

Sexual reproduction in British populations of *Adoxa moschatellina* L.

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ABSTRACT

Adoxa moschatellina L. is a small perennial herb found mainly in damp woodlands. Little is known about the ecology and biology of this species. The aim of this investigation was to examine seed production and the factors that influence seed set in natural populations in Worcestershire and Shropshire. Phenological studies in this species suggest the possibility of limited protogyny. Self-pollination within an inflorescence is likely, due to the overlap in anther maturation within the several flowers in an inflorescence, coupled with the foraging behaviour of the pollinators. This species is apparently adapted for pollination by small flying insects, mainly Diptera; caddis flies and moths also appear to be acting as pollinators. Potential seed set was less than 10% of that possible. Very few (about 20%) inflorescences survived to fruit. This loss of reproductive potential can be explained in terms of herbivore activity and pathogens. Of the inflorescences that did survive until mid-late June, very few (10%) produced seed. This may be due to a sporophytic and/or late acting self-incompatibility mechanism. Pollen viability (68%) does not appear to be a limiting factor.

KEYWORDS: phenology, pollination, self-incompatibility, pathogens, herbivores.

INTRODUCTION

Adoxa moschatellina was thought to be the only member of the family Adoxaceae until the early 1980s when two rare new species were recorded in China (Hara 1981; Wu 1981; Wu *et al.* 1981). Another new species from the Amur region of Russia was described in 1984 (Nepomnyashchaya 1984). The family is now thought to consist of three genera (*Tetradoxa*, *Sinadoxia* and *Adoxa*) and the *Adoxa* genus comprises two species, *A. moschatellina* and *A. orientalis* (Jianquan *et al.* 1999).

A. moschatellina has a circumpolar distribution and is present in the north-temperate zone to 2400 m (Blamey & Grey Wilson 1989). In the U.K. it is found predominantly in deciduous woodlands. Rodwell (1991) records *A. moschatellina* in woodlands with oak-hazel (W8), ash (W9) and birch-juniper (W19) communities. It is also found in other shaded habitats e.g. in the U.S. it has been recorded in coniferous woodland, on shady north-facing sandstone slopes, limestone bluffs and talus slopes (Cochrane & Salamun 1974). It has been identified as an indicator of ancient woodland but is also known to become established in secondary woodlands (Brunet & von Oheimb 1998; Honnay *et al.* 1998; Bossuyt *et al.* 1999; Hill 2004). Although widespread this is a rare and protected species across most of its range (e.g. Cochrane & Salamun 1994, Envfor 2003, Northfield 2003, Ulster museum 2003). In the U.K. it is found in the south, West Midlands, Wales and as far north as the Caithness. (Preston *et al.* 2002). It is only locally frequent to scarce throughout its U.K. range (Rodwell, 1991).

Adoxa moschatellina L. (moschatel, muskroot, townhall clock) is a small (5–15 cm), delicate, hairless perennial with white musk-scented rhizomes and stolons. The above-ground parts of the plant grow in spring from the swollen scales on the rhizomes. The basal leaves have relatively long petioles and the leaves are ternately compound with deeply toothed, ovate/obovate leaflets. The cauline leaves occur as a pair on slightly shorter petioles and tend to be smaller and less deeply toothed than the basal leaves. An inflorescence arises from the axil between the cauline leaves and is usually composed of five (2–6) hermaphrodite flowers. The flowers are arranged as five sides of a cube, reminiscent in shape to a townhall clock.

The top flower is usually tetramous with four styles and four divided anthers, each part having its own anther sac. The side flowers usually have 5–6 styles and 5–6 anthers (10–12 anther sacs). The flowers are small, simple and bowl shaped. About 20–30 multicellular hairs form a cushion-like nectary at the base of the upper side of each corolla lobe. When fruiting, the gynoecea of adjoining flowers combine to form a small drupe-like fruit. The seeds have an oily endosperm and a small embryo (Cochrane & Salamun 1974; Erbar 1994).

The production of seeds is the result of a complex series of processes that are influenced by environmental and genetic factors. The structure of the inflorescence, the viability of pollen, the effectiveness and type of pollinator and the nature of the breeding system all impact on seed production (e.g. Proctor, Yeo & Lack 1996). The aim of this investigation is to examine the seed production of *Adoxa moschatellina* and the factors that influence seed set.

STUDY SITES

Populations of *Adoxa moschatellina* were studied in 1995 and 2002 at three sites in Worcestershire and Shropshire, U.K.: Knapp and Papermill (KP) (1995 only) (SO755515), Shrawley Wood (SW) (SO655807) and Bensons Brook (BB) (SO580771). The Knapp and Papermill site is floristically diverse and includes meadows, a stream, orchard and woodland: *A. moschatellina* was present alongside a footpath between the stream and meadows. Shrawley Wood is dominated by *Tilia cordata* with the floristic community typical of a W8 woodland (Rodwell 1991). The population at Bensons Brook is found within a small area of oak-ash W8-type woodland (Rodwell 1991) which encompasses the brook. *A. moschatellina* has a dispersed distribution at each of these sites and these separate clumps have been indicated as BB1, BB2 etc.

METHODS

FLOWERING PHENOLOGY

Three 0.25 m² quadrats were placed at random in late January 2002 at BB7 and the flowering phenology (growth of leaves, initiation and development of the inflorescence, floral maturation, duration of flowering, fruit production, causes of loss of plant material) of the 38 ramets within these quadrats was observed every three days until die back in June. Supporting notes were also recorded at BB, KP and SW in 1995. Eight plants were set aside for observation in the breeding system study in 2002 (below). These inflorescences were observed every four hours during the day for a three week period (mid March–early April). During this period the inflorescences completed the flowering process.

INFLORESCENCE AND SEED PRODUCTION

A. SW AND BB 1995

On 16 May 1995 BB2 was surveyed and, for all plants with above ground parts, the numbers of inflorescences and fruits were recorded. A further three clumps in SW (SW1, SW2 and SW3) were examined on 8 June 1995. Observations at the sites indicated that all inflorescences had matured beyond flowering. Fruits from BB2, SW1 and SW3 were collected and dissected to establish the level of seed set. To reduce the impact of the research on SW no fruits were taken from SW2. The relative numbers of inflorescences present at the BB and SW sites were compared using chi-squared tests (Fowler & Cohen 1990).

B. BB 2002

During the study of flowering phenology at BB7 (above) the numbers of inflorescences and fruits produced were also recorded every three days between January and June.

POLLEN VIABILITY

The pollen viability was estimated at five locations (BB1, BB2, KP1, KP2 and SW1) in March 1995. To ensure that different genets were examined the sampling was systematic and one mature anther was removed from a single inflorescence no closer than 1 m between plants. The pollen from each anther was mounted immediately on a microscope slide in Alexander's stain (Alexander

1969) and stored at 4°C on return from the field. The % viability, as indicated by pink staining, was recorded within 3–48 hours of sampling. The difference between the populations was examined using the Kruskal-Wallis test (Fowler & Cohen 1990).

POLLINATORS

DAY-FLYING POLLINATORS

A limited survey of all insects landing on *Adoxa moschatellina* at BB7 was carried out for a two hour period on a bright cool day on 16 April 2002. These pollinators and the inflorescences on which they had alighted were collected.

NIGHT-FLYING POLLINATORS

A Heath type light trap was set for a single, relatively warm night on 12 April 2002 within population BB7. In the morning all insects within the trap were removed and identified. Parts from each specimen were examined under a light microscope ($\times 400$) for the presence of *A. moschatellina* pollen. A reference collection of pollen was prepared from the eight species (insect and wind pollinated) that were flowering at that time in the vicinity. The pollen from each species was mounted on a slide, in the field, in Alexander's stain and stored subsequently at 4°C. The reference collection was used both to positively identify *A. moschatellina* pollen and to exclude other species. The presence of a locally very rare moth in this first sample precluded further work.

BREEDING SYSTEM

A systematic sample of flowering *A. moschatellina* was collected from the BB population in March 2002. Plants sampled were at least 3 m apart. The original soil was supplemented by John Innes No. 1 and the plants were grown in seed trays outside in a shaded cold frame. The plants received no additional watering.

As inflorescences developed they were assigned at random to one of two categories – experimental or observation. The phenological details of the second group of inflorescences were recorded. These plants had a minimum of handling.

In the experimental group each inflorescence was covered in a cellophane pollen bag and assigned at random to one of four treatments: apomixis, cross-pollination, self-pollination or control. In the 'apomixis' and cross-pollination treatments all anthers were carefully removed from the flowers whilst immature. To test for apomixis in this species, the 'apomixis' inflorescences were then left. Pollen from one other genet was applied to each flower in the cross-pollinated inflorescences. In the self-pollinated inflorescence pollen from a mature anther within the inflorescence was transferred. In the cross-pollination and self-pollination treatments, as it was not possible to know when the stigmas were receptive, each flower was examined twice a week and pollen applied to the stigma from the time that the petals opened until when the petals began to curl back again. The control inflorescences once bagged were not handled again. Comparisons were made between the numbers of inflorescences producing seed in self- and cross-pollinated inflorescences (χ^2 test); the numbers of mature ovaries per inflorescence (Mann Whitney U test) and the number of seeds per mature ovary (z test) (Fowler & Cohen 1990).

RESULTS

PHENOLOGY

In this study the aerial parts were present from late January until mid-June. By mid-February a small compact inflorescence was produced in the axil between two leaves. The peduncle supporting the inflorescence extended to an average length of 22 mm by mid March. Normally the flower at the top of the cube-shaped inflorescence opened first (mid-March), followed an average of 2 days later (range 2–5 days) by one pair of flowers on opposite sides to each other. These were followed, on average, 4 days later (range 4–6 days) by the other opposite pair of flowers.

When the petals on the flowers first opened they were green and the style showed no further structural change once the petals opened. In the top flower all the petals opened together. The anthers started to dehisce on average 2.5 days (range 2–7 days) after the petals first opened and at this time a small volume of free nectar was seen at the base of the petals. On the side flowers the top petals opened first and the anthers matured in a similar order after 3 days (range 4–6 days).

The petals turned yellow after about 2 weeks and after a further 2.5 weeks (range 2–3 weeks) they became papery and closed back over the ovaries. At the same time (early–mid April) the petiole corkscrewed down to the ground. This process did not involve any further growth and occurred irrespective of fertilisation success. Fruits were also seen to develop and swell at this stage.

There was some variation in flowering phenology within a population and some plants flowered as late as May. The rhizomes and stolons were also observed to grow extensively during mid-May. By mid-June no plant remains were visible above ground.

INFLORESCENCE AND SEED PRODUCTION

INFLORESCENCES

In the natural populations of SW and BB in May 1995 the rhizomes produced between 670–1357 ramets but very few of these produced an inflorescence (range 1.2%–10.8%) (Table 1). This figure is likely to be a minimum since some inflorescences will have died back earlier in the growing season. The relative numbers of inflorescences produced varied significantly from one population to another ($\chi^2 = 111.1$, $p < 0.001$) (Table 1), with those plants growing in the *Tilia cordata* W8 woodland (SW) producing fewer inflorescences than those in the oak-ash W8 woodland at BB. Of the inflorescences present at SW and BB, signs of grazing, presumably by molluscs, were recorded on a considerable number of inflorescences (Table 1). This was more prevalent in SW than in BB ($\chi^2 = 81.8$, $p < 0.001$).

TABLE 1. INFLORESCENCE PRODUCTION IN *ADOXA MOSCHATELLINA* AT SHRAWLEY WOOD (SW) AND BENSONS BROOK (BB2) IN MAY 1995

	Populations			
	SW1	SW2	SW3	BB2
Total no. ramets	670	1215	131	1357
No. ramets/m ² in the population	26.25	13.75	5.0	18.25
Total no. inflorescences	8	35	3	146
Inflorescences per plant (%)	1.2	2.9	2.3	10.8
Evidence of grazing (%)	25	69	100	6

At BB7 from April–June 2002 the total number of inflorescences declined from 55 to only 11 (20% survival) (Table 2). Throughout this period up to 47% of inflorescences (average 19%) were seen to have been removed by grazing leaving only the peduncle. A further 22% of inflorescences were infected by *Puccinia* spp. (smut fungi) which led to early die back.

FRUITING.

Each fruit usually comprises five ovaries. Each ovary is partitioned into 3–5 locules and one ovum is present per locule. In the apparently fruiting inflorescences, which were collected from BB2, SW1 and SW3 in 1995 (Table 3), it was evident that the ovaries fell into one of three states: 'immature'; 'intermediate' and 'mature'. The 'mature' ovaries were swollen and translucent, apparently fully developed at 4–7 mm in diameter. Only 6% of all ovaries were 'mature'. The 'immature' ovaries were small (1–2 mm), dark green and showed no sign of development following flowering and no seeds. The 'intermediate' ovaries appear to have initiated development of the fruit but there was an air space within the locule in place of a seed. This form was either deep green or crystalline in appearance and did not develop further than 2–4 mm in diameter. That there appear to be three distinct morphological states was confirmed in observations at BB7 and in crosses in 2002 (Tables 2 and 6). Those inflorescences with ovaries in an 'immature' or 'intermediate' state never developed into a 'mature' state and since there are usually five ovaries in each drupe these different states were sometimes observed in combination (Table 3).

SEED PRODUCTION

Of the 161 inflorescences examined at BB2, SW1 and SW3 (1995) only 16 (10%) had any seeds and only two of these fruits had seeds in all the ovaries (Table 3). The remaining 14 fruits had seeds in one to four ovaries (2.6 ± 0.2). Only ovaries containing seeds had the 'mature' appearance. In these 'mature' ovaries average seed set was just over half (3.2 ± 0.2) compared to the usual maximum of five seeds.

TABLE 2. INFLORESCENCE AND FRUIT DEVELOPMENT IN *ADOXA MOSCHATELLINA*, APRIL TO JUNE 2002, AT BENSONS BROOK (BB7).

Date	29.4.02			8.5.02		
Quadrat	A	B	C	A	B	C
% grazed (Petiole only remaining)	20	47	0	23	40	0
% infected by <i>Puccinia adoxa</i>	15	12	17	8	13	0
% infected by <i>Puccinia albescens</i>	0	0	0	8	0	0
% In flower	65	41	78	38	40	50
% Fruiting ('Intermediate'/'Immature')	0	0	0	15	0	0
% Fruiting ('Mature'/'Immature')	0	0	1	8	7	50
Total no. inflorescences	20	17	18	13	15	14

Date	21.5.02			12.6.02		
Quadrat	A	B	C	A	B	C
% grazed (Petiole only remaining)	40	0	0	25	0	33
% infected by <i>Puccinia adoxa</i>	0	0	0	0	0	0
% infected by <i>Puccinia albescens</i>	0	0	0	0	0	0
% In flower	0	25	25	0	0	0
% Fruiting ('Intermediate'/'Immature')	27	50	25	0	75	0
% Fruiting ('Mature'/'Immature')	33	25	50	75	25	64
Total no. inflorescences	15	4	8	4	4	3

'In flower' indicates an inflorescence where the flowers are open and nectar evident. The terms 'intermediate', 'immature' and 'mature' describe the appearance of the ovaries (see text).

TABLE 3. APPEARANCE OF OVARIES OF *ADOXA MOSCHATELLINA* AT BENSONS BROOK (BB2) AND SHRAWLEY WOOD (SW1 AND SW3) 1995

Population		Immature only	Intermediate only	Mature only	Immature + Intermediate	Immature + Mature	Intermediate + Mature	Total
BB2	Inflorescence development	6	29	2	87	11	0	134
BB2	No. seeds in each fruiting ovary ($\bar{x} \pm SE$)	0	0	2.8 \pm 0.6	0	3.5 \pm 0.3	-	
SW1	Inflorescence development	2	0	0	0	5	0	7
SW1	No. seeds in each fruiting ovary ($\bar{x} \pm SE$)	0	-	-	-	2.3 \pm 0.5	-	
SW3	Inflorescence development	0	0	0	0	2	0	2
SW3	No. seeds in each fruiting ovary ($\bar{x} \pm SE$)	-	-	-	-	3.3 \pm 0.4	-	
Totals	Inflorescence development	8	29	2	87	18	0	161

The number of fruits dissected (Table 3) is not equal to the number recorded (Table 2) since grazing had reduced some inflorescences to only a peduncle.

POLLEN VIABILITY

Pollen viability at the five populations was found to be very variable (range 28–100%) (Table 4). However no significant difference was observed between the populations ($K = 2.45$, $p > 0.05$). The average across all populations was 68%.

TABLE 4. % POLLEN VIABILITY IN ANTHERS FROM *ADOXA MOSCHATELLINA*, AT 5 POPULATIONS, MARCH 1995

	Populations				
	KP1	KP2	BB1	BB2	SW1
No. anthers	22	6	22	24	17
Average viability (%)	65	67	65	68	73
Range (%)	30–100	56–86	28–94	28–98	92–48

POLLINATION

DAY-FLYING POLLINATORS

Five of the seven species observed apparently acting as day-time pollinators were Diptera (Table 5). The most frequently observed species were the caddis fly *Stenophylax permistus* (30%) and the flesh fly *Scathophaga stercoraria* (20%). All the insects observed showed foraging behaviour, moving around a flower and then from one flower to its neighbour within the inflorescence. The inflorescences visited by these probable pollinators were in mid to late flowering with three to five of the five flowers in each inflorescence open with mature anthers. Pollen was present on the stigmas of these flowers.

TABLE 5. APPARENT POLLINATORS OF *ADOXA MOSCHATELLINA* AT BENSONS BROOK (BB7) IN APRIL 2002

Day (D) Night (N)	Order	Family	Species	Male (M) Female (F) Unknown (U)	Frequency of observation
D	Coleoptera	Nitidulidae	<i>Meligethes viridescens</i>	U	1
D	Trichoptera	Limnophilinae	<i>Stenophylax permistus</i>	M	3
D	Diptera	Mycetophilidae	<i>Cordyla crassicornis</i> Meigon	M	1
D	Diptera	Chironomidae	<i>Diamesa tonsa</i> (Haliday = <i>culicoides</i> Heegar).	F	1
D	Diptera	Mycetophilidae	<i>Exechia</i> sp.	F	1
D	Diptera	Scathophagidae	<i>Scathophaga stercoraria</i>	F	2
D	Diptera	Psychodidae	sp.	U	1
N	Trichoptera	Limnophilinae	<i>Stenophylax permistus</i> .	U	2
N	Lepidoptera	Noctuidae	<i>Orthosia gothica</i> L.	U	33
N	Lepidoptera	Noctuidae	<i>Orthosia incerta</i> Hufnagel	U	5
N	Lepidoptera	Noctuidae	<i>Orthosia munda</i> Denis & Schiffermuller	U	1
N	Lepidoptera	Noctuidae	<i>Orthosia cerasi</i> Fabricius	U	1
N	Lepidoptera	Noctuidae	<i>Lithophane hepatica</i> Clerck.	U	1

NIGHT-FLYING POLLINATORS

Five of the species found with *A. moschatellina* pollen adhering to their antenna or on leg hairs were Lepidoptera (moths) and of these *Orthosia gothica*, Hebrew character, was the most abundant in the light trap (Table 5). One species *Selenia tetralunaria*, purple thorn, was not found to be carrying any *A. moschatellina* pollen. Another species found to be carrying *A. moschatellina* pollen was again the caddis fly *Stenophylax permistus*.

BREEDING SYSTEM

In total, 101 inflorescences were bagged. However a third of these died or became infected with *Puccinia adoxa* and/or *P. albescens* and were excluded from the experiment. The remaining 63 inflorescences were assigned at random to one of four treatments. The control and 'apomixis' group of inflorescences set no seeds and remained 'immature' in appearance (Table 6). In the cross-pollinated group all but one set seed and in each inflorescence most, and usually all, ovaries did so. These fruits developed the swollen translucent appearance of the 'mature' ovaries seen in the natural populations. By contrast the inflorescences that were self-pollinated showed a very highly significant reduction in the numbers setting seed (5/30) compared to the cross-pollinated plants (23/24) ($\chi^2 = 34.9$, $p < 0.001$). The numbers of mature ovaries in these inflorescences was also significantly lower in the self-pollinated inflorescences (2 ± 0.5) compared to the cross-pollinated plants (4 ± 0.2) ($U = 9$, $p = 0.05$). The numbers of seeds produced per fruiting ovary in the self-pollinated group was 1.3 ± 0.5 compared to 3.5 ± 1.0 in the cross-pollinated group, again a very highly significant difference ($z = 12.7$, $p < 0.001$). Interestingly, the self-pollinated ovaries took on one of two morphological forms. Those ovaries containing seed became swollen and translucent whilst the majority of those without seed took on an 'intermediate' form.

TABLE 6. FRUIT AND SEED PRODUCTION IN EXPERIMENTAL CROSSES OF *A. MOSCHATELLINA*, 2002

Treatment	No. inflorescences	No. inflorescences with at least one mature ovary	No. 'mature' ovaries in these* inflorescences ($\bar{x} \pm SE$).	No. seed in fruiting ovaries ($\bar{x} \pm SE$).
Control	3	0	-	-
Apomixis test	6	0	-	-
Self-pollination	30	5*	2.0 ± 0.5	1.0 ± 0.1
Cross-pollination	24	23*	4.0 ± 0.2	3.5 ± 0.1

DISCUSSION

The development of *A. moschatellina* in the field is in accord with that described in part by, for example, Fukuoka (1974) and Nepomnyashchaya (1984). The sequence of flower maturation reflects that seen in the embryo. Studies of early buds using a scanning electron microscope have shown that the top flowers develop first and the lateral flowers show 'top down' development (Erbar 1994; Roels & Smets 1994). The detailed observations of flowering phenology in this study would indicate the possibility of limited protogyny since the style showed no further structural change when the petals opened yet the anthers did not dehisce until several days later. Nectar was not usually evident until the anthers dehisced, however, so pollinators may not be attracted until this stage. The overlap of flowering times between the top flower and the two, staged openings of opposite pairs of flowers means that a high level of self-fertilisation is possible, the top flower being in anthesis when the side flowers first open and the first opposite pairs of flowers shed pollen when the second opposite pair of flowers opens.

In this study *A. moschatellina* has been observed to have a low level of seed set in natural populations. Very few inflorescences survived to fruit. In BB7 (2002) only 6% to 15% of inflorescences remained at the end of the flowering period. This loss of reproductive potential can be explained to some extent in terms of herbivore activity and pathogens (Tables 2, 3). Of the inflorescences that did survive until mid-late June very few (10%) produced seed. This low seed set cannot be accounted for in terms of pollen viability where the consistent average across four populations was 68% viability.

A. moschatellina is found in damp shady places and has relatively small flowers and inflorescences with freely exposed nectar and short stamens. As such it would appear to be adapted for pollination by small flying insects. Of the trn day-time visitors collected at BB7 six were small Diptera (Table 5). *A. moschatellina* is known to be entomophilous with records for

visits by Mycetophilidae (fungus gnats), Cecidomyiidae (gall midges), Simuliidae (black flies), and Chironomidae (non-biting midges) (Proctor & Yeo 1973). Flowers pollinated predominantly by Diptera usually smell aminoid and *A. moschatellina* does have a musky scent rather than the 'flowery' scent typical of plants pollinated by Lepidoptera or Hymenoptera. The flowers also have green to pale yellow petals, which is a common feature of fly-pollinated flowers (Proctor, Yeo & Lack 1996).

This species is clearly a source of resources for a number of diverse organisms since, as well as the Diptera, the plants were seen to be visited both during the day and night by the caddis fly (*Stenophylax permistus*) and during the day by a beetle (*Meligethes viridescens*) (Table 5). The presence of *S. permistus* is presumably restricted to habitats with proximity to water such as BB. *M. viridescens* is a common pollen beetle and may be an agent for pollination. *A. moschatellina* has also been recorded as being visited by small chalcid wasps and ichneumon wasps (Hymenoptera) (Proctor & Yeo 1973).

One surprising result was the presence of *A. moschatellina* pollen caught within the hairs on the antennae and legs of a number of moths (Table 5). Moths, like most Lepidoptera, tend to have a long slender proboscis which is adapted for reaching nectar at the base of narrowly tubular flowers, though it can be used for sucking up exposed liquids (Proctor, Yeo & Lack 1996). The flat bowl of an *A. moschatellina* flower may therefore provide an accessible source of nectar for these species.

Given the limitations of the survey of day-time apparent pollinators it is likely that the number of species visiting this plant is higher than ten. In this study it is also not possible to assess the effectiveness of these flower visitors as pollinators of *A. moschatellina*.

In the study of the breeding system 14.5% of the inflorescences initially bagged became infected with one of the *Puccinia* species. This is comparable to the levels of infection found within the natural populations (Table 2). Experimental crosses indicate that *A. moschatellina* is not apomictic and has reduced seed set when self-pollinated. Seed set is reduced significantly both in terms of the numbers of ovaries producing seed and the numbers of seed these ovaries produced (Table 6). This could be the result of inbreeding depression although such low numbers of seed set per ovary is thought to indicate partial self-incompatibility (Weller *et al.* 1995). Self-incompatibility of this type is usually either sporophytic or gametophytic and determined by S alleles at several genes. Although it is not known which system determines the incompatibility reaction of *A. moschatellina*, a review of self-incompatible species indicated that one key characteristic of plants with sporophytic incompatibility systems is that they have tricellular pollen unlike plants with gametophytic incompatibility systems which have bicellular pollen (Dafni 1992). Since *A. moschatellina* has tricellular pollen (Donoghue 1985), it is possible that this species has a sporophytic incompatibility system.

Three morphological forms of fruit have been observed in this species. The 'immature' form appears to be the result of 'no pollination' (Table 6). The 'mature' and 'intermediate' forms appear to be associated with pollination. Given the proposal that a self-incompatibility mechanism exists in this plant it is tempting to suggest that these latter forms in some way relate to the level of compatibility between the S alleles. Alternatively, it may be that the existence of the 'intermediate' form in self-fertilised inflorescences only indicates that the self-incompatibility system is not sporophytic but late-acting and arises following the abortion of ova after fertilisation. This is clearly an area that requires further investigation.

A. moschatellina is globally an uncommon or rare plant, existing in small fragmented populations. Vegetative reproduction does occur in this species but sexual reproduction via seed is limited. The factors that negatively affect seed set in the U.K. populations studied are inflorescence production per plant, pathogens, herbivores and one (or possibly more) self-incompatibility mechanisms. Pollen viability does not appear to be a limiting factor. The behaviour of the apparent pollinators and the phenological sequencing of anther maturation within the inflorescences may facilitate self-pollination which combined with a self-incompatibility system may be another factor contributing to the low seed set observed.

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