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PLANT ANIMAL INTERACTIONS

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Does pollination limit tolerance to browsing in *Ipomopsis aggregata*?

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Abstract Ungulate browsing of flowering stalks of the semelparous herb *Ipomopsis aggregata* leads to regrowth of lateral inflorescences, a response that has been reported to yield overcompensation in some cases (browsed plants with higher reproductive success than unbrowsed), but undercompensation in others. Little is known about the mechanisms that cause such variable tolerance to herbivory. We explored one possible mechanism—variation in effects of browsing on pollination—by clipping *I. aggregata* inflorescences to mimic browsing, observing subsequent visits by pollinators and nectar-robbers, and adding pollen by hand to flowers of some clipped and unclipped plants. Clipping reduced floral display size and increased inflorescence branching, but neither hummingbirds, the primary pollinators, nor nectar-robbing bumblebees showed any preference for unclipped versus clipped plants. Clipping delayed flowering; this shift in phenology caused clipped plants to miss the peak of hummingbird activity and to have lower per-flower visitation rates than unclipped controls in one year, but to have greater overlap with birds and higher visitation rates in the subsequent year. In three sites and 2 years, clipped plants exposed to natural pollination suffered extreme undercompensation, producing on average only 16% as many seeds as unclipped controls. This was not directly attributable to clipping effects on pollination, however, because clipped plants were unable to increase fecundity when provided with supplemental pollen by hand. Taken altogether, our results suggest that compensation was constrained less by indirect effects of browsing on pollination than by its direct impacts on resource availability and hence on the ability of plants to regrow lost inflorescence tissue and to fill seeds. Exploring the physiological and developmental processes involved in regrowth of inflorescences and provisioning of seeds is a promising future direction for research designed to understand variation in browsing tolerance.

Keywords Herbivory · Flowering phenology · Overcompensation · Pollen limitation · Mechanism of compensation

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Introduction

Plants do not remain passive in the face of attack by herbivores. Instead, they respond actively in numerous ways to resist attack (resistance; Schultz 2002) and to reduce detrimental fitness effects of herbivory once it occurs (tolerance). Tolerance usually involves replacement of lost tissue through physiological responses such as increased photosynthetic rates, mobilization and reallocation of resources, and activation of dormant meristems (McNaughton 1983; Stowe et al. 2000; Tiffin 2000). Understanding mechanisms of tolerance and what constrains the extent of tolerance are critical for developing a predictive understanding of the evolutionary and ecological consequences of plant-herbivore interactions (Marquis 1992; Tiffin 2000).

The degree to which physiological responses decrease the fitness differential between damaged and undamaged plants—that is, the degree of compensation, or tolerance—varies widely from undercompensation (damaged plants less fit than undamaged plants) to overcompensation (damaged plants more fit than undamaged plants), depending on the type of herbivory, mechanism of tolerance, and ecological context (Belsky 1986; Maschinski and Whitham 1989; Whitham et al. 1991). Compensation can be affected directly by availability of the plant structures, resources, and favorable abiotic conditions needed for regrowth, but may also be affected indirectly by herbivory-mediated changes in a plant's interactions with competitors, pathogens and parasites, or pollinators and other mutualists (Maschinski and Whitham 1989; Juenger and Bergelson 1997, 1998; Strauss and Agrawal 1999).

Ipomopsis aggregata, a monocarpic perennial herb, has been a focus of work on tolerance ever since Paige and Whitham (1987a) reported that ungulate browsing of elongating flowering stalks enhances individual reproductive success. Subsequent studies of this species and its congener, *I. arizonica,* have suggested that the degree of compensation varies from overcompensation in some Arizona populations (Paige and Whitham 1987a; Paige 1994, 1999; Gronemeyer et al. 1997) to under- or equal compensation in populations ranging from Colorado to Oregon (Bergelson and Crawley 1992a, 1992b; Juenger and Bergelson 1997, 2000a; Paige 1999). Observational and experimental studies indicate that the degree to which *Ipomopsis* compensates for browsing depends on factors that directly affect regrowth, such as the timing and severity of browsing, availability of nutrients, initial plant size, temperature and length of the growing season, or the competitive environment (Maschinski and Whitham 1989; Paige 1994; Juenger and Bergelson 1997; Freeman et al. 2003).

The degree of compensation in *Ipomopsis* might also be constrained by pollination, a possibility that has not yet been explored directly. Flower and leaf damage have been shown to reduce pollinator visitation in other species (Strauss et al. 1996; Krupnick et al. 1999; Mothershead and Marquis 2000). In *Ipomopsis*, browsing might affect pollination by changing traits that affect pollinator preferences, such as plant height, floral display size, flower quality, and inflorescence architecture (Strauss 1997; Gronemeyer et al. 1997; Juenger and Bergelson 1997, 2000a; Paige et al. 2001), or by delaying flowering and hence changing the temporal overlap with pollinators (Waser 1979; Juenger and Bergelson 1997, 2000a; Santandreu and Lloret 1999).

Indirect evidence exists to suggest that pollination may indeed play a role in tolerance to browsing in *Ipomopsis.* Juenger and Bergelson (1997) found that pollen supplementation increased seed set in plants that were clipped experimentally, as well as in control plants, suggesting overall pollen limitation. An insignificant trend for clipped plants to benefit more strongly than unclipped plants from pollen supplementation further suggested that browsing may increase the degree of pollen limitation. Juenger and Bergelson (2000a) subsequently found that emasculation increased seed production in unclipped plants, but not in clipped plants. Emasculation prevents transfer of self pollen among flowers on a plant (geitonogamy), and should increase seed production in this self-incompatible species. Juenger and Bergelson (2000a) speculated that clipped plants did not benefit from emasculation because they received too few pollinator visits for geitonogamy to be important.

Virtually all studies of compensation in *Ipomopsis* have been phenomenological, in the sense that they have not explicitly addressed ecological or physiological mechanisms that determine the degree of response (Tilman 1987; Tiffin 2000). No previous study of which we are aware has directly observed effects of browsing on pollination. As a result, we lack a detailed understanding of the relative importance of pollination relative to alternative processes that may determine tolerance in this focal species. The goal of our study was to help fill this gap. We asked (1) whether browsing affects phenological overlap with and visitation rates by major pollinators and nectar-robbers; and (2) whether browsing affects the degree to which pollen limits whole-plant seed production.

Materials and methods

Study system

Ipomopsis aggregata ssp. *aggregata* is a self-incompatible, semelparous, perennial herb of montane meadow habitats in the western United States. In the vicinity of the Rocky Mountain Biological Laboratory, Colorado, USA (RMBL, 2,900 m elevation) seeds germinate soon after snowmelt or the onset of summer rains and establish a single vegetative rosette that grows for 2–6 years before sending up a single flowering stalk and dying. Multiple flowering stalks and iteroparity are rare, and are generally restricted to instances where multiple rosettes form after the primary rosette has been damaged while the plant is a vegetative rosette (Juenger and Bergelson 2000b; Waser et al. 2000; M. Price, N. Waser, A. Brody, and D. Campbell, unpublished data).

The primary pollinators of *I. aggregata* at the RMBL are broad-tailed (*Selaphorus platycercus*) and rufous (*S. rufus*) hummingbirds, although long-tongued bumblebee queens (*Bombus appositus*), solitary bees, swallowtail butterflies, and white-lined sphinx moths (*Hyles lineata*) serve as co-pollinators of this species in some years and at some sites (Waser 1978; Elam and Linhart 1988; Mayfield et al. 2001). Broad-tailed hummingbirds are breeding residents that establish territories at RMBL 4–6 weeks after snowmelt, when their first major food plant (*Delphinium nuttallianum*) begins to flower, and start migrating south in August after young have fledged, whereas rufous hummingbirds pass through on their southward migration in July and August (Waser 1976; Calder 1987, 1991; Price and Waser, unpublished data). Thus there is a midseason peak in hummingbird abundance after rufous start arriving and before broadtails migrate south.

Pollination of *I. aggregata* is affected by a number of floral characters, including inflorescence height, flower number, corolla length and width, stigma position, nectar production rate, and timing of flowering (Waser 1978; Campbell 1991; Campbell et al. 1991; Wolf and Hainsworth 1991; Mitchell 1994; Brody and Mitchell 1997). Experimental addition of pollen and/or fertilizer to plants shows that reproductive success, as measured by total seed set, is often limited both by pollen and resources (Hainsworth et al. 1985; Campbell 1991; Campbell and Halama 1993; De Jong et al. 1993; Juenger and Bergelson 1997, 2000a). Pollen supplementation can increase percent fruit set and seeds per fruit, whereas fertilizer addition leads to maturation of additional flowers (Campbell and Halama 1993; Juenger and Bergelson 1997). Production of viable seeds is reduced by a pre-dispersal seed predator, *Hylemya* sp. (Diptera, Anthomyiidae) that oviposits on elongated floral buds, as well as by lepidopteran herbivores (Brody 1992, 1997; Juenger and

Bergelson 1998; Anderson and Paige 2003). Seed set and pollen donation are also reduced by nectar-robbing bumblebees (*B. occidentalis*) because robbed flowers are avoided by hummingbirds (Irwin and Brody 1999), and perhaps also by pollen-eating flies and beetles.

Elongating flowering stalks of *I. aggregata* plants are frequently browsed by ungulates (Paige and Whitham 1987; Bergelson and Crawley 1992a, 1992b; Paige 1999). At RMBL, where deer and elk are abundant, 50–70% of stalks are browsed. Most browsing occurs early in the season, during the 5–8 week period after snowmelt when flowering stalks are bolting but flowers have not opened. Stalks are clipped to a height of 1−5 cm; stalks browsed after flowering has begun are clipped to a height of ca. 10 cm. Browsing disrupts the strong apical dominance characteristic of undamaged flowering stalks, releasing lateral meristems and producing bushy, multiple-stalked inflorescences (Paige and Whitham 1987; Maschinski and Whitham 1989; Juenger and Bergelson 1997). Because regrowth takes time, flowering of browsed plants is delayed relative to unbrowsed plants (Bergelson and Crawley 1992b; Juenger and Bergelson 1997, 2000a).

Field experiments

We investigated browsing effects on pollination and compensation with full-factorial clipping and hand-pollination field experiments in the growing seasons of 1999 and 2000. We used experimental rather than natural browsing to control for initial plant size and timing of clipping; experimentally clipped plants resemble naturally browsed plants in patterns of regrowth (Paige and Whitham 1987; Juenger and Bergelson 1997). We characterized effects of clipping on stalk height and number, total flower production, floral display size, and flowering phenology. Direct observation of visitation to focal clipped and unclipped plants allowed us to evaluate treatment effects on temporal overlap with pollinators, per-flower visitation rates, and pollinator preference. To evaluate the impacts of pollination on compensation, we assessed how pollen supplementation changed reproductive success, measured as total seed set, of clipped versus unclipped plants.

In each year we selected 30−40 single-stalked bolting plants at each of three sites. The sites, 200–600 m apart, are representative of habitats that support *Ipomopsis* populations at the RMBL, ranging from clearings in aspen forests to open dry meadows dominated by sagebrush or bunchgrass. We measured initial stalk height as an index of plant size and assigned plants to one of the factorial combinations of clipping and pollen-supplementation treatments at random with respect to size, and in such a way that treatments were not spatially segregated. ANOVA verified that initial stalk height did not vary among treatments (1999: $F_{3,\ 94}$=0.058, P=0.982; 2000: $F_{3,\ 90}$=0.034, P=0.992). In 2000 we measured root crown diameter just below rosette leaves as well. Initial stalk height was highly correlated with root crown diameter at all sites (R^2=0.31, $F_{1,\ 120}$ =54.4, P<0.001).

Plants were clipped to 1–2 cm when stalks were elongating but before buds were visible—19–22 June in 1999 (25–28 days after snowmelt at the RMBL reference site) and 11 June in 2000 (32 days after snowmelt). These clipping times were well within the period when most natural browsing takes place; stalk height at the time of clipping averaged 45 and 64% of final stalk height for 1999 and 2000, respectively, for unclipped plants. Two levels of pollination were included in both years: natural pollination (natural) and supplemental hand pollination (supplemented). Pollen was applied by hand to stigmas of female-phase flowers of supplemented plants every 2 days throughout the flowering season. Because flowers spend at least 2 days on average in female phase (Campbell 1991), virtually all flowers were supplemented under this schedule. Donor pollen was obtained by collecting freshly dehisced anthers from plants growing 3−10 m from the recipient, in an attempt to maximize the quality of outcross pollen (Waser and Price 1989). We brushed a single anther across each receptive stigma until the receptive surface of the stigma was saturated with pollen.

We counted stalk number and measured the height of the tallest stalk of each plant at the end of the flowering season. A lateral shoot was counted as a stalk if its length exceeded 3 cm and it included at least one node subtended by a lobed stem leaf (see figures in Paige and Whitham 1987; Brody and Morita 2000). The number of open flowers on each plant was counted approximately every other day throughout the flowering season to characterize floral display size and flowering phenology.

In both years visitation to 15−25 focal plants at each site was recorded for 3 h per week, 1 h in the morning (between 0830 and 1000 hours), 1 h at midday (between 1100 and 1300 hours), and 1 h in the afternoon (between 1500 and 1700 hours). The order in which sites were observed was rotated between observation days during each week, for a total of 9 h of observation per week. In 1999 we observed visitation for 6 weeks (4 July to 13 August), and in 2000 for 8 weeks (18 June to 10 August). During observations, we recorded the number of open flowers on each focal plant and noted all visits to *I. aggregata* plants within view of the observer, focal or not, recording the visitor identity, number of approaches to plants, and the number of flowers probed per approach. Visitation to focal plants was expressed as number of probes per open flower per hour of observation. Overall activity of each visitor type was expressed as the number of separate bouts of foraging per hour of observation; a bout began when a visitor entered the observation area and started foraging on *Ipomopsis* and ended when the visitor left the area. Because observers were ca. 15 m from focal plants, visitors could only be identified to the following groups: hummingbirds (female broad-tailed and rufous are difficult to distinguish), long-tongued bumblebees, nectar-robbing bumblebees, and small insects (flies and solitary bees). No swallowtail or hawk moth visits to *Ipomopsis* were seen in either year. Because it was difficult to see when small insects entered or left the observation area, we did not calculate bout frequencies for these visitors.

We collected all mature fruits of experimental plants just before they dehisced and placed them individually into envelopes. Aborted buds were counted and removed from the plants but were not included in estimates of flower production. A collected fruit was scored as an “abort” if the ovary had not expanded beyond its size in female-phase flowers; “parasitized” if it had expanded and contained a fly larva or other evidence of parasitism; and “expanded unparasitized” if there was no evidence of a parasite. Only mature, full-sized seeds were counted. We calculated the total number of seeds produced by each plant, the proportion of flowers that produced an expanded fruit, the proportion of expanded fruit parasitized, and the average number of seeds produced per expanded, unparasitized fruit.

Analyses

We used ANOVA to evaluate the potential for browsing to affect pollination, by analyzing treatment effects on flowering stalk height and number, floral display size (the maximum number of flowers simultaneously open on a plant at any time of the season), onset of flowering (characterized as the number of days from clipping until the first flower on a plant opened), and phenological overlap with pollinators and nectar-robbers. This last measure was calculated for each visitor group by summing, over weeks, the product of each week’s mean activity at a site by the proportion of each plant’s total flowering that occurred during that week. Each week’s mean activity was the average of the number of foraging bouts observed over the three observation periods per week at a site, and proportion of each plant’s total flowering was the sum of open flowers counted on these same 3 observation days divided by the sum of such “flower-days” over the entire flowering season. Thus, phenological overlap was expressed for each plant and visitor type as a per-flower average visitor activity.

We also analyzed per-flower visitation rates to evaluate the actual impacts of browsing on pollination. Total-season visitation rates per flower were calculated by averaging rates for each plant across observation days within each week, and then summing these weekly average rates, weighted by the proportion of each plant's total flowering that occurred during each week.

We used total flower and seed production, proportion aborted or parasitized fruits, and average number of seeds per expanded, unparasitized fruit to indicate effects of clipping and pollination treatments on size and reproductive success. The overall degree of pollen limitation is reflected in differences between supplemented and control plants, and effects of browsing on degree of pollen limitation are reflected in interactions between pollination and clipping treatments. If the ability of a plant to compensate for browsing is constrained by indirect effects via pollination, then the pollination by clipping interaction should be significant.

All analyses were conducted using JMP statistical software (SAS 2000). For most analyses a mixed-model ANCOVA design was used, treating site and year and their interactions as random effects, clipping and hand-pollination treatments as fixed effects, and initial stalk height or total flower number as the size covariate. Treatment effects were tested over synthetic denominator mean squares as appropriate to the model design (SAS 2000). Significance was assessed using Bonferroni correction for multiple tests as appropriate given the hypothesis being tested (Cabin and Mitchell 2000).

We transformed variables as needed to achieve homogeneity of variances and to reduce skew. Initial and final stalk heights were log-transformed. Square root transformations were applied to stalk number, flower number, maximum floral display size, total seed number, number of days to first flower, overlap with bee visitors, and weighted visitation rates. Proportion aborted and parasitized fruits were arcsine-transformed. Seeds per expanded fruit, overlap with hummingbirds, and unweighted visitation rates did not require transformation.

Results

Effects of clipping on plant size, architecture, and reproductive success

Clipping significantly changed inflorescence size, inflorescence architecture, and reproductive success in both years (Table 1). Final stalk height was shorter for clipped than unclipped plants (24.7 vs 49.2 cm overall). Inflorescences of clipped, naturally pollinated plants were more branched than unclipped controls, producing 4.83 versus 1.04 stalks on average, respectively. On average, clipped plants produced fewer than half as many total flowers as unclipped plants (36.7 vs 77.5 flowers), and a greater proportion of their flowers (0.52 vs 0.26) failed to set fruit. Clipping also more than halved the mean number of seeds produced per expanded, unparasitized fruit (1.92 vs 4.57 seeds). Clipping did not significantly affect the proportion of expanded fruits that were parasitized, although there was a slight tendency for clipped plants to have lower proportions of flowers parasitized (0.27 vs 0.31). Because they produced fewer flowers, aborted a greater fraction of them, and produced fewer seeds per fruit, clipped plants produced only 16% as many seeds on average as unclipped, naturally pollinated controls (32.9 vs 202.6 seeds).

Table 1 Effect of experimental clipping and pollen supplementation treatments on variables related to plant size (*Final stalk height*, *Total flowers produced*), inflorescence architecture (*Number of flowering stalks*), and reproductive success (*Total expanded fruits*, *Proportion of flowers aborted*, *Proportion of expanded fruits parasitized*, *Total seed number*, *Seeds per expanded, unparasitized fruit*). Top: Means across plants±1 SE. *n* number of plants, *Suppl.* supplemented. Bottom: Significant effects from univariate ANCOVAs of variables transformed as described in the text. *S* Site, *Y* Year, *C* Clipping treatment, *P* Pollination treatment, *Ht* or *Fl* plant size covariate (initial stalk height or total flower number, respectively). Effects including Site and Year were treated as random

Clipping	Pollen	*n*	Final stalk height	Stalk number	Total flowers	Total expanded fruits	Proportion aborted	Proportion parasitized	Total seeds	Seeds per expanded fruit
Means, S. E., and N of plants										
1999										
Clipped	Suppl.	18	22.81±1.57	4.89±0.47	30.11±5.26	12.94±2.77	0.607±0.044	0.200±0.046	4.11±1.59	0.367±0.112
Clipped	Natural	32	24.11±1.78	5.13±0.41	39.88±6.09	17.44±3.67	0.572±0.042	0.309±0.047	15.44±4.76	0.974±0.225
Unclipped	Suppl.	17	42.47±2.91	1.00±0.00	66.53±8.49	43.88±5.44	0.336±0.047	0.307±0.030	115.59±19.44	3.438±0.360
Unclipped	Natural	30	44.72±2.71	1.00±0.00	62.03±7.15	36.67±5.10	0.412±0.047	0.309±0.029	79.80±19.59	2.503±0.310
2000										
Clipped	Suppl.	13	27.15±1.54	4.23±0.51	33.23±9.55	25.15±8.43	0.329±0.067	0.199±0.049	57.84±16.18	4.116±0.708
Clipped	Natural	15	26.07±1.54	4.67±0.57	40.67±8.57	26.67±7.55	0.449±0.066	0.330±0.060	69.80±26.47	4.110±0.783
Unclipped	Suppl.	32	52.41±1.95	1.13±0.13	83.06±8.67	70.97±7.50	0.144±0.013	0.306±0.019	284.63±32.69	6.094±0.303
Unclipped	Natural	33	53.45±2.42	1.00±0.00	91.76±9.82	74.03±8.23	0.187±0.021	0.329±0.027	314.30±46.42	5.543±0.336
Summary of ANOVA results										
			Model***	Model***	Model***	Model***	Model***	Model**	Model***	Model***
			S×Y***	C***	C***	Y**	C***	Fl**	Y***	C***
			C***		Ht***	C***	Fl*		C***	C×Ht***
			Ht***		C×Ht***	P*			Fl***	
			C×Ht***			Fl***				
						Y×Fl**				

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

In addition to these treatment effects, the initial size of plants, indexed by their inflorescence height at the time of clipping, strongly influenced most of the measured attributes, including final stalk height, flower number, seeds per fruit, and (via effects on flower number), final total number of seeds produced (cf. size covariate effects and interactions in Table 1). Initial height also modulated the effect of clipping on final stalk height, total flowers, and seeds per fruit; these variables increased more rapidly with initial height in clipped than unclipped plants (C × Ht effects in Table 1), indicating that larger plants compensated better than small plants.

Effects of clipping on pollination

Clipping significantly affected several plant characteristics in addition to stalk number that are likely to be important for pollination (Table 2). Floral display size, indicated by the maximum number of flowers open at the same time on a plant, was reduced by about 25% in clipped versus unclipped plants on average (10.7 vs 14.3 flowers). Flowering onset also differed; clipped plants flowered later than unclipped plants (48 vs 18 days after clipping averaged over years; Fig. 1, Table 2). In each treatment, initially larger plants had larger displays and flowered earlier (Height effects, Table 2).

Activity of floral visitors varied seasonally (Fig. 1). In both years, hummingbird activity peaked in July. In 1999, the only year in which bumblebees visited *Ipomopsis*, long-tongued queens were active early in the season, and nectar-robber activity peaked in late August. Because clipping affected flowering phenology, it also affected overlap with pollinators and nectar-robbers (Fig. 1; Table 2). Clipping did not significantly affect overlap with hummingbirds overall, but there was a significant year × clipping interaction. In 1999 clipped plants had greater overlap with hummingbirds than unclipped plants, whereas the situation was reversed in 2000 (Fig. 1). In 1999, but not in 2000, pollinating and nectar-robbing bumblebees both visited *Ipomopsis*, and unclipped plants had significantly greater overlap with the long-tongued *B. appositus* pollinators at all sites, and lower overlap with robbing *B. occidentalis*, although the magnitude of the treatment effect on overlap with robbers varied significantly among sites (S × C interaction; Table 2).

Phenological overlap was reflected in per-flower rates of visitation by hummingbirds. Because of high variance among sites in hummingbird activity, there were no significant overall clipping effects; the only significant effect was a site × year × clipping interaction (Table 2, Fig. 1). In 1999, clipped plants received higher per-flower visitation rates from hummingbirds than unclipped plants; the pattern was reversed in 2000. These differences apparently stemmed from the degree of phenological overlap between flowering and hummingbird activity, since there was no difference in per-flower visitation rates ($F_{1,\ 27.7}=0.0006$, $P=0.98$) when observations were blocked by site-year-week observation periods in which clipped and unclipped plants were flowering and hummingbird visits were observed (0.0731 vs 0.0725 visits per flower per hour for clipped and unclipped plants, respectively, with site-year-week effects removed).

Visitation by bumblebees in 1999 also reflected patterns of phenological overlap. The weighted per-flower visitation rate of *B. occidentalis* nectar-robbers to clipped plants was more than 10 times greater than the rate to unclipped plants in 1999 (Table 2). This effect disappeared

Table 2 Effect of experimental clipping on floral display size (maximum number of flowers open on a plant at one time), flowering phenology (days from clipping to first flower), overlap with floral visitors, and rates of visitation by birds and nectar-robbers (visits per flower per hour). Top: Means across plants±1 SE. *n* number of plants. Bottom: Significant effects from univariate ANCOVAs of variables transformed as described in the text. *S* Site, *Y* Year, *C* Clipping treatment, *P* Pollen treatment, *Ht* plant size covariate (initial stalk height). Effects including Site and Year were treated as random

Clipping Treatment	*n*	Maximum number of flowers	Days from clipping to first flower	Bird overlap[a]	Bumblebee overlap[a]	Robber overlap[a]	Bird visits per flower per hour	Robber visits per flower per hour
Means, S. E., and N of plants								
1999								
Clipped	32	11.08±0.836	49.22±2.652	1.21±0.04	0.08±0.04	5.55±0.37	0.051±0.020	0.142±0.020
Unclipped	33	14.47±0.881	25.60±1.520	0.81±0.04	0.32±0.01	1.22±0.19	0.016±0.005	0.012±0.004
2000								
Clipped	15	9.93±1.276	45.68±1.06	1.46±0.09	n/a	n/a	0.044±0.212	n/a
Unclipped	35	14.11±0.994	13.31±0.59	2.29±0.03	n/a	n/a	0.056±0.061	n/a
Summary of ANOVA results								
		Model*** C*** S×Y×C* Ht***	Model*** Y×C*** Ht***	Model*** Y×C**	Model*** C**	Model*** S×C**	Model*** S×Y×C**	Model*** C***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

[a] Values are overlaps $\times 10^2$

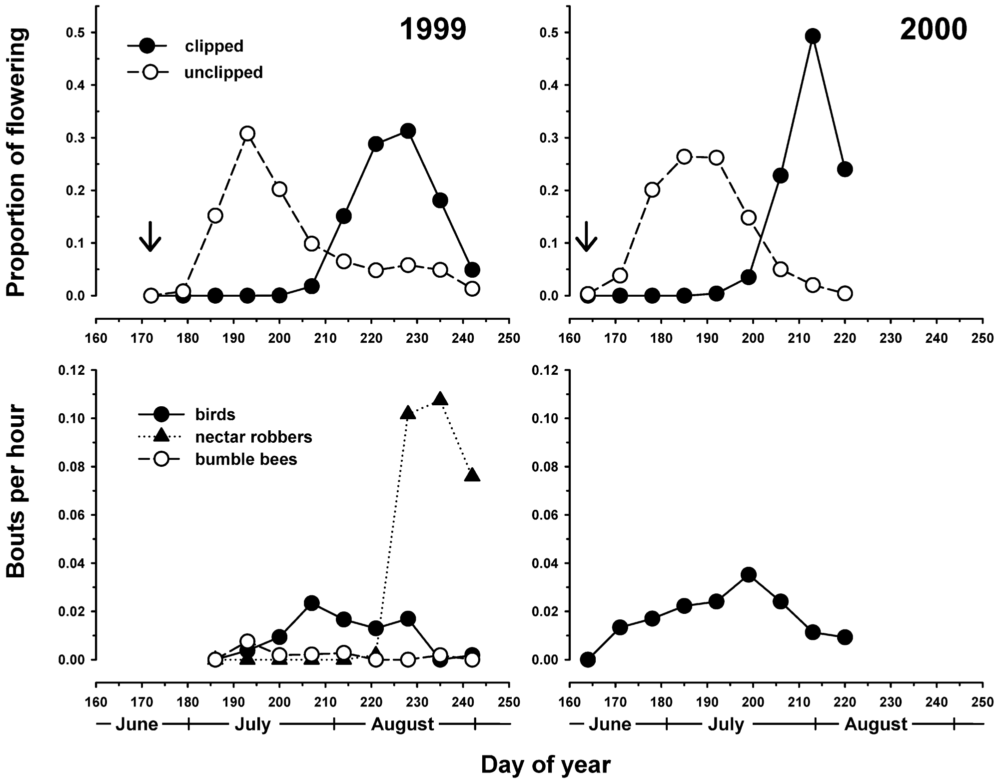

Fig. 1 Phenology of flowering of clipped and unclipped plants (*top*) and activity of pollinators and nectar-robbers (*bottom*) for 1999 (*left*) and 2000 (*right*). *Arrows* indicate clipping date. *Proportion of flowering* is the average, across plants, of the number of flowers open during a week's block of observations, divided by the sum of open flowers across the entire flowering season. *Bouts per hour* indicates the average number of times per hour visitors entered an observation area, foraged on *Ipomopsis*, and then left the area. Phenology was advanced in 2000 relative to 1999

($F_{1,\ 2.02}$=0.0, P=0.999) when observations were blocked by site-week observation periods in which both clipped and unclipped plants were flowering and nectar-robbers were observed visiting focal plants (0.224 visits per flower per hour for both clipped and unclipped plants). Visitation by *B. appositus* pollinators was too rare for statistical analysis, but all four observed visits in 1999 were to unclipped plants, which is not surprising given that these animals were active early in the season, when only unclipped plants were in flower (Fig. 1).

Pollen limitation

Effects of pollen supplementation were generally much smaller than effects of clipping. Pollen supplementation did not significantly affect plant size, onset of flowering, or visitation rates (Tables 1, 2). Although supplementation increased total seed production across both clipping treatments from 130 seeds for naturally pollinated plants to 149 seeds for supplemented plants, this difference was not significant. There was a nonsignificant trend, due to patterns in 1999, for supplementation to increase total seed production of unclipped plants (from 203 to 226 seeds), but to slightly decrease seed production of clipped plants (from 33 to 27 seeds).

Consistent with the small effects of pollen supplementation on total seed production, effects on its components (number of expanded fruits, abortion and parasitization rates, seeds per expanded, unparasitized fruit) were not significant, with the exception of a marginally significant effect on total expanded fruits (Table 1).

Discussion

Our results are consistent with previous reports that experimental clipping of *Ipomopsis* outside of Arizona generally delays flowering, increases inflorescence stalk number, reduces flower production and stalk height, and decreases seed production (Bergelson and Crawley 1992a, 1992b; Juenger and Bergelson 1997, 1998, 2000a; Paige 1999). The overall level of undercompensation we observed was dramatic: clipped, naturally pollinated plants achieved only about 16% of the lifetime female reproductive success of unclipped, naturally-pollinated controls. This level of compensation is low compared to the average of 56% (range 13–237% relative fruit production) observed in 22 comparisons of experimentally clipped versus control *Ipomopsis* in populations from Colorado to Washington (Bergelson and Crawley 1992a, 1992b; Juenger and Bergelson 1997, 1998, 2000a; Paige 1999).

The degree to which a plant compensates for browsing can be constrained by two basic processes (Juenger and Bergelson 1997, 2000a). First, degree of compensation can be determined directly through constraints on acquisition or reallocation of resources to replace lost tissue. Resource limitation can constrain the number of flowers produced, ovules per flower, or fertilized ovules that are provisioned. Second, when clipping modifies flowering phenology and plant attributes that affect attractiveness to pollinators, the degree of compensation might be determined indirectly through pollinator availability or pollinator preference, which influences pollen receipt and therefore potential seed production. Our explicit examination of pollination suggests that both direct and indirect processes occur in

Ipomopsis, but that pollen limitation places far weaker and less consistent constraints on compensation than does resource limitation.

We found that clipping significantly affected phenological overlap with pollinators and nectar-robbers, but that effects differed between years. In 1999 clipping reduced overlap with long-tongued bumblebees, which are very effective pollinators (Mayfield et al. 2001), and increased overlap with nectar-robbers, which in turn reduce visitation by hummingbirds, the most common pollinators (Irwin and Brody 1999). Clipping affected overlap with hummingbirds differently in the 2 years, increasing overlap in 1999, and decreasing it in 2000. Thus, overlap with hummingbirds averaged across our 2 years of study was not significantly different for clipped versus unclipped plants.

Differences between years in flowering phenology and overlap with pollinators reflected differences in timing of snowmelt, which determines when the growing season begins, and summer precipitation, which affects duration of flowering (Price and Waser 1998). Neither year was climatically extreme, suggesting that such variation is to be expected. In 1999, snowmelt date at RMBL's reference site was 25 May, close to the 29-year average of 22 May (range 25 April to 19 June), reflecting a winter with 92% of the 13-year average winter precipitation of 825 mm water. Summer precipitation was 132% of the 8-year average of 195 mm. In 2000, snowmelt was early (10 May), reflecting a winter with 70% of average precipitation, while summer precipitation was 184 mm, 95% of average. Earlier snowmelt caused flowering to begin earlier in 1999 than in 2000, and the wetter and cooler summer of 1999 extended the flowering season relative to 2000.

Our results further suggest that clipping effects on pollination were mediated primarily through the seasonal timing of flowering relative to pollinator or robber activity, not through effects on floral attributes that determine attractiveness to pollinators, such as inflorescence size, corolla length, width, or nectar production rate (Campbell et al. 1991). We found no evidence that flowers of clipped or unclipped plants were visited at different rates by hummingbirds or nectar-robbers during periods when both were in flower and the visitors were active.

This lack of herbivory impacts on pollinator preference in *Ipomopsis* differs from studies of other species. When flowers or buds themselves are damaged, pollinator service can be seriously impacted, as has been shown with *Isomeris arborea* (Krupnick et al. 1999; Krupnick and Weis 1999) and *Oenothera macrocarpa* (Mothershead and Marquis 2000). In other cases, such as in wild radish and *Oenothera macrocarpa*, pollinators have been shown to respond to changes in flower number and flower size that result from leaf damage (Strauss et al. 1996; Lehtila and Strauss 1997; Mothershead and Marquis 2000).

Even though clipping in *Ipomopsis* influenced visitation patterns, we found little evidence that tolerance was higher when pollen was less limiting. In 1999, clipped plants should have been less pollen limited than unclipped plants by virtue of greater phenological match with hummingbirds and higher hummingbird visitation rates; visitation rates to clipped plants in 1999 approached the highest values observed between 1996 and 2000 (Mayfield et al. 2001). Yet, undercompensation of clipped plants was more extreme in 1999 (ca. 10% of the fecundity of unclipped plants) than in 2000 (ca. 21%), even though pollen should have been more limiting to clipped plants in 2000 than 1999. In both years, pollen supplementation should have reduced pollen limitation. Yet, in both 1999 and 2000, pollen-supplemented clipped plants achieved if anything lower, not higher, reproductive success relative to unsupplemented controls (4% and 20% for 1999 and 2000, respectively) than did clipped plants that were naturally pollinated (19 and 22%). The apparent slight detrimental impact of supplemental pollination on clipped plants was not due to damage inflicted during hand pollination, because the same treatments tended to increase seed production in unclipped plants. Our observation of strong undercompensation in pollen-supplemented clipped plants, and in 2 years that differed considerably in expected relative pollen limitation of clipped and unclipped plants, suggests that compensation for browsing in *Ipomopsis* is limited more strongly and consistently at the RMBL by capacity for regrowth than by pollination.

If seed production is jointly limited by pollen and resources, as Campbell and Halama (1993) have cogently argued, then variation in either pollination or in growth conditions can be expected to influence observed levels of compensation, and one expects their relative importance to vary temporally and spatially. Results to date from studies of compensation or seed production in *Ipomopsis* under experimentally manipulated pollen or resource environments indeed have yielded variable results. Several studies have found stronger resource than pollen limitation. In the absence of pollen supplementation, *Ipomopsis arizonica* could compensate completely for clipping when fertilizer was added (Maschinski and Whitham 1989), suggesting that compensation was entirely determined by acquisition of nutrients needed for regrowth. Similarly, Campbell and Halama (1993) observed that fertilizer addition alone increased seed production of unclipped *I. aggregata* much more than did pollen supplementation alone. Juenger and Bergelson (2000a) found that emasculation, which reduces self-pollination, increased fitness of unclipped plants but not of clipped plants, perhaps because clipped plants lacked resources to fill additional seeds. Juenger and Bergelson (1997), however, found a trend for clipped plants to compensate more completely with than without pollen supplementation (45% vs 26% the seed production of unclipped plants), although the pollen × clipping interaction was not statistically significant. They also found that fertilizer addition had no effect on compensation.

Because in a semelparous plant like *Ipomopsis* the loss of the bolting inflorescence means loss of resources stored over several years, few environments are likely to permit plants to capture new resources fast enough to compensate completely during their single flowering season. Hence it is not surprising that the growth environment can place a primary constraint, and pollination a secondary and variable constraint, on compensation. Pollination would be relatively more important if plants withheld stored resources from the initial flowering attempt, leaving some behind for regrowth after browsing occurs. Such bet-hedging is predicted under conditions of high herbivory by some theoretical models (Vail 1992; Stowe et al. 2000), and may explain patterns of overcompensation in the field gentian (Lennartsson et al. 1997). Why *Ipomopsis* in Colorado appears not to hold back resources, whereas the field gentian does in mown fields, is an interesting question. Perhaps the value of bet-hedging is reduced by repeat herbivory, which is likely to be more intense as more resources are allocated to regrowth and the resulting tissues become more attractive to herbivores. Ungulates do browse the lateral inflorescences produced by clipped *Ipomopsis* plants, but such late-season browsing is less intense than early-season browsing. It is also possible that ungulate densities, and browsing rates, are much higher now than they were before large predators were exterminated, and there has been too little time for evolution of characters involved in browsing tolerance. Deer and elk densities near the RMBL certainly seem to have increased dramatically over the past 30 years (personal observation). Finally, net selection on tolerance characters may involve herbivores other than ungulates (Juenger and Bergelson 1998; Anderson and Paige 2003).

Understanding mechanisms is an important strategy for reaching generalizations in evolutionary ecology, because mechanisms often provide the key to causal factors that underlie otherwise inexplicable variation. A close examination of pollination leads us to suggest that tolerance to browsing in *Ipomopsis* is likely to be constrained more consistently by the ability of browsed plants to produce flowers and ovules and to provision seeds, than by the quantity or quality of pollination those flowers receive. To improve understanding of the variable expression of tolerance, therefore, we need to turn our attention toward the physiological and developmental processes involved in growth and regrowth of inflorescences and seed provisioning, about which little is known.

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