



Entomophily of the cloudberry (*Rubus chamaemorus*)

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Abstract

Cloudberry (*Rubus chamaemorus* L.), a dioecious perennial plant of boreal circumpolar distribution, is greatly prized for its berries. We crossed two treatments, pollinator exclusion and supplementary hand-pollination, to determine i) the relative importance of insects as pollinators, ii) if pollinator activity was a limiting factor for the sexual reproduction of the plant, and iii) the relative contribution of diurnal vs. nocturnal visitors to pollination. The activity of natural pollinators resulted in 97.5 and 88.5% fruit set, along with 76.7 and 62.5% seed set in 1998 and 1999, respectively. When insects were excluded, fruit-set dropped significantly to 18.4 (1998) and 12.8% (1999) and seed-set to 5.4 (1998) and 5.0% (1999) showing the importance of mid- and large-sized insects as pollinators. Natural levels of insect activity were sufficient to ensure complete pollination in both years as supplementary hand-pollination did not significantly increase either parameter in plots where pollinators had free access. Nocturnal insects may serve as pollinators (fruit-set = 41%), although they were less effective than diurnal pollinators (fruit-set = 93%).

Introduction

Cloudberry (*Rubus chamaemorus* L., Rosaceae) is a dioecious perennial plant of boreal circumpolar distribution. The plant spreads mainly by means of an extensive rhizome system (Rapp et al., 1993), with 94% of total biomass going to underground organs responsible for vegetative propagation and only 0.05% allocated to sexual reproduction (Dumas & Maillette, 1987). While the plant allocates few resources to sexual reproduction, the fruits are a prized commodity and so the factors limiting fruit production have received some attention.

Frost appears to be an important abiotic factor limiting fruit production, as late spring frosts can kill a significant proportion of the flowers and fruits (Dumas & Maillette, 1987; Rapp et al., 1993), which occurred in Baie Saint-Ludger, Qc, in 1998 (pers. observ.). The availability of nutrients may also limit yield as fertilization of bogs with superphosphate or complete

fertilizers increased yields several fold (Rapp et al., 1993). Herbivory and parasites may also limit the reproductive success (see Ågren, 1987, 1989), as well as insufficient pollination, especially if the plant is principally entomophilous since female flowers are reported to offer little reward (no pollen and small amounts of nectar) to insect visitors (Ågren et al., 1986).

There is an abundant and diversified diurnal insect fauna known to frequent cloudberry flowers. In Fennoscandia, Hippa et al. (1976, 1981a–c) differentiated two groups of insects found on cloudberry flowers: flower stayers and flower visitors. Flower stayers, such as thrips and staphylinids, were very poor cloudberry pollen-carriers while the best pollen-carriers were considered to be the diurnal medium- and large-sized flower visitors such as hymenopterans (Apidae and Formicidae) and dipterans (Syrphidae, Muscidae, Empididae, Fanniidae, Coelopidae, and Heleomyzidae) (Hippa et al., 1981c). However, these observations only provide indirect evidence that in-

sects serve as pollinators as anemogamy may also play a role, especially in windswept coastal areas (Rapp et al., 1993).

We therefore conducted a study to examine cloudberry pollination and the three objectives of this study were to experimentally determine:

(1) the relative importance of insect visitors vs. wind and small insects in the pollination of cloudberry by comparing the reproductive success in quadrats with and without cages that excluded medium and large-sized insects;

(2) if natural pollination, presumably by insects, was sufficient to ensure that all flowers were pollinated by comparing the reproductive success in quadrats where all flowers had supplemental hand pollination with those with natural pollinators only; and

(3) if nocturnal visitors contributed to the pollination, and quantify their relative effectiveness to diurnal ones by comparing the reproductive success in quadrats that were accessible to pollinators only during the photophase or the scotophase.

Materials and methods

We conducted our experiments in a *Sphagnum fuscum* peat bog at Hâvre-Saint-Pierre (50°16' N, 63°34' W), on the north shore of the St-Lawrence River, Québec, Canada. The site is a wet, coastal windswept area, bordered to the north by boreal forest. The cloudberry is one of the first plants to flower in the bog, with the peak of female flowering occurring about a week after that of males (pers. observ.).

To address the first two objectives, we crossed the exclusion of pollinators and supplemental hand-pollination treatments. Each of the four combinations of treatments was assigned randomly to ten 1.2 m × 1.2 m (2.44 m²) quadrats, for a total of 40 quadrats. We marked all female flowers within quadrats with a numbered vinyl tag attached around the base of the shoot. Only quadrats with more than three female flowers were kept for the analysis. We conducted this experiment twice, in 1998 and in 1999.

To address the third objective, we added ten insect enclosure quadrats in 1999. Flowers in five enclosures were available to pollinators only during the day (official photophase from about 04:00 to 20:30), and the other five only at night (official scotophase from about 20:30 to 04:00). This was accomplished by removing the exclusion cages for the designated pollination period each day. We compared the reproductive success

of these two groups with that of the permanently open and permanently closed quadrats.

To hand-pollinate flowers, we collected pollen by brushing dehiscent anthers of male flowers with a small paintbrush (damp in 1998, dry in 1999), and checked for the presence of pollen with a 10× magnifying glass before brushing the stigmas of the female flower several times. For each female flower, we repeated the operation with the pollen of at least two males. Different individuals hand-pollinated flowers in the two years of the study.

Insect enclosure cages (1.2 m long × 1.2 m wide × 0.4 m high) were made of a wooden frame and vinyl insect netting (size of mesh = 1.4 mm). The surface area of openings in the insect netting (1.96 mm²) was 2700 times larger than the planar surface of a cloudberry pollen grain (7.1 × 10⁻⁴ mm²) based on an approximate diameter of 30 μm (Eide, 1981). Hence, openings in the netting were large enough to allow pollen to pass. Furthermore, there were male plants within the enclosure cages that could serve as a source of pollen.

To reduce any possible beneficial or deleterious effect of enclosing plants on fruit development we minimised enclosure time. We set up quadrats and enclosures on 3–5 June 1998 and 1 June 1999, with marking and hand-pollination occurring a week later (10–12 June 1998, 6–16 June 1999). We removed enclosures two weeks after set-up, after the last female flowers had shut. We harvested the fruits prior to complete maturation (5–6 July 1998, 7–8 July 1999) as locals would harvest mature fruits, despite notices that these were research plots.

Variables. We used three variables as indices of the reproductive success of cloudberry flowers per quadrat:

(a) Fruit-set: the proportion of female flowers which developed into a fruit.

(b) Mean seed-set: the average of the individual seed-sets. The individual seed-set being the ratio of the number of drupes (a fruit being composed of drupes and each drupe contains one seed) on the total number of ovules for each fruit. The total number of ovules was determined by dissecting the unripe fruits under the binocular (10×).

(c) The mean fresh weight of unripe fruits. Within a given year, we collected all of the fruits at the same date and weighed them on a pan balance (precision = 1 × 10⁻² g). As noted above it was not possible to wait and harvest ripe fruit, so our estimate of fresh weight should only be considered as complementary

information to the other two measures of reproductive success.

Statistics. We tested the effect of hand-pollination, insect exclusions, of the year, and of all possible interactions among these factors, on fruit set with multiway analyses of variance (ANOVA). We used the general linear model (GLM) procedure in SAS (SAS Institute, 1996) to conduct the ANOVAs because of the unequal cell sizes. The unbalanced design resulted from the fact that we did not include quadrats with less than three flowers in the analyses. We used the same analyses for seed set and fresh weight but the data for the two years were analysed separately due to difference in hand pollination efficacy and the fact that fruits were collected before they were fully mature. We tested the effect of the regime of day or night exclusion on fruit-set in 1999 with a one-way ANOVA.

We transformed proportions of the fruit-set per quadrat with the arcsine transformation of Freeman & Tukey (1950, cited in Zar, 1999):

$$p' = \frac{1}{2} \left[\arcsin \sqrt{\frac{X}{n+1}} + \arcsin \sqrt{\frac{X+1}{n+1}} \right], \quad (1)$$

where p' is the transformed proportion and $X/n = p$ is the actual proportion. We transformed proportions of the mean seed-set per quadrat with the common arcsine transformation (Zar, 1999): $p' = \arcsin \sqrt{p}$. Standard errors (s.e.) were calculated using the transformed data and then reconverted to proportions with $p = (\sin(p'))^2$. We checked normality in the distribution of residuals with the Kolmogorov-Smirnov's test and homoscedasticity with Levene's test. We used three multiple comparisons tests: the Tukey's studentized range test, the Bonferonni t-tests, and the Student-Newman-Keuls (SNK) test. We set the level of significance to $P < 0.05$ for all statistical tests.

Results

Medium to large sized insects are necessary for effective cloudberry pollination (significant effect of exclusion in Table 1), as in both 1998 and 1999 fruit-set (Table 2), seed-set (Table 3), and the fresh weight of unripe fruits (Table 4) were greatly and significantly reduced in quadrats with exclusion cages compared with those in open controls. Wind and small insects (e.g., blackflies, thrips) only pollinated a very small fraction of flowers, as seen in exclusion quadrats

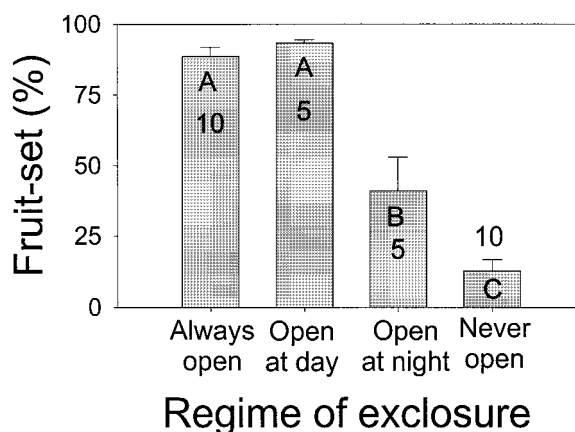


Figure 1. Effect of four different regimes of exclusion of insect visitors on the fruit-set of cloudberry in Håvre-Saint-Pierre in 1999. Mean fruit-set per combination of treatments \pm standard error (error bars) and number of quadrats. Means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical.

without supplemental pollination (Table 2). Furthermore, the flowers pollinated by these vectors gave poor quality fruits as shown by the comparison between open habitats and exclusions of the seed-set and fresh weight of unripe fruits of flowers which succeeded to develop into a fruit (Table 5).

Insect activity was sufficient to fully pollinate female flowers in both years of the study as supplementary hand-pollination in open habitats did not significantly increase any of the parameters of reproductive success (Tables 2–4). This was not due to an ineffective technique as hand-pollination significantly increased fruit production in exclusions (Tables 2–4).

There is a highly significant difference in the efficacy of nocturnal and diurnal pollinators, as measured by fruit set (one-way ANOVA: $F = 45.90$, $df_{\text{model}} = 3$, $df_{\text{error}} = 26$, $P = 0.0001$) (Figure 1). While nocturnal pollinators significantly increased fruit set over the controls where insects were always excluded, they were not as effective as diurnal pollinators (Figure 1). The activity of diurnal pollinators, such as bumblebees and syrphids, was sufficient to fully pollinate cloudberry flowers, for fruit-set was as high in quadrats open only during the day as in those always open (Figure 1).

Discussion

Fruit set was high (>94%) in our study when compared with previous reports in northern Quebec (57%)

Table 1. Multiway ANOVAs of the effect of the year, insect exclosures, hand-pollination, and all possible interactions among these factors, on three different measures of reproductive success: (a) the fruit-set (combined analysis for 1998 and 1999), and (b) the seed-set and the fresh weight of unripe fruits (distinct analyses for 1998 and 1999, see Materials and methods for details) of cloudberry in Håvre-Saint-Pierre. Power of the test provided when not significant

		df	F	P	Power		
Fruit-set	Model	7	36.44	0.0001***			
	Year	1	0.86	0.3575 n.s.	0.534		
	Exclosure	1	125.92	0.0001***			
	Hand pollination	1	47.63	0.0001***			
	Year × Exclosure	1	3.30	0.0746 n.s.	0.503		
	Year × Hand pollination	1	8.89	0.0042**			
	Exclosure × Hand pollination	1	42.46	0.0001***			
	Year × Exclosure × Hand pollination	1	1.92	0.1707 n.s.	0.505		
	Error	59					
Total	66						

		1998			1999		
		df	F	P	df	F	P
Seed-set	Model	3	34.88	0.0001***	3	40.42	0.0001***
	Exclosure	1	82.64	0.0001***	1	40.88	0.0001***
	Hand pollination	1	4.47	0.0447*	1	46.42	0.0001***
	Exclosure × hand pollination	1	8.76	0.0067**	1	30.07	0.0001***
	Error	25					
	Total	28					
Fresh weight	Model	3	21.19	0.0001***	3	20.01	0.0001***
	Exclosure	1	48.51	0.0001***	1	21.85	0.0001***
	Hand pollination	1	4.64	0.0411*	1	24.57	0.0001***
	Exclosure × hand pollination	1	4.91	0.0361*	1	11.82	0.0016**
	Error	25					
	Total	28					

*P < 0.05; **P < 0.01; ***P < 0.001; n.s. non-significant.

Table 2. Effect of insect exclosures and supplementary hand-pollination on the fruit-set (%) of cloudberry in Håvre-Saint-Pierre in 1998 and 1999. Mean per combination of treatments (\bar{X}) ± standard error (s.e.) and number of quadrats (*n*). Means with the same letters are not significantly different ($P \geq 0.05$). Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

	Exclosure			Open habitat		
	<i>n</i>	$\bar{X} \pm$ s.e.		<i>n</i>	$\bar{X} \pm$ s.e.	
Hand-pollination						
1998	7	61.1% ± 12.0%	B	6	95.4% ± 2.5%	A
1999	9	89.4% ± 4.7%	A	9	94.3% ± 2.2%	A
Control						
1998	9	18.4% ± 7.6%	C	7	97.5% ± 1.6%	A
1999	10	12.8% ± 5.0%	C	10	88.5% ± 3.2%	A

Table 3. Effect of insect exclosures and supplementary hand-pollination on the seed-set (%) of cloudberry in Håvre-Saint-Pierre. Separate analyses for 1998 and 1999 (see methods for explanations). Mean per combination of treatments (\bar{X}) \pm standard error (s.e.) and number of quadrats (n). Within years, means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

		Exclosure			Open habitat		
		n	$\bar{X} \pm \text{s.e.}$		n	$\bar{X} \pm \text{s.e.}$	
1998	Hand-pollination	7	31.3% \pm 7.6%	B	6	70.1% \pm 5.6%	A
	Control	9	5.4% \pm 2.9%	C	7	76.7% \pm 3.3%	A
1999	Hand-pollination	9	67.4% \pm 5.2%	A	9	73.2% \pm 3.2%	A
	Control	10	5.0% \pm 2.7%	B	10	62.5% \pm 7.1%	A

Table 4. Effect of insect exclosures and supplementary hand-pollination on the fresh weight (g) of unripe cloudberry fruits in Håvre-Saint-Pierre. Separate analyses for 1998 and 1999 (see methods for explanations). Mean per combination of treatments (\bar{X}) \pm standard error (s.e.) and number of quadrats (n). Within years, means with the same letters are not significantly different. Results of the SNK, Tukey, and Bonferonni multiple comparisons tests are identical

		Exclosure			Open habitat		
		n	$\bar{X} \pm \text{s.e.}$		n	$\bar{X} \pm \text{s.e.}$	
1998	Hand-pollination	7	0.15 \pm 0.04 g	B	6	0.28 \pm 0.02 g	A
	Control	9	0.03 \pm 0.02 g	C	7	0.26 \pm 0.03 g	A
1999	Hand-pollination	9	0.44 \pm 0.06 g	A	9	0.50 \pm 0.04 g	A
	Control	10	0.04 \pm 0.02 g	B	10	0.43 \pm 0.06 g	A

Table 5. T-tests of the effect of insect exclosures on the seed-set (%) and fresh weight (g) of unripe fruits of the cloudberry flowers that gave rise to a fruit at Håvre-Saint-Pierre in 1998 and 1999. Mean per treatment (\bar{X}) \pm standard error (s.e.) and number of quadrats (n)

		Exclosure		Open habitat		t	P
		n	$\bar{X} \pm \text{s.e.}$	n	$\bar{X} \pm \text{s.e.}$		
1998	Seed-set	6	32.4 \pm 11.4%	7	78.6 \pm 2.5%	3.86	0.0096**
	Fresh weight	6	0.20 \pm 0.06 g	7	0.29 \pm 0.03 g	1.46	0.17 <i>n.s.</i>
1999	Seed-set	10	31.6 \pm 5.6%	8	84.1 \pm 7.8%	5.21	<0.0001***
	Fresh weight	10	0.26 \pm 0.06 g	8	0.55 \pm 0.05 g	3.99	0.0011**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. non-significant

(Dumas & Maillette, 1987) and in spruce mires of northern Sweden and Finland (72.5–75.6%) (Ågren, 1989), although values >90% have been reported (Ågren, 1987). The results of the present study clearly demonstrate the importance of insects relative to anemogamy for the pollination of the cloudberry. Thus some of the site and year differences reported in fruit-set may well be due to factors influencing the density and/or activity of potential pollinators. This is particularly true when one considers that cloudberry grows in

circumboreal regions where the climatic conditions in spring are highly variable, and that individual flowers only last for 2–3 days (Ågren, 1987). The high fruit-set obtained in the present study may, at least in part, be attributed to the mild spring conditions encountered in both years. Higher temperatures would not only have decreased losses due to spring frost but would also have favoured the activity of potential insect pollinators.

It is clear from the comparison of fruit-set in quadrats open only at night and those always closed (Figure 1) that nocturnal pollinators do exist in the habitat. The fact that we found unidentified lepidopteran larvae (Tortricidae) feeding on leaves and fruits in both years make different Lepidoptera potential candidates. However, their relative contribution to cloudberry reproductive success was minor compared to diurnal pollinators (Figure 1). In other words, in 1999 diurnal visitors were sufficient for pollination, and nocturnal visitors could be seen as complementary pollinators. Nonetheless, if the short duration of enclosure opening at night (7.5 h) compared to the duration of opening at day (16.5 h) were taken into account, nocturnal insect visitors could prove to be as efficient pollinators as diurnal visitors. In years where overall insect numbers are low in the habitat of the cloudberry, the activity of nocturnal pollinators could significantly contribute to the sexual reproduction of the plant. Furthermore, nocturnal pollinators are believed to have a lower frequency of flower visitations than diurnal ones during foraging and to transfer pollen over greater distances (Herrera, 1987). If true, then this could be important as it might increase the genetic heterozygosity of a plant with a high degree of clonal propagation.

The next step will be to determine experimentally the effectiveness and efficiency of the most important groups of diurnal and nocturnal insects visiting cloudberry flowers.

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