The reproductive biology of boreal forest herbs. I. Breeding systems and pollination

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Detailed observations and experimental studies of the reproductive biology of 12 boreal forest herbs were conducted over a 3-year period (1978–1980) in spruce–fir forests of central New Brunswick. The species examined were Aralia nudicaulis, Chimaphila umbellata, Cliftonia borealis, Cornus canadensis, Cypripedium acaule, Linnaea borealis, Maianthemum canadense, Medeola virginiana, Oxalis montana, Pyrola secunda, Trientalis borealis, and Trillium undulatum. All taxa are insect-pollinated perennial herbs, and most exhibit clonal growth. Floral syndromes of the understorey community are relatively unspecialized with many species possessing small white or green flowers. A total of 103 taxa of insects were collected from flowers during the 1979 season. Bombus spp. are the major pollinators of 5 of the 12 species. Syrphid flies, bee flies, and halictid and andrenid bees were also commonly observed. Controlled pollinations were undertaken to determine the breeding systems of herbs. Bagged, self-, cross- and open-pollinated treatments were used to investigate the capacity for self-pollination, compatibility status, and factors influencing fecundity in each species. A diversity of reproductive systems was revealed. Six species are completely dependent on insects for pollination, four species are weakly autogamous, one is strongly autogamous, and one appears to be apomictic. Comparisons of fruit-set and seed set from controlled self- and cross-pollinations indicate that four species are strongly self-compatible, one is dioecious, and the remainder display varying degrees of self-incompatibility. Despite this variation, outbreeding appears to be the most common reproductive mode. In several species there is evidence that low pollinator service limits fruit-set.


[Traduit par la revue]

Introduction

The boreal forest covers extensive areas of the cool north temperate regions of North America and Eurasia and is composed primarily of different assemblages of coniferous trees (Hare 1954; La Roi 1967; Komaš 1972; Rowe 1972). At its southern boundary in North America, the forest gives way to a mixture of various coniferous and broad-leaved deciduous trees (e.g., Maycock and Curtis 1960). Despite considerable variation in the species composition and vegetation structure of northern forests (e.g., Carleton and Maycock 1978, 1981), virtually all of the tree species exhibit uniform methods of pollination and seed dispersal. Anemophily (wind pollination) and anemochory (wind dispersal) predominate, a feature common to many cool temperate forests of the world (Regal 1982) and a striking contrast to tropical forests, where biotic influences are often closely involved with the reproductive biology of tree species (e.g., Bawa 1974; Ashton 1976; Zapata and Arroyo 1978; Sobrevilla and Kalin-Arroyo 1982; Baker et al. 1983; Bawa et al. 1985a, 1985b).

The herbaceous understorey of the boreal forest is composed almost exclusively of perennial species, many of which are clonal and have showy floral displays and conspicuous, fleshy fruits. This suggests that animals play an important role in pollination and seed dispersal. To investigate this possibility we initiated a study of the reproductive biology of 12 common, widespread boreal forest herbs occurring in spruce–fir forests of central New Brunswick. The study was designed to provide basic descriptive information on the breeding systems, pollination biology, and phenology of understorey herbs. Such information is important for the design of more detailed studies of the population biology of individual taxa and can also provide insights into the selective forces shaping reproductive adaptations at the community level.

In this paper we (i) provide quantitative estimates of reproductive traits associated with the breeding systems of herbs under study, (ii) by controlled pollinations conducted under field conditions, determine the compatibility status of species, their capacity for self-pollination, and whether natural levels of
fruit-set may be limited by pollen availability; and (iii) identify the major insect visitors to flowers and comment on their roles as pollinators. Following the presentation of results we evaluate some of the factors that are likely to influence the reproductive success of herbaceous plants occurring in the boreal forest.

Materials and methods

Study site

All field studies were conducted on two plots 3 km apart located on the property of R. Jona, 5 km east of Doaktown, Northumberland County, central New Brunswick, from May to September 1978, 1979, and 1980. Vegetation at the two sites is composed of elements of boreal forest, and common species include Picea rubens Sarg., Picea mariana (Mill.) BSP., Abies balsamea (L.) Mill., Pinus strobus L., Tsuga canadensis (L.) Carr., Thuja canadensis L., Populus tremuloides Michx., with occasional Larix laricina (Du Roi) K. Koch, Acer saccharum, and Betula alleghaniensis Britton. The vegetation of the area is classified as Acadian Forest by Rowe (1972), where further details of the structure, species composition, and climate of the region are available.

The two plots were each approximately 1000 m² and were chosen because their understorey vegetation was representative of the surrounding spruce–fir forests and each contained sufficient numbers of the 12 common boreal forest herbs under study to allow experimental studies. The 12 species comprised >95% of the herbaceous cover at each site. Other species of low frequency at the sites were Gaultheria procumbens, Moneses uniflora, and Monotropa hypopitys. The average frequency of the 12 herbs in the two plots was estimated by recording the number of times a given species occurred in twenty-five 1-m² quadrats positioned at random within each plot. Because the results of studies conducted at both sites were similar, data from the two plots were pooled in analyses and data presentations. 

Reproductive traits

Observations and measurements of reproductive traits for each species were made by sampling plants at random from the two study plots. Traits examined were flower colour, blossom type, number of flowers per inflorescence, pollen production, ovule number, seed weight, fruit weight, and reproductive effort. Estimates of pollen production involved 10 replicates of one to five unopened buds, depending on the amount of pollen produced per flower. Pollen counts were made using a haemocytometer, using the method of Lloyd (1965). Reproductive effort was estimated by sampling reproductive stems (ramets) of each species just prior to fruit maturity, drying the above-ground portion in an oven at 75°C for 3 days, and weighing the aboveground vegetative parts separately from reproductive structures. Reproductive effort in this study is defined as the percentage of aboveground dry weight allocated to reproductive structures per ramet. Means and sample sizes are given in Table 3.

Insect visitors

Observations of insect visitors to flowers were made throughout the 3-year study period. During summer 1979, insect visitors to flowers of each plant species were observed and collected at daily intervals, weather permitting. Sampling was not quantitative, but an attempt was made to make systematic collections of insects visiting each of the 12 species during their peak flowering period. Differences in the total number of individuals collected on each plant species provide a rough estimate of the level of visitation that each species received. All insects were pinned, mounted, and later sent to specialists for identification.

Breeding systems

Information on the breeding system of each species was obtained by controlled hand-pollinations and bagging experiments. Flowers were protected from insect visitors by the use of fine mesh nylon net (Bridal Temptation, eight strands per centimetre) that enclosed the entire inflorescence and were secured by drawstrings encircling the peduncle. For some species (e.g., Oxalis montana) visited by small Coleoptera, several thicknesses of net prevented insects from entering bags. In species with inflorescences that were unable to support the weight of the net bags (particularly after rain), exclosures were made with wire or wooden frames to surround the entire plant with netting (Cornus canadensis, Linnaea borealis, Maianthemum canadense, Medeola virginiana, Oxalis montana, Pyrola secunda, Trientalis borealis), or net bags were supported by wire hooks (Clintonia borealis and Trillium undulatum).

To determine the dependence of each species on insect visitation for seed set, comparisons of fruit and seed production in bagged and open-pollinated flowers were made. waxed jeweller’s tags attached around the peduncle were used for marking individuals. To assess the compatibility relationships of each species, individual plants were self- and cross-pollinated. Emasculation was undertaken for all cross-pollinated flowers and plants in both treatments were bagged prior to the pollinations being applied. In some species with multiple-flowered inflorescences not all flowers within an inflorescence were used in cross-pollinations. Flowers not pollinated were excised from inflorescences. Because of the extensive clone size of many of the herbs under study a special effort was made in cross-pollinations to use pollen from conspecific patches occurring at least 10 m away from the pollen recipient. After controlled crosses plants were immediately rebagged to prevent pollen contamination and herbivory. Pollination treatments were conducted only on chasmogamous flowers of Oxalis montana.

Fruit-set and seed set data from the four pollination treatments (unmanipulated and bagged, self-pollinated, cross-pollinated, and open-pollinated) were compared statistically by chi-square tests, tests for equality of percentages based on the arcsin distribution (Sokal and Rohlf 1969), and Student’s t-tests.

Results

Floral biology

The 12 species of boreal forest herb that were examined at the two study plots in central New Brunswick are all entomophilous (Table 1). Floral complexity varied among the species, ranging from the relatively simple unspecialized bowl-shaped flowers of Oxalis montana and Trientalis borealis to more structurally complex flowers as in the orchid Cypripedium acaule. Most floral syndromes were, however, relatively unspecialized. Most species display either uniformly white flowers or perianth parts with a combination of white with green or pink (Table 1). Blue, deep yellow, red, or orange flowers are rare or absent from the forest floor, although these floral colours are well represented in meadows and disturbed areas adjacent to the forest.

Insect visitors

Peak flowering of the most abundant species in the community was in June. Detailed phenological data for each species are presented in Helenurm and Barrett (1987). Visitation by insects to flowers of the 12 species varied greatly during the season. During cool or wet weather, particularly in May and June, no insects were observed on flowers for extended periods lasting for up to 7 consecutive days. The major insect visitors to flowers are listed in Table 1. A total of 552 insects comprising 103 taxa were collected from flowers during summer 1979. The mean number of insect taxa and individuals visiting each plant species was 14 and 46, respectively. Visitation levels to Aralia nudicaulis, Cornus canadensis, and Maianthemum canadense were relatively high in comparison with the remaining species. The former were among the most common at the study sites. Not all visitors were effective pollinators; those listed in Table 1 are considered likely pollinators based on observations of their foraging activities.
<table>
<thead>
<tr>
<th>Flower colour</th>
<th>Blossom type</th>
<th>Major insect visitors</th>
<th>Peak flowering</th>
<th>Frequency at study sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green—white</td>
<td>Brush</td>
<td>Bumblebees, solitary bees, and syrphids</td>
<td>Early June</td>
<td>0.24</td>
</tr>
<tr>
<td>Pink—white</td>
<td>Bowl</td>
<td>Bumblebees and staphylinid beetles</td>
<td>Late July</td>
<td>0.03</td>
</tr>
<tr>
<td>Yellow—green</td>
<td>Bell</td>
<td>Bumblebees and solitary bees</td>
<td>Early June</td>
<td>0.61</td>
</tr>
<tr>
<td>White</td>
<td>Brush</td>
<td>Bumblebees, solitary bees, bee flies, and syrphids</td>
<td>Mid-June</td>
<td>0.83</td>
</tr>
<tr>
<td>White—pink</td>
<td>Gullet</td>
<td>Bumblebees?</td>
<td>Mid-June</td>
<td>0.02</td>
</tr>
<tr>
<td>Pink</td>
<td>Bell</td>
<td>Solitary bees and syrphids</td>
<td>Early July</td>
<td>0.55</td>
</tr>
<tr>
<td>White</td>
<td>Brush</td>
<td>Solitary bees, bee flies, and syrphids</td>
<td>Early June</td>
<td>0.92</td>
</tr>
<tr>
<td>Green—red</td>
<td>Dish</td>
<td>Flies?</td>
<td>Late June</td>
<td>0.08</td>
</tr>
<tr>
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<td>Bowl</td>
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</tr>
<tr>
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<td>Bowl</td>
<td>Bumblebees and solitary bees</td>
<td>Mid-July</td>
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</tr>
<tr>
<td>White</td>
<td>Bowl</td>
<td>Syrphids</td>
<td>Early June</td>
<td>0.45</td>
</tr>
<tr>
<td>White</td>
<td>Bowl</td>
<td>Solitary bees</td>
<td>Late May</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Based on blossom classification of Fougri and Van der Pijl (1971).
*From collections listed in Appendix 1.
*From quantitative data detailed in Helenurm and Barrett (1987).

Table 2. Species of bumblebees (Bombus spp.) collected from flowers of boreal forest herbs during summer 1979 in central New Brunswick

<table>
<thead>
<tr>
<th>Bumblebees</th>
<th>Bombus perplexus</th>
<th>Bombus ternarius</th>
<th>Bombus terricola</th>
<th>Bombus vagans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aralia nudicaulis</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Chimaphila umbellata</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Clintonia borealis</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Cornus canadensis</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Cypripedium acaule</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Linnaea borealis</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Meianthemum canadense</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pyrola secunda</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

*Not observed on C. acaule but carrying pollinia.

Species of Bombus (bumblebees) were major pollinators of five species at the study sites and visited 8 of the 12 species (Table 2). On cool days they were frequently the only insects to be observed visiting flowers. Of the remaining insects collected from flowers, syrphids, bombylids, and various solitary bees (e.g., halictids and andrenids) were most commonly observed. Despite long periods of observation no insects that might have been significant to the pollination of Cypripedium acaule and Meioedola virginiana were recorded from these species during the 3-year period. Two queens of Bombus vagans were, however, collected from flowers of other plant species and were carrying pollinia of Cypripedium acaule. This suggests that they may play a role in the pollination of the species.

Floral traits
Quantitative measures of floral traits are presented in Table 3. Inflorescence size varied among species, although most exhibited a relatively small number of flowers per inflorescence. Five species had, on average, either one or two flowers, and only three species possessed more than 20 flowers per inflorescence. In the dioecious Aralia nudicaulis, male inflorescences produce nearly twice as many flowers as female inflorescences (Barrett and Helenurm 1981). In Oxalis montana, two flower types are produced, cleistogamous and chasmogamous. Cleistogamous flowers were not examined in detail.

Pollen:ovule (P:O) ratios ranged from 58 in Chimaphila umbellata to 6291 in Clintonia borealis (Table 3). The relatively low P:O ratios of the two members of the Pyrolaceae (Chimaphila umbellata and Pyrola secunda) are mainly the result of the extremely large number of ovules produced in flowers of the two species (Table 3). The P:O ratio of Linnaea
borealis was calculated on the basis of one ovule per flower. No case of more than a single seed per fruit was recorded from the range of pollination treatments applied to the species including controlled cross-pollinations, so we assume that the remaining ovules are either nonfunctional or display fixed levels of abortion. By comparing ovule number per flower with the number of seeds per fruit in cross-pollinated flowers, an estimate of the number of functional and (or) nonaborted ovules per flower can be obtained. The seed:ovule ratios for species in which data are available are Clintonia borealis, 0.52; Cornus canadensis, 0.40; Linnaea borealis, 0.10; Medeola virginiana, 0.76; Oxalis montana, 0.72; and Trillium borealis, 0.70.

Pollination experiments

Percentage fruit-set, mean seeds per fruit, and statistical comparisons of fruit and seed production following the four pollinations treatments are presented in Tables 4 and 5, respectively.

The exclusion of insect visitors to flowers of six species (Aralia nudicaulis, Cornus canadensis, Cypripedium acaule, Medeola virginiana, Maianthemum canadense, Trillium borealis) resulted in virtually no seed formation, indicating that they are dependent on insects for pollination. In four species small amounts of seed were produced in undisturbed flowers (Chimaphila umbellata, Clintonia borealis, Linnaea borealis, Oxalis montana), some automatic self-pollination. However, the seed set of bagged flowers in the four species was well below what was found in the plants in the remaining treatments, indicating a major role for insects as pollen vectors. Cleistogamous flowers in Oxalis montana are completely autogamous. Significant levels of seed production from bagged flowers were obtained in only two species (Pyrola secunda, Trillium undulatum), indicating that they are highly autogamous or ampicotic. Although the latter condition seems unlikely for Pyrola secunda, the observations of Swamy (1948) and P. Keenan (unpublished data) on apomixis in Trillium undulatum suggest a role for this reproductive system in the New Brunswick populations.

Comparisons of fruit-set and seed-set data from controlled self- and cross-pollinations of Cornus canadensis, Maianthemum canadense, Medeola virginiana, and Trillium borealis suggest that these species are highly self-incompatible. Self-pollinations generally resulted in little seed production in comparison with cross-pollinations (Table 4). Self-pollinations of the remaining species (with the exception of Aralia nudicaulis, which is dioecious) resulted in various amounts of seed, indicating self-compatibility.

Although Clintonia borealis is clearly self-compatible, cross-pollinations consistently resulted in higher amounts of fruit and seed production compared with that from self-pollinations. The reduced seed set from self-pollinations may have resulted from weak self-incompatibility and (or) postzygotic abortion of developing embryos caused by inbreeding depression (see Galen et al. (1985) and Galen and Weger (1986) for further details). Flowers of Clintonia borealis are strongly protogynous, and under field conditions it is possible that, despite self-compatibility, clones are largely outcrossed.

A notable feature of the data from controlled cross-pollinations is the low level of fruit-set obtained in Cornus canadensis (21.5%) and Maianthemum canadense (12.5%). Although the values are significantly higher than those obtained from self-pollinations, they are considerably lower than cross-pollinations of the other species (Table 4). The low fecundity

<table>
<thead>
<tr>
<th>Flowers per inflorescence</th>
<th>Pollen grains per flower</th>
<th>Surviving ovules per flower</th>
<th>Fruit per flower</th>
<th>Seed per flower</th>
<th>Seed weight (mg)</th>
<th>Seed weight (mg) n = 20</th>
<th>Reproductive effort (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.5</td>
<td>100</td>
<td>50</td>
<td>5,0</td>
<td>0 (20)</td>
<td>0.1</td>
<td>0.8</td>
<td>23.3</td>
</tr>
<tr>
<td>20.5</td>
<td>100</td>
<td>50</td>
<td>5,0</td>
<td>0 (20)</td>
<td>0.1</td>
<td>0.8</td>
<td>23.3</td>
</tr>
<tr>
<td>30.5</td>
<td>100</td>
<td>50</td>
<td>5,0</td>
<td>0 (20)</td>
<td>0.1</td>
<td>0.8</td>
<td>23.3</td>
</tr>
<tr>
<td>40.5</td>
<td>100</td>
<td>50</td>
<td>5,0</td>
<td>0 (20)</td>
<td>0.1</td>
<td>0.8</td>
<td>23.3</td>
</tr>
</tbody>
</table>

Notes: Values are the mean, standard deviation, and sample size in parentheses. NR, not recorded.
is unlikely the result of sampling error or faulty pollination technique. Sample sizes were large and ample pollen from distant clones was applied to stigmas. In both years the results of cross-pollinations for both species were similar. It is more likely that the low fecundity of these species is associated with postzygotic processes associated with resource limitation (see below).

In eight species (Aralia nudicaulis, Clintonia borealis, Cornus canadensis, Cypripedium acaule, Medeola virginiana, Oxalis montana, Pyrola secunda, Trisetalis borealis) fruit-set was significantly higher in controlled cross-pollinations in comparison with natural levels of fruit-set (Table 5). In Chimaphila umbellata, Linnaea borealis, and Maianthemum canadense, although fruit-set was higher in the cross-pollinated treatment that in the open-pollinated treatment, the differences were not significant. Fruit-set and seed set values following cross-pollination of Trillium undulatum were significantly lower than the corresponding values from the open-pollinated treatment. Nearly twice as many seeds per fruit were produced in open-pollinated flowers in comparison with each of the three experimental treatments (Table 3). The difference is difficult to interpret but may be associated with the possible apomictic mode of reproduction in the species, the influence of multiple pollen donors on fecundity in open-pollinated flowers (see Schemske and Pautler 1984), or damage to flowers as a result of hand-pollinations.

**Fecundity**

The natural levels of fruit and seed production varied greatly among the species of boreal forest herbs that were examined. Few flowers of Cornus canadensis, Cypripedium acaule, and Trisetalis borealis set fruit, whereas most flowers of Aralia nudicaulis, Chimaphila umbellata, Clintonia borealis, and Trillium undulatum produced fruit. These differences were found in both years. In 8 of the 10 species that were examined, the percentage fruit-set of flowers was significantly lower in 1979 than 1978 (Fig. 1). In Cornus canadensis, the reverse pattern occurred; maximum fruit-set was recorded in both years for Trillium undulatum, although it is worth noting that considerable predation of flowers and fruits was evident in the plants used for phenological studies reported in Helenurm and Barrett (1987). No fruits were produced by Cypripedium acaule in 1978, whereas two were recorded the following year.

**Discussion**

Most understory herbs of the boreal forest are perennial and many are long lived with well-developed clonal growth. Reproduction usually involves occasional episodes of seedling recruitment as a result of disturbance (e.g., fire, logging) followed by long periods of vegetative growth. Once individuals attain reproductive status, flowering and seed production occur regularly as long as local site conditions are favourable. Heavy shade, waterlogging, and the poor nutrient regime of soils can all reduce the flowering capacity of populations.

At our study sites, the 12 species chosen for study comprised most of the herbaceous cover and were common in spruce-fir forests throughout the region. Most of the species have widespread distributions throughout the boreal forest region of North America. Two species (Medeola virginiana, Trillium undulatum) are, however, more characteristic of mixed coniferous-deciduous forests of southern Canada and the United States. Several taxa (Chimaphila umbellata, Linnaea borealis, Oxalis montana (= O. acetosella), and Pyrola secunda) are known from boreal forest regions of Eurasia or have close relatives there (Kornas 1972).

Although members of the herbaceous understory community in central New Brunswick share the perennial, clonal habit, they display a diversity of reproductive systems. These range from obligate outbreeding enforced by dioecism (Aralia nudicaulis) or physiological self-incompatibility (e.g., Medeola virginiana) through species with self-compatibility and presumably mixed mating systems (e.g., Pyrola secunda) to autogamy and possible apomixis (Trillium undulatum). Despite this variation the predominant reproductive mode appears to be outbreeding with insect pollinators playing a central role in regulating the levels of self- and cross-pollination in taxa with self-compatibility. Isozyme markers could be profitably used in boreal forest herbs to obtain quantitative estimates of the mating systems of individual species (Clegg 1980).

Surveys of pollen:ovule ratios in flowering plants indicate that they are associated with the breeding system of species (Cruden 1977). In general, outcrossers exhibit relatively high P:O ratios in comparison with species that display a high degree of self-fertilization. Comparison of P:O ratios of boreal forest herbs with values published in Cruden's survey is instructive in this regard. All species investigated, except Chi-

<table>
<thead>
<tr>
<th></th>
<th>Bagged</th>
<th></th>
<th></th>
<th>Self-pollinated</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of flowering stems</td>
<td>No. of flowers</td>
<td>% fruit-set</td>
<td>No. of seeds/ fruit ± SD (n)</td>
<td>No. of flowering stems</td>
<td>No. of flowers</td>
</tr>
<tr>
<td><strong>Aralia nudicaulis</strong></td>
<td>7</td>
<td>203</td>
<td>0</td>
<td></td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td><strong>Chimaphila umbellata</strong></td>
<td>55</td>
<td>260</td>
<td>10.8</td>
<td>NR*</td>
<td>69</td>
<td>168</td>
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<tr>
<td><strong>Clintonia borealis</strong></td>
<td>81</td>
<td>247</td>
<td>28.3</td>
<td>4.6±4.4 (70)</td>
<td>28</td>
<td>347</td>
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<tr>
<td><strong>Cornus canadensis</strong></td>
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<td>1777</td>
<td>1.5</td>
<td>1.0±0 (27)</td>
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<td>15</td>
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<td><strong>Cypripedium acaule</strong></td>
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<td>0</td>
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<td>19</td>
<td>30</td>
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<td>28</td>
<td>54</td>
<td>35.2</td>
<td>1.0±0 (19)</td>
<td>21</td>
<td>24</td>
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<td><strong>Maianthemum canadense</strong></td>
<td>76</td>
<td>1082</td>
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<td>1.0±0 (3)</td>
<td>14</td>
<td>166</td>
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<td>4.0±0 (1)</td>
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<td>223</td>
<td>63.2</td>
<td>NR</td>
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<td>34</td>
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<tr>
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</tr>
<tr>
<td><strong>Trillium undulatum</strong></td>
<td>48</td>
<td>48</td>
<td>95.8</td>
<td>27.3±14.3 (46)</td>
<td>17</td>
<td>17</td>
</tr>
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</table>

*NR, not recorded.
maphila umbellata and Pyrola secunda, have P:O ratios that fall into his outcrossing classes (facultative xenogamy $x = 796.6$, xenogamy $x = 5859.2$). The P:O ratios of these two exceptions are more suggestive of facultative autogamy ($x = 168.5$) or obligate autogamy ($x = 27.7$). Pyrola secunda is moderately autogamous and Chimaaphila umbellata is weakly autogamous. The low P:O ratios of these species may partly result from their extremely high ovule numbers per flower. Both produce capsules containing very large numbers of tiny seeds (Table 3), and it is possible that this feature makes the P:O ratios less indicative of their actual breeding system. Because of this, we feel that it is premature to attempt any interpretation of the breeding systems of these species. A similar difficulty arises in Cypripedium acaule, although in this case the complex floral mechanism prohibits self-pollination and the species is entirely dependent on insect pollinators for cross-pollination (Stoutamire 1967).

Peak flowering for most understory herbs in central New Brunswick is June (Helenurm and Barrett 1987). During this period, cool, wet weather is frequent and at our study sites little pollinator activity was observed. Under these conditions flowers receive few pollinator visits and fruit-set and seed set may be reduced accordingly. Comparison of data from controlled cross-pollinations and open-pollinations has been used to investigate whether plant reproductive success is pollen limited (reviewed in Rathcke 1983; Willson and Burley 1983). In 8 of the 12 species examined, fruit-set following cross-pollinations is significantly higher than in open-pollinated flowers. However, interpretation of these findings is not straightforward since in six of the species, fewer flowers per inflorescence were used in the cross-pollinated treatment than in the open-pollinated treatment. This was done in some species because of the difficulties encountered in cross-pollinating all flowers with multiflowered inflorescences. This procedure could artificially inflate fruit-set values for the cross-pollinated treatment in species in which low resource levels and high abortion rates limit the number of fruits matured (see Stephenson 1981). Two observations are relevant to this problem. First, comparison of total fruit number per inflorescence (rather than percentage fruit-set) in the two pollination treatments can be used as a conservative test of whether fruit-set is pollinator limited, as long as inflorescences in the two treatments are selected at random. When this approach was adopted it was found that 3 of the 6 species with smaller samples for
cross-pollinated inflorescences (*Cornus canadensis*, *Medeola virginiana*, and *Trientalis borealis*) have significantly higher total fruit-set per inflorescence in this treatment in comparison with that from open-pollinated flowers. This indicates that, irrespective of the number of flowers used per inflorescence, cross-pollinations are capable of elevating fruit-set above that achieved in open-pollinated flowers.

The second observation concerns the frequency of fruit abortion in the species examined. If resource limitation largely restricts the percentage of fruits matured, this should be observable under field conditions. Our censuses of fruit development in 11 of the species indicated that in only 2 (*Cornus canadensis* and *Maianthemum canadense*) was there any significant degree of fruit abortion, suggesting that in these two species resources play a major role in regulating fruit-set (Helenurm and Barrett 1987).

Elsewhere, several authors have reported pollen limitation to fruit-set and seed set (Bierzychudek 1981; Rathcke 1983). Although some controversy exists as to the interpretation of data (Bawa and Webb 1984; Garwood and Horvitz 1985), it seems generally agreed that the flowers of early flowering entomophilous species occurring in cool temperate climates may be prone to pollen limitation owing to reduced pollinator activity during inclement weather (Schemske et al. 1978). Since these species are frequently perennial, it is unlikely that fluctuations in pollinator numbers will have any dramatic effect on the overall fitness of individuals. Resources not used for fruit and seed production within a given year may be reallocated to growth and flowering in later years.

The most striking examples of pollen limitation of fruit-set in our data occur in *Oxalis montana* and *Cypripedium acaule*. Plants of both species are self-compatible and controlled cross-pollinations yielded maximum fruit-set, while open-pollinated flowers produced no more fruits than did bagged flowers. Interpretation of these data is unambiguous because each species produces only a single flower per inflorescence, although *Oxalis montana* additionally produces cleistogamous flowers. No pollinators were observed visiting flowers of *Cypripedium acaule* during the 3-year period. A parallel study close to our study site of pollinium removal in *C. acaule* also provides evidence that these plants receive very little pollinator visitation. Of 236 flowers censused during 1979, only 26 had pollinia removed (Plowright et al. 1980). *Cypripedium acaule* is occasionally visited by bumblebees (Stoutamire 1967), but at our study site they largely concentrate their activities on other co-occurring species that flower at the same time as *C. acaule* (see Helenurm and Barrett 1987). Floral complexity and absence of nectar in the orchid presumably makes other species in the community more profitable food sources for bumblebees.

The five species showing pollen limitation (*Cornus canadensis*, *Cypripedium acaule*, *Medeola virginiana*, *Oxalis montana*, *Trientalis borealis*) may be indirectly affected to some extent by aerial spraying of insecticide for spruce bud-
worm control in New Brunswick. The effect of spraying on plant fecundity depends on the type of spray used, the time of flowering, and the insects that act as pollinators (Kevan 1975; Plowright et al. 1978; Kevan and La Berge 1979; Plowright and Rodd 1980; Thomson et al. 1985). Pollinator populations may be significantly reduced as a result of spraying, although recovery can occur later in the season. Several of the plant species we examined (Aralia nudicaulis, Clintonia borealis, Cornus canadensis, Melanthemum canadense) that flower immediately after spray applications in New Brunswick have been shown to have significantly lower fecundity in sprayed compared with unsprayed areas close to our study sites (Thaler and Plowright 1980). However, our plots were not in areas that were sprayed directly during the study period, and of the five pollen-limited species, only Trientalis borealis flowers at the time when pollinator populations are at their nadir. Nevertheless, since extensive insecticide spraying has been used for spruce budworm control in New Brunswick since 1952, it is possible that populations of pollinators are reduced over large areas despite absence of spraying at a local level over short time periods.

Since this study was completed, several of the understory herbs we examined have been investigated more intensively. As similar aspects of the reproductive biology were examined, it is worthwhile comparing the results and interpretations of these studies with our own. Anderson and Beare (1983) studied the breeding system and pollination ecology of Trientalis borealis in Michigan. They report three findings relevant to the present study: (i) the species is self-incompatible with 2.0% fruit-set upon selfing and 84.3% fruit-set in cross-pollinations; (ii) open-pollinated fruit-set averaged 66.1% and pollinator availability (halictid and andrenid bees) limits seed set; and (iii) the P:o ratio = 3,755. These results are in general agreement with our own but suggest that populations in New Brunswick may receive less pollinator service than those in Michigan. This may be associated with a reduced insect fauna caused by aerial spraying of insecticide over much of the province, as discussed previously. Further evidence to support this suggestion comes from a comparison of fruit-set data in Oxalis montana. Jasieniuk (1985) censused the fruit-set of chasmogamous flowers over a 2-year period (1980–1981) at five sites in Quebec. Her values average two to three times higher than the corresponding values for Oxalis montana in New Brunswick.

Galen et al. (1985) and Galen and Weger (1986) have recently reported on a detailed investigation of the pollination biology of Clintonia borealis in central Ontario. Their study shows that considerable variability in the strength of self-incompatibility occurs among clones ranging from a high degree of self-compatibility to strong self-incompatibility. Similar patterns were evident in New Brunswick, suggesting that C. borealis possesses quantitative variation for self-incompatibility and (or) that the variation reflects different amounts of embryonic abortion of selfed zygotes as a result of inbreeding depression. A detailed analysis of the complete reproductive cycle in Clintonia borealis, from pollination to seed set, is required to distinguish between incompatibility phenomena and inbreeding depression. In the former case, we are dealing with a mechanism operating in maternal tissue and controlled by its genotype and that of the pollen or pollen donor. In contrast, inbreeding depression is a process acting in the progeny zygote determined by its own genotype. Although it is conceptually straightforward to distinguish between these factors, in practice it may not always be clear which of these processes is responsible for the reduced seed set after selfing as opposed to outcrossing. Further discussion of the difficulties in distinguishing incompatibility phenomena from inbreeding depression are reviewed in Seavey and Bawa (1986) and Barrett (1987).

In addition to our own studies of dioecism in populations of Aralia nudicaulis from central New Brunswick (Barrett and Helenum 1981; Barrett and Thomson 1982), Bawa et al. (1982) and Flanagan and Moser (1985) have investigated various aspects of the reproductive biology of this species in Massachusetts and Alberta, respectively. Flanagan and Moser (1985) provide evidence, based on controlled cross-pollinations, that at certain periods during the blooming period, inflorescences are pollen limited.

Amounts of fruit-set varied greatly among the 12 species that were examined. In general, values in 1979 were lower than in 1978. This may have been the result of unfavourable conditions for pollination and fruit maturation and (or) reduced pollinator visitation as a result of the spray programme. Unfortunately, quantitative information on pollinators was not collected and the meteorological information for the 2 years (see Helenum and Barrett 1987) is not particularly informative. Comparison of fruit-set levels in the five obligately outbreeding herbs is of interest. Although Aralia nudicaulis had high percentage fruit-set in 1978 and 1979, the four self-incompatible species all displayed less than 50% fruit-set in both years (Fig. 1). All these species, with the exception of Medeola virginiana, flower during the same period, have small, white flowers, and are visited by similar pollinators. The high flower:fruit ratios of the self-incompatible species may result in part from selection for increased flower production to enhance pollen removal and male success (Willson and Burley 1983). According to this hypothesis, self-incompatible species maintain a significant number of flowers which function solely as males. However, females of dioecious species are not subject to these selective pressures and, as a result, a greater proportion of flowers may set fruit. Survey data of the fecundity of dioecious and self-incompatible species support this interpretation (Sutherland and Delph 1984) and it may help to explain the differences in fruit-set among some of the outbreeders observed in our study.

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Appendix 1

The following are a list of insects collected from the flowers of boreal forest herbs at Doaktown, central New Brunswick, during May—August 1979. The number of individuals of each taxon caught is indicated in parentheses.

1. Aralia nudicaulis (Araliaceae); total taxa 17, total individuals 66
   Coleoptera: Stephylidae; Eusphaelurrum convexus Fw. (7), E. pothos Mann (2), Eusphaelurrum spp. (31). Nitidulidae; Catelates (Palliion) pennis (Murray) (1). Cerambycidae; Trachysydes aspera brevifrons (Hodg.) (1). Diptera; Syrphidae; Mallota posticata Fab. (1), Chelisota tirsitis LW. (1). Hymenoptera; Apidae; Bombus terricola Kirby (2 queens), B. perplexus Cress. (3 workers), B. vagans Smith (8 queens). Halictidae; Halicus rubicundus (Christ.) (1), Dialisit spp. (1), D. cressoni (Rbsten.) (3), D. imitatus (Smith) (1), Euryalaque quebecensis (Cwfd.) (1). Andrenidae; Andrena w-scripta Vier. (1), A. wheeleri Gracn (1)

2. Chiminula umbrallata (Pyraloidea); total taxa 3, total individuals 70
   Diptera; Milichidae; Paramyia nitens LW. (61). Hymenoptera; Apidae; Bombus terricola Kirby (4 workers), B. vagans Smith (4 workers, 1 male)

3. Clintonia borealis (Liliaceae); total taxa 15, total individuals 24
   Coleoptera; Cerambycidae; Pidonia ruficornis (Say) (1). Diptera; Syrphidae; Parasyrophus relictus Zett. (2), Carposcelis confusus Cn. (1), Spyphaeophora sp. (1), Sarcophagidae; Sarcophaga nearctica Park (1). Hymenoptera; Apidae; Bombus terricola Kirby (3 queens), B. vagans Smith (6 queens), B. ternarius Say (2 queens). Halictidae; Dialisit spp. (1), D. cressoni (Rbsten.) (1), D. imitatus (Smith) (1), Andrenidae; Andrena wiktella (Kby.) (1). Megachilidae; Osmia lignaria Say (1). Cntrabridae; Rhopalum sp. (1). Lepidoptera; Noctuidae; Poanes hormomobok (Harris) (1)

4. Cormus canadensis (Cormaceae); total taxa 65, total individuals 169

5. Gyrispredium acaula (Orchidaceae); total taxa 2, total individuals 8
   Coleoptera; Staphylidae; Eusphaelurrum spp. (6). Hymenoptera; Apidae; Bombus vagans Smith (2 queens)

6. Linnaea borealis (Caprifoliaceae); total taxa 21, total individuals 37
   Coleoptera; Staphylidae; Eusphaelurrum spp. (2). Diptera; Bombyliidae; Euclymis harrisi (O.S.) (3). Syrphidae; Carposcelis confusus Cn. (1), Spyphaeophora rossum Fab. (1), Spyphaeophora bifurcata Knut. (1), Spyphaeophora sp. (1), Melangyna lasiopthalma Zett. (1). Muscidae; Phaonia serva (Meig.) (2), Fannia unguulata Chll. (2). Hymenoptera; Apidae; Bombus vagans Smith (1 worker). Colletidae; Hylaeus stennis Ckl. (1), E. ellipticus (Kby.) (5). Halictidae; Dialisit spp. (6), D. cressoni (Rbsten.) (1), Euryalaque quebecensis (Cwfd.) (3). Megachilidae; Osmia proxima Cress. (1), O. pulissa Cress. (1). Cranidae; Cerbrob (1). Pemphredonidae; Passalococcus sp. (1). Tenthredinidae; Zeraea americana Cress. (1). Lepidoptera; Noctuidae; Euphyes vestris metacomment (Harris) (1)

7. Maianthemum canadense (Liliaceae); total taxa 26, total individuals 85
   Coleoptera; Staphylidae; Eusphaelurrum sp. (1). Elateridae; Agiotes stabilis (LeC.) (2). Diptera; Bombyliidae; Bombylius major (L.) (3), B. pygmaeus (1). Syrphidae; Parasyrophus relictus Zett. (41), Carposcelis confusus Cn. (1), Spyphaeophora sp. (1), Spyphaeophora bifurcata Knut. (1), Melangyna umbelitarum Fab. (1), Orthoneura pulchella Will. (1), Tropidula quadrala Say (2), Chelisota slossoana Shan. (1), Xylota hinei Cn. (1). Anthomyiidae; Eremomyioides setosa (Stevea) (1), Pogodyelmyra digax (Mg.) (1). Hymenoptera; Apidae; Bombus vagans Smith (1 queen). Halictidae; Halicus rubicundus (Christ.) (1), Dialisit spp. (14), E. divergens (Lovell). (1). Andrenidae; Andrena nivalis Smith (1), A. miranda Smith (1), Anthophoridae; Nomaed Eunana (Rbsten.) (1). Lepidoptera; Noctuidae; Phalaenophora pyramusalis Willk. (1). Homoptera; Cikadellidae; Thamnotettix conifinis (Zett.) (1)

8. Medeola virginiana (Liliaceae); total taxa 1, total individuals 1
   Ephemeroptera; Ephemerella sp. (1)
9. *Oxalis montana* (Oxalidaceae); total taxa 6, total individuals 81
 COLEOPTERA: Staphylidae; *Eusphalerum convexum* Fvl. (1), *E. pothos* Mann. (11), *Eusphalerum* spp. (64).
 DIPTERA: Syrphidae; *Melanostoma* sp. (2), *Cheliosia pallipes* Lw. (2). HYMENOPTERA: Halictidae; *Dialictus* sp. (1)

10. *Pyrola secunda* (Pyrolaceae); total taxa 4, total individuals 4
 HYMENOPTERA: Apidae; *Bombus vagans* Smith (1 male). Halictidae; *Dialictus* sp. (1). Megachilidae; *Hoplitis producta* Cress. (1). Vespidae; *Vespula arenaria* Fabr. (1)

11. *Trientalis borealis* (Primulaceae); total taxa 3, total individuals 3
 DIPTERA: Syrphidae; *Carposcelis confusus* Cn. (1), *C. obscura* Say (1), *Sphaerophoria bifurcata* Knut. (1)

12. *Trillium undulatum* (Liliaceae); total taxa 4, total individuals 4
 COLEOPTERA: Buprestidae; *Anthisia expansa* LeC. (1). DIPTERA: Syrphidae; *Carposcelis confusus* Cn. (1), *Sphaerophoria bifurcata* Knut. (1). HYMENOPTERA: Halictidae; *Dialictus* sp. (1)