SHORT COMMUNICATION

Reduced maternal fecundity of the high Andean perennial herb *Alstroemeria umbellata* (Alstroemeriaceae) by aphid herbivory

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Abstract: Assessments of the effects of invertebrate herbivores on high-altitude plants have seldom taken into account both mutualistic and antagonistic interactions. To evaluate the effect of herbivores (antagonists) and pollinators (mutualists) on the female reproductive success of the high-Andean perennial herb *Alstroemeria umbellata,* we separately and simultaneously excluded aphids (herbivores), and bees and bumblebees (pollinators) in a 2×2 factorial design. In flowers with pollinators excluded, aphids did not reduce seed set per flower (i.e., a direct effect). However, in flowers exposed to pollinators, aphids reduced seed set by 1.7 times (i.e., a pollinator-mediated indirect effect). Likewise, both types of animals exerted non-additive effects on maternal fecundity. These results suggest a modulating role for herbivores on the selection pressures exerted by pollinators on *A. umbellata*.

Keywords: Pollinators; aphids; direct effect; indirect effect; seed set.

Introduction

Plant biomass removal by herbivores is an important selection pressure faced by plants (Hendrix, 1987; Strauss and Zangerl, 2002; Danell and Bergström, 2002). Herbivores may modify plant survivorship and reproductive success. Their action may have a significant impact on the reproductive ecology of plants by directly reducing seed production, or by indirectly modifying floral attractiveness to pollinators, which in turn may also reduce seed production (Hendrix, 1987; Herrera, 2000; Herrera *et al*., 2002; Strauss and Zangerl, 2002; Danell and Bergström, 2002). The combined action of herbivores and pollinators may influence the ecology of plant reproduction and the evolution of pollination-related traits (Karban and Strauss, 1993; Armbruster, 1997). Herbivores can modulate the nature, strength, and fitness consequences of interactions between plants and pollinators through their effects on plant and flower characteristics. Thus, by modifying factors such as pollen quantity and quality, and floral display, herbivores may change plant attractiveness towards pollinators (Hendrix, 1987; Herrera, 2000; Herrera *et al*., 2002; Strauss and Zangerl, 2002; Danell and Bergström, 2002). For instance, Herrera (2000) and Herrera *et al*. (2002) proposed a herbivore-dependent selection by pollinators based on the non-additive effects exerted by pollinators and herbivores on the fitness of the perennial herbs *Paeonia broteroi* and *Helleborus foetidus*.

Plants growing at high altitudes have only a narrow seasonal window available in which to interact with mutualistic and antagonistic animals, exhibit comparatively high pollinator dependency for seed setting (Arroyo *et al*., 1982; Arroyo and Squeo, 1990), and are exposed to low levels of herbivory (Kelly, 1998; Susuki, 1998; Scheidel *et al*., 2003). Hence, herbivores and pollinators are likely to interact to modulate the reproductive success of high altitude plants. We address this question using a high Andean perennial herb *Alstroemeria umbellata* (Alstroemeriaceae); this species was selected because it bears conspicuous protandrous flowers which may be intensely infested by aphids. A factorial design in which pollinators and aphids were excluded separately and simultaneously was used in order to determine possible direct and indirect effects of aphids on the maternal fecundity of *A. umbellata*, and to determine whether these effects are additive or non-additive.

Methods

Study site and species

Fieldwork was carried out between late January and late February 2004 near Valle Nevado in the Andes of Central Chile (33º21'S; 70º16'W; 2800 m a.s.l.). *Alstroemeria umbellata* is a perennial, rhizomatous, cushion-type herb occurring between 2500 and 3000 m a.s.l. in the Southern Andes (Bayer, 1987). Blooming and fruit-growing periods occur from December to February. Its breeding system is unknown. Pollinators are mainly hymenopterous insects, the most common in the study site being the bumblebee, *Bombus dahlbomii* (Apidae), and the bee, *Megachile semirufa* (Megachilidae). The only herbivore detected in the study site was *Aphis alstroemeriae* (Aphididae), which was found on some individuals, mainly at the base of the flowers.

Herbivore and pollinator exclusion experiments

In order to assess the combined and isolated effects of pollinators and herbivores on the female reproductive success of *A. umbellata*, 30 individual plant cushions each composed of at least one individual plant were selected at random. Inflorescences of *A. umbellata* were either exposed to natural pollination, or they were enclosed inside a tulle bag to prevent pollinator access. Plants chosen had aphids either naturally present or absent and did not differ in any other apparent way (i.e. in size, height, colour, etc). Plants without aphids were sprayed with a malathion-based systemic insecticide during the blooming and fruit-growing periods whereas aphid-infested plants were sprayed with an equivalent amount of water. Approximately the same number (n = 6) of inflorescences in each cushion were marked with cream-coloured masking tape hidden below the inflorescences at the beginning of the experiment, and were re-examined just prior to seed dispersal. These treatments were factorially combined to give four combinations of plants: 1) plants with both pollinators and aphids excluded from flowers and developing fruits; 2) plants with no aphids present but exposed to pollinators; 3) plants with aphids present and pollinators absent until seed production; and 4) plants exposed to the action of both aphids and pollinators (i.e. controls).

Because some plants were destroyed by livestock, a fully balanced design could not be obtained; thus, 256 individual flowers were excluded from both aphids and pollinators, 232 flowers were excluded from aphids but not pollinators, 171 flowers were excluded from pollinators but not aphids, and 153 flowers were exposed to both aphids and pollinators. The total number of seeds per flower in each treatment was determined at the end of the experiment. Because the seed-distribution data were right-skewed, with numerous flowers producing very few or no seeds, comparisons among treatments were made using a non-parameric two-way ANOVA (the Scheirer-Ray-Hare extension of the Kruskal-Wallis test; Sokal and Rohlf, 1998), and pairwise comparisons were made with Tukey's HSD test for unbalanced data. Analysis was made by applying the Type III SS error because this procedure is appropriate for testing hypotheses with unbalanced data and for testing main effects in the presence of interactions (Statistica software package v. 6.0).

Petal biomass at the end of the experiment was determined by severing the petals from all flowers in each inflorescence, drying them (40°C, 72 h), and weighing them.

Results

Nearly half of the plant cushions growing at the study site had inflorescences infested by aphids. The number of aphids per inflorescence was negatively correlated with total petal biomass of inflorescences (r_s= -0.441, P = 0.015, n = 30).

Seed set was 4.5-fold higher when pollinators were present in plants exposed to herbivores (Tukey HSD test, $P << 0.001$, Fig. 1). When herbivores were excluded, seed set was 4.0-fold higher in the presence of pollinators ($P << 0.001$, Fig. 1).

Aphids did not reduce seed set significantly in those plants excluded from pollinators (i.e., a direct effect) (P = 0.708, Fig. 1), but in the presence of pollinators, seed set was reduced significantly by herbivores (i.e., an indirect pollinator-mediated effect) ($P << 0.001$, Fig. 1).

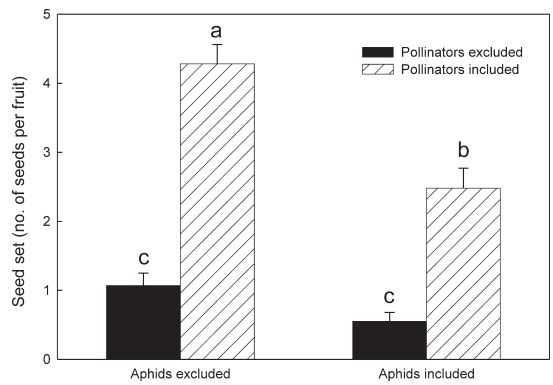

Figure 1. Effect of aphids and pollinators on the reproductive success (mean ± SE number of seed set per fruit) of the alpine plant *Alstroemeria umbellata*. Different letters above means represent significant differences (Tukey HSD test).

Table 1. Results of a non-parameric two-way ANOVA (the Scheirer-Ray-Hare extension of the Kruskal-Wallis test) testing the effects of aphids and pollinators on female reproductive success of the alpine plant *Alstroemeria umbellata*.

Source	df	SS	MS	*H*	*P*
Aphids	1	734402	734402	19.14	<0.001
Pollinators	1	4655606	4655606	121.38	<0.001
Aphids x Pollinators	1	321395	321395	8.38	<0.02
Error	808	24703150	30573		

Flowers excluded from aphids, but with pollinators present, produced significantly more seeds per fruit than flowers excluded from pollinators and with aphids present ($P << 0.001$, Fig. 1). In contrast, flowers excluded from both aphids and pollinators produced significantly fewer seeds per fruit than flowers exposed to both pollinators and aphids ($P << 0.001$, Fig. 1).

Our results show that both aphids and pollinators elicited a significant impact on the female reproductive success of *A. umbellata* (Table 1). Furthermore, there was a statistical interaction between aphids and pollinators, (i.e., a non-additive effect) (Table 1), demonstrating a modulating role for herbivores on the selection pressures exerted by pollinators on *A. umbellata*.

Discussion

Our study shows that herbivory by aphids had a negative effect on the reproductive success of the alpine plant *A. umbellata*, by lowering seed set in the presence of pollinators, but not in their absence. Self-pollination is not an uncommon phenomenon in the genus *Alstroemeria* (Souto *et al*., 2002) and explains the low level of seed set in plants with pollinators excluded. This pollinator-mediated indirect effect detected may have been the result of lower attractiveness of flowers due to a reduction in their biomass in response to aphid infestation. Thus, differences in floral attractiveness to pollinators triggered by herbivores have been shown to strongly influence female reproductive success of some hermaphroditic plants (Krupnick and Weiss, 1999; Krupnick *et al*., 1999).

Even though plants growing at high altitudes have fewer oportunities to interact with herbivores than those at low altitudes (Kelly, 1998; Susuki, 1998; Scheidel *et al*., 2003), the negative effect of aphids reported in this paper points to the importance of considering the consequences of mutualistic and antagonistic plant-animal relationships in high mountain environments. Even though the ultimate mechanisms accounting for such relationships remain to be determined, our report highlights the occurrence of a possible pathway for correlational selection on mutualistic- and antagonistic-linked traits in plants, even in high altitude environments.

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