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NOTE

Potential nocturnal insect pollinators of lowbush blueberry

Paul Manning and G. Christopher Cutler

Lowbush blueberry, *Vaccinium angustifolium* Aiton (Ericaceae), is a wild plant native to north-eastern North America that is intensively managed on more than 27 thousand hectares of land in Canada, at an annual worth exceeding 56 million dollars (Statistics Canada 2013). Because it has relatively large pollen size, downward opening urn-shaped blossoms, and poricidal anthers, lowbush blueberry flowers must be cross-pollinated by insects to achieve fruit set (Usui et al. 2005; Cutler et al. 2012). Like many other crops dependent on insect-mediated pollination, bees (Apoidea) are considered the most important pollinators of lowbush blueberry.

Although most blueberry pollination can be attributed to bees, nocturnal insect activity may contribute significantly to lowbush blueberry yield (Cutler et al. 2012). Several insect families have been captured in a lowbush blueberry field at night using Malaise traps, but it was not determined if these insects were carrying pollen (Cutler et al. 2012). In this study, we collected insects from a lowbush blueberry field at night using sweep-netting and light-trapping, and examined individuals for the presence of lowbush blueberry pollen.

Insects were captured on six cool (4-10°C), dry nights from a fruit-bearing lowbush blueberry field during peak bloom (24 May – 3 June 2013) at a site in Debert, Nova Scotia (45°26'35''N, 63°26'57''W). For light trap captures, a white cotton sheet stretched and fastened over a 2.4 x 1.0 m sheet of plywood was rested against a fence facing towards the blueberry field. A 120-W mercury-vapour lamp attached to a post was placed 1 m above the sheet. Insects that were attracted to the light and landed on the sheet were captured and quickly placed in a kill-jar containing ethyl acetate. Multiple kill-jars were used, but any given jar was used to capture several insects. A folded Kimwipe® (Sigma-Aldrich, Oakville, Ontario) was placed in each jar after every insect capture to minimize contact between specimens. Insects were captured with light trapping during a two hour period that began approximately at, or three hours after the onset of scotophase. Sweep-netting was done with a 35 cm diameter canvas net in randomly selected locations of the field that were at least 15 m away from artificial light and 5 m from the field edge. Sweeps of 180° were made over an area approximately 5 m in length and the captured insects were dumped into a 6-L plastic box containing ethyl acetate. Sweep-netting was performed three times during each sampling period, stratified equally within the duration of the two hour period of light trapping.

Collections were returned to the laboratory and stored at -4°C until processing. Insects were removed from containers individually using clean forceps. Pollen was removed from the eyes, antennae, and mouthparts of each specimen by swabbing these parts with a 1-mm³ cube of fuchsin-stained glycerin jelly, on a sterilized steel, blunt surgical probe using a technique modified by Beattie (1972). Cubes of jelly were transferred to individual microscope slides, covered with a cover-slip, heated over a Bunsen burner, and labeled with an identification number. Each insect was thereafter pinned, labeled, and identified to taxonomic family, unless damaged and unidentifiable. Slides were randomized to eliminate potential bias, and were analyzed for blueberry pollen tetrads using a light microscope under 400X magnification. Pollen tetrads were compared to a prepared standard. A specimen corresponding to a slide found to contain one or more pollen tetrads matching the standard was considered to be a carrier of lowbush blueberry pollen.

Within the most commonly collected taxa, logistic regression was used to determine if the probability of an insect carrying pollen tetrads differed among taxonomic families. Using a Walds test, we compared taxonomic families by examining 95% confidence intervals of the respective Odds ratio (SAS 2010).

A total of 588 specimens representing 47 families were captured. The light trap captured 400 specimens from 31

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Paul Manning and G. Christopher Cutler: Department of Environmental Sciences, Faculty of Agriculture, Dalhousie University, PO Box 550, Truro, Nova Scotia, B2N 5E3, Canada

Corresponding author (email chris.cutler@dal.ca).

families, and sweep-netting captured 188 specimens from 25 families. Several collected Diptera (37), Lepidoptera (13), and Trichoptera (2) were unidentifiable due to damage sustained to key morphological features during capture or pollen removal. Sixty-three specimens (11.9%) identified to family and seven unidentifiable Diptera (4), Trichoptera (2), Lepidoptera (1), carried blueberry pollen tetrads. Forty-nine insects (12.3%) of the total captured using light trapping (Table 1) and 21 insects (12.2%) from the total sweep capture were found to be carrying pollen tetrads (Table 2).

Table 1. Pollen carrying insects captured in a lowbush blueberry field at night by light-trapping, Debert NS.

Order	Family	Total individuals	Individuals carrying pollen	% pollen carriers
Coleoptera	Scarabaeidae	25	3	12.0
Diptera	Bibionidae	2	1	50.0
	Chironomidae	21	2	9.5
	Culicidae	32	1	3.1
	Tachnididae	57	8	14.0
	Tipulidae	26	3	11.5
	Undetermined	20	4	20.0
Hymenoptera	Apidae	1	1	100.0
	Icheunomidae	32	2	6.3
Lepidoptera	Geometridae	41	8	19.5
	Noctuidae	31	5	16.1
	Pyralidae	8	2	25.0
	Sphingidae	3	2	66.7
	Undetermined	11	1	9.1
Thysanoptera	Thripidae	1	1	100.0
Trichoptera	Lepidostomatidae	44	5	11.4
Total		**355**	**49**	**13.8**

Table 2. Pollen carrying insects captured in a lowbush blueberry field at night by sweep-netting, Debert NS.

Order	Family	Total individuals	Individuals carrying pollen	% pollen carriers
Coleoptera	Curculionidae	38	12	31.6
Collembola	Entomobryidae	4	1	25.0
Diptera	Phoridae	28	1	3.6
	Undetermined	12	2	16.7
Hemiptera	Lygaeidae	5	1	20.0
Hymenoptera	Siricidae	1	1	100.0
Lepidoptera	Noctuidae	8	2	25.0
Orthoptera	Tetigoniidae	35	2	5.7
Total		**131**	**22**	**16.8**

The likelihood of insects to be carrying pollen differed among families ($X^2 = 20.2$, $df = 11$, $P = 0.0432$). By examining the confidence intervals determined through the Walds test, we found that Curculionidae was a more frequent pollen carrying family than Culicidae, Icheunomidae, Phoridae and Tetigoniidae. Geometridae was a more frequent pollen carrying family than Culicidae and Phoridae. Noctuidae was a more frequent pollen carrying family than Culicidae (Table 3).

Table 3. Logistic regression and associated Wald's confidence intervals to determine likelihood of most commonly collected insect families to be carriers of lowbush blueberry pollen tetrads.

Family	n	% with pollen	Odds ratio (95% CI)	Statistically different from:
Curculionidae (Cur)	38	32	0.28 (0.21 – 1.41)	Ich, Tet, Pho, Cul
Geometridae (Geo)	37	22	0.47 (0.36 – 2.46)	Pho, Cul
Noctuidae (Noc)	32	19	0.57 (0.44 – 3.09)	Cul
Tachinidae (Tac)	59	14	0.83 (0.63 – 4.26)	-
Scarabaeidae (Sca)	25	12	0.96 (0.78 - 6.21)	-
Tipulidae (Tip)	26	12	-----[a]	-
Lepidostomatidae (Lep)	44	11	1.02 (0.80 – 5.68)	-
Chironomidae (Chi)	26	8	1.57 (1.33 – 11.81)	-
Ichneumonidae (Ich)	32	6	1.96 (1.66 – 14.65)	Cur
Tetigoniidae (Tet)	35	6	2.15 (1.82 – 16.06)	Cur
Phoridae (Pho)	27	4	3.39 (3.06 – 38.30)	Cur, Geo
Culicidae (Cul)	32	3	4.04 (3.65 – 45.46)	Cur, Geo, Noc

[a]The Tipulidae value was selected as a dummy variable with no associated odds ratio or confidence interval.

Previous work indicated that pollination of lowbush blueberry blossoms can occur at night (Cutler et al. 2012). Our results indicate that a diverse assemblage of insects carry pollen of lowbush blueberry, reflecting other studies that found a diversity of insects may be involved in nocturnal pollination (Stephenson and Thomas 1977; Morse and Fritz 1983; Devoto et al. 2011). It is possible that insects we collected did not remove pollen directly from anthers while feeding, but instead encountered the pollen elsewhere in the environment (e.g., plant foliage) after it was dislodged by other insects. Pollen on insects collected by sweep-netting also may have moved on to the insects while in the net. Nonetheless, a significant proportion of pollen carrying insects were removed from light traps, in which case indirect transfer of pollen would have been very unlikely. Moreover, by removing pollen only from eyes, mouth-parts, and antennae, which are most likely to come into contact with pollen during feeding, we had hoped to minimize indirect pollen encounters.

Other plants produce tetrad-shaped pollen, which potentially could be incorrectly identified as lowbush blueberry pollen. Approximately 75 m adjacent to our study area we found an oblong (25 x 1 m) patch of *Rhododendron canadense* (L. Torr.) (Ericaceae) blooming simultaneously with lowbush blueberry. A small patch of cranberry (*Vaccinium macrocarpon* Ait.) (Ericaceae) less than 1 m² was also found 50 m away from our study site. These species produce tetrad shaped pollen of a similar size to lowbush blueberry (Crompton and Wajtas 1993). Though the influence on the results would have been small, misidentification of pollen from *Rhododendron canadense* and *Vaccinium macrocarpon* as *Vaccinium angustifolium* might have resulted in an overestimate of insects acting as pollinators of lowbush blueberry.

Insects captured in this study may not normally be active during nocturnal hours. Light trapping may have attracted primarily diurnally-active insects (Umar et al. 2012). Sweep-netting may have collected diurnal insects resting in the foliage. This may have caused us to overestimate the abundance, and diversity of insects potentially responsible for nocturnal pollination.

Our results suggest that Curculionidae, Geometridae, and Noctuidae may be of interest for further study with regards to lowbush blueberry pollination. Curculionidae are significant pollinators of a variety of tropical plants including several species of *Anthurium* (Araceae) (Franz 2007), the cycad *Zamia furfuracea* Aiton (Norstog and Fawcett 1989), the oil palm *Elaeis guineensis* Jacq. (Dhileepan 1994), and snake fruit *Salacca edulis* Reinw (Mogea 1978). Likewise, Geometridae and Noctuidae are nocturnal pollinators for a variety of plants including cigar tree (*Catalpa speciosa* (Warder) Warder ex Engelm.) (Stephenson and Thomas 1977), white campion (*Silene alba* Poiret) (Young 2002), and common milkweed (*Asclepias syrica* L.) (Morse and Fritz 1983). Geometridae and Noctuidae have previously been captured at night in a lowbush blueberry field during bloom (Cutler et al 2012). Nocturnal Sphingidae moths pollinate various plants in different ecosystems (Haber and Franke 1982; Morse and Fritz 1983), but were relatively rare in our collections. The potential importance of nocturnal pollinators should not be overlooked within other agro-ecosystems dependent on insect pollination.

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