FINAL PROJECT REPORT

Project Title: Mechanical pollination for yield security

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Cooperators: OnTarget Spray Systems, Firman Pollen Co., Olsen Brothers, Hayden Farms, Russ LeSage; Jason Matson; Allan Brothers

Total Project Request: **Year 1:** 30,123 **Year 2:** 31,086

Percentage time per crop: Apple: 25% Pear: 25% Cherry: 50% Stone Fruit: 0%

Other funding sources

Notes: In kind support is provided by Firman Pollen Co (pollen donations) and OnTarget Spray Systems (donation of sprayer and technician for support)

Budget 1
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: 509 335-4564 **Email address: carriej@wsu.edu**

Item	2014	2015
Salaries	13,028	13,549
Benefits	1,173	1,219
Wages	9,360	9,734
Benefits	562	584
Equipment		
Supplies	3,000	3,000
Travel	2,500	2,500
Plot Fees	500	500
Miscellaneous		
Total	30,123	31,086

Footnotes:

OBJECTIVES:

1. Ensure consistent fruiting in sweet cherry, apple, and pear through the development of an effective mechanical pollination system.
2. Pursue further funding to build upon this project's outcomes

SIGNIFICANT FINDINGS

- Artificial pollination with pollen suspensions applied electrostatically is capable of setting fruit in apple, pear, and sweet cherry
- We documented the deposition of mechanically-applied pollen to the stigmatic surface in sweet cherry – increasing pollen density per stigma by about 3-fold compared to open pollinated
- A single supplementary application of a pollen suspension can improve fruit set
- Replacement pollination tests (i.e., no pollen applied other than via our system) are promising in apple and sweet cherry – tree yield was similar from two applications of pollen compared to open pollinated
- Inconsistent and very weak correlation found between flower density, fruit set, shoot length, shoot angle and shoot diameter
- Including sucrose and boron in pollen suspension media can improve pollen viability for more than 1 hr
- Pure pollen is required for our pollination system
- Pure pollen can be prepared on a commercial scale for artificial pollination systems
- Percentage of pollen germination varied for same sucrose concentration in medium, across the genotypes used in current study
- Minimal variation of pollen deposition variation by flower location in the tree was noticed, receiving electrostatic pollen suspension spray
- It is feasible to incorporate growth regulator ReTain® to aid against premature ovule senescence in sweet cherry, without further affecting satisfactory germination rate in pollen suspension
- Sweet cherry stigmas exhibit high receptivity on the second day after bloom

RESULTS AND DISCUSSION

This project has evaluated the potential to pollinate and fertilize tree fruit flowers with applications of liquid pollen suspensions through commercial electrostatic sprayers. This research project has combined small-scale lab/research plot trials with larger-scale field trials. Our proposed precision pollination system is comprised of 3 steps:

1. Collect and purify pollen
2. Suspend pollen in liquid
3. Apply pollen through electrostatic application system

This research project has addressed the second and third aspects directly, and we have worked in collaboration with Firman Pollen Company to address pollen collection and purification.

1. ***Pollen collection and purification***.

Through our field trials and lab analyses of suspension materials, we learned that it is necessary to utilize pure pollen in our pollination system. Field trials in 2014 revealed problems with rehydration of non-pollen floral parts (e.g., filament, anthers) that caused filters in the sprayer to clog rapidly. The standard pollen that is used commercially for hive inserts or to be applied through dusting means contains too many of these non-pollen materials to be useful for field application through a sprayer. We investigated the potential to modify filtration systems in the On Target Spray Systems sprayer but it became clear that further pollen purification would be necessary. Firman Pollen Company has developed an additional filtration step that yields pure pollen. Our field tests in 2015 utilized this purified pollen product, and we had no difficulties with filtration.

Pollen collection systems will need to be improved to keep up with demand. The current process of hand-harvesting flowers in commercial orchards is too laborious and time-consuming. This is largely due to the pickers' inability to harvest all available flowers – the process is selective because flower must remain for the grower to harvest a crop. This critical issue is addressed in our new proposal. Orchards should be planted strictly for pollen collection – this will require investigation of new training systems (or modification of existing systems) to optimize pollen yield.

2. ***Suspend pollen in liquid***

In 2014 we conducted lab studies on pollen viability and the role of suspension media components. From our tests of three sweet cherry pollen genotypes (Lapins, Rainier, Sweetheart), we found that we could improve pollen viability (i.e., germination) when pollen was suspended in a modified medium, compared to water. For each genotype, the following suspension mediums were evaluated: solution 1: 5% (w/v) sucrose + 0.15% (w/v) pollen grain, solution 2: 10% sucrose (w/v) + 0.15% (w/v) pollen grain, solution 3: 15% (w/v) sucrose + 0.15 % (w/v) pollen. Pollen germination declined over time in the control treatment with only water. This occurred for all cultivars similarly, losing about 20% germination from initial suspension to 60 minutes later. In contrast, the suspension solutions improved pollen germination and extended viability over time. There appears to be a benefit to higher rates of sucrose, with viability improved more than 600% after one hour in suspension. Suspension medium containing 15% sucrose (w/v) improved germination significantly in all pollen genotype irrespective of how long the pollen was in suspension. This is likely due to the favorable osmotic balance in the medium with sucrose. In contrast, we documented approximately a 15-20% decrease in the viability of pollen grains in control between the first and last sampling time. The highest pollen germination was observed with Sweetheart (67%) followed by Rainier (59%) after 40 minute suspension time in media enriched with 15% sucrose (w/v).

In 2015, pollen suspension development continued with multiple experiments carried out to investigate the role of candidate suspension components on pollen viability. For our in vitro pollen germination experiments, we utilized one sweet cherry, apple and pear genotype (Rainier, Red Delicious and Bartlett). Cultivar selections were based on most suitable and compatible pollen genotypes to source pollen for our field trial pollen receiving cultivars. In every case, pure pollen was utilized. Candidate suspension components were selected from a literature review of relevant scholarly articles. Initially we evaluated pollen viability after incubation of 1 hour at a concentration gradient of each the components. Afterwards, we assessed combinations of components on pollen viability. In each case we used 0.18% w/v of pollen for the experiments. Replicate tubes of suspension materials were created, pollen was added, and the tubes were agitated briefly (<5 sec) before incubated at room temperature. Suspension aliquots were withdrawn after 5, 30 , and 60 minutes and pollen germination was evaluated to assess viability. Following suspension, our modified medium maintained or significantly improved pollen germination percentage compared to only water, irrespective of pollen genotypes and cultivars. Recent findings revealed that, using sucrose at lower rate could maintain favorable osmotic balance retention with optimal pollen

viability, either alone or in combination of other components in suspension, across all three genotypes.

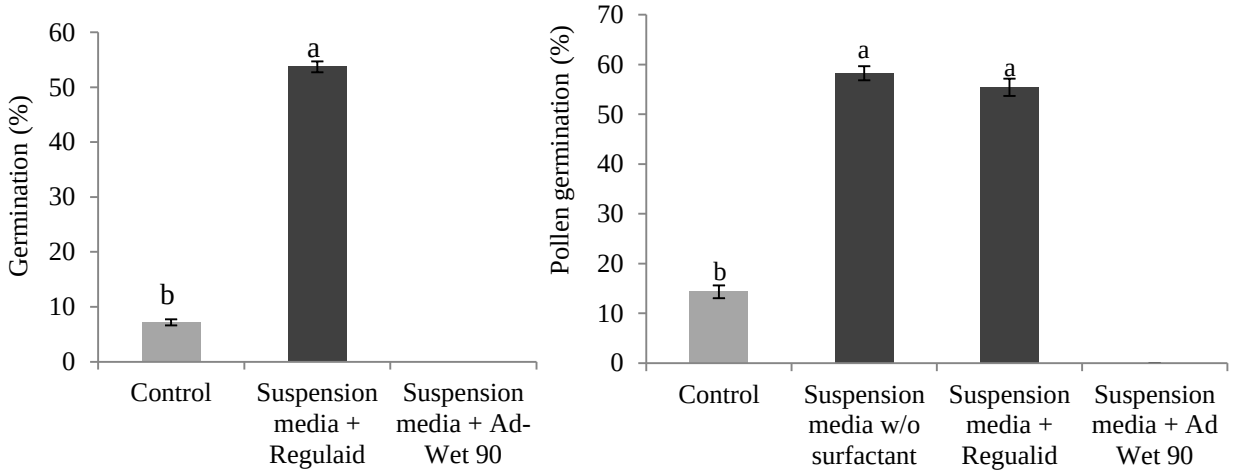

Figure 1: Effects of suspension media on sweet cherry (left) and apple (right) *in vitro* pollen germination after 60 minutes in suspension (both p-values: 0.000).

Pollen + water alone is not an effective combination. First, pollen is not easily wettable (i.e., difficult to suspend); secondly, pollen losses viability in water. Germination rate of pollen suspended in water consistently decreased over the incubation periods, and reached a minimum after 60 minutes. We evaluated candidate suspension components individually and in combination for their effect on pollen germination. After 5 minutes in suspension with water + a single suspension ingredient, several suspension components improved germination – rates were 151% and 144% of control for 'Red Delicious' pollen. Similar results were found with cherry and pear pollen. We then combined suspension constituents at their optimum concentration and found improved pollen germination (206% of control). After 30 minutes of pollen in suspension, we found great improvements in pollen germination. Several suspension components increased germination by 220% to 230% of control. The greatest improvements in pollen viability were seen after 60 minutes of pollen incubation, when our suspension improved pollen germination rates that were more than three-fold greater that the control of water + pollen. Our combined suspension media improve pollen germination by more than 4x compared to the control after 60 minutes (Figure 1).

Future work is needed to transition from lab-scale tests to large-scale suspensions in commercial application equipment. Lab tests need to be repeated in the field, collecting pollen that has been loaded in suspension in the spray tank, and sprayed through the system. We do not know how the application system will affect pollen viability, nor how easily suspensions will be maintained under constant agitation, nor the effect of agitation on the suspension and pollen viability. In addition, suspension creation in the lab is fairly straight forward using lab agitation tools (e.g., vortex system), in our field trials of 2015 we encountered some difficulty in creating a uniform suspension in the sprayer tank. This process needs further development.

***In vivo* pollen deposition**

We also evaluated pollen deposition to the stigma using cut limbs of 'Rainier' sweet cherry in the lab. *In vivo* pollen deposition tests were carried out on emasculated flowers was carried out with two pollen rates applied at 2 and 3 days after bloom (DAB) under greenhouse conditions. Flowering limbs were positioned vertically in buckets of water. Using a single-nozzle electrostatic application

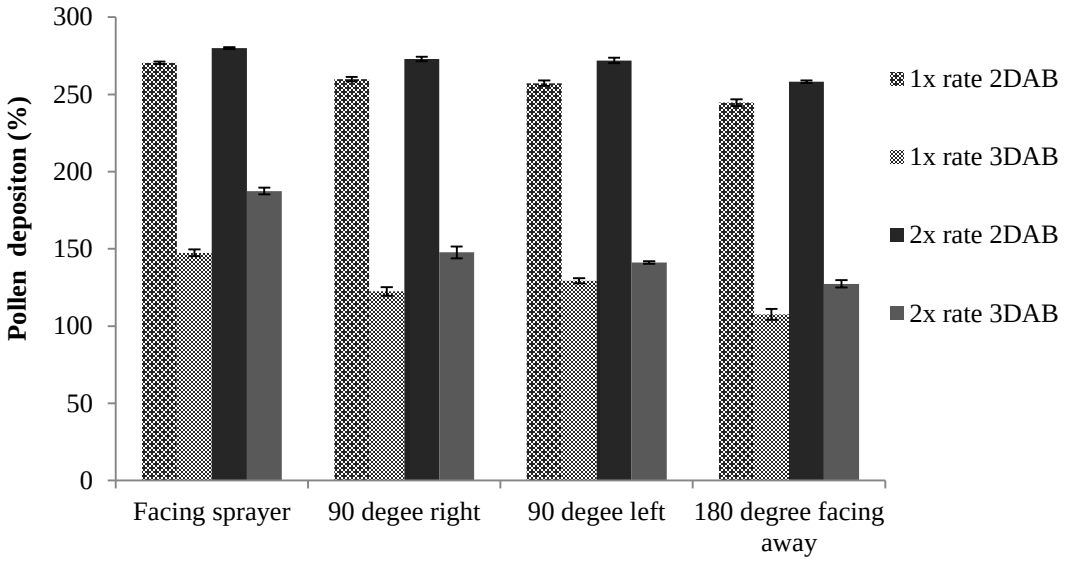

Figure 2: Percent pollen deposition after mechanical pollination only, according to the total number of pollen collected from emasculated sweet cherry flower stigma at 2 and 3 days after bloom under controlled condition. Pollen deposition represented as the average of 5 flowers from each of five replicated flowering limbs, *i. e.* for each treatment n=25/direction, per sampling day.

setup we applied two rates of pollen in suspension to replicate limbs moving the nozzle past the limbs at about 2 mph (to mimic application in the field). Post application, sample flowers from two different bloom dates were collected and the deposition of pollen on the stigmatic surfaces was assessed microscopically on flowers that faced the sprayer as well as those that were 90 degrees away, and those that were 180 degrees from the sprayer (i.e., on the 'back' of the limb). Pollen deposition was consistently higher when pollen was applied at 2DAB, at both pollen rates (Figure 2). Highest pollen deposition on flower stigmas facing nozzles was found from double rate (30g/acre) of pollen applied at 2DAB (279%), and lowest from single rate (15g/acre) applied at 3DAB at similar angle. Minimal differences observed in pollen deposition on flowers stigmas located at 90^{o} from the direction of the spray at both 2DAB and 3DAB applications, separately, with either pollen rate. However, at this angle flowers from 2DAB has shown 125% higher deposition compared to 3DAB. Flowers located at straight angle (180^{o} facing away from the spray) received lowest pollen deposition (108%), when single rate pollen applied at 3DAB. These results support our lab's previous studies of stigmatic receptivity which revealed maximum receptivity of sweet cherry stigmas on flowers that were open for two days.

Field trials

In 2014 we conducted several field trials to evaluate the potential for improving fruit set with supplemental applications of pollen as well as replacing the use of pollenizers and pollinators (i.e., replacement pollination). Cherry field trials were established in an Early Robin orchard (Pasco) and two Tieton orchards (Roosevelt and Benton City). We are unable to collect data from the Early Robin and Roosevelt Tieton orchards due to difficulties with the application system. We discovered that our pollen mixture was clogging the sprayer's filter and we had trouble with the nozzles getting

plugged too. We attributed this to two factors: the quality of the pollen used and the characteristics of the pollen suspension media used. We have learned that only pure pollen will work with the current application system because anthers and filament material commonly present in standard "off-the-shelf" pollen mixes will hydrate rapidly and plug the filters and nozzles. In addition, the pollen suspension media we used was too viscous and contributed to the clogging of the sprayer. This media was provided by PollenTech, a startup company trying to develop mechanical pollination systems. We did not use their proprietary slurry in 2015 trials. In our third field trial, we were able to use pure pollen, in the Tieton orchard in Benton City, and the system performed well. Our supplemental pollen applications at about 50% and 90% of full bloom increased fruit set by 15% compared to open-pollinated trees (Fig. 1). The application system was clearly effective at placing pollen in suspension on the stigmatic surface – we documented nearly a 3-fold increase in pollen deposition on treated stigmas compared to open-pollinated stigmas (Figure 3).

We also conducted a full replacement pollination (i.e., pollen applied through bee exclusion netting) trial, applying pollen suspension twice (about 25% and 75% full bloom) to 'Bing' trees. The artificial pollination system was as effective as natural open pollination (Fig. 2). Interestingly, yield was less variable among trees that were pollinated artificially compared to those open pollinated trees. Furthermore, in a 'Gala' apple trial of replacement pollination to limbs covered with netting, we recorded very high fruit set from electrostatic pollen application. We evaluated two pollen suspension solutions and recorded approximately 56% and 75% higher fruit set over natural pollination (see continuing report from 2015 for these results).

In 2015 we established four sweet cherry trials were designed at multiple locations including Tieton (Grandview), Early Robin (Pasco), Benton (Prosser) and Regina (Brewster). We also conducted one apple trial and one pear trial (D'Anjou near Naches). Each of these trials was supplemental pollination – we applied pollination treatments to trees or entire rows in orchards where both pollenizers and pollinators were present.

In a 9th leaf Tieton orchard trained to a vertical UFO architecture, supplemental pollen application using a single pollen rate of 15g/acre, with two applications (once at 50% and again at 100% bloom, increased fruit set by 10% compared to natural pollination. In this orchard we recorded a 2 to 3 fold increase in pollen deposition (Figure 1) on flower stigma sprayed with our liquid pollen suspension compared to naturally pollinated stigmas. We have also noticed that pollen deposition was superior in treated than non-treated stigmas, irrespective of flower position and training systems of cultivars under investigation.

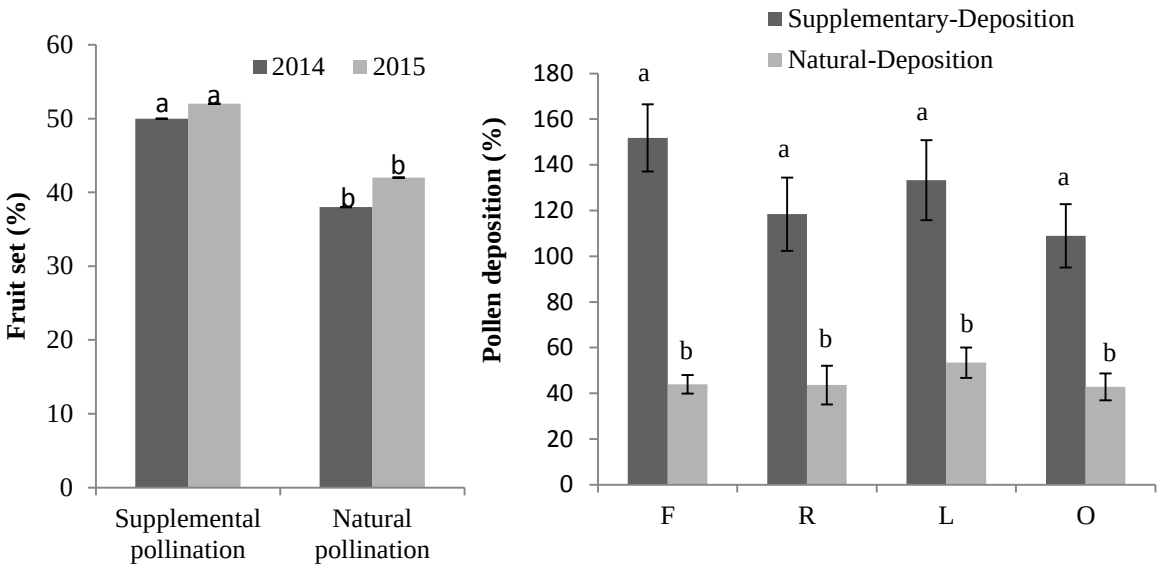

Figure 3. Effect of supplemental pollination (two applications) on fruit set in 'Tieton' sweet cherry (both year p-value: 0.041) (left) and pollen grain deposition on the stigma (year 2015; p-value: 0.000) (right). F=Facing sprayer, R and L= 90º from the direction of sprayer right and left, respectively, and O= 180 º from the direction of sprayer.

In a Y-trellised 'Early Robin' orchard we evaluated pollination efficacy of two application timings and two rates of pollen. Supplemental pollination was made at about 50% and 100% full bloom. Each supplemental pollination treatment improved fruit set. The greatest response was in response to applying the high pollen rate (30g/acre) applied twice – this treatment increased fruit set by 65% compared to natural pollination. Including the growth regulator ReTain® in the spray did not improve fruit set (our lab tests revealed no toxic effect of ReTain® on pollen viability). Single applications of pollen were similarly effective – the timing of application and the rate of pollen made no difference. In each case, fruit set was increase by about 40%.

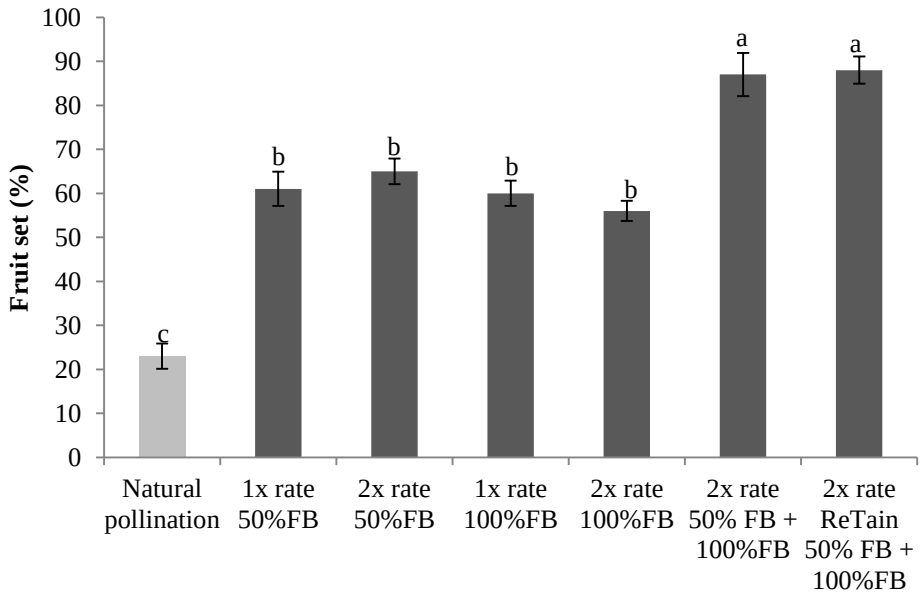

Figure 4. Fruit set in 'Early Robin' sweet cherry upon two supplemental pollen suspension applications (at 50% and 100% bloom) with single and double rate and natural pollination (non-supplemental) (p-value:0.000).

In a Regina orchard near Brewster a single application of 15g pollen was applied at about 75% full bloom. This supplemental pollination treatment improved fruit set by about 20% (Figure 5). The improvement in fruit set (and subsequently yield) from our supplemental pollination treatment was enough to convince the orchardist to keep the orchard (this block was set to be removed due to poor production).

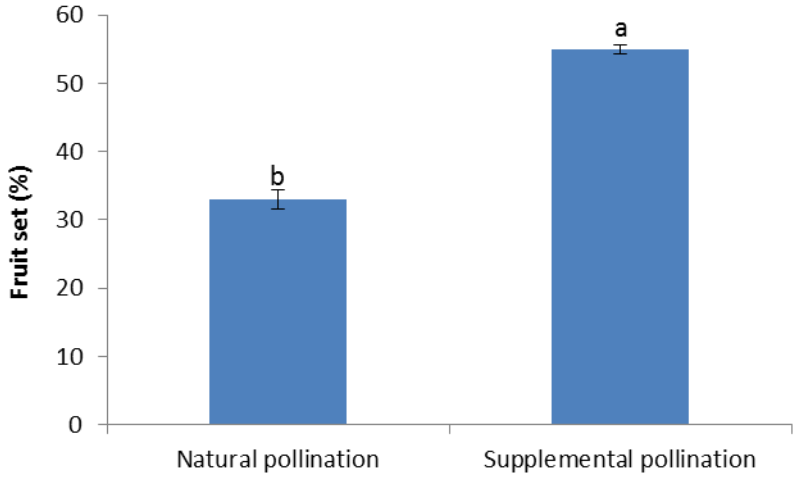

Figure 5. Fruit set (% of available flowers) for open-pollinated trees and those treated with supplemental pollination at ca. 75% full bloom and 15 g/acre of pollen.

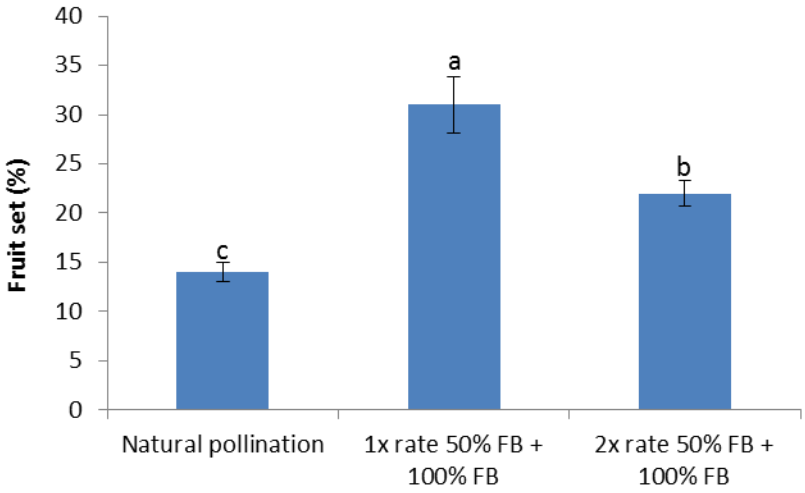

Figure 6. Fruit set (% of available flowers) in D'Anjou pear as affected by pollination treatment. 1x rate = 15 g/acre, 2x = 30 g/acre.

Supplemental pollination treatment to D'Anjou pear resulted in significant improvements in fruit set. Natural fruit set in this orchard was less than 15% of available flowers (Figure 6). Two applications of pollen at roughly 50% and 100% full bloom improved fruit set by 200% and 150% for single and double pollen rates, respectively. Interestingly, our application of a 1x rate (15 g/acre) was more effective than the double rate. This underscores the importance of further investigation into the pollen rate response. It will be prudent to utilize as little pollen as necessary to set the desired crop since pollen will be in short supply. We propose to evaluate fertilization rate response to pollen rate in sweet cherry and apple in a new proposal.

In a Jazz/M9 block we treated trees with two pollen rates (15 or 30 g/acre) at roughly 50% and 100% full bloom. We also treated rows where manual blossom thinning was complete, leaving only one flower per cluster in comparison to rows that were unthinned. Both unthinned and thinned fruiting limbs of Jazz apple receiving replacement pollination had fruit set despite the absence of any bee-mediated pollination (Figure 7). We documented no fruit set in untreated and bagged limbs whereas limbs treated with our pollination system exhibited fruit set rates between about 50% and 65% (unthinned limbs) or 40% and 55% (thinned limbs). The double rate induced 17% higher fruit set compared to single rate. Percent of open flower at pollen application was same in thinned and unthinned block. This is further evidence that we are able to fertilize flowers successfully with our pollination system. This may be particularly important when growers go to the expense of hand-thinning flowers. It will be critical to fertilize each remaining flower, and this may be accomplished with artificial pollination. Our vision of precision pollination systems in the absence of pollinators and pollenizers has the potential to revolutionize crop load management if we are able to successfully pollinate only a portion of the flowers.

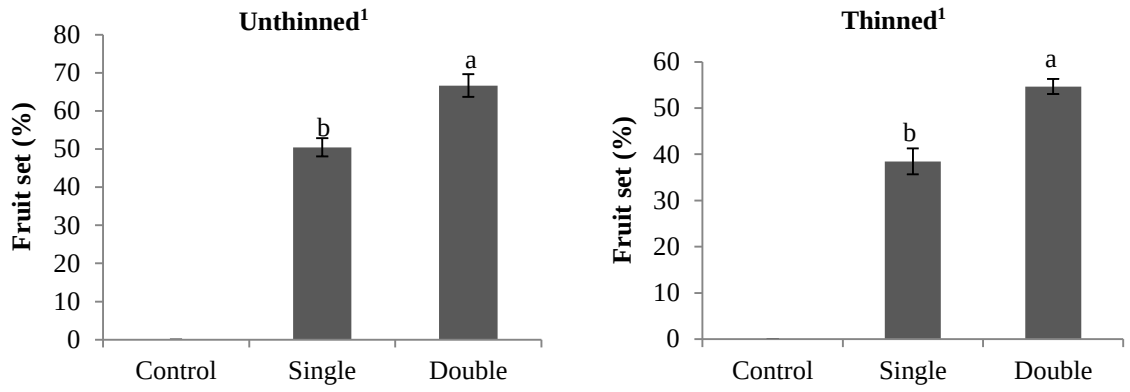

Figure 7. Fruit set in 'Jazz'/M®9 apple branches upon pollen suspension treatment with two pollen rates- single (15g/acre) and double (30g/acre), applied to unthinned (left) and thinned (right) flowering limbs at about 50% and 100% full bloom. All treated limbs including control were enclosed by bee boxes throughout flowering (p-value: 0.000). [1]Percent of open flower at pollen application was same in thinned and unthinned block.

EXECUTIVE SUMMARY

This research project has provided preliminary data and proof of concept data on the possibility to pollinate tree fruit crops artificially with pollen suspensions applied with commercially available electrostatic sprayers. Herein we report on successful pollination of apple, pear, and sweet cherry using a pollen suspension that is capable of maintaining or improving pollen viability for up to 1 hour. We have proven that one can take previously harvested pollen, incorporate it in a suspension, and spray it through commercially available sprayers to effect pollination in tree fruit crops. The implications are considerable – ensuring consistent fruiting in the face of declining bee populations, increasingly variable spring weather conditions, and the perennial challenges growers face with both pollinators and pollenizers. Further, the ability to artificially pollinate tree fruit holds the potential to revolutionize crop load management. Our vision for precision pollination systems that do not include pollenizers nor pollinators appears plausible. Clearly these results should be considered promising yet preliminary. More research is needed into the role of pollen rate, application timing, and suspension development to extend pollen viability. We have collaborated with interested growers across Washington, Firman Pollen Company, and On Target Spray Systems – all to facilitate the commercial adoption of our research. As a result of this research, there is significant interest in precision pollination systems. Every grower we worked with in 2015 is interested to collaborate further, seeing promising results – this underscores the importance of pollination/fertilization in commercial fruit production as well as the confidence our collaborators have in our research approach and vision.