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Research

Managed Bumble Bees (*Bombus impatiens*) (Hymenoptera: Apidae) Caged With Blueberry Bushes at High Density Did Not Increase Fruit Set or Fruit Weight Compared to Open Pollination

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Abstract

Highbush blueberry (*Vaccinium corymbosum* L.) is an important crop grown throughout Florida. Currently, most blueberry growers use honey bees (*Apis mellifera* L.) to provide pollination services for highbush blueberries even though bumble bees (*Bombus* spp.) have been shown to be more efficient at pollinating blueberries on a per bee basis. In general, contribution of bumble bees to the pollination of commercial highbush blueberries in Florida is unknown. Herein, we determined if managed bumble bees could contribute to highbush blueberry pollination. There were four treatments in this study: two treatments of caged commercial bumble bee (*Bombus impatiens* Cresson) colonies (low and high weight hives), a treatment excluding all pollinators, and a final treatment which allowed all pollinators (managed and wild pollinators) in the area have access to the plot. All treatments were located within a highbush blueberry field containing two cultivars of blooming plants, 'Emerald' and 'Millennia', with each cage containing 16 mature blueberry plants. We gathered data on fruit set, berry weight, and number of seeds produced per berry. When pollinators were excluded, fruit set was significantly lower in both cultivars (<8%) compared to that in all of the other treatments (>58%). Berry weight was not significantly different among the treatments, and the number of seeds per berry did not show a clear response. This study emphasizes the importance of bumble bees as an effective pollinator of blueberries and the potential beneficial implications of the addition of bumble bees in commercial blueberry greenhouses or high tunnels.

Key words: *Bombus impatiens*, bumble bee, highbush blueberry, *Vaccinium corymbosum*, pollination

The highbush blueberry (*Vaccinium corymbosum* L.) is native to eastern North America and may have originated in Florida during the Pleistocene (Vander Kloet 1980). However, due to chill requirements, highbush blueberries could not be grown commercially in Florida until the 1970s. During the mid-1970s, southern-adapted varieties of highbush blueberries were developed for Florida's climate, and a small blueberry industry was established by the 1980s (Williamson and Lyrene 2004). Since then, numerous cultivars of highbush blueberries have been established for use within Florida and other areas (Williamson and Lyrene 2004, Williamson et al. 2004). Highbush blueberries ripen 4–6 wk earlier than do rabbiteye blueberries (*Vaccinium ashei* Reade), another popular blueberry grown in the southeastern United States. This allows Florida blueberry growers to monopolize the market during early spring (Williamson and Lyrene 2004). On the other hand, this blueberry blooms early in the year, making it subject to bloom freezes and a general lack of available pollinators. Regardless, it remains the preferred blueberry species among commercial blueberry growers in all parts of Florida (Williamson et al. 2004).

Highbush blueberries are self-fertile, but crossing with different varieties can alter fruit development (Lang and Danka 1991). Several studies about self-pollinating and cross-pollinating various *Vaccinium* species have yielded different results, though they generally show that cross-pollination in highbush blueberry cultivars results in larger berry size, increased fruit set, and early ripening (Chavez and Lyrene 2009). In contrast, there are some cultivars of highbush blueberries that experience decreased fruit set or berry weight when cross-pollinated (Ehlenfeldt 2001). This shows that, overall, pollination requirements are poorly understood for many of the highbush blueberry cultivars in the southeastern United States due to their complex ancestries (Lang and Danka 1991, Dogterom et al. 2000). In Florida, in order to obtain good fruit set, most southern highbush cultivars require cross-pollination from a different cultivar of the same species (Williamson et al. 2004). Hence most commercial blueberry growers grow at least two cultivars within a field. Thus, a highbush blueberry grower needs to consider the pollination requirements of a cultivar before planting (MacKenzie 1997).

Pollination of highbush blueberry varieties is dependent primarily on insects. In fact, decreases in fruit production have been observed when there was a deficiency in insect pollination of highbush blueberries (Benjamin and Winfree 2014). Historically, blueberry growers depended on native bees for the pollination services they provided, but they are now more reliant on western honey bees (*Apis mellifera* L.) to provide these services (Sampson and Cane 2000, Isaacs and Kirk 2010, Gibbs et al. 2016). Although numerous native bees have been shown to visit blueberries in other parts of the United States (Tuell et al. 2009), bumble bees (*Bombus* spp.), carpenter bees (*Xylocopa* spp.), and the southeastern blueberry bee (*Habropoda laboriosa* F.) are the few native bee species that are common visitors to blueberries in the southeastern United States (Cane and Payne 1993, Delaplane 1995). Honey bees have been shown to provide adequate pollination services to blueberries (Danka et al. 1993, Dedej and Delaplane 2003), but alternative pollinators, such as other managed bees (e.g., *Bombus* spp.), should be explored due to the problems affecting managed honey bee colonies (Ellis et al. 2010, Gibbs et al. 2016).

Managed bumble bees (*Bombus impatiens* Cresson) may be a good candidate as an alternative pollinator of highbush blueberries. Within small fields (<30 acres), managed bumble bees were shown to pollinate lowbush blueberries (*Vaccinium angustifolium* Aiton) as effectively as managed honey bees do and may be a better contributor to outcrossing than are honey bees (Stubbs and Drummond 2001, Drummond 2012, Drummond 2016). Javorek et al. (2002) found that *Bombus* spp. queens are much more efficient pollinators of lowbush blueberries than are honey bees on a bee-to-bee basis. They can pollinate 3.6–6.5 flowers in the time it takes a honey bee to pollinate one flower. Prior bumble bee visitation to blueberry flowers has also been shown to improve the pollination efficiency of honey bees (Drummond 2016).

Bombus impatiens also have been shown to be more active during poor weather conditions than honey bees and pollinated highbush blueberries better than did honey bees which generally foraged during good weather (Tuell and Isaacs 2010). In Florida, commercial blueberry growers use honey bees to provide pollination services and a growing number also use *B. impatiens*. Blueberries grown in greenhouses or high tunnels may also benefit from managed bumble bees because they readily acclimate inside tunnels (Bal 1997, Sampson and Spiers 2002). Although the addition of *B. impatiens* to Florida blueberry fields is common, their contribution to highbush blueberry pollination is not well studied. Herein, we present data from a cage study we conducted to determine the potential contribution of managed *B. impatiens* to highbush blueberry pollination.

Materials and Methods

Study Site and Experimental Design

For this study, we used a large commercial blueberry farm in Alachua County, FL (29.791482, −82.119505), that had two highbush cultivars ('Emerald' and 'Millennia') planted in double rows. The blueberry bushes from both cultivars were 12 yr old and approximately 1.5 m in height.

Bumble bee (*B. impatiens*, Koppert Biological Systems, Howell, MI) quads (Fig. 1) each contained four hives which were subdivided into individual hives and weighed without the food reservoir. Koppert Biological Systems states that a minimum of 150 worker bees are within each hive. Some hives weighed significantly more than did others. We assumed that the heavier hives contained more bees per brood than did the lighter ones. Due to the weight discrepancy, we separated hives into two groups: 1) hives with a weight of 381–412 g and 2) others with a weight of 258–287 g, hereafter referred to as high and low, respectively. Individual bumble bee hives were placed back into a Koppert quad box, and polyester fiber-fill (Fairfield Poly-fil) was placed around three sides of the hive within the quad box to help maintain warmth.

Cages (Lumite screen portable field cages, 5.4 by 2.5 by 2.4 m, l by w by h, BioQuip, Rancho Dominguez, CA) were positioned linearly along a double row of 16 blueberry plants (eight plants each of 'Millennia' and 'Emerald' highbush cultivars), separated from neighboring double rows by a minimum of 35 m. Four treatments were used in this experiment (Fig. 2), with nine cage replicates allocated per treatment: 1) pollinator exclusion cages (negative control), 2) high weight bumble bee colony cages, 3) low weight bumble bee colony cages, and 4) an open area with no cage (same size as the cages and including the same number of blueberry plants, positive control). The farm was stocked with bumble bee hives (~7.5 per ha or 3 per acre) and honey bee hives (4 per ha or 1.6 per acre). Adequate stocking rates depend on a number of factors (Stubbs et al. 2002), and the stocking rates in our experimental field are within a normal range that growers use for blueberry (Delaplane and Mayer 2000). We hypothesized that the open area treatment would represent the best case pollination scenario, as it contained two managed pollinators (bumble bees and honey bees) at a high stocking rate and allowed visitation by native pollinators such as carpenter bees and southeastern blueberry bees. Managed bumble bee colonies were at equidistant distances from each plot, but honey bee colonies were up to 50 m further away from some plots.

A pallet was placed on the ground in the cage for replicate cages containing bumble bee colonies (low and high weight treatments). A quad box containing one hive was placed on the wooden pallet and protected from rain and irrigation using a sheet of corrugated plastic (Fig. 1). Cages were positioned into the field 15 January 2016, approximately four weeks before bloom. Bumble bee hives were placed

Fig. 1. Photo of Koppert quad box that contained one bumble bee hive placed within the high and low weight treatment cages.

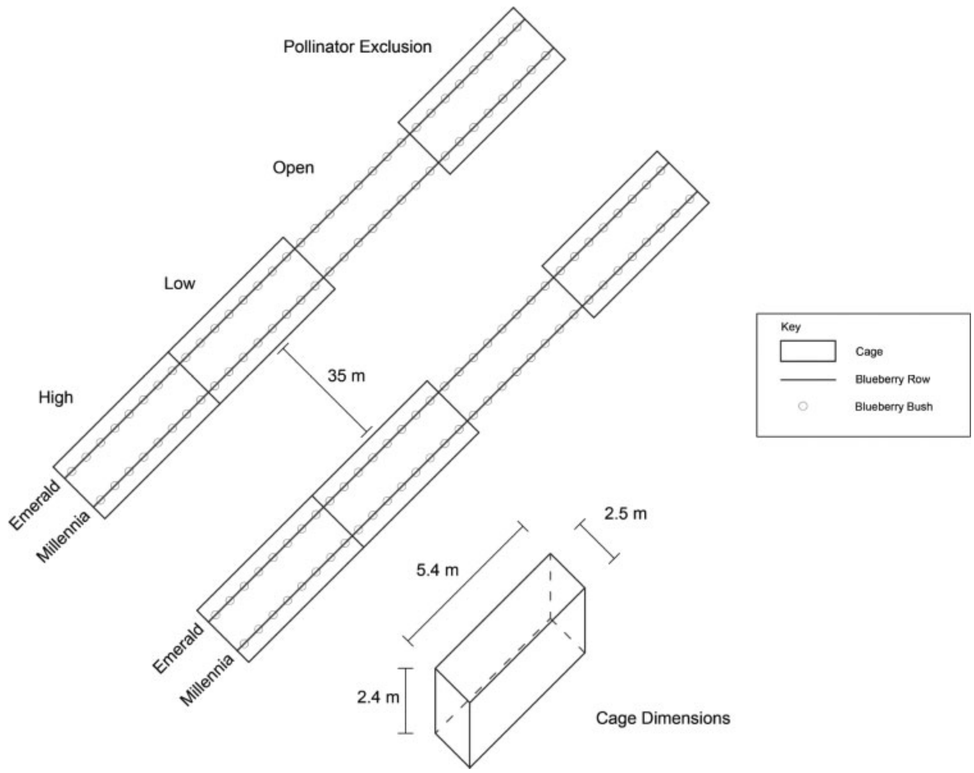

Fig. 2. Schematic drawing of blueberry field depicting the two cultivars of blueberry ('Emerald' and 'Millennia') and the four treatments used in this experiment.

into their respective field cages once plants began to bloom (15 February 2016) and within 48 h of arrival from Koppert Biological Systems. The managed bumble bee colonies and cages were removed from the field once flowers had senesced (22–23 March 2016).

Within each treatment cage, five flower clusters from each cultivar were tagged for a total of 10 clusters per cage. Individual flowers were counted within each cluster. Each cluster was enclosed in a mesh bag to prevent bird predation and loss of fallen berries once the petals had fallen from the flowers. The resulting blueberries were picked from the mesh bags upon ripening. The first blueberries were picked 10 April 2016 and the last on 19 May 2016. Blueberries were weighed individually on a digital scale, and all seeds were counted.

Statistical Analysis

A Kruskal–Wallis one-way nonparametric analysis of variance test was used to determine if there was an impact of treatment on berry weight, seed count, and fruit set (Statistix 9.0 Analytical Software, Tallahassee, FL). Kruskal–Wallis tests were used after square-root and logarithmic transformations applied to the data failed to eliminate heteroscedasticity. A Wilcoxon rank-sum test was used to compare bumble bee hive weights between the high and low bumble bee treatments. A linear regression was performed to determine the correlation between berry weight and number of seeds within the two cultivars (JMP Pro 12).

Results

Percent fruit set was significantly lower in the pollinator excluded treatment cages than in cages of all other treatments in both 'Emerald' ($\chi^2 = 57.15$, df = 3, $P < 0.01$) and 'Millennia' ($\chi^2 = 84.8$, df = 3, $P < 0.01$) blueberries. There were no significant differences in berry weight among the treatments for either 'Emerald' (N = 518 berries) or 'Millennia' (N = 726 berries) cultivars ($\chi^2 = 1.21$, df = 3, $P = 0.75$; $\chi^2 = 1.25$, df = 3, $P = 0.74$, respectively, Table 1). 'Emerald' berries from pollinator-excluded cages had significantly fewer seeds per berry than did berries from the other treatments ($\chi^2 = 18.7$, df = 3, $P < 0.01$, Table 1). Berries from the 'Millennia' open treatments contained significantly more seeds per berry than did berries from the low bumble bee colony cage treatment, but contained a similar number of seeds per berry as berries from the other treatments ($\chi^2 = 13.06$, df = 3, $P < 0.01$, Table 1). Overall, there was a weak, positive correlation between berry weight and seed counts for 'Emerald' ($R^2 = 0.38$, $P < 0.01$; Fig. 3) and 'Millennia' ($R^2 = 0.34$, $P < 0.01$; Fig. 4) berries.

The managed bumble bee colonies were housed within treatment cages for 37–38 d. All of the bumble bee colonies used within the cages were still alive at the completion of the experiment. Although all hives declined in weight during the experiment, hive weights were significantly different between the high and low treatments prior to placing them within the field ($z = 3.53$, $P < 0.01$) and after removing them from the field ($z = 2.56$, $P < 0.01$).

Discussion

Overall, and within the parameters of our study, we found that highbush blueberry is highly dependent on bees for pollination services and managed *B. impatiens* can provide adequate pollination services. Our pollinator exclusion cages emphasized that pollinator limitation in highbush blueberry can lead to reduced fruit set (<8% in this study). Pollinator limitation has been found in highbush blueberries within other regions (Benjamin and Winfree 2014). In our study, fruit set was statistically similar in both bumble bee treatments and the open treatment. Interestingly, fruit set in these three

Table 1. Mean berry weight, number of seeds per berry, and % fruit set (± SE) in the four treatments of 'Emerald' and 'Millennia' highbush blueberry cultivars

Cultivar	Treatment	N	Mean berry weight (g)	Mean number of seeds per berry	Mean % fruit set
Emerald	High	146	1.21 (0.04)a	7.64 (0.65)a	58.2 (5.7)a
	Low	164	1.24 (0.05)a	8.62 (0.79)a	58.9 (5.3)a
	Poll. Excl.	16	1.37 (0.18)a	3.81 (1.81)b	7.9 (3.5)b
	Open	192	1.26 (0.04)a	10.6 (0.90)a	63.5 (5.0)a
Millennia	High	221	1.16 (0.04)a	4.75 (0.23)ab	64.5 (3.7)a
	Low	238	1.21 (0.03)a	4.30 (0.21)a	58.9 (4.4)a
	Poll. Excl.	13	1.22 (0.19)a	4.69 (1.28)ab	4.5 (1.6)b
	Open	254	1.20 (0.03)a	5.82 (0.35)b	69.1 (5.1)a

Within a cultivar, columnar data with the same letter are not significantly different at $\alpha \leq 0.05$. High, bumble bee colonies weighing 381–412 g; low, bumble bee colonies weighing 258–287 g. Poll. Excl, pollinator excluded. Open, open cages.

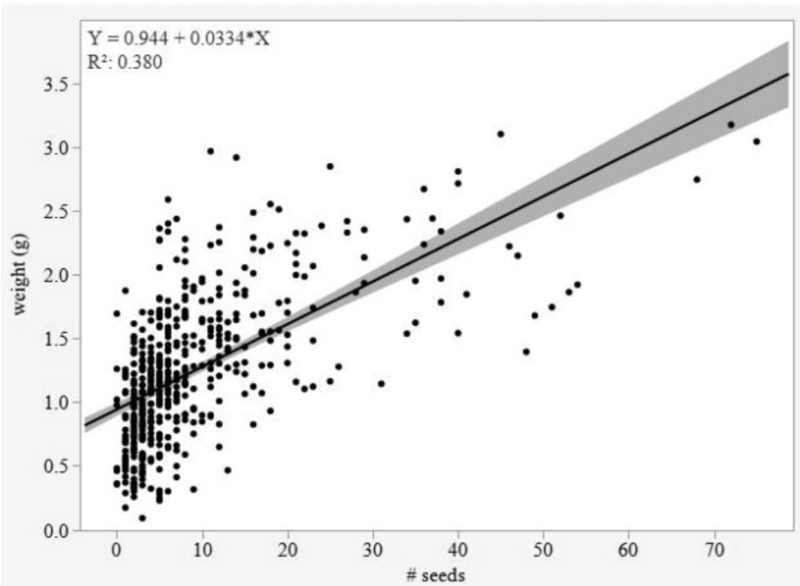

Fig. 3. Berry weight (g) in highbush blueberry cultivar 'Emerald' in relation to number of seeds ($R^2 = 0.38$, $P < 0.01$). The best fit line with 95% confidence bands connects predicted values from the linear model.

treatments was close to 60% despite the high stocking rate of bumble bees and honey bees. Although lack of fruit set has been attributed to poor pollination (MacKenzie 1997), other abiotic factors also could play a role (Tuell and Isaacs 2010). Danka et al. (1993) found that fruit set of a highbush cultivar peaked at 70% even when visitation rates by honey bees were very high. Therefore, it is likely that our cultivars reached their physiological limit for fruit set within our bumble bee and open treatments.

Highbush blueberry weight and seed count have been shown to be correlated positively to pollination visitation rate (e.g., Eaton 1967, Brewer and Dobson 1969, Moore et al. 1972). However, other studies have suggested that a correlation does not exist (Ehlenfeldt and Martin 2010). The two cultivars that we tested showed a weak positive relationship between berry weight and number of seeds. Berry weight alone did not differ among the treatments and it did not seem to be related to the stocking rates of bumble bees and honey bees. The cultivar 'Emerald' is known to have low self-compatibility and cross pollination is needed to yield adequate fruit set (Lyrene 2008). Since adequate fruit set was obtained, we can assume that cross pollination occurred.

Seed count in the pollinator excluded treatment was significantly lower than that in the other treatments for 'Emerald' plants but showed confounding results in 'Millennia'. However, we caution that only 29 berries across both cultivars developed within our clusters in the pollinator exclusion treatment, thus making conclusions tentative regarding berry size and seed numbers within this one treatment. Although the low-chill highbush cultivars are still considered *V. corymbosum*, they are hybrids that may contain genes from *V. darrowii* Camp and *V. virgatum* Aiton (Williamson et al. 2004). Therefore, seed set (and potentially other characteristics) may have genetic variability that mask environmental factors (e.g., pollinators), possibly producing the inconsistent results we saw for some of the data between 'Emerald' and 'Millennia' varieties. Alternatively, seed counts have been shown to decrease when bee density is very high or low (Dedej and Delaplane 2003), which may explain low seed set in some of our treatments. However, our data further emphasize the need to study the pollination requirements of individual cultivars.

During our experiment, bumble bees inside the two bumble bee cage treatments (high and low) were observed to visit blueberry flowers frequently. However, we rarely noticed a bumble bee on a plant within the open treatments despite the field being stocked with managed *B. impatiens*. In contrast, honey bees were very common in the open treatment and were observed to visit flowers regularly

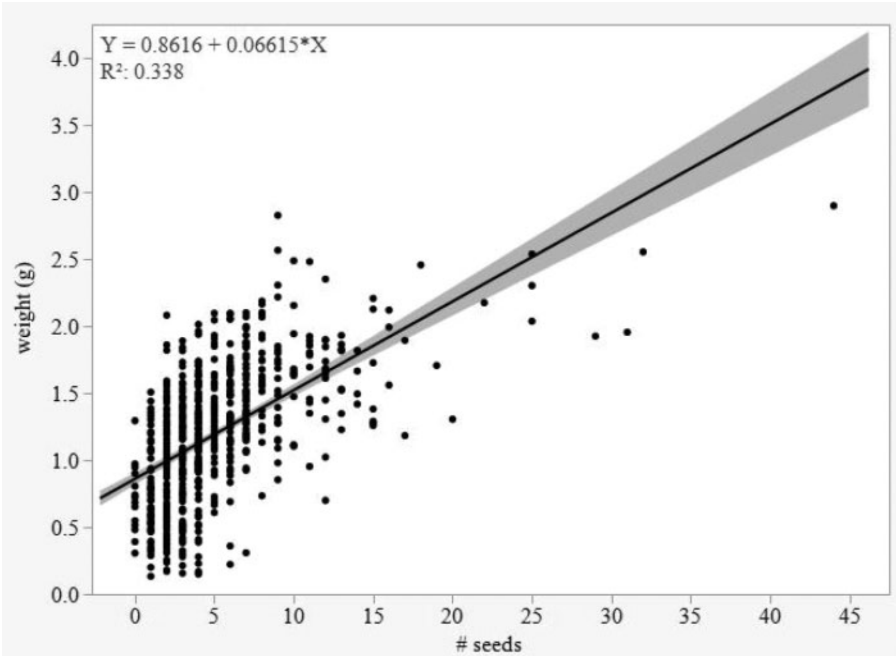

Fig. 4. Berry weight (g) in highbush blueberry cultivar 'Millennia' in relation to number of seeds ($R^2 = 0.34$, $P < 0.01$). The best fit line with 95% confidence bands connects predicted values from the linear model.

within the open treatment. Anecdotally, we noticed no differences in bumble bee visitation frequency within the high and low weight bumble bee treatments, potentially explaining the lack of significant differences found between these treatments. The commercial blueberry farm we utilized was large (~178 hectares). Because of our data, we hypothesize that managed *B. impatiens* colonies disperse within large fields but can be stocked in high densities and potentially used as surrogate pollinators in small fields, greenhouses, and tunnels. In Michigan, native bees (including *Bombus*) have been shown to contribute greatly to highbush blueberry pollination in small fields compared to large fields that were primarily dominated by managed honey bees (Isaacs and Kirk 2010).

Overall, we found that bumble bees can pollinate 'Emerald' and 'Millennia' varieties of highbush blueberry to produce similar berry formation as that of open pollinated plots. The bumble bee stocking rate recommended for lowbush blueberry is ~3 hives/0.4 ha (Stubbs et al. 2002, Artz and Nault 2011, Drummond 2012). Based on this, our bumble cage treatments contained at least 100 times more bees than the standard recommended stocking rate. Stocking rates for bumble bees are not well-defined and may depend on a number of factors (e.g., size of field, abundance of other pollinators, etc.; Stubbs et al. 2002). Whether a much lower stocking rate of bumble bees would have resulted in a fruit set similar to that seen in our bumble bee and open treatments is unknown. Koppert Biological Systems recommends that individual colonies not be manipulated to reduce their population. Thus, we were unable to put fewer bees in our cages than those found in a single colony. Nonetheless, our high and low bumble bee and open treatment data show that bees cause a ~7-fold increase in berry formation in 'Emerald' and 14-fold increase in 'Millennia' varieties of highbush blueberry compared to our pollinator exclusion treatment, thus displaying the high dependency of bees for pollination services. Blueberries are often grown in high tunnels and greenhouses and have been shown to be adequately pollinated by bumble bees in tunnels (Sampson and Spiers 2002). Due to their foraging behavior, honey bees are not the best pollinators for blueberry within tunnels (Spivak 2012), making growers primarily dependent on bumble bees in tunnels. Therefore, despite our cages containing bumble bee densities well over recommended stocking rates, within in tunnels and greenhouses, similar densities could be common.

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