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CARPENTER BEE NECTAR ROBBERY EFFECTS ON FRUIT QUALITY AND NESTING PROVISIONS IN RHODE ISLAND

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BY

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ABSTRACT

Eastern carpenter bees, *Xylocopa virginica* L., are among the most abundant native bee visitors to highbush blueberry flowers in Rhode Island, and they frequently slit corollas to rob nectar. My objective was to assess if nectar robbery offsets the possible value of *X. virginica* as a native pollinator of blueberries in Rhode Island. I studied foraging behavior of *X. virginica* at the Rhode Island Agricultural Experiment Station planting which consists of 14 highbush blueberry cultivars. I assessed plant and environmental factors related to slitting behavior, and the effects of slitting on fruit set and blueberry quality. The average number of flowers that *X. virginica* visited per minute was significantly influenced by time of day, temperature, and sex, but the time spent per flower was not. The frequency of corolla slitting by carpenter bees among 14 cultivars during bloom averaged 35% slit flowers (range 16 – 67% 'Earliblue' and 'Lateblue' respectively) in 2017, and 39% (range 20 – 62% 'Bluecrop' and 'Collins' respectively) in 2018. Plant and environmental factors that affected the proportion of corollas slit included cultivar, anther length, flower volume, and number of days in bloom at or above 15^{o}C. Corolla slitting did not affect fruit set. Average fruit weight and percent soluble solids resulting from slit and non-slit corollas did not differ significantly in two early- ('Bluehaven', 'Earliblue'), two mid- ('Collins', 'Bluecrop'), and two late-season ('Herbert', 'Lateblue') ripening cultivars in 2017. In 2018, average fruit weight and percent soluble solids resulting from slit and non-slit flowers did not differ significantly in most cultivars, but slit flowers resulted in berries with greater mass in two cultivars,

‘Bluehaven’ and ‘Collins’. ‘Collins’ fruit from non-slit corollas had a significantly higher percentage of soluble solids at maturity than fruit from slit corollas in 2018. Corolla slitting and nectar robbery by *X. virginica* did not have a significant negative effect on fruit quality under our growing conditions and pollinator community.

Understanding the nesting and foraging habits of *Xylocopa virginica* can aid in efforts to recruit natural populations to crops for pollination services. Measurements of *Xylocopa virginica* nest tunnels and cells were similar to those reported in previous studies. Analysis of pollen loaves showed that *X. virginica* provisioned pollen loaves from 21 different genera of plants in 2016, 19 in 2017, and 39 in 2018. *Antirrhinium majus* (Garden snapdragon) made up the majority (21.4%) of pollen collected in all three years. Blueberry pollen was a minor component of pollen loaves (0.1%). Only two of 168 trap nests deployed in 2017 were occupied by a total of ten *X. virginica* bees. However, 33 nests (19.6%) hosted 230 *Osmia taurus*, 73 *Osmia cornifrons*, and 8 *Osmia lignaria* Thirty-four nests (20.2%) were occupied by 151 grass-carrying wasps, *Isodontia* sp. and 6 vespid wasps occupied two nests (1.2%) in 2017. In 2018, four of ninety-six trap nests were occupied by carpenter bees.

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PREFACE

The chapters of this thesis are being submitted in manuscript format. Chapter one, "Effect of Corolla Slitting and Nectar Robbery by the Eastern Carpenter Bee (*Xylocopa virginica* L.; Hymenoptera: Apidae) on Fruit Quality of Highbush Blueberry (*Vaccinium corymbosum*, L.; Ericaceae)" will be submitted for publication to Environmental Entomology. Chapter two, "Eastern Carpenter Bee (*Xylocopa virginica* L., Hymenoptera: Apidae) Nest Structure, Nest Cell Provisions, and Trap Nest Acceptance in Rhode Island" will also be submitted for publication to Environmental Entomology.

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CHAPTER 1

"Effect of Corolla Slitting and Nectar Robbery by the Eastern Carpenter Bee (*Xylocopa virginica* L.; Hymenoptera: Apidae) on Fruit Quality of Highbush Blueberry (*Vaccinium corymbosum*, L.; Ericaceae)"

by

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In preparation for submission to Environmental Entomology

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ABSTRACT

Carpenter bees, *Xylocopa virginica* L., are frequent visitors to highbush blueberry flowers in the northeastern U.S., and they sometimes perform corolla slitting behavior to rob nectar. My objective was to assess if nectar robbery offsets the possible value of *X. virginica* as a native pollinator of blueberries in Rhode Island. I studied foraging behavior of *X. virginica* on 14 blueberry cultivars in an experimental plot in Rhode Island, and assessed factors related to slitting behavior, and the effects of slitting on fruit set and blueberry quality. The average number of flowers that *X. virginica* visited per minute was significantly influenced by time of day, temperature, and sex, but the time spent per flower was not. The frequency of corolla slitting by carpenter bees among 14 cultivars during bloom showed an average of 35% slit flowers (range 16 – 67% 'Earliblue' and 'Lateblue' respectively) in 2017, and 39% (range 20 – 62% 'Bluecrop' and 'Collins' respectively) in 2018. Plant and environmental factors that affected the proportion of corollas slit included cultivar, anther length, flower volume, and number of days in bloom at or above 15ºC. Corolla slitting did not affect fruit set. Average fruit weight and percent soluble solids resulting from slit and non-slit corollas did not differ significantly in two early- ('Bluehaven', 'Earliblue'), two mid- ('Collins', 'Bluecrop'), and two late-season ('Herbert', 'Lateblue') ripening cultivars in 2017. In 2018, average fruit weight and percent soluble solids resulting from slit and non-slit flowers did not differ significantly in most cultivars, but slit flowers resulted in berries with greater mass in 'Bluehaven' and 'Collins'. 'Collins' fruit from non-slit corollas had a significantly higher percentage of soluble solids at maturity than fruit from slit

corollas in 2018. Corolla slitting and nectar robbery by *X. virginica* did not have a significant negative effect on fruit quality under our growing conditions and pollinator community.

INTRODUCTION

Much of modern agriculture relies on the critical activity of insect pollinators, namely bees, for pollination services that enhance crop production (Klein et al. 2007). In the last century, the European honey bee, *Apis mellifera*, has been managed as the primary pollinator for cultivated crops (Southwick and Southwick Jr. 1992). Managed bees, primarily *A. mellifera*, contribute an estimated $11.53 billion to US agriculture each year (Koh et al. 2016). Widespread declines in *A. mellifera* populations could lead to future agricultural instability, particularly in agroecosystems with insufficient wild pollinators (Allen-Wardell et al. 1998). Therefore alternative, managed pollinator species are being explored for sustainable crop pollination services (Bosch and Kemp 2002, Javorek et al. 2002, Westerkamp and Gottsberger 2000).

Highbush blueberries depend on pollinators for fruit production (Brewer and Dobson 1969, Dogterom et al. 2000, MacKenzie 1997) and many growers stock their blueberry plantings with *Apis mellifera* colonies to meet this need. At least 80% of highbush blueberry flowers must set fruit to result in a commercial crop (MacKenzie 1997) and berry production is known to benefit from sonication and cross-pollination (De Luca and Valleho-Marin 2013, Free 1993, Brewer and Dobson 1969). Sonication, also referred to as buzz pollination, is a pollination syndrome that allows a pollinator to effectively release pollen from the small pores in blueberry anthers (De Luca and Valleho-Marin 2013, Free 1993). Cross-pollination enhances fruit set, seed number, and fruit mass (Brewer and Dobson 1969). Cross-pollination from more distantly related cultivars results in larger berries that ripen earlier (Dogterom et al. 2000).

Blueberry cultivars vary in flowering phenology, and cultivars must bloom at the same time for cross pollination (Eck et al. 1990, McGregor 1976).

Bees that buzz pollinate include the Andrenidae, Halictidae, *Bombus* spp., and *Xylocopa virginica* (Hogendoorn et al. 2000, Javorek et al. 2002). Honey bees do not sonicate, and are known to transport few blueberry pollen tetrads (Javorek et al. 2002, Benjamin and Winfree 2014). In lowbush blueberry crop systems, species from the genera *Bombus* and *Andrena* are known to collect large amounts of blueberry pollen, with some *Andrena* species collecting nearly 100 percent lowbush blueberry pollen (Bushmann and Drummond 2015, Moisan-Deserres et al. 2014, Stubbs et al. 1992). The efficacy of *Bombus* spp. as lowbush blueberry pollinators has already been documented (Javorek et al. 2002, Drummond 2012).

In Rhode Island, Scott et al. (2016) identified 41 species of native bees collected from highbush blueberry flowers during bloom throughout the state. *Andrena* spp., *Bombus* spp., and *Xylocopa virginica* were among the top ten most often collected bees. *X. virginica* was found to carry the third largest mean pollen grain load of the species sampled (233,500). Abundance at highbush blueberry plantings and sonication behavior suggest that *X. virginica* might be an effect pollinator of highbush blueberry. Indeed, many of the roughly 400 species of *Xylocopa* around the world are already appreciated as agriculturally-significant pollinators of some plants and crops (Gikungu 2014).

However, the Eastern carpenter bee, *Xylocopa virginica*, is a known nectar robber of blueberry flowers (Sampson et al. 2004). Blueberry flowers that experience nectar robbery by *X. virginica* have observable vertical slits in the corollas. It is assumed that

nectar robbers are more likely to exhibit this behavior in flowers with long corollas like blueberry (Maloof and Inouye 2000). Charles Darwin (1872) wrote that plants "must suffer" when bees rob nectar by accessing a flower's nectary from the outside of the corolla. However, Maloof and Inouye (2000) challenged this long-standing assumption by counting the number of studies showing negative, neutral, or positive effects of nectar robbery on fruit. The number of studies were about equal for each category. The potential for *X. virginica* to be an effective alternative blueberry pollinator may be compromised by the possible negative effects of their nectar robbery. The objectives of this study were to: 1) observe the foraging behavior of *X. virginica* on blueberry, 2) determine if *X. virginica* had a preference for slitting the corollas of certain highbush blueberry cultivars, 3) identify plant and environmental characteristics that might influence the percentage of corolla slitting, 4) assess if slitting and nectar robbery is detrimental to fruit quality, and 5) determine if slitting and nectar robbery affects the proportion of flowers that set fruit.

METHODS

Study Site. All experiments were conducted at a 0.15 ha highbush blueberry planting at the University of Rhode Island's East Farm, Kingston, RI. The planting consists of early- ('Earliblue', 'Bluehaven', 'Bluetta') mid- ('Blueray', 'Bluejay', 'Bluecrop', 'Collins', 'Northland', 'Bluegold', 'Jersey', 'Chandler') and late- ('Darrow', 'Herbert', 'Lateblue') ripening cultivars of different ages planted in a grid, 1.5 by 2.4 m apart.

***X. virginica* Foraging Time on Blueberry.** The time that individuals spent on each blueberry flower was recorded on 14 May, 24 May, and 30 May 2018. The number of blueberry flowers that individuals visited up to 3 min. was recorded and converted to a one-minute rate, and the sex of each bee was recorded. Ninety-two males and 23 females were observed for visit duration. Forty-three males and 5 females were recorded for visitation rates. Foraging observations were recorded in both morning and afternoon, and at 16°, 17°, 20°, 21°, and 22°C.

Percent Slit Corollas. Fifty to 100 corollas that had dropped naturally from 14 different blueberry cultivars were haphazardly collected weekly during bloom from 9 May to 6 June 2017 and 14 May to 5 June 2018. Bushes were sampled three to five times depending on bloom duration. Flowers from each bush were evaluated for corolla slitting and percentages were recorded. A total of 32,661 flowers were sampled in 2017, and 13,639 in 2018.

Flower Morphology and Bush Height. Twenty flowers from each cultivar were brought back to the laboratory and measured for corolla opening width, corolla length, anther length, style length, the distance between the top of the anther to the top of the stigma, and the volume of the flower (Fig. 1). The height of each of the blueberry bushes was recorded (10-15 bushes per cultivar).

Bloom Period and Weather Data. In 2018, the date that each blueberry bush started to bloom and the duration of bloom (days) were recorded. The following weather data were recorded: minimum temperature, maximum temperature, average temperature, and precipitation during the bloom period of each bush. The number of

bloom days at or above 9° and 15°C was recorded based on the minimum foraging temperature range (9°-15°C) of *X. virginica* (Skandalis et al. 2011).

Fruit Mass and Soluble Solids (% Brix) Resulting from Slit and Non-Slit Flowers. In 2017, one hundred open flowers on each of 2 early- ('Earliblue' and 'Bluehaven'), 2 mid- ('Collins' and 'Bluecrop'), and 2 late-season ('Herbert' and 'Lateblue') cultivars were tagged with different colored thread indicating whether the flower was slit or non-slit (Fig. 2). We selected slit and non-slit flowers adjacent to each other and in the same cluster. Both slit and non-slit flowers had an equal opportunity of being pollinated prior to tagging and netting. Following tagging, each bush was covered with 80 g (1.0 x 0.6 mm mesh) ProtekNet (Tek-Knit Industries, Mont-Royal, QC, Canada) 2-10 days after first bloom to prevent any further pollinator visitation. In isolating each bush from further visitation, we were able to ensure that tagged non-slit flowers did not experience subsequent slitting and nectar robbery. Exclusion netting was removed from bushes after fruit set. Tagged flowers from each bush were followed to fruit maturity. Berries were harvested when they were entirely blue and had no indications of immaturity. Berries from slit and non-slit flowers were weighed and sugar content (% Brix, mostly sucrose) was measured using a refractometer (Vee Gee BTX-1, Great Lakes IPM, Vestaburg, MI). In 2018, this procedure was replicated for one hundred to two hundred open flowers per bush on the same cultivars that were evaluated in 2017. Two bushes per cultivar were tagged for a total of twelve tagged bushes. Each bush was isolated with exclusion netting as in 2017 after three days of open pollination.

Percent Fruit Set from Slit and Non-slit Flowers. Individual blueberry flower clusters on caged bushes were labelled with weather-resistant adhesive labels (Chartpak, Inc., Leeds, MA) (Fig. 3). Each cluster was uniquely labelled with numbers and letters to indicate how many slit and non-slit flowers were in the cluster at the time of caging. Slit and non-slit flowers were tagged with colored thread as previously described. Each labelled cluster was monitored until harvest, when the number of berries from slit and non-slit flowers were recorded. Percent fruit set for slit flowers was determined by dividing the total number of berries resulting from slit flowers by the number of slit flowers (x100). The percent fruit set for non-slit flowers was determined by dividing the total number of berries resulting from non-slit flowers by the total number of non-slit flowers (x100).

Statistical Analysis. Differences between cultivar floral part measurements and bush heights were analyzed by ANOVA and mean separation by Tukey's HSD test (JMP, ver. 12, SAS Institute, Cary, NC). Multiple stepwise logistic regression analysis was used to analyze the relationship of floral variables, bloom timing, and weather measurements to percent slit corollas (SAS 9.4, SAS Institute, Cary, NC). The Williams method was used to account for over dispersion where the Hosmer-Lemeshow test showed poor model fit. Analysis of variance for the mass and soluble solids (% Brix) of fruit from slit and non-slit flowers was performed using a generalized linear model (PROC GLM, SAS 9.4). Logistic regression analysis was used to compare fruit set between slit and non-slit flowers (SAS 9.4, SAS Institute, Cary, NC).

RESULTS

***X. virginica* Foraging Time on Blueberry.** The time that *X. virginica* spent visiting each blueberry flower did not differ between sexes ($F = 2.37$, df = 1, $P = 0.127$) (Fig. 4) or between AM and PM ($F = 0.39$, df = 1, $P = 0.532$) (Fig. 5). Temperature at the time of observation had a marginal effect on visit duration ($F = 3.63$, df = 1, $P = 0.059$) (Fig. 6). There were no interactions among variables. With respect to flowers visited per minute, there were significant interactions among these variables (temperature-time interaction, $P < 0.01$; temperature-sex interaction, $P < 0.01$). Even with these significant interactions, the number of flowers visited per minute differed significantly for the main effects of sex ($F = 6.48$, df = 1,41, $P = 0.014$) (Fig. 7), time of day ($F = 10.56$, df = 1, 41, $P < 0.01$) (Fig. 8) and temperature ($F = 10.57$, df = 1, 41, $P < 0.01$) (Fig. 9). These results suggest that foraging rate (flowers visited per minute) of *X. virginica* on highbush blueberry is affected more by movement between flowers than by handling time per individual flower.

Percent Slit Corollas. Of 32,661 sampled highbush blueberry flowers among 14 cultivars in 2017, nectar robbery slits were recorded from 11,447 flowers (35%). Average percentages of slit corolla ranged from 16% (‘Earliblue’) to 67% (‘Lateblue’), with statistically significant differences among some cultivars (Table 1). In 2018, 13,639 flowers were sampled, and nectar robbery slits were recorded from 5,311 flowers (39%). Average slit corolla percentages ranged from 20% (‘Bluecrop’) to 62% (‘Collins’), again with significant differences in slitting percentage among some cultivars (Table 1).

Correlation of Slit Corollas to Flower Dimensions, Bloom Time, and Weather. There were significant differences among cultivars in all of the flower dimensions measured and in bush height (Table 1). In 2017 we included cultivar, flower volume (cc), corolla opening width (mm), corolla length (mm), style length (mm), and anther length (mm) in a stepwise logistic regression of percent slit corollas, and cultivar was the only significant variable (Wald $\chi 2 = 200.33$, df = 13, $P < 0.01$). In 2018 we included variables related to weather and timing of flowering in the analysis (Table 3). The logistic regression model in 2018 that best fit slitting frequencies observed in the field included cultivar, anther length, flower volume, and the number of bloom days at or above 15°C (Table 6). To assess the factors that influenced slitting differences among cultivars, we ran the stepwise logistic regression without cultivar as a class variable, and corolla opening width (Fig. 10), pistil length (Fig. 11), bloom season (Fig. 12), date of first flowering (Fig. 13), average temperature (Fig. 14), bloom days above 9°C (Fig. 15), and days (Fig. 16) and proportion of bloom days above 15°C (Fig. 17) were all significantly related to the proportion of corollas slit ($P < 0.05$ in all cases).

Fruit Mass and Soluble Solids (% Brix) Resulting from Slit and Non-Slit Flowers. Fruit mass (Table 4) and sugar content (% Brix) (Table 5) of berries from slit and non-slit corollas were not significantly different among any of the cultivars in 2017. In 2018, analysis of variance for fruit mass revealed a significant two-way interaction between class variables cultivar and the slit or non-slit condition ($F = 3.21$, df = 5, $P = 0.0071$). Thus, we analyzed differences in fruit mass separately for each cultivar. There were no significant differences in mass between fruit that resulted from

slit and non-slit corollas in four of the six cultivars. Berries that resulted from slit corollas of 'Bluehaven' and 'Collins' had a higher average berry mass at the time of harvest (Table 4). A generalized linear model of fruit soluble solids (% Brix) revealed a significant two-way interaction between class variables cultivar and the slit or non-slit condition ($F = 3.47$, df = 5, $P = 0.0041$). Thus, we analyzed differences in fruit soluble solids (% Brix) separately for each cultivar. Berries that resulted from 'Collins' corollas that were non-slit had significantly higher average soluble solids (% Brix) (Table 5).

Percent Fruit Set. The average percentage of slit blueberry flowers that set fruit was 88% and the percentage of non-slit blueberry flowers that set fruit was 82% (Fig. 18). The difference in average percent fruit set did not differ between slit and non-slit flowers on the same cluster overall (Wald $\chi^2 = 0.0292$, df = 1, $P = 0.864$) or among cultivars (Wald $\chi^2 = 8.755$, df = 5, $P = 0.119$).

DISCUSSION

Our results indicate that corolla slitting for nectar robbery by carpenter bees did not affect fruit set, berry size, or sugar content of most highbush blueberry cultivars in our planting. These results suggest that nectar robbery does not offset the possible value of *X. virginica* as a native pollinator of blueberries in southern New England.

Observations of *X. virginica* foraging on blueberry flowers suggest that the number of flowers visited per minute is affected more by movement between flowers than foraging time on individual flowers. The results of our logistic regression model suggest that *X. virginica* does not necessarily slit corollas and rob nectar from

blueberry flowers because of a physical barrier like a narrow corolla opening or a long distance to the nectary. *X. virginica* robs nectar from blueberry flowers due to a combination of cultivar, anther length, flower volume, and the number of days at or above 15°C (Table 3). The consistent significance of cultivar in the frequency of nectar robbery suggest that there is a difference between cultivars that may extend outside of flower morphology alone. It is known that 'Earliblue' flowers are less attractive to honey bees (Pavlis 1991). It appears that carpenter bees are also less attracted to this cultivar. Isaacs et al. (2016) also noted that the cultivar 'Jersey' had a low attractiveness to bees. Lower attraction could result from differences in nectar volume, nectar sugar concentration, or floral scents. Rodriguez-Saona et al. (2011) selected bagged (pollinator-excluded) and unbagged (open-pollinated) blueberry flowers to measure volatiles given off (mostly by the petals) and bee visitations to each treatment. They found that pollinator-excluded flowers emitted a 46% higher amount of volatiles than open-pollinated flowers, which suggests that pollination had a significant effect on volatile emissions. They also found that after removing the exclusion netting, honey bees and bumble bees visited approximately two times as many flowers on the previously pollinator-excluded bushes compared to visits to the previously open-pollinated bushes. Flowering plants provide nutrients and scent signals to attract pollinators to visit flowers while also minimizing investment in these signals. Pollinators on the other hand, maximize their nutritional uptake from flowers by quickly determining which flowers will reward them with pollen and/or nectar. Rodriguez-Saona et al. (2011) also hypothesized that a decrease in floral scent

emissions after pollination might be adaptive both to conserve the costs of odor production and to reduce the likelihood of attracting consumers.

Fruit weight and sugar content between slit and non-slit corollas were not significantly different in all but two cultivars with respect to mass and one cultivar with respect to soluble solids. In 2018, 'Collins' and 'Bluehaven' fruits from slit corollas had significantly higher mass than berries from non-slit flowers. Interestingly, berries from slit corollas of 'Collins' had significantly less soluble solids at harvest in 2018. Since we covered 'Collins' bushes after seven days of open pollination in 2017 and after three days in 2018, the fewer days of open pollination in 2018 may account for the significantly lower soluble solids. These results suggest that pollination by other bees and insects prior to our exclusion netting (even as soon as two days after the start of bloom), was sufficient to overcome any negative effects of nectar robbery by *X. virginica.*

Benjamin and Winfree (2014) studied honey and native bee pollination in commercial highbush blueberry in New Jersey. They found that the European honey bee, *Apis mellifera* L. deposited a median of 18.5 tetrads of pollen during a nectar-collecting visit, 24 tetrads during a pollen-collecting visit and 0.5 tetrads during a secondary nectar-robbing visit (through punctures made by *X. virginica*). They also found that pollen tetrads deposited by *Bombus* spp., large *Andrena* spp., medium *Andrena* spp. and *Xylocopa virginica* were 23.5, 9.0, 11.5, and 2.5 tetrads respectively. All their study sites were stocked with domesticated honey bees at densities of 2.5-7.5 hives ha^{-1}. Honey bees provided 86% and native bees 14% of the pollination. Conversely, Winfree et al. (2007) found that native bees were the most important

pollinators and alone were sufficient to pollinate commercially grown watermelons in New Jersey and Pennsylvania.

Previous studies have shown that native bees contribute to crop pollination at farms near natural habitat, but not in more intensively used agricultural areas (Kremen et al. 2004, Klein et al. 2007). European honey bees do not perform buzz pollination and are not considered efficient pollinators of lowbush or highbush blueberries (Drummond 2016, Benjamin and Winfree 2014). Increasing honey bee stocking densities could unnecessarily increase the production costs of commercial plantings where native bee populations might provide sufficient pollination (Garibaldi et al. 2013, Benjamin et al. 2014).

Xylocopa virginica has a long colony life cycle, with many females living two years (Gerling and Hermann 1978). In March and April males defend areas near the nest and mate with females. Females construct nests in unfinished wood, and nests can be reused for many generations (Gerling and Hermann 1978). *Xylocopa virginica* has nectar robbing tendencies, relatively low blueberry pollen loads, and pollen transfer efficiency is low (2.5 pollen tetrads deposited per visit, Benjamin and Winfree 2014). Despite these shortcomings, the natural abundance of these pollinators and possible ease of increasing numbers by providing unfinished wood nesting sites around blueberry plantings, suggests more research on the importance of this bee as a blueberry pollinator is needed. The results of our study indicate that corolla slitting and nectar robbery by Eastern carpenter bees does not have a significant negative effect on fruit yield under the described growing conditions and pollinator community.

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Table 1. Mean (± SE) dimensions of 14 highbush blueberry cultivar flowers and percentage with slit corollas, 2017 and 2018.

	$\bar{x}$ ± SE[a]						
Cultivar	Flower Volume (cc)	Corolla Opening (mm)	Corolla Length (mm)	Style Length (mm)	Anther Length (mm)	% Slit Corollas 2017	% Slit Corollas 2018
Early-Season							
Bluegold	0.184 ± 0.005ef	4.3 ± 0.09abc	8.5 ± 0.08fg	7.2 ± 0.08h	3.83 ± 0.07f	32 ± 10bcd	37 ± 16abcd
Bluehaven	0.136 ± 0.136h	3.8 ± 0.08e	7.9 ± 0.10hi	8.3 ± 0.10f	4.31 ± 0.10cde	34 ± 10bcd	49 ± 19ab
Bluetta	0.203 ± 0.006def	4.2 ± 0.08bcd	8.8 ± 0.04ef	8.6 ± 0.08f	3.56 ± 0.04g	28 ± 6cd	59 ± 21a
Earliblue	0.289 ± 0.008b	4.7 ± 0.10a	9.1 ± 0.08de	8.6 ± 0.10ef	4.17 ± 0.06de	16 ± 8d	38 ± 14bc
Northland	0.151 ± 0.151gh	4.0 ± 0.07cde	7.6 ± 0.08i	7.7 ± 0.08g	4.15 ± 0.06e	25 ± 5d	41 ± 17bcd

Table 1 (continued).

Cultivar	$\bar{x} \pm SE$[a]						
	Flower Volume (cc)	Corolla Opening (mm)	Corolla Length (mm)	Style Length (mm)	Anther Length (mm)	% Slit Corollas 2017	% Slit Corollas 2018
Mid-Season							
Bluecrop	0.211 ± 0.007cde	4.0 ± 0.08de	8.9 ± 0.10ef	9.0 ± 0.05de	4.53 ± 0.03abc	30 ± 7cd	20 ± 5d
Bluejay	0.293 ± 0.008b	4.0 ± 0.07de	10.5 ± 0.05b	10.4 ± 0.07a	4.58 ± 0.05ab	41 ± 7bc	40 ± 15bcd
Blueray	0.284 ± 0.006b	4.5 ± 0.07ab	9.4 ± 0.10cd	9.0 ± 0.06e	4.43 ± 0.04bcd	29 ± 8cd	25 ± 5cd
Collins	0.213 ± 0.006cde	3.8 ± 0.074e	10.3 ± 0.08b	9.8 ± 0.08b	4.56 ± 0.06abc	35 ± 8bc	62 ± 15a
Chandler	0.344 ± 0.007a	4.4 ± 0.05ab	11.2 ± 0.06a	9.5 ± 0.05bc	4.09 ± 0.04e	46 ± 14abc	24 ± 8bcd
Darrow	0.215 ± 0.006cd	3.3 ± 0.05f	9.7 ± 0.09c	9.0 ± 0.09e	4.34 ± 0.04bcde	38 ± 2bc	32 ± 13d

Table 1 (continued).

Cultivar	$\bar{x} \pm SE^a$						
	Flower Volume (cc)	Corolla Opening (mm)	Corolla Length (mm)	Style Length (mm)	Anther Length (mm)	% Slit Corollas 2017	% Slit Corollas 2018
Late-Season							
Herbert	0.235 ± 0.006c	3.8 ± 0.11e	8.9 ± 0.08e	9.7 ± 0.13bc	4.48 ± 0.04bc	40 ± 8bc	31 ± 4cd
Jersey	0.177 ± 0.004fg	3.4 ± 0.04f	8.1 ± 0.11gh	9.4 ± 0.06cd	4.79 ± 0.05a	47 ± 4b	31 ± 13bc
Lateblue	0.208 ± 0.004cde	4.0 ± 0.05cde	8.3 ± 0.07g	8.6 ± 0.09f	4.75 ± 0.05a	67 ± 3a	30 ± 7bcd

Table 1 (continued).

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[a]Means in the same column followed by the same letter are not significantly different, $\alpha = 0.05$, Tukey's-HSD test. Flower Volume: $F = 94.2$; df = 13, 262; $P < 0.01$. Corolla Opening: $F = 28.4$; df = 13, 262; $P < 0.01$. Corolla Length: $F = 159.0$; df = 13, 262; $P < 0.01$. Style Length: $F = 103.2$; df = 13, 262; $P < 0.01$. Anther Length: $F = 40.1$; df = 13, 262; $P < 0.01$. % Slit Corollas 2017: $F = 16.0$; df = 13, 155; $P < 0.01$. % Slit Corollas 2018: $F = 10.9$; df = 13, 136; $P < 0.01$.

Table 2. Mean (± SE) bush height and percentage of flowers with slit corollas, 2017 and 2018.

	$\bar{x}$ ± SE[a]		
Cultivar	Bush Height (cm)	% Slit Corollas 2017	% Slit Corollas 2018
Early Season			
Bluegold	93 ± 6.4h	32 ± 10bcd	37 ± 16abcd
Bluehaven	148 ± 4.1e	34 ± 10bcd	49 ± 19ab
Bluetta	142 ± 8.6cde	28 ± 6cd	59 ± 21a
Earliblue	177 ± 5.3bc	16 ± 8d	38 ± 14bc
Northland	129 ± 8.0g	25 ± 5d	41 ± 17cde
Mid Season			
Bluecrop	156 ± 7.6f	30 ± 7cd	20 ± 5f

Table 2 (continued).

	$\bar{x} \pm SE^a$		
Cultivar	Bush Height (cm)	% Slit Corollas 2017	% Slit Corollas 2018
Bluejay	163 ± 10.1ab	41 ± 7bc	40 ± 15bcd
Blueray	177 ± 4.0bc	29 ± 8cd	25 ± 5cd
Collins	150 ± 5.9g	35 ± 8bc	62 ± 15a
Chandler	120 ± 3.6g	46 ± 14abc	24 ± 8bcd
Darrow	153 ± 5.6cd	38 ± 2bc	32 ± 13d
Late Season			
Herbert	171 ± 8.3de	40 ± 8bc	31 ± 4cd
Jersey	133 ± 6.4e	47 ± 4b	31 ± 13bc

Table 2 (continued).

	$\bar{x} \pm SE$[a]		
Cultivar	Bush Height (cm)	% Slit Corollas 2017	% Slit Corollas 2018
Lateblue	184 ± 4.7a	67 ± 3a	30 ± 7bcd

[a]Means in the same column followed by the same letter are not significantly different, α = 0.05, Tukey's-HSD test. Bush height: F = 109.8; df = 13, 261; $P < 0.01$. % Slit Corollas 2017: F = 16.0; df = 13, 155; $P < 0.01$. % Slit Corollas 2018: F = 10.9; df = 13, 136; $P < 0.01$.

Table 3. Variables included in stepwise logistic regression model for correlation with percent slitting per bush, 2018.

Plant-Related Variables	Bloom Period-Related Variables	Weather-Related Variables
Cultivar (categorical variable)*	Season (categorical variable)	Rain (inches during bloom)
Corolla Opening Width (mm)	Bloom Duration (days)	Temperature (average during bloom)
Flower Volume (cc)*	Bloom Start Date	Number of Bloom Days ≥ 9°C
Length of Corolla (mm)		Proportion of Bloom Days ≥ 9°C
Length of Pistil (mm)		Number of Bloom Days ≥ 15°C*
Length of Anther (mm)*		Proportion of Bloom Days ≥ 15°C
Distance From Top of Anther to Tip of Stigma (mm)		

Variables followed by * were included in final model.

Table 4. Mean (± SE) mass (g) of blueberries resulting from slit and non-slit flowers on six highbush blueberry cultivars, 2017 and 2018.

	$\bar{x}$ ± SE[a]			
	2017		2018	
Cultivar	Slit Flowers	Non-Slit Flowers	Slit Flowers	Non-Slit Flowers
Early Season				
Bluehaven	1.24 ± 0.06	1.34 ± 0.07	2.39 ± 0.08*	1.89 ± 0.12*
Earliblue	1.06 ± 0.08	1.26 ± 0.06	1.75 ± 0.04	1.75 ± 0.05
Mid-Season				
Collins	1.39 ± 0.05	1.40 ± 0.05	1.86 ± 0.04*	1.70 ± 0.04*
Bluecrop	1.74 ± 0.06	1.72 ± 0.07	2.13 ± 0.06	2.00 ± 0.05
Late Season				
Herbert	0.92 ± 0.08	1.14 ± 0.11	1.91 ± 0.06	1.80 ± 0.06

Table 4 (continued).

	$\bar{x} \pm SE^a$			
	2017		2018	
Cultivar	Slit Flowers	Non-Slit Flowers	Slit Flowers	Non-Slit Flowers
Lateblue	1.05 ± 0.08	0.95 ± 0.07	1.63 ± 0.06	1.56 ± 0.05

[a]2017: Bluehaven: $F = 1.18$; df = 1, 94; $P = 0.28$. Earliblue: $F = 3.81$; df = 1, 11; $P = 0.08$. Collins: $F = 0.003$; df = 1, 77; $P = 0.96$. Bluecrop: $F = 0.03$; df = 1, 61; $P = 0.87$. Herbert: $F = 2.49$; df = 1, 34; $P = 0.12$. Lateblue: $F = 0.94$; df = 1, 29; $P = 0.34$. 2018: Bluehaven: $F = 12.08$; df = 1, 85; $P < 0.01$. Earliblue: $F = 0.006$; df = 1, 127; $P = 0.94$. Collins: $F = 7.33$; df = 1, 175; $P < 0.01$. Bluecrop: $F = 2.88$; df = 1, 116; $P = 0.09$. Herbert: $F = 1.60$; df = 1, 122; $P = 0.21$. Lateblue: $F = 0.57$; df = 1, 123; $P = 0.45$. Means in the same year and in the same row marked with * were significantly different. $\alpha = 0.05$, Tukey's-HSD test.

Table 5. Mean (± SE) soluble solids (% Brix) of fruit resulting from slit and non-slit flowers on six highbush blueberry cultivars, 2017 and 2018.

	$\bar{x}$ ± SE[a]			
	2017		2018	
Cultivar	Slit Flowers	Non-Slit Flowers	Slit Flowers	Non-Slit Flowers
Early Season				
Bluehaven	12.83 ± 0.27	12.86 ± 0.33	11.80 ± 0.24	12.04 ± 0.36
Earliblue	10.83 ± 0.004	11.43 ± 0.007	12.18 ± 0.14	12.08 ± 0.18
Mid-Season				
Collins	11.32 ± 0.29	10.93 ± 0.23	11.53 ± 0.20*	12.52 ± 0.21*
Bluecrop	10.16 ± 0.21	10.00 ± 0.20	10.65 ± 0.18	10.96 ± 0.16
Late Season				
Herbert	12.77 ± 0.47	13.01 ± 0.38	12.19 ± 0.25	11.94 ± 0.22

Table 5 (continued).

	$\bar{x} \pm SE^a$			
	2017		2018	
Cultivar	Slit Flowers	Non-Slit Flowers	Slit Flowers	Non-Slit Flowers
Lateblue	11.40 ± 0.49	12.21 ± 0.48	11.59 ± 0.20	11.30 ± 0.17

[a]2017: Bluehaven: $F = 0.005$; df = 1, 94; $P = 0.95$. Earliblue: $F = 0.48$; df = 1, 11; $P = 0.50$. Collins: $F = 1.11$; df = 1, 77; $P = 0.30$. Bluecrop: $F = 0.35$; df = 1, 61; $P = 0.56$. Herbert: $F = 0.15$; df = 1, 34; $P = 0.70$. Lateblue: $F = 1.39$; df = 1, 29; $P = 0.25$. 2018: Bluehaven: $F = 0.3185$; df = 1, 85; $P = 0.57$. Earliblue: $F = 0.21$; df = 1, 128; $P = 0.64$. Collins: $F = 11.58$; df = 1, 175; $P < 0.01$. Bluecrop: $F = 1.6137$; df = 1, 117; $P = 0.21$. Herbert: $F = 0.58$; df = 1, 126; $P = 0.45$. Lateblue: $F = 1.29$; df = 1, 124; $P = 0.26$. $\alpha = 0.05$, Tukey's-HSD test.

Table 6. Stepwise logistic regression model on proportion of flowers slit, 2018.

Variable	Wald χ^2	df	*P*
Cultivar	289.96	13	<0.01
Flower Volume (cc)	4.311	1	0.04
Anther Length (mm)	14.96	1	<0.01
Bloom Days ≥ 15°C	3.94	1	0.05

Model fit: Hosmer and Lemeshow, $\chi^2 = 5.305$, df = 8, $P = 0.725$.

Table 7. Comparison of slit and non-slit blueberry flowers that set fruit, 2018.

Variable	Wald χ^2	df	*P*
Cultivar	8.76	5	0.12
Slit/Non-Slit	0.03	1	0.86
Interaction	2.48	5	0.78

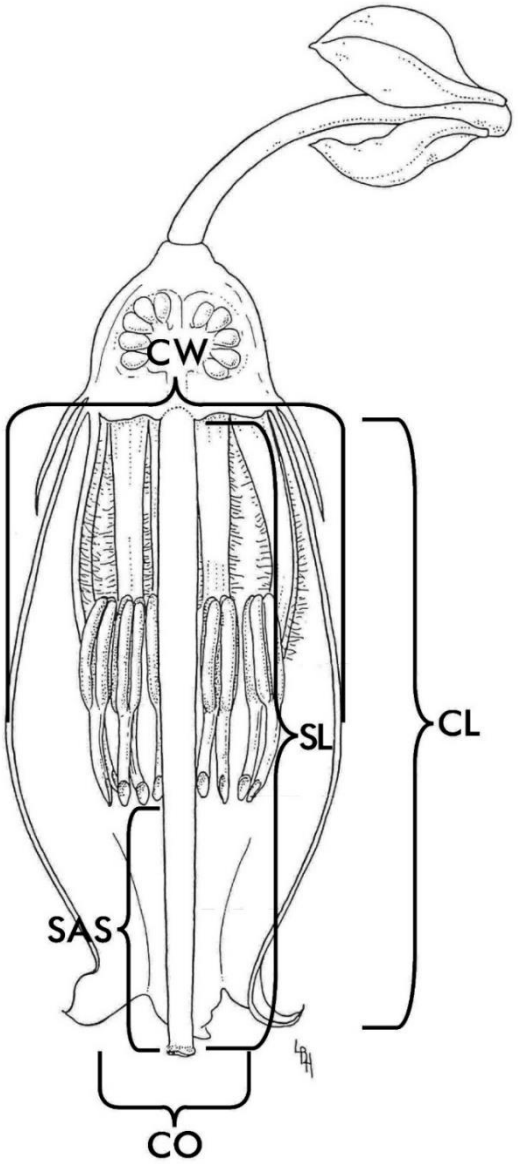

Figure 1. Blueberry flower parts: CW = corolla width, CO = corolla opening, CL = corolla length, SL = style length, SAS = stigma-anther separation, SL-SAS = anther filament length.

Figure 2. Tagged slit and non-slit blueberry flowers.

Figure 3. Weather resistant label to determine fruit set of slit and non-slit flowers.

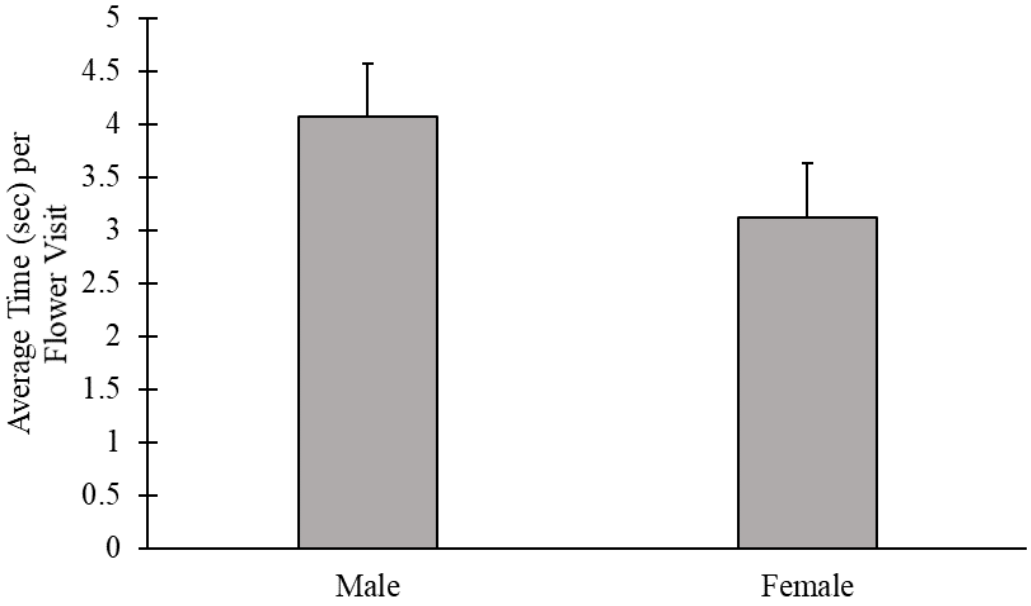

Figure 4. Average time (seconds) per flower visit by males and females (F = 2.37; df = 1, 111; P = 0.12).

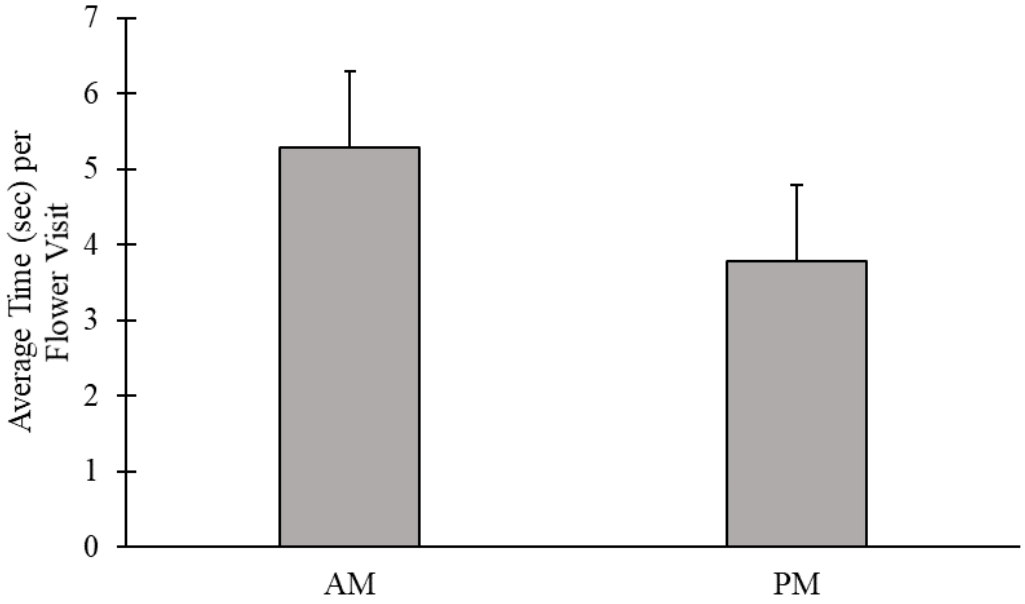

Figure 5. Average time (seconds) per flower visit, AM and PM (F = 0.39; df = 1, 111; P = 0.53).

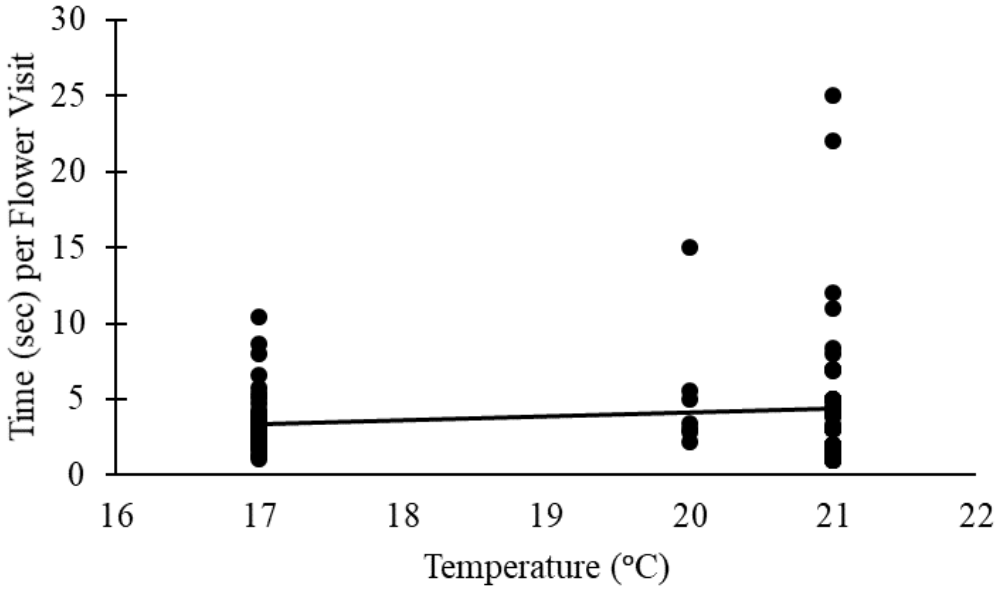

Figure 6. Average time (seconds) per flower visit at 17°, 20°, and 21°C (F = 3.63; df = 1,111; P = 0.059).

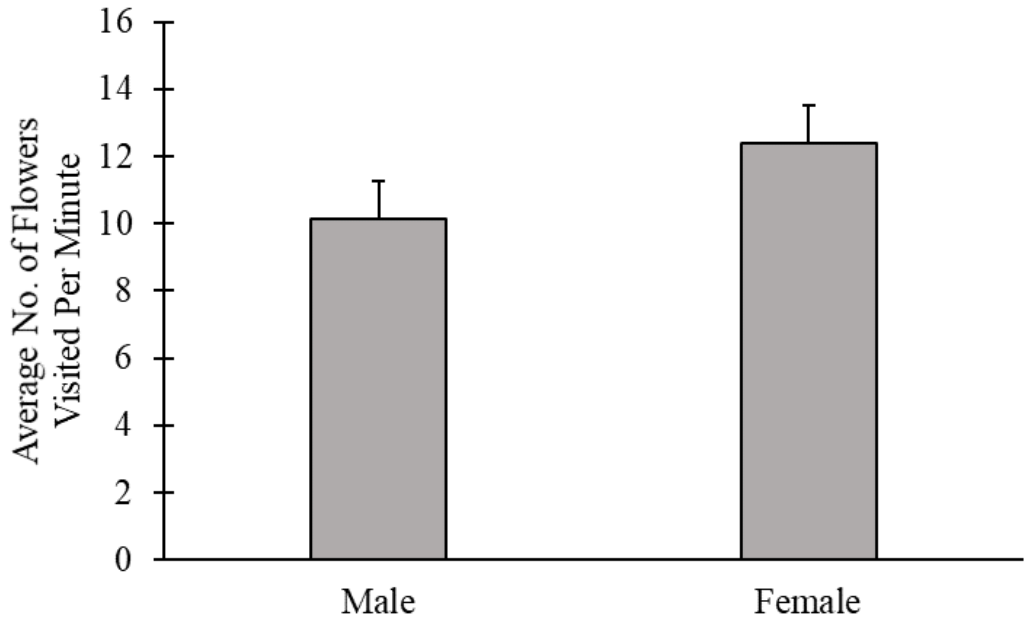

Figure 7. Average number of flowers visited per minute by males and females (F = 6.48; df = 1,41; P = 0.014).

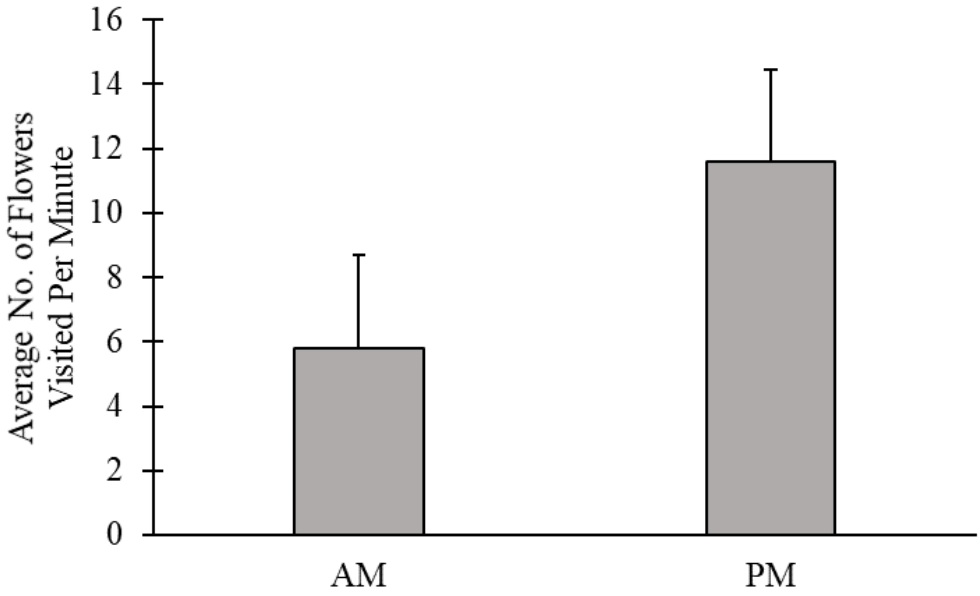

Figure 8. Average number of flowers visited per minute, AM and PM (F = 10.56; df = 1,41; $P < 0.01$).

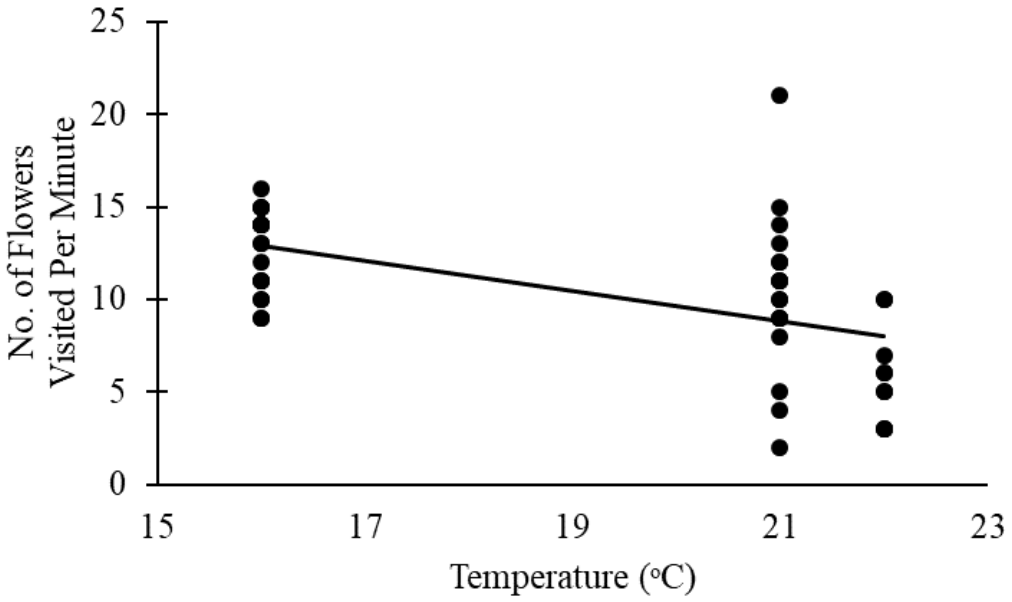

Figure 9. Average number of flowers visited per minute at 16°, 21°, and 22°C (F = 10.57; df = 1,41; $P < 0.01$).

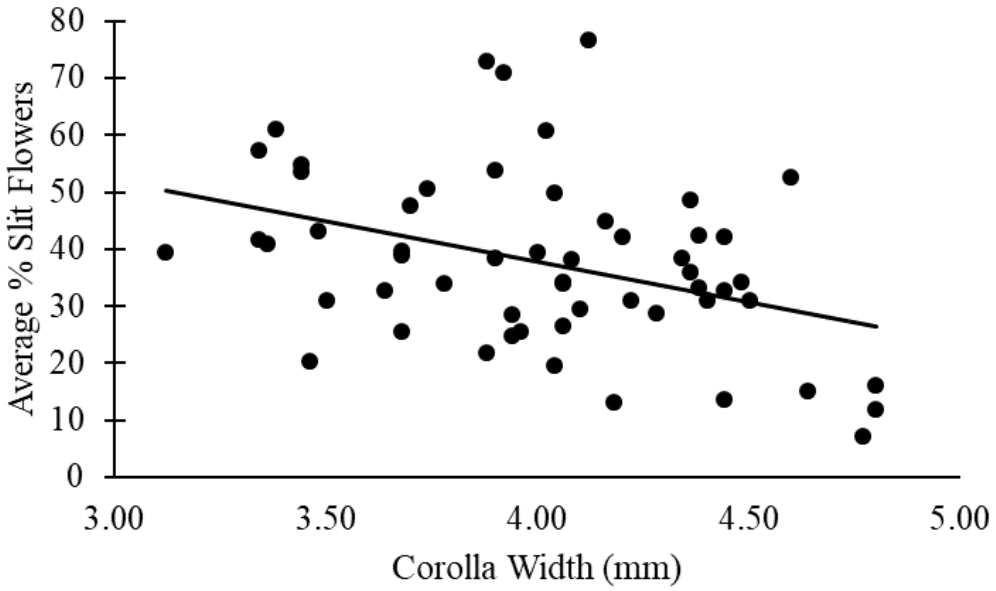

Figure 10. Average percent slit flowers as a function of corolla opening width (mm) (F = 9.36; df = 1, 54; P < 0.01).

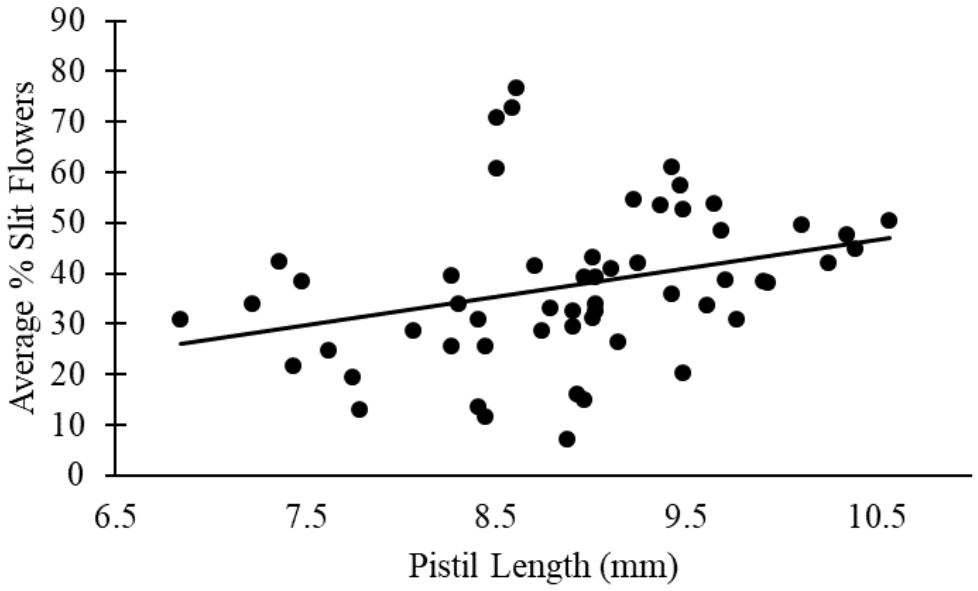

Figure 11. Average percent slit flowers as a function of pistil length (mm) (F = 5.69; df = 1, 54; P = 0.02).

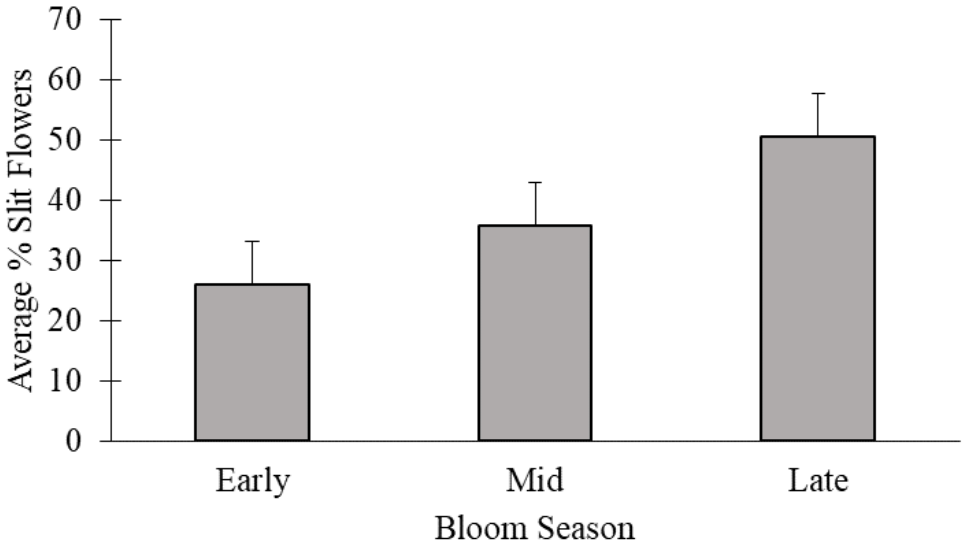

Figure 12. Average percent slit flowers as a function of bloom season ($F = 9.46$; df = 2, 53; $P < 0.01$).

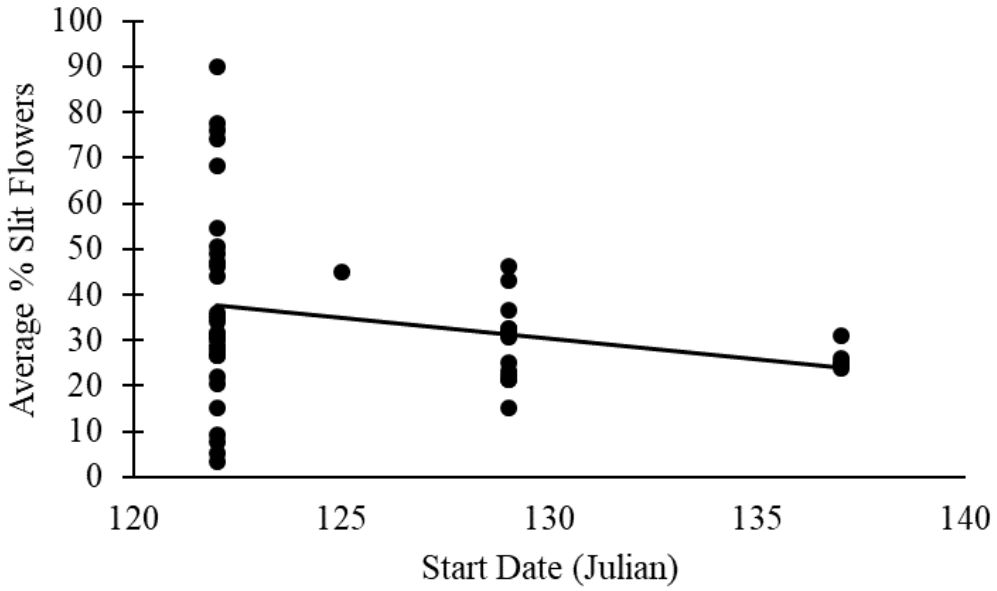

Figure 13. Average percent slit flowers as a function of bloom start date (F = 3.03; df = 1, 48; P = 0.09).

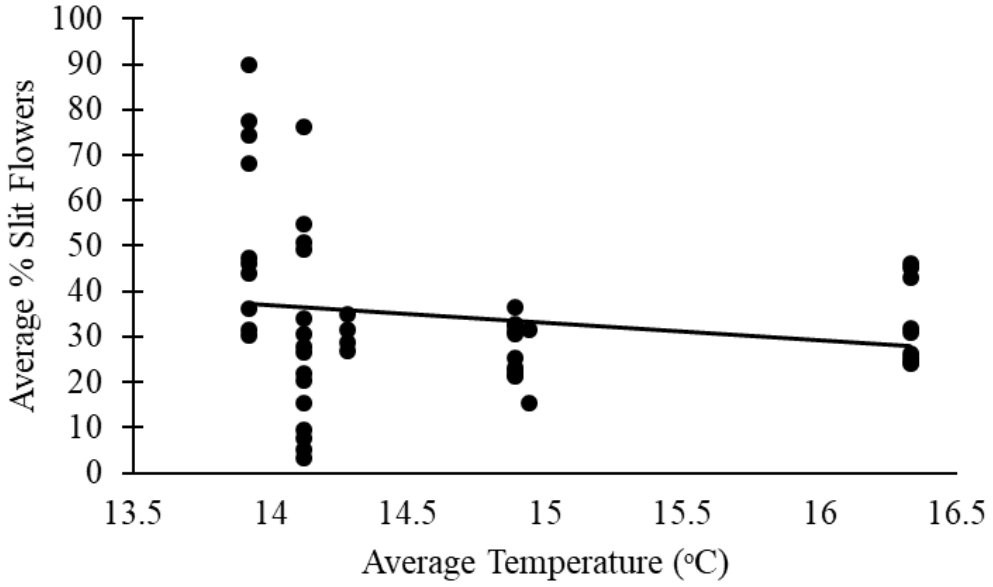

Figure 14. Average percent slit flowers as a function of temperature during bloom at 13.9°, 14.1°, 14.3°, 14.9°, and 16.3°C (F = 1.64; df = 1, 48; P = 0.21).

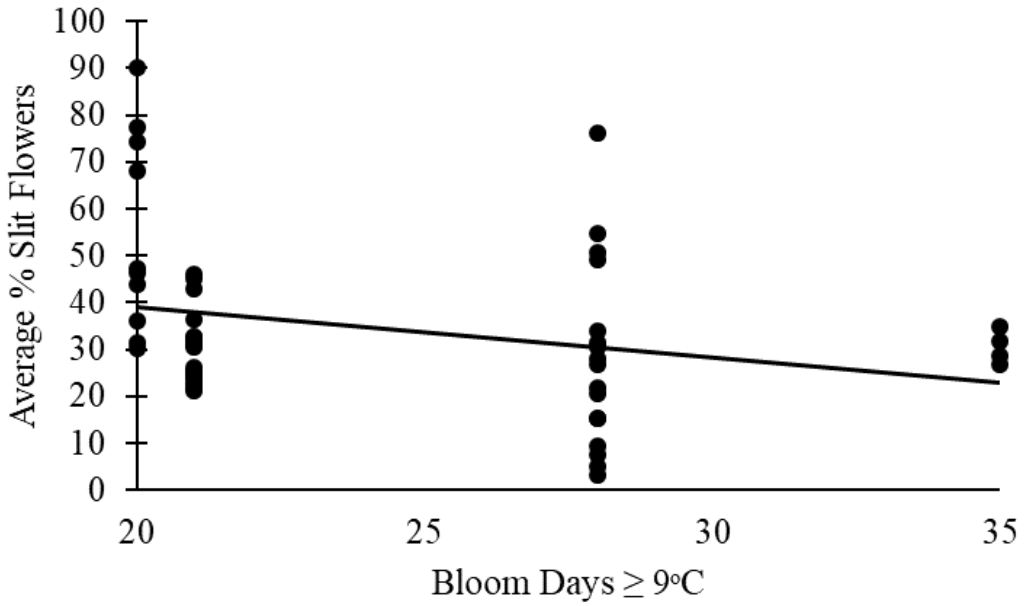

Figure 15. Average percent slit flowers as a function of the number of bloom days ≥ 9°C (F = 3.86; df = 1, 48; P = 0.06).

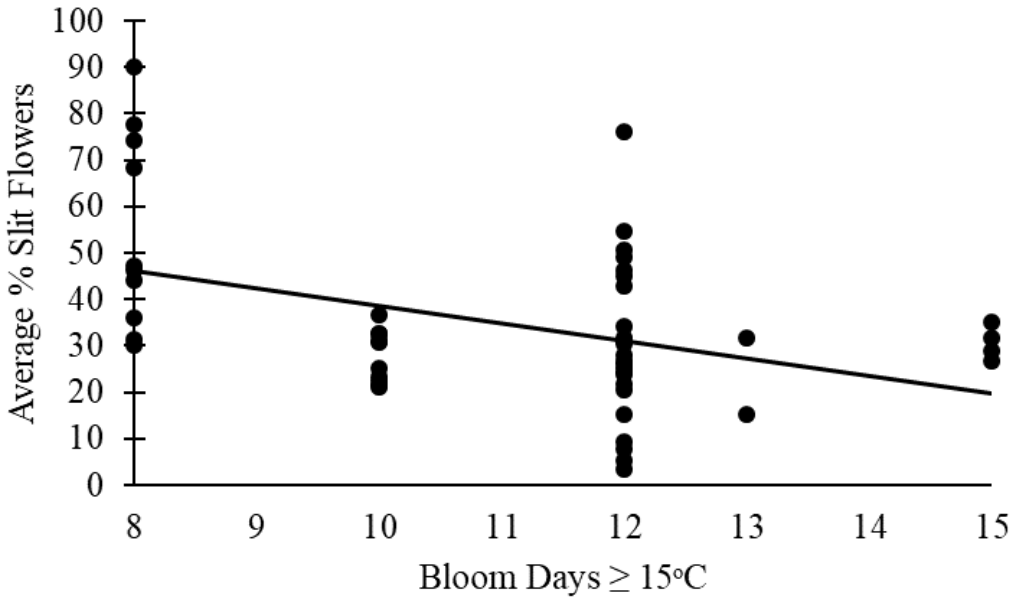

Figure 16. Average percent slit flowers as a function of the number of bloom days ≥ 15°C (F = 9.68; df = 1, 48; P < 0.01).

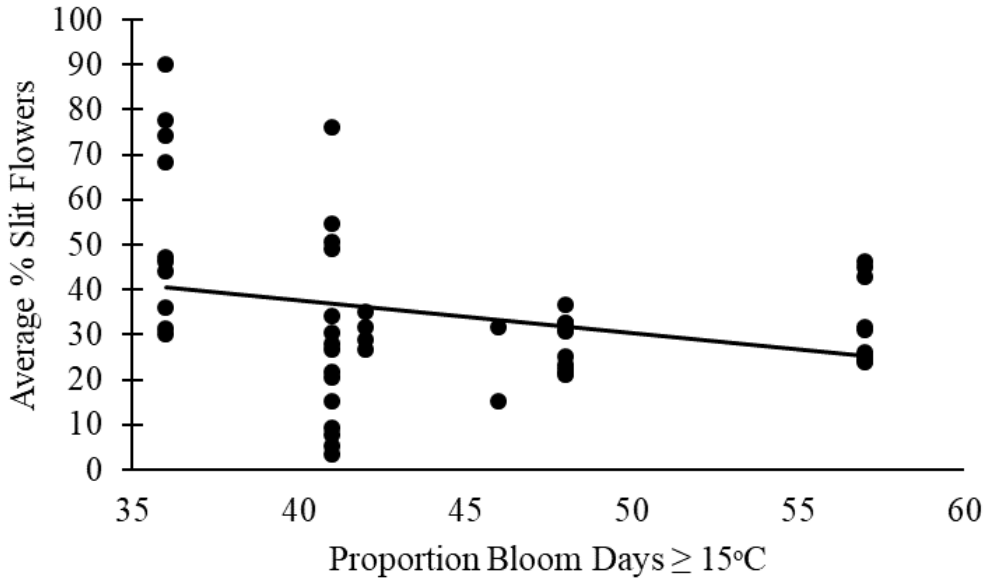

Figure 17. Average percent slit flowers as a function of proportion of bloom days ≥ 15°C (F = 4.16; df = 1, 48; P = 0.05).

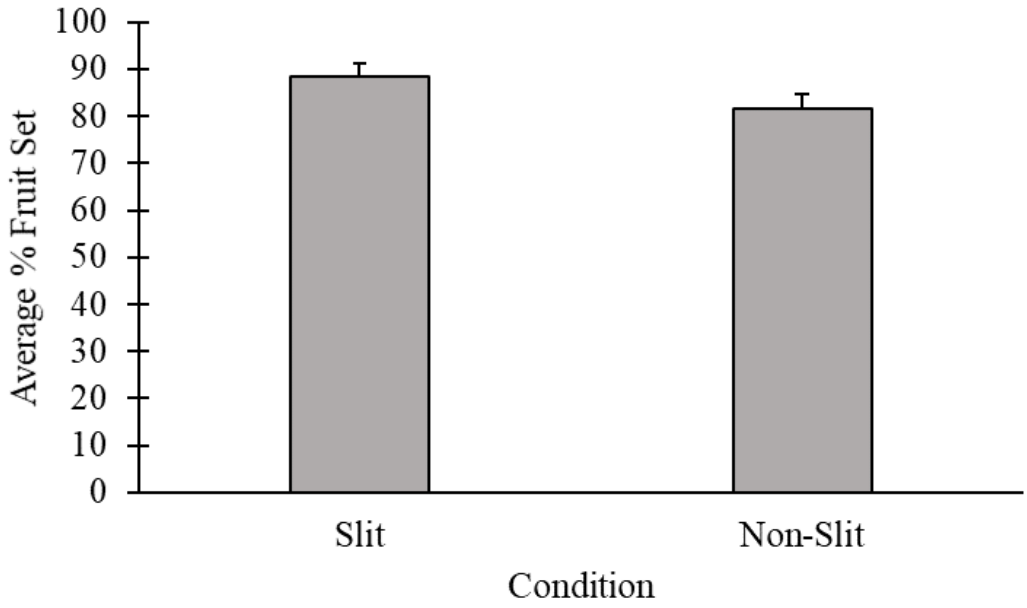

Figure 18. Average percent fruit set resulting from slit and non-slit flowers (F = 2.8; df = 1, 174; P = 0.096).

CHAPTER 2

"Eastern Carpenter Bee (*Xylocopa virginica* L., Hymenoptera: Apidae) Nest Structure, Nest Cell Provisions, and Trap Nest Acceptance in Rhode Island"

by

Sara K. Tucker, Howard Ginsberg, and Steven R. Alm

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ABSTRACT

Mean values of *Xylocopa viginica* nest tunnel and cell measurements were similar to those reported in previous studies. Analysis of pollen loaves showed that *X. virginica* provisioned pollen loaves from 21 different genera of plants in 2016, 19 in 2017, and 39 in 2018. *Antirrhinium majus* (Garden snapdragon) made up the majority (21.4%) of pollen collected in all three years. Overall, wind-pollinated tree pollen made up 22.13% of all pollen loaves. Blueberry pollen was a minor component of pollen loaves (0.1%). Only two of 168 trap nests deployed in 2017 were occupied by a total of ten *X. virginica* bees. However, 17 nests hosted 230 *Osmia taurus*, six nests hosted 73 *Osmia cornifrons*, and one nest hosted 8 *Osmia lignaria*. Thirty-four nests (20.2%) were occupied by 151 grass-carrying wasps, *Isodontia* sp. and 6 vespid wasps occupied two nests (1.2%) in 2017. In 2018, four of ninety-six trap nests were occupied by carpenter bees.

INTRODUCTION

The heavy reliance of modern agriculture on the European honey bee, *Apis mellifera*, for crop pollination poses a serious risk of food insecurity as honey bee populations decline at alarming rates (Allen-Wardell et al. 1998, Klein et al. 2007; Southwick and Southwick Jr. 1992). As demand for pollination services increases, the need for alternative pollination strategies is evident. The annual benefit of bees to agricultural production in the U.S. alone is upwards of $14.6 billion, with native bees contributing at least 20% of this value (Koh et al. 2016). Some studies suggest that wild native bee populations may provide adequate pollination services where suitable habitat exists (Garibaldi et al. 2013, Winfree et al. 2007). The commercial application of native bees for crop pollination may depend on both the effectiveness of bees to pollinate crops and the potential of bees to be managed (Velthuis and van Doorn 2006). Recent studies have explored the effectiveness of native bees including *Bombus*, *Osmia*, and *Andrena* as crop pollinators (Morandin et al. 2001, Bosch and Kemp 2015, Park et al. 2016). In some agro-ecosystems, native bees may be even better pollinators than honey bees (Westerkamp 1991).

Honey bees may not provide sufficient pollination to crops in cold and rainy climates, or to crops requiring particular pollination syndromes (Willmer et al. 1994). Bees that buzz-pollinate are especially effective at pollinating blueberry, where flowers only release pollen from small pores in the anthers (De Luca and Valleho-Marin 2013, Free 1993). Many native bee species, including those in the genera *Andrena*, *Bombus* and *Xylocopa*, and family Halictidae have evolved this adaptation (Javorek et al. 2002). All of the native bee genera that are known to perform buzz

pollination have been identified throughout Rhode Island (Scott et al. 2016). Between 2014 and 2016 the Eastern carpenter bee, *Xylocopa virginica*, was among the top five most commonly collected bee species in highbush blueberry plantings (Scott et al. 2016).

The potential for the commercial application of native bees for crop pollination may be limited by the ability to maintain populations. *Bombus terrestris* and *Bombus impatiens* have been utilized for commercial crop pollination since 1987 because populations have been successfully domesticated, are adaptable, and are easy to transport (Velthuis and van Doorn 2006). Other managed native bees include *Nomia melanderi* and *Megachile rotundata* for alfalfa pollination. *Osmia cornifrons* and *Osmia lignaria* are managed for apple and almond pollination. Man-made structures like the increasingly popular "bee hotel" may recruit naturally-occurring native bees to crop plantings (MacIvor and Packer 2015). Structures and nest traps could ideally be set up within agro-ecosystems to maintain native bee populations for crop pollination requirements.

The natural abundance of *X. virginica* populations in Rhode Island presents an opportunity to explore the adaptability of carpenter bees for crop pollination. Because *X. virginica* is one of the most common bees visiting blueberry flowers and has the ability to buzz-pollinate, we focused on the potential application of *X. virginica* as a managed blueberry pollinator. Our objectives were to 1) determine the nesting habits of *X. virginica* based on field observations and nest dissections, 2) identify the forage requirements for *X. virginica* by analyzing pollen provisions, and 3) evaluate several nest designs to recruit and maintain *X. virginica* populations.

METHODS

Nest architecture and pollen loaf composition. Wooden boards (3.6 x 8.8 cm) with active *X. virginica* nest entrances were removed from a pole barn in Kingston, RI and planed to reveal nest architecture and pollen provisions. Twenty-eight tunnels and twenty cells were measured. A total of 27 pollen loaves were sampled over three years. Eight pollen loaves were collected from one *X. virginica* nest on June 20, 2016; six pollen loaves from one nest on June 8, 2017; and 2, 8, and 3 pollen loaves from three nests collected on June 8, 15 and 22, 2018 respectively. Pollen loaves were removed from their cells and examined for any eggs or larvae accompanying them. If no larva was found in a cell with a loaf, it was considered to be a full loaf. Full loaves were weighed and analyzed for pollen cell composition via acetolysis processing (Faegri et al. 1989). Pollen cells were identified to the lowest taxon possible.

The eight full loaves collected in 2016 were weighed and dissolved in a 20 ml dye solution (92.5% water, 5% Tween 20, 2.5% Gram fuchsin solution). One microliter of this solution was placed onto a hemocytometer and the pollen grains were counted under a microscope. This value was then extrapolated to find the number of pollen grains in the pollen loaf. We used the average number of pollen grains calculated from the top 10% of pollen loads carried by *X. virginica* bees collected in 2015 to calculate an average "full" pollen load of 1,207,333 pollen grains. We were then able to calculate the approximate number of foraging trips required for a bee to complete a full pollen loaf.

Discarded Pollen Composition. In July 2017, one female *X. virginica* was observed noticeably pushing pollen out of a nest with her head. A container was

placed directly below the entrance of the nest. After one week, the pollen contents from the container were collected and analyzed for pollen cell composition.

Manufactured nests attached to a barn, 2017. Eighty-one pine boards (36.5 x 2 x 9 cm) were routered in each of two boards to create two halves of both 9 mm and 12 mm tunnel widths 15 cm long on each side of a 12 diam. entrance hole 15 mm deep (Fig. 1). The two halves of a nest were held together with four 3.8 cm screws. Twelve nests of each 9 mm and 12 mm tunnel widths were randomly assigned and attached to 3.6 x 8.8 cm boards 2.5 m from ground level where carpenter bees have been nesting for at least 30 years 54 cm from the edge of an aluminum roof (Fig. 2). Twelve nests of each of the tunnel widths again were randomly assigned and attached to 3.6 x 8.8 cm boards 2.3 m from ground level and 13 cm from the edge of the aluminum roof (Fig. 2). All entrance holes were directed downward.

Manufactured nests attached to a lean too storage area, 2017. Twelve 9 mm and twelve 12 mm tunnel width pine nests as above were randomly assigned and attached to the fascia of a lean too storage area 2.1 m from ground level and 18 cm from the edge of the roof (Fig. 3). Carpenter bees had also been seen nesting in this area for at least 30 years. All entrance holes were directed downward.

Manufactured nests attached to posts, 2017. Fourty-eight 9 mm and fourty-eight 12 mm tunnel width pine nests as above were randomly assigned and attached to 10.2 x 10.2 cm pressure treated posts with nest entrances at 1.5 and 3 m above ground level (Fig. 4). Posts were 6 m from the edge of a 0.3 ha blueberry planting and 4.5 m between posts. There were 12 posts on each of the east (sunny) and west (shady) sides of a 0.15 ha blueberry planting (Fig. 4).

Manufactured nests attached to posts, 2018. Twenty-four nests each of four different designs were deployed in 2018 along the east and west sides of the blueberry planting previously described. The nest designs were as follows: A) Two pieces of weathered pine boards (36.5 x 2 x 9 cm) were overlapped in the middle and attached with screws. A 12 mm. diam. entrance hole was drilled in the middle of the boards just below the top board at a 60^{o} angle and 2 cm deep (Fig. 5A), B) two pieces of weathered pine boards were attached at right angles with a 15 mm overhang at the 12 mm diameter and 3 cm deep entrance (Fig. 5B), C) two pieces of weathered pine boards as above were attached with screws at right angles (Fig. 5C), D) nests with 12 mm wide tunnels that were constructed and used in 2017 were reused in 2018 (Fig. 5D). As in the previous year, pine nests were randomly assigned and attached to 10.2 x 10.2 cm pressure treated posts with nest entrances at 1.5 and 3 m above ground level.

RESULTS

Nest architecture and pollen loaf composition. Twenty-eight nest tunnels averaged 15.4 $\pm$ 1.2 cm in length. Twenty cells averaged 17.7 $\pm$ 0.3 mm in length and first year tunnels were 13.2 $\pm$ 0.4 mm in width. Multi-year tunnels were 16.8 $\pm$ 0.3 mm in width.

The average (± SE) weight of a pollen loaf was 1.29 ± 0.06 g. The average (± SE) number of grains per loaf was 60,260,000 ± 8,403,295. The average full pollen load carried by *X. virginica* was determined to be 1,207,333 grains. The estimated number of foraging trips needed to complete a pollen loaf was 50.

The majority (69.9%) of the pollen cells in pollen loaves collected June 20, 2016 were from *Antirrhinium majus* L. (garden snapdragon) (26.5%), *Toxicodendron radicans* (L.) Kuntze (poison ivy) (16.4%), *Trifolium campestre* Schreb. (hop clover) (14.7%), *Polygonatum pubescens* (Willd.) Pursh (hairy Solomon's seal) (6.7%) and *Ilex* sp. (Holly or Winterberry) (5.8%) (Table 1). Pollen cells from 21 different genera were recorded in 2016. The majority (71.2%) of the pollen cells in pollen loaves collected June 8, 2017 were from *Antirrhinium majus* L. (Garden snapdragon) (23.9%), *Ajuga reptans* L. (Carpet bugle) (23.7%), *Quercus* sp. (Oak) (23.6%), *Lonicera* sp. (honeysuckle) (6.4%), and *Acer platanoides* (Norway maple) (4.8%) (Table 2). Pollen cells from 19 different genera were recorded in 2017. The majority (69.4%) of the pollen cells in pollen loaves collected in June 2018 were from *Antirrhinium majus* L. (garden snapdragon) (17.3%), *Toxicodendron radicans* (L.) Kuntze (poison ivy) (16.4%), *Quercus* (Oak) (13.3%), *Rubus* sp. (brambles) (12.0%), and *Trifolium campestre* Schreb. (hop clover) (10.4%). Pollen cells from 39 different genera were recorded in 2018.

Across three years of samples, a total of 47 plants were represented in analysis of pollen loaf composition. *Antirrhinium majus* L. (garden snapdragon) was the primary source of pollen in all three years of sampling (21.4%) and in three of five *X. virginica* nests. Where *A. majus* was not the most abundant pollen source it was among the top five sources. Pollen cell types from the following plants were identified each year of sampling: *Antirrhinium majus* (21.4% of all samples), *Quercus* sp. (13.1% of all samples), *Ajuga reptans* (8.2% of all samples), *Lonicera* sp. (3.6% of all samples), *Carya cordifolis* (2.4% of all samples), *Juglans* sp. (1.9%

of all samples), and *Rumex* sp (0.3% of all samples). Of these, all of the sampled nests contained pollen from *A. majus, A. reptans, Lonicera* sp., and *Juglans* sp. Four out of five (80%) of the sampled nests contained pollen cell types from *Toxicodendron radicans, Trifolium campestre*, *Quercus* sp., *Carya cordiformis*, *Rumex* sp., and *Wisteria* sp. Pollen from the following sources was only identified in three of five sampled nests: *Ilex* sp., *Aesculus hippocastanum*, *Iris* type, *Scutellaria* type, *Acer platanoides*, and *Elaeagnus* sp. Nine genera of plants were represented in *X. virginica* nests twice and 21 genera of plants were represented once.

Discarded Pollen Composition. The majority (66.04%) of discarded pollen sampled in July 2017 was from *Viburnum* sp. Also identified in the sample were pollen types from *Stachys* sp. (23.4%), Ilex sp. (8.1%), *Lotus corniculatus* (0.9%), *Quercus* sp. (0.6%), *Ajuga reptans* (0.3%), *Lonicera* sp. (0.3%), and *Nymphaea* sp. (0.3%). Pollen from *Viburnum* sp. was only identified in one of five *X. virginica* nests in 2018. Pollen from *Stachys* sp. was only identified in one of five nests in 2016. Pollen from *Nymphaea* sp. was not found in any sampled pollen loaves.

Manufactured nests attached to a barn, 2017. Of the ninety-six nests that were constructed and set-up along the pole barn, a total of seventeen nests (17.7%) hosted 283 megachilid bees that emerged between 26 April 2018 and 4 May 2018. Twelve nests were occupied by 209 *Osmia taurus* and four nests were occupied by 68 *Osmia cornifrons*. One nest was occupied by 6 individuals of both species. Thirteen megachilid-occupied nests were in 9 mm width tunnels and four were in 12 mm width tunnels. Seven of the nests (7.3%) along the barn were occupied by 26 grass-carrying wasp *Isodontia* sp. These wasps emerged between 29 June and 13 July 2018.

Two nests (2.1%) hosted 13 dermestid beetles. One nest at this same site was occupied by two individuals belonging to the family Vespidae, emerging on 13 July 2018. Seventy-one nests (74%) at this site were not occupied by any insects.

Manufactured nests attached to a lean too storage area, 2017. Of the twenty-four nests that were constructed and set-up on the outside of a lean too storage area, a total of twelve nests (50%) hosted 79 megachilid bees. Five of the twelve occupied nests (42%) supported 21 individuals of *Osmia taurus.* One nest was occupied by 3 *Osmia cornifrons.* Five nests were occupied by 47 individuals of both *O. taurus* and *O. cornifrons*. Individuals of *Osmia cornifrons* and *Osmia taurus* emerged between 26 April 2018 and 9 July 2018. One of the eleven occupied nests supported eight *Osmia lignaria* individuals, all emerging between 26 April 2018 and 2 May 2018. Eight of the megachilid-occupied nests were in 9 mm width tunnels and four were in 12 mm width tunnels. Eight of the twenty-four nests (33.3%) at this same site were occupied by 29 *Isodontia* sp. Two nests hosted 32 flies in the family Milichiidae. One nest was occupied by one vespid wasp. Eight of the twenty-four nests (33.3%) at this site were not occupied by any insects.

Manufactured nests attached to posts, 2017. Of the forty-eight nests that were attached to posts along parallel sides of a blueberry planting, 4 nests (8.3%) were occupied by 9 individuals in the family Megachilidae. One nest was occupied by two *Osmia cornifrons*. The species of Megachilidae that emerged from the remaining three nests could not be confirmed. Two of the Megachilidae-occupied nests were in 9 mm width tunnels and two were in 12 mm width tunnels. Nineteen nests (39.6%) were occupied by 97 *Isodontia* sp. One out of forty-eight nests (2.1%)

was occupied by three vespid wasps. Twenty-six nests (54.2%) were not occupied by any insects.

Manufactured nests attached to posts, 2018. Of the forty-eight nests that were attached to posts along parallel sides of a blueberry planting, four nests (8.3%) were occupied by *Xylocopa virginica* (three nests of design B and one nest of design C). Three of four (75%) occupied nests were at the 1.5 m height and one of four (25%) occupied nests was at the 3 m height. Two nests of design A had observable excavation to suggest that *X. virginica* had visited, but nests were incomplete and were occupied by grass-carrying wasps at the end of the season. The nest which was occupied by *X. virginica* in 2017 (design D) showed additional excavation in 2018, but was empty at the end of the season in 2018. The three nests that showed signs of *X. virginica* visitation but were not occupied were at 3 m height. Two nests were occupied by grass-carrying wasps.

DISCUSSION

Nest architecture. Manufactured wooden nests have been used to "trap" bees and wasps to study their nest provisions and structure as well as to provide nesting sites in addition to those available naturally (Krombein 1967). Gerling and Hermann (1978) studied *X. virginica* nests in the vicinity of Athens, GA. Entrance holes were 10 mm in diam. and 13-15 mm in length. At the end of the entrance holes, tunnels are constructed at more or less right angles and follow the grain of the wood. There was an average of 2.4 tunnels per nest (range: 1-4). Tunnel length averaged 17.5 (3.9 –

47) cm. There were an average of 3.8 (1-8) cells per active tunnel. Cells averaged 17.5 (14.5 – 20) mm in length.

Krombein (1967) studied nests in the Plummers Island, MD and found that coniferous wood is preferred to deciduous wood, although bees will nest in either. Cells were 21-22 mm long with partitions 3-4 mm thick. The partitions were made from tiny wood chips rasped from the tunnel walls and cemented together, presumably by a salivary secretion. Pollen masses were well saturated with nectar and were 14 mm long. Nest measurements from Rhode Island were comparable to those reported by Gerling and Hermann (1978) and Krombein (1967).

Pollen Loaf Composition. *Xylocopa virginica* has a long colony life cycle, with many females living two years. Discoverlife.org (2018) lists 59 genera of host plants for *X. virginica*. Ten genera of documented host plants were represented in pollen loaf composition. Our research adds 37 more host plants based on pollen loaf analysis (*Antirrhinium majus*, *Toxicodendron radicans, Trifolium campestre,* etc., Tables 1-6). In March and April males defend areas near the nest and mate with females. Females construct nests in unfinished wood, and nests can be reused for many generations (Gerling and Hermann 1978). *Xylocopa virginica* has nectar robbing tendencies, relatively low blueberry pollen loads, and pollen transfer efficiency is low (2.5 pollen tetrads deposited per visit, Benjamin and Winfree 2014). Despite these shortcomings, the large number of these pollinators and possible ease of increasing numbers by providing unfinished wood nesting sites around blueberry plantings, suggests more research on the importance of this bee as a blueberry pollinator is needed.

Discarded Pollen. Corbet and Willmer (1980) describe behavior by *Xylocopa mordax* Smith where bees are often seen grooming off and discarding *Passiflora* sp. pollen from the nest entrance. Only trace amounts of *Passiflora* pollen were found in sampled pollen loaves, while pollen from *Gliricidia* sp. composed nearly all of the pollen loaves. We observed similar behavior at the entrance of one *Xylocopa virginica* nest in July 2017. Nearly all (97.5%) of the discarded pollen was from *Viburnum* sp (66.0%)., *Stachys* sp. (23.4%), and *Ilex* sp. (8.1%) which supports the hypothesis that *Xylocopa* can differentially select pollen for inclusion in pollen loaves. Pollen from *Nymphaea* sp. (0.3%) was only identified in discarded pollen, suggesting that this pollen source is not acceptable for inclusion in pollen loaf provisions.

Manufactured Nest Acceptance. We believe that our pre-routered tunnels and two piece manufactured nests did not attract more carpenter bees for two reasons. The two boards warped somewhat upon deployment and there was a gap between the two pieces allowing moisture to enter the tunnels which may have deterred nesting by carpenter bees. Also, the ready-made tunnels used in 2017 were quickly occupied by sphecid wasps and megachilid bees (75% of all manufactured nests in 2017) and therefore were not available for carpenter bees.

Benjamin and Winfree (2014) studied honey and native bee pollination in commercial highbush blueberry in New Jersey. They found that the European honey bee, *Apis mellifera* L. deposited a median of 18.5 tetrads of pollen during a nectar-collecting visit, 24 tetrads during a pollen-collecting visit and 0.5 tetrads during a secondary nectar-robbing visit. They also found that pollen tetrads deposited by

Bombus spp., large *Andrena* spp., medium *Andrena* spp. and *Xylocopa virginica* were 23.5, 9.0, 11.5, and 2.5 tetrads respectively. All of their study sites were stocked with domesticated honey bees at densities of 2.5-7.5 hives ha^{-1}. Honey bees provided 86% and native bees 14% of the pollination. Conversely, Winfree et al. (2007) found that native bees were the most important pollinators and alone were sufficient to pollinate commercially grown watermelons in New Jersey and Pennsylvania. Previous studies have shown that native bees contribute to crop pollination at farms near natural habitat, but not in more intensively used agricultural areas (Kremen et al. 2004, Klein et al. 2007).

Numerically, *X. virginica* was one of the top five bees collected at 15 commercial blueberry plantings in Rhode Island from 2014-16. Benjamin and Winfree (2014) found *X. virginica* deposited a median number of 2.5 pollen grains per blueberry flower visit. This is rather low compared to a median number of 23.5 pollen grains for the bumble bee *Bombus bimaculatus*. Sampson et al. (2004), however, found that carpenter bees are benign or even potentially beneficial floral visitors to rabbiteye blueberry.

Because each *X. virginica* tunnel system is only 15 cm in length, it is feasible to manufacture many nest structures and deploy them around blueberry or other crop plantings (Fig. 4). Future research will be aimed at resolving the issue of *X. virginica's* value as a blueberry pollinator and evaluating artificial nests which may also provide clues as to how to prevent them from infesting homes, etc. We will also continue to identify other pollen cells collected by *X. virginica* to see what forage plants are important for this species.

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Table 1. Number of pollen cell types from eight *Xylocopa virginica* pollen loaves collected June 20, 2016.

Pollen Cell Type	Sample Number									
	1	2	3	4	5	6	7	8	Total	%
Antirrhinium majus L. (Garden snapdragon)	0	111	0	51	0	212	246	152	772	26.5
Toxicodendron radicans (L.) Kuntze (poison ivy)	129	0	157	105	73	13	0	0	477	16.4
Trifolium campestre Schreb. (Hop clover)	147	0	85	91	102	3	0	0	428	14.7
Polygonatum pubescens (Willd.) Pursh (Hairy Solomon's seal)	2	44	30	29	0	36	37	16	194	6.7
Ilex sp. (Holly or Winterberry)	29	0	0	0	139	0	0	0	168	5.8
Medicago type (Medick)	22	0	26	25	7	10	11	51	152	5.2
Quercus sp. (Oak)	0	81	0	0	0	0	0	55	136	4.7
Juglans sp. (Walnut)	11	22	0	0	0	20	0	39	92	3.2

Table 1 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	7	8	Total	%
Aesculus hippocastanum L. (Horse chestnut)	0	22	0	0	0	0	33	20	75	2.6
Iris type (Iris)	2	19	0	0	29	13	11	0	74	2.5
Lonicera morrowii Gray (Morrow's honeysuckle)	0	52	0	0	0	10	4	0	66	2.3
Carya cordiformis (Wangenh.) K. Koch (Bitternut hickory)	0	0	11	11	0	13	11	16	62	2.1
Stachys type (Mint)	11	0	15	18	11	0	0	0	55	1.8
Sambucus sp. (Elderberry)	0	0	26	25	0	0	0	0	51	1.7
Scutellaria type (Skullcap)	0	0	0	0	3	0	0	31	34	1.1
Ajuga reptans L. (Carpet bugle)	4	0	11	0	0	0	15	0	30	1.0
Acer platanoides L. (Norway maple)	0	15	0	0	0	0	0	0	15	<1.0

Table 1 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	7	8	Total	%
Rumex sp. (Dock)	0	0	7	7	0	0	0	0	14	<1.0
Rhodendron catawbiense Michx. (Catawba rosebay)	0	0	0	0	2	0	0	12	14	<1.0
Betula sp. (Birch)	0	4	0	0	0	0	0	0	4	<1.0
Wisteria sp. (Wisteria)	0	0	0	0	0	0	4	0	4	<1.0

Table 2. Number of pollen cell types from six *Xylocopa virginica* pollen loaves collected June 8, 2017.

Pollen Cell Type	Sample Number						Total	%
	1	2	3	4	5	6		
Antirrhinium majus L. (Garden snapdragon)	251	19	0	0	91	165	526	23.9
Ajuga reptans L. (Carpet bugle)	34	108	70	172	76	62	522	23.7
Quercus sp. (Oak)	17	153	160	147	0	41	518	23.6
Lonicera sp. (Honeysuckle)	0	15	82	20	22	3	141	6.4
Acer platanoides L. (Norway maple)	0	15	43	49	0	0	107	4.8
Juglans sp. (Walnut)	3	60	0	0	43	0	106	4.8
Medicago type (Medick)	0	0	0	0	76	14	90	4.1

Table 2 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	Total	%
Polygonatum pubescens (Willd.) Pursh (Hairy Solomon's seal)	14	2	0	0	11	21	48	2.2
Carya cordiformis (Wangenh.) K. Koch (Bitternut hickory)	10	0	0	0	29	0	39	1.7
Capsella sp. (Shepherd's-purse)	0	0	12	8	0	0	20	<1.0
Scutellaria type (Skullcap)	0	0	12	4	0	0	16	<1.0
Iris type (Iris)	7	0	0	0	7	0	14	<1.0
Rumex sp. (Dock)	0	0	0	0	7	7	14	<1.0
Vaccinium corymbosum L. (Highbush blueberry)	0	2	4	2	0	2	10	<1.0
Elaeagnus sp. (Russian or Autumn olive)	0	0	4	2	0	3	9	<1.0
Lonicera morrowii Gray (Morrow's honeysuckle)	2	0	4	0	0	0	6	<1.0

Table 2 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	Total	%
Ranunculus sp. (Crowfoot)	0	0	0	4	0	0	4	<1.0
Kalmia angustifolia L. (Sheep-laurel)	0	2	0	0	0	0	2	<1.0
Prunus sp. (Cherry)	2	0	0	0	0	0	2	<1.0

Table 3. Number of pollen cell types from two *Xylocopa virginica* pollen loaves collected June 8, 2018.

	Sample Number			
Pollen Cell Type	1	2	Total	%
Toxicodendron radicans (L.) Kuntze (poison ivy)	243	159	402	52.7
Trifolium camprestre Schreb. (Hop clover)	85	30	115	15.1
Ajuga reptans L. (Carpet bugle)	38	58	96	12.6
Antirrhinium majus L. (Garden snapdragon)	5	37	42	5.5
Wisteria sp. (Wisteria)	33	8	41	5.4
Lonicera sp. (Honeysuckle)	0	41	41	5.4
Juglans nigra L. (Eastern black walnut)	12	12	24	3.1

Table 3 (continued).

Pollen Cell Type	Sample Number 1	2	Total	%
Rumex sp. (Dock)	1	0	1	<1.0
Pinus resinosa (Red pine)	0	1	1	<1.0

Table 4. Number of pollen cell types from eight *Xylocopa virginica* pollen loaves collected June 15, 2018.

	Sample Number									
Pollen Cell Type	1	2	3	4	5	6	7	8	Total	%
Antirrhinium majus L. (Garden snapdragon)	205	325	130	0	0	0	0	9	669	22.4
Quercus sp. (Oak)	0	0	49	220	253	0	0	0	522	17.5
Rubus sp. (Brambles)	0	0	0	0	0	153	163	157	473	15.8
Toxicodendron radicans (L.) Kuntze (poison ivy)	130	0	0	0	0	0	96	99	325	10.9
Trifolium campestre Schreb. (Hop clover)	0	0	0	0	0	87	38	49	174	5.8
Ajuga reptans L. (Carpet bugle)	2	1	11	48	43	9	28	16	158	5.3
Lonicera morrowii Gray (Morrow's honeysuckle)	10	10	43	27	18	0	1	0	138	4.6
Carya cordiformis (Wangenh.) K. Koch (Bitternut hickory)	0	17	104	2	0	0	0	11	134	4.5
Juglans nigra L. (Eastern black walnut)	10	24	10	58	27	0	0	0	129	4.3

Table 4 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	7	8	Total	%
Syringa vulgaris L. (Lilac)	0	0	0	0	0	74	0	0	74	2.5
Salix alba L. (White willow)	0	0	40	13	0	0	0	0	53	1.8
Wisteria sp. (Wisteria)	0	0	0	0	0	11	19	6	36	1.2
Elaeagnus sp. (Russian or autumn olive)	0	0	9	14	13	0	0	0	36	1.2
Hypericum sp. (St. John's wort)	0	0	0	0	0	36	0	0	36	1.2
Lilium sp. (Lily)	0	0	0	0	0	1	0	9	10	<1.0
Rumex sp. (Dock)	1	4	0	0	0	0	0	0	5	<1.0
Prunus sp. (Cherry)	0	5	0	0	0	0	0	0	5	<1.0
Aesculus hippocastanum L. (Horse chestnut)	0	0	0	2	1	0	0	0	3	<1.0

Table 4 (continued).

Pollen Cell Type	Sample Number 1	2	3	4	5	6	7	8	Total	%
Pinus resinosa Aiton (Red pine)	0	0	0	1	0	0	0	0	1	<1.0
Ilex sp. (Holly or Winterberry)	0	0	0	1	0	0	0	0	1	<1.0
Vaccinium sp.	0	0	0	1	0	0	0	0	1	<1.0
Fraxinus americana L. (White ash or American ash)	0	0	0	0	1	0	0	0	1	<1.0
Morus sp. (Mulberry)	0	0	0	0	0	0	0	1	1	<1.0

Table 5. Number of pollen cell types from three *Xylocopa virginica* pollen loaves collected June 22, 2018.

Pollen Cell Type	Sample Number				
	1	2	3	Total	%
Trifolium camprestre Schreb. (Hop clover)	83	108	43	234	18.8
Antirrhinium majus L. (Garden snapdragon)	53	37	67	157	12.6
Quercus sp. (Oak)	2	3	142	147	11.8
Rubus sp. (Brambles)	64	55	12	131	10.5
Toxicodendron radicans (L.) Kuntze (poison ivy)	59	36	3	98	7.9
Ilex sp. (Holly or Winterberry)	26	30	6	62	5.0
Viburnum sp. (Viburnum)	20	36	3	59	4.7

Table 5 (continued).

Pollen Cell Type	Sample Number 1	2	3	Total	%
Paeonia sp. (Peony)	11	31	5	47	3.8
Amorpha fruticose L. (False Indigo-Bush)	35	12	0	47	3.8
Lonicera sp. (Honeysuckle)	0	1	45	46	3.7
Hypericum sp. (St. John's wort)	17	10	0	27	2.2
Ajuga reptans L. (Carpet bugle)	7	10	8	25	2.0
Lilium sp. (Lily)	18	7	0	25	2.0
Salix alba L. (White willow)	13	5	0	18	1.4
Iris type (Iris)	10	7	0	17	1.4

Table 5 (continued).

Pollen Cell Type	Sample Number 1	2	3	Total	%
Juglans nigra L. (Eastern black walnut)	2	2	12	16	1.3
Aesculus hippocastanum L. (Horse chestnut)	0	2	13	15	1.2
Elaeagnus sp. (Russian or Autumn olive)	0	0	12	12	<1.0
Acer sp. (Maple)	0	2	9	11	<1.0
Urtica sp. (Nettle)	0	0	11	11	<1.0
Brassica sp. (Mustard)	0	5	5	10	<1.0
Carya cordiformis (Wangenh.) K. Koch (Bitternut hickory)	1	5	3	9	<1.0
Wisteria sp. (Wisteria)	2	1	1	4	<1.0
Rhamnus sp. (Buckthorn)	2	2	0	4	<1.0

Table 5 (continued).

Pollen Cell Type	Sample Number 1	2	3	Total	%
Scutellaria type (Skullcap)	0	2	2	4	<1.0
Allium sp. (Onion)	0	0	3	3	<1.0
Phryma leptostachya L. (Lopseed)	0	2	0	2	<1.0
Amaranthus sp. (Amaranth)	0	0	2	2	<1.0
Morus sp. (Mulberry)	1	0	0	1	<1.0
Lotus corniculatus L. (Bird's-foot trefoil)	0	0	1	1	<1.0

Table 6. Number of pollen cell types from pollen sample discarded from *Xylocopa virginica* nest July, 2017.

Pollen Cell Type	Total	%
Viburnum sp. (Viburnum)	212	66.0
Stachys type (Mint)	75	23.4
Ilex sp. (Holly or Winterberry)	26	8.1
Lotus corniculatus L. (Bird's-foot trefoil)	3	<1.0
Quercus sp. (Oak)	2	<1.0
Ajuga reptans L. (Carpet bugle)	1	<1.0
Lonicera sp. (Honeysuckle)	1	<1.0
Nymphaea sp. (Water lily)	1	<1.0

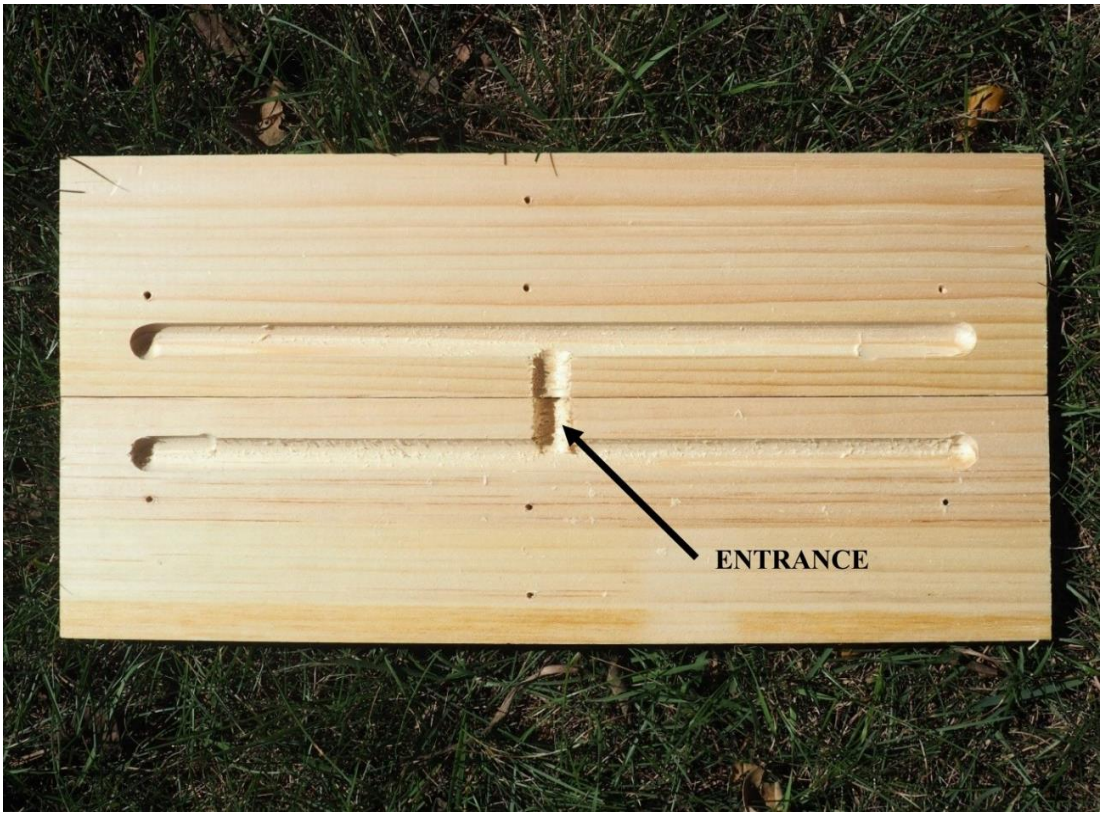

Figure 1. Manufactured nest, 2017. Pine boards (36.5 x 2 x 9 cm) were routered in each of two boards to create two halves of both 9 mm and 12 mm tunnel widths 15 cm long on each side of a 12 mm diam. entrance hole 15 mm deep.

Figure 2. Manufactured nests attached to barn, 2017. Twenty-four nests each of 9 mm and 12 mm tunnel widths attached to 3.6 x 8.8 cm boards 2.3 and 2.5 m from ground level and 13 and 54 cm respectively from the edge of an aluminum roof.

Figure 3. Manufactured nests attached to lean too storage area, 2017. Nests attached to the fascia of a lean too storage area 2.1 m from ground level and 18 cm from the edge of the roof. All entrance holes were directed downward.

Figure 4. Manufactured nests attached to posts, 2017. Ninety-six pine nests were randomly assigned and attached to 10.2 x 10.2 cm pressure treated posts with the each of the 9 mm and 12 mm tunnel width nest entrances at 1.5 and 3 m above ground level. Posts were 6 m from the edge of a 0.3 ha blueberry planting and 4.5 m between posts. There were 12 posts on the east (sunny) and west (shady) sides of a 0.15 ha blueberry planting.

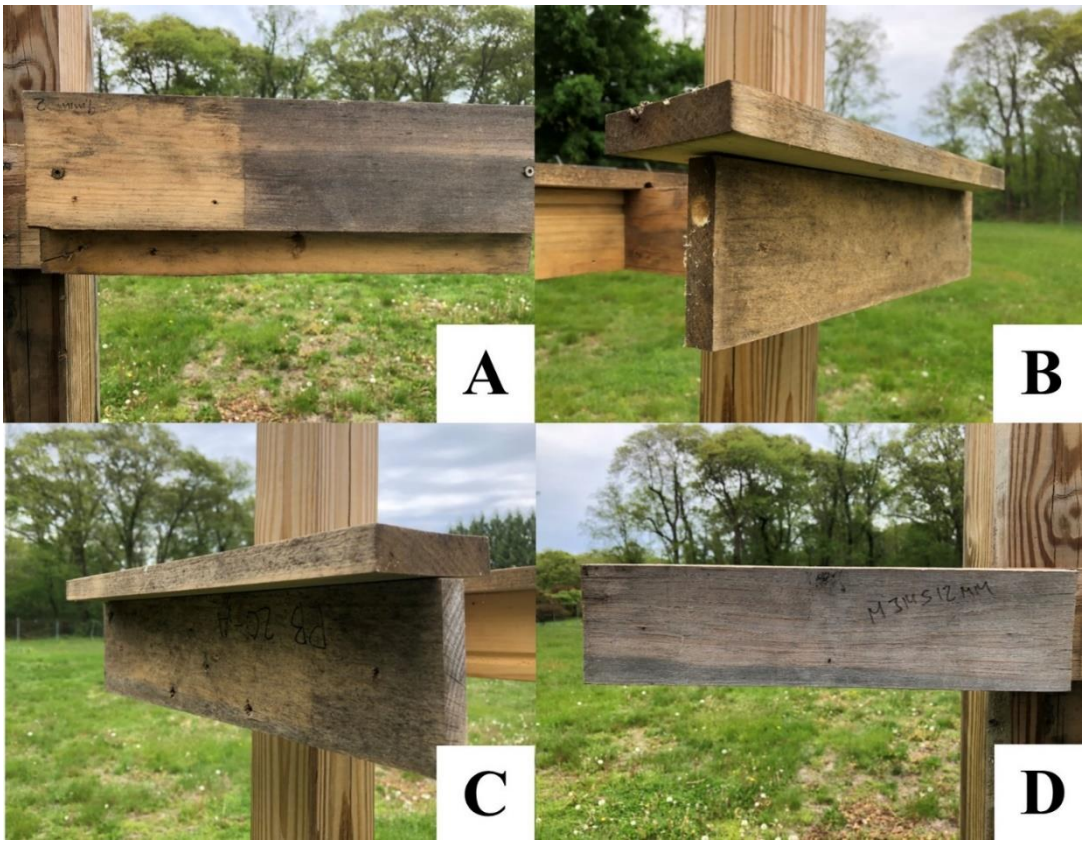

Figure 5. Manufactured nests attached to posts, 2018. A) Two pieces of weathered pine boards (36.5 x 2 x 9 cm) were overlapped in the middle and attached with screws. A 12 mm. diam. entrance hole was drilled in the middle of the boards just below the top board at a 60^{o} angle and 2 cm deep. B) Two pieces of weathered pine boards as above were attached at right angles with a 15 mm overhang at the entrance end. The entrance was 12 mm in diam. and 3 cm deep. C) Two pieces of weathered pine boards as above were attached with screws at right angles. D) Weathered pine boards as above were routered in each of two boards to create two halves of 12 mm tunnel widths 15 cm long on each side of a 12 mm diam. entrance hole 15 mm deep.