

FINAL PROJECT REPORT

Project Title: Advancing precision pollination systems to improve yield security

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Other funding sources

Firman Pollen company provided pollen for this project at no cost – estimated at \$5,000; OnTarget Spray Systems is providing a technician (estimated 140 hrs/ year for this project) and a sprayer (retail value of \$20,000) for this research

Total Project Funding: **Year 1:** 74,566 **Year 2:** 74,624

Budget History

Organization Name: Washington State University **Contract Administrator:** Katy Roberts
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Item	2017	2018	2019
Salaries	\$40,856		\$44,191
Benefits	\$5,482		\$6,053
Wages	\$12,480		\$13,500
Benefits	\$1,248		\$1,350
Equipment	\$0		\$0
Supplies	\$8,000		\$2,500
Travel	\$6,500		\$7,030
Plot Fees			
Miscellaneous			
Total	\$74,566		\$74,624

Objectives:

Our long term goal is to improve yield security and yield by developing and deploying a reliable precision pollination system that can 1) supplement current grower pollination practices or, 2) replace the current (ancient) system of planting pollinizers and renting pollinators. We will continue to work with grower collaborators, Firman Pollen Company, and On Target Spray Systems to be sure that research progress is easily translatable to commercial-scale solutions.

1. Refine pollen rate and application timing to improve efficiency of precision pollination systems
2. Optimize pollen suspension constituents to preserve pollen viability and improve solubility
3. Investigate pollen production systems
4. Use commission funded work to strengthen regional and national research proposals

Significant findings:**Overall:**

- There is tremendous variability within and among orchards in fruit set
- Supplemental pollination treatment effects should be assessed on large-scale
- Supplemental pollination treatments can reduce variability in fruit set
- Supplemental pollination treatments, at 15 – 40 g/acre, can improve fruit set and yield
- Under favorable environmental conditions, pollination may be maximized with 4-5 hives per acre
- Pollen viability can be maintained in suspension media, in a commercial sprayer, for 100 minutes
- Pollen germinability is improved in the suspension media for up to 90 minutes
- There is high variability in pollen viability among genotypes, both within a year and among years

Finley ‘Chelan’/Mazzard, mature block (2018 & 2019)

- Low fruit set overall, ca. 9% (2018) 25% (2019)
- High variability in branch-level fruit set (0% - 35%)
- No statistically significant effect on fruit set and yield (2018)
- 20% increase in fruit set with 20 g pollen per acre (2019)

Finley ‘Chelan’/Mazzard, young block (2018 & 2019)

- High fruit set overall, ca. 47% in both years
- No statistically significant effect on fruit set and yield due to application timing being late (ca. 95% open flowers)
- 15% increase in fruit set from 20 g/acre treatment (2019)

Brewster ‘Chelan’/‘Gisela6’ (2018)

- Moderate fruit set overall at ca. 20%
- High variability in branch-level fruit set (2% - 48%)
- No statistically significant effect on fruit set
- 10% increase in yield overall; 20% yield increase in top of tree

Angol, Chile ‘Regina’ (2018)

- Moderate fruit set overall at ca. 29%
- Range in branch-level fruit set of 15% - 40%
- Significant increase in fruit set from ca. 15 and 30 g pollen/ac

Pasco ‘Benton’ (2019)

- High fruit set overall, ca. 46% in control
- 7% and 28% increase in fruit set from 20 g and 40 g/acre, respectively

Pasco ‘Skeena’ (2019)

- 25% fruit set in control
- 46% increase in fruit set from 20 g/acre applied with electrostatic or airblast

Pollen viability

- Viability varies over time in suspension media and spray tank
- Viability improved or maintained for ca. 100 minutes
- Viability varies significantly among cultivars and between years

RESULTS & DISCUSSION

1. Refine pollen rate and application timing

Over two years, we conducted 10 trials, 8 across Washington, and 2 in Chile. Each supplemental pollination trial was conducted in a commercial orchard with grower established pollenizers and 4 to 5 hives per acre of pollinators. Our treatments examined the potential to improve fruit set and yield in these different orchards. Each orchard site was different in terms of pollenizer density and distribution (Table 1).

Table 1. Summary of orchard trial sites for 2018 and 2019.

Location	Brewster	Benton City	Finley	Finley	Pasco	Pasco
Year	2018	2018	2018 & 19	2018 & 19	2019	2019
Cultivar	‘Chelan’	‘Chelan’	‘Chelan’	‘Chelan’	‘Benton’	‘Skeena’
Rootstock	‘Gisela 6’	Mazzard	Mazzard	Mazzard	‘Gisela 6’	Mazzard
Pollinizer	‘Santina’	‘Index’	2017 ‘Coral’	‘Coral Champagne’ & ‘Lapins’	‘Cristalina’	none
Year planted	2008	2007	1996	2014	2014	2004
Architecture	steep leader	steep leader, 3 leaders/ tree	open center	steep leader, 2 leaders/ tree	steep leader, 2 leaders/ tree	steep leader, 3 leaders/ tree
Spacing	8’ x 15’	10’ x 15’	9’ x 18’	6’ x 18’	6’ x 16’	9’ x 18’
Treatment block size	2 rows x 35 trees	2 full rows	2 full rows	4 rows x 25 trees	3 rows x 42 trees	3 rows x 25 trees
# of trees/ block	70	N/A	200	100	126	75

Trial Site 1 (Finley, Old ‘Chelan’/Mazzard): In 2018 we compared two pollen rates (15 and 30 g/acre) with untreated control. The grower collaborator used their electrostatic sprayer (100 gallon tank @ ca. 20 gallons/acre). Two applications were made (4 and 7 April) to 3 replicate blocks of two complete rows. Overall, fruit set was low in this block at about 9% across treatments (Table 2). Among the branches we evaluated for fruit set there was tremendous variability – a range from 0% - 35%. This variability was not associated with branch location (E vs. W sides of tree) nor the bloom density. There was no significant treatment effect on fruit set. Average fruit set was 8.9, 9.5, and 8.2% for the control, 15 g/acre, and 30 g/acre treatments, respectively. This lack of treatment effect may be related to the environmental conditions during application. It was cold during the first application (51 F) and it rained following the second application. Low temperatures reduce pollen tube germination and growth (Zhang et al., 2018).

Fruit yield was similarly unaffected by pollen application. We weighed all fruit harvested from each two-row block on the day of commercial harvest. Tree yield was determined from total weight from each block divided by the number of trees per block. Yield varied slightly among treatments: 32, 33, and 27 lbs per tree for control, 15 g/acre, and 30 g/acre, respectively. These data translate to 4.3, 4.5, and 3.6 tons per acre.

In 2019 we setup a trial in the same orchard. Applications were made on April 12th and 13th using a 100-gallon On Target Systems electrostatic sprayer calibrated to ca. 20 gallons/acre. This year, fruit set in the control trees of the mature ‘Chelan’ orchard was 25%, nearly a 3-fold increase compared to the previous year, but again, significantly less than the adjacent, younger ‘Chelan’ orchard (see below). In this orchard two applications of 20 g pollen per acre had higher fruit set (29.6%) than the 40 g pollen per acre (24.6%) treatment and the control trees (25.3%). There was no similar effect on fruit yield however, with control, 20 g, and 40 g treatments all yielding about 80 lbs per tree, or roughly 10 tons/acre.

Table 2. Summary of fruit set (% available flowers) across all trials in 2018.

	Finley young ‘Chelan’*	Finley old ‘Chelan’	Brewster ‘Chelan’	Benton City ‘Chelan’	Angol ‘Regina’
Control	48%	9%	19%	5%	21% b
Mix Only	55%				
15 G	48%	10%			34% a
30 G	45%	8%	20%	2%	32% a
30 G AB**				3%	
60 G	58%				24% b
Overall	48%	9%	20%	3%	29%

Fruit set = # fruit at harvest/ # flowers at full bloom. Finley young $n = 20$, $p = 0.19$; Finley old $n = 20$, $p = 0.71$; Brewster $n = 30$, $p = 0.36$; Angol $n = 25$, $p = 0.02$. *second application only; **AB=airblast (all other pollen treatments applied with electrostatic sprayer).

Trial Site 2 (Finley, Young ‘Chelan’/Mazzard): In 2018 at this site we compared 3 rates of pollen, the pollen suspension media alone, and a water-sprayed control. Pollen was applied on two days, the 7th and 11th of April. Unfortunately the pollen rates on the first application date were miscalculated and applied at ¼ of the intended rate (i.e., about 4, 8, and 15 g/acre instead of 15, 30, and 60 g/acre). The proper rates were applied on the second date, so our analyses are from those data only. We again recorded a tremendous variability in fruit set among branches. Irrespective of treatment, the range was about 23% - 83%. Overall, across treatments, fruit set was high in this trial – just under 48%. This is particularly interesting because the same genotype in an adjacent block (older Chelan block data above) exhibited much lower fruit set (about 9% overall). Bloom timing, tree age, pollinizer density, pollinator activity, etc., all will impact fruit set (Whiting et al., 2005; Sagredo et al., 2017). Climatic conditions influence ovule viability and longevity, pollen viability, pollen deposition, and stigma receptivity (Zhang et al., 2018). The effective pollination period, estimated at 3-6 days in sweet cherry (Sanzol and Herrero, 2001; Ughini and Roversi, 1996; Sagredo et al., 2017) was cool and windy, with some precipitation. Temperatures were between 50° - 55° F (Table 4). Rain occurred on the day of application in approximately half of our trials, and wind visibly influenced spray deposition in several of our trials. Further research is necessary to identify the key factors influencing fruit set across years and locations. Yield per tree was variable in this block, ranging from 20 to 56 lbs per tree. The highest mean yield was for the 15 g/acre treatment at 43 lbs/tree. Other treatment yields were 42 lbs (60 g/acre), 37 lbs/tree (30 g/acre), 36 lbs/tree (control), and 34 lbs/tree (suspension media only) though differences among treatments were not statistically significant.

In 2019, the orchard was split into 12 treatment blocks of four rows by 25 trees separated by buffer sections of 15 – 20 trees and a buffer row between sections. Treatments were a no-spray control, 20 grams pollen/acre, or 40 grams pollen/acre assigned using a CRD. Each treatment was replicated four times. Applications were made on April 13th and 14th using a 100-gallon On Target Systems electrostatic sprayer at a rate of 15 gallons/acre. In 2019, untreated, control fruit set was nearly identical to the previous year at 45%. Both 20 g and 40 g per acre supplemental pollination treatments had higher fruit set – 52% and 49%, respectively ($P < 0.295$). This apparently did not improve yield however, as all trees were similar at about 43 lbs/tree, or ca. 8.8 tons/acre.

Trial Site 3 (Benton City, 'Chelan'/Mazzard): In this trial we compared treatments of 30 g pollen/acre applied by electrostatic sprayer (ca. 15 gallons/acre) and airblast sprayer (ca. 85 gallons/acre) with untreated control. Fruit set was particularly low in this trial – an average of 3% across all treatments; ranging from a low of 0 to 17%. This is likely related to relatively poor environmental conditions during bloom and the lack of pollenizers. 'Index' trees were planted as every third tree in every third row as pollenizers and these trees were weakened with poor bloom density (one of the reasons we selected this block for a trial). Poor fruit set in treated blocks may be related to timing of application. During the first and second applications, the percent of bloom on labeled branches was 87% and 89%, respectively. There was a slight negative linear relationship between fruit set and the percent bloom during application. This block was left unharvested due to poor yield.

Trial Site 4 (Brewster, 'Chelan'/'Gisela6'): In this trial we compared a single application of 30 g pollen/acre vs. water-sprayed control. The application was made on 23 April using the 50 gallon electrostatic system at ca. 15 gallons/acre. Fruit set in this block was moderate overall at ca. 20%. This may be related to the relatively high pollinizer ratio of 33%. The block was setup with 6 rows of 'Chelan' and 2 rows of 'Santina'. Among the labeled branches, irrespective of treatment, we documented a large variability in fruit set, from 2 – 48%. This is consistent with the range observed in other blocks. There was no statistical difference in fruit set between treated (20%) and control (19%) branches (Table 2). This may be related to the timing of application – average percent open flowers on labeled branches was ca. 86%.

Yield data were collected by weighing bins of fruit during commercial harvest. Trees were harvested over two dates (20 and 23 June) with the first pick targeting ripe fruit in the upper portions of the canopies. From the treated blocks, about 65% of the fruit were harvested on the first date, from the tops of the trees. In control trees, about 58% was harvested on the first date. This difference may have been due to pollen application since our treatment would have been most effective in the upper regions of the trees – flowers in the lower halves of the trees were fully open and likely past optimum receptivity. Extrapolated to a per acre basis, there was about 680 lbs more fruit from the upper portions of treated trees compared to control though this was not statistically significant ($p = 0.35$). Again, the lack of a clear improvement in tree yield may be related to the effect of application timing, or, in this case, insufficient replication. We observed a negative linear relationship between the percent open flowers during pollen application and final fruit set – this suggests that applications made earlier may have been more effective.

Trial Site 5 (Angol, Chile, 'Regina'/Colt): This trial was setup with collaborators who used their own application equipment (On Target Systems electrostatic sprayer). A single application (made at 60-70% full bloom) of ca. 15, 30, or 60 g/acre of pollen was compared to the untreated control. Fruit set overall was moderate at about 29% across treatments, with a range in set among individual branches of about 15 – 40%. Fruit set was improved significantly by treatments with both 15 and 30 g/acre of pollen, but interestingly, not with the highest pollen rate (Table 2). Compared to control fruit set of 21%, treatment with 15 or 30 g/acre led to fruit set of 34% and 32%, respectively. This represents an improvement of about 59% over the control. It is possible that the positive treatment effects in this trial were due to the

timing of application. Again, our results suggest that applications made at later stages of flowering are less effective.

Table 3. Summary of fruit set (% of available flowers) from trial sites in 2019.

Site	Cultivar	Treatment			
		Control	20 g/ac	40 g/ac	P-value
Finley, WA	Young 'Chelan'	45%	52%	49%	0.295
	Mature 'Chelan'	25% ab	30% a	25% b	0.04 ^z
Pasco, WA	'Benton' ^y	46%	49%	59%	0.114
		Control	Electrostatic	Airblast	
	'Skeena'	25%	34%	36%	0.168

^yThe 40g application was applied only once in this trial. All other applications were made on two separate days during the bloom time period.

^zStatistical significance at $P \leq 0.05$; means separation was performed with a Tukey's honest significant difference test; means with no letter or the same letter are not different at $P \leq 0.05$.

Trial Site 6 (Pasco, 'Benton'/'Gisela 6'): Treatment blocks were three rows by approximately 42 trees with ~15-tree buffer sections between blocks and two buffer rows between treatment rows. There were 12 blocks with treatments assigned using a CRD. Treatments were a no-spray control, 20 grams pollen/acre applied twice, and 40 grams pollen/acre applied once. 20 gram and 40 gram rates were applied on April 18, and a second application to the 20 gram treatment blocks was on April 19. An On Target Spray Systems 100-gallon electrostatic sprayer was used at a rate of 15 gallons/acre. In this trial fruit set of untreated control was high, at ca. 46% (Table 3). Trees treated twice with supplemental pollination treatments at 20 g per acre had 49% fruit set, and those treated with 40 g per acre had 59% fruit set, a significant improvement compared to the control ($P < 0.11$). The single application of 40 g/acre represents an increase of ca. 28% over the control. This translated into an increase in yield per tree of ca. 4 lbs, or about 1700 lbs per acre. Combined, these data also reveal a potential cause for low yield in 'Benton' – low flower density. This orchard had similar fruit set to the young 'Chelan' block in Finley (both nearly 46%) yet had a much lower yield – 9 tons/acre in the 'Chelan' orchard compared to 3.6 tons/acre in the 'Benton' orchard. These orchards were the same age, and a similar training system. In addition, the improvement in fruit set and yield with a single application in 'Benton' shows that supplemental pollination treatments can be effective in self-fertile orchards, despite there being an abundance of compatible pollen. Our previous data have shown greater fruit set from outcrossing 'Benton' compared to selfing (Whiting, unpublished).

Trial Site 7 (Pasco, 'Skeena'/'Mazzard'): In 2019 we also setup a trial comparing application technologies in a 'Skeena' orchard north of Pasco. There were no pollinizers in this block. Treatment blocks were comprised of 3 rows by 25 trees with a 15-tree buffer section between blocks and two buffer rows between treatment rows. There were 16 treatment blocks and 3 treatments, each replicated 5 times. Treatments were a no-spray control, 40 grams pollen/acre applied with an airblast sprayer at a rate of ca. 100 gallons/acre, and 40 grams pollen/acre applied with an On Target Spray Systems electrostatic sprayer at a rate of ca. 15 gallons/acre. A single application was made on April 18, 2019. In this orchard, fruit set

of the untreated control trees was moderate at 25% (Table 3). Both application technologies applying 40 g per acre had higher fruit set: 34% and 36% for electrostatic and airblast, respectively ($P < 0.17$). This represents an increase of about 40% over the untreated control. We did not collect yield data in this block.

Interestingly, the greatest improvements in fruit set across both years came from single applications at the higher rate of 40 g/acre in self-fertile cultivars ('Skeena' and 'Benton'). Application timing will be important for single applications to be effective. From our first year we concluded that, in many cases, supplemental pollination treatments were applied too late (e.g., >80% open flowers). Depending on the environmental conditions, it is likely that a single application timed when there is a large population of recently-opened flowers may be sufficient to set a commercially acceptable crop. This is an area deserving of further study. In addition, we conclude that the lack of improvement in fruit set in some trials may simply be due to the high natural pollination in those blocks. Each trial site was stocked with 4-5 hives per acre, bloom overlap with pollinizers was generally quite good, and environmental conditions were favorable. In these conditions, it may be difficult to improve upon natural pollination with any technique. To be sure, supplemental pollination will only improve fruit set & yield when there is a limitation to pollination in the pollinator + pollinizer model. Our long-term goal of replacement pollination systems (i.e., in the absence of pollinizers and pollinators) will require a different approach –

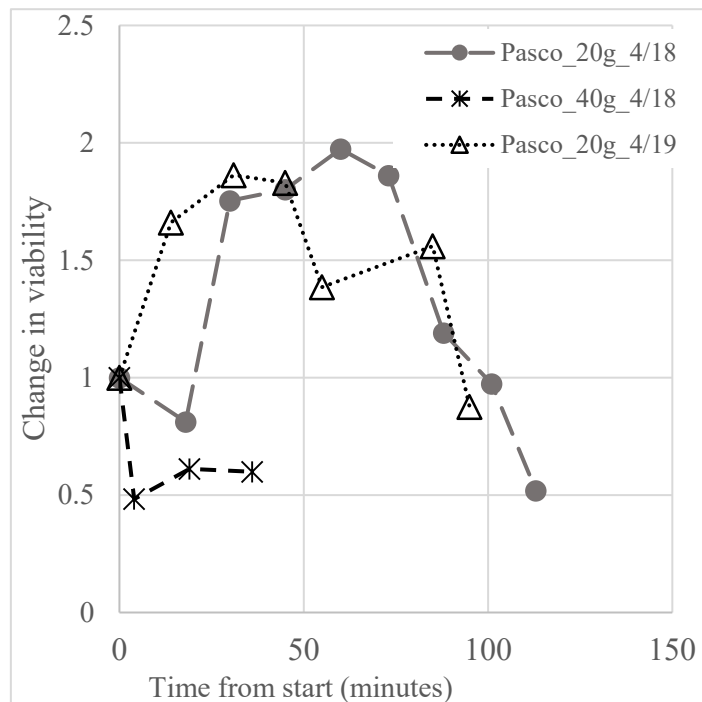


Figure 1. Relative change in pollen viability (time 0 = 1) by tree plate collection method in 3 separate electrostatic pollen applications in commercial sweet cherry orchards in eastern Washington during April, 2019. Mean sample size, n , for each point was 3.5 (Pasco_20g_4/19) and 2 for the other 2 trials. Starting viability, taken prior to liquid mixture, was 38% for Pasco_20g_4/18; 47% for Pasco_40g_4/18; and 41% for Pasco_20g_4/19.

sampling techniques (data not shown). Compared to the germination rate of dry pollen on the germination media plates at the outset of the assessment, there was an increase in germination over the first twenty minutes and an arc that peaked at 40 – 60 minutes (Fig. 3). Pollen viability was maintained

one in which blocks can be isolated from pollinators. This is the next step for this work.

2. Optimize pollen suspension constituents

The pollen suspension media used for all trials was developed in Whiting's lab, based on previous studies of viability over time in a laboratory setting. However, further development of the suspension media will occur privately, not through this project – in 2018 PI Whiting licensed the IP through WSU and is preparing it commercially (the budget request for 2019 was reduced to reflect this change).

To evaluate the impact of the application system on pollen viability during commercial application, samples were collected at various intervals during application. In 2019 pollen samples were collected via three means: 1) liquid suspension samples withdrawn from the sprayer tank, 2) pollen spray collected by holding petri dishes with germination media in the spray cloud, and 3) hanging petri plates with germination media in the tree canopy during application. The latter was determined to be the preferred method because of high moisture in the other

for 80 minutes and decreased to the level of the dust at approximately 95 minutes and continued to decline for the duration of the spray tank, 113 minutes (Fig. 3). Pollen is in a dehydrated state at time of anthesis and dispersal, which sustains viability until rehydration occurs on the stigma and triggers germination (Heslop-Harrison, 1979; Edlund et al., 2004; Radunić et al., 2017). Similarly, pollen is desiccated in storage conditions to preserve viability (Vaknin et al., 1999; Dafni & Firmage, 2000). Thus, a rehydration of the pollen grain is an important process expected to increase viability from the pollen dust. Zhang et al (2018) reported that rehydration levels on the stigmatic surface were optimum at anthesis and reduced when pollen was applied to stigmas open for 3 days or more. Vaknin et al. (1999) reported that controlled rehydration allows reorganization of the plasmalemma, increasing pollen germination rates. A period of rehydration could explain the increasing viability in the first thirty minutes of solution exposure.

3. Investigate pollen production systems

In 2017 and 2018 we worked with Firman Pollen Co. in establishing two new orchards for the purpose of pollen production. We are developing pruning and training strategies to promote high flower production and pollen yield. We anticipate the first significant harvest in 2020 from the ‘Regina’ and ‘Black Gold’ orchards planted in 2017 on ‘Gisela5’ rootstock. A block planted in 2018 is comprised of ‘Regina’ and ‘Benton’, both on ‘Gisela5’.

Table 4. Percent of germinated pollen grains collected from sweet cherry trees in the WSU Roza Orchard in Prosser, WA in 2018 and 2019; analysis was by germination within 20 hours on an agar medium.

Cultivar	Germination (%)		P – value (between years)
	2018	2019	
‘Chelan’	56 a ^z	68 a	0.024*
‘Sweetheart’	59 a	60 ab	0.81
‘Regina’		59 ab	
‘Bing’	62 a	57 bc	0.32
‘Lapins’	63 a	53 bc	0.022*
‘Selah’	52 a	50 bc	0.64
‘Van’	64 a	49 bcd	0.07
‘Skeena’	56 a	48 cde	0.06
‘Santina’	59 a	38 de	< 0.001*
‘Benton’	53 a	37 e	0.001*
‘Ulster’	18 b	12 f	0.006*

The bulk of our work in this area has been on determining the variability among genotypes in pollen viability. This information will be useful for future pollen production systems, and for new orchards being planted and decisions on the best pollenizer to use. Overall, average pollen germination rate across all genotypes was ca. 54% in 2019 and 47% in 2018. Across years and 11 genotypes, pollen germination exhibited significant variability (ca. 5-fold), ranging from 12% to 68%. Interestingly, the variability in germination was different in 2018 and 2019. In 2018, pollen viability was relatively consistent among the ten cultivars examined with a means separation test only yielding two groups: ‘Van’ (64% viable pollen), ‘Lapins’ (63%), ‘Bing’ (62%), ‘Santina’ (59%), ‘Sweetheart’ (59%), ‘Chelan’ (56%), ‘Skeena’ (56%), ‘Benton’ (53%), and ‘Selah’ (52%) were in the same statistical group for germination, with only 12% between the highest and lowest germinating cultivars (Table 2). The overall mean for this group of cultivars was 58%. By contrast, ‘Ulster’ comprised the lower viability group, with mean germination of 18%, ca. 40% lower than the cultivars with higher germination (Table 2; $P < 0.0001$).

In 2019, pollen germination was considerably more variable among cultivars tested – means separation testing identified six distinct viability groups among the eleven cultivars tested. The range between the

highest and lowest germination was 56%, compared to 46% in 2018. ‘Chelan’ (68%), ‘Sweetheart’ (60%), and ‘Regina’ (59%) had the highest viability. ‘Bing’ (57%), ‘Lapins’ (53%), and ‘Selah’ (50%) had lower viability than ‘Chelan’ and were equal to ‘Van’ (49%) and ‘Skeena’ (48%). ‘Skeena’ and ‘Santina’ (38%) had viability the same as ‘Benton’ (38%). And Ulster (12%) was again the cultivar with the least viable pollen in 2019, only about 17% the viability of ‘Chelan’ (Table 2).

Five cultivars exhibited different germination rates between the two years. Of those 5, ‘Chelan’ was the only cultivar that exhibited higher viability in 2019 (68%) than 2018 (56%), a 21% increase (Table 2; $P = 0.024$). In 2019, germination rates for ‘Ulster’, ‘Lapins’, ‘Benton’, and ‘Santina’ were lower than in 2018 by ca. 6%, 10%, 15%, and 21%, respectively (Fig. 4; $P = 0.006$, $P = 0.022$, $P = 0.001$, $P < 0.001$). In contrast, five cultivars (‘Bing’, ‘Selah’, ‘Skeena’, ‘Sweetheart’, and ‘Van’) exhibited similar germination between the two years. Of these 5, all, except for Skeena (S_1S_4), contained an S_3 allele. The only cultivar with higher germination in 2019 was ‘Chelan’ (S_3S_9), also containing the S_3 allele. Of the 4 cultivars exhibiting lower viability in 2019, ‘Ulster’ (S_3S_4) was the only one containing the S_3 allele and had the lowest germination rate in both years. Ulster and Bing are in the same incompatibility group, as are ‘Lapins’, ‘Santina’, and ‘Skeena’ (S_1S_4), ‘Sweetheart’ and ‘Selah’ (S_3S_4), and ‘Regina’ and ‘Van’ (S_1S_3) (Schuster, 2012). ‘Benton’ is $S_4 S_9$ (Olmstead et al., 2011). It seems unlikely with these 11 cultivars that S-alleles had significant influence on germination rates. Environmental effects on pollen viability will be important to study in the future. If orchards continue to be established with a fixed quantity of pollenizers, variability in pollen viability may affect fruit set and yield. It would be advantageous to plant pollenizers with consistently high pollen viability to maximize pollination potential.

4. Use this work to strengthen larger proposals

We submitted a proposal to the Washington State Specialty Crop Block Grant program (request of ca. \$250,000) to expand our research into precision pollination systems and the potential to improve yield security in tree fruit. The proposal was well-rated but not selected for funding.

Literature cited

- Dafni, A., & Firmage, D. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Systematics and Evolution*. 222(1):113–132.
- Edlund, Anna F., Swanson, Robert, & Preuss, Daphne. 2004. Pollen and stigma structure and function: the role of diversity in pollination. *The Plant Cell*. 16:S84 – S97.
- Heslop-Harrison, J. 1979. An interpretation of the hydrodynamics of pollen. *American Journal of Botany*. 66(6):737–743.
- Olmstead, J., Whiting, M., Ophardt, D., Oraguzie, N., & Lang, G. 2011. 'PC7146-8' (Benton (R)) Sweet Cherry. *Hortscience* 46(1):121-122.
- Radunić M, Jazbec A, Ercisli S, Čmelik Z, Ban SG. 2017. Pollen-pistil interaction influence on the fruit set of sweet cherry. *Scientia Horticulturae*. 224:358–366.
- Schuster, M. 2012. Incompatible (S-) genotypes of sweet cherry cultivars (*Prunus avium* L.). *Scientia Horticulturae* 148:59-73.
- Vaknin Y, Gan-Mor S, Bechar A, Ronen B, Eisikowitch D. 1999. Effects of desiccation and dilution on germinability of almond pollen. *The Journal of Horticultural Science and Biotechnology*. 74(3):321–327.
- Zhang, L., Ferguson, L., & Whiting, MD. 2018. Temperature effects on pistil viability and fruit set in sweet cherry. *Scientia Horticulturae*. 241:8–17.

Executive Summary

Project Title: Advancing precision pollination systems to improve yield security

Keywords: fruit set, yield, pollen viability, pollination, germination

The project set out to 1) further investigate the potential to improve fruit set & yield security with supplemental pollination in commercial sweet cherry orchards, and 2) better understand the potential for pollen production systems. We documented improvements in fruit set and, in some cases, yield in several orchards. In ‘Skeena’, for example, we increased fruit set by 40% compared to untreated control trees with a single application of 40 g pollen/acre. We also documented an increase in yield of ca. 1700 lbs/acre in ‘Benton’ orchard treated with a single application of 40 g pollen/acre. In other trials, we did not record a significant effect on fruit set, nor yield. We draw two main conclusions for these results: 1) supplemental pollination treatments were made too late in bloom (ca. 90% open flowers), and 2) supplemental pollination treatments may not be effective when pollinizer bloom overlap is good, pollinizer density is high, pollinator activity is good, and pollinator density is high (5 hives/acre). Indeed, our approach of using supplemental pollination treatments to evaluate the potential for artificial pollination will be effective only if there is a pollination shortcoming under the growers’ best management practices. Our treatments, in some cases a single pass alone, will only be effective in the population of flowers that are open, receptive, and, not already pollinated by the ‘natural’ process. Interestingly, the greatest improvements in fruit set across both years came from single applications at the higher rate of 40 g/acre in self-fertile cultivars (‘Skeena’ and ‘Benton’). Application timing will be important for single applications to be effective. From our first year we concluded that, in many cases, supplemental pollination treatments were applied too late (e.g., >80% open flowers). Depending on the environmental conditions, it is likely that a single application timed when there is a large population of recently-opened flowers may be sufficient to set a commercially acceptable crop. This is an area deserving of further study. Our long-term goal of replacement pollination systems (i.e., in the absence of pollinizers and pollinators) will require a different approach – one in which blocks can be isolated from pollinators. This is the next step for this work.

Our investigations of variability in pollen viability across genotypes and years has revealed significant discrepancies for both factors. These data (Table 4) will be useful for pollen companies interested in planting orchards strictly (or predominantly) for pollen production, as well as growers considering pollinizer choices for new orchards. Clearly, genotypes that exhibit both high and consistent pollen viability will be preferred. ‘Chelan’ is one example of a cultivar having high pollen viability both years. We intend to continue these evaluations for an additional year and include various collection sites (e.g., low chill areas) to better understand the variability in pollen viability.

Our studies of pollen viability through the commercial application system showed that pollen remains viable for ca. 100 minutes from initial loading. Interestingly, pollen viability increases, nearly doubling, after 45 – 60 mins in suspension media (Fig. 1), then declines to initial levels by about 100 mins. We hypothesize that pollen in suspension media goes through the initial stages of germination (i.e., hydration) in the spray tank, improving its germinability. Importantly, commercial application of supplemental pollination treatments can be completed within about 90 minutes, before any significant loss of pollen viability.

With these results, we are optimistic for the potential to utilize full replacement pollination in the future – we have shown that pollen can be applied through commercial sprayers, and pollinate sweet cherry flowers, improving fruit set and yield. In addition, the application of supplemental pollination in existing orchards is useful for improving set and yield when the current pollinizer + pollinator model fails. Importantly, the entire process is now commercially available, with several thousand acres treated in 2019.