Washington Blueberry Council (WBC) Progress Report - 2015

Project No. 3455-4640

Title: Relating Honey Bee Activity to Fruit Set and Yield in Washington Highbush Blueberry

Year Initiated: 2014 **Terminating Year:** 2015

Reporting Period: FY 2014-2015

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Objectives:
The main objective of this project is to measure and evaluate honey bee (*Apis mellifera*) activity and its influence on yield of highbush blueberry (*Vaccinium corymbosium*) within the conditions of the PNW, specifically Washington State. Additionally, this project is surveying colony stocking rates and landscape features in order to assess the range and potential relationships between stocking densities and yield components of blueberry within Washington.

Methodology:
One of the primary objectives of this project was to survey honey bee activity across commercial plantings of highbush blueberry in Washington State and to assess the relationship between honey bee activity and select yield components. To accomplish this, we evaluated honey bee visitation and subsequent yields at 16 and 18 commercial grower sites in 2014 and 2015, respectively. Additional organic sites located in western Washington were included in 2015 due to an observation in 2014 that organic sites had overall greater honey bee visitation rates relative to conventionally managed sites. However, it was unclear at the time if this relationship was related to a regional effect, given most organic sites are located in eastern Washington and eastern Washington tends to have greater honey bee activity due to climactic conditions being more favorable.

All sites included in the study had established 'Duke' plants (approximately six years old or older), although the size of the plants themselves varied across sites. Sites of data collection were distributed across Washington and included Whatcom, Skagit, and Benton counties (**Figs. 1-5**). Sites 1-6 represent eastern Washington, whereas sites 7-18 represent western Washington. Sites 3-6 and 17-18 were managed organically, while remaining sites were managed conventionally. All sites included in the study were a minimum of 2 miles apart from one another, which helped maintain independence in data collection.

Measurements of honey bee activity followed scientifically referenced methods provided by scientific collaborators (Courcelles et al., 2013). At each site, three 330-ft transects proceeding down a row were identified. Within each transect, ten randomly selected bushes were identified and tagged so that they could be re-measured throughout the experiment (30 bushes total per site; 480 and 540 plants total in 2014 and 2015, respectively). All sites were revisited during both years of the study, with the exception of sites 12, 17, and 18. The grower at site 12 did not want to participate in 2015 and sites 17 and 18 were new additions in 2015. Transects began at the natural edge of a field and proceeded towards the interior of the planting.

Honey bee activity was measured at each site from 10:00 AM to 4:00 PM and when weather conditions were conducive to their activity (> 55 °F, with low wind, full-to-partial sun), which minimizes artificial differences due to environmental effects and makes the data more comparable. Honey bee activity was recorded when plants were in 15-100% bloom and only "legitimate" pollinations were measured (i.e., honey bees foraging within flowers and entering through the corolla, no "nectar robbing"). These data were collected by counting the number of flower visitations per tagged bush within one-minute intervals, repeated three times per day for three days. Data were collected April 30 to May 7 in eastern Washington and April 30 to May 15 in western Washington in 2014. In 2015, data were collected April 16 to May 1 in eastern Washington and April 8 to May 18 in western Washington.

Colony strength was evaluated concurrent to measures of honey bee activity at each site. These data were collected by enumerating the number of honey bees entering their colony within one minute intervals, repeated twice per day for two days in 2014 and five times per day for three days in 2015. These data were video-recorded so that accuracy could be later verified. Previous reports have indicated that good pollinating colonies have uniform flight and approximately 100 or more bees return to their colonies per minute when temperatures are 65 °F or above (Sagili and Burgett, 2011).

Select yield components, including berry number, berry size, and estimated yield were measured in order to evaluate the effects of honey bee activity on blueberry productivity across the sites and years. Average seed number per berry was also determined, as number of healthy, fertile seeds ($\geq$ 1.7 mm in length) is indicative of fertilization and can serve as a proxy for effective pollination in highbush blueberry (Dogterom et al., 2000). Average berry number was estimated for every bush utilized in the study and the equation is provided below (**Equation 1**). Note that the number of fruiting clusters per cane was determined from one randomly selected cane per bush and average berry number per cluster was determined by taking the average of two randomly selected clusters for one cane. Yield per bush was subsequently estimated using the formula provided in **Equation 2**. Average berry weight was determined from a sample of 30 berries randomly collected from the study sites.

Equation 1: Berry # per Bush = (Cane # per Bush) x (# Fruit Clusters per Cane) x (Average Berry # per Cluster)

Equation 2. Estimated Yield = (Berry # per bush) x (Average berry weight)

Summaries of the preliminary data from 2014 and 2015 have been shared with grower cooperators. A survey verifying colony stocking rates, source of honeybees, yields, and patterns of pesticide application during the bloom time was also included in the distributed summaries. We had a response rate of 50% in 2014 and 44% in 2015. We are still waiting for

more survey responses and have sent a follow-up message requesting survey responses to growers that have not yet provided information. Grower confidentiality is and will continue to be maintained throughout the project and presentation of project results. As a result of the low survey response rate, results regarding stocking rates and their relationship to yield components are still preliminary.

Data were first evaluated for normality and homogeneity of variance before being analyzed using analysis of variance (ANOVA). Instances of unequal variance were corrected by taking a log transformation of the response variable. All data presented are reported in their original units. Modeling and analysis of the data was performed using RStudio (RStudio Team, 2015). Tests of significance were done at $\alpha \leq 0.05$ using a least significant differences (LSD) option with a Duncan's test for multiple comparisons. Tests of individual variables' relationship to yield components were assessed by examination of the coefficient of determination (R^2). Individual variables were considered significant at $\alpha \leq 0.05$.

Results and Discussion:

Honey bee visitation rates across sites and years are provided in **Fig. 1**. Sites differed in average honey bee visitation rates, but there was no year effect. Differences based on region (i.e., eastern versus western Washington) and management practices (i.e., organic versus conventional) are presented in **Tables 1 and 2**, respectively. Honey bee visitation rates were consistently greater in eastern Washington relative to western Washington (**Table 1**). Western Washington honey bee visitation rates were consistently below the recommendation of having four to eight honey bees per bush during the warmest part of the day, whereas sites in eastern Washington on average fell within this recommended range (Isaacs et al., 2014). Organically managed sites also had greater visitation rates, but these data must be interpreted cautiously (**Table 2**). Despite our inclusion of more organic sites in western Washington in 2015, elevated visitation rates may still be associated with regional effects given eastern Washington has more organic sites and higher visitation rates relative to western Washington. However, we did observe two sites managed by the same grower in western Washington having consistently greater honey visitation rates in the organic field relative to the conventional field (**Fig. 1**, sites 16 and 17). These data suggest further attention to management effects on honey bee visitation rates is warranted.

Honey bee colony strength, which was measured as the average number of incoming honey bees entering a hive per minute, was greater in 2014 versus 2015 and greater in eastern Washington relative to western Washington (**Table 1**). Colony strength was also observed to be greater in organically managed fields relative to conventionally managed fields (**Table 2**). Data presenting colony strength for all sites and years are presented in **Fig. 2**. Despite regional and management effects, none of the sites met the recommendation of having 100 or more bees enter a hive per minute (Sagili and Burgett, 2011).

Estimated average number of berries per bush and yield are presented in **Figs. 3 and 4**. A year effect was observed, with estimated yield being greater in 2014 relative to 2015 (**Fig. 4**). The greater yield in 2014 is attributed to greater average berry size, despite berry number being greater in 2015 (**Figs. 3** and **5**). No regional nor management effects were detected for berry number or estimated yield. Overall, our observations suggest that estimated yield for these data was largely influenced by size of plant and corresponding productivity. This was mainly suggested in 2014 for site 10, as this site had comparatively large plants that had high yields. However, exclusion of site 10 from the analyses leads to the same results as presented here.

Average berry size across sites and years is presented in **Fig. 5**. As previously indicated, berries sampled for this project were on average larger in 2014 relative to 2015. Berry size was also greater in eastern Washington compared to western Washington in 2014 (**Table 2**). However, this trend did not continue in 2015. Berry size was not influenced by management for any year of the study (**Table 2**). Seeds extracted from berries revealed that eastern Washington had more fertile seeds per berry relative to western Washington (**Table 1**). This is likely attributed to the greater honey bee visitation rates and colony strengths observed in eastern Washington. Regression analyses revealed seed number per berry was positively related to honey bee visitation rates ($R^2 = 0.25$). Seed number per berry was not found to be influenced by management (**Table 2**).

Regression analyses revealed few other statistically significant relationships. Berry size was positively related to colony strength ($R^2 = 0.63$), indicating a stronger/more active hive can promote berry size through enhanced pollination. Berry size also demonstrated a weak, but positive relationship with seed number ($R^2 = 0.16$). The relationship between seed number and berry size varies by cultivar, as some cultivars of highbush blueberry are more-or-less parthenocarpic (Ehlenfeldt and Vorsa, 2007). 'Duke', for example, can produce large berries even if fertilization and seed development is low (Ehlenfeldt and Martin, 2010). Landscape features [e.g., adjacent crop fields (crop types were specified), woodland, urban structures, etc.] were not related to honey bee visitation rates nor colony strength. Yet, it was observed that sites adjacent to wooded areas did have a greater abundance of bumble bees (*Bombus* spp.) in western Washington and these bumble bees were found to forage on blueberry. We observed few *Bombus* spp. in eastern Washington. Although we are waiting for more survey responses to verify stocking rates, we have presently not found a statistically significant relationship between hive stocking densities and visitation rates nor colony strength.

Weather data, specifically average temperatures, solar radiation, and precipitation during the pollination period are presented in **Figs. 6-8**. These data were collected from local WSU weather stations that were in close proximity to the sites of data collection. Averages confined to the pollination period (April-May) and between times of pollination data collection (10 AM to 4 PM) are presented in order to represent average conditions during peak pollinator activity for both years and across all sites. Prosser is located in Benton County, whereas Lynden and Mount Vernon are located in Whatcom and Skagit counties, respectively. The eastern Washington location of Prosser was consistently warmer during the pollination period relative to Lynden and Mount Vernon (**Fig. 6**). Prosser also received move solar radiation and less precipitation (**Figs. 7 and 8**). Combined, these climactic variables are more conducive to honey bee activity relative to western Washington, as honey bees tend to be more active at temperatures $\geq$ 55 °F and under sunny-to-partly sunny conditions with few winds. Precipitation was greatly reduced in 2015 for Lynden and Mount Vernon, but visitation rates were still not influenced by year and this is likely due to temperatures still being too low for optimal honey bee activity in this region (**Figs. 8 and 6**).

Anticipated Benefits and Information Transfer:
This project provided valuable information regarding the status of honey bee activity across Washington State blueberry fields. Moreover, this project provided information about the regional differences and potential influence management may have on honey bee activity. Although most sites included in this project fell below recommendations in terms of honey bee visitation rates and colony strength, results have demonstrated that western Washington has exceptionally low pollination rates and this influences several corresponding variables. Information on how to promote honey bee activity, as well as the activity of other pollinators, is

viewed as being especially important for western Washington growers given the consistent regional effects observed in this study. This project has provided the justification to continue studying ways to promote pollination and corresponding fruit set and yield components for blueberry grown in Washington State. The WSU Small Fruit Horticulture program is currently planning for continued work on this topic and will be submitting research proposals directly related to this endeavor.

Through doing this project, we have also discovered that education about honey bees and other pollinators is lacking. We feel that a better understanding of honey bees and other pollinators will enable growers to take further measures to promote the activity of these organisms in their fields. As such, we have been participating in and coordinating the delivery of this information at various workshops and conferences, including the Small Fruit Conference in Lynden on Dec. 3, 2015. Additional information from this completed project will be shared through bulletins, regional reports, the WSU Small Fruit Horticulture website (http://smallfruits.cahnrs.wsu.edu/). The goal of this information sharing is to help Washington blueberry growers become more knowledgeable about pollination needs specific to their crop and location so that they may ultimately optimize production. The data from this project is also intended to go into a peer-reviewed scientific journal.

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Carrie Scott, Ben Guadagnoli, China Moss, Matt Arrington, Rachel Rudolph, Doug Walsh, Tora Brooks, Yajun Li, and Marina.

Table 1. Regional effects on honeybee visitation rates, hive strength, berry size, and seed number in Washington blueberry, 2014-2015.

	Average no. honeybee visits/min	Average no. honeybees entering a hive/min		Berry size (g)		Average seed no./berry
Location	2014 - 2015	2014	2015	2014	2015	2014 - 2015
East	5.7 a[z]	65 a	27 a	2.02 a	1.04	31.1 a
West	1.2 b	46 b	6 b	1.84 b	1.00	21.3 b
Significance	***	**	***	*	NS	***

[z]Mean separation in columns by Duncan's multiple range test; means with the same letter are not different at $P \leq 0.05$; data are presented by year if a year effect was observed.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Management effects on honeybee visitation rates, hive strength, berry size, and seed number in Washington blueberry, 2014-2015.

	Average no. honeybee visits/minute	Average no. honeybees entering a hive/min		Berry size (g)		Average seed no./berry
Management	2014 - 2015	2014	2015	2014	2015	2014 - 2015
Conventional	1.9 b[z]	48.0 b	10.0 b	1.87	1.02	24.4
Organic	5.4 a	68.7 a	18.3 a	2.08	0.93	26.1
Significance	***	**	*	NS	NS	NS

[z]Mean separation in columns by Duncan's multiple range test; means with the same letter are not different at $P \leq 0.05$; data are presented by year if a year effect was observed.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

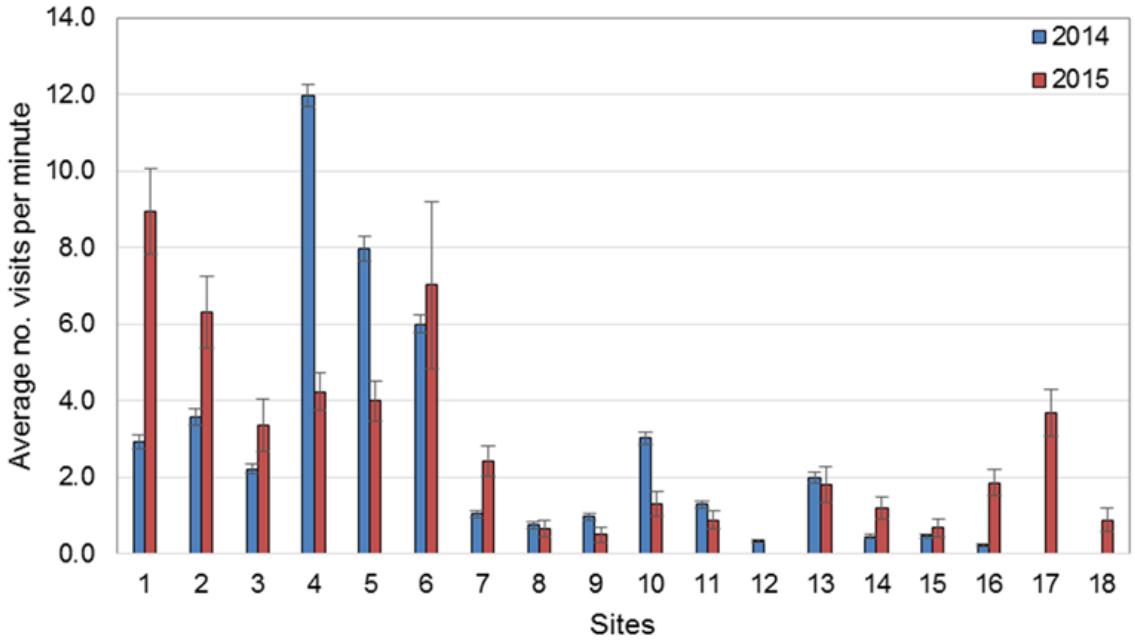

Figure 1. Average number of honeybee visitations per minute in eastern and western Washington blueberry, 2014-2015. Sites 1-6 were located in eastern Washington, while sites 7-18 were located in western Washington. Sites 3-6 and 17-18 were certified organic. No data were collected from sites 17 and 18 in 2014 and site 12 in 2015.

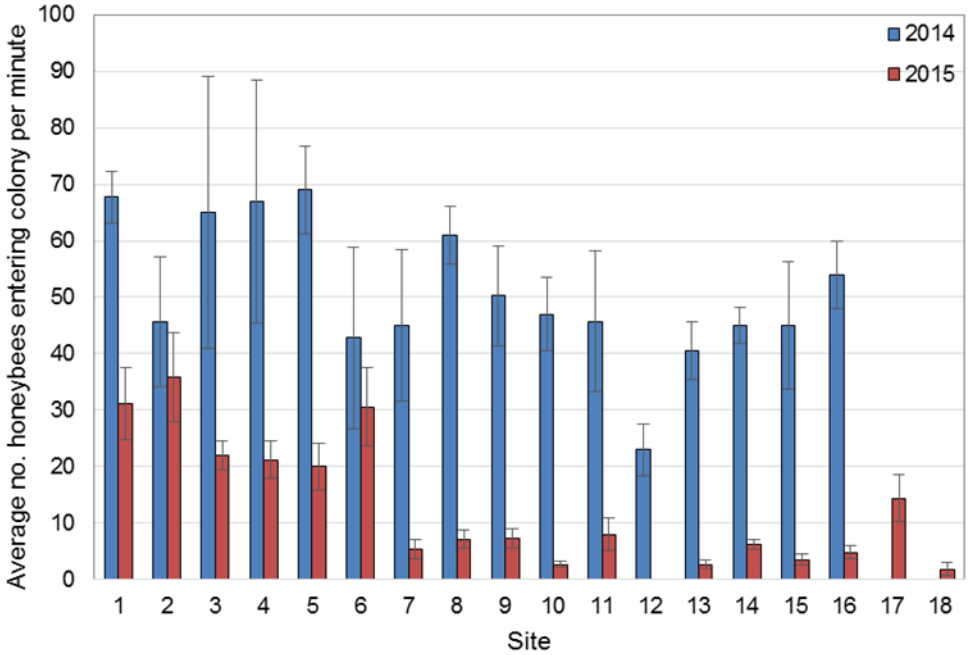

Figure 2. Average number of honeybees entering a colony per minute in eastern and western Washington blueberry, 2014-2015. Sites 1-6 were located in eastern Washington, while sites 7-18 were located in western Washington. Sites 3-6 and 17-18 were certified organic. No data were collected from sites 17 and 18 in 2014 and site 12 in 2015.

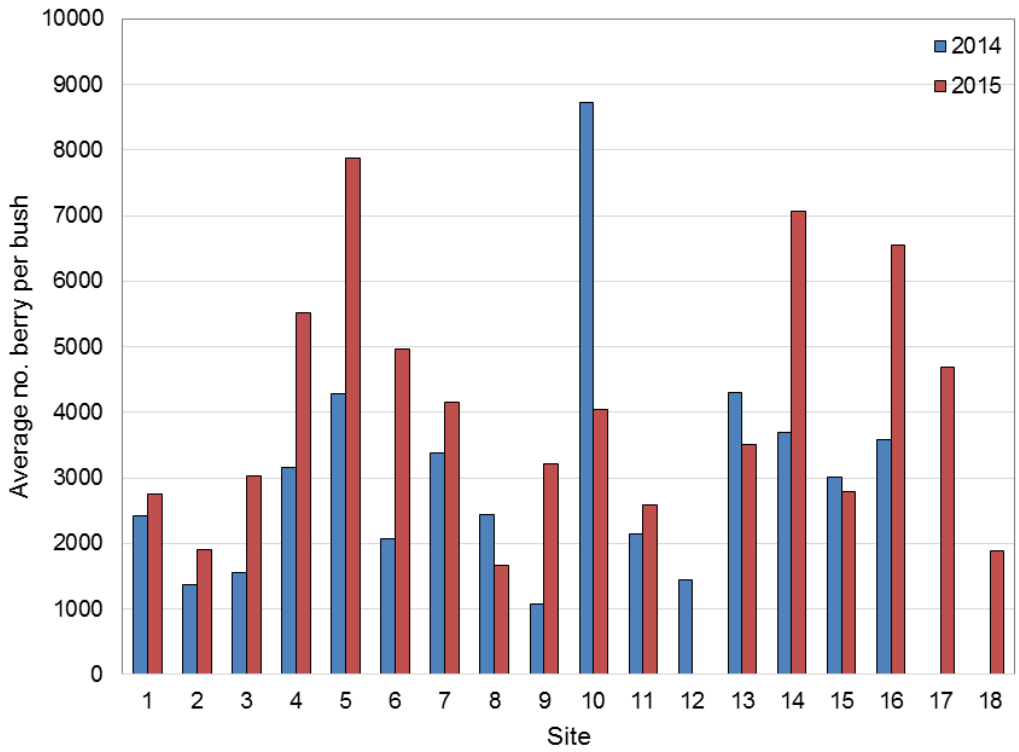

Figure 3. Estimated average number of berries per bush in eastern and western Washington blueberry, 2014-2015. Sites 1-6 were located in eastern Washington, while sites 7-18 were located in western Washington. Sites 3-6 and 17-18 were certified organic. No data were collected from sites 17 and 18 in 2014 and site 12 in 2015.

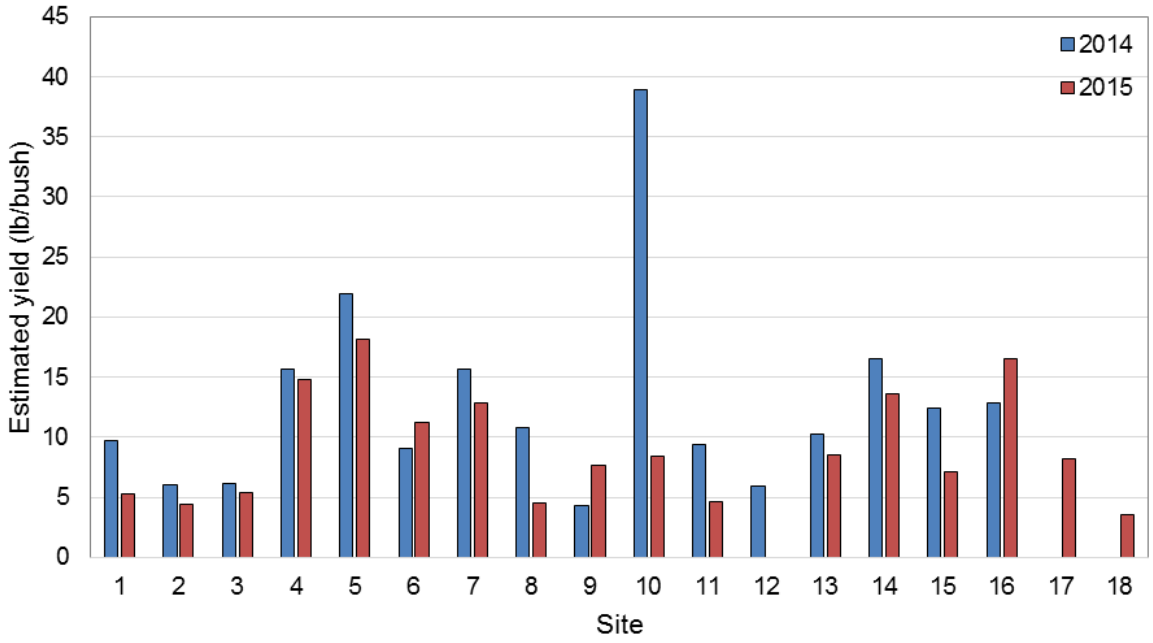

Figure 4. Estimated yield in eastern and western Washington blueberry, 2014-2015. Sites 1-6 were located in eastern Washington, while sites 7-18 were located in western Washington. Sites 3-6 and 17-18 were certified organic. No data were collected from sites 17 and 18 in 2014 and site 12 in 2015.

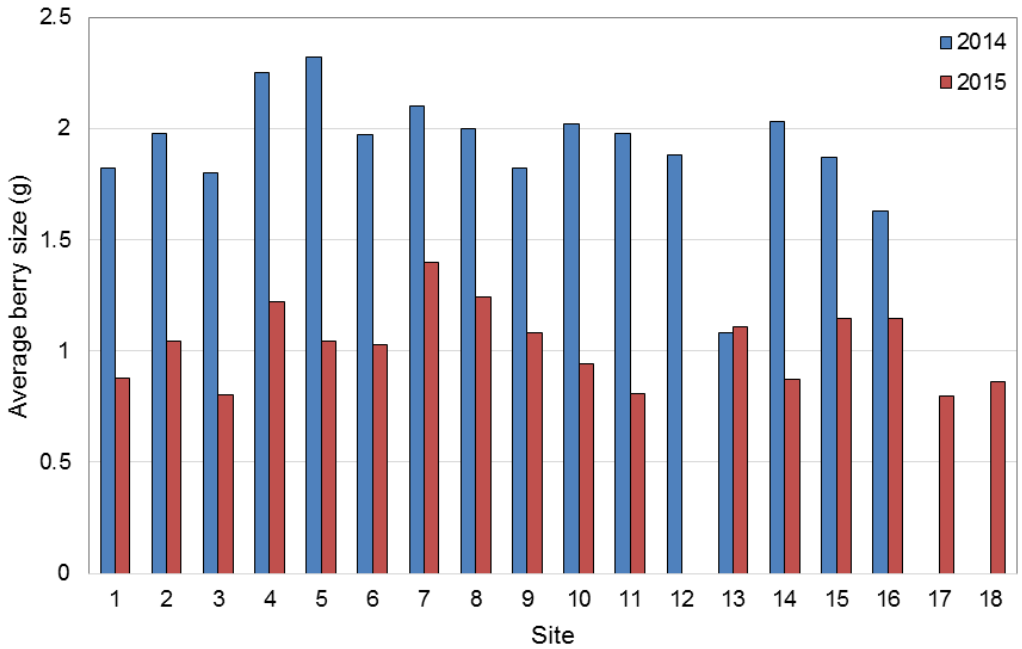

Figure 5. Average berry size in eastern and western Washington blueberry fields, 2014-2015. Sites 1-6 were located in eastern Washington, while sites 7-18 were located in western Washington. Sites 3-6 and 17-18 were certified organic. No data were collected from sites 17 and 18 in 2014 and site 12 in 2015.

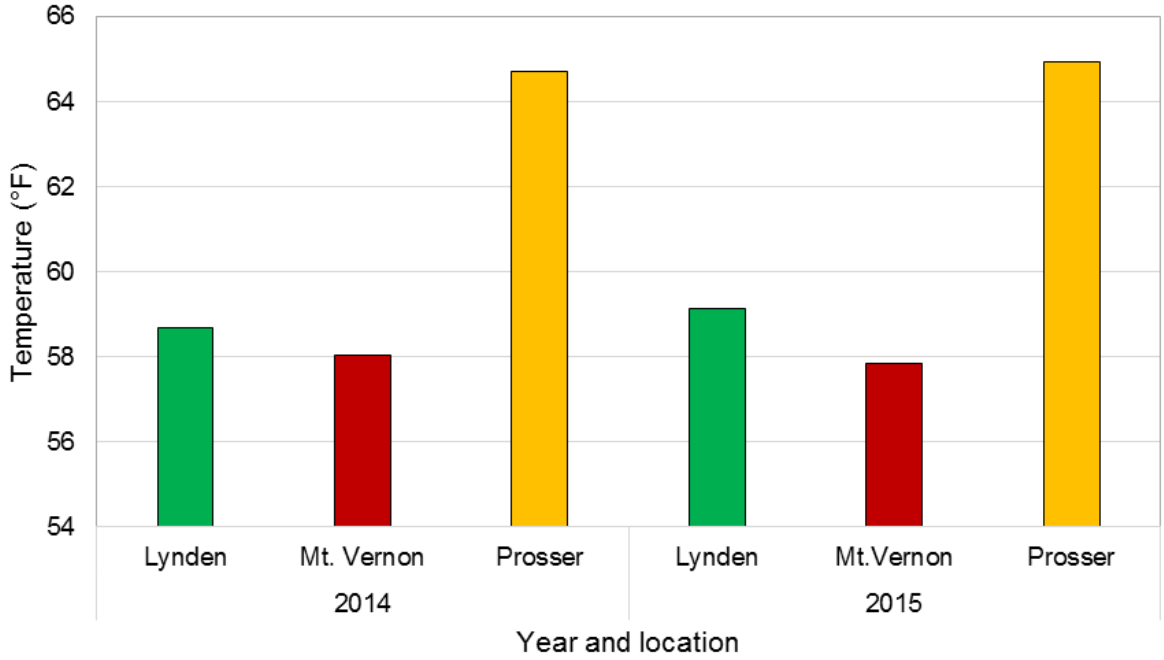

Figure 6. Average temperatures during the blueberry pollination period (April-May) for Lynden, Mount Vernon, and Prosser, Washington, 2014-2015. Presented averages were determined from temperatures recorded during 10 AM to 4 PM, which corresponds to when honeybee visitation data were collected. Data provided courtesy of Washington State University AgWeatherNet. Data are copyright of Washington State University.

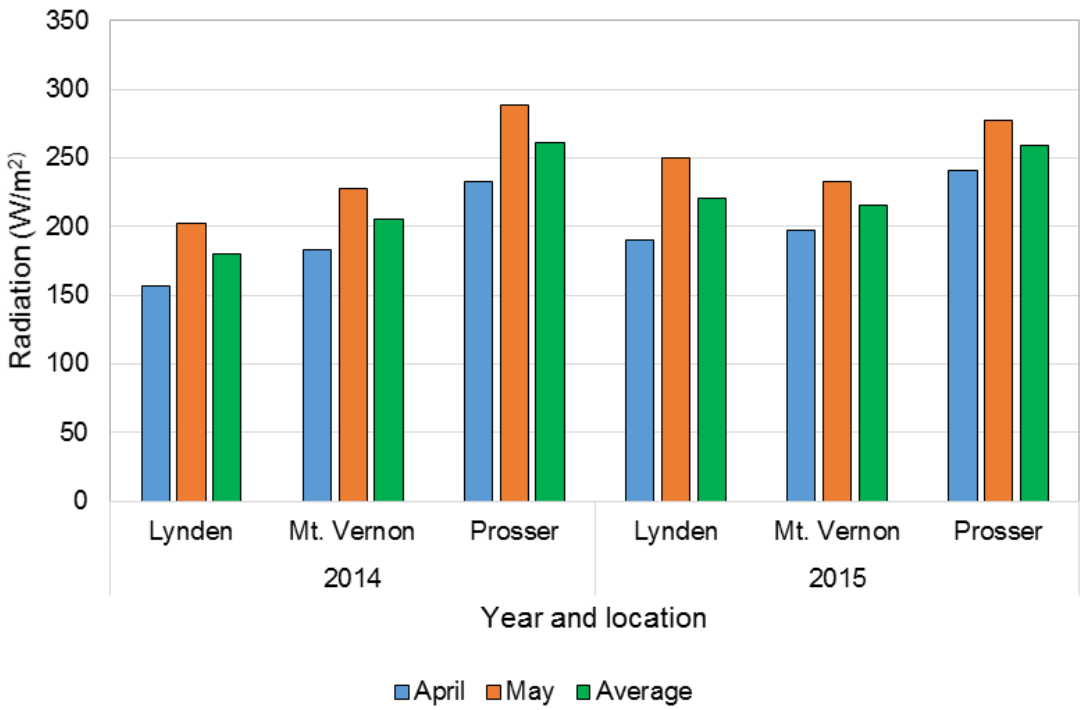

Figure 7. Average solar radiation during the blueberry pollination period (April-May) for Lynden, Mount Vernon, and Prosser, Washington, 2014-2015. Solar radiation data were determined from measurements recorded during 10 AM to 4 PM, which corresponds to when honeybee visitation data were collected. Data provided courtesy of Washington State University AgWeatherNet. Data are copyright of Washington State University.

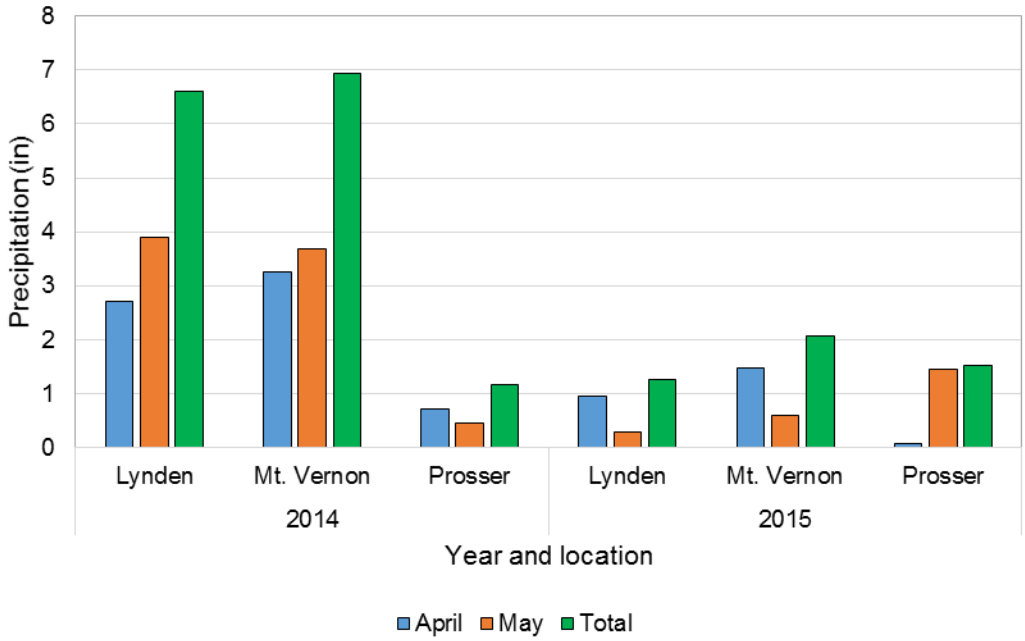

Figure 8. Precipitation during the blueberry pollination period (April-May) for Lynden, Mount Vernon, and Prosser, Washington, 2014-2015. Precipitation data were determined from measurements during 10 AM to 4 PM, which corresponds to when honeybee visitation data were collected. Data provided courtesy of Washington State University AgWeatherNet. Data are copyright of Washington State University.