Bulletin of Insectology **77** *(2): 193-205, 2024*
ISSN 1721-8861 eISSN 2283-0332

Tree architecture impacts the effectiveness of exclusion netting for *Cydia pomonella* mating suppression in pome fruit

Michele Preti[1], Esteban Basoalto[2], Alan L. Knight[3]
[1]*ASTRA Innovazione e Sviluppo, Faenza, Italy*
[2]*Universidad Austral de Chile, Valdivia, Chile*
[3]*Instar Biologicals, Yakima, WA, USA*

Abstract

Mechanical control involving nets and screens to physically exclude insect pests from pome fruit is an effective and sustainable method to suppress insect damage reducing the insecticide usage. The successful of these netting systems can be affected by several factors, including the tree crop architecture. Studies were conducted in 2022-2023 to evaluate the effectiveness of single-row exclusion netting in the management of codling moth, *Cydia pomonella* L., in pome fruit orchards. White netting (6.0 mm × 1.8 mm mesh) was placed in plots of apple and pear of different canopy structures (central leader, spindle, and V-trellis). Releases of sterilized male and female moths were conducted inside and outside netting. Delta traps baited with a sex pheromone or kairomone-based lure were used to monitor *C. pomonella* flight inside and outside replicated plots. Wild and sterile females caught in traps were dissected to determine their mating status. The exclusion netting significantly reduced the occurrence of fruit injury from *C. pomonella* and birds compared to unnetted adjoining trees. The recapture of sterile moths was very low in the netted spindle trees, while a moderate number of moths were recaptured under the other two training systems. Similarly, the proportion of mated sterile moths was higher in the V-trellis plots compared with the spindle trellis. The catch and level of mating of wild moths in these plots were also highest with the V-trellis. Fruit injury recorded in both the V-trellis and central leader canopies were primarily from the first generation and only small clusters of injury occurred in the second half of the season. These data demonstrate the relative effectiveness of exclusion netting for *C. pomonella* management can be influenced by the canopy structure and the degree of open space remaining under the netting for successful moth flight and sexual communication.

Key words: codling moth, integrated pest management, pest monitoring, fruit injury, mechanical control, organic agriculture.

Introduction

The use of netting in apple orchards serves several important management roles ranging from protection from hail, UV radiation, frugivorous birds, and insect pests (Middleton and McWaters, 2002; Chouinard *et al.*, 2017; Manja and Aoun, 2019; Nelson *et al.*, 2023). Not surprisingly, nets also have a range of secondary effects on orchard's microclimate (temperature, relative humidity, and wind speed) impacting disease epidemiology, photosynthesis, and various fruit quality parameters (Iglesias and Alegre, 2006; Bogo *et al.*, 2012; Bastías *et al.*, 2012; Mupambi *et al.*, 2018; Chouinard *et al.*, 2019). Tilting the balance towards increased benefits from netting versus the costs of construction and potential negative effects has been the goal in utilizing this technology (Iglesias and Alegre, 2006; Castellano *et al.*, 2008; Chouinard *et al.*, 2016).

The impact of orchard netting systems on the management of codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), has evolved with their development over the past 30+ years. The initial use of overhead flat-roof permanent structures for hail protection was shown to have significant positive effects in managing *C. pomonella* (Graf *et al.*, 1999). However, the high cost of these structures, and the infrequent occurrence of hail in many production regions was often not cost-effective (Apáti and Soltész, 2011). Instead, the adoption of overhead permanent structures in the arid region of eastern Washington State has been primarily for sunburn protection of fruit, which is a significant yearly cull factor (Mupambi *et al.*, 2019; Morales-Quintana *et al.*, 2020). Exclusion netting in Europe has been more directed for *C. pomonella* management and in response to insecticide resistance developing to both organic and conventional insecticides (Sauphanor *et al.*, 2006; Reyes *et al.*, 2009). In addition, the mechanical control methods involving nets and screens, which in tree fruit crops act as physical barriers for the exclusion of several insect pests' infestation, are a sustainable component of the integrated pest management approach in order to overall reduce the insecticide usage (Barzman *et al.*, 2015; Karuppuchamy and Venugopal, 2016) and have become a pillar in both the organic and conventional agricultural systems (Shaw *et al.*, 2021). In southern France, where successful management of *C. pomonella* had become problematic for some growers, a less expensive approach that did not interfere with orchard operations was the application of single-row netting (Alt'Carpo) that completely enclosed each row separately and was removed each year, with more than 3,000 ha in France and 2,000 ha in Italy (Romet *et al.*, 2010; Caruso *et al.*, 2017). Single-row netting was shown to be more effective against *C. pomonella* than the traditional hail netting in two studies (Kelderer *et al.*, 2010; Baiamonte *et al.*, 2016). This difference in efficacy of the two systems could be due to their relative permeability for moths due to differences in the mesh sizes (3.0 mm × 7.4 mm *versus* 2.2 mm × 5.4 mm) or the proximity of the netting over the tree's canopy (Basedow *et al.*, 2018).

Studies have shown that a range of mesh sizes can significantly affect the exclusion of pests, including adult *C. pomonella* (Chouinard *et al.*, 2022), and natural enemies (Marshall and Beers, 2022). Interestingly, netting has been somewhat more permeable to moth emigration out of the net enclosure (Sauphanor *et al.*, 2012; Marshall and Beers, 2023). Under experimental conditions, *C. pomonella* was seen to lay eggs through the netting on exposed fruit or leaf surfaces and females readily passed through screened cages toward a source of sugar water (Sauphanor *et al.*, 2012).

Netting is either placed directly over the canopy with the Alt'Carpo method or attached to poles and suspended at some distance above the top of the canopy in permanent structures. However, even the single-row netting used in trellised orchards is typically suspended from the top wire and horizontal supports may be included to keep the netting from crushing the canopy (Chouinard *et al.*, 2017). Thus, a variable amount of open space exists above the tree canopy under single-row netting in different orchards according to tree architecture. Unlike with hail netting, the single-row netting is generally in close contact with each tree's canopy on top and along the sides of the row and is tied off at or near the ground. One hypothesis is that the proximity of the netting to the foliage and the available air space for moths to fly around and within the canopy impacts the sexual behaviors and oviposition of moths and females (Tasin *et al.*, 2008; Sauphanor *et al.*, 2012). Therefore, the more air space that is available for moth flight over the canopy, the less effective the behavioral disruption.

The impacts of netting on female *C. pomonella* behaviors including mating and oviposition have not been characterized in relation to tree architecture under the nets. For example, no studies have dissected free-flying female *C. pomonella* to assess their mating status under nets. One study under hail nets used virgin female-baited traps and tethered females, caught few males, yet had significant levels of mating low in the canopy (Tasin *et al.*, 2008). Marshall and Beers (2023) released both sexes of marked, sterilized *C. pomonella* inside and outside permanent enclosures but data for females caught in traps and their mating success in either location have not been reported. Under laboratory conditions males (smaller) escaped more frequently than females through two mesh sizes, 3.0 mm × 7.4 mm (hail net) and 2.5 mm × 5.5 mm mesh (Alt'Carpo). Interestingly, virgin females were more mobile than mated females (Sauphanor *et al.*, 2012). However, in their field experiments with single-row netting system these authors caught only one female moth under the netting compared with 44 individuals outside the nets, and its mating status was not ascertained. Both studies primarily relied on significant reductions in male catch in traps and fruit injury level to establish the success of netting and did not measure the impact of netting on female moth sexual behaviors. Importantly, both studies reported a sizeable and unexplained variability in moth catch and fruit injury in relation to the netted areas proximity to open rows, among netted blocks, across generations, and over repeated seasons (Sauphanor *et al.*, 2012; Marshall and Beers, 2023).

Apple production systems have been radically transformed over the past 20 years towards high-density trellised systems with variable canopy shapes, i.e. primarily V-trellis and conic shapes on dwarfing rootstocks (Robinson *et al.*, 2007; Lauri, 2009). This variability among orchard plantings may have significant effects on how netting impacts *C. pomonella* infestations. Not all studies of netting have clearly stated (often a photo or drawing is shown) the canopy dimensions under netting versus the height of the netting structure. The first published experimental study with single-row netting in France placed poles that suspended the netting either 30-40 cm or 1-m above the canopy of the trees (Sauphanor *et al.*, 2012). However, this key point was not provided for the 23 growers' orchards utilizing Alt'Carpo netting. The most recent experimental study with *C. pomonella* under nets used both 3-m and 4.5-m tall frames placed over trellised rows and the dimensions of the trees were not provided (Marshall and Beers, 2021; 2023). Hail netting is generally flat-roofed and encloses the entire block (Middleton and McWaters, 2002). Thus, the drive rows provide repetitive open spaces for moth flight around the canopy. Also, these structures are usually built taller than the expected tree canopy and this provides open space for moths to fly over the trees. *C. pomonella* adults tend to be most abundant in the upper canopy during dusk (Weissling and Knight, 1995; Epstein *et al.*, 2011).

Herein, we report studies examining the catch and mating status of wild and released sterilized female *C. pomonella* under single-row "Drape-Net" covering three common types of apple tree architectures: central leader, spindle, and V-trellis. Levels of fruit injury from *C. pomonella* and birds were collected from organic apple and pear orchards in both 2022 and 2023. The aim was to evaluate how the tree architecture impacts the netting effectiveness to control *C. pomonella* not only by physically exclude this pest from the orchard, but also by changing the female moths' behavior and their mating status under the nets.

Materials and methods

Studies were conducted with white netting (model "Drape-Net", 6.0 mm × 1.8 mm) provided by Drapenet North America (Prosser, WA, USA) in six organic pome fruit orchards located near Zillah (46.24°N 120.15°W) and near Tieton (46.70°N 120.76°W), in Washington State, USA. Netting was placed following petal fall (ca. at the end of April) in all blocks except in 2022 in one apple block cultivar Honeycrisp located near Zillah, where the acreage of netting was expanded in mid-June to include an additional hectare of orchard. The start of moth flight based on *C. pomonella* catches in traps was established in Tieton and Zillah as 31 May and 3 May in 2022 and 25 May and 3 May in 2023, respectively. Netting was either hung over plots by the grower with the use of a specialized machine ("NetWizz Applicator", Drapenet Inc.) or by a small crew of workers lifting the netting using PVC poles. Nets were closed underneath the canopy with variably spaced cable ties.

Sites were treated with mating disruption dispensers for *C. pomonella* except for the two Zillah sites in the second year of study (2023). The five pome fruit blocks located near Tieton were treated with Cidetrak CMDA Combo PP dispensers loaded with 90 mg pheromone and 60 mg pear ester and applied at 500 dispensers ha^{-1} (Trécé Inc., Adair, OK). The apple blocks cultivar Honeycrisp located near Zillah were treated with the Cidetrak CMDA Combo Meso-A dispensers loaded with 850 mg sex pheromone and 500 mg pear ester and applied at 60 dispensers ha^{-1} (Trécé Inc., Adair, OK). The apple blocks cultivar Cripps Pink located near Zillah were treated with the Isomate CM Flex dispenser loaded with 158 mg of sex pheromone and applied at 1,000 dispensers ha^{-1} (Pacific Biocontrol, Vancouver, WA). No sex pheromone dispensers for mating disruption were used in the two Zillah sites under nets in 2023.

Orchards were managed with a certified organic management program for *C. pomonella*, and this was identical for trees inside and outside the netting in each orchard, except in the second year of the two Zillah studies. Tactics in addition to sex pheromone dispensers included repeated spray applications of horticultural oil (1%) alone and in combination with insecticides, such as *Cydia pomonella* granulosis virus (4 - 12 applications per season), and a spinosyn insecticide (1 - 4 applications per season). Insecticide use was reduced approximately 75% in the blocks netted for two seasons in the Zillah sites in the second year of study.

All plots were monitored with orange delta-shaped traps (Pherocon Delta VI, Trécé Inc.) baited with one of two commercial lures (Trécé Inc.): the Pherocon Mega-Lure CM Dual 4K lure (4K), and in 2022 in the Tieton orchards and one site in Zillah with additional traps baited with Pherocon CM 1X septa (PH1X). The first lure (4K) is a 4-component kairomone-based blend including (*E*,*Z*)-2,4-ethyl decadienoate (pear ester), acetic acid, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and pyranoid linalool oxide; the second lure (PH1X) is a sex pheromone-based bait containing (*E*,*E*)-8,10-dodecadien-1-ol (codlemone). Cumulative male catch per replicate was from the sum of these two traps (baited either with 4K or PH1X lure). Female catch was the total from only traps with the 4K lure. The total number of traps varied across trials, as detailed hereafter; however, in each plot a minimum of one trap baited with the 4K lure was used to monitor the *C. pomonella* flight. Lures were replaced after 8 weeks. Liners (Clean Brake, Trécé Inc.) were checked and replaced every 4 weeks. Levels of fruit injury were sampled in all replicates of netting and unnetted areas. In each block, by visually inspecting groups of 25 fruits per sample, the total sample size ranged from 1,200 to 3,300 fruits. Damage incidence (percent of injured fruits out of the total observed) was recorded for both insect pests' and birds' feeding activity. Fruit injury caused by *C. pomonella* comprised larvae stings and deep entries, while pecked fruits were scored as bird injury. In addition, any fruit injury possibly caused by secondary pests such as leafrollers (erosion of the fruit surface), aphids, mirids or stink bugs (fruit deformation), was also recorded.

Six studies were conducted in the first year of the project in 2022. Trials #1-4 were conducted in Tieton apple orchards with a free-standing central leader architecture of cultivars Ambrosia, Honeycrisp, and Golden Delicious, and a pear orchard of cultivar Bartlett (figure 1). Both the Ambrosia and Honeycrisp cultivar blocks had been previously grafted and had assumed a vase-shaped structure. Each trial consisted of 4-5 paired replicates of small netted and unnetted plots of 3-10 plants each, according to the trial (table 1). Replicate pairs of plots (netted and without net) were spaced 20-30 m apart. Trial #5 was conducted near Zillah with 4 apple replicates of 1-row length each, of variable sized slender spindle trellised cultivar Cripps Pink. Two replicates had a narrow canopy with a maximum of 0.3 m spacing between the netting and canopy and the canopy was pruned as a near vertical wall of foliage (figure 2A). The other two replicates had up to 0.8 m of space between the netting and the canopy and had some open spacings of 1.3 - 1.6 m between trees within a row (figure 2B). The four unnetted comparison plots were adjacently planted apples of cultivar Royal Gala with a similar architecture as the wider rows of cultivar Cripps Pink. Each replicate was on a different row and traps were spaced 30 m apart. In Trials #1-5, each replicated plot was monitored with two traps baited with either the 4K or the PH1X lures. Trial #6 was conducted in a V-trellised block of cultivar Honeycrisp situated near Zillah in 2022 (figure 3). In Trial #6, 14 traps baited with the 4K lure were arrayed within a solid 2-ha block under single-row netting. Four traps with the 4K lure were also placed in the adjacent unnetted orchard. The grower decided to expand his netted area on 17 June and traps in the previously unnetted area were moved to a new area. However, two traps with 4K lures were kept in this plot with late deployment of the nets, and monitored for the rest of the season.

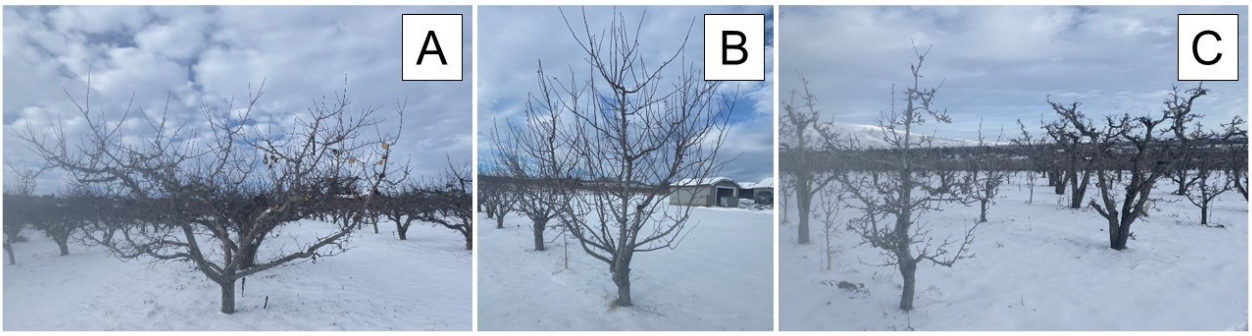

Figure 1. Central leader architecture in Tieton (WA, USA) orchards netted in 2022, (**A**) Golden Delicious apple, (**B**) Honeycrisp apple, and (**C**) Bartlett pear.

Table 1. Summary of research plots used to evaluate single-row netting for *C. pomonella* management, in Tieton and Zillah (WA, USA), in 2022-2023.

Trial #	Year, location	Cultivar	# plots netted [a]	Training system	Row × tree spacing (m × m)	Canopy height × width (m × m)	Plot size (m^2)
1	2022, Tieton	Ambrosia	5	Central leader	4.0 × 2.7	2.9 × 1.8	43
2	2022, Tieton	Honeycrisp	4	Central leader	4.9 × 2.4	2.7 × 2.7	120
3	2022, Tieton	Golden Delicious	4	Central leader	4.9 × 2.7	3.5 × 3.0	100
4	2022, Tieton	Bartlett	5	Central leader	4.3 × 4.0 4.3 × 4.0	2.7 × 1.5 2.1 × 1.2	43 - 156
5, 7	2022-2023, Zillah	Cripps Pink	4	Spindle, trellis	3.1 × 0.5 4.7 × 2.4	3.5 × 1.2 3.5 × 2.5	950 - 7,600
6, 8	2022-2023, Zillah	Honeycrisp	2 [b], 4	V-trellis	4.3 × 2.7	2.7 × 1.8	20,000 - 50,000
9	2023, Tieton	Cosmic Crisp	8	Spindle, trellis	3.2 × 1.0	2.1 × 1.0	43 - 476

[a] In each trial, in order to place the monitoring traps, the same number of replicates with a comparable area was considered for both the treated (# plots netted) and the control (# plots with no nets).
[b] Nets applied late in one of two blocks in mid-June 2022, four blocks included in 2023.

Figure 2. Netting placed over the narrow (**A**) and wide (**B**) slender spindle trellis architecture on apple orchards cultivar Cripps Pink located in Zillah (WA, USA), in 2022-2023.

Trials were continued with the same two growers in the Zillah area in the second year of the project, in 2023, using only traps baited with the 4K lure (table 1). Trial #7 carried out in 2023 on cultivar Cripps Pink was the same as Trial #5 conducted in 2022. In Trial #7, each plot was monitored with one trap baited with the 4K lure. Similarly, Trial #8 carried out in 2023 on cultivar Honeycrisp was the same as Trial #6 conducted in 2022. However, netting in 2023 was added to two more areas in this orchard, with a moderate and severe *C. pomonella* infestation, respectively. The only remaining portion of the orchard was the cultivar Granny Smith and was used as the unnetted comparison. In Trial #8, 16 traps were placed within the netted plots and 5 traps outside the nets, all baited with the 4K lure. Finally, in Trial #9 eight variable-sized single-rows of an apple orchard of cultivar Cosmic Crisp in Tieton were enclosed using the netting previously used in the four Tieton orchards in 2022.

Figure 3. V-trellis canopy in the apple orchards cultivar Honeycrisp without the netting (**A**) and inside a row with netting (**B**) located in Zillah (WA, USA), in 2022-2023.

One trap baited with the 4K lure was placed in each of the eight netted plots. Fourteen traps baited with the 4K lure were placed in the adjacent apple blocks including cultivars Cosmic Crisp, Golden Delicious and Delicious monitored as the unnetted areas.

Sterilized laboratory-reared and unsexed *C. pomonella* adults were obtained from the Okanagan-Kootenay Sterile Insect Release Program's mass-rearing facility in Osoyoos (British Columbia, Canada), through a donation from M3 Consulting Group (Dayton, OH, USA). Sterilized moths were chilled, packaged in cardboard cups, and marked with an internal red dye, which allowed them to be easily differentiated from wild moths when examined on trap sticky liners (Esch *et al.*, 2021). Two moth releases were conducted in Zillah orchards during 2022. Sterilized moths were released on 6 June at the Zillah orchard cultivar Honeycrisp (trial #6) and on 13 June at the orchard cultivar Cripps Pink (trial #5). Subsamples of chilled moths were counted and weighed (20 g = ca. 350 unsexed moths), placed in a cardboard cup, and released within 2 m on either side of 14 and 4 of the traps under netting in the two orchards, respectively. In addition, moths were released around 4 traps outside the netting in the cultivar Honeycrisp block but not outside the netting in the cultivar Cripps Pink. A sample of the sterilized moths released on the two dates in 2022 was sexed (N = 150) and a sample of female moths (N = 60) was dissected prior to each release to determine the males/females sex ratio (1 : 0.86) and the mating status (< 10.0% females were already mated).

The protocol for releasing sterile moths was changed somewhat in 2023, with fewer moths released around each trap (10 g = ca. 175 unsexed moths). Cohorts of sterile moths were released twice per season, on 15 July and 15 August, around traps placed inside and outside nets in the Zillah blocks with cultivars Cripps Pink (trial #7) and Honeycrisp (trial #8), and in the Tieton block with cultivar Cosmic Crisp (trial #9). Dissections of sterile female moth samples (N = 30) on both dates found that the proportion of mated females was < 0.20. In 2023, the males/females sex ratio of the released sterile moths was comparable to what observed in the previous season (1 : 0.85).

All *C. pomonella* catches were counted and sexed in the laboratory directly on the sticky liners using a stereomicroscope (Optech Stereomicroscopes Zoom series SZ-N, Exacta + Optech Labcenter Spa). Female moths were then dissected to determine their mating status by observing the *bursa copulatrix* using the same stereomicroscope. All data presented in tables are the mean catch ± standard error (SE) per trap over the length of each trial.

Statistical analyses of the *C. pomonella* catches and mating status were carried out with R software v. 4.0.3 (R Core Team, 2024). Data were analyzed with non-parametric statistics due to the variability of the data, often low catch, and relatively low number of replicates. Male and female moth catch data in 2022 from the four Tieton orchards, with paired netted and unnetted plots, were analyzed with Wilcoxon signed-rank test. The Wilcoxon rank-sum test was used to compare moth catches when the netted and unnetted replicates were not paired, such as the Zillah sites and Tieton in 2023. Total males or female catches, either wild or sterile, in netted and unnetted plots of different trials were analyzed using a GLMER model with Poisson distribution and considering the trial factor as random effect. Fisher's Exact test was used to compare the proportion of mated females caught both inside and outside netting. The female mating status were analyzed together according to the tree architecture when trials provided a sufficient number of replicates, using the two-sample *t*-test since data were found to fit normality, which was tested with Shapiro-Wilk's test. For all female moth catches, both wild and released sterile, the mating status was analyzed considering together the net factor (net, no net) and the tree architecture factor (spindle trellis, V-trellis) and their interaction, using a GLMER model with the moth type factor as random effect; the moth type factor (either wild or released sterile) was previously tested and resulted not to have an impact on the mating status ($\chi^2 = 2.8$, $p = 0.094$). Finally, the Wilcoxon rank-sum test was used to compare fruit injury levels in the netted and unnetted plots. Significant differences were considered at the 95% confidence level ($p < 0.05$).

Results

Catches of male and female wild *C. pomonella* adults inside and outside netting were collected from 12 comparisons over the two-year project. In general, very few wild moths of either sex (≤ 3) were caught per trap under nets across all the trials and were significantly lower than the number of moths caught outside these nets (table 2). Male and female catch was 98.8% and 96.0% lower under nets than outside across all blocks, respectively. Across the nine trials where wild *C. pomonella* adults were caught (table 2), total wild catches inside the nets were overall 36.5-fold and significantly lower ($\chi^2 = 2452.6$; $p < 0.001$) than total wild catches outside the net (1.9 ± 0.3 and 69.4 ± 8.6, respectively).

The relative recapture of the released sterile moths inside and outside nets were much more variable among trials (table 3). Overall, sterile males were recaptured at a higher rate than sterile females, but not in every trial. Across the five trials where sterile *C. pomonella* adults were released (table 3), total sterile catches inside the nets were overall 1.4-fold and significantly lower ($\chi^2 = 165.33$; $p < 0.001$) than total sterile catches outside the net (11.9 ± 2.0 and 17.1 ± 3.0, respectively).

The mating status of female *C. pomonella* caught in traps largely differed across trials, considering the different factors involved (training system, wild or sterile moth, trial period) (table 4). Analyzing the wild moths collected from all trials (in total 613 females), under the nets the level of female virginity was overall significantly higher compared to that recorded dissecting wild females caught in traps with no net. Analyzing the sterile moths, the level of female virginity was in general numerically higher under nets compared to no nets and, considering the sterile moths collected from all trials (in total 402 females), this difference was close to the significance threshold ($p = 0.051$).

Levels of *C. pomonella* virginity were analyzed also according to the tree architecture for both wild (figure 4A) and sterile (figure 4B) female catches. The very low number of wild females caught under the nets in the central leader tree architecture (Trials #1-4) has not allowed to statistically analyze the data. Nevertheless, a numerical higher proportion of unmated females was observed in the netted plots compared to the unnetted plots considering the central leader tree architecture. The same numerical trend was observed for the spindle trellis architecture (Trials #5,7,9), where the proportion of unmated wild females was 4-fold higher under the nets compared to the unnetted plots. Regarding the sterile moths released

Table 2. Summary of wild *C. pomonella* males and females caught in orange delta traps baited with the kairomone lure Pherocon Megalure CM Dual 4K placed inside and outside nets, Tieton and Zillah (WA, USA), in 2022-2023.

Trial #	Trap replicates		Mean ± SE wild moth catch per trap [a]			
	Under nets	No nets	Males		Females	
			Under nets	No nets	Under nets	No nets
1 [b]	5	5	0.6 ± 0.2 a	88.6 ± 11.3 b	0.0 ± 0.0 a	15.8 ± 4.1 b
2 [b]	4	4	0.0 ± 0.0 a	22.8 ± 5.5 b	0.0 ± 0.0 a	4.8 ± 0.5 b
3 [b]	4	4	0.3 ± 0.3 a	46.3 ± 5.0 b	0.0 ± 0.0 a	25.0 ± 5.7 b
4 [b]	5	5	1.4 ± 1.0 a	68.8 ± 12.9 b	1.8 ± 1.1 a	29.6 ± 9.0 b
5 [b]	4	4	1.8 ± 0.9 a	57.5 ± 1.3 b	1.3 ± 0.5 a	23.8 ± 3.4 b
6a	14	4	3.1 ± 0.7 a	159.0 ± 12.6 b	1.9 ± 0.4 a	55.5 ± 5.0 b
6b [c]	2	2	1.0 ± 0.0	80.5 ± 34.5	2.5 ± 0.5	23.0 ± 7.0
7a	5	5	0.0 ± 0.0 a	19.4 ± 4.0 b	0.0 ± 0.0 a	21.8 ± 4.7 b
7b	5	5	0.0 ± 0.0 a	10.2 ± 1.9 b	0.0 ± 0.0 a	13.6 ± 3.2 b
8a	16	5	0.1 ± 0.1 a	136.8 ± 11.5 b	0.8 ± 0.3 a	48.0 ± 11.4 b
8b	16	5	0.9 ± 0.3 a	17.0 ± 3.1 b	1.5 ± 0.6 a	23.6 ± 5.8 b
9	8	14	0.4 ± 0.3 a	6.1 ± 2.0 b	0.8 ± 0.4 a	2.4 ± 0.6 b
All [d]	86	60	0.9 ± 0.2 a	48.9 ± 6.6 b	1.0 ± 0.2 a	20.5 ± 2.5 b

[a] Trials #1-4 were analyzed with Wilcoxon signed-rank test and Trials #5-9 were analyzed with Wilcoxon rank-sum test. Means for males and females separately followed by a different letter were significantly different, $p < 0.05$.

[b] Male catch in Trials #1-5 was the sum from two traps baited with either the 4K or the PH1X lures.

[c] Nets in Trial #6b were not applied until 17 June 2022, so cumulative mean counts were those < 17 June (No nets) versus counts after that date (Under nets). These data were not analyzed.

[d] Data for all wild moths were analyzed with GLMER model with Poisson distribution and considering the trial factor as random effect (males: $\chi^2 = 1460.9$, $p < 0.001$; females: $\chi^2 = 894.1$, $p < 0.001$); Trial #6b was not included in this analysis.

Table 3. Summary of sterile *C. pomonella* males and females caught in orange delta traps baited with the kairomone lure Pherocon Megalure CM Dual 4K placed inside and outside nets, in Tieton and Zillah (WA, USA), in 2022-2023.

Trial #	Trap replicates: Under nets	Trap replicates: No nets	Mean ± SE sterile moth catch per trap [a]: Males, Under nets	Males, No nets	Females, Under nets	Females, No nets
5 [b]	4	4	3.4 ± 0.9	-	2.0 ± 0.9	-
6	14	4	6.5 ± 0.8 a	30.8 ± 2.8 b	3.1 ± 0.5 a	13.0 ± 1.4 b
7a [c]	5	5	0.6 ± 0.4 a	20.4 ± 2.8 b	0.4 ± 0.2 a	12.0 ± 1.3 b
7b	5	5	11.2 ± 3.5 b	2.8 ± 1.2 a	4.4 ± 1.2 a	6.8 ± 3.0 a
8a [d]	16	5	1.4 ± 0.6 a	4.0 ± 1.6 a	2.4 ± 0.8 a	1.8 ± 0.5 a
8b	16	5	20.0 ± 4.2 a	21.4 ± 6.5 a	9.1 ± 1.8 a	16.2 ± 5.0 a
9	8	14	1.5 ± 0.5 a	1.9 ± 0.4 a	0.4 ± 0.3 a	1.6 ± 0.5 a
All [e]	68	42	7.9 ± 1.4 a	10.3 ± 2.0 b	4.0 ± 0.6 a	6.8 ± 1.2 b

[a] Data for Trials #5-9 were analyzed with Wilcoxon rank-sum test. Means for males and females separately followed by a different letter were significantly different, $p < 0.05$.
[b] Male catch in Trial #5 was the sum from two traps baited with either the 4K or the PH1X lures.
[c] Sterile moths were released on 6 and 13 June 2022 in Trials #5 and #6, respectively.
[d] Sterile moths were released on 15 July 2023 in Trials #7a and #8a and 15 August 2023 in Trials #7b, #8b, and #9.
[e] Data for all wild moths were analyzed with GLMER model with Poisson distribution and considering the trial factor as random effect (males: $\chi^2 = 81.4$, $p < 0.001$; females: $\chi^2 = 86.7$, $p < 0.001$).

Table 4. Summary of mating status of wild and sterile female *C. pomonella* catch in traps baited with the kairomone lure Pherocon Megalure CM Dual 4K placed inside and outside single-row netting, in Tieton and Zillah (WA, USA), in 2022-2023.

Trial #	Training system	Moth type	Trial period	Proportion of unmated female *C. pomonella* [# females]: Under nets	No nets	Fisher's Exact test
1	Central leader	Wild	2022	- [0]	0.28 ± 0.02 [79]	-
2	Central leader	Wild	2022	- [0]	0.28 ± 0.06 [32]	-
3	Central leader	Wild	2022	- [0]	0.27 ± 0.02 [100]	-
4	Central leader	Wild	2022	0.50 ± 0.16 [9]	0.37 ± 0.14 [33]	$p = 0.451$
5	Spindle, trellis	Wild	2022	1.00 ± 0.00 [5]	0.13 ± 0.06 [186]	$p < 0.001$
7	Spindle, trellis	Wild	July 2023	- [0]	0.30 ± 0.00 [30]	-
		Wild	August 2023	- [0]	0.32 ± 0.04 [68]	-
9	Spindle, trellis	Wild	August 2023	0.72 ± 0.15 [6]	0.08 ± 0.04 [33]	$p = 0.011$
6	V-trellis	Wild	2022	0.18 ± 0.09 [26]	0.22 ± 0.01 [95]	$p = 1.000$
8	V-trellis	Wild	July 2023	0.05 ± 0.05 [11]	0.12 ± 0.04 [157]	$p = 1.000$
		Wild	August 2023	0.38 ± 0.07 [22]	0.20 ± 0.00 [30]	$p = 0.353$
	All	Wild	2022-2023	0.36 ± 0.07 [79]	0.21 ± 0.02 [534]	$p < 0.001$
5	Spindle, trellis	Sterile	June 2022	1.00 ± 0.16 [10]	- [0]	-
7	Spindle, trellis	Sterile	July 2023	1.00 ± 0.00 [2]	0.30 ± 0.00 [30]	$p = 0.111$
		Sterile	August 2023	0.66 ± 0.12 [22]	0.56 ± 0.19 [34]	$p = 0.171$
9	Spindle, trellis	Sterile	August 2023	0.75 ± 0.25 [3]	0.18 ± 0.07 [21]	$p = 0.194$
6	V-trellis	Sterile	June 2022	0.58 ± 0.09 [44]	0.36 ± 0.02 [52]	$p = 0.410$
8	V-trellis	Sterile	July 2023	0.54 ± 0.10 [34]	0.22 ± 0.00 [9]	$p = 0.059$
		Sterile	August 2023	0.36 ± 0.05 [121]	0.40 ± 0.0 [30]	$p = 0.671$
	All	Sterile	2022-2023	0.58 ± 0.05 [226]	0.34 ± 0.06 [176]	$p = 0.051$

The proportions of unmated females caught inside and outside nets in 12 comparisons were analyzed with Fisher's Exact test. Mating status data were also analyzed cumulated across all comparisons of the two-year study (all wild and all sterile female moth catches separately).

inside and outside the nets, the netting placed on the spindle trellis training system confirmed a significant effect on the *C. pomonella* female mating status, increasing the level of virginity (figure 4B). The V-trellis tree architecture (Trials #6,8) had not shown a significant effect on the female mating status neither considering the wild females (figure 4A) nor the sterile females (figure 4B), with a comparable level of unmated moth both inside and outside nets. Data from both spindle trellis and V-trellis orchards collected from both wild and sterile moths were analyzed together to test the interaction between the two factors involved (occurrence of nets and tree architecture). The level of *C. pomonella* mating was significantly reduced by the nets ($\chi^2 = 28.6$, $p < 0.001$), with an overall

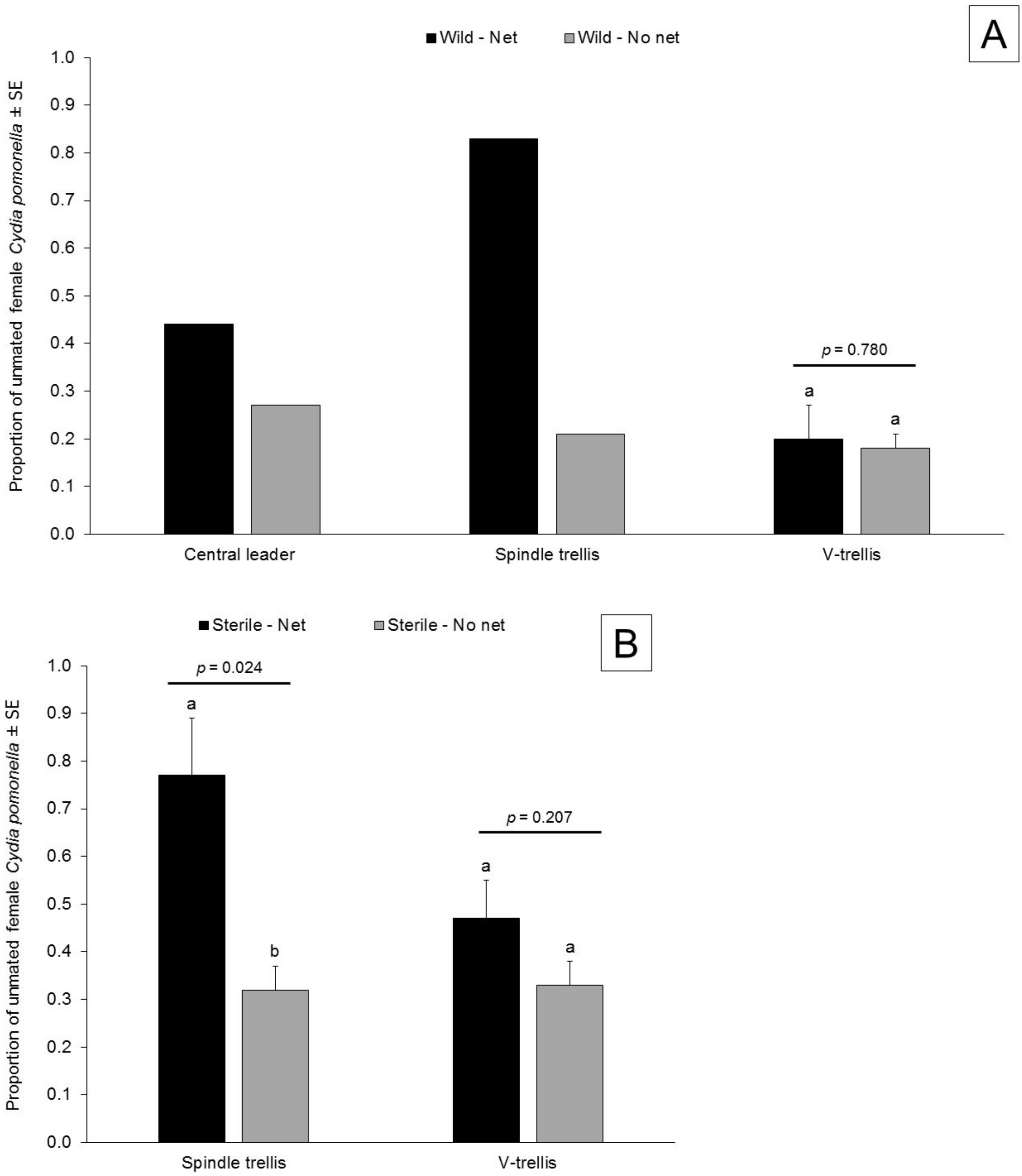

Figure 4. Comparison of the mating status of wild (**A**) and released sterilized (**B**) female *C. pomonella* catch inside and outside netting in pome fruit orchards during 2022-2023. Central leader (Trials #1-4) and spindle trellis (Trials #5,7,9) data for wild moths were not analyzed due to the lack of a sufficient number of replicates (not enough catches of wild female moths under the nets). Spindle trellis (Trials #7,9) data for sterile moths and V-trellis (Trials #6,8) data for both wild and sterile moths were analyzed with the two-sample *t*-test.

proportion of unmated females of 0.25 ± 0.03 outside the net compared to 0.54 ± 0.09 under the netting. The tree architecture was also a factor significantly affecting the level of female virginity (χ^2 = 14.2, $p < 0.001$), with an average proportion of unmated females of 0.29 ± 0.04 in the V-trellis versus 0.48 ± 0.09 in the spindle trellis. Finally, the effect of the interaction between nets occurrence and tree architecture on the level of *C. pomonella* female moth virginity was also significant (χ^2 = 15.4, $p < 0.001$), showing among these four tested combinations how only the net deployed in the spindle trellis (proportion of unmated females of 0.79 ± 0.08) significantly differed from all the other cases (0.25 ± 0.04 in both the spindle trellis without net and the V-trellis without net, and 0.34 ± 0.08 in the V-trellis with net).

Levels of fruit injury under nets were in general very low, except for a few instances (figure 5). The Tieton pear study with variable tree canopies within replicates had instances of nets not being secured under the canopy and had some injury (< 0.5%). Also, one replicate of the Tieton apple block cultivar Ambrosia had some injury (1.3%), and this replicate was positioned on the edge of the orchard and had much higher levels of fruit injury compared with the other four replicates in the previous year, which had no injury. Injuries found under nets were all from the first-generation larvae of *C. pomonella*. In Tieton, the use of nets in 2022 significantly reduced the fruit injury in all trials, showing an overall damage reduction with the nets also from the previous and following year (figure 5A). The Zillah apple block cultivar Honeycrisp added in mid-June 2022 had moderate levels of fruit injury (2.5%). This block had a damage higher than

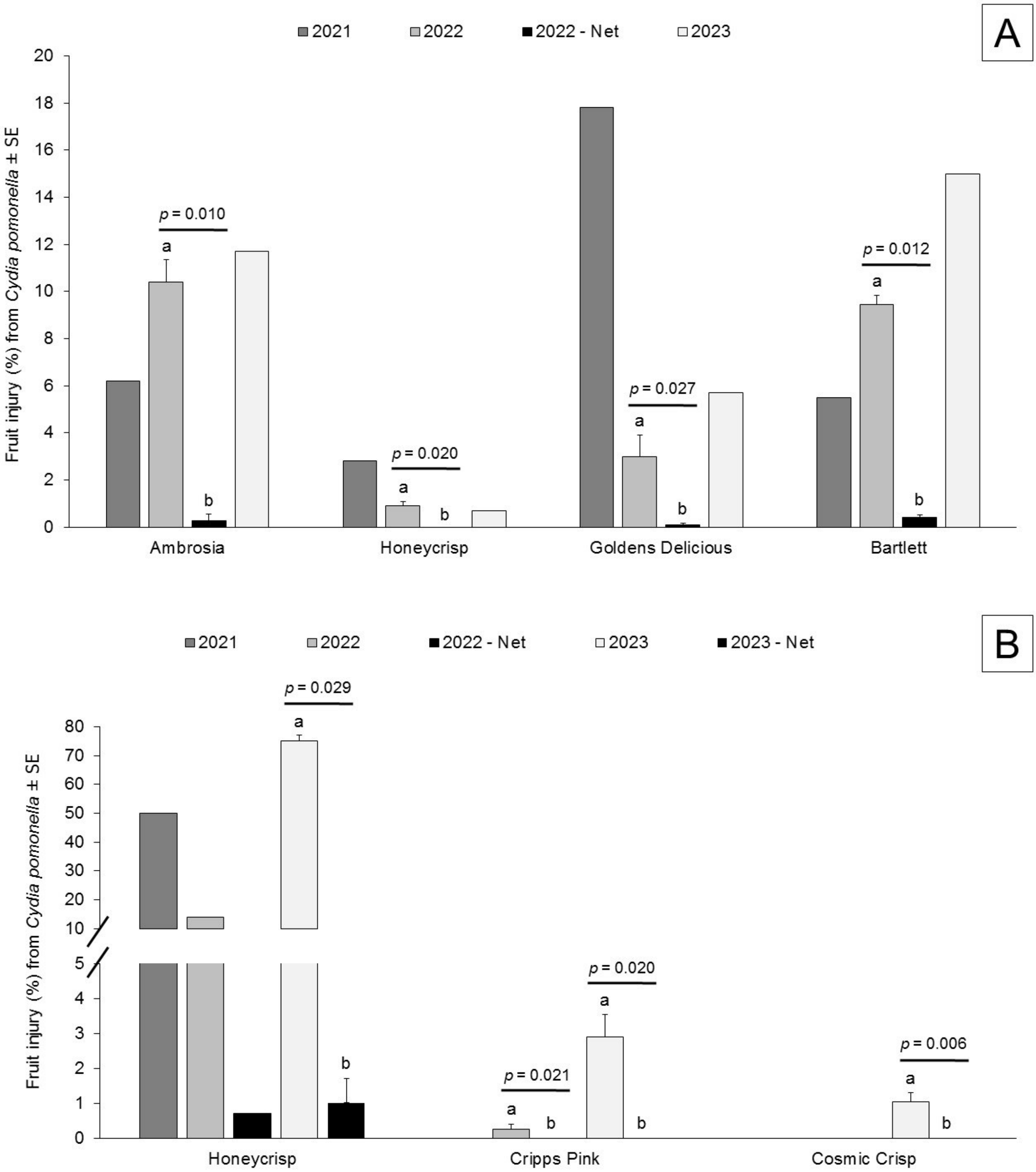

Figure 5. Comparison of fruit injury level from *C. pomonella* inside and outside single-row nets: (**A**) in replicated plots (N = 4-5) inside pome fruit orchards near Tieton in 2022 (Trials #1-4), considering a fruit injury assessment carried out also in 2021 and 2023 in the same unnetted plots; and (**B**) in four blocks of cultivar Honeycrisp near Zillah in 2023 (Trial #8), four blocks of cultivar Cripps Pink near Zillah in 2022-2023 (Trials #5,7), and six plots of cultivar Cosmic Crisp near Tieton in 2023 (Trial #9). In Zillah, the unnetted blocks of cultivar Honeycrisp were monitored also in 2021 and 2022, considering also the two netted blocks in 2022 (Trial #6).

the block where nets were deployed in early season (0.7%) but considerably lower than the unnetted block (14.0%) (figure 5B). The use of two years of netting in the Zillah blocks cultivar Honeycrisp cleaned up the orchard, while the adjacent unnetted block cultivar Granny Smith have become heavily infested over this period. In 2023, the injury under nets in one of the newly netted blocks cultivar Honeycrisp in Zillah (3.1%) were found only in discrete clusters (where the severe pest pressure was located). Levels of fruit injury from *C. pomonella* increased to nearly 3.0% outside of netting in the Zillah apple blocks cultivar Cripps Pink in 2023; in comparison, there was no injury sampled inside the nets (figure 5B). The Tieton apple blocks cultivar Cosmic Crisp had no injury compared with > 1.0% injury on the trees surrounding the nets (figure 5B).

Fruit feeding by birds was a variable factor impacting the pome fruit orchards in our study. Pressure from large assemblages of several key species of frugivorous and omnivorous birds such as European starlings, *Sturnus vulgaris* L. (Passeriformes Sturnidae), and black-billed magpies, *Pica hudsonia* Sabine (Passeriformes Corvidae), were present around the Tieton orchards in both years. Fruit injury (% mean ± SE) from birds was present both inside and outside netting in several of these orchards, including cultivars Golden Delicious (0.3 ± 0.2 inside *versus* 1.9 ± 0.4 outside) and Honeycrisp (no injury inside *versus* 2.8 ± 0.3 outside) in 2022; and Cosmic Crisp (0.3 ± 0.2 inside *versus* 5.8 ± 0.8 outside) in 2023. A diverse assemblage of birds was initially trapped inside netted rows when the netting was applied by machine in the two Zillah locations in 2022, including few specimens belonging to several common species of sparrows and finches (Passeriformes) such as the American robin, *Turdus migratorius* L. (Passeriformes Turdidae). In response, both growers in 2023 made concerted efforts to exclude birds from the netted rows during the net deployment. Levels of fruit injury inside nets in Zillah was incidental and < 0.3% both inside and outside the netting prior to harvest in both years.

Discussion

Our studies clearly support the use of single-row exclusion nets as mechanical control tool to effectively manage *C. pomonella* and to clean up heavily infested orchards. In the organic orchards, levels of *C. pomonella* fruit injury in plots prior to and after discontinuing the use of nets were above action threshold and unacceptably high for the growers. Conversely, over a two-year period, *C. pomonella* under nets was well managed, while surrounding blocks had increasing levels of fruit injury. These findings are consistent with previous studies using the same netting system in Canada and Minnesota as physical barrier to exclude the insect pests (Chouinard *et al.*, 2017; Nelson *et al.*, 2023).

The timing of net application is a key factor affecting *C. pomonella* management. Growers have three alternative timings: install the netting either before or after bloom or later after hand fruit thinning in June. Pollinators such as bumblebees (*Bombus* spp.) can be placed under netting and the use of nets to manage crop load has been considered (Elsysy *et al.*, 2019). However, this would not be practical with hives of honey bees (*Apis mellifera* L.). Concerns about netting interfering with hand fruit thinning operations has been a justification for late applications (Basedow *et al.*, 2018). However, the general phenological overlap of the start of spring moth flight and full bloom to petal fall supports an early as possible application timing, i.e. once the bees are removed from an orchard (Knight, 2007). For example, the delay in applying the net until June in one portion of the Zillah block cultivar Honeycrisp in 2022 resulted in high levels of fruit injury. Also, not all apple blocks require hand fruit thinning every season. Further, with increasing adoption of exclusion netting, growers and workers have adapted by either working in the row under the net or by opening portions of nets sequentially to hand thin the fruits.

Both resident and immigrant moths attack pome fruit orchards (Basoalto *et al.*, 2010). Fruit injury that occurred under nets in our studies was in general limited to the first *C. pomonella* generation in the first year and occurred in scattered pockets throughout the canopies. *C. pomonella* larvae overwinter as mature larvae under bark on the lower trunk and major scaffold branches (MacLellan, 1960). Overwintering larval distribution in these sites is aggregated due to release of a larval pheromone, which has been hypothesized to aid in the future success of mating due to earlier male eclosion and male attraction to sex pheromone released from female pupae (Duthie *et al.*, 2003). Thus, the deployment of nets over the canopy in the spring can trap the adults emerging from these pupation sites. Young plantings, which are more often trellised, have smoother bark and are less likely to have suitable sites for diapausing larvae compared to older orchards with loose bark (Blomefield and Giliomee, 2012). Thus, netting young, trellised orchards is likely to be effective primarily by blocking moth immigration.

Some studies have suggested that *C. pomonella* adults can pass through the netting or females could lay eggs inside netted structures (Sauphanor *et al.*, 2012; Marshall and Beers, 2023). Female *C. pomonella* are somewhat larger than males and moth length averages 10 mm with a 15-22 mm wingspan (Pajaĉ *et al.*, 2011). The tight weave of the netting used in our studies (6.0 mm × 1.8 mm) would seem to be an adequate barrier to prevent moth movement through nets. This was clearly demonstrated with netted rows surrounded by heavily infested unnetted rows (up to 75% injury) remaining clean. However, the disrepair of netting or incomplete covering of the canopy with netting remain important concerns when moth immigration is likely to occur. Interestingly, Marshall and Beers (2023) reported that with a mesh size (5.0 mm × 2.0 mm) similar to our study, the net was partially permeable to moth emigration and immigration, with an overall higher capacity of the release moth to disperse from the netted cage rather than to enter in a netted cage.

Our data suggests that the use of netting in a block with a history of *C. pomonella* injury requires an integrated management program. All the organic growers in our studies chose to maintain their use of mating disruption and spray programs for *C. pomonella* in the first year with nets. However, the two growers we monitored for two years did not continue to use sex pheromone dispensers for mating disruption and reduced their number of late-season sprays in the second year of study. This response was similar to what occurred in French orchards following adoption of the Alt'Carpo netting (Sauphanor *et al.*, 2012).

Studies have suggested that mating disruption is more effective when used under netting due to the effect on reproductive behaviors of both sexes, as well as microclimate impacts on temperature and wind speed (Kührt *et al.*, 2006; Ioratti and Tasin, 2018). Our studies did not directly consider the interaction of mating disruption and exclusion netting. However, the impact of the netting on the mating of wild and sterile *C. pomonella* females was consistent with or without the use of sex pheromones in the two-year studies in Zillah. Gradually, growers using single-row netting have discontinued their use of sex pheromones for *C. pomonella* (Sauphanor *et al.*, 2012).

No secondary pests occurred above action thresholds in any of our studies and sprays were not applied for any pest other than *C. pomonella*. The positive impact of exclusion netting on several other orchard pests have been reported, such as some tortricid leafrollers, stink bugs, temperate fruit flies, pear psylla, and aphids (Dib *et al.*, 2010; Vergnani *et al.*, 2013; Chouinard *et al.*, 2017; Candian *et al.*, 2021; Nelson *et al.*, 2023). Conversely, a few pests have become a more significant problem under nets, such as oblique banded leafroller, *Choristoneura rosaceana* (Harris), the wooly apple aphid, *Eriosoma lanigerum* (Hausmann), and spider mites (Chouinard *et al.*, 2017, Marshall and Beers, 2021; 2022). These pests all occur in apple orchards in Tieton and Zillah but have not been of significant concern to the organic growers in our study, likely due to a complex of natural control agents enacting biological control.

Irrigated orchards, especially when surrounded by a dry shrub-steppe ecosystem, are utilized by a diverse array of bird species (Katayama, 2016; Mangan *et al.*, 2017). Orchard pest management programs can have a significant impact on bird abundance and diversity (Bouvier *et al.*, 2010). Fruit injuries from birds can be a significant cull factor in pome fruit orchards of certain cultivars, such as Honeycrisp (Anderson *et al.*, 2013). Economists have found there can be tradeoffs in the benefits and costs associated with birds in orchards depending on the feeding niche of the major species, e.g. insectivorous versus frugivorous (Peisley *et al.*, 2016). Single-row nets have both been shown to affect or not specific bird species (Brambilla *et al.*, 2015; Bouvier *et al.*, 2022). Nevertheless, trapping birds inside nets can be avoided during installation and restricting any subsequent entry through effective closures.

The most interesting result from our study was the demonstration that the success of *C. pomonella* females in mating is impacted by tree architecture. Basedow *et al.* (2018) first mentioned a potential impact of tree canopy structure on the success of exclusion netting in a brief report. They had better results with spindle than central leader canopies and hypothesized that it was due to the differences in the size of the opening of the netting along the bottom of the canopy. Our data found the greatest disruption of mating by wild and sterile moths occurred in trellised spindle canopies. We suggest that the tight netting placed over the canopy precludes moth sexual activity. In contrast, exclusion netting placed over V-trellised rows was the least effective in disrupting mating. The V-trellis canopy had a wide pathway down the middle of each net (ca. 1.2 m width), which likely increased the flight of moths and their opportunity for mating (figure 3).

Immigration of *C. pomonella* females is a key factor influencing orchard management success (Basoalto *et al.*, 2010; Wearing, 2021). Exclusion techniques to keep moths out of an orchard are essential, especially for organic production. The successes of area-wide management programs where neighboring growers work together to clean up *C. pomonella* infestations over a large area have been demonstrated (Knight *et al.*, 2008). Exclusion netting provides a similar approach, but for individual growers and at a farm scale. A final point is that any type of exclusion netting that blocks immigration to the crop should be effective once the resident *C. pomonella* population is reduced to zero. Of course, this would also be true regardless of the tree canopy structure under the netting. Our studies suggest that tree architecture under the netting is an important factor for resident *C. pomonella* populations, and growers should consider this in evolving their management programs.

Acknowledgements

The authors would like to thank Bill Lingren (Trécé Inc., Adair, OK) for kindly providing trap and lure materials, M3 consulting for the sterile *C. pomonella*, and the growers in Tieton and Zillah (WA, USA) for their permission in conducting these on-farm studies.

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Authors' addresses: Michele PRETI (corresponding author: michele.preti@astrainnovazione.it), ASTRA Innovazione e Sviluppo, via Tebano 45, 48018 Faenza (RA), Italy; Esteban BASOALTO (esteban.basoalto@uach.cl), Facultad de Ciencias Agrarias y Alimentarias, Instituto de Producción y Sanidad Vegetal, Universidad Austral de Chile, Valdivia, Chile; Alan L. KNIGHT (uncfencer76@hotmail.com), Instar Biologicals, 4602 Snowmountain, Yakima 98908, Washington, USA.

Received March 7, 2024. Accepted July 31, 2024.