FINAL PROJECT REPORT **PERIOD:** 2 year of 2 years
(AP-21-109A)
Project Title: **Maximize pollination window to improve fruit set in WA 38**

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Cooperators: Chelan Fruit - Monument Hills Orchard (Quincy, WA), Columbia Reach (Yakima, WA), AgroFresh, Extenday USA, Valent Biosciences.

Other funding sources/in-kind support:
We will use commercial orchards established by growers in the Royal City and Quincy areas in WA. The WA 38 orchard blocks available cover approximately 2 acres for an estimated value of $80,000 to be considered as in-kind support for the current project. Valent Biosciences is donating ReTain® and providing technical support for this project. AgroFresh has agreed to donate Harvista™ as well as technical staff support for material application for the two years of research. Extenday USA has donated reflective ground cover and technical support for material installation for this project.

Total Project Request: Year 1: $ 72,022, Year 2: $ 73,813 (Total: $ 145,835)

BUDGET
Primary PI: Sara Serra
Organization Name: Washington State University
Contract Administrator: Stacy Mondy/ Jason Hansen
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Budget 1

WSU: Serra-Musacchi		
Costs	Year 1 (2021)	Year 2 (2022)
Salaries[1]	$ 33,840	$ 35,194
Benefit[2]	$ 11,306	$ 11,758
Wages[3]	$ 4,800	$ 4,992
Benefit[4]	$ 1,076	$ 1,119
Supplies [5]	$ 4,000	$ 4,000
Fruit reimbursement for sampling	$ 5,000	$ 5,000
Travel[6]	$ 5,000	$ 5,000
Serra-Musacchi Total	$65,022	$67,063

Footnotes:
[1] Salary for a 50% Assistant Research Professor (Serra-Musacchi)
[2] Benefit on salary at 33.41%
[3] One non-Student temporary for 8 wks: 40hrs/wk at $15/hr (Serra-Musacchi).

[4] Benefits on temporary at 22.4%
[5] Labware/consumable, field products (Serra-Musacchi)
[6] 8,696 miles/year for domestic travel ($0.575/mile) to go to the orchards. Adjusted for COVID19 distancing (independent cars).

WTFRC Collaborative Expenses

WTFRC: Schmidt		
Costs	Year 1 (2021)	Year 2 (2022)
Wages/Benefits	$4,000	$3,750
Supplies[1]	$500	$500
Equipment costs[2]	$1,000	$1,000
Travel[3]	$1,500	$1,500
WTFRC Total	$7,000	$6,750

Footnotes:
[1] Spray suits, lab supplies for fruit quality analysis
[2] Fuel, maintenance, wear and tear on trailer, tractor, sprayer
[3] In-state travel to research plots

RECAP OBJECTIVES:

1. **Determine the AVG and 1-MCP effect on fruit set in WA 38**
2. **Explore the effect of pre-bloom deployment of reflective fabric on WA 38 fruit set**

SIGNIFICANT FINDINGS:

1. Determine the AVG and 1-MCP effect on fruit set in WA 38

a. No significant differences in flower bud return and tree growth across the 5 treatments in 2022

b. At 8 weeks after full bloom, the fruitlet shedding ended at 91% in 2021 with no differentiation between ethylene inhibitor treatments or application timings and at 82% in 2022 with Retain treatments at both application times, reporting the lowest fruit drop.

c. While average number of apples per tree, production per tree, and crop load at harvest were all comparable across treatments in 2021, in 2022, both ReTain treatments reported the highest number of apples per tree, and crop load significantly differed across the five treatments, despite similar yield per tree.

d. "ReTain 56% bloom" and "ReTain +7 days" significantly penalized the average fruit weight in 2022 (~150 apples/box) with respect to untreated control trees ("CTRL"), and "Harvista 56%" fruit (~113 apples/box). In 2021, only "ReTain +7 days" penalized the average fruit weight.

e. For two consecutive years, the natural crop load in the orchard of untreated control trees was very high with limited potential for further enhancement (10.2 and 9.9 fruit/cm^2 TCSA).

f. "ReTain 56% bloom" showed 55% of the production as a single apple/flower cluster (lowest proportion), 30% as a double and 13% as a triple (significantly higher than the other treatments), and a 2% as a quadruple, suggesting an improved fruit set on a flower cluster basis.

g. "ReTain 56% bloom" produced 99.7% of 2022 apples in the small size (almost 17% more than control), while "Harvista 56% bloom" performed very similarly to "CTRL". In 2021, "ReTain +7 days" negatively impacted the packout towards small fruit (+13.4% smaller than in "CTRL").

h. The triple fruit/cluster category reported the highest proportions of smaller fruit in all treatments except for "ReTain 56% bloom", suggesting considering the option of a mid-summer hand thinning of triple apples at least down to double/cluster when the crop load in medium-high to minimize the smaller fruit.

2. Explore the effect of pre-bloom deployment of reflective fabric on WA 38 fruit set

a. Reflective material deployed in 2021 did not impact tree trunk growth, the vegetative growth of 1-year-old shoots, or the number of flower buds per tree when compared to control.

b. An abnormal frost event occurred in April 2022, which may have contributed to a confounding effect in the fruit retention assessment along the season as some compromised flowers abscised due to cold damage.

c. The last fruit retention assessment reported similar fruit drop for "RM until harvest", "no RM (CTRL)" and "RM for 2M" (90%, 90%, and 91%, respectively), while "RM for 1M" showed the lowest proportion of fruit left on tree (7%).

d. The reflective material employed for 2 years in this trial proved to alter the tree microclimate towards a drier and hotter canopy but a cooler and wetter soil.

e. Monthly photosynthesis measurements from May to September of both years did not show significant differences in carbon assimilation rates for trees with reflective material from early bloom and "no RM (CTRL)" trees.

f. Across the four treatments, yield/tree was not significantly different, ranging from 15.0 kg/tree to 17.9 kg/tree for "no RM (CTRL)" and "RM until harvest", respectively (similarly to 2021).

g. "RM until harvest" trees presented the highest proportion of 2022 apples belonging to large size class (46%), followed by "RM for 1 M" (41%), while "no RM (CTRL)" showed the largest proportion in the smallest size class (38%). The season-long deployment of RM from early bloom to harvest promoted fruit size and red coloration rather than fruit retention.

Objective 1) Determine the AVG and 1-MCP effect on fruit set in WA 38

RESULTS AND DISCUSSION

The return bloom assessment was carried out in March 2022 in the same WA 38/Nic29 orchard (planted in 2018) in Quincy as the first year of this study. The number of flower buds per tree was counted before pruning in a subsample of 9 trees/trt (out of 12) and did not produce significant differences (p=0.0705) between the 5 treatments imposed in 2021. Despite the lack of statistical discrimination, "ReTain 31% bloom" showed the highest average, equal to 201 flower buds/tree, while "Harvista +7 days" produced the lowest average with 171 flower buds/tree (Figure 1). Similarly, no significant difference emerged in terms of TCSA annual growth across the 5 treatments (Figure 1).
In the second year (2022), we repeated the same experiment as in 2021 in the same orchard rows keeping the same treatments on the same plots to account for a 2 year-cumulative effect. Each treatment was represented by 12 trees (4 trees/row × 3 rows × 5 treatments) chosen in the same plots treated in 2021, for a total of 60 trees in the trial. The experimental trees were selected in spring 2022 to ensure a uniform starting point for the second season. The selection criteria were similar TCSA and a narrow range of flower buds (FB)/TCSA.TCSA was measured 4/6/2022 on a new set of trees and averaged 16.4 ± 2.2 per cm^2 with no differences across treatments. The count of flower buds/tree was done after pruning on 4/18/22, and the calculated flower bud loads averaged 11.1 ± 1.7 FB/ cm^2.

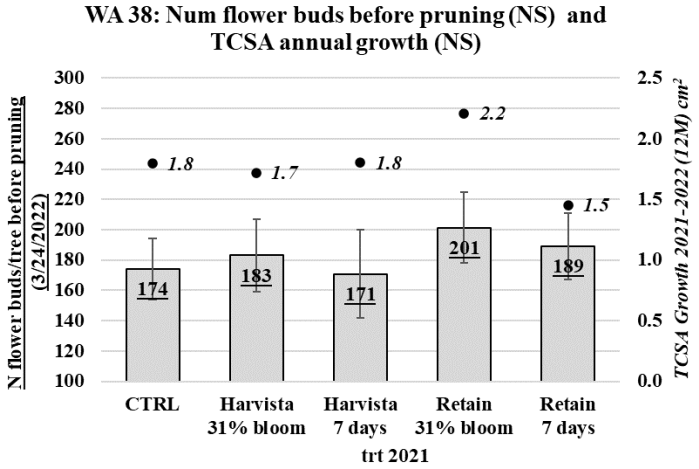

***Figure 1**. WA 38 return bloom in 2022 on trees in trial in 2021 (as N flower buds/tree before pruning) and annual trunk cross sectional growth (TCSA, cm^2) in 12 months in Quincy block across the 5 treatments in trial: "CTRL", "Harvista 31% (king) bloom", "Harvista +7 days" and "ReTain 31% (king) bloom" and "ReTain +7 days". (The "+" in the two treatments at 7 days is omitted in the x-axis from now on). Each column represents the mean yield of 9 trees per trt and the error bar indicates the standard deviation. Each circle marker related to the secondary Y axis represents the TCSA growth of 12 trees per trt. Differences reported are not significant (NS, p>0.05).*

A severe frost event hit the Wenatchee and Quincy areas during the second and third weeks of April 2022. On 04/15/22, a minimum air temperature of 26.9 °F was reported in Quincy (avg. daily temperature for April 2022 was 44.6 °F, while 52.9 °F in 2021). A survey on green cluster-early pink tip flowers conducted on 4/19/2022 by dissecting them to examine the potential impact of cold damage did not show significant browning of styles nor ovaries that would lead to a possible significant loss, indicating the frost protection system in the block was effective. For each experimental tree, 5 branches were labeled (total 300 branches) and tracked for their phenology from swollen flower bud stage until harvest, following the same protocol reported for 2021 (with minor modifications). The precise tracking of the phenology evolution was crucial to target the suitable time for the chemical applications. In 2022, the first spray of ReTain® (Valent Biosciences) and Harvista™ (AgroFresh) was on 5/3/2022, corresponding to 56% king flower open (or 17% of total flower open). The chemical application followed the same methodology described for the previous year. The utilized doses complied to the recommended label rates for both products: 1 pouch of 333 g/acre (123.4 g AI/ha; AI= active ingredient) of ReTain® and 60 g AI/acre (148.2 g AI/ha) for Harvista™. Seven days later, on 5/10/2022 (full bloom), the second spray ("ReTain +7 days" and "Harvista +7 days") was administered to the designated plots for each of the two ethylene inhibitors at 89% total flowers open (97% king open and

king petal fall had begun). To investigate the effect of the two ethylene inhibitors and their application time in improving the fruit set and on-tree retention of WA 38 fruitlets, fruitlet retention assessment on the 300 branches was carried out from June through October 2022. Fruitlet retention assessment began on 6/1/2022 (=21 days after full bloom (DAFB), avg. fruit diameter = 14 mm) and was repeated on 5 subsequent dates until harvest (6/21, 7/8, 7/18, 8/4, 10/7/2022). At 3 weeks after full bloom (6/1/2022), the "ReTain 56% bloom" showed 80% of fruitlets still on tree – a significantly higher proportion relative to the other 4 treatments, with the lowest being "CTRL" and "Harvista 56% bloom" with only 50% and 57% retention, respectively (data not shown). At 41 DAFB, on 6/21/2022 (avg. fruit diameter = 33 mm), the overall fruitlet drop reached 79% across the 5 treatments with significant differences between them. "ReTain 56% bloom" and "ReTain +7 days" showed 26 and 25% retention, respectively, while "CTRL" showed only 18% drop, statistically similar to both "Harvista" treatments (data not shown). Figure 2 (A to D) reports fruit retention/fruit drop for two-time points for each year of the trial. At approximately 8 weeks AFB (7/8/2022, avg. fruit diameter = 42 mm), the overall drop reached 82%, and the retention of fruit showed significant differentiation across the treatments (Figure 2). In fact, "Retain 56% bloom" and "Retain +7 days" maintained the highest proportions of apples on tree (21 and 19 %, respectively, Figure 2B), while "CTRL" recorded 16%. Compared to 2021, at 56 DAFB, the general fruit drop was 9% more intense (91%) than in 2022 at similar DAFB (Figure 2A and B) but did not report significant differences across the treatments. Weather conditions, in particular temperature, in the first 8 weeks AFB (avg. 2022: min 50.5 °F, avg. 60.5 °F, max 70.8 °F and avg. 2021: min 52.2 °F, avg. 63.2 °F, max 74.4 °F, Figure 3) could have had a meaningful impact on the fruit shedding dynamics and retention in the two years. In the two years of this project, we are able to corroborate that WA 38 natural shedding lasts 8 weeks after full bloom, as previously observed during the project on WA 38 pollination and fruit development (# AP-19-10) and published in Serra *et al.,* 2022.

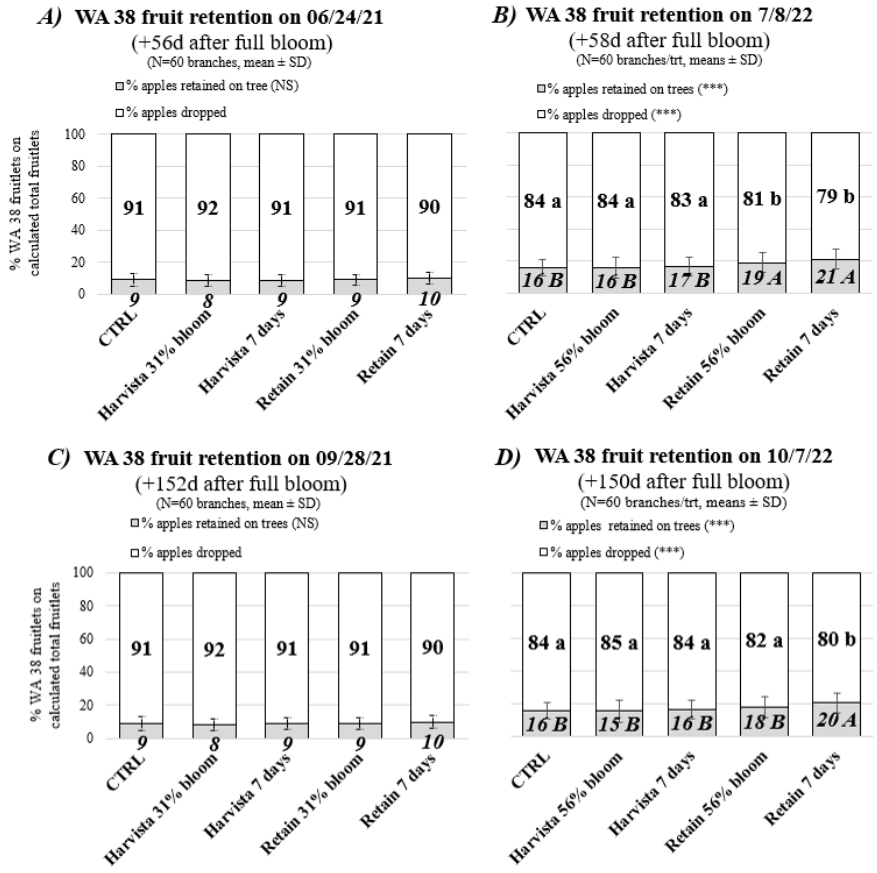

Figure 2*. WA 38 fruit retention assessments in the Quincy block across the 5 treatments in trial: "CTRL" (control, no treatment), "Harvista 31 or 56%¥ king bloom", "Harvista +7 days", "ReTain 31 or 56% king bloom", "ReTain +7 days" on 2 different dates in the 2 seasons in June 2021 (A= 8 weeks after full bloom), July 2022 (B=8 weeks after full bloom), September 2021 (C=22 weeks after full bloom) and October 2022 (D=22 weeks after full bloom). Each bar represents the mean of fruit retention (%) and fruit drop (%) in 60 branches/treatment each year. Error bars represent standard deviation of retained fruit percentage (in gray). NS= not significant, ***p≤0.001. ¥ The two percentages indicate the proportion of king flowers open in the tree at time of first chemical spray each year (31% in 2021 and 56% in 2022).*

At 10 weeks AFB (7/18/2022, avg. fruit diameter = 49 mm), once the natural shedding period typical of the variety had ended, the pattern of retention was similar to the 7/8/2022 assessment, confirming the highest proportions of retained apples in "ReTain +7 days" (+5% than "CTRL") and "ReTain 56% bloom" (+3% than "CTRL"), with significant differences with "CTRL" (data not shown). The following assessments on 8/4/22 (~12 weeks AFB; avg. fruit diameter = 57 mm; data not shown) and 10/7/2022 (~21 weeks AFB; avg. fruit diameter = 76 mm; Figure 2D), were also comparable to previous assessments, with the **highest retention of fruit in "Retain +7 days**" which was significantly different from all other treatments. The final assessment before harvest (10/7/2022) showed an overall average fruit drop of 83%, the same percentage of dropped fruit as reported in Serra *et al.*, 2022 for the year 2020 in a different block. Compared to 2021, which had higher temperatures during the shedding period, the fruitlet drop was higher at 91% (Figure 2C and 3). "ReTain +7 days" in 2022 contributed to a +4% retention with respect to "CTRL" (20% and 16%, respectively) in the experimental branches tracked along the season (Figure 2D). A survey conducted on a subsample of the branches (N=251) in 8/4/2022 in this block regarding the type of fruit present on tree by cluster occupancy showed 57% being single king apples, 26% single lateral apples, 9% king+lateral, 7% double laterals, and 1% king+2 laterals (data not shown).

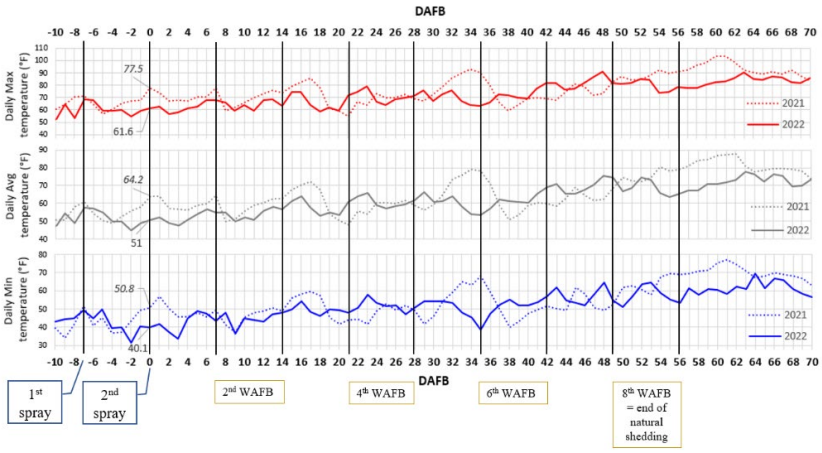

Figure 3. *Comparison between daily minimum, average, and maximum air temperature (°F) in Quincy in 2021 and 2022 from AWN station. Temperatures are presented in DAFB (x-axis), where 0 DAFB indicates "full bloom" for both years in the WA 38 experimental block. In both years, the first Harvista™ and ReTain® sprays were applied at -7 DAFB (corresponding to 4/22/2021 and 5/3/2022), while the second spray at 0 DAFB (corresponding to 4/29/2021 and 5/10/2022). WAFB= weeks after full bloom.*

The trial was harvested as a single pick on 10/13/2022 for the entire commercial block. The 5 treatments resulted in a significant difference in the number of apples picked/tree, with 223 apples on average in "Retain 56% bloom", 183 for "Retain +7 days", 169 in "Harvista +7 days", and 158 for both "CTRL" and "Harvista 56% bloom" (letters for mean discrimination: A, B, BC, C, C respectively; data not shown). The number of apples per tree in 2022, regardless of treatment, was on average 22 apples higher than in 2021. Similarly to 2021, production per tree did not reveal any significant differences across the 5 treatments with an average yield/tree ranging from 26.0 kg/tree to 27.1 kg/tree (57-60 lb/tree, Figure 4). However, average fruit weight in 2022 did significantly differ across treatments ($p \leq 0.001$), with "CTRL" and "Harvista 56% bloom" showing the highest and most similar fruit masses (173 g, 167 g, respectively, ~113 apples/box), followed by "Harvista +7 days" (157 g, ~125 apples/box). In the lower spectrum of fruit size, "Retain +7 days" yielded a smaller average fruit weight (142 g, ~125-138 apples/box), while "ReTain 56% bloom" produced the smallest fruit (121 g; ~150 apples/box, Figure 4). All treatments in both years had very high crop loads associated with general reductions in average fruit size in an optimum crop load scenario for WA 38 (5-6 apples/TCSA cm^2, 80-64 apples/box). While the crop load at harvest was not statistically different across treatments in 2021 (avg. 10.6 apples/TCSA cm^2), in 2022 the highest crop load of 13.3 apples/TCSA cm^2 for "ReTain 56% bloom", followed by "Retain +7 days" with 11.8. The other 3 treatments were similarly lower ("Harvista +7 day" 10.5, "CTRL" 9.9, and "Harvista 56% bloom" 9.6 apples/TCSA cm^2, data not shown).

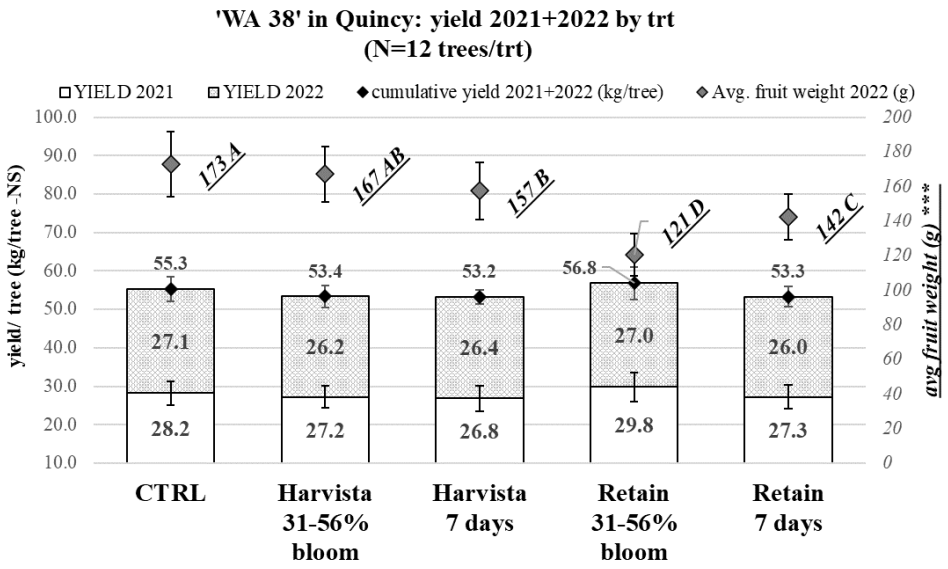

Figure 4. *WA 38 yield in kg/tree in 2021 and 2022 and 2022 average apple weight (g) in the Quincy block across the 5 treatments in trial: "CTRL" (control, no treatment), "Harvista 31-56% bloom", "Harvista +7 days", "ReTain 31-56% bloom", "ReTain +7 days". The 2 treatments at bloom are labeled with the 2 real percentage of king flowers that were open at time of spray in 2021 and 2022 (the target was in theory 50%). Each chemical spray was applied to the same plots as in 2021.Each column represents the mean yield of 12 trees/trt and the error bar indicates standard deviation. Each gray diamond marker related to the secondary Y axis represents the mean fruit weight of 12 trees/trt and the error bar indicates standard deviation. The 5 treatments are presented in X-axis in order of application time for each product after CTRL. ***=p<0.001, NS=not significant.*

A hailstorm hit the experimental block on 06/05/2022 causing significant damage to the small fruitlets (~ 16-21 mm size), impacting the final packout at harvest. In general, 33% of graded apples were culled, and both treatments at "+7 days" showed the highest proportion of culled fruit, while "Retain 56% bloom" and "CTRL" showed the lowest (data not shown). As performed in 2021, each apple harvested in 2022 was labeled at the time of pick based on the cluster pattern of origin as being single (S=1 apple/cluster), double (D=2 apples/cluster), triple (T=3 apples/clusters) or quadruple (Q=4 apples/cluster). All 2022 production was graded tree by tree, keeping apples separated from the 4 different cluster patterns within each tree. Significant differences in the proportion of fruit by cluster occupancy emerged across the treatments (Figure 5). "CTRL" and "Harvista 56% bloom" presented 70% of the crop as a single apple/cluster, which was shown to be significantly different from both treatments with ReTain. "ReTain 56% bloom" showed 55% of the production as single, 30% as double, and significantly higher proportion in the triple category, 13%, in comparison with the other treatments, and 2% in the quadruple (Figure 5). The same analysis of apple by cluster pattern in 2021 resulted in no significant differences.

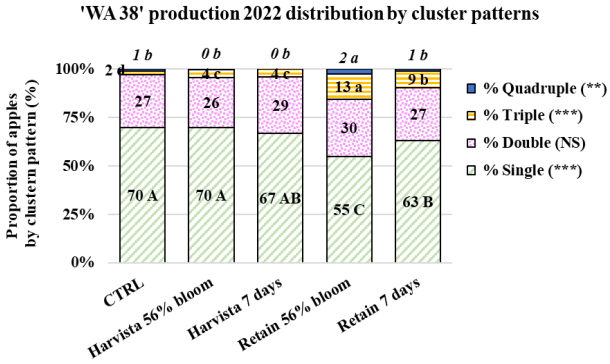

Figure 5*. WA 38 fruit grading by cluster patterns (or cluster occupancy) for the whole crop of Quincy trees (N=60) across the 5 treatments in 2022. Each tree production was graded by a sorting machine and apples were run separately among cluster categories: single, double, triple, quadruple. NS= no statistically significant differences, **=p≤0.01, ***= p≤0.001.*

Fruit size distribution was reported in proportion (%) of apples belonging to each of the three size categories for each of the treatments in trial (total 60 trees were graded= 10,685 apples, Figure 6). "ReTain 56% bloom" produced significantly more apples in the small classes (<215 g) than the other 4 treatments with 99.7% (Figure 6), while "CTRL", "Harvista 56% bloom" and "Harvista +7 days" reported higher proportions of medium apples (216-263 g, ~80 apples/box) in comparison to both treatments with ReTain (Figure 6). In 2022, the "ReTain 56% bloom" significantly penalized the fruit size leading to almost 100% of the crop in the smallest category, while in 2021, the application of ReTain at bloom (31% king bloom) led to the highest proportion of medium-sized fruit.

Many factors might have contributed to the contrasting results between the two years, in particular, the weather conditions at the time of application (for stigmatic receptivity and ovule longevity), as well as flower phenological stage, pollination, flower cluster quality, and resource availability. Machine grading by color criteria (Extra-Fancy & Fancy = red overcolor >50%, Grade 1 = red overcolor 30-50% and utility = red overcolor < 30%) highlighted a significant difference among the treatments. "ReTain 56% bloom", indeed, produced the lowest proportion of fruit in the Extra-Fancy & Fancy class (78% versus 92% in "CTRL") and the highest percentages in grade 1 and utility apples (data not shown).

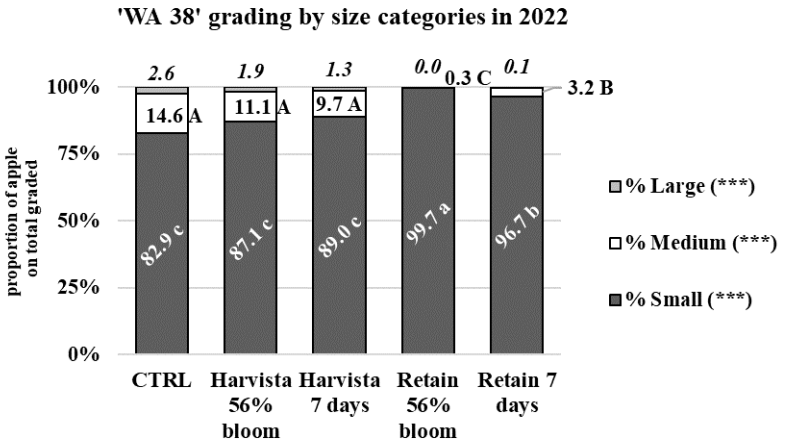

***Figure 6**. WA 38 fruit grading in size categories for the Quincy block across the 5 treatments in 2022. Each tree production was graded by the sorter machine and apples were divided in 3 size categories: small (≤ 215 g), medium (216-263 g), and large (264-339 g) corresponding to ≥88, 80, and 72-64 apples/box, respectively. WA 38 fruit in the extra-large (≥ 340 g) class were absent from this trial. ***=p<0.001 for significant differences.*

Additionally, in this second year, we can confirm that differences in terms of proportions of fruit in size categories are strongly influenced by cluster patterns (S, D, T) within each treatment (data not shown). The triple fruit/cluster (T) category reported the highest proportions of smaller fruit in "CTRL", "Harvista 56% bloom", "Harvista +7 days", and "ReTain +7 days" with respect to S (or S and D), while single fruit/cluster (S) showed significantly higher proportions of fruit in the medium size class than what was found in T (data not shown). These results corroborate 2021 findings and sound support in considering mid-summer hand thinning of triple apples when the crop load is medium-high.

Objective 2) Explore the effect of pre-bloom deployment of reflective fabric on WA 38 fruit set

RESULTS AND DISCUSSION

In March 2022, the number of flower buds before pruning was counted on the experimental trees selected for 2021 in the WA 38/G935 block (planted in 2018) to assess the effect of treatments on return bloom. As reported in Figure 7, the average number of flower buds ranged between 131 in the no reflective material ("CTRL") to 161 in "RM until harvest" with no significant differences. Despite the lack of statistical significance, "RM until harvest" tended to have 23% more flower buds than "CTRL". This same tendency was found for TCSA annual growth (in 12 months), where no significant difference emerged across the 4 treatments (Figure 7). Since the deployment of reflective material for longer duration, such as the treatment "RM until harvest", could have had an impact on the tree physiology, such as growth, the total length of 1-year-old shoots was measured on 3 trees/treatment before pruning and the number of shoots counted. Results on vegetative growth of 1-year-old shoots did not reveal significant differences across treatments, with an average of 23 m (~75 ft) of shoot growth/tree and 127 shoots of approximately 17 cm (6.7 inches) length (data not shown). The "RM until harvest" average of the total length of 1-year-old shoots was 5 m (16 ft) higher than "CTRL" (NS, data not shown).

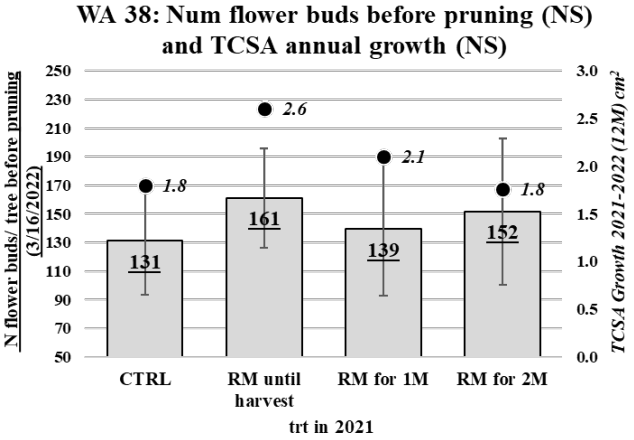

***Figure** 7. WA 38 return bloom in 2022 on trees in trial in 2021 (as N flower buds/tree before pruning) and annual trunk cross sectional (TCSA) growth (cm^2) in 12 months in Royal City block across the 4 treatments in trial: "CTRL", "RM until harvest", "RM for 1M" and "RM for 2M". Each column represents the mean yield of 12 trees per trt and the error bar indicates means ± standard deviation. Each circle marker related to the secondary Y axis represents the TCSA growth of 12 trees per trt and the error bar indicates standard deviation. Differences reported are not significant (NS =p>0.05).*

The plots designated for each treatment in 2021 were maintained the same in 2022, but new trees were selected for each treatment (4 east-facing trees/row × 3 V system rows) in April 2022 for a total of 48 trees to guarantee a uniform starting point for the second season of trial. Trees were selected for their similar number of flower buds (9.4 ± 2.1 FB/cm^2 TCSA) and TCSA (14.2 ± 1.9 cm^2) on 4/8/2022. Abnormally cold temperatures were recorded throughout April, including 25.3°F on 04/15/22 (avg. daily temperature for April 2022 was 44°F). While a frost protection system was activated, the orchard suffered a bit of cold damage, particularly in the lower canopy of the trees. A few days following the frost event, a subsample of flower clusters at green cluster-early pink tip stage were sampled and dissected. Approximately 46% of flowers dissected showed some browning in the styles, and 12%, some browning in the ovary (data not shown). This survey gave us more awareness about branch selection for 2022, knowing that the lower part of the canopy could have been compromised. Five branches/tree were tagged before bloom and described by position in the canopy and type of bearing wood. In each branch, the number of flower clusters was recorded following the 2021 protocol with minor modifications. Reflective material was installed on 4/28/22, when bloom was at ~10% king flowers open (~2% total flowers open). The reflective material used in the trial was the same as utilized in 2021 (open weave reflective fabric provided by Extenday® with 80% diffuse reflection). The 4 treatments evaluated in trial were: "RM for 1M" = RM deployment for 1 month (from 4/28/22 early bloom to 5/26/22), "RM for 2M" =RM deployment for 2 months (from 4/28/22 early bloom to 6/28/22), "RM until harvest" = RM deployment for all-season until harvest (from 4/28/22 early bloom to 10/12/22 harvest), and no reflective material ("CTRL"). Full bloom for the experimental block was 5/07/2022 (9 days later than 2021). Recording of fruitlet retention started 19 DAFB (on 5/26/22) as the

first assessment before the "RM for 1M" removal on 5/26/22, followed by 5 subsequent assessments before harvest. Fruitlet retention was calculated as described in the 2021 project report. On 5/26/22 (avg. fruit diameter = 13.3 mm), an average of 49% of fruitlets were still retained on tree with significant differences across the treatments; "RM for 2 M" showed the lowest percentage of fruit on tree significantly smaller than the other 3 treatments (data not shown). Twenty days later, on 6/15/2022 (6 weeks AFB, avg. fruit diameter = 29.9 mm), the average fruit retention dropped to 18% with "RM until harvest" producing the highest proportion of apples on tree (21%) with respect to the other treatments (16% and 17%; data not shown). Thirteen days later, on 6/28/22 (8 weeks AFB, date of "RM for 2 M" reflective material removal, avg. fruit diameter = 40.0 mm), the average fruit retention was 10%, with "RM for 1M" conferring the lowest proportion of fruit on tree (7%) - significantly different from "RM until harvest" and "CTRL" (12 and 11% respectively; Figure 8B). Figure 8 reports a visual comparison between two similar timings for fruit retention assessments in the two years; in general, the fruit drop tended to be more intense in 2022 (91%) than in 2021 (86%).

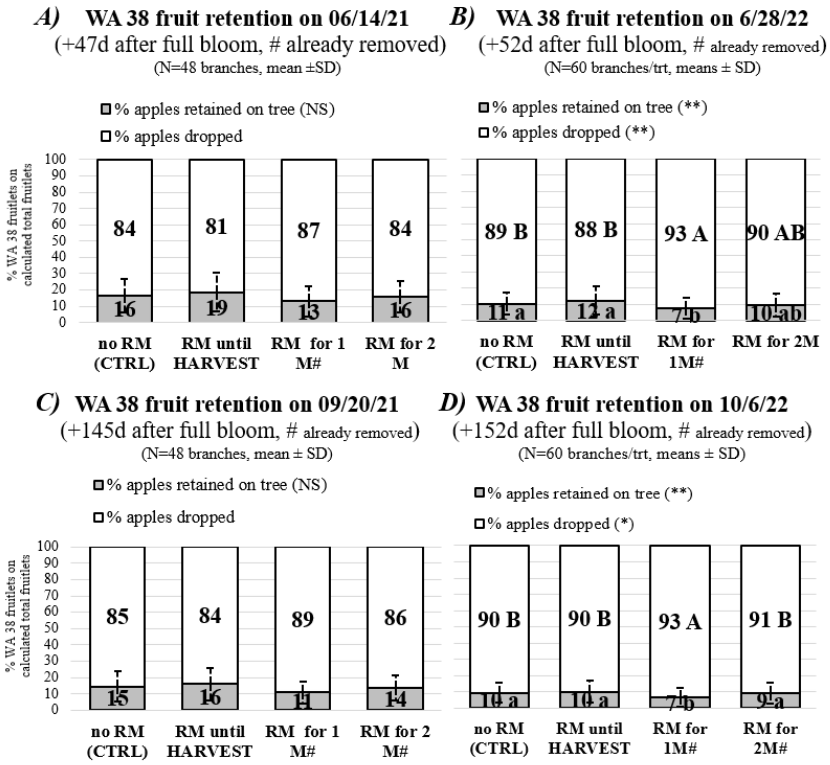

***Figure 8**. WA 38 fruit retention assessments in Royal City block across the 4 treatments in trial: no reflective material ("CTRL)"), RM deployed at bloom until harvest ("RM until harvest"), RM deployed for 1 month from bloom ("RM for 1M") and RM deployed for 2 months from harvest to June ("RM for 2M") on 2 different dates in the 2 seasons in June 2021 (A =7 weeks after full bloom), June 2022 (B= 8 weeks after full bloom), September 2021 (C=21 weeks after full bloom), and October 2022 (D=21 weeks after full bloom). Each bar represents the mean of fruit retention (%) and fruit drop (%) in 48 or 60 branches/treatment depending on the year. Error bars represent standard deviation of retained fruit percentage (in gray). NS= not significant, * $p \leq 0.05$, ** $p \leq 0.01$.*

The same statistical differences across the 4 treatments were maintained in the following 3 assessments: 7/12/22, 8/2/22, and 10/6/2022 (avg. fruit diameters = 50.4, 61.6, 80.4 mm, respectively). The last assessment reported similar fruit retentions for "RM until harvest", "CTRL", and "RM for 2M" (10%, 10%, and 9%, respectively), while "RM for 1M" showed the lowest proportion of fruit left on tree (7%, Figure 8D). In both years of trial, the treatment "RM for 1M" always recorded the lowest average of fruit retention relative to the other treatments (difference significant only in 2022; Figure 8C and D). The early removal of the reflective material after 1 month was confirmed to have negatively impacted fruitlet retention, probably perceived as a sudden shading or deprivation of resources altering the tree microenvironment. **Season 2022 ended up with an average of 9% of total fruit retained (regardless of the treatment); in 2021 the final proportion was 14%.** This may be attributed to the cold event in April 2022, increasing fruit drop in a magnitude not possible to discern from the natural shedding.

The second year of the trial confirmed some significant differences in canopy temperature/RH found in 2021 between "CTRL" trees and "RM until harvest". While the two treatments did not differ for daily minimum temperature in the 6 months, the average daily temperature was 0.9 °C (~34 °F) higher in "RM until harvest" than "CTRL" in May (as in 2021), but not in the other months (Figure 9). Moreover, daily maximum temperatures were significantly higher in "RM until harvest" from May to September compared to "CTRL" (Figure 9). The installation of the reflective material affected the mid-canopy microclimate with +3.3 °C (~6 °F) daily maximum temperature, similar to the +3.5 °C (~6.3 °F) recorded in 2021. These treatments also differed for daily minimum RH in May and June 2022 (data not shown), with "RM until harvest" canopies recording respectively 5.4 and 3.4 lower RH% than "CTRL". In July and August, the daily maximum RH was significantly higher in "RM until harvest" than "CTRL". September 2022 showed significant differences in average daily RH% with 78.1% in "RM until harvest" and 75.5% "CTRL" (data not shown). Additionally, significant differences were found for soil daily average, minimum, and maximum temperatures, and soil moisture comparing the two treatments (Figure 10). "RM until harvest" experienced cooler daily average soil temperatures than "CTRL" soil (ranging between 1.1 and 1.6 °C cooler, depending on the month) at a depth of 20 cm (8"), 40 cm (16") from the trunk on the east aspect.

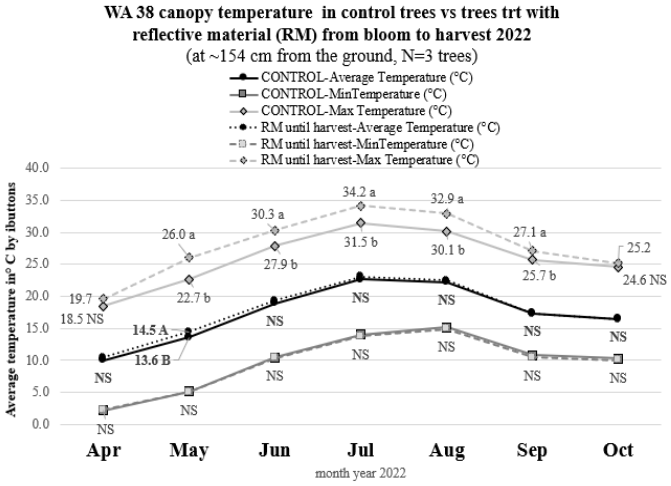

***Figure 9**. WA 38 monthly averages of daily average canopy temperatures (average, max, and min) and relative humidity (average, max, and min) measured in 2022 by iButtons hanging in the canopies at 150 cm from ground in Royal City. Three trees for "CTRL" and three trees for "RM until harvest" were monitored. NS =not significant. Letters discriminate means within each month for p=0.05*

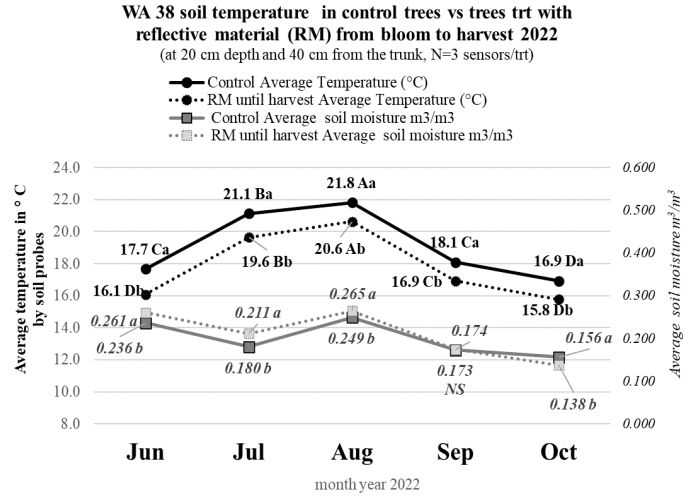

***Figure 10**. WA 38 monthly averages of daily average soil temperatures and soil moisture (VWC in m^3/m^3) measured by Meter Teros-11 sensors in 2022 in Royal City. 3 probes were buried in "CTRL" and 3 in "RM until harvest" at 20 cm (8") depth and 40 cm (16") from the east-sided trunks. NS =not significant. Lowercase letters discriminate means vertically pairwise for p=0.05, while capital letters discriminate means within trt along the season (comparison between months) within each month.*

This difference was also observed for average soil minimum and average maximum temperatures for each month (data not shown). Similarly, "RM until harvest" soil moisture was significantly higher than "CTRL" in the warmer months of the year (June to August, Figure 10). Overall, the reflective material utilized for 2 years modified the tree microclimate towards a drier and hotter canopy but a cooler and wetter soil. Therefore, warmer temperature and drier conditions during bloom (here in May 2022) could have negatively impacted the flower longevity speeding the flower senescence.

Photosynthesis measurements were taken monthly on one leaf/tree on 9 trees/treatemtn following the same protocol as in 2021 to evaluate the impact of reflective materials installed at bloom on foliar carbon assimilation. Across the 5 months of measurement, no significant differences emerged among the treatments, with net photosynthesis rates ranging on average from 13.2 µmol CO_2 m^{-2} s^{-1} in May to 18.6 µmol CO_2 m^{-2} s^{-1} in June, while the other months reported intermediate values (data not shown).

At harvest 2022 (10/12/22), the average number of apples per tree was very similar across treatments, ranging between 63 for "RM for 1M" and 68 for "RM for 2M", with yield/tree varying from 15.0 kg/tree to 17.9 kg/tree, for "CTRL" and "RM until harvest", respectively. No significant differences emerged from the statistical analysis (Figure 11). Anyway, despite the lack of statistical significance among the treatment, probably related to the high variability between trees, we want to point out that RM maintained until the harvest was ~20% more productive than control (direct comparison between the two treatments resulted in p=0.0839). The lack of discrimination between average fruit weight for the 4 treatments, when analyzing just "CTRL" and "RM until harvest", a significant difference emerged for fruit mass in 2022. Indeed, "RM until harvest" were 29 g on average larger than "CTRL"; this difference can be translated in 72-64 apples/box and ~ 80 apples/box, respectively. The crop load at harvest 2022 did not differ across treatments and averaged 4.7 fruit/TCSA cm^2, while in 2021 was 5.3 fruit/TCSA cm^2. This lower crop load could be partially due to the frost event that hit the orchard in April 2022. Despite the lack of significance between productivity/treatment across the 2 years, numerically, "RM until harvest" reached 35.1 kg/tree (77 lb), while "no RM (CTRL)" just 29.2 kg/tree (64 lb, Figure 11), corresponding to a difference of 11 Mton/Acres in the two years (data not shown).

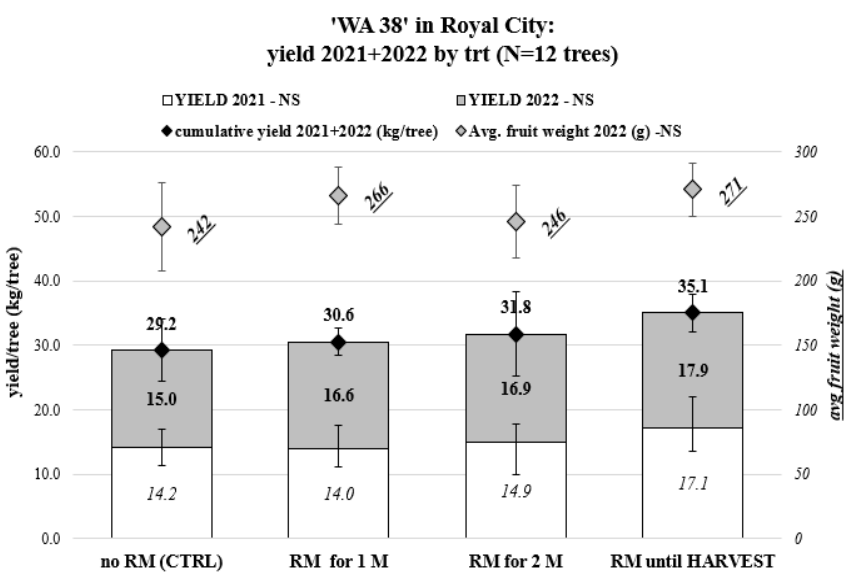

***Figure 11**. WA 38 yield in kg/tree in 2021 and 2022 and 2022 average apple weight (g) in Royal City block across the 4 treatments in trial: "CTRL", "RM for 1M", "RM for 2M"and "RM until harvest" (sorted by ascending cumulative yield in 2 years. Each column represents the mean yield of 12 trees per trt and the error bar indicates standard deviation. Each diamond marker related to the secondary Y axis represents the mean avg. fruit weight of 12 trees per trt and the error bar indicates means ± standard deviation. Differences in average fruit weight are reported as not significant (NS).*

Fruit grading for the 2022 crop revealed significant differences in the proportion of culled and marketable apples across the 4 treatments; the proportion of good-markatable apples was overall 83% (data not shown) with the best treatment being "CTRL". Similar to 2021, no major differences were found in the proportions of production by cluster pattern as single (S), double (D), triple (T) among the treatments, while a significant difference emerged for quadruple (Q) with "RM for 2 M" reporting slightly higher percentage of fruit in that category than the other three treatments (data not shown). **As an average of all treatments, 67.4% of apples were harvested as singles, 28.9% as doubles, 3.4% as triples, and only 0.3% as quadruple** (data not shown). Our grading program sorted apples into four size categories: small (≤ 215 g), medium (216-263 g), large (264-339 g), and extra-large (≥ 340 g), corresponding to ≥88, 80, 72-64, and ≤56 apples/box, respectively. Fruit size distribution for each of the treatments in the trial was expressed as a proportion (%) of apples belonging to each of the 4 size

categories (total 48 trees were graded= 3,146 apples). In contrast to 2021, where no significant differences were found in the size distribution, the grading in 2022 showed a significant difference in the large class (~72-64 apples/box, Figure 12). Indeed, "RM until harvest" trees showed the highest proportion of apples belonging to large size (46%), followed by "RM for 1 M" (41%), while "CTRL" the smallest (26%; Figure 12). Moreover, "RM until harvest" and "RM for 1 M" reported the lowest proportion of fruit (17%) in the smallest size class (≥88 apples/box), significantly different from "CTRL" (38%; Figure 12).

Fruit color was assessed as 3 major categories: Extra-fancy & fancy, grade 1, and utility (respectively with red overcolor >50%, 30-50% and < 30%). In general, color was very satisfactory, ranging from 94% to 98% for Extra-fancy & fancy, respectively, for "RM for 1 M" and "RM until harvest" (data not shown), though there were so significant differences among treatments (same as in 2021).

Grading data were collected for each tree, keeping apples separated based on cluster pattern (S, D, T, Q) to identify differences in fruit size by type of fruit. While in 2021, 73% of triple (T) apples (regardless of the treatment) belonged to the small size class, representing the highest proportion in that category and significantly different from single (S) and double (D), in 2022, the difference was not quite significant (p=0.074, data not shown). Despite the lack of significance, there was a confirmed tendency of T apples to have a higher proportion in the smallest apple category (≥88 apples/box). In 2022, single (S) produced the highest proportion of extra-large fruit (6%), significantly greater than the proportion found in D and T (2 and 1%; data not shown). Another significant difference is worth reporting when looking at each treatment independently from the others: the proportion of triple apples in the medium size category (~80 apples/box) was 15% in comparison to 32 and 33% for double and single, respectively, in "CTRL". Based on the two-year data, it could be advisable in case of a high crop load year to thin down the triple clusters to double clusters, as single and double clusters indeed presented similar size distribution at harvest.

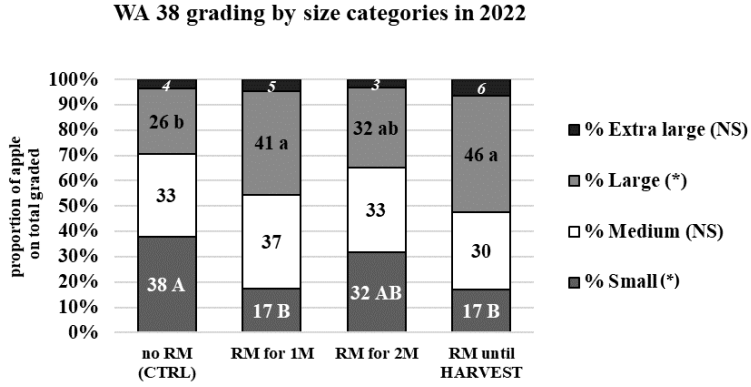

***Figure 12**. WA 38 fruit grading in size categories for Royal City block across the 4 treatments in 2022. Each tree production was graded by a sorting machine and apples were divided in 4 size categories: Small (≤ 215 g), Medium (216-263 g), Large (264-339 g) and Extra-large (≥ 340 g) corresponding to ≥88, 80, 72-64, and ≤56 apples/box, respectively. NS= no statistically significant differences and *=p≤0.05.*

FIELD DAYS

Two WA 38 field days were organized by WSU researchers and the Tree Fruit Extension Team before harvest in September 2021 and 2022. The participants were 109 and 89 in the 2 years (not counting speakers and WSU/WTFRC organizers). The tour was planned in different stations to cover different topics, not only the present project. At the Quincy station (location of obj.1), significant findings of this project were presented by Serra S. for both objectives (the Royal City block was not visited). Field day evaluations indicated that part of the attendees (in 2021) found information relating to this project useful. The Good Fruit Grower published about the events: A) "WSU leads Cosmic Crisp field days as harvest approaches" by Prengaman K., Courtney R., Mullinax TJ // September 23, 2021, B) "Cosmic Crisp field day focuses on horticulture research and commercial experience" by Prengaman K.//September.

Executive Summary

Project title: **Maximize pollination window to improve fruit set in WA 38**

Keywords: *fruit retention, ethylene inhibitors, fruit abscission, reflective material, photosynthesis*

WA 38 is demonstrated to be a variety with abundant and prolonged bloom, though these characteristics do not necessarily translate to a satisfactory fruit set. The variety is self-thinning, naturally abscising 83-91% of fruitlets within 8 weeks from bloom. Some historical data on pilot trials reported inconsistencies in annual yield. Based on these aspects, the present project aimed to maximize WA 38 fruit set by testing different ethylene regulators and reflective material applications to manage post-bloom fruit drop. The first approach explored was the adoption of ethylene inhibitors to disrupt ethylene signaling at bloom. We tested AVG (ReTain®) and 1-MCP (Harvista™) at different timings in bloom to determine their effectiveness in improving WA 38 fruit set. Their mode of action is different: 1) AVG blocks ethylene biosynthesis, while 2) 1-MCP reduces ethylene receptor sensitivity – both with the effect of reducing fruit senescence initiated by ethylene. Because the timing of these applications can be critical in influencing the fruit set, we tested an early bloom application (~50 % king bloom) and another application 7 days later (+7 days, petal fall). The two phenological stages of application could delay the beginning of natural floral senescence, extending the pollination period (~50 % king bloom) and reducing the ethylene signaling responsible for early green fruitlet drop (+7 days, petal fall). The natural WA 38 crop load of the experimental block was ~10 fruit/ TCSA cm^2 across two consecutive years (2021 and 2022) with no artificial pollination implemented. This scenario limited the understanding of the full potential of applying both ethylene inhibitors due to a high fruit set already established naturally (recommended crop load of WA 38 is ~ 5-6 fruit/ TCSA cm^2). In the second year of the trial, both treatments with Retain showed the highest number of fruit/tree and crop load but similar yield with respect to the control (no ethylene inhibitor). This increased fruit number corresponded to a less desirable average fruit weight for both Retain treatments with an average size equal to 138-150 apples/box (control was, on average 113 apples/box). Harvista applied at bloom reported similar performances as control with a slight decrease in fruit size.

The second approach studied in this project investigated the magnitude of fruitlet abscission driven by competition between fruitlets, where the ones dropping precociously are those with a lower sink strength demonstrated by decreased growth rate. For this aspect, we utilized reflective material deployed at early bloom (<30% king open) in the orchard inter-rows with the aim to increase diffuse light in the canopy to increase carbon assimilation and photosynthate availability to support greater fruitlet retention. The reflective material was tested for 3 deployment durations from early bloom (1 month, 2 months, and until harvest, ~5 months). No significant improvement in either photosynthetic assimilation rate or fruit set was recorded in the two trial years across any reflective material applications concerning untreated control. RM maintained until harvest increased by 20% yield compared to the control, despite this difference resulted not statistically significant. However, we demonstrated that the reflective material utilized in the study did modify the tree microclimate towards a drier and hotter canopy but a cooler and wetter soil. The warmer canopies during bloom could have negatively impacted the flower longevity without gaining any benefit from an enhanced light environment to mitigate fruitlet competition. The season-long deployment of reflective material from early bloom to harvest did, however, result in improved fruit size and red coloration.

PROJECT OUTCOMES

- Outreach: 2 field days: September 2021 and 2022
- Literature (generated from project award #AP-19-102 but relevant for the present study as well):

Serra, S., Sheick, R., Roeder, S. and Musacchi, S. (2022). 'WA 38' abscission and fruit development in an open pollination scenario. Acta Hortic. 1346, 129-138.

- Presentations:

Serra S., Sheick R., Schmidt T., Musacchi S. "Preliminary results on the effect of AVG (ReTain®) and 1-MCP (HarvistaTM) applied at bloom on fruit set, yield, and apple size in WA 38 cultivar" (Poster presented by Serra S.). 31st IHC (International Horticultural Conference), S16 Innovative Perennial Crops Management, August 19, 2022.

FUTURE DIRECTIONS

Assess the effect of both ethylene inhibitors tested in 2021-2022 in a 3-tier-pruning severity trial to seek potential, improvement of fruit set in scenarios with different crop loads, in particular in a low crop load study case that did not occur in the present 2-year trial.