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Net Enclosure of 'Honeycrisp' and 'Gala' Apple Trees at Different Bloom Stages Affects Fruit Set and Alters Seed Production

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Received: 16 July 2019; Accepted: 20 August 2019; Published: 23 August 2019

Abstract: Thinning is a critical but challenging practice in apple production, especially for organic producers. The objective of this experiment was to determine if exclusion netting, used to manage insect pests and stress, could reduce fruit set and thinning requirements of 'Gala' and 'Honeycrisp' apple trees in Michigan and Washington, U.S.A., respectively. Nets were closed around whole canopies at different timings based on a predetermined percentage of open flowers. In 2017 and 2018, netted trees set a full commercial crop regardless of percentage of open bloom at the time of netting, including prebloom enclosures. Fruit set and yield of netted 'Honeycrisp' trees were significantly lower than non-netted, nonthinned controls but similar to non-netted hand-thinned controls. 'Gala' fruit set and yield did not differ among treatments. Exclusion netting markedly reduced the number of mature seeds and increased the number of nonfertilized seeds in both cultivars. Pollinator exclusion to 'Gala' in a frost year increased parthenocarpic fruit set two-fold compared to non-netted trees. Fruit size, shape, and quality attributes of 'Gala' were were similar among treatments, but 'Honeycrisp' fruit were significantly smaller than hand-thinned, non-netted controls. Netting may constitute an alternative, viable strategy to manage fruit set but requires testing on different cultivars.

Keywords: fruit set; insect exclusion netting; thinning; crop load management; organic production; and parthenocarpy

1. Introduction

Global organic fruit production increased 109% between 2008 and 2013 (on a land area basis) with *Malus* x *domestica* Borkh. (apple) and *Musa acuminate* L. (banana) showing the largest gains [1]. Developing novel, crop load management methods for organic apple systems will improve producer profitability while meeting increasing consumer demand for organically produced fruit. Flower and fruit thinning are essential field practices for sustainable tree fruit production. Inadequate thinning leads to a high number of fruit per tree, which compromises fruit size, fruit quality, postharvest storage life, flower bud formation (threatening the following year's crop), accumulation of tree resources, limb integrity, and cold hardiness [2,3]. Hand thinning is the most accurate method to reduce crop load but is no longer practical due to labor shortages, cost, and time. Moreover, thinning of apple cultivars that are prone to biennial bearing must occur early (far before reaching 25 mm fruitlet diameter) to ensure sufficient return bloom [4]. Mechanical thinning has been applied to different apple varieties with varying levels of success [5,6] but this practice may increase the risk of fire blight infection [7].

Ultimately, the use of chemical thinners has become an essential field practice for apple production systems to help maintain a consistent crop year after year [2,8,9]. Success of chemical thinning depends on several factors such as thinner chemistry, rate, environmental conditions, and a myriad of biological and cultural factors. Organic producers, in particular, face severe challenges for managing crop load, primarily due to a dearth of efficacious chemical thinners. Compatible compounds for use in organic systems tend to be caustic and act by damaging flower tissues and thus are limited to bloom time applications (i.e., lime sulfur, fish oil) [10].

Self-fertility is of great interest for many fruit and nut trees. Self-fertility simplifies management and facilitates consistent production [11]. Unfortunately, most apple cultivars are self-incompatible (i.e., not self-fertile) and require donor pollen from compatible pollinizers to set a commercial crop [12,13]. Nevertheless, some apple cultivars show self-compatible alleles [14]. In other cases, varying levels of self-compatibility were observed among 'Golden Delicious', 'Fuji', 'Gala', 'Cox's Orange Pippin', and 'Elstar' [15]. When S-RNAase gene expression was repressed in the pistil, self-fertility occurred in two transgenic apple lines leading to self-pollination and fertilization [16]. There is evidence that environmental factors affect the self-compatibility of some apple cultivars and facilitate production of a commercial seeded crop without pollinizers [17].

Parthenocarpy, i.e., development of seedless fruit, is a naturally occurring phenomenon in several species from diverse genera such as *Citrus*, *Vitis* (grape), *Pyrus* (pear), and *Musa* (banana) [18]. Parthenocarpy can be divided into three general classifications: obligatory or vegetative, stimulative, and facultative [19,20]. Stimulative parthenocarpy can be artificially induced by treating flowers with plant growth regulators [18], applying incompatible pollen sources [21], or pollinating with irradiated pollen. There is evidence for a stimulative role of auxin in the growth and development of unfertilized ovaries resulting in parthenocarpic fruit [22]. Induction of seedless fruit in several apple cultivars by different mixtures of growth regulators has been reported [23]. Facultative parthenocarpy can occur under adverse pollination or fertilization conditions. For example, preventing bee pollination of entire 'Cox's Orange Pippin' trees resulted in a considerable number of seedless apples per tree [24].

Effects of parthenocarpy on fruit quality vary. Negative morphological characteristics are associated with parthenocarpy and/or reduced seed numbers, most notably, misshapen apples and/or reduced fruit size [25,26]. On the other hand, the induction of parthenocarpy may reduce or eliminate issues associated with pollinators (e.g., adverse climatic conditions for pollination and fertilization; oversetting of fruit and subsequent need for thinning; direct expenses for bee rental and/or supply issues related to bee health) in addition to facilitating single cultivar orchard blocks. Apple orchard blocks comprised of a single cultivar, whose cross-pollination needs are met by the use of crabapple pollinizers, can simplify many management practices compared to blocks containing multiple cultivars. Parthenocarpic fruit set purportedly produced crops below optimum commercial levels [27]; however, practical strategies to manipulate parthenocarpic fruit set in modern, high-density systems have not been explored.

Global use of netting in tree fruit production systems is increasing [28]. Alt' Carpo exclusion netting as a mechanical barrier to diverse threats of tree fruit is also receiving wide attention [29–31]. Exclusion netting has been effectively used to prevent fruit damage from climatic events such as hail [32], solar radiation, and wind [33,34]. In addition, exclusion netting reduced or eliminated insecticidal applications [35], and improved resource efficiency, i.e., via reduced evapotranspiration [36]. Kelderer et al. (2014) [37] investigated the application of black hail-nets to reduce fruit set and thinning of 'Golden Delicious' and 'Gold Rush' apple trees. Two net timings (before bloom and once during bloom) were compared to non-netted control trees. Netting produced smaller crops of larger fruit for both cultivars compared to non-netted trees. In other cases, tree response to exclusion netting varied considerably based on biological (e.g., genotype) and environmental factors. For example, alteration of fruit quality under exclusion netting differed among seasons as a function of the environmental conditions [38,39]. Further, nets vary considerably in material construction (type of polymer, density, color, weave, etc.); thereby differentially affecting plant developmental processes [40].

The objective of this experiment was to enclose entire apple trees in netting at specific percentages of open bloom to reduce pollination, fruit set, and thinning. We hypothesized that netting could potentially produce a range of crop loads based on the percentage of open bloom accessible to pollinators prior to the time of canopy enclosure. A secondary objective was to evaluate the effect of nets on productivity, fruit size, and quality.

2. Materials and Methods

2.1. Plant Material

These experiments were conducted in consecutive years, 2017 and 2018, in the states of Washington (WA) and Michigan (MI), U.S.A. on commercial farms. A 9-year-old 'Honeycrisp'/M9 'Pajam 2' orchard, planted in 2008 at 0.9 m × 3.7 m (2988 trees per ha) and trained to a spindle was selected in the 'Quincy' WA fruit growing district (47.2 latitude −119.9 longitude). The orchard was managed for organic production. A 10-year-old 'Brookfield Gala'/M9 'Nic 29' orchard, planted in 2007 at 0.9 m × 3.7 m (2988 trees per ha) and trained to a spindle was selected in Sparta, MI (43.1 lat.−85.7 long.). The orchard was managed for conventional fruit production. Aside from netting treatments, all management practices were performed according to industry standards (from both a regional and cultural (organic and conventional) perspective), with the exception of chemical thinning, which was omitted. The two orchards were treated as separate experiments. Subsequently, only within-orchard comparisons by treatments and year were assessed. Direct comparisons between the two experiments were not considered.

2.2. Experimental Design and Treatments

The treatment timing and number of trees per replication differed at each site based on availability of trees and the developmental rate at which bloom progressed. Treatment trees receiving complete exclusion netting (Alt-carpo, 10% shading, 2.8 mm × 4 mm weave, Helios® antihail systems, Bergamo, Italy) were monitored by calculating the percentage of open king bloom. Netting was installed over portions of tree rows as Alt' Carpo, enclosing whole trees, recently termed complete exclusion [38], ensuring that the bottom of the netting was tightly secured around each tree trunk to prevent bee entry (Figure 1). When a predefined target bloom was attained and/or deemed adequate based on the progression of flowering (in combination with forecasted weather at each site), entire canopies of multiple tree replicates within a row were fully enclosed by nets. Daily bloom monitoring within each replicate was performed to determine net enclosure timings by counting the number of open king flowers (i.e., with visible anther sacs) and dividing by either the total number of flower buds on a single tree (WA) or from a sample population of 100 flower buds dispersed over eight preselected fruiting limbs per replicate (MI). At both sites, a randomized complete block design was employed. Treatments are provided in Table 1. For 'Honeycrisp' trees in WA, five treatments were replicated four times in 2017: non-netted control without hand-thinning (natural set), non-netted control plus hand-thinning (standard commercial bloom thinning leaving 1 flower/cluster), netting at 0% open bloom (i.e., 'pink' phenology stage), 23% open 'king' bloom (KB), and 58% open KB. In 2018, a new unthinned, non-netted control was added, since the 2017 control could no longer be considered a true control in 2018 due to the alternative bearing tendency and marked reduction in flowering. Therefore, the WA treatments for 2018 included a non-netted control without hand-thinning (natural set in 2018), non-netted control without hand-thinning (natural set in 2017 and 2018), non-netted control plus hand-thinning (standard commercial bloom thinning leaving one flower/cluster in 2017 and 2018), netting at 0% open bloom (i.e., 'pink' phenology stage), 28% open 'king' bloom (KB), and 68% open KB. For 'Gala' trees in MI, in 2017, five treatments were replicated three times: non-netted control without hand-thinning, netting at 0% open bloom (i.e., 'pink'), 26% open KB, 60% open KB, and 95% open KB. Approximately 50% of the side (i.e., lateral) flowers were open when the latter treatment was applied. A non-netted, hand thinning treatment was not employed for 'Gala' due to relatively low fruit set in non-netted control trees due to spring frost event during bloom. In 2018, six treatments were replicated three times, non-netted control without hand-thinning, non-netted control plus hand-thinning (standard commercial fruitlet thinning leaving 1 fruit/cluster), netting at 0% open bloom (i.e., 'pink'), 20% open KB, 40% open KB, and 80% open KB. A single replicate comprised 10 contiguous trees in both years (MI) or 20 trees in 2017 and 24 trees in 2018 (WA), regardless of the treatment. Nets remained over canopies until harvest for both years. Trees were not rerandomized between years in either plot. Meteorological data were collected from weather stations within 100 m of the experimental sites.

A

B

Figure 1. Experimental sites of 10-year-old 'Gala' in Michigan (**A**) and 9-year-old 'Honeycrisp' in Washington (**B**) for Alt' Carpo exclusion netting. Tree density was 2988 trees per ha at both sites.

Table 1. Net enclosure treatments for 'Gala' and 'Honeycrisp' experiments in 2017 and 2018. Data are percent of open king bloom at time nets were fully closed around multitree replicates.

Targeted King Bloom%	Actual King Bloom %			
	'Gala'		'Honeycrisp'	
	2017	2018	2017	2018
Non-netted	(Nonthinned)	(Nonthinned)	(Nonthinned)	(Thinned 2017)
Non-netted		(Hand thinned)	(Hand thinned)	(Nonthinned)
Non-netted				(Hand thinned)
0%	0%	0%	0%	0%
20%	26%	20%	23%	28%
40%	60%	40%	58%	68%
80%	95%	80%		

2.3. Preharvest Measurements and Yield Components

For 'Honeycrisp', the total number of flowers on one entire tree was counted per replicate. Percent fruit set was calculated by dividing the number of fruit harvested per tree (in September 2017 and August 2018) by the total number of flowers (calculated by multiplying the number of flower clusters by 5, which was the average number of flowers per cluster for 'Honeycrisp'). For 'Gala' trees, eight representative fruiting limbs were selected at 'pink' from multiple trees within each replicate. Limbs were positioned in the plane of the tree row, 1.4 to 2 m in height. Percent fruit set was calculated by dividing the number of fruit remaining after 'June' drop by the number of flowers on the each limb. Limbs were treated as subsamples and pooled to generate replicate means. Measured limb circumferences were converted to cross-sectional area (lcsa); average lcsa was ~2.5 cm^2, ±2.5 mm^2.

'Gala' and 'Honeycrisp' trees were harvested in a single event, a few days prior to their respective commercial harvests. All fruit from entire trees were harvested from multitree replicates as follows, n = 8 trees and n = 1 tree for 'Gala' and 'Honeycrisp', respectively. The total number of fruit harvested from experimental units was weighed to determine tree yield and counted. Average fruit weight was calculated by dividing the yield per representative tree (kg) by the number of fruit harvested from that tree and expressed in grams (g). Seeds were examined from all harvested fruit from one ('Honeycrisp') or five ('Gala') trees per replicate in 2017 or from a random sample of 50 apples per replicate (both cultivars) in 2018. Fruit were categorized as "seedless" if no mature seeds were observed in a given fruit.

2.4. Postharvest Measurements

For 'Gala', fruit quality attributes were determined on a 15-fruit sample of randomly selected fruit of similar size for each treatment replicate for 2017 and 2018. Flesh firmness (FF) was determined using a computerized penetrometer (Mohr MDT-2 series, Richland, WA, USA) on opposite sides of the fruit near the equator after removing a 1-cm^2 disc of peel. A composite sample of peeled slices of cortical tissue from five fruit (~100 g) were blended for 30 s in a juice extractor (Acme 6001, Acme Juicer Manufacturing Co, Sierra Madre, CA, USA) equipped with a uniform strip of milk filter. The soluble solids concentration, SSC, (%) of juice was measured using a digital refractometer (PAL-1, ATAGO, Tokyo, Japan). Titratable acidity was quantified using an automated titration system (Model DL15, Mettler-Toledo, LLC., Columbus, OH, USA) by titrating a 10 mL juice sample in 10 mL of DI H_2O to an endpoint pH of 8.1 with 0.1 N NaOH. For acidity and SSC, three composite samples of five fruit were averaged to generate replicate means. Starch was evaluated on fruit freshly sectioned laterally through the seed cavities and immediately dipped in an iodine solution (10 g KI and 40 g I_2 per 4 L DI H_2O). Starch was then rated according to the staining pattern using the Cornell starch–iodine staining pattern index [41]. Red color of fruit was determined with a Compac single-lane sorting line equipped with the Spectrim Apple Grading System (Compaq, Auckland, New Zealand). All fruit from individual trees (n = 8 per replicate) were quantitatively assessed for percent red color. Fruit shape of 'Gala' was characterized by the ratio of width to height on 50 fruit per tree (eight trees per rep). In 2018 only, postharvest (3 months storage) fruit quality attributes were measured using the same procedure described above using fruit boxed at harvest and stored in regular air (RA) at 0 °C.

For 'Honeycrisp', the highest quality apples at harvest, ranging from 70 to 85 mm, were sampled from all treatment replicates to represent a subsample for instrumental quality analysis. Fruit were boxed in the field the day of harvest and then stored in regular air cold storage at 1 °C until fruit quality assessments at +1 month (+1M) after harvest and +6 month (+6M) after storage (RA, 0–1 °C) for both 2017 and 2018. Forty-eight apples were sorted by the index of absorption difference between 670 and 720 nm (IAD index) [42] into two homogenous groups (n = 24 per treatment replicate) in 2017. In 2018, when possible, 40 apples were evaluated per treatment replicate and fruit quality assessment (+1 M and +6M; N = 240 total fruit). Red color was assessed by a trained operator and expressed as the percentage of blush red coverage over the whole fruit surface. Dry matter (pDM; %) was nondestructively predicted by a Felix F750 Produce Quality Meter (Felix Instruments, Camas, WA, USA) with the "apple demo" model (developed for apple varieties produced in the Pacific Northwestern U.S.A.) provided by the manufacturer. Each apple was scanned using near-infrared spectroscopy on the sun exposed and shade sides and averaged to generate a predicted p DM. Flesh firmness (FF) was determined using a computerized penetrometer (Mohr MDT-2 series, Richland, WA, USA) as described for 'Gala' above. The SSC (%) was measured on each single fruit from two equatorial wedges taken from opposite sides of the fruit and pressed in a garlic press directly over the lens of a digital refractometer (PAL-1, ATAGO, Tokyo, Japan). 'Honeycrisp' shape was assessed by trained operators to identify misshapenness in the vertical and horizontal plane of the apple and reported as the incidence of misshapen fruit in both planes for both years. Six months after storage, fruit were also assessed for average weight loss (g) during the storage duration.

2.5. Statistical Analysis

The experimental designs were randomized complete block designs (RCBD). The data were subjected to one-way analysis of variance (ANOVA) using R software 3.4.3 (2017-11-30 ("Kite-Eating Tree")) (R Foundation, Vienna, Austria) and PROC GLM SAS v.9.4 (SAS Institute, Cary, NC, USA). Data were evaluated within cultivar and within year for a given cultivar. Means were separated by Tukey's honestly significant difference HSD test at $p < 0.05$ when the F-tests generated from ANOVA were significant at $p < 0.05$.

3. Results

3.1. Fruit Set

'Gala' fruit set did not differ among treatments, irrespective of the timing of net closure or the year (Figure 2A,B). 'Honeycrisp' fruit set was similar among netting treatments in both years; however, netting at 23% and 58% of full KB in 2017 reduced fruit set relative to non-netted (nonthinned) control trees by ~42% (Figure 2C). In 2018, 28% KB and 68% KB netting treatments reduced fruit set of non-netted (nonthinned) control trees by ~61% (Figure 2D). The non-netted 2017–2018 no thin treatment had 49% fruit set due to the biennality. Interestingly, enclosing canopies before flowering (i.e., 'pink') did not reduce fruit set compared to trees netted that had higher percentages of open bloom present before netting, regardless of cultivar or year (Figure 2A–D).

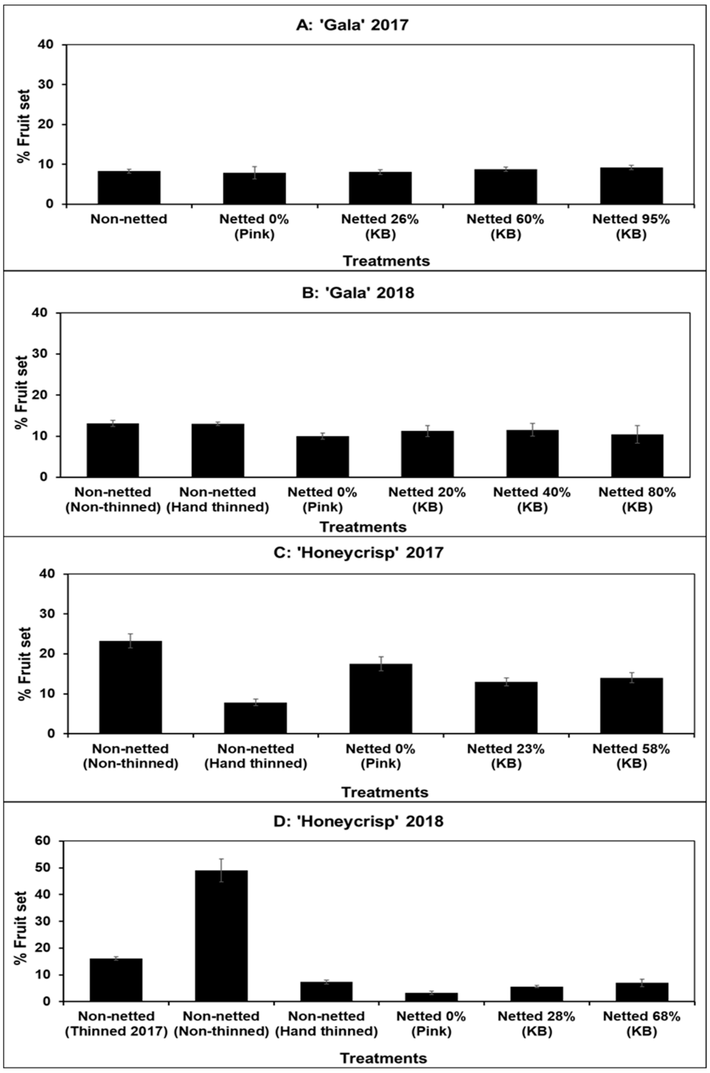

Figure 2. Effect of 2017 and 2018 netting treatments on average fruit set (%) of 'Gala' (**A**,**B**) and 'Honeycrisp' (**C**,**D**) limbs and whole trees, respectively. Vertical bars represent SE. Each treatment is the average of three replicates for 'Gala' and four replicates for 'Honeycrisp'. Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. (Pink) pink stage of bud development. (KB) king flower bloom.

3.2. Seed Number

Netting significantly reduced the number of mature seeds per fruit compared to non-netted trees, regardless of cultivar or year (Figure 3A–D). All seeds were classified as mature, nondeveloped or nonfertilized according to representative images of seeds extracted from experimental fruit (Figure 3E). In 2017, non-netted 'Gala' trees had 4.5 mature seeds per fruit, which was significantly higher than trees enclosed with nets at 0% (Pink), 26%, 60%, and 95% KB, which had 1.9, 1.4, 1.5, and 1.7 mature seeds per fruit, respectively (Figure 3A). 'Gala' showed the same trend in 2018 but with a higher number of mature seeds per fruit for all treatments: non-netted 'Gala' trees had 7.5 mature seeds per fruit, which was significantly higher than trees enclosed with nets at 0% (Pink), 20%, 40% and 80% KB, which had 4.3, 4.1, 4.2, and 5 seeds per fruit, respectively (Figure 3B). For 'Honeycrisp' fruit in 2017, mature seed counts were significantly higher in control fruit (5.8 and 4.5 mature seeds per fruit for non-netted/nonthinned and non-netted/hand-thinned trees, respectively), compared to 1.7, 2.6, and 2.3 mature seeds per fruit for trees netted at 0%, 23%, and 58% KB, respectively (Figure 3C). 'Honeycrisp' seed counts in 2018 were nearly equivalent to those observed in 2017: the number of mature seeds was significantly higher in control fruit (5.4 and 4.4 mature seeds per fruit for non-netted/nonthinned and non-netted/hand-thinned trees, respectively), compared to the 0%, 28% and 68% KB treatment fruit which had 1.6, 2.7, and 2.6 mature seeds per fruit, respectively (Figure 3D). The highest number of mature seeds per fruit was recorded in the non-netted 2017–2018 (nonthinned) treatment which also contained the fewest nonfertilized seeds per apple (Figure 3D). The number of nondeveloped seeds was relatively low in both cultivars, regardless of treatment or year.

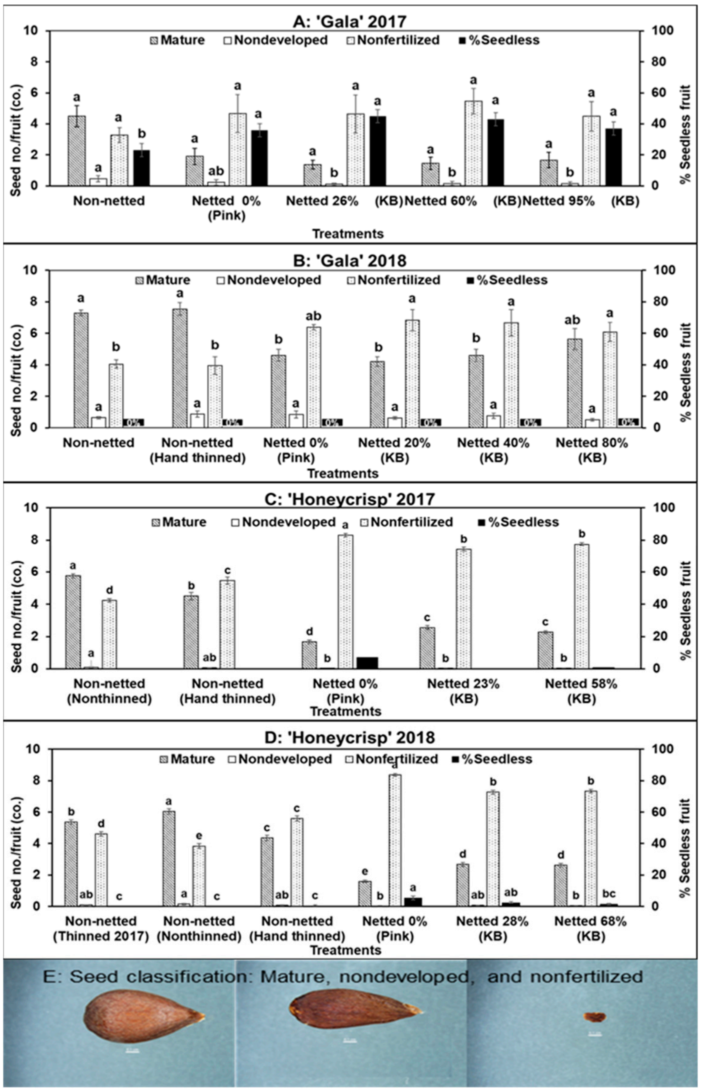

Figure 3. Effect of 2017 and 2018 netting treatments on number of mature, nondeveloped and nonfertilized seeds, and the percentage of seedless fruit of 'Gala' (**A**,**B**) and 'Honeycrisp' (**C**,**D**) using classifications of mature, nondeveloped and nonfertilized seeds (**E**) from left to right, respectively. Vertical bars in A and B represent SE (n = 100 to 1200). Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. (Pink) pink stage of bud development. (KB) king flower bloom. For (**E**), all seeds were imaged at 7.5 ×; horizontal bars below seeds represent 0.1 cm.

In 2017, all 'Gala' netting treatments led to a significantly higher percentage of seedless fruit (up to 64%) compared to control non-netted trees (~33% of fruit) (Figure 3A). The relatively high percentage of seedless fruit in 2017 may have been attributed to freeze events just after full bloom (Figure 4A). This was supported by the absence of any seedless fruit in 'Gala' 2018. In 'Honeycrisp', the highest number of nonfertilized seeds/fruit was observed in netted 0% KB for both years (8.31 of 10 seeds in 2017 and 8.38 of 10 seeds in 2018). The highest incidence of seedless fruit for 'Honeycrisp' was 7.2% and 5.5% reported for trees netted at 0% (Pink), respectively, in 2017 and 2018, while the other treatments ranged from 0.0 to 2.5% (Figure 3C,D).

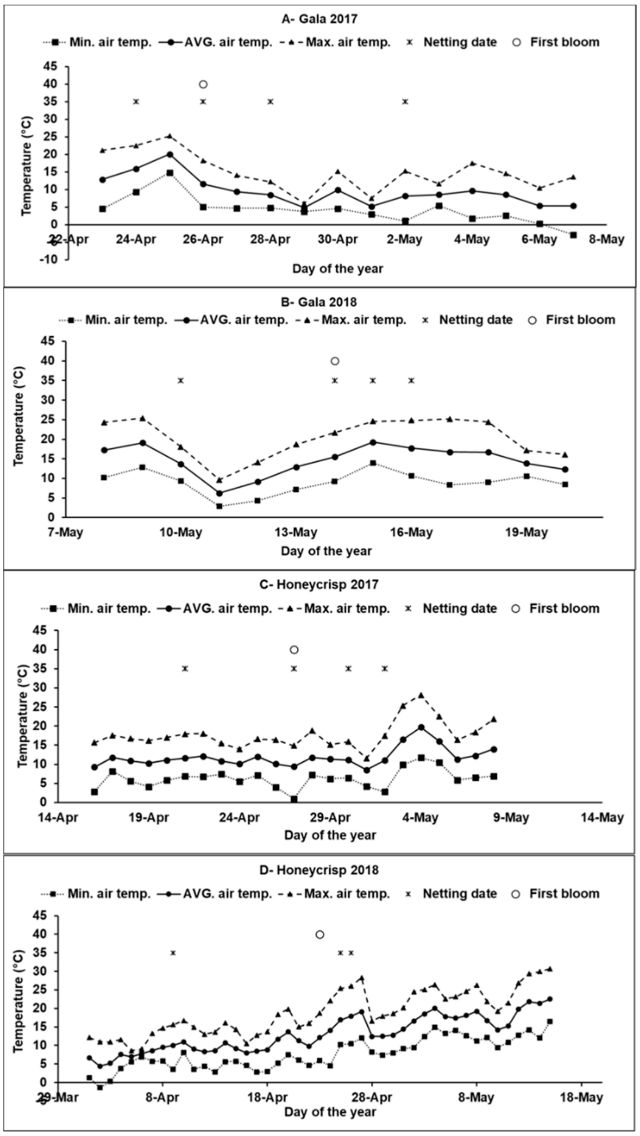

Figure 4. Maximum, minimum, and average daily air temperatures for 'Gala' in (**A**) Sparta, Michigan 2017, (**B**) Sparta, Michigan 2018, and 'Honeycrisp' in (**C**) Quincy, Washington 2017 and (**D**) Quincy, Washington 2018 corresponding with the application of netting treatments pre-bloom through petal fall in 2017 and 2018. Asterisks at the top of each panel signify the dates of net enclosure treatments. Open circles signify first bloom dates.

3.3. Yield and Fruit Weight

Average yield, individual fruit weight, and number of fruit per tree for 'Gala' were not significantly different among treatments (Table 2). Net treatments for 'Honeycrisp', on the other hand, had significantly reduced yield and fruit number and increased fruit weight compared to nonthinned, non-netted controls for both years (Table 3). Yield of netted trees in 2017 and 2018 was similar to hand-thinned control trees and was commercially acceptable (Table 3). Thinning markedly increased fruit weight compared to net treatments and nonthinned control in 2017 but not 2018. Non-netted 2017–2018 (nonthinned) trees had the lowest yield among the six 'Honeycrisp' treatments in 2018 due to reduced return bloom (Table 3).

Table 2. Effect of netting treatments in 2017 and 2018 on average tree yield and number of fruit per tree, yield efficiency (YE), crop load, average fruit weight, and fruit quality attributes; red color (percentage red), flesh firmness, soluble solids concentration (SSC), and shape of 'Gala' fruit at harvest.

Treatments	Avg. Tree Yield (kg)	(Fruit No.)	YE ($kg \cdot cm^{-2}$)	Cropload (Fruit No./cm^2)	Fruit Weight (g)	Red Color (%)	Firmness (kg)	SSC (%)	Shape [z] Height/Width
					'Gala' 2017				
Non-netted (Nonthinned)	23	181.1	0.64	4.9	127.2	39	3.8 a [y]	12.1	1.1
Netted 0% (Pink [w])	20.6	160.3	0.66	5.1	128.2	41	3.7 ab	12	1.1
Netted 26% (KB)	21.5	164.5	0.6	4.9	130.5	36	3.6 bc	11.9	1.1
Netted 60% (KB)	21.5	159	0.6	4.6	135.5	33	3.5 c	11.8	1.5
Netted 95% (KB)	20.9	162.6	0.6	4.9	128.4	35	3.8 a	11.8	1.1
					'Gala' 2018				
Non-netted (Nonthinned)	29.8	212.1	0.82	5.8	139.9	43.5	6	12.4	0.93
Non-netted (Hand thinned)	26.8	190.6	0.74	5.3	140.4	54.1	5.2	11.6	0.94
Netted 0% (Pink)	26.6	215.1	0.86	6.9	126	48	5.5	11.3	0.97
Netted 20% (KB)	27.9	233	0.84	6.9	119.5	40.1	5.6	11.1	0.98
Netted 40% (KB)	25.2	209.8	0.73	6.1	121.1	42.6	5.9	11.6	0.96
Netted 80% (KB)	25.7	218.8	0.80	6.8	120.7	45.6	5.4	11.4	0.92

[z] Shape was expressed as the ratio of fruit width to height. [y] Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. [w] Pink, pink stage of bud development; KB, king flower bloom. [y] Data are means of three replicates. Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. $n = 8$ for yield, YE, crop load, and fruit no.; $n = 15$ firmness; $n = 3$ (each a composite sample comprising five fruit) for SSC; $n = 8$ for shape and fruit weight (average of 50 individually measured fruit from each of 8 trees per replicate); $n = 8$ for red color (all fruit from each of eight trees per replicate).

Table 3. Effect of netting treatments in 2017 and 2018 on average tree yield and number of fruit, average fruit weight, and fruit quality attributes; red color (percentage red), flesh firmness, soluble solids concentration (SSC), nondestructively predicted dry matter (%), and shape of 'Honeycrisp' fruit 1 month after harvest (RA storage).

Treatments	Avg. Tree Yield (kg)	(Fruit no.)	YE ($kg \cdot cm^{-2}$)	Cropload (Fruit no./cm^2)	Fruit Weight (g)	Red Color (%)	Firmness (kg)	Dry Matter (%)	SSC (%)	Shape [z] % Misshapen
					'Honeycrisp' 2017					
Non-netted (Nonthinned)	35.1 a [y]	366 a	1.4 a	14.5 a	96 c	53 a	6.5	14.5 d	11.1 b	47.6
Non-netted (Hand thinned)	27.8 ab	139 b	1 b	5 c	204 a	46 a	6.6	15.2 bc	11.4 ab	33.8
Netted 0% (Pink [w])	22.3 b	148 b	0.9 b	6.3 bc	155 b	44 ab	6.7	15.8 a	12 a	45.1
Netted 23% (KB)	31.7 ab	213 b	1.1 b	7.1 bc	151 b	32 b	6.5	15.6 ab	11.5 ab	47.7
Netted 58% (KB)	31.6 ab	217 b	1.2 ab	8.4 b	146 b	31 b	6.3	14.8 cd	11 b	48.4
					'Honeycrisp' 2018					
Non-netted (Thinned 2017)	31.7 a	338 a	1 a	11.1 a	94 c	55 ab	7.1 c	15.1 b	12.1 d	45.1
Non-netted (Nonthinned)	5.1 c	35 b	0.18 c	1.2 b	152 b	68 a	8.3 a	17.6 a	14.7 a	32.9
Non-netted (Hand thinned)	16.9 b	74 b	0.58 b	2.5 b	234 a	52 bc	7.6 b	17.3 a	14.2 a	48
Netted 0% (Pink)	12.1 bc	50 b	0.47 bc	2 b	240 a	40 c	7.6 b	17.3 a	13.6 b	54.5
Netted 28% (KB)	14.9 bc	59 b	0.46 bc	1.8 b	257 a	45 bc	7.5 bc	17.1 a	13 c	56.4
Netted 68% (KB)	16.9 b	71 b	0.58 b	2.4 b	242 a	49 bc	7.6 b	17.2 a	13.3 bc	51.6

[z] Shape was expressed as percentage of fruit asymmetrical in both horizontal and vertical plane. [y] Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. [w] Pink, pink stage of bud development; KB, king flower bloom. [y] Data are means of four replicates. Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. $n = 4$ for yield, YE, crop load, and avg. fruit wt.; $n = 24$ and 40 for individual fruit quality attributes for 2017 and 2018, respectively.

3.4. Fruit Quality Attributes

No differences were found for fruit quality attributes of 'Gala' among treatments except firmness in 2017. The lack of statistical differences among treatments for harvested 'Gala' apples in 2018 was maintained after storage (Table 4). For 'Honeycrisp', fruit from the 23% and 58% netted treatments had significantly lower percentage of red color than non-netted fruit +1 M after harvest in 2017, while in 2018 the values were not statistically different (Table 3). No clear discrimination in 2018 postharvest data was evident, though the most colored fruit belonged to the non-netted (no thin 2017–2018) treatment with very low yield, possibly an effect from the excessive crop load in 2017 (Table 5). 'Honeycrisp' apples from trees netted at 'pink' had the highest SSC and predicted dry matter content than other treatments in 2017 both 1 M after harvest as well as 6 M storage (Tables 3 and 5). These results were not repeated, however, in 2018 where the non-netted, nonthinned new control had significantly less predicted DM% compared to all other treatments, which had similar and higher DM% (Table 3). After 6 M storage from 2018 harvest, the predicted dry matter varied among treatments showing the highest mean value for netted 28% KB and the lowest for non-netted (no thin 2018) (Table 5). The lowest SSC 1 M after harvest was measured in apples harvested in 2018 from the overcropped non-netted, nonthinned new control (12.1% SSC), followed by the three netted treatments (13.0 to 13.6%), and the hand thinned control non-netted (14.2%). A similar trend was observed for SSC 6 M after harvest (Table 5). Non netted 2017–2018 (nonthinned) apples had the highest percentage of red color, SSC, and DM% compared to all other treatments in both fruit quality assessment time points (Tables 3 and 5). Netting did not significantly affect fruit shape of either cultivar in either year. Differences in misshapenness incidence across the six treatments in 2018 were not statistically significant.

Table 4. Effect of netting treatments in 2018 on 'Gala' postharvest quality (3 months storage) as determined by average fruit weight and fruit quality attributes; flesh firmness, soluble solids concentration (SSC), starch, and titratable acidity.

Treatments	Fruit Weight (g)	Firmness (kg)	SSC (%)	Starch (1–10)	Acidity (%)
Non-netted (Nonthinned)	115.1	6.3	12.4	7.1	0.27
Non-netted (Hand thinned)	137.1	6.5	11.6	7.7	0.27
Netted 0% (Pink ^{z})	123.9	6.5	11.3	7.8	0.28
Netted 20% (KB)	116.8	6.2	11.1	7.7	0.23
Netted 40 (KB)	124.8	6.2	11.6	8	0.24
Netted 80% (KB)	135.4	6.3	11.4	7.6	0.25

^{z} Pink, pink stage of bud development; KB, king flower bloom. Data are means of three replicates. Fruit weight, Firmness and Starch, n = 15; SSC and acidity, n = 3 (each observation was a composite sample comprising five fruit).

Table 5. Effect of netting treatments in 2017 and 2018 on 'Honeycrisp' postharvest quality (6 months storage) as determined by average weight loss of fruit, red color (percentage red), flesh firmness, dry matter, and soluble solids concentration (SSC).

Treatments	Avg. Weight Loss (g)	Red Color (%)	Firmness (kg)	Dry Matter (%)	SSC (%)
		'Honeycrisp' 2017			
Non-netted (Nonthinned)	2.8 c ^{z}	49 a	6.3 b	14.2 c	10.8 c
Non-netted (Hand thinned)	4 a	44 a	6.5 ab	15.4 ab	11.6 ab
Netted 0% (Pink) ^{y}	3.8 ab	42 a	6.7 a	15.7 a	12 a
Netted 23% (KB)	3.4 b	23 b	6.5 ab	15.3 ab	11.1 bc
Netted 58% (KB)	3.7 ab	31 b	6.5 ab	15.1 b	11.1 bc

Table 5. *Cont.*

Treatments	Avg. Weight Loss (g)	Red Color (%)	Firmness (kg)	Dry Matter (%)	SSC (%)
			'Honeycrisp' 2018		
Non-netted (Thinned 2017)	2.9 c	55 b	7.2 c	15.4 c	12 e
Non-netted (Nonthinned)	4.7 a	67 a	8.1 a	17.5 ab	14.5 a
Non-netted (Hand thinned)	4 b	53 b	7.6 b	17.5 ab	14.1 a
Netted 0% (Pink)	4.3 ab	45 bc	7.5 bc	17 ab	13.4 b
Netted 28% (KB)	4.7 a	47 b	7.6 b	17.6 a	13.3 c
Netted 68% (KB)	4.4 ab	51 b	7.5 bc	16.7 b	12.6 d

[z] Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. [y] Pink, pink stage of bud development; KB, king flower bloom. Data are means of four replicates. Mean separation among treatments by Tukey HSD ($p < 0.05$), whereby means associated with different letters are significantly different. N = 24 and 40 for individual fruit quality attributes for 2017 and 2018, respectively.

4. Discussion

Numerous benefits associated with netting include the exclusion of insect pests [29,35], hail protection [32], and mitigation of abiotic stress [33,34]. Little has been described, however, with respect to the use of exclusion netting as a potential crop load management strategy [30,37], particularly for organic production systems. In two consecutive years, we evaluated the potential for netting to reduce pollination and fruit set of two different cultivars in vastly different climatic regions of the Unites States. We observed consistent fruit set and crop levels between years for a given cultivar under nets. At least in the case of 'Honeycrisp', we suggest that netting may provide an alternative approach to crop load management as previously suggested for 'Gold Rush' and 'Golden Delicious' [37]. Surprisingly, for 'Gala', netting did not markedly alter fruit set and harvest parameters compared to non-netted trees, though interesting differences between years provided insight into the mechanisms controlling 'Gala' fruit set.

Commercial fruit set was achieved under netting for both cultivars but was not markedly influenced by the percentage of open flowers at the time of canopy enclosure (Figure 2). 'Honeycrisp', which can naturally set fruit on all of the flowers of a cluster (Elsysy, personal observation), showed a significantly reduced fruit set when nets were applied at either 23% or 58% KB and 28% or 68% KB (respectively in 2017 and 2018) compared to nonthinned, non-netted trees. Hand thinning (leaving the strongest flower in the cluster) of non-netted trees produced slightly lower crop loads than achieved by netting in 2017, indicating that a full, commercial crop of 'Honeycrisp' was set under nets, irrespective of the timing (Figure 2B). In 2018, fruit set of the three netted treatments ranged from 3.3% to 7.0% (Netted 0% to Netted 68%), which were statistically comparable to the hand thinned control (7.3% fruit set) for commercial 'Honeycrisp' organic production in WA. 'Gala' fruit set, in contrast, was not affected by any of the netting treatments compared to the non-netted control. Similar observations were drawn by Dorigoni and Micheli (2015) whose experimental trials report 'Gala' as recalcitrant to thinning by a single-row netting system. Our data, in combination with [30], support the previously described partial self-fertility of 'Gala' [15]. In 2017, fruit set of non-netted 'Gala' trees was lower than normal and did not require thinning, presumably due to a spring frost event that occurred between full bloom and petal fall (−4 °C sustained for 4 h, Figure 4A). Crop loads were close to commercial targets for all treatments.

Surprisingly, in both cultivars, netting at 0% KB did not lead to a severe reduction in fruit set compared to non-netted controls (Figure 2), nor did this timing eliminate seeded fruit (Figure 3), despite exclusion of pollinators. While we cannot unequivocally dismiss the possibility that honeybee and/or native pollinators may have been trapped during net closure, we did not observe active pollinators inside closed nets, and the relatively low percentage of mature and nondeveloped seeds and concomitant high percentage of nonfertilized seeds per fruit under nets (at both sites) does not indicate the presence of pollinators acting in the fertilization of flowers. In the tropical region in east

Java, environmental factors stimulated self-compatibility, and produced a commercial, seeded 'Rome Beauty' apple crop without pollinizers [17]. High temperatures contribute to parthenocarpic fruit set of 'Bartlett' (i.e., 'Williams') pears in California, U.S. [43], though we would not expect marked alteration in temperature or other climatic factors between netted and non-netted trees of our experiments to have been responsible for these differences. In terms of light, the experimentally measured PAR below nets on several dates in MI was ~92% of PAR incident on control trees (data not shown), which was similar to the manufacturer's light transmission data (≈approx. 10% shading). Alternatively, fruit set under nets may have been due to partial self-fertility. Self-fruitful cultivars of apple do exist, the most notable being 'Golden Delicious' and 'Idared' [44] as well as 'Gala' (15). Indeed, fruit set of 'Golden Delicious' approached full crop potential when netted prior to bloom, though information on the number and type of seeds present in those fruit was not reported [30,37].

An alternative explanation for seeded fruit under nets is the transfer of pollen into netted canopies by wind [45], despite the general assumption that apple pollen is too heavy for dissemination [46]. Electrostatic forces in combination with wind dissemination of pollen have been linked to pollination of flowers of different species in the absence of pollinators [47,48]. Whether these forces could be responsible for pollination of apple remains to be elucidated. Incidentally, we did trap pollen in nets via suspended wax-coated petri dishes but their identity was not confirmed. The high number of nonfertilized seeds observed for both cultivars in netting treatments may not indicate high efficiency for the dissemination of pollen by wind, but fruit set was clearly achievable with low seed content. Facultative parthenocarpy was inducible under conditions adverse for pollination and fertilization [19,49]. The existence of naturally occurring parthenocarpic 'Cox's Orange Pippin' fruit tended to be highest when the number of seeded fruit was low, indicating that seedless fruit may be at a competitive disadvantage for retention [24]. This appeared to be true for 'Gala' when examining the differences in seedless fruit between 2017 and 2018 and for 'Honeycrisp', where delayed netting reduced the number of seedless fruit, especially in 2018. Maximum temperatures at both locations throughout the bloom period were presumed generally sufficient for bee activity prior to net closure [44] and would have expectedly led to higher percentages of seeded fruit as the time of netting was delayed, a trend that was evident in 'Honeycrisp' but only to a small degree. In MI, in 2017 average temperatures between 60% and 95% KB may have adversely affected pollen germination and/or tube growth [9]. When maximum daytime temperatures were 13° C or lower, no pollen tubes had reached the base of 'Gala' styles by 4 d irrespective of pollen source [10]. Low maximum temperatures (i.e., within the range observed in the present experimental sites) may not have markedly shortened the effective pollination period, since retarded growth of pollen tubes would be accompanied by delayed senescence of ovules [50]. The effect of netting on seed production could have additionally been due to the change in auxin activity [49] though we do not have data to support this contention. High auxin levels in fruiting spurs the year following a seeded crop of 'd'Anjou' pear were correlated with set of seedless fruit [20]. Thus, multiple seasons of netting are required to assess carryover effects of seediness on yield consistency.

The change in environmental conditions caused by exclusion nets can lead to a change in yield and fruit size and this change can be stimulative or inhibitive based on several factors [40]. 'Gala' yield was not significantly affected by netting treatments, nor was fruit weight. Given the similar within-year fruit set, yield and yield efficiency among 'Gala' treatments, any differences in fruit weight would have plausibly been attributed to fertilization status and hormonal regulation, especially given the marked differences in seed content. We did not measure vegetative growth parameters, aside from trunk size (data not shown), but light levels were only reduced by ~8% and no observable differences in canopy growth and development were visibly evident. While this reduction of light would not limit fruit set, 90% shade nets applied at 12 mm fruitlet diameter for a period of 7 d have effectively thinned apples [51]. There were no treatment effects on 'Gala' fruit quality at harvest or following postharvest storage except firmness, which did not follow a clear trend. Average fruit weight from 2017 'Honeycrisp' trees netted at 0% bloom (pink), however, was 24% reduced than non-netted hand-thinned control trees despite having a similar crop load, while in 2018 all three netted treatments reported comparable average fruit weight as in hand thinned commercial standard. These treatment differences may in fact be related to seed number and auxin content since seeds have been linked to cell size of cortical tissue [52]. Additional fruit quality differences between net treatments and controls were found for 'Honeycrisp'. For example, trees netted at 23% or 58% KB (2017) and 28% or 68% KB (2018) had less color than either of the 'Honeycrisp' control treatments, yet canopies netted at 0% bloom were intermediate in 2017. In 2018, only the nonthinned control fruit had significantly higher overcolor, though this was attributed to their significantly lower crop load (Table 2). The percentage of red color under black hail net was significantly reduced compared to a control [53]. Chouinard et al., showed reduced fruit color of 'Honeycrisp' in netted trees compared to non-netted trees in several years of a six-year netting study, though annual variation in fruit quality attributes was considerably high [38]. For 'Honeycrisp', trees netted at 'pink' had ~15–30% fewer fruit than their later-netted counterparts in both years, albeit non-significantly. These numeric differences were larger in 2017 than in 2018 and may have contributed to differences observed in SSC and DM%. Development of a near infrared spectroscopy (NIR) model specifically for Honeycrisp under Washington growing conditions would be valuable. Importantly, the effects of netting on fruit quality of both cultivars were not altered following postharvest storage indicating that reduced seed content did not affect postharvest quality.

5. Conclusions

Exclusion netting represents a potential alternative to hand and chemical thinning for managing crop load, particularly in organically managed systems. Both 'Gala' and 'Honeycrisp' apples set a commercial crop when netting trees before bloom (i.e., 'pink' phenology stage) and throughout flowering. Netting affected seed formation with minimal effects on fruit quality and fruit shape in 'Gala' and 'Honeycrisp'. Exclusion of pollinators to 'Gala' during a frost year led to a high percentage of parthenocarpic fruit set, indicating the capacity of this cultivar to set fruit under adverse conditions. The effects of exclusion netting on fruit set, growth, and development requires further investigation under different environmental conditions and in combination with diverse cultivars to determine the broad applicability of the net enclosure technique.

Author Contributions: Conceptualization, S.M., T.E.; methodology, S.S., M.E., S.M., and T.E.; formal analysis, S.S. and M.E.; investigation, S.S., M.E., S.M., T.E., P.S.; resources, P.S.; data curation, M.E.; writing—original draft preparation, M.E., S.S.; writing—review and editing, T.E., S.M. and P.S.; project administration, T.E. and S.M.; funding acquisition, T.E. and S.M.

Funding: This research was funded by Michigan Apple Committee (grant number MAC18-001) and MSU AgBioResearch ProjectGREEEN (grant number GR17-035). This work was supported by the USDA National Institute of Food and Agriculture Hatch project 1014919, titled "Crop Improvement and Sustainable Production Systems" (WSU reference 00011).

Acknowledgments: We are grateful to the Michigan Apple Committee for their generous financial support. We thank Helios® Anti-Hail Systems for supplying netting and Ag-Liner, Inc. for supplying monofilament and help with net installation. S. Musacchi and S. Serra thank Stefan Roeder, Brendon Anthony, Ryan Sheick, Alex Goke and Angela Knerl for the technical assistance in net installation and data collection.

Conflicts of Interest: The authors declare no conflict of interest.

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