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PLASTIC GROWTH RESPONSES TO SIMULATED HERBIVORY

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Plants rely on phenotypic plasticity to maintain performance and survive in changing environments. Stress such as herbivory has the potential to cause short- and long-term changes to a plant's phenotype. In response to simulated herbivory by clipping, plants either may show lower growth and fecundity or may overcompensate and exceed the size and fecundity of undamaged individuals. This study examined the short- and long-term responses to the timing and frequency of clipping in *Barbarea vulgaris*. In the greenhouse, clipping resulted in elevated growth rates, and individuals eventually matched the size of undamaged individuals. In the field, clipping had negligible effects on size or fitness components. Aboveground clipping also reduced the belowground biomass. One possible explanation for the size convergence and equal fecundity despite clipping 50% of the leaves is that the belowground biomass supports the regrowth of the aboveground biomass. Stored belowground resources allowed plants to regenerate lost photosynthetic tissue. These results illustrate the ability of *B. vulgaris* to tolerate clipping. Regardless of the timing or frequency of clipping, plants that experienced biomass removal displayed remarkable plasticity and were able to maintain performance relative to undamaged plants.

Keywords: absolute growth rate, relative growth rate, simulated herbivory, belowground biomass, plasticity, *Barbarea vulgaris*.

Introduction

Phenotypic plasticity can play an important role for plants when they experience variable or heterogeneous environments (Bradshaw 1965; Sultan 1992). One type of variable stress that plants frequently experience is herbivory. There is a long history of studies of the interactions between plants and their herbivores (Ehrlich and Raven 1964), and it is now clear that there is a range of plastic responses to damage. Plants may tolerate and be able to compensate for herbivory (Painter 1958) such that there is no long-term reduction in individual fitness (e.g., Paige and Whitham 1987). Alternatively, plants may evolve defensive resistance traits to reduce levels of herbivory (Karban and Myers 1989; Agrawal et al. 1999; Hochwender et al. 2000).

Research on herbivory initially focused on plant growth and development (McNaughton 1983; Belsky 1986), but studies have also investigated the defensive mechanisms plants use to tolerate or resist herbivory (Karban and Myers 1989; Agrawal et al. 1999). Despite being studied extensively, there is no consensus on how plants are expected to respond after biomass removal (Belsky 1986; Simons and Johnston 1999; Huhta et al. 2000; Hawkes and Sullivan 2001). Many plants fail to recover after damage and are smaller or produce fewer offspring than undamaged individuals (Nascimento and Hay 1994; Bergelson and Juenger 1996; Stowe 1998; Hanley and Fegan 2007; Klimesova et al. 2007). However, herbivory may also be a positive stress in the sense that it may stimulate plant growth, longevity, or reproduction (McNaughton 1983; Belsky 1986). A full range of responses has been observed, from full compensation, where damaged and undamaged plants have the same size or fitness, to overcompensation, where damaged individuals exceed the size or fecundity of undamaged individuals (Paige and Whitham 1987; Lennartsson et al. 1998; Hochwender et al. 2000; Hawkes and Sullivan 2001). The degree of recovery can depend on resource levels (Hochwender et al. 2000; Hawkes and Sullivan 2001), the type or concentration of damage (Marquis 1992; Mauricio et al. 1993; Gavloski and Lamb 2000), or the particular population or species (Bilbrough and Richards 1993; Lennartsson et al. 1997). An assessment of growth compensation after the loss of biomass is further complicated by the fact that plants allocate resources to both above- and belowground tissues (McConnaughay and Coleman 1999; Hochwender et al. 2000), and changes in aboveground growth patterns can affect belowground biomass (Oesterheld 1992). Moreover, it is not clear whether these short-term changes in growth rate have survival or fecundity costs associated with them.

One way to study response to herbivores in a controlled setting is to simulate herbivory by clipping biomass (Bergelson and Juenger 1996). Although clipping may underestimate the effect of herbivory because it should not induce as many secondary chemical defenses as natural herbivory, it does allow for the study of the direct effect of biomass removal (Baldwin 1990). This study will address the following three questions: (1) Do responses to biomass removal vary across time, and to what extent does repeated biomass removal influence growth rates? (2) Does aboveground biomass removal

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influence belowground biomass? (3) Does biomass removal during the vegetative stage have consequences during the reproductive stage? Given the life history plasticity of our study species, we predict that it will show a high level of tolerance to these biomass removal manipulations.

Material and Methods

Species Description

Barbarea vulgaris (Brassicaceae) is an exotic herbaceous plant that is likely Mediterranean in origin, but it currently has a cosmopolitan distribution (MacDonald and Cavers 1991). Given ample moisture and light exposure, germination can occur year-round (Schreiber 1962; Baskin and Baskin 1989). *Barbarea vulgaris* blooms in the summer and can exhibit a variety of life history strategies: a winter annual, a biennial, and both a monocarpic and a polycarpic perennial (Lindsay and Bassett 1951; Schreiber 1962; MacDonald and Cavers 1991). Plants overwinter as rosettes and must achieve a size threshold and be vernalized to transition to a reproductive stage, where reproduction is primarily outcrossing (Lindsay and Bassett 1951; Schreiber 1962; MacDonald 1977; MacDonald and Cavers 1991). In Virginia, *B. vulgaris* is likely a polycarpic perennial (E. M. Yoshizuka, personal observation) and can be found in recently disturbed habitats (such as roadsides) as well as in gaps and margins of woodlands.

Experiment 1: Repeated Biomass Removal

This experiment was conducted to assess the effect of the timing and frequency of biomass removal on plant size and growth. Seeds collected from the field were planted on the soil surface and placed under a mist bench in a greenhouse for 2 wk. Although this study did not focus on the genetic basis of herbivory tolerance, it was desirable to account for genetic effects. To this end, seeds from nine different individuals were collected along a roadside <1 mi from Mountain Lake Biological Station, Giles County, Virginia. A total of 460 plants were used in this experiment, with relatively equal distribution between the families (~50 individuals). Plants were grown in a 1 : 1 combination of Metro-Mix (Sun Gro Horticulture, Bellevue, WA) and Pro-Mix (Premiere Horticulture, Quakertown, PA) in 4-in pots and were watered regularly but not fertilized. A total of 115 individuals were randomly assigned to each of the four biomass removal treatment groups: control, early, late, or both early and late (hereafter, "both"). Plants in the early and late treatment groups were each defoliated once, with early plants being defoliated when they were 3 mo old and late plants being defoliated when they were 4 mo old, and plants that received both treatments were defoliated at each of these time points.

Before biomass removal, leaf number was recorded, and there were no differences in size across the treatment groups. In a preliminary study, rosettes were destructively sampled, and it was found that leaf number was a strong indicator of biomass (ln (biomass) $= -2.14 + 0.16 \times$ leaf number, $R^2 = 0.75$, $P < 0.0001$, $n = 35$). Because this study was concerned with the effect of biomass removal, it was decided that removing the most valuable leaves should produce the most pronounced effect. Because removing younger leaves is more detrimental than removing older leaves (Barto and Cipollini 2005), all biomass removal treatments completely removed 50% of the leaf number, starting with the youngest leaves. In month 3, 50% of the leaves were removed for individuals in the early treatment group and the group that received both treatments. Four weeks later, in month 4, size was measured again, and 50% leaf removal was applied for individuals in the late treatment group and the group that received both treatments. The timing of the second round of leaf removal was done so that plants that received both treatments would have time to partially recover but would not have time to completely compensate for lost biomass. Leaf number for all plants was recorded when plants were 5, 7, and 8 mo old.

There are a number of different ways to calculate and measure growth (McGraw and Garbutt 1990). Absolute growth rate (AGR) is the actual change in biomass over a time interval. Relative growth rate (RGR) is the proportional change in size over an interval. AGR can be biased if individuals add biomass in a size-dependent manner. In this case, RGR can be used to compare growth between individuals of different sizes. AGR and RGR measure different aspects of growth, and one measurement cannot be used to infer the other. All experiments used both measures to evaluate growth after biomass removal.

A repeated-measures analysis (Proc Mixed, SAS 9.1; SAS Institute, Cary, NC) was conducted to detect differences in the overall growth trajectory between treatments with family as a random factor. Individual one-way ANOVAs (Proc Mixed, SAS 9.1) were used to evaluate differences between treatments in growth rates and size for each month. Four monthly growth rates (months 3–4, months 4–5, months 5–7, and months 7–8) were calculated, and for each individual both the AGR and the RGR were calculated for every interval. For the size analyses, size measurements were natural-log transformed to meet the assumptions of normality for ANOVA. In each analysis, treatment was considered a fixed factor. To assess whether growth responses after biomass removal were age dependent, growth rates 1 mo after biomass removal were compared for early treatment, late treatment, and both treatments.

Experiment 2: Carryover Effects of Biomass Removal

This experiment was conducted to determine whether responses to biomass removal depend on an individual's history of biomass removal. Individuals from experiment 1 were assigned to receive either control or removal treatments (fig. 1). In other words, individuals in the removal treatment group in experiment 2 either were naive to biomass removal (if previously in the control group) or had experienced biomass removal once (if previously in the early or late treatment group) or twice (if previously in the group that received both treatments). When plants were 8 mo old, plant size was measured, and there were no differences in size across treatment groups. Individuals in the removal group had 50% of their leaves removed. Leaf number was then recorded when plants were 9 and 10 mo old.

A repeated-measures analysis was used to detect differences in growth rates over time with family as a random factor.

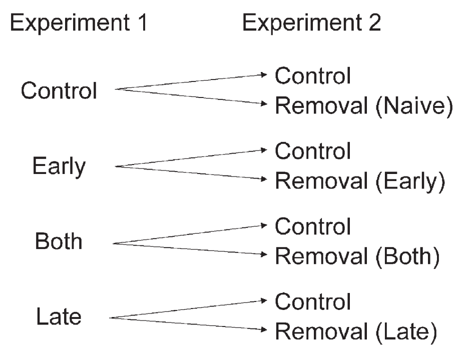

Fig. 1 Design for experiments 1 and 2. Each individual in experiment 1 was assigned to a new treatment group in experiment 2. For experiment 2, individuals in the removal treatment group are shown with their previous exposure to biomass removal in parentheses.

One-way ANOVAs were then used to assess differences between treatments within each time interval. AGR and RGR were calculated for two intervals (months 8–9 and months 9–10). Size measurements were natural-log transformed to meet the assumptions of normality for ANOVA. In experiment 2, it was of particular interest to determine whether plants that had previously experienced biomass removal in experiment 1 would respond to biomass removal to the same degree as plants that were naive to biomass removal. Given this objective, the means for plants that were naive to biomass removal were compared with the means for all other plants that received removal treatment (early, both, and late).

Experiment 3: Belowground Responses to Aboveground Biomass Removal

This experiment was conducted to determine whether aboveground biomass removal influences belowground biomass. Plants were grown in the same manner as in experiment 1. Seeds were germinated from a haphazard selection of field-collected maternal families. Individuals were randomly assigned to receive either control or removal treatment; there were 45 individuals in each treatment group. When the plants were 3 mo old, the number of leaves was counted for each individual, and there were no differences in size across treatment groups. Then, 50% of the leaves were removed for individuals in the removal group. Four weeks later, when the plants were 4 mo old, size was measured again, and all individuals were harvested. The aboveground biomass was clipped at the base of each rosette, and the belowground biomass was obtained by washing soil from the roots of each plant. All biomass was dried at 60°C for a week until it had completely dried, at which time it was weighed.

To assess the effect of aboveground removal on belowground biomass, a one-way ANOVA was used (Proc Mixed, SAS 9.1) with treatment as a fixed factor. AGR and RGR were calculated for one interval (months 3–4). To meet the assumptions of normality for ANOVA, both the aboveground and belowground biomass weights were natural-log transformed, and the proportion of biomass allocated to either above- or belowground tissue was arcsine-square-root transformed.

Experiment 4: Consequences of Vegetative Biomass Removal for Reproduction

A fourth experiment was conducted to determine whether biomass removal during the vegetative stage influenced reproduction under field conditions. Field-collected seeds were germinated and grown with the same methods as in experiment 1. Six weeks after germination, plants were vernalized for 8 wk in a cold room held at 4°C. After vernalization, in April, plants were randomly assigned to receive either control ($n = 160$) or removal ($n = 170$) treatment and planted in a randomized block design at a forest edge near Mountain Lake Biological Station.

In May, when the plants were 6 mo old, the leaf number for each individual was recorded, and there were no differences in size across treatment groups before manipulation. Then, 50% of the leaves were removed from individuals in the removal treatment group. Size and life history traits were recorded throughout the season. During June and July, the day on which plants started to flower was recorded. In June, stem width was measured 5 cm from the soil surface as an estimate of aboveground biomass. In a preliminary experiment, it was found that stem width was an excellent predictor of aboveground biomass (aboveground biomass $= -3.19 + 0.89 \times$ stem width, $R^2 = 0.90$, $P < 0.0001$, $n = 20$). In July, the number of branches was counted as measure of architecture, and the total length (height) of each individual was recorded as an estimate of final size. In August, individual fruits were counted and collected from plants as they matured. Several fruits (3–4) were subsampled from 10 different individuals in each treatment group, and these fruits were used to estimate the number of seeds per fruit and the seed mass for each treatment.

A MANOVA (Proc GLM, SAS 9.1) was initially performed to detect differences in overall phenotype between the different treatments. The result of the MANOVA was significant ($F = 2.35$, Wilks's $\lambda = 0.041$, df $= 5, 310$), so stem width, branch number, total length, time to flowering, and fruit number were each examined separately using one-way ANOVAs (Proc Mixed, SAS 9.1). Branch number and fruit number were square-root transformed and stem width and time to flower were natural-log transformed to meet the assumptions of normality for ANOVA. Block was considered a random variable, whereas treatment was considered a fixed variable.

Results

Experiment 1: Repeated Biomass Removal

Repeated-measures analysis revealed that over the course of the experiment there was a month-by-treatment interaction for size ($F = 65.90$, df $= 9, 445$, $P < 0.0001$), RGR ($F = 43.86$, df $= 9, 455$, $P < 0.0001$), and AGR ($F = 19.95$, df $= 9, 455$, $P < 0.0001$). Family significantly influenced size ($z = 1.97$, $P = 0.024$) and AGR ($z = 1.95$, $P = 0.026$) but not RGR ($z = 1.57$, $P = 0.059$). At the beginning of month 4, 1 mo after early biomass removal, plants that had received early treatment and both treatments had an elevated RGR ($F = 105.87$, df $= 3, 443$, $P < 0.0001$) and AGR ($F = 70.01$, df $= 3, 443$, $P < 0.0001$) relative to the control plants and the plants that received late treatment. At the beginning of month 5, 1 mo after late biomass removal, RGR and AGR differed between all treatment groups. Plants that received both treatments had the highest RGR, followed by plants in the late, early, and control groups, respectively ($F = 100.67$, df $= 3, 444$, $P < 0.0001$; fig. 2*A*). There were no differences in AGR between plants that received both treatments and those that received late treatment for months 4–5; however, plants that received both treatments and late treatment produced significantly more leaves than plants in the early treatment and control groups ($F = 52.35$, df $= 3, 445$, $P < 0.0001$; fig. 3). Plants differed in size in month 5, with control plants being the largest, followed by those that received early treatment, late treatment, and both treatments ($F = 24.33$, df $= 3, 445$, $P < 0.0001$; fig. 3).

Between months 5 and 7, plants that received both treatments had the highest RGR, followed by those that received late treatment, those that received early treatment, and finally the control plants ($F = 16.72$, df $= 3, 439$, $P < 0.0001$; fig. 2*B*). During this time, plants that received both treatments, early treatment, and late treatment produced the same number of leaves, but control plants produced fewer ($F = 6.77$, df $= 3, 439$, $P = 0.0002$; fig. 3). In month 7, control plants and plants that had received early or late treatment were of equal size and were larger than plants that had received both treatments ($F = 5.08$, df $= 3, 439$, $P < 0.0001$; fig. 3).

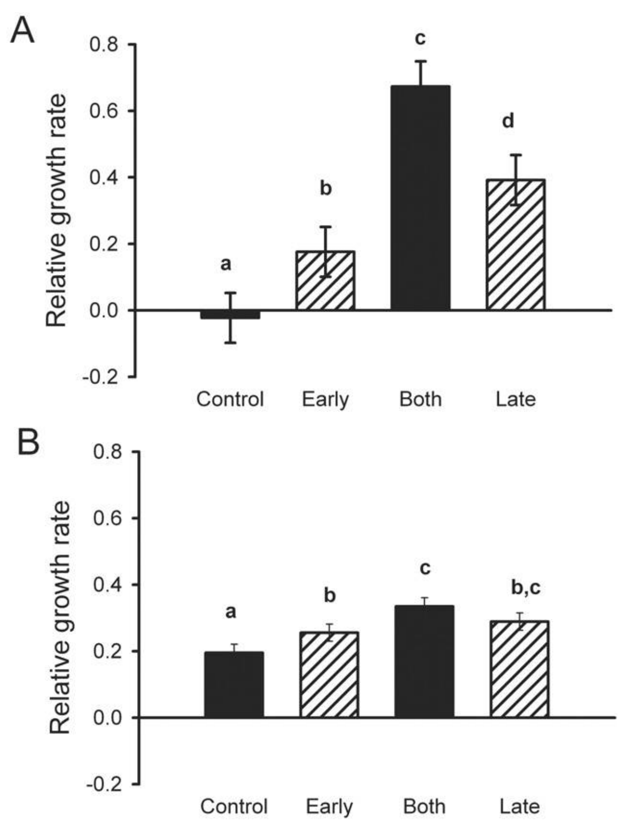

Fig. 2 Relative growth rates (mean ± 1 SE) for experiment 1. *A*, Relative growth rate for months 4–5, 1 mo after late biomass removal; *B*, relative growth rate for months 5–7, 3 mo after late biomass removal. Multiple comparisons were tested using a post hoc Tukey test; letters denote significant differences in means.

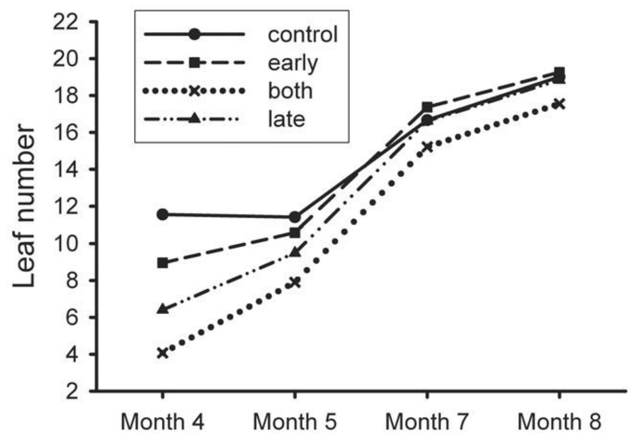

Fig. 3 Size and growth trajectories for experiment 1, after late biomass removal. Absolute growth rates can be observed as the slope between intervals.

Between months 7 and 8, RGR was equal across treatments except for the comparison between early treatment and both treatments, for which plants in the early treatment group had the lowest RGR ($F = 3.34$, df $= 3, 392$, $P = 0.0194$). Treatments did not differ with respect to AGR ($F = 1.48$, df $= 3, 392$, $P = 0.218$; fig. 3). Similar to the pattern seen in month 7, control plants and plants that received early or late treatment were equal in size and were larger than plants that received both treatments ($F = 4.07$, df $= 3, 401$, $P = 0.0072$; fig. 3).

With respect to the timing of biomass removal, AGR and RGR did not differ between early treatment and late treatment (fig. 4*A*, 4*B*). For months 3–4, plants that received both treatments did not differ from those that received early or late treatment. For months 4–5, plants that received both treatments had an elevated AGR and RGR relative to those that received early treatment or both treatments in months 3–4 (fig. 4*A*, 4*B*). However, for months 4–5, plants that received both treatments and those that received late treatment had an equal AGR (Tukey test, $t = 2.26$, $P = 0.11$).

Experiment 2: Carryover Effects of Biomass Removal

Over the course of this experiment, size depended on month ($F = 67.30$, df $= 1, 842$, $P < 0.0001$) and treatment ($F = 22.42$, df $= 4, 842$, $P < 0.0001$). There was a significant interaction between month and treatment ($F = 3.64$, df $= 4, 842$, $P = 0.006$). Families also differed in size ($z = 1.83$, $P = 0.033$). AGR and RGR differed between the two time intervals (AGR: $F = 76.35$, df $= 1, 838$, $P < 0.0001$; RGR: $F = 65.68$, df $= 1, 838$, $P < 0.0001$) but did not vary between treatments. The month-by-treatment interaction was significant for AGR ($F = 5.39$, df $= 4, 838$, $P < 0.0001$) and RGR ($F = 6.93$, df $= 4, 838$, $P < 0.0001$). Families did not differ with respect to AGR ($z = 1.55$, $P = 0.068$) or RGR ($z = 1.45$, $P = 0.074$).

Plants differed in RGR 1 mo after biomass removal (months 8–9: $F = 25.89$, df $= 4, 422$, $P < 0.0001$; fig. 5*A*). In the planned comparison, plants that experienced biomass removal for the first time (naive) had the lowest RGR, com-

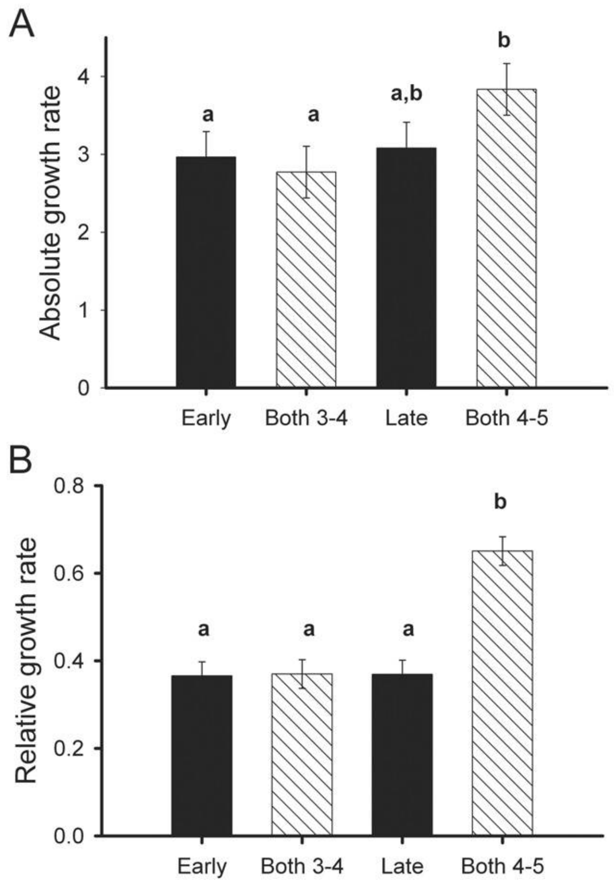

Fig. 4 Growth rates (mean ± 1 SE) 1 mo after biomass removal in experiment 1. Shown are (*A*) absolute and (*B*) relative growth rates. All pairwise comparisons were done using a post hoc Tukey test; letters denote significant differences in means.

pared with plants that received all other removal treatments ($F = 7.84$, df = 1, 1422, $P = 0.0053$). AGR also varied between treatments ($F = 1.60$, df = 3, 168, $P = 0.1907$; fig. 5*B*), and there were differences in the a priori comparison between the plants that were naive to biomass removal with those that had experienced removal ($F = 4.25$, df = 1, 405, $P = 0.0398$). Between months 9 and 10, treatments differed with respect to RGR ($F = 3.3$, df = 4, 386, $P = 0.0112$) and AGR ($F = 6.36$, df = 4, 386, $P < 0.0001$), but the a priori comparisons were not significant ($P > 0.05$). Across all treatments, biomass declined from months 9 to 10 (fig. 5*B*), possibly because of the shorter days in winter.

Experiment 3: Belowground Responses to Aboveground Biomass Removal

One month after biomass removal, treated plants had a higher RGR and produced more leaves than unmanipulated plants (table 1). Plants that experienced biomass removal had lower aboveground biomass and belowground biomass than control plants (table 1). Given this difference in above- and belowground biomass, the percentage of biomass allocated above- and belowground was examined, and the analysis showed that belowground biomass made up a larger proportion of total biomass in plants that experienced biomass removal than in control plants (table 1).

Experiment 4: Consequences of Vegetative Biomass Removal for Reproduction

In the field, removing 50% of the leaves did not alter stem width, a proxy for aboveground biomass, but did influence total plant length (height; table 2). At the end of the season, plants that experienced biomass removal were significantly shorter than control plants (table 2), and biomass removal had no effect on the number of branches (table 2). There was a significant family effect for branch number, flowering time, and fruit number (table 2).

Although biomass removal influenced plant length, flowering time and fecundity did not differ between treatments (ta-

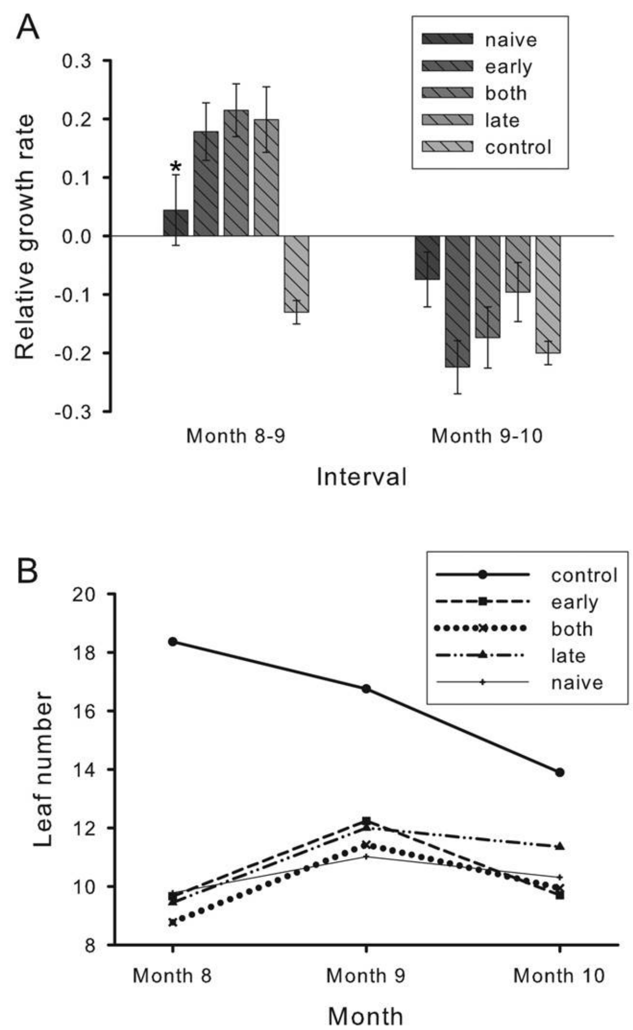

Fig. 5 Results from experiment 2: carryover effects of biomass removal. Shown are (*A*) relative growth rates (mean ± 1 SE; one asterisk = $P = 0.03$ for the a priori comparison) and (*B*) size and growth trajectories for both the control and removal treatments.

Table 1

Experiment 3 Trait Means (±1 SE)

Trait	Treatment		df	*F* statistic
	Control	Removal		
Relative growth rate	.085 ± .023	.46 ± .028	1, 87	108.04***
Absolute growth rate (leaf no.)	.80 ± .180	2.71 ± .180	1, 87	53.00***
Aboveground biomass (g)	.56 ± .027	.36 ± .016	1, 87	44.19***
Belowground biomass (g)	.77 ± .027	.69 ± .021	1, 87	5.38*
Belowground biomass (%)	58.42 ± .007	66.16 ± .008	1, 87	48.13***

Note. For each treatment, $n = 45$.
* $P < 0.05$.
*** $P < 0.001$.

ble 2). In a subsample of 30 fruits from each treatment, the number of seeds per fruit did not differ significantly between control plants and plants that experienced biomass removal (11.77 ± 0.50 and 11.15 ± 0.49 seeds per fruit, respectively; $F = 0.78$, df $= 1, 62$, $P = 0.379$). Seed masses also did not differ between treatments (0.366 ± 0.01 and 0.367 ± 0.01 g for control and removal treatment, respectively; $F = 0.00$, df $= 1, 62$, $P = 0.975$).

Discussion

Taken together, these experiments illustrate the remarkable ability of plants to persist and perform despite removal of 50% of the leaves. In the greenhouse, individuals that experienced biomass removal had an elevated AGR relative to control plants; this pattern was present across experiments 1, 2, and 3, suggesting that the repeatability of this result is strong. The constant AGR in plants after biomass removal was most striking when also considering RGR. In experiment 1, between months 4 and 5 plants that received both treatments and those that received late treatment had an equal AGR, but those that received both treatments had the highest RGR. These plants were small because they had experienced biomass removal twice but were still able to produce the same number of leaves as plants that experienced biomass removal only once. By the end of experiment 1, plants in the early treatment group and the late treatment group had compensated for the lost biomass and were the same size as the unmanipulated control plants, suggesting that there were no long-term consequences of removing 50% of the leaves. In experiment 2, plants had equivalent AGRs regardless of whether it was the first, second, or third time they had experienced biomass removal, suggesting that growth responses are independent of biomass removal history.

In the field, biomass removal during the vegetative stage produced plants that were smaller at the end of the season but did not result in differential fruit production. Although somewhat counterintuitive, plant damage does not always significantly influence fecundity (Nascimento and Hay 1994; Stowe 1998; Mauricio 2000; Rooney and Waller 2001). In this experiment, the loss of biomass during the vegetative stage could be mitigated by the fact that the plants bolted and flowered shortly after defoliation, at which time the rosettes senesced. When 100% of the aboveground biomass is removed, *Barbarea vulgaris* is able to resprout and perform relatively well the following year, further attesting to the species' ability to tolerate biomass removal (Martinkova et al. 2008). The results reported here extend these findings and show that removing 50% of the leaves during the vegetative stage does not significantly influence fecundity within the same growing season.

Elevated AGR (and, in turn, RGR) after biomass removal allowed plants from all treatment groups to eventually converge toward a common size. One reason for the stable AGR after biomass removal could be the fact that *B. vulgaris* is quite weedy; it establishes itself and grows quickly and has the ability to recover even after severe bouts of aboveground damage (Lindsay and Bassett 1951; MacDonald and Cavers 1991; Klimesova et al. 2008; Martinkova et al. 2008). The constant rate of biomass accumulation is somewhat surprising given the size differences between treatments, but damage can result

Table 2

Experiment 4 in the Field Trait Means (±1 SE)

Trait	Treatment				Family, *F* statistic
	Control	Removal	df	*F* statistic	
June stem width (cm)	2.00 ± .03	1.97 ± .03	1, 319	.35	1.14
Branch no.	1.74 ± .09	1.87 ± .10	1, 315	.56	1.70*
Total length (cm)	48.04 ± .68	46.00 ± .59	1, 299	10.40*	1.64
Time to flower (days)	56.09 ± .33	55.36 ± .32	1, 317	.00	1.89*
Fruit no. per plant	37.95 ± 1.89	34.95 ± 1.60	1, 310	1.59	1.75*

Note. For control, $n = 160$; for removal, $n = 170$.
* $P < 0.05$.

in elevated photosynthesis rates, which may contribute to biomass accumulation (Morrison and Reekie 1995). Another possible reason for the stable AGR is that regrowth after biomass removal might be supplemented by the belowground biomass.

Belowground biomass was lower in treated plants that had aboveground biomass removed (experiment 3). Removal of 50% of the leaves reduced the belowground biomass by 11%. This reduction in belowground biomass could be the result of root death or a decrease in belowground biomass growth (Oesterheld 1992), both of which could be caused by the removal of aboveground photosynthetic tissue. A decrease in belowground biomass could be the result of a shift in biomass allocation, where newly acquired resources are shunted toward regenerating the aboveground biomass instead of producing new roots. Decreases in belowground biomass could also occur if stored resources are being used to supplement the regrowth of the aboveground tissues. Moreover, across both treatments belowground biomass made up a greater proportion of the total biomass than the aboveground biomass. This difference in allocation suggests that there are potentially large amounts of stored nutrients belowground that can be used to support the growth and maintenance of the aboveground biomass. Alternatively, it is possible that AGR or size could be limited under greenhouse conditions. However, we found that the range of size of both above- and belowground biomass was very large for both manipulated and unmanipulated plants (manipulated: 0.168–0.657 g for aboveground and 0.456–0.990 g for belowground; unmanipulated: 0.199–1.039 g for aboveground and 0.497–1.314 g for belowground). This large variation in size within treatments suggests that this phenomenon is not an artifact of the greenhouse and that pot size was not the primary factor limiting the size of individual plants.

There are numerous consequences of biomass removal that can potentially influence *B. vulgaris* phenotype, growth, survival, and fecundity that were not investigated in this study. For example, the composition of chemical defenses in *B. vulgaris* are known to vary greatly between populations (Agerbirk et al. 2001, 2003; van Leur et al. 2006, 2008*a*, 2008*b*). Moreover, these chemical defenses vary through ontogeny (Agerbirk et al. 2001) and could play a role in reducing biomass loss in the field. Studies of other Brassicaceae have found that clipping and biomass removal from herbivores can have sex-specific fitness consequences (Agrawal et al. 1999) and induce secondary chemicals and trichomes (Traw and Dawson 2002). Studies of other plant species have shown that repeated aboveground biomass removal can interact with the belowground biotic community to produce complex patterns of diversity in fungi and other organisms (Mikola et al. 2005) or alter herbivore behavior (Strengbom et al. 2003). Last, it has been suggested that stress in plants may result in epigenetic changes (Bruce et al. 2007; Boyko and Kovalchuk 2008; Whittle et al. 2009). Epigenetic changes could represent a mechanism that allows plants to alter their phenotype for long periods of time.

This study applied damage in the middle of life, after individuals had established and passed the seedling stage but before they reproduced. The timing of biomass removal relative to life history is important because the timing of damage has the potential to affect a variety of fitness components (Tiffin 2002; Marshall et al. 2005). Plants have a number of ways of tolerating herbivory (Tiffin 2000), but these mechanisms may vary across ontogeny (Bowers and Stamp 1993; Boege and Marquis 2005). Previous theoretical and empirical studies suggest that early (Tiffin 2002; Hanley and Fegan 2007; Barton 2008) and late (Tiffin 2002; Marshall et al. 2005) life stages should be susceptible to damage but that middle life stages are relatively resilient to damage (Boege and Marquis 2005). Damage early in life is detrimental because the plant has relatively few nutrients stored and must devote resources to regenerate lost photosynthetic tissues. As a result, young plants can be very sensitive to damage, and early life damage can affect the adult phenotype (Knight 2003; Boege 2005; Brody et al. 2007; Hanley and Fegan 2007; Poelman et al. 2008). Meanwhile, damage late in life occurs at a critical time because plants have used much of their stored nutrients preparing for reproduction and do not have enough time to produce the new photosynthetic tissues required to complete reproduction (Lapointe et al. 2010); damage during reproduction can alter flower, fruit, and seed number as well as seed mass (Marshall et al. 2005). Although this study focused on biomass removal in the middle of life, other life history stages may be more sensitive. Clearly, if plants differ in the way they tolerate biomass loss across ontogeny and in which aspects of fitness are affected, it is important to take a longitudinal experimental approach to studying biomass loss (Garcia and Ehrlen 2002; Barton 2007, 2008).

The goal of these experiments was to examine the effects of biomass removal on growth and fecundity in *B. vulgaris*. In the greenhouse, removing 50% of the leaves elicited short-term changes in AGR and RGR and reduced belowground biomass. However, regardless of the timing and frequency of biomass removal, AGR was constant after biomass removal, and over time individuals converged toward a common size. In the field, biomass removal during the late vegetative stage had relatively little effect on size and no effect on reproduction. These experiments illustrate the remarkable plasticity of a plant species to tolerate aboveground biomass removal.

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