Developing methods of monitoring techniques to measure insecticidal impacts on solitary bees and the fecundity of selected forest wildflowers.

File Report No. 82 February 1987

K.N. Barber and P.D. Kingsbury

Forest Pest Management Institute
Canadian Forestry Service
Sault Ste. Marie, Ontario
P6A 5M7

GENERAL INTRODUCTION.

The role of native pollinators in pollination is well known for many species of native forest plants but as yet undetermined in many more (Kevan 1975a, NRCC 1981). The vulnerability of these pollinators to chemical insecticides applied to control insect pests of timber stands has been demonstrated (Plowright and Rodd 1980, Thomson *et al.* 1985). Concern over potential reductions in plant reproduction and food production for wildlife has stimulated research of means to monitor and minimize such non-target impacts of forestry practices (review in NRCC 1981, Thaler and Plowright 1980, Barrett and Helenurm in prep., Thomson *et al.* 1985, Somer 1985, Hansen and Osgood 1984, Plowright *et al.* 1980).

The present study was conducted to provide some preliminary investigation of two aspects of insecticidal impacts of pollinators. The major objective of the work was to identify and extend the list of plant species which are suitable candidates for monitoring indirect impacts on plant fecundity. Secondarily, the development of an alternative method of using solitary bees as impact monitoring tools in portable nesting 'sandbox' units was initiated. These two parts of the study are reported separately.

PLANT FECUNDITY AND POLLINATORS.

Introduction.

Relative measures of fruit-set and/or seed-set in pollinator-dependent plants can provide an indirect measure of pollinator activity in sprayed *versus* unsprayed areas (Miliczky and Osgood 1979, Thomson *et al.* 1985, Thaler and Plowright 1980, Somer 1985). Before similar work can proceed with any degree of confidence on other candidate plant species, they must be critically assessed as to their relative reproductive response to isolation from pollinators. The more dramatic the depression of reproductive output of a species is when restricted from access by pollinators, the more likely is a similar response to be of a measurable level in the usually less dramatic situation where pollinators have been impacted by insecticides. As well, the greater the specializations of the plant-pollinator relationships (i.e. approaching monolectic/monophilous) the greater are the risks of incurring impacts.

Barrett and Helenurm (in prep.) provide the most recent and critical assessment of the role of insects in the pollination of several species of plants occurring in New Brunswick, while other less rigorous studies have been conducted in the northwestern United States (Robinson and Johansen 1978, Olson-Elliott 1978). This aspect of the study attempts to extend the list of potential indicator plant species by investigating the effects of pollinator-exclusion and manual pollinations on fruit-set and seed-set, as well as qualitatively assessing the spectrum of pollinators associated with each plant species. Baseline

data generated from this preliminary study will guide the future development of implementation protocols for appropriate sentinel plant species.

Materials and Methods.

Study sites.

The study was carried out during the 1986 season in or near the Icewater Creek watershed research area located about 50 km north of Sault Ste. Marie, Ontario. Since 1980, this area has been utilized for long-term environmental impact research by personnel of the Environmental Impact Project, Forest Pest Management Institute, through the cooperation of the Sault Ste. Marie District of the Ontario Ministry of Natural Resources.

The majority of the botanical work was accomplished in a mixed forest predominated by *Acer saccharum* Marsh. (Sugar Maple) where four species of woodland lilies (Liliaceae) were studied -- *Trillium cernuum* L. (Nodding Trillium), *Streptopus roseus* Michaux (Rose Twisted Stalk), Polygonatum pubescens *(Willd.) Pursh (Hairy Solomon's Seal)*, and *Smilacina racemosa* (L.) Desf. (Racemed False Solomon's Seal). *Heracleum lanatum* Michx. (Cow Parsnip; Apiaceae) was studied in a dense patch on the edge of an open meadow overlooking the Goulais River near the mouth of Icewater Creek. *Sorbus americana* Marsh. (American Mountain-ash; Rosaceae) occurred sporadically on a sandy upland site described by Kingsbury *et al.* (1980; site 1) and predominated by *Populus tremuloides* Michx. (Trembling Aspen) and *Prunus pensylvanica* L.f. (Pin-cherry). The last site was several miles east of the others and consisted of a low, open, hummock area where *Vaccinium myrtilloides* Michx.(Velvet-leaf Blueberry; Ericaceae) grew in a mixed population with the more predominant *V.angustifolium* Ait. (Low Sweet Blueberry), and together provided the dominant cover. Other vegetation on this site included other ericads and sphagnum.

Field Methods

The seven species of plants studied were primarily selected on the basis of availability on the study sites. As well, selections were made to provide a range of inflorescence and habitat types, and flowering period (Fig. 1). *Vaccinium myrtilloides* was included because its fruit-set is well known to be dependent upon pollinators and preliminary study would provide a baseline for future experimental studies in the Icewater Creek watershed research area. *Heracleum lanatum* was included to replace *Sorbus americana* which suffered extensive frost damage early in its flowering period.

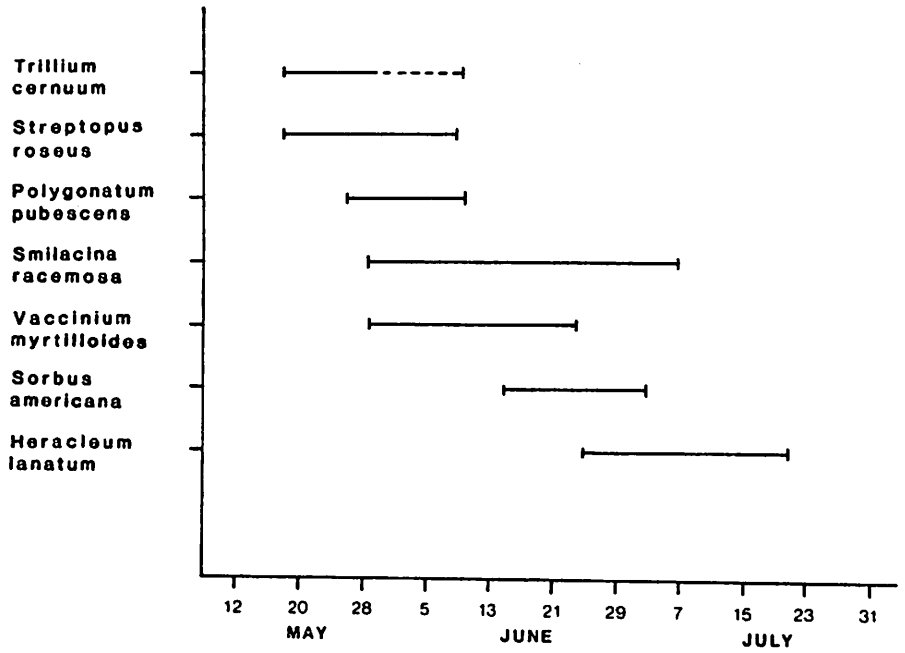


Figure 1. Flowering periods for seven species of plants in or near the Icewater Creek watershed, 1986 (dashed line indicates gradual, indiscrete senescence of corolla).

The experimental procedures used were classical pollinator-exclusion experiments accompanied by manual pollinations and qualitative observations and collections of pollinating insects. Each species displayed particular attributes (Table I) which required specific considerations in manipulation and analysis. These are discussed separately after the following general discussion.

Pollinator-exclusion Experiments.

Bags were made from 100% white polyester fabric which was stitched to form a tube of an appropriate diameter and closed at one end. Mesh openings were about 1.0x0.5mm. (Caprice) except for *H. lanatum* where the openings were about 0.3x0.3mm. (drapery sheer). These were secured on the plant with a paper and wire twist-tie.

Individual plants (flowering stems) were recruited into the experiments when encountered during a hike through the respective sites. Approximately 75% were bagged after the individual flower buds were determined to be closed but nearing blossom. Plants with no flower buds were overlooked, and in *S. roseus*, those individuals subsequently found to have only staminate flowers were excluded from the experiment. The remaining 25% of the plants were left unbagged. Each plant was marked with a numbered section of coloured flagging tape and its position mapped for future reference. The bags on the four species of lily were supported with crossed twigs pushed into the soil.

Table I. Attributes of seven plant species studied in or near the Icewater Creek watershed, 1986.

ATTRIBUTE	SPECIES						
	T.cernuum	*S.roseus*	*P.pubescens*	*S.racemosa*	*V.myrtilloides*	*S.americana*	*H.lanatum*
gender	monoecious	andro-monoecious [a]	monoecious	monoecious	monoecious	monoecious	andromonoecious protandrous [b]
flowers per plant	1	several total(2-56) hermaph.(1-36)	several (1-16)	many (ca.10-200)	many (ca.100-200)	very many (ca.1000-10000)	very many ca.400-10000 [b]
inflorescence type	solitary	solitary or doubles	solitary, doubles, triples	panicle	racemes	corymbiform cyme	compound umbels
locules or cells per fruit	3	3	3	3	4-5	2-4	2
ovules per fruit	many (6-164)	many (12-22)	several (4-12)	6 (NR)	many (NR)	? (NR)	2 (NR)
fruit type	pink berry	red, subglobose berry	blue-black, globose berry	speckled red on greenish globose berry	blue,globose berry	orange-red pome	2, 1-seeded mericarps
seeds per fruit	many (0-105)	many (1-16)	several (0-12)	usually 1-2 (0-3)	many (NR)	4-8 (NR)	usually 2 (0-2)
fruit requires dissection	yes	no	no	yes	no?	no?	yes
study site	forest	forest	forest	forest	blueberry bog	open poplar	meadow edge

Sources: Gleason and Cronquist (1963).
Fernald (1950).
bracketed figures - this study.

[a] this study.
[b] Hendrix (1984).
NR - not recorded.

The experimental unit varied from species to species ranging from all the flowers on a plant to only a portion of an inflorescence. Portions of inflorescences were marked with short sections of twist-tie. The individual flowers of the experimental unit were then enumerated and, where possible, mapped to enable the fate of the individual flowers to be followed. Those flowers dislodged or damaged while handling were removed from the tallies of treated flowers where possible.

The treatments applied were: *bagged x not manually pollinated* (bagged), *bagged x manually self-pollinated* (self-pollinated), *bagged x manually cross-pollinated* (cross-pollinated), and *not bagged x not manually pollinated* (open-pollinated) (see exceptions in *T. cernuum*, *P. pubescens*, and *S.americana)*. The open-pollinated plants were selected from the plants which had not been previously bagged. The other three treatments were assigned in a relatively *ad hoc* manner such that a running total of individual treatments guided subsequent treatment assignments. This procedure attempted to reduce the influence of local effects in the serpentine transect and sequence in which the plants were initially recruited. More than one treatment was assigned to individual plants or clones in *S. americana* and *V. myrtilloides*.

The manual pollinations were roughly timed to coincide with pollen availability within individual flowers and not from a direct measure of stigmatal receptivity. Donor pollen for cross-pollinations was obtained from other bagged or not bagged individuals at least five metres distant. This was particularly important with the rhizomatous lilies. Flowers included in the experimental units of the open-pollinated and bagged treatments were not used as sources of pollen but were left unmolested save for flower enumeration and assessment. Pollen for self-pollinations was usually taken from the same flower (autogamous pollinations) or from other flowers on the same plant (geitonogamous pollinations) but no distinctions were made between these two types of self-pollinations. Pollen was applied in excess to the stigmatal surface(s) either directly from excised anthers, or from forceps sprinkled with pollen, or from fingers sprinkled with pollen. Forceps were cleaned after each treatment by wiping on clothing and plunging the tips into the duff while fingers were wiped clean on clothing. The manual pollinations were carried out over a period of time as the flower buds opened and became available for treatment. Individual flowers were pollinated once except in those species *(S.racemosa, H. lanatum*, and *V. myrtilloides)* where individual flower mapping was not attempted and some repeat pollinations were required to ensure that all newly recruited flowers were pollinated.

Bags were removed as soon as stigmatal receptivity was judged to be past, usually using a visual assessment of the deteriorating condition or loss of the corolla or perianth. The plants were then left to develop but field evaluation of fruit-set and/or harvest of the fruits occurred in advance of full maturation to minimize subsequent losses to herbivores or frugivores.

Harvesting involved the collection of all the above-ground parts of the plants *(T. cernuum, S. roseus,* and *P. pubescens)* or only the infloresence including the experimental unit *(S. racemosa* and *H. lanatum)*. No harvesting nor subsequent dissections of the fruits were carried out with *S. americana* or *V. myrtilloides*. Generally, fruits were brought into the laboratory and kept at about 4-10°C until a convenient time for dissection, usually within a week of harvest. The fleshy fruits of *T. cernuum, S. roseus, P. pubescens,* and *S. racemosa* were dissected with forceps under a dissecting binocular microscope and the firm, inflated seeds enumerated. Undeveloped ovules, and a small number of intermediate classes of ovule development were scored as unsuccessful. A total ovule count was not taken for *S. racemosa* nor *H. lanatum* (see below).

Fruit-set is defined as the number of successful (usually seed-bearing) fruit as a proportion of the treated flowers per plant. "Observed" fruit-set refers to field-evaluated success rates judged by outward appearance of enlarging ovaries. "Harvest" fruit-set relies on the results of dissection to determine seed content and scores only seed-bearing fruits as successful (one seedless fruit incorporated in *P. pubescens)*.

Seed-set is defined as the number of seeds as a proportion of the ovules available per seed-bearing fruit (one seedless fruit in *P. pubescens)* and is generally computed on an individual fruit basis.

Trillium cernuum.

Bags were made large enough to enclose virtually the entire plant since the reflexed peduncle was considered too fragile to withstand the weight of a small bag and the application of a twist-tie. The enclosed leaves assisted in isolating the reproductive parts of the flower from the bagging material, thus minimizing abrasion by the bag as well as the possibility of pollinator contact through the bag. Since aging flowers changed little besides the gradual but considerable enlargement of the ovary over the experimental period (Fig. 1; dashed line), no clear indication of loss of stigmatal receptivity was evident. Therefore the bags were left in place for a period of time considered adequate to ensure loss of receptivity. This coincided with the maximum bagging period of *S.roseus* (Table II). For similar reasons, a successful fruiting event was not distinguishable from an unsuccessful one in the field and had to await the results of dissection.

In addition to the usual four treatments, a fifth was included which had an intentionally smaller sample size. This emasculated treatment consisted of gently opening a flower bud and plucking the anthers at a stage before pollen had begun to be shed. The plant was then bagged.

Two measures were made on this single-flowered species which were not carried out on the other species. A relative measure or index of leaf area was obtained by tracing the outline of one leaf on 0.4 cm^2 grid paper and then estimating the total number of grids to the nearest whole grid. Dry weight of each fruit after dissection, including the seeds, was obtained with a Sartorius balance after drying at 50°C for five days.

Streptopus roseus.

The entire plant was enclosed in a bag in the appropriate treatments.

An obvious dimorphism in the flowers necessitated floral mapping of each plant to enable determination of the fate of each flower. Flowers with a normally elongate style (hermaphroditic) were treated and subsequently monitored through the experimental period. A very few of the flowers with a shortened or no style (staminate) were treated but not considered in any analyses. The number of treated flowers is essentially equivalent to the number of hermaphroditic flowers less a small number of damaged or lost individuals.

Because of a cumulative loss of plants and fruits to herbivores, particularly of the open-pollinated treatment, five fruit-set estimates were calculated representing three variations of "observed" and two variations of "harvested" fruit counts. The figures are based on only those plants surviving to initial fruit count. The "observed 1" fruit-set was determined in the field (June 13) based on an evaluation of the enlargement of the ovary several days after the perianth had dropped. Twenty-one small fruits, which were difficult to classify, were later checked (June 29) and the original tallies modified to reflect the fact that eight were indeed fruits and eight had aborted (turned yellow) thus providing the "observed 2" fruit-set. A third, more conservative "verified observed" fruit-set codes the five missing small fruits as being unsuccessful or aborting. Thus these three fruit-set estimates are based on sequentially more conservative refinements of the same initial fruit count.

The "harvest 1" fruit-set is based on the same number of plants in the treatments as the three "observed" fruit-set measures. The "harvest 2" fruit-set is computed using only those plants surviving to harvest date.

Polygonatum pubescens.

The flowers of this species have a fused perianth tube and the relatively elongate stamens converge medially beyond the stigma. The anthers thus preclude clear access to the stigma. This is of prime importance with the cross-pollinated treatment where the intent is to provide only foreign pollen and not an admixture with self-pollen.

Rather than plow through the stamens with foreign pollen, or to introduce emasculations, a flap was ripped in the side of the flowers and folded back to gain access to the stigma. This procedure was applied to the two manual pollination treatments but necessitated the addition of a *bagged x slit x not pollinated* treatment in order to control for the effect of this partial dissection and to maintain the intact *bagged x not pollinated* treatment for comparisons with the open-pollinated treatment.

Two measures of fruit-set were obtained - "observed" and "harvest". Sample sizes differed considerably in this trial resulting from an accumulation of bagged and open-pollinated plants at one end of the study area. These had been added (open-pollinated) or represented the residuum of bagged plants not recruited for manual pollinations (bagged). Again, bags enclosed the entire plant.

Smilacina racemosa.

Bags were applied only to the solitary terminal inflorescence but included the terminal one to three leaves to assist in supporting the bag and reducing contact with the flowers.

The flowers on only the basal one to three branches of the inflorescence were enumerated and treated. This experimental subunit of the inflorescence was selected since these flowers were the first to mature and could provide an objective comparative base between plants. There was an aim to incorporate about 20 flowers into each experimental unit and not a predetermined proportion of the total flowers or inflorescence branches. The number of flowers on non-experimental parts of the inflorescence were later estimated by counting fruits and scars left by dehiscence of flowers or groups of flowers.

Three fruit-set estimates were obtained. Two of these were based on field evaluations, the initial "observed 1" fruit-set (23 June) and the "observed 2" fruit-set estimated on date of havest (21 July), with no reference made to seed content. The entire inflorescence was harvested and those fruits derived from experimental flowers were dissected to provide a "harvest" fruit-set based on seed-bearing fruit. Nine of the 219 harvested ("observed 2") fruits dislodged during handling along with several others from the non-experimental portions of the infructescences. Seed counts were thus made on only the 210 unconfounded experimental fruits. Total ovules were not counted but are not expected to be the limiting factor in determining the number of seeds. Rather the fruits appear to be limited to a maximum of three seeds per fruit although having six ovules (Table I). Thus, seed-set is computed as the number of seeds per fruit divided by a constant of three (maximum seed potential per fruit). This was of particular concern for implementation of the G-test (see discussion of "Analysis" below).

Vaccinium myrtilloides.

The sturdiness and growth pattern of this species allowed the application of four treatments on a single plant or clone. Four flower-bearing branches were chosen on which ten to twenty flowers were enumerated, and three of these branches were bagged.

Ripe anthers of this species release dry pollen though pores which necessitated tapping the flowers with a fingertip to apply manual pollinations to the sticky stigma. Because of the efficiency of active pollinators, donor pollen for the cross-pollinations had to be obtained from the bagged non-experimental flowers of the manually pollinated treatments on other distant individuals or from additional plants bagged expressly for the purpose of supplying pollen.

Fruit-set was evaluated in the field only once and no harvest nor subsequent fruit dissections or seed-set estimates were made.

Sorbus americana.

Initially, this species was handled in such a fashion that one to three multiples of four treatments were applied to each of twelve plants or clones, one treatment per inflorescence. The bags were placed over individual inflorescences and included one or two of the terminal leaves. A sub-perimeter portion of each inflorescence was tagged and counted. However, before manual pollinations could be applied, a large number of staphylinid beetles (probably *Anthobium* sp.) were found to be so strongly attracted to the flowers that they became 'gill-netted' in the open mesh of the bags. A significant number of the beetles successfully entered the bags and penetrated between the overlapping petals of the flower buds to contact the reproductive parts. The experiment was readjusted to eliminate the time-consuming manual pollinations and all bagged inflorescences were then pooled as a 'bagged' but selective pollinator-exclusion treatment. An additional twelve plants were then recruited to increase the sample size of the open-pollinated treatment.

Fruit-set was determined only once based on a count of fruits in the field. No harvest of fruits nor dissections were made.

Heracleum lanatum.

Only the primary terminal umbel on each plant was investigated and a subumblet located just medial to the perimeter subumblets was chosen as the experimental unit after it was judged to be healthy and undamaged. The individual umbels were bagged while still ensheathed by the leaf bases requiring frequent readjustment of the bags during the subsequent growth period prior to pollen availability. The tighter mesh of the drapery sheers was used to avoid the problems of beetle penetration encountered with *S.americana.* In addition, surveillance was maintained for presence of and damage caused by *Depressaria pastinacella*

(Duponchel) (Lepidoptera: Oecophoridae), the Parsnip Webworm. Caterpillars and webbing were removed as discovered and, where damage occurring below the experimental unit was considered severe, the plant was removed from the study if an adequate replacement subumblet was unavailable.

Fruiting success was evaluated only in the laboratory since a continuous range of size and condition of fruits was evident. The entire umbel was harvested and the fruits removed from the experimental subumblet. Where necessary, the larger fruits were split apart and each of the two mericarps was examined on a light table for the presence of a developing seed. Only those fruits bearing at least one developing seed were considered successful.

Pollinators

Insects visiting the various flowers were collected as encountered with an aim to obtain representatives of each species in a non-quantitative manner. An insect was considered to be visiting a flower if it alighted on its surface and did not immediately move away. These were aspirated or netted and immediately killed with cyanide vapours. In the laboratory, each specimen was mounted and later identified, where possible, to the level of family, genus, or species. For the purposes of this study, these specimens are referred to as pollinators with the qualification that actual participation in or contribution to the process of pollination has not been demonstrated (see review by Kevan and Baker 1983).

Analysis.

Data analyses were performed with a Digital VAX 8500 computer primarily using program packages of BMDP. A one-way analysis of variance was performed upon selected parameters measured on a per plant (fruit-set) or per fruit (seed-set) basis (unless otherwise stated), accompanied by the non-parametric Kruskal-Wallis H-test. Where data could be scored in a binary fashion (e.g. hermaphroditic flower *vs.* staminate flower; fruit *vs.* not fruit; seed *vs.* not seed) an unplanned test of the homogeneity of replicates (G-test) was computed following the procedure described by Sokal and Rolph (1981; Box 17.5; 2 X C program). The G-test, however, analyzes fruit-set on a per flower basis and seed-set on a per ovule basis and are not directly comparable to those of the ANOVA (per plant and per fruit bases, respectively).

The recommendations made by Milliken and Johnson (1984) guided the parametric analyses and are summarized here. An ANOVA was run with four embedded tests, first on the untransformed data. These were Levene's F-test of homogeneity of variance, Welch's F-test to compare means, Bonferroni's multiple comparisons test, and a natural logarithmic plot of the mean against standard deviation. Data transformation

selections, where necessary, were guided by the recommendation of Box and Cox (1964) to minimize the dependence of the standard deviation on the mean (slope of natural logarithmic plot) as well as to maximize homogeneity of variance (Levene's test). Analyses of only arcsine-, square-root-, natural logarithm-, and reciprocal-transformed data were attempted and compared with each other and that of the untransformed data. Once an optimal balance of homogeneity of variance and independence of the means and standard deviations was achieved, Bonferroni's multiple comparisons test was applied. If heterogeneity of variance could not be removed with transformation, Welch's test was used to compare means and a G-test employed. Alternatively, the source of the heterogeneity was located and excluded to allow computation of a parametric analysis on the data subset leading up to Bonferroni's multiple comparisons test.

Bonferroni's test was applied even when the ANOVA F-test was significant. This test is more conservative than the recommended Fisher's LSD (Milliken and Johnson 1984) and was considered appropriate for the purposes of screening prospective indicator plant species. An appropriate indicator species will show dramatic depressions in reproductive success (see "Interpretation" below) in this experimental design but considerably less dramatic depressions in an insecticidal impact situation. The more conservative approach of Bonferroni's test to recognizing statistically significant differences will enhance assessment of biologically dramatic differences in reproductive success of these plants.

The means and standard deviations are tabulated and/or figured only as computed on the untransformed data and the statistics and multiple comparisons based on transformed data are reported in the text or figured below the untransformed means and standard deviations. The reported confidence levels of 95 and 99% for Bonferroni's test represent minimum levels of confidence, or maximum levels of experiment-wise error rates. This is because the actual alpha value cannot be determined but is usually much less than the selected test alpha (Milliken and Johnson 1984). The comparison-wise error rate equals the alpha level divided by the number of comparisons made.

Interpretation.

A hypothetical model response of a plant subjected to the four treatments described above is graphically depicted in Fig. 2. This pattern of response indicates two things. First, the bagged plants are less successful than the open-pollinated plants. The cross-pollinated treatment provides a control for possible lethal effects of the bags and in this example indicates these to be negligible, thus implicating a primary role of pollinators excluded by the bags. Second, a comparison of the differential response to exposure to self-pollen or foreign (cross) pollen gives a relative measure of self-incompatibility. The example demonstrates a high level of self-incompatibility since the

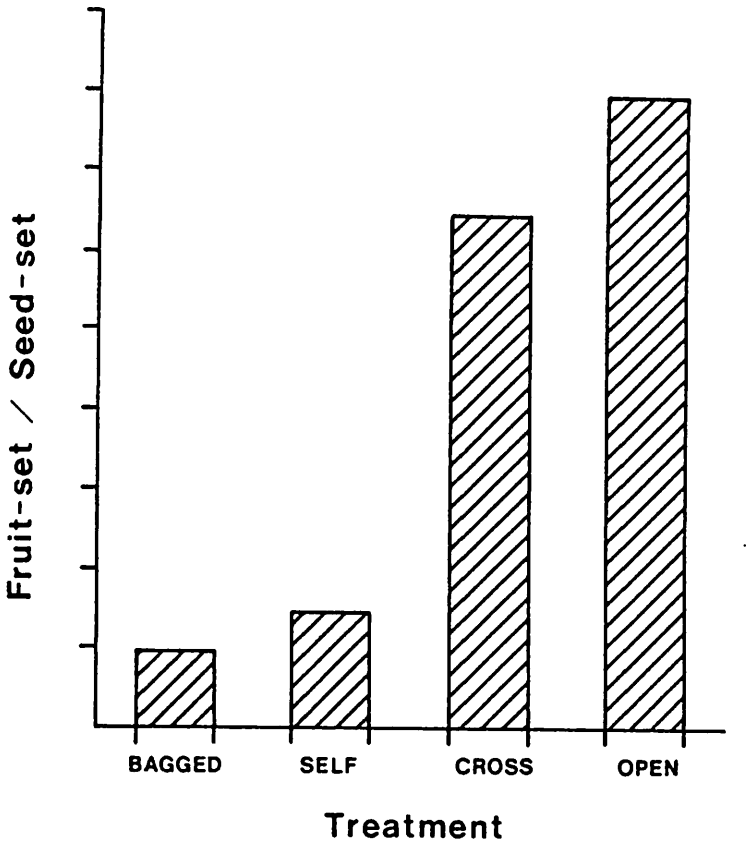


Figure 2. Model response of hypothetical pollinator-dependent plant with low level of self-compatibility.

self-pollinated plants are less successful than the cross-pollinated plants. This further implicates a dependency upon pollen vectors to provide more highly compatible foreign pollen. Thus the model response we are searching for is a bimodal one -- low success rates in bagged and self-pollinated plants and high success rates in cross- and open-pollinated plants.

Results.

Table I summarizes some pertinent anatomical and ecological characteristics of the seven plant species. Table II displays the sequence of events through the duration of the experiments. Table III provides a coarse comparison of the estimated numbers of species and taxonomic distribution of insects visiting each species of plant. The results of the bagging experiments and observations of pollinators are discussed separately below.

Table II. Experimental procedures applied to plant species in or near Icewater Creek watershed, 1986.

EVENT/PROCEDURE	SPECIES						
	T.cernuum	*S.roseus*	*P.pubescens*	*S.racemosa*	*V.myrtilloides*	*S.americana*	*H.lanatum*
treatments per plant	1	1	1	1	4	1-2	1
replicates per plant	1	1	1	1	1	1-15	1
bagging	May 15	May 15	May 22-23	May 22-23	May 27	June 4	June 23
first blossoms	May 18	May 18	May 26	May 29	May 29	ca.June 15	ca.June 25
first pollen	May 21	May 22	May 27	June 2	May 29	ca.June 17	July 1
manual pollinations applied	May 21	May 22-26	May 27-June 4	June 5-18	May 30-June 19	NA	July 2-9
first blossoms finished	? [a]	ca.May 30	May 29	ca.June 7	June 2 (frost)	June 19 (frost June 16)	July 7
bags removed	June 10	June 3-9	June 7-10	June 23-July 7	June 10-24	July 3	July 9-21
preliminary fruit count	NA	June 13/29	June 24	June 23	August 1	July 15	NA
fruits harvested	July 10	July 10	July 21	July 21	NA	NA	July 28-August 1
fruits ripe in field	late August	early August	mid September	late August	mid August	late August	mid August

[a] gradual, indiscrete senescence of corolla precluded estimation.

Table III. Estimated number of species for each taxon of potential pollinator for each plant species (excluding *T.cernuum* for which there were no pollinators observed) in or near the Icewater Creek watershed, 1986.

POLLINATOR TAXON	PLANT SPECIES					
	S.roseus	*P.pubescens*	*S.racemosa*	*V.myrtilloides*	*S.americana*	*H.lanatum*
DIPTERA						
Syrphidae	1	1	6	2	10	34
Muscidae			2		3	5
Asilidae				1		
Milichiidae					1	
Calliphoridae					1	1
Tachinidae						8
Empididae						4
Ephydridae						3
Anthomyiidae						3
Ceratopogonidae						3
Chloropidae						3
Phoridae						2
Stratiomyidae						1
Therevidae						1
Tabanidae						1
Sarcophagidae						1
Simuliidae						1
Sciaridae						1
Scatopsidae						1
Lonchaeidae						1
Psilidae						1
Total Diptera	1	1	8	3	15	75
HYMENOPTERA						
Apidae		2		4	1	3
Andrenidae				2	2	5
Halictidae				2	1	3
Vespidae				3		2
Megachilidae				1		
Ichneumonidae					1	3
Formicidae				1		1
Braconidae					1	1
Pompilidae					1	1
Larridae						3
Pemphredonidae					1	
Colletidae						1
Gasteruptiidae						1
Chrysididae						1
Argidae						1
Pteromalidae						1
Eurytomidae						1
Scelionidae						1
Eucoilidae						1
Total Hymenoptera	0	2	0	13	8	30

(continued)

Table III. Estimated number of species for each taxon of potential pollinator for each plant species (excluding *T.cernuum* for which there were no pollinators observed) in or near the Icewater Creek watershed, 1986. (concl.)

POLLINATOR TAXON	PLANT SPECIES					
	S.roseus	*P.pubescens*	*S.racemosa*	*V.myrtilloides*	*S.americana*	*H.lanatum*
COLEOPTERA						
Cerambycidae			2		1	8
Cantharidae			2		1	1
Mordellidae			1		1	2
Staphylinidae			1		1	1
Oedemeridae					1	1
Elateridae					1	1
Scarabaeidae					1	1
Chrysomelidae					1	1
Buprestidae			1			
Nitidulidae			1			
Pedilidae						1
Dermestidae						1
Lampyridae						1
Total Coleoptera	0	0	8	0	8	19
HEMIPTERA						
Miridae						2
Pentatomidae						2
Psyllidae						1
Total Hemiptera	0	0	0	0	0	5
LEPIDOPTERA						
Papilionidae						1
Nymphalidae						1
Noctuidae						1
undetermined			1			
Total Lepidoptera	0	0	1	0	0	3
THYSANOPTERA						
undetermined	1			1		
Total Thysanoptera	1	0	0	1	0	0
MECOPTERA						
Panorpidae						1
Total Mecoptera	0	0	0	0	0	1
Total Insecta	2	3	17	17	31	133

Trillium cernuum.

Table IV summarizes the results for the five treatments applied to this species.

The leaf area index did not differ significantly among the five treatments (1/X-transformed: ANOVA F=0.95 P=0.445; H=9.13 P=0.058).

Fruit-set differed very significantly among treatments (untransformed: ANOVA F=11.57 P=0.000; H=26.16 P=0.000) and was attributed by Bonferroni's test entirely to the singularly low level of the emasculated treatment at the 95% and 99% (Fig. 3). The G-test supported this singularity of the emasculated treatment at the 95% level but could notdistinguish it from the self- and cross-pollinated treatments at the 99level.

Total ovules per fruit differed significantly among treatments whether computed based on all fruits (untransformed: ANOVA F=3.51 P=0.013; H=10.91 P=0.028) or on seed-bearing fruits only (untransformed ANOVA F=3.69 P=0.011; H=11.64 P=0.020). Bonferroni's test revealed thathe self-pollinated treatment was significantly higher than the bagged and the open-pollinated treatments at the 95% confidence level.

The seed-set measures based on only the seed-bearing fruits were not found to differ significantly among treatments by the parametric (Fig. 4) and nonparametric tests (arcsine-transformed: ANOVA F=1.24 P=0.308; H=4.77 P=0.311). However, the G-test at the 99% confidence level detected that the bagged treatment was less than all but the self-pollinated treatment and that the cross-pollinated treatment was greatethan the self-pollinated and the bagged treatments. At the 95% level, the bagged treatment was considered singularly lower than all others while the cross-pollinated treatment was greater than all but the emasculated treatment.

Dry weight and seeds per fruit (including seedless fruits) were highly correlated (r=0.96). There were highly significant differences among treatments in fruit dry weight (ln(100X)-transformed: ANOVA F=9.6P=0.000; H=20.14 P=0.001) and seeds per fruit (squareroot-transformed: ANOVA F=6.76 P=0.000; H=14.54 P=0.006). Bonferroni's tests indicated that these were entirely due to the emasculated treatment being lower than all others at the 95% and 99% levels for fruit dry weight and at the 95% level for seeds per fruit.

No insects were ever observed visiting the flowers of *T. cernuum.*

Table IV. Summary of bagging experiment with *Trillium cernuum* in the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT				
	Emasculated	Bagged	Self-pollinated	Cross-pollinated	Open-pollinated
treated flowers (=plants)	6	14	12	12	12
leaf area index (0.40 cm^2)	124.8 ± 32.4	117.2 ± 26.5	150.3 ± 36.3	134.1 ± 44.6	141.8 ± 38.8
fruit dry weight (mg.) [a]	0.012 ± 0.011	0.039 ± 0.023	0.082 ± 0.039	0.060 ± 0.037	0.062 ± 0.036
total ovules [a]	382	857	1219	801	762
total ovules/ fruit [a]	63.7 ± 19.7	61.2 ± 35.0	101.6 ± 32.3	66.8 ± 31.0	63.5 ± 30.1
seeds/ fruit [a]	5.8 ± 14.3	31.9 ± 22.4	58.3 ± 32.7	45.8 ± 28.8	41.4 ± 25.3
seed-bearing fruit	1	13	11	11	12
fruit-set [b]	0.167 ± 0.408	0.929 ± 0.267	0.917 ± 0.289	0.917 ± 0.289	1.000 ± 0.000
total ovules [c]	48	851	1164	752	762
total ovules/ fruit [c]	48.0 ± 0.0	65.5 ± 32.5	105.8 ± 30.2	68.4 ± 32.0	63.5 ± 30.1
seeds	35	446	700	549	497
seed-set [c]	0.729 ± 0.000	0.535 ± 0.208	0.615 ± 0.236	0.717 ± 0.133	0.631 ± 0.243

[a] includes seedless fruits (n= 6,14,12,12,12).
[b] seed-bearing fruit per treated flower.
[c] seed-bearing fruit only.

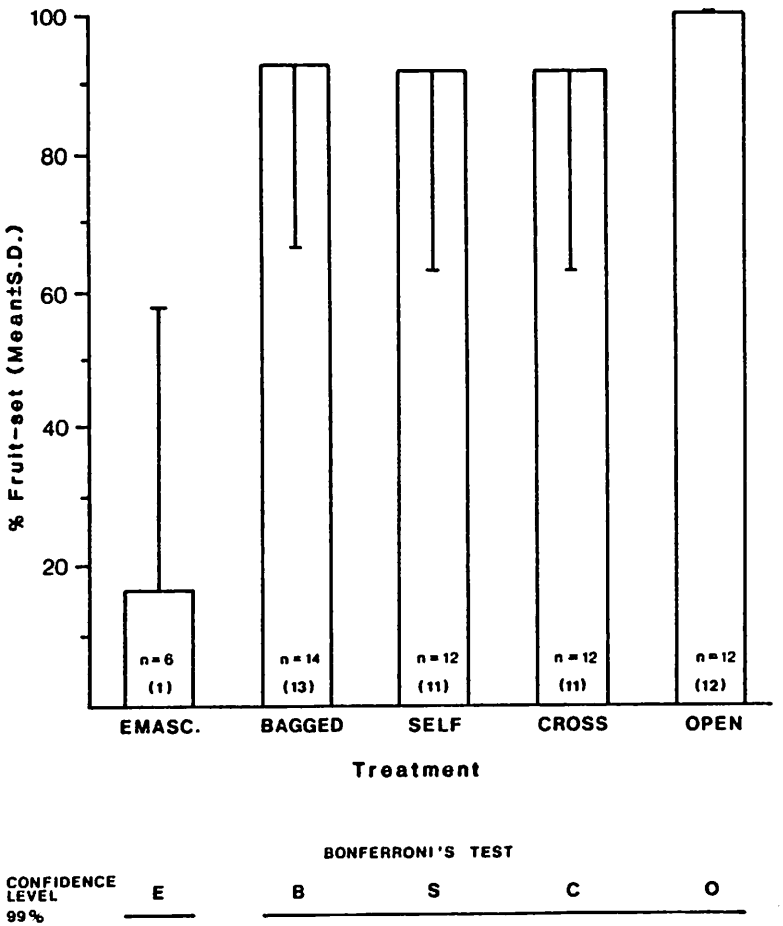


Figure 3. Harvest fruit-set in *Trillium cernuum* (n= number of plants or treated flowers; (a)= number of seed-bearing fruit).

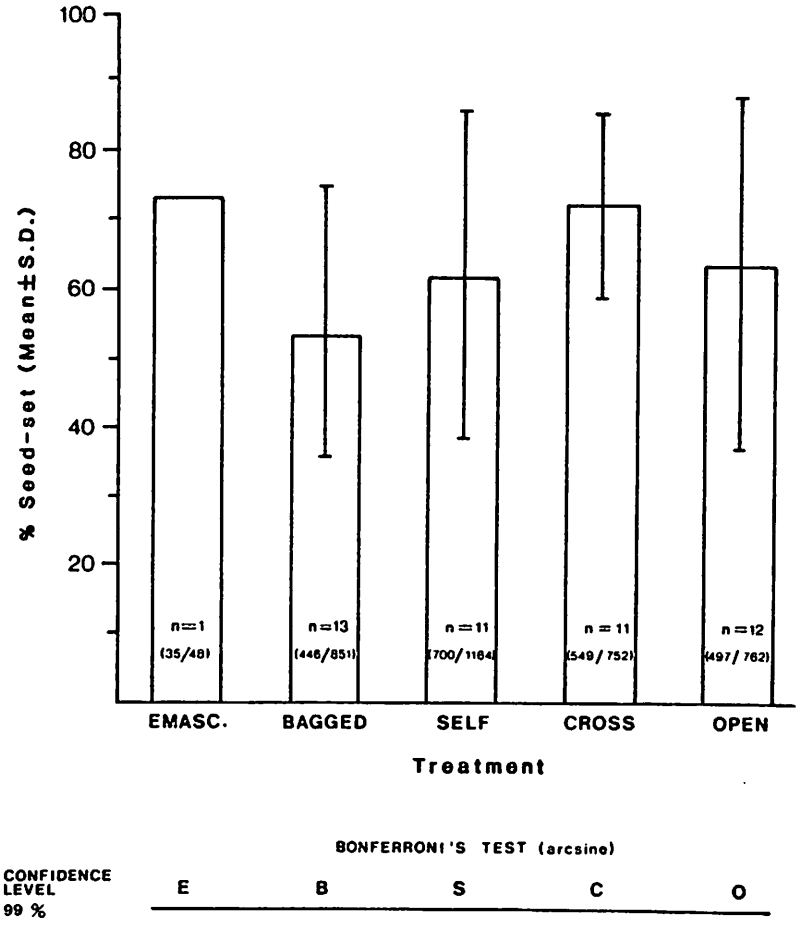


Figure 4. Seed-set in *Trillium cernuum* (n= seed-bearing fruit; (a/b)= seeds/ total ovules).

Streptopus roseus.

Table V summarizes the data for *S. roseus*. Neither total flowers (untransformed: ANOVA F=0.74 P=0.535; H=2.17 P=0.538) nor hermaphroditic flowers per plant (untransformed: ANOVA F=0.59 P=0.625; H=2.77 P=0.423) differed significantly among treatments. Although the differences in the ratios of hermaphroditic flowers to total flowers were considerably more significant (arcsine-transformed: ANOVA F=2.45 P=0.074; H=7.18 P=0.066) the G-test could not discern any significant differences even at the 90% level.

Analyses of the five fruit-set estimates were hampered by the large and heterogenous variances (Levene's tests $P \leq 0.0001$). Welch's and Kruskal-Wallis tests indicated significant differences among the treatments in the three "observed" fruit-sets ($P \leq 0.0002$). Ln(100X+1)-transformations satisfied Levene's tests (P=0.136-0.980) and the subsequent ANOVA's indicated highly significant differences among treatments (P=0.0000). Bonferroni's tests (95 and 99% levels) attributed this entirely to the significant difference between the two pairs of bagged and self-pollinated *versus* the cross- and open-pollinated treatments (Fig.5; "verified observed" fruit-set). The G-tests supported the results of the Bonferroni's tests.

The two "harvest" fruit-sets, however, pitted a significant Kruskal-Wallis test (H=17.10 P=0.001) against insignificant Welch's tests (P=0.21-0.22). Levene's tests could not be satisfied even with very strong transformations but maximization of independence of the mean and standard deviation did provide significant Welch's tests (harvest 1: F=3.19 P=0.044; harvest 2: F=4.05 P=0.023). The G-tests at the 99% level attributed this entirely to the cross-pollinated treatment being significantly greater than the bagged and self-pollinated treatments. Additionally, at the 95% level, the open-pollinated treatment was greater than the bagged treatment but was not distinguishable from the self-pollinated treatment.

No fruits were available for dissection in the bagged treatment. The following figures are based on all the harvested fruits except one misplaced and two damaged fruits (n=1,22,3) from one plant in the cross-pollinated treatment. Ovules per fruit did not differ significantly among treatments (untransformed: ANOVA F=0.42 P=0.662; H=1.64 P=0.440). The seed-set data indicated significant differences among treatments (arcsine-transformed: ANOVA F=6.66 P=0.005; H=6.00 P=0.050). Bonferroni's test attributed this entirely to the open-pollinated treatment being greater than the cross-pollinated treatment at the 99% level while at the 95% level the open-pollinated treatment was significantly greater than both the cross- and self-pollinated treatments (Fig. 6). The G-test singled out the open-pollinated treatment as being larger than the other two treatments at the 99% level and all three treatments were different from each other at the 95% level.

Table V. Summary of bagging experiment with *Streptopus roseus* in the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT			
	Bagged	Self-pollinated	Cross-pollinated	Open-pollinated
plants	18	18	17	6
total flowers	323	320	238	85
total flowers/ plant	17.9 ± 8.5	17.8 ± 11.7	14.0 ± 8.1	14.2 ± 10.1
treated flowers [a]	145	157	114	30
treated flowers/ plant [a]	8.1 ± 6.5	8.7 ± 8.5	6.7 ± 4.5	5.0 ± 6.8
treated flowers/ total flowers	0.388 ± 0.203	0.441 ± 0.202	0.494 ± 0.164	0.265 ± 0.181
observed 1 fruit [b]	8	11	66	11
observed 2 fruit [c]	5	9	63	11
verified observed fruit [d]	3	8	61	11
harvested fruit	0	1	25	3
plants surviving to harvest	12	12	12	5
observed 1 fruit-set [b]	0.059 ± 0.135	0.098 ± 0.257	0.598 ± 0.407	0.810 ± 0.401
observed 2 fruit-set [c]	0.044 ± 0.132	0.071 ± 0.237	0.529 ± 0.408	0.810 ± 0.401
verified observed fruit-set [d]	0.036 ± 0.121	0.066 ± 0.237	0.518 ± 0.405	0.810 ± 0.401
harvested 1 fruit-set	0.000 ± 0.000	0.056 ± 0.236	0.282 ± 0.404	0.333 ± 0.516
harvested 2 fruit-set [e]	0.000 ± 0.000	0.083 ± 0.289	0.400 ± 0.432	0.400 ± 0.548
dissected fruit [f]	0	1	22	3
total ovules	—	13	341	46
total ovules/ fruit	—	13.0 ± 0.0	15.5 ± 2.5	15.3 ± 4.0
seeds	—	1	138	39
seed-set 8	—	0.077 ± 0.000	0.406 ± 0.271	0.856 ± 0.125

[a] equivalent to the number of hermaphroditic flowers.
[b] field observations, 13 June - includes 8 fruits eventually aborting and 5 fruits of unknown fate.
[c] field observations, 29 June - includes 5 fruits of unknown fate.
[d] scores 5 fruits of unknown fate as unsuccessful.
[e] based upon only those plants surviving to harvest date (10 July).
[f] harvested fruit excluding two damaged and one lost fruits.
8 seeds per total ovules of seed-bearing fruit (=dissected fruit).

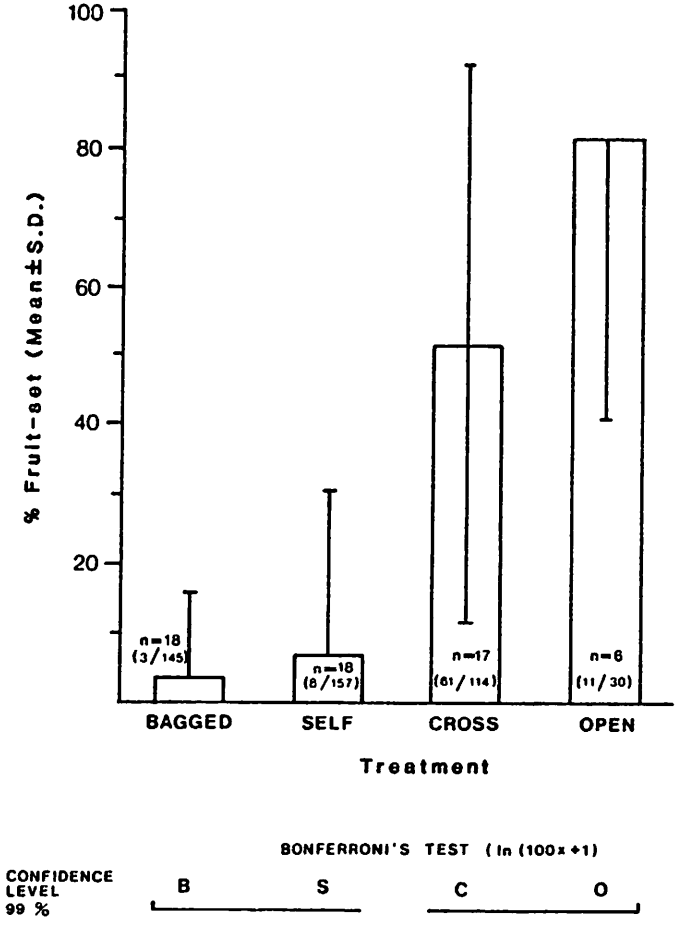


Figure 5. Pre-harvest ("verified observed") fruit-set in *Streptopus roseus* (n= plants; (a/b)= seed-bearing fruit/ treated flowers).

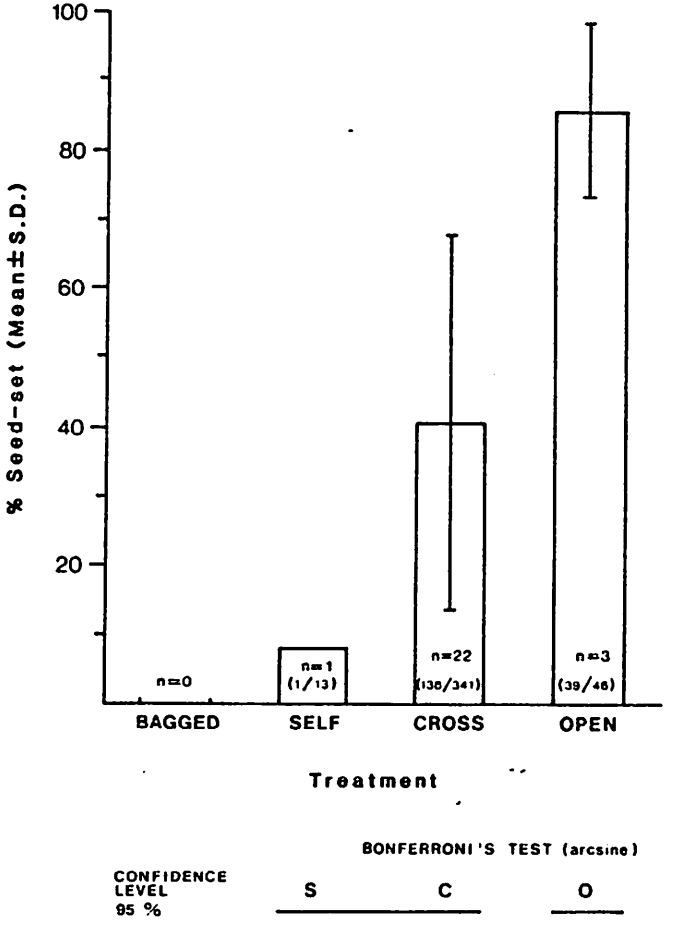


Figure 6. Seed-set in *Streptopus roseus* (n= seed-bearing fruit; (a/b)= seeds/ total ovules).

A single hover fly, *Dasysyrphus venustus* (Mg.), was observed visiting a flower of *S. roseus*. Thrips commonly occurred within the flowers of bagged and not bagged plants.

Polygonatum pubescens.

Table VI summarizes the data for *P.pubescens*. The total flowers (untransformed: ANOVA F=10.60 P=0.0000; H=32.98 P=0.0000) and treated flowers per plant (untransformed: ANOVA F=11.53 P=0.0000; H=34.97 P=0.0000) differed significantly among treatments. Bonferroni's tests attributed this primarily to the higher number of total flowers per plant in the bagged treatment than all other treatments except the slit treatment at the 95 and 99% levels. The treated flowers per plant reflected similar differences at the 99% level but at the 95% level the bagged treatment was greater than all other treatments including the slit treatment. These differences were only partially dampened by eliminating the data for additional plants in the bagged and open-pollinated treatments to provide more equitable sample sizes (n=15,14,15,15,15) (total flowers - untransformed: ANOVA F=4.08 P=0.005; H=14.41 P=0.006. treated flowers - untransformed: ANOVA F=3.97 P=0.006; H=13.38 P=0.010). Bonferroni's tests attributed this to a difference between only the extremes of the open-pollinated and the bagged treatments at the 99% level, while at the 95% level the open-pollinated treatment was also significantly lower than the slit treatment.

All harvested fruits except one from the open-pollinated treatment bore seeds. The analyses of fruit-set and seed-set incorporate this single seedless fruit in the respective data sets.

Difficulties arose in applying the ANOVA to fruit-set estimates whether calculated with the initial "observed" fruit or the final "harvested" fruit counts. Because of the relative invariability of the bagged, slit, and self-pollinated treatments, Levene's test revealed highly significant heterogeneity of variance despite transformations. However, in all analyses Welch's tests were highly significant ($P<0.001$) and Kruskal-Wallis tests also supported these findings (observed H=52.22 P=0.0000; harvested H=42.92 P=0.0000). The G-tests of the fruit-set estimates each indicated highly significant differences (95 and 99% levels) only between the cross- and open-pollinated treatments on the one hand and the other three treatments (Fig.7; "harvest" fruit-set).

Only three treatments produced fruits for dissections, the bagged, cross- and open-pollinated treatments. Although total ovules per fruit did not differ significantly among treatments (1/X-transformed: ANOVA F=8.82 P=0.457; H=0.28 P=0.869) seed-set was calculated as seeds/total ovules. Maximization of homogeneity of variance and independence of mean and standard deviation were obtained and no significant differences in seed-set were revealed (1/(X+0.01)-

Table VI. Summary of bagging experiment with *Polygonatum pubescens* in the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT				
	Bagged	Slit	Self-pollinated	Cross-pollinated	Open-pollinated
plants	45	14	15	15	29
total flowers	447	104	97	85	150
total flowers/ plant	9.9 ± 3.6	7.4 ± 3.6	6.5 ± 4.0	5.7 ± 2.5	5.2 ± 3.0
treated flowers	446	96	92	81	150
treated flowers/ plant	9.9 ± 3.7	6.9 ± 3.2	6.1 ± 3.8	5.4 ± 2.5	5.2 ± 3.0
observed fruit [a]	3	0	0	17	44
observed fruit-set [a]	0.006 ± 0.028	0.000 ± 0.000	0.000 ± 0.000	0.190 ± 0.173	0.300 ± 0.340
harvested fruit	3	0	0	14 [b]	29 [c]
harvested fruit-set	0.006 ± 0.028	0.000 ± 0.000	0.000 ± 0.000	0.177 ± 0.172 [b]	0.202 ± 0.288 [c]
total ovules	29	--	--	129	256 [c]
total ovules/ fruit	9.7 ± 0.6	--	--	9.2 ± 1.5	8.8 ± 2.5 [c]
seeds	3	--	--	44	137 [c]
seed-set [d]	0.104 ± 0.006	--	--	0.351 ± 0.153	0.549 ± 0.291 [c]

[a] field observations, 24 June - includes some enlarging ovaries eventually aborting.
[b] based upon 14 plants and 73 treated flowers as one plant had disappeared by harvest.
[c] includes one seedless fruit.
[d] seeds per total ovules of harvested fruit.

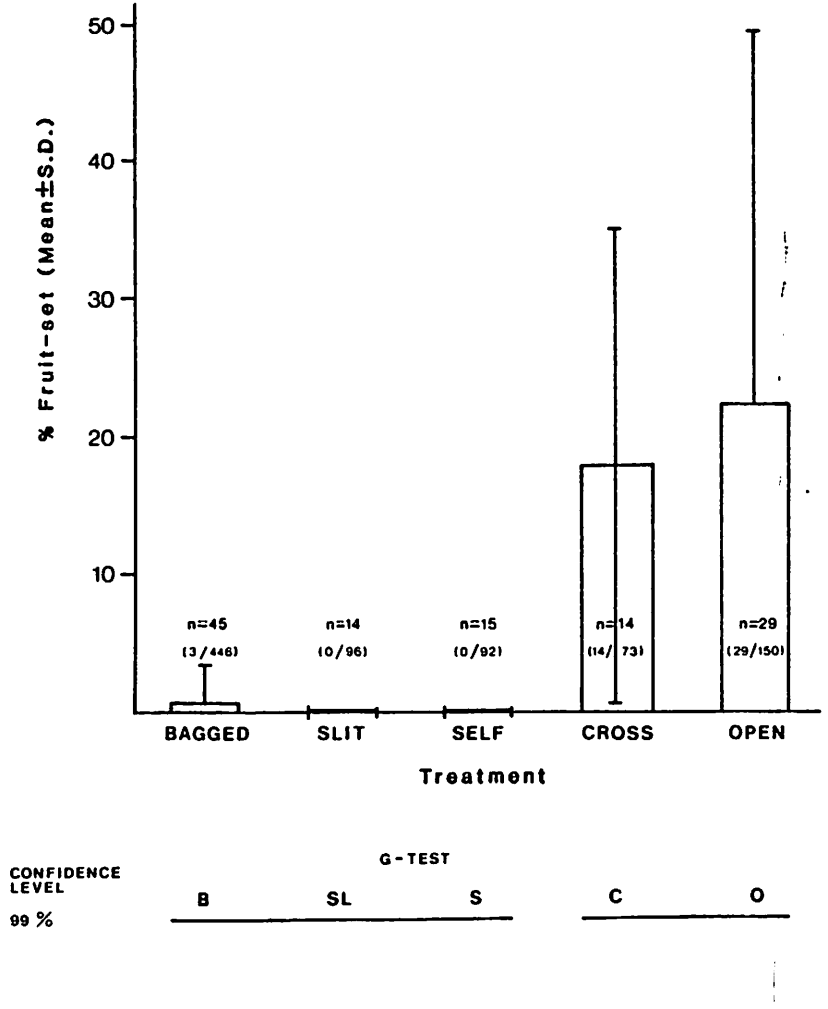


Figure 7. Harvest fruit-set in *Polygonatum pubescens* (n= plants; (a/b)= seed-bearing fruit/ treated flowers).

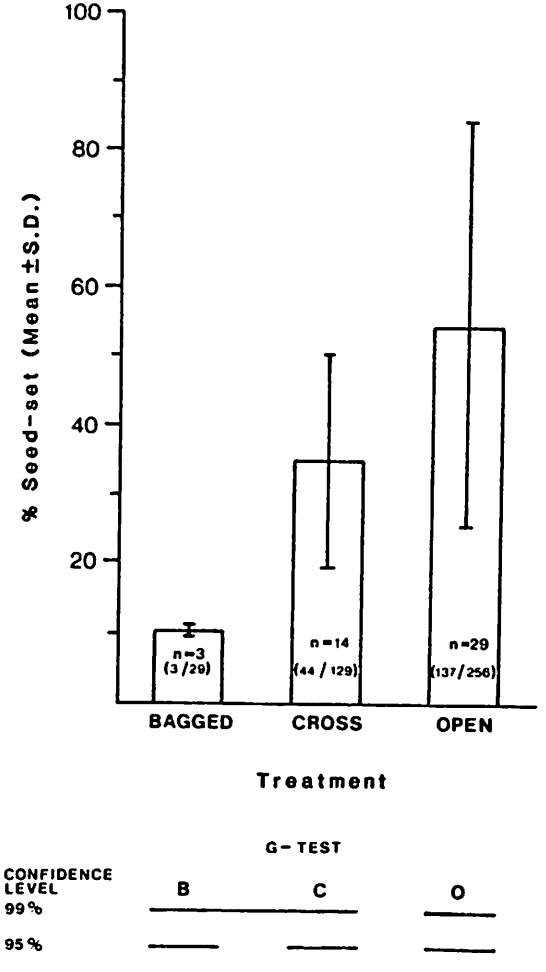


Figure 8. Seed-set in *Polygonatum pubescens* (n= seed-bearing fruit; (a/b)= seeds/ total ovules).

transformed: ANOVA F=0.19 P=0.825). However, both Welch's test (F=25.28 P=0.0000) and Kruskal-Wallis' test (H=10.63 P=0.005) revealed highly significant differences. The untransformed data, for which there was significant heterogeneity of variance (Levene's F=6.39 P=0.004), indicated highly significant differences (ANOVA F=6.21 P=0.004; Welch's F=50.10 P=0.0000) among treatments. Bonferroni's test, to be used with care with heterogeneous variances, revealed no significant differences at the 99% level and a difference between only the extremes of the bagged and open-pollinated treatments at the 95% level. The G-test recognized the open-pollinated treatment as being significantly greater than the other two treatments at the 99% level while at the 95% level, all three treatments were different from each other (Fig. 8).

The queens of two species of bumblebees, *Bombus perplexus* Cresson and *B. vagans* Smith, were very active visitors of flowers of *P. pubescens*. A single specimen of a hover fly, *Platycheirus inversus* Ide, was also observed to contact a flower.

Smilacina racemosa.

The results for *S.racemosa* are presented in Table VII. The analyses of the estimated total flowers per plant revealed significant differences among treatments (untransformed: ANOVA F=3.39 P=0.025; H=8.06 P=0.045) attributed by Bonferroni's test to a difference between the bagged and open-pollinated treatments at the 95% level. The treated flowers per plant did not differ significantly among treatments (untransformed: ANOVA F=0.51 P=0.680; H=3.95 P=0.266).

The initial "observed 1" fruit-set indicated significant differences among treatments (1/(X+0.1)-transformed: ANOVA F=2.73 P=0.054; H=9.73 P=0.021) attributed by Bonferroni's test to a difference between the extremes of self- and open-pollinated treatments at the 95% level. This difference remained qualitatively the same but had become statistically insignificant by the time of the "observed 2' fruit-set (at harvest) (1/(X+0.1)-transformed: ANOVA F=2.23 P=0.097; H=6.84 P=0.077). The G-test of "observed 1" fruit-set indicated highly significant differences (95 and 99% levels) such that the open-pollinated treatment was singularly greater than the other three treatments in addition to the self-pollinated treatment being singularly less than the other three treatments. The G-test could detect only the singularly depressed self-pollinated treatment in the "observed 2" fruit-set (95 and 99% levels).

The "harvest" fruit-set figures revealed no significant differences among treatments (ln(1000X+1)-transformed: ANOVA F=0.98 P=0.408; H=4.49 P=0.214) (Fig. 9). The G-test, however, still recognized the singular depression of the self-pollinated treatment at the 95% level but could not distinguish it from the cross-pollinated treatment at the 99% level.

Table VII. Summary of bagging experiment with *Smilacina racemosa* in the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT			
	Bagged	Self-pollinated	Cross-pollinated	Open-pollinated
plants	13	14	12	14
est. total flowers [a]	1176	1054	892	656
est.total flowers/ plant	90.5 ± 45.9	75.3 ± 32.6	74.3 ± 31.6	46.9 ± 33.7
treated flowers	245	247	212	223
treated flowers/ plant	18.9 ± 4.4	17.6 ± 8.0	17.7 ± 4.4	15.9 ± 6.9
observed 1 fruit [b]	74	34	73	118
observed 1 fruit-set [b]	0.263 ± 0.289	0.097 ± 0.143	0.319 ± 0.391	0.509 ± 0.354
observed 2 fruit [c]	64	28	51	76
observed 2 fruit-set [c]	0.225 ± 0.227	0.077 ± 0.139	0.226 ± 0.325	0.333 ± 0.290
harvest fruit [d]	54	27	44	58
harvest fruit-set [d]	0.188 ± 0.184	0.073 ± 0.139	0.196 ± 0.282	0.249 ± 0.245
dissected fruit [e]	61	28	49	72
0-,1-,2-,3-seeded fruit	7,38,14,2	1,7,14,6	5,22,17,5	14,42,12,4
potential seeds/ fruit [f]	3	3	3	3
seeds	72	53	71	78
seed-set [g]	0.444 ± 0.183	0.654 ± 0.235	0.538 ± 0.230	0.448 ± 0.203

[a] rough estimate including counts of pedicel scars.
[b] field observations, 23 June - includes seedless fruit and others eventually aborted.
[c] field observations, 21 July - includes seedless fruit at harvest date.
[d] based on dissected fruits.
[e] observed 2 fruits not including small number of loose fruits confounded with non-experimental fruits.
[f] invariable limit of seed production to replace total ovules/ fruit which is invariably six (Fernald 1950, Gleason & Cronquist 1963).
[g] seeds per seed-bearing fruit divided by 3 (the maximum potential seeds/ fruit).

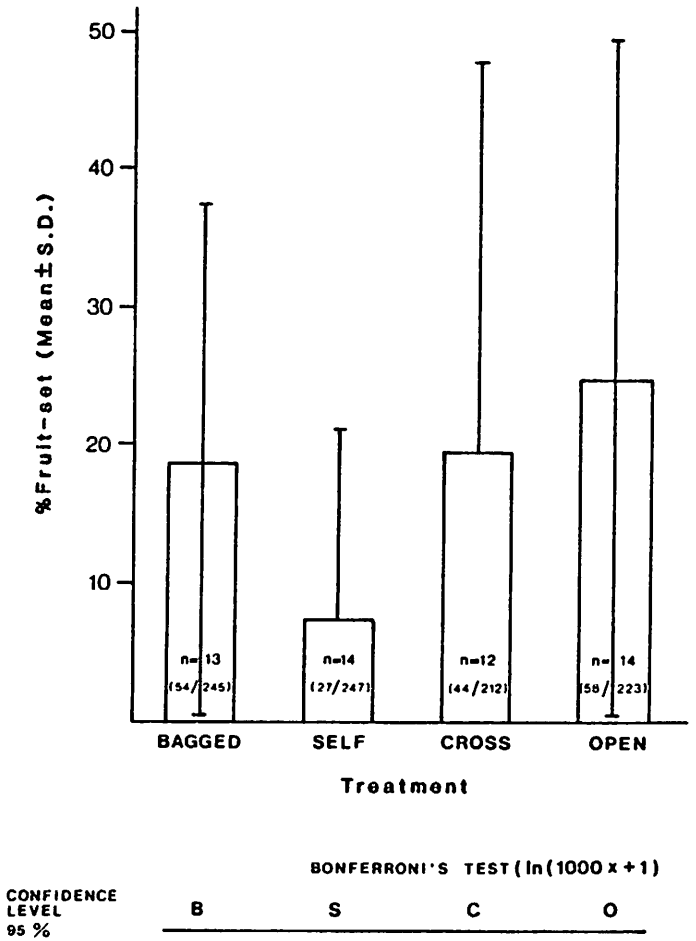


Figure 9. Harvest fruit-set in *Smilacina racemosa* (n= plants; (a/b)= seed-bearing fruit/ treated flowers).

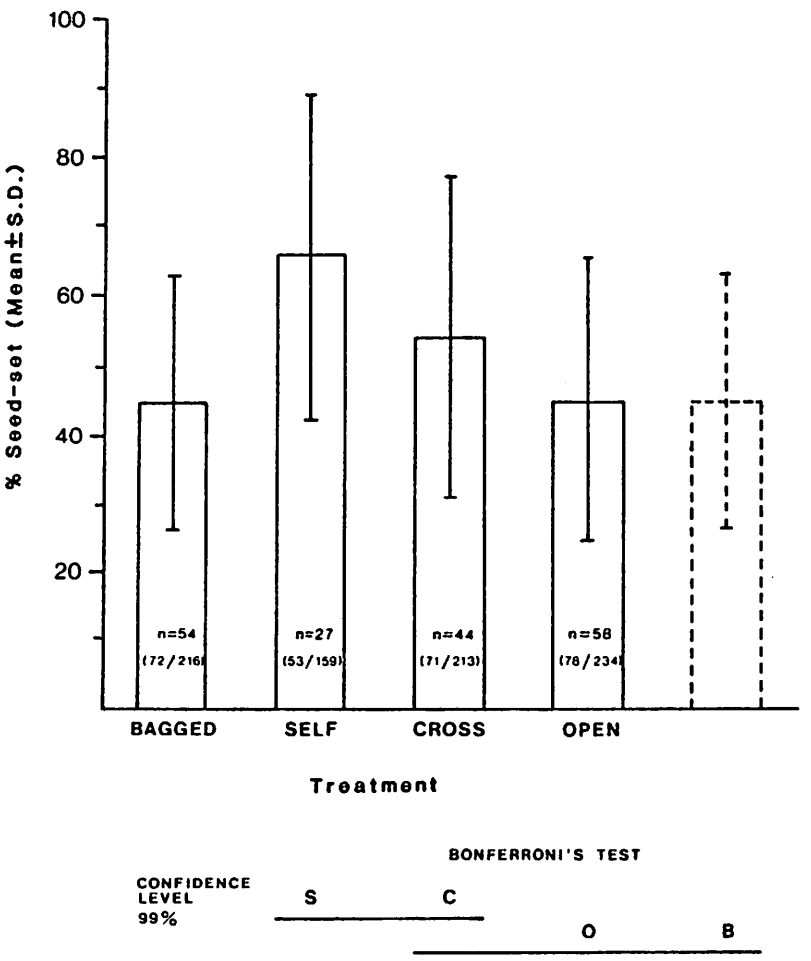


Figure 10. Seed-set in *Smilacina racemosa* (broken bar at far right represents the bagged treatment in its properly ranked position) (n= seed-bearing fruit; (a/b)= seeds/ total ovules).

Highly significant differences in seed-set among treatments were found (untransformed: ANOVA F=7.83 P=0.0001; H=21.88 P=0.0001). Bonferroni's test attributed this entirely to the self-pollinated treatment being significantly higher than the bagged and open-pollinated treatments at the 95 and 99% levels (Fig. 10). The G-test revealed that the self-pollinated treatment was significantly greater than the bagged treatment at the 99% level as well as the open-pollinated treatment at the 95% level.

The seed contents of *S. racemosa* fruits are presented in Table VIII. The relative production rates of 1-seeded and 0-seeded fruits as proportions of treated flowers are particularly low in the self-pollinated treatment and also, to a lesser extent, in the cross-pollinated treatment.

The estimated number and taxonomic distribution of insect species visiting *S. racemosa* are summarized graphically in Fig. 11.

Vaccinium myrtilloides.

Table IX presents the results for *V. myrtilloides.* Because of frost damage, a very poor fruit-set (<3%) was obtained and no analyses were carried out.

The estimated number and taxonomic distribution of insect species visiting *V. myrtilloides* are summarized graphically in Fig. 12.

Sorbus americana.

The results for *S.americana* are summarized in Table X. Because staphylinid beetles penetrated the bags and the flower buds, no manual pollinations were applied. Due to an overall poor fruit-set (<1%) presumably resulting from frost damage, no analyses were carried out.

The estimated number and taxonomic distribution of insects visiting flowers of *S. americana* are summarized in Fig. 13.

Heracleum lanatum.

The results for *H. lanatum* are summarized in Table XI. The number of total flowers (untransformed: ANOVA F=0.98 P=0.408; H=2.50 P=0.476) and treated flowers per subumblet (untransformed: ANOVA F=4.63 P=0.201; H=4.63 P=0.201) did not differ significantly among treatments.

Analysis of the fruit-set data indicated significant differences among treatments (ln(100X+1)-transformed: F=5.16 P=0.003; H=16.53 P=0.001). Bonferroni's test attributed this entirely to a difference between the extremes of the bagged and the open-pollinated treatments at

Table VIII. Seed content of dissected fruits[a] in *Smilacina racemosa* in the Icewater Creek watershed, 1986.

FRUITS	TREATMENT			
	Bagged	Self	Cross	Open
0-seeded	7 (2.9)[b]	1 (0.4)	5 (2.4)	14 (6.3)
1-seeded	38 (15.5)	7 (2.8)	22 (10.4)	42 (18.8)
2-seeded	14 (5.7)	14 (5.7)	17 (8.0)	12 (5.4)
3-seeded	2 (0.8)	6 (2.4)	5 (2.4)	4 (1.8)

[a] including seedless fruits.
[b] bracketed figures represent percentage of treated flowers (n= 245,247,212,223, respectively).

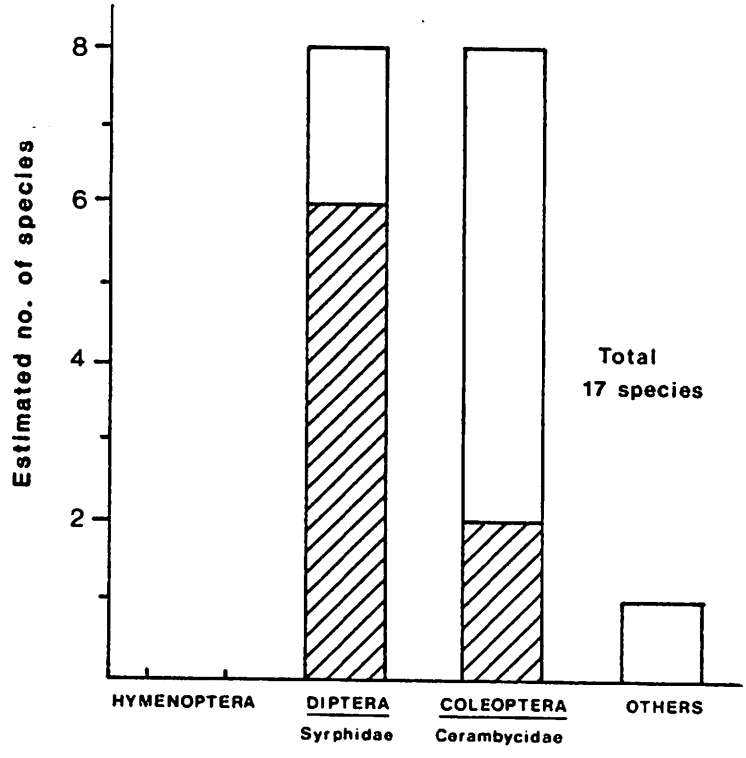


Figure 11. Estimated number of species of pollinators of *Smilacina racemosa* (infra-ordinal taxon represented by hatched portion of bar).

Table IX. Summary of bagging experiment with *Vaccinium myrtilloides* near the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT			
	Bagged	Self-pollinated	Cross-pollinated	Open-pollinated
plants or clones	24	23	24	24
treated flowers	229	218	242	209
treated flowers/ plant	9.5 ± 4.3	9.5 ± 3.6	10.1 ± 4.7	8.7 ± 5.4
observed fruit [a]	1	1	3	7
observed fruit-set [a]	0.004 ± 0.019	0.003 ± 0.015	0.008 ± 0.038	0.029 ± 0.070

[a] field observations, 1 August - no harvest nor fruit dissections made because of very poor fruit-set.

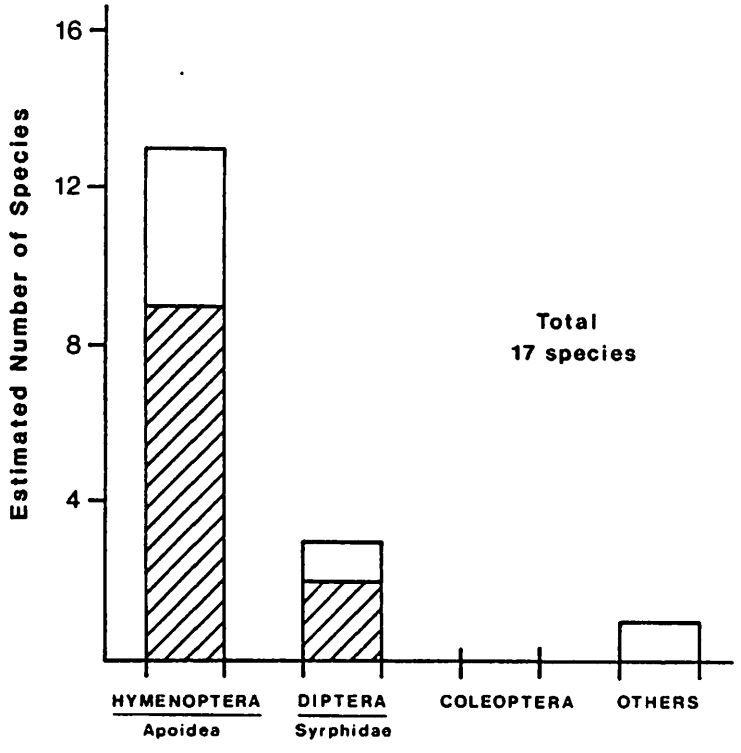


Figure 12. Estimated number of species of pollinators of *Vaccinium myrtilloides* (infra-ordinal taxon represented by hatched portion of bar).

Table X. Summary of bagging experiment with *Sorbus americana* in the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT	
	Bagged [a]	Open-pollinated
plants or clones	12	24
inflorescences	63	86
treated flowers	1709	1994
treated flowers/ inflorescence	27.1 ± 12.8	23.2 ± 8.4
observed fruit	11	4
observed fruit-set [b]	0.006 ± 0.029	0.002 ± 0.014

[a] bagged flowers were not protected from staphylinid beetles which penetrated the bags and contacted the flowers.
[b] field observations, 15 July - no harvest nor fruit dissections made because of very poor fruit-set.

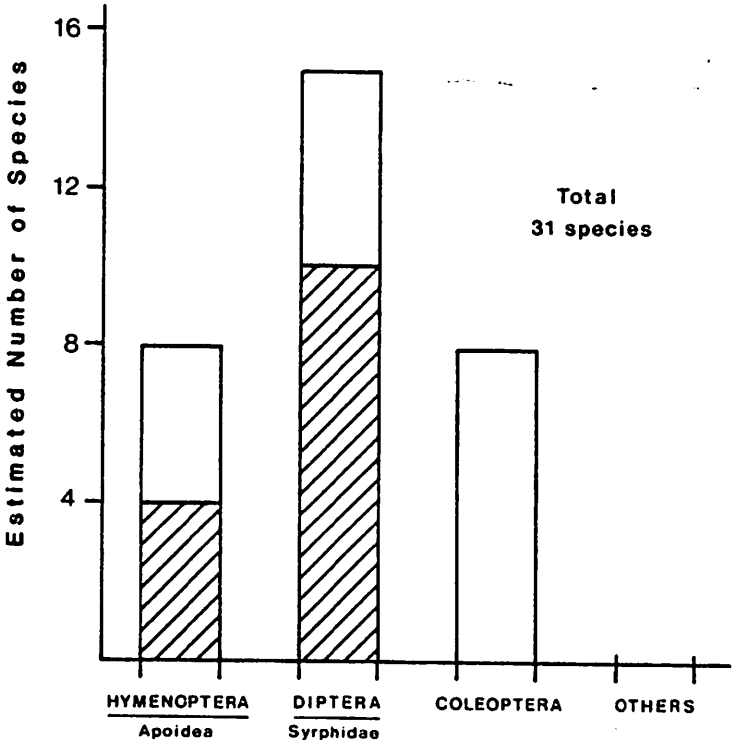


Figure 13. Estimated number of species of pollinators of *Sorbus americana* (infra-ordinal taxon represented by hatched portion of bar).

Table XI. Summary of bagging experiment with *Heracleum lanatum* near the Icewater Creek watershed, 1986.

ATTRIBUTE	TREATMENT			
	Bagged	Self-pollinated	Cross-pollinated	Open-pollinated
plants or subumblets	20	17	17	15
total flowers [a]	439	367	390	333
total flowers/ subumblet [a]	22.0 ± 2.7	21.6 ± 2.0	22.9 ± 2.2	22.2 ± 2.7
treated flowers [a]	439	330	373	333
treated flowers/ subumblet [a]	22.0 ± 2.7	19.4 ± 5.3	21.9 ± 4.9	22.2 ± 2.7
1-,2-seeded fruit	27,6	55,35	63,47	70,102
seed-bearing fruit	33	90	110	172
fruit-set	0.078 ± 0.111	0.290 ± 0.251	0.313 ± 0.261	0.506 ± 0.343
total ovules/ fruit [b]	2	2	2	2
seeds	39	125	157	274
seed-set [c]	0.591 ± 0.196	0.694 ± 0.245	0.714 ± 0.248	0.797 ± 0.246
seed-set [d]	0.599 ± 0.170	0.640 ± 0.119	0.691 ± 0.156	0.762 ± 0.120

[a] for experimental subumblets only; 1 subumblet per plant.
[b] an invariable 2 ovules per fruit (Fernald 1950, Gleason and Cronquist 1963).
[c] seeds per total ovules of seed-bearing fruit calculated on an individual fruit basis (n= 33, 90,110,172).
[d] seeds per total ovules of seed-bearing fruit calculated on a subumblet basis (n= 20,17,17,15).

the 99% level but at the 95% level the bagged treatment was significantly less than the other three treatments (Fig. 14). The G-test discriminated three distinct groups at the 95 and 99% levels -- the bagged treatment was singularly less than the others, the open-pollinated treatment was singularly greater than the others, and the two manually pollinated treatments were intermediate and equivalent.

The analysis of the untransformed data for seed-set revealed highly significant heterogeneity of variances (Levene's F=27.83 P=0.0000) while indicating significant differences among treatments (Welch's F=10.40 P=0.0000; H=24.58 P=0.0000). Because seed-set in this species is bimodal (two ovules per fruit) the transformations attempted did not influence any of the previous test statistics. The G-test revealed that at the 99% level, the open-pollinated treatment was significantly greater than the bagged treatment while at the 95% level, the open-pollinated treatment was also greater than the self-pollinated treatment (Fig. 15).

Seed-set based on per plant measures, though not directly comparable, provided a parametric analysis of treatment effects satisfying Levene's test (F=0.77 P=0.516) and indicated significant differences (untransformed: ANOVA F=3.01 P=0.039; H=9.52 P=0.023). Bonferroni's test attributed this entirely to the extreme treatments, the open-pollinated treatment being greater than the bagged treatment at the 95% level.

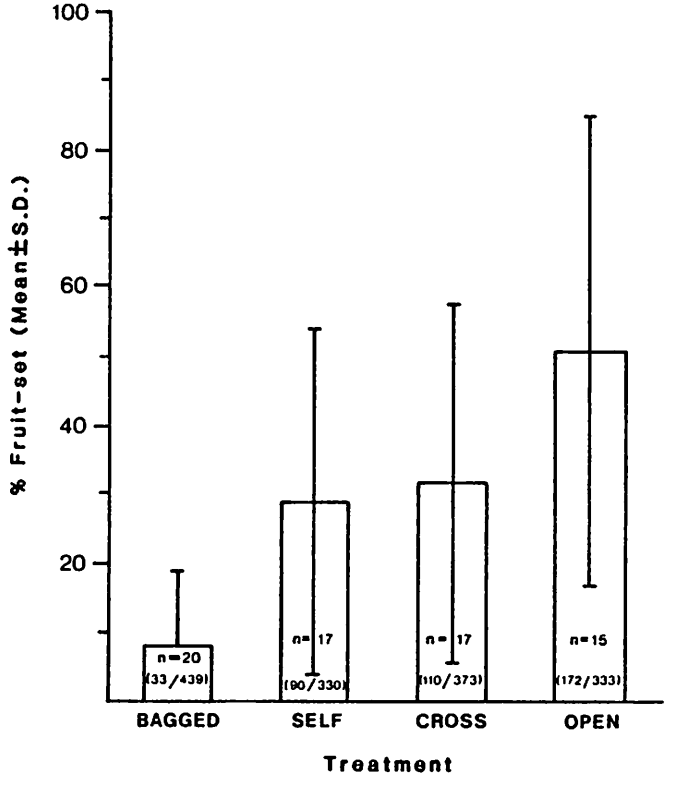
The assemblage of insects visiting *H.lanatum* is summarized graphically in Fig. 16.

Discussion and Conclusions.

Trillium cernuum.

The significantly higher number of ovules per fruit in the self-pollinated treatment relative to the bagged and open-pollinated treatments cannot be attributed to anything other than chance. This was a hidden characteristic and the leaf area index used as a measure of size did not suggest any bias in that regard. Fruit-set would not be expected to be affected by this since only one ovule needs to develop to produce a "successful" (seed-bearing) fruit while seed-set incorporates the number of total ovules to provide a proportional measure or rate of success. However, measures such as seed number and the presumably dependent fruit dry weight are more likely to be affected and are not recommended even though they reflected the same relative differences among treatments as the fruit-set measures.

Trillium cernuum is apparently an autogamous species which does not require a vector to transfer pollen to its stigma since the fruit-set measures reveal no differences between the bagged and the three pollinated treatments. This is similar to the findings of Barrett and



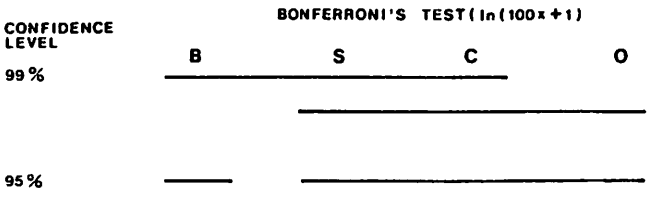
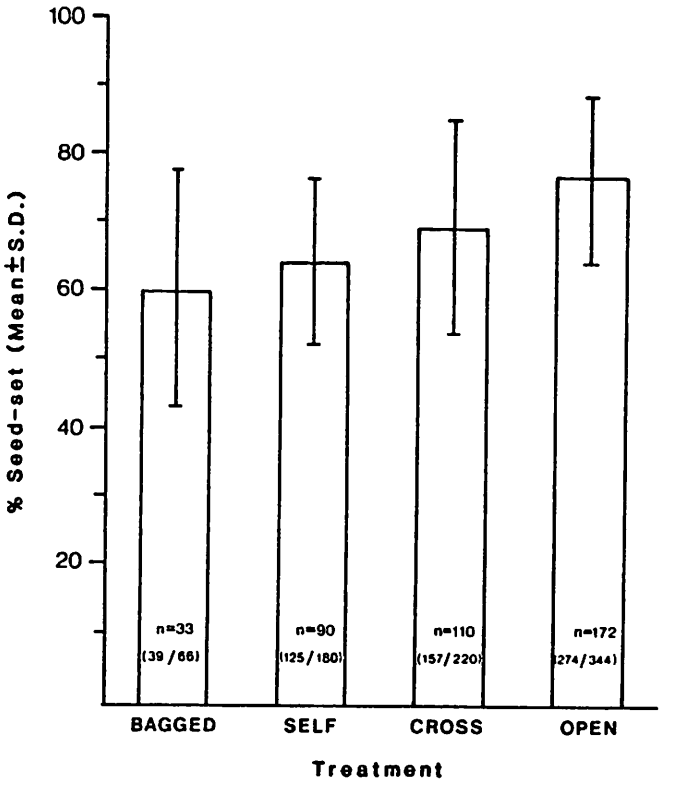


Figure 14. Harvest fruit-set in *Heracleum lanatum* (n= plants; (a/b)= seed-bearing fruit/ treated flowers).



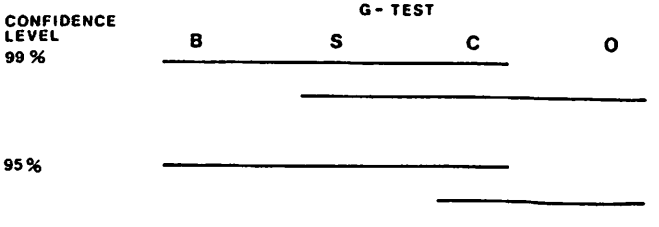


Figure 15. Seed-set in *Heracleum lanatum* (n= seed-bearing fruit; (a/b)= seeds/total ovules).

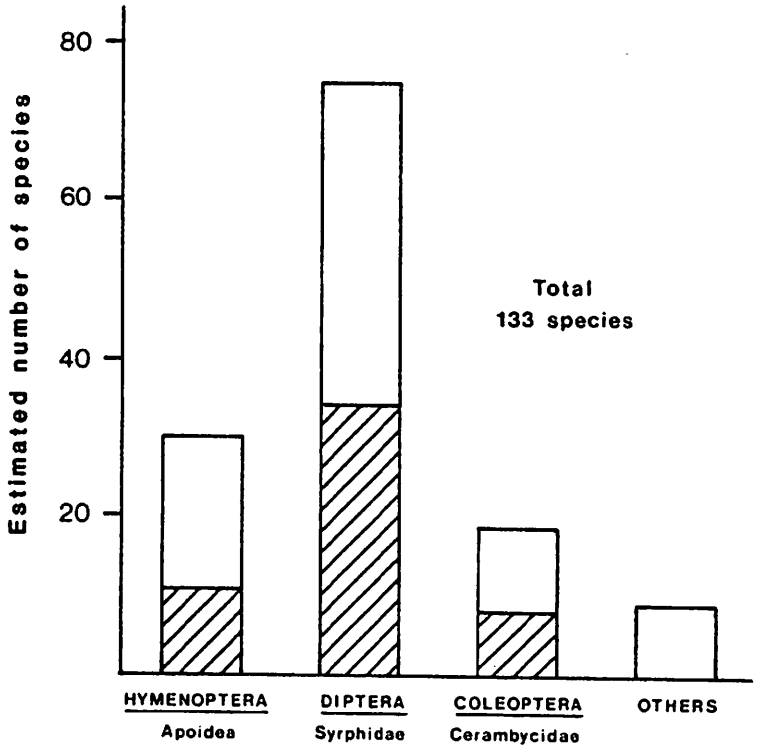


Figure 16. Estimated number of species of pollinators of *Heracleum lanatum* (infra-ordinal taxon represented by hatched portion of bar).

Helenurm (in prep.) for *T. undulatum* Willd. but contrasts with those of Kevan (1975a) for *T. grandiflorum* (Michx.) Salisb. The highly significant depression of fruit-set in the emasculated treatment demonstrates that pollen is indeed required to initiate the development of seeds and it is therefore unlikely that apomixis is a possible mechanism of reproduction. The single seed-bearing fruit in the emasculated treatment likely represents extraneous contamination of the stigmatal surfaces with pollen during the emasculation procedure or subsequent to this.

The actual mechanism by which pollen is transferred by bagged flowers is not known but the distance between the stigmatal surfaces and the anthers is not great and in fact decreases with the contortions of the aging stigmatal branches. Nesom and LaDuke (1985) indicate that for *T. nivale* Riddell, the corolla closes nightly and during inclement weather "forcing contact between the anthers and stigmas". The pollen itself is very dry and dislodgement by wind or animals may be a sufficient means to accomplish the tranfer. Uchino (1985) observed limited insect activity and windborne pollen with *T. tschonoskii* Maximowicz while Nesom and LaDuke (1985) also noted low insect activity with *T. nivale*. The lack of observations of flower visitors to *T. cernuum* is likely to be a fair representation of the usual level of pollinator activity at this site.

The analyses of seed-set are interpreted to indicate relatively insignificant differences among treatments by relying on the ANOVA and Kruskal-Wallis statistics. The significant differences revealed by the G-test are considered suggestive of the relative increase in rate of seed-set with the presumably increased exposure to foreign pollen but further study is required before a definitive stand can be made on this point. Barrett and Helenurm (in prep.) documented a relatively larger seed-set in open-pollinated flowers of *T. undulatum* compared to bagged and manually pollinated flowers. The works of Uchino (1985) and Nesom and LaDuke (1985) suggest reduced seed-set in emasculated plants of the respective species of *Trillium*, but their figures were based on all plants treated and not just seed-bearing plants as in the present study. Their figures of seed-set are thus more appropriately compared to the fruit-set figures presented here and reveal the same general trends.

The high level of autogamy (pollination within a single flower), let alone the paucity (absence) of pollinators, makes *T. cernuum* an inappropriate choice for monitoring insecticidal impacts on pollinators.

Streptopus roseus.

In the present study the three measures of fruit-set based on early counts of fruits in the field provide a picture of significant reductions in the bagged and self-pollinated treatments relative to the cross- and open-pollinated treatments. These relationships are obscured by the time of harvest primarily because of the reductions in sample size imposed by herbivores. The "verified observed" fruit-set (Fig.5) was chosen as the definitive fruit-set measure since it is the most conservative estimate of fruiting success at an early stage of loss to herbivores. The field assessments of fruiting success (ie. seed-bearing) based on external appearance at this stage of development is clear and agrees very well with the results of dissection after harvest of "surviving" fruits.

The differences in seed-set require confirmation before any assessment of their reliability and significance can be made. *Streptopus roseus* fits the model of an obligate out-crossing plant which requires pollinators to achieve its natural rate of pollination in terms of fruit-set and probably seed-set.

The single potential pollinator encountered, *D. venustus*, a hover fly, provides little information about the natural pollinator assemblage associated with this species. However, the relative position of hermaphroditic flowers basal to staminate flowers suggests co-adaptation with bumblebee pollinators which forage from bottom to top (Wyatt 1982). The many thrips observed were capable of penetrating the bags yet their activity did not obscure the pattern of response and indicates that they play no discernible role in the pollination of *S. roseus*.

Robinson and Johansen (1978) documented that in *S. amplexifolius* (L.) fruit-set was reduced to 5% in plants under pollinator exclosures compared to 60% in open-pollinated plants. They also indicated that *S. amplexifolius* fruits were an important food for wildlife. A very pervasive problem with *S. roseus* in this study was its susceptibility to nearly total above-ground removal by herbivores. This may have been accentuated by the presence of a highly visible flagging tape around the base of each plant but if so was certainly countered by the presence of a bag since the open-pollinated plants were much more susceptible to loss.

Loss of plants to herbivores and the dimorphism of flowers are two recognized handling problems that may be encountered in the context of utilization of *S. roseus* in an insecticidal impact monitoring program. Both of these might be surmounted by sufficient sample sizes to reduce sampling error given that compared populations are similar in ratios of hermaphroditic and staminate flowers.

Polygonatum pubescens.

The single seedless fruit incorporated in the analysis for this species does not reflect on the definition of fruits but is rather an artifact of timing of harvest and the open-pollinated treatment. This fruit was shrivelled and nearly dry, a condition commonly seen in the other treatments but usually resulting in loss of the fruit from the plant. The lack of abrasion of a bag and the larger sample size of the open-pollinated treatment probably account for this one dry fruit remaining until harvest. The inclusion of this fruit in the analyses slightly inflates the fruit-set estimate and deflates the seed-set estimate in the open-pollinated treatment but is insignificant. Future work with this species should interpret any dry fruit as unsuccessful.

The significantly higher number of flowers in the bagged treatment can only be partly explained by the addition of plants at one end of the study area since the artificial removal of additional plants proved to dampen these differences only slightly. As well, additional plants for the open-pollinated treatment were also located here and this treatment had the lowest number of flowers per plant. These discrepancies may be an artifact of the non-random assignment of treatments but should not reflect on the conclusions drawn considering the relative magnitude of the differences.

The fruit-set estimates for this species are obviously bimodal -- the highly unsuccessful bagged, slit, and self-pollinated treatments *versus* the highly successful cross- and open-pollinated treatments. Despite the difficulties in analysis, there is consistancy among the various approaches to statistically support this conclusion. *Polygonatum pubescens* therefore appears to be exclusively dependent upon insects for effective pollination to achieve usual rates of fruit-set.

The relative effects of pollinator-exclusion upon seed-set are more difficult to tease apart owing primarily to the low sample size (n=1) and the concommitant invariablility of the bagged treatment. As such, no conclusive statement can be made as to the effect of only bagging flowers as compared to the unmanipulated open-pollinated flowers. The seed-set estimates are suggestive of a significantly graded seed-set response from the bagged to cross-pollinated to open-pollinated treatments. With the relatively high visitation rate by *Bombus* queens and, secondarily, hover flies, it is reasonable to suggest that a relatively higher proportion of repeat visits to the open-pollinated flowers contributed to an increase in seed-set in this treatment when compared to the single pollination events in the manual cross-pollinations. This was also reflected by the field observations that only the bagged flowers accumulated a pool of nectar and these were highly attractive to the bees which would land upon the bags and apparently search for a means to access the enclosed flowers. Mitchell (1962) lists *B. vagans* and *B. pennsylvanicus* (Degeer) as visiting flowers of the genus *Polygonatum* while Krombein *et al.* (1979) record three species of Anthophoridae and one species of Halictidae visiting *Polygonatum* sp(p). Virtually no anthecological data have been published for *P. pubescens*.

Polygonatum pubescens holds considerable potential for incorporation into a monitoring program since fruit-set and possibly seed-set estimates will provide an indirect measure of the relative activity of its primary pollinators, bumblebees. Further work must be done to verify these preliminary findings and extend knowlege of the pollinator/plant relationship before specific recommendations can be made.

Smilacina racemosa.

The effect of the differences in total flowers per plant in the data for this species cannot be assessed. However, it is emphasized that this measure includes counts of pedicel scars after initial flower-drop and onset of ovarial enlargement which may have introduced errors into the data set. The lack of differences in treated flowers per plant is the result of the arbitrary recruitment of basal flowers up to a pre-selected number of approximately twenty flowers.

The initially significant differences in "observed" fruit-set are primarily the result of an unexplained depression in the self-pollinated treatment. The relatively high variances obscure most other real effects of the treatments if they do in fact exist.

The depression in fruit-set in the self-pollinated treatment can be contrasted with its higher seed-set relative to the bagged and open-pollinated treatments. This should not be construed as a measured response by these plants to compensate for decreased fruit-set but is more likely the result of differential loss of single-seeded fruits.

The reason for this is unknown but could be due to frost, beetle penetration of bags, or abrasion of flowers and fruits by the bags. The latter factor is especially pertinent since the cross-pollinated treatment followed the self-pollinated treatment in having relatively low numbers of single-seeded and seedless fruits (Table VIII) which may not have been as firmly held by the pedicels. Selective loss of single-seeded fruits in these, the most highly handled treatments, would serve to deflate fruit-set and increase seed-set.

This data set provides no clear resolution of the breeding system of *S. racemosa* especially when considering the possibility of staphylinids penetrating bags. Although these beetles were present on the bags and unprotected flowers, they were never observed inside the bags. Subsequent problems with bag penetration in *Sorbus americana* suggested this possibility after the fact. Nonetheless, the unpollinated bagged plants produced fruit and seeds at a similar rate to the cross- and open-pollinated plants. This can be contrasted with the findings of Olson-Elliott (1976) who reported clear reductions in bagged (0.5±0.3%) as compared to open-pollinated (32.3±2.7%) *S. racemosa* flowers. Perhaps the most striking difference with their study is the exceptionally low variation they obtained although they did not indicate sample sizes. The relative abrasion factor of the bags used in this present study, a problem recognized in the field, may account for some of the variability in the response of bagged plants, but would not explain the equally high variability in open-pollinated plants. Perhaps frost had an unrecognized influence in this trial as well.

Smilacina racemosa is a species which, because of the composition of its pollinator assemblage being weighted toward Coleoptera and Diptera (Fig. 11), holds some promise for use in an impact monitoring program only if future pollinator exclusion experiments incorporating manual pollinations can clearly demonstrate a level of pollinator dependence similar to that provided by other studies (Olson-Elliott 1976). The predominance of Hymenoptera-dependent species among early-flowering herbs could allow *S. racemosa* to provide another facet of impact-monitoring. This may require qualification since Krombein *et al.* (1979) record Andrenidae, Halictidae, and Anthophoridae from unspecified species of *Smilacina*.

Vaccinium myrtilloides.

The very poor fruit-set in *V. myrtilloides* is attributed to frost-kill of the flowers. Other plants in the area were also obviously affected. The corollas and pistils of many of the *V. myrtilloides* flowers began to turn brown within two days of the primary frost event of June 2. A qualitative comparison with the co-occurring *V. angustifolium* did not show such a dramatic effect most likely due to the more advanced stage of development and the presumed inherently greater cold-hardiness of this earlier blossoming species.

The preliminary accounting of insect visitors to *V. myrtilloides* demonstrates that the primary group of pollinators is Hymenoptera, particularly bees (Fig. 12) as has been found elsewhere for *V. myrtilloides* or mixed populations with *V. angustifolium* (Boulanger *et al.* 1967, Finnamore and Neary 1978, Vander Kloet and Hall 1981, Morrissette *et al.* 1985). This was also borne out by unquantified observations of relative activity.

The dependence of blueberry species upon insect pollinators is well known and has provided the classical model for demonstrating indirect insecticidal impacts upon fruit-set (Kevan and Collins 1974, Kevan 1975b, 1977, Kevan and LaBerge 1979, Miliczky and Osgood 1979). The work of Reader (1977) suggests non-genetic, graded levels of compatibility correlated with spatial separation of individual flowers in *V. myrtilloides*. The low fruit-set demonstrated in this study illustrates the need for controlled experiments and the pervasive cause/effect problems which could otherwise creep into an already difficult situation. Future study of pollination of *V. myrtilloides* in this area will be approached with some trepidation and concern about losses to frost.

Sorbus americana.

The penetration of the bags by staphylinid beetles was obvious and severe before the flower buds had opened. These beetles penetrated between the overlapping petals to presumably contact the reproductive and secretory structures of the flowers. The subsequent dramatically low fruit-set is difficult to attribute to any one factor. The very high activity of the beetle visitors may have inflicted sufficient damage during feeding to weaken the pedicels. However, the concurrent frosts experienced in early to mid-June are more likely to have contributed to the majority of flower mortality as experienced earlier in *V. myrtilloides*.

Sorbus scopulina (?= *S. aucuparia* (L.) Gaertn.) has been demonstrated to incur reduced fruit-set (0% *vs.* 70%) within pollinator exclosures and is judged to be an important food for wildlife (Robinson and Johansen 1978). Any further trials with *S. americana* would definitely require the closed-mesh bags used on *Heracleum lanatum*. But the risk of loss to late frost would still remain on this study site. The wide-spectrum pollinator assemblage associated with *S. americana* may also limit its ultimate utility in an impact monitoring program since an impacted pollinator component may be obscured by compensatory activity of another component. Companion pollinator censuses would be required to identify such an impacted component and/or compensatory activity.

Heracleum lanatum.

The depression of fruit-set in the bagged treatment relative to the open-pollinated treatment indicates that *H. lanatum* is dependent upon insect pollinators to achieve usual levels of fruit production, as has been previously demonstrated (6% vs 100%; Robinson and Johansen 1978). At the same time, the equivalent levels achieved by the two manually pollinated treatments suggest a very high level of self-compatibility.

The relative levels of seed-set among treatments reflect a similar pattern to that of fruit-set. The discrepancies lie in the discrimination of the intermediate levels of the manually pollinated treatments and the relative significance of their separations from the bagged and open-pollinated treatments. In both measures of success, an increase in sample size might increase the statistical significance of the differences found in this preliminary study.

This species is not an obligate out-crosser but insect vectors of pollen are required as the flowers within an individual inflorescence are synchronously protandrous (Cruden and Hermann-Parker 1977) - the flowers function first as males and then as females. This restricts or eliminates the occurrence of autogamous (within a single flower) and intra-inflorescence pollinations. Insects are required to move pollen from another "younger" inflorescence on the same plant (geitonogamous) or from another plant. This situation could greatly influence the success rate in the manually pollinated treatments where essentially single pollination events occurred when the flowers were actively shedding pollen. However, it also suggests the ability of pollen in these situations to survive until stigmatal receptivity is achieved.

Heracleum lanatum produces both hermaphroditic and staminate flowers (Hendrix 1984), but no attempt was made to distinguish these in the present study. The primary umbels, those studied here, have been determined to have a higher proportion (75-90%) of hermaphroditic flowers than secondary umbels (12-52%) (Cruden 1976 in Hendrix 1984). This would mean that the fruit-set measures reported here are underestimated but still retain value as relative measures. These considerations should not unduly affect the results discussed here other than to point out some limitations in obtaining accurate measures of reproductive potential. It also reflects on the 100% fruit-set estimate achieved for open-pollinated plants by Robinson and Johansen (1978).

Heracleum lanatum produces "very great quantities of concentrated nectar" (Faegri and vander Pijl 1979). It is certainly a highly attractive and undoubtedly an important plant for a large number and variety of foraging insects. The abundance and wide array of taxa of pollinators associated with *H. lanatum* in this study is very similar to that obtained by others (Bell 1971, Grace and Nelson 1981) for *H. sphondylium* L., a species considered by Brummitt (1971) to be con-

specific with *H. lanatum*. This diversity limits the suitability of *H. lanatum* as an insecticidal impact monitoring species since significant impacts on at least the major pollinator taxa would be necessary to affect the fecundity of this species. However, if such impacts are incurred, this species may be at risk. Considering the lack of data pertaining to the relative contribution of each pollinator taxon (eg. Syrphidae) the possibility exists for this species to provide an independent measure of impacts on pollinators. Accompanying censuses might identify which components of the pollinator assemblage were impacted upon.

Summary and Evaluation.

The five species for which data analyses have been provided are listed in rank of decreasing suitability for insecticidal impact-monitoring (Table XII).

Trillium cernuum is certainly not an appropriate species since it is autogamous and not dependent upon pollen vectors.

Table XII. Summary of evaluation of five species of plant.

PLANT SPECIES	ATTRIBUTE	
	Pollinator-dependent	Self-incompatible
Polygonatum pubescens	Yes	Yes
Streptopus roseus	Yes	Yes
Heracleum lanatum	Yes	"Yes" [a]
Smilacina racemosa	No? [b]	No? [b]
Trillium cernuum	No	No

[a] synchronously protandrous within individual inflorescences.

[b] data confounded by frost, beetle penetration of bags, and/or abrasion by bags.

Smilacina racemosa presents an enigmatic situation since data presented here may be confounded by extraneous factors and are in contradiction to data previously published elsewhere (Olson-Elliott 1976). However, there is still potential for this species to serve as a sentinel of insecticidal impacts on primarily Diptera and Coleoptera if pollinator-dependency can be confirmed.

The remaining three species have been demonstrated to be dependent upon insects. Two of these, *Polygonatum pubescens* and *Streptopus roseus*, are also highly self-incompatible and are thus dependent upon pollen vectors to provide foreign pollen (inter-plant). *Heracleum lanatum* displays a similar requirement but at a different organizational level (inter-inflorescence) achieved through synchronous protandry within individual inflorescences. The apparent "promiscuity" of *H. lanatum* is its greatest disadvantage since individual components of the wide array of pollinators would be expected to be variably affected by an insecticidal impact. Such an apparently polyphilous relationship undoubtedly reduces the vulnerability of *H. lanatum* to limitations of sexual reproduction due to depressions in populations of pollinating insects.

The two remaining species hold the greatest potential for incorporation in an impact monitoring program with *P. pubescens* holding certain advantages over *S. roseus*. These are: monomorphic flowers removing the necessity for individual flower assessments; the linear arrangement of flowers and fruits easing their enumeration; dehiscence of unsuccessful flowers at an early stage of ovary development allowing identification of seed-bearing fruit without dissection; and known identity and narrow spectrum of presumably major pollinators (*Bombus* spp.) allowing determination of affected pollinator components in impact situations.

Deficiencies which remain to be addressed before *Polygonatum pubescens* can be incorporated in a monitoring program include the following.

1. The dramatic depressions in fruit-set of treatments which did not receive foreign pollen detected in this preliminary study must be verified. An increase in the present sample size of approximately 15 plants each for the three manipulated treatments will increase confidence in the conclusions drawn from the present data set.

2. A more rigorous and quantitative study of the pollinators is required to uncover the relative roles played by the bumblebee queens and other pollinators. This should be approached with timed observations of flowers as well as with selective exclusion experiments to protect flowers from contact by the relatively large bumblebees.

3. Lastly, the pedicels of aborted and lost flowers and fruits are apparently persistent in *P. pubescens*. The suitability of using late-

season counts of pedicels to replace early-season flower counts in estimating fruit-set must be evaluated. This could potentially eliminate the need to be on-site during the flowering period and still provide a retrospective relative measure of pollinator activity.

PORTABLE SANDBOXES.

Introduction.

Previous monitoring procedures have included direct censuses of pollinators using relative activity measures before and after spraying compared to unsprayed areas, or have used caged pollinators to measure relative mortality rates (Thomson *et al.* 1985, Plowright *et al.* 1978). These measures have been performed on natural populations or on artificially established colonies of bumblebees moved into the study areas a period of time before spraying occurs (Plowright *et al.* 1978). The latter procedure removes the need to locate and assess natural populations in an often unfamiliar area and provides some control over manipulation of the bees and subsequent assessment of impacts.

Along this line, no work has been reported on developing a means of transporting ground-nesting solitary bees (Andrenidae, Halictidae) into spray areas for use as sentinels of insecticidal impacts despite their known vulnerability (Plowright and Rodd 1980, Thomson *et al.* 1985). This is most likely due to the obvious difficulties associated with providing suitable nesting substrates, sandy soils, in a manageable portable system. The successes achieved by western workers in promoting artificial populations of the alkali bee, *Nomia melanderi* Cockerell (Halictidae) (Parker and Potter 1974) provide a model for modification. The following reports the results of preliminary attempts to intercept breeding solitary bees in a modular, portable 'sandbox' system.

Materials and Methods.

Honeybee hive boxes with inside dimensions of 38 x 46 x 24 cm deep were modified by attaching a plywood bottom with right angle corner braces. Several holes were drilled through the plywood for drainage and plastic window screen was stapled to the inside to reduce loss of sand through the drainage holes. The boxes were filled on site with available sand and tamped by foot and the surface then smoothed by hand. An attempt was made to place a layer of the surrounding surface stratum on top of the sandboxes to mimic local conditions. These boxes provided 24 cm depth of sand considered to be adequate for the reported burrow depths of *Andrena carlini* Cockerell (13.5-26.0 cm; Schrader and LaBerge 1978) and *A. erythronii* Robertson (7.5-23.0 cm; Michener and Rettenmeyer 1956).

Most boxes were situated alongside an access road through a sandy upland area (site 1) of the Icewater Creek watershed where there were large actively nesting populations of a variety of Halictidae and Andrenidae, particularly *A. erythronii*. Twenty-one boxes were put in place May 14 in three linear groups of five and one group of six (2x3). On June 6, twenty-two more boxes were added. One of the previous groups of five was increased to ten (2x5), while six more were dug into the sand nearly flush with the surface at the end of the original group of six (2x6). Six (2x3) boxes were placed in an open area dominated by grasses and American Mountain-ash (*Sorbus americana* Marsh.) (site 2) while three others were placed in a mixed forest dominated by Sugar Maple (*Acer saccharum* Marsh.) (site 3) (refer to site descriptions in the report on "Plant Fecundity and Pollinators").

Two attempts, May 26 and June 20, were made to actively encourage andrenids to colonize the sandboxes. Specimens of mostly *A. carlini* were collected in the early evening (1800-2000 hours) with sweep nets several kilometers from the study sites and transported in plastic tubs provided with some foliage for traction. Plastic window screen was stapled to the tops of the boxes along three sides and was centrally supported against collapse by a short twig pushed into the sand. The bees were approximately equally distributed amongst the number of boxes used and the screens completely closed with staples. The screens were left in place overnight and removed the following morning.

Successful colonization was defined as nest-initiation determined by the observation of repeat visits of a provisioning female to an excavated burrow.

Results.

No colonization was achieved either by passive attraction or by restraining bees overnight with window screen. One observation was made of a halictid bee burrowing into a sandbox immediately upon putting it into place but this was considered the result of a temporary disorientation since this particular sequence was never repeated.

The results of the restraining trials are summarized in Table XIII. In all, 98 *Andrena* were caged among five sandboxes in site 1 on May 26, while on June 20, 41 Andrena were caged among five sandboxes, three in site 1 and one each in sites 2 and 3.

Restrained *Andrena* constructed short burrows in the sandboxes immediately or a short time after being confined. On the morning of May 27, the screens were removed at about 1000 hours when the bees were already out of their burrows and they immediately flew and dispersed. The second trial of June 21 encountered cool and overcast weather such that the bees were lethargic and crawled away from the sandboxes or remained in the burrows during the surveillance period (0700-1200

hours). Observed mortality rates for the two twelve-hour periods were estimated to be approximately 7% (n=98) for May 26-27 and 10% (n=41) for June 20-21.

Table XIII. Results of restraining *Andrena* on sandboxes in the Icewater Creek watershed, 1986.

	May 26-27	June 20-21
Total boxes	21	43
Restraint boxes	5	5
Total *Andrena*	98	41
12-hr mortality (%)	7	10
Established nests	0	0

Discussion and Conclusions.

The lack of success reported here could be due to a number of different factors, singly or in combination: the disturbance of the stratification of sand and its microflora/fauna; the presentation of the sandbox surfaces elevated above the surrounding substrate (although six were effectively buried); the handling of the restrained bees causing undue stress (7-10% mortality); the residues in the old hive boxes deterring the solitary bees (though attracting bears); the relatively high availability of suitable natural nesting sites in the immediate surroundings.

The sandboxes have been left in place to overwinter with the expectation that they will become more attractive to ground-nesting bees after some weathering and settling. The possibility of transplanting immature bees is being considered, but the successes summarized by Bohart (1972) using this technique for *Nomia melanderi*, may rest in the fact that the *innoculae* were placed in large-scale artificial beds or natural but underutilized nesting sites surrounded by unnacceptable nesting areas. In this light, the sandboxes could be moved to an area without any nearby sites suitable for ground-nesting bees but the logistics of providing large nesting areas in a sandbox container system, if indeed a large minimum area is required, are limiting. This aspect of the study program will remain on a "wait and see" holding pattern.

ACKNOWLEDGEMENTS.

Major support for this study was provided through a grant from the Ontario Pesticides Advisory Committee, Ministry of the Environment, administered by Sault College of Applied Arts and Technology, Sault Ste. Marie. The superb technical assistance of M.Harvey and W.Inkster was central to the successes achieved. The willing consultations with Dr. P.G.Kevan, Dept. of Environmental Biology, University of Guelph provided critical guidance and expertise in all botanical aspects of this study. D.W.Beilhartz is also thanked for her advice regarding data analyses.

REFERENCES.

Barrett, S.C.H. and K. Helenurm. (in preparation). The reproductive biology of boreal forest herbs. I. Breeding systems and pollination. Can. J. Bot.

Bell, C.R. 1971. Breeding systems and floral biology of the Umbelliferae or evidence for specialization in unspecialized flowers. *In* V.H. Heywood (Ed.), the biology and chemistry of the Umbelliferae. pp. 93-107. Academic Press, London.

Bohart, G.E. 1972. Management of wild bees for the pollination of crops. Ann. Rev. Entomol. 17: 287-312.

Boulanger, L.W., G.W. Wood, E.A. Osgood, and C.O. Dirks. 1967. Native bees associated with the lowbush blueberry in Maine and eastern Canada. Me. agric. Exp. Stn. and Canada agric. Res. Stn., Fredericton, N.B. Tech. Bull. 26. 22 pp.

Box, G.E.P. and D.R. Cox. 1964. An analysis of transformations. J. roy. stat. Soc., Ser.B, 26: 211-243.

Brummitt, R.K. 1971. Relationships of *Heracleum lanatum* Michx. of North America to *H. sphondylium* of Europe. Rhodora 73: 578-584.

Cruden. R.W. 1976. Intraspecific variation in pollen-ovule ratios and nectar secretion - preliminary evidence of ecotypic adaptation. Ann. Miss. bot. Garden 63: 277-289.

Cruden, R.W. and S. Hermann-Parker. 1977. Temporal dioecism: an alternative to dioecism? Evolution 31: 863-866.

Faegri, K. and L. vander Pijl. 1971. The principles of pollination ecology. Pergamon Press, Oxford, England. 244 pp.

Fernald, M.L. 1950. Gray's manual of botany. Eighth (Centennial) edition - illustrated. American Book Company, New York, New York. 1632 pp.

Finnamore, A.T. and M.E. Neary. 1978. Blueberry pollinators of Nova Scotia, with a checklist of the blueberry pollinators in eastern Canada and northeastern United States. Ann. ent. Soc. Que. 23:168-181.

Gleason, H.A. and A. Cronquist. 1963. Manual of vascular plants of northeastern United States and adjacent Canada. D. Van Nostrand Company, New York, New York. 810 pp.

Grace, J. and M. Nelson. 1981. Insects and their pollen loads at a hybrid *Heracleum* site. New Phytol. 87: 413-423.

Hansen, R.W. and E.A. Osgood. 1984. Effects of a split application of Sevin-4-oil on pollinators and fruit set in a spruce-fir forest. Can. Ent. 116: 457-464.

Hendrix, S.D. 1984. Reactions of *Heracleum lanatum* to floral herbivory by *Depressaria pastinacella*. Ecology 65: 191-197.

Kevan, P.G. 1975a. Pollination and environmental conservation. Environ. Conserv. 2:293-298.

Kevan, P.G. 1975b. Forest application of the insecticide fenitrothion and its effect on wild bee pollinators (Hymenoptera: Apoidea) of lowbush blueberries (*Vaccinium* spp.) in southern New Brunswick, Canada. Biol. Conserv. 7:301-309.

Kevan, P.G. 1977. Blueberry crops in Nova Scotia and New Brunswick - pesticides and crop reductions. Can. J. agric. Econ. 25: 61-64.

Kevan, P.G. and H.G. Baker. 1983. Insects as flower visitors and pollinators. Ann. Rev. Entomol. 28: 407-453.

Kevan, P.G. and M. Collins. 1974. Bees, blueberries, birds, and budworm. Osprey (Nfld. nat. Hist. Soc. Newsl.) 5: 54-62.

Kevan P.G. and W.E. LaBerge. 1979. Demise and recovery of native pollinator populations through pesticide use and some economic implications. *In* D.M. Caron (Ed.), Proc. IV Int. Symp. Pollination. Md. Agric. Exp. Stn. Spec. Misc. Publ. 1: 489-508.

Kingsbury, P.D., B.B. McLeod, and W.J. Beveridge. 1980. Environmental impact studies in the Icewater Creek watershed. A progress report for 1980. Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario. File Report No.6.

Krombein, K.V., P.D. Hurd, and D.R. Smith, eds. 1979. Catalog of Hymenoptera in America north of Mexico. Smithsonian Institution Press, Washington, D.C. Vols.I-III, 2735 pp.

Michener, C.D. and C.W. Rettenmeyer. 1956. The ethology of *Andrena erythronii* with comparative data on other species (Hymenoptera, Andrenidae). Kans. Univ. Sci. Bul. 37: 645-679.

Miliczky, E.R. and E.A. Osgood. 1979. The effects of spraying with Sevin-4-oil on insect pollinators and pollination in a spruce-fir forest. Life Sci. agric. Exp. Stn., Univ. Maine Orono, Tech. Bul. #90. 21 pp.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data. Life time Learning Publications, Belmont, California. 473 pp.

Mitchell, T.B. 1962. Bees of the eastern United States. Volume II. N.C. agric. Exp. Stn. Tech. Bul. 141: 1-557.

Morrissette, R., A. Francouer, et J.-M. Perron. 1985. Importance des abeilles sauvages (Apoidea) dans la pollinisation des bleutiers nains (*Vaccinium* spp.) en Sagamie, Québec. Rev. Entomol. Qué. 30: 44-53.

National Research Council Canada, Associate Committee on Scientific Criteria for Environmental Quality. 1981. Pesticide-pollinator interactions. NRCC No. 18471. Ottawa, Canada. 190 pp.

Nesom, G.L. and J.C. LaDuke. 1985. Biology of *Trillium nivale* (Liliaceae). Can. J. Bot. 63: 7-14.

Olson-Elliott and Associates. 1978. Effect of spruce budworm control on pollinating insects. 65 pp. *In* Western spruce budworm, a pilot project with carbaryl and trichlorfon, 1975. U.S.D.A. Forest Service, Northern Region, Missoula, Montana. Report No. 78-5.

Parker, F.D. and H.W. Potter. 1974. Methods of transferring and establishing the alkali bee. Environ. Entomol. 3: 739-743.

Plowright, R.C. and F.H. Rodd. 1980. The effect of aerial insecticide spraying on hymenopterous pollinators in New Brunswick. Can. Entomol. 112: 259-269.

Plowright, R.C., B.A. Pendrel, and I.A. McLaren. 1978. The impact of aerial fenitrothion spraying upon the population biology of bumble bees (*Bombus* Latr.; Hymenoptera) in south-western New Brunswick. Can. Entomol. 110: 1145-1156.

Plowright, R.C., J.D. Thomson, and G.R. Thaler. 1980. Pollen removal in *Cypripedium acaule* (Orchidaceae) in relation to aerial fenitrothion spraying in New Brunswick. Can Entomol. 112: 765-769.

Reader, R.J. 1977. Bog ericad flowers: self-compatibility and relative attractiveness to bees. Can. J. Bot. 55:2279-2287.

Robinson, W.S. and C.A. Johansen. 1978. Effects of control chemicals for Douglas-fir tussock moth *Orygia pseudotrugata* (McDonnough) on forest pollination (Lepidoptera: Lymantriidae). Melanderia 30: 9-56.

Schrader, M.N. and W.E. LaBerge. 1978. The nest biology of the bees *Andrena (Melandrena) regularis* Malloch and *Andrena (Melandrena) carlini* Cockerell (Hymenoptera: Andrenidae). Ill. nat. Hist. Surv., Biol. Notes No. 108.

Sokal, R.R. and F.J. Rolph. 1981. Biometry. Second edition. W.H. Freeman and Company, New York, New York. 859 pp.

Somer. 1985. Effets des insecticides fénitrothion et aminocarbe sur la fructification de quatre espèces végétales en milieu forestier. Gouv. du Quebec, Min. Energie et Ressources, Dir. de la Con servation. 207 pp.

Thaler, G.R. and R.C. Plowright. 1980. The effect of aerial insecticide spraying for spruce budworm control on the fecundity of entomophilous plants in New Brunswick, Canada. Can. J. Bot. 58: 2022-2027.

Thomson, J.D., R.C. Plowright, and G.R.Thaler. 1985. Matacil insecticide spraying, pollinator mortality, and plant fecundity in New Brunswick forests. Can. J. Bot. 63: 2056-2061.

Uchino, A. 1985. Structure and dynamics of natural populations of polyploid *Trillium*. III. A tetraploid species *Trillium tschonoskii*. Jpn. J. Genetics 60: 545-556.

Vander Kloet, S.P. and I.V. Hall. 1981. The biological flora of Canada: 2. *Vaccinium myrtilloides*, velvet-leaf blueberry. Can. Field-Nat. 95:329-345.

Wyatt, R. 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. Amer. J. Bot. 69: 585-594.