

Honey Bee Activity in Northern Highbush Blueberry Differs across Growing Regions in Washington State

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Additional index words. *Vaccinium corymbosum*, *Apis mellifera*, fruit set, pollination efficiency, pollination deficit

Abstract. Commercial production of northern highbush blueberry (*Vaccinium corymbosum*) is dependent upon rented colonies of honey bees (*Apis mellifera*) for meeting pollination requirements. Despite the prevalent use of honey bees, growers in Washington State and the greater Pacific Northwest (PNW), particularly those located in the western regions, claim pollination is limited and yield potential is subsequently reduced due to pollination deficits. However, there have been no studies or surveys that document this occurrence for this economically important region of blueberry production. The objective of this study was to survey honey bee activity in commercial plantings of ‘Duke’ highbush blueberry in western and eastern Washington and to assess the relationship between honey bee activity, growing region, and select yield components. Honey bee colony strength was also assessed to evaluate this variable’s relationship to honey bee activity and measured yield components. Sixteen and 18 commercial ‘Duke’ blueberry fields across Washington State were surveyed in 2014 and 2015, respectively. Average number of honey bee visitations per plant and honey bee colony strength were determined to evaluate overall honey bee activity. Estimated yield, berry number per plant, berry size (mass), and seed number per berry were also determined and analyzed to determine their relationship to honey bee activity through regression analysis. Honey bee visitation rates differed between western and eastern Washington, with western Washington sites consistently below recommended honey bee densities. Colony strength was also below recommended levels, but was lower for western Washington relative to eastern Washington. Estimated yield and berry number differed across sites and years, but were not related to honey bee visitation rates. Regression analysis revealed few significant relationships, although honey bee visitation rates were positively related to seed number per berry and seed number was positively related to berry size ($R^2 = 0.25$ and 0.16 , respectively). Berry size was also positively related to colony strength ($R^2 = 0.63$). This study demonstrates that honey bee activity is limited in Washington blueberry production, particularly in western Washington, when compared with recommendations for optimal honey bee activity in blueberry. However, yields were unaffected between the compared regions. The lack of a relationship between honey bee visitation rates and yields suggests that pollination is sufficient for ‘Duke’ blueberry in Washington State and pollination deficits do not limit yield for this cultivar under the conditions of the study.

Commercial production of northern highbush blueberry (*Vaccinium corymbosum*) is concentrated in the PNW, which includes Washington State, Oregon, and British Columbia. Washington State is the leading national producer, representing $\approx 18.6\%$ (4452 ha) of total national cultivated blueberry production in the United States of America in

2015 (NASS, 2016). Despite the significance of the industry, production is reportedly limited by pollination deficits, particularly in western Washington (Washington Highbush Blueberry Commission, personal communication). Effective pollination is essential for optimal fruit set and large berry size in most commercial cultivars of northern highbush

blueberry and is insect mediated (MacKenzie, 1997). Several tactics have been employed in the past to enhance bee pollination in crops including blueberry, where environmental conditions are often suboptimal during bloom or the crop is not attractive to bees (Sagili et al., 2015). Most of the blueberry plantings in Washington and elsewhere employ honey bees (*Apis mellifera ligustica*) for pollination, which are rented from commercial beekeepers and placed in fields at $\approx 5\%$ bloom. These rented colonies are particularly valuable for large plantings given native bee populations and their pollination contributions are relatively low (Isaacs and Kirk, 2010).

Blueberry flowers primarily exhibit protandry, whereby flowers release pollen before stigmas are receptive (Vander Kloet, 1988). Receptivity of flowers to pollination is related to flower age, with receptivity typically limited to 5 d after anthesis (Merrill, 1936; Moore, 1964). These features limit the effective pollination period of blueberry. Pollination and fruit set may be further constrained in Washington due to unfavorable weather conditions that limit pollinator activity. Tuell and Isaacs (2010) demonstrated that foraging activity of honey bees was reduced in Michigan-grown blueberry during poor weather. Honey bee flight and foraging activity begins between 12 and 14 °C, which is frequently not achieved during the typical cool, wet springs in western Washington and the greater PNW (Winston, 1987). Additionally, high wind velocities can further reduce honey bee activity. Pollination declines over time due to poor health of both domestic and wild pollinators is also suspected to contribute to pollination deficits in blueberry. As reviewed by Potts et al. (2010), pollinator health and subsequent crop pollination declines are likely due to an array of integrated and interacting factors, including pests and pathogens [e.g., ectoparasitic varroa mites (*Varroa destructor*)], malnutrition, loss of habitat, decreasing genetic diversity, improper pesticide use in both hives and fields, and climate change.

Research on promoting pollination within highbush blueberry and the variable environmental conditions in which they are produced is limited. Pritts and Hancock (1992) recommended stocking densities of 0.2 to 0.8 healthy honey bee colonies per hectare in northeastern United States. Recommendations from research performed in Oregon range from 0.2 to 1.2 healthy colonies per hectare (Sagili and Burgett, 2011; Strik et al., 2006). In Washington, we have observed stocking densities ranging from 0.4 to 3.2 hives per hectare within a given cultivar. This wide range across farms reflects uncertainty in pollinator management and appears to be the result of a lack of adequate research-based information in formulating recommendations for optimizing pollination. Recommended stocking densities should vary based on cultivar, which differ in their degree of self-incompatibility, attractiveness to pollinators, and flower morphology (Courcelles et al., 2013; MacKenzie, 1997; Strik et al., 2006). Very little is known about cultivar-based honey bee stocking densities

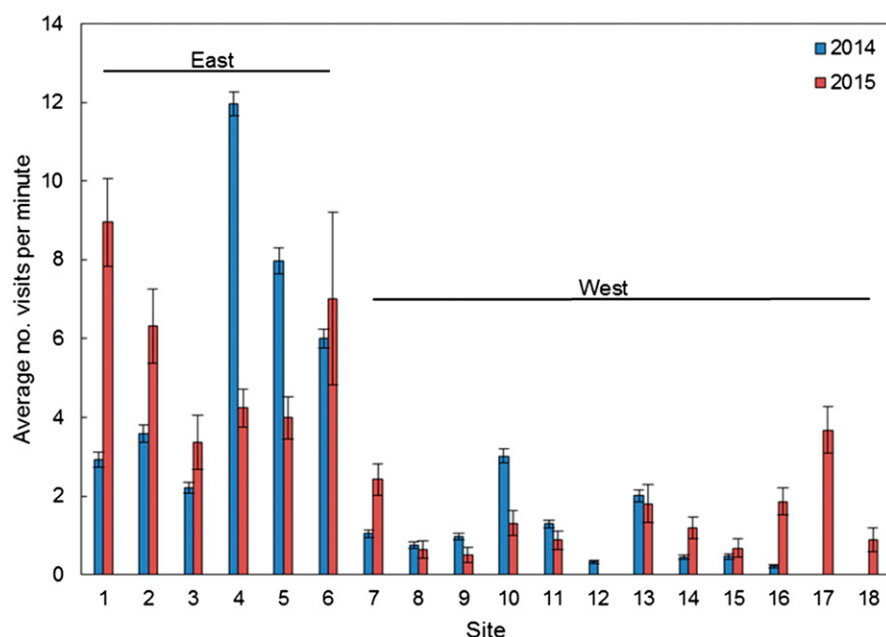


Fig. 1. Average number of honey bee visitations per minute in eastern and western Washington blueberry fields, 2014 to 2015. Sites 1 to 6 were located in eastern Washington, whereas sites 7 to 18 were located in western Washington. No data were collected from sites 17 and 18 in 2014 or site 12 in 2015. Bars represent standard error.

and none of the reviewed recommendations include ‘Duke’, which is one of the most widely planted cultivar in Washington. Furthermore, recommendations may need to be optimized according to climatic conditions due to the influence environmental conditions have on pollination. This observation is relevant to Washington State given the differences between the cool and humid maritime climate of western Washington and the semiarid climate of eastern Washington (DeVetter et al., 2015).

Investigating and developing a comprehensive understanding of the factors limiting pollination and fruit development would be beneficial to the blueberry industry in Washington and the PNW region in general, as it seeks to overcome these limitations and improve yields. The objective of this study was to survey honey bee activity in commercial plantings of ‘Duke’ highbush blueberry in western and eastern Washington and to assess the relationship between honey bee

activity, growing region, and select yield components. Additionally, we also surveyed honey bee colony strength to monitor this variable’s relationship to honey bee activity and measured yield components. Documentation and knowledge of honey bee activity, colony strength, and potential pollination limitations would enable blueberry growers to work with researchers and other entities to refine their approaches to enhance pollination, as well as overall crop yields.

Materials and Methods

Data collection occurred within 16 and 18 commercial grower sites in 2014 and 2015, respectively. All sites included in this study had established ‘Duke’ plants (about 6 years old or older), although the size of the plants themselves varied across sites. Sites were distributed across Washington State and were classified as western or eastern sites depending on whether they resided west or east of the Cascade Range. The reason for this distinction is because of the climatological differences created by the mountain range, which results in different climate, soils, and production practices (DeVetter et al., 2015). Sites were assigned an identification number and this identification was maintained throughout the project. All sites included in the study were a minimum of 3.2 km apart from one another, which helped maintain independence in data collection by ensuring the majority of honey bees being observed were from the hives placed on the specific grower site. Honey bees were stocked according to commercial grower practices and ranged from 0.6 to 2 hives per hectare. Growers rented honey bee (Italian subspecies) colonies

for pollination from the same commercial beekeeper (Belleville Honey & Beekeeping Supply, Burlington, WA) for all sites included in this study, except for sites 10 and 12.

Measurements of honey bee activity followed protocols described by Courcelles et al. (2013). At each site, three 100-m transects proceeding down a row were identified. Within each transect, 10 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 three sites (the grower at site 12 was unable 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 toward the interior of the planting. Honey bee activity was measured three times at each site from 1000 to 1600 hr and when weather conditions were conducive to their activity ($>12.8^{\circ}\text{C}$, with low wind, full-to-partial sun), which minimized variability due to environmental effects and made the data more comparable. Nectar reward production in blueberry is known to peak between 0900 and 1100 hr, but foraging is often limited during morning hours in western Washington due to cool morning temperatures (Rodriguez-Saona et al., 2011). Temperature, precipitation, and radiation (light), data were collected from regional weather stations through the Washington State University (WSU) AgWeatherNet program (<http://weather.wsu.edu/awn.php>; data provided courtesy of WSU AgWeatherNet and are copyright of WSU). Data were recorded throughout the season, but only seasonal averages were determined by location for the extended pollination period of both years (1 Apr. to 31 May; between 1000 and 1600 hr). Honey bee activity was recorded when plants were in 15% to 100% bloom and only “legitimate” visits were measured (i.e., honey bees foraging within flowers and entering through the corolla; no “nectar robbing” whereby honey bees bypass the stigma and anthers to access nectar rewards). Bloom conditions were consistent between eastern and western Washington. Honey bee activity data were collected by counting the number of flower visitations per tagged bush within 1-min intervals, repeated three times per day for 3 d per year. Data were collected on 30 Apr. to 7 May in eastern Washington and 30 Apr. to 15 May in western Washington in 2014. In 2015, data were collected on 16 Apr. to 1 May in eastern Washington and 8 Apr. to 18 May 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 1-min intervals, repeated twice per day for 2 d in 2014 and five times per day for 3 d in 2015. These data were video-recorded so that accuracy could be later verified.

Received for publication 17 May 2016. Accepted for publication 15 Aug. 2016.

This work was funded by the Washington Highbush Blueberry Commission.

We acknowledge the assistance of our grower cooperators and Rachel Weber, as well as Doug Walsh, Tora Brooks, Marina Mireles, and Yajun Li for project assistance in eastern Washington. We also acknowledge Elizabeth Elle for assistance with project design.

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Table 1. Regional effects on honey bee visitation rates, hive strength, estimated yield, berry size, and seed number in Washington blueberry fields, 2014–15. Site location was determined by their relative location east or west of the Cascade Range.

Location	Avg no. honey bee visits/min	Avg no. honey bees entering a hive/min		Yield (kg/plant)		Berry size (g)		Avg seed no./berry
	2014–15	2014	2015	2014	2015	2014	2015	2014–15
East	5.7 ± 0.7 a ^z	65 ± 5.3 a	27 ± 2.7 a	5.2 ± 1.2	4.5 ± 1.1	2.02 ± 0.1 a	1.04 ± 0.1	31.1 ± 1.5 a
West	1.2 ± 0.2 b	46 ± 3.6 b	6 ± 1.1 b	6.2 ± 1.4	3.9 ± 0.6	1.84 ± 0.1 b	1.00 ± 0.1	21.3 ± 1.0 b
Significance	***	**	***	NS	NS	*	NS	***

^zValues are means and standard errors (SE); means with the same letter within a column are not different at $P \leq 0.05$ using Duncan's multiple range test; means were combined across both years when analyses revealed no significant interaction due to year.

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

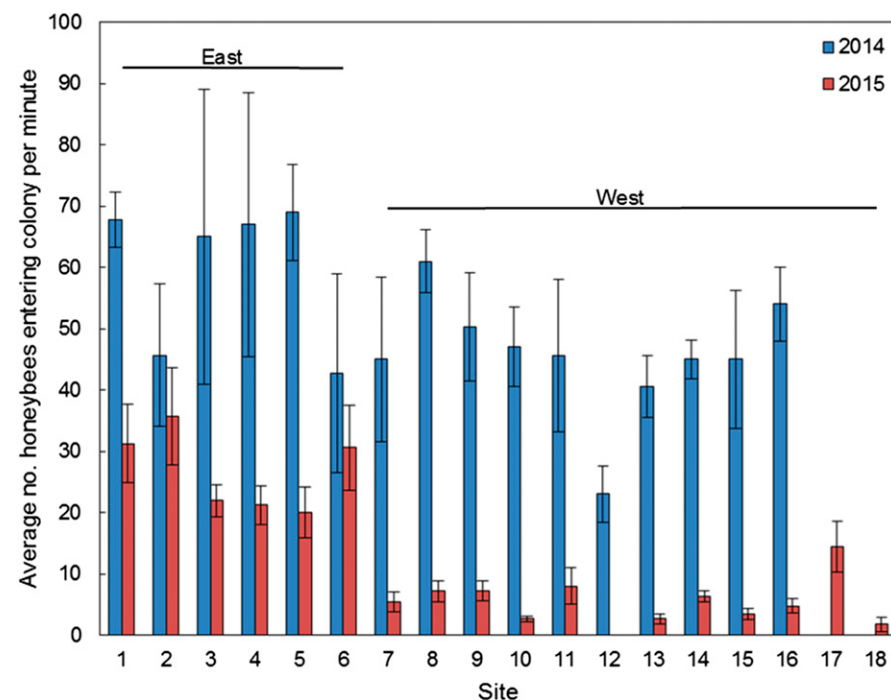


Fig. 2. Average number of honey bees entering a colony per minute in eastern and western Washington blueberry fields, 2014 to 2015. Sites 1 to 6 were located in eastern Washington, whereas sites 7 to 18 were located in western Washington. No data were collected from sites 17 and 18 in 2014 or site 12 in 2015. Bars represent standard error.

Previous reports have indicated that good pollinating colonies have uniform flight and ≈ 100 or more bees return to their colonies per minute when temperatures are 18 °C or above (Sagili and Burgett, 2011).

Select yield components, including berry number, berry size, and estimated yield per bush were measured to evaluate the effects of honey bee activity on blueberry productivity across the sites and years. Average seed number per berry was also determined from a sample of 30 berries per transect (90 berries per site), as number of healthy, fertile seeds (≥ 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). Seeds were extracted by crushing berries partially submerged in 550 mL of deionized water over a fine wire mesh screen placed above a 600-mL beaker for 30 to 45 s with the bottom of a glass beaker (Strik, unpublished data). Seeds that were released in the beaker were collected by decanting the water three to four times per sample, which removed remaining skin and pulp that did not adhere to the mesh

screen. Seeds remaining in the beaker were then washed into a commercial coffee filter placed in a funnel. Seeds were counted after air-drying for a minimum of 48 h.

Average berry number per bush was estimated for every plant included in the study using the equation: Berry number per bush = (cane number per bush) $\times$ (number fruit clusters per cane) $\times$ (average berry number per cluster). 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 by using the following formula: Estimated yield = (berry number per bush) $\times$ (average berry weight). Average berry weight was determined from a sample of 30 berries randomly collected from the study sites immediately before the first harvest. Sampling berries before the first picking has the potential to overestimate average berry weight, as berries from the first harvest tend to be larger than berries from subsequent

harvests, but was a constraint of the experiment due to geography and grower harvest schedules.

Data were first evaluated for normality and homogeneity of variance before being analyzed using analysis of variance. Instances of unequal variance were corrected by taking a log transformation of the response variable. All data presented are reported in their original units. Means were combined when analyses revealed no significant interaction due to year. Modeling and analysis of the data were performed using RStudio (RStudio Team, 2015). Tests of significance were done at $\alpha \leq 0.05$ using a least significant differences 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 differed among sites and regions (i.e., eastern vs. western Washington), but there was no year effect (Fig. 1; Table 1). 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 4 to 8 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). Honey bee colony strength, which was measured as the average number of honey bees entering a hive per minute, was greater in 2014 compared with 2015 and greater in eastern Washington relative to western Washington (Table 1). Despite regional differences, none of the sites met the recommendation of having 100 or more bees enter a hive per minute, indicating colony strength was not optimal according to current guidelines (Fig. 2; Sagili and Burgett, 2011).

Estimated yield was greater in 2014 relative to 2015, with the greater yield being attributed to greater average berry size, despite berry number being higher in 2015 (Table 1; Figs. 3–5). We observed very high yields at Site 10 in 2014, which was attributed to the large size of the plants (Fig. 4). Minimal pruning also likely impacted yield of these plants between years, as unpruned or minimally pruned plants can be induced

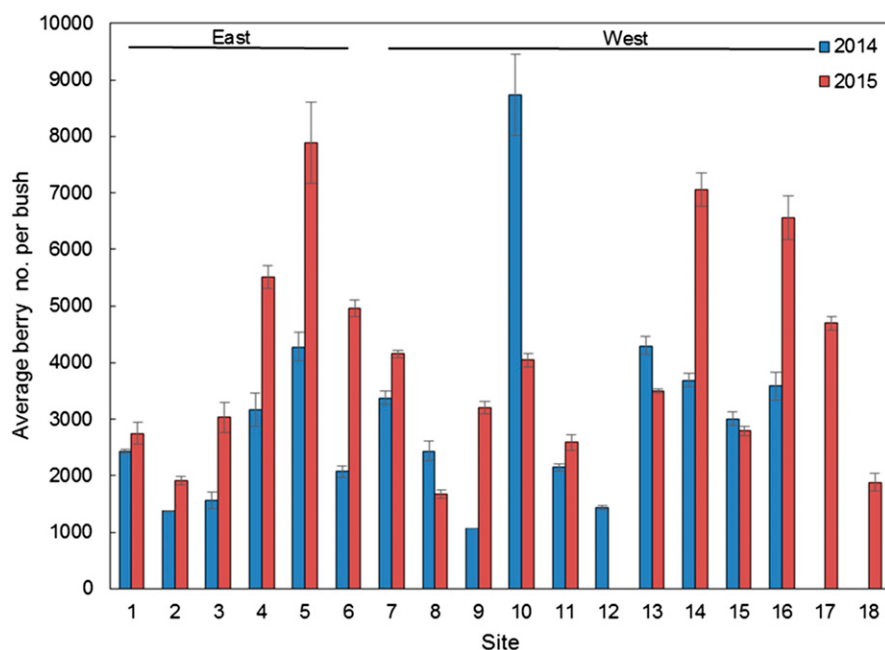


Fig. 3. Estimated average number of berries per bush in eastern and western Washington blueberry fields, 2014 to 2015. Sites 1 to 6 were located in eastern Washington, whereas sites 7 to 18 were located in western Washington. No data were collected from sites 17 and 18 in 2014 or site 12 in 2015. Bars represent standard error.

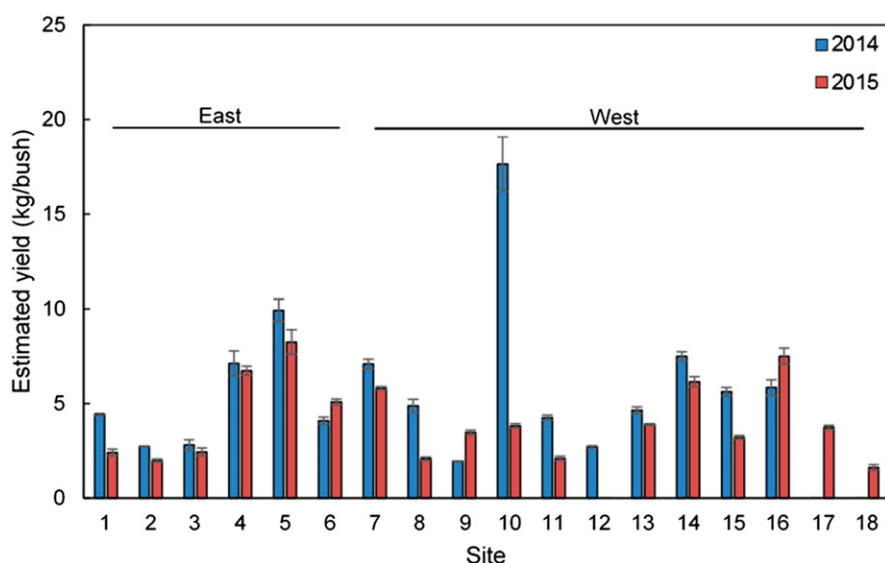


Fig. 4. Estimated yield in eastern and western Washington blueberry fields, 2014 to 2015. Sites 1 to 6 were located in eastern Washington, whereas sites 7 to 18 were located in western Washington. No data were collected from sites 17 and 18 in 2014 or site 12 in 2015. Bars represent standard error.

to bear biennially (Siefker and Hancock, 1987). Despite this, our results for the study were consistent whether Site 10 was included or excluded in the analyses. No regional effects were detected for berry number nor estimated yield (Table 1) and overall estimated yield was not related to honey bee visitation rates ($R^2 = 0.03$). 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 with western Washington in 2014 (Table 1). However, this trend did not continue in 2015. Eastern

Washington blueberries contained more fertile seeds per berry relative to western Washington, which may be attributed to greater honey bee visitation rates and colony strength (Table 1).

Regression analyses indicated that seed number per berry was positively related to honey bee visitation rates ($R^2 = 0.25$). Regression analyses showed few other statistically significant relationships. No relationships were found between hive stocking density and visitation rates or colony strength (data not presented). Berry size was positively related to colony strength ($R^2 =$

0.63), indicating that a stronger and more active hive can increase berry size through enhanced pollination. Berry size also demonstrated a weak, but positive relationship with seed number ($R^2 = 0.16$). This relationship varies by cultivar, as some cultivars of highbush blueberry are more parthenocarpic than other cultivars (Ehlenfeldt and Vorsa, 2007). 'Duke', for example, can produce large berries even if fertilization and seed development is low (Ehlenfeldt and Martin, 2010). The high berry sizing capacity and plasticity of 'Duke', even under conditions of low pollination and fertilization, may partially explain why few differences in yield components were observed in our study. Despite our findings, provision of pollinators is still important for fruit set and berry development in blueberry, particularly for cultivars that have a greater seed set requirement for berry development.

Weather data indicated that the two regions and pollination periods differed with respects to temperature, precipitation, and solar radiation. Temperature varied less across the years, but differed by region. The average temperatures during the pollination period in western and eastern Washington in 2014 were 14.6 and 18.2 °C, respectively, whereas in 2015 they were 14.7 and 18.3 °C, respectively. Precipitation differed across regions and the two seasons, with a drought experienced in western Washington in the 2015 season. Total precipitation during the pollination periods in western Washington was 172 and 42 mm in 2014 and 2015, respectively. Precipitation was 30 and 45 mm in 2014 and 2015, respectively, in eastern Washington. Eastern Washington growers are more accustomed to high temperatures combined with low rainfall and, as a consequence, have established systems of irrigation and evaporative cooling. Solar radiation from 1 Apr. to 31 May was greater in 2015 in western Washington, averaging 218 W/m² compared with 192 W/m² in 2014. Average solar radiation in eastern Washington did not differ across the 2 years and averaged 260 W/m². These environmental variables demonstrate the differences in climate between the two regions. Additionally, these data demonstrate that eastern Washington is environmentally more conducive to honey bee activity relative to western Washington, as honey bees tend to be more active at higher temperatures and only begin foraging between 12 and 14 °C (Winston, 1987). Although precipitation was greatly reduced in 2015 for western Washington, honey bee visitation rates were not influenced by the year and this is likely due to temperatures still being too low for optimal honey bee activity in this region.

This study highlights differences in honey bee visitation rates, colony strength, and potential implications on pollination and corresponding yield components across the diverse growing regions of Washington State. Although colony strength was below recommended levels in both regions, western

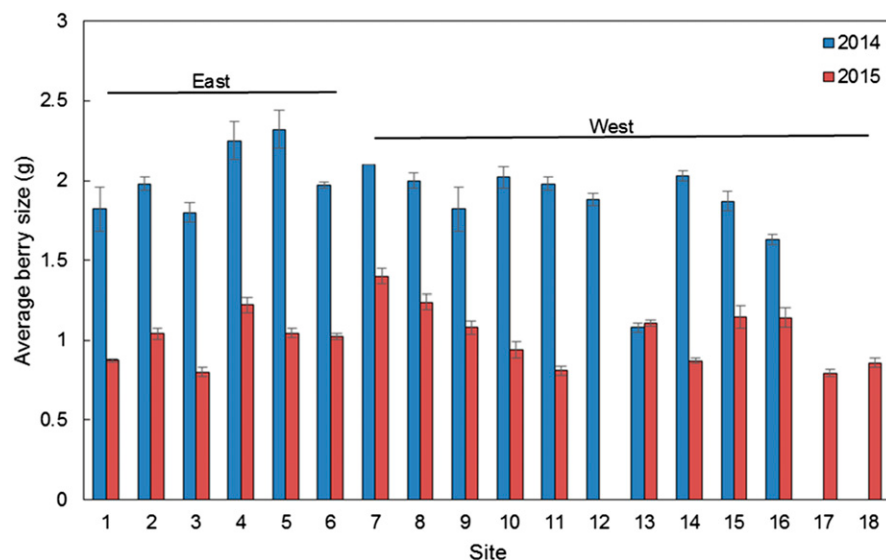


Fig. 5. Average berry size in eastern and western Washington blueberry fields, 2014 to 2015. Sites 1 to 6 were located in eastern Washington, whereas sites 7 to 18 were located in western Washington. No data were collected from sites 17 or 18 in 2014 and site 12 in 2015. Bars represent standard error.

Washington exhibited the greatest number of deficits with regard to honey bee visitation rates and colony strength, which may contribute to reduced seed number per berry and berry size (Table 1). Differences in honey bee visitation rates may also influence yield, which is characteristically lower in western vs. eastern Washington (DeVetter et al., 2015). However, estimated yield of ‘Duke’ did not differ between the regions during the years in which the study was conducted and the relationship between honey bee visitation rates and yield was found to be insignificant. These findings indicate that pollination may, in fact, not be a yield limiting factor for ‘Duke’ grown in western Washington. Other source-sink, climatic, and management factors may be responsible for the regional yield differences observed in other studies (Brady et al., 2015; DeVetter et al., 2015). Pollination deficits may still be an issue for other cultivars of blueberry that have a higher pollination requirement for berry development or are less appealing to pollinators. To better understand and overcome potential pollination deficits in blueberry, future research investigating stocking densities of honey bees and augmentation with alternative pollinators, including native bumble bee (*Bombus* spp.) species, should be explored with other economically important cultivars that exhibit greater sensitivity to pollination and fruit development. Higher stocking densities may help growers achieve greater honey bee visitation/foraging and optimize overall pollination in western Washington. Use and availability of native pollinator species and other honey bee subspecies may also enhance pollination given these insect pollinators have coevolved with blueberry and/or have several

adaptations to effectively pollinate blueberry flowers under a range of environmental conditions (Delaplane et al., 2000). Additionally, the grower community has been experimenting with honey bee pheromones to stimulate honey bee foraging and enhance pollination (Sagili et al., 2015). These practices and their potential utility should be further explored through research.

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