

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

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## 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 Vittoni, 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 *et 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<sup>-1</sup>) 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.	<i>Prunus persica</i> (L.) Batsch	Amiga*	0.6	13
2	Savigliano	44°37'20.8"N 7°37'31.6"E 321 m a.s.l.	<i>Prunus persica</i> (L.) Batsch	Fire Top®	0.6	13
3	Cervignasco	44°41'35.7"N 7°30'47.0"E 280 m a.s.l.	<i>Malus domestica</i> Borkh.	Baigent Brookfield®	3.9	13
4	Revello	44°39'51.1"N 7°24'33.5"E 351 m a.s.l.	<i>Malus domestica</i> 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	<i>Grapholita molesta</i> (Busck)	2.92 L	1	1 <sup>st</sup> July
		Etofenprox	Trebon up	<i>Grapholita molesta</i> (Busck)	0.73 L	1	18 <sup>th</sup> July
2	Fire Top <sup>®</sup>	Chlorpyrifos methyl	Reldan	<i>Grapholita molesta</i> (Busck)	2.92 L	1	1 <sup>st</sup> July
		Etofenprox	Trebon up	<i>Grapholita molesta</i> (Busck)	0.73 L	1	18 <sup>th</sup> July
3	Baigent Brookfield <sup>®</sup>	Chlorpyrifos	Terial 75 WG	Tortricidae	0.76 kg	2	20 <sup>th</sup> June, 3 <sup>rd</sup> July
		Chlorpyrifos methyl	Reldan	Tortricidae	3 L	1	29 <sup>th</sup> July
		Etofenprox	Trebon Star ECC	Tortricidae	0.75 L	1	10 <sup>th</sup> August
4	Galaval*	Chlorpyrifos methyl	Runner M	Tortricidae	3.23; 2.45 L	2	5 <sup>th</sup> and 20 <sup>th</sup> July
		Etofenprox	Trebon up	Tortricidae	0.57 L	1	16 <sup>th</sup> 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<sup>®</sup>: August 2 and 8), while in apple orchards three picking dates occurred (Baigent Brookfield<sup>®</sup>: 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<sup>®</sup> Jet, Bayer Crop-Science AG, Monheim am Rhein, Germany, 120 mL hL<sup>-1</sup>) 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 laid 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 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 (Martinez-Las Heras *et al.*, 2016). The firmness was measured using a manual standard penetrometer (52200 Fruit penetrometer, Turoni, Forli, 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 supernatant 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 (C<sub>3G</sub>) 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.

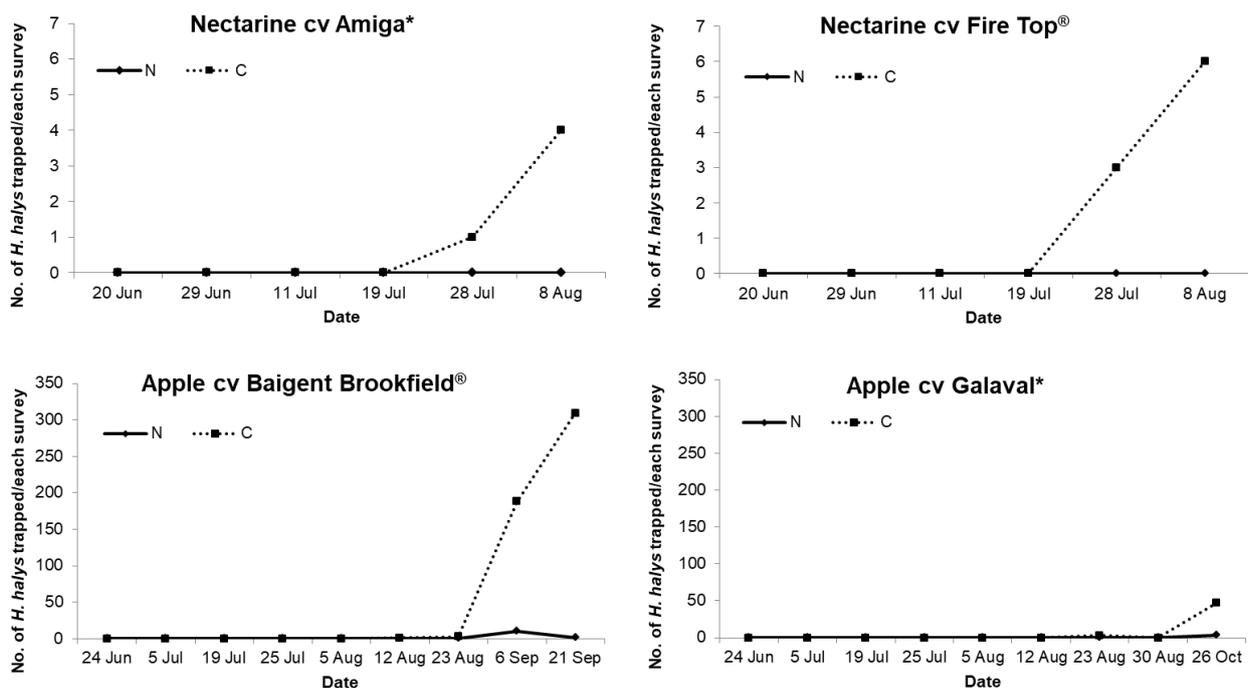
### Data analysis

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 Brook-

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

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\*, statistical 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  $\pm$  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	<i>H. halys</i>
16 August	Amiga*	N	23.00 $\pm$ 4.00	3.00 $\pm$ 0.00 <b>b</b>	0.00 $\pm$ 0.00
		C	112.33 $\pm$ 16.68	11.67 $\pm$ 4.25 <b>a</b>	19.33 $\pm$ 11.26
		I	23.33 $\pm$ 0.67	2.00 $\pm$ 0.58 <b>b</b>	8.00 $\pm$ 3.21
09 August	Fire Top®	N	354.33 $\pm$ 304.36	8.67 $\pm$ 3.18	6.67 $\pm$ 6.17 <b>b</b>
		C	155.67 $\pm$ 37.82	15.33 $\pm$ 6.44	35.33 $\pm$ 3.53 <b>a</b>
		I	52.67 $\pm$ 6.64	6.67 $\pm$ 1.20	9.33 $\pm$ 5.46 <b>b</b>
20 September	Baigent Brookfield®	N	199.00 $\pm$ 162.56	42.00 $\pm$ 30.50	0.33 $\pm$ 0.33
		C	57.00 $\pm$ 2.89	25.00 $\pm$ 3.61	2.67 $\pm$ 2.67
		I	50.33 $\pm$ 9.96	30.33 $\pm$ 8.45	0.33 $\pm$ 0.33
19 October	Galaval*	N	36.33 $\pm$ 3.48 <b>ab</b>	23.67 $\pm$ 4.10	0.33 $\pm$ 0.33
		C	40.33 $\pm$ 5.78 <b>a</b>	28.00 $\pm$ 7.37	1.33 $\pm$ 1.33
		I	22.67 $\pm$ 2.03 <b>b</b>	12.67 $\pm$ 1.45	0.00 $\pm$ 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  $\pm$  SE) of the two picking dates for nectarine orchards. No significant differences were found by ANOVA.

Cultivar	Treatment	Colour index	Firmness (g cm <sup>-2</sup> )	Tot. solid soluble (°Brix)	Tot. polyphenols (mg <sub>GAE</sub> 100g <sup>-1</sup> )	Tot. anthocyanins (mg <sub>C3G</sub> 100g <sup>-1</sup> )
Amiga*	N	33.11 $\pm$ 2.41	5.45 $\pm$ 0.11	8.62 $\pm$ 0.09	34.23 $\pm$ 3.49	8.74 $\pm$ 0.30
	C	35.03 $\pm$ 2.56	5.63 $\pm$ 0.08	8.88 $\pm$ 0.08	33.52 $\pm$ 1.80	7.54 $\pm$ 1.55
	I	39.42 $\pm$ 2.87	5.83 $\pm$ 0.09	8.57 $\pm$ 0.08	38.98 $\pm$ 3.76	8.74 $\pm$ 12.17
Fire Top®	N	49.12 $\pm$ 1.91	4.23 $\pm$ 0.11	8.15 $\pm$ 0.07	38.85 $\pm$ 3.12	17.49 $\pm$ 1.47
	C	48.05 $\pm$ 1.58	4.49 $\pm$ 0.06	8.41 $\pm$ 0.06	43.73 $\pm$ 3.69	20.93 $\pm$ 3.29
	I	50.59 $\pm$ 2.46	4.20 $\pm$ 0.07	8.24 $\pm$ 0.11	44.30 $\pm$ 3.98	11.60 $\pm$ 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  $\pm$  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 <sup>-2</sup> )	Total solid soluble (°Brix)	Total polyphenols (mg <sub>GAE</sub> 100g <sup>-1</sup> )		Total anthocyanins (mg <sub>C3G</sub> 100g <sup>-1</sup> )	
					Pulp	Peel	Pulp	Peel
Baigent Brookfield®	N	39.09 $\pm$ 0.66	7.45 $\pm$ 0.06	13.85 $\pm$ 0.09	0.00 $\pm$ 0.00	61.72 $\pm$ 2.22 <b>a</b>	1.15 $\pm$ 0.16	32.96 $\pm$ 0.80 <b>b</b>
	C	40.01 $\pm$ 0.93	7.58 $\pm$ 0.06	13.45 $\pm$ 0.09	0.00 $\pm$ 0.00	44.91 $\pm$ 0.48 <b>b</b>	1.17 $\pm$ 0.33	58.02 $\pm$ 2.96 <b>a</b>
	I	49.79 $\pm$ 1.00	7.51 $\pm$ 0.07	14.00 $\pm$ 0.08	0.00 $\pm$ 0.00	39.14 $\pm$ 2.74 <b>b</b>	2.10 $\pm$ 0.44	32.84 $\pm$ 1.63 <b>b</b>
Galaval*	N	44.46 $\pm$ 1.15	7.99 $\pm$ 0.08	12.89 $\pm$ 0.09	13.99 $\pm$ 4.29 <b>a</b>	28.51 $\pm$ 2.99	2.71 $\pm$ 41.8	19.28 $\pm$ 1.71
	C	50.06 $\pm$ 0.96	7.72 $\pm$ 0.09	13.59 $\pm$ 0.08	9.16 $\pm$ 2.48 <b>ab</b>	36.98 $\pm$ 3.23	5.94 $\pm$ 1.83	17.47 $\pm$ 1.70
	I	54.25 $\pm$ 1.26	8.15 $\pm$ 0.40	13.03 $\pm$ 0.09	0.00 $\pm$ 0.00 <b>b</b>	25.11 $\pm$ 2.22	1.56 $\pm$ 0.43	18.09 $\pm$ 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 (chlorpyrifos and chlorpyrifos 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 photosensitive 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 photosensitive 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 physiologi-

cal 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.

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