2018 PROGRESS REPORT

Year Initiated: 2018  Current Year: 2018  Terminating Year: 2019

Title: Enhancing Blueberry Pollination through an Improved Understanding of Pollen Biology and Implementation of In-Field Practices in Western Washington

Personnel:
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OBJECTIVES:
The overarching goal of this project and the WSU Small Fruit Horticulture Program is to learn more about the biology of blueberry pollination and evaluate in-field practices or tools that have the potential to promote pollination and subsequent yields in pollination-limited western Washington.

The specific objectives of this project are to:

Objective 1: Improve the current understanding of pollination biology for blueberry grown in western Washington by studying pollen functionality (germination and vigor) and longevity at different temperatures among four commercially important highbush cultivars; and

Objective 2: Evaluate commercial strategies that have the potential to promote pollination, fruit set, yield, and fruit quality attributes in western Washington blueberry. Specific strategies that we focused on include further testing of modified honey bee hive densities and the application of commercial pheromones and attractants.

PROCEDURES:
Objective 1 - Pollen experiment. ‘Aurora’, ‘Draper’, ‘Duke’, and ‘Liberty’ blueberry pollen were collected from four commercial and conventionally managed sites in Skagit County in May 2018. Collection occurred weekly for a total of three weeks (except ‘Aurora’, which was only collected for two weeks because of difficulties with pollen release from the anthers), which spanned 20-100% bloom for all cultivars considered. Pollen was incubated at five different temperature treatments (35, 45, 55, 65, and 75°F) on pollen-specific growth media. Pollen grains were observed under a microscope (Nikon Eclips 50i, Nikon Instruments Inc., Melville, NY) at 40x magnification every 24 hours for four days. After four days, pollen growth ceased. Pollen germination rate, pollen tube number per tetrad, and pollen tube length were determined from 30 randomly selected tetrads per quadrant and for all four quadrants.
per petri dish. There was one petri dish per cultivar and temperature treatment for each sampling time.

**Objective 2 - Hive Density Experiments.** Hive density experiments were conducted in ‘Duke’ and ‘Draper’. We wanted to complete hive density experiments in ‘Liberty’, which is known to have chronic pollination deficits, but there is an insufficient number of fields for proper experimental set-up and replication. For ‘Duke’, nine commercial, conventionally managed field sites in Skagit and Whatcom counties were identified. Hives were placed in the study sites at approximately 5% bloom and honey bees were provided by Belleville Bees and Grigg Honey. To maintain independence, field sites were a minimum of one mile apart. The treatments were as follows, with each treatment replicated three times per year.

1. 4 hives/acre of honey bees (control)
2. 8 hives/acre of honey bees
3. 10 hives/acre of honey bees

For ‘Draper’, six commercial, conventionally managed field sites in Skagit and Whatcom counties were identified. Hives were placed in the study sites at approximately 5% bloom and bees were provided by Belleville Bees and Grigg Honey. To maintain independence, sites were a minimum of one mile apart. The treatments were as follows, with each treatment replicated two times per year.

1. 4 hives/acre of honey bees (control)
2. 8 hives/acre of honey bees
3. 10 hives/acre of honey bees

Honey bee visitation data were collected during the bloom period following the procedures described by Courcelles et al. (2013). Honey bee activity was assessed at each site from 9:30 AM to 4:00 PM when temperature were $\geq 55 \, ^\circ F$, conditions were sunny to partly cloudy, and there was no precipitation. Measures of honey bee visitation only considered “legitimate” pollination events, which occurs when a honey bee forages within the flower and enters through the corolla (i.e. no “nectar robbing”). Honey bee visitation data were collected from 30 flagged bushes/site (10 plants per transect x 3 transects per site) within one-minute intervals repeated three times per bush per day for three days within the bloom period.

Fruit set was measured from the 30 flagged bushes/site and estimated yield/bush was determined. Fifty randomly selected ripe berries per transect were collected prior to harvest for assessment of average berry mass and seed number per berry, which is indicative of fertilization and can serve as a proxy for effective pollination in blueberry. Fruit firmness was also determined from a sample of 50 berries per transect (150 berries/site) using a FirmTech II (Bioworks, FirmTech II; Bioworks, Wamego, KS). °Brix was measured from a sample of 40 berries per transect using a digital refractometer (H19680 Refractometer, Hanna Instruments, Woonsocket, RI). These data were analyzed using JMP Statistical Software (SAS Institute Inc.; Cary, NC).
**Objective 2 – Pheromone Experiment.** One ‘Draper’ field site located in Skagit County was used for this experiment. The experimental design was a randomized complete block with four replications of six treatments. Four different pheromone or pollinator attractant treatments plus two controls were applied to 30-foot-long plots. Treatments include: 1) Bee-Scent (Scentry Biologicals, Inc., Billings, MT), 2) Pollinate Pro (Instar Naturals LLC, Yakima, WA), 3) Honey Bee Magnet (AgBio Inc., Westminster, CO), 4) SureSet-Apex (Fusion360, Inc., Turlock, CA), 5) Water control (treated with distilled water), and 6) No-water control. A buffer of 60 feet was maintained on all sides of individual plots so to avoid confounding effects of having multiple pheromones and attractants applied in a single field site. Buffer selection was based upon the products’ label and listed active space. Treatments were applied according to the manufacture’s guidelines. Honey bees were stocked at 4 hives/acre and were sourced from Bellville Bees. Honey bee visitation data were collected from six bushes per plot and followed the same procedure described above. Fruit set was determined from six bushes per plot and average berry mass was determined from 30 berries per plot. Seed number, firmness, and Brix were measured as described above for the hive density experiments. Data were analyzed using JMP Statistical Software (SAS Institute Inc., Cary, NC).

**Outreach** - We have presented preliminary information at two field days and two WSU classes in 2018 (see below in the “Output” section) and delivered project information at the Small Fruit Conference as part of a pollination session on Nov. 30, 2018. Once full data analyses are complete, these data will be shared with our grower cooperators by December 2018. Data will be part of Weixin Gan’s thesis and chapters will be submitted for publication after a second year of data collection in 2019.

**2018 PRELIMINARY RESULTS:**

**Objective 1 - Pollen experiments.** For all cultivars considered, the temperatures with the highest germination rates were 55, 65, and 75 °F (Fig. 1). The optimal temperature for maximum pollen tube length was 75 °F (Fig. 2). Pollen tube number per tetrad was lowest at 35 and 45 °F, and highest at 75 °F (Fig. 3). Among all cultivars, ‘Duke’ has the highest pollen germination rate. The highest germination rate in ‘Duke’ was observed at 65 °F and was 81.75%, while the lowest germination rate in ‘Duke’ was 9% at 35 °F (Fig. 1). In contrast, ‘Liberty’ had the lowest germination ranging from 3.25% - 68.5% across the experimental temperatures. Pollen tube length was greatest in ‘Aurora’ and measured 1601 µm at 75 °F, whereas it was greatest in ‘Duke’ at 45 and 55 °F (Fig. 2). Pollen tube length was on average lowest in ‘Liberty’ followed by ‘Draper’, but peaked at 65 °F in ‘Liberty’ and 75 °F in ‘Draper’. Pollen tube number per tetrad was on average greatest in ‘Duke’ followed by ‘Draper’, ‘Aurora’, and ‘Liberty’ (Fig. 3). One tetrad has the capacity to produce four pollen tubes, but this was seldom observed in our study.

Results suggest that the optimal temperature range for these four cultivars to reach maximum pollen germination rate and pollen tube number per tetrad is 55 – 65 °F, except for ‘Aurora’ which reached maximum germination and pollen tube number per tetrad at 75 °F (Figs. 1 and...
3). Maximum pollen tube length was achieved at 75 °F (Fig. 2). ‘Liberty’ also had relatively lower germination and pollen tube growth (Figs. 1, 2, and 3). ‘Liberty’ was also more sensitive to low and high temperatures. These observations suggest that some of the pollination challenges with ‘Liberty’ may be due to the biology of ‘Liberty’ pollen itself, as it has a reduced capacity to germinate. Furthermore, lower pollen tube number/tetrad and length could translate into challenges with fertilizing ovules to induce seed set during blueberry’s short bloom period. These data will be useful in building an effective pollination period model for blueberry.

**Figure 1.** Percent pollen germination of ‘Aurora’, ‘Draper’, ‘Duke’, and ‘Liberty’ blueberry by temperatures (°F). Note the coefficient of determination (R²) describes the proportion of the variance in the dependent variable (germination) that is predicted from the independent variable (temperature). Values range from 0 to 1, with values closer to 1 suggesting a stronger relationship. Data are from 2018.
Figure 2. Pollen tube length for ‘Aurora’, ‘Draper’, ‘Duke’ and ‘Liberty’ blueberry by temperatures (°F). Note the coefficient of determination ($R^2$) describes the proportion of the variance in the dependent variable (pollen tube length) that is predicted from the independent variable (temperature). Values range from 0 to 1, with values closer to 1 suggesting a stronger relationship. Data are from 2018.
Figure 3. Pollen tube number per tetrad for ‘Aurora’, ‘Draper’, ‘Duke’, and ‘Liberty’ blueberry by temperature (°F). Note the coefficient of determination (R²) describes the proportion of the variance in the dependent variable (pollen tube number per tetrad) that is predicted from the independent variable (temperature). Values range from 0 to 1, with values closer to 1 suggesting a stronger relationship. Data are from 2018.

Objective 2 - Hive density experiments. For ‘Duke’ blueberry, the highest hive density significantly increased honey bee visitation and fruit set, while fruit firmness decreased (Table 1). There were no statistical differences in average berry mass, yield/bush, and seed number per berry, but these data show an important numerical trend of increasing with increasing hive density. These numerical differences are noteworthy and may be economical significant for a grower. °Brix also increased with increasing density.

Table 1. Honey bee visitation rate, fruit set, average berry mass, firmness, seed number, °Brix, and yield of ‘Duke’ blueberry with different levels of honey bee hives densities in 2018.

<table>
<thead>
<tr>
<th>Treatment (hives/acre)</th>
<th>Visitation (honeybees/ bush/min)</th>
<th>Fruit set (%)</th>
<th>Average berry mass (g/berry)</th>
<th>Firmness (g/mm of deflection)</th>
<th>Seed number / berry</th>
<th>°Brix</th>
<th>Yield (lb/bush)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.00 c^d</td>
<td>79.37 b</td>
<td>1.70</td>
<td>176.62 a</td>
<td>40</td>
<td>12.51 b</td>
<td>11.63</td>
</tr>
<tr>
<td>8</td>
<td>1.41 b</td>
<td>74.48 b</td>
<td>1.77</td>
<td>171.15 b</td>
<td>43</td>
<td>13.51 ab</td>
<td>12.35</td>
</tr>
<tr>
<td>10</td>
<td>2.03 a</td>
<td>96.87 a</td>
<td>1.93</td>
<td>172.05 b</td>
<td>46</td>
<td>14.04 a</td>
<td>18.70</td>
</tr>
</tbody>
</table>

P-value <0.0001 <0.0001 NS 0.0067 NS 0.04 NS

Means separations were performed with Tukey’s Honest Significant Difference (HSD) test; means with the same letter are not different at P ≤ 0.05 and NS denotes not statistically significant.
For ‘Draper’ blueberry, 10 hives/acre significantly increased honey bee visitation and fruit set (Table 2). Firmness was greatest in the 10 hive/acre treatment. There are no statistical differences in fruit mass, seed number per berry, °Brix, and yield, which is attributed to variation among sites. However, there is still an important numerical trend whereby the 8 and 10 hives/acre treatment sites have numerically greater yields. Again, although not statistically significant, this may be economically significant for growers.

We plan to repeat these hive density experiments in 2019 and provide an economic assessment of our treatments in our final report.

**Table 2.** Honey bee visitation rate, fruit set, average berry mass, firmness, seed number per berry, °Brix, and yield of ‘Draper’ blueberry with different levels of honey bee hive densities in 2018.

<table>
<thead>
<tr>
<th>Treatment (hives/acre)</th>
<th>Visitation (honeybees/bush/minute)</th>
<th>Fruit set (%)</th>
<th>Average berry mass (g/berry)</th>
<th>Firmness (g/mm of deflection)</th>
<th>Seed number / berry</th>
<th>°Brix</th>
<th>Yield (lb/bush)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.65 c</td>
<td>67.45 b</td>
<td>2.17</td>
<td>207.49 b</td>
<td>24</td>
<td>17.57</td>
<td>4.45</td>
</tr>
<tr>
<td>8</td>
<td>1.35 b</td>
<td>73.35 b</td>
<td>2.37</td>
<td>204.20 b</td>
<td>33</td>
<td>16.10</td>
<td>8.60</td>
</tr>
<tr>
<td>10</td>
<td>1.69 a</td>
<td>85.25 a</td>
<td>2.42</td>
<td>216.29 a</td>
<td>32</td>
<td>16.26</td>
<td>7.35</td>
</tr>
</tbody>
</table>

*Means separations were performed with Tukey’s Honest Significant Difference (HSD) test; means with the same letter are not different at *P* ≤ 0.05.

**Objective 2 - Pheromone experiment.** Pollinator activity was extremely low for this particular field site. Observations suggest that the honey bees found blooming maple trees (*Acer* spp.) more attractive, which lead to reduced honey bee activity in the blueberry field. Thus, conditions were good for an assessment of the efficacy of these materials given the bloom competition. Pollinate Pro (*P*-value < 0.0001) had the highest honey bee visitation rate, while our no water control (*P*-value < 0.0001) and Sureset Apex (*P*-value < 0.0001) had the lowest visitation rate (Table 3). Although we detected these statistical differences in honey bee visitation rates, there were no observable effects on the blueberry plants. No differences in fruit set, average berry mass, seed number per berry, nor °Brix were observed, although we did see differences in firmness. Fruit collection from Pollinate-Pro, Bee-Scent, and no water control treated plots were less firm than fruit from the water control, Honey Bee Magnet, and Sureset Apex treatments. We would like to repeat these studies in 2019 to validate the results and also test plant growth regulator in addition to pheromones and attractants.
Table 3. Honey bee visitation rate, fruit set, firmness, average berry mass, seed number per berry, and °Brix of ‘Draper’ blueberry treated with pheromones and attractants in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visitation rate (bee/bush/min)</th>
<th>Fruit set (%)</th>
<th>Firmness (g/mm of deflection)</th>
<th>Average berry mass (g/berry)</th>
<th>Seed number / berry</th>
<th>°Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee Scent</td>
<td>0.11 b</td>
<td>59.63</td>
<td>180.17 ab</td>
<td>1.62</td>
<td>13</td>
<td>16.49</td>
</tr>
<tr>
<td>Honey Bee Magnet</td>
<td>0.06 bc</td>
<td>66.26</td>
<td>183.92 a</td>
<td>1.77</td>
<td>11</td>
<td>16.39</td>
</tr>
<tr>
<td>Pollinate Pro</td>
<td>0.18 a</td>
<td>71.68</td>
<td>172.35 ab</td>
<td>1.65</td>
<td>12</td>
<td>17.71</td>
</tr>
<tr>
<td>SureSet-Apex</td>
<td>0.03 c</td>
<td>70.31</td>
<td>181.59 a</td>
<td>1.66</td>
<td>12</td>
<td>15.97</td>
</tr>
<tr>
<td>Distilled water (control 1)</td>
<td>0.06 bc</td>
<td>64.71</td>
<td>184.33 a</td>
<td>1.69</td>
<td>11</td>
<td>15.69</td>
</tr>
<tr>
<td>No water (control 2)</td>
<td>0.03 c</td>
<td>62.55</td>
<td>177.12 ab</td>
<td>1.65</td>
<td>7</td>
<td>16.23</td>
</tr>
</tbody>
</table>

P-value <0.0001 NS 0.001 NS NS NS

*Means separations were performed with Tukey’s Honest Significant Difference (HSD) test; means with the same letter are not different at $P \leq 0.05$.

ANTICIPATED BENEFITS AND INFORMATION TRANSFER:

This project is providing information on pollination biology specific to the unique conditions of western Washington and potential strategies to increase pollination and subsequent effects on fruit set, berry size (mass), and yield. Results from the pollen experiment will inform growers if pollination may be restricted due to innate cultivar differences or temperature conditions, which can enable them to implement targeted strategies to enhance pollination through modified hive densities, application of pheromones, or other strategies tested and validated by research when environmental conditions are unconducive for their pollination. Furthermore, this project is demonstrating the limitations commercial pheromones and attractants have in blueberry, which will be important in justifying their application on commercial operations.

Information from this project may result in changes to current recommended practices for promoting pollination of blueberry in western Washington. Data collected will also provide a continued foundation of research on how to improve pollination and fruit set for Washington-grown blueberries, especially during unfavorable weather conditions which are typical during blueberry bloom times in western Washington. Information from this project will be part of Weixin Gan’s MS thesis and shared with grower cooperators, at regional horticulture events (e.g. Small Fruit Horticulture Conference), and at national horticulture meetings. Information will also be published online on the Small Fruit Horticulture Website (http://smallfruits.wsu.edu/) in the Whatcom Ag. Monthly (http://extension.wsu.edu/wam/), and in peer-reviewed journals.
OUTPUTS:

- Individual grower cooperator reports. 2018.
- Reports also shared with honey bee and bumble bee suppliers.
- Updates being added to the Small Fruit Horticulture website.

REFERENCES