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Pest Management Science



## Photoselective exclusion netting in apple orchards: effectiveness against pests and impact on beneficial arthropods, fungal diseases and fruit quality

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Complete List of Authors:	Candian, Valentina; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Pansa, Marco; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Santoro, Karin; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Spadaro, Davide; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Tavella, Luciana; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) Tavella, Luciana; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA) TEDESCHI, Rosemarie; Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA)
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	Photoselective exclusion netting in apple orchards: effectiveness against pests
	and impact on beneficial arthropods, fungal diseases and fruit quality
	Effectiveness of photoselective exclusion nets in apple orchards
	Valentina Candian, Marco G Pansa, Karin Santoro, Davide Spadaro, Luciana Tavella, Rosemarie
	Tedeschi
	Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Largo
	Paolo Braccini 2, 10095 Grugliasco (TO), ITALY
	Corresponding author
1	Rosemarie Tedeschi
1	E-mail: rosemarie.tedeschi@unito.it
	Phone number: +39 011 6708675
	Fax: +39 011 2368675
	Co-authors' email address
	Valentina Candian: valentina.candian@unito.it
	Marco G Pansa: marco.pansa@unito.it
	Karin Santoro: <u>santorokarin@gmail.com</u>
	Davide Spadaro: <u>davide.spadaro@unito.it</u>
	Luciana Tavella: <u>luciana.tavella@unito.it</u>
I	Author Contribution Statement
1	DS, LT and RT conceived and designed research. VC, MGP, KS conducted experiments. VC, KS

and DS analysed data. VC wrote the manuscript. All authors assisted in the follow up of the work,

22 read and approved the manuscript.

### 23 Abstract

BACKGROUND: Frequent pesticide treatments in fruit orchards increase hazards for workers, consumers and environment. Moreover, their indiscriminate and excessive use often induces resistance in pests. In the last few years, physical exclusion strategies have been proposed as an alternative for the control of insect pests. The goal of this study was to evaluate the effectiveness of an anti-hail photoselective net in protecting apples against key and emerging pests as well as the impact on beneficial arthropods, fungal diseases and fruit quality.

RESULTS: In netted plots, a significant reduction of pest populations [i.e. fruit moths, *Halyomorpha halys* (Stål) and *Drosophila suzukii* (Matsumura)] was recorded in comparison with un-netted controls. Moreover, the damage on fruits caused by *H. halys* was reduced up to 62% compared with insecticidal treatments. The net did not negatively affect the abundance of predators and the incidence of postharvest rots. In addition, the incidence of bitter pit on apple was reduced up to 52%. Furthermore, the fruit quality was unaffected by the net coverage (both at harvest and after 4 months of storage).

37 CONCLUSION: The anti-hail photoselective pearl net proved to be a promising exclusion system 38 that can prevent the attack of more than one insect pest at a time, allowing a strong reduction of 39 insecticide treatments and the relative costs. At the same time, the net did not negatively influenced 40 the presence of predators, the incidence of fungi disease and the fruit quality.

Keywords: fruit moths, Halyomorpha halys, fruit damage, predators, bitter pit.

## **1. Introduction**

In the past, crop protection was mainly based on the use of synthetic pesticides to prevent or limit pest damage.<sup>1</sup> In particular, in fruit orchards the application of several pesticides has been required

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46 to ensure the quality of the fruits, which is a key point for commercialization. Nowadays, it is well 47 established that pesticides increase hazards for workers, consumers and environment. The 48 indiscriminate and excessive use induces both resistance in the treated populations<sup>2</sup> and pest 49 outbreaks.<sup>3</sup>

Over the last few years, there has been an increasing interest in the development of alternative control strategies in order to reduce environmental impact. For apple orchards, microbiological insecticides<sup>4</sup> and sex-pheromone-mediated mating disruption technologies<sup>5</sup> were developed against tortrix moths. Although these methods are widely implemented, they often target a single key pest, and insecticides are still needed under high pest densities<sup>6</sup> and to contain other pests.<sup>7</sup> As an alternative, exclusion nets, known as the Alt'Carpo system, have been successfully designed and applied in apple orchards against Cydia pomonella L. (Lepidoptera: Tortricidae) in France since the early 2000s<sup>8</sup> allowing a significant reduction in insecticide inputs. More recently, the nets have been revalued as a multi-target strategy,<sup>9,10</sup> in particular in relation to the introduction of exotic pests such as *Halvomorpha halvs* (Stål) (Hemiptera: Pentatomidae).<sup>11,12</sup> Indeed, the chemical management required to control this invasive pest disrupts the established integrated pest management (IPM) programmes for many crops, especially for fruit orchards. The aim of this study was to assess the effectiveness of the exclusion nets in apple orchards in a multi-target approach by monitoring different pest populations, their damage on fruits, and their possible impact on natural enemies, in particular predators. Moreover, the effects on fruit quality as well as on postharvest diseases and bitter pit were evaluated at harvest and after the storage period.

66 2. Materials and methods

67 2.1 Experimental sites

Field trials were carried out in 2 apple orchards equipped with an anti-hail net system and located in
Cervignasco (cv. Baigent Brookfield<sup>®</sup>, area: 3.9 ha, age: 13 YR) and in Revello (cv. Galaval<sup>\*</sup>, area:

1.1 ha, age: 3 YR), in the province of Cuneo (NW Italy), in 2016 and 2017. Trials were arranged in a randomized complete block design with 3 replicates for each of the following treatments: 1) netted plots (N); 2) un-netted control plots (C); 3) un-netted plots treated with insecticides following the farmer schedule (I). Un-netted insecticide-treated plots were included to evaluate the effectiveness of the net in comparison with the insecticide treatments in reducing pest fruit damage at harvest.

In each orchard, 9 plots of 20 neighbouring trees on the row were selected. The 3 netted plots were covered by the pearl anti-hail photoselective net Tenax Iridium (mesh 2.4×4.8 mm) [AGRINTECH] S.r.l., Eboli (SA), Italy] set up hooking their upper side to the anti-hail net support and fixing the lower side to the ground with metal pegs. The net was placed at the petal fall (mid-May) and removed after harvesting, at the end of the trials (mid-October). A knock-down treatment with the pyrethroid deltamethrin (Decis<sup>®</sup> Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL<sup>-1</sup>) was performed immediately after the net closing to eliminate pest populations. Later in the trials, no insecticides were applied in netted plots and un-netted control plots, while fungicides were applied in the same way in netted plots (directly through the net coverage) and in both un-netted plots, including control and insecticide-treated plots. The schedule of all pesticide treatments is reported in Table 1.

87 2.2 Insect monitoring

Every 10 days from the net setting-up until the harvest, pest populations [i.e. fruit moths, *H. halys*, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) and aphids] and beneficial arthropods
were monitored by traps and visual inspections.

2.2.1 Fruit moths

92 Fruit moths were sampled using sticky delta traps (CSALOMON<sup>®</sup>, Budapest, Hungary). In each
93 orchard, a trap for *C. pomonella* and another one for *Grapholita molesta* (Busck) (Lepidoptera:

Tortricidae) baited with sex pheromones (CSALOMON<sup>®</sup>, Budapest, Hungary) were placed in each netted and un-netted control plot. Moreover, traps for *C. pomonella* were baited with the feeding attractant CSALOMON<sup>®</sup> "BISEX" (CSALOMON<sup>®</sup>, Budapest, Hungary) in order to collect also females of this species as well as other moths. Sex pheromones and feeding attractants were replaced every 4 weeks to ensure their consistent effectiveness. Caught adults were transferred to the laboratory for the species identification by analysis of morphological features and, when necessary, of the aedeagus shape following dichotomous keys [Gilligan TM and Epstein ME (http://idtools.org/id/leps/tortai/Fact\_Sheet\_Index.htm)], and counted.

## 02 2.2.2 Halyomorpha halys

The abundance of *H. halys* was monitored through DEAD-INN<sup>TM</sup> Stink Bug Traps (AgBio, Westminster, CO, USA) (high 121.92 cm), baited with the Xtra Combo lure provided with the trap as described in Candian *et al.*<sup>12</sup> In each orchard, a trap was placed in a netted plot and an un-netted control plot from mid-June in 2016 and from mid-May in 2017, until the end of the harvest time. The lure was changed every 4 weeks according to manufacturer's instructions. The specimens collected into the traps during each survey were identified and counted. Moreover, in each netted and un-netted control plot, 5 branches of 3 randomly selected trees were shaken on a beating tray (1×1 m) to assess the presence and the abundance of the pest during the growing season.

### 111 2.2.3 Drosophila suzukii

Although at the moment *D. suzukii* is not a key pest in apple orchards, the abundance of this pest was monitored during the trials. A trap filled with the feeding attractive Droskidrink (74.5% apple vinegar, 25% red wine and sugar) [Prantil, Priò di Vervò (TN), Italy] was hung in each netted and un-netted control plot at 1.50 m from the ground. It consisted in a transparent plastic bottle filled with 250 mL of Droskidrink and a drop of soap as surfactant. The bottle was closed, and 4 symmetrical holes were applied in its upper part in order to allow the insect entrance. At each

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survey, the collected *D. suzukii* adults were determined [Vlach J
(http://liebegg.ch/upload/cms/user/150730\_Identifikation2.pdf)] and counted, and the attractive
solution was replaced with new Droskidrink.

21 2.2.4 Other arthropods

Other arthropods including aphids and beneficials were monitored every 10 days during the trials. Aphid population levels were sampled by visual inspection of 30 shoots (10 shoots from 3 trees) in each netted and un-netted control plot. The abundance of beneficial arthropods was evaluated thanks to sticky traps. A Glutor YELLOW (25×20 cm) [BIOGARD® Division, Cesena (FC), Italy] sticky trap was placed in each netted and un-netted control plot. The collected specimens were examined under a stereomicroscope for their identification, and at the same time, the predators were separated and counted.

Moreover, in 2017, after harvesting, a knock-down treatment with the pyrethroid deltamethrin (Decis<sup>®</sup> Jet, Bayer CropScience AG, Monheim am Rhein, Germany, 120 mL hL<sup>-1</sup>) was applied on a tree per plot in all the treatments (N, C, I) to assess the arthropod fauna, as described in Candian *et al.*<sup>12</sup> After 3 h and a final beating of the canopy, all the arthropods killed were collected on a nylon tarpaulin ( $3 \times 2$  m) lying under the tree canopy. The collected specimens were examined and sorted in the following clusters: 1) total catches, 2) predators.

2.3 Evaluation of fruit damage caused by pests

The damage on fruits caused by tortrix moths and *H. halys* was evaluated along the growing season and at harvest. Since the net setting-up, 30 fruits in each netted and un-netted control plot (10 fruits on 3 randomly selected trees) were visually inspected every 10 days to evaluate the damage during the growing season. Overall, 270 and 300 apples per replicate were checked in 2016 and 2017, respectively. Page 7 of 29

At harvest, apples were sampled in all the 3 treatments (N, C, I). Fruits were picked in 3 dates in 2016 and 2 dates in 2017. Overall, 510 apples in 2016 and 480 apples in 2017 were picked in each treatment (N, C and I), with 8,910 fruits totally harvested in each apple orchard in the 2 years. The number of fruits damaged by tortrix moths and *H. halys* was recorded. In particular, the damage caused by *H. halys* was identified according to Acebes-Doria *et al.*<sup>13</sup>

46 2.4 Evaluation of postharvest rots and bitter pit

In both years, samples of harvested fruits were selected to evaluate the incidence of postharvest diseases and bitter pit after the storage period. For each netted, un-netted control and un-netted insecticide treated plot, 150 apples were collected in 3 plastic boxes (50 fruits per box) for a total of 2,700 apples (900 per treatment) per orchard. Apples were stored in normal atmosphere (0°C, 98% RH) for 4 months. The incidence of postharvest rots and bitter pit was evaluated after storage and after further 14-day of shelf life at 15°C. Fungal isolation was performed from fruit showing disease symptoms. Pathogens were isolated by transferring small pieces of symptomatic fruit tissues,<sup>14</sup> previously washed in 1% sodium hypochlorite and rinsed in sterile deionized water, onto potato dextrose agar (PDA, Merck, Darmstadt, Germany) plates amended with 25 mg L<sup>-1</sup> streptomycin sulfate (Merck). A 7-day-old culture was used for observation of the fungal structures under optical microscope.

### 2.5 Fruit quality parameters

Quality parameters (firmness, soluble solid content and titratable acidity) were measured on 90 fruits per orchard (10 fruits plot<sup>-1</sup> × 3 plots × 3 treatments), both at harvest and after a 4-month storage period in normal atmosphere (0°C, 98% RH). Quality parameters were determined on healthy fruits with an average grade ranging from 75 to 85 mm. The firmness was determined on 2 sides of each fruit using a FT 327 manual penetrometer (Turoni, Forlì, Italy) (diameter of the probe 8 mm) with a kg scale. For each measure, a slice of skin was removed using a cutter, and the probe

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was pushed into the flesh tissue to a depth of 9 mm. The total soluble solids (TSS) were measured on fresh prepared juice with a DBR 95 digital refractometer (XS Instruments, Carpi, Italy). Titratable acidity was determined by titrating to an end point of pH 8.0 with 0.1N NaOH. For each sample, 10 mL of pressed juice were diluted with 40 mL of distilled water. Titratable acidity was calculated as percent malic acid.<sup>15</sup>

0 2.6 Statistical analyses

The statistical analyses were performed using SPSS v24.0 [SPSS Inc., Chicago, IL, USA] and outcomes were considered significant at *P*<0.05. Captures of fruit moths, *D. suzukii* and other beneficial arthropods effected with traps were compared using a *t*-test for two independent samples. The numbers of damaged fruits per treatment and orchard at harvest 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. The data on arthropods collected by the knock-down treatments and on the incidence of postharvest rots, bitter pit and fruit quality 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 for the arthropods were separated by Tukey's test, while the others were separated by Duncan's multiple range test.

### 2 **3. Results**

3.1 Populations and damage of fruit moths

Low catches of Tortricidae by traps were recorded in both apple orchards. In Baigent Brookfield<sup>®</sup>, *C. pomonella* was never trapped in both years. In 2016, 1 and 13 *G. molesta* were totally collected in netted plots and in un-netted control plots, respectively; in the following year, 2 *G. molesta* were collected in netted plots and 11 in un-netted control plots. In Galaval<sup>\*</sup>, only 1 *C. pomonella* and 2 Page 9 of 29

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G. molesta were recorded in un-netted control plots in 2016, while only 2 C. pomonella were 188 189 collected in un-netted control plots and no G. molesta was trapped in 2017. By contrast, high infestations of Synanthedon myopaeformis (Borkhausen) (Lepidoptera: Sesiidae) were observed in 190 traps for C. pomonella in both orchards, especially in 2017. In Baigent Brookfield<sup>®</sup>, 2 and 38 192 specimens were collected in netted plots and in un-netted control plots, respectively, in 2016, while 18 and 111 specimens were trapped in netted plots and in un-netted control plots, respectively, in 2017. In Galaval<sup>\*</sup>, 4 specimens were recorded in netted plots in both years, while 71 and 108 195 specimens were trapped in un-netted control plots in 2016 and in 2017, respectively. In order to evaluate the efficacy of the net, all adults of C. pomonella, G. molesta and S. myopaeformis caught with traps were grouped before the statistical analysis. Significant differences between the 2 treatments (N and C) were observed both in 2016 (Baigent Brookfield<sup>®</sup>: t = -7.224, P = 0.002; Galaval<sup>\*</sup>: t = -6.515, P = 0.003) and in 2017 (Galaval<sup>\*</sup> t = -5.550, P = 0.005) with always a lower number of catches under net (Table 2).

Damage on fruits caused by Tortricidae was never observed in the apple orchards all along the
 growing season but 4, 6 and 6 fruits damaged by *C. pomonella* were recorded in 2017 at harvest in
 Galaval\* in netted, control and un-netted insecticide-treated plots, respectively.

### 41 204 3.2 Population and damage of *Halyomorpha halys*

*Halyomorpha halys* was collected by traps but never by using the beating tray. In netted plots, only 2 nymphs of *H. halys* were caught in Baigent Brookfield<sup>®</sup> in 2017 at the end of July, otherwise no catches were recorded in both cultivars (Fig. 1). In un-netted control plots, a variable population density was observed between the years. In 2016, only 3 adults and 1 nymph in Baigent Brookfield<sup>®</sup> and 3 adults in Galaval<sup>\*</sup> were totally collected by the traps (Fig. 1). By contrast, in 2017, *H. halys* was detected along all the growing season, with peaks of catches close to the fruit 2017, *H. halys* was detected along all the growing season, with peaks of catches close to the fruit

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ripening. Overall, 42 nymphs and 23 adults were caught in Baigent Brookfield<sup>®</sup>, while 180 nymphs 211 212 and 18 adults were trapped in Galaval\* (Fig. 1).

In 2016, during the growing season, a low number of damaged fruits was recorded. In Galaval\*, no 213 10214 and only one damaged fruit were found in netted and un-netted control plots, respectively. In 11 12 215 Baigent Brookfield<sup>®</sup>, 3 damaged fruits (0.4%) were observed in netted plots and 4 (0.5%) in un-14 15 216 netted control plots. In 2017, a higher number of damaged fruits was recorded. In Baigent 16 17217 Brookfield<sup>®</sup>, 25 (2.8%) and 21 (2.3%) damaged fruits were found in netted and in un-netted control 18 <sup>19</sup>218 plots respectively, while in Galaval\*, 12 (1.2%) and 17 (1.7%) damaged fruits were observed in 20 21 22 219 netted plots and in un-netted control plots, respectively.

24 2 20 The damage on fruits at harvest is reported in Table 3. Significant differences between the 3 25 26 221 treatments (N, C, I) were only observed in 2017, both in Baigent Brookfield<sup>®</sup> (F = 9.117, P =27 <sup>28</sup> 29</sub> 222 0.006), where damage rate was similar in netted and insecticide-treated plots, and in Galaval<sup>\*</sup> (F =30 31 223 9.462, P = 0.005), where damage rate was lower in netted plots (Table 3). Significant differences 32 33 224 between the picking dates were found both in Baigent Brookfield<sup>®</sup> (2017: F = 5.022, P = 0.049) and 34 <sup>35</sup><sub>36</sub> 225 in Galaval<sup>\*</sup> (2016: F = 5.933, P = 0.012; 2017: F = 6.444, P = 0.029) with a significantly lower 37 38 226 damage in the second picking date in Baigent Brookfield<sup>®</sup> and in the first picking date in Galaval<sup>\*</sup>. 39 40 227 No interactions between the treatments and the picking dates were recorded in any orchard. 41 42 43 228 Moreover, the block effect was analysed in order to assess if the damage by *H. halys* was higher on 44 45 229 the borders or in the middle of the orchards. Significant differences for the block effect were 46 recorded only in Baigent Brookfield<sup>®</sup> in 2017 (F = 10.749, P = 0.003) with a higher concentration 47 230 48 <sup>49</sup>231 of the damage along the borders. 50

<sup>52</sup>232 3.3 Population of Drosophila suzukii

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55 233 Drosophila suzukii was collected starting from June in 2016 and from May in 2017. Abundant 56 <sup>57</sup> 234 catches were recorded in 2016 when 118 D. suzukii were totally collected in netted plots and 631 in 59 60

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un-netted control plots in Baigent Brookfield<sup>®</sup>, while in Galaval<sup>\*</sup> 117 and 394 specimens were trapped in netted plots and un-netted control plots, respectively. Significant differences between the 2 treatments (N and C) were recorded in both cultivars with always a lower number of *D. suzukii* collected in netted plots (Baigent Brookfield<sup>®</sup>: t = 7.071, P = 0.002; Galaval<sup>\*</sup>: t = 0.561, P = 0.025) (Table 2).

In 2017, catches well below than the previous year were recorded. In Baigent Brookfield<sup>®</sup>, 21 specimens were totally collected in netted plots and 111 in un-netted control plots, while 50 specimens were trapped in netted plots and 147 un-netted control plots in Galaval<sup>\*</sup>. The number of *D. suzukii* was always significantly lower in netted plots in both cultivars (Baigent Brookfield<sup>®</sup>: t =4.472, P = 0.011; Galaval<sup>\*</sup>: t = 6.364, P = 0.003) (Table 2).

245 3.4 Other arthropods

29 2 4 6 Aphis pomi De Geer (Hemiptera: Aphididae) and Eriosoma lanigerum (Hausmann) (Hemiptera: <sup>31</sup> 247 Aphididae) were recorded in both orchards in 2016, but colonies were mainly composed by E. 34 248 lanigerum in Baigent Brookfield<sup>®</sup> and by A. pomi in Galaval<sup>\*</sup>. Overall, 20 infested shoots (10 for each aphid species) in netted plots and 5 infested shoots in un-netted control plots (one by A. pomi 36 2 4 9 <sup>38</sup>250 and 4 by E. lanigerum) were observed in Baigent Brookfield<sup>®</sup>. In Galaval<sup>\*</sup>, only 4 infested shoots 40 41 251 by A. pomi in netted plots and 38 infested shoots in un-netted control plots (35 by A. pomi and 3 by 43 252 E. lanigerum) were recorded, respectively. In 2017, only A. pomi was observed in both orchards 45 2 5 3 with 56 infested shoots in netted plots and 107 in un-netted control plots in Baigent Brookfield<sup>®</sup>, 47 48 254 while 23 in netted plots and 81 in un-netted control plots were sampled in Galaval\*. Significant ... 50<sup>255</sup> differences between the 2 treatments (N and C) were found only in Galaval<sup>\*</sup> (2016: t = 2.909, P =52 2 56 0.044; 2017: t = 5.469, P = 0.005) with a lower number of aphids recorded in netted plots.

Predators collected by sticky traps belonged to Aranaeidae (Araneae), Anthocoridae (Hemiptera)
 [only in Baigent Brookfield<sup>®</sup> in 2016], Hemerobiidae [except in Galaval<sup>\*</sup> in 2017] and Chrysopidae
 (Neuroptera), Staphylinidae and Coccinellidae (Coleoptera), Syrphidae (Diptera). These predators

were together grouped and statistically analysed. Higher catches were always obtained in un-netted control plots but significant differences between the 2 treatments (N and C) were recorded only in Galaval\* in 2016 (t = 6.993, P = 0.002) and in Baigent Brookfield® in 2017 (t = 4.628, P = 0.010) Galaval\* (Table 2).

In the final knock-down treatment, all the specimens killed by the insecticide were considered in the 15 265 cluster total catches. Specimens belonging to Aranaeidae (Araneae); Acarina; Forficulidae 17 266 (Dermaptera); Psocoptera; Thripidae (Thysanoptera); Anthocoridae, Nabidae, Tingidae, Coreidae, <sup>19</sup> 267 Lygeidae, Pentatomidae, Cicadellidae and Aphidoidea (Hemiptera); Hemerobiidae and Chrysopidae <sup>21</sup> 22 268 (Neuroptera); Staphylinidae and Coccinellidae, Chrysomelidae and Curculionidae (Coleoptera); 24 269 Syrphidae and Drosophilidae (Diptera); Lepidoptera; and Hymenoptera were collected. Significant 26 270 differences between the 3 treatments (N, C, and I) were not recorded for this cluster in both <sup>28</sup> 29 271 orchards. Moreover, Aranaeidae (Araneae); Allothrombium fuliginosum (Hermann) (Acarina); 31 272 Forficulidae (Dermaptera); Anthocoridae and Nabidae (Hemiptera); Hemerobiidae and Chrysopidae (Neuroptera); Staphylinidae and Coccinellidae (Coleoptera); Syrphidae (Diptera) were grouped in 33 273 <sup>35</sup> 274 predators, but no significant differences between the 3 treatments (N, C, and I) were recorded.

### 275 3.5 Evaluation of postharvest rots and bitter pit

41 276 During the trials, the incidence of postharvest rots was not significantly different between the 3 <sup>43</sup> 277 treatments in either cultivar of apple (Table 4). The main postharvest pathogens isolated from 45 46 278 diseased apples at the end of storage were counted. On Galaval<sup>\*</sup>, *Botrytis cinerea* Persoon (92%) 48 279 was the major pathogen isolated, followed by low levels of Alternaria spp. and Penicillium 50 280 expansum Link. In Baigent Brookfield<sup>®</sup>, the main pathogens isolated were B. cinerea (77%), <sup>52</sup> 53 281 Alternaria spp. (15%) and P. expansum (8%). Moreover, the incidence of bitter pit was significantly 55 282 reduced in netted plots in Galaval\* both in 2016 (4-month storage: F = 3.928, df = 2, P = 0.042; 14day storage: F = 8.034, df = 2, P = 0.004) and in 2017 (14-day storage: F = 6.012, df = 2, P = 0.004) 57 283 58

0.012) (Table 5). No differences between the 3 treatments (N, C, and I) were recorded in Baigent
Brookfield<sup>®</sup> (Table 5).

286 3.6 Fruit quality parameters

The fruit quality evaluated on fruits sampled at the first picking date in 2017 at harvest and after 4month storage was not affected by the presence of photoselective net. Differences between the the 3 treatments (N, C, and I) were not statistically significant except for the firmness in Baigent Brookfield<sup>®</sup> (harvest: F = 7.412, df = 2, P = 0.023) and for the total soluble solid in Galaval<sup>\*</sup> (harvest: F = 6.432, df = 2, P = 0.006; 4-month storage: F = 8.034, df = 2, P = 0.013) (Table 6).

### **4. Discussion**

The growing attention to food safety and environmental protection, the increasing occurrence of resistance in insects as well as the introduction of exotic pests require the implementation of new methods for crop protection in accordance with IPM principles. In this scenario, exclusion netting represents a valid multi-purpose system against several key and emerging apple pests. The impact of insect exclusion nets is not only confined to being an actual physical barrier against the in-out movement of insects from the orchard but they also interfere with their behaviour. The nets indeed hamper the flight of male moths during their approach towards the females reducing mating success, and may interfere by causing a visual disturbance to the searching males.<sup>16, 17</sup> Even if *C*. *pomonella* and *G. molesta* were not very abundant in the surveyed orchards, our trials confirmed the effectiveness of the nets against these moths<sup>18</sup> as well as against *S. myopaeformis* collected thanks to the feeding attractant added in the *C. pomonella* pheromone traps. In our trials, the number of damaged fruits was too low to assess the effectiveness of the used net in containing *C. pomonella* damage, as reported by other anti-hail nets.<sup>8</sup>

Particularly interesting are the results obtained against *H. halys* whose density has increased worrisomely in NW Italy in the last years.<sup>19</sup> The net always prevented the entry of the adults while

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only 2 nymphs were collected under the net, allowing a reduction of fruit damage, in particular in 2017, in comparison not only to the un-netted control plots, but also, in Galaval<sup>\*</sup>, to the un-netted plots treated with insecticides. *Halyomorpha halys* is a perimeter-driven threat<sup>20</sup> and, as expected, the damage was higher on netted and un-netted trees closer to the edges. Moreover, the highest damage rate was observed in the netted and un-netted control plots where the pheromone trap was placed, confirming the fact that adults aggregate around a pheromone source within a radius of ca. 2.5 m without contacting the source.<sup>21</sup>

Although previous research reported that only nets with a mesh thinner than 1 mm<sup>2</sup> are effective in excluding *D. suzukii*,<sup>22, 23</sup> satisfactory results in reducing *D. suzukii* populations, and generally the Drosophilidae abundance (data not shown), were recorded under the net in our trials, in which a 2.4×4.8 mm net was used. This may be due more to the optical properties of the photoselective net than to its physical activity. In fact, it was shown that the light reflected by the photoselective nets may cause an optical disruption and as a consequence, negatively affect the pest in distant host finding and landing.<sup>24</sup>

The influence of the exclusion nets on aphid populations is quite controversial and seems mainly related to the species,<sup>25,10</sup> to the microclimate under the nets and to the preclusion of natural enemies<sup>26</sup> as well as to the exclusion system (single row or single-plot).<sup>27</sup> In our trials, a low number of infested shoots was observed under the net, except in Baigent Brookfield<sup>®</sup> in 2016 when colonies were mainly composed by *E. lanigerum*. As already observed, the net favours the development of this aphid species<sup>28</sup> while no clear effect of the nets has been yet demonstrated for *A. pomi.*<sup>29</sup>

As observed by other authors,  $^{25,30,31}$  a significant lower number of predators was collected by sticky traps under the net, in both orchards, during the growing seasons. Generally, the net mesh was large enough to allow tiny beneficial insects to pass through (mainly Anthocoridae, Staphylinidae and Coccinellidae). Larger size insects, such as Hemerobiidae, Chrysopidae and Syrphidae, were

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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 installation. However, the results obtained with the final knock-down treatments (here referred only to 2017, but 2016 data are available in Candian *et*  $al.^{12}$ ) showed that the net did not negatively influence the abundance of predators. These contrasting results may be due to different aspects, such as the timing of the survey and the impact of the net on the visual cues of predators. The disrupting insect fly orientation, visual detection, and colour perception might reduce the attractiveness of yellow sticky traps and render this monitoring technique ineffective, <sup>32</sup> as already observed in Candian *et al.*<sup>12</sup>

The mesh size is a very critical factor not only for the exclusion effectiveness, but also for the consequences on the microclimate occurring under the net. Despite the expectations, under the pearl net the temperature and the relative humidity were similar to the ones recorded outside net (data not shown). Therefore, the pearl photoselective net did not favour the development of fungal pathogens. Neither significant differences were found in the incidence of apple scab in the field (data not shown), nor in the incidence of postharvest rots. The main agents of postharvest rots isolated were *B. cinerea, Alternaria* spp. and *P. expansum*, which are the most common postharvest pathogens on apple in northern Italy.<sup>33</sup> High relative humidity could be conducive to the occurrence of russeting and other physiological disorders in apples during postharvest.<sup>34</sup> Apples subjected to cold storage after harvest revealed an interesting effect of the nets on bitter pit. For both cultivars, and in particular for Galaval\*, the presence of the nets reduced the incidence of bitter pit, as previously observed on Gala and Fuji apples grown under white net.<sup>35</sup> Various papers show that the influence of protective shade netting on the incidence of bitter pit varies depending on cultivar, net colour, shading percentage, net mesh size and timing of deployment relative to full bloom.<sup>36,37</sup>

Finally, the fruit quality was not affected by the net coverage as already reported by several authors.<sup>38-40</sup> A lower level of total soluble solids in Galaval<sup>\*</sup> apples grown under net is comparable with the previously reported reduction of total soluble solids in 'Gala' apples, but not in 'Fuji'

apples grown under whiteshade net.<sup>35</sup> Actually, in some cases, the pearl photoselective net was also 358 able to enhance some nutraceutical properties of the fruits.<sup>12</sup> 359

Overall, photoselective exclusion nets are able to preserve apple production and quality with a 360 10 361 strong reduction of insecticide treatments, in our trials up to 7 less. This aspect is particularly <sup>12</sup> 362 important in the case of invasive pests with typical severe outbreaks. For instance, in the case of H. 15 363 halvs, the use of frequent and broad spectrum insecticides are required to reduce fruit injury,<sup>41,42</sup> but this prejudices the principles of IPM. On the contrary, exclusion nets allow obtaining apples 17 364 19 365 without insecticidal residues as confirmed by a multiresidual analysis performed on fruits at harvest <sup>21</sup> 22 366 (data not shown). Moreover, the use of exclusion nets can even have an added value considering the 24 367 reduction of costs associated with insecticide use: in our trials, the omission of 7 insecticidal 26 368 treatments in the netted plots gave rise to a saving up to  $1,050 \in ha^{-1}$  (ca.  $150 \in ha^{-1}$  per treatment). <sup>28</sup> 369 In terms of costs, in orchards in which an anti-hail net system is already present, a single plot 31 370 exclusion-net system is more feasible, entailing a 2,300 € ha<sup>-1.12</sup> The reduction of insecticide costs, 33 371 associated with possible public contributions to the growers can easily amortize in few years the <sup>35</sup><sub>36</sub> 372 Z.C upfront fixed costs of nets setting up.

#### 38 373 5. Conclusion

41 374 The pearl photoselective exclusion net proved to be effective in controlling more than one apple 42 43 44 375 pest species at a time and their damage on fruits, and in reducing the occurrence of some 45 physiological disorders and diseases. Moreover, in some cases, it has been more effective than 46 3 7 6 47 48 377 chemical treatments representing a great-value alternative for the management of pests not 49 <sup>50</sup> 378 effectively controlled by insecticide treatments and in organic farming. It can be a great resource as 52 53 379 an environment-friendly strategy for a healthier fruit production in face of climate change issues, 54 55 380 which are favouring the increasing occurrence of invasive exotic pests. 56

#### <sup>58</sup> 381 6. Acknowledgments 59

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2 3 382	We th	ank Luca Rivoira for his support in experimental activities and the growers Silvano Ezio
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<sup>5</sup> 383	Bertor	ello and Giuseppe Borretta for their hospitality. This work was supported by the EU LIFE13
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#### 8. Tables

Table 1. Insecticidal and fungicidal treatments applied in the orchards from the net setting-up until harvest in 2016 and

2017. 

Cultivar	Target	Treatment				
		Applied on	Active ingredient	Trade name	Year	N
Baigent	Aculus schlechtendali	Ι	Abamectin	Zoro <sup>®</sup> 1,9 EV	2017	1
Brookfield®	Cydia pomonella	Ι	Chlorpyrifos	Terial <sup>®</sup> 75 WG	2016	2
			Chlorpyrifos methyl	Reldan <sup>TM</sup> LO	2016	1
			Etofenprox	Trebon <sup>®</sup> STAR	2016	1
			Etofenprox	Trebon <sup>®</sup> UP	2017	1
			Phosmet	Spada <sup>®</sup> 50 WG	2017	2
	Halyomorpha halys	Ι	Chlorpyrifos methyl	Reldan <sup>TM</sup> LO	2017	1
	Synanthedon myopaeformis	Ι	Chlorpyrifos	Terial <sup>®</sup> 75 WG	2017	1
	Tortricidae	Ι	Methoxyfenozide	Intrepid <sup>TM</sup>	2017	1
	Heart-rot	N, C, I	Pyraclostrobin-boscalid	Bellis®	2016	2
			Pyraclostrobin-boscalid	Bellis®	2017	1
	Postharvest rots	N, C, I	Captan	Merpan <sup>®</sup> 80 WDG	2016	1
			Captan	Santhane® WG	2017	1
			Fludioxonil	Geoxe®	2017	1
	Venturia inaequalis	N, C, I	Dodine	Syllit 355 SC	2016	1
			Dithianon	Delan <sup>®</sup> 70 WG	2016	1
			Dodine	Syllit 355 SC	2017	3
			Fluazinam	Banjo®	2017	1
Galaval*	Anthonomus pomorum	Ι	Phosmet	Spada <sup>®</sup> 50 WG	2017	1
	C. pomonella	Ι	Chlorpyrifos methyl	Runner <sup>®</sup> M	2016	2
			Etofenprox	Trebon <sup>®</sup> UP	2016	1
			Chlorpyrifos methyl	Runner <sup>®</sup> LO	2017	2
			Etofenprox	Trebon <sup>®</sup> UP	2017	1
			Methoxyfenozide	Prodigy®	2017	2
	Podosphaera leucotricha	N, C, I	Sulfur	Tiovit <sup>®</sup> Jet	2016	1
			Sulfur	Thiopron <sup>®</sup>	2017	1
	Postharvest rots	N, C, I	Captan	Merpan <sup>®</sup> 80 WDG	2017	1
	Venturia inaequalis	N, C, I	Captan	Captan arvesta 80 WG	2016	2
			Sulfur	Thiopron <sup>®</sup>	2016	1

Treatment: N = netted plots, C = un-netted control plots, I = un-netted plots treated with insecticides.

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**Table 2.** Percentage of Tortricidae + Sesiidae and *Drosophila suzukii* per trap on the total catches (mean  $\pm$  SE), and total number of predators per sticky trap (mean  $\pm$  SE) in the apple orchards. For each cultivar, means followed by different letters are significantly different (*t*-test, *P*<0.05).

Cultivar	Treatment	Tortricidae + Sesiidae (%)		Drosophila suzukii (%)		Predators (no.)	
		2016	2017	2016	2017	2016	2017
Baigent	Ν	1.85±1.07 <b>b</b>	$4.70 \pm 0.80$	5.30±2.00 <b>b</b>	5.30±1.90 <b>b</b>	74.67±27.51	2.33±0.88 k
Brookfield®	С	31.48±3.21 <b>a</b>	28.60±11.10	28.10±2.20 <b>a</b>	28.80±4.30 a	137.00±9.45	15.33±2.66 <b>s</b>
Galaval*	Ν	1.71±1.71 <b>b</b>	1.20±0.30 <b>b</b>	7.60±0.90 <b>b</b>	8.50±1.80 <b>b</b>	7.00±1.00 <b>b</b>	$0.66 \pm 0.66$
	С	31.62±2.26 <b>a</b>	32.10±8.00 <b>a</b>	25.70±4.10 a	24.90±2.80 a	67.33±8.56 <b>a</b>	$7.00 \pm 3.05$

16 508 Treatment: N = netted plots, C = un-netted control plots.

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**Table 3.** Percentages of apples damaged by *Halyomorpha halys* (mean  $\pm$  SE) on fruits sampled at harvest in 2016 (no. = 510 fruits per repetition) and in 2017 (no. = 480 fruits per repetition). For each cultivar, means followed by different letters are significantly different (GLMM, Bonferroni correction, P<0.05). Cultivar Treatment Damaged apples Baigent Brookfield® Ν 5.8±1.4 1.9±0.8 **b** С 7.1±1.6 7.7±2.1 a I 5.5±1.4 1.2±0.7 b Galaval\* Ν 4.3±1.3 6.3±1.4 b С 9.0±1.9 17.7±2.3 a I 7.4±1.7 16.4±2.4 **a** Treatment: N = netted plots, C = un-netted control plots, I = un-netted plots treated with insecticides. 20<sup>9</sup>514 

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**Table 4.** Incidence of postharvest fungal diseases (mean  $\% \pm SE$ ) in apples harvested in 2016 and 2017 after 4-month storage and after additional 14-day in shelf life. For each cultivar, means followed by different letters are significantly different (Duncan's multiple range test, P<0.05).

Cultivar	Treatment	2016		2017		
		4-month storage	14-day shelf life	4-month storage	14-day shelf life	
Baigent	N	2.00±0.86	11.11±6.28	4.67±1.73	13.55±2.43	
Brookfield®	С	1.11±0.44	8.89±0.87	4.28±1.22	14.78±2.58	
	Ι	$0.89 \pm 0.28$	6.56±0.63	2.83±1.13	12.05±1.68	
Galaval*	N	2.22±0.92 <b>b</b>	13.97±4.14	1.35±0.44	7.42±1.95	
	С	4.25±1.06 ab	17.07±4.22	2.85±0.62	14.42±3.29	
	Ι	7.34±1.44 <b>a</b>	24.91±2.28	6.00±3.51	30.92±12.55	

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<sup>18</sup> 518 Treatment: N = netted plots, C = un-netted control plots, I = un-netted plots treated with insecticides.

20 519 Percent rotten apples = (no. of infected apples/total no. of apples) $\times 100$ .

**Table 5.** Incidence of bitter pit (mean  $\% \pm SE$ ) in apples harvested in 2016 and 2017 after 4-month storage and after additional 14-day in shelf life. For each cultivar, means followed by different letters are significantly different (Duncan's multiple range test, P<0.05).

Cultivar	Treatment	2	016	2017		
		4-month storage	14-day shelf life	4-month storage	14-day shelf life	
Baigent	N	14.67±3.71	14.89±4.44	1.78±0.66	3.57±1.12	
Brookfield®	С	9.56±1.39	10.44±4.02	1.78±0.71	3.85±1.22	
	Ι	6.22±2.14	8.22±2.69	0.52±0.52	1.53±0.78	
Galaval*	N	27.29±2.64 ab	32.25±2.91 b	11.03±2.90	19.45±3.36 <b>b</b>	
	С	33.21±5.29 <b>a</b>	42.37±6.45 ab	16.07±3.59	32.85±3.80 a	
	Ι	24.91±2.28 b	55.20±3.99 <b>a</b>	22.18±5.54	40.45±4.06 a	

Treatment: N = netted plots, C =un-netted control plots, I = un-netted plots treated with insecrticides.

Percent apples with bitter rot = (no. of diseased apples/total no. of apples) $\times 100$ . 

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**Table 6.** Firmness, total soluble solids and titratable acidity (mean  $\pm$  SE) recorded on fruits at the first picking date in 2017 at harvest and after 4-month storage. For each cultivar, means followed by different letters are significantly different (Duncan's multiple range test, *P*<0.05).

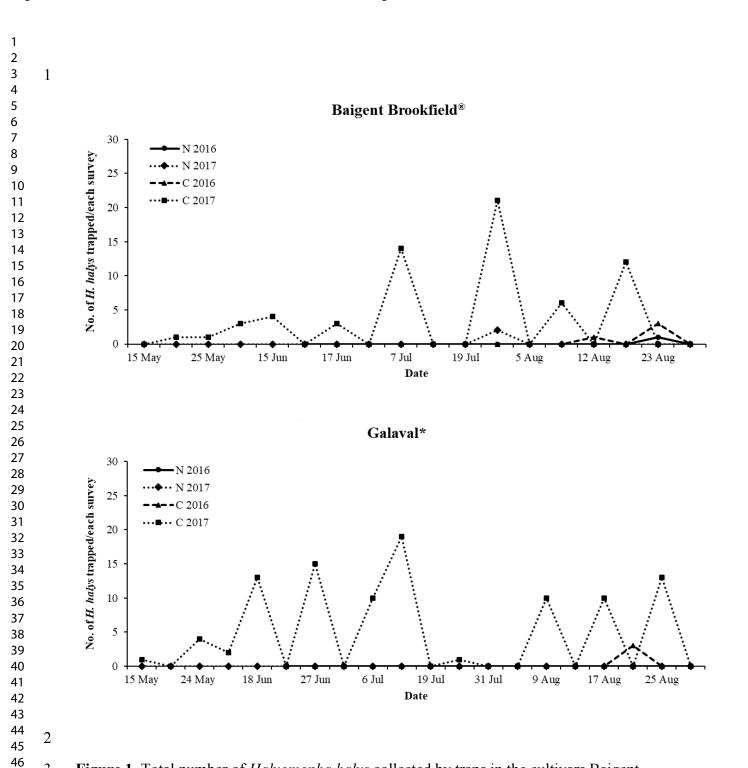
8	-	-						
9	Cultivar	Treatment	Firmness		Total soluble so	lids	Titratable a	cidity
10			(g cm <sup>-2</sup> )		(°Brix)		(% malic ac	eid)
11			Harvest	4-month storage	Harvest	4-month storage	Harvest	4-month storage
12	Baigent	Ν	7.63±0.23 a	6.33±0.21	13.83±0.16	14.37±0.19	0.27±0.03	0.28±0.02
13 14	Brookfield®	С	7.41±0.16 <b>b</b>	6.54±0.12	13.88±0.13	13.80±0.17	$0.27 \pm 0.03$	$0.27 \pm 0.02$
15		Ι	7.50±0.09 <b>ab</b>	6.15±0.15	13.55±0.19	13.46±0.23	$0.27 \pm 0.01$	$0.26 \pm 0.03$
16	Galaval*	Ν	8.28±0.36	6.39±0.33	12.38±0.11 c	12.53±0.14 <b>b</b>	0.32±0.01	0.30±0.03
17		С	8.01±0.34	6.57±0.37	13.43±0.21 <b>b</b>	13.51±0.16 <b>a</b>	$0.30 \pm 0.03$	$0.28 \pm 0.02$
18 19		Ι	7.81±0.31	6.27±0.23	13.71±0.13 <b>a</b>	13.35±0.11 <b>a</b>	$0.34 \pm 0.03$	$0.28 \pm 0.02$

 $\frac{1000}{20}$  530 Treatment: N = netted plots, C = un-netted control plots, I = un-netted plots treated with insecticides.

# 532 9. Figure Legends

Figure 1. Total number of *Halyomopha halys* collected by traps in the cultivars Baigent Brookfield<sup>®</sup>
and Galaval<sup>\*</sup> in treatments N (netted plots) and C (un-netted control plots) in 2016 and in 2017.

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**Figure 1.** Total number of *Halyomopha halys* collected by traps in the cultivars Baigent Brookfield<sup>®</sup> and Galaval<sup>\*</sup> in treatments N (netted trees) and C (un-netted control trees) in 2016 and in 2017.