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ARTICLE

Pollination ecology of lowbush blueberry (*Vaccinium angustifolium* Aiton) in an island ecosystem

Rachel E. Noone, Stéphanie M. Doucet, and Patricia L. Jones

Abstract: Plant pollination is influenced by plant traits, pollinator community, plant community, and abiotic conditions. Island ecosystems, which often have reduced pollinator communities, provide unique insights into pollination ecology. Lowbush blueberry, *Vaccinium angustifolium* Aiton, has predominantly been studied in managed agricultural areas with introduced honeybees. We examined diurnal and nocturnal pollination of unmanaged lowbush blueberry patches on an 80 ha island in New Brunswick without honeybees. We restricted pollinator access to blueberry stems in five different treatments. Using mesh bags, we made stems accessible to pollinators 24 h a day (positive control), only during the day, only at night, or never (negative control), with an additional sham control. Blueberry stems accessible only to diurnal visitors had 70.55% fruit set and a mean 7.33 viable seeds per fruit. Stems accessible only to nocturnal visitors had 63.76% fruit set and 5.87 viable seeds, significantly higher than the continuously bagged negative control stems. The most common diurnal insects observed visiting flowers were bumblebees and two wasp species. Insects collected from plants at night were beetles and flies. There was substantial variation between blueberry patches in fruit set and fruit size. We examined whether flower color could be driving patch differences in pollination, and quantified flower color using spectrophotometry. We found no effect of flower color on metrics of pollination. As well as demonstrating substantial nocturnal pollination, we record unusually high fruit set, especially for an island without managed bees. We discuss some of the potential drivers of this high fruit set.

Key words: diurnal, nocturnal, pollination, fruit set, fruit size, lowbush blueberry, spectrophotometry, flower color, seed set, *Vaccinium angustifolium*.

Résumé : La pollinisation dépend de certains caractères de la plante, de la population d'insectes pollinisateurs, de la composition de la végétation et de conditions abiotiques. Les écosystèmes insulaires, où on trouve habituellement peu d'insectes pollinisateurs, offrent un point de vue unique sur l'écologie de la pollinisation. On a surtout étudié le bleuet nain (*Vaccinium angustifolium*) dans les zones agricoles aménagées où avait été introduite l'abeille mellifère. Les auteurs ont étudié la pollinisation diurne et nocturne de peuplements sauvages de bleuet nain sur une île de 80 ha sans abeilles, au Nouveau-Brunswick. L'accès des insectes aux tiges de bleuet a été restreint de cinq façons. Un sac-filet a rendu les tiges accessibles 24 heures par jour (témoin positif), le jour seulement, la nuit uniquement ou jamais (témoin négatif), À ces quatre traitements s'ajoutait un leurre, comme témoin également. Les tiges de bleuet visitées uniquement par les insectes diurnes ont porté des fruits à raison de 70,55 %, avec une moyenne de 7,33 graines viables par fruit. Celles fécondées par les insectes nocturnes seulement ont fructifié à raison de 63,76 %, avec 5,87 graines viables, soit significativement plus que les plants témoins négatifs, ensachés en permanence. Les insectes diurnes qui visitent le plus souvent les fleurs sont le bourdon et deux sortes de guêpe; ceux recueillis la nuit appartiennent aux coléoptères et aux diptères. La nouaison et le calibre des fruits varient passablement d'un peuplement de bleuets à l'autre. Les auteurs ont tenté de voir si la couleur de la fleur peut expliquer la variation de la pollinisation en la mesurant avec un spectrophotomètre. Les résultats indiquent que la couleur n'a aucune incidence sur la pollinisation. En plus de constater une importante pollinisation nocturne, les auteurs ont relevé une nouaison inhabituelle, surtout sur une île dépourvue d'abeilles. Suit une discussion sur les raisons à l'origine de cette mise à fruits particulièrement élevée. [Traduit par la Rédaction]

Mots-clés : diurne, nocturne, pollinisation, nouaison, calibre du fruit, bleuet nain, spectrophotométrie, couleur de la fleur, grenaison, *Vaccinium angustifolium*.

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Introduction

Widespread pollinator declines are a critical issue facing cultivated and wild plants that depend on insect pollination (Dicks et al. 2021). Offshore islands can provide key insights into the potential impacts of pollinator declines as they can serve as models for fragmented habitats (Preston 1962; Kruess and Tscharntke 1994), and typically have reduced pollinator communities (Barrett 1996; Traveset et al. 2016). The majority of research on island pollination, however, has focused on non-crop plants (Barrett 1996; Traveset et al. 2016). Lowbush blueberry, *Vaccinium angustifolium* Aiton, is common on offshore islands but is also an economically important crop in New Brunswick, Nova Scotia, and Maine, providing a unique opportunity to study the pollination ecology of a crop species in an unmanaged island ecosystem. Insect-mediated pollination is a critical component of the reproduction of lowbush blueberry (Stubbs and Drummond 2001; Usui et al. 2005; Cutler et al. 2012). Lowbush blueberry with its heavy, sticky pollen and a bell-shaped flower morphology is considered largely self-incompatible, and therefore relies on cross-pollination by insects to achieve robust fruit set (Usui et al. 2005; Bell et al. 2010; Cutler et al. 2012; Drummond 2012; Bushmann and Drummond 2015; Asare et al. 2017). Previous research has shown that diurnal pollinators of lowbush blueberry consist of bees, flies, beetles, butterflies, and wasps (Stubbs et al. 1997; Cutler et al. 2012; Manning and Cutler 2013; Bushmann and Drummond 2015; Drummond et al. 2017). The most efficient of these pollinators are bees that sonicate, or "buzz pollinate," flowers and shake pollen grains from the anthers (Drummond 2012). A study from mainland Nova Scotia, however, suggests that significant pollination is also carried out by nocturnal insects, potentially including geometer and owlet moths, as well as true weevils (Cutler et al. 2012; Manning and Cutler 2013). Lowbush blueberry varies in flower color from white to pink (Drummond and Rowland 2020), which could be influencing attractiveness to diurnal and nocturnal pollinators. For example, nocturnally pollinated flowers tend to be white (Van der Pijl 1961; Yokota and Yahara 2012), and we might therefore expect more nocturnal pollination of lowbush blueberry clones with white flowers than clones with pink flowers. In other *Vaccinium* species researchers have investigated the effects of floral color on pollination (Stournaras and Schaefer 2017), but this has not been studied in lowbush blueberry.

Previous research has shown that despite their widespread use, honeybees are not as efficient at lowbush blueberry pollination on a per bee basis as wild bees or commercial bumblebees (Wood 1961; Stubbs and Drummond 2001; Isaacs and Kirk 2010). With managed and native pollinator communities under stress (Potts et al. 2010; Goulson et al. 2015; Powney et al. 2019; Soroye et al. 2020), and habitat fragmentation increasing globally, it is important to understand the dynamics of pollination in areas without managed pollinators, and particularly in small, isolated habitat patches. We investigated lowbush blueberry pollination at the Bowdoin Scientific Station on Kent Island in the Bay of Fundy, New Brunswick, Canada. Kent Island has no managed or feral honeybee colonies. Additionally, the bumblebee species most frequently used for commercial pollination, *Bombus impatiens* Cresson, is not present on Kent Island. We hypothesized that because diurnal bees are considered to be the most efficient at sonication, blueberry stems visited by diurnal insects would have the highest fruit sets (Drummond 2012). Moths and beetles are expected to be less effective than diurnal bees in pollinating blueberry, and we therefore expected nocturnal-only exposure stems to have lower fruit yields (Dogterom et al. 2000; Javorek et al. 2002; Bushmann et al. 2012; Manning and Cutler 2013). We additionally hypothesized that lowbush blueberry clones with white flowers would be more detectable at night and therefore have higher levels of nocturnal pollination than clones with pink flowers. As offshore islands typically have reduced pollinator communities (Barrett 1996; Traveset et al. 2016), for which there is support from previous pollination studies on Kent Island (Zink and Wheelwright 1997; Wheelwright et al. 2006), we expected lower fruit set than similar studies at mainland sites.

Materials and Methods

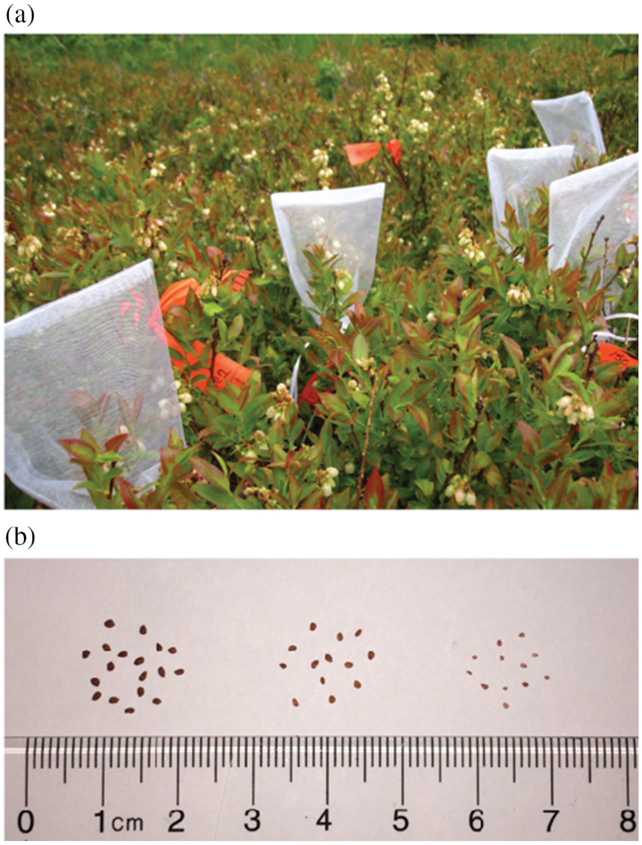
Study site

This research was conducted on Kent Island (44.58°N, -66.76°W), an 80-ha island about 20 km offshore of mainland Maine and Nova Scotia, with cool summer temperatures (6–22 °C) and strong ocean winds reaching gusts of 47 kph (Zink and Wheelwright 1997). We selected 20 lowbush blueberry patches at the end of May in 2018. We have examined pollination ecology at the patch level, as it is difficult to distinguish genets (clones) of blueberry within a continuous patch and therefore patches may have contained more than one genet. Patches ranged in size from 2 to 75 m^2. In large patches, experimental stems were all placed within an area of 2 m^2 to maximize the likelihood that stems belonged to the same genet. Patches were a minimum of 3 m from one another and therefore likely contained distinct genets (Bell et al. 2010). Patches were selected for study if ≥15 individual stems had completely unopened flowers at the start of data collection.

Pollination treatments

We used mesh bags to restrict pollinator access to flowering stems of lowbush blueberry in five different pollination treatments. We selected individual stems from all 20 patches within a 24-h period when buds were unopened from 31 May to 1 June 2018. Pollination exclusion bags were 10 × 15 cm and made of sheer white synthetic organza with a drawstring

Fig. 1. (*a*) The pollinator exclusion bags used for pollination treatments. (*b*) Seed size categorization for fruit measurements. Only the leftmost (large and viable) seeds were counted in seed counts. [Colour online.]



mouth (HRX Package, Fig. 1). Stem selection criteria required a minimum of four healthy unopened buds per inflorescence. Stems with over 35 buds were avoided in an effort to reduce bias in fruit quality measurements due to extreme fruit load (Bajcz and Drummond 2017*a*, 2017*b*). We note that avoidance of stems with very high bud counts may increase our fruit set relative to commercial fields, where stems can average 70 buds (Drummond 2019). We randomly assigned experimental stems to one of five pollinator exclusion treatments such that there were three replicate stems per treatment in each of the 20 patches (60 stems per treatment, 300 stems total). The five treatments consisted of: (*i*) negative control: left continually bagged for the duration of the flowering period with no access for pollinators; (*ii*) diurnal-only exposure: bagged during nighttime hours (1900–2100 to 0700–0900), to exclude the majority of nocturnal but not diurnal pollinators; (*iii*) nocturnal-only exposure: bagged only during daytime hours (0700–0900 to 1900–2100), to exclude the majority of diurnal but not nocturnal pollinators; (*iv*) positive control: no bag treatment, left continually exposed during both diurnal and nocturnal periods; and (*v*) sham control: left continually exposed during both diurnal and nocturnal periods, but with bags placed and immediately removed from stems once daily to simulate the degree of flower manipulation experienced in diurnal-only and nocturnal-only treatments. To reduce the risk of pollen contamination between flowers, each experimental stem had a designated pollination exclusion bag. Once flowers had begun to open, patches were visited three times daily: once in the morning between 0700 and 0900 when diurnal treatment bags were removed and nocturnal treatment bags were placed onto stems; once around midday to perform the sham control; and once in the evening between 1900 and 2100 to place diurnal treatment bags onto stems and remove nocturnal treatment bags. During both morning and evening bag exchange periods insect pollinator activity was noticeably quiet, which helped to assure us that diurnal pollinators were not contaminating nocturnal treatment stems with either early-morning or late-evening pollination (Cutler et al. 2012; Scopece et al. 2018). Treatments were carried out until the floral senescence of all study stems (Van Doorn 1997). We then bagged and tied off all stems for the duration of the post-flowering period, regardless of treatment, to prevent predation of ripened fruit until harvest (Balmford et al. 2006). We harvested ripened blueberry fruits between the end of July and mid-August. Harvested fruits were left attached to stems whenever possible, placed into labeled bags, and carried in a cooler in the field for no more than one hour. Samples were frozen at −20 °C for preservation until lab analysis (Chiabrando et al. 2009; MacLean and NeSmith 2011; Turmanidze et al. 2017).

Fruit measurements

We counted flower buds for each of the tagged stems. Bud clusters from one stem each in patches 9, 16, and 20 were lost or compromised as a result of wind damage such that the results reported here are from a reduced sample of 297 stems ($N = 59$ for the nocturnal-only treatment, negative control, and sham control instead of 60 stems). After harvesting the stems, we counted the number of berries per stem to determine percent fruit to bud ratio (i.e., fruit set). All statistical analyses were performed in R version 4.0.2 (R Core Team 2020). Fruit to bud ratio was converted to a percentage and analyzed with an ANOVA with factors of treatment, patch, and the interaction between treatment and patch. We conducted Tukey's honestly significant difference (HSD) post hoc tests to determine statistical differences between treatments.

Fruit were categorized into three groups by color: ripe fruit (deep blue), turning fruit (pinkish or purplish), and green fruit. To estimate fruit size, we took two diameter measurements with calipers along the central horizontal axis of all ripe and turning fruits (Soots et al. 2017). We calculated fruit size as area (in mm^2) of an ellipse formed by the two measurements. We analyzed fruit size using a linear mixed-effect model with treatment, patch, and the interaction between treatment and patch as fixed effects and individual stem as a random effect using the lme4 package (Bates et al. 2014), and Type II Wald chi square tests using the car package (Fox and Weisberg 2018). Tukey-corrected post hoc tests were conducted using the glht command in the multcomp package (Hothorn et al. 2008). To determine seed set, we manually dissected 67 ripe fruits distributed across a size range of 17–137 mm^2 (Irwin and Brody 1998; Stubbs and Drummond 2001; Javorek et al. 2002; Kolb 2008; Artz and Nault 2011; Ollerton et al. 2011; Holzschuh et al. 2012; Garibaldi et al. 2013). We dissected at least 15 fruits per treatment (except the negative control), with a minimum of 2 fruits dissected per patch. All six negative control fruits were assessed for seed set. Following established protocol guidelines (Dogterom et al. 2000), seeds were scored as "large" if plump and dark brown in color (viable), versus "small" if not quite as plump and golden brown in color, or "flat" if very small, flattened, and pale in color (Castro et al. 2012) (Fig. 1). The number of viable seeds per berry was compared using a two-way ANOVA with patch, treatment, and their interaction as predictor variables.

Pollinator surveys

We surveyed insects visiting lowbush blueberry flowers on Kent Island in the summer of 2019. Surveys were conducted mid-day every non-rainy day in the month of June. To create as comprehensive a survey of insect visitors as possible, insects were collected from all the lowbush blueberry on Kent Island, not just the 20 study patches. Insects seen entering flowers were captured in nets in the field and brought back to the lab where they were identified to the most precise taxonomic level feasible using field guides and taxonomic keys (Buck et al. 2008; Williams et al. 2014; Wilson and Carril 2015) and vouchers were posted on iNaturalist for confirmation of identification. Nocturnal visitors were observed on two nights during the blueberry flowering period in 2018. We resampled nocturnal visitors by beating lowbush blueberry bushes with insect nets on two nights in June 2019. We photographed insects collected at night in 2019 and posted them on iNaturalist for identification confirmation.

Flower color

Lowbush blueberry varies in flower color from entirely white, to pink and white striped, to almost entirely pink flowers. We were interested in whether this color variation might lead to differences in nocturnal versus diurnal pollination (Elam and Linhart 1988). We categorized blueberry patches by flower color into four groups: (*i*) predominantly white flowers (four patches), (*ii*) predominantly striped flowers (four patches), (*iii*) a mixture of white and striped flowers (seven patches), and (*iv*) predominantly ombre flowers that fade from pink closest to the sepals to white at the edge of the petals (five patches; including one patch where some flowers were uniformly pink). We used these categories to reanalyze fruit to bud ratio, fruit size, and number of large seeds using separate ANOVAs with factors of flower color, treatment, patch, and the interactions between treatment and flower color and treatment and patch.

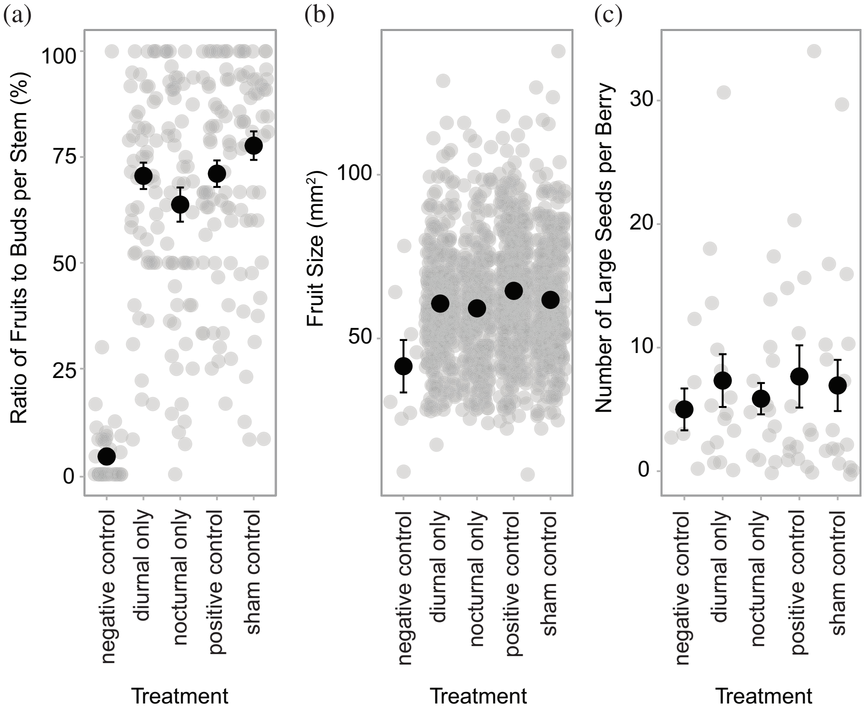
To ensure our flower color categories were quantifiably different, we collected flowers from 11 representative patches and used an Ocean Optics Jaz Spectrophotometer with an integrated Xenon light source to collect reflectance measurements, which were calculated relative to a white Spectralon standard. Our reflectance probe was sheathed in a rubber stopper to maintain a 90 degree angle to and fixed distance of ~3 mm from the measurement surface. From each stem, we measured two fully opened flowers. For flowers, we gently pressed the probe onto the petal offset from the center of the flower so as not to measure the color of the stamens through the slightly transparent petals. For striped flowers, we separately measured the pink stripe and the white surrounding the stripe. For ombre flowers we separately measured the pink part closer to the sepals and the whiter part at the edge of the petals. For all petal parts, we collected three readings, each of which was made up of three measurements averaged by the Jaz operating software. We averaged all measurements to obtain a single reflectance spectrum for each petal part. We calculated the brightness, hue, and saturation of each petal part. We calculated brightness as the average reflectance along the reflectance spectrum from 400 nm (in the near ultraviolet) to 700 nm (red). We calculated hue as: $\arctan \{[(B_y - B_b) /B_1]/[(B_r - B_g)/ B_1]\}$, where B refers to brightness, B1 refers to the total brightness across the reflectance spectrum from 300–700 nm, and subscripts refer to the following segments of the reflectance spectrum: red (r) = 600–700 nm, yellow (y) = 500–600 nm, green (g) = 400–500 nm, and blue (b) = 300–400 nm. We calculated saturation as the square root of $(B_r{-}B_g)^2 + (B_y{-}B_b)^2$ (Montgomerie 2006). We examined whether our flower color categories were quantitatively different from each other in terms of brightness, hue, and saturation using separate ANOVAs in which we included all measures for each flower part within a category.

Results

Fruit measurements

Fruit to flower bud ratio (i.e., fruit set) varied by treatment ($F_{4,197} = 165.84$, $P < 0.001$; Fig. 2*a*) and patch (ANOVA; $F_{19,197} = 10.34$, $P < 0.001$), with an interaction between treatment and patch ($F_{76,197} = 1.93$, $P < 0.001$). The negative control produced less fruit than all other treatments (Tukey HSD; $P < 0.001$) with 4.26% fruit set, while the sham control had the highest at 77.68%

Fig. 2. Effects of pollination treatment on fruit measurements. Gray dots represent raw data and larger black points indicate means and standard errors for each treatment. In Fig. 2*b* error bars of treatments other than the negative control are very small due to the large sample size.



(Fig. 2*a*). While the nocturnal-only treatment yielded lower fruit set (63.76%) than the sham control ($P < 0.001$), it did not set significantly less fruit than the diurnal treatment (70.55%) or positive control (71.06%).

Fruit size (mm^2) varied by treatment (linear mixed-effect model (LMM); Type II Wald $\chi^2 = 22.92$; degrees of freedom (df) = 4, $P = 0.0001$; Fig. 2*b*), and patch ($\chi^2 = 250.26$; df = 19, $P < 0.001$), but there was no interaction between treatment and patch ($\chi^2 = 60.44$; df = 61, $P = 0.50$). None of the treatments were significantly different from each other in post hoc tests. Viable seed set was not affected by treatment ($F_{4,57} = 0.33$, $P = 0.86$; Fig. 2*c*), patch ($F_{1,57} = 1.97$, $P = 0.17$), or the interaction between treatment and patch ($F_{4,57} = 0.43$, $P = 0.79$).

Pollinator surveys

In 2019, $N = 275$ insects were collected from lowbush blueberry flowers on Kent Island (Table 1). Sonicating bees represented 35% of these visits, vespid wasps 31%, flies 25%, and small numbers of visits were seen from beetles and butterflies. Visits by bumblebees in the subgenus *Pyrobombus* (either *Bombus vagans* Smith or *Bombus sandersoni* Franklin, we could not determine the species) were the most common (N = 77), followed by the two wasp species *Dolichovespula arenaria* Fabricius (N = 38) and *Dolichovespula norvegicoides* Sladen (N = 23), and kelp flies in the family Coelopidae (N = 28). From two separate night observations during the blueberry flowering period in the summer of 2018, moths of unknown species were recorded on patch flowers. In June 2019 we collected nocturnal visitors to blueberry flowers by bushes at 2000 with insect nets. The 23 insects collected included 7 flies (30%) in the families Coleopidae (N = 2), Lauxaniidae (N = 4), Tephritidae (N = 1), and 16 beetles (70%) in the families Elateridae (N = 10), Curculionidae (N = 3), Carabidae (N = 2), and Chrysomelidae (N = 1). These data suggest that pollination occurring at night was most likely carried out by moths, beetles, and flies.

Flower color

Our flower color categories were quantifiably different in measures of hue ($F_{3,394} = 73.91$, $P < 0.001$; Fig. 3), saturation ($F_{3,394} = 30.99$, $P < 0.001$), and brightness (ANOVA; $F_{3,394} = 43.66$, $P < 0.001$). All categories were significantly different in post hoc tests (Tukey HSD; $P < 0.05$), except that the stripe and ombre categories were not significantly different in measures of saturation ($P = 0.86$). Patch flower color categories did not affect fruit to bud ratio (ANOVA; $F_{4,197} = 1.69$, $P = 0.15$), or have an interaction with treatment to affect fruit to bud ratio ($F_{16,197} = 0.97$, $P = 0.48$). There was an effect of flower color on fruit size (LMM; Type II Wald $\chi^2 = 40.06$; df = 3, $P < 0.001$), with patches that had striped flowers and patches that had a mix of striped and white flowers having slightly larger fruit than ombre or all white patches, but none of the color categories were different from each other in post hoc tests. There was no interaction

Table 1. Numbers and percentages of insects observed visiting lowbush blueberry flowers in 2019. Insects were identified to the lowest taxonomic level possible given available resources.

Taxonomic Group	Number of individuals observed	% Of individuals observed
Coleoptera: Cerambycidae: *Evodinus monticola* Randal	7	2.5%
Diptera	23	8.4%
Diptera: Calliphoridae	4	1.4%
Diptera: Coelopidae	28	10.2%
Diptera: Syrphidae: *Toxomerus marginatus* Say	11	4.0%
Diptera: Tipulidae	2	0.7%
Hymenoptera: Anthophila: *Andrena* spp.	4	1.5%
Hymenoptera: Anthophila: *Bombus flavidus* Eversmann	10	3.6%
Hymenoptera: Anthophila: *Bombus ternarius* Say	3	1.1%
Hymenoptera: Anthophila: Halictidae: *Dialictus* spp.	2	0.7%
Hymenoptera: Anthophila: *Bombus vagans/sandersoni*	77	28.0%
Hymenoptera: Crabronidae: *Pemphredon* spp.	1	0.4%
Hymenoptera: Ichneumonidae: Ophioninae	1	0.4%
Hymenoptera: Siricidae	3	1.1%
Hymenoptera: Vespidae: *Ancistrocerus waldenii* Viereck	6	2.2%
Hymenoptera: Vespidae: *Dolichovespula arenaria* Fabricius	38	13.8%
Hymenoptera: Vespidae: *Dolichovespula maculata* L.	3	1.1%
Hymenoptera: Vespidae: *Dolichovespula norvegicoides* Sladen	23	8.4%
Hymenoptera: Vespidae: *Vespula acadica* Sladen	1	0.4%
Hymenoptera: Vespidae: *Vespula consobrina* de Saussure	14	5.1%
Lepidoptera: Lycaenidae: Polyommatini	2	0.7%
Lepidoptera: Nymphalidae: *Vanessa atalanta* L.	8	2.9%
Lepidoptera: Nymphalidae: *Vanessa cardui*	2	0.7%
Lepidoptera	2	0.7%
Total	275	

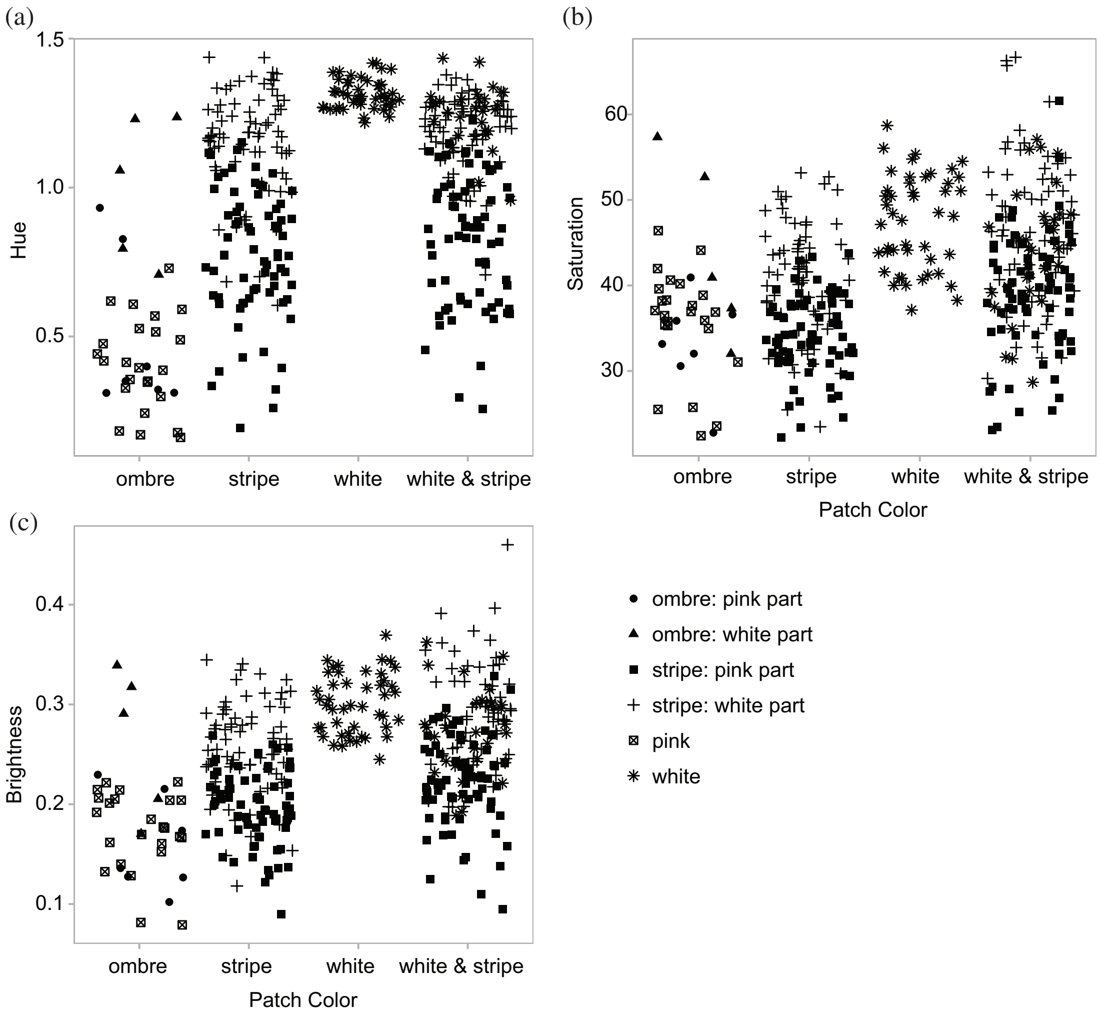
between color category and treatment for fruit size (LMM; Type II Wald $\chi^2 = 6.82$; df = 12, $P = 0.87$). The number of large seeds was not affected by flower color category (ANOVA; $F_{3,54} = 1.03$, $P = 0.39$) or an interaction between flower color category and treatment ($F_{11,43} = 0.85$, $P = 0.60$).

Discussion

Recent declines in managed and wild pollinators have motivated new avenues of research into pollinator ecology, particularly for crops dependent on insect pollination (Kolb 2008; Potts et al. 2010; Cutler et al. 2012; Macgregor et al. 2019). Our data corroborate that lowbush blueberry requires insect-mediated pollination (Javorek et al. 2002; Bell et al. 2010; Cutler et al. 2012; Garibaldi et al. 2013) because our negative control treatment had low fruit set, fruit size, and numbers of viable seeds. The few fruits observed on negative control stems (4.26%) suggest that lowbush blueberry may self-pollinate in rare instances. Previous research has indicated that diurnal pollinators, particularly sonicating bees, represent the most effective method of pollination for lowbush blueberry (Maust et al. 1999; Dogterom et al. 2000; Javorek et al. 2002; Bushmann et al. 2012; Manning and Cutler 2013), and we therefore predicted higher fruit set in stems accessible to diurnal pollinators. While the nocturnal-only exposure treatment did have slightly lower fruit sets than treatments that were accessible to diurnal pollinators, this was not a statistically significant difference. That the diurnal and nocturnal treatments produced similar fruit sets indicates that pollen transfer is occurring during both photoperiods as has been shown in mainland Debert, Nova Scotia (Cutler et al. 2012). Ripe berries produced through nocturnal pollination were similar in size and number of viable seeds to berries produced through diurnal pollination, as was also shown in the Debert study (Cutler et al. 2012). It is possible that nocturnal insects on Kent Island, which are not known to sonicate, still facilitate the movement of already-dislodged pollen grains as has been shown with non-sonicating honeybees (Drummond 2016).

For example, while nectar-foragers like Lepidoptera do not actively harvest pollen, several families have been shown to carry pollen from multiple plant taxa (Wood and Wood 1963; Javorek et al. 2002; Cutler et al. 2012; Macgregor et al. 2019). In both our study and the Debert study (Cutler et al. 2012), treatments exposed to pollinators 24 h a day did not have higher fruit set than treatments only accessible to pollinators during the day. Therefore, while nocturnal pollination did occur, it did not boost fruit set beyond diurnal-only levels, potentially

Fig. 3. Spectrophotometry measures of patch color categories. Shapes indicate the part of the flower petal measured, particularly relevant for ombre and striped petals. Pink and white indicate measurements from flowers that were uniformly one color.



explained by the expected lower efficiency of nocturnal pollinators (Javorek et al. 2002).

Kent Island lowbush blueberry offers an opportunity to better understand the influences of island dynamics on pollination success and fruit quality. Islands differ from mainland habitats in several ways including having lower pollinator densities, unique pollinator communities, and distinct abiotic factors such as increased exposure to ocean winds and storm frequency, which could potentially constrain pollinators (Spears Jr 1987; Zink and Wheelwright 1997; Balmford et al. 2006; Artz and Nault 2011; Chamorro et al. 2012). While we might expect a less dense and diverse community of pollinators in island environments like Kent Island, we observed the same orders of nocturnal insects as Cutler et al. (2012) in mainland Nova Scotia including Diptera, Coleoptera, and Lepidoptera. The most common diurnal visitors to blueberry on Kent Island were bumblebees, *Bombus* spp., followed by multiple wasp species. A previous study on Kent Island with blueflag iris, *Iris versicolor* L., indicated a reduced pollinator community, describing that while bumblebees have been reported as the predominant pollinators for mainland iris, bumblebees comprised only 1% of the visits to Kent Island iris (Zink and Wheelwright 1997). Our study, in contrast, found the majority of visits to blueberry from bumblebees. It is not clear if this difference is a product of survey methodology, plant preferences by bees, or time (the iris study was conducted in 1994 and 1995 and bumblebee populations may have changed in the following 20 yr). Kent Island also differs from mainland sites in its lack of introduced honeybees and no history of widespread pesticide use. The Debert site had commercial honeybees, *Apis mellifera* L., and colonies of the bumblebee *B. impatiens*, but also recorded visits from wild *Bombus* and andrenid, megachilid, and halictid bees (Cutler et al. 2012). Future studies on Kent Island should examine individuals observed visiting flowers during diurnal and nocturnal photoperiods for pollen grains to determine which species are transferring the most lowbush blueberry pollen.

Individual genets, or clones, of lowbush blueberries are known to vary in yield by as much as 15-fold even when grown nearby under managed conditions (Bell et al. 2012). Our 20 lowbush blueberry patches varied substantially in fruit set and fruit size. Factors driving these differences may include the genetic similarity of neighboring plants that are donating pollen (Bell et al. 2012), variation in self-compatibility (Bell et al. 2010), timing of flowering (White et al. 2012; Drummond and Rowland 2020), and frost tolerance of flowers (Hicklenton et al. 2000). Lowbush blueberry genets can vary substantially in their self-compatibility, and the degree of self-compatibility can be affected by phenology and associated pollinator availability (Drummond and Rowland 2020). Additionally, self-compatible genets tend to have the highest fruit sets and berry size (Drummond and Rowland 2020). It is possible that our patch variation in fruit parameters may be correlated with variation in self-compatibility.

To our knowledge, variation in flower color and resulting attractiveness to pollinators has not been previously studied in lowbush blueberry. Flower color serves as an important signaling factor for attracting pollinating insects (Hoballah et al. 2005; Miller et al. 2011; Rodriguez-Saona et al. 2011), and our patches fell into different flower color categories that significantly differed in hue, saturation, and brightness. There were, however, no significant effects of our flower color categories on fruit set, or an interaction between treatment and flower color. While there was an overall effect on fruit size, with patches with striped flowers having slightly larger fruits, none of the color categories were significantly different from each other in post-hoc tests. As lowbush blueberry predominantly requires cross-pollination (Drummond and Rowland 2020) but grows in large clonal patches, it could be disadvantageous if pollinators had strong preferences for one color over another as that might reduce cross-pollination. We had nonetheless hypothesized that color variation might affect detectability for nocturnal versus diurnal pollinators. We did not find support for this hypothesis. The role of this color variation in lowbush blueberry is in need of further investigation.

Unmanaged lowbush blueberry in Ontario boreal forests can set 12%–86% of their fruits, with an average of 50% (Usui et al. 2005; Cutler et al. 2012), while 75% fruit set is believed to be the upper limit when stocking commercial fields with honeybees in Nova Scotia (Kinsman 1993). In the Debert study, stems open diurnally or 24 h were exposed to commercial honeybees and wild bumblebees and achieved 40%–45% fruit set (Cutler et al. 2012). Studies in Maine fields where honeybees have not been added have documented fruit sets of 42% (Desjardins and De Oliveira 2006; Bushmann and Drummond 2020), 28% (Drummond 2012), 33% (Asare et al. 2017), and 55% (Stubbs and Drummond 1997). Kent Island produced very high fruit sets in all four exposed treatments, with positive and sham controls achieving 71% and 78% fruit set, respectively. This higher fruit set could be explained by our bagging of all blueberry stems after complete corolla fall, preventing the loss of fruit to drop, frugivory, and parasitism (Drummond 2020). A previous study conducted on Kent Island in 1993, found 53.9% fruit set in lowbush blueberry when accessible to pollinators and 23.4% when pollinators were excluded (Wheelwright et al. 2006). In this previous study the pollination exclusion bags were removed after floral senescence; therefore, fruit may have been lost to drop or frugivory by birds, unlike in our study where stems remained bagged until the fruit was collected. Another possible explanation for the high fruit set in our study is that we excluded stems with more than 35 buds, while commercial fields can average 70 buds per stem, and stems with lower numbers of buds have been shown to have higher fruit sets (Drummond 2019). Pesticides are a factor driving insect declines worldwide (Whitehorn et al. 2012; Stuligross and Williams 2020; Tosi et al. 2021). Many lowbush blueberry growers in Maine and New Brunswick spray the fungicide Propiconazole as well as insecticides (Rose et al. 2013; Colwell et al. 2017). Insecticides and fungicides, including Propiconazole, can have negative effects on bees both in isolation and combination (Thompson et al. 2014; Sgolastra et al. 2018; Han et al. 2019; Chandler et al. 2020). The lack of insecticide or fungicide usage on Kent Island may be a potential explanation for the high pollination success of our lowbush blueberry. Another possibility is that the patchy habitat of Kent Island with blueberry interspersed between spruce forest and other plant communities may support more wild pollinators. For example, in commercial Nova Scotia blueberry fields, several wild bee species are more abundant closer to forest edges and decline towards the interior of the fields (Cutler et al. 2015). The high fruit set on Kent Island, however, occurs in the absence of introduced bees despite what is likely to be a reduced pollinator community due to island dynamics. Genetic variation in self-compatibility has been shown to affect yield in lowbush blueberry (Bell et al. 2010), and plants on islands in particular are expected to have higher rates of self-compatibility (Schueller 2004). It is possible that blueberry on Kent Island is more tolerant of self-pollination than blueberry in mainland commercial fields, as has been shown for other plant species on Kent Island (Wheelwright et al. 2006). However, as our negative control stems indicate, lowbush blueberry still requires insect-mediated pollination even if self-fertilizing. Lowbush blueberry often co-occurs with velvetleaf blueberry, *Vaccinium myrtilloides* Michx., and cross-pollination between the two species can negatively affect fruit production (Aalders and Hall 1961; Bell et al. 2010; Fournier et al. 2020). Velvetleaf blueberry is not present on Kent Island, and its absence may benefit fruit production of lowbush blueberry in comparison to mainland populations with both species. Since the eradication of snowshoe hares from Kent Island in 2007 (Wheelwright 2016), the only remaining non-volant mammals are muskrats and occasional otters, who do not appear to browse lowbush blueberry. The high fruit set of Kent Island blueberry is therefore additionally surprising given this lack of regular browsing or pruning and burning which are conducted to increase fruit yield in managed lowbush blueberry (Warman 1987; Eaton et al. 2004).

The increasing prevalence of habitat fragmentation will have important impacts on ecological communities, particularly plant-pollinator dynamics (Grass et al. 2018; Lázaro et al. 2020). Islands have long been used by ecologists as model systems in which to study the long-term impacts of habitat fragmentation (MacArthur and Wilson 1963). Given current pollinator declines (Gallai et al. 2009) and ongoing habitat fragmentation in agricultural systems (Montoya et al. 2021), studying crop pollination on an offshore island can provide insights into crop pollination dynamics. Lowbush blueberry is a native plant in New Brunswick, and has a co-evolutionary history with native pollinator species. We found robust pollination of lowbush blueberry on an offshore island, despite the absence of introduced honeybees or managed pruning. This research highlights both the usefulness of offshore island studies, and the complexity of pollination dynamics even in reduced island communities.

Competing Interests

The authors declare there are no competing interests.

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Data AvailabilityData will be made available on Dryad.

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References

Aalders, L.E., and Hall, I.V. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. **3**: 300–307. doi:10.1139/g61-034.

Artz, D.R., and Nault, B.A. 2011. Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as pollinators of pumpkin. J. Econ. Entomol. **104**: 1153–1161. doi:10.1603/EC10431. PMID:21882677.

Asare, E., Hoshide, A.K., Drummond, F.A., Criner, G.K., and Chen, X. 2017. Economic risk of bee pollination in Maine wild blueberry, *Vaccinium angustifolium*. J. Econ. Entomol. **110**: 1980–1992. doi:10.1093/jee/tox191. PMID:28981673.

Bajcz, A., and Drummond, F. 2017*a*. Flower power: Floral and resource manipulations reveal the consequences of reproductive effort in lowbush blueberry (*Vaccinium angustifolium*). Ecol. Evol. **2017**: 1–15.

Bajcz, A.W., and Drummond, F.A. 2017*b*. Bearing fruit: flower removal reveals the trade-offs associated with high reproductive effort for lowbush blueberry. Oecologia **185**: 13–26. doi:10.1007/s00442-017-3908-2.

Balmford, B., Balmford, J., Balmford, A., Blakeman, S., Manica, A., and Cowling, R. 2006. Diurnal versus nocturnal pollination of Brunsvigia gregaria RA Dyer (Amaryllidaceae) at a coastal site. S. Afr. J. Bot. **72**: 291–294.

Barrett, S.C.H. 1996. The reproductive biology and genetics of island plants. Phil. T. R. Soc. B. **351**: 725–733. doi:10.1098/rstb.1996.0067.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2014. Fitting linear mixed-effects models using lme4. arXiv:1406.5823.

Bell, D.J., Rowland, L.J., Stommel, J., and Drummond, F.A. 2010. Yield variation among clones of lowbush blueberry as a function of genetic similarity and self-compatibility. J. Amer. Soc. Hort. Sci. **135**: 259–270. doi:10.21273/JASHS.135.3.259.

Bell, D.J., Rowland, L.J., and Drummond, F.A. 2012. Does pollen 'neighborhood' affect berry yield In lowbush blueberry (*Vaccinium angustifolium* Ait)? Int. J. Fruit Sci. **12**: 65–74.

Buck, M., Marshall, S.A., and Cheung, D.K. 2008. Identification atlas of the Vespidae (Hymenoptera, Aculeata) of the northeastern Nearctic region. Can. J. Arth. ID. **5**: 1–492.

Bushmann, S.L., and Drummond, F.A. 2015. Abundance and diversity of wild bees (Hymenoptera: Apoidea) found in lowbush blueberry growing regions of Downeast Maine. Environ Entomol. **44**: 975–989. doi:10.1093/ee/nvv082. PMID:26314043.

Bushmann, S.L., and Drummond, F.A. 2020. Analysis of pollination services provided by wild and managed bees (Apoidea) in wild blueberry (*Vaccinium angustifolium* Aiton) Production in Maine, USA, with a literature review. Agronomy **10**: 1413. doi:10.3390/agronomy10091413.

Bushmann, S.L., Drummond, F.A., Beers, L.A., and Groden, E. 2012. Wild bumblebee (Bombus) diversity and Nosema (Microsporidia: Nosematidae) infection levels associated with lowbush blueberry (Vaccinium angustifolium) production and commercial bumblebee pollinators. Psyche **2012**.

Castro, C., Olarte, Y., Rache, L., and Pacheco, J. 2012. Development of a germination protocol for blueberry seeds (Vaccinium meridionale Swartz). Agron. Colombiana. **30**: 196–203.

Chamorro, S., Heleno, R., Olesen, J.M., McMullen, C.K., and Traveset, A. 2012. Pollination patterns and plant breeding systems in the Galápagos: a review. Ann. Bot. Lond. **110**: 1489–1501. doi:10.1093/aob/mcs132.

Chandler, A.J., Drummond, F.A., Collins, J.A., Lund, J., and Alnajjar, G. 2020. Exposure of the common eastern bumble bee, Bombus impatiens (Cresson), to Sub-lethal doses of Acetamiprid and Propiconazole in wild blueberry. J. Agric. Urban Entomol. **36**: 1–23.

Chiabrando, V., Giacalone, G., and Rolle, L. 2009. Mechanical behaviour and quality traits of highbush blueberry during postharvest storage. J. Sci. Food Agric. **89**: 989–992. doi:10.1002/jsfa.3544.

Colwell, M.J., Williams, G.R., Evans, R.C., and Shutler, D. 2017. Honey bee-collected pollen in agro-ecosystems reveals diet diversity, diet quality, and pesticide exposure. Ecol. Evol. **7**: 7243–7253. doi:10.1002/ece3.3178. PMID:28944014.

Cutler, G.C., Reeh, K.W., Sproule, J.M., and Ramanaidu, K. 2012. Berry unexpected: nocturnal pollination of lowbush blueberry. Can. J. Plant Sci. **92**: 707–711. doi:10.4141/cjps2012-026.

Cutler, G.C., Nams, V.O., Craig, P., Sproule, J.M., and Sheffield, C.S. 2015. Wild bee pollinator communities of lowbush blueberry fields: Spatial and temporal trends. Basic Appl. Ecol. **16**: 73–85. doi:10.1016/j.baae.2014.11.005.

Desjardins, È.-C., and De Oliveira, D. 2006. Commercial bumble bee *Bombus impatiens* (Hymenoptera: Apidae) as a pollinator in lowbush blueberry (Ericale: Ericaceae) fields. J. Econ. Entomol. **99**: 443–449. doi:10.1093/jee/99.2.443. PMID:16686145.

Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., et al. 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat. Ecol. Evol. **5**: 1453–1461.

Dogterom, M.H., Winston, M.L., and Mukai, A. 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv.'Bluecrop' (Vaccinium corymbosum; Ericaceae). Am. J. Bot. **87**: 1584–1591.

Drummond, F. 2012. Commercial bumble bee pollination of lowbush blueberry. Int. J. Fruit Sci. **12**: 54–64. doi:10.1080/15538362.2011.619120.

Drummond, F. 2019. Reproductive biology of wild blueberry (*Vaccinium angustifolium* Aiton). Agriculture **9**: 69. doi:10.3390/agriculture9040069.

Drummond, F.A. 2016. Behavior of bees associated with the wild blueberry agro-ecosystem in the USA. Int. J. Entomol. Nematol. **2**: 21–26.

Drummond, F.A. 2020. Wild Blueberry fruit drop: a consequence of seed set? Agronomy **10**: 939. doi:10.3390/agronomy10070939.

Drummond, F.A., Dibble, A.C., Stubbs, C., Bushmann, S.L., Ascher, J.S., and Ryan, J. 2017. A natural history of change in native bees associated with lowbush blueberry in Maine. Northeast. Nat. **24**: 49–68. doi:10.1656/045.024.m1502.

Drummond, F.A., and Rowland, L.J. 2020. The ecology of autogamy in wild blueberry (*Vaccinium angustifolium* Aiton): does the early clone get the bee? Agronomy **10**: 1153. doi:10.3390/agronomy10081153.

Eaton, L.J., Glen, R.W., and Wyllie, J.D. 2004. Efficient mowing for pruning wild blueberry fields. Small Fruit. Rev. **3**: 123–131. doi:10.1300/J301v03n01_12.

Elam, D.R., and Linhart, Y.B. 1988. Pollination and seed production in Ipomopsis aggregata: differences among and within flower color morphs. Am. J. Bot. **75**: 1262–1274.

Fournier, M.-P., Paré, M.C., Buttò, V., Delagrange, S., Lafond, J., and Deslauriers, A. 2020. How plant allometry influences bud phenology and fruit yield in two Vaccinium species. Ann. Bot. Lond. **126**: 825–835.

Fox, J., and Weisberg, S. 2018. An R companion to applied regression. SAGE Publications.

Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B.E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. **68**: 810–821. doi:10.1016/j.ecolecon.2008.06.014.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science **339**: 1608–1611. doi:10.1126/science.1230200. PMID:23449997.

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E.L. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science **347**.

Grass, I., Jauker, B., Steffan-Dewenter, I., Tscharntke, T., and Jauker, F. 2018. Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks. Nat. Ecol. Evol. **2**: 1408–1417. doi:10.1038/s41559-018-0631-2. PMID:30082735.

Han, W., Yang, Y., Gao, J., Zhao, D., Ren, C., Wang, S., et al. 2019. Chronic toxicity and biochemical response of *Apis cerana cerana* (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. Ecotoxicology **28**: 399–411. doi:10.1007/s10646-019-02030-4.

Hicklenton, P., Reekie, J., MacKenzie, K., Eaton, L., and Havard, P. 2000. Freeze damage and frost tolerance thresholds for flowers of the lowbush blueberry (*Vaccinium angustifolium* Ait). Acta Hortic. **574**: 193–201. doi:10.17660/ActaHortic.2002.574.29.

Hoballah, M.E., Stuurman, J., Turlings, T.C., Guerin, P.M., Connetable, S., and Kuhlemeier, C. 2005. The composition and timing of flower odour emission by wild *Petunia axillaris* coincide with the antennal perception and nocturnal activity of the pollinator *Manduca sexta*. Planta **222**: 141–150. doi:10.1007/s00425-005-1506-8. PMID:15891900.

Holzschuh, A., Dudenhöffer, J.-H., and Tscharntke, T. 2012. Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biol. Conserv. **153**: 101–107. doi:10.1016/j.biocon.2012.04.032.

Hothorn, T., Bretz, F., and Westfall, P. 2008. Simultaneous inference in general parametric models. Biom. J. **50**: 346–363. doi:10.1002/bimj.200810425.

Irwin, R.E., and Brody, A.K. 1998. Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia **116**: 519–527. doi:10.1007/s004420050617. PMID:28307521.

Isaacs, R., and Kirk, A.K. 2010. Pollination services provided to small and large highbush blueberry fields by wild and managed bees. J. Appl. Ecol. **47**: 841–849. doi:10.1111/j.1365-2664.2010.01823.x.

Javorek, S., Mackenzie, K., and Vander Kloet, S. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Ann. Entomol. Soc. Am. **95**: 345–351. doi:10.1603/0013-8746(2002)095[0345:CPEABH]2.0.CO;2.

Kinsman, G. 1993. The history of the lowbush blueberry industry in Nova Scotia 1950–1990. Nova Scotia Department of Agriculture & Marketing.

Kolb, A. 2008. Habitat fragmentation reduces plant fitness by disturbing pollination and modifying response to herbivory. Biol. Conserv. **141**: 2540–2549. doi:10.1016/j.biocon.2008.07.015.

Kruess, A., and Tscharntke, T. 1994. Habitat fragmentation, species loss, and biological control. Science **264**: 1581–1584. doi:10.1126/science.264.5165.1581. PMID:17769603.

Lázaro, A., Fuster, F., Alomar, D., and Totland, Ø. 2020. Disentangling direct and indirect effects of habitat fragmentation on wild plants' pollinator visits and seed production. Ecol. Appl. **30**: e02099. doi:10.1002/eap.2099.

MacArthur, R.H., and Wilson, E.O. 1963. An equilibrium theory of insular zoogeography. Evolution, **17**: 373–387.

Macgregor, C.J., Kitson, J.J., Fox, R., Hahn, C., Lunt, D.H., Pocock, M.J., and Evans, D.M. 2019. Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. Ecol. Entomol. **44**: 17–29. doi:10.1111/een.12674.

MacLean, D.D., and NeSmith, D.S. 2011. Rabbiteye blueberry postharvest fruit quality and stimulation of ethylene production by 1-methylcyclopropene. HortScience **46**: 1278–1281. doi:10.21273/HORTSCI.46.9.1278.

Manning, P., and Cutler, G.C. 2013. Potential nocturnal insect pollinators of lowbush blueberry. J. Acadian Entomol. Soc. **9**: 1–3.

Maust, B., Williamson, J., and Darnell, R. 1999. Flower bud density affects vegetative and fruit development in field-grown southern highbush blueberry. HortScience **34**: 607–610. doi:10.21273/HORTSCI.34.4.607.

Miller, R., Owens, S.J., and Rørslett, B. 2011. Plants and colour: flowers and pollination. Opt. Laser Technol. **43**: 282–294. doi:10.1016/j.optlastec.2008.12.018.

Montgomerie, R. 2006. Analyzing colors. Pages 90–147 in G.E. Hill and K.J. McGraw, eds. Bird coloration: mechanisms and measurements. Harvard University Press.

Montoya, D., Haegeman, B., Gaba, S., De Mazancourt, C., and Loreau, M. 2021. Habitat fragmentation and food security in crop pollination systems. J. Ecol. **109**: 2991–3006. doi:10.1111/1365-2745.13713.

Ollerton, J., Winfree, R., and Tarrant, S. 2011. How many flowering plants are pollinated by animals? Oikos **120**: 321–326. doi:10.1111/j.1600-0706.2010.18644.x.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. **25**: 345–353. doi:10.1016/j.tree.2010.01.007. PMID:20188434.

Powney, G.D., Carvell, C., Edwards, M., Morris, R.K., Roy, H.E., Woodcock, B.A., and Isaac, N.J. 2019. Widespread losses of pollinating insects in Britain. Nat. Commun. **10**: 1–6. doi:10.1038/s41467-019-08974-9.

Preston, F.W. 1962. The canonical distribution of commonness and rarity: part II. Ecology **43**: 410–432. doi:10.2307/1933371.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rodriguez-Saona, C., Parra, L., Quiroz, A., and Isaacs, R. 2011. Variation in highbush blueberry floral volatile profiles as a function of pollination status, cultivar, time of day and flower part: implications for flower visitation by bees. Ann. Bot. Lond. **107**: 1377–1390.

Rose, A., Drummond, F.A., Yarborough, D.E., and Asare, E. 2013. Maine wild blueberry growers: A 2010 economic and sociological analysis of a traditional Downeast crop in transition. University of Maine: College of Natural Sciences, Forestry & Agriculture.

Schueller, S.K. 2004. Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). Am. J. Bot. **91**: 672–681. doi:10.3732/ajb.91.5.672. PMID:21653422.

Scopece, G., Campese, L., Duffy, K.J., and Cozzolino, S. 2018. The relative contribution of diurnal and nocturnal pollinators to plant female fitness in a specialized nursery pollination system. AoB Plants **10**: ply002.

Sgolastra, F., Blasioli, S., Renzi, T., Tosi, S., Medrzycki, P., Molowny-Horas, R., Porrini, C., and Braschi, I. 2018. Lethal effects of Cr (III) alone and in combination with propiconazole and clothianidin in honey bees. Chemosphere **191**: 365–372. doi:10.1016/j.chemosphere.2017.10.068. PMID:29049960.

Soots, K., Krikmann, O., Starast, M., and Olt, J. 2017. Determining the dimensional characteristics of blueberries. Agron. Res. **15**: 886–896.

Soroye, P., Newbold, T., and Kerr, J. 2020. Climate change contributes to widespread declines among bumble bees across continents. Science **367**: 685–688. doi:10.1126/science.aax8591. PMID:32029628.

Spears, E.E., Jr 1987. Island and mainland pollination ecology of *Centrosema virginianum* and *Opuntia stricta*. J. Ecol. **75**: 351–362. doi:10.2307/2260423.

Stournaras, K.E., and Schaefer, H.M. 2017. Does flower and fruit conspicuousness affect plant fitness? Contrast, color coupling and the interplay of pollination and seed dispersal in two *Vaccinium* species. Evol. Ecol. **31**: 229–247. doi:10.1007/s10682-016-9864-1.

Stubbs, C.S., and Drummond, F.A. 1997. Management of the alfalfa leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae), for pollination of wild lowbush blueberry. J. Kansas Entomol. Soc. **70**: 81–93.

Stubbs, C.S., and Drummond, F.A. 2001. Bombus impatiens (Hymenoptera: Apidae): an alternative to *Apis mellifera* (Hymenoptera: Apidae) for lowbush blueberry pollination. J. Econ. Entomol. **94**: 609–616. doi:10.1603/0022-0493-94.3.609. PMID:11425013.

Stubbs, C.S., Drummond, F.A., and Allard, S.L. 1997. Bee conservation and increasing *Osmia* spp. in Maine lowbush blueberry fields. Northeast. Nat.: 133–144. doi:10.2307/3858708.

Stuligross, C., and Williams, N.M. 2020. Pesticide and resource stressors additively impair wild bee reproduction. Proc. R. Soc. B. **287**: 20201390.

Thompson, H.M., Fryday, S.L., Harkin, S., and Milner, S. 2014. Potential impacts of synergism in honeybees (*Apis mellifera*) of exposure to neonicotinoids and sprayed fungicides in crops. Apidologie **45**: 545–553. doi:10.1007/s13592-014-0273-6.

Tosi, S., Nieh, J.C., Brandt, A., Colli, M., Fourrier, J., Giffard, H., et al. 2021. Long-term field-realistic exposure to a next-generation pesticide, flupyradifurone, impairs honey bee behaviour and survival. Comm. Biol. **4**: 1–9. doi:10.1038/s42003-021-02336-2.

Traveset, A., Tur, C., Trøjelsgaard, K., Heleno, R., Castro-Urgal, R., and Olesen, J.M. 2016. Global patterns of mainland and insular pollination networks. Global Ecol. Biogeogr. **25**: 880–890. doi:10.1111/geb.12362.

Turmanidze, T., Jgenti, M., Gulua, L., and Shaiashvili, V. 2017. Effect of ascorbic acid treatment on some quality parameters of frozen strawberry and raspberry fruits. Ann. Agrarian Sci. **15**: 370–374. doi:10.1016/j.aasci.2017.02.017.

Usui, M., Kevan, P.G., and Obbard, M. 2005. Pollination and breeding system of lowbush blueberries, *Vaccinium angustifolium* Ait. and V. myrtilloides Michx.(Ericacaeae), in the boreal forest. Can. Field-Nat. **119**: 48–57. doi:10.22621/cfn.v119i1.80.

Van der Pijl, L. 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. Evolution **15**: 44–59. doi:10.1111/j.1558-5646.1961.tb03128.x.

Van Doorn, W.G. 1997. Effects of pollination on floral attraction and longevity. J. Exp. Bot. **48**: 1615–1622.

Warman, P. 1987. The effects of pruning, fertilizers, and organic amendments on lowbush blueberry production. Plant Soil. **101**: 67–72. doi:10.1007/BF02371032.

Wheelwright, N.T. 2016. Eradication of an ecosystem engineer. Front. Ecol. Environ. **14**: 53–54. doi:10.1002/fee.1221.

Wheelwright, N.T., Dukeshire, E.E., Fontaine, J.B., Gutow, S.H., Moeller, D.A., Schuetz, J.G., et al. 2006. Pollinator limitation, autogamy and minimal inbreeding depression in insect-pollinated plants on a boreal island. Am. Midl. Nat. **155**: 19–38. doi:10.1674/0003-0031(2006)155[0019:PLAAMI]2.0.CO;2.

White, S.N., Boyd, N.S., and Van Acker, R.C. 2012. Growing degree-day models for predicting lowbush blueberry (*Vaccinium angustifolium* Ait.) ramet emergence, tip dieback, and flowering in Nova Scotia, Canada. HortScience **47**: 1014–1021. doi:10.21273/HORTSCI.47.8.1014.

Whitehorn, P.R., O'connor, S., Wackers, F.L., and Goulson, D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science **336**: 351–352. doi:10.1126/science.1215025. PMID:22461500.

Williams, P.H., Thorp, R.W., Richardson, L.L., and Colla, S.R. 2014. Bumble bees of North America. Princeton University Press.

Wilson, J.S., and Carril, O.J.M. 2015. The bees in your backyard. Princeton University Press.

Wood, G. 1961. The influence of honeybee pollination on fruit set of the lowbush blueberry. Can. J. Plant Sci. **41**: 332–335. doi:10.4141/cjps61-045.

Wood, G., and Wood, F. 1963. Nectar production and its relation to fruitset in the lowbush blueberry. Can. J. Bot. **41**: 1675–1679. doi:10.1139/b63-153.

Yokota, S., and Yahara, T. 2012. Pollination biology of *Lilium japonicum* var. *abeanum* and var. *japonicum*: evidence of adaptation to the different availability of diurnal and nocturnal pollinators. Plant Spec. Biol. **27**: 96–105. doi:10.1111/j.1442-1984.2011.00336.x.

Zink, R.A., and Wheelwright, N.T. 1997. Facultative self-pollination in island irises. Am. Midl. Nat. **137**: 72–78. doi:10.2307/2426756.