FINAL PROJECT REPORT Year: 2 of 2

Project Title: Pollination, flower biology and fruit development in 'WA38' apples

Project Award: AP-19-102

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Total Project Request: Year 1: $67,156 Year 2: $69,262

In-kind support:

- TASC (2014-2019): "Identifying and Managing Sources of Quarantine Significant Post-Harvest Diseases in Pacific Northwest Apple and Pear Orchards" total funds $ 1,913,832 provided several of the pollinizers we will use and contributed to develop expertise in flower biology.
- Project #AP14-103A: "WA38 rootstocks and training systems" (2014-2016+1yr NCE) total funds $ 242,519 provided the support to maintain the orchard for this project.

Budget: WSU

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Serra-Musacchi		
Costs	Year 1 (2019)	Year 2 (2020)
Salaries [1]	$ 36,000	$ 37,440
Benefit [2]	$ 14,033	$ 14,594
Wages [3]	$ 2,400	$ 2,496
Benefit [4]	$ 223	$ 232
Supplies [5]	$ 10,000	$ 10,000
Plot fee [6]	$ 1,500	$ 1,500
Travel [7]	$ 3,000	$ 3,000
Total	$ 67,156	$ 69,262

Footnotes:

[1] Salary for a 75% Research assistant/Research intern ($4,000/month)
[2] Benefit on salary at 38.98%
[3] One non-student temporary for 4 wks: 40hrs/wk at $15/hr
[4] Benefits on temporary wage
[5] Labware/consumable, includes $ 1,200 membership for Franceschi Microscopy & Imaging Center (Pullman, WA) and $ 600 for electronic fruit sizer.
[6] Plot fee for the orchard
[7] 5,556 miles/year for domestic travel (0.54$/mile).Travel to orchard and to Pullman for microscopy work.

Recap objectives:

1. Assess the effective pollination period for 'WA38' and identify limiting factors (2019).
2. Evaluate pollen tube growth of different crabapples in 'WA38' flowers (2019-2020).
3. Analyze seed set, fruit drop, and fruit growth potential based on pollen source (2020).

Significant findings:

1. Assess the effective pollination period for 'WA38' and identify limiting factors (2019).

- Effective pollination period (EPP) for 'WA38' apples was at least 2 days in 2019:
    - Stigmatic receptivity: 9 days
    - Pollen tube growth (‘Granny Smith’) from stigma to ovule: 7 days
    - Ovule longevity: 9 days.

2. Evaluate pollen tube growth of different crabapples in 'WA38' flowers (2019-2020).

- Pollen tubes of ‘Snowdrift’ tend to grow faster inside ‘WA38’ styles than other pollinizers tested in 2019.
- In 2020, no significant difference was found across the pollen tube growth of the 5 pollen sources three days after pollination.
- Between Day 4 and Day 5, after pollination, all pollen sources passed the style base (between 2 years).
- No significant differences in the fruit set (%) between the 5 pollen sources in May, June nor September (2020).
- ‘WA38’ fruit weight and diameter and number of healthy seeds did not significantly differ comparing the 5 pollen sources.

3. Analyze seed set, fruit drop, and fruit growth potential based on pollen source (2020).

- When only one stigma (out of 5) in the flower gets pollinated, there is a higher possibility that apples are misshapen and have incomplete seed set.
- By the end of May, 82% of flowers/fruitlets naturally dropped, and no significant shedding occurred in the following weeks.
- At harvest (150 DAFB), 51% of tracked clusters ended up with single fruit, 12% with double fruit, 35% empty (no fruit), and 2% broken (out of trial).
- Imposing king and lateral occupancy treatments within a cluster at the end of April did not result in significant differences in the proportion of clusters that retained fruit at harvest.

Results and Discussion

Objective 1: Effective pollination period

Stigmatic receptivity

The effective pollination period (EPP) is defined as the time period in which a pollination event with compatible pollen can result in ovule fertilization (Sanzol and Herrero, 2001, *Scientia Horticulturae* 90(1):1-17). This period can be limited by the stigmatic receptivity, pollen tube growth, and ovule longevity. We observed a significant decrease in pollen germination on the stigmatic surface area on Day 4; this is probably related to the quality of ‘Granny Smith’ pollen used on this specific day, since the ability of the stigmas to support pollen adhesion was not affected (80% but not statistically different from Day 1 to Day 3; data not shown). On Day 10, only 8% of the stigma samples analyzed contained adhered pollen, and none of the stigmas pollinated on Day 10 carried germinated pollen. Thus, in our 2019 study, stigmas of WA38 were considered receptive for nine days (data not shown).

Pollen tube growth

'Granny Smith' pollen tubes reached the ovules seven days after pollination (data not shown). There was no variation between sample replicates; however, pollen tubes from different pollinizers could potentially reach the ovules earlier or later (see Objective 2: Pollen tube growth of five different pollinizers for details). Pollen tube growth rates in WA38 pistils could differ depending on the location because environmental factors also influence pollen tube growth. In general, higher temperatures increase pollen tube growth rate. Pollen tube growth from the style to the ovule in 2020 was reported in the paragraph "Objective 2A: Pollen tube growth" below.

Ovule viability

Ovules were classified by microscopy assessment either as viable or senescent based on the fluorescence signal's absence/presence (data not shown). In our 2019 sampling, the first fluorescent 'WA38' ovules were observed on Day 9 (out of 10 days of samples available). Not all ovules from Day 9 and 10 showed fluorescence, which suggests there is some variability between flowers in the timing of ovule senescence. Day 9 and Day 10 samples showed an "ovule senescence index" with an estimated mean of probability of 0.3 and 0.7, respectively, indicating that ovules started becoming senescent (index=1 means ovules are senescent).

Overall, based on the present data, the effective pollination period for 'WA38' in 2019 was two days (Figure 1). This window of time was calculated by subtracting the time required for the pollen tube to reach the ovules (7 days) from the longevity of the ovules (9 days), knowing that the stigmatic receptivity was not a liming factor until Day 10. All three components of the pollination period are temperature-dependent. Usually, a higher temperature increases pollen tube growth but decreases ovule longevity. In this experiment, we decided to use 'Granny Smith' as a fully compatible pollen source based on bloom phenology, full compatibility, and availability; however, pollen tube kinetics may vary depending on the pollen source (see results Objective 2).

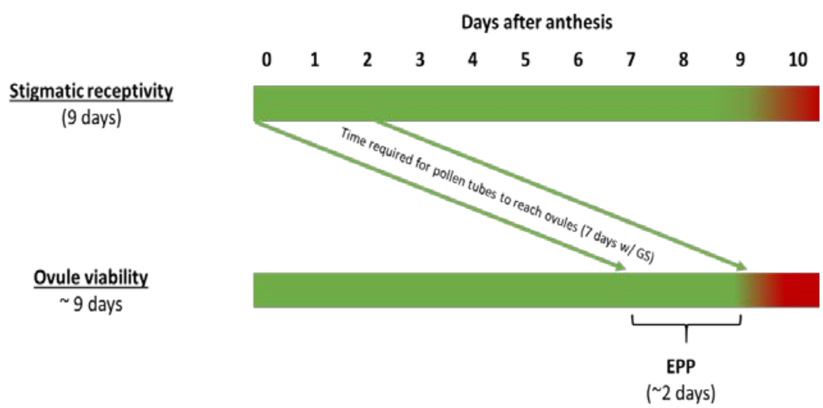

Figure 1: *Effective pollination period (EPP) in WA38 flowers. EPP is based on duration of stigmatic receptivity, pollen tube kinetics, and ovule longevity. The bracket indicates the EPP approximately 2 days with the present experimental conditions in 2019.*

Objective 2: Evaluate pollen tube growth of different crabapples in 'WA38' flowers

<u>Objective 2A: Pollen tube growth</u>

In 2019, WA38 flowers were cross-pollinated with 5 different pollen sources: 'Evereste', 'Indian Summer', 'Granny Smith', 'Snowdrift', and 'Frettingham' where the last pollen source was substituted for 'Mt Blanc' due to an off-blooming year in our 'Mt. Blanc' collection. Blossoms were harvested from pollinizers planted in Sunrise Research Orchard in 2016 and trained to a spindle (spacing 5 ft × 12 ft). In 2020, the 5 pollen sources used for this objective were 'Evereste', 'Indian Summer', 'Granny

Smith', 'Snowdrift', and 'Mt Blanc' as originally planned. Anthers were manually separated from blossoms and dried at 25 ± 1°C (RH: 24.4 ± 5.3 %) for 2-3 days (until anther dehiscence) prior to pollination. Fifty-four flowers on spurs (best lateral available) at pink balloon stage (4/15/2020) were tagged, emasculated, and cross-pollinated with each of five pollen sources across 27 WA38/NIC29 biaxis trees, for a total of 270 flowers. After hand-pollination, flowers were isolated with Kleenguard^{TM} A20 protective sleeves. Six flowers pollinated from each of the five pollen sources (2 flowers/tree rep) were harvested in 24 h intervals for 9 days. Flowers were fixed and prepared following the protocol described in report Year 1.

In 2019, pollen tubes from 'Snowdrift' reached the base of the 'WA38' style four days after pollination **(**data not shown**),** while the other four genotypes required one additional day (Day 5, data are shown in previous report Year 1). 'Frettingham' and 'Indian Summer' were slower than 'Evereste' and 'Granny Smith' at Day 3 (=72 h after pollination (AP)). The same trial repeated in 2020 showed some different results. Firstly, by Day 4 all the pollen had already reached the base of the style, one day earlier than in 2019. The difference in pollen tube lengths between pollen sources in 2020 (as proxy for pollen tube growth rate) was significant only at 48 hours AP (Day 2), where Snowdrift was confirmed to be the fastest pollen tube grown among the 5 pollen sources compared, as reported in 2019 for Day 3. In order of descending pollen tube length at Day 2 we found 'Indian Summer', 'Granny Smith' and 'Mt Blanc' (statistically similar), and the slowest was 'Evereste' in 2020. After 72 hours AP, however, there were no significant differences in pollen tube length between the 5 pollen sources, and by the following Day (4) all of them had passed the base of the style (Table 1). A high pollen tube growth rate is recommended for a good pollinizer to ensure flowers get fertilized in the shortest period of time: the more time it takes for pollen tubes to reach the ovule, the shorter the EPP becomes. Upon feedback from the first-year project report, the sampling of pollinated flowers by 5 different pollen sources was extended to Day 9 in 2020 (up to Day 6 in 2019) to dig further into the pollen tube journey after surpassing the base of the style. In general, from the microscopy observation of pollinated flowers on Day 5 we have not found any pollen tubes reaching the ovules, while on Day 6 and Day 7, about the 64% and 95% of the discerned pollen tubes, respectively (excluding flowers where pollen tubes were not able to be visualized) were in close proximity of the ovules (data not shown). On Day 8, 100% of discernable pollen tubes reached the ovules. Pollen tube growth is highly dependent on temperature, so a comparison between 2019 and 2020 is necessary to draw some conclusions. To quantify and display the potential year-to-year

Table 1: *'WA38' pollen tube length measurements in 2020 for obj. 2A: comparison between 5 pollen sources for 3 days and across the 3 days within each pollen source. Significance reported in the legend: *= p<0.05, **=p<0.001, ***p<0.001.Means are separated with post-hoc SNK test within each parameter, where different letters indicate significant difference between those means.*

Pollen sources 2020	N pistils (avr 5 styles)	Day 1 (=24h AP)		Day 2 (=48 h AP)		Day 3 (=72h AP)		*Signif. across days*
Evereste	6	0.96	C	2.66	c B	8.33	A	***
Mt Blanc	6	0.83	C	3.81	b B	7.66	A	***
Granny Smith	6	0.91	C	3.67	b B	7.58	A	***
Indian Summer	6	0.91	C	4.09	ab B	8.57	A	***
Snowdrift	6	0.92	C	4.47	a B	7.96	A	***
Significance across pollens		***NS***		***		***NS***		

AP= after pollination. Capital letters discriminate means horizontally for each pollen source (significance across days)

Small letter discriminate means vertically for each day (significance across pollen sources). WA38 styles = 10.2 mm on average in 2020. The number of styles measured range from 25 to 30 for each time point and pollen source.

variation in weather, we plotted the growing degree hours (GDH) with a baseline of 41°F from Sunrise AWN weather station from Day 1 to Day 9 (AP) in 2019 and 2020 (Figure 2). The 24 hours of difference between 2019 and 2020 for the average (avg) pollen tube to reach the base of the style can be due to: 1) higher GDH in 2020 (warmer AP week) from Day 3 to Day 9 with respect to 2019 and 2) the average style length of 'WA38' in 2020 was 1 mm shorter than in 2019 (10.2 mm and 11.2 mm respectively). An approximate amount of GDH ranging from 1440 to 1700 (GDH °F) was needed for the pollen tube to successfully reach the base of the style based on the two years of the experiment (Figure 2).

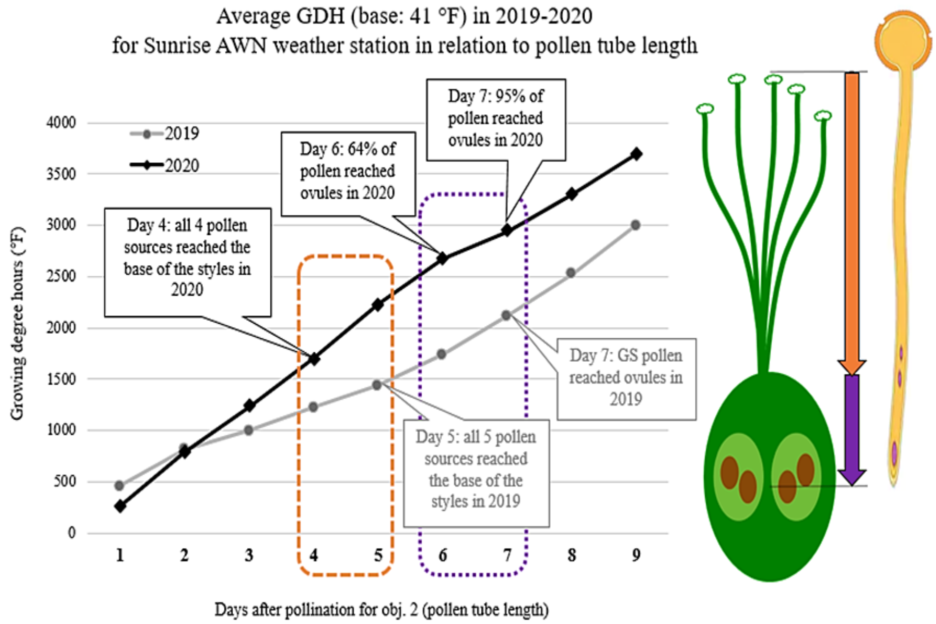

Figure 2: *Comparison between 2019 and 2020 in growing degree hours (GDH) with a base temperature of 41°F from Sunrise AWN weather station from Day 1 to Day 9 (days after pollination) for objective 2A. Dashed-line box marks the days AP when the pollen tubes reached the base of the 'WA38' styles, while the dotted-line box indicates when pollen tubes were in general proximity of ovules. Arrows on the right indicate the two meaningful position (based of the style and ovule proximity) in the "pollen tube journey".*

<u>*Objective 2B: Fruit set*</u>

The pollen performance was also assessed in terms of 'WA38' fruit set after hand pollination with the 5 pollen sources in trial. Fifteen mature trees of 'WA38'/NIC29 trained to V (planted in 2013 at 2,997 trees/Acre) were selected for this objective. Pollination was performed on 4/16/20 at balloon stage, and the best lateral flower on spur was selected and thinned to a single (10 flowers x 3 trees x 5 pollen sources). Starting one month after pollination (5/15/20), fruit set ranged between 70% and 87%, with no significant differences across the 5 pollen sources (data not shown). No significant differences, neither in June nor in September (at harvest), were found related to the fruit set. Despite the lack of statistical significance between the fruit set for the different pollen sources, a clear reduction in fruit set, regardless of the pollen source, was evident between May and June. For instance, 'Granny Smith' reported a decrease in fruit set from 87 % in May to 43 % in June, but after the "shedding wave," all the apples left on the tree were retained until harvest (data not shown). Seed analysis was conducted on all apples harvested from this objective, and no significant differences were found comparing the 5 pollen sources regarding average 'WA38' fruit weight, fruit diameter, number of good/healthy seeds, number of underdeveloped seeds. The average number of "healthy" (as determined by morphology) seeds/fruit ranged from 8.7 in 'Mt. Blanc' to 9.6 for 'Snowdrift', and the average number of seeds/fruit was 9.2 across all the pollen used.

Objective 3: Analyze seed set, fruit drop, and fruit growth potential based on pollen source

This set of experiments designed to investigate Objective 3 was carried out in 2020, according to the original project proposal. The present objective is divided in 3 sub-objectives: A) pollination intensity and seed set analysis, B) tracking natural shedding inside clusters from pre-bloom to harvest, and C) fruit development with or without king flower in the cluster.

Objective 3A: pollination intensity and seed set analysis

In this experiment, we simulated different pollination situations by removing stigmas and saturating the remaining stigmas with compatible pollen to investigate if varying degrees of "pollination intensity" can affect the fruit shape and size. Eighteen mature trees of WA38/NIC29 trained to bi-axis (planted in 2013 at a density of 1,499 trees/acre and headed back in 2014) were selected for this objective. For each of the six treatments, thirty king flowers on spurs (10 flowers × 3 trees) were singularized (no laterals) at late balloon stage, emasculated and pollinated with compatible pollen of 'Granny Smith' on 4/14/20. The six treatments were established to test the number of stigmas and therefore, the level of pollination intensity needed to achieve a full seed set. Treatments were applied by removing 0, 1, 2, 3, 4, or 5 stigmas and then hand pollinating the remaining stigma(s) in each flower. 'Granny Smith' pollen utilized for this pollination was previously collected, sieved, and dried at room temperature for 48 hours. Pollen was germinated on 1% agar plates in the field on the day of pollination to confirm its viability. No pollination bags were installed for this task. Starting from 1 month after pollination day, the fruit set was assessed for each of the 180 king flowers as presence or absence. The assessment was repeated after June drop and then at harvest on 9/15/20 (150 DAFB). Apples were picked and stored at 34 °F until further analysis. Six weeks after harvest, apple dimensions were measured, including equatorial diameter, maximum and minimum diameter, maximum and minimum height, and individual fruit mass. Additional parameters were calculated based on fruit dimensions: Symmetry Index A = Min diameter/Max diameter, Symmetry Index B = Min height/Max height, H/D ratio = average height/average diameter. Moreover, the seed analysis included the following parameters: number of carpels/fruit, distribution of seeds among the carpels (following the template from Sheffield 2014, *Journal of Pollination Ecology*, 12(13):120-128), number of seeds per carpel, seed types (healthy and underdeveloped) and healthy seed weight.

Starting at one month after pollination (5/15/20), the significant difference between the six treatments highlighted a lower fruit set when all 5 stigmas were cut before hand pollination in comparison to 0 stigmas cut, and a slight decrease when only 1 stigma remained (Figure 3). In June, a similar fruit set confirmed the significant difference between the fruit set of 93% in flowers with 5 stigmas remaining (no cut) and the flowers from which all 5 stigmas were removed (17%). In September, the final % fruit left per treatment did not result in statistically significant differences (Figure 3). The uneven pollen deposit between the five stigmas during bee visitation can result in variable seed distribution that directly affects fruit size and shape. Regarding apple dimensions and weight, the treatment that reported the lowest symmetry indices was "4 stigmas cut, 1 left" confirming our hypothesis (data not shown). The average differences between maximum and minimum diameter and maximum and minimum height were confirmed to be larger in the "4 stigmas cut, 1 left" (followed by "5 stigmas cut" and "3 stigmas cut"), suggesting this condition can lead to more asymmetric and misshapen fruit. Treatments equal to and fewer than "3 stigmas cut" produced apples with the least variation in dimensions. No significant differences emerged in relation to Height/Diameter (H/D) ratio nor average fruit weight across the 6 treatments (avg H/D=0.91 and avg apple weight 290 g). The seed occupancy in the carpels showed that leaving at least 3 stigmas in the flower resulted in 100% of fruit with a full seed set (100% apples had "healthy" seeds in the 5 carpels). When the number of stigmas remaining in the flowers decreased (2 or fewer stigmas), we observed carpels without "healthy" seeds (data not shown). The treatments with the highest number of stigmas remaining (3 to 5 remaining/flower) showed the best seed set where on average, 9.7-9.8 of "healthy" seeds were found in each apple **(**data not shown**)**. WA38 often exceeded the standard 10 seeds/fruit and sometimes carried as many as 14 seeds.

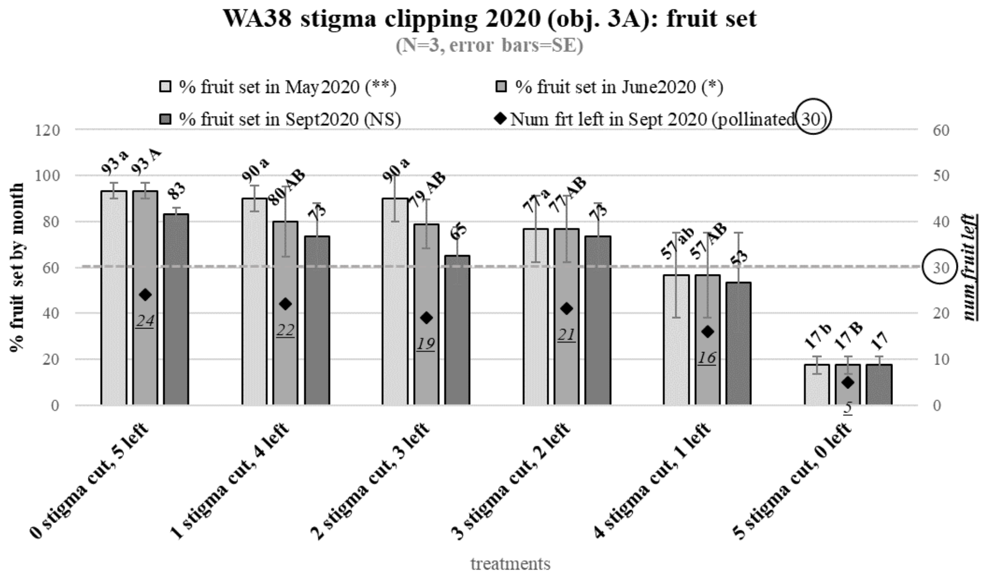

Figure 3: *'WA38' fruit set (%) in May, June and September 2020 based on the numbers of stigma left on the flower (0, 1, 2, 3, 4 and 5) and then hand pollinated with 'Granny Smith' pollen. Three trees with 10 flowers each were used as replication (N=3), error bars represent the standard error of the mean. For each of the 6 treatments 30 flowers were selected and in the secondary Y axis the number of fruit retained until harvest/treatment is reported. Significance reported in the legend: *= p<0.05, **=p<0.001, NS= not significant. Means are separated with post-hoc SNK test within each month, where different letters indicate significant difference between those means.*

Objective 3B: tracking natural shedding inside clusters from pre-bloom to harvest

One hundred WA38 clusters were selected across 2 days on 4/15/20 and 4/16/2020 across 14 WA38/NIC29 bi-axis trees planted in the same rows described for objective 3A. Each cluster was numbered, and each flower within a cluster was marked by acrylic paint to identify it by cardinal position from that moment on as follows: King (no color), blue (west lateral), yellow (northwest lateral), red (northeast), white (east), black (south). The assessment of phenology and presence/absence of each flower in the clusters were carried out daily from 4/16/20 to 4/29/20, then every 2-3 days from 4/29/20 to 6/6/20, then weekly until harvest on 9/15/2020 (150 DAFB). The WA38 natural shedding of flowers/fruitlets was the primary focus of this objective, as this variety is characterized by a self-thinning tendency that reduces the need for chemical thinning. This 22-week long cluster assessment allowed us to track the natural shedding and define the duration of the different phenological stages from bloom to fruit set. In contrast to objectives 2A-B and 3A, the data collected in objective 3B represents fruit set under open-pollination conditions instead of hand-pollination. Starting at 32 DAFB, maximum apple diameters of attached fruitlets were measured with digital calipers, and a fruit growth curve was plotted for fruit that were retained on the tree throughout the season; the frequency of the measurements were first every 2-4 days for a month, then weekly until harvest.

Tracking 100 clusters from balloon stage until harvest allowed us to observe several peculiar traits of WA38. The time window from first bloom to all open flowers in our experimental condition was from 4/15/20 to 4/20/20; in the 5 days, 549 flowers (across 100 clusters) opened. At the time of cluster selection flowers were at the balloon stage. However, by 4/16/20, 94% of the king blooms had opened, followed by lateral flowers facing southwest and southeast (34% and 25%, respectively), while just 7% of the north-facing lateral flowers had opened. Within 48 hours from first bloom, the percentages of open flowers ranged from 77% (lateral north-facing flowers) to 97% king flowers (data not shown). April 18th was recorded as the "full bloom date" for king blossoms and served as the reference date for counting days after full bloom (DAFB). Petal fall also started early for the king flowers (starting in the first 24-48h), and within 48 hours from the beginning of bloom, 38% of the king flowers were in petal fall while the lateral flowers had just begun shedding petals after 72 hours (=4/19/20). The petal fall phase lasted 9 days and ended on 4/24/20 (data not shown). The difference in phenology between king and lateral flowers was around 48-72 hours, with the king flowers blooming earlier as reported in the literature and regularly observed.

The flower/fruitlet drop started on 4/21/20 and ended 49 days later. Within the first 3 weeks post-bloom, the drop was around 23% regardless of the flower's specific position in the cluster, and in the following week (5/11/20= week 4) almost doubled up to 45% (Figure 4).

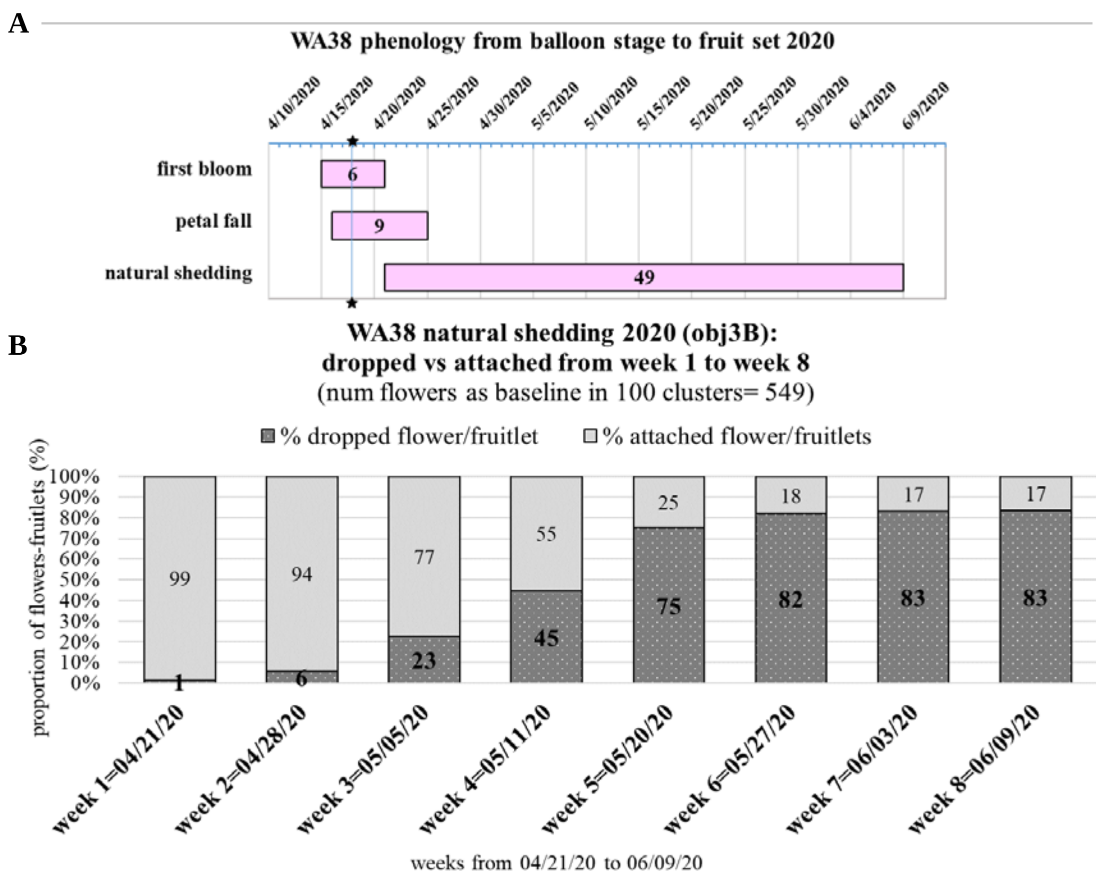

Figure 4: *A) 'WA38' phenology from balloon to fruit set in 2020, length of bars represents duration of each stage in days. B) 'WA38' natural shedding from 4/21/20 to 6/09/20 (=8 weeks) considering 100 clusters in trial.*

During the second half of May, the natural shedding of flowers/fruitlets reached 82% and did not change much for the following two weeks (83%). We consider the "shedding wave" of the variety ending on 6/9/20 (=52 DAFB=8 weeks of shedding); the remaining fruitlet drop until harvest was minimal and likely due to random causes unrelated to genetic factors (broken branch, bird damage, etc.). A survey was conducted to understand the destiny of the clusters up to harvest. Of the one hundred clusters selected before bloom, 52% consisted of 6 flowers (1 king and 5 laterals), 45% of 5 flowers, and 3% were made of only 4 flowers. At 150 DAFB, 35% of the clusters had completely dropped (no fruit retention), and 2% were broken during evaluation and excluded from the trial. Clusters supported two apples until harvest in 12% of the inflorescences monitored, whereas 51% of the clusters yielded only one fruit at harvest (Figure 5). Of the clusters that supported a single apple until harvest, the king was set in 78% of "single" clusters, whereas 22% were set on laterals (most frequently the blue, west-facing lateral, Figure 5). King fruitlets reported a higher diameter in comparison to lateral fruitlets for 8 weeks, between 5/20/20 and 7/8/20 (data not shown**)**, but after 7/8/20 the diameter of the two types of fruit did not statistically differ (average size 86 mm at harvest). A difference in fruit size emerged when comparing apples that were growing in double in the same cluster with respect to apples grown in single-fruit clusters **(**data not shown). The "double" apples were penalized from 35 to 42 DAFB, reporting a significantly lower diameter on average in comparison to single fruit/cluster, but this aspect did not impact the final fruit size.

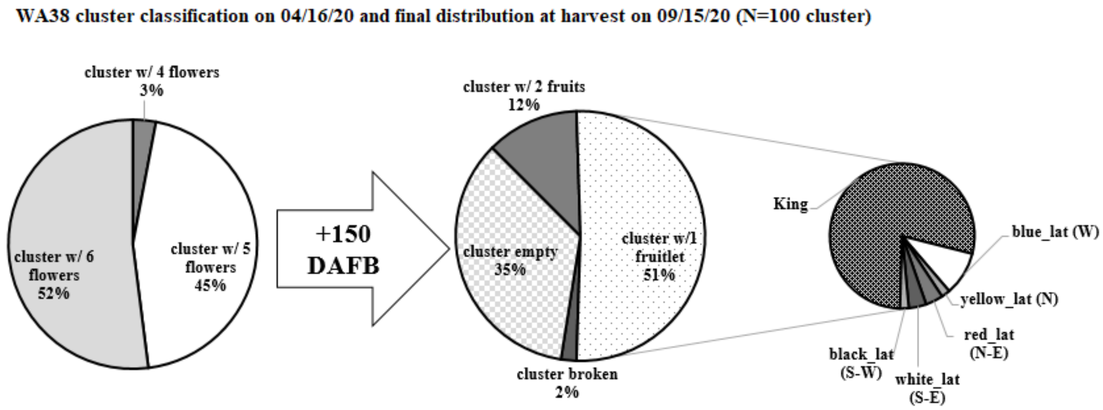

Figure 5*: 'WA38' clusters classification from bloom (4/16/20) to harvest (9/15/20) for obj. 3B.*

Analyzing the characteristics of the apples after harvest, we identified some major differences comparing firstly, apples originating from king flowers versus apples borne from lateral flowers (regardless of their position inside the cluster). Apples produced by king flowers and those coming from lateral flowers did not differ significantly in average diameter, nor for any of the symmetry indices (Table 2). The main parameters that highlighted differences between these two types of fruit were the average apple height, and the H/D ratio, for which fruit originating from king flowers showed high values. Those apples indeed tended to be more spherical than the apples from lateral flowers, which were shorter in height and had lower average H/D ratios. Despite the absence of statistical differences in diameter (avg 85 mm) between those types of fruit, the "king apple" registered a higher average fruit weight than the "lateral apple" with a difference of 33 g between average values (Table 2). Seed counts and weights did not reveal significant differences between the two types of fruit (data not shown), with an average number of healthy seeds/apple of 9.9 and 9.5 respectively for "king apples" and "lateral apples" (67 mg/seed for both, NS). This comparison did not consider the number of apples per cluster.

Looking at the comparison between fruit that were retained until harvest as single apples in the cluster (named "single", N=52) versus apples that were retained as doubles (named "double", N=24) allowed us to shed more light into the cluster dynamics. "Double" apples were less round and shorter in height than "single" apples, which were more spherical with a significantly higher H/D ratio. The latter also tended to have a higher average fruit weight than the former with 292 g for the "single" and 267 g for the “double” (but this difference was not statistically significant, data not shown). Interestingly, “single” apples presented a more complete seed set with respect to the “double”; single fruit in clusters presented an average of 9.9 healthy seeds/apple versus 9.3 for the “double.” Moreover, the number of underdeveloped seeds were significantly higher in the “double” fruit with respect to the “single” (0.8/apple vs 0.4/apple).

***Table 2**: WA38 apple dimensions and weights for obj. 3B: comparison between flower of origin (king versus lateral) regardless to the number of fruits/cluster. Significance reported in the legend: *= p<0.05, **=p<0.001, ***p<0.001, NS= not significant. Means are separated with post-hoc SNK test within each parameter, where different letters indicate significant difference between those means.*

FLOWER TYPE	N=	Avr. apple diameter (mm)	Avr. apple height (mm)		Avr. Symmetry Index A	Avr. Symmetry Index B	Avr. H/D		Avr. apple weight (g)	
KING	46	85.50	77.55	A	0.96	0.94	0.91	A	297	A
LATERAL	30	84.08	71.18	B	0.96	0.93	0.85	B	264	B
Significance		*NS*	***		*NS*	*NS*	***		**	

Objective 3C: fruit development with or without king flower in the cluster

This part of the study was focused on attaining a deeper understanding of the fate of the flower clusters with and without the presence of the king flowers. We started with 30 clusters for each possible pattern of king flower with 0, 1, 2, 3, 4, or 5 laterals (6 combinations with king; K+0L to 5L) and a duplicate set of clusters without the king flower (5 combinations without king; 1L to 5L) for a total of 11 combinations and 330 flower clusters selected. On 4/17/20, eighty WA38/NIC29 V-system trees were chosen, and king flowers were removed the same day for the 5 combinations without king (noK). On 4/29/20, clusters were labeled by combination and excess of laterals manually clipped to meet the target number flowers/cluster. From 4/29/20 to 5/20/20, the clusters were tracked weekly to assess the drop and from 5/20/20 to harvest on 9/18/20, fruit(lets) were also measured for their diameter at their widest point. At harvest (9/18/20), all the apples remaining from the original 330 clusters were picked and brought back to the lab. Overall, at harvest, 31% of clusters were empty (fruit dropped prematurely), 55% supported a single fruit and only 14% retained two apples (“double” clusters). Comparing the 11 combinations the highest percentage of clusters with single fruit was found in K+0L

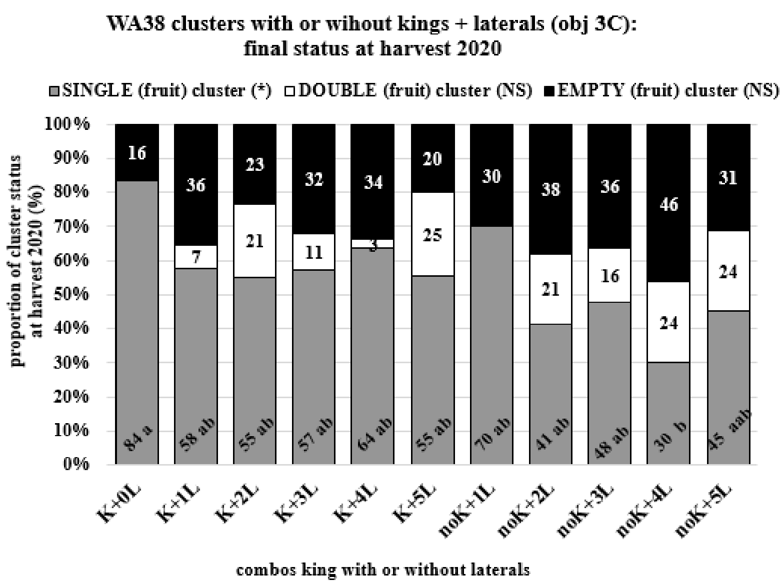

***Figure 6:** WA38 proportion (%) of clusters at harvest classified as empty (no fruit), single (one apple/cluster) and double (two apples/cluster). The comparison is presented between the 11 combinations. Significance: *= p<0.05, no asterisks means=NS. Means separation by SNK.*

(84%) and the lowest in noK+4L (30%, Figure 6). The proportions of empty cluster and “single” cluster across the combinations did not show specific statistical trends (Figure 6). Manipulating king and lateral occupancy treatments within a cluster at the end of April did not result in significant differences in the proportion of clusters that retained fruit at harvest.

To understand the fruit growth along the season based on the presence or absence of the king inside the cluster, we considered only the measurable fruit that were retained until harvest (N=264). Then, we classified those apples based on all possible patterns recorded at harvest as follows: K_K_single, K_king_w_lat, K_lat_single, K_lat_w_king, K_two_lats, noK_lat_single, noK_two_lats, where “K” or “noK” indicates the original treatment at the beginning of the trial, while K_single or king_lat indicate apples that originated from king flowers alone in the cluster (without laterals) until harvest, and apples that originated from king flowers, but from clusters that also carried lateral fruit, respectively. The highest proportions of fruit that arrived to harvest belonged to apples originating from king flowers alone in the cluster (32% K_K_single), followed by apples produced by a lateral ending alone in the cluster (27% noK_lat_single, data not shown). The fruit diameters measured for 18 weeks showed only a few differences across the combinations in the early part of the season from 5/20/20 to 6/3/20, where K_K_single fruit were larger since the beginning of measurements and significantly different than fruitlets grown in shared clusters, such as K_lat_w_king, K_two_lats, noK_two_lats. On the other hand, both treatments in which a single lateral ended up alone in a cluster at harvest despite the original “K/noK” treatment (such as K_lat_single and noK_lat_single) resulted in statistically similar diameters to the “king” apples alone on 5/27 and 6/3/20 (data not shown). No further significant differences in diameters emerged from June to harvest 2020 with a final diameter of 87 mm as average across the 7 combinations at harvest.

Another way to look at the fruit growth for this sub-trial is comparing apples that ended up as “double” in the same cluster with respect to “single” apples retained in the cluster at harvest within each of the original scenarios: clusters with king and clusters without king (data not shown). In both scenarios, we observed significantly higher diameters for fruit that ended up being “single” at harvest versus apples that were picked as “double” from 5/20/20 to 6/3/20. Looking at the average daily fruit growth (mm/day) calculated from the weekly measurements and comparing the four “simplified” scenarios at harvest such as K_single, K_double, noK_single, noK_double, we can report some differences between 6/24/20 and 7/15/20 (approx. 2 and 3 months after full bloom, data not shown). Both combinations with double fruit in the cluster with or without king at bloom showed higher growth rates after “June” drop in the weeks 5, 6, and 8 of measurements (6/24/20, 7/1/20, and 7/15/20) than “single” apples in cluster with or without kings (data not shown).

‘WA38’ apples picked from "double" clusters at harvest exhibited a faster average growth rate throughout the growing season (18 weeks) than fruit detached from "single" clusters at harvest in the scenario where king was removed.

EXECUTIVE SUMMARY

Project Title: Pollination, flower biology and fruit development in 'WA38' apples

Keywords: *pollinizers, effective pollination period (EPP), fruit set, natural shedding*
The project originally sought to address WA growers' concerns regarding the selection of the most suitable pollinizers for the new 'WA38' variety as well as to gain knowledge about the timing of fruitlet drop and potential fruit growth based on flower of origin. This investigation firstly focused on understanding the *effective pollination period* (EPP) of 'WA38' which is defined as the time window during which a pollination event turns into fertilization and successful fruit set. EPP is linked to the duration of three other factors occurring in the flowers during bloom. The first is the *stigmatic receptivity* expressed as the number of days the stigmas support pollen adhesion, second is the *pollen tube growth* (rate) which is the time the pollen tube takes to reach the ovules and last is the *ovule longevity,* namely, the duration of the ovules' viability. We learned that in 2019 'WA38' stigmatic receptivity lasted 9 days and was not a limiting factor in the successful pollination event. The EPP was calculated to be approximately 2 days by subtracting the duration of time required for the pollen tube to reach the ovules (7 days for 'Granny Smith' pollen) from the duration of ovule viability (9 days; 9 days - 7 days = 2 days). Therefore, the first 2 days after anthesis are the most important to have a successful pollination event that leads to fruit set (assuming adequate flower visitation). *Pollen tube growth* of 5 potential compatible pollinizers commonly planted in the apple orchards and selected with a similar bloom window with 'WA38' were tested for two years. In 2019, 'Snowdrift' pollen tubes were fastest to reach the base of the style, while 'Indian Summer' pollen tubes were the slowest; however, all 5 pollen sources reached the base of the style by the fifth day after pollination. In 2020 the same distance was covered by the pollen tube in 24 hours less than the previous year, and by 4 days post-pollination all the pollen had passed the base of the style with no significant differences across pollen sources. Also, *fruit set from hand pollination* experiments confirmed the 5 pollen sources were equally efficient in fertilizing 'WA38' flowers with no relevant differences in fruit set throughout the growing season. Moreover, 'WA38' fruit mass and diameter and number of healthy *seeds* did not significantly differ based on the pollen source. Fruitlet drop typical in the early stages after bloom (approx. 8 weeks) was particularly evident in 'WA38'; this self-thinning variety, indeed, tends to retain one or two fruit per cluster until harvest. In this study we aimed to investigate the dynamics of the natural shedding and quantify the drop of 'WA38' fruitlets throughout the season. By the end of May, 82% of flowers/*fruitlets naturally dropped* and no significant shedding occurred in the following weeks. About 30% of the clusters ended up being unfruitful at harvest 2020. This investigation provided information about 'WA38' fertility and fruitlet abscission traits that could be valuable to WA38 growers when making management decisions in the orchard.

PROJECT OUTCOMES

Presentations:

- Serra S., Musacchi S., Roeder S., Sheick R..: "Pollination, flower biology, and fruit development in 'WA38' apples" (oral presentation by Serra S. continuing report). January 29th 2020, Yakima.
- Serra S., Roeder S., Sheick R., Musacchi S. "Assessing 'WA38' Pollination and Fruit Development" (invited oral presentation by Serra S.) during the Pomology Professional Interest Group workshop: "Reproductive Development and Environmental Stress: Tree Fruit Crops". ASHS Annual Conference. August 10th-13th, 2020.

FUTURE DIRECTIONS

The natural evolution of this study would be using the acquired knowledge about the 'WA38' natural fruitlets shedding and aim to mitigate it.