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EFFECTS OF BUMBLEBEE VISITS ON THE SEED SET OF PEDICULARIS, RHINANTHUS AND MELAMPYRUM (SCROPHULARIACEAE) IN THE NETHERLANDS

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SUMMARY

The pollination ecology of five species of Rhinanthoideae (the annual species *Rhinanthus serotinus*, *R. minor* and *Melampyrum pratense*, the biennial *Pedicularis sylvatica* and the annual and biennial *P. palustris*) has been investigated.

All species contain pollen and nectar and are frequently visited by sternotribically and nototribically pollinating bumblebees. The effectiveness of pollen transfer has been measured by the use of fluorescent powder. In *R. serotinus* and *M. pratense* no differences exist in percentages of fluorescent stigmas of flowers nototribically or sternotribically visited by bumblebees. *R. minor* flowers, visited sternotribically, have very low percentages of fluorescent stigmas. This indicates that the pollen-covered venter cannot touch stigmas enclosed by the galea; the movements of the bumblebees probably caused self-pollination. *P. palustris*, *R. serotinus* and *M. pratense* flowers are very frequently perforated by nectar-collecting short-tongued bumblebees. *P. sylvatica* and *R. minor* flowers are very rarely perforated. On these species nectar is mainly collected by nototribically pollinating bumblebees. Seed production and dependence upon pollination by bumblebees (*Bombus* Latr. spp.) are considered.

A range from high dependence upon bumblebee visits for seed production in *P. palustris*, to medium dependence in *P. sylvatica* and *R. serotinus* and virtual independence in *R. minor* and *M. pratense* is established. No species is completely self-sterile. Seed set in caged plants is due to favourable morphology and position of flowers. Close proximity of thecae and stigma or a downward curving of the pistil under pollen chamber in *Melampyrum* and *Rhinanthus* insure seed set in caged plants. In *Pedicularis* these characteristics for self-pollination are absent.

The importance of bumblebees for the five *Rhinanthoideae* and the reciprocal importance of these pollen and nectar providing plants for bumblebees is discussed. The importance of alternative pollination by honeybees, thrips and wind is evaluated.

1. INTRODUCTION

The present study was undertaken, as part of a general survey, to extend our knowledge of the floral ecology of *Rhinanthoideae* by detailed analysis of pollinator behaviour in its general ecological context. The pollination mechanisms have been described in a previous paper (KWAK 1977). The zygomorphic, nectariferous flowers are mainly visited by bumblebees (*Bombus* Latr. spp.), nototribically and sternotribically.

This paper deals with the significance of bumblebee visits for the seed set of five hemiparasites in the northern Netherlands: *Pedicularis palustris* L., *P. sylvatica* L., *Rhinanthus serotinus* (Schönh.) Oborny (= *R. angustifolius* Gmelin), *R. minor* L., *Melampyrum pratense* L. These species are annual or biennial. In addition, the

importance of alternative pollination by honeybees, thrips and wind, will be evaluated.

Honey bees, collecting pollen and nectar on alpine *Rhinanthus* species, are mentioned by FOSSEL (1974). Their pollination efficiency is unknown.

In the literature the ability to self-pollinate in the five species depends on the view of the authors. Several mechanisms of selfing are described: the close proximity of anthers and stigma, the curving of the style so that the stigma is under the anthers at the end of the flowering period, and the decrease of the pressure with which the pollen chamber is kept closed at the end of the flowering period (MÜLLER 1881; KNUTH 1899; WAGNER 1908; WARMING 1908; HEUKELS 1910; VON KIRCHNER 1911; SCHOENIGEM 1922; KERNER VON MARILAUN 1902; WERTH 1940; LAGERBERG et al. 1957; FAEGRI & VAN DER PIJL 1966). The presence of hairs on the thecae is mentioned in relation to the ability of self-pollination and of the prevention of lateral pollen release during bumblebee visits (KNUTH 1899; HEUKELS 1910; SCHOENIGEM 1922). No comparative figures are available of crossing and selfing. In this context the remark by SMITH (1963) that *Melampyrum pratense* had a good seed set in the greenhouse, probably in absence of bumblebees, is most pertinent. Other *Melampyrum* species also exhibit autogamy (CANTLON et al. 1963; HARTL 1974). Alpine *Rhinanthus* and *Pedicularis* species are able to self-pollinate, according to MÜLLER (l.c.) and KNUTH (l.c.). Self-pollination in buds of *R. minor* in the Faroes is mentioned by HAGERUP (1951). *Pedicularis* species in America produced very few or no seeds when caged (SPRAGUE 1962; MACIOR 1970, 1973, 1974, 1975, 1977). MACINNES (1972) mentions arctic *Pedicularis* populations consisting of plants which self-pollinate and are self-fertile and plants which neither self-pollinate nor are self-fertile.

2. METHODS AND MATERIALS

Field studies were conducted from May until October in 1974–1978 in populations of *Pedicularis palustris*, *P. sylvatica*, *Rhinanthus serotinus*, *R. minor* and *Melampyrum pratense* in the northern Netherlands including the West Frisian island of Schiermonnikoog. A *R. minor* population in the southern Netherlands was also visited. Self-pollination potentials for each species were determined by comparing fruit development on plants caged to exclude pollinating insects with fruit development on uncaged plants in the immediate vicinity of the caged ones. Lace covered (meshes 1 × 1 mm) cages 60 × 60 × 60 cm were placed over whole plants, or bags were placed over individual stems. Except for *M. pratense*, insect enclosures were used both in the field and in the experimental garden. One of the *R. minor* populations, grown in the experimental garden, was of French origin; seeds had been collected in a dune population near Portbail (Normandy).

To analyze the possibility of autodeposition of pollen or self-sterility, stigmas of caged flowers of *P. palustris* and *P. sylvatica* were dipped in ripe pollen from the same flower. The effectiveness of pollen transfer by bumblebees was studied by touching the pollen sites on the bodies of the visiting bumblebees with a brush containing a fluorescent powder ("Daylight" Fluorescent Heffner and Co.) These

bumblebees continued their foraging trips. Visited flowers were collected and the styles removed and examined with a UV lamp for the presence of fluorescent powder. Pollen germination and pollen tube growth were observed by UV fluorescence microscopy (LINSKENS & ESSER 1957; MARTIN 1959) using a 2% solution of water-soluble aniline blue dye in 20% K_3PO_4 (WILMS 1974).

The possibility of honeybee pollination was examined. Two hives were placed at the edge of a dense stand of *Rhinanthus*. Pollen loads were collected by using a pollen trap and examined by light microscopy. Hives were also present at a distance of about 30 m from the experimental garden.

The possibility of wind dispersal of pollen was examined in a very dense stand of flowering *R. serotinus* when weather conditions were favourable. Test slides, coated with silicon oil on one side to catch air-borne pollen grains, were placed at different levels above the ground: 15, 25, 35, 45 and 55 cm, with the coated side facing the wind. Test slides were left for 12 hours, from 23.00 till 11.00 hours and from 11.00 till 23.00 hours. They were examined microscopically for *Rhinanthus* pollen.

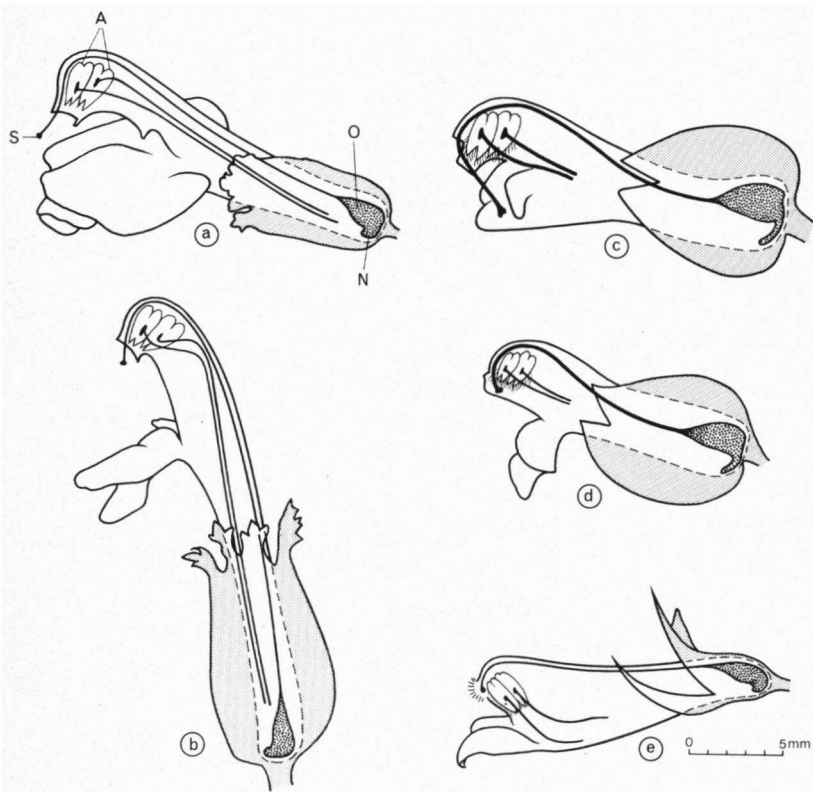


Fig. 1. Flowers of the five plant species studied in natural positions, under insect-exlosures: A = anthers; S = stigma; O = ovary; N = nectary.
 a. *P. palustris*; b. *P. sylvatica*; c. *R. serotinus*; d. *R. minor*; e. *M. pratense*.

Table 1. Selected characteristics of *Pedicularis*, *Rhinanthus* and *Melampyrum*.

	life form	flowering-time	flower color	corolla length in mm	tube length in mm	stigma	hairs on thecae	anther first ripe	max. number observed seeds/caps.
<i>P. palustris</i>	biennial	$\frac{1}{2}$ May- $\frac{1}{2}$ July	purplish/pink	17-20	9-20	protr.	absent	short	38
	annual	$\frac{1}{2}$ August-September		15-18	7-10				25
<i>P. sylvatica</i>	biennial	$\frac{1}{2}$ May-July	pink	20-24	14-17	protr.	absent	short	34
<i>R. serotinus</i>	annual	$\frac{1}{2}$ May-September	yellow (blue tooth)	17-22	9-11	protr.	present	long	18
<i>R. minor</i>	annual	May-July	yellow/ (white tooth)	13-17	7-9	encl.	present	long	18
<i>M. pratense</i>	annual	June-July	yellow/white	13-16	10-13	encl.	present	long	4

Table 2. Distribution of bumblebee species on populations of *Pedicularis*, *Rhinanthus* and *Melampyrum*; first part based on observation of 1974-1977; + + + : very frequent; + + : frequent; + : occasional; (+) : rare; second part based on amount of pollen loads, taken in 1974 from bumblebees foraging on these plant species (- no observation).

	<i>B. hortorum</i>	<i>B. pascuorum</i>	<i>B. muscorum</i>	<i>B. equestris</i>	<i>B. rudertanus</i>	<i>B. lapidarius</i>	<i>B. pratorum</i>	<i>B. hypnorum</i>	<i>B. terrestris/</i> <i>B. lucorum</i>	<i>B. jonellus</i>	number of loads	% of sterno- tribic
<i>P. palustris</i>												
Kappersbulten	+	+	+			+	+		+		36	100
Zuidlaardermeer	+	+	+	(+)		+	+	+	+		38	86.8
Schiermonnikoog	+	+	+			+	+	+	+		75	96.0
Molkwerum	+	+	+			+	+	+	+		16	87.5
<i>P. sylvatica</i>												
Hooerveen		+	+			(+)	+		+		15	86.7
Zeegeerdunen		+	+			+			+	(+)	5	80.0
Peest		+	+						+		-	-
<i>R. serotinus</i>												
Hooholtje	+	+	+				+		+		21	85.7
Populierenlaan	+	+	+				+		+		-	-
Smaibroek	+	+	+			+	+		+		40	97.5
Zeegeertloopje	+	+	+	(+)		+	+		+		18	100
Hornbulten	+	+	+			+	+		+	(+)	-	-
Hortus de Wolf	+	+	+			+	+		+		59	37.3
Taarlo	+	+	+	(+)		+	+		+		10	20.0
Balloërveld	+	+	+			+	+		+		6	0
<i>R. minor</i>												
Kantens	+	+	+	(+)		+	+		+		-	-
Zeegeertloopje	+	+	+			+	+		+		-	-
<i>M. pratense</i>												
Amen		+	+						+		4	75.0
Annen	+	+	+				+		+		24	83.3
Tonckensbos	+	+	+				+		+		26	80.8
Linthorst Homan bos	+	+	+				+		+		7	42.9
Appelbergen	+	+	+				+		+		-	-

3. OBSERVATIONS

3.1. *Pedicularis palustris* – Marsh Lousewort, Red Rattle

Pedicularis palustris occurs in wet, grassy places, dune slacks, damp reed land and meadows. The biennial is most common. Annual plants, known in the Netherlands only on the West-Frisian coast, differ in several respects from the biennials (TER BORG & KOEMAN-KWAK 1973, and *table 1*).

The flowers were frequently visited by nototribically and sternotribically pollinating bumblebees. Sternotribically pollinating bumblebees, mainly *Bombus terrestris* and *B. lucorum* workers were dominant in June and in August–September on the biennial and annual plants, respectively (*table 2*). *P. palustris* flowers were often perforated for nectar; sometimes 100% of the flowers exhibited holes. On the annual plants bumblebee males were observed securing nectar. In open-pollinated plants 63–100% of the flowers produced fruits, with 6.6–15.1 seeds per flower. Seed production varied in different years. 1975 was a good seed production year, and 1974 a poor year (*table 3*). Seed set in caged plants was very low (*fig. 2*). The two methods of insect enclosures, cages or bags, gave different results. Fruiting was 12.3% in cages and 27.2% in individual bags. In individual bags flowers may have been pressed together possibly resulting in artificially high fruiting. Fruits developed within the insect enclosures may also have been the result of incidental success by thysanopteran inhabitants. The low fruiting in *P. palustris* plants under cages indicated the unimportance of *Thysanoptera*. The lack of autodeposition possibly combined with self-sterility was responsible for low fruiting under cages. Partial self-sterility may occur: 59.4% of the seeds developed under insect enclosure were empty. Only 0.7 viable seed per flower (total 1.7 seeds per flower) were produced. Caged flowers, self-pollinated by dipping their stigmas through their own ripe pollen, set seed (maximum 10 seeds per capsule, see also *table 1*).

Table 3. Fruit- and seed production in *Pedicularis palustris* (– no value available).

population	life form	year	open pollinated			insect-enclosure		
			number of flowers	% fruiting	seeds/flower	number of flowers	% fruiting	seeds/flower
Kappersbulten	biennial	1974	135	64.5	6.6	1215	10.0	0.3
		1975	65	96.9	14.5	290	27.2	1.5
		1975				73	12.3	0.3
Zuidlaardermeer	biennial	1975	64	92.2	10.3	–	–	–
		1977	397	99.5	15.1	316	31.3	1.7
Schiermonnikoog Molkwerum	biennial	1974	487	63.6	7.5	1586	0.4	0.02
	annual	1974	169	62.6	8.2	178	10.1	0.2
		1975	231	97.4	9.8	–	–	–

The sequence in ripening of the anthers can influence self-pollination positively. In *Pedicularis* the thecae of the longest stamens, nearest to the stigma, ripen late (fig. 6a). The escape of pollen grains from the pollen chamber is restricted by the compressed galea. The position of the stigma in relation to the thecae is not favourable for selfing (figs. 1 and 6).

3.2. *Pedicularis sylvatica* – Lousewort

Pedicularis sylvatica is a plant of moist heath, marshes and moorland. It occurs in the Netherlands in rather small populations in remnants of heath and along small paths across heath.

The flowers were visited by nototribically and sternotribically pollinating bumblebees (table 2). Perforations were rarely observed. Flowers still in bud with unfolded lower lip and unripe pollen were already visited by pollen collecting

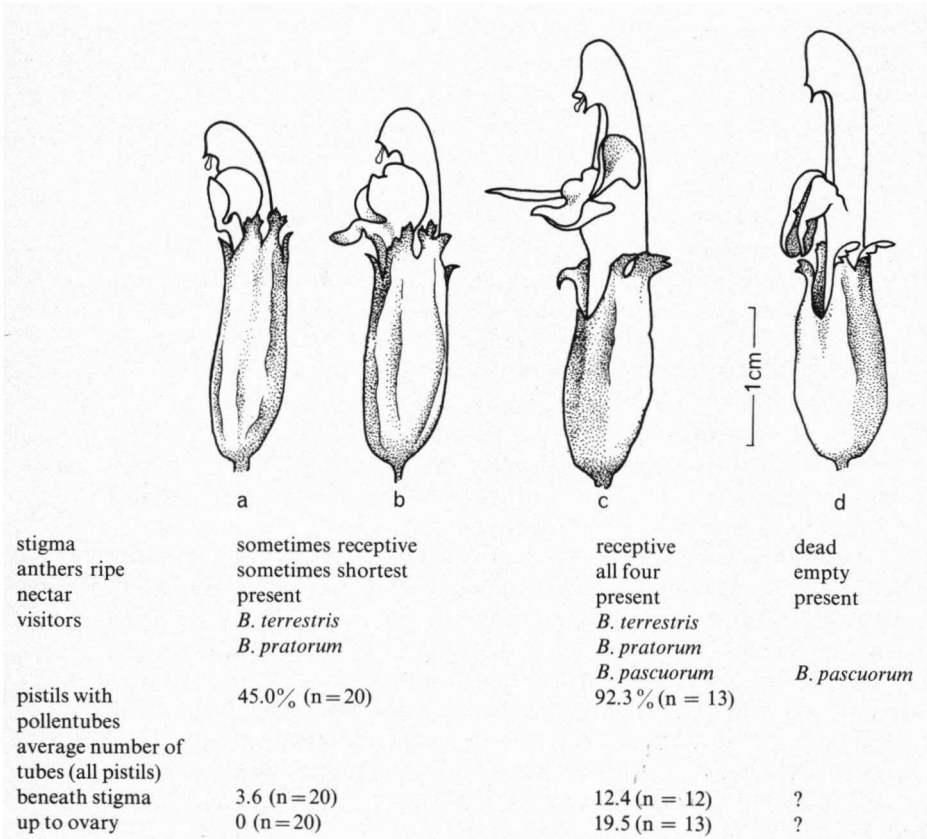


fig. 3. *P. sylvatica* flowers and their characteristics during different phases of anthesis; phase b. was very short, *B. terrestris* and *B. pratorum* were sternotribic pollen collectors, *B. pascuorum* was a nototribic visitor.

sternotribically visiting bumblebees. These bumblebees spent a lot of time on the buds. They did not receive pollen grains because the anthers were not ripe, but at this stage the stigma is receptive since pollen tubes could be distinguished, growing just below the stigmatic surface. The period of maturity of only the short anthers was very limited. Nototribic *B. pascuorum* workers were observed when all anthers or the two long ones contained ripe pollen grains. In this phase many pollen tubes in the style had reached the ovary. *B. pascuorum* continued collecting nectar in flowers whose pistils were already brown and dried (fig. 3). On *P. sylvatica* the sternotribically visiting bumblebees collected pollen, while the nototribically visiting bumblebees collected pollen and nectar. The last group was not seen collecting nectar on buds.

Open-pollinated flowers produced 73–92 % fruits and 5.4–9.7 seeds per flower. Caged flowers produced moderate (36 %) numbers of fruits and seeds per flower (1.5–3.2). These were probably the result of self-pollination (table 4). In caged flowers, self-pollinated by dipping stigmas through their own ripe pollen, 82.7% developed fruits. This percentage is similar to that in open-pollinated flowers. The position of the *P. sylvatica* flower was more favourable for self-pollination than that of *P. palustris*. The stigma was more or less under the galea and under the thecae of the long anthers (fig. 1). Hairs on the thecae were absent (fig. 6b). One can wonder whether self-pollination did occur in the field since the flowers were visited frequently, and no pollen grains were left in their anthers.

3.3. *Rhinanthus serotinus* – Greater Yellow Rattle

Populations of *Rhinanthus serotinus* with different flowering times and morphology are present in various habitats such as grasslands, dunes and roadsides. Early flowering ecotypes (aestivals) are characterized by few branches and a low number of flowers. Autumnals, on the contrary, are richly branched with nearly every branch bearing flowers. Intermediately flowering plants are morphologically intermediate (TER BORG 1972, HARTL 1974).

Table 2 shows that all ecotypes were pollinated both by nototribically and sternotribically pollinating bumblebees. The frequency of nototribically pollinating bumblebees increased during the season. Perforations in calyces and corollas of *R. serotinus* were often observed, up to 100%.

Pollen analyses of corbicular loads of honeybees returning from their foraging trips showed the absence of *Rhinanthus* pollen. Direct observations on foraging honeybees also showed the nearly complete neglect of *Rhinanthus*. They visited *Taraxacum* spec., *Trifolium repens* L. and *Lychnis flos-cuculi* L.

Analysis of test slides measuring possible wind pollination showed very many pollen grains but no *Rhinanthus* grains indicating the absence of wind pollination. Wind, however, could play a role in self-pollination by shaking the flower.

Sternotribically visiting bumblebees, with fluorescent powder on their venter, were at least as good vectors as nototribically visiting bumblebees (table 5).

In open-pollinated plants fruit production was almost 100%, with 5.9–10.8 seeds per flower. In caged plants fruit and seed production was decreased. Plants of early blooming populations produced, if caged, more seeds per flower than plants

Table 4. Fruit- and seed production in *Rhinanthoideae*, ranges of data observed in different populations and years. n = number of populations observed and/or years of observation. Maximum number of seeds per capsule, as given in *table 1*, used as capacity.

species	open pollinated				insect-exclosure			
	n	% fruiting	seeds/ flower	% capacity	n	% fruiting	seeds/ flower	% capacity
<i>P. palustris</i>								
annual	2	62.6– 97.4	8.2– 9.8	32.8–39.2	1	10.1	0.2	0.8
biennial	5	63.6– 99.5	6.6–15.1	17.4–39.7	1	0.4–31.3	0.02– 1.7	0.1– 4.5
<i>P. sylvatica</i>	6	73.1– 92.0	5.4– 9.7	15.9–28.5	2	35.1–35.7	1.5 – 3.2	4.4– 4.9
<i>R. serotinus</i>								
early	1	98.4	8.3	46.1	1	75.4	5.5	30.6
middle	5	96.7–99.0	8.3–10.8	46.1–60.0	2	?	2.8 – 6.0	15.6–33.3
late	1	?	5.9	32.8	1	?	1.3	7.2
<i>R. minor</i>	9	95.7–100.0	9.0–13.3	50.0–73.9	6	89.4–94.3	7.1 –10.3	40.6–57.2
<i>M. pratense</i>	5	74.5– 84.2	2.4– 3.0	60.0–75.0	1	84.2	2.5	62.5

of later blooming populations (*table 6*). Minimum and maximum values of seed production per plant were always higher in open-pollinated plants than in caged ones. In caged plants a mechanism insuring self-pollination was observed. During anthesis the style curved downwards (*figs. 1, 4, 5*), and the stigma moved into a more suitable position for pollen deposition. The lower lip, closing the galea, then bent downwards so that a free fall of pollen grains out of the galea was possible; meanwhile the pressure with which the pollen chamber was closed, decreased. The thecae were more or less kept together by curly white hairs on their surface. Pollen grains were retained among the hairs (*figs. 6c, d*).

Table 5. Transfer of fluorescent powder, dusted on bumblebee bodies, to stigmas of *Rhinanthoideae*; nototribic: powder on head: *B. hortorum*, *B. pascuorum*; sternotribic: powder on venter: *B. terrestris*, *B. pratorum*.

	nototribic		sternotribic	
	number of flowers visited	% fluorescent stigmas	number of flowers visited	% fluorescent stigmas
<i>R. serotinus</i>	92	42.4	319	55.2
<i>R. minor</i>	182	48.4	127	13.4
<i>M. pratense</i>	40	57.5	41	58.5

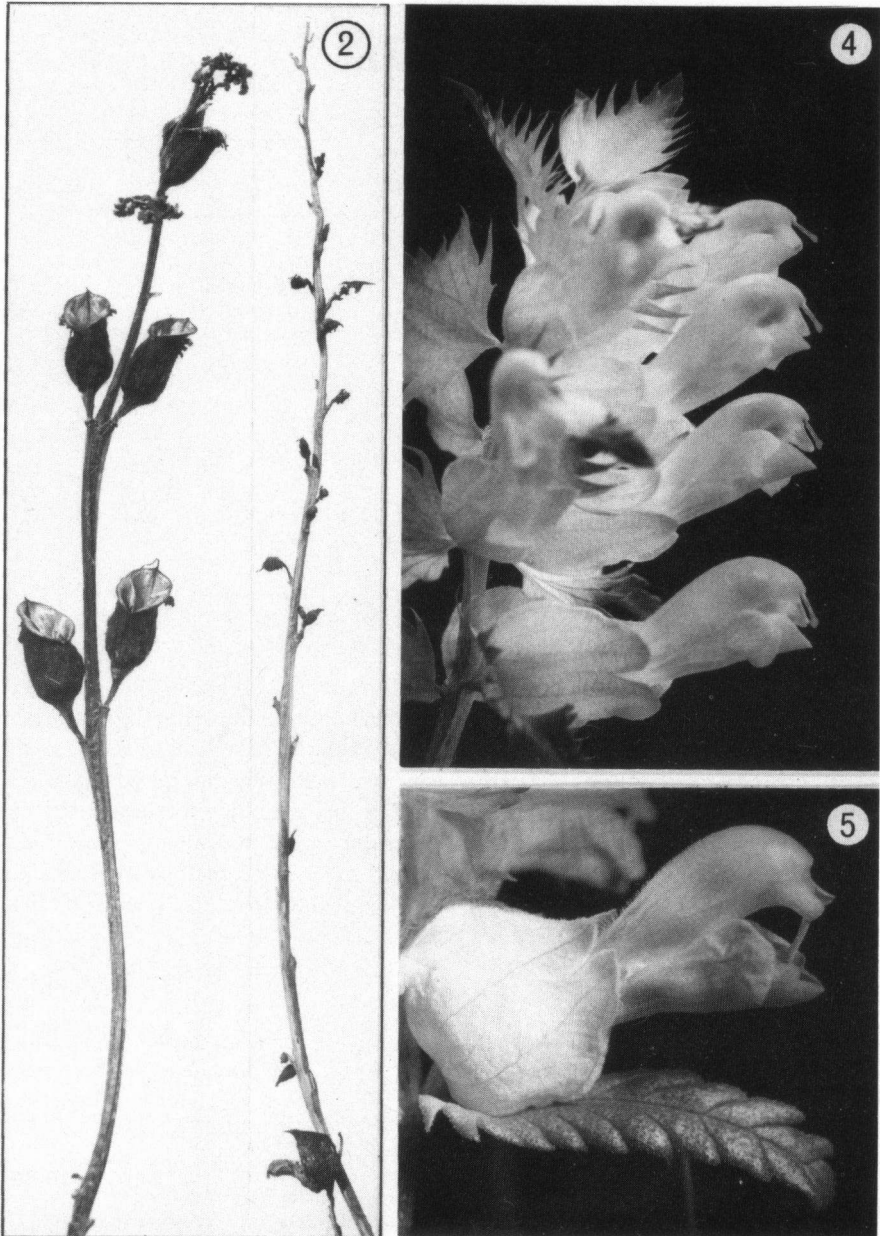


Fig. 2. Silhouettes of an open pollinated (left) and a caged (right) *P. palustris* inflorescence.

Fig. 4. An inflorescence of *R. serotinus* (caged); uppermost flower with a protruding pistil, the other flowers show a backwards curving of the pistil.

Fig. 5. *R. serotinus* flower with a backwards curved pistil and a stigma touching the underlip, during self-pollination.

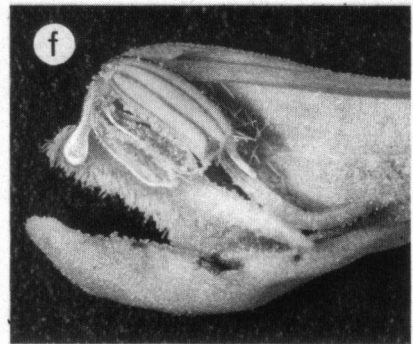
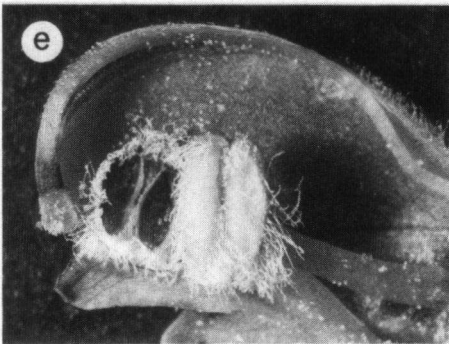
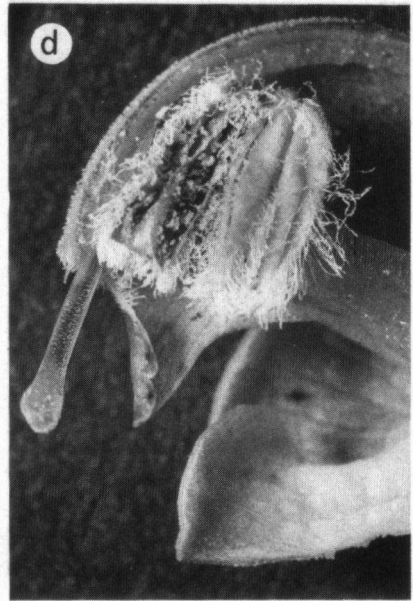
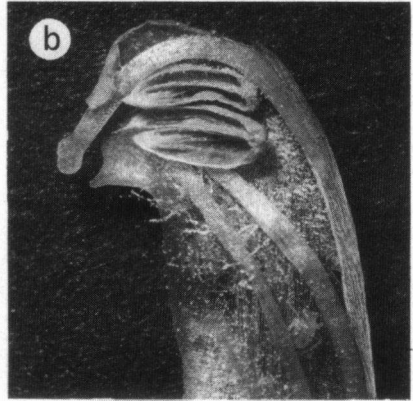
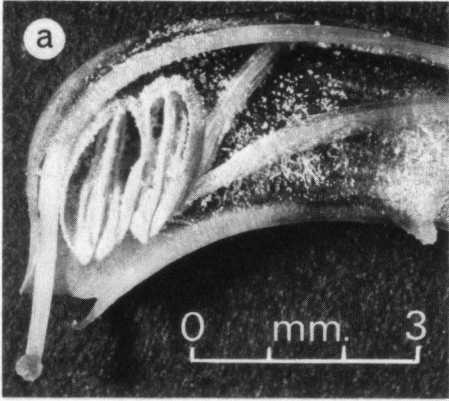
3.4. *Rhinanthus minor* – Yellow Rattle

Rhinanthus minor grows in grasslands, roadsides and dunes. Plants with blue- and white-toothed flowers may occur in separate populations or mixed ones. *R. minor* flowers were visited nototribically and sternotribically by bumblebees. Though plants of a certain population were in full bloom in May, bumblebees preferred to visit *Symphytum officinale* L., *Lamium album* L. and later *Trifolium pratense* L. instead of *R. minor*. In meadows where *R. minor* grew together with *R. serotinus*, the latter was preferred (KWAK 1978). Nectar was collected mainly by nototribically visiting bumblebees. Perforations in calyces and corollas of *R. minor* were rarely observed. Sternotribically visiting bumblebees did not transfer fluorescent powder at the same rate as did nototribically visiting bumblebees (table 5). The consequence is that pollen grains were removed from the thecae without cross-pollination.

Self-pollination during the visits of sternotribic bumblebees is very likely because of the very close proximity of thecae and stigma (fig. 6e). A certain amount of cross-pollination is established by nototribically visiting bumblebees, mainly *B. pascuorum* workers in populations flowering in June. 96–100% of open-pollinated flowers produced fruits and 9.0–13.3 seeds per flower; 89–94% of caged flowers produced fruits and 7.1–10.3 seeds per flower (table 4). Minimum and maximum numbers in seed production per plant were always higher in open-pollinated plants than in caged ones. Maximum fruit development per plant was in all cases 100%. By the very close proximity of thecae and stigma and a curving backwards of the stigma inside the galea, pollen grains dropped from the thecae can be deposited on the stigma. Hairs on the thecae were present like in *R. serotinus* (fig. 6e).

Table 6. Fruit and seed production in *R. serotinus*, grown in the experimental garden, except Smalbroek 1978, Hornbulten, Zeegserloopje 1977, Oudemolen 1974.

population	year	open pollinated			insect-exclosure		
		number of flowers	% fruiting	seeds/flower	number of flowers	% fruiting	seeds/flower
Smalbroek (early)	1975	275	98.4	8.3	189	75.4	5.5 (cages)
	1978	50	100.0	9.9	–	–	–
Hornbulten (intermediate)	1975	57	?	10.8	–	–	–
	1977	118	98.3	7.9	–	–	–
Zeegserloopje (intermediate)	1977	99	99.0	8.3	–	–	–
Oudemolen (intermediate)	1974	70	?	9.0	20	?	6.0 (bags)
	1975	301	96.7	8.3	359	46.3	2.8 (cages)
Meppel (intermediate-late)	1977	156	?	5.9	139	?	1.3 (bags)



3.5. *Melampyrum pratense* – Common Cow-wheat

Melampyrum pratense is rather common in woodlands and their margins, and in heaths on acid humus. Growing in a vegetation poor in other flowering plants, providing pollen and nectar, the flowers were frequently visited by bumblebees, mainly workers. Sternotribically visiting bumblebees were the most frequent pollen collectors (table 2). Many flowers, up to 100% in some populations, were perforated for nectar. These flowers exhibited two slits near the tube base corresponding to the spacing of the mandibles of the bumblebees. The transfer efficiency of fluorescent powder by nototribically and sternotribically visiting bumblebees was similar (table 5). Nototribically visiting *B. terrestris* workers (usually sternotribically) also transferred the powder, if dusted on their heads (50% fluorescent stigmas).

75–84% of open-pollinated flowers produced fruits and 2.4–3.0 seeds per flower. Seed set in caged flowers was similar (table 4). By the very close proximity of thecae and stigma, pollen grains dropped out of the thecae can be deposited on the stigma. The position of the flowers more or less hanging downwards, positively influenced autodeposition (fig. 1). Although some hairs on the thecae were present, these were probably not very important in restricting the free fall of pollen on the stigma (fig. 6f).

4. DISCUSSION

Bumblebees are the most frequent visitors of the five *Rhinanthoideae* species studied. No species in this study is visited by only a single *Bombus* species or by a single caste. High frequencies of sternotribic *B. terrestris* and *B. lucorum* were evident in all sites studied (table 2).

Habitat preferences exhibited by the plant species will determine, to a great extent, the availability of sternotribic and nototribic pollinators as BEATTIE (1974) found in *Viola*. This was demonstrated with *R. serotinus* growing in the Botanical Garden "De Wolf", which is very rich in bumblebee species. Flowering time also influenced the proportion of nototribic visitors. The later establishment of colonies of the longer-tongued bumblebee species resulted in the later emergence of workers, the most important nototribic visitors, e.g. *B. pascuorum* versus *B. lucorum*.

Nototribic and sternotribic visits did not have the same effect on pollination of plant species with an enclosed stigma. In *R. minor* sternotribic visitors cross-pollinated only 13% of the flowers visited. They had more effect on self-

Figs. 6. a/f. Longitudinal sections through upperlips, all same magnification: a. *P. palustris* two anthers, all thecae open; hairs on thecae absent; only few hairs on filament visible; b. *P. sylvatica*, all thecae open; only few hairs on filament visible; c. *R. serotinus* four anthers, hairs on thecae present; d. *R. serotinus* two anthers; thecae of longest stamen empty; e. *R. minor* two anthers, thecae of longest stamen empty; f. *M. pratense* two anthers, thecae of longest stamen open; few short hairs on thecae present.

pollination within one flower. Although they did not touch the stigmas, as for instance pollen thieves in *Cassia* do (THORP & ESTES 1975), the term pollen thief is not completely applicable. In species with a protruding stigma (*R. serotinus*) or with a rather accessible stigma by the broad galea (*M. pratense*) transfer of fluorescent powder by bumblebees was clear.

Long-tongued bumblebees generally visit more flowers per minute than short-tongued ones (STAPEL 1933; BENEDEK et al. 1973). In the species studied, in which two methods of collecting were strongly related to tongue length, the same rule appeared to exist. The speed of the nototribic visitors, however, did not balance the higher frequency of sternotribic pollinating events due to the greater number of sternotribically visiting bumblebees (KWAK 1978).

The specializations in pollen gatherers and nectar gatherers occurred probably more often in short-tongued bumblebee species than in long-tongued species. In the *Rhinanthoideae* species studied, the methods of pollen and nectar collecting by short-tongued bumblebees were very distinct because the flowers were perforated for nectar. On some dates 100% of the flowers in *R. serotinus*, *P. palustris* and *M. pratense* were perforated. *R. minor* and *P. sylvatica* both contained nectar; the impression exists that this nectar was mainly collected nototribically by *B. pascuorum* (see also KWAK 1978). In *P. sylvatica* it was very difficult for a perforating bumblebee to assume an appropriate position. Only the corolla tube outside the calyx was perforated. Because of the long calyx (10–13 mm) it would still be difficult to reach the nectar with a 6.7 mm tongue. The negative effect of perforation behaviour on seed set could be expected to be limited as long as the pistil is not bitten by perforating bumblebees. The latter was observed in *Melampyrum arvense* (unpublished data). SOPER (1952) mentioned the possibility of pollen transfer in a self-fertile flower (*Vicia faba*) by movements of the perforating bee. It is comprehensible that BILIŃSKI (1970) found no decrease in seed set in highly perforated *M. pratense* populations. HEINRICH & RAVEN (1972) postulated, on the basis of energetics, that under conditions of ample nectar secretion long-tongued bumblebees visited more flowers when less nectar remained per flower after robbing than without robbing. If this is true, the unprofitable effect of perforating bumblebees on the seed set of a certain plant species depends on the visits of other insect species and on the self-pollination and self-fertilization potentials of the plant species.

The presence of pollen tubes in pistils of unopened flowers of *P. sylvatica* demonstrated the early visits of sternotribically visiting bumblebees on the one hand and the necessity of pollen for the bumblebee colony on the other. Since *B. pascuorum* workers visited older flowers, they could serve as pollinators on flowers which had not been visited in bud stage.

Cross-pollination by wind in *Rhinanthus* was not observed. Wind might play a role in shaking and moving the plant resulting in escape of pollen which can be deposited on stigmas if flower morphology is favourable. If the stigma is protruding this is less likely by the loss of pollen grains passing through the air.

FOSSEL (1974) mentioned *Rhinanthus* pollen in honey from Alp meadows. The

proportion of *Rhinanthus* nectar was possibly underestimated by the behaviour of honeybees as secondary thieves, using perforations made by bumblebees. Honeybees with corbicular loads of pale-yellow *Rhinanthus* pollen were also observed. It came from *R. major* (= *R. serotinus*), *R. hirsutus* (= *R. alectorolophus*) and *R. angustifolius* (= *R. aristatus*). It is striking that we did not observe frequent visits of honeybees in the field or in the experimental garden despite the fact that bee hives were present within 30 meters or less. On *R. alectorolophus* and *R. aristatus* in the Alps I have also observed honeybees visiting flower buds of which only one theca was ripe and where nectar was present. The number of bumblebees was very low if compared with numbers observed in the Netherlands. Perhaps the nearly complete absence of bumblebees permitted honeybees to visit typical bumblebee flowers (unpublished data).

A range in seed production of open-pollinated plants existed. In *P. palustris* the realized seed production varied from 17–40% of the capacity, in *P. sylvatica* 16–29%, in *R. serotinus* 33–60%, in *R. minor* 50–74% and in *M. pratense* 60–75% of the capacity (table 4). Within the five *Rhinanthoideae* studied, a range from high dependence on to independence from bumblebee visits for seed set was observed. The seed set ratio of caged and open-pollinated plants varied from 0.003 to 0.11 in *P. palustris*, a species very dependent on bumblebee visits; from 0.25 to 0.59 in *P. sylvatica*; from 0.28 to 0.65 in *R. serotinus*, two species rather insect-dependent; and from 0.81 to 0.97 in *R. minor* and 0.93 in *M. pratense*, two species nearly independent of bumblebee pollination. Species, able to self-pollinate do not have such great differences in seed production in open pollinated flowers as the species that are dependent on bumblebee visits for their seed set. In *M. pratense* and in *R. minor*, both with a close proximity of stigma and anthers, the percentages of realized seed production of caged plants are the highest, followed by *R. serotinus* with a downward curving of the pistil and finally by *Pedicularis*. In *Pedicularis* no mechanism of self-pollination by curving of the pistil was observed. Both *Pedicularis* species are at least partially self-fertile.

Some bagged plants in *P. palustris* produced more seeds than others. This was due to some external circumstances, as, for instance, removing of the bag to photograph unpollinated flowers. Seed production of this plant was 2.5 seeds per flower and that of the control 1.7. The difference in seed production in plants caged and individually bagged was influenced by movements of the plants and the narrowness of the bags resulting in autodeposition. The observation by MAC-INNES (1972) that some caged plants of a *Pedicularis* population had a higher seed production than others could not be confirmed. Germination capacity of seeds of self-pollinated plants was lower than that of seeds of cross-pollinated plants (KOEMAN-KWAK 1973). In caged *P. palustris* plants the style can elongate resulting in a still greater distance between thecae and stigma and in an unfavourable position. The position of the flower and the sequence of ripening of the thecae have also an effect on self-pollination. The drawings of *P. oederi*, *P. lapponica* and of *P. sudetica* (KAIGORODOVA 1976) showed that the position of the flower influenced self-pollination. In *P. sylvatica* the position of the flower makes autodeposition more likely.

In *Rhinanthus* there exists a wide difference between caged plants producing a few or a large number of seeds. This was probably due to a more or less favourable position of the stigma, reached by different rates of recurving of the style. In some flowers the stigma touched the underlip, where pollen grains were dropped; in others the stigma remained exerted (figs. 4 and 5). In *Rhinanthus* and *Melampyrum* the sequence in thecae ripening is reversed. The stigma of the curving pistil in *Rhinanthus* must come under the anthers of the short stamens to receive pollen grains (figs. 1 and 6). A decrease of the pressure of the thecae on each other and a downward bending of the lower lip also occurs. Stigma and thecae are in close proximity in *M. pratense* and *R. minor*. Hairs on the thecae keep them more together. A function of the hairs in the prevention of lateral pollen release during bumblebee visits is doubtful. If the flower was visited, the head of the bumblebee directly contacted the hairs of the thecae or the venter was curved under the pollen chamber contacting both edges of the galea. The hairs retained pollen grains which might serve at a later stage for self-pollination; the importance of this is unclear. Field observations indicated that the flowers of the *Rhinanthoideae* were frequently visited by pollen collecting bumblebees. At the time when self-pollination should take place, it is likely that few or no pollen grains were left. Longer periods of weather unfavourable for bumblebee activity can particularly influence the seed set of *Pedicularis* species (table 3).

The chance of a flower in an early blooming population being visited and cross-pollinated was expected to be smaller than in later blooming populations due to the scarcity of bumblebees. RUST & CLEMENT (1977) estimated, based on insect behaviour, the probability of a *Collinsia sparsiflora* flower (*Scrophulariaceae*) not being cross-pollinated by bees in an early blooming population to be 0.60 and 0.36 for mid- and late season blooming populations, respectively. Both LLOYD (1965) and RUST & CLEMENT (1977) concluded that the absence of pollinators in early flowering populations would select for self-compatible individuals. In early blooming populations autogamy in *R. serotinus* insures seed production (table 6). This was less important in later blooming populations. Both minimum and maximum average seed production in caged plants of the early flowering *R. serotinus* population are higher than in the later blooming populations.

R. serotinus and *R. minor* grew together in the experimental garden. Flowers could have been cross-pollinated with pollen of the other species. There exists a physiological reproductive isolation resulting in the production of fewer seeds in interspecific than in intraspecific crosses (KWAK 1979, in prep.). Since the number of seeds was higher in cross-pollinated plants than in caged ones, this cross-pollination with pollen of the other species probably had not inferred to a high extent (table 6). Hybridization between *R. minor* and *R. serotinus* occurred in the field. Hybrid swarms and introgression towards *R. serotinus* was observed (KWAK 1978, 1979 in prep.). Although *P. palustris* and *P. sylvatica* can grow within several meters of each other no hybrids were found nor are they mentioned by HARTL (1974). HARTL mentioned many hybrids between alpine *Pedicularis* species. In America the potential for hybridization between two *Pedicularis* species was discovered by partial fertility in artificial crosses (MACIOR 1975).

Much attention has been paid to morphological adaptation between flower and pollinator. BRIAN (1954) and HOBBS et al. (1961) believe that the preferential utilization of certain flowers by bumblebees is directly attributable to the ease with which they can collect nectar. MACIOR (1971) cautions that morphological suitability of the pollinator to floral structure is not the sole criterion determining utilization and cross-pollination, but that a complex of factors is involved. LIU et al. (1975) indicate that nectar-gathering is more influenced by floral structure than was pollen-gathering. According to these authors the independent evolution of the two behavioural patterns and their differing manifestations indicate that the acquisition of pollen is not a mere coincidence of the collection of nectar. Much work has been done on the role played by odours, colours and nectar-guides in attracting bees to flowers, particularly for nectar. Little is known regarding the mechanisms which attract a bee searching for pollen. In this context the tooth of *Rhinanthus* is to be considered. In *R. serotinus*, *R. alectorolophus*, *R. aristatus* and *R. antiquus* it is usually blue-violet. The yellow-blue contrast is known to be attractive to bumblebees. The thecae are localized in the immediate vicinity of the tooth. For the tooth to function as nectar-guide this is a rather strange site. To function as a pollen-guide for unexperienced workers is another possibility. To prove this more experimental work must be done. In *R. minor* the tooth is much smaller and generally white. In this species autogamy is important to insure seed production. In the experimental garden, caged plants of *R. minor* with white teeth had a higher seed production than caged blue-toothed plants. It is striking that populations of *R. minor* on the Wadden Islands have a small dark blue-violet tooth.

According to CLIFFORD (1973) sufficiency of food in spring is more important for the successful establishment of a bumblebee colony than good nest sites or hibernation places. Willows are frequently visited in spring. Only a few plant species, flowering over a large area after willows have finished, are known in the research areas. TERÄS (1976) found in her study field that *Geum rivale* was a food source when willows had finished flowering. *Taraxacum*, flowering at the same time was soon disregarded by the the bumblebees. *Rhinanthus* and *Pedicularis* started flowering when *Taraxacum* was in full bloom or at its end. The *Rhinanthoideae* species play an important role for bumblebees in providing pollen and nectar. *P. sylvatica* is important in a habitat with *Erica tetralix* before the latter starts flowering. *M. pratense* grows in a habitat very poor in other nectar- and/or pollen-providing plants. The importance of *P. palustris* as a food source was observed on Schiermonnikoog. After the road margins had been mown, individually marked bumblebees, foraging on *Trifolium pratense*, were recovered on *P. palustris* more than 2 km from the site of marking (unpublished data).

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