POLLEN REMOVAL IN *CYPRIPEDIUM ACAULE* (ORCHIDACEAE) IN RELATION TO AERIAL FENITROTHION SPRAYING IN NEW BRUNSWICK

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Abstract

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Measures of pollen removal in the orchid *Cypripedium acaule* Ait. provided a retrospective method for estimating the activity of bumble bee pollinators in New Brunswick spruce-fir forests. Significantly lowered activity was observed in fenitrothion-sprayed forest as compared with unsprayed control areas. The difference is probably attributable to direct mortality of bees in sprayed forest.

Résumé

Des mesures du prélèvement de pollen sur l'orchidée *Cypripedium acaule* Ait. ont été utilisées pour évaluer rétrospectivement l'activité des bourdons pollinisateurs dans des boisés d'épinette et de sapin au Nouveau-Brunswick. L'activité s'est révélée significativement moindre dans une forêt arrosée au fénitrothion que dans des boisés témoins non-arrosés. La différence est probablement due à la mortalité directe des bourdons dans la forêt arrosée.

Our investigations of the effects of aerial insecticide spraying for spruce budworm control on forest pollination in New Brunswick have demonstrated reduced fecundity among entomophilous plants in fenitrothion-sprayed areas which bloom soon after insecticide application (Thaler and Plowright, in press). To work out the quantitative relationships between such reductions and insecticide-induced pollinator mortality (Plowright *et al.* 1978), we have sought information on the activity of pollinating insects on various plant taxa. The prohibitive labour demands inherent in direct measurement of insect activity have led us to employ retrospective methods of assessing visitation rates to insect pollinated flowers. We have shown that nectar levels may be used for this purpose (Plowright and Rodd 1980), and recently one of us (JDT) has investigated methods involving the dispersal of fluorescent dyes.

Some plant species (orchids and milkweeds) conveniently preserve a record of insect visitation in that they bear detachable pollinia. The absence of pollinia may be taken as indubitable evidence of visitation (cf. Willson and Rathcke 1974, Willson and Price 1977 on *Asclepias*). One such plant, *Cypripedium acaule* Ait., is a "high risk" species with respect to the potential impact of insecticide spraying in New Brunswick because it blooms shortly after spray application (late May – early June) and is wholly dependent on insect visitation to set fruiting capsules (Stoutamire 1967; Barrett and Helenurm in prep.). However, the flowers are never visited heavily and fruit set is so rare in central New Brunswick (but see Case 1964, p. 40) that an impractically large sampling effort would be required to distinguish insect visitation rates in sprayed versus control habitats by direct observation. (Of 74 flowers whose phenology was monitored in 1978 and 1979, none set fruit.) Therefore we decided to let the plant "do its own bookkeeping" for us: in this paper we report the use of the removal rate of *Cypripedium* pollen masses as a convenient estimator of insect visitation.

Unlike those of other orchids, flowers of the tribe Cypripedieae bear two anthers, one on either side of the column. The pollen of *C. acaule* is considered "granular" (Correll 1950; Case 1964), and is not presented, as in many other orchids, in the form of a perfectly discrete waxy pollinium. Instead it comprises a

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Table I. Removal of *Cypripedium acaule* pollen masses in various habitats

Site	Sample area	No. of fls. with pollen intact	No. of fls. with 1 mass removed	No. of fls. with both removed	% of fls. with 1 or 2 removed
Jonah					
property	1	180	22	1	11.3
(unsprayed)	2	17	2	0	10.5
	3	23	0	0	0
	4	16	1	0	5.9
Site totals		236	25	1	9.9
Porter					
Cove	1	17	1	0	5.6
(unsprayed)	2	137	8	0	5.5
Site totals		154	9	0	5.5
Crooked					
Bridge	1	7	0	0	0
Brook	2	13	0	0	0
Road	3	5	2	0	28.6
(sprayed)	4	57	0	0	0
	5	301	4	0	1.3
Site totals		383	6	0	1.5

dense, coherent, viscid mass which is usually removed as a unit when the column is brushed with a fingertip, a stiffish brush, or a vigorous insect such as a bumble bee. Correll (1950) describes this process as follows:

> "The insect enters the (longitudinal) fissure in front of the lip but usually finds it impossible to leave the pouch through this fissure because of the infolded margins. The only other possible exit is through an aperture at the base of the lip. The column is so placed over this opening that the insect must first strike the stigma and deposit there any pollen which it may be carrying at the time. Then, upon leaving the lip, the insect receives a load of pollen from either of the two anthers which it happens to touch. Thus the pollen will be transported to the next flower visited, and in this way, cross pollination is assured."

The pollen masses are borne on a sticky flaplike supporting structure that is part of the anther. This piece usually comes off with the pollen masses. Although it is impossible to determine whether an apparently intact flower may have had a small portion of its pollen removed, the presence or absence of the whole mass together with its underlying supporting structure is an unambiguous character which can be immediately scored in the field by direct observation after peeling back the staminode.

Although we did not have the opportunity to witness the process of visitation as described by Correll, we found several bumble bee queens (*Bombus vagans* F. Sm.) carrying the pollen masses of *C. acaule* on the dorsal surfaces of their thoraces during the blooming period of the plant. Also, we observed one *B. terricola* Kby. queen investigating the lip of a *C. acaule* flower, though she ultimately left without entering the fissure. These observations, together with the fact that the dimensions of the flower match the body size of bumble bees rather closely, lead us to suspect that bumble bees are the major removers of pollen. Stoutamire (1967) reports similar difficulties observing pollination on *C. acaule* in Michigan, but also records *B. vagans* as a definite visitor, and *B. borealis* Kby. as a probable visitor, based on pollen found on museum specimens.

Methods

Because the pollen masses remain fresh longer than the rest of the flower, it is possible to score wilted flowers whose inflated lips have begun to collapse. Pollination by the usual mechanism cannot occur in a flower of this condition. From 18 June to 23 June 1979 we scored such wilted *C. acaule* flowers at several sampling areas within three major sites. We attempted to examine the entire population at each area. Two sites served as unsprayed controls: (1) the Jonah property, approximately 4.2 km SSE of Doaktown, N. B. (Northumberland Co.) on Hwy. 123; (2) an area ~1 km E of the Porter Cove settlement, approximately 15 km SSW of Doaktown on the Priceville Road. The third site, approximately 12 km W of Doaktown on the Crooked Bridge Brook Road, received aerial sprays of fenitrothion (210 g/ha) on 1 June and 7 June. Similar vegetation occurred in all the sampling areas, consisting of spruce fir forest with small amounts of white pine and some hardwoods. The Porter Cove site had a uniform dense canopy, while the

Table II. Habitat comparisons by contingency table: the Jonah property vs. the sprayed Crooked Bridge Brook Road

	Pollen intact	One or both removed	
Jonah property	236	26	262
CBBR	383	6	389
	619	32	651

$\chi^2 = 23.53 \quad p < .001$

Table III. Habitat comparisons by contingency table: the unsprayed Porter Cove site vs. the sprayed CBBR

	Pollen intact	One or both removed	
Porter Cove	154	9	163
CBBR	383	6	389
	537	15	552

$\chi^2 = 5.99 \quad .01 < p < .025$

Table IV. Habitat comparisons by contingency table: the two unsprayed sites

	Pollen intact	One or both removed	
Jonah property	236	26	262
Porter Cove	154	9	163
	390	35	425

$\chi^2 = 2.58$, not significant

sampling areas within the Jonah site ranged from dense to rather sparse canopy. The areas within the Crooked Bridge Road site were similarly variable, but the majority of the flowers scored were from one area with sparse canopy. At each sampling area, searchers fanned out to cover at least one hectare of forest, and when flower populations were large enough to warrant, several hectares were sampled. All sampling areas were located within large blocks of forest, and although roadsides and cleared land were close enough to be possible sources of site differences, at the time of the *Cypripedium* bloom there are relatively few weedy flowers blooming to attract pollinators away from the forest.

Results and Discussion

The results are shown in Table I. When the data for each site are combined, and pollen removal compared between sites by 2×2 contingency tables, removal is significantly lower in the sprayed site than in either of the control sites (Tables II, III). The Porter Cove site had a lower removal rate than the Jonah property (5.5% as compared with 9.9%). Although this difference was not significant (Table IV), it may perhaps be a result of the denser canopy at Porter Cove, which tends to reduce flower-visiting insect activity.

Clearly, there are likely to be site-to-site variations in the abundance of bumble bees which are unrelated to spray treatment, and it would be ideal to have independent data regarding bee densities before and after the spray. However, extensive previous work has shown that foraging bee densities in fenitrothion-sprayed blocks, as judged by various means, are reliably lower than in unsprayed blocks of similar habitat, i.e., the spray-induced mortality is generally strong enough to override other sources of site variability (Plowright *et al*. 1978; Plowright and Rodd 1980). In view of the great difficulties of direct bee density assessment, we have depended on this past work in taking it as given that densities will be lower in sprayed blocks. With respect to openness of the forest canopy, a potentially important site variable, the sites varied in such a manner as to make our conclusions conservative. Sample area 5, where most of the sprayed flowers were observed, had one of the more open canopies and had the highest density of flowers, both of which would be expected to increase visitation rate relative to darker and sparser stands. However, pollen removal remained low in spite of favourable conditions.

The reduced removal rate in the fenitrothion-sprayed area could be a direct and simple result of reduction of visitor numbers. In this case, a 10-fold drop in removal rate would indicate a bee reduction of similar magnitude. However, behavioural changes may also be involved. A comparatively moderate reduction in bees might reduce competition for food, allowing the remaining bees to specialize on a few rewarding plants, and reducing the likelihood of individuals sampling unrewarding plants like *C. acaule* (cf. Plowright 1977). Whatever the mechanism, the lowered removal rate, given the breeding system of *C. acaule* (Barrett and Helenurm in prep. and pers. comm.) and its mechanical requirements for fertilization (Stoutamire 1967), is almost certain to reduce fruit set, unless the incidence of fertilizations by incompletely removed pollen-masses is (1) higher than expected and (2) somehow increased by spraying. The latter is implausible.

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References

Case, F. W. 1964. Orchids of the western Great Lakes region. Cranbrook Inst. Sci., Bloomfield Hills, Mich. 147 pp.

Correll, D. S. 1950. Native orchids of North America. Chronica Botanica, Waltham, Mass. 400 pp.

Plowright, R. C. 1977. The effect of fenitrothion on forest pollinators in New Brunswick. pp. 335-341 *in* Proceedings of the symposium on fenitrothion, the long-term effects of its use in forest ecosystems. Nat. Res. Council Can., Ottawa.

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.: Hym.) in south-western New Brunswick. *Can. Ent.* **110**: 1145-1156.

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

Stoutamire, W. P. 1967. Flower biology of the lady's-slippers (Orchidaceae: Cypripedium). *Mich. Bot.* **6**: 159-175.

Thaler, G. R. and R. C. Plowright. The effect of aerial insecticide spraying for spruce budworm control on the fecundity of entomophilous plants in New Brunswick. *Can. J. Bot.* (in press).

Willson, M. F. and P. W. Price. 1977. The evolution of inflorescence size in *Asclepias* (Asclepiadaceae). *Evolution* **31**: 495-511.

Willson, M. F. and B. J. Rathcke. 1974. Adaptive design of the floral display in *Asclepias syriaca* L. *Am. Midl. Nat.* **92**: 47-57.

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