

No evidence for simultaneous pollen and resource limitation in *Aciphylla squarrosa*: A long-lived, masting herb

ROWAN H. BROOKES[†] AND LINLEY K. JESSON^{*}

School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand

Abstract For successful reproduction animal pollinated plants must provide resources for both pollinator attraction and offspring production, and theory suggests that resources and pollen delivery limit reproduction simultaneously. We conducted a series of experiments involving supplemental pollination, flower removal, fertilizer addition and foliage removal to investigate the interaction of resources and pollen on fruit-set of *Aciphylla squarrosa*, a long-lived, dioecious, masting herb in Wellington, New Zealand. Reducing floral display decreased open-pollinated fruit-set, suggesting that display size is a reflection of an optimal investment between attraction and fecundity. In combination with supplemental pollination, resource reduction and fertilization addition did not alter fruit-set, suggesting that changes in resource availability did not limit reproduction in the current year. In addition, supplemental pollination of non-manipulated treatments did not increase fruit-set, demonstrating that plants were not naturally pollen limited. While we found that simultaneous pollen and resource limitation did not occur within a season, this is possibly mitigated by life history patterns including mast flowering and a storage taproot. Multiple year studies are required to further examine simultaneous resource and pollen limitation.

Key words: Bateman's principle, Haig and Westoby model, masting, pollen limitation, resource limitation.

INTRODUCTION

In animal-pollinated plants, resources must be allocated for both pollinator attraction and fruit and seed maturation. As a result, seed production can be constrained by pollen or resource availability, or by both (Lloyd 1988; Ashman *et al.* 2004). Low levels of seed production are generally attributed to pollen limitation if plants receiving supplemental pollen are more reproductively successful than naturally pollinated plants (Ashman *et al.* 2004). However, widespread pollen limitation fails to provide a complete explanation for the high reproductive failure observed in many natural systems (Lee & Bazzaz 1982). As an alternative to pollen limitation, resource limitation has been inferred when supplemental pollination does not increase seed production (Bierzychudek 1981; de Jong & Klinkhamer 1989; Zimmerman & Aide 1989; Griffin & Barrett 2002).

Traditionally, reproduction has been examined under a premise by Bateman (1948) (Bateman's Principle) stating that the reproductive success of males is limited by access to mates, whereas females are limited by the amount of resources required for

offspring provisioning. In flowering plants, Bateman's Principle can be applied when the resources available for seed provisioning limit maternal reproductive success (Charnov 1982). However, the application of Bateman's Principle to plants has been criticized as being simplistic, because pollination is only one of a range of ecological (proximate) and evolutionary (ultimate) factors that cause variation in reproductive success (Stephenson 1981; Ehrlen 1992; Larson & Barrett 2000; Ashman *et al.* 2004).

Haig and Westoby (1988) proposed a model in which resources are finite and therefore must be allocated to balance the conflicting demands between resources required for pollinator attraction and those needed to provision seeds successfully. This selective pressure would lead to the simultaneous limitation of reproductive success by both pollen and resources (Haig & Westoby 1988). At this equilibrium, supplemental pollination results in no, or only minor, increase in seed set (as resources would then be limiting), while additional resources would also fail to increase seed production (because no additional fertilized ovules are available for maturation). Haig and Westoby's (1988) model allowed for minor fluctuations around the equilibrium as a consequence of pollinator and resource stochasticity.

Even though Haig and Westoby's (1988) model has become one of the foundation models of pollen-limitation theory few manipulative experiments have simultaneously tested for an interaction between pollen and resources on fruit-set (but see Galen

^{*}Corresponding author. Present address: Department of Biology, University of New Brunswick, PO Bag Service 45111 Fredericton, Canada E3B 6E1 (Email: jesson@unb.ca)

[†]Present address: School of Biological Sciences, Monash University, Victoria 3800, Australia.

Accepted for publication July 2006.

1985; Campbell & Halama 1993; Corbet 1998). Furthermore, abundant reports of pollen limitation contradict Haig and Westoby's (1988) hypothesis. For example, in a literature review of supplemental pollination experiments involving 258 species, Burd (1994) found 62% were pollen limited, and in an expanded literature review Knight *et al.* (2005) found a similarly high level of pollen limitation. In addition, the Haig and Westoby model also predicts that allocation to attraction will influence both levels of pollination and the resources available for ovule maturation. While many studies have demonstrated that increased allocation to attraction increases fruit-set (for review see Ohashi & Yahara 2001), it is less clear if this change in allocation results in subsequent resource limitation.

In this study, we manipulated floral attraction, and resource and pollen levels to examine the interaction of pollen and resource limitation in *Aciphylla squarrosa* J.R. et Forst, a long-lived, mast seeding herb with many features that might result in simultaneous selection by resources and pollen availability. Mast seeding (the synchronous, irregular output of seeds by a plant population; Silvertown 1980) might mitigate the effects of pollen limitation, as resources can be stored for future reproductive events (Kelly 1994), or the pattern of mast seeding might even become entrained with pollinator activity (Crone *et al.* 2005). In addition, *Aciphylla* is also dioecious, which removes the confounding effects of self-fertilization and within-plant competition between male and female function, yet pollinator service is necessary for current reproduction. Finally, *A. squarrosa* is found in alpine and grassland areas in New Zealand that are characterized by variable weather patterns, likely influencing opportunities for pollination. These characteristics undoubtedly combine to influence pollen and resource limitation during a season. We empirically examined Haig and Westoby's (1988) hypothesis by assessing whether seed production by females is limited by resources alone (Bateman's Principle), or simultaneously by pollen and resources and whether attraction effort, as determined by the number of flowers displayed, influences fecundity.

METHODS

Study species and site

Aciphylla squarrosa is a dioecious herb, with a dense rosette of glaucous, pungent leaves reaching up to 1 m high. Flowers are held on a peduncle with lateral umbellules from the base to the apex of the peduncle, with an average of 4090 ± 355 ($n = 50$) flowers on each inflorescence (R. H. Brookes, unpubl. data, 2004). Females flower only during mast years (Mark

1970; Campbell 1981; Haase 1986), whereas males flower during most years. Fruits consist of two mericarps, forming a laterally compressed schizocarp (Webb & Simpson 2001). Although female inflorescences present all of their flowers simultaneously, fruits mature basipetally, so that upper fruits mature before lower fruits.

We studied a population of *A. squarrosa* at Hawkin's Hill, South Wellington, New Zealand ($174^{\circ}44'80''\text{N}$; $41^{\circ}19'70''\text{W}$). During 2003 this population consisted of 62% flowering plants and 38% non-flowering (mostly juvenile) individuals. Of the flowering plants, 31% were male and 69% were female ($n = 1055$; R. H. Brookes unpubl. data, 2004). We conducted experimental pollinations at sites scattered along roughly 2 km of roadside, ranging from 360 to 495 m above sea level.

Experimental methods

We conducted two experiments manipulating pollinator attraction and resource levels. For each experiment, we included five replicates of three treatments crossed with two levels of open or supplemental pollination. Thirty female plants were used in each experiment. Treatments were applied to all inflorescences on a plant (usually one to four), and treatments in a replicate were grouped in a block (within 20 m), to ensure similar environmental conditions. It was not always possible to include all plants in a replicate in each block, and so eight blocks were established in the first experiment and seven blocks in the second.

The first experiment examined the effect of display size on pollinator attraction and consisted of plants with no flowers removed, 20% flowers removed, or 80% flowers removed (Fig. 1a). Flower removal was conducted after the inflorescence had presented all juvenile flowers. The removal of flowers from the inflorescence could have a secondary effect of allowing resources to be directed to the remaining flowers (Bell 1985). Therefore, the pollinator attraction component of this study also tested the interaction between pollinator attraction and resource availability. As an assessment of the pollinating environment, we made *ad hoc* observations of pollinators when possible. Pollinating insects observed visiting female plants were caught with a hand net and identified.

The second experiment investigated the potentially interacting effects of pollen and resource limitation. To assess resource limitation, we both increased and decreased resource availability (see Fig. 1b). We decreased resources by removing between 60% and 70% of the foliage. To increase resources, we reduced competition by weeding all competing plants surrounding the base of *A. squarrosa*. This treatment should decrease interspecific competition for soil held

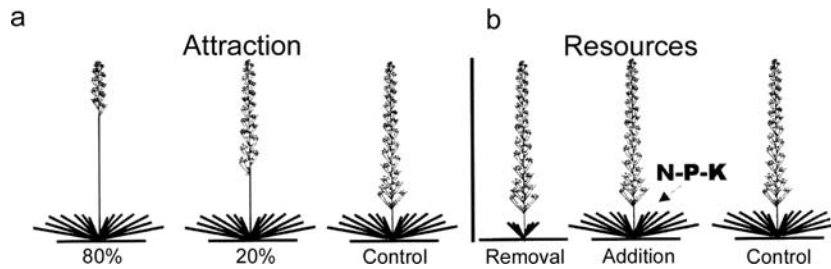


Fig. 1. Schematic drawing showing treatments. (a) Inflorescences with 80%; 20% and none of the flowers removed. (b) 50–60% of foliage stripped; fertilizer added and competitors weeded from base of plant; no resources added or removed. To test for pollen limitation half of the treated inflorescences also received supplemental pollination. Picture of umbel modified from Dawson (1971). $n =$ five replicates in each treatment.

resources. A general 7:5:7, N-P-K fertilizer (Tui brand) was then added to the base of the plants at the manufacturer's recommendations of 250 g m^{-2} . The plants with altered resources were compared with control plants with no resource changes.

We replicated the above experiments and supplementally pollinated the plants to test for pollen limitation and the interactive effects of pollen and resources. To access the flowers for hand-pollination, bracts surrounding the umbellules were removed on both control and manipulated plants in all treatments. If bracts aid pollinator attraction, fruit-set in open-pollinated plants with removed bracts would be lower than in pollinated individuals, but this was not observed (see *Results*). Supplemental pollination was performed by brushing male umbellules over female flowers. To reduce biparental inbreeding, pollen from several male plants was applied to each female. Experimental pollinations began during November 2003, when 20 female plants had begun flowering. Pollen was reapplied weekly until fruit-set began, by which time plants had received two to five pollinations. We stopped supplemental pollination on 4 December, when no females remained receptive in the population. All female inflorescences were harvested between 15 December and 17 January, when fruits were mature.

Fruits were separated into large and small seeds (presumably from pollinated and unpollinated flowers), and all seeds from five replicates were counted for each plant. To ensure we identified pollinated flowers correctly, we used a seed viability test on 10 large seeds from five plants and 10 small seeds from three plants to check that large seeds had indeed been fertilized. Each mericarp was cut longitudinally, stained in a 0.5% tetrazolium solution, and placed in an incubator at 40°C for 1 h. This test showed that 50% ($\pm 10.5\%$ SE, $n = 50$) of large seeds were viable, whereas no small seeds were viable, indicating that we had correctly identified pollinated flowers, although the number of viable seeds was less than that estimated for fruit-set.

Data analysis

To analyse fruit-set among different treatments, fruit number from both experiments were arc-sine transformed to satisfy assumptions of homoscedasticity of variances. Each observation represented the mean for all inflorescences sampled on a plant. To determine whether pollen or resource limitation affected per cent fruit-set, we performed ANOVAS in S+ (Mathsoft 4.0, Mathsoft, Washington) and examined the effect of total flower number (as a covariate), block, resource treatment, pollen supplementation, the interaction between resource and pollen treatment and the interaction between total flower number with pollen addition. The data were subsequently back-transformed for graphical presentation. To examine the influence of sample size on the significance of our results, we conducted a power analysis (Neter *et al.* 1996).

RESULTS

Pollinator observations

Most of the insects that we observed visiting *A. squarrosa* were flies (Diptera), primarily Calliphoridae, Muscidae and Tachinidae, although we also observed some apid bees (Hymenoptera). Pollinators crawled across flowers before flying to the next plant. Our *ad hoc* observations suggested that there were numerous pollinators on the flowers of both sexes, although abundance was affected by weather conditions – pollinators were most abundant on calm, sunny days, rare during windy conditions, and absent during wet weather.

Pollinator attraction

Total ovule number ranged from 249 to 13 830 ovules per inflorescence ($\bar{x} \pm \text{SE} = 3763 \pm 322$ ovules per

Table 1. Analysis of variance of the effects of flower removal on open pollinated *versus* supplemental pollination and their interactions on proportion fruit-set in *Aciphylla squarrosa*

Source of variation	d.f.	Sum of squares	Mean square	<i>F</i>	<i>P</i>
Total flower number	1	0.0004	0.0004	0.009	0.92
Block	7	0.51	0.07	1.64	0.19
Flower removal	2	0.13	0.07	1.47	0.26
Pollen	1	0.13	0.13	2.97	0.10
Pollen × flower removal	2	0.34	0.17	3.88	0.04
Pollen × total flower number	1	0.02	0.02	0.50	0.48
Residuals	15	0.67	0.05		

Where possible, plants in a replicate were located in a Block. Pollination treatments (Pollen) were either open or supplemental pollination, while flower removal treatments (Flower removal) were no flowers removed, 20% flowers removed, or 80% flowers removed.

Table 2. Analysis of variance of the resource addition and removal on open pollinated *versus* supplemental pollination and their interactions on proportion fruit-set in *Aciphylla squarrosa*

Source of variation	d.f.	Sum of squares	Mean square	<i>F</i>	<i>P</i>
Total flower number	1	0.01	0.01	0.61	0.45
Block	6	0.19	0.03	1.48	0.45
Resources	2	0.07	0.04	1.61	0.23
Pollen	1	0.05	0.05	2.37	0.14
Resources × pollen	2	0.05	0.03	1.23	0.31
Pollen × total flower number	1	0.001	0.001	0.006	0.98
Residuals	16	0.24	0.02		

Where possible, all plants in a replicate were located in a Block. Pollination treatments (Pollen) were either open or supplemental pollination, while resource treatments (Resources) were no resources added, fertilized and weeded, or foliage stripped.

inflorescence, $n = 80$ inflorescences). Despite this large range, total flower number did not influence per cent fruit-set, and there were no significant interactions between total flower number and pollen treatment (Tables 1,2). The interaction of flower removal with supplemental pollination significantly influenced fruit-set (Table 1). When we removed flowers, fruit-set decreased under open pollinated conditions more than with supplementary pollen (Fig. 2a). A power analysis of the flower removal experiment suggested that the probability that an *F*-test will detect differences in fruit-set for the interaction of pollen and resources was 100% ($\phi = 5$, $v_1 = 4$, $v_2 = 36$, $\alpha = 0.05$, $P = 1$).

Pollen and resource manipulations

There were no significant effects of resource addition or removal or the interaction between resources and pollen addition (Table 2). Consistent with the patterns observed in the pollinator attraction experiment, supplemental pollination did not increase fruit-set (see Fig. 3a). Further, there was no difference in fruit-set between all treatments (stripped, control, fertilized) following supplemental pollination

(all $P > 0.05$). There was also no significant effect of block, or total flower number, on fruit-set (Table 2). A power analysis of the fertilizer experiment suggested that the probability that an *F*-test will detect differences in fruit-set for the interaction of pollen and resources was 99% ($\phi = 2.3$, $v_1 = 4$; $v_2 = 36$, $\alpha = 0.05$, $P = 0.99$).

DISCUSSION

Floral display and pollinator attractiveness

Plants that attract more pollinators should be less likely to experience pollen limitation. Therefore, a greater floral display has been associated with increasing pollinator visits in both hermaphroditic and dioecious plants (Bawa 1980; Klinkhamer & de Jong 1993; Pickering 2001; Gómez 2003). Conversely, a reduction in floral display decreases reproductive success (Harder & Barrett 1996; Corbet 1998). When resources are not limiting, a plant's reproductive success can be increased through greater allocation to floral display (Ehlers *et al.* 2002). However, because of

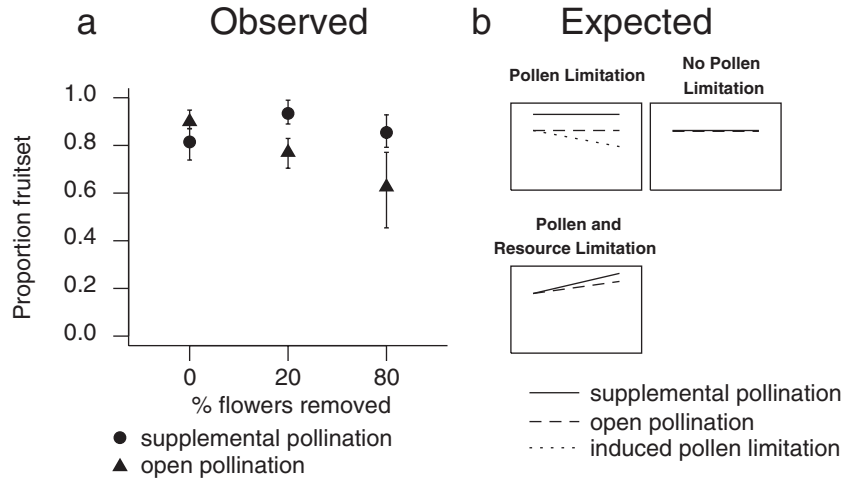


Fig. 2. (a) Observed proportion (mean \pm 1 SE) fruit-set of open pollinated (grey squares) *versus* supplementally pollinated (black circles) plants following 0%, 20% or 80% flower removal. Error bars are asymmetric due to back transformation of data. (b) Schematic of expected scenarios of pollen and resource limitation following supplemental pollination and flower removal. Under a scenario of pollen limitation, supplemental pollination with no flower removal would result in increases to fruit-set. Removal of flowers with no pollen limitation would result in no increase in fruit-set after pollination. When the plant is simultaneously resource and pollen limited, an increase of resources to the remaining flowers and supplemental pollination would induce greater increases in fruit-set than only being limited by one factor.

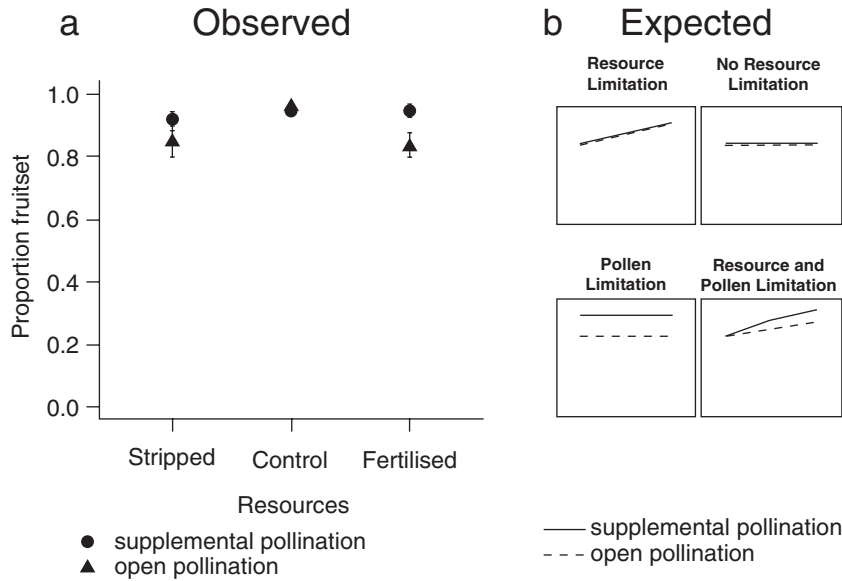


Fig. 3. (a) Observed proportion fruit-set (mean \pm 1 SE) of open pollinated (grey squares) *versus* supplementally pollinated (black circles) plants following resource manipulations. Error bars are asymmetric due to back transformation of data. (b) Schematic of expected scenarios of pollen and resource limitation following supplemental pollination. If resource limitation is successfully induced, yet plants are not pollen limited then fruit-set should increase equally in both pollination treatments. No resource or pollen limitation can be demonstrated when resource addition or removal and supplemental pollination have no effect on seed set. Pollen limitation can be demonstrated when supplemental pollination increases fruit-set, but resource manipulations produce no response. Supplemental pollination and resource addition should increase fruit-set if the plant is simultaneously resource and pollen limited.

maternal reproductive requirements, such as ovule production and seed provisioning, females are constrained in the size of their floral display (Vaughton & Ramsey 1998). This conflict between resources and

pollen limiting factors results in a trade-off between pollinator attraction and seed provisioning, and is central to expectations presented in the Haig and Westoby (1988) model.

We found partial support for the expectation that pollen and resources are at equilibrium. Decreasing the floral display of *A. squarrosa* reduced fruit-set in open-pollinated plants (Fig. 2a), suggesting that small displays are less attractive to pollinators. If *A. squarrosa* was simultaneously resource and pollen limited, fruit-set in treatments with resources increased should be greater than control levels following supplemental pollination (e.g. supplemental pollinated with 20% and 80% flowers removed; Fig. 2b). Contrary to the expectations from the Haig and Westoby model, supplemental pollination resulted in equivalent fruit-set to control levels in all treatments. These results suggest that the current floral display of the female plants (e.g. $\bar{x} = 4090$ flowers per plant, $n = 20$ plants) might be an evolutionary stable strategy, whereby resources are not limiting and reproductive success is maximized through greater display. Thus, rather than pollen and resources being simultaneously limiting, reproduction in this study system was limited by other unknown factors.

The large floral display observed in *A. squarrosa* might be the result of pollinator-mediated selection (see Fægri & van der Pijl 1979). Interestingly, in open-pollinated plants there was no correlation between total flower number and fruit-set, yet when flowers were removed, a reduction in proportional fruit-set occurred (Fig. 2a). This discrepancy may be either due to a disruption in the pollinator's search image due to the change in floral form, or to damage to the inflorescences when flowers were removed. However, the latter suggestion is unlikely, as fruit-set was not reduced when these treatments received supplemental pollination.

Generally male, but not female, reproductive success is considered to be proportional to display size (Barrett & Harder 1996; Vaughton & Ramsey 1998; Bond & Maze 1999), yet in this study we find clear evidence that female reproductive success is also limited by display size. Most studies examining selection for increased display size have focused on male function, because sexual selection theory suggests that male fitness depends on pollen removal (Bell 1985; Queller 1997). However, display size is also important for female fitness (Morgan & Schoen 1997; Morgan 1998). If resources are not limiting, increased display size would produce more offspring. The fitness gains curve of display size *versus* fecundity is likely to be less linear for females than for males, and it is possible there is a threshold size for females over which increases in size do not increase seed set. Tests of this hypothesis would require the experimental addition of flowers.

Pollen and resource limitation

Supplemental pollination did not increase fruit-set above control levels in *A. squarrosa* (Figs 2,3). Therefore, this study provided no evidence for pollen

limitation in this study species. The study season was in a mast flowering year, resulting in a prominent floral display. This display might have attracted more pollinators than during non-mast flowering years (Crone *et al.* 2005). Further, *Aciphylla* has a generalist pollination syndrome with Diptera as the principal pollinator (Primack 1983), perhaps further releasing *Aciphylla* from pollen limitation. Multiple year studies would reveal whether this trend occurred only during mast flowering years, or if visitation by a high number of generalist pollinators resulted in the present finding of no pollen limitation.

Despite resource and pollen addition, no plant attained 100% levels of fruit-set. The population structuring might have resulted in biparental inbreeding, which reduced fruit-set. Furthermore, recent work by Porcher and Lande (2005) has suggested that a plant may regularly produce more ovules than it is able to support to minimize the fertility costs of less fit gametophytes by maturation of fitter ovules (reproductive compensation). This can occur even in dioecious species as the expression of deleterious alleles is still possible. The role of biparental inbreeding or reproductive compensation in restricting fruit-set is unknown in *Aciphylla* and is potentially a mechanism that might mediate the interactive effect of pollen and resources, especially if resource addition or flower removal results in similar levels of embryo abortion, but preferential resource allocation to fitter embryos. Estimates of seed weight and the subsequent success of offspring from fertilized and non-fertilized treatments would test for other effects of resource limitation.

Both resource manipulations (pollinated and fertilized and 80% flowers removed and pollinated) suggested the reproductive output of *A. squarrosa* is not resource limited (although the effect of lifetime reproduction is not known). We could not ascertain if resource addition had an effect, as no explicit examination of changes in soil or plant-held resources was undertaken. In other studies, manipulating plant resources through defoliation or resource addition can influence fruit-set during the first experimental season (Lee & Bazzaz 1982; Delph 1990; Campbell & Halama 1993; Juenger & Bergelson 1997; Susko & Lovett-Doust 1999; Litrico *et al.* 2005). However, the influence of resource addition can also be recorded in subsequent seasons, or over lifetime fitness (Worley & Harder 1999).

The reason for the apparent lack of resource limitation might lie in the life history strategies of *A. squarrosa*. First, *A. squarrosa* is a masting species flowering only during sporadic years. Masting might enable plants to accumulate resources during non-flowering years in preparation for a masting episode (Kelly 1994). Second, *A. squarrosa* has a deep taproot that assists in energy storage (Wyka 2000), which

could contribute to more stored resources when reproduction does take place. In combination with mast flowering, this could mitigate the effect of resource addition, as the plant already has sufficient stored resources needed for reproduction, and extra resources are likely stored for future reproductive events. Mechanistic models of mast-seedling in *Astragalus scapoides* suggest that pollen limitation and heterogenous resource acquisition can interact to cause mast-seeding (Crone *et al.* 2005). It may be that the interaction between mast-seeding and pollen and resource limitation is also important in *A. squarrosa*. Therefore, a multiple year study examining the effect of resource and pollen addition might show the effects of resource limitation.

ACKNOWLEDGEMENTS

We thank S. Eppley, E. Minot, K. Abbott and M. Burd for discussion and comments on this manuscript, S. Newman for her assistance with fieldwork, and A. Tritt and T. Best who helped with seed counting. Special thanks to P. Taylor for his assistance throughout this project. This project was funded by the Betty Loughhead Soroptomist Scholarship Trust, School of Biological Sciences Graduate Grant, E.L. Hellaby Indigenous Grasslands Research Trust and the Wellington Botanical Society to RB.

REFERENCES

- Ashman T., Knight T. M., Steets J. A. *et al.* (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**, 2408–21.
- Barrett S. C. H. & Harder L. D. (1996) Ecology and evolution of plant mating. *Trends Ecol. Evol.* **11**, 73–9.
- Bateman A. J. (1948) Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–68.
- Bawa K. S. (1980) Evolution of dioecy in flowering plants. *Annu. Rev. Ecol. Syst.* **11**, 15–39.
- Bell G. (1985) On the function of flowers. *Proc. R. Soc. London, Ser. B* **224**, 223–65.
- Bierzychudek P. (1981) Pollinator limitation of plant reproductive effort. *Am. Nat.* **117**, 838–40.
- Bond W. J. & Maze K. E. (1999) Survival costs and reproductive benefits of floral display in a sexually dimorphic dioecious shrub, *Leucadendron xanthoconus*. *Evol. Ecol.* **13**, 1–18.
- Burd M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot. Rev.* **60**, 83–139.
- Campbell A. D. (1981) Flowering records for *Chinochloa*, *Aciphylla*, and *Celmisia* species in the Cragieburn Range, South Island, New Zealand. *N. Z. J. Bot.* **19**, 97–103.
- Campbell D. R. & Halama K. J. (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* **74**, 1043–51.
- Charnov E. L. (1982) *The Theory of Sex Allocation*. Princeton University Press, Princeton.
- Corbet S. A. (1998) Fruit and seed production in relation to pollination and resources in bluebell, *Hyacinthoides non-scripta*. *Oecologia* **114**, 349–60.
- Crone E. E., Polansky L. & Lesica P. (2005) Empirical models of pollen limitation, resource acquisition, and mast seeding by a bee-pollinated wildflower. *Am. Nat.* **166**, 396–408.
- Dawson J. W. (1971) *Relationships of the New Zealand Umbelliferae*. Linnean Society, London.
- de Jong T. & Klinkhamer P. G. J. (1989) Limiting factor for seed production in *Cynoglossum officinale*. *Oecologia* **80**, 167–72.
- Delph L. (1990) Sex-differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology* **71**, 1342–51.
- Ehlers B. K., Olesen J. M. & Ågren J. (2002) Floral morphology and reproductive success in the orchid *Epipactis helleborine*: regional and local across-habitat variation. *Plant Syst. Evol.* **236**, 19–32.
- Ehrlen J. (1992) Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. *Ecology* **73**, 1820–31.
- Fægri K. & van der Pijl L. (1979) *The Principles of Pollination Ecology*. Pergamon Press, Oxford.
- Galen C. (1985) Regulation of seed-set in *Polemonium viscosum*: floral scents, pollination, and resources. *Ecology* **66**, 792–7.
- Gómez J. M. (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *Am. Nat.* **162**, 242–56.
- Griffin S. R. & Barrett S. C. H. (2002) Factors affecting low seed: ovule ratios in a spring woodland herb, *Trillium grandiflorum* (Melanthiaceae). *Int. J. Plant Sci.* **163**, 581–90.
- Haase P. (1986) Flowering records of some subalpine trees and shrubs at Arthur's Pass, New Zealand. *N. Z. J. Ecol.* **9**, 19–23.
- Haig D. & Westoby M. (1988) On limits to seed production. *Am. Nat.* **131**, 757–9.
- Harder L. D. & Barrett S. C. H. (1996) Pollen dispersal and mating patterns in animal-pollinated plants. In: *Floral Biology* (eds D. G. Lloyd & S. C. H. Barrett) pp. 140–90. Chapman and Hall, New York.
- Juenger T. & Bergelson J. (1997) Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* **78**, 1684–95.
- Kelly D. (1994) The evolutionary ecology of mast seeding. *Trends Ecol. Evol.* **9**, 465–70.
- Klinkhamer P. G. L. & de Jong T. J. (1993) Attractiveness to pollinators: a plant's dilemma. *Oikos* **66**, 180–4.
- Knight T. M., Steets J. A., Vamosi J. C. *et al.* (2005) Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Syst.* **36**, 467–97.
- Larson B. M. H. & Barrett S. C. H. (2000) A comparative analysis of pollen limitation in flowering plants. *Biol. J. Linn. Soc.* **69**, 503–20.
- Lee T. D. & Bazzaz F. A. (1982) Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology* **63**, 1363–73.
- Litrico I., Pailler T. & Thompson J. D. (2005) Gender variation and primary succession in a tropical woody plant, *Antirhea borbonica* (Rubiaceae). *J. Ecol.* **93**, 705–15.
- Lloyd D. G. (1988) A general principle for the allocation of limited resources. *Evol. Ecol.* **2**, 175–87.
- Mark A. F. (1970) Floral initiation and development in New Zealand alpine plants. *N. Z. J. Bot.* **9**, 67–75.
- Morgan M. T. (1998) Female fertility per flower and trade-offs between size and number in *Claytonia virginica* (Portulacaceae). *Am. J. Bot.* **85**, 1231–6.

- Morgan M. T. & Schoen D. J. (1997) Selection on reproductive characters: floral morphology in *Asclepias syriaca*. *Heredity* **79**, 433–41.
- Neter J., Kutner M. H., Nachtsheim C. J. & Wasserman W. (1996) *Applied Linear Statistical Models*. McGraw-Hill, Boston.
- Ohashi K. & Yahara T. (2001) Behavioural responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution* (eds L. Chittka & J. D. Thomson) pp. 274–96. Cambridge University Press, Cambridge.
- Pickering C. M. (2001) Size and sex of floral display affect insect visitation rates in the dioecious Australian alpine herb, *Aciphylla glacialis*. *Nord. J. Bot.* **21**, 401–9.
- Porcher E. & Lande R. (2005) Reproductive compensation in the evolution of plant mating systems. *New Phytol.* **166**, 673–84.
- Primack R. B. (1983) Insect pollination in the New Zealand mountain flora. *N. Z. J. Bot.* **21**, 317–33.
- Queller D. (1997) Pollen removal, paternity, and the male function of flowers. *Am. Nat.* **149**, 585–94.
- Silvertown J. W. (1980) The evolutionary ecology of mast seeding in trees. *Biol. J. Linn. Soc.* **14**, 235–50.
- Stephenson A. G. (1981) Flower and fruit abortion: proximate causes and ultimate function. *Annu. Rev. Ecol. Syst.* **12**, 253–79.
- Susko D. J. & Lovett-Doust L. (1999) Effects of resource availability, and fruit and ovule position on components of fecundity in *Alliaria petiolata* (Brassicaceae). *New Phytol.* **144**, 295–306.
- Vaughton G. & Ramsey M. (1998) Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* **115**, 93–101.
- Webb C. J. & Simpson M. J. A. (2001) *Seeds of New Zealand Gymnosperms and Dicotyledons*. Manuka Press, Christchurch.
- Worley A. C. & Harder L. D. (1999) Consequences of preformation for dynamic resource allocation by a carnivorous herb, *Pinguicula vulgaris* (Lentibulariaceae). *Am. J. Bot.* **86**, 1136–45.
- Wyka T. (2000) Effect of nutrients on growth rate and carbohydrate storage in *Oxytropis sericea*: a test of the carbon accumulation hypothesis. *Int. J. Plant Sci.* **161**, 381–7.
- Zimmerman J. K. & Aide T. M. (1989) Patterns of fruit production in a neotropical orchid: pollinator *versus* resource limitation. *Am. J. Bot.* **76**, 67–73.