AN ABSTRACT OF THE THESIS OF

Kimberly N. Phillips for the degree of Master of Science in Entomology presented on November 29, 2011.

Title: A Comparison of Bumble Bees (*Bombus spp.*) and Honey Bees (*Apis mellifera*) for the Pollination of Oregon Cranberries (Ericaceae: *Vaccinium macrocarpon*).

Abstract approved:

Sujaya Rao

Abstract. In cranberry (*Vaccinium macrocarpon* Aiton) cultivation, farmers typically rent colonies of honey bee (*Apis mellifera*) for pollination. However, the efficiency of this bee at pollinating cranberries in Oregon, as in other regions, is questionable. Bumble bees (*Bombus* sp.) are reportedly effective in other regions, but their impact in Oregon is unknown. My objectives were to: (i) Compare bumble bee and honey bee pollination efficiencies under caged conditions; (ii) Estimate the abundance of bumble bees, honey bees, and other pollinators on an Oregon cranberry farm; and iii) Analyze and compare sources of pollen collected by bumble bees and honey bees in Oregon cranberries.

In comparing pollination efficiencies of bumble bees and honey bees under caged conditions, the analysis of variance of data from the cage study indicated that bumble bee and honey bee pollinated plants yielded statistically equivalent average numbers of cranberries (1421 ± 302.5 and 1405 ± 347.6 berries/m^2, respectively) and weight of berries (11.5 ± 2.42 and 11.5 ± 2.77g/m^2). However, bumble bees may have increased fruit set in honey bee treatments. On one occasion, bumble bees were found in the honey bee treatment, and may have contributed to the pollination of flowers in these plots.

To estimate the abundance of pollinators, visual observations and were blue vane traps were utilized. Thirty-four timed visual observations in transects of cranberry beds were performed over on four dates during cranberry bloom. Blue vane traps were set-up on five occasions during bloom for two day periods. In the visual observations, honey bees (3.5 ± 0.58/min) were observed more frequently than bumble bees (1.2 ± 0.20/min). Bumble bees of four species made up 69.1% of trapped bees while honey bees made up 16.6% of bees caught in blue vane traps.

On an Oregon cranberry farm during bloom periods in 2009, 2010 and 2011, pollen was collected from honey bee colonies using pollen traps. In 2010 and 2011, pollen was hand collected from reared bumble bee (*B. vosnesenkii*) colonies at the same farm. A total of 2937 honey bee pollen loads and 171 bumble bee pollen samples (137 scopal pollen loads, and 34 samples from with the colony) were individually acetolyzed and compared to a reference collection using light microscopy. Each pollen load was homogenized and 100 pollen grains were identified and counted to determine the percentage of each pollen type. Pollen collected by honey bees consisted of 29.1 ± 1.4% (2009), 18.3 ± 2.4% (2010), and 23.0 ± 1.1% (2011) cranberry pollen. Cranberry pollen contributed a higher percentage (56.0 ± 6.1%, and 70.4 ± 4.3% in 2010, and 2011, respectively) in bumble bee collected pollen. Both bee species collected pollen from non-target plants including those in the following families: Asteraceae, Fabaceae, Ranunculaceae, and Roseaceae. Native bumble bees (*B. vosnesenskii*) collected more cranberry pollen than pollen from non-target plants, and consistently collected a higher proportion of cranberry pollen than honey bees.

The results of these studies suggest that native bumble bees may be adequate for cranberry pollination in Curry County, Oregon. However, the size of bumble bee populations may vary from year to year due to climactic conditions, availability of nesting sites, and forage before and after cranberry bloom. Thus the dependability and consistency pollination services rendered to cranberry crops by bumble bees needs to be further investigated in relation to population fluxuations.

A Comparison of Bumble Bees (*Bombus spp.*) and Honey Bees (*Apis mellifera*) for the Pollination of Oregon Cranberries (Ericaceae: *Vaccinium macrocarpon*)

by
Kimberly N. Phillips

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APROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Kimberly N. Phillips, Author

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CONTRIBUTION OF AUTHORS

Dr. Rao helped with experimental design, writing and statistical analysis in Chapters 2 and 3. Dr. Stephen assisted with experiment set-up, design and bee identification. Linda White helped with field site selection, grower cooperation and data collection.

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CHAPTER 1

Comparison of honey bees and bumble bees for the pollination of Oregon cranberries

Introduction

Cranberries (Ericaceae: *Vaccinium macrocarpon* Aiton), one of a few commercially produced berry crops native to North America, are grown on 38,200 acres in the Unites States, 4,000 of which are in the Pacific Northwest region (NASS, 2008). The crop is valued at approximately $444 million annually (NASS, 2008).

Cultivation of cranberries involves the construction of below-grade beds surrounded by dikes to facilitate flood harvesting. The low-growing, and long-lived perennial plant establishes an evergreen mat. In Oregon, cranberries typically bloom in spring, over a six week period fromn late May or early June to early July. The flowers are pendant consisting of four basally fused pink or green sepals, four white to pink unfused petals, and eight brown-orange porose anthers forming a ring around a single pistil. The anthers of cranberries, like many Ericaceous plants, are porose, and release pollen most readily when vibrated, or buzz pollinated, at a frequency of 262 Hz (the musical note Middle C) (Buchman, 1983; Loose et al., 2005). The heavy, sticky pollen grains are not produced singly; rather they form groups of four in a tetrad configuration. Cranberry flowers form on upright branches of the mat-forming plant. There are typically five to seven solitary flowers per flowering upright (Eck, 1986; Brown and McNeil, 2006), and there are typically 4,306 to 5,382 uprights per square meter in Oregon 'Stevens' cultivar cranberries (Roper, 2006). It is typical, and not necessarily a sign of insufficient pollination, for only 1-3 flowers per upright to set fruit (Brown, 2006). Nevertheless, insufficient pollination is often a challenge for cranberry growers to overcome, including in Oregon (Eck, 1990; MacFarlane and Patten, 1997). A cranberry flower is sufficiently pollinated in terms of subsequent percent fruit set and berry mass when eight or more pollen tetrads are deposited on the stigma, and 16 tetrads in terms of seed set (Cane and Schiffhauer, 2003). In a greenhouse experiment, during individual visits by pollen foraging honey bees in which stigmatic contact was made, Cane and Schiffhauer found that 10 pollen tetrads were typically deposited (2003). However, in a study in Massachusetts and New Jersey cranberry farms, pollen foraging honey bees consisted of only 2-20% of honey bee foragers observed (Cane et al., 1993).

A study by Evans and Spivak (2006) showed that, in Wisconsin rented honey bees introduced to cranberry farms during bloom increased yield. However, honey bees may not be the ideal pollinator for this crop in Oregon due to climactic conditions. Honey bees tend to forage less frequently in temperatures cooler than 21 ˚C, in rain, high winds, or on cloudy days (Burril and Dietz, 1981; Broussard et al., 2011). In this region, these unfavorable conditions are common during cranberry bloom; on average 18 days in May, 11 days in June and 3 days in July are rainy, and average daily high temperatures are below 21 ˚C for all three months (Taylor et al., 2011). Additionally, because they do not buzz pollinate, honey bees are inefficient movers of pollen on flowers with porose anthers, including cranberries (Buchman, 1983). Honey bees are also becoming more difficult for Oregon growers to rent because of decreasing availability and increasing costs (K. Andersson, personal communication).

Bumble bees (*Bombus* spp.) may be a viable alternative for cranberry pollination in Oregon and elsewhere. Being a native organism, bumble bees found in coastal Oregon are adapted to prevailing weather conditions more so than the non-native honey bee. Bumble bees transfer more pollen (60 tetrads) than honey bees (10 tetrads) per visit to a cranberry flower, though both amounts are sufficient for satisfactory fruit set (Cane and Schiffhauer, 2003). The larger number of pollen grains transferred by bumble bees is likely a result of several characteristics exhibited by bumble bees; their large size and thick pile compared to honey bees, their tendency to forage in cooler conditions (Broussard et al., 2011), and their ability to buzz pollinate and extract large amounts of pollen from porose anthers (Cane et al., 1993).

In the study conducted in Wisconsin, Evans and Spivak (2006) found that rented honey bee colonies were necessary during peak cranberry bloom to deposit the requisite eight pollen tetrads, but during early and late cranberry bloom native bees were sufficient. Besides the number of pollen grains deposited in bumble bee visits, little is known about how bumble bees compare to the more traditional pollinator of cultivated cranberries, particularly in Oregon. Hence, my objectives were to compare bumble bees and honey bees for the pollination of cranberries in Oregon.

In Chapter 2 of this thesis, using cages to control access to plants, the differences in cranberry fruit set when plants were pollinated exclusively by either honey bees or bumble bees were investigated. The fruit set induced by each type of pollinator was compared to that of two controls; no pollinators and all pollinators. The relative abundance of each pollinator was also investigated on the research site using blue vane traps and observational counts of foragers.

In Chapter 3, the pollen foraging habits of uncaged honey bees and bumble bees are described. The goal with this study was to compare the proportion of cranberry pollen collected by each pollinator, and to discover what non-target plants were attracting their pollen foraging attention. The pollen used in this study was collected from foragers as they returned to their hives and nest boxes rather than from foragers on flowers. Chapter 4 interprets and integrates the findings of both studies and explores possible implications for growers and policy makers.

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CHAPTER 2

Comparison of pollination by bumble bees and honey bees in Oregon cranberries

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Abstract

Cranberries (*Vaccinium macrocarpon* Aiton) require bee pollination for fruit-set, for which farmers typically rent honey bees (*Apis mellifera*). However, their efficiency at pollinating cranberries in Oregon, as in other regions, is questionable. Bumble bees (*Bombus* spp.) are reportedly effective in other regions, but their impact in Oregon is unknown. The objectives of this study were to: (i) Compare bumble bee and honey bee pollination efficiencies under caged conditions; and (ii) Estimate their abundance on an Oregon cranberry farm. In comparing pollination by bumble bees and honey bees under caged conditions, the analysis of variance of data indicated that bumble bee and honey bee pollinated plants yielded statistically equivalent average numbers of cranberries (1421 ± 302.5 and 1405 ± 347.6 berries/m^2, respectively) and weight of berries (11.5 ± 2.42 and 11.5 ± 2.77g/m^2). However, bumble bees may have increased fruit set in honey bee treatments. On one occasion, a bumble bee was found in the honey bee treatment, and may have contributed to the pollination of flowers in these plots. Thirty-four timed visual observations in transects of cranberry beds were performed over on four dates during cranberry bloom. In the visual observations, honey bees (3.5 ± 0.58/min) were observed more frequently than bumble bees (1.2 ± 0.20/min). . Blue vane traps were set-up on five occasions during bloom for two day periods. Bumble bees

of four species made up 69.1% of trapped bees while honey bees made up 16.6% of bees caught in blue vane traps. This study indicates that native bumble bee populations may be adequate for Oregon cranberry pollination. However, populations vary from year to year so conservation programs may be needed to ensure their sustainability.

Introduction

Cranberries (*Vaccinium macrocarpon* Aiton; family Ericaceae) are grown in numerous regions in North America, including Massachusetts, New Jersey, Wisconsin, Prince Edward Island, New Brunswick, Newfoundland, Quebec, Ontario, British Columbia, Washington, and Oregon (Agriculture and Agri-Food Canada, 2009; United States Department of Agriculture, 2011). The plant is a low-growing evergreen which establishes a mat. This long-lived perennial crop is grown in sand, peat, or other organic material, below grade, and surrounded by ditches and dikes. In Oregon, cranberries are raised on approximately 1,100 hectares along the southern coast in Coos and Curry counties (NASS, 2008).

Oregon cranberry growers face challenges in achieving successful pollination of their crops. Cranberry plants require deposition of at least 8 pollen tetrads by insect visitation for sufficient fruit set (Eck, 1990; Cane and Schiffhauer, 2003; Brown, 2006). Like many producers of pollination-dependant crops, cranberry growers depend upon the services of the European honey bee (*Apis mellifera* L.) (Eck, 1990). However, a study in Wisconsin found that the presence of honey bees does increase yield, especially when the cranberry farm is large or surrounded by other agricultural lands (Evans and Spivak, 2006). Perhaps because pollen from cranberry flowers is largely inaccessible to honey bees (Cane et al., 1993; Cane and Schiffhauer, 2001), honeybees may not the most effective pollinator of cranberries. The heavy, sticky cranberry pollen is released in tetrads from a single terminal pore in each anther (Buchman, 1983; Eck, 1986; Eck, 1990). Porose anthers tend to release more pollen when buzz pollinated, a behavior which bumble bees (*Bombus* spp.) exhibit and honey bees do not (Buchman, 1983; Cane et al., 1993; Cane and Schiffhauer, 2001). Honey bees forage on cranberries more frequently for nectar than for pollen (Cane and Schiffhauer, 2001). Nectar foraging bees do not typically deposit enough pollen for sufficient fruit set, and this has raised questions about the efficiency of honey bees in pollination of cranberries (Cane and Schiffhauer, 2001).

Climatic conditions also affect the performance of honey bees. They prefer to forage at temperatures higher than those typically experienced during cranberry bloom in Oregon (Burrill and Dietz, 1981; Broussard et al., 2011). Here, cranberries bloom over a six-week period between mid-May and mid-July, during which the southern Oregon coast experiences cool, windy, cloudy and rainy weather conditions. Average rainfall in May, June and July is 3.85 inches, 2.11 inches and 0.63 inches, respectively (Taylor et al., 2011). There are an average of 18.1 days of rain in May, 10.5 in June and 3.3 days in July (Taylor et al., 2011). Mean high temperatures for May, June and July are 17, 18 and 20 °C, respectively (Taylor et al., 2011). Due to the limited number of days when conditions favor foraging by honey bees, growers rent 1 or more hives per hectare. However, the availability of honey bees has decreased while the cost of hive rentals has increased in recent years, possibly due to diseases caused by the tracheal and Varroa mites, and Colony Collapse Disorder. Hence, there is a need for alternative pollinators for Oregon cranberries.

In contrast to honey bees, bumble bees, are known to forage in cool, wet conditions. Broussard et al. (2011) correlated temperatures during Oregon cranberry bloom with foraging behaviors and found most bumble bees foraged at temperatures between 18.3 and 22.2 °C while honey bees foraged at 21.1 to 26.7 °C. Additionally, bumble bees have large, fuzzy bodies that hold copious amounts of pollen (Cane and Schiffhauer, 2003). In a typical visit, bumble bees will deposit 60 pollen tetrads while honey bees only deposit 10 (Cane and Schiffhauer, 2003).

In regions where bumble bee populations are low, growers can use *B. impatiens* Cresson, which is available commercially, for cranberry pollination. However, Oregon prohibits the introduction of non-native bumble bee species (Oregon Department of Agriculture, 2011), and hence growers in this state cannot use *B. impatiens* for cranberry pollination. Bumble bees are the most frequently observed native visitor to cranberry flowers in Oregon (Broussard et al., 2011). Four native bumble bee species are commonly observed on the southern Oregon cranberry farms (from most frequent to least): *B. vosnesenskii* Radoszkowski, *B.*

mixtus Cresson, *B. melanopygus* Nylander, and *B. caliginosis* Frison, (Broussard et al., 2011). However, their impact on cranberry pollination in Oregon is not known.

Although, bumble bees in other cranberry growing regions have been shown to be highly efficient, little is known about the population size in this region. Thus their efficiency could potentially be made irrelevant if their numbers are insufficient. Doehlert (1940) estimated that 0.118 bumble bees per m^2 is enough to provide sufficient pollination to cranberries. Only one study (Broussard et al., 2011) estimated bee abundance in Oregon, but more data would increase understanding of the current and potential impact of native bees on cranberry crop pollination.

The current study was conducted to determine whether bumble bees are a viable alternative to honey bees for cranberry pollination in Oregon. My objectives were to: (i) Compare the pollination of cranberry flowers by bumble bees and honey bees under caged conditions; and (ii) Estimate the abundance of bumble bees and honey bees on an Oregon cranberry farm.

Materials and Methods

Study site. A 65 hectare cranberry farm located south of Langlois in Curry County, Oregon, was selected for this study. Cranberry beds at this farm are typically 200m X 50m and are constructed with surrounding dikes approximately 3m wide and 1.5-2m tall. Habitat surrounding this farm is largely undeveloped forest land dominated by Douglas fir (*Pseudotsuga menziesii*) and shore pine (*Pinus contorta*). During this study, the grower rented 1 hive/hectare for cranberry pollination.

Comparison of the pollination efficiencies of bumble bees and honey bees under caged conditions. The experiment was set up as a randomized block design with four replications over two adjacent cranberry beds. Nine sturdy cages, 1m X 1m X 1m, constructed with polyvinyl chloride pipes and covered with amber Luminite screen (BioQuip Products, Rancho Dominguez, CA) were placed over cranberry plants ('Stevens' cultivar) on May 14, 2009, prior to bloom. The edges of the screen material were tightly secured to the ground using tent stakes. The following treatments were compared in 2009: 1) bumble bee, *B. vosnesenskii* (one colony per cage); 2) honey bee (one 3-frame nucleus per cage); 3) closed (no bees added); and 4) open pollinated (plots that were 1m X 1m, staked in un-caged cranberry). Locally captured queens of *B. vosnesenskii* were reared at Oregon State University, and colonies of approximately 20 individuals were used for the study. The honey bees in this study were reared in Corvallis, Oregon, and consisted of approximately 1000 individuals per hive. On May 27, 2009, at 10% bloom, honey bee hives or bumble bee nests were directly connected to the cages using tubing through a zippered opening in the cage. On the assumption that $1m^2$ of cranberry plants would not provide enough forage for one colony of bumble bees or honey bees, the study design allowed the bees to forage both from within the cage area and in the surrounding vegetation. All bumble bee and honey bee colonies had two openings; one to the interior of the cage and one to the outside the cage. Closed treatments were zipped closed to prevent floral visitation by insects. The open pollinated plots were likely visited not only by honey bees and bumble bees, but by other native pollinators as well. Bloom within the cages lasted longer than bloom outside the cages. Outside the cages, 1% of flowers remained on July 8, 2009. However, inside the cages, flowers remained viable until July 22, 2009 at which point, the cages were removed.

Cages were monitored 1-2 times per week, and when no bumble bees were observed within their respective cages, three wild bumble bees were introduced into the cage. On one occasion each, one bumble bee was found in a honey bee cage and a closed cage. Upon discovery of each offending bumble bee, it was promptly removed. No attempt was made to mimic honey bee and bumble bee concentrations found outside the cage since the objective was primarily to evaluate whether each bee species was able to pollinate cranberries in Oregon.

To evaluate pollination efficiency in each treatment, all berries were hand-picked within a 0.09 m^2 square shape centered in the middle of each plot. Later, these berries were weighed and counted to determine yield (eg. g/m^2 and number of berries/m^2). Though g/m^2 and berry count/m^2 are the typical indicators of good quality pollination and fruit set, successful pollination can also result in larger fruit and high numbers of seeds (Cane and Schiffhauer, 2003; Ratti et al., 2008). Thus, random sub-samples from each plot were taken from the previously picked berries to measure berry size (n=25), and count the number of seeds/berry (n=12). Berry diameters were measured using calipers in two dimensions: pole to pole (blossom end to pedicel end) and around the largest part of the equator perpendicular to the first measurement. Berry size was calculated as the volume of an ellipsoid.

Supplementary pollination prevention. Additionally, the following year, in May 2010, 13 individual, unopened, virgin cranberry flowers were bagged in glassine envelopes to prevent pollinator visitation, and inspected for fruits during the harvest period (October, 2010).

Estimation of the abundance of bees on cranberry farms. To estimate the relative abundances of bees on the cranberry farm, the following two methods were used: 1) trapping, and 2) visual observations.

Trapping: Four blue vane traps (Spring Star LLC, Woodinville, WA) that have been observed to be effective for recording the presence of native bees (Stephen and Rao, 2005), were placed on 1m metal posts at the research site. These traps were set-up five times during bloom for two day periods on June 10, June 19, June 24, July 1, and July 8, 2009. The captured bees were collected, frozen, and later identified to species in the case of *A. mellifera, Bombus, Agapostemon*, and *Halictus*; and to genus in the case of other bees.

Visual observations: The numbers of bees on cranberry flowers were estimated during a timed period while walking slowly along a 50m transect of a cranberry bed and counting all pollinators observed within a 1m wide swath of cranberries. For the first week of the study five two-minute visual counts were performed in each of the two cranberry beds, but for every subsequent week, four five-minute counts were performed in each bed. Observations were converted to number of bees/minute. Visual observations occurred June 10 (5, 2-minute), June 24 (4, 5-minute), July 1 (4, 5-minute) and July 7 (4, 5-minute), 2009 between 1100 HR and 1430 HR.

Statistical analysis. The data on berry yield, weight, and seed number required no transformations and were analyzed by one-way analysis of variance (ANOVA). Because of unequal variance among the treatments, the non-parametric Kruskal-Wallis test was used for comparing berry size. Means were separated using Tukey Kramer test and differences accepted at $\alpha \leq 0.05$.

Walking count data for honey bees and bumble bees was square-root transformed and analyzed using a paired t-test. All analyses were performed using ‘R’ (R development Core Team, 2011) v. 2.13.0.

Results

Comparison of pollination by bumble bees and honey bees under caged conditions.

While monitoring the cages, bumble bees were observed in both the honey bee and the control cages on one occasion each even though the cages were tightly secured to the ground with many stakes on every side. The bumble bees were removed as soon as they were noticed.

Yield: There was a significant difference in berry yield (g/ m^2) across the four treatments (F = 4.25; df = 3, 12; p = 0.0290) (Figure 2.1). A Tukey-Kramer pair-wise comparison indicated one significant difference in yield among treatments; open pollinated plots yielded, on average, 13.5 g more berries than the closed plots (95% C.I 1.66 to 25.3). However, the yields in the honey bee, bumble bee, and closed cages all produced statistically equivalent yields.

Berry counts: The ANOVA indicated that there was a significant difference in the numbers of berries (berries/m^2) among treatments (F = 5.50; df = 3, 12; p = 0.0130) (Figure 2.2). One significant difference among treatments was detected in berry number; there were 1520 more berries in the open pollinated compared with the closed cages (95% C.I. 340.2 to 2701). However, the berry counts in the honey bee and bumble bee cages did not differ statistically from each other nor did either differ from closed pollinated treatments.

Berry size: The non-parametric Kruskal-Wallis indicated that there were significant differences in the size of berries across the four treatments ($p < 0.001$) (Figure 2.3). Closed pollinated plots had significantly smaller berries (829 mm^3) than any other treatment. Berries from open pollinated plots (1170 mm^3) were significantly larger than those in the bumble bee cages (1000 mm^3) but not those in the honey bee cages (1080 mm^3). However, the sizes of berries in the bumble bee and honey bee pollinated cages were not significantly different.

Seed counts: The ANOVA indicated that there was a significant difference in the numbers of seeds/berry ($F = 2.83$; df = 3, 187; $P = 0.0399$) (Figure 2.4). However, Tukey-Kramer pair-wise comparisons revealed no significant differences in seed number between any two treatments at a confidence level of $\alpha = 0.05$ (all 95% C.I.s bracketed zero). However, at a confidence level of $\alpha = 0.10$, differences between open plots and bumble bee pollinated plots as well as between open and closed plots emerged. Berries from open pollinated plots had on average 2.9 more seeds than those in bumble bee pollinated plots (90% C.I. 0.27 to 5.5). Closed pollinated plots had 2.8 less seeds per berry than open pollinated plots (90% C.I 0.14 to 5.4).

Supplementary pollination prevention. Of the 13 individually bagged flowers from 2010, none produced fruits.

Estimation of the relative abundance of bees on cranberry farms.

Trapping: Overall, 755 bees were caught in the traps (Table 2.1). Of these, bumble bees (522) comprised 69.1% of the bees, while honey bees (125) comprised 16.6% of the trapped bees. Bumble bee species present included: *B. vosnesenskii* (304), *B. mixtus* (99), *B. melanopygus* (84), and *B. calginosus* (35). Other native bee genera caught in the traps include *Lassioglossum* (52), *Agapostemon* (31), *Mellissiodes* (16), *Colletes* (5), *Halictus* (3), and *Megachile* (1). The halictids *Lasioglossum* and *Agapostemon* comprised 11.0% of bees caught. Besides bees, 22 other insects were caught including: Diptera (8); Opiliones (4); Hymeoptera (4): (Vespidae (3) and Formicidae (1)); Lepidoptera (2); Coleoptera (2); and Dermaptera (2).

Visual observations: In the 34 visual observations made in the study, there were 0.24 more honey bees observed per minute than bumble bees (95% C.I. 0.025 to 0.68, p = 0.00525, df = 33) (Figure 2.5). Other pollinators observed (0.088 ± 0.032 per minute) were syrphid fly adults and native bees belonging to the family Halictidae. Due to their small size and quick flight it was not possible to identify these to the genus level.

Discussion

This is the first study that documents pollination services under caged conditions provided in cranberries by a non-commercial, western native bumble bee species. Despite the differences in the number of bees per colony/hive, there was no difference in yield, berry number and size and seed count between the bumble bee and the honey bee cages. The study suggests that the native *B. vosnesenskii* may be an efficient pollinator of cranberries.

There were difficulties in assuring the integrity of some of the caged treatments. Because of the unexpectedly high pollination rates observed in all repetitions of the closed cages, bumble bees could have entered the cages more often than they were detected, and this may be due to their tendency to crawl between cranberry flowers (and under cages) (personal observation, unpublished data). The presence of the intruding bumble bees likely served to enhance the apparent pollination success of the honey bee and the closed treatments.

To determine if cranberries can be pollinated without bees, 13 virgin unopened cranberry flowers were individually bagged, on the same farm in May 2010, and inspected them for fruits during the harvest period (October, 2010). None of the 13 bagged flowers produced fruit, thus corroborating the widely understood need for cranberry flowers to receive insect pollination to produce fruit (Eck, 1986, 1990; Marucci, 1997; Cane and Schiffhauer, 2003; Loose et al., 2005; Brown and McNeil, 2006; Evans and Spivak, 2006; Ratti, 2008).

The open pollinated plots had the highest yield, berry size and berry and seed count compared to the other treatments even though berry size and seed count were not significantly different from the honey bee cages. This is not surprising as the plots were open not only to honey bees and bumble bees but to all other potential pollinators in the area. Also, because the cages blocked a certain amount of water, nutrients, and light from reaching nearby cranberry vines, they had a noticeable detrimental effect on the apparent health of the plants. Though the open pollinated plots were not surrounded by cages, they were adjacent to the cages. Thus these plants also suffered from the blockage of resources by neighboring cages, and this was observed in the yield data. The irrigation sprinklers at the research site are on low risers (approximately 10-15cm above the plants), and are not designed to irrigate over or around 1m tall objects. Also, much of the plant’s nutrition is delivered via fertigation, thus plants beneath and near cages were probably denied some nutrients. Plants near the cages also suffered from increased foot traffic in monitoring the experiment. As a comparison, the open pollinated plants in this study yielded 24,882 kg/hectare while cranberry beds in which this study was conducted produced about 33,625 kg/hectare overall in 2009 (K.Andersson, personal communication).

This study also corroborates that *B. vosnesenskii* is the most dominant bumble bee species in the region (Broussard et al., 2011). In this study, it comprised 58.2% of the bumble bees in the traps and is likely a key cranberry pollinator in southern Oregon. For every one honey bee, there were 4.2 bumble bees found in the traps. However, previous studies have documented that while the traps capture a great diversity of native bees, few honey bees are captured in them (Stephen and Rao, 2005). In contrast, there were greater numbers of honey bees compared to bumble bees observed during the visual observations. However, as indicated earlier, honey bees forage on cranberries more frequently for nectar than for pollen which ineffectively pollinates the flowers (Cane and Schiffhauer, 2001). Solitary bees collected in the traps could well contribute to the pollination of cranberries in Oregon. However, because of their size, and similarity in appearance of many species, they are a challenge to detect and identify in the field. More detailed studies are required to assess their impact and determine whether it is worth developing strategies to enhance their populations.

Successful cranberry production in Oregon may be due, at least in part, to native pollinators. To determine if cranberry growers in Oregon can depend on native bumble bees to achieve adequate pollination without investing in honey bee rentals, correlations are needed between their abundance and yield. While bumble bees were observed to be abundant in this study, their populations vary year to year. Hence, to sustain cranberry pollination in the absences of access to commercial colonies, efforts to conserve bumble bees are justified. There is evidence that proximity to relatively undisturbed "native areas" has a positive impact on the presence of native bees (Evans and Spivak, 2006). Therefore, setting aside areas as undisturbed native bee habitat may be beneficial to cranberry growers for maintaining current pollinator population levels. Many Oregon cranberry farms are in close proximity to areas of native vegetation communities composed of shore pine (*Pinus contortata*), Douglas fir (*Pseudotsuga menziesii*), evergreen huckleberry (*Vaccinium ovatum*), deciduous huckleberry (*Vaccinium parvifolium*), salal (*Gaultheria shallon*), and rhododendron (*Rhododendron macrophyllum*). While the importance of these plant communities to the pollinator population on cranberry farms is not known, they may be beneficial and thus their conservation may be warranted. Meanwhile cranberry producers need to be encouraged to preserve them.

There are global reports of declines in bumble bees due to improper pesticide use, diseases, or habitat destruction and fragmentation that results in loss of refugia and nesting sites (Goulson et al., 2008; Cameron et al., 2011). Hence to enable cranberry growers to benefit from local native bumble bees, options for encouraging bumble bees on cranberry farms, by providing or maintaining suitable nesting sites and season-long forage, should be explored. Build-up of bumble bee populations will be facilitated if one or more floral sources that bloom before and after cranberry flowers are provided in cranberry producing areas. Also, because native bees may be assisting in Oregon cranberry production new pesticides registered for use in cranberries should be evaluated for their toxicity to native bees, including bumble bees.

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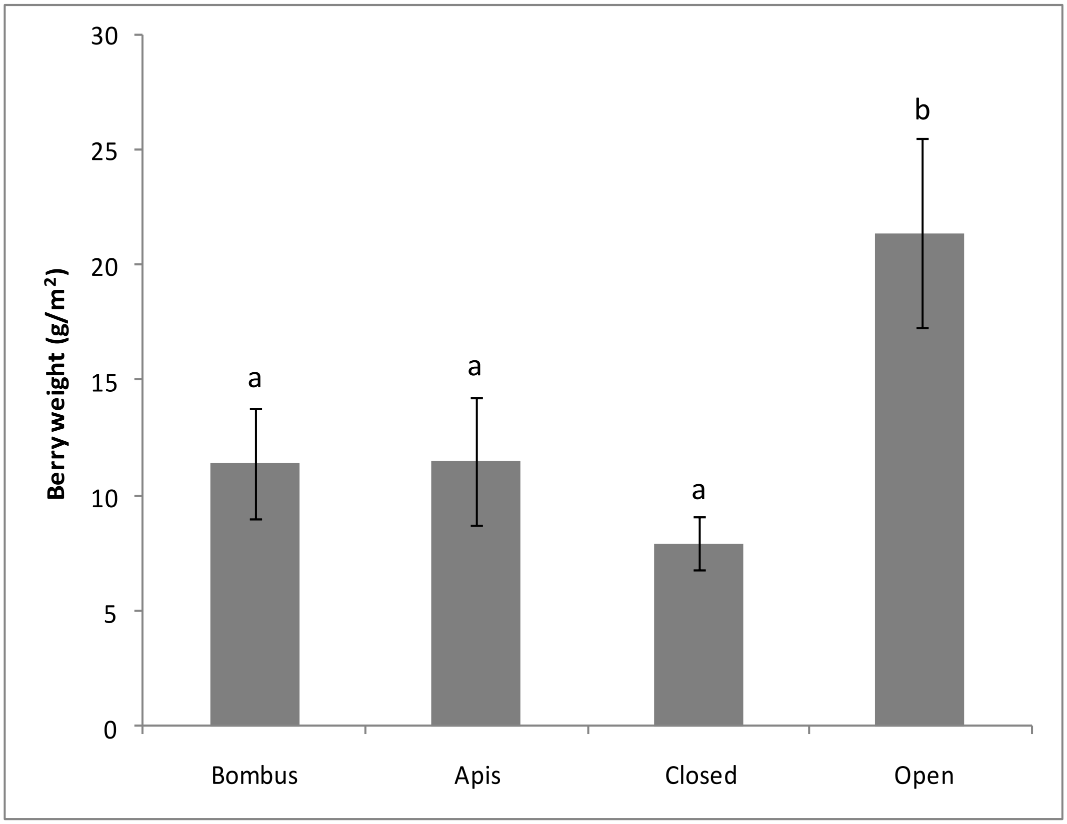

Figure 2.1: Comparison of cranberry weight (Mean g/m^2 ± SE) in cages with bumble bees (*Bombus*), honey bees (*Apis*), closed (no bees), and open pollinated plots in southern coastal Oregon in 2009. Bars with different letters represent significant differences in means separated using Tukey-Kramer pair-wise comparisons (α = 0.05).

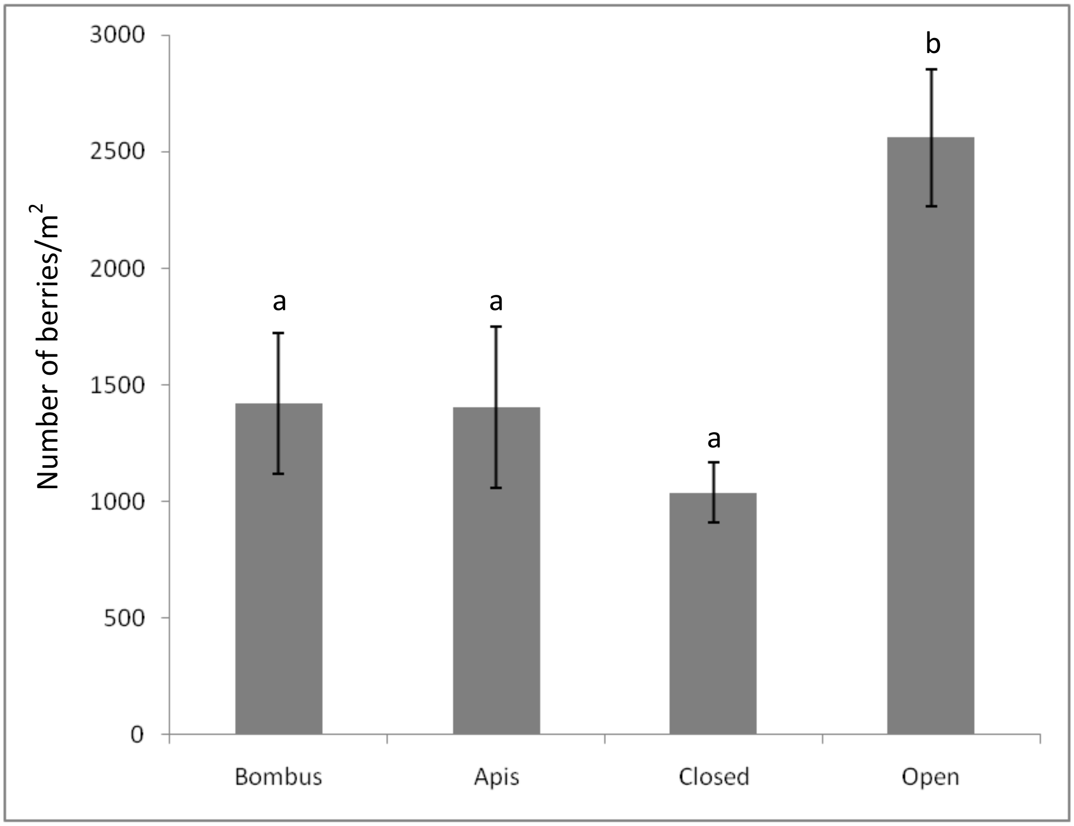

Figure 2.2: Comparison of cranberry fruit numbers (Mean number/m^2 ± SE) in cages with bumble bees (*Bombus*), honey bees (*Apis*), closed (no bees), and open pollinated plots in southern coastal Oregon in 2009 Bars with different letters represent significant differences in means separated using Tukey-Kramer pair-wise comparisons ($\alpha = 0.05$).

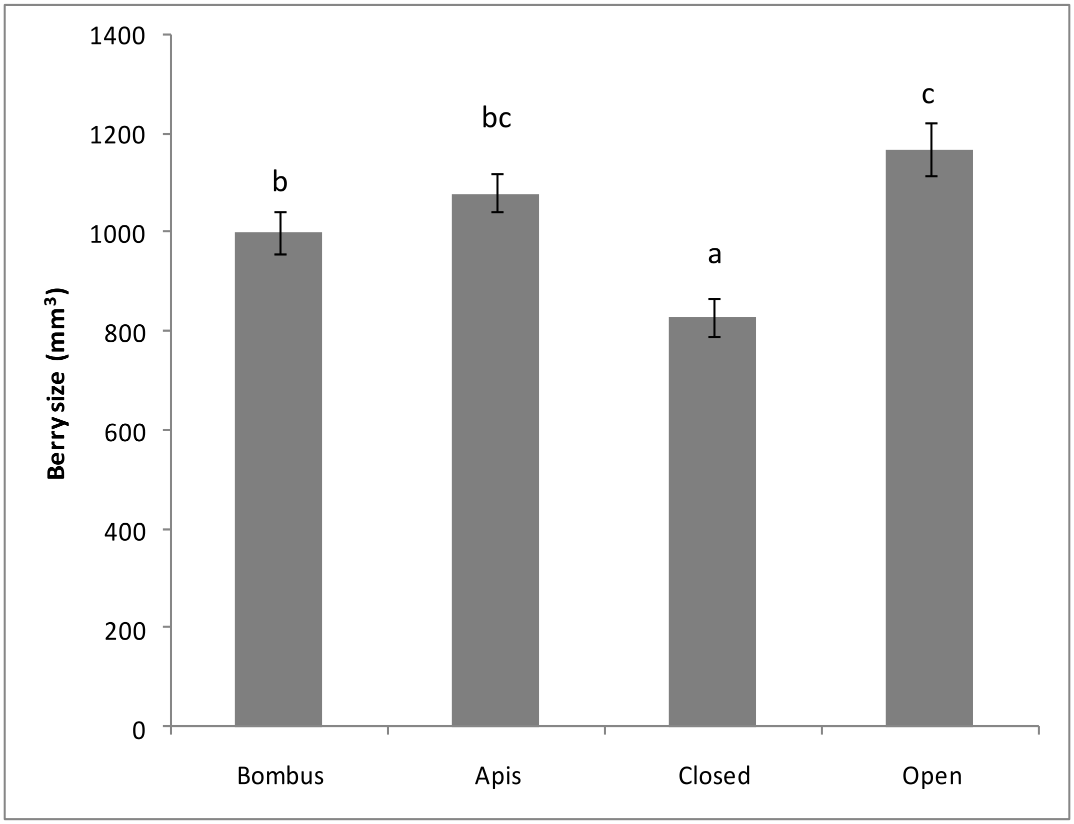

Figure 2.3: Comparison of the size of individual cranberries (Mean mm^3 ± SE) in cages with bumble bees (*Bombus*), honey bees (*Apis*), closed (no bees), and open pollinated plots in southern coastal Oregon in 2009 Bars with different letters represent significant differences in means separated using Tukey-Kramer pair-wise comparisons ($\alpha = 0.05$).

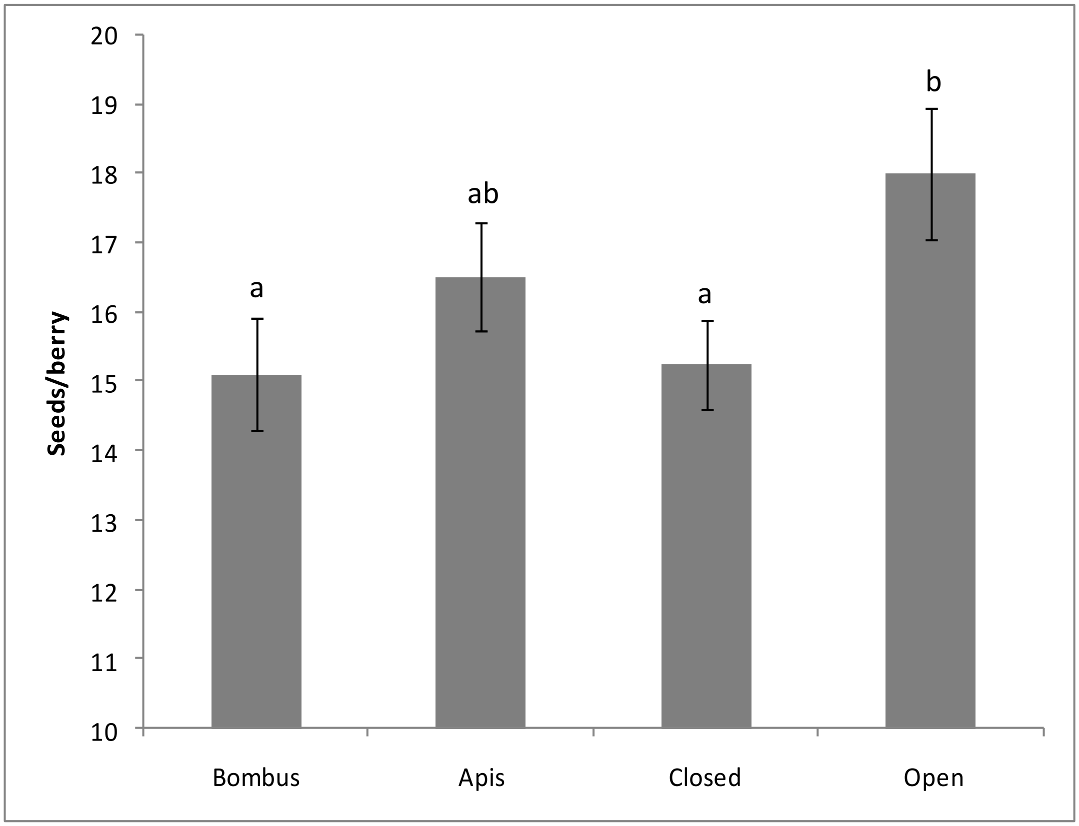

Figure 2.4: Comparison of the number of seeds per cranberry (Mean ± SE) in cages with bumble bees (*Bombus*), honey bees (*Apis*), closed (no bees), and open pollinated plots in southern coastal Oregon in 2009 Bars with different letters represent significant differences in means separated using Tukey-Kramer pair-wise comparisons (α = 0.10).

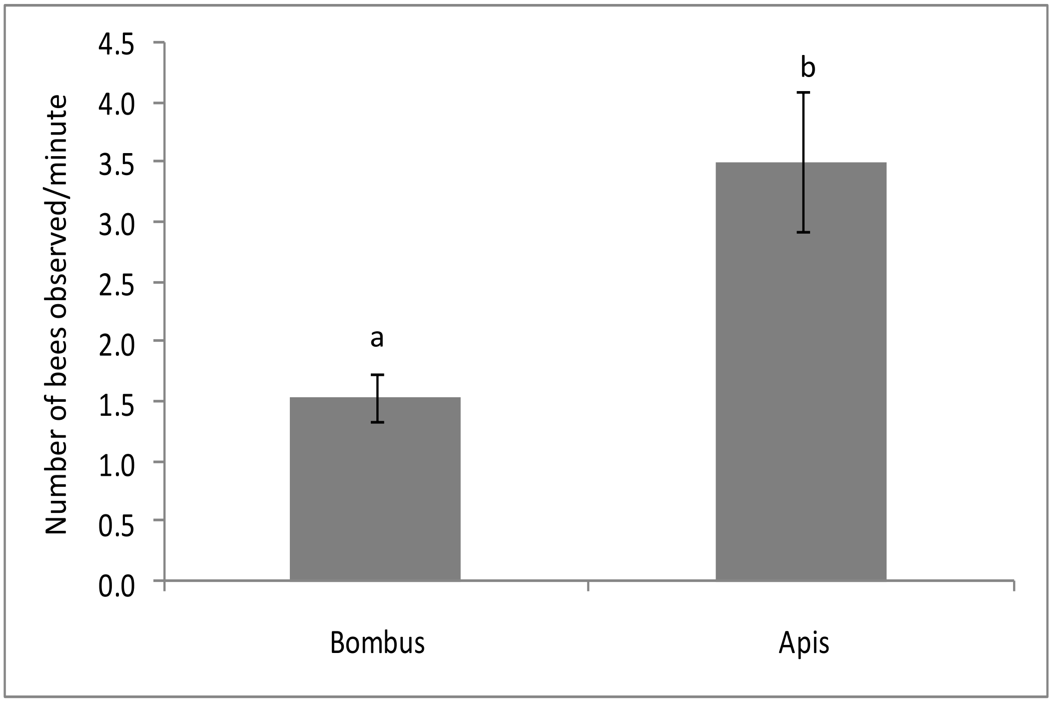

Figure 2.5: Numbers of bumble bees (*Bombus*) and honey bees (*Apis*) observed per minute (Mean ± SE) during visual counts (n = 34) made while walking 50 m transects in cranberry beds in Curry County, Oregon, 2009.

Table 2.1: Total bees caught in blue vane traps at four locations at the cranberry farm research site over five sampling dates (June 10, June 19, June 24, July 1, and July 8, 2009).

Family	**Species**	**Number trapped ***	**Percentage of bees caught**
Apidae	*Apis mellifera*	125	16.6%
	Bombus caliginosis	35 (32, 3, 0)	4.6%
	Bombus melanopygus	84 (31, 48, 5)	11.1%
	Bombus mixtus	99 (64, 33, 2)	13.1%
	Bombus vosnesenskii	304 (242, 60, 2)	40.3%
	Melissodes sp.	16	2.1%
Colletidae	*Colletes* sp.	5	0.6%
Halictidae	*Agapostemon* texanus	28	3.7%
	Agapostemon virescens	3	0.4%
	Halictus rubicundus	2	0.3%
	Halictus tripartitus	1	0.1%
	Lasioglossum sp.	52	6.9%
Megachilidae	*Megachile* sp.	1	0.1%
	Total bees	**755**	

*For *Bombus*, numbers in parentheses represent workers, males, and queens, respectively.

CHAPTER 3

Pollen collection by bumble and honey bees on an Oregon cranberry farm

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Abstract

For cranberry (*Vaccinium macrocarpon* Aiton) production, farmers typically rent honey bees (*Apis mellifera*) for pollination. However, the efficiency of this bee at pollinating cranberries in Oregon, as in other regions, is questionable. Earlier research suggests that bumble bees (*Bombus* spp.) are effective cranberry pollinators, but their pollen foraging patterns are not well known. The objective of this study was to analyze and compare pollen collected by bumble bees and honey bees in hives/nest boxes placed adjacent to an Oregon cranberry farm. Pollen was collected from both types of bees as they returned to their hive or nest box and analyzed using acetolysis. Pollen grains were identified using light microscopy and the identities were confirmed by comparison with a reference collection. Pollen collected by honey bees consisted of 29.1 ± 1.4% (2009), 18.3 ± 2.4% (2010), and 23.0 ± 1.1% (2011) cranberry pollen while the remaining percentage was derived from non-target plants. Cranberry pollen contributed a higher percentage in bumble bee collected pollen: 56.0 ± 6.1%, and 70.4 ± 4.3% in 2010, and 2011, respectively. Both bees collected pollen from non-target plants including those in the following families: Asteraceae, Fabaceae, Ranunculaceae, and Roseaceae. Native bumble bees (*B. vosnesenskii*) collected more cranberry pollen than pollen from non-target plants, and consistently collected a higher proportion of cranberry pollen than honey bees.

This study shows that bumble bees may be more loyal to cranberry flowers than honey bees.

Introduction

Cranberries (Ericaceae: *Vaccinium macrocarpon* Aiton) are a specialty crop native to North America. This crop is grown commercially several regions of the United States and Canada including the New England area, the Great Lakes region, and the Pacific Northwest. This high value perennial berry is grown for several purposes such as fresh market, dried berries, canned berries, frozen berries, and juice. In North America, the Pacific Northwest region (Oregon, Washington, and British Columbia) produces 54% of Canadian cranberries and 6-9% of cranberries in the United States (Agriculture and Agri-Food Canada, 2009; United States Department of Agriculture, 2011). In Oregon, cranberries are grown in the southwestern corner of the state, in Coos and Curry counties (NASS, 2008).

Pollination can be one limiting factor to fruit set in cranberry production (Eck, 1990; Macfarlane, 1995; Loose et al., 2005; Brown and McNeil, 2006). Cranberry bloom in Oregon typically lasts six weeks, while other regions have shorter bloom periods of 2-4 weeks (Brown and McNeil, 2006; Evans and Spivak, 2006). A prolonged bloom period may indicate sub-optimal pollination (MacFarlane 1995). Pollination of cranberry crops by honey bees is particularly a problem in Oregon because of prevailing weather conditions during bloom and uncertainty in availability and cost of honey bees (*Apis mellifera* L.). There is evidence in Oregon (Broussard et al., 2011), and in other regions (Marucci and Moulter, 1977; MacFarlane, 1995; Stubbs and Drummond, 1997; Evans and Spivak, 2006) that bumble bees contribute to cranberry pollination, but information is lacking about pollen collection behavior of these native bees as pollinators of this crop.

Honey bee colonies are typically rented by Oregon cranberry growers as pollinators for cranberries. Despite the fact that placing honey bees on cranberry farms has been shown to increase cranberry pollination (Evans and Spivak, 2006), honey bees may not be the optimal pollinators for cranberries. Honey bees visit cranberry flowers for nectar more often than for pollen, reducing their efficacy at transferring pollen among flowers (Cane and Schiffhauer, 2001). In fact, pollen foraging honey bees are infrequent and often absent from cranberry beds (Cane et al., 1993; Cane and Schiffhauer, 2001). The porose anthers of cranberry flowers impede pollen collection by bees that do not buzz pollinate, such as honey bees (Buchman, 1983; Cane et al., 1993; Cane and Schiffhauer, 2001). Instead, when honey bees visit cranberry flowers for pollen, they drum the anthers with their legs which may be less effective (Cane et al., 1993). In addition, honey bees are known to forage infrequently in temperatures below 21 ˚C. Broussard et al., (2011) reported that the majority of honey bees forage in temperatures above 24 ˚C. In Oregon, during cranberry bloom, average temperatures are: 17 ˚C in May, 18 ˚C in June, and 20 ˚C in July (Taylor et al., 2011). Precipitation events occur frequently in this region during cranberry bloom; on average 58% of days in May, 35% of days in June, and 10.6% of days in July have precipitation events (Taylor et al., 2011). The few days favorable to honey bees foraging, coupled with their inefficient pollen collection and transfer has lead growers to compensate by stocking their farms with high numbers of honey bees: typically 1 to 1.5 hives per hectare. Moreover, beekeepers claim that their stocks fare poorly while located on cranberry farms (Cane et al., 1993; personal communications with beekeeper). Rising costs and decreasing availability of honey bee colonies, plus inquiries by growers have led us to investigate whether and to what extent honey bees are visiting and gathering pollen from cranberry flowers.

Bumble bees are present in high numbers on cranberry farms in many cranberry growing regions including Oregon. In an earlier study (Chapter 2), bumble bees were the most frequently trapped native bee at more than 69% of bees caught, of which *Bombus vosnesenskii* Radowszkowski was the most common. Bumble bees are also known to forage most frequently in a temperature range more compatible to that which is typically experienced during cranberry bloom; 18 ˚C to 22 ˚C (Broussard et al., 2011). For bees that buzz pollinate, cranberry flowers may be more attractive to pollen foragers than to nectar foragers because nectar, although high in sugar, is produced in small quantities whereas pollen is produced in large amounts (Cane et al., 1993). Also, because they buzz pollinate, bumble bees are more effective than honey bees at extracting pollen from the porose anthers of cranberry flowers

(Buchman, 1983; Cane et al., 1993; Cane and Schiffhauer, 2001). Bumble bees deposit more cranberry pollen (60 pollen tetrads per visit) than honey bees (10 tetrads per visit) (Cane and Schiffhauer, 2003). In a greenhouse flight cage study that compared honey bees, bumble bees (commercial *B. impatiens* Cresson), *Megachile rotundata* Fabricus, and *Osmia atriventris* Cresson, the bumble bees were the most effective at pollinating cranberries (Stubbs and Drummond, 1997). Because bumble bees are currently present on cranberry farms in apparently high populations and due to their ability to buzz pollinate, ability to transfer large amounts of pollen, and ability to forage in cooler temperatures appears to make bumble bees ideal pollinators for cranberries — at no cost to growers. However, little is understood about bumble bee pollen foraging preferences: whether and to what extent they collect cranberry pollen, and how they compare to honey bees in this respect.

The objectives of this study were to: i) Compare proportions of pollen collected from cranberry flowers by bumble bees and honey bees; and ii) Identify non-target sources of pollen collected by each bee on a cranberry farm in Oregon. This analysis will enable a further comparison of honey bees and bumble bees as pollinators for Oregon cranberries.

Materials and Methods

Study site. The study was conducted over three years on a cranberry farm south of Langlois in Curry County, Oregon (Longitude: 42.8744, Latitude: -124.4835). The grower rented 400 honey bee hives in 2009, 354 in 2010, and 400 in 2011 for 66 hectares in production in 2009, and 70 in 2010 and 2011. Cranberry beds at this farm are typically 200m X 50m, and all are surrounded by dikes approximately 3m wide and 1.5 to 2m tall. This farm is surrounded by undeveloped, and primarily forested natural areas.

Pollen collection: honey bees. Honey bee pollen was collected from four 10-frame Langstroth honey bee hives in 2009 using four pollen traps of different designs. Pollen was trapped for 48 to 72 hours, and collected on four dates during cranberry bloom; June 8 (early), June 19 (mid), July 1 (late), and July 8 (very late).

Based on preliminary observations, the Sundance pollen trap was the most practical for this project and thus these were the traps I used in the following two seasons. In 2010, Sundance pollen traps were placed on four 10-frame Langstroth honey bee hives. Pollen was trapped for 48 to 72 hours and collected on four dates: June 26 (mid), July 2 (late), July 14 (very late), July 22 (post-bloom). Because of late delivery of honey bees by the cooperating beekeeper, it was not possible to collect honey bee pollen during early bloom of 2010.

In 2011, six, eight-frame Langstroth hives were used (Dirk Olsen of Olsen Honey Farms, Albany, Oregon). Pollen trapping frequencies were increased to more precisely determine pollen collection patterns: one 24 hour period once per week for each of the six weeks of cranberry bloom.

Pollen collection: bumble bees. In both 2010 and 2011, six commercially reared bumble bee (*B. vosnesenskii*) colonies were placed near a cranberry bed (Bee Man Exterminators, LLC., Olympia Washington). Each of the six bumble bee colonies were observed for one 60 minute period once per week in 2010. In 2011, each colony was observed for 30 minutes, one to two times per week, except on June 24 when the bumble bee colonies were inaccessible due to fungicide application nearby. Observations were made of the following: i) arrivals with scopal pollen loads, ii) arrivals without scopal pollen loads, and iii) bumble bee departures. Bumble bees arriving with pollen loads were captured in a clean vial, and chilled in a cooler until immobilized. Later, the pollen loads were removed and the bumble bees were released. Because bumble bee activity slowed in 2010 before bloom ceased (Table 3.1 pollen was also collected from pollen pots within the bumble bee nest box during that season. In 2011, bumble bee activity continued throughout the duration of bloom (Table 3.1), thus pollen pot was not collected from pollen pots.

Pollen identification. A process modified from that originally developed by Erdtman (1943) was used to clean and prepare individual pollen loads. Acetolyzed pollen loads were homogenized individually, three separate drops from each sample were slide mounted in silicon oil, viewed under a compound microscope for uniformity, and 100 grains were counted and identified by comparison with a reference collection. The reference collection, prepared by Broussard et al. (2011), consisted of pollen samples taken directly from flowering plants in 2008 and 2009 at the same study site. When possible, subsamples of 100 honey bee pollen loads were examined per hive and collection date from 2009, and 50 per hive and collection date from 2010 and 2011. The total number of honey bee pollen loads examined each year was 1,145 in 2009, 253 in 2010, and 1,539 in 2011. The smaller number of pollen loads examined from honey bees in 2010 was due to: 1) “leaky” honey bee colonies—the bees were able to avoid the pollen traps by entering the hive through alternative openings; and 2) late delivery of honey bee colonies. All collected bumble bee pollen from 2010 and 2011 was examined. In 2010, there were 28 pollen loads collected and analyzed from foraging bumble bees, 27 pollen loads collected from within the nest box, and 7 pollen pots. One hundred nine pollen loads were collected and analyzed from scopal loads of foraging bumble bees in 2011.

Statistical analysis. Data was analyzed using R version 2.13.0 (R Development Core Team, 2011). A Wilcoxon rank-sum test was used to determine the difference in season averages of percent cranberry pollen collected between honey bee and bumble bees in 2010. Wilcoxon rank-sum tests were used to determine the difference in percent cranberry pollen collected between honey bee and bumble bees for each week in 2011. Bumble bee pollen loads that were collected on consecutive days in the same week in 2011 were grouped together in the analysis to compare with honey bee pollen collected over the same period. Differences in cranberry pollen collection rates were accepted at $\alpha \leq 0.05$.

Results

Pollen collection from cranberry flowers. Both bumble bees and honey bees collected pollen from cranberry flowers, but there were differences in the proportions that cranberry pollen was represented in the samples collected from each type of bee. On June 8, 2009 (early bloom) and June 19, 2009 (mid bloom), the majority of pollen collected by honey bees originated from cranberry flowers (91.9 ± 2.0% and 35.7 ± 2.3%, respectively), (Fig. 3.1). By July 1, 2009 (late bloom) the amount of the pollen that honey bees collected from cranberries dropped to 7.6 ± 1.6%, and then on July 8, 2009 (post bloom) no cranberry pollen was collected by honey bees.

Pollen collected from bumble bees and honey bees in 2010 was statistically compared. The Wilcoxon rank-sum test revealed strong evidence for a higher percentage of cranberry pollen collected by bumble bees compared with honey bees over the 2010 cranberry bloom period as a whole ($W = 5103$, p-value < 0.001), (Fig. 3.2). Of all pollen types collected by honey bees in the 2010 season, cranberry pollen represented 18.3 ± 2.4% while cranberry pollen represented 56.0 ± 6.1% of that collected by bumble bees. On June 24, 2010 (mid bloom) the ten bumble bees that were caught with pollen loads as they returned to the nest box all carried exclusively cranberry pollen. On June 26, 2010 (mid bloom) and July 2, 2010 (late bloom), cranberry pollen made up the majority of pollen collected by honey bees (71.4 ± 10.1% and 38.9 ±5.8%, respectively). At July 14, 2010 (late bloom) and July 22, 2010 (post bloom) cranberry bloom, no cranberry pollen was found in samples from honey bees.

Wilcoxon-rank sum tests on the data from each week in 2011 that a comparison could be made revealed that cranberry pollen made up a consistently higher percentage of pollen collected by bumble bees than by honey bees (Fig. 3.3). On June 3, 2011, while cranberries were in very early bloom, honey bees collected 0.60 ± 0.6% cranberry pollen. Cranberry pollen was in the majority for the following two weeks (June 10, 2011 (early bloom): 50.3 ± 3.4%, and June 16, 2011 (mid bloom): 44.6 ± 2.7%). On June 24, 2011 (mid bloom) cranberry pollen (33.0 ± 2.8%) was found in a lower proportion than Rosaceaous pollen (42.6 ± 2.9%) in collections by honey bees. During the final two weeks of cranberry bloom, low amounts of cranberry pollen were collected by honey bees. On July 1, 2011 (late bloom) cranberry pollen comprised 4.5 ± 1.2% and on July 7, 2011 (late bloom) 0.4 ± 0.4%.

In every week of cranberry bloom in 2011, except for the last (July 7, 2011), bumble bees collected a higher percentage of cranberry pollen than that of non-target plants (Fig. 3.4). The 2011 season average for bumble bee collected cranberry pollen collection was 70.4 ± 4.3%.

Pollen collection from non-target plants. Both bumble bees and honey bees collected pollen from non-target plants, though there were differences in proportions over the cranberry bloom period. On June 8, 2009, Fabaceous pollen (7.5 ± 1.9 %) made up the second highest percentage of pollen collected by honey bees (cranberry pollen was collected in higher proportions) (Fig. 3.1). On June 19, 2009, though cranberry pollen made up the majority of that collected by honey bees, Fabaceous pollen collection composed 32.7 ± 2.3% and Ranunculaceous pollen consisted of 17.9 ± 1.8%. In the last two pollen collection events of 2009, two different plant families were represented at higher rates than cranberry in pollen collected by honey bees; Roseaceae (July 1: 43.8% ± 3.1, July 8: 57.9 ± 2.78) and Asteraceae (July 1, 2009: 37.3% ± 3.0, July 8, 2009: 41.0 ± 2.8). Other pollen made up 1.5% of pollen collected over all dates in 2009 including; Liliaceae (0.4 ± 0.18%), Apiaceae (0.3 ± 0.15%), Pineaceae (0.1 ± 0.08%), and pollen from unknown sources (0.5 ± 0.25%).

Honey bee colonies were unavailable during early cranberry bloom in 2010, thus no pollen was collected during part of bloom. Cranberry was the preferred pollen source for honey bees during on June 26, 2010 (mid bloom) and July 1, 2010 (late bloom) (Fig. 3.5). During late to post cranberry bloom in 2010, instead of cranberry pollen, honey bees exhibited a preference for Rosaceous (July 14: 65.9 ± 5.1%; July 22: 68.5 ± 5.4%) and Asteraceous (July 14: 22.7 ± 4.6%; July 22: 20.7 ±

4.7%) pollen. Smaller amounts of other pollen types were collected by honey bees in 2010 and means over all collection dates were; Ranunculaceae (0.4 ± 0.04%), Apiaceae (0.8 ± 0.06%), Pineaceae (2.0 ± 0.09%), and pollen from unknown sources (3.6 ± 0.12%).

On June 3, 2011, honey bees exhibited a preference for Fabaceous (50.3 ± 3.9%) and Ranunculaceous (46.9 ± 3.9%) pollen (Fig. 3.7). During the June 10, 2011 collection date, honey bees collected pollen from plants in the following families: Ranunculaceae (23.0 ± 3.0%), Fabaceae (12.9 ± 2.4%), Liliaceae (7.4 ± 1.5%), and Rosaceae (5.4 ± 1.6%). On June 16, 2011 non-target pollen collected by honey bees included: Fabaceae (17.0 ± 2.2%), *Eucalyptus* (11.5 ± 1.8%), Rosaceae (9.1 ± 1.6), Liliaceae (4.8 ± 1.1%), Asteraceae (2.5 ± 0.9%), and Apiaceae (2.1 ± 0.8%). On June 24, Rosaceaous pollen (42.6 ± 2.9%) was collected in higher proportions than cranberry pollen (33.0 ± 2.8%), and other non-target pollen included; Asteraceae (13.5 ± 2.0%), Ranunculaceae (7.3 ± 1.5%), and Fabaceae (2.1 ± 0.8%). Non-target pollen collected by honey bees on the July 1, 2011 and July 7, 2011 included; Roseaceae (55.4 ± 2.8% and 52.1 ± 3.1% respectively), Asteraceae (32.1 ± 2.6% and 38.8 ± 3.1%), and Ranunculaceae (2.3 ± 0.9% and 1.2 ± 0.7%).

Although bumble bees in 2010 collected cranberry pollen more than other pollen types, they also collected pollen from non-target plants (Fig. 3.6). Over the entire season, bumble bees also collected pollen from the following plant taxa;

Fabaceae (21.3 ± 5.1%), Asteraceae (10.9 ± 3.9%), Rosaceae (9.0 ± 3.5%), *Rhododendron* (1.3 ± 1.0%), Pineaceae (0.08 ± 0.06%), and Ranunculaceae (0.02 ± 0.02%).

Cranberry pollen was the pollen type most frequently collected by bumble bees in 2011, but non-target pollen was also collected (Fig. 3.4). Non-target pollen collected by bumble bees included the following taxa; Fabaceae (14.3 ± 3.4%), *Rhododendron* (5.8 ± 2.2%), Rosaceae (5.5 ± 2.0%), Asteraceae (2.0 ± 1.1%), and *Vaccinium ovatum* (1.9 ± 1.3%).

Discussion

This is the first study that compares pollen collection by native bumble bees and commercial honey bees in Pacific Northwest cranberry production. It demonstrated that while commercial honey bees do collect cranberry pollen, the native bumble bee, *B. vosnesenskii*, was more consistent in collecting a higher proportion of cranberry pollen than pollen from non-target plants. Though pollen gathered into pollen loads by either bee is no longer available to pollinate a flower, the proportion pollen collected by each bee reflects the pollination services each bee provides. Probably as a result of sophisticated communication about forage sources (von Frisch, 1966), honey bee pollen collection in this study tended to reflect the phenology of local plants.

This study offers further evidence that bumble bees are significant contributors to cranberry pollination. Honey bee rentals on cranberry farms for pollination may be redundant because of the native bumble bees already present. However, little is known about native bee population levels and year-to-year fluctuations on cranberry farms and how this corresponds with cranberry pollination rates. More research needs to be conducted to quantify the relationship between native bee abundance and cranberry yield in the absence of honey bees. Meanwhile, options to encourage consistent populations of native bees need to be considered by providing forage pre- and post- cranberry bloom. Thus, protocol needs to be outlined for creating and conserving bumble bee nesting habitat and forage sources. Food resources may be limiting for bumble bee populations near cranberry operations (Macfarlane and Patten, 1997). Providing suitable forages close (within 100m) to cranberry plantings that bloom before and after, but not concurrently with cranberries may help reduce this limitation on bumble bee populations (Macfarlane and Patten, 1997). Additionally, pesticides, especially those commonly used in cranberry cultivation should be evaluated for their effects on bumble bees.

Though honey bees do collect cranberry pollen, they appear to be less "loyal" to the crop than bumble bees. Honey bees utilize sophisticated dance language to share information with workers in their colony about the best floral forage (von Frisch, 1966). Many flowers besides cranberry appear to attract the pollen collecting efforts of honey bees on cranberry farms, especially plants in the legume, rose, and buttercup families. Pervasive weeds in this region in these taxa include gorse (*Ulex europaeus* Linnaeus), Scotch broom (*Cytisus scoparius* Linnaeus), Himalyan blackberry (*Rubus armeniacus* Focke), and creeping buttercup (*Ranunculus repens* Linnaeus). Efforts to reduce populations of these plants could serve to increase honey bee "loyalty" to cranberries. The redeeming quality of honey bees is the ability for growers and beekeepers to introduce large numbers of honey bees at the optimal time in relation to cranberry bloom. Until bumble bee populations are well documented to be consistently at levels high enough to provide cranberry pollination, growers will likely need to continue to rely on honey bees.

Bumble bees appear to be more “loyal” collectors of cranberry pollen than honey bees. During the 2010 season, and for every week of cranberry bloom in 2011, bumble bees collected a higher percentage of cranberry pollen than honey bees. Bumble bees lack communication methods comparable to honey bees (Dornhaus and Chittka, 1999). Thus foraging habits may change more slowly than honey bees in response to forage availability. Cranberry flowers are more accessible as a pollen source for bumble bees than for honey bees (Cane et al., 1993) and this may contribute to their tendency to be more consistent in collecting cranberry pollen over the bloom period. It is not known whether current bumble bee populations are sufficient to pollinate the 1,100 hectares of cranberries currently cultivated in Oregon (NASS, 2008). By Doehlert’s (1940) calculations in New Jersey cranberries, 0.12 bumble bees per m^2 are enough to adequately pollinate cranberries. A comparable and current calculation for bumble bees in Pacific Northwest cranberry cultivation would be invaluable.

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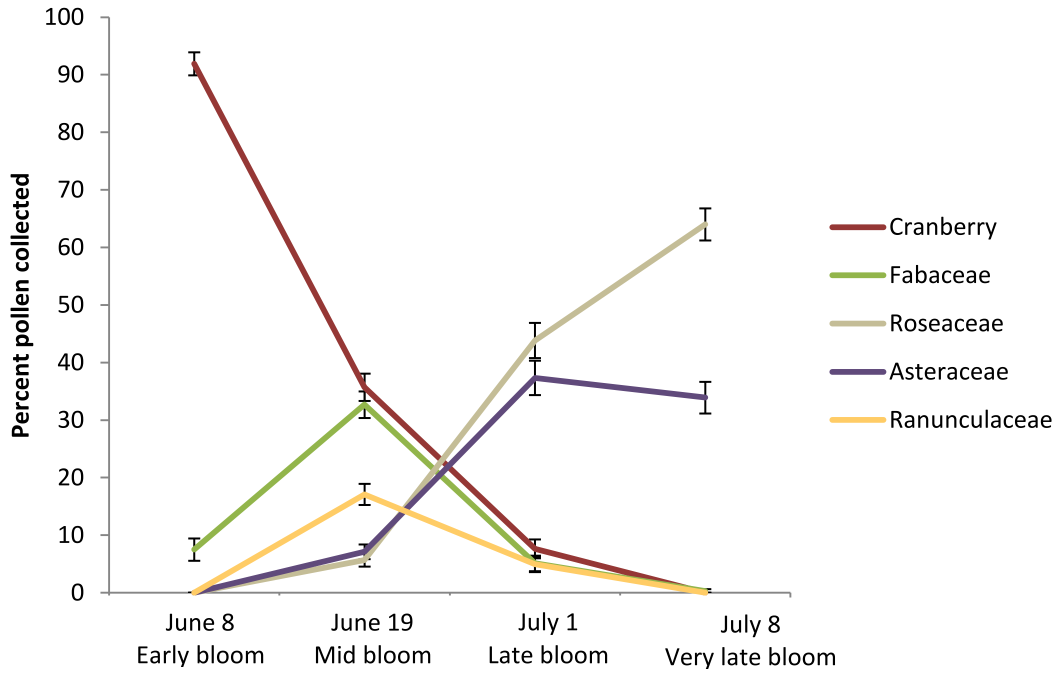

Figure 3.1: Composition (mean percent ± SE) of pollen collected from honey bees using pollen traps on their hives in 2009. Collection dates correspond to progress of cranberry bloom: early (June 8, 2009), mid (June 19, 2009), late (July 1, 2009), and very late/post (July 8, 2009). 100 pollen grains were counted from homogenized sub-samples of individually acetolyzed pollen loads (n= 1162 pollen loads) to create percentages of pollen collected on each date.

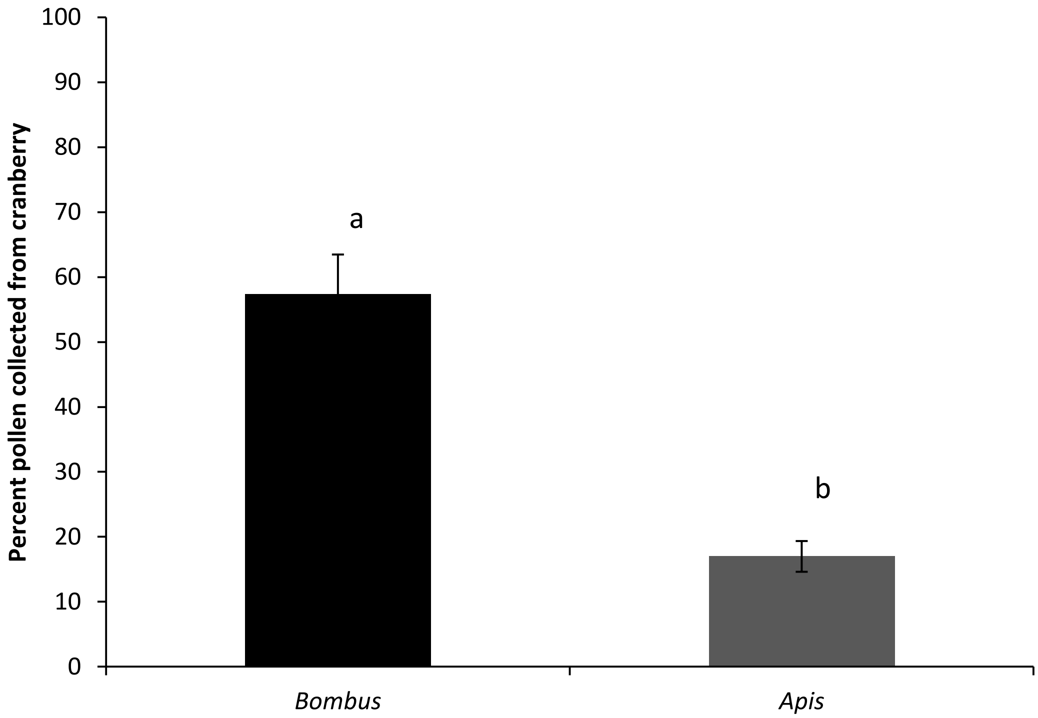

Figure 3.2: Percentage of cranberry pollen in pollen collected by bumble bee (Bombus) and honey bee (*Apis*) workers returning to nests placed near cranberry beds in Curry County, Oregon in 2010.

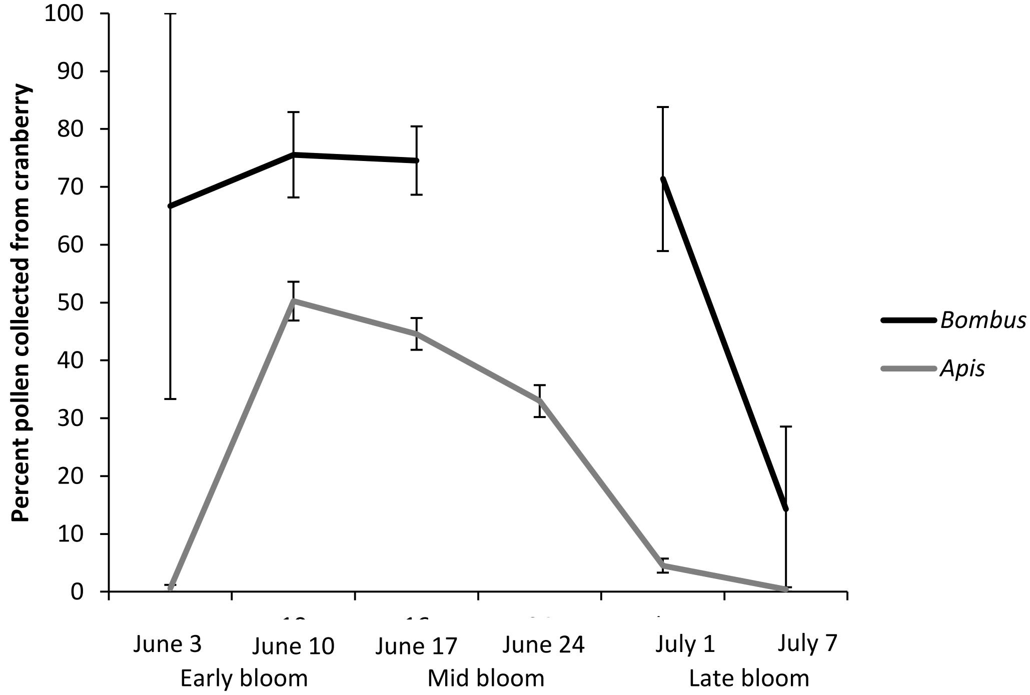

Figure 3.3: A comparison of the percentage of pollen originating from cranberry flowers collected by honey bees and bumble bees. Bumble bees collected significantly higher proportions of cranberry pollen on each date than did honey bees (Wilcoxon rank-sum test, W= 5103, p-value < 0.001). No data was collected from bumble bees on June 24, 2011 due to inaccessibility to the area because of a fungicide application.

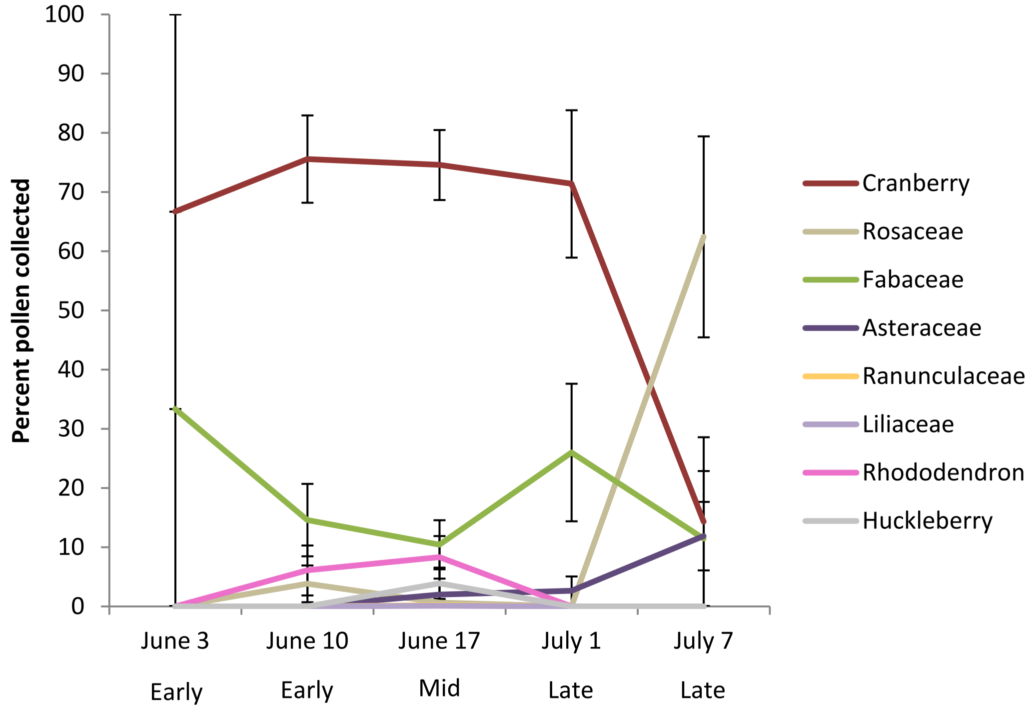

Figure 3.4: Composition (mean percent ± SE) of pollen collected from bumble bee scopal pollen loads. Collection dates correspond to progress of cranberry bloom: early (June 3, 2011), early (June 10, 2011), mid (June 16, 2011), late (July 1, 2011), and late (July 7, 2011). 100 pollen grains were counted from homogenized sub-samples of individually acetolyzed pollen loads (n=108 pollen loads) to create percentages of pollen collected on each date.

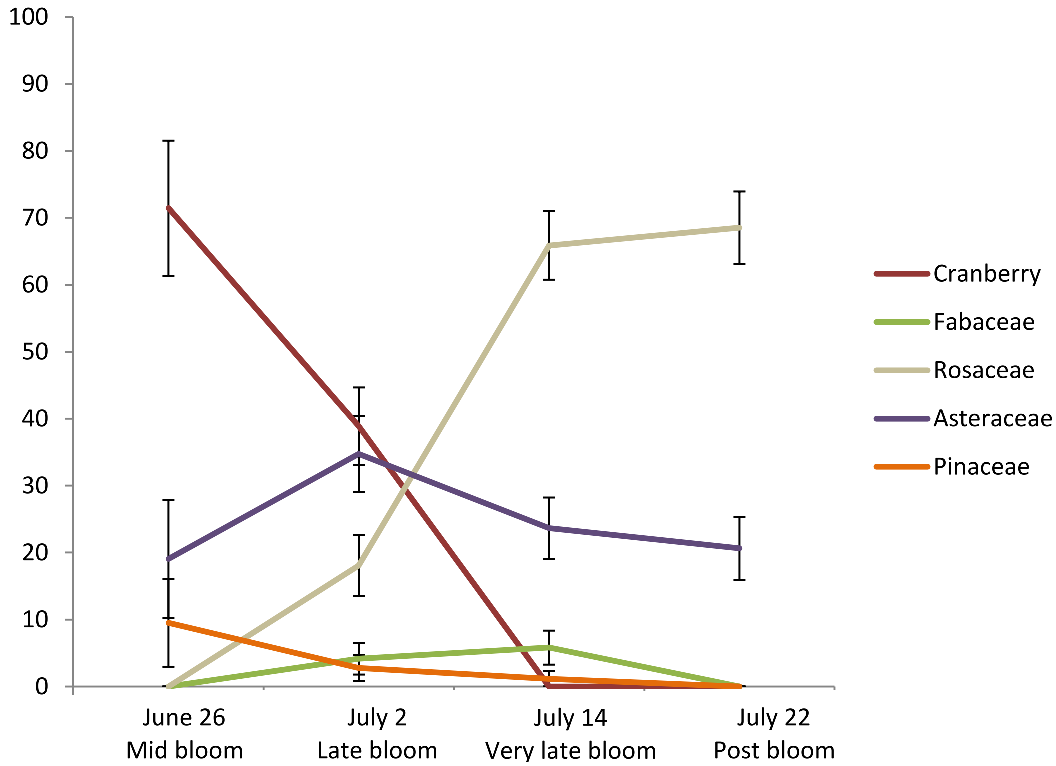

Figure 3.5: Composition (mean percent ± SE) of pollen collected from honey bees using pollen traps on their hives. Collection dates correspond to progress of cranberry bloom: mid (June 26, 2010), late (July 2, 2010), very late (July 14, 2010), and post (July 22, 2010). 100 pollen grains were counted from homogenized sub-samples of individually acetolyzed pollen loads (n= 253 pollen loads) to create percentages of pollen collected on each date.

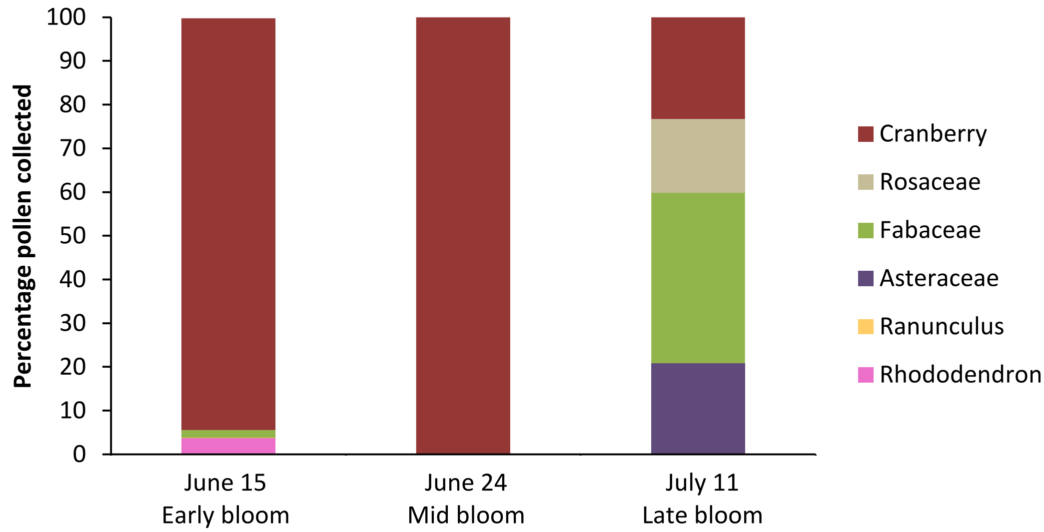

Figure 3.6: Plant origins of pollen collected from bumble bee scopal pollen in 2010 at early (June 15), mid (June 24) cranberry bloom. Pollen collected during late (July 11) cranberry bloom was collected from within colonies. 100 pollen grains were counted from homogenized sub-samples of individually acetolyzed pollen loads (n=64 pollen loads and pollen pots) to create percentages of pollen collected on each date.

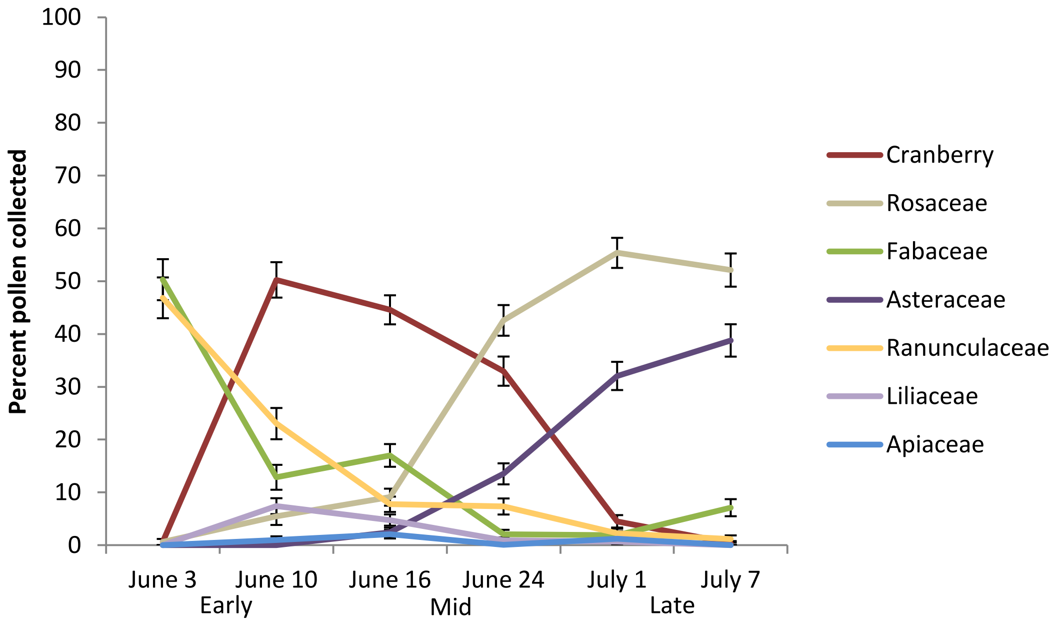

Figure 3.7: Composition (mean percent ± SE) of pollen collected from honey bees using pollen traps on their hives. Collection dates correspond to progress of cranberry bloom: early (June 3, 2011), early (June 10, 2011), mid (June 16, 2011), mid (June 24, 2011), late (July 1, 2011), and late (July 7, 2011). 100 pollen grains were counted from homogenized sub-samples of individually acetolyzed pollen loads (n= 253 pollen loads) to create percentages of pollen collected on each date.

Table 3.1: Numbers of bumble bees arriving and departing nests with corresponding dates and cranberry bloom period.

Bloom period	Date	Arriving with pollen	Arriving without pollen	Departing nest	Total bees
Early	6/15/2010	17	Data not collected	Data not collected	17
Early	6/20/2010	0	46	Data not collected	46
Mid	6/24/2010	9	21	10	40
Mid	6/25/2010	0	13	11	24
Mid	6/30/2010	0	8	5	13
Late	7/5/2010	0	0	2	2
Late	7/11/2010	2	0	0	2
Early	6/2/2011	3	3	7	13
Early	6/9/2011	8	3	5	16
Early	6/10/2011	25	3	8	36
Mid	6/16/2011	24	3	5	32
Mid	6/17/2011	28	1	11	40
Late	6/30/2011	14	48	17	79
Late	7/7/2011	7	28	8	43

CHAPTER 4

Discussion

Review of findings

The studies conducted in Chapters 2 and 3 were intended to compare bumble bees and honey bees for pollinating commercial cranberries in Oregon. The cage study in chapter 2 suggests that both honey bees and bumble bees have the ability to contribute to cranberry pollination. The results of the honey bee treatment were not as clear due to inadvertent entry of bumble bees. Although an unknown number of bumble bees may have contributed to the pollination in honey bee and closed treatments, pollination of cranberry flowers by either bee yielded berries indistinguishable in the parameters of grams per square meter, number of berries per square meter, size of berries, and seed number. Despite great differences in numbers of individuals, as a unit one 3-frame honey bee nucleus (500-1000 individuals) provided equivalent pollination services as one reared bumble bee colony (approximately 20 individuals). The results of the closed treatment in chapter 2 also were unclear because flowers in this treatment were likely pollinated by invading bumble bees. To document whether cranberry flowers self pollinate, in the following year (2010), I bagged 13 individual cranberry flowers in a second attempt to exclude all pollinators. The bagged flowers yielded no fruits.

A comparison of pollen foraging habits of the two prominent cranberry pollinators, honey bees and bumble bees (Chapter 3), showed that both bees collect pollen from cranberry flowers though they exhibit different patterns of doing so. The study documented foraging behaviors and “loyalty” to target crop relative to presence of other pollen sources in the landscape. Pollen was collected from the scopae of foragers as they returned to the nest. Other examinations of pollen collection habits compared pollen loads taken from bees as they were foraging, and usually reflect the floral resources upon which the bee was caught (personal experience, unpublished data). Though honey bees are inefficient collectors and transporters of cranberry pollen, they do utilize the plant as a pollen source, though seldom in late bloom. Cranberry flowers, as a pollen resource, appear to be most attractive to honey bees closest to peak bloom. Honey bees also collect considerable proportions of pollen from non-target plants in the legume, rose, buttercup, and aster families. Reducing populations of non-target flowers, especially noxious weeds, may serve to improve cranberry pollen collection by honey bees. Bumble bees, on the other hand, collect a considerably higher proportion of their pollen resources from cranberry flowers as compared to honey bees.

The examination of bee relative abundance on the cranberry farm research site in Chapter 2 did not reveal whether population numbers of bumble bees or honey bees were higher. Both *Apis* and *Bombus* were observed in high numbers in visual observations and blue vane traps. In visual observations *Apis* was more common than *Bombus* however, in the blue vane traps, the opposite was true. There may be some unknown biases against *Apis* in the blue vane traps that explain the difference.

Limitations of the studies and future directions

Improvement to the cage study in Chapter 2 could have been achieved by repetition in a second season to correct unexpected problems of invading unsolicited bumble bees and a detrimental cage effect on plants. If a second season would have been permitted for this project, burying the perimeter of the cages into the sand substrate slightly, and surrounded the cages with a chemical deterrent may have eliminated the problem of unwanted bumble bees. Another improvement which could have been added in a second year would be to add an “open pollination” treatment with the same cage as the bumble bee, honey bee, and “closed pollination” treatments except with zippered openings left unfastened. This type of “open pollination” treatment would have eliminated the unintended variable of the cage effect on the cranberry plants. Repeating the cage study with these improvements would allow a fair comparison among all the pollination treatments (bumble bee, honey bee, closed, and open) without the probability that the bumble bees were boosting the pollination output of other treatments. Unfortunately, the cages proved difficult for the grower to operate farm machinery around and thus he was unwilling to allow a repeated year of this project.

The pollen study in Chapter 3, did not impact the farm operations at the research site to the extent of the cage study in Chapter 2. This allowed for repetitions and refinements to the field methods over three seasons, and produced more informative results in comparison to the cage study. One improvement to the pollen study would be to engineer a type of pollen trap that would consistently collect pollen from bumble bees in a way comparable to the Sundance pollen traps on the honey bee hives. The challenge in this would be to engineer a device that would remove pollen loads from the legs of returning pollen foraging bumble bees which are normally a wide range of sizes. While honey bee workers tend to be fairly uniform in size, bumble bee sizes vary greatly (Couvillon et al., 2010). In addition, inclusion of multiple farm sites would be valuable for determining differences, in any, in pollen foraging among Oregon cranberry farms.

Both honey bees and bumble bees appear to contribute to the production of this high value crop in Oregon, but the two studies generated new questions. One key question is -- how many bumble bees are necessary to pollinate the crop in absence of imported honey bees? What can policy makers, land managers, and growers do to conserve or build native bumble bee populations in coastal Coos and Curry Counties? Should efforts be made to rear bumble bees native to Oregon for use by growers to augment wild populations, or is the benefit not worth the risk of disease outbreak (de Ruijter, 1997)?

Implications for growers

Growers in many other states in which cranberries are grown have the option of importing commercially reared bumble bees (*B. impatiens*). However, because of existing laws preventing importation of non-native bumble bees, this option is not available to Oregon growers. For this reason, growers in Oregon especially would benefit from long-term investigations of potential conservation protocol for bumble bee populations in this region. Meanwhile, determining protocol for economically providing additional nesting sites and year-round forage resources compatible to cranberry cultivation would be advantageous for growers and bumble bee populations alike.

There is current evidence that bumble bee populations are declining worldwide (Cameron et al., 2011), but little is known about Oregon populations. Furthermore, there is evidence that honey bees may be negatively impacting bumble bee populations (Goulson and Sparrow, 2008). Lessening grower dependence on non-native honey bees may have two-fold benefits: reducing the cost to growers of renting honey bees, and easing strains on bumble bee populations due to competition with honey bees.

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