Development of a monitoring protocol for using the fecundity of *Polygonatum pubescens* as an indicator of impacts of forest sprays on bumblebees.

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K.N. Barber

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, first suggested by Kevan and Collins (1974), has been demonstrated (Kevan 1975b, 1977, Plowright et al. 1978, Kevan and LaBerge 1979, 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, Plowright et al. 1980, Hansen and Osgood 1982, 1984, Thomson et al. 1985, SOMER 1985). Barrett and Helenurm (1987) and Helenurm and Barrett (1987) provide the most recent 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).

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 plant species when pollinators are restricted from access to the flowers, 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 (ie. approaching monolectic/monophilous) the greater are the risks of incurring impacts.

The major objective of the work reported here is to study the suitability of Polygonatum pubescens (Willd.) Pursh. (Liliaceae), or Hairy

Solomon's Seal, as an indicator species for monitoring indirect impacts of forestry insecticides on plant fecundity as the result of direct impacts on pollinators. Preliminary evidence (Barber and Kingsbury 1987) had suggested that this species held considerable potential for implementation in this manner. These findings needed to be verified and a much closer investigation of the roles of the insects visiting the flowers was necessary to determine which insects visiting the flowers were effective pollinators (Kevan and Baker 1983).

The taxonomy and distribution of the North American species of Polygonatum were treated by Ownbey (1944). Since that time others have provided general distributional summaries and keys for identification (Fernald 1950, Gleason and Cronquist 1963). Polygonatum pubescens is distinguished from other Nearctic species by the presence of hairs on the veins on the ventral surface of the leaves. Its distribution in Canada follows the Great Lakes - St. Lawrence and the Acadian forest regions of Hosie (1969), south to Pennsylvania, Indiana, Minnesota, and through the mountains to Georgia (Gleason and Cronquist 1963).

Polygonatum has been generally regarded in Europe as being pollinated by bumblebees. Knuth (1909) describes Polygonatum species as "humble-bee and bee flowers" although records are given of a wider range of visitors including Lepidoptera and Rhingia rostrata L. (Diptera: Syrphidae). He also suggests that some European species of Polygonatum undergo automatic self-pollination if bee visits fail. Proctor and Yeo (1973) indicate that Polygonatum is visited by long-tongued species of Anthophora bees (Hymenoptera: Anthophoridae) and by bumblebees.

In North America, Mitchell (1962) lists Bombus vagans Smith, B.pennsylvanicus (Degeer), three species of Anthophoridae, and one species of Halictidae as visiting flowers of the genus Polygonatum. These records are all based on those originally published by Robertson (1929). More recently, Krombein et al. (1979) have repeated these records.

Published information on the ecology of P.pubescens has been primarily

restricted to growth patterns and nutrient dynamics (see Boerner 1986). Virtually no anthecological data have been published specifically for P.pubescens.

The present study is reported in four parts preceded by a general discussion of the study site and analytical procedures common to all parts. The investigations begin with basic plant breeding trials (pollinator-exclusion), followed by studies of insect visitation, nectar removal, and then some preliminary considerations for implementation in a monitoring situation are discussed. Cited literature is consolidated in one section at the end.

Materials and Methods.

Study site.

The study was carried out during the 1986 and 1987 seasons in 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 this work was accomplished in a mixed forest predominated by Acer saccharum Marsh. (Sugar Maple) where Polygonatum pubescens was common along with other woodland lilies such as Trillium cernuum L. (Nodding Trillium), Streptopus roseus Michaux (Rose Twisted Stalk), and Smilacina racemosa (L.) Desf. (Racemed False Solomon's Seal). This forest was situated at the level of mile 10.5 on Whitman Dam Road, 12.7 kmNNE of Searchmont, Ontario.

Analysis.

Data analyses were performed with a Digital VAX 8500 computer primarily

using program packages of BMDP (Dixon 1983). These included one-way and two-way analysis of variance accompanied by the non-parametric Kruskal-Wallis H-test and Mann-Whitney U-test (for comparisons of two treatments). Where data could be scored in a binary fashion 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 recommendations made by Milliken and Johnson (1984) guided the parametric analyses and are summarized here. An ANOVA was run with three embedded tests, namely, Levene's F-test of homogeneity of variance, Bonferroni's multiple comparisons test, and a 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 when Levene's test was not satisfied with linear data. Once homogeneity of variance was achieved, Bonferroni's multiple comparisons test was applied.

Bonferroni's test was applied 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 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.

Fruit-set data were prone to considerable heterogeneity and a G-test was employed here as well as with other frequency data.

The means and standard deviations are tabulated and/or figured only as computed on the untransformed, linear data and the statistics and multiple comparisons based on transformed data are reported as such in the text. The reported confidence levels for Bonferroni's test ($P<0.01$, $P<0.05$) 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.

POLLINATOR-EXCLUSION

Objectives.

The breeding system of P.pubescens was investigated to qualitatively describe the degree of dependence upon pollinators as well as the expression of self-incompatibility. Only if pollinator-dependency has been demonstrated could a potential for insecticidal disruption of pollination be expected to exist. If self-incompatibility is evident, then a physiological requirement implicates the critical role of pollen vectors in maintaining this flow of foreign or donor pollen.

Materials and Methods.

This experiment employed classical pollinator-exclusion and manual pollination techniques. Bags were made from 100% white polyester fabric (Caprice; mesh openings about 1.0x0.5mm.) which was stitched to form a tube of an appropriate diameter and closed at one end. These were secured over the entire plant (flowering stem) with a paper and wire twist-tie.

Five treatments were applied in each year. These are referred to as bagged (bagged with no other manipulation), slit (bagged and flowers partially dissected but no pollen transfer (see below)), self-pollinated (bagged, flowers partially dissected and manually self-pollinated), cross-pollinated (bagged, flowers partially dissected and manually cross-pollinated), and open-pollinated (not bagged nor manipulated).

The flowers of P.pubescens 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 into the experimental design, a flap was ripped in the side of the flowers and

folded back to gain clear 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 (the slit treatment above) 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.

In 1987, a sixth treatment was intended to exclude large insects (primarily queen bumblebees) and was dubbed the bee-cone treatment. It consisted of placing a cone (height - 40cm.; diameter - 46cm.) constructed of hardware cloth, comprised of a grid of 6.4mm. (0.25 in.) square holes, over an individual plant. This mesh size had been determined to be the maximal, readily available size which was still capable of restricting access by all but the smallest bumblebee queens. This was determined by enclosing individual bumblebee queens of several species with hardware cloth and observing penetration rates. Leaf litter and debris were gathered and pressed around the base to minimize access to the enclosed plant along the cone-ground interface. These plants were not further manipulated. Some of the bee-cone treatments had to be reassigned to the nearest neighbour when there was insufficient room to enclose, individually, plants growing in close proximity.

Individual plants were recruited into the experiments when encountered during a hike through the site. On 22-23 May 1986, approximately 75% were bagged after the individual flower buds were determined to be closed but nearing blossom. Plants with no flower buds were intentionally overlooked. Treatments were assigned in a relatively ad hoc manner, recruiting plants evenly to treatments as encountered in an attempt to minimize local effects. The remaining 25% of the plants were left unbagged and were used for assignments of the open-pollinated treatment. In 1987, all plants were bagged initially, before bloom. Treatments were then assigned randomly using computer-generated assignments and the bags removed from the open-pollinated and bee-cone treatments. Each plant was marked and numbered with a section of coloured flagging tape and its position mapped for future reference. The bags were supported with crossed twigs pushed

into the soil. The individual flowers of each plant were then enumerated and mapped to facilitate monitoring of their individual fates. Those flowers dislodged or damaged while handling were removed from the tallies of treated flowers where possible and any plant lost during the study was also removed from consideration.

Sample sizes differed considerably in 1986 resulting from a concentration or accumulation of plants assigned to the bagged and open-pollinated treatments 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). Sample size for the bee-cones was approximately double that of the other treatments in 1987 since this treatment had not been applied the previous year.

The manual pollinations were roughly timed to coincide with pollen availability within individual flowers and not from a direct measure of stigmatal receptivity (attempts at quantification in 1987 were unsuccessful since a dye was not incorporated with the peroxide; see Galen and Plowright 1987). Donor pollen for cross-pollinations was obtained in the form of excised anthers from plants outside the study area (1986) or from other bagged individuals at least ten metres distant (1987). This is particularly important with clonal, rhizomatous lilies. Flowers 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 taken from the same flower (autogamous pollinations). Pollen was applied in excess to the stigmatal surface directly from excised anthers. Forceps were cleaned after each treatment by wiping on clothing and plunging the tips into the duff. The manual pollinations were carried out at two-day intervals over a period of time (27 May - 4 June 1986; 23 May - 4 June 1987) as the flower buds opened and became available for treatment. Individual flowers were pollinated once only.

Because of the increased number of manual pollinations required in 1987, a modification of pollen-handling and source was made. Two or three

anthers were collected from slit, self- and cross-pollinated flowers when treated as they were recruited into the bloom over the experimental period. The anthers were kept in separate snap-cap vials according to location of collection and used as pollen sources for flowers to be cross-pollinated in more distant parts (>10 metres) of the site on the same day. This procedure reduced pollen collection efforts and depletion of flowers in the peripheral area that would otherwise be sacrificed for pollen.

Bags were removed as soon as all flowers had senesced as judged by the apical closing of the perianth. The plants were then left to develop although harvest took place well in advance of full maturation (21 July 1986; 29 June 1987) to minimize subsequent losses to herbivores or frugivores.

Harvesting involved the collection of all the above-ground parts of the plants. Fruits were brought into the laboratory and kept at about 4-10°C. until a convenient time for dissection within a week of harvest. The fleshy fruits 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 and contributed to an enumeration of total ovules.

The total number of flowers on each plant was recorded as well as the number of flowers eventually treated. Fruit-set is computed as the number of successful fruit as a proportion of the treated flowers for each treatment, thus yielding single point estimates. Seed-set is computed as the number of seeds as a proportion of the ovules available per seed-bearing fruit in each treatment, thus yielding a mean and standard deviation.

Results and Discussion.

Tables I-III summarize some attributes of the 1986, 1987, and combined data.

Table I. Summary of pollinator-exclusion and manual pollination with *P. pubescens*, 1986.

ATTRIBUTE	TREATMENT					
	Bagged	Slit	Self-pollinated	Cross-pollinated	Open-pollinated	Bee-Cone
plants	45	14	15	14	29	—
total flowers	447	104	97	77	150	—
total flowers/plant $\bar{x}$ (s.d.)	9.9 (3.6)	7.4 (3.6)	6.5 (4.0)	5.5 (2.5)	5.2 (3.0)	—
treated flowers	446	96	92	73	150	—
treated flowers/plant $\bar{x}$ (s.d.)	9.9 (3.7)	6.9 (3.2)	6.1 (3.8)	5.2 (2.5)	5.2 (3.0)	—
fruit	3	0	0	14	28	—
% fruit-set	0.7	0.0	0.0	19.2	18.7	—
total ovules	29	—	—	129	252	—
total ovules/fruit $\bar{x}$ (s.d.)	9.7 (0.6)	—	—	9.2 (1.5)	9.0 (2.4)	—
seeds	3	—	—	44	137	—
% seed-set $\bar{x}$ (s.d.)	10.4 (0.6)	—	—	35.1 (15.3)	56.8 (27.6)	—

Table II. Summary of pollinator-exclusion and manual pollination with P. pubescens, 1987.

ATTRIBUTE	TREATMENT					
	Bagged	Slit	Self-pollinated	Cross-pollinated	Open-pollinated	Bee-Cone
plants	30	27	28	28	25	58
total flowers	155	170	188	146	211	371
total flowers/plant $\bar{x}$ (s.d.)	5.2 (2.9)	6.3 (3.3)	6.7 (5.2)	5.2 (3.6)	8.4 (5.6)	6.4 (4.1)
treated flowers	155	168	186	141	210	371
treated flowers/plant $\bar{x}$ (s.d.)	5.2 (2.9)	6.2 (3.3)	6.6 (5.0)	5.0 (3.5)	8.4 (5.7)	6.4 (4.1)
fruits	3	1	4	9	81	17
% fruit-set	1.9	0.6	2.2	6.4	38.6	4.6
total ovules	30	12	38	88	865	183
total ovules/fruit $\bar{x}$ (s.d.)	10.0 (2.6)	12.0 (0.0)	9.8 (2.6)	9.8 (2.6)	10.7 (2.2)	10.8 (1.6)
seeds	4	11	12	50	321	54
seed-set $\bar{x}$ (s.d.)	15.3 (11.5)	91.7 (0.0)	30.2 (21.2)	58.0 (18.2)	37.6 (17.5)	29.4 (28.6)

Table III. Summary of pollinator-exclusion and manual pollination with P. pubescens, 1986 and 1987 combined.

ATTRIBUTE	TREATMENT					
	Bagged	Slit	Self-pollinated	Cross-pollinated	Open-pollinated	Bee-Cone
plants	75	41	43	42	54	58
total flowers	602	274	285	231	361	371
total flowers/plant $\bar{x}$ (s.d.)	8.0 (4.1)	6.7 (3.4)	6.6 (4.7)	5.3 (3.3)	6.7 (4.7)	6.4 (4.1)
treated flowers	601	264	278	214	360	371
treated flowers/plant $\bar{x}$ (s.d.)	8.0 (4.1)	6.4 (3.3)	6.5 (4.6)	5.1 (3.2)	6.7 (4.7)	6.4 (4.1)
fruits	6	1	4	23	109	17
% fruit-set	1.0	0.4	1.4	10.7	30.3	4.6
total ovules	59	12	38	217	1117	183
total ovules/fruit $\bar{x}$ (s.d.)	9.8 (1.7)	12.0 (0.0)	9.8 (2.6)	9.4 (2.0)	10.2 (2.3)	10.8 (1.6)
seeds	7	11	12	94	458	54
seed-set $\bar{x}$ (s.d.)	12.9 (7.8)	91.7 (0.0)	30.2 (21.2)	44.1 (19.7)	42.6 (22.1)	29.4 (28.6)

In 1986, there were significant differences in total flowers/plant (linear ANOVA F=10.68, d.f.=4,112, P<<0.001; H=32.98, d.f.=4, P<<0.001) and treated flowers/plant (linear ANOVA F=11.64, d.f.=4,112, P<<0.001; H=34.97, d.f.=4, P<<0.001). This was principally attributed by Bonferroni's tests to the bagged treatment having a greater number of total and treated flowers than all but the slit treatment (P<0.01) and significantly more treated flowers than all treatments (P<0.05).

In 1987, analysis of total flowers/plant and treated flowers/plant indicated significant differences (linear ANOVA F=2.17, d.f.=5,190, P=0.0586; F=2.28, d.f.=5,190, P=0.0485, respectively) while marginally satisfying Levene's test for homogeneity of variances (F=2.82, d.f.=5,190, P=0.0174; F=2.89, d.f.=5,190, P=0.0154). However, Bonferroni's test could not discriminate any treatment differences (P>0.05) and Kruskall-Wallis tests (H=7.18, d.f.=5, P=0.2076; H=7.63, d.f.=5, P=0.1779) indicated no significant differences amongst the six treatments.

The combined data for both years demonstrated some differences amongst treatments for total flowers/plant (linear ANOVA F=2.57, d.f=5,307, P=0.0270; H=15.28, d.f.=5, P=0.0092) and for treated flowers/plant (linear ANOVA F=2.98, d.f.=5,307, P=0.0120; H=17.13, d.f.=5, P=0.0043). These were attributable to the larger number of total flowers (P<0.05) and of treated flowers (P<0.01) in the bagged treatment compared to the cross-pollinated treatment.

The treatment differences in flowers/plant observed in 1986 were presumably due to the non-systematic means of assigning treatments. This was redressed in 1987 by using randomized generations of treatment assignments to plant numbers and the treatments are considered to be applied to similar samples of the population in that year. The combined data set is influenced by the original bias in the data from 1986 to the point where the bagged treatment again has an inflated number of flowers/plant. However, in the context of this study, these discrepancies are expected to have a minor influence on the parameters of fruit-set and seed-set.

Parametric testing of differences in fruit-set was beset with difficulties in satisfying Levene's test for homogeneity of variances even with transformations. Thus, G-tests were utilized to compare the frequency data of fruits vs. not fruits (unsuccessful treated flowers). A graphical representation of the fruit-set responses is presented in Figure 1 and the results of the multiple comparisons in Figure 2.

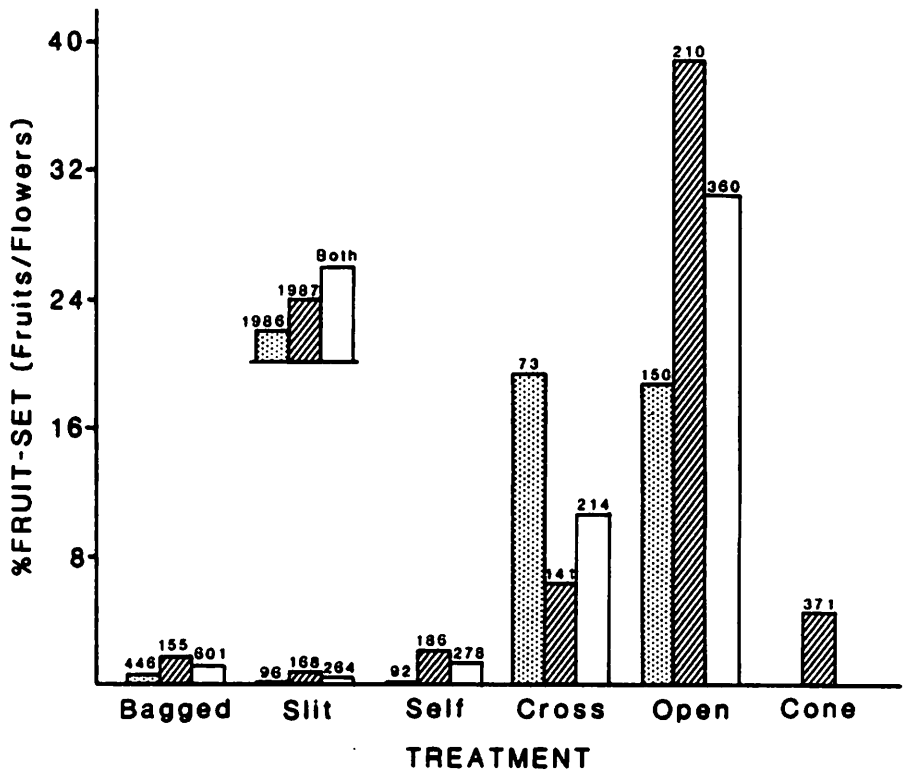

Figure 1. Fruit-set in P.pubescens under different treatments of pollinator-exclusion and manual pollination in 1986, 1987, and both years combined

In 1986, two subsets of treatments were clearly discriminated (G^2=99.153, d.f.=4, P<0.01). The cross- and open-pollinated treatments set significantly more fruit than the other two treatments (Fig.2).

In 1987, the G-test discriminated two subsets with the open-pollinated

treatment being greater than all others (G^2=198.153, d.f.=5, P<0.01). The cross-pollinated treatment was significantly greater than only the slit treatment (P<0.05) (Fig.2).

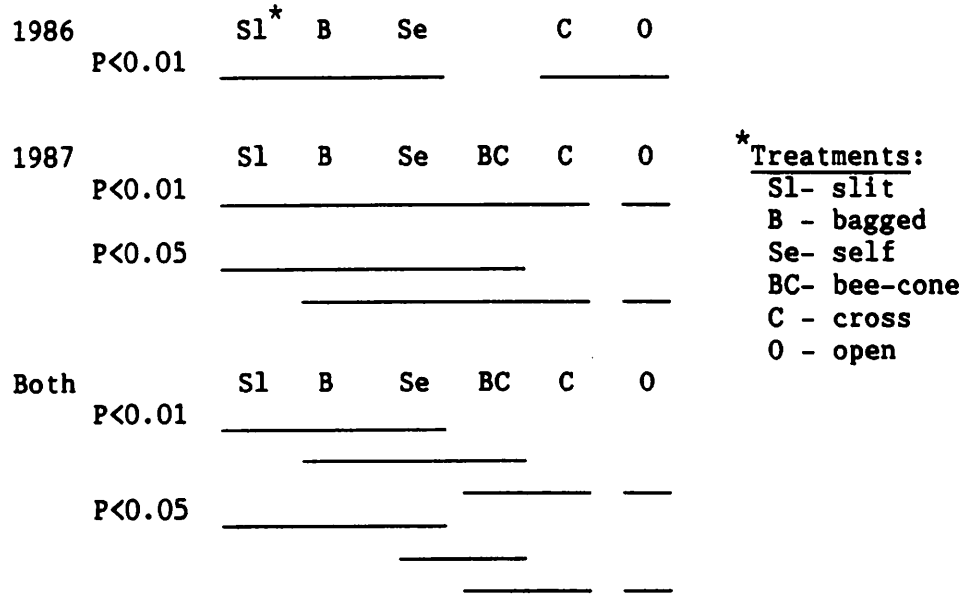

Figure 2. Significant differences in fruit-set among treatments of P.pubescens in 1986, 1987, and both years combined as determined by G-test at two confidence levels.

When the two years' data are combined, the G-test discriminated the open-pollinated treatment as discretely larger than all others while the cross-pollinated is greater than the bagged, slit, and self-pollinated treatments, and the bee-cones are greater than the slit treatment (G^2=281.611, d.f.=5, P<0.01). Additionally, the bee-cones are discriminated as larger than the bagged treatment (P<0.05). The general topology of comparisons amongst the five treatments common to both years remains the same as for 1986 except the cross- and open-pollinated treatments are discriminated (Fig.2).

What appears to have occurred in 1987 is a relatively low rate of fruit-set in the cross-pollinated treatment with only about 6% of the treated flowers producing fruit. This is compared with the 19% fruit-set

of cross-pollinated flowers in 1986 while the open-pollinated flowers enjoyed an increased rate of return over the two years (19% to 39%). A full explanation for the lack of success with cross-pollinations in 1987 is not available but the difference in handling of the pollen source might explain part of this. Pollen originating at a greater proximity to the recipient stigmas may have influenced the response rate in 1987. Alternatively, the success of manual cross-pollinations may well be a stochastic phenomenon which is subject to influences beyond experimental control.

No significant differences were found in total ovules/fruit in 1986 (linear ANOVA $F=0.17$, d.f.=2,42, $P=0.8471$; $H=0.22$, d.f.=2, $P=0.8957$), in 1987 (linear ANOVA $F=0.57$, d.f.=5,109, $P=0.7262$; $H=3.30$, d.f.=5, $P=0.6533$), or in the combined data set (linear ANOVA $F=0.99$, d.f.=5,154, $P=0.4234$; $H=7.33$, d.f.=5, $P=0.1974$).

The seed-set figures are presented graphically in Figure 3 (excluding the single fruit in the slit treatment in 1987). In 1986, significant treatment differences were obtained in seed-set (10/x-transformed ANOVA $F=13.09$, d.f.=2,42, $P<<0.001$; $H=12.42$, d.f.=2, $P=0.0020$). Bonferroni's test indicated that seed-set in the cross- and open-pollinated treatments was significantly greater than in the bagged treatment ($P<0.01$).

Significant differences in seed-set in 1987 (linear ANOVA $F=4.97$, d.f.=5,109, $P=0.0004$; $H=21.38$, d.f.=5, $P=0.0007$) were determined by Bonferroni's test to be attributable to the cross-pollinated treatment setting more seeds than the bee-cone treatment ($P<0.01$) and all treatments except the open-pollinated and slit (n=1) treatments ($P<0.05$).

The combined data set also demonstrated significant treatment differences in seed-set (linear ANOVA $F=4.25$, d.f.=5,154, $P=0.0012$; $H=23.09$, d.f.=5, $P=0.0003$). These were attributed by Bonferroni's test to the lower seed-set in the bagged treatment compared to the slit (n=1), cross- and open-pollinated treatments ($P<0.05$).

Generally, seed-set increased with the "quality" of pollen or pollination service. This is not clear-cut but simply represents a trend.

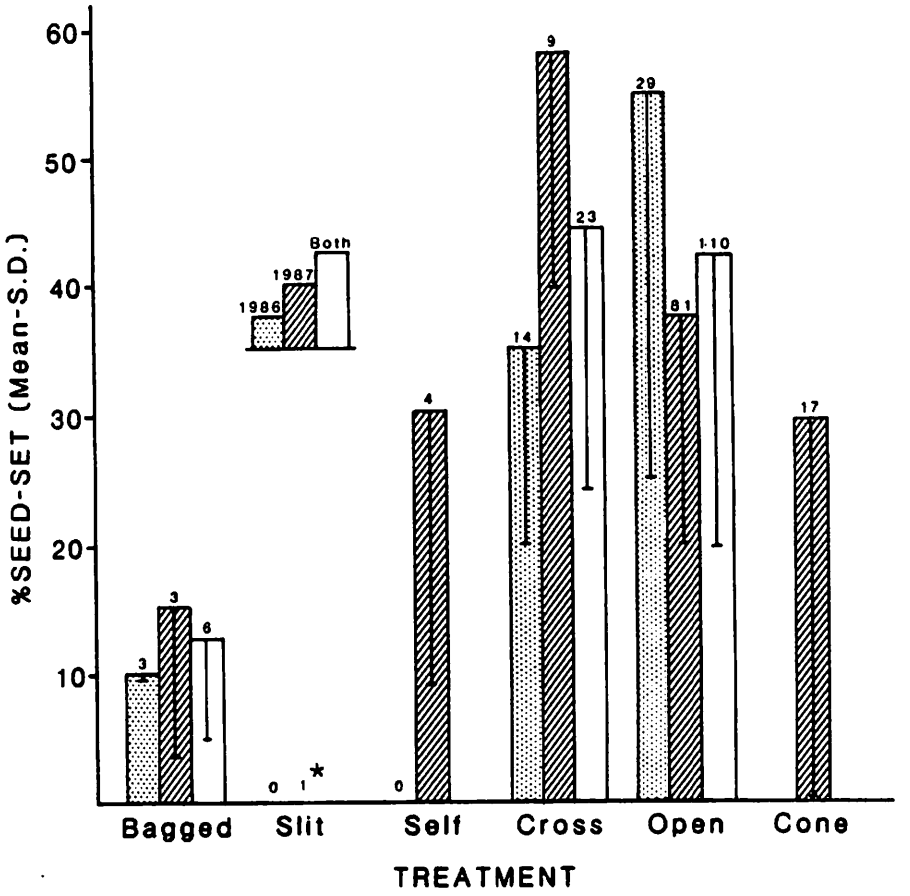

Figure 3. Seed-set in P.pubescens under different treatments of pollinator-exclusion and manual pollination in 1986, 1987, and both years combined (* single fruit not depicted - 91.7%)

The experimental cross-pollinations achieved elevated rates of seed-set compared to the foreign pollen-starved bagged and self-pollinated treatments in 1987. However, the achievement rate of the first ovule fertilization in any one flower, the definitive criterion of a successful (seed-bearing) fruit, was low in the cross-pollinations of 1987. This is consistent with what might be expected in an all-or-none incompatibility response or, alternatively, when manual-pollination techniques are applied inconsistently.

An aspect of the 1987 data set that helps to interpret the failure of the cross-pollinations is the fact that the bee-cone treatment drastically depressed fruit-set values below the open-pollinated treatment and was intermediate to the cross-pollinated and the three remaining treatments. This is in spite of the fact that these flowers were relatively exposed, accessible, and received a minimum of manipulation. These results suggest that relatively small insects (eg. small beetles and flies), capable of flying or crawling through the mesh of the hardware cloth, are not responsible for effecting fruit-set in P.pubescens. Larger insects (eg. bumblebees and syrphids), which cannot fly directly through the mesh but must land and walk or squeeze through, are more likely to be the key pollinators. However, the plants and flowers under bee-cones are not to be considered completely free from contact with bumblebees. Not all of these larger insects were excluded since one observation of a small B.vagans queen inside a bee-cone was made. This particular individual eventually squeezed through the mesh and departed when we approached the bee-cone.

Conclusions.

Polygonatum pubescens is dependent upon insects to achieve ambient levels of fruit-set and seed-set, showing no indication of automatic (or autogamous) self-pollination as suggested for some European species (Knuth 1909). This species is also considered to be highly self-incompatible despite a methodological breakdown with manual cross-pollinations in 1987. The circumstantial evidence provided by the bee-cones suggests that larger-sized insects, probably bumblebees, are the key pollinators. This is pursued further in the natural visitation study.

NATURAL VISITATION

Objectives.

In order to better understand the interrelationships of specific insect visitors with P.pubescens, some assessment of the relative value or effectiveness as pollen vectors must be made of each taxon. Only then can the pollinator(s) which contribute(s) the most to the pollination process be identified. This pollinator would then represent the key component which must be impacted upon in order for an insecticidal disruption to be reflected in a response by the plant.

Materials and Methods.

Twenty-five previously bagged plants were selected primarily for their ease of observation. They were roughly arranged in three groups (presumably three clones) in a line and separated by about three metres each. This facilitated vigilant observation of experimental flowers in two groups by one observer or three groups by two observers.

Non-experimental plants were intermixed and continuously available (not bagged) to serve as additional attractants to pollinators during experimental periods as well as maintaining an attraction between these periods. Observations were conducted on three dates in 1987 during the following hours: 24 May, 1440-1555 (Day1); 25 May, 1410-1530 (Day2); and 29 May, 1025-1320 (Day3), for a total of about 5.5 hours (approximately 9.5 man-hours) of observation.

A number of experimental plants were unbagged each experimental period as determined by the number of plants and flowers that could be confidently observed. Initially (Day1 and Day2), plants with recorded visits were immediately bagged after departure of the visitor in order to provide fruit-set data for flowers visited exclusively by a single taxon or category (eg. bumblebee) of visitor. Subsequently (Day3), multiple-taxon

visits (eg. bumblebee and fly) were allowed to occur.

A visit was considered to begin when an insect landing on a flower made internal contact of the flower with its head/mouthparts, and to end when the insect left the flower. An individual flower could be visited more than once during one or more visits to the plant, by more than one taxon on the same day, or visited on more than one day (only one occurrence).

Records included notation of individual flowers and the best identification of the visitor. Some observations of the behaviour of the insect visitors were made along with rough estimates of visit duration. Qualitative, representative collections of specimens were also made, particularly of previously unidentified taxa. These were generally collected after they departed from the experimental flowers or neighbouring, non-experimental flowers.

Bags were left in place on the experimental plants and then removed when all flowers were no longer open. Plants were harvested on 6 July and the fate of all flowers was determined by scoring as "seed-bearing fruit" or "not seed-bearing fruit" (invariably absent since unsuccessful flowers dehisce and are lost). Seed-set estimates were not calculated because of small sample sizes.

Pollen samples were taken from a few specimens of bumblebees observed to actively collect pollen. These were applied as a slurry in 70% ethanol to a microscope slide, stained with aniline blue, dehydrated with ethyl acetate, and mounted in Canada balsam. Reference was made to pollen taken directly from flowers and to Kapp (1969) in order to identify Polygonatum pollen.

Results and Discussion.

Pollinator effectiveness.

Table IV provides a list of the taxa observed visiting P.pubescens and are primarily hover flies and bumblebees. Besides these insect visitors, a male ruby-throated hummingbird (Archilochus colubris (L.)) was observed in the area and on one occasion visited several flowers of P.pubescens. No particular level of biological significance can be attached to these observations.

Table IV. Insects visiting flowers of P.pubescens

COLEOPTERA
Staphylinidae
Anthobium sp.
DIPTERA
Syrphidae
Rhingia nasica Say
Meliscaeva cinctella (Zett.)
Melanostoma mellinum (L.)
Platycheirus obscurus Say
Platycheirus inversus Ide*
Muscidae
Thricops sp.
Tachinidae
Siphona sp.
HYMENOPTERA
Apidae
Bombus vagans Smith
Bombus perplexus Cresson

* observed only in 1986

Table V displays the frequency distributions of the fate of flowers grouped according to qualitative exposure-visitor histories as follows. "Never exposed, not visited" flowers were never open during the exposure

periods being either young, unopened flowers, or senescent, closed flowers. "Exposed, not visited" flowers were unbagged and open during an exposure period or part of an exposure period but not observed to have been visited. These two groups serve as controls. The "flies" group comprises flowers visited only by flies (primarily syrphids), the "beetles or unknown" group by staphylinids or unidentified/unrecorded visitors only, the "bumblebees" group by bumblebees only (primarily P.vagans), while the "bumblebees + flies" group comprises flowers visited by both bumblebees and flies (including one unsuccessful flower visited by a bumblebee and an unidentified visitor).

Table V. Relative pollinating effectiveness of insects visiting flowers of P.pubescens

EXPOSURE-VISITOR	FATE OF FLOWERS		
	n	Fruit : Not fruit	%Fruit-set
Flowers not visited:			
Never exposed	79	1 : 78	1.3 a*
Exposed	90	5 : 85	5.6 a
Flowers visited by:			
Flies	27	0 : 27	0.0 a
Beetles or unknown	4	1 : 3	25.0 ab
Bumblebees	21	7 : 14	33.3 b
Bumblebees + flies**	8	5 : 3	62.5 b

* different letters indicate significant differences (G^2=39.185, d.f.=5, P<0.05)
** includes one unidentified/unknown visitor

A G-test of these proportions revealed significant differences (G^2=39.185, d.f.=5, P<0.05) primarily attributable to the relatively good success rate of flowers visited by bumblebees as compared to controls or to those visited only by flies. The intermediate position of the "beetles or unknown" category is presumably a function of low sample size overriding the relatively high success rate. This particular grouping consists of two

flowers visited by staphylinids which were unsuccessful while one of two flowers visited by unidentified visitors (identity not recorded and potentially bumblebees) produced a fruit. This is a very artificial grouping and will not be further discussed. Certainly the results of the bee-cone trial in the previous section clearly demonstrates that small insects like these staphylinids are not responsible for the majority of successful pollination achieved in P.pubescens.

The common element of the last two categories in Table V is the bumblebees (primarily B.vagans). The very high rate of success (62.5%) for flowers visited by both bumblebees and flies is not significantly different from bumblebees alone and is interpreted to be an insignificant artifact of sampling error, not the expression of some synergistic phenomenon (but see comments below).

As a check for bias within the categories discussed, the relative proportions of single:multiple visits within the "flies" (24:3) and "bumblebees" (14:7) were tested. There were no or marginally significant differences between these two main groups of flower visitors (G^2=3.557, d.f.=1, 0.10>P>0.05), suggesting a slight bias toward more multiple visits in the flowers visited only by bumblebees as compared to those visited only by flies. Even so, a G-test of fruit-set in single vs. multiple visits revealed no significant differences (bumblebees only - 4:10 vs 3:4, G^2=0.421, d.f.=1, P>0.10; bumblebees and bumblebees + flies combined - 8:13 vs. 4:4, G^2=0.336, d.f.=1, P>0.10). The data at hand cannot discriminate a difference in effectiveness of single or multiple visits by bumblebees.

Activity and behaviour.

Rigourous quantification by means of timed observations of visitation rates was not attempted but the following comments can be made. Flies generally visited a flower for a period ranging from a few seconds to in excess of ten minutes. Most of this time appeared to be spent contacting the anthers with the labellum, presumably feeding on pollen. This was also true of Rhingia nasica, the most frequently encountered fly, which

possesses a relatively long, geniculate proboscis. But even this species is physically incapable of accessing the nectar at the base of the flower. Pollen is presumably the only floral reward for flies although no crop dissections have been undertaken. It is interesting to note that Knuth (1909) reports a record of Rhingia rostrata L. visiting European Polygonatum.

Bumblebees spent considerably less time per flower, usually about five seconds, and never more than 10-15 seconds. Bumblebees could be seen, through the transluscent perianth, to extend the proboscis to the accumulated nectar at the base of the flower. Only one specimen within the study and three specimens outside the study, were ever observed to collect pollen. The low frequency of this foraging behaviour illustrates the primary role that nectar plays in attracting bumblebees, particularly B.vagans, to flowers of P.pubescens. Three of these specimens were of B.vagans (one specimen in 1986) and were determined to be carrying pure loads of P.pubescens pollen. One specimen of B.perplexus collected in 1986 bore a pollen load of an unidentified mixture containing a few P.pubescens pollen grains.

Table VI summarizes information on visitation in the three main groups of visitors over the three-day period. For the most part, the "beetles or unknown" (no recorded identification) visitors play a minor role and will generally be ignored. The majority of records were made on Day3 in all categories and was likely due to warmer temperatures and possibly the earlier hours of observation.

Bumblebees appear to visit more flowers per visit to a plant (39/14=2.8) compared to flies (38/28=1.4) and this relates back to the differences in handling time and behaviour as previously mentioned.

Two possible behaviour biases or differences could be present as evidenced by these data. Bumblebees visited plants with about 5.4 (54/10) open flowers while those plants visited by flies bore 8.3 (116/14) open flowers. However, this disparity was not as great on Day3 (bumblebees -

42/5=8.4; flies - 110/12=9.2) when the majority of the observations were made. Also, a much larger proportion of total fly activity occurred on Day3 compared to that of bumblebees as illustrated by, for example, the number of plants visited (12/14 vs. 5/10), or the number of flowers visited (31/34 vs. 17/29).

Table VI. Activity of insect visitors at flowers of P.pubescens.

VISITOR	ACTIVITY: # of flowers visited / # of flowers open* : # of flower visits / # of plants visited			
	Day 1	Day 2	Day 3	All days
Bumblebees**	5 : 2 / 4 / 2	11 : 6 / 10 / 3	23 : 6 / 42 / 5	39 : 14 / 54 / 10
Flies**	2 : 1 / 4 / 1	1 : 1 / 4 / 1	35 : 26 / 110 / 12	38 : 28 / 116 / 14
Beetles or unknown**	0	0	5 : 5 / 39 / 5	5 : 5 / 39 / 5
All taxa combined	7 : 3 / 8 / 3	12 : 7 / 14 / 4	63 : 37 / 124 / 14	82 : 47 / 60 / 20

* only of those plants visited
** includes mixed-taxon visits and are not mutually exclusive as evidenced by non-additivity in some categories (eg. # of flowers visited)

If a date bias is related to differential receptivity or attractiveness of flowers such that low receptivity on Day3 negatively affected pollinator efforts, this should be reflected in the time distribution of successful

flowers (fruits). Table VII displays this information and indicates that over half (7/13) the fruits produced by flowers with recorded visits resulted from activity on Day3 demonstrating that there were receptive flowers available on Day3. When considering the rate of fruit production from bumblebee visits (# fruits/ # flowers visited) as a measure of daily effectiveness, no significant differences were revealed amongst days (2/5, 4/11, 6/23, G^2=0.592, d.f.=2, P>>0.10). With the narrow overlap of flowers visited by both bumblebees and some other taxon on Day3 (8/46), the possibility for differential attraction exists but does not affect the principal conclusions of the pollinator evaluation. If flies are attracted to flowers with lower potential for successful fruit-set, then they suffer loss of efficiency as pollinators and this further describes them as a less important pollinator component. A larger study will be necessary to investigate further the interaction of effects of bees and flies on the same flowers.

Table VII. Distribution over time of fruits resulting from flowers with recorded visitors.

TAXON		TIME			
		Day1	Day2	Day3	Total
Bombus vagans		2	3	2	7
B.vagans + syrphid		0	1*	4	5
unknown	0	0	1	1	
		—	—	—	—
Total	2	4	7	13	

* bumblebee on Day2 + syrphid on Day3

No nocturnal observations were made of P.pubescens. Certainly if night-flying moths visit this flower there is a potential for a

contribution to fruit-set. In light of the evidence provided, it would appear that diel bumblebees provide 41.4% (12/29) fruit-set success rate of flowers visited compared to 38.6% of all flowers in the open-pollinated treatment (pollinator-exclusion study). This would require that bumblebees visit approximately 93% of all flowers. The additional possibility of other long-tongued bees being involved at other localities cannot be excluded.

Conclusions.

There is an underlying qualitative difference in the behaviour of bumblebees and flies at flowers of P.pubescens which overrides the superficial effects of abundance and frequency of contact. The concerns of possible bias in the data set have been considered but cannot detract from the disparate fruit-set success rates of flowers visited by these two groups. Bumblebees are the main driving force in the pollination of P.pubescens at this site and the possible synergistic effect of bumblebees and flies awaits testing with a larger data set. This supports the description of Polygonatum as a bumblebee flower (Knuth 1909).

The floral resources being utilized can be broadly summarized as nectar and pollen for bumblebees but pollen only for flies. The structure of the flowers reduces the chances of a fly contacting the stigma as it grazes pollen from the anthers converging beyond the recessed stigma.

NECTAR REMOVAL

Objectives

As an extension to the study of resource use by insect visitors to P.pubescens, a comparison of nectar availability and quality was made in flowers accessible to visiting nectarivores and in flowers protected from such depletion. This simple experimental procedure attempted to demonstrate that nectar was indeed being utilized and the effectiveness with which nectarivores remove the nectar.

Materials and Methods

A portion of the previously bagged and numbered plants was arbitrarily selected and one of two treatments, protected and unprotected, was randomly assigned to each on 21 May when all flowers were still unopened. Those plants assigned to the unprotected treatment had their bag removed. Microcapillary tubes (Microcaps®, Drummond Scientific Co.) were used to extract nectar individually from all flowers considered to be open and accessible to penetration. All flowers not yet open and those senescing and closed at sampling time were not sampled and were considered not available even if nectar was evident through the perianth.

A microcapillary tube was probed beyond the converging anthers to several points along the inside perimeter of the base of the perianth. Accumulated nectar could usually be observed through the translucent perianth and often guided extraction. The length of each tube that was occupied by nectar was measured to the nearest 0.5mm and recorded alongside the size (1µl, 5µl, 10µl) of tube used since some flowers required more than one tube, often of different sizes. These values were later converted to total volume measures for each flower on each sample date.

All tubes from the same flower were placed in the same plastic screw-cap vial (8 ml) and stored on ice in a thermos. Samples of less than 4µl were

usually pooled into common vials since these were less than the minimum sample volume required by the refractometer (Erma Optical Works, Ltd., Tokyo) taking into account some loss due to leakage into the vials during transport. The samples on ice were transported to the laboratory and refractometer readings taken that evening.

Samples were first taken when a substantial number of flowers were open on 24 May (Day1), and subsequently on 27 May (Day2) and 30 May (Day3). Sampling was conducted during the hours of 1330-1630, 1045-1645, and 1030-1300 on the three days, respectively.

Each plant was scored for its total number of flowers and the number of flowers open and sampled on each sampling date to provide measures of available bloom.

Results and Discussion

Table VIII presents information on the flowers/plant and the bloom characteristics for the three sample dates.

Table VIII. Bloom characteristics of P.pubescens sampled for nectar.

TREATMENT	CHARACTERISTICS							
	No. of plants (flowers)	$\bar{x}$ Flowers/ plant (S.D.)	No. of flowers open (1st:2nd)*			Mean % bloom (S.D.)		
			Day1	Day2	Day3	Day1	Day2	Day3
Protected	17 (117)	6.158 (4.272)	22 (NA)	73 (51:22)	42 (41:1)	22.2 (27.0)	68.3 (24.4)	30.5 (23.2)
Unprotected	18 (109)	6.056 (2.667)	35 (NA)	68 (34:34)	25 (25:0)	29.9 (19.9)	65.6 (23.8)	21.8 (17.5)

* proportion of first-sampled ("virgin") flowers to second-sampled
NA not applicable

The two treatments did not differ in total flowers/plant (linear ANOVA F=0.01, d.f.=1, P=0.9313; H=0.18, U=157.00, P=0.6688). A two-way ANOVA (Table IX) indicated no significant treatment (P=0.7758) or interaction (P=0.2962) effects on bloom but a highly significant influence of sample date (P<<0.001). Bonferroni's test and the G-test (G^2=87.960, d.f.=1) both discriminated the %bloom on Day2 as significantly higher (P<0.01) than Day1 and Day3. In these attributes, the two treatments can be regarded as comparable subsamples of the local population.

Table IX. Two-way ANOVA table for treatment and sampling date effects on % bloom (linear data)

Source	S.S.	d.f.	M.S.	F-value	Tail probability
Treatment	0.0042	1	0.0042	0.08	0.7758
Sample Date	4.1155	2	2.0577	39.64	0.0000
Interaction	0.1278	2	0.0639	1.23	0.2962
Error	5.4506	105	0.0519		

It should be noted that the three sample dates roughly represent early, peak, and late portions of the phenology of total available bloom. The cumulative bloom over the experimental period represents 97.4% (protected) and 86.2% (unprotected) of the total available flowers.

Analyses of nectar volume and sugar concentration are applied to values taken from individual flowers.

Sugar concentration

It is less circuitous to begin the discussion of nectar removal by first considering the sugar concentration estimates (gm sucrose/ 100gm solution). Figure 4 displays the distribution of sugar concentration according to treatment and sample date. Table X summarizes the sugar concentration

values for various categories of treatment, sample date, and past-sampling.

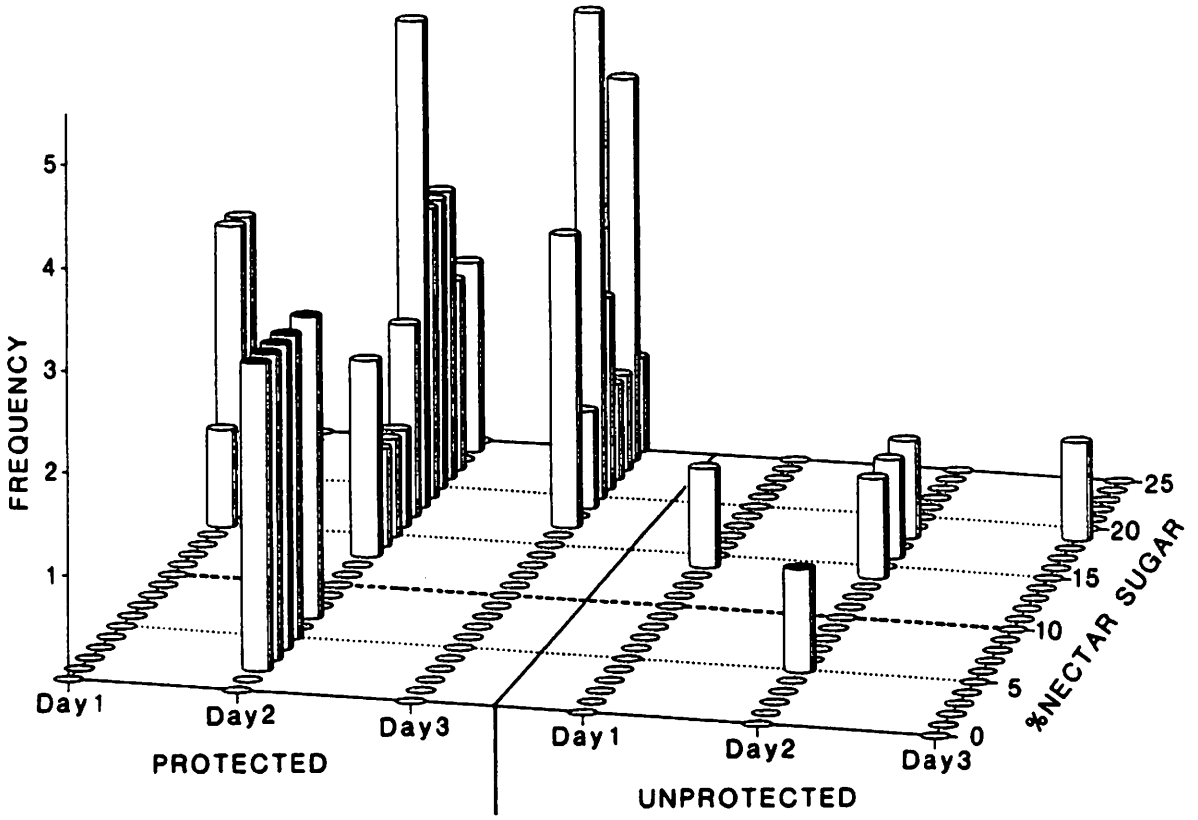

Figure 4. Frequency histogram of nectar sugar concentration (gm sucrose/ 100gm solution) from protected and unprotected flowers of P.pubescens sampled at three-day intervals (solid-topped columns represent flowers previously sampled on Day1).

Refractometer readings required a minimum sample of about 4µl which limited the number of readings for the unprotected treatment on each sample date (n=1,4,1, respectively) and subsequent analytical options. When considering all readings for each treatment irrespective of sample date (n=65,6), no significant differences due to treatments were recognized (linear ANOVA F=0.22, d.f.=1, P=0.6425; H=1.32, U=250.50, d.f.=1, P=0.2512). This was also the conclusion when comparing only the readings obtained for Day2 (n=40,4) (linear ANOVA F=0.00, d.f.=1, P=0.9934; H=0.11, U=88.00, d.f.=1, P=0.7439).

Table X. Sugar concentration* in nectar from protected and unprotected flowers by sample date and past-sampling.

TREATMENT	% NECTAR SUGAR* (linear $\bar{x}$ (s.d.) n)					
	Day1	Day2			Day3	All dates combined
		1st	2nd	Both		
Protected	16.400 (0.966) 7	18.724 (2.949) 25	4.240 (1.772) 15	13.293 (7.544) 40	21.028 (2.511) 18	15.769 (6.935) 65
Unprotected	14.300 (0.000) 1	15.933 (1.626) 3	5.500 (0.000) 1	13.325 (5.383) 4	18.900 (0.000) 1	14.417 (4.729) 6

* (gm sucrose/ 100gm solution)

A striking bimodality in sugar concentration was noted on Day2 (Fig. 4). This was due to the fact that all but one (unprotected treatment) of the flowers sampled on Day1 was also sampled on Day2, of which a subset provided large enough samples for refractometer readings. The horizontal line in Figure 4 (at 10µl) represents the separation of the flowers sampled for the first time (above the line) and those sampled for the second time (below the line). This distinction between first-sampled and second-sampled flowers was supported by a two-way ANOVA of treatment and past-sampling effects on Day2 sugar concentration (n=25,15,3,1) as summarized in Table XI. There were no significant interaction ($P=0.1913$) or treatment ($P=0.6182$) effects on sugar concentration. However, there was a highly significant effect due to past-sampling ($P<<0.001$) and Bonferroni's test discriminated these readings into two pairs based on past-sampling ($P<0.01$).

Guided by the previous observation, more refined comparisons can be made of first-sampled flowers only, ignoring the previously sampled flowers. The results of a two-way ANOVA of treatment and sampling date effects on sugar concentration (n=7,25,18,1,3,1) are summarized in Table XII.

Interaction effects were virtually non-existent (P=0.9635) but the effect of sample date was borderlining on significance (P=0.0611) suggesting some changes over time in nectar sugar concentration. There were no significant treatment effects (P=0.0965). If only the flowers sampled for the first time on Day2 are compared (n=25,3), no significant differences attributable to treatment are uncovered (linear ANOVA F=2.53, d.f.=1, P=0.1235; H=2.67, U=59.50, d.f.=1, P=0.1021). Larger sample sizes would be needed to further investigate these effects over the entire time period.

Table XI. Two-way ANOVA table for treatment and past-sampling effects on nectar sugar concentration (linear data).

Source	S.S.	d.f.	M.S.	F-value	Tail probability
Treatment	1.6271	1	1.6271	0.25	0.6182
Past-sampling	431.1622	1	431.1622	66.86	0.0000
Interaction	11.3944	1	11.3944	1.77	0.1913
Error	257.9483	40	6.4487		

Table XII. Two-way ANOVA table for treatment and sampling date effects on nectar sugar concentration (linear data) in flowers sampled for the first time.

Source	S.S.	d.f.	M.S.	F-value	Tail probability
Treatment	19.1538	1	19.1538	2.87	0.0965
Sample date	39.4982	2	19.7491	2.96	0.0611
Interaction	0.4963	2	0.2481	0.04	0.9635
Error	326.7884	49	6.6692		

Thus, the quality of nectar, as measured by sugar concentration (sucrose equivalents), is not demonstrably different between treatments - only

past-sampling and likely sampling date have an effect. However, visual inspection of Figure 4 suggests a slight depression in the unprotected treatment which is not statistically supported ($0.12>P>0.09$), presumably because of the small sample sizes. Such a difference might be expected since at least some of the protected nectar had accumulated over a period of up to three days and might be subject to greater evaporative water loss (Plowright 1985). This is to be contrasted with the unprotected treatment where sequential depletion by insect visitors would be expected to increase the relative proportion of "fresh" (recently secreted) nectar available for experimental sampling.

Nectar volume

Figure 5 displays the frequency distribution of nectar volume over the three sample dates distinguishing between first- and second-sampled flowers and excluding the one observation of a second-sampled flower on Day3 (0.141µl, protected treatment). These data are further summarized in Table XIII. Knowing that the nectar obtained from second-sampled flowers is low in sugar compared to first-sampled flowers, only these first-sampled flowers are considered here initially.

A two-way ANOVA to test for treatment and sample date effects on nectar volume of only first-sampled flowers (n=22,51,41,35,34,25) could not be made to satisfy Levene's test of homogeneity for both main effects and interaction despite transformation, as might be expected from the distributions. Therefore, separate Kruskal-Wallis and Mann-Whitney tests were applied to each sample date and these distinguished significantly ($P<<0.001$) higher nectar volumes in the protected treatment on all three sample dates (Day1: $H=18.97$, $U=650.50$, d.f.=1, $P<<0.001$. Day2: $H=46.67$, $U=1628.50$, d.f.=1, $P<<0.001$. Day3: $H=30.97$, $U=933.00$, d.f.=1, $P<<0.001$).

A two-way ANOVA of treatment and past-sampling effects on nectar volume for the Day2 readings (n=51,22,34,34) also could not be made to satisfy Levene's test of homogeneity for all effects. Kruskal-Wallis and Mann-Whitney tests were applied to past-sampling effects on Day2 in each

treatment separately. The two classes of unprotected flowers on Day2 were not significantly different (H=0.07, U=600.00, d.f.=1, P=0.7872) while those in the protected treatment were (H=4.08, U=393.00, d.f.=1, P=0.0434).

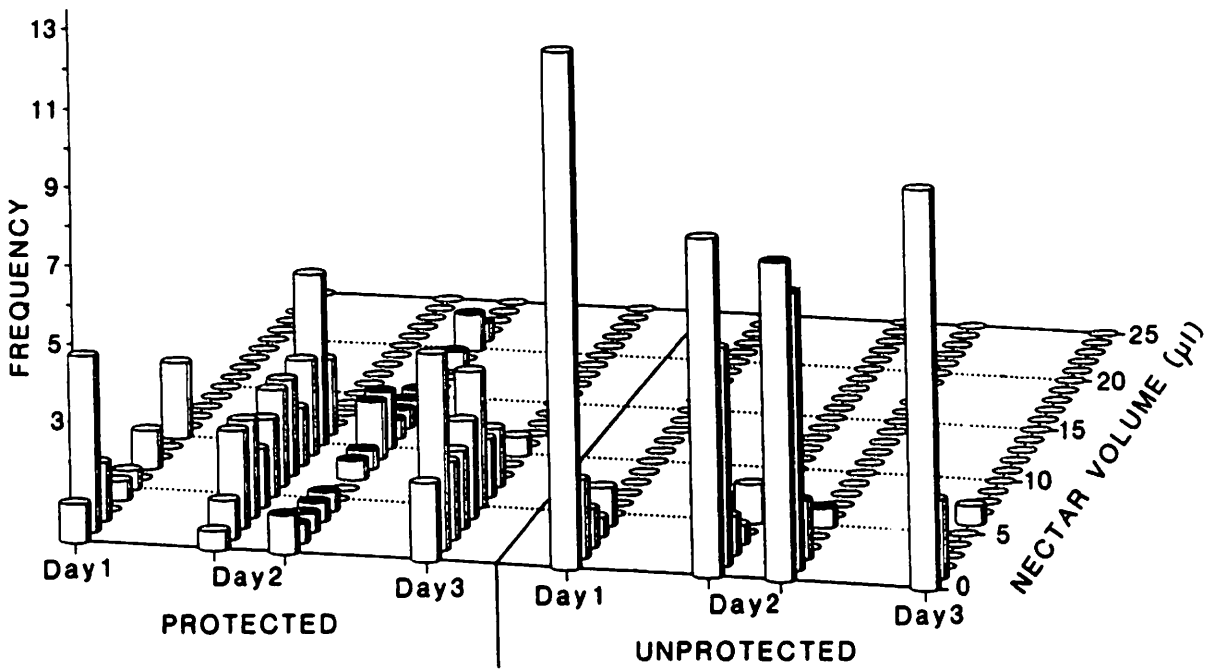

Figure 5. Frequency histogram of nectar volume from protected and unprotected flowers of P.pubescens sampled at three-day intervals (solid-topped columns represent flowers previously sampled on Day1).

The differences amongst the distributions of nectar volume in Figure 5 are perhaps best discussed narratively in light of the difficulties in analysis. Besides the demonstrated treatment differences on each sampling date implicating an overall depletion in nectar in the unprotected treatment, several observations can be made. Considering only the first-sampled flowers, similar trends over time can still be seen in both treatments despite depletion in the unprotected treatment with the greatest volume measured on Day2 (Table XIII). This sampling date effect is ultimately tied to extrinsic factors such as temperature and humidity and

intrinsic factors such as phenological differences in nectar secretion rates and cannot be discussed within the context of this experimental design.

Table XIII. Nectar volumes from protected and unprotected flowers by sample date and past-sampling.

TREATMENT	NECTAR VOLUME (µl) (linear $\bar{x}$ (s.d.) n)					
	Day1	Day2			Day3	All dates combined
		1st	2nd	Both		
Protected	3.505 (3.650) 22	6.404 (3.252) 51	9.585 (6.481) 22	7.362 (4.664) 73	3.306 (2.400) 41	5.516 (4.401) 136
Unprotected	0.630 (1.045) 35	1.029 (1.499) 34	0.791 (1.027) 34	0.910 (1.280) 68	0.446 (1.145) 25	0.743 (1.200) 128

The most striking feature is the distinctive distribution of nectar volume in the protected, second-sampled flowers. These flowers exhibit the greatest individual values and range of nectar volumes. The second-sampled flowers in the unprotected treatment do not have a corresponding distribution since they have been sequentially depleted by nectarivores which apparently did not discriminate amongst sugar-rich and sugar-poor nectars. Such learned behaviour is unlikely to have developed in the relatively small scale of this experiment and has been suggested for nectar volume and not specifically for concentration in conspecific flowers (Corbet et al. 1984).

The second-sampled flowers remained open and accessible over the initial three-day period from day1 to day2. Therefore, they are flowers of maximal age in the day2 samples as well as having the unique quality of being previously sampled. This raises doubts as to the suitability of comparing

them with the other flowers sampled only once. The reduction in sugar concentration could possibly be: 1. elicited by the depletion of the initially sugar-rich nectar; 2. the result of heightened susceptability to dilution by rainwater as the perianth softens and begins to close; or 3. the direct result of mechanical damage to the tissue of the nectaries by the glass microcapillary tubes which might incapacitate normal sugar secretion or increase "leakage" of less concentrated fluids.

None of these hypothetical explanations has been pursued experimentally but a few comments are in order. If the depletion of initially sugar-rich nectar was occurring, a reflection of this might have been expected in the unprotected plants. These flowers were being depleted naturally and no reduction in sugar concentration was evident (n=5). However, these samples were necessarily biased toward relatively large volume and smaller sample volumes resulting from recent depletion could be less sugar rich. Nonetheless, four samples of pooled low-volume nectar indicated no reduction in sugar concentration (see comments below). The possibility of rainwater contamination is real but this would again have been expected to show up in the unprotected flowers. The small sample size does not allow a firm conclusion to be drawn on either of these two points. Just the same, a conversion method to estimate absolute sugar content (volume x concentration x correction factor; where the correction factor = 0.0046(concentration) + 0.9946; Cruden and Hermann 1983) demonstrates that there was significantly ($P<<0.001$) less sugar present in the second-sampled flowers (Day2 protected flowers: first-sampled 1.746±0.504ug (n=25), second-sampled 0.583±0.392ug (n=15); linear ANOVA $F=11.28$, d.f.=1, $P<<0.001$; $H=22.96$, $U=359.00$, d.f.=1, $P<<0.001$). This suggests that an explanation cannot entirely be made with dilution by rainwater.

The opportunity for damage to have been caused by the glass micro-capillary tube to the secretory and adjacent tissues in the base of the flower is, in the author's opinion, the most likely or plausible. This is prompted by the observations made, while extracting the nectar, of the frequent difficulty in reaching into the narrow confines of the flower base, especially when a persistent drop of nectar could be seen through the

perianth. Also, as evidenced by the volume estimates, these second-sampled flowers provided a greater proportion of larger volume samples and displayed the greatest range of volumes. This suggests an unregulated "leakage" of fluid low in sugar content from the damaged tissues.

General Comments and Conclusions

The limiting of sugar concentration estimates to those of higher (>4µl) volume was the result of restricting analyses to individual flowers. Since these required individual volume estimates it was decided not to pool nectar samples over several flowers to accumulate sufficient volumes for the refractometer. A check was made of unprotected, non-experimental flowers in the periphery of the study area. A total of four samples was taken and each represented a cumulative sample of small volumes from several flowers. The low number of samples was a reflection of the difficulty of obtaining nectar from exposed flowers which were being well serviced by bumblebees. One sample was taken on 28 May and the other three were obtained on 30 May (Day3) giving sugar concentration readings of 21.4, 22.6, 20.8, and 12.1 (gm sucrose/ 100gm solution). These are well within the ranges of the other first-sampled experimental flowers except perhaps for the last reading of 12.1. This could possibly be the result of having sampled some of these peripheral flowers a second time since only two days had separated the two sample dates and no record or identification was made of previous sampling of these flowers. It appears safe to say that the sugar concentration readings of experimental flowers, though biased toward larger volumes, are representative of all nectar secreted by similarly treated flowers.

The principal restriction of analysis to first-sampled flowers primarily affects Day2 samples since only one other second-sampled flower exists from Day3. Applying a G-test of the unprotected flowers not previously sampled as a proportion of the total flowers (protected - 51/117, unprotected - 34/109; $G^2=3.714$, d.f.=1) indicated no or marginally significant differences ($0.10>P>0.05$). These proportions represent a modified %bloom

("virgin" bloom) and confirm that there is an insignificant bias in this regard. However, there is a significant difference ($P<0.05$) in the relative frequencies of first- and second-sampled flowers (protected - 51:22, unprotected - 34:34; $G^2=5.837$, d.f.=1) between treatments on Day2. This probably represents a slight phenological shift between the two groups of flowers since a higher proportion of the Day2 flowers in the unprotected treatment were older, previously sampled flowers.

Despite this, the evidence is clear that there are treatment effects on nectar volume but not on nectar quality. The effects of sampling date and past-sampling are secondary to these main observations and do not alter the conclusion that nectarivores are effectively utilizing the nectar of P.pubescens.

IMPLEMENTATION.

Objectives

Having established baseline data that demonstrate the potential for disruptions in the pollination of P.pubescens if bumblebee populations are sufficiently impacted upon, certain considerations must be made regarding the implementation of this system in an insecticidal impact monitoring program. No data are available on what level of variation one might expect to find when comparing different populations at disjunct sites. The following section reports data including estimates of fruit-set and seed-set from three sites in addition to those made at Icewater Creek. These are then discussed and compared, along with other pertinent attributes, to those of another bumblebee-pollinated lily, Clintonia borealis (Ait.) Raf..

Materials and Methods

The three additional sites were found by scouting the local area for mixed forest predominated by Acer saccharum. These were called the Stokely site (30kmWSW of the Icewater Creek site (IWC)), the Tower site (22kmSW of IWC), and the Whitman site (1.8kmS of IWC). These were sampled on 16 June, 15 July, and 22 July, respectively, while the sample from the Icewater Creek site was made on 29 June and comprised the open-pollinated treatment of the pollinator-exclusion experiment. The plants from the other five treatments of the pollinator exclusion experiment were also incorporated in an investigation of correlates of total flower counts

Plants were collected in an ad hoc manner as they were encountered while hiking through the sites. Plants with no pedicels were not included in the analysis. The plants were snipped off at ground level and transported back to the laboratory for assessment. Approximately 100 plants was the goal set for each of the three new sites (only 26 at IWC).

Plants were scored for a number of growth characteristics including stem length below basal leaf (stemlo), stem length above basal leaf (stemhi), the sum of these representing total stem length (stemtot), number of axils (axils), number of pedicel-bearing axils (pedaxils), number of pedicels (pedicels), number of flowers (totflow, IWC only), number of fruit, number of seeds, and number of ovules.

Pedicel counts used in this study excluded only those pedicels $\leq$1mm. in length since these had not borne a flower. However, any pedicel which remains parallel to the stem and does not arch away from it should also be excluded. Records kept in this study were not consistent for all four sites and thus precluded implementing this additional stricter definition over all four sites sampled. The advantage will be to provide a much more accurate, retrospective estimate of the flowers that were available during the blooming period and the subsequent estimates of fecundity by removing those pedicels representing early flower bud abortion.

Results and Discussion

Table XIV provides some insight into the correlative interrelationships of some of the characteristics scored for the 196 plants from the Icewater Creek pollination-exclusion experiment of 1987. Of particular interest at this time is the progressively greater correlations with total flower counts as more and more refined measures are taken of the upper part of the stem, as might be expected. The pedicels counted on a plant after fruiting has begun and unsuccessful flowers have dropped is highly correlated (R=0.96) with the number of flowers originally counted. Figure 6 graphically displays the relationship between pedicels and total flowers. Although there is not a 1:1 relationship, pedicel counts provide the best retrospective, relative measure of available bloom. Subsequently, there is a conservative bias in the eventual estimates of fruit-set including that for the Icewater Creek site which reflects modification resulting from the use of pedicels rather than total flowers to estimate fruit-set.

The estimates of the characters previously introduced are summarized for all plants collected (Table XV) and for only those plants bearing at least one fruit (fruit-bearing; Table XVI). Figure 7 graphically displays the fruit-set estimates.

Table XIV. Correlation matrix of growth characteristics in P.pubescens, Icewater Creek, 1987.

	stemlo	stemhi	stemtot	axils	pedaxils	pedicels
stemhi	0.73 (195)					
stemtot	0.93 (195)	0.93 (195)				
axils	0.59 (194)	0.88 (194)	0.79 (194)			
pedaxils	0.62 (195)	0.79 (195)	0.76 (195)	0.84 (194)		
pedicels	0.61 (195)	0.85 (195)	0.78 (195)	0.90 (194)	0.93 (195)	
totflow	0.59 (196)	0.79 (195)	0.74 (195)	0.84 (194)	0.90 (195)	0.96 (195)

The fruit-set estimates for all plants (Table XV) were discriminated into two groups (G^2=495.023, d.f.=3, P<0.01) with the plants from the Stokely and Icewater Creek sites setting significantly more fruit (Fig.7). When considering only fruit-bearing plants (Table XVI) in an effort to evaluate only those plants which had received pollinator service, the results were the same (G^2=148.719, d.f.=3, P<0.01). These differences could not be directly attributed to the mensural characteristics summarized in Tables XV-XVI since the Stokely and Tower sites ranked one and two in largest size (all plants), and counts of axils, pedicel-bearing axils, and pedicels, yet represented the extremes in fruit-set. These differences in

fruit-set would appear to be attributable to differences in pollinator service.

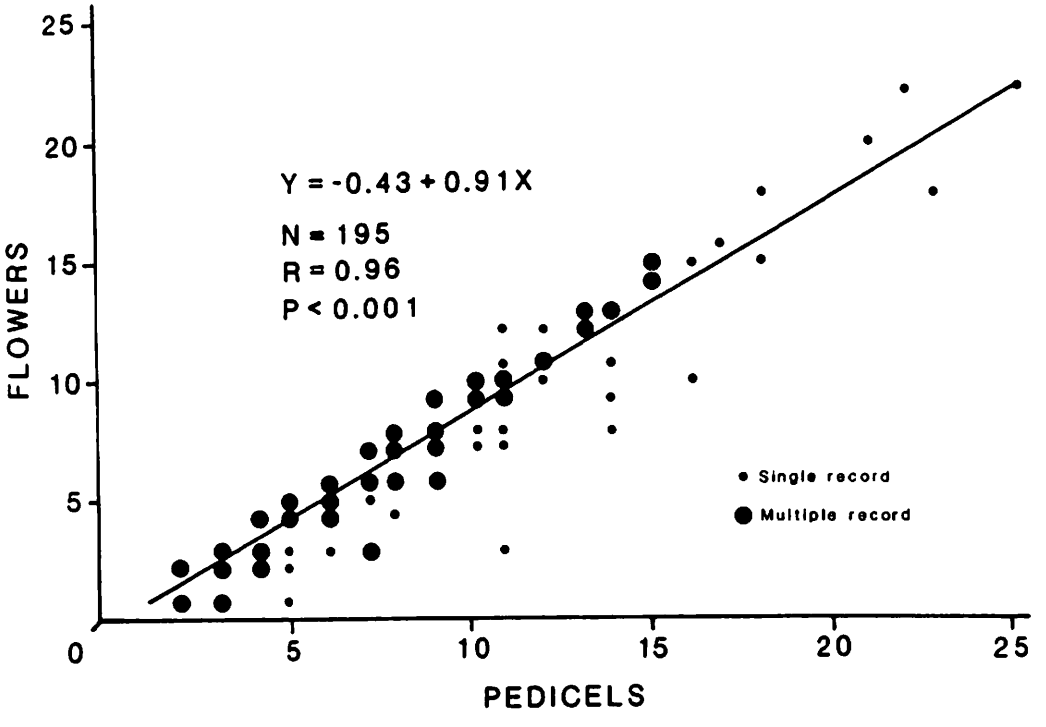

Figure 6. Scatter plot of relationship between number of flowers counted during bloom and number of pedicels counted after fruiting initiated for P.pubescens plants, Icewater Creek (pollinator-exclusion study).

The results of the fruit dissections are presented in Table XVII. Site differences in seed-set (Fig.8) were determined to be significant (ln-transformed ANOVA $F=6.90$, d.f.=3,889, $P=0.0001$; $H=29.07$, d.f.=3, $P<<0.001$) and attributed by Bonferroni's test entirely to the discretely greater seed-set at the Stokely site ($P<0.05$). The number of seeds/fruit showed the same general topography as seed-set (ln-transformed ANOVA $F=12.89$, d.f.=3,889, $P<<0.001$; $H=48.3$, d.f.=3, $P<<0.001$) although the relative postions of the Icewater Creek and Whitman sites were reversed (Table XVII) and Bonferroni's test discriminated greater seeds/fruit at the Stokely site at a higher level of significance ($P<0.01$).

Table XV. Attributes of naturally pollinated P.pubescens from four sites in the Searchmont, Ontario area, 1987 (all plants).

ATTRIBUTE	SITE			
	IWC	Stokely	Tower	Whitman
plants	25	130	100	80
stemlo cm. $\bar{x}$ (s.d.)	22.4 (4.2)	22.3 (8.0)	22.9 (5.9)99*	22.0 (5.0)
stemhi cm. $\bar{x}$ (s.d.)	11.8 (4.4)	15.4 (6.4)127	14.8 (5.5)	13.1 (5.4)
stemtot cm. $\bar{x}$ (s.d.)	34.2 (8.3)	37.6 (13.2)127	37.7 (10.7)99	35.1 (9.9)
axils/plant $\bar{x}$ (s.d.)	8.6 (2.2)	10.3 (2.4)129	9.6 (2.2)98	9.1 (2.2)79
pedaxils/plant $\bar{x}$ (s.d.)	6.0 (2.4)	7.2 (2.9)	6.5 (2.7)	5.6 (2.6)
pedicels/plant $\bar{x}$ (s.d.)	9.1 (5.5)	13.3 (8.1)	11.9 (7.0)	8.5 (5.5)
seeds/flower $\bar{x}$ (s.d.)	1.1 (1.0)	2.0 (1.7)	0.3 (0.5)	0.3 (0.6)
pedicels	227	1725	1191	676
fruits	81	657	88	70
% fruit-set	35.7	38.1	7.4	10.4

* reduction in sample size

Table XVI. Attributes of naturally pollinated P.pubescens from four sites in the Searchmont, Ontario area, 1987 (fruit-bearing plants only).

ATTRIBUTE	SITE			
	IWC	Stokely	Tower	Whitman
plants	19	107	37	26
stemlo cm. $\bar{x}$ (s.d.)	23.6 (3.6)	23.4 (8.2)	24.3 (5.7)	25.6 (3.9)
stemhi cm. $\bar{x}$ (s.d.)	13.3 (3.9)	16.6 (6.2)105*	17.2 (5.2)	16.8 (4.9)
stemtot cm. $\bar{x}$ (s.d.)	36.9 (7.1)	40.0 (13.0)105	41.5 (10.0)	42.4 (8.5)
axils/plant $\bar{x}$ (s.d.)	9.4 (1.9)	10.8 (2.3)106	10.5 (2.2)	10.4 (1.9)
pedaxils/plant $\bar{x}$ (s.d.)	6.9 (1.7)	7.9 (2.4)	7.6 (2.5)	7.3 (2.0)
pedicels/plant $\bar{x}$ (s.d.)	11.0 (4.8)	15.0 (7.7)	15.0 (7.2)	12.1 (4.7)
seeds/flower $\bar{x}$ (s.d.)	1.5 (0.9)	2.4 (1.6)	0.7 (0.5)	0.9 (0.7)
pedicels	209	1603	555	314
fruits	81	657	88	70
% fruit-set	38.8	41.0	15.9	22.3

* reduction in sample size

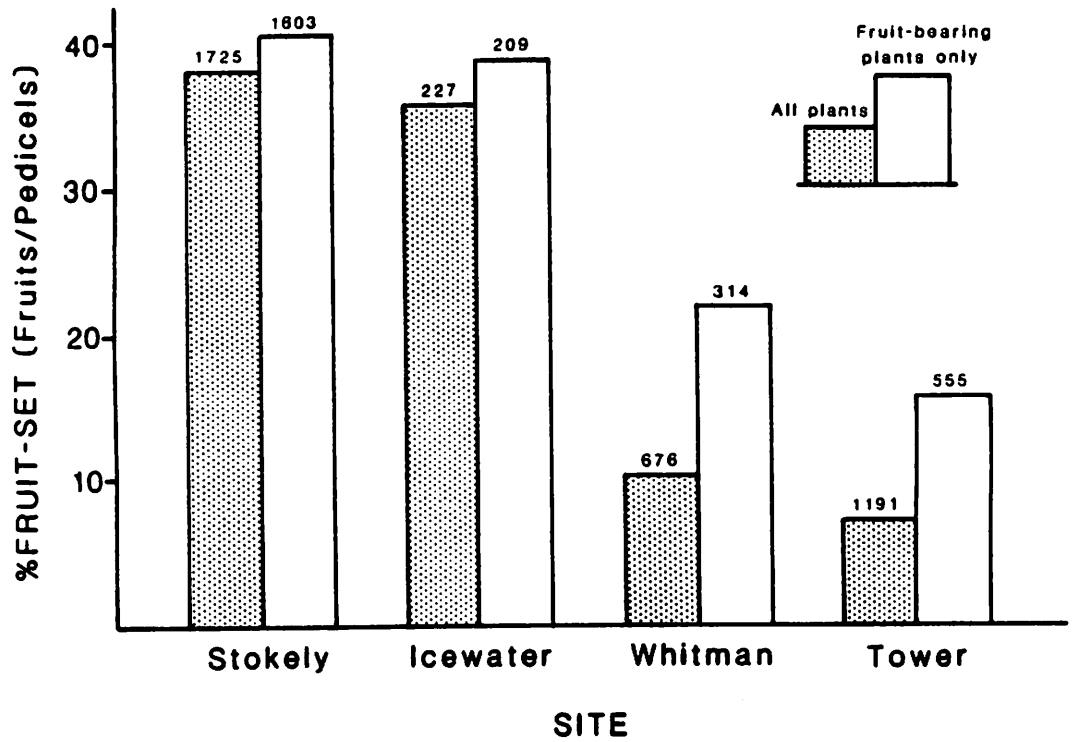

Figure 7. Fruit-set estimates for P.pubescens collected at four sites including all plants or only fruit-bearing plants.

The two parameters, seed-set and seeds/fruit, are highly correlated (all sites - R=0.94 (n=893); IWC - 0.88 (81); Stokely - 0.95 (654); Tower - 0.87 (88); Whitman - 0.82 (70)). This is in spite of the fact that there appear to be site differences in ovules/fruit (H=10.02, d.f.=3, P=0.0184). Despite transformation of the ovules/fruit data, homogeneity of variance could not be achieved and Bonferroni's test was not applied. However, estimates of seeds/fruit were not highly correlated with ovules/fruit (all sites - R=0.26 (n=893); IWC - 0.31 (81); Stokely - 0.26 (654); Tower - 0.14 (88); Whitman - 0.16 (70)). Thus, there do appear to be site differences in both seed-set and seeds/fruit. Seeds/fruit may well be a sufficient estimate of within-fruit success without having to laboriously search and count undeveloped ovules to obtain measures of seed-set.

Table XVII. Results of fruit dissections of P.pubescens from four sites in the Searchmont area, 1987.

ATTRIBUTE	SITE			
	IWC	Stokely	Tower	Whitman
fruits	81	654 *	88	70
seeds	321	3624	358	261
seeds/fruit $\bar{x}$ (s.d.)	4.0 (2.0)	5.5 (3.1)	4.1 (2.5)	3.7 (2.0)
ovules	868	7424	953	718
ovules/fruit $\bar{x}$ (s.d.)	10.7 (2.1)	11.4 (1.7)	10.8 (2.3)	10.3 (2.7)
% seed-set $\bar{x}$ (s.d.)	37.5 (17.3)	48.8 (25.7)	39.2 (24.0)	38.4 (20.3)

* three of the original 657 fruits had been damaged with some loss of contents

When comparing the estimates of fruit-set and seed-set (Figs.7-8) there is an obvious difference in the relative amount of between-site variation. Fruit-set is a much more variable estimate which is presumably a reflection of the all-or-none criterion of success hinging on the production of the first seed. If plants are situated so as not to be visible or attractive to bumblebees, samples could be unintentionally biased toward these areas and low estimates of fruit-set obtained. This is the justification for partitioning the samples obtained in this study to consider a complementary measure of fruit-set in those plants "known" to have been pollinated (fruit-bearing).

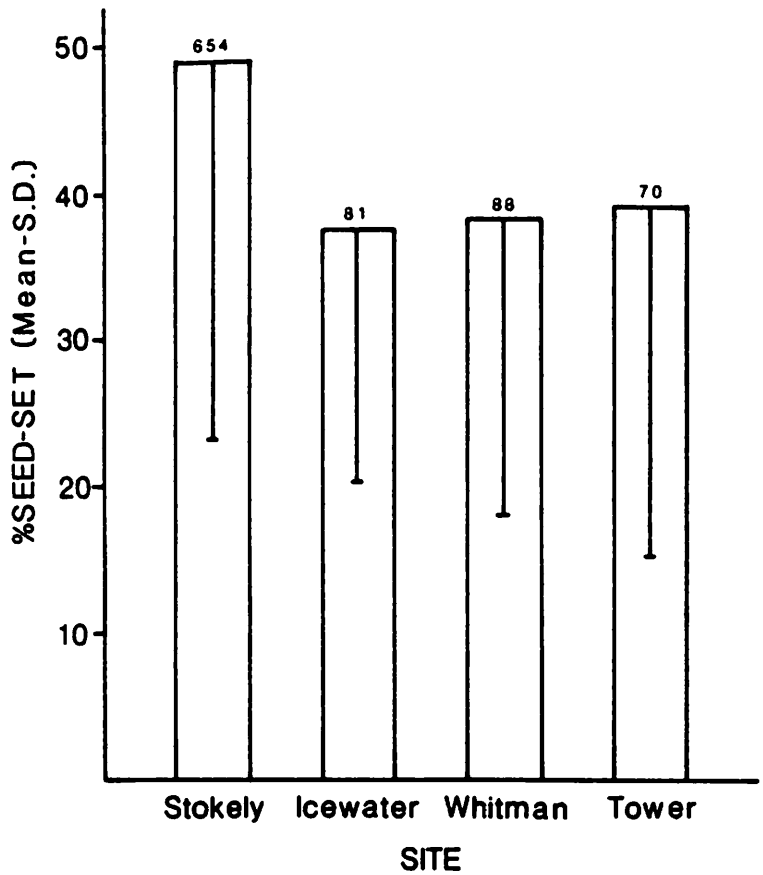

Figure 8. Seed-set estimates for P.pubescens collected at four sites.

This is to be contrasted with the stochastic seed counts (seed-set, seeds/fruit) which are a second level response using the different sampling universe of only successful flowers (seed-bearing fruits) and are not necessarily subject to the same sampling problems. Estimates of this sort could be loosely regarded as measures of the "quality" of the activity of bumblebees but assumes that multiple visitation or greater distances travelled between plants increases seed production as more or higher "quality" pollen (outcross) is deposited. This has not been demonstrated in P.pubescens. Just the same, in the context of insecticidal disruption, the expectation of demonstrating depressions in seed-set are likely to be lower than for fruit-set (see Holmes et al. 1981). As has been stressed before (Thaler and Plowright 1980) these problems can be circumvented by maximizing the number of flowers sampled. Also, a measure of seeds/flower,

which is essentially an arithmetic product of fruit-set (fruits/flower) and seeds/fruit, has been used successfully with Clintonia borealis (SOMER 1985, Hansen and Osgood 1984, Thaler and Plowright 1980) in monitoring programs.

Comparisons with Clintonia borealis

As an initial evaluation of the potential for utilization of P.pubescens in a monitoring program, it is worthwhile comparing it with another species. Clintonia borealis has been successfully used to demonstrate insecticidally mediated depressions in fruit-set and/or seeds/flower (Table XVIII). This flower is a woodland lily considered to be pollinated primarily by bumblebees (Barrett and Helenurm 1987, Thomson and Plowright 1980, Thaler and Plowright 1980, Galen et al. 1985). Twelve other species of insects are known to visit these flowers in New Brunswick of which four species of solitary bees may also be important (Barrett and Helenurm 1987). Table XIX summarizes some of the pertinent characteristics for each species and these will each be discussed further.

Table XVIII. Past use of Clintonia borealis in monitoring for insecticidal impacts on measures of fecundity.

INSECTICIDE	FECUNDITY REDUCTION (*)		
	Fruit-set	Seeds/flower	Seed-set
Fenitrothion	Yes (1,3,4)	Yes (1,3)	—
Aminocarb	Yes (1) No (4)	Yes (1) No (3)	—
Carbaryl	No (2)	Yes (2)	No (5)

* References - 1, SOMER (1985); 2, Hansen and Osgood (1984); 3, Thaler and Plowright (1980); 4, Plowright and Thaler (1979); 5, Holmes et al. (1981).

Insect-dependency has been demonstrated by Barrett and Helenurm (1987) who showed that 28% of bagged flowers produced fruit compared to 62% of the open-pollinated flowers. This is even more dramatically illustrated in P.pubescens where as little as 1% of the bagged flowers produced fruit. The differences between species may be partly explained by the apparent differences in self-incompatibility. This is known to vary greatly amongst clones of C.borealis (Galen and Weger 1986) but its strong protogyny would serve to delay self-pollination in self-fertile clones. This automatic selfing can apparently account for as much as 28% fruit-set in protected flowers (Barrett and Helenurm 1987). These workers showed a similar increase in seeds/fruit with increased "quality" of pollination (from bagged and self-pollinated to cross- and open-pollinated flowers) as was demonstrated in P.pubescens (pollinator-exclusion experiment). Nothing is known of the temporal gender of P.pubescens but an unsuccessful attempt to quantify stigmatal receptivity suggested constant but increasing peroxidase activity with the age of the flower (personal observation).

The number of flowers borne by each plant is considerably greater in P.pubescens. This may make some difference in handling and sampling with such a large range possible in P.pubescens.

Each species shows a similar range of approximately 30-40% in estimates of natural fruit-set but they are at opposite ends of the scale. The 62% fruit-set estimate in C.borealis was reported by Barrett and Helenurm (1987) where 28% of unvisited (bagged) flowers set fruit. This represents a difference of only 34% that can be roughly attributed to pollinator service alone. A similar effect in P.pubescens is negligible (1%). Another factor contributing to the discrepancies in fruit-set could be the larger number of flowers in P.pubescens. There may well be some production of "extra" flowers in this species which serve only as pollen sources (evidenced by personal observations of reduced styles in some flowers, particularly those developing later on individual plants) and not destined to produce fruit.

Table XIX. Comparative attributes of the two bumblebee-pollinated forest wildflowers, Polygonatum pubescens and Clintonia borealis.

ATTRIBUTE	CONDITION	
	Polygonatum pubescens	Clintonia borealis
Insect-dependency (%fruit-set B:O)*	high (1 : 30 %)	moderate to high (28 : 62 %) (a)**
Self-incompatibility	low but variability and temporal gender not known	clonally variable but protogynous (b)
Flowers/plant ($\bar{x}$ ± s.d.)	1 - 22 (7.4 ± 4.4)	2 - 8 (c) (3.6 ± 0.9) (a)
Mean %fruit-set	7 - 38	62 - 100 (a,d)
Mean %seed-set	38 - 55	39 - 58 (e)
Mean seeds/fruit	4.0 - 5.5	11.2 (a)
Mean seeds/flower	0.3 - 2.0	6 - 16 (d)
Flowering period	late May to early June (12-16 days)	early to mid-June (10-20 days) (f,g)

* B-bagged; O-open-pollinated

** References - a, Barrett and Helenurm (1987); b, Galen and Weger (1986); c, Fernald (1950); d, SOMER (1985); e, Holmes et al. (1981); f, Galen et al. (1985); g, Helenurm and Barrett (1987).

The only available information on seed-set in C.borealis is provided by Holmes et al. (1981). Their estimates are very similar to those found for P.pubescens although theirs represent three sequential estimates over the blooming period rather than a composite measure as used in P.pubescens.

The mean number of seeds/fruit in C.borealis is about twice that found in P.pubescens. This measure has not been used in a monitoring program probably because it is not sensitive to the influence of flowers which

produce no fruit. This measure, coupled with fruit-set, provides an estimate of seeds/flower.

An estimate of fecundity used by several reports for C.borealis is seeds/flower. This was not discussed at length in the present report but estimates are provided in Table XIX. Again, the differences between the two species are subject to the same influences as discussed for fruit-set and are further accentuated by the larger number of seeds/fruit in C.borealis.

The calendar flowering period of P.pubescens is likely to vary between years as has been reported for C.borealis (Helenurm and Barrett 1987). The two species have similar durations of bloom and, at least in the Icewater Creek area, can have widely overlapping flowering phenologies (unpublished data but comparisons of peak blooming times cannot be made with existing data). Hansen and Osgood (1981) indicate that C.borealis flowers as early as 24 May and as late as 24 June in Maine but did not provide any data for P.pubescens. Polygonatum pubescens is likely to be found to bloom somewhat earlier than C.borealis which may or may not be advantageous in any given monitoring program considering the range of dates and conditions under which operational sprays are undertaken. These two species will serve in concert to widen the window for monitoring insecticidal impacts on plant fecundity.

Another consideration is local availability of the two plants. These two species share similar northeastern distributions, associations with "rich moist woods and wooded bogs" (C.borealis) and "moist woods and thickets" (P.pubescens; Gleason and Cronquist 1963) and can occur together locally as in the Icewater Creek site. However, a survey of co-occurrence has not been made. Polygonatum pubescens may in fact be more common in mixed forest habitat (personal observation) than in spruce-fir forests. Thus, this pollination system might lend itself more to impact-monitoring during insecticidal control operations for pests of broad-leaved species.

No obvious disadvantage has surfaced to indicate that P.pubescens would

be limited in its implementation in an insecticidal impact monitoring program. What the present study has provided is an alternative or complementary pollination system to be used in a program to monitor for insecticidally induced depressions in plant fecundity mediated by impacts on bumblebees.

SUMMARY

Polygonatum pubescens has been determined, using pollinator-exclusion and manual pollination techniques, to be to be highly self-incompatible and to be dependent upon insect pollinators for effective production of fruits and seeds. The primary pollinators in this study were bumblebees which primarily utilized the nectar while flies, mostly syrphids, were ineffectual while grazing pollen from the anthers. The structure and orientation of the flower preclude utilization of nectar by insect groups having a relatively short proboscis and also minimize contacts with the recessed stigma.

Fruit-set and seed-set vary from site to site suggesting a need for adequate replication and sample sizes. Pedicel counts provide a good estimate of the number of flowers formerly present on the plants during blooming period. Seeds/fruit is probably an adequate parameter to replace seed-set as an estimate of within-fruit success and obviates the need to enumerate undeveloped ovules. Fruit-set and seeds/fruit are recommended as companion measures of fruiting success. Comparisons made with Clintonia borealis suggest that P.pubescens will also serve as an appropriate indicator species when monitoring for impacts of insecticidal sprays in forestry on the pollination service provided by bumblebees.

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