2019 FINAL REPORT

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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

Principle Investigator:
PI: Lisa W. DeVetter, Small Fruit Horticulturist, WSU Mount Vernon Research and Extension Center, 16650 State Route 536, Mount Vernon, WA, 98273, email: lisa.devetter@wsu.edu

Co-PIs:
Weixin Gan, Master Graduate Student in Horticulture, Washington State University Northwestern Washington Research and Extension Center, 16650 State Route 536, Mount Vernon, WA 98273, email: weixin.gan@wsu.edu

Andony Melathopoulos, Assistant Professor in Pollinator Health and Extension, Oregon State University, 2750 SW Campus Way, Corvallis, OR 97331, email: andony.melathopoulos@oregonstate.edu

Justification and Background:
Single-species pollination by honey bees (*Apis mellifera*) is the primary method of insect-mediated pollination in highbush blueberry (*Vacinium corymosum*) in Washington State. However, honey bees are morphologically not well adapted for the efficient pollination of blueberry. This is in part due to honey bee's short tongue length, which may not extend down the long corolla tube of blueberry flowers, and the inability of honey bees to sonicate to cause effective pollen release from poricidal anthers. The climate in western Washington is another limiting factor (Courcelles et al., 2013; Delaplane et al., 2000). In fact, DeVetter et al. (2016) conducted statewide surveys and found honey bee visitation rates in western Washington were below the recommended 4-8 honey bees/minute/bush guideline (Isaacs et al., 2019), demonstrating that pollination by honey bees is below current guidelines and that this may be a factor that can reduce crop productivity.

Strategies that promote pollination are being investigated in DeVetter's Small Fruit Horticulture program. Arrington and DeVetter (2018) demonstrated that honey bee visitation rates and subsequent berry mass and estimated yield can be increased through modified hive stocking densities in 'Duke'. Results from this two-year experiment suggests that yield may be increased by ~2.65 lbs/plant by doubling stocking densities from 4 to 8 hives/acre. However, cultivars differ in flower morphology and consequent ability to be effectively pollinated by honey bees. Courcelles et al. (2013) showed 'Duke' is visited more frequently by honey bees than 'Draper', 'Liberty', and 'Bluecrop'; these differences in honey bee visitation rates were attributed to differences to flower size and morphology. Therefore, the

potential benefits of increased hive densities should be further evaluated in other commercially important blueberry cultivars. Additionally, continued evaluations in 'Duke' are warranted in order to determine the point of diminishing returns with regards to honey bee stocking density and to develop more advanced recommendations for growers.

Pheromone and pollinator attractant application is another strategy being employed by growers in order to promote honey bee activity. Arrington (2017) experimented with Bee Scent pheromone in 'Draper' in 2017 and found no effect on honey be visitation rates, fruit set, berry size, or estimated yield. As is standard for field experiments and because pheromone application is such a widespread practice in commercial blueberry production in western Washington, additional years of field trails and experimentation with more commercially available pheromones and attractants is recommended. In addition, plant growth regulators (such as those that mimic gibberellins) may promote berry development and compensate for insufficient pollination. Yet, they remain unexplored for highbush blueberry.

The bloom period of many commercially important cultivars poses another constraint to blueberry pollination, as it is relatively short and sometimes lasts only 5-12 days (Dogterom et al., 2000). This short bloom window means there is a limited period of time to successfully transfer pollen, fertilize ovules, and initiate fruit development. Yet, pollen functionality and longevity in highbush blueberry is not well characterized across commercially important cultivars and under environmental conditions typical for western Washington. Cultivars may differ in pollen functionality and how they respond to environmental conditions, which will impact the effective pollination period of this crop. Knowing what cultivars are most sensitive and threshold environmental conditions that impact pollen functionality and longevity can help growers implement targeted pollination strategies that are being tested and developed through research.

This proposal continued our work investigating methods to improve pollination in highbush blueberry and contributed to our understanding of pollination limitations. Specifically, we studied pollen performance to develop a better understanding of pollen biology among commercially important blueberry cultivars in western Washington conditions and tested in-field strategies that have the potential to enhance honey bee pollination and resultant yields.

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 were to:

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.

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 (2a) further testing of modified honey bee hive densities and the application of (2b) commercial pheromones and (2c) plant growth regulators.

Procedures:

Objective 1 - Pollen Biology Experiments: '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 Eclipes 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.

Each Petri dish was divided into quadrants. Thirty clearly visible tetrads were randomly selected in each quadrant for further measurement. Tetrads were observed under a compound microscope (Eclipse 50i, Nikon, Japan) at 40x magnification every 24 hours. Pollen germination rate, tube length, and tube number per tetrad were determined for each quadrant containing 30 tetrads. A tetrad that produced one or more pollen tubes was considered germinated.

Statistical analyses were conducted using Statistical Analysis Software (SAS; SAS Institute Inc., Cary, NC). The experimental design was completely randomized and data were evaluated for normality and equal variance before conducing regression analysis using PROC REG. An analysis of covariance approach was first used to determine if there was a cultivar x temperature interaction for the variables measured with cultivar as the indicator variable and temperature as the covariate. After determining the interaction was significant (with $\alpha = 0.05$), linear and quadratic models were fitted for each cultivar. The significance of the model (P-value), coefficient of determination (R^2), and adjusted coefficient of determination (R^2_{adj}) were calculated and are presented for each cultivar. Only pollen germination, tube length, and tube number per tetrad data collected on the fourth day were used in the analyses, which was when pollen growth ceased for all cultivars. All data are presented in original units.

Objective 2a - Hive Density Experiment – 'Duke' and 'Draper': Hive density experiments were conducted in 'Duke' and 'Draper' in 2018 and 2019. 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 sited 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 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/total soluble solids (TSS) 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 2b – Pheromone Experiment. One ‘Draper’ field site located in Skagit County was used for this experiment from 2018-2019. 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 Nuturals LLC, Yakima, WA), 3) Honey Bee Magnet (AgBio Inc., Westminster, CO), 4) SureSet-Apex (Fusion360, Inc., Turlock, CA; *this treatment was only applied in 2018 because of application equipment failure in 2019*), 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 form 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).

Objective 2c - Plant Growth Regulator Experiment: This experiment was conducted in a conventionally managed 'Liberty' field located in Skagit County in 2019. Treatments were applied to 30-foot-long plots replicated four times in a randomized complete block design. Treatments include: 1) ProGibb® [gibberellic acid (GA3)]; 2) Promalin® [mixture of gibberellic acid 4 and 7 (GA4+7) and 6-benzyladenine (6-BA)]; 3) ReTain® (ethylene inhibitor meant to extend flower life); 4) ProGibb + Retain; and 5) Promalin + Retain; and 6) Control (distilled water) applied according to the label.

Unfortunately, the grower harvested the 'Liberty' plots a day before anticipated, so we were unable to collect fruit set and yield data. Therefore, we have no data to report from this experiment. However, we did apply ProGibb® to conventionally managed 'Reka' grown in Whatcom County in 2019. The experimental design was a randomized complete block with single row plots serving as the experimental unit replicated three times. ProGibb® LV Plus (20-fl oz/a.i. acre per application) was applied with an organosilicone surfactant at 75% bloom and reapplied 14 days after. Fruit set, berry size, firmness, and yield were monitored. Symptoms of phytotoxicity were also observed for in both experiments.

RESULTS:

Objective 1 - Pollen Biology Experiments: Results from this project were reported in the 2018 commission progress report and is scheduled to be published in 2020 in the *Journal for the American Pomological Society*. The manuscript is in press and we are happy to share the publication with the commission and growers upon request. Overall, results showed that the optimal temperature range to reach maximum pollen germination, tube length, and tube number per tetrad in vitro is 55-75 °F for 'Aurora', 'Draper', and 'Duke', but 55-64 °F for 'Liberty'. 'Liberty' had a relatively lower pollen germination rate and tube growth than the other evaluated cultivars. 'Liberty' was also more sensitive to low and high temperatures. These observations suggest that some of the pollination and fruit development challenges with 'Liberty' may be due to the biology of the pollen itself, as it exhibited a reduced capacity to germinate and grow. These data also demonstrate pollen performance differs across commercially important cultivars of highbush blueberry and suggest developing cultivar-

specific effective pollination period models may be useful. Additionally, these findings indicate breeders should consider phenotyping pollen characteristics to better understand adaptation and potential intrinsic pollination constraints at the genetic level.

Objective 2a - Hive Density Experiment – 'Duke' and 'Draper': Honey bee visitation in 'Duke' was greatest at the 10 hives/acre treatment in both years of the experiment (Table 1). Visitation rates decreased linearly with decreasing hive density, demonstrating increased hive densities has a positive effect on honey bee visitation in 'Duke' under the conditions of our experiment. Fruit set was greatest at the 10 hives/acre treatment in both years of the experiment and was the same at the 4 and 8 hives/acre treatments. However, berry mass and seed number per berry did not respond to our treatments (Table 1 and Fig. 1). Firmness was greatest at the 4 hives/acre treatment, which may be due to the smaller diameter of berries in this treatment (diameter data not presented), but firmness was still overall high across all treatments. TSS showed a treatment effect and was greater at the 10 hives/acre treatment relative to the 4 hives/acre treatment. While yield showed not treatment effect, numerical yield values tended to increase with increasing hive density. Yield effects are difficult to capture due to other in-field practices, plant variability across farms (despite trying to control for it), and the difficulty in controlling hive quality. However, our results continue to demonstrate 'Duke' tends to respond positively to increasing hive densities. Therefore, we recommend growers struggling with pollination experiment with higher hive densities in small blocks to test whether this is an economical approach for them to increase pollination and yield components on their farms.

'Draper' was less responsive to our hive density treatments (Table 2). In 2018 and 2010, honey bee visitation rates were greatest in the 10 hives/acre treatment, but were lowest in the 8 hives/acre treatment in 2019. This divergence in our usual trends may be due to hive quality. Fruit set was greatest at 10 hives/acre at ~88%, but all plots suffered some fruit loss at the berry coloring stage due to premature 'Draper' fruit drop. 'Draper' fruit drop tends to be due to a calcium deficiency (Gerbrandt et al., 2019), not a pollination effect, but did impact our fruit set and yield results by making it difficult to quantify treatment impacts. In fact, no yield effects were observed among 'Draper' and may be due to 'Draper' fruit drop (Fig. 2). Berry mass, TSS, and seed number per berry also did not respond to our hive density treatments, whereas firmness was greatest among plants treated with 10 hives/acre (Table 2). While we tend to observe a mild benefit to increased hive densities in 'Draper', we are cautious about recommending this practice unless growers are able to also manage 'Draper' fruit drop. In essence, any economic benefit from increased hive densities could be offset by premature fruit drop if this is not managed for.

Objective 2b – Pheromone Experiment. Honey bee visitation differed between years, but was overall very low in this field (Table 3). Pollinate Pro had the highest honey bee visitation rate in 2018, but there results were not repeated in 2019 and were still overall low, questioning biological and commercial significance. Fruit set ranged from ~59-71% across both years and did not differ by treatment. Berry mass, TSS, and seed number per berry also showed no treatment effect across the years. Firmness did differ across treatments, with firmness being

highest among fruit collected from the Honey Bee Magnet, SureSet-Apex, and distilled water treated plots. However, firmness across all treatments was high and ranged from ~172-184 g/mm of deflection. Results from this experiment do not support the use of the evaluated pheromones and attractants for enhancing honey bee visitation and resultant yield and fruit quality components.

Objective 2c - Plant Growth Regulator Experiment: No differences in fruit set, berry size, firmness, nor diameter were detected (Table 4). The grower also reported no differences in yield. Results do not support use of ProGibb® in 'Reka' for improvement of fruit set, berry size, and yields in northwest Washington.

ANTICIPATED BENEFITS AND INFORMATION TRANSFER:
This project is provided 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 have indicated that pollination in cultivars, like 'Liberty', may be restricted due to innate cultivar differences or temperature conditions. In turn, this can inform growers, extension specialists, and crop advisors about how to implement strategic and 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 demonstrated the limitations commercial pheromones and attractants have in blueberry, which will be important in justifying their application on commercial operations. While our PGR experiment did not go as planned, results from the ProGibb® experiment demonstrated no impact from application at label rates, questioning the utility of this product.

While we are not applying for continued funding to the Washington Blueberry Commission in 2020, we are still looking at opportunities to continue our research on ways to improve pollination and yield components in highbush blueberry. Currently, we have teamed up with Dr. Isaac's program at Michigan State University and have submitted a multi-million dollar federal grant that seeks to develop a regional pollination model for blueberry. Furthermore, we have observed a high incidence of foul brood which impacts hive quality. Collaborative work with Dr. Andony Melathopoulos at Oregon State University is seeking ways to understand this disease in attempts to manage it.

OUTPUTS:
- Gan, W., H. Zhang, N. Bostan, and L.W. DeVetter. 2019. Pollen germination and growth rates differ among cultivars of northern highbush blueberry (*Vaccinium corymbosum*). Journal of the American Pomological Society. *In press*.
- Gan, W. 2019. Enhancing blueberry pollination through an improved understanding of pollen biology and implementation of in-field practices in western Washington. Washington State University. MS Thesis.
- Gan, W. and L.W. DeVetter. 2019. Commercial pheromones and attractants have no contribution to increasing pollination, fruit set, and berry mass in highbush blueberry.

American Society for Horticultural Sciences Conference. Poster Presentation. Las Vegas, NV.

- Gan, W. and L.W. DeVetter. 2019. Increasing honey bee stocking density improves pollination in two blueberry cultivars in western Washington. American Society for Horticultural Sciences Conference. Oral Presentation. Las Vegas, NV.
- W. Gan. (presenter) and L.W. DeVetter. 2019. Improving pollination in blueberry. Small Fruit Conference, Lynden, WA. Oral presentation.
- W. Gan. (presenter) and L.W. DeVetter. 2019. Pollen functionality. Small Fruit Conference, Lynden, WA. Oral presentation.
- W. Gan. (presenter) and L.W. DeVetter. 2018. Improving pollination in blueberry. Washington State University Northwestern Research & Extension Center Field Day. Washington State University Northwestern Research & Extension Center. Mount Vernon, WA. Oral presentation.
- W. Gan. (presenter) and L.W. DeVetter. 2018. Improving pollination in blueberry through modifying honey bee hive density and applying pheromones and attractants. Blueberry Field Day. Oregon State University North Willamette Research & Extension Center. Aurora, OR. Oral presentation.
- W. Gan. (presenter) and L.W. DeVetter. 2018. Enhancing blueberry pollination through an improved understanding of pollen biology and implementation of in-field practices in western Washington. Class Presentation for Hort 310 (Pomology) Class. Washington State University Northwestern Research & Extension Center. Mount Vernon, WA. Invited oral presentation.
- W. Gan. (presenter) and L.W. DeVetter. 2018. Improving pollination in blueberry. Class Presentation for AFS 201 (Systems Skills Development for Agricultural & Food Systems). Washington State University Northwestern Research & Extension Center. Mount Vernon, WA. Invited oral presentation.
- W. Gan (presenter) and L. W. DeVetter. 2018. Promising strategies that can improve blueberry pollination. Washington Small Fruit Conference. Lynden, WA. Invited oral presentation.
- W. Gan (presenter) and L. W. DeVetter. 2018. Comparison of blueberry pollen vigor and germination between tour different cultivars. Washington Small Fruit Conference. Lynden, WA. Invited oral presentation.
- Individual grower cooperator reports. 2018 and 2019.
- Reports have also been shared with honey bee suppliers.
- Updates being added to the Small Fruit Horticulture website.

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- Grower cooperators
- Kurtis Plaisted, Grigg Honey
- Belleville Bees
- Valent BioSciences
- Pollinate Pro
- Fusion360
- Skagit Farmers Supply

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Table 1. Honey bee visitation, fruit set, berry mass, firmness, total soluble solids (TSS), and seed number per berry in 'Duke' highbush blueberry treated with different honey bee hive densities in western Washington, 2018-2019.

Density (hives/acre)	Visitation rate (honeybees/bush/minute)		Fruit set (%)	Berry mass (g/berry)	Firmness (g/mm of deflection)	TSS	Seed no./berry
	2018	2019					
4	1.0 c[z]	1.5 c	79.4 b	1.7	176.6 a	12.5 b	40
8	1.4 b	2.8 b	74.5 b	1.8	171.2 b	13.5 ab	43
10	2.0 a	3.5 a	96.9 a	1.9	172.1 b	14.0 a	46
Significance[y]	<0.0001	<0.0001	<0.0001	0.19	<0.01	0.04	0.56

[z]Means separations were performed with and Tukey's Honest Significant Difference (HSD) test or non-parametric Wilcoxon test; means with the same letter are not different at $P \leq 0.05$.
[y]*P*-value with significance at $\alpha = 0.05$.

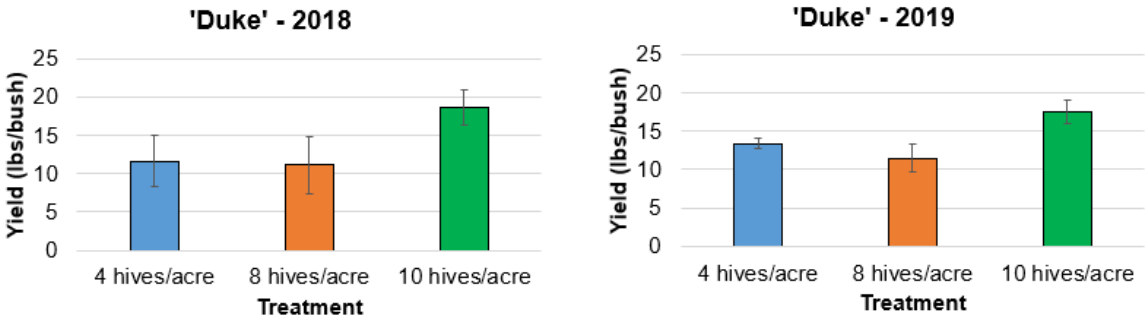

Figure 1. Yield of 'Duke' highbush blueberry treated with different honey bee hive densities in western Washington, 2018-2019.

Table 2. Honey bee visitation, fruit set, berry mass, firmness, total soluble solids (TSS), and seed number per berry in 'Draper' highbush blueberry treated with different honey bee hive densities in western Washington, 2018-2019.

Density (hives/acre)	Visitation rate (honey bees/bush/minute)		Fruit set (%)	Berry mass (g/berry)	Firmness (g/mm of deflection)	TSS	Seed no./berry
	2018	2019					
4	0.7 c[z]	1.2 b	67.5 b	2.2	207.5 b	17.6	24
8	1.4 b	1.0 c	73.4 b	2.4	204.2 b	16.1	33
10	1.6 a	1.7 a	87.6 a	2.4	216.3 a	16.3	32
Significance[y]	<0.0001	<0.0001	<0.0001	0.12	<0.001	0.09	0.16

[z]Means separations were performed with and Tukey's Honest Significant Difference (HSD) test or non-parametric Wilcoxon test; means with the same letter are not different at $P \leq 0.05$.
[y]*P*-value with significance at $\alpha = 0.05$.

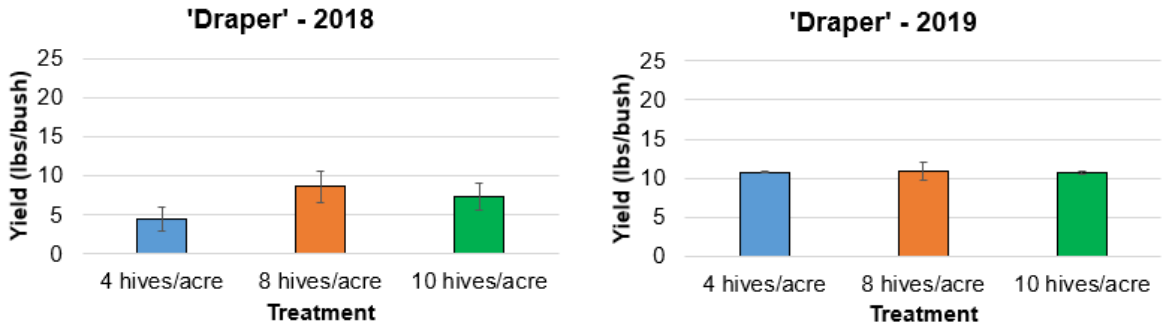

Figure 2. Yield of 'Draper' highbush blueberry treated with different honey bee hive densities in western Washington, 2018-2019.

Table 3. Honey bee visitation, fruit set, berry mass, firmness, total soluble solids (TSS), and seed number per berry in 'Draper' highbush blueberry treated with different pheromones and attractants in western Washington, 2018-2019.

Treatment	Visitation rate (honey bees/bush/minute)		Fruit set (%)	Berry mass (g/berry)	Firmness (g/mm of deflection)	TSS	Seed no./berry
	2018	2019					
Bee-Scent	0.11 b[z]	0.41	59.6	1.6	180.2 ab	16.5	13
Honey Bee Magnet	0.06 bc	0.37	66.3	1.8	183.9 a	16.4	11
Pollinate Pro	0.18 a	0.48	71.7	1.7	172.4 bc	17.7	12
SureSet-Apex	0.03 c	/[y]	70.3	1.7	181.6 a	16.0	12
Distilled Water	0.06 bc	0.50	63.7	1.8	184.3 a	15.7	11
No Water	0.03 c	0.45	62.6	1.7	177.1 ab	16.2	7
Significance[y]	<0.0001	0.50	0.12	0.18	0.001	0.07	0.12

[z]Means separations were performed with and Tukey's Honest Significant Difference (HSD) test or non-parametric Wilcoxon test; means with the same letter are not different at $P \leq 0.05$.
[y]*P*-value with significance at $\alpha = 0.05$.

Table 4. Average berry size, firmness, and berry diameter in 'Reka' blueberry treated with ProGibb®, 2019.

Treatment	Average berry sample (g)	Firmness (g/mm deflection)	Diameter (mm)
ProGibb®	1.9	176.6	16.7
Control	1.9	182.4	16.7