damage. It is proved that *H. halys* feeding on apples during the last 1-2 weeks before harvest may not be expressed as injury at harvest; however, apples showing no surface injury at harvest may develop both surface and internal injuries following a period in post-harvest cold storage (Bergh *et al*., 2016). It should also be taken into account that our trials were carried out on early ripening apple cultivars, and that probably late ripening apple cultivars may be subject to a higher pressure by this pest.

Statistical differences for the block effect were recorded only in Amiga*, but in general the damage was higher on netted and unnetted trees closer to the edges, and mainly when the orchard borders on other peach orchards or soybean fields. In particular, in Galaval*, the damage was higher on netted and unnetted apples close to the edge bordering a peach orchard, while the nectarines were more damaged in the edge bordering soybean. Leskey *et al*. (2012) already found that the *H. halys* is a perimeter-driven threat. In their research, injury was usually significantly greater at the exterior of orchard relative to the interior, suggesting an adult emigration from overwintering sites in the early season and from wood lots or cultivated hosts later in the season.

Although the use of the exclusion net is increasing, there are still too few studies on the impact that the net can have on the beneficial arthropods. In complete exclusion systems, Marliac *et al*. (2013) reported side-effects of codling moth exclusion netting on Miridae, Anthocoridae, Syrphidae and Coccinellidae, natural predators of the rosy apple aphid *Dysaphis plantaginea* (Passerini). Similarly Dib *et al*. (2010) and Romet *et al*. (2010) reported a lower abundance of Syrphidae and Coccinellidae under apple netted plots than uncovered ones. We evaluated the possible effect of the nets on the orchard arthropod fauna, in particular predators, with a final knock-down treatment. In general, the presence of the net did not have negative influences on the abundance of the 'total catches' and on 'predators', although the net coverage caused a reduction in *H. halys* population. Probably, these results are due to the mesh of the net we used: it is thin enough to keep out *H. halys* (at least the adults), but at the same large enough to allow most beneficial insects to pass through.

The mesh size is a very critical issue not only for the exclusion effectiveness, but also for the consequences on the microclimate occurring under the net, which can affect fruit quality and yield. Changes to the orchard microclimate are significantly greater where nets with small mesh size are used. Net colour also influences fruit quality and yield. Positive effects of photoselective nets were already reported by several authors (Shahak *et al*., 2004; Retamales *et al*., 2008; Basile *et al*., 2012). Our qualitative analyses showed that the fruit quality was not negatively influenced by the net coverage; actually, in some cases, the pearl photoselective net was able to enhance the nutraceutical properties. A greater source of total polyphenol compounds in the peel of the Baigent Brookfield® apples grown under net (N) compared to the unnetted treatments (C and I) may have therapeutic value (Scalbert *et al*., 2005; Almeida *et al*., 2008; Mileo and Miccadei, 2015; Zhang and Tsao, 2016).

Moreover, apples coming from the trials and subjected to cold storage after harvest revealed interesting preliminary results on the effect of the nets on physiological disorders such as bitter pit. For both cultivars, and in particular for Galaval*, the nets reduced the incidence of bitter pit (Davide Spadaro, DISAFA, personal communication) as already observed by do Amarante *et al*. (2011) supposing that a lower leaf transpiration under the nets might increase the xylem transport of calcium to the fruits in detriment to the shoots, therefore reducing bitter pit. Moreover in our trial, no differences between the treatments were found as regards the incidence of apple scab and brown rot (Davide Spadaro, DISAFA, personal communication).

In terms of costs, in all the cases in which an anti-hail net system is already present in the orchard, a single-plot exclusion-net system is more feasible, entailing a 2,300 €/ha cost increase depreciable in 15 years. This strategy allows to save approximately 280 €/ha per year compared with chemical control against *C. pomonella* in areas with a high moth pressure (Pavarino and Vittone, 2014). All the more reasons, this saving will be even higher considering the cost of chemical control against *H. halys*. Naturally, it is necessary to ensure the uniformity of the closure of the anti-hail net on the top to prevent any entrance of the pest. As a consequence, an easy opening system and a sufficient space for the entry and the manoeuvre of the machineries should be provided. By contrast, in orchards without an anti-hail net coverage the single row strategy could be economically more advantageous. However, in this case, farming operations such as pruning and harvesting will be harder, whereas fungicide treatments will be easier because of their application through the net.

A good exclusion net system could be even more cost-effective considering that it can prevent more than one pest species at a time, reducing or eliminating costs associated with insecticide use, and open up new opportunities as a "ready to use" tool against other worrisome emerging pests, such as the highly polyphagous *Popillia japonica* Newman (Coleoptera Rutelidae) recently reported in North Italy (Pavesi, 2014). Finally, although in areas of landscape value, exclusion nets may have a strong visual impact, in highly specialized fruit-growing areas, already equipped with anti-hail systems and subjected to a high pressure of the phytophagous, they can be a great resource.

Acknowledgements

We thank Luca Rivoira and Enrico Tomatis for their support in experimental activities, and the growers Silvano Ezio Bertorello, Giuseppe Borretta and Andrea Ingaramo for their hospitality. This work was supported by the EU LIFE13 ENV/HR/000580 project "Low pesticide IPM in sustainable and safe fruit production".

References

ACEBES-DORIA A. L., LESKEY T. C., BERGH J. C., 2016.- Injury to apples and peaches at harvest from feeding by *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs early and late in the season.- *Crop Protection*, 89: 58-65.

Aigner B. L., Herbert D. A., Dively G. P., Venugopal D., Whalen J., Cissel B., Kuhar T. P., Brewster C. C., Hogue J. W, Seymore E., 2016.- Comparison of two sampling methods for assessing *Halyomorpha halys* (Hemiptera: Pentatomidae) numbers in soybean fields.- *Journal of Economic Entomology*, 109: 2586-2589.

Almeida I. F., Valentão P., Andrade P. B., Seabra R. M., Pereira T. M., Amaral M. H., Costa P. C., Bahia M. F., 2008.- *In vivo* skin irritation potential of a *Castanea sativa* (chestnut) leaf extract, a putative natural antioxidant for topical application.- *Basic & Clinical Pharmacology & Toxicology*, 103: 461-467.

Bariselli M., Bugiani R., Maistrello L., 2016.- Distribution and damage caused by *Halyomorpha halys* in Italy.- *EPPO Bullettin*, 46 (2): 332-334.

Basile B., Giaccone M., Cirillo C., Ritieni A., Graziani C., Shahak Y., Florani M., 2012.- Photo-selective hail nets affect fruit size and quality in hayward kiwifruit.- *Scientia Horticulturae*, 141: 91-97.

Ben-Yakir D., Antignus Y., Offir Y., Shahak Y., 2012.- Colored shading nets impede insect invasion and decrease the incidences of insect-transmitted viral diseases in vegetable crops.- *Entomologia Experimentalis et Applicata*, 144 (3): 249-257.

Bergh C., Acebes-Doria A., Leskey T., Morrison R., Short B., Krawczyk G., Walgenbach J., Agnello A., Jentsch P., Hamilton G., Nielsen A., Blaauw B., Walton V., Wiman N., Hedstrom C., Shearer P., Beers B., 2016.- *Integrated pest management for brown marmorated stink bug in orchard crops*.- Brown Marmorated Stink Bug SCRI CAP Orchard Crop Commodity Team and Northeastern IPM Center. [online] URL: http://www.stopbmsb.org/where-is-bmsb/crop-by-crop/orchard-crops/

Blaauw B. R., Polk D., Nielsen A. L., 2015.- IPM-CPR for peaches: incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach: behaviorally-based methods to manage *H. halys* and key pests in peach.- *Pest Management Science*, 71 (11): 1513-1522.

Blaauw B. R., Jones V. P., Nielsen A. L., 2016.- Utilizing immunomarking techniques to track *Halyomorpha halys* (Hemiptera: Pentatomidae) movement and distribution within a peach orchard.- *PeerJ*, 4: e1997.

Bosco L., Moraglio S. T., Tavella L., 2017.- *Halyomorpha halys*, a serious threat for hazelnut in newly invaded areas.- *Journal of Pest Science*, (online first) doi: 10.1007/s10340-017-0937-x

Candian V., Pansa M. G., Tavella L., Tedeschi R., 2016.- Il progetto LIFE+ SU.SA.FRUIT - Utilizzo delle reti escludi-insetto per una frutticoltura sostenibile: l'esperienza in Piemonte, p. 168. In: *Atti XXV Congresso Nazionale Italiano di Entomologia*, 20-24 June 2016, Padova, Italy.

Castellano S., Scarascia Mugnozza G., Russo G., Briassoulis D., Mistriotis A., Hemming S., Waaijenberg D., 2008.- Plastic nets in agriculture: a general review of types and applications.- *Applied Engineering in Agriculture*, 24 (6): 799-808.

Cesari M., Maistrello L., Ganzerli F., Dioli P., Rebecchi L., Guidetti R., 2015.- A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug *Halyomorpha halys* populations in Italy.- *Journal of Pest Science*, 88 (1): 1-7.

Cesari M., Maistrello L., Piemontese L., Bonini R., Dioli P., Lee W., Park C-G., Partsinevelos G. K., Rebecchi L., Guidetti R., 2017- Genetic diversity of the brown marmorated stink bug *Halyomorpha halys* in the invaded territories of Europe and its patterns of diffusion in Italy.- *Biological Invasions*, (online first) doi: 10.1007/s10530-017-1611-1

Charlot G., Weydert C., Millan M., Brachet M. L., Warlop F., 2014.- Nets and covers to protect cherry trees from rain and insects, pp. 222-227. In: *Proceedings of Ecofruit. 16th International Conference on Organic-Fruit Growing*, Hohenheim, 17-19 February 2014. Fördergemeinschaft Ökologischer Obstbau e.V., Weinsberg, Germany.

Cheng G. W., Breen P. J., 1991.- Activity of phenylalanine ammonia-lyase (pal) and concentrations of anthocyanins and phenolics in developing strawberry fruit.- *Journal of the American Society for Horticultural Science*, 116 (5): 865-869.

Chouinard G., Firlej A., Cormier D., 2016.- Going beyond sprays and killing agents: exclusion, sterilization and disruption for insect pest control in pome and stone fruit orchards.- *Scientia Horticulturae*, 208: 13-27.

Cissel W. J., Mason C. E., Whalen J., Hough-Goldstein J., Hooks C. R. R., 2015.- Effects of brown marmorated stink bug (Hemiptera: Pentatomidae) feeding injury on sweet corn yield and quality.- *Journal of Economy Entomology*, 108: 1065-1071.

Cormier D., Veilleux J., Firlej A., 2015.- Exclusion net to control spotted wing *Drosophila* in blueberry fields.- *IOBC/wprs Bulletin*, 109: 181-184.

Dib H., Sauphanor B., Capowiez Y., 2010.- Effect of codling moth exclusion nets on the rosy apple aphid, *Dysaphis plantaginea*, and its control by natural enemies.- *Crop Protection*, 29 (12): 1502-1513.

do Amarante C. V. T., Steffens C. A., Argenta L. C., 2011.- Yield and fruit quality of 'Gala' and 'Fuji' apple trees protected by white anti-hail net.- *Scientia Horticulturae*, 129: 79-85.

Kriticos D. J., Kean J. M., Phillips C. B., Senay S. D., Acosta H., Haye T., 2017.- The potential global distribution of the brown marmorated stink bug, *Halyomorpha halys*, a critical threat to plant biosecurity.- *Journal of Pest Science*, 90: 1033-1043.

Kuhar T. P., Kamminga K. L., Whalen J., Dively G. P., Brust G., Hamilton G., Hooks C., Herbert D. A., 2012.- The pest potential of brown marmorated stink bug on vegetable crops.- *Plant Health Progress*, 2012 (May).

Lee D. H., Short B. D., Joseph S. V., Bergh J. C., Leskey T. C., 2013.- Review of the biology, ecology and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan and the Republic of Korea.- *Environmental Entomology*, 42 (4): 627-641.

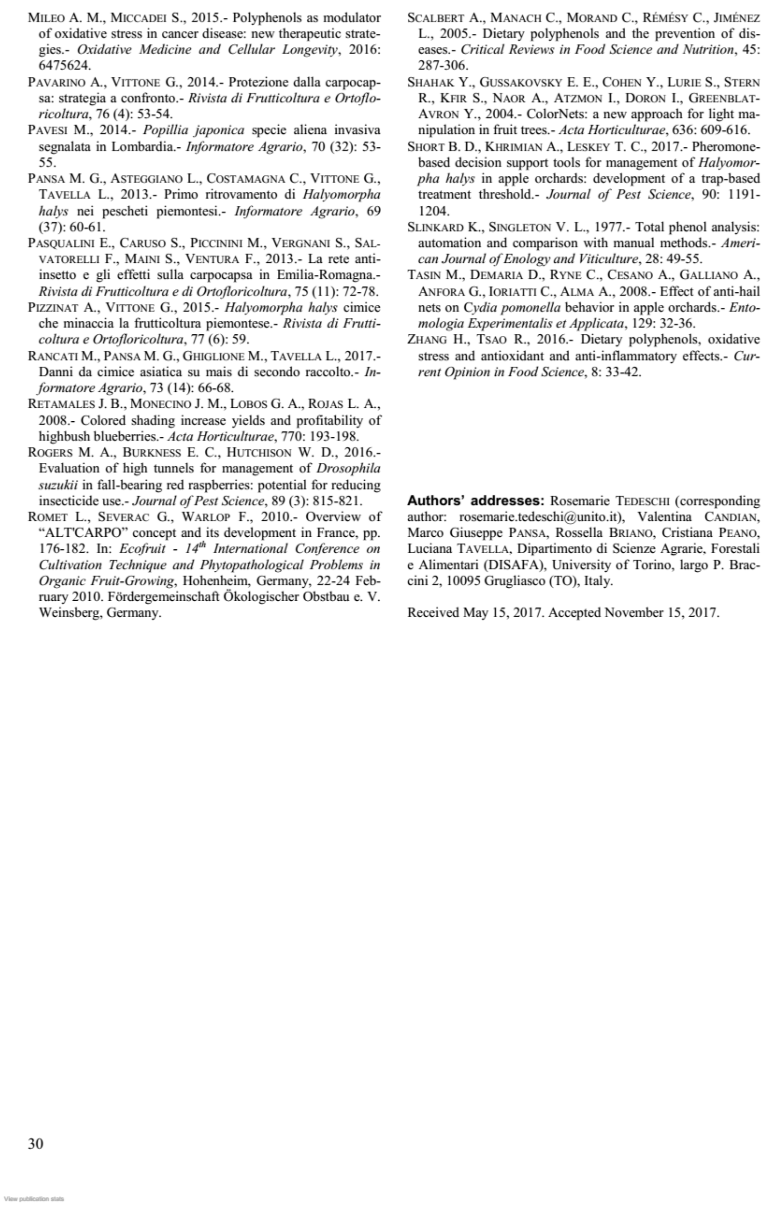
Leskey T. C., Short B. D., Butler B. R., Wright S. E., 2012.- Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-atlantic tree fruit orchards in the United States: case studies of commercial management.- *Psyche*, 2012: 535062.

Leskey T. C., Short B. D., Lee D. H., 2014.- Efficacy of insecticide residues on adult *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards.- *Pest Management Science*, 70 (7): 1097-1104.

Marliac G., Simon S., Fleury A., Alaphilippe A., Dib H., Capowiez Y., 2013.- Contrasting effects of codling moth exclusion netting on the natural control of the rosy apple aphid.- *IOBC/wprs Bulletin*, 91: 81-85.

Martínez-Las Heras R., Amigo-Sánchez J. C., Heredia A., Castelló M. L., Andrés A., 2016.- Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage.- *CyTA - Journal of Food*, 14 (2): 333-339.

Martinson H. M., Venugopal P. D., Bergmann E. J., Shrewsbury P. M., Raupp M. J. 2015.- Fruit availability influences the seasonal abundance of invasive stink bugs in ornamental tree nurseries.- *Journal of Pest Science*, 88 (3): 461-468.



Mileo A. M., Miccadei S., 2015.- Polyphenols as modulator of oxidative stress in cancer disease: new therapeutic strategies.- *Oxidative Medicine and Cellular Longevity*, 2016: 6475624.

Pavarino A., Vittone G., 2014.- Protezione dalla carpocapsa: strategia a confronto.- *Rivista di Frutticoltura e Ortofloricoltura*, 76 (4): 53-54.

Pavesi M., 2014.- *Popillia japonica* specie aliena invasiva segnalata in Lombardia.- *Informatore Agrario*, 70 (32): 53-55.

Pansa M. G., Asteggiano L., Costamagna C., Vittone G., Tavella L., 2013.- Primo ritrovamento di *Halyomorpha halys* nei pescheti piemontesi.- *Informatore Agrario*, 69 (37): 60-61.

Pasqualini E., Caruso S., Piccinini M., Vergnani S., Salvatorelli F., Maini S., Ventura F., 2013.- La rete anti-insetto e gli effetti sulla carpocapsa in Emilia-Romagna.- *Rivista di Frutticoltura e di Ortofloricoltura*, 75 (11): 72-78.

Pizzinat A., Vittone G., 2015.- *Halyomorpha halys* cimice che minaccia la frutticoltura piemontese.- *Rivista di Frutticoltura e Ortofloricoltura*, 77 (6): 59.

Rancati M., Pansa M. G., Ghiglione M., Tavella L., 2017.- Danni da cimice asiatica su mais di secondo raccolto.- *Informatore Agrario*, 73 (14): 66-68.

Retamales J. B., Monecino J. M., Lobos G. A., Rojas L. A., 2008.- Colored shading increase yields and profitability of highbush blueberries.- *Acta Horticulturae*, 770: 193-198.

Rogers M. A., Burkness E. C., Hutchison W. D., 2016.- Evaluation of high tunnels for management of *Drosophila suzukii* in fall-bearing red raspberries: potential for reducing insecticide use.- *Journal of Pest Science*, 89 (3): 815-821.

Romet L., Severac G., Warlop F., 2010.- Overview of “ALT'CARPO” concept and its development in France, pp. 176-182. In: *Ecofruit - 14th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing*, Hohenheim, Germany, 22-24 February 2010. Fördergemeinschaft Ökologischer Obstbau e. V. Weinsberg, Germany.

Scalbert A., Manach C., Morand C., Rémésy C., Jiménez L., 2005.- Dietary polyphenols and the prevention of diseases.- *Critical Reviews in Food Science and Nutrition*, 45: 287-306.

Shahak Y., Gussakovsky E. E., Cohen Y., Lurie S., Stern R., Kfir S., Naor A., Atzmon I., Doron I., Greenblat-Avron Y., 2004.- ColorNets: a new approach for light manipulation in fruit trees.- *Acta Horticulturae*, 636: 609-616.

Short B. D., Khrimian A., Leskey T. C., 2017.- Pheromone-based decision support tools for management of *Halyomorpha halys* in apple orchards: development of a trap-based treatment threshold.- *Journal of Pest Science*, 90: 1191-1204.

Slinkard K., Singleton V. L., 1977.- Total phenol analysis: automation and comparison with manual methods.- *American Journal of Enology and Viticulture*, 28: 49-55.

Tasin M., Demaria D., Ryne C., Cesano A., Galliano A., Anfora G., Ioriatti C., Alma A., 2008.- Effect of anti-hail nets on *Cydia pomonella* behavior in apple orchards.- *Entomologia Experimentalis et Applicata*, 129: 32-36.

Zhang H., Tsao R., 2016.- Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects.- *Current Opinion in Food Science*, 8: 33-42.

Authors' addresses: Rosemarie Tedeschi (corresponding author: rosemarie.tedeschi@unito.it), Valentina Candian, Marco Giuseppe Pansa, Rossella Briano, Cristiana Peano, Luciana Tavella, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

Received May 15, 2017. Accepted November 15, 2017.