



Insect netting: effect of mesh size and shape on exclusion of some fruit pests and natural enemies under laboratory and orchard conditions

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Received: 5 April 2022 / Revised: 19 November 2022 / Accepted: 24 November 2022 / Published online: 13 December 2022
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Abstract

To improve exclusion systems for fruit trees, insect nets of various types were evaluated for their permeability to different beneficial and pest species, under laboratory and field conditions. Pests studied were the apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae) and the spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). Beneficials were *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), *Aphidius matricariae* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). Mesh nets with five different apertures (square, rectangle, triangle, rhombus and hexagon) and six different sizes (from 0.4 to 2.8 mm) were 3D-printed from strands of polylactic acid and tested in the laboratory along with two commercially available nets made of polyethylene. The physical and behavioral characteristics of the six studied species affected their ability to cross the nets. For an equal size (open area), the intrusion rate was generally greater through the square- and/or hexagonal-shaped meshes. Rectangular-shaped apertures totally excluded the apple maggot in both laboratory and field trials, provided their shortest side did not exceed 1.9 mm. For the spotted wing drosophila, a maximum of 1.0 mm was similarly required for exclusion in the laboratory. The shape factor (length/width ratio) of the apertures appeared to affect net selectivity. Field trials confirmed that more aphid predators and leafroller parasitoids colonized trees covered with larger mesh nets (2.3 × 3.4 mm), while still excluding the apple maggot. Thus, for a similar aperture size (area), an elongated rectangular-shaped mesh appears to facilitate access for beneficials, while continuing to provide effective protection against apple pests.

Keywords Protected cultivation · Exclusion · Pest management · Orchards · Biological control

Introduction

The use of exclusion nets for pest management of vegetable and fruit crops is increasing worldwide (Mukherjee et al. 2019; Alaphilippe et al. 2013; Chouinard et al. 2016). While there are several reasons for this, the most commonly cited are a) the arrival of exotic pests that are difficult to control

with pesticides (e.g., *D. suzukii*, *H. halys*); b) social pressure to reduce the use of chemicals in agriculture; c) the effectiveness of nets against pests that attack many agricultural commodities, as well as the sustainability of the protection they offer.

Nonetheless, despite the fact that orchards are highly susceptible to damage from insect pests, the exclusion technique remains far less used in them than for other crops, except for hail protection. The challenges posed by large 3D exclusion structures (acquisition and operating costs, durability, and end-of-life recovery) and by the perennial nature of fruit trees (impact of netting on growth and long-term profitability) have long limited implementation, but the large body of work published in recent years suggests that some of these challenges have been or can be addressed (Mukherjee et al. 2019). For example, insect nets are most often made of high-density polyethylene (HDPE), which, even taking their long lifespan into account, is not environmentally responsible, based on greenhouse gas emissions, energy costs and end-of-life

Communicated by Nicolas Desneux.

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management. However, bio-polymer nets such as those made from polylactic acid (PLA), which can be generated from bacterial fermentation, are becoming available and can be used instead of HDPE.

In apple orchards, nets have recently been found to effectively prevent damage caused by the vast majority of fruit pests without the use of insecticides and without any detrimental effect on photosynthesis—despite reducing light interception by up to 7% (Chouinard et al. 2016, 2017, 2019). They can also positively affect tree physiology, which could be used to increase fruit quality once their impacts can be controlled more precisely (Mupambi et al. 2018). However, net use can sometimes be accompanied by increased population levels of specific leafrollers (i.e., *Choristoneura rosaceana*) and aphids of various species (e.g., *Aphis pomi*, *Dysaphis plantaginea*), possibly due to the exclusion of some of their natural enemies (Dib et al. 2010; Capowiez et al. 2013; Romet et al. 2010; Marshall and Beers 2021, 2022). Smaller-sized meshes have generally been identified as the culprit, but mesh shape can also affect the composition of the pest population (Bethke and Paine 1991; Bethke et al. 1994; Álvarez and Oliva 2015), and hence beneficial species (Hanafi et al. 2007).

In apple orchards of northeastern North America, the guild of natural enemies of *Dysaphis* and *Aphis* spp. is composed of multiple species of predators, including a cecidomyid fly and several species of braconid and chalcid parasitoid wasps (Carroll and Hoyt 1984; Bergh and Stallings 2016). The guild of natural enemies of *C. rosaceana* is diverse and similarly includes a variety of braconid and chalcid wasps (Cossentine et al. 2004; Sarvary et al. 2007; Vanoosthuyse and Cormier 2008; Tremblay et al. 2018).

Commercially available nets are mostly mesh with rectangular-shaped apertures, which can vary greatly in size and shape. Mesh size is also a determinant factor of net cost, as smaller ones generally require more material and also increase net weight and wind resistance, which has a direct impact on the supporting infrastructure required (Álvarez et al. 2019). The present study was undertaken to examine the impacts of size and shape of meshes on the exclusion of selected beneficials representative of the abovementioned guilds, both in the laboratory and in row-by-row systems recently developed (by our team) for orchards. The impact on two key fruit pests was included in the study, in the hope that this knowledge could be used to improve net selectivity (its capacity to block pests while facilitating access or continued presence of beneficials inside the system) and overall effectiveness of exclusion systems for tree fruit production.

Materials and methods

Laboratory experiments

Test insects Based on commercial availability, ease of rearing and relevance to the tree fruit agroecosystem, we selected the following three beneficials and two pests for testing: the predatory midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), the parasitic wasps *Aphidius matricariae* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae), the apple maggot *Rhagoletis pomonella* (Diptera: Tephritidae) and the spotted wing drosophila *Drosophila suzukii* (Diptera: Drosophilidae). The first three (beneficials) were obtained from commercial sources (wasps: Crop Defenders, Leamington, ON, Canada; midges: Anatis Bioprotection, Saint-Jacques-le-Mineur, QC, Canada) and comprised individuals of both sexes (ca. 50% females). Insect pests were obtained from rearings maintained at the Research and Development Institute for the Agri-environment (IRDA) and comprised both sexes (*D. suzukii*), or females only (*R. pomonella*). Average thorax width of each species (obtained from measurements taken under the microscope from a random sample of ten individuals) was as follows: 0.55 ± 0.03 mm for *A. aphidimyza*, 0.43 ± 0.01 mm for *A. matricariae*, 0.50 ± 0.01 mm for *A. abdominalis*, 1.76 ± 0.02 mm for *R. pomonella*, 1.15 ± 0.03 mm for *D. suzukii* females and 1.03 ± 0.01 mm for *D. suzukii* males.

Test materials Net samples of different patterns (mesh shape) and sizes were custom-made from translucent polylactic acid (PLA) polymer strands (Materio3D, Longueuil, Canada) using a 3D printer (FlashForge Creator Pro) and printing software (Simplify 3D). Extrusion of the filament was performed at 212 °C. Commercially available polyethylene net samples from Artes Politecnica (Schio, Italy: mesh size 2.2×3.4 mm) and Dubois Agrinovation (Saint-Rémi, QC Canada: mesh sizes 0.95×1.90 mm and 0.85×1.4 mm) were also included for comparison.

Experimental setup In the first experiment, square meshes with apertures of five different sizes were compared for their capacity to exclude each species. Discriminant dimensions were chosen based on the average width of the thorax of the targeted insects and on the results obtained in preliminary tests. A common mesh dimension (2×2 mm) was included for all species.

In the second experiment, five geometric patterns (shapes) were compared: triangle square, rhombus, hexagon and rectangle (Fig. 1). All shapes were adjusted to the same discriminant size (area), selected for each species based on the results of the first experiment.

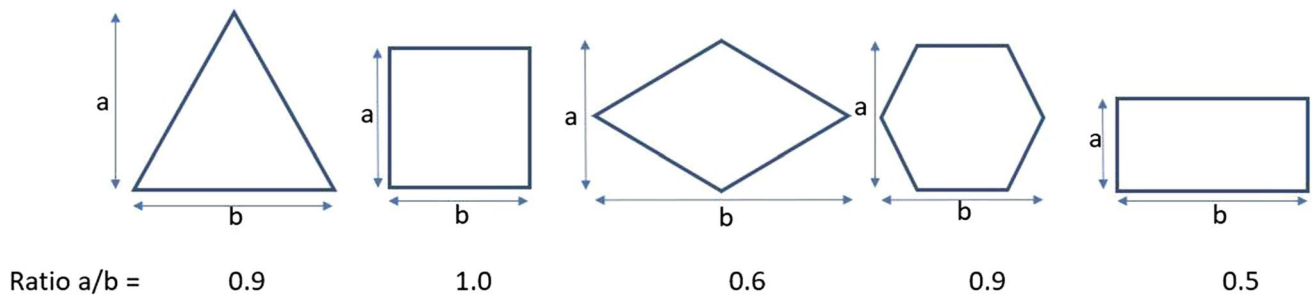


Fig. 1 Relative measurements of each of the geometric shapes tested (triangle, square, rhombus, hexagon, and rectangle), for an equal area (a = maximum height; b = maximum width). Hexagon was tested only with pest species for reasons of material availability

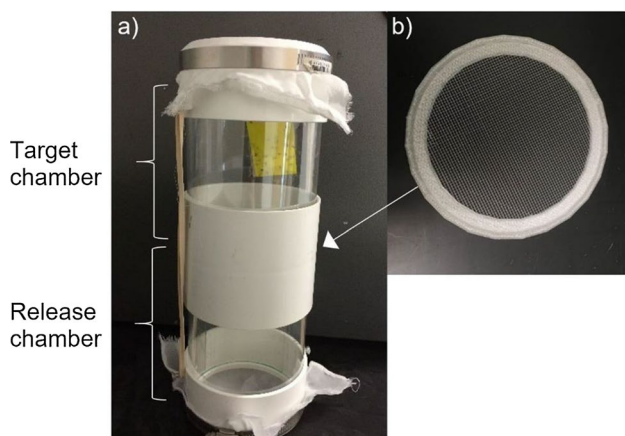


Fig. 2 Experimental setup used in the laboratory (**a** = cylindrical cage; **b** = net positioned inside a custom printed double ring to tightly divide the cage into two enclosures)

Insects were inserted in the lower compartment of a cylindrical cage (height 30.7 cm; diameter 11.5 cm) separated at mid-height by a net sample that differed according to the treatment (Fig. 2). Depending on the species, between 15 and 50 individuals were released in each of the six cages used for each treatment. Adult specimens were used, but since beneficial species were inserted at their pre-emergence stage, the exact number of individuals released was therefore adjusted according to the emergence rate observed in preliminary trials. Devices were placed vertically and covered with aluminum foil (except for the top end), to use positive phototropism and negative geotropism as stimuli to entice the insects to cross the net. A yellow sticky trap was suspended in the upper compartment to collect those who crossed after 24 h (or 6 days for beneficials, to allow adult stages to emerge). For tests with *A. aphidimyza*, cages were placed horizontally after failure of the insects to move was noticed in the vertically oriented design. All tests ($n = 6$ repetitions) were performed under controlled conditions (23°C, 70% RH and a photoperiod of 16:8 L:D).

Field experiment

Site An insecticide-free plot of cv. ‘Honeycrisp’ dwarf apple trees were selected at IRDA’s experimental orchard (45.543572°, –73.341501° lat long.). This plot had historical records (Chouinard et al. 2019) of presence of various pest and beneficial species which included most of those under study—or close relatives, in the case of beneficials.

Nets Since 3D custom-printing was not possible for field-sized replicates, commercial nets made of rectangular mesh were used, i.e., 1) 0.95 × 1.90 mm (Proteknets 60™), the reference size used in studies previously published by our team, from Dubois Agrinovation (Saint-Rémi, QC Canada) and 2) 2.2 × 3.4 mm (Artes 5X4™), a larger mesh size, from Artes Politecnica (Schio, VI, Italy). The performance of both types of nets was compared to a net-free control.

Setup Nets were deployed in spring 2018 over experimental units (single row sections of 10–12 apple trees) at bud break, closed with clips above ground level to reproduce a complete exclusion system (Chouinard et al. 2016) and kept closed until harvest, except for two pollination days (Chouinard et al. 2019) during bloom (May 21 and 23, between ca. 9AM and 6PM). Five blocks (=replicates) were defined, based on the previous year’s *C. rosaceana* population size, and the two net treatments were randomly allocated within each block. The net-free control units had been net-free the previous year, and, similarly, the netted units had been netted the previous year. This design allowed comparisons to be made between the performance of the two nets during the current season, while considering the cumulative effect of netting when comparing netted vs unnetted trees.

Assessments were focused on predominant pests (aphids and leafrollers) and associated beneficials in this exclusion system (Chouinard et al. 2017). They were performed by removing clips and entering the netted zone without lifting the net up. Population density of aphids was assessed twice with methods adapted to the microhabitats of the species present. In early June, when most aphids were *Dysaphis*, 120 spur-leaf clusters and 60 growing shoots (newest ten leaves of each)

were randomly sampled in each experimental unit, while at the end of June, when most aphids were *Aphis*, 180 growing shoots were similarly sampled (no sampling on leaf clusters). The number of aphids per colony was visually assessed and categorized for each species (Dib et al. 2010) as follows: 0 = no aphids; 1 = 1–5; 2 = 6–25; 3 = 26–50; 5 = 51–125; 5 = more than 125. Aphid colonies of category 2 or more (exclusively *A. pomi*) were also selected, marked and monitored weekly throughout the season (from June 7 to September 12) in order to assess the abundance and composition of the natural enemies present on the growing shoots in the experimental units (50 colonies/treatment, at a rate of ca. 10 colonies/unit). For each assessment, the presence and number of aphid predators (any life stage) or parasitoids (mummified aphids) were noted in each colony. The number of ants (aphid allies) was also visually assessed and categorized in the same way as for aphids.

Parasitoids of occurring Lepidoptera (almost exclusively *C. rosaceana*) were similarly assessed by sampling 120 spur-leaf clusters and 60 growing shoots in each experimental unit—on May 31 for the overwintering generation larvae, and on July 26 for the summer generation larvae. Half of the larvae were collected and sent to the laboratory and then maintained on a wheat germ-based artificial diet (Southland Product, Lake Village, Arkansas). They were observed once or twice a week under a binocular lens to assess the parasitism rate, and the parasitoids that emerged were collected and identified.

Fruit was also sampled at different times during the season (June 19th for plum curculio damage and July 24th for codling moth damage) and collected at harvest (September 14) to assess quality (yield, color, size and damage from discernable causes). For yield, all fruits from six trees per experimental unit were collected, counted and weighed. Fruit size was measured using an electronic Vernier ($n = 120$ fruits/unit). Color was measured on 30 marketable fruits/unit, for a total of 150 fruits per treatment. Damage was assessed as in Chouinard et al. (2017), by sampling 180 fruits/unit. All types of damage observed were identified, whether from insects and diseases, or from physiological or physical causes. Although not found on fruit, fire blight was also assessed weekly on shoots after being observed at bloom. Air temperature and relative humidity sensors (Hobo U23 Pro v2, Onset, MA, USA) were installed at a height of 1.25 m in the tree canopy to monitor hourly conditions in two replicates of each treatment. The photosynthetic activity of trees was also measured with a chlorophyll fluorometer (OS30p, Opti-Sciences, NH, USA) as described in Chouinard et al. (2019) on three occasions between June 22 and August 30.

Analysis

For laboratory experiments, the percentage of individuals that crossed each net was compared using an ANOVA followed by a Tukey–Kramer HSD test (or nonparametric Kruskal–Wallis test, followed by a Steel–Dwass test when assumptions for normality and homoscedasticity were not met). A linear regression was also performed to test the relation between the length/width ratio of the apertures (Fig. 1) and the exclusion rate of the net.

For the field experiment, differences in the percentage of apple damage, insect pest density and fruit quality data were compared by two-way ANOVAs (treatment, block) and means were separated with a Tukey–Kramer HSD test. When the data did not meet the ANOVA assumptions, a nonparametric Kruskal–Wallis test was performed, followed by post hoc comparison tests. To assess differences in abundance of aphid natural enemies and aphid allies among treatments, observations on selected infested shoots for each sampling date were pooled across the season. A Kruskal–Wallis test was then used on pooled data, since assumptions for homogeneity of variance were not met. Parasitism of *C. rosaceana* was compared among larvae collected from trees covered with nets of different mesh sizes and from unnetted trees using a Likelihood Ratio Chi-square test (G^2).

Results

Laboratory experiments

Mesh size. Under laboratory conditions, both pests and parasitoids were able to pass through net apertures that were only slightly larger than the width of their thorax. This resulted in only one commercial net (with a smaller mesh size) effectively excluding the apple maggot (Fig. 3A) and the spotted wing drosophila (Fig. 3B) in the laboratory. For the predatory species *A. aphidimyza*, the aperture had to be more than twice its thorax width (Fig. 3C).

Based on these results, the following aperture dimensions were used for the mesh shape experiment: a) 5.29 mm^2 for the apple maggot (corresponding to a square mesh of $2.3 \times 2.3 \text{ mm}$), 1.69 mm^2 for the spotted wing drosophila (corresponding to a square mesh of $1.3 \times 1.3 \text{ mm}$), 0.49 mm^2 for the two parasitoids (corresponding to a square mesh of $0.7 \times 0.7 \text{ mm}$) and 7.84 mm^2 for the predatory *A. aphidimyza* (corresponding to a square mesh of $2.8 \times 2.8 \text{ mm}$).

Mesh pattern Among the shapes under study, the exclusion rate of apple maggot females (ANOVA, $F_{4, 25} = 22.58$; $p < 0.0001$) and spotted wing drosophila (Kruskal–Wallis, $\chi^2 = 20.87$; $df = 4$; $p = 0.0003$) was lowest through hexagonal apertures, followed by square apertures. Rectangular apertures totally excluded these two pests (Table 1). Exclusion

Fig. 3 Percentage of individuals (mean $\pm$ SEM) of pest (**A**, **B**) and beneficial (**C**, **D**, **E**) species not crossing nets made of square or rectangular mesh of different sizes under laboratory conditions. Different letters indicate significant differences (ANOVA (*D. suzukii* males) or Kruskal–Wallis (all others), $\alpha=0.05$)

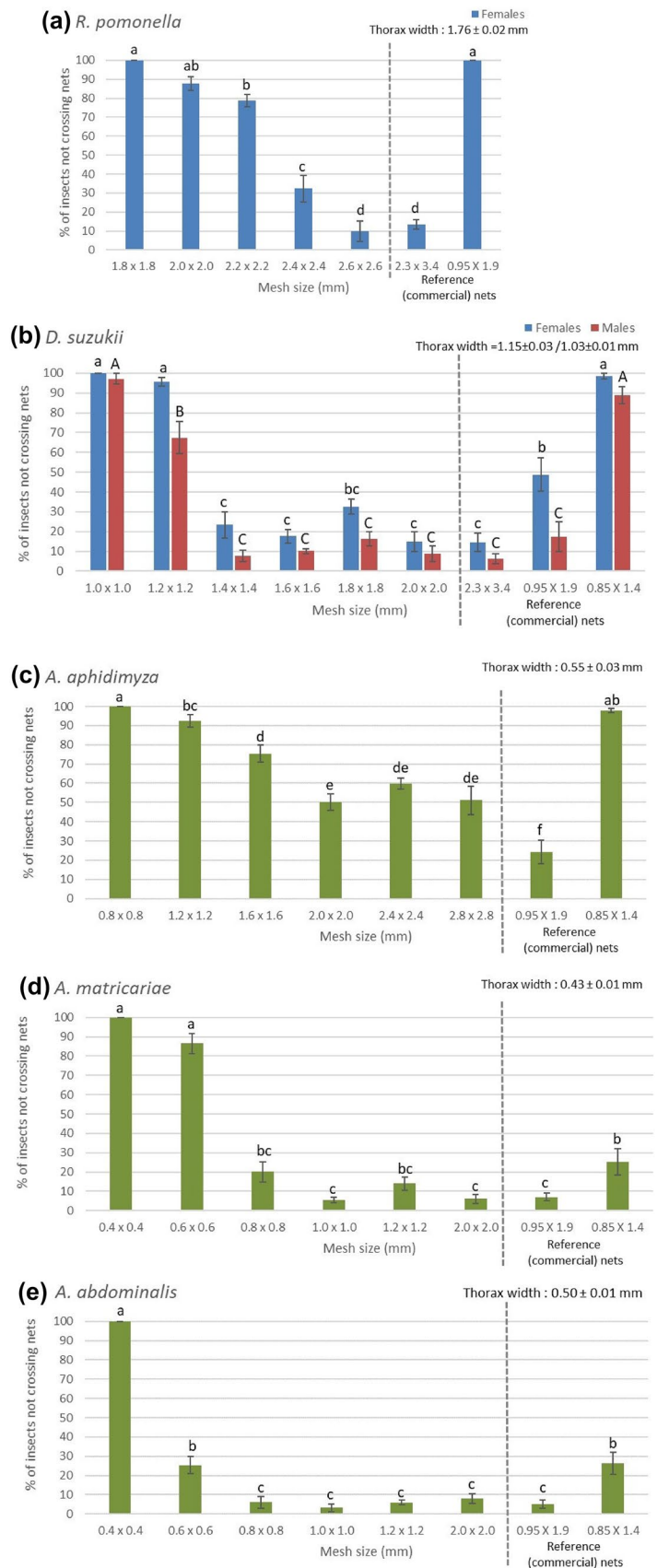


Table 1 Percentage of individuals of selected pest species (mean $\pm$ SEM) not crossing nets of different geometric patterns with an equal aperture size (area). Different letters indicate significant differences (ANOVA (*R. pomonella*) or Kruskal–Wallis (*D. suzukii*), $\alpha = 0.05$). *a* = height; *b* = width

Mesh pattern	Area: 5.29 mm ²		<i>R. pomonella</i> (females)	Area: 1.69 mm ²		<i>D. suzukii</i> (females)	<i>D. suzukii</i> (males)
	<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		
	mm			mm			
Hexagon	2.5	2.9	13.3 $\pm$ 9.4 a	1.4	1.6	32.1 $\pm$ 4.0 a	15.7 $\pm$ 6.4 a
Square	2.3	2.3	41.1 $\pm$ 3.6 ab	1.3	1.3	52.1 $\pm$ 12.4 ab	24.8 $\pm$ 9.5 a
Rhombus	2.5	4.3	69.9 $\pm$ 7.4 bc	1.4	2.4	79.0 $\pm$ 3.8 b	50.2 $\pm$ 6.2 a
Triangle	3.0	3.5	75.6 $\pm$ 9.5 cd	2.0	1.7	80.5 $\pm$ 5.6 b	40.8 $\pm$ 9.9 a
Rectangle	1.6	3.3	100.0 $\pm$ 0.0 d	0.9	1.8	100.0 $\pm$ 0.0 c	100.0 $\pm$ 0.0 b

rate was positively related to the shape factor (length/width ratio) of the apertures ($r^2 = 0.42$, $p < 0.0001$).

For the aphidiphagous predator *A. aphidimyza*, square apertures allowed a greater number of individuals to pass through than the other shapes tested (ANOVA, $F_{3,20} = 9.47$; $p = 0.0004$). In the case of parasitoid wasps, the area selected for the test did not enable measurement of significant effects of mesh shape on ability to cross (Fig. 3D–E; Table 2).

Field experiment

Aphids and their natural enemies. More than 85% (163/183) of aphid colonies observed in early June were *A. pomi*, and the remainder were *D. plantaginea*; in late June, > 99% of colonies were *A. pomi* (Table 3). Aphid density on the second assessment was significantly greater on trees covered with nets made of smaller-sized mesh (0.95×1.9 mm), compared to unnetted trees (ANOVA; $F_{2,8} = 4.68$; $p = 0.0221$). The effect of nets on aphids was only apparent for the green apple aphid, and only with the smaller-sized mesh. The larger-sized mesh (2.2×3.4 mm) did not have an overall effect on aphids compared to control plots. The same pattern was also observed following the monitoring of aphids on selected colonies (Kruskal–Wallis; $\chi^2 = 11.29$; $df = 2$; $p = 0.0035$).

Ants were observed in large numbers on growing shoots throughout the season, which possibly reduced the presence of predators and parasitoids. However, nets were not found to have an observable effect on the abundance of ants (Kruskal–Wallis; $\chi^2 = 0.38$; $df = 2$; $p = 0.8268$). Predatory midges were the most abundant natural enemies, representing more than 80% of beneficials observed within aphid colonies in control plots, and nearly all of those observed under netted plots. As for aphids, this predator was observed in similar numbers in trees covered with large mesh netting and in unnetted trees. However, although aphidiphagous cecidomyiids were also present in plots covered by small mesh nets, their abundance in those was significantly lower (about three times) than in the other plots with or without nets (Kruskal–Wallis; $\chi^2 = 46.17$; $df = 2$; $p < 0.0001$), despite aphids being more abundant.

Syrphid fly larvae and *Leucopis* relatives were the second most important among aphid predators and were observed almost exclusively in unnetted plots. A very small number of lady beetles and lacewings were observed, again only in unnetted plots. A few parasitized aphids were observed, but a significant effect of the net could not be detected (Kruskal–Wallis; $\chi^2 = 3.08$; $df = 2$; $p = 0.2141$). Most parasitoids that emerged from the collected mummies belonged to the genus *Binodoxys* (Hymenoptera: Braconidae) and were found in all three types of plots. A colony of the rosy apple aphid *D. plantaginea* parasitized by braconids of the genus *Ephedrus* was also observed

Table 2 Percentage of individuals of selected beneficials (mean $\pm$ SEM) not crossing nets of different geometric patterns with an equal aperture size (area). Different letters indicate significant differences (ANOVA, $\alpha=0.05$). a =width; b =length

Mesh pattern	Area: 7.84 mm ²		Area: 0.49 mm ²		<i>A. aphidimyza</i> (males and females)	<i>A. maricariae</i> (males and females)	<i>A. abdominalis</i> (males and females)
	$\frac{a}{b}$	mm	$\frac{a}{b}$	mm			
Hexagon ¹					NA	NA	NA
Square	2.8	2.8	0.7	0.7	13.1 $\pm$ 3.3 a	16.1 $\pm$ 3.3 a	9.4 $\pm$ 2.7 a
Rhombus	3.0	5.2	0.8	1.3	29.4 $\pm$ 3.2 b	21.2 $\pm$ 3.2 a	7.0 $\pm$ 0.6 a
Triangle	3.7	4.3	1.1	0.9	34.1 $\pm$ 5.4 b	16.0 $\pm$ 5.4 a	5.7 $\pm$ 1.2 a
Rectangle	2.0	4.0	0.5	1.0	34.2 $\pm$ 4.8 b	31.7 $\pm$ 4.8 a	4.1 $\pm$ 2.3 a

¹The hexagon pattern was tested only with pest species for reasons of material availability

in a control plot in mid-June. Some hyperparasitic wasps were also identified, belonging to the family Figitidae.

Leafrollers and their natural enemies. Leafroller populations (exclusively *C. rosaceana*) were significantly more abundant in netted trees than in unnetted ones, but this difference was significant only in the summer generation (Kruskal–Wallis; $\chi^2=6.02$; $df=2$; $p=0.0494$). No difference could be detected between the two mesh sizes (Table 4).

A significantly higher rate of parasitism was observed in larvae collected from control trees than from netted trees, both for the overwintering and the summer generation ($G^2=25.08$; $df=2$; $p<0.0001$ and $G^2=40.71$; $df=2$; $p<0.0001$). Mesh size had no significant effect on the parasitism rate of the overwintering generation, which remained very low (0–3.6%) under nets, while it exceeded 40% in the control plots (Table 4). Parasitism among larvae was much higher for the summer generation than for the one that overwintered, both in the unnetted trees (73%) and in trees covered with large mesh netting (29%). The smaller mesh netting, on the other hand, strongly reduced the parasitism rate (to 3%).

Eight different species of leafroller parasitoids were identified, *Actia interrupta* (Diptera: Tachinidae) being the most abundant overall (Table 4). Parasitism by this species was also more important in trees covered with large mesh netting than in those covered by small mesh netting ($G^2=24.81$; $df=2$; $p<0.0001$). The second most abundant parasitoid species was *Apophua* sp. (Hymenoptera: Ichneumonidae).

Fruit damage and quality. Damage from most insect pests was significantly reduced by nets, with almost no difference according to mesh size. Both mesh sizes almost totally excluded the apple maggot (ANOVA; $F_{2,8}=13.28$; $p=0.0029$) and the codling moth (ANOVA; $F_{2,8}=13.00$; $p=0.0024$), while damage to 3 and 13% of fruits was due to these pests, respectively, in unnetted plots (Table 5).

Plum curculio and leafrollers (almost exclusively the obliquebanded leafroller *Choristoneura rosaceana*) were the most damaging pests of netted apples (7–15%), while plum curculio (*Conotrachelus nenuphar*) and hemipterans (mostly from families Miridae and Pentatomidae) were the most damaging in unnetted control plots (18–22%). Nearly 10% of fruits observed in netted trees was damaged by plum curculio. When present, diseases affecting fruit were equally important in all treatments, although fire blight was observed in significantly lower numbers on netted trees, regardless of mesh size (Kruskal–Wallis; $\chi^2=7.10$; $df=2$; $p=0.0288$). Surveyed fruit quality parameters appeared unaffected by nets, regardless of mesh size (Table 5).

Table 3 Mean density ($\pm$ SEM) of aphids per leaf cluster/growing shoot versus mean abundance ($\pm$ SEM) of aphids, ants and aphid natural enemies on selected infested apple shoots from trees covered with nets of different mesh sizes and uncovered trees. Different letters indicate significant differences (ANOVA (aphid density) or Kruskal–Wallis (abundance on selected shoots), $\alpha=0.05$)

	Large mesh (2.2 $\times$ 3.4 mm)	Small mesh (0.95 $\times$ 1.9 mm)	No net
<i>Population density</i> ¹			
Early June			
<i>D. plantaginae</i>	0.53 $\pm$ 0.29 a	0.11 $\pm$ 0.07 a	0.92 $\pm$ 0.54 a
<i>A. pomi</i>	5.95 $\pm$ 3.76 a	2.78 $\pm$ 1.15 a	0.52 $\pm$ 0.24 a
Late June			
<i>D. plantaginae</i>	0 a	0.46 $\pm$ 0.31 a	0.06 $\pm$ 0.04 a
<i>A. pomi</i>	14.1 $\pm$ 3.94 ab	20.57 $\pm$ 6.13 a	8.08 $\pm$ 2.12 b
<i>Abundance on selected infested shoots</i> ²			
Aphids			
<i>A. pomi</i>	82.58 $\pm$ 3.66 b	97.71 $\pm$ 4.02 a	80.28 $\pm$ 4.21 b
Aphid allies			
Formicidae	3.20 $\pm$ 0.16 a	3.42 $\pm$ 0.20 a	3.52 $\pm$ 0.25 a
Natural enemies			
Cecidomyiidae	1.48 $\pm$ 0.21 a	0.48 $\pm$ 0.07 b	1.49 $\pm$ 0.17 a
Chamaemyiidae/Syrphidae	0.008 $\pm$ 0.004 b	0.003 $\pm$ 0.002 b	0.278 $\pm$ 0.073 a
Chrysopidae	0 a	0 a	0.004 $\pm$ 0.003 a
Coccinellidae	0 b	0 b	0.01 $\pm$ 0.003 a
Braconidae	0.002 $\pm$ 0.002 a	0.003 $\pm$ 0.002 a	0.01 $\pm$ 0.006 a

¹ Average number of aphids per leaf cluster/shoot ($n = 180$ samples per experimental unit)

² Average number of individuals per selected infested shoot per sampling date ($n = 606, 604$ and 479 , respectively, for large mesh, small mesh and unnetted control)

Table 4 Mean larval density ($\pm$ SEM) of *C. rosaceana*, parasitism rate and number of natural enemies that emerged from larvae collected from apple trees covered with nets of different mesh sizes and uncovered trees. Different letters indicate significant differences (Kruskal–Wallis (larval density) or Likelihood Ratio Chi-square test (parasitism), $\alpha=0.05$)

	Large mesh (2.2 × 3.4 mm)	Small mesh (0.95 × 1.9 mm)	No net
Assessment of <i>C. rosaceana</i> larval density			
Overwintering generation	8.6 ± 4.2 a	6.2 ± 1.8 a	1.8 ± 0.5 a
Summer generation	3.6 ± 1.4 a	5.0 ± 1.9 a	0.0 b
Assessment of larval parasitism			
Overwintering generation ¹			
Parasitism (% total)	0.0% b	3.6% b	41.4% a
Ichneumonidae (Hymenoptera)			
<i>Apophua</i> sp	0 b	1 b	7 a
<i>Itoplectis</i> sp	0 a	0 a	1 a
Braconidae (Hymenoptera)			
<i>Macrocentrus</i> sp	0 b	0 b	3 a
<i>Meteorus</i> sp	0 a	0 a	1 a
Summer generation ²			
Parasitism (% total)	28.6% b	3.0% c	72.7% a
Tachinidae (Diptera):			
<i>Actia interrupta</i>	3 b	0 c	14 a
Other tachinids	0 b	0 b	4 a
Ichneumonidae (Hymenoptera)			
<i>Apophua</i> sp	3 a	1 a	3 a
<i>Exochus</i> sp	2 a	0 a	0 a
Braconidae (Hymenoptera)			
<i>Macrocentrus</i> sp	0 a	0 a	2 a
Chalcidoidea (Hymenoptera)			
<i>Sympiesis</i> sp	0 a	0 a	1 a

¹n = 29, 28, 29, respectively, for large mesh, small mesh and unnetted control²n = 28, 33, 33, respectively, for large mesh, small mesh and unnetted control

Table 5 Fruit damage, yield and fruit quality measurements ($\pm$ SEM) from apple trees covered with nets of different mesh sizes and unnetted (control) trees. Different letters indicate significant differences (ANOVA or Kruskal–Wallis (hemipterans and fire blight), $\alpha=0.05$)

	Large mesh (2.2 $\times$ 3.4 mm)	Small mesh (0.95 $\times$ 1.9 mm)	No net
Insect damage¹			
Codling moth (July)	0.1 $\pm$ 0.1 b	0.0 b	4.6 $\pm$ 1.7 a
Codling moth (harvest)	0.1 $\pm$ 0.1 b	0.0 b	3.1 $\pm$ 0.8 a
Apple maggot	0.1 $\pm$ 0.1 b	0.0 b	12.9 $\pm$ 3.5 a
Plum curculio (June)	8.8 $\pm$ 2.3 b	7.1 $\pm$ 2.8 b	22.2 $\pm$ 7.0 a
Plum curculio (harvest)	12.3 $\pm$ 3.5 b	9.9 $\pm$ 3.9 b	26.8 $\pm$ 8.1 a
Tarnished plant bug	0.6 $\pm$ 0.3 b	0.8 $\pm$ 0.1 b	9.3 $\pm$ 6.0 a
Other plant bugs	1.4 $\pm$ 0.4 b	0.7 $\pm$ 0.2 b	8.9 $\pm$ 4.1 a
Stink bugs	1.1 $\pm$ 0.4 a	0.3 $\pm$ 0.2 a	7.4 $\pm$ 3.3 a
Total hemipterans	3.1 $\pm$ 0.7 b	1.8 $\pm$ 0.3 b	25.8 $\pm$ 11.8 a
Spring feeding caterpillars	0.4 $\pm$ 0.3 a	0.2 $\pm$ 0.1 a	1.3 $\pm$ 0.8 a
Leafrollers	15.0 $\pm$ 1.8 ab	9.8 $\pm$ 2.6 b	18.3 $\pm$ 4.5 a
Disease damage¹			
Apple scab	0.0	0.0	0.0
Sooty blotch/flyspeck	1.8 $\pm$ 1.1 a	3.9 $\pm$ 2.2 a	2.2 $\pm$ 0.8 a
Rust disease	0.1 $\pm$ 0.1 a	0.1 $\pm$ 0.1 a	0.1 $\pm$ 0.1 a
Summer rot	0.8 $\pm$ 0.4 a	0.8 $\pm$ 0.4 a	3.6 $\pm$ 1.4 a
Fire blight ²	0.0 b	0.0 b	7.2 $\pm$ 6.0 a
Other damage¹			
Mechanical injury	5.4 $\pm$ 0.9 a	6.1 $\pm$ 0.9 a	10.0 $\pm$ 1.7 a
Fruit asymmetry/deformity	0.8 $\pm$ 0.5 a	0.2 $\pm$ 0.1 a	0.3 $\pm$ 0.1 a
Russetting	1.3 $\pm$ 0.6 a	0.3 $\pm$ 0.1 a	1.1 $\pm$ 0.3 a
Bitter pit	3.1 $\pm$ 1.0 a	2.7 $\pm$ 0.6 a	4.2 $\pm$ 1.2 a
Yield and fruit quality measurements³			
Red skin color (%)	27.2 $\pm$ 2.7 a	20.1 $\pm$ 2.4 a	26.1 $\pm$ 2.4 a
Firmness (kg)	7.1 $\pm$ 0.2 a	7.1 $\pm$ 0.1 a	15.9 $\pm$ 0.3 a
Fruit diameter (mm)	79.7 $\pm$ 2.7 a	77.2 $\pm$ 1.1 a	75.3 $\pm$ 1.4 a
Total yield (kg/tree)	10.7 $\pm$ 0.7 a	11.9 $\pm$ 1.0 a	9.3 $\pm$ 1.2 a
In-canopy measurements			
Chlorophyll fluorescence (Fv/Fm) ⁴	0.78 $\pm$ 0.002 a	0.78 $\pm$ 0.005 a	0.77 $\pm$ 0.013 a
Air temperature (°C) ⁵	21.1 $\pm$ 0.02 a	21.1 $\pm$ 0.02 a	21.1 $\pm$ 0.02 a
Relative Humidity (%) ⁵	74.5 $\pm$ 0.08 a	74.9 $\pm$ 0.08 a	74.1 $\pm$ 0.08 a

¹Percentage of damage at harvest (unless otherwise specified) ($n=180$ apples per experimental unit)²Number of infected shoot and fruit clusters (pooled from weekly inspections from mid-June to mid-August)³Average value from 15 to 30 apples (fruit quality) or 6 trees (yield) per experimental unit⁴Average value from 6 leaves located on two shoots (from opposite sides of tree) measured on three occasions between mid-June and late August⁵Average value from hourly measurements at a height of 1.25 m, recorded between fruit set (late May) and harvest (mid-September) from two replicates per treatment

Discussion

Mesh size and pattern. Regarding the geometry of the mesh, results suggest that the closer to one the a/b ratio, the more the aperture area allows insects to pass through. Thus, for nets with square or hexagonal apertures, a smaller size is necessary to achieve a pest exclusion efficacy similar to that obtained with nets with rectangular, rhomboid or triangular shapes. Most exclusion nets on the market are indeed rectangular in shape, with an a/b ratio generally less than 0.5 (Álvarez and Oliva 2015). In our laboratory tests, the greatest exclusion efficacy was actually obtained with the rectangle, which had the smallest a/b ratio (0.5) among tested shapes. It should be noted, however, that a triangular shape with an a/b ratio lower than the one we used (0.9) could have increased the exclusion efficacy of this pattern. Parasitoids *A. matricariae* and *A. abdominalis* and insect pests *R. pomonella* and *D. suzukii* were able to pass through net apertures only slightly larger than the width of their thorax. For predatory species *A. aphidimyza*, the aperture had to be more than twice its thorax width. Exclusion efficacy was not solely predicted by mesh size relative to the thorax size of insects, however (as similarly noted by Bethke and Payne 1991). Other physical and/or behavioral characteristics are probably involved, because while the thoraxes of the three beneficials tested were relatively similar in width, the tested nets were much more permeable to parasitoid wasps than to the predator *A. aphidimyza*. Although other morphological characteristics could be involved, it is also possible that, for the latter species, the phototropism stimuli used in the laboratory to stimulate insect crossing through the nets were inadequate. As a matter of fact, while *A. aphidimyza* was almost completely excluded in the laboratory by the commercial net made of smaller mesh (0.95×1.90 mm), permeability was greater in the orchard, with eggs or larvae being observed on more than 40% of infested shoots from trees covered with the same netting.

Conversely, it is also possible for an insect to be more effectively excluded by a specific netting installed in the field than in the laboratory. When alternative choices are available, the physical presence of the net itself may be sufficient to divert the insect to other more easily accessible hosts. For example, in our laboratory tests, the commercial net made of larger-sized mesh was ineffective in preventing *R. pomonella* from passing through, whereas in orchards, the same net offered excellent protection against this pest. A similar finding was also reported for *D. suzukii* by Charlot et al. (2014). According to their work, mesh sizes up to 1.37×1.71 mm were sufficient to exclude flies, while a much finer mesh (0.81×1.37 mm) was required

in the laboratory. Our laboratory results concur with their observations but differ from those of Grassi et al. (2016), who reported that a mesh size of 1.0×1.0 mm allows some *D. suzukii* to pass through (possibly smaller males, since unsexed individuals were used in their trials). In our laboratory tests, 1.0×1.0 mm mesh net, as well as the commercial 0.85×1.4 mm mesh net currently used in Quebec to control *D. suzukii* in berry crops, also almost completely excluded females, although a very small number of males did pass through the latter.

While larger mesh sizes can facilitate pest colonization of netted trees, they obviously can also help beneficials to access their prey or hosts. The outcome of those interactions cannot be generalized; in our field tests, for example, aphids were not observed to benefit much from increased mesh size, possibly because predatory *A. aphidimyza* were present in higher numbers when mesh size was larger. Other studies have also shown contrasting effects of netting on aphids and/or their natural enemies (Dib et al. 2010; Marliac et al. 2013; Alaphilippe et al. 2016). Determining appropriate mesh size in a given system requires consideration of the relative contribution of multiple factors, including effectiveness against pests and selectivity toward the beneficials present in that system, as well as the agronomic and meteorological effects of varying mesh sizes. In our field study, the larger mesh size offered a similar protection to that obtained with the smaller one (which is the current reference netting) against the apple pests present in 2018. It did not differ either in terms of effects on temperature, relative humidity, photosynthetic activity, yield or fruit quality. These results confirm our previous findings (Chouinard 2017, 2019) that nets can be used with no significant impact on yield or fruit quality, while providing adequate protection against many insect pests. It also identified the following possible benefits and concerns regarding pests and beneficials in complete exclusion systems:

Plum curculio the observed level of damage in netted trees (9.9–12.3%) was higher than expected, considering it never exceeded 2% in our previous studies. Higher pest pressure in that year (as observed in the control) may partly explain these results, considering that, in such cases, there is an increased risk that some females will colonize the trees when nets are left open during pollination and then cause damage later.

Stink bugs despite the fact that overall damage from hemipterans was significantly reduced by netting (Kruskal–Wallis, $p = 0.0084$), only numerically lower levels (Kruskal–Wallis, $p = 0.0681$) were observed for stink bugs. This differs from findings in Italian studies (Candian et al. 2018, 2021) that demonstrated the effectiveness of nets to protect rows of apple and nectarine trees against Pentatomidae such as *Halyomorpha halys*. However, it should be noted that despite being significant in those

studies, efficacy was not always agronomically significant and that pesticides were used in conjunction with netting. Among the possible explanations for the relatively weak efficacy of netting against stink bugs, we suggest imperfect net closure around tree trunks, net opened up during pollination, neonates entering following egg deposition on the exterior of the nets (which was repeatedly observed during our assessments) and confounding factors (e.g., bitter pit being misidentified as stink bug damage).

Leafroller parasitoids surprisingly, the most common parasitoid found in netted plots (*Apophua* sp.), was an insect whose adult size is much larger than the apertures of the nets used in our study. Moreover, it was almost exclusively found in our second sampling, which targeted the summer generation of OBLR. Since *Apophua* overwinters inside its host (Cossentine et al. 2004), these results suggest that adults can parasitize caterpillars in the fall (after the nets are removed). In the following season, the parasitoids that emerge find themselves confined by nets and parasitize the larvae of the next (summer) generation of leafrollers. A parasitoid of the genus *Exochus* (Hymenoptera: Ichneumonidae) was also collected from trees covered with large mesh netting. Little information is available in the literature on this genus (period of activity, parasitized larval stages, diapause stage). It is therefore difficult to determine whether its presence under the nets was due to its ability to pass through the 2.2 mm X 3.4 mm apertures or to parasitism that occurred during pollination or after harvest, when the nets were not in place. Increased parasitism in the summer generation of *C. rosaceana* was observed only with larger mesh size, which is supported by significantly higher associated numbers of the tachinid *A. interrupta* in parasitized larvae. This suggests that this fly can cross the larger-sized mesh. It also suggests that some nets actively impact leafroller parasitism and that they should be retracted as soon as possible to benefit from the parasitic activity of species that are active in the fall. Since those parasitoids overwinter in the host and emerge from the hibernating larvae next spring, they would then be confined under the nets and hopefully parasitize larvae present in the system. The other parasitoids recorded in the study included tachinids, ichneumonids, braconids and chalcids and were only found in unnetted control plots.

A follow-up over a longer period would be required to establish more solidly the effectiveness of larger mesh sizes over smaller ones. Such studies would enable verification of whether a greater abundance of certain natural enemies is observable year after year and would result in better natural control of certain pests present in exclusion systems. These could, in turn, lead to possible changes in the current standards used for exclusion netting in apple orchards of Québec and elsewhere.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by FP, ML and CP. The first draft of the manuscript was written by GC, and all authors commented on previous version of the manuscript. All authors read and approved the final manuscript.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10340-022-01582-5>.

Acknowledgements The authors would like to thank the following persons for their involvement in field and/or laboratory work during the study: Jessica Champagne, Valérie Roy, Josiane Morrisette, Franz Vanoosthuyse, Audrey Charbonneau, Benoit Gadbois, Annabelle Firlej, Elisabeth Ménard, Kim Ostiguy, Éric Ménard, and many others.

Funding This research was funded by the Innov'Action Agroalimentaire program, supported by the ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec and Agriculture and Agri-Food Canada, through the Growing Forward 2 initiative and with contributions from Dubois Agrinovation and Les Producteurs de pommes du Québec.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This declaration is not applicable.

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