

Berry unexpected: Nocturnal pollination of lowbush blueberry

G. Christopher Cutler, Kevin W. Reeh, Jason M. Sproule, and Krilen Ramanaidu

Department of Environmental Sciences, Nova Scotia Agricultural College, PO Box 550, Truro, Nova Scotia, Canada B2N 5E3 (e-mail: ccutler@nsac.ca). Received 3 February 2012, accepted 23 March 2012.

Cutler, C. G., Reeh, K. W., Sproule, J. M. and Ramanaidu, K. 2012. **Berry unexpected: Nocturnal pollination of lowbush blueberry.** *Can. J. Plant Sci.* **92**: 707–711. Lowbush blueberry, *Vaccinium angustifolium*, is an economically important crop of eastern North America that is critically dependent on insect-mediated cross-pollination for successful fruit set and high yields. It is generally assumed that bees are responsible for the vast majority of lowbush blueberry pollination, and producers usually augment the natural pollination force with managed bees. Little is known, however, of the potential role of nocturnal pollinators in lowbush blueberry production. We conducted a field experiment where patches of blooming blueberry were exposed to flying insects 24 h a day, only during the day (sunrise to sunset), only at night (sunset to sunrise), or 0 h a day. We found that significant fruit set occurred on blueberry stems that were exposed only at night, although it was higher on stems exposed during the day or 24 h a day. However, ripe fruit produced on stems exposed only at night weighed just as much as those exposed 24 h. Captures with Malaise traps activated only at night consisted mainly of several families of Lepidoptera and Diptera, although we do not know if these taxa pollinated blueberries. We conclude that nocturnal pollination may contribute significantly to lowbush blueberry fruit set.

Key words: *Vaccinium angustifolium*, pollination, fruit set, berry yield, nocturnal insects

Cutler, C. G., Reeh, K. W., Sproule, J. M. et Ramanaidu, K. 2012. **Une surprise: la pollinisation nocturne du bleuets nain.** *Can. J. Plant Sci.* **92**: 707–711. Économiquement, le bleuets nain (*Vaccinium angustifolium*) est une importante culture de l'est de l'Amérique du Nord et dépend considérablement de la pollinisation croisée par les insectes pour une bonne nouaison et un rendement élevé. On présume habituellement que la vaste majorité des plants sont fécondés par les abeilles, à tel point que les producteurs rehaussent la pollinisation naturelle en recourant à l'apiculture. On sait toutefois peu de choses sur le rôle éventuel de la pollinisation nocturne dans la production du bleuets nain. Les auteurs ont procédé à une étude sur le terrain en vertu de laquelle ils ont exposé des bleuets en fleur aux insectes volants 24 h par jour, le jour uniquement (de l'aube au crépuscule), la nuit seulement (du crépuscule à l'aube) ou pas du tout. Ils ont constaté une importante nouaison chez les bleuets qui n'étaient exposés aux insectes que la nuit, même si les plants exposés le jour ou exposés 24 h portaient plus de fruits. Les fruits mûrs des bleuets exposés uniquement la nuit pesaient autant que ceux des plants exposés pendant 24 h. Les insectes capturés la nuit dans les pièges Malaise font principalement partie de diverses familles des Lépidoptères et des Diptères, mais les auteurs ignoraient si ces taxons fertilisent le bleuets. Ils en concluent néanmoins que la pollinisation nocturne pourrait sensiblement concourir à la nouaison chez le bleuets nain.

Mots clés: *Vaccinium angustifolium*, pollinisation, nouaison, rendement en petits fruits, insectes nocturnes

Lowbush blueberry (syn. “wild blueberry”), *Vaccinium angustifolium* Aiton (Ericaceae), is a native plant of north-eastern North America. Over the past 100 yr, lowbush blueberry has become a major horticultural crop with thousands of hectares of intensively managed wild stands in production in the Atlantic provinces, Quebec, and the state of Maine (Kinsman 1986, 1996). Lowbush and highbush species of blueberries constitute approximately 50% of Canada's land area in fruit and nut production, and blueberries are now the most important horticultural crop in the country in terms of farmed area and value (Robichaud 2006)

The importance of insect-mediated pollination for successful lowbush blueberry production has long been appreciated. The relatively large size of lowbush blueberry pollen, poricidal anthers, and the tendency of urn-shaped blueberry blossoms to hang downward are thought to negate the role of wind pollination for the

plant. These characters also have repercussions for the relative efficiencies of different insect pollinators, with pollen-foraging “buzz pollinator” species, that sonicate flowers, typically achieving the highest rates of pollen transfer (Javorek et al. 2002). In addition, lowbush blueberry fields generally contain large numbers of clones that are predominately self-sterile (Aalders and Hall 1961; Usui et al. 2005). Thus, cross-pollination by insects is required for appreciable fruit set.

Bees (Apoidea) are the most important insect pollinators of blueberries. Wild bees play an important role, but their populations are often inadequate to satisfy yield demands of commercial operations. Most producers therefore supplement the natural pollination force with managed honey bees (*Apis mellifera* L.), bumble bees (*Bombus impatiens* Cresson) (Hymenoptera: Apidae) or alfalfa leafcutting bees [*Megachile rotundata* (Fabricius)] (Hymenoptera: Megachilidae). In terms of commercial

production, Kinsman (1957) considered inadequate pollination the most common cause of poor yields of lowbush blueberry in Nova Scotia.

Although the important contribution of bees in lowbush blueberry production is well recognized, far less is known about potential non-Apoidea pollinators in this agroecosystem. Hunter et al. (2000) showed that black flies (Diptera: Simuliidae) do not pollinate wild blueberries, but we were unable to find any other published literature that examined pollination of *Vaccinium* spp. by insects other than bees. Moreover, we are unaware of studies into nocturnal pollinators of lowbush blueberry or other *Vaccinium* spp. Indeed, nocturnal insects are largely overlooked in pollination studies, particularly in cropping systems. In the boreal forest, nocturnal lepidopterans transfer pollen for a variety of plant taxa, including several Ericaceae (Devoto et al. 2011). Nocturnal pollinators may only make minimal contributions to some plant species, such as milkweed, *Asclepias syriaca* L. (Asclepiadaceae) (Morse and Fritz 1983), but may make significant contributions to others. For example, Pelletier et al. (2001) found nocturnal pollinators of cloud berry (*Rubus chamaemorus* L.) (Rosaceae), which grows in habitats similar to that of wild blueberry (Pelletier et al. 2001), may contribute up to 41% fruit set. Herein we describe an experiment that examined the occurrence of nocturnal pollination in lowbush blueberry. Our a priori hypothesis was that pollination and berry production would be insignificant in blueberry flowers that were exposed to insects only at night.

METHODS AND MATERIALS

Experimental Design

The experiment was done from late May to August 2011 at the Wild Blueberry Research Centre in Debert, Nova Scotia (lat. 45°26'35"N, long. 63°26'57"W). At less than 5% bloom (May 30), galvanized steel rings (30 cm high, 76 cm diameter) were placed in the blueberry field. Within each ring there were at least two different clones of *V. angustifolium*. This was done in order to mediate potential problems of inter-clone incompatibility. On May 31 and as required by treatment, a double layer of 1.0-mm craft netting was placed over the aluminum ring and held in place with a bungee cord and twine cinch, thereby serving as an insect exclusion cage. Experimental treatments were arranged in a completely randomized design, with seven replicate cages for each of four exposure scenarios: (1) open 24 h per day; (2) closed 24 h per day; (3) open only during the day; (4) open only at night. Each day during the entire blueberry bloom period designated cages were opened or closed at sunrise and sunset based on times given for the town of Debert on the Environment Canada Weather Office website (www.weatheroffice.gc.ca). During June when bloom occurred, sunrise and sunset were at approximately 0530 and 2100, respectively, each day. Prior to bloom, four random stems within each cage were marked with

flagging tape. The number of flower buds or blossoms (June 01) and subsequently the number of green unripe berries (June 30) per stem was recorded. Berries were harvested and weighed in August.

During the experiment we sampled for active nocturnal insects using Townes-style Malaise traps (BioQuip, Rancho Dominguez, CA). Six Malaise traps were placed randomly in and around the field where test cages were located. Traps were activated at sunset and deactivated at sunrise each day during bloom at the same time mesh was removed from or placed over the aluminum rings. Captured insect specimens were returned to the lab, frozen and later identified to family.

Data Analysis

Analysis of variance was used to compare percent fruit set among treatments, calculated for each stem as the number of berries per number of fruit buds and blossoms. Normal distribution and constant variance assumptions on the error terms were verified for these data by examining the residuals (Shapiro-Wilk test, $P > 0.05$). Data on the number of ripe berries, percent ripe berries (number ripe per total berries per stem) and ripe berry weight were $\log(X+1)$ transformed to normalize residuals (Zar 1999), and treatments were compared by analysis of variance. Means were compared with Tukey's HSD test. Back-transformed means and 95% confidence intervals are presented. All analyses were done using JMP 9.0 statistical software (SAS Institute, Inc. 2012) at $\alpha = 0.05$.

RESULTS

Treatment had a significant effect on percent fruit set ($F_{12,99} = 5.22$, $P < 0.0001$). There was no difference in percent fruit set on stems that were exposed 24 h or open during the day only but fruit set for these treatments were significantly higher than cages that were closed 24 h per day. In the latter treatment fruit set was very low (approximately 7%). Cages that were only open at night had an intermediate amount of fruit set that was significantly greater than that of cages permanently closed, and less than that in cages open only during the day, but not different than percent fruit set seen in cages that were open 24 h per day (Fig. 1A). There were also significant differences among treatments in the number of ripe berries collected ($F_{3,24} = 5.18$, $P = 0.0067$), with a trend similar to that seen in percent fruit set; very few ripe berries were collected from enclosures that were closed 24 h per day, while an intermediate number of ripe berries were collected from cages that were opened only at night (Fig. 1B). However, we did not detect a treatment effect for the percent ripe berries produced ($F_{3,24} = 0.62$, $P = 0.61$) (Fig. 1C) or the weights of those ripe berries ($F_{3,24} = 0.53$, $P = 0.67$) (Fig. 1D).

During the day, we observed a variety of bees on blueberry blossoms within cage rings that were open, and on flowers throughout the field outside experimental enclosures. Commercial honey bee and bumble bee

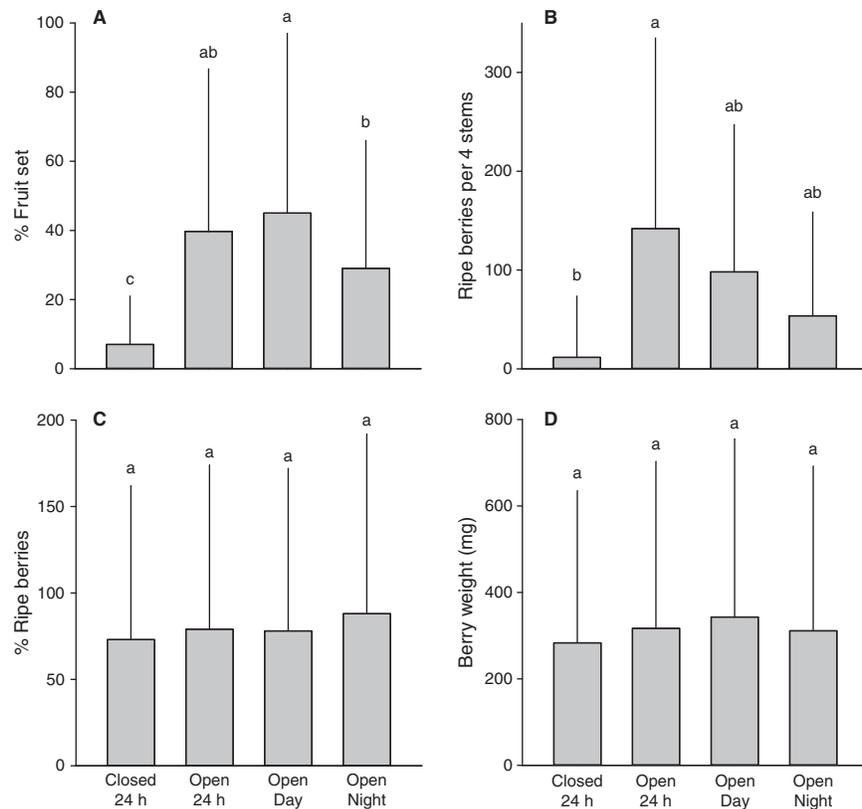


Fig. 1. Mean ($\pm 95\%$ CI) lowbush blueberry percent fruit set (A), number of ripe berries (B), percent ripe berries (C), and ripe berry weight (D) when blossoms were exposed to insect pollinators 0 h per day, 24 h per day, only during the day, or only at night ($n = 7$). Number of ripe berries, percent ripe berries, and ripe berry weight data were $\log(X+1)$ transformed. Back-transformed means and confidence intervals are presented. Bars with different letters are significantly different (Tukey test, $\alpha = 0.05$)

colonies were on site at the Research Centre, and *A. mellifera* and *B. impatiens* were often observed on blossoms. Wild *Bombus* spp., Andrenidae, Megachilidae and Halictidae (Hymenoptera) were also seen pollinating blossoms within open experimental units and throughout the field during the day.

We collected a variety of insects in nightly activated Malaise traps. Over the 4-wk bloom period, Diptera were the most collected group. The dominant dipterans were Chironomidae (66 specimens), Cecidomyiidae (19) and Ceratopogonidae (16). Mycetophilidae (6), Culicidae (4), Muscidae (2), Sciariidae (2), single specimens of Heleomyzidae, Psychodidae, Rhagionidae, Sarcophagidae, and Tipulidae, and six unidentifiable (damaged) dipterans were also collected in Malaise traps. Several families of Lepidoptera were found in our nightly collections. Multiple specimens of Gelechiidae (13 specimens), Tortricidae (10) and Geometridae (7), and single specimens of Elachistidae, Nepticulidae, Yponomeutidae, and an unidentifiable moth were recovered. The Coleoptera were represented by two families, Scarabaeidae (5) and Elateridae (2). One family each of Homoptera [Cicadellidae (3)], Hymenoptera [Ichneumonidae (4)], and Plecoptera [Perlidae (1)] were also found.

DISCUSSION

Our experiment demonstrated several things about the general nature of lowbush blueberry pollination. As previously shown, we found that insect-mediated pollination is required to achieve significant fruit set. Usui et al. (2005) reported that there is little natural fruit-set of *V. angustifolium* or *V. myrtilloides* Michx. without insect-mediated cross-pollination and that cross-pollination among clones within each species provides much better fruit and seed-set than does self-pollination. In our experiment, blossoms within cages that had pollinators excluded 24 h per day had poor fruit set. The low amount of fruit set and berry production that we did observe within these cages may have been the result of some wind-dispersal of pollen, or movement of pollen to flowers by undetected non-flying insects within the enclosure. Because a small number of blossoms (<5%) were already open at the start of our experiment, it is also possible that some pollination had occurred before cages were closed.

As we expected, cages that were open 24 h per day or only during daylight hours (sunrise to sunset) had good fruit and berry production. *Bombus* spp., Andrenidae, and Megachilidae, are very effective blueberry

pollinators (Javorek et al. 2002), and many growers in large fields supplement the natural pollination force with managed bees in order to achieve high yields (Aras et al. 1996; Kinsman 1996; Stubbs and Drummond 2001). We observed all of these taxa pollinating blueberry flowers during the day within our open experimental units. Fruit set in these treatments was nonetheless only 40–45%. It is possible that the steel rings deterred some bees from visiting these flowers, but in natural settings or commercial fields lowbush blueberry fruit set can be highly variable. In the boreal forest of central Ontario, Usui et al. (2005) observed that natural pollination of *V. angustifolium* gave 12–86% fruit-set. Kinsman (1996) suggested that around 75% fruit set is an achievable upper limit for commercial operations incorporating honey bees. In the state of Maine, fruit set in lowbush blueberry fields can exceed 80%, but in Nova Scotia less than 50% fruit set is often seen, even when high stocking rates of honey bees are used (G. Wood, Manager, Bragg Lumber Company, Collinwood Corner, Nova Scotia, personal communication).

We did not expect to see the significant levels of fruit set and berry production recorded in treatments that were only open at night. If one considers treatments that were open during the day (exposed to bees) to be representative of normal expected fruit set (~45%) and berry production (~30 berries per stem), our results indicate nocturnal pollination can account for approximately two-thirds of fruit set, and subsequently one-third of ripe berries produced. In addition, though lower fruit set was observed overall, ripe berries produced through nocturnal pollination were equal in weight to those produced diurnally, indicating that nocturnal pollinators can efficiently transfer the number of pollen tetrads required to achieve a normal sized fruit. We suspect that the 30% fruit set seen in cages opened at night was due to insects that were active or flying between sunset and sunrise. Wind pollination of blossoms in this treatment seems unlikely as a major contributor given the poricidal anthers, urceolate and pendulous flowers, and high incidence of self-sterility of *V. angustifolium* (Aalders and Hall 1961; Wood 1968; Usui et al. 2005). We captured a number of nocturnal Diptera and Lepidoptera in our Malaise traps. Chironomidae, Cecidomyiidae and Ceratopogonidae were most abundant in our collections and are known to provide pollination services for a variety of plants (Kevan 1972; Larson et al. 2001), although we were not able to find reports of these families pollinating *Vaccinium* or Ericaceae. We did not capture many mosquitoes (Culicidae) in our Malaise traps, but these were very active nocturnally at our experimental site and are known to be common flower visitors, mostly as nectarophages but also in pollination (Larson et al. 2001), often visiting flowers at night (Parmenter 1958; Sandholm and Price 1962).

Several lepidopterans, mainly Gelechiidae, Tortricidae and Geometridae, were collected in Malaise traps,

although at lower numbers than Diptera. There is evidence that species in these families can pollinate (Patt et al. 1989; Guédot et al. 2008; Luo et al. 2011), but little work is published on these groups as pollinators. In a pine forest in Scotland, Devoto et al. (2011) found that many moths, including members of the Geometridae and Noctuidae, carried pollen of multiple plant taxa, including *Erica cinerea* L. (Ericaceae), which has a floral arrangement and anatomy very similar to *V. angustifolium*. We did not collect bees in our night-activated Malaise traps. There are night-active bees in the region – for example, among the Andrenidae, *Calliopsis*, *Perdita*, and *Protandrena* are found in Nova Scotia and are known to be vespertine (active at dusk or evening) – but these are summer flying taxa and therefore probably are not active during blueberry bloom (Sheffield et al. 2003).

Although the insects we collected at night have been reported as pollinators of other plants, we have no direct evidence that any of the insects captured in our Malaise traps pollinated blueberry in our experiment. It is possible that non-flying insects within the aluminum rings pollinated blueberry, or that there were other groups of flying insects not captured. Malaise traps are considered good for trapping a wide variety of insects, but are less likely to capture some taxa, such as Coleoptera and Hemiptera (Juillet 1963). It would be worthwhile collecting nocturnal insects from blueberry fields using sweep netting, sticky traps, or light traps, and to compare with Malaise traps the diversity and abundance of groups collected. It would also be important to examine pollen on individual insects collected at night to confirm which nocturnal species are carriers of blueberry pollen. Buzz pollination or other means of moving the anthers is required to release pollen in this plant, and this is probably always done by bees. It may be that nocturnal insects moved pollen that was dislodged by bees during the day from blossoms outside our experimental cages, to blossoms in our open-at-night treatment. That is, although our data indicate pollen is moved to blueberry flowers at night, it is unclear whether or not pollen is removed from anthers by insects at night. As with efforts to better manage agricultural habitats to promote wild bee pollinators and natural enemies of insect pests (Landis et al. 2000; Kremen et al. 2004), it might be possible and beneficial to develop management strategies to enhance contribution of nocturnal pollinators in lowbush blueberry.

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