

Pollination and Breeding System of Lowbush Blueberries, *Vaccinium angustifolium* Ait. and *V. myrtilloides* Michx. (Ericaceae), in the Boreal Forest

MASAYUKI USUI¹, PETER G. KEVAN¹, and MARTYN OBBARD²

¹Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1 Canada (Corresponding author: Kevan)

²Ontario Ministry of Natural Resources, Wildlife Research and Development Office, 300 Water Street, Peterborough, Ontario K9J 8M5 Canada

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Breeding systems and pollination requirements of two wild lowbush blueberries, *Vaccinium angustifolium* and *V. myrtilloides*, in the Canadian boreal forest in the Chapleau Crown Game Preserve, Ontario, were tested. Fruit production, size and seediness were significantly higher in samples exposed to natural pollination than in those cross- or self-pollinated by hand. There were no significant differences among artificial treatments (variously hand-pollinated and bagged) except when cross-pollination (xenogamy) was done by insect pins. In *V. angustifolium*, the density of flowering varied with forest age (canopy closure). It was most in open areas and least in the sites with the most mature forest. Although fruit-set and seediness varied among forest habitats of different ages, there were no significant differences between sites in forests of different ages. Thus, pollination seems to be similarly effective no matter the age of the forest. In both species, fruit-set in 1992, which had severe June frosts, was markedly poorer than that in 1993 when the flowers suffered little frost damage. The combined number of complete and incomplete seeds from the fruit among the breeding and pollination systems tested were similar; however, the ratio of complete seeds to total seeds was greater from cross-pollinated than from self-pollinated flowers. Our observations indicate that there is little natural fruit-set without insect-mediated cross-pollination and that cross-pollination provides much better fruit and seed-set than does self-pollination.

Key Words: boreal forest, lowbush blueberries, *Vaccinium angustifolium*, *V. myrtilloides*, breeding system, fruit-set, seed-set, bumblebees, pollination, pollinators, Charleau, Ontario.

Lowbush blueberries (*Vaccinium angustifolium* and *V. myrtilloides*) are wide spread throughout the North American boreal forest. Their fruit is important for wildlife (Usui et al. 1994). They grow best under acidic and well-drained soils in open habitats (Eck and Childers 1966), although they persist and may thrive in clearings in darker, old forests.

Although it is generally accepted that lowbush blueberries usually depend on insects, mostly bees (Hymenoptera: Apoidea) for pollination (Eck and Childers 1966; McGregor 1976; Reader 1977; Hall et al. 1979; Morrisette et al. 1985; Mohr and Kevan 1987; Aras et al. 1996; Stubbs and Drummond 1997, 1999, 2001; Javorek et al. 2002), most research on pollination is from commercial lowbush blueberry barrens and there is little information from unmanaged habitats, completely natural stands, and especially the boreal forest (Kevan et al. 1993; Mohr and Kevan 1987).

Even in horticultural studies, the effects of self- and cross-pollination have not been thoroughly investigated. Aalders and Hall (1961), and Wood (1968) noted a high incidence of self-sterility in *V. angustifolium*. Hall and Aalders (1961) stated that about 5% of the plants they observed in Nova Scotia and New Brunswick were male-sterile and 45% produced less than a complete complement of normal pollen. Hall et al.

(1966) also noted female-sterility associated with breakdown of the integument and the nucellus of the ovules. There is little published information on *V. myrtilloides*, presumably because it is of little commercial importance. It is regarded as an obligate entomophilous outcrosser (Vander Kloet and Hall 1981) and well pollinated by various bees (Morrisette et al. 1985; Aras et al. 1996). Reader (1977) examined the breeding strategies of *V. myrtilloides* in natural bogs of southern Ontario and reported that this species was self-compatible with 40% of flowers setting seed autogamously, 60–80% setting seed when pollination occurred between flowers of the same plant (geitonogamy), and when pollination occurred between two different plants (xenogamy), the percentage of flowers setting seed was 70%. Thus, it is possible that the breeding systems of the species are different in different parts of their ranges, as is known for other species of plants (Richards 1997). Nevertheless, in nature, pollination of most *Vaccinium* spp. by bees is essential because of the urceolate (urn-shaped) and pendulous flowers, which require that pollinators hang onto the flowers and enter them from below to obtain nectar or pollen, or both. The most effective pollinators (Javorek et al. 2002), such as bumblebees (*Bombus* spp. (Apidea)) and an array of solitary bees (Apoidea) (Finna-

more and Neary 1978) vibrate the anthers to extract pollen (vibratile or “buzz” pollination) (Buchmann 1983).

The aim of our study was to examine the effects of cross- and self-pollination on fruit-set in these two species of wild lowbush blueberries in the boreal forest and the fruiting success of *V. angustifolium* in forests of different ages.

Materials and Methods

The research was done as a part of blueberry fruit productivity project in the Chapleau Crown Game Preserve (CCGP), northern Ontario (48°N, 83°W) from 1990 to 1993. Of the 31 study sites used (Figure 1), the main one was Chaplin Number 4, studied during the flowering period (20 May to 5 July) of 1993 (Figure 2). Chaplin Number 4 site is a typical blueberry

habitat with acidic, sandy soil covered by organic matter. The area was logged until 1990, burned, and the soil prepared for tree planting in 1990. Jack Pine seedlings were hand-planted in 1991 (Figure 3).

Twenty-five plots (1 m × 1 m) for *V. angustifolium* and 20 plots (1 m × 1 m) for *V. myrtilloides* were selected for experiments of the breeding system early in the blooming season in 1993 throughout Chaplin Number 4 (Figure 4). The plots were separated by at least 15 metres to assure studies on different clones (Vander Kloet 1988).

To elucidate the breeding systems of *Vaccinium angustifolium* and *V. myrtilloides* naturally occurring in the boreal forest, five treatments were set in place in each plot (1 m × 1 m) before blooming began. Sprigs of blueberries with flower buds were selected for each of the following treatments: a) Free: all flowers were

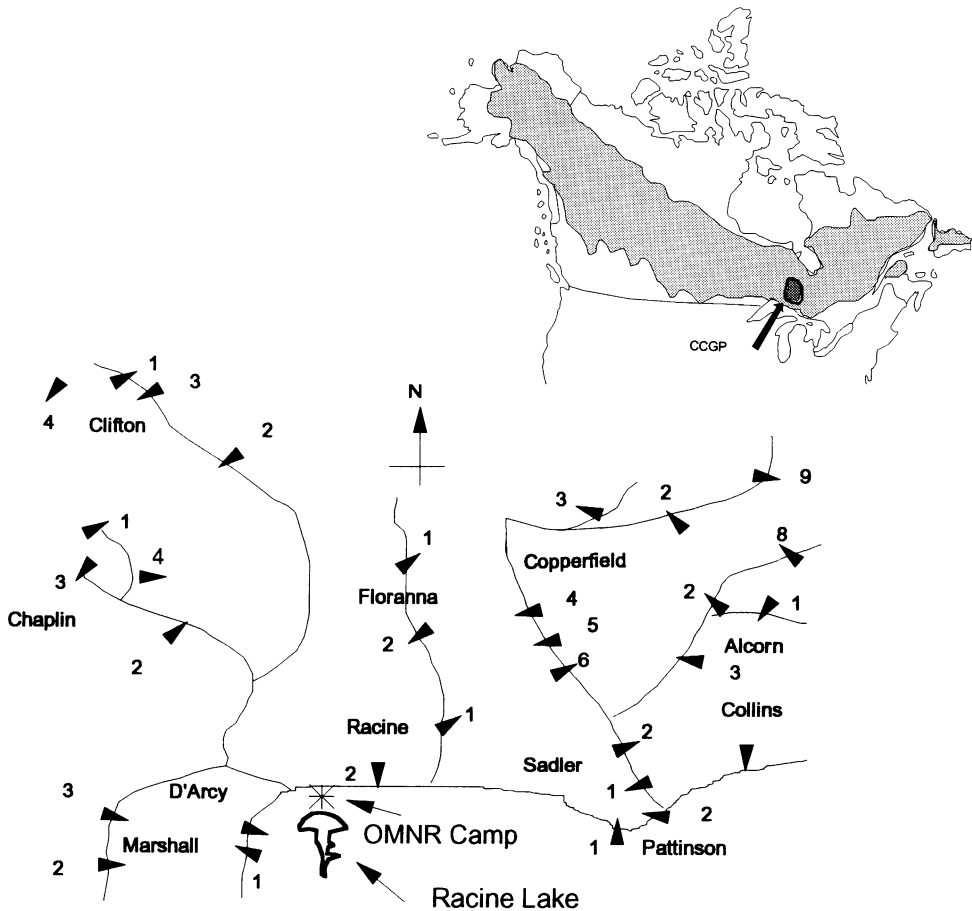


FIGURE 1. Location of Chapleau Crown Game Preserve within the boreal forest of Canada (inset) and of 31 study sites for lowbush blueberry in the area of the Ontario Ministry of Natural Resources Camp at Racine Lake.

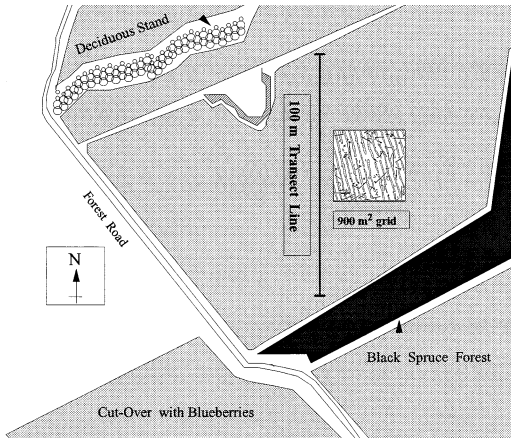


FIGURE 2. Map of the Chaplin Number 4 (Chapleau Crown Game Preserve) study site, showing the location of the 900 m² study grid within the burned area (see also Figure 3).

free to be visited by any pollinators; b) Bagged: flowers were enclosed in a white, fine mesh, bag to exclude pollinators; c) Geitonogamy: bagged (as in b) but all fresh flowers were individually pollinated by hand with pollen from other flowers of the same plant; d) Xenogamy: bagged, but all fresh flowers were pollinated individually by hand with pollen from other plants more than 15 metres away. The sprigs in each plot were marked by pink flagging tape with identification numbers at the base until the harvesting of fruit was complete (1 September).

Three methods were used to transfer pollen grains in *V. angustifolium*: toothpicks, cotton balls (approximately 2.0 cm in diameter), and Number 4 insect pins. For *V. myrtilloides*, only cotton balls were used because there were too few plants to try all methods. Anthers from fresh flower(s) were placed on a clean Petri dish and cut with toothpicks or insect pins. One torn anther was attached to a toothpick or insect pin and then touched to a stigma. Fresh, clean toothpicks or insect pins were used for each pollination. Individual corollas were touched with a small piece of cotton ball and the flower was knocked by fingers ten times onto the piece of cotton ball. The cotton ball was found to be the quickest and easiest method to use, and toothpicks the most difficult.

Fruit-set of *V. angustifolium* under different forest conditions (age of forest) was compared from 1991 to 1993. The soils in all these areas were acidic, sandy, and covered with a layer of organic duff. Fruit-set was measured in total of 100 evenly spaced plots (100 cm² = 10 cm × 10 cm) set along 100 m transects from the edge of, but within the forest (1 m² = 100 × 100 cm²), and into the forest. The study area contained forests of all ages from newly regenerating to mature with

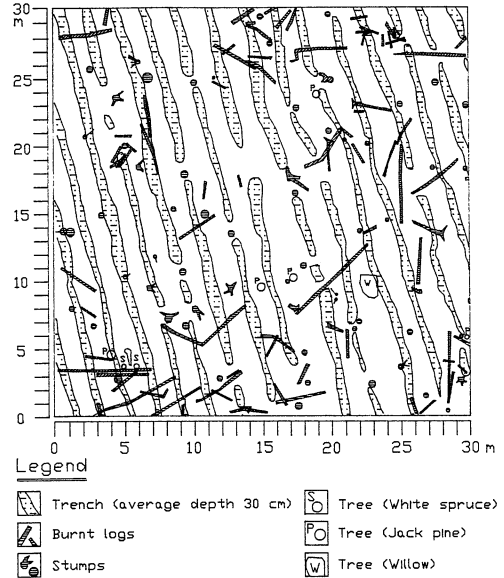


FIGURE 3. Sketch map of the 900 m² study grid at Chaplin Number 4 (Chapleau Crown Game Preserve) showing trenches, burnt logs, stumps and living trees.

closed canopy. All the flowers produced in each plot were counted and the fruits later harvested, counted and examined for seediness from the same plots during 1991-1993. These data were compared among forest ages and years.

During the blooming and fruiting season, some marked plants were damaged by Moose (*Alces alces*) and Black Bears (*Ursus americanus*). To reduce the effects of such damage, fresh ripened fruits from all sites, treatments, and years were hand-picked every five days. Their diameters were measured and the seeds counted.

Before the hand pollination experiments were started, a preliminary study of pollen availability and extraction from blueberry anthers was made at the start of bloom (20 May to 4 June, 1993) to assure that pollen was being produced in sufficient quantity for our experiments. We found that individual anthers from fresh flowers (10 anthers per flower) and large buds contained from 0 to over 300 pollen grains, thus a blueberry flower may contain up to 3000 pollen grains available for removal by pollinators (Table 1). The average production of pollen grains was 166.95 ± 6.03 SE in large buds. For experimental pollinations, only fresh flowers and large buds which had had no contact with insect visitors were used as sources of pollen.

The weather from 25 May to 5 July in 1993 (the main flowering period) was cool and free from severe frosts (only 3 times were freezing temperatures, ranging -1.0 to -1.5°C, recorded at the weather screen at the Chapleau station). From 5 July until early September (the fruiting season) the weather was relatively mild

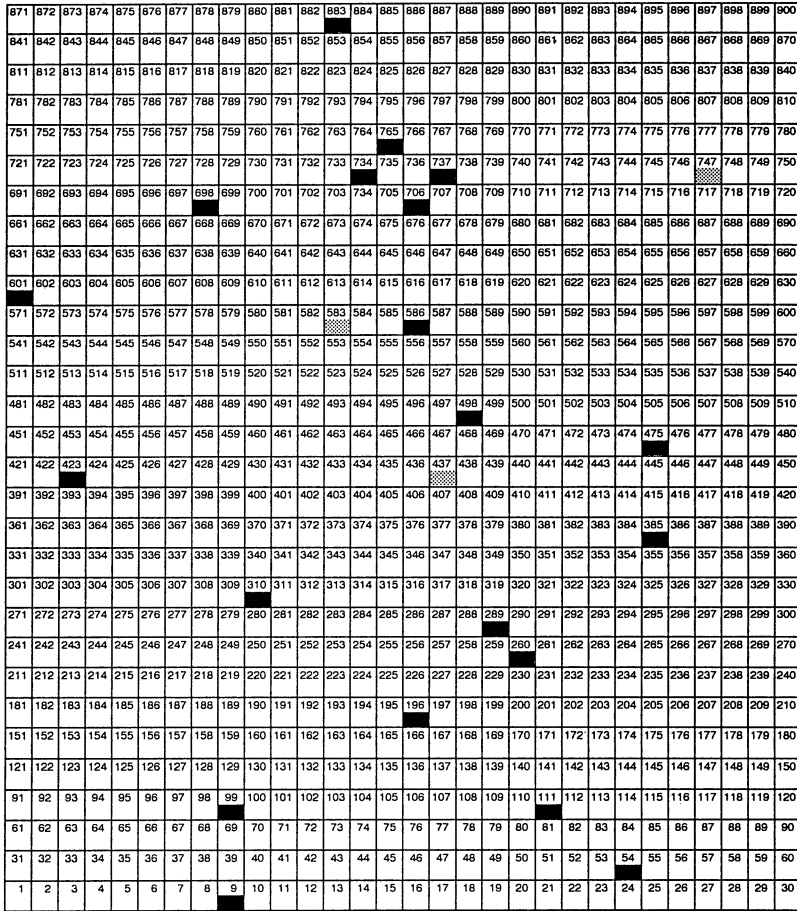


FIGURE 4. Locations of the 20 randomized plots used for pollination studies within the 900 m² study grid (Chapleau Crown Game Preserve).

compared to long-term average (an average monthly mean temperature was 17.2°C in July and 17.1°C in August) and wet (monthly rainfall was 106 mm in July and 54.8 mm in August). However, the weather in the spring of 1992 was cold with six occasions of below freezing temperatures, as low as -4.0 and -2.5°C, between 21 May and 22 June.

Statistical analyses of fruit-sets, seed-set, and fruit size were made. The numbers of fruits were compared in size (mm in diameter) among treatments on the two species of lowbush blueberries, respectively. Data were subjected to ANOVA and means separated by Tukey's Studentized Range Test at *P* = 0.05 level (SAS Institute 1985), or subjected to a modified ANOVA for data sets with unequal variances (Zar 1998 :187) (Toms and Almond, personal communication, see Acknowledgments). The latter approach required application of Bonferroni corrections to the critical probability value

TABLE 1. Number of pollen grains extracted from large buds of *Vaccinium angustifolium* collected from four locations in the Chapleau Crown Game Preserve. Pollen grains were extracted from one anther tube of each flower.

Range of number of approximate pollen grains	Total observations Freq.	Mean ± SE (Number of pollen grains)
Large bud (grains/anther)		
0	7	3.1
10-49	8	3.6
50-99	17	7.6
100-199	64	28.7
200-299	82	36.8
< 300	45	20.2
Total number of flowers and anthers	223	166.95 ± 6.03

TABLE 2. The comparison of fruit-set ratio among eleven pollination treatments of *V. angustifolium*.

Treatment	Number of sprigs examined	Number of sprigs with fruits (% fruit-set)	Total flowers among sprigs	Total fruits among sprigs	Mean \pm SE of fruit-set %	Range of fruit-set per sprig %
Free (natural condition)	56	56 (100.0)	5570	2837	50.5 \pm 2.6a*	12 to 86
Bagged (no pollination)	25	2 (8.00)	1692	2	0.1 \pm 0.1c	0 to 1
Xenogamy using insect pins	8	8 (100.0)	193	40	21.3 \pm 3.5a,b	5 to 33
Xenogamy using cotton balls	16	12 (75.0)	453	48	10.5 \pm 2.8b	0 to 38
Xenogamy using toothpicks	16	8 (50.0)	542	25	3.4 \pm 1.3b	0 to 16
Geitonogamy using insect pins	7	4 (57.1)	302	47	3.1 \pm 1.5b	0 to 11
Geitonogamy using cotton balls	15	7 (46.7)	477	26	2.4 \pm 0.8b	0 to 7
Geitonogamy using toothpicks	16	1 (6.3)	481	1	0.2 \pm 0.2b	0 to 3
Autogamy using insect pins	8	3 (37.5)	112	12	5.3 \pm 2.8b	0 to 18
Autogamy using cotton balls	16	4 (25.0)	262	4	2.1 \pm 1.0b	0 to 11
Autogamy using toothpicks	16	1 (6.3)	279	1	0.4 \pm 0.4b	0 to 7

*Values in a column followed by the same letter are not significantly different.

for rejection of our null hypotheses: for *V. angustifolium* $p < 0.0026$; for *V. myrtilloides* $p < 0.0071$ (Toms and Almond, personal communication).

Results

In *V. angustifolium* (Table 2), the fruit-sets, under pollination regimes were significantly different ($F_{10,97} = 128$, $P < 0.001$). Significantly the least amount of fruit-set was obtained from flowers enclosed in bags and thereafter untouched (2 fruit from 1692 flowers). The most fruit was produced by the flowers open to natural pollination. Although artificial xenogamy produced more fruit than did autogamy or geitonogamy there were no significant differences in fruit-set among hand-pollinated and bagged treatments, regardless of the method used (t ranged from 0.6 to 4.8; p ranged from 0.8 to 0.1). Our statistical analysis indicates that artificial xenogamy done with an insect pin was not significantly different from the open pollinated controls in terms of fruit-set ($t = 6.7$; $p = 0.12$). In *V. myrtilloides* (Table 3), fruit-sets under pollination regimes were significantly different ($F_{4,23} = 17$; $p < 0.001$). Again, open pollination produced more fruit than any of the other four treatments among which there were no significant differences (t ranged from 1.1 to 1.9; p ranged from 0.31 to 0.47) except for bagged and left alone, which again had markedly few fruits (6 from 1794 flowers).

In *V. angustifolium*, the fruits that developed from free pollinations had the greatest number of seeds (Table 4), but those that developed from the hand-done xenogamy had significantly fewer (ANOVA $F_{6,93} = 7.26$, $p = 0.05$), and those from hand done geitonogamy and autogamy were by far the least and were not significantly different from each other (ANOVA $F_{4,14} = 1.08$, $p = 0.05$; $F_{3,10} = 1.26$, $p = 0.05$). Fruit diameters were also affected by treatment (Table 4). Free pollinations resulted in the largest and seediest fruits (31.3 \pm 5.9 SE seeds in the 12.5 mm diameter class of fruits) with the other treatments significantly different from each other and the average size of fruit being smaller (Table 4) (ANOVA $F_{7,113} = 8.20$, $p = 0.05$). The fruits from geitonogamy and xenogamy were disproportionately smaller with fewer seeds than those from free pollination. There were no significant differences in fruit size and seediness from flowers pollinated by geitonogamy and autogamy (Table 4). The few fruits from flowers bagged and left alone were very small (5 mm in diameter) and seedless; they were omitted from analyses.

In comparing seediness and fruit size (in 1 mm diameter classes from 4.5 to 12.5 mm) of free pollination and xenogamy treatments on *V. angustifolium*, some regression relationships were calculated and found to be significantly different from zero (Table 4 and Figure 5). How-

TABLE 2. The comparison of fruit-set ratio among five pollination treatments of *V. angustifolium*.

Treatment	Number of sprigs examined	Number of sprigs with fruits (% fruit-set)	Total flowers among sprigs	Total fruits among sprigs	Mean \pm SE of fruit-set %	Range of fruit-set per sprig %
Free (natural condition)	38	38 (100.0)	4201	2336	57.85 \pm 0.3a*	11 to 86
Bagged (no pollination)	18	4 (22.2)	1794	6	0.2 \pm 0.1c	0 to 1
Xenogamy using cotton balls	18	11 (61.1)	602	61	11.3 \pm 4.7b	0 to 73
Geitonogamy using cotton balls	17	6 (35.3)	590	15	2.1 \pm 0.9bb	0 to 9
Autogamy using cotton balls	17	4 (23.5)	209	11	5.3 \pm 2.5b	0 to 31

*Values in a column followed by the same letter are not significantly different.

TABLE 4. The comparison of the complete seed production and fruit size produced from 4 pollination treatments of *V. angustifolium*.

Fruit Size diameter (mm)	Free		Xenogamy		Geitonogamy		Autogamy	
	Number of fruits	Mean \pm SE of complete seeds	Number of fruits	Mean \pm SE of complete seeds	Number of fruits	Mean \pm SE of complete seeds	Number of fruits	Mean \pm SE of complete seeds
12.5	8	31.250 \pm 5.918a*						
11.5	10	26.300 \pm 3.173a						
10.5	22	25.000 \pm 2.764a	3	16.000 \pm 8.737a				
9.5	18	22.611 \pm 2.836ab	11	8.455 \pm 2.436ab			4	3.750 \pm 0.854a
8.5	24	13.875 \pm 1.989bc	26	4.192 \pm 0.433bc	1	2.000a	1	1.000a
7.5	15	10.200 \pm 1.522c	31	3.355 \pm 0.647c	10	1.600 \pm 0.267a	7	2.286 \pm 0.680a
6.5	14	10.143 \pm 1.421c	15	2.133 \pm 0.236c	3	1.333 \pm 0.333a	2	1.500 \pm 0.500a
5.5	10	9.500 \pm 3.027c	8	2.000 \pm 0.378c	3	0.667 \pm 0.333a		
4.5			6	1.833 \pm 0.654c	2	2.000 \pm 1.000a		
Total	121		100		19		14	
F - values and df:		F _{7,113} = 8.20; p < 0.05	F _{6,93} = 7.26; p < 0.05		F _{4,14} = 108; p < 0.05		F _{3,10} = 1.26; ns	

*Values in columns followed by the same letter are not significantly different

ever, no regression relationship was found for geitonogamy and autogamy mostly because of the small number of fruits (for example, $r^2 = 0.023$ and 0.039 for linear regression for each treatment respectively). The comparison regression lines between free pollination and xenogamy were significantly different from each other for all models, i.e., the regression line in free pollination is higher than that in xenogamy.

Between sites in CCGP, percent fruit-set and seediness of *V. angustifolium* did not differ significantly with different forest ages (Tables 5, 6) even though the numbers of flowers and fruits in younger forests were greater than those in the older forests (ANOVA $F_{3,6} = 0.16$ in 1991, $F_{4,11} = 0.70$ in 1992, $F_{4,7} = 0.32$ in 1993, and $F_{4,33} = 0.22$ on average for 1991 to 1993, inclusive). For example, there were 683 ripened fruits from 2108 flowers per 1 m² during 1991 to 1993 from patches in the young forests (A: 1 to 5 year-old after clear-cut or burned), compared to 63 ripened fruits from 190 flowers per 1 m² in old forest sites (E: 36 to 100 year-old forests after clear-cut), during the same years; these differences were statistically significant (ANOVA $F_{4,33} = 20.67$, for flower production, and $F_{4,33} = 5.42$, for fruit production).

Discussion

The urceolate flower structure restricts flower visitors and the most effective pollinators are large buzz-pollinating bees such as bumblebees (*Bombus* spp.) and larger andrenids (*Andrena* spp.) (Finnamore and Neary 1978; Mohr and Kevan 1987; Stubbs and Drummond 1999, 2001; Javorek et al. 2002), although other, commercially available, bees (e.g., *Apis mellifera* L. (Aras et al. 1996; Javorek et al. 2002) and *Megachile rotundata* F. (Stubbs and Drummond 1997)) may be useful. In fact, there was almost no fruit-set without manipulation of some sort on the flowers of *Vaccinium angustifolium*

and *V. myrtilloides* (Table 2 and 3). Natural pollination resulted in 12 to 86% fruit-set in *V. angustifolium* and 11 to 86% fruit-set in *V. myrtilloides*. Cross-pollination by hand resulted in a fruit-set up to 38% in *V. angustifolium* and 73% in *V. myrtilloides*. From self-pollinations, fruit-set was low (less than 18% and 31% in both species, respectively).

Fruit size and seediness were greater in the natural conditions of free pollination. In the experiments, the few fruits produced by self-pollination were small with few seeds (1 to 6); more complete seed production was obtained by cross-pollination by hand (1 to 33 seeds), but even this was low by comparison by open pollination (Table 4). Eck and Childers (1966) also noted that natural pollinations produced larger berries and more seeds than did artificial cross-pollinations. Blueberry stigmata remain receptive for 5 to 8 days (Merrill 1936; Moore 1964; Wood 1962), however, fruit-set is not initiated if pollination does not occur within 3 days of anthesis (Chandler and Mason 1935). Thus, the longer period of pollination and greater amounts of pollen available under natural conditions would be expected to give rise to more, larger, seedier fruits.

Our results confirm the importance of natural xenogamy by insect pollinators in the fruit-set and seed-set of *V. angustifolium* and *V. myrtilloides* in the boreal forest of the CCGP. The small amount fruit and seed-set by experimental geitonogamy, autogamy and bagging alone may be the result of small amounts of wind-dispersed pollen (bagging alone) or accidental contamination or both (geitonogamy and autogamy).

Fruit-set ratio under natural, free pollination, was variable, ranging from 11% to 86% in *V. angustifolium* and *V. myrtilloides*. This suggests that many flowers are not pollinated or that other factors operate such as male- and female-sterility, or both (Aalders and Hall 1961; Hall and Aalders 1961; Hall et al. 1966; Reader 1977).

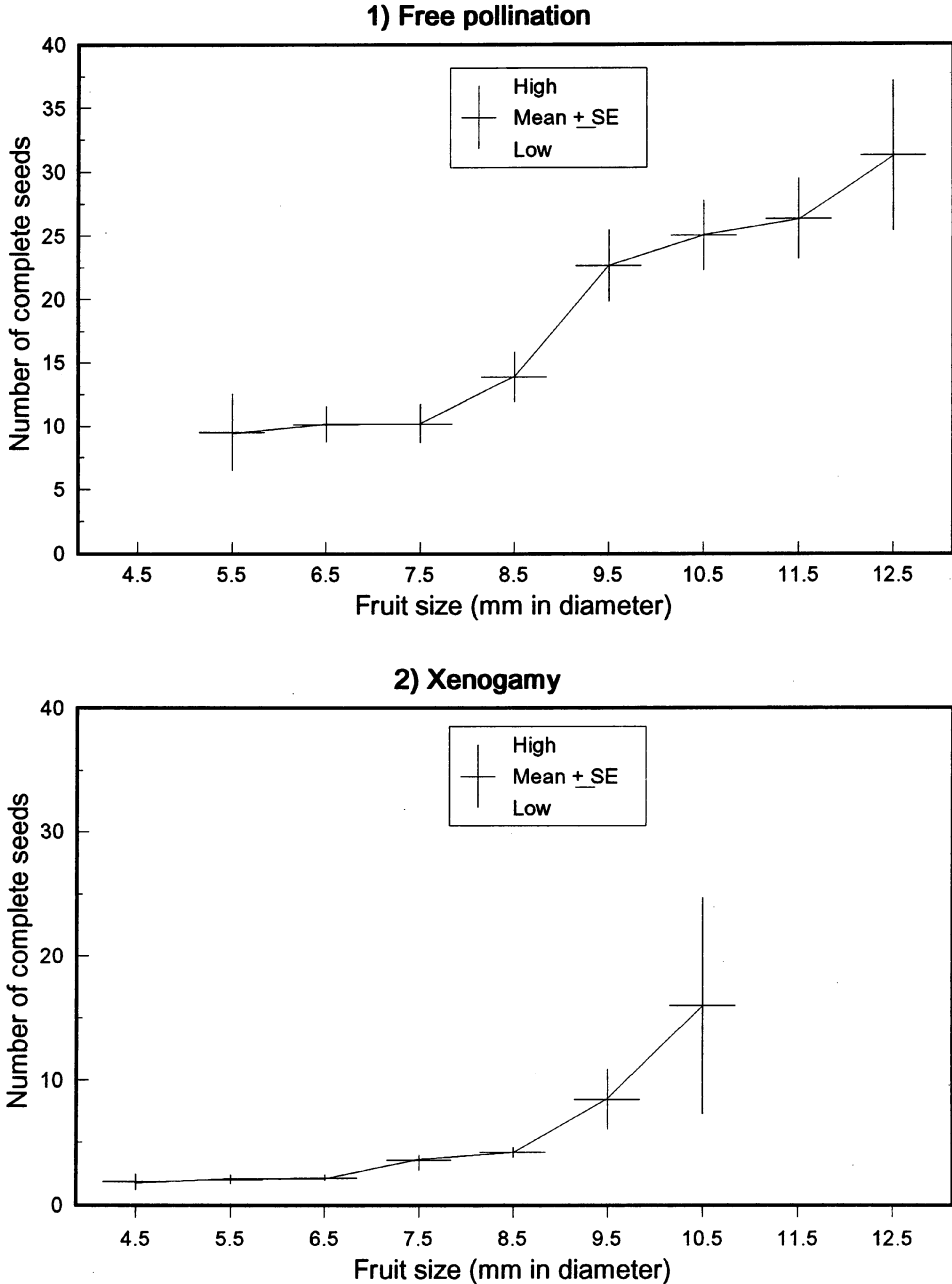


FIGURE 5. Comparison of several regression models of seediness on fruit size between free pollination and xenogamy for *Vaccinium augustifolium* in Chapleau Crown Game Preserve in 1993. 1) Free Pollination: Linear regression: $r^2 = 0.3061$, $Y = -13 + 3.6X$; Exponential regression: $r^2 = 0.3297$, $Y = 1.6e^{0.24X}$; Logarithmic regression: $r^2 = 0.2904$, $Y = -46 + 29.5(\log X)$; Power regression: $r^2 = 0.3296$, $Y = 0.15X^{2.1}$, $N = 121$. 2) Xenogamy: Linear regression: $r^2 = 0.2156$, $Y = -9.0 + 1.7X$; Exponential regression: $r^2 = 0.3436$, $Y = 0.2e^{0.35X}$; Logarithmic regression: $r^2 = 0.1839$, $Y = -19 + 11.3(\log X)$; Power regression: $r^2 = 0.3221$, $Y = 0.02X^{2.4}$, $N = 100$.

TABLE 5. Comparison of flower and fruit production and percent fruit-set among *V. angustifolium* in forests of different ages from 1991 to 1993. Forest age groups (years after clear-cut or burned): A (1-5); B (6-10); C (11-20); D (21-35); E (36-100).

Year and forest age group	Number of Observations (Sites)	Number of Flowers/m ²	Number of Fruits/m ²	% Fruit-set/m ² (fruits/flowers)
		Mean ± SE	Mean ± SE	Mean ± SE
1991				
A	3	1493.0 ± 181.18a	414.67 ± 15.84a	28.67 ± 4.41a
B	3	652.0 ± 192.99b	225.00 ± 78.36 ab	33.00 ± 8.72a
C	0	—	—	—
D	3	104.7 ± 17.05b	36.67 ± 16.83b	42.33 ± 23.15a
E	1	124.0b	37.00b	30.00a
F-value and df:		F _{3,6} = 15.57; p < 0.05	F _{3,6} = 12.30; p < 0.05	F _{3,6} = 0.16; ns
1992				
A	4	2169.5 ± 535.03a	424.00 ± 206.47a	18.00 ± 5.79a
B	2	1721.0 ± 113.00ab	352.00 ± 145.00a	21.00 ± 10.00a
C	3	962.3 ± 236.30ab	247.00 ± 85.78a	26.00 ± 5.13a
D	2	140.3 ± 20.73b	19.00 ± 4.04a	13.67 ± 2.73a
E	4	118.8 ± 42.63b	29.50 ± 14.80a	21.75 ± 4.25a
F-value and df:		F _{4,11} = 8.52; p < 0.05	F _{4,11} = 2.14; ns	F _{4,11} = 0.70; ns
1993				
A	3	2642.7 ± 167.41a	1297.33 ± 157.79a	48.67 ± 3.18a
B	2	1403.5 ± 160.50b	590.00 ± 286.00ab	44.50 ± 25.50a
C	4	1053.8 ± 212.30b	410.00 ± 116.66b	38.25 ± 7.25a
D	1	415.0b	—	142.00b 34.00a
E	2	366.5 ± 166.50b	144.50 ± 132.50b	29.00 ± 23.00a
F-value and df:		F _{4,7} = 17.54; p < 0.01	F _{4,7} = 7.62; p < 0.05	F _{4,7} = 0.32; ns
Average during 1991 and 1993				
A	10	2108.5 ± 254.02a	683.20 ± 159.13a	30.40 ± 4.95 a
B	7	1172.1 ± 208.27b	365.57 ± 97.94ab	32.86 ± 7.73a
C	7	1014.6 ± 145.59b	340.14 ± 77.61ab	33.00 ± 4.99a
D	7	164.3 ± 43.52c	44.14 ± 17.89b	28.86 ± 10.38a
E	7	190.3 ± 62.53c	63.43 ± 36.58b	25.00 ± 5.72a
F-value and df		F _{4,33} = 20.67; p < 0.01	F _{4,33} = 5.42; ≈ 0.05	F _{4,33} = 0.22; ns

*Values in column followed by the same letter are not significantly different (Tukey's Studentized Range Test at $P < 0.05$).

TABLE 6. The comparison of the seediness of naturally produced fruits on *V. angustifolium* at six study sites.

Study Site	Number of fruits measured	Number of seeds/fruit
		Mean ± SE
Clifton Number 1	18	22.667 ± 2.894a*
Clifton Number 2	12	18.500 ± 3.115a
Clifton Number 3	49	19.265 ± 2.045a
Copperfield Number 3	9	13.222 ± 3.894a
Copperfield Number 5	3	13.615 ± 2.793a
Copperfield Number 9	20	16.150 ± 2.404a

*values in column followed by the same letter are not significantly different (Tukey's Studentized Range Test at $P < 0.05$).

In 1993, we found a few strange flowers (e.g., no pistil (one sample), short style nearly the same length of stamens (two), three-divided stigmata (one), and little or no pollen within the anthers (Table 1)), but the incidence of these aberrations is too low to explain all our records of diminished fruit and seed-set under natural pollination. Nevertheless, the fruit-sets we observed under natural conditions (means: 50.5% in *V. angustifolium*

and 57.8% in *V. myrtilloides*) at Chaplin Number 4 and other study sites in 1993, indicates that the wild bee populations (or pollinator forces (Kevan et al. 1986)) were ample by comparison with those on some commercially managed lowbush blueberry barrens (an excellent commercial crop for a fruit-set of 50%, but it is usually less 10 to 20% (Karmo 1957)). Our results are different from those obtained by Reader (1977) for *V. myrtilloides*. The importance and variability in fruit-set in open pollinated flowers are demonstrated by both studies, with similar results. However, Reader's results indicate much greater capacity for self-pollination (autogamy and geitonogamy (60–80%)) in the plants he studied than we found (0–31%). That large discrepancy suggest that the two populations, his in two bogs in southern Ontario and ours in the boreal forest, have different sexual reproductive strategies. Perhaps the relatively isolated and smaller populations in southern Ontario have adapted by having lesser reliance on entomophily in the face of less diverse guilds and smaller populations of pollinating bees; Richards (1997) provides examples of other plants with variable sexual reproductive strategies. Nevertheless, fruit-set varies

from year to year and location to location. Weather during bloom and the size of the pollinator force may be important. At CCGP, 1991 and 1993 were relatively good for blueberry production because there was little severe frosty weather to damage the flowers in June (Environment Canada 1994). However, in 1992 there were several severe June frosts that killed many flowers and buds, especially in the Clifton Number 1 study site where from 1800 flowers only 12 fruits resulted vs. 1094 fruits from 2540 flowers in 1993. At Clifton Number 1 the young open, forest is susceptible to severe June frosts by advective cooling (Cochran 1969a).

Our hypothesis that forest age affects fruit-set and seed-set was not validated. We found no significant differences in percent fruit-set among the forests at different ages, but did note significantly fewer flowers per unit area in older forests than in open habitats (Table 6). Pollinator populations were not as great in older forests sites as in the opened area (Usui 1994), however, we observed some shade-loving bumblebees such as *B. vagans* in the former (Usui 1994) as also noted by Heinrich (1979) in Maine. The relative paucity of flowers and paucity of pollinators seem to offset each other to allow for adequate pollination and pollinator forage. As suggested in Table 6, some locations, which have relatively larger fruits and more complete seed productions, have greater bee diversity and population.

During the blueberry flowering season at CCGP and Chaplin Number 4 study site during 1993, many bees (53 species (Usui 1994)), especially the bumblebees, *Bombus ternarius* and *B. terricola*, visited many flowers in rapid succession. They showed several flower-searching patterns; e.g., mainly toward the sun or windward in straight lines, in zig-zags, or in circles. For example, one queen of *B. ternarius* visited 1297 flowers among 55 *V. myrtilloides* plants within 30 m² (5 m × 6 m) in only 24.5 minutes (1600 h on 16 June) flying roughly in two large circles and re-visiting the same inflorescence of the plants (Usui 1994). Such behaviour would promote cross-pollination among different clonal plants as she transferred many different sources of viable pollen grains in a short time.

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