Influence of nutrient availability on the mechanisms of tolerance to herbivory in an annual grass, *Avena barbata* (Poaceae)[1]

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Tolerance, or the capacity of a genotype to survive and reproduce following herbivore damage, varies widely across the plant kingdom. One proximate cause of this variation is resource availability, which can influence tolerance through mechanisms such as growth rate and photosynthesis. We examined the effect of high and low soil nutrient levels on the relationship between tolerance and two of its underlying mechanisms, biomass regrowth and photosynthetic upregulation, among genotypes of the Mediterranean annual grass *Avena barbata*. Although defoliated plants did not reach the same biomass as controls, biomass regrowth was higher at high nutrients. However, increased seed abortion at high nutrients caused tolerance to be the same in both nutrient treatments. Increased seed abortion also uncoupled biomass regrowth from tolerance at high nutrients. We found no evidence for photosynthetic upregulation in defoliated compared to control plants in either nutrient treatment. However, tolerance was positively correlated with predefoliation photosynthetic efficiency at high nutrients. Thus, constitutive photosynthetic efficiency may be a better predictor of tolerance than photosynthetic responses following herbivory in *A. barbata*. More generally, our results highlight the possibility that the mechanisms of tolerance can differ across resource environments even if tolerance is the same.

Key words: *Avena barbata*; biomass regrowth; chlorophyll fluorescence; fitness; herbivory; nutrients; seed abortion.

It has long been recognized that herbivore damage can cause reductions in plant biomass, fitness, and net primary production (Painter, 1958; Mattson and Addy, 1975; Crawley, 1983; Rosenthal and Kotanen, 1994; Hawkes and Sullivan, 2001). Tolerance, or the capacity of a plant to survive and reproduce following herbivory, is operationally defined by the response of fitness to damage for a genotype exposed to contrasting levels of herbivory (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Simms, 2000; Stowe et al., 2000). Tolerance varies widely among plant lineages, and recent studies of the evolution of tolerance suggest that this variation is caused by differences in natural selection by herbivores (Juenger and Bergelson, 2000; Stinchcombe and Rausher, 2002), differential constraints on evolutionary change associated with the amount of quantitative genetic variation for tolerance (Fornoni and Núñez-Farfán, 2000; Juenger and Bergelson, 2000; Pilson and Decker, 2002) or costs associated with the expression of tolerance (Mauricio et al., 1997; Strauss and Agrawal, 1999).

The evolution of interspecific variation for tolerance also depends on its underlying mechanisms (Crawley, 1983; Tiffin, 2000; Fornoni et al., 2003). One potentially important mechanism of tolerance is the upregulation of photosynthesis following herbivore damage, which would increase carbon availability for new leaf growth and seed production (Gifford and Marshall, 1973; Crawley, 1983; Trumble et al., 1993; Mabry and Wayne, 1997; Tiffin, 2000). This response is usually caused by a decreased carbon source–sink ratio within the plant (Caldwell

et al., 1981; Trumble et al., 1993; Mabry and Wayne, 1997; Thomson et al., 2003) as well as increased light penetration (Caldwell et al., 1981; Nowak and Caldwell, 1984; Mabry and Wayne, 1997) following defoliation. Most experimental tests of photosynthetic upregulation as a mechanism of tolerance rely on documenting photosynthetic changes following damage or by comparing groups of damaged and undamaged plants (Gifford and Marshall, 1973; Nowak and Caldwell, 1984; Meyer, 1998; Thomson et al., 2003). However, because tolerance is a property of the genotype, researchers testing for the importance of a mechanism of tolerance must also evaluate if that mechanism is genetically correlated with the degree of tolerance (Tiffin, 2000).

Another potential mechanism that confers tolerance is an increase in the amount of leaf biomass regrowth after defoliation. Although increased leaf regrowth may itself be caused by higher photosynthetic rate, a rapid increase in leaf area following herbivory could increase seed production by increasing the total surface area available for photosynthesis (Lambers and Poorter, 1992; Stowe et al., 2000; Tiffin, 2000). Because of the hypothesized positive link between the amount of regrowth and tolerance, studies of herbivore effects on plants often report the amount of biomass regrowth as an indicator of tolerance (Tiffin, 2000). Nevertheless, it is also possible that increased biomass regrowth following herbivory may not increase tolerance if leaf production competes with seed production for resources such as nutrients and carbon (Mabry and Wayne, 1997; Tiffin, 2000). As a result, biomass regrowth following herbivory may not necessarily be a good predictor of tolerance.

Tolerance to herbivory also varies with environmental resource availability because many of the underlying mechanisms are affected by these resources (McNaughton et al., 1983; Rosenthal and Kotanen, 1994; Hamilton et al., 1998; Hochwender et al., 2000). Historically, two hypotheses have been proposed to explain the response of tolerance to resources. First, the compensatory continuum hypothesis (CCH; Maschinski and Whitham, 1989) predicts that tolerance should be greater in resource-rich environments because photosynthesis and regrowth will not be limited by resources such as nitrogen.

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In contrast, the growth rate model (GRM; Hilbert et al., 1981) predicts that tolerance should be lower in resource-rich environments because plants would already be operating at near maximum growth rate and may not have the physiological capacity to upregulate photosynthesis and growth following tissue loss. Empirical results, however, do not consistently support either model (Hawkes and Sullivan, 2001). Recently, Wise and Abrahamson (2005, 2007) proposed the more successful limiting resource model (LRM) in which specific predictions about the effects of resources on tolerance depend on whether herbivory influences uptake of the experimentally manipulated focal resource or an alternate resource. Regardless of the success of these models in predicting how resources influence tolerance, the effect of resource availability on the relationship between tolerance and its mechanisms remains poorly understood (Hochwender et al., 2000; Fornoni et al., 2003).

To examine how photosynthetic upregulation and biomass regrowth contribute to tolerance, we grew a population of genotypes of the C_3 Mediterranean annual grass *Avena barbata* Pott. ex. Link in the absence or presence of simulated herbivory. Plants were grown under either high or low nutrients to determine if the relationship between tolerance and its underlying mechanisms varied with resource availability. We expected that photosynthetic upregulation would be positively correlated with tolerance in both treatments, but that this relationship would be stronger in the high nutrient treatment because ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme content depends strongly on nitrogen availability (Chapin, 1980). In contrast, we predicted that biomass regrowth and tolerance would be poorly correlated because biomass allocated to vegetative growth would be more likely to constrain the opportunity for reproduction in an annual plant. Based on the LRM, we expected that defoliation would not change access to the focal resource, nutrients, but instead affect an alternate resource: carbon. Defoliation should exacerbate carbon limitation by removing leaf surface area, but this limitation should be less severe in the low focal resource environment because plant growth would already be limited by nutrients. Thus, tolerance should be weaker in the high focal resource environment because plant growth would be more limited by the alternate resource, carbon, than by nutrients (Wise and Abrahamson, 2005, 2007).

MATERIALS AND METHODS

We used *A. barbata* as a study species to examine tolerance and its mechanisms for two reasons. First, it has a long history of use as a forage grass for cattle (>200 yr; Hamrick and Allard, 1972; Latta et al., 2004), suggesting that tolerance could be an important mechanism for coping with herbivore damage. In addition, *A. barbata* is self-fertilizing (>95%) and completes its life cycle in <6 mo, which allowed us to estimate lifetime reproductive fitness in a single growing season. In our experiments, we used recombinant inbred genotype lines (RIL) of crosses of two homozygous genotypes associated with moist (mesic) and dry (xeric) habitats in California (Hamrick and Allard, 1972; Garcia et al., 1989; Latta et al., 2004). Parental ecotypes differ for a suite of quantitative traits including seed size, flowering time, root depth, competitive ability, and fecundity (Hamrick and Allard, 1975; Latta et al., 2004; Gardner and Latta, 2006). F1 progeny, which are heterozygous for all loci that differ between the parents, were self-fertilized to yield F2 families. To reduce within genotype variation, F2 individuals were selfed for five generations via single seed descent (Latta et al., 2004; Gardner and Latta, 2006). Six RILs from the F7 generation were selected to encompass variation in time to flowering, a correlate of fitness.

To induce germination, we removed the outer lemma of ripened seeds and placed them on moistened filter paper in Petri dishes at 4°C. After 3 d of imbibi-

tion, seeds were moved into a dark location at room temperature. Germination took place within 24 h, and seedlings were transplanted into plastic cylindrical 15-cm pots filled with Promix (Premier Tech, Rivière-du-Loup, Quebec, Canada) and placed on a greenhouse bench. Pots were watered to saturation three to four times a week. The experiment was conducted during the winter and spring (19 November 2003 to 28 May 2004), approximating the winter annual habit of *A. barbata*. To compensate for shorter day lengths at Guelph compared to the more southern latitude of *A. barbata*'s home populations, daylight was artificially maintained at 12 h with supplemental high intensity discharge (HID) lighting.

To study the effects of nutrient levels (high or low), simulated herbivory (clipped or not clipped) and genotype on growth and seed production of *A. barbata*, we used a three-factor, completely randomized block design. To account for environmental heterogeneity in the greenhouse, we set up five blocks of 24 plants on a single greenhouse bench. Each pot was randomly positioned within a block. Eight plants were lost because of poor seed germination prior to commencing the experiment. For the high nutrient treatment, 250 mL of 1.5 g/L 20 : 20 : 20 fertilizer was applied to half of the pots in each block every two weeks, for a total of 11 applications. For the low nutrient treatment, 250 mL of 0.5 g/L of 20 : 20 : 20 fertilizer was applied every five weeks on average for a total of four applications. Sixty-five days after transplanting, half of the plants in each of the high and low nutrient treatments were clipped by removing all biomass 2 cm above the soil level. The clipping treatment simulated the intensity of ungulate grazing experienced by grasses (Hicks and Reader, 1995).

To examine physiological changes induced by the treatments, we measured photosynthetic efficiency as the ratio of dark-adapted variable to maximum fluorescence (F_v/F_m) with a portable continuous-excitation type chlorophyll fluorescence analyzer (Handy PEA, Hansatech Instruments, Norfolk, UK). F_v/F_m reflects the maximum amount of photons absorbed by photosystem II (quantum efficiency of PSII) and is correlated with leaf-level quantum efficiency or the initial slope of the response of photosynthesis to increasing irradiance (Lambers et al., 1998). It has been used previously to determine whether foliar herbivory alters the photosynthetic efficiency of leaves (e.g., Thomson et al., 2003) and is often correlated with plant growth (Cavender-Bares and Bazzaz, 2004). Chlorophyll fluorescence measurements from each plant were made on two randomly selected leaves and averaged. Measurements were made between 8 am and 1 pm when the photosynthetic rate of *A. barbata* is at its daily peak (M. E. Sherrard and H. Maherali, University of Guelph, unpublished data). Leaves were dark-acclimated for 10 min before measurement to ensure that the reaction center for photosystem II was fully oxidized. Chlorophyll fluorescence was measured six times: first at 59 d after germination, 6 d prior to the clipping treatment, and then on days 80, 97, 115, 136, and 162.

To determine whether allocation to leaf nitrogen was affected by our treatments, we measured chlorophyll concentration (Chl), which is correlated with leaf nitrogen content in many species (Chapman and Barreto, 1997; Herrick and Thomas, 2003). We used Chl as a proxy for leaf N content because it allowed us to nondestructively sample this parameter over the course of the experiment. Chlorophyll concentration was measured on three randomly selected leaves per plant using a hand-held spectrometer (SPAD 502 chlorophyll meter, Spectrum Technologies, Plainfield, Illinois, USA) at the same times that we measured F_v/F_m.

We determined total aboveground biomass by harvesting shoots as they senesced during the course of the experiment and by harvesting the remaining vegetative and reproductive tissues after 190 d of growth. Though *A. barbata* plants were still alive, we terminated the experiment at 190 d because this simulated the approximate 6-mo growing season of this winter annual in California, which ends when rainfall stops in the late spring (Hamrick and Allard, 1975; Latta et al., 2004; Sherrard and Maherali, 2006). All tissues were dried to constant mass in a forced air convection oven at 60°C for at least 96 h and weighed. To determine lifetime reproductive fitness for calculating tolerance, we counted the number of seeds each plant produced by harvesting florets as the seeds within them matured during the experiment. Because not all florets produced seeds, we visually inspected the florets for seeds. Only florets that contained seeds were counted to determine fitness. We estimated the proportion of aborted seeds by randomly sampling 100 florets on each plant and counting the florets without seeds.

We used a three-way analysis of variance (ANOVA) to test for the effect of clipping, nutrient level, and genotype and all interactions on aboveground biomass, seed abortion and seed number (Systat 8.0, Systat software, Point Richmond, California, USA). We included block as a random factor in the model and treated main effects as fixed factors. Although some authors treat genotype as a random factor in tolerance studies, we nonrandomly selected a subset of RIL genotypes that encompassed the range of fitness variation in our experiment.

Because genotypes were sampled nonrandomly, genotype was treated as a fixed factor in the ANOVA. We used Levene's test to asses whether the data met the homogeneity of variance assumption for ANOVA (Zar, 1999). This assumption was met in every case except for the clipping × nutrient (c × n) interaction for aboveground biomass and seed number (Levene's test: $P_{cxn,biomass} < 0.001$ and $P_{cxn,seed\ no.} = 0.049$). Because log transformation did not homogenize variance across groups for this interaction, we analyzed the untransformed data (e.g., Stanton and Thiede, 2005). Therefore, P values for the clipping × nutrient interaction for these traits could be inaccurate. To examine if clipping, nutrient level, and genotype influenced chlorophyll concentration and F_v/F_m over the four months of the study, we used a three-way repeated measures ANOVA. Data used in this analysis met the homogeneity of variance assumption, but violated the assumption of sphericity. Because the sphericity violation was modest (i.e., epsilon was between 0.8 and 1), we report Huynh–Feldt corrected probabilities for this analysis (Zar 1999).

We used regression to determine whether photosynthetic upregulation, seed abortion, and aboveground biomass responses were correlated with tolerance among genotypes within each nutrient treatment. For this analysis we expressed tolerance as a fitness ratio for each genotype across clipping treatments (i.e., [clipped fitness/unclipped fitness]). Fully tolerant genotypes would have a ratio of 1, whereas genotypes expressing reduced tolerance would have a ratio <1. Defining tolerance in relative terms using a fitness ratio was necessary because tolerance defined in absolute terms (as the mean difference in fitness between clipping treatments) is biased by the magnitude of fitness. We also used ratios to calculate the response of photosynthetic parameters, seed abortion, and the level of biomass regrowth so that these parameters were expressed on the same scale as tolerance. To calculate this ratio for tolerance, seed abortion and biomass regrowth, we used fitness (seed number), proportion seeds aborted and aboveground biomass values, respectively. To calculate the response ratio for photosynthetic parameters, we used measurements of F_v/F_m and Chl made on clipped and unclipped plants 15 d after the clipping treatment, after new leaves had been produced. We used analysis of covariance (ANCOVA) to determine if the relationship between tolerance and its mechanisms differed between nutrient treatments. This was done by examining the statistical significance of the nutrient × tolerance mechanism interaction term. If this term was significant, then the null hypothesis that slope coefficients between nutrient treatments were homogeneous could be rejected (Zar, 1999).

RESULTS

We found that *A. barbata* did not fully tolerate simulated herbivory. Clipped plants produced significantly fewer seeds than unclipped plants (Table 1, Fig. 1). The effect of clipping on seed number, and therefore on tolerance, was about the same at both nutrient levels (i.e., a 49% reduction at high nutrients and a 53% reduction at low nutrients; nonsignificant c × n interaction, Table 1, Fig. 1C). Aboveground biomass was also lower in clipped plants, but simulated herbivory had a greater impact on aboveground biomass at low compared to high nutrients (a significant c × n interaction, Table 1). Clipped plants had 39% lower total aboveground biomass than unclipped plants at high nutrients, but 55% lower aboveground biomass than unclipped

plants at low nutrients (Fig. 1A). Overall, seed abortion did not differ between clipped and unclipped plants and was significantly higher at high nutrients than low nutrients (Table 1, Fig. 1B). However, clipped plants had 13% higher seed abortion than unclipped plants at high nutrients, whereas seed abortion was similar between clipped and unclipped plants at low nutrients (a significant c × n interaction, Table 1, Fig. 1B).

Recombinant inbred genotype lines (RILs) differed for all three response variables (i.e., a significant genotype effect, Table 1). The RILs also differed in how they responded to the nutrient and clipping treatments, but significant interactions were not consistently observed across response variables. For example, there was no variation among RILs in how seed number and aboveground biomass responded to increased nutrients (Table 1, Fig. 2). In contrast, RILs differed in how seed abortion responded to increased nutrients (Table 1, Fig. 2). There was also significant variation among *A. barbata* RILs in their response to clipping with respect to aboveground biomass and seed abortion (Table 1, Fig. 2) but not for tolerance (based on seed number; Table 1, Fig. 2). Overall, the tolerance of genotypes (g) to simulated herbivory did not differ significantly between resource environments (i.e., no c × n × g interaction for seed number, Table 1), though our power to detect interactions associated with genotype was low. Nevertheless, there was a more than twofold range of variation for tolerance among RILs within each nutrient treatment. The tolerance ratio ranged from 0.29 to 0.73 in the high nutrient treatment and from 0.32 to 0.71 in the low nutrient treatment.

There was no photosynthetic upregulation following clipping. Although both chlorophyll concentration (Chl) and chlorophyll fluorescence (F_v/F_m) changed with time (Chl: $F_{4,336} = 37.96$, $P < 0.0001$; F_v/F_m: $F_{4,336} = 62.90$, $P < 0.0001$), simulated herbivory did not cause these variables to increase in *A. barbata* leaves. The clipping treatment generally reduced Chl ($F_{1,84} = 61.20$, $P < 0.0001$; Fig. 3A) but had no effect on F_v/F_m ($F_{1,84} = 0.15$, $P = 0.69$; Fig. 3B). On average, plants grown at high nutrients had higher Chl levels than those grown at low nutrients ($F_{1,84} = 92.6$, $P < 0.0001$). In addition, the clipping treatment more strongly reduced Chl for plants grown at high nutrients relative to those grown at low nutrients ($F_{1,84} = 29.4$, $P_{cxn} < 0.0001$; Fig. 3A). In contrast, F_v/F_m was not influenced by the nutrient treatment ($F_{1,84} = 1.34$, $P = 0.25$), nor was the effect of clipping on this trait dependent on nutrient level ($F_{1,84} = 1.10$, $P_{cxn} = 0.30$; Fig. 3B). There was variation among RILs for both Chl ($F_{5,84} = 9.31$, $P < 0.0001$) and F_v/F_m ($F_{5,84} = 8.02$, $P < 0.001$), but there was no variation in how RILs responded to the clipping treatment (Chl: $F_{5,84} = 0.99$, $P_{cxg} = 0.42$; F_v/F_m: $F_{5,84} = 0.70$, $P_{cxg} = 0.62$).

TABLE 1. Three way analysis of variance (ANOVA) for aboveground biomass, proportion seeds aborted and number of seeds of *Avena barbata*. Fixed factors were genotype (g), nutrient level (n) and clipping (c). Statistically significant relationships ($P < 0.05$) are in boldface type.

| Source of variation	Aboveground biomass				Proportion of seeds aborted				Number of seeds			
	df	MS	F	P	df	MS	F	P	df	MS (×10⁵)	F	P
Block	4	68.38	2.994	**0.023**	4	0.08	6.628	**<0.001**	4	1.08	7.084	**<0.001**
g	5	70.49	3.086	**0.013**	5	0.69	55.478	**<0.001**	5	1.62	10.686	**<0.001**
n	1	6764.57	296.146	**<0.001**	1	1.73	139.558	**<0.001**	1	6.76	44.542	**<0.001**
c	1	4481.44	196.193	**<0.001**	1	0.04	2.998	0.087	1	10.43	68.708	**<0.001**
n × g	5	7.90	0.346	0.883	5	0.09	7.113	**<0.001**	5	0.32	2.112	0.072
n × g	5	60.68	2.657	**0.028**	5	0.07	5.983	**<0.001**	5	0.28	1.858	0.110
c × n	1	115.06	5.037	**0.027**	1	0.05	4.049	**0.047**	1	0.49	3.260	0.075
c × n × g	5	72.91	3.192	**0.011**	5	0.01	1.048	0.395	5	0.22	1.426	0.223
Error	83	22.84			84	0.01			84	0.15		
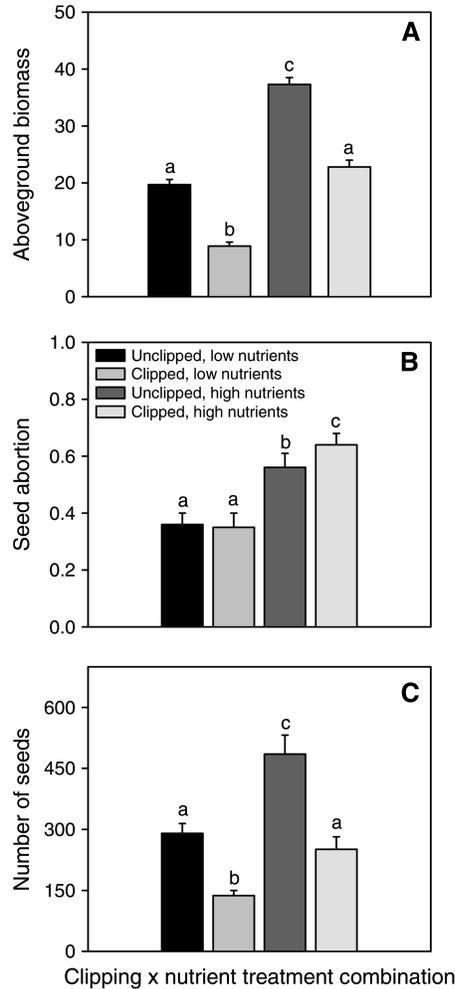
Fig. 1. Mean (±1 SE) for aboveground biomass (A), proportion seeds aborted (B) and number of seeds (C) for *Avena barbata* in the nutrient by clipping treatment combination. Overall statistical differences for each response variable are shown in Table 1. Different letters above bars within each variable indicate that treatment combinations differed statistically ($P < 0.05$), based on a Tukey post hoc test.

Tolerance was positively associated with biomass regrowth responses to simulated herbivory. The relationship was strongly significant in the low nutrient environment, but only marginally so in the high nutrient environment ($P = 0.07$; Table 2). Tolerance was not associated with seed abortion response to clipping in the low nutrient environment. However, tolerance was negatively correlated with increased seed abortion in response to clipping at high nutrients, though only marginally ($P = 0.07$; Table 2). There was no relationship between tolerance and the response of F_v/F_m and Chl to clipping. Nevertheless, tolerance was positively associated with preclipping F_v/F_m at high nutrients (Table 2). There was no relationship between tolerance and preclipping Chl (Table 2). In addition, tolerance was not correlated with fitness in the undamaged state in either nutrient environment (data not shown). Though some regression slopes were statistically significant in one nutrient treatment but not the other, regression slopes did not differ statistically between nutrient treatments for any relationship (ANCOVA, Table 2). We note, however, that our power to detect these differences was low.

DISCUSSION

Our results suggest that biomass regrowth could be a mechanism of tolerance in *A. barbata* because it was positively correlated with tolerance. However, the relationship between biomass regrowth and tolerance was much stronger at low nutrients than at high nutrients, suggesting that resource availability influences the mechanisms of tolerance, even though tolerance itself was the same in each nutrient treatment. The weaker relationship between biomass regrowth and tolerance at high nutrients may be explained by significantly higher seed abortion in plants in the high nutrient vs. low nutrient treatment. Seed abortion also increased in response to clipping at high nutrients, and this response was negatively correlated with tolerance among genotypes. Though this outcome conflicts with the expectation that seed abortion should be higher in resource poor environments (e.g., Stephenson, 1981) our results are similar to another selfing Mediterranean annual (*Hordeum spontaneum*), in which an over-commitment to reproductive allocation when resources were abundant caused higher seed abortion (Volis et al., 2004). Such an over-commitment to reproduction is more likely to occur in species that are not pollen-limited and reproduce only once per lifetime (Wiens, 1984; Harper and Wallace, 1987). Thus, biomass regrowth and tolerance may not necessarily be interchangeable in selfing annuals.

The upregulation of photosynthetic efficiency following herbivory was not a mechanism of tolerance in *A. barbata* because F_v/F_m did not increase following herbivory. A potential explanation for this result is that because plant root growth was constrained in pots, reduced sink demand from roots prevented the upregulation of photosynthesis (Thomas and Strain, 1991). However, we removed nearly all the aboveground photosynthetic tissue of clipped plants, which would have greatly decreased the capacity of the photosynthetic sources (the leaves) to meet the large respiratory sink demands of the remaining roots. Thus, our treatment would have minimized the source to sink ratio regardless of whether root growth was constrained (Caldwell et al., 1981; Trumble et al., 1993; Mabry and Wayne, 1997; Thomson et al., 2003). Our results suggest that two alternate explanations were more likely. Either nutrient resources required for photosynthetic upregulation were not available or were allocated to other functions. We rule out the first explanation because plants continued to receive fertilizer treatments after the simulated herbivory treatment. In support of the second explanation, we found that Chl, which is indicative of leaf N content (Chapman and Barreto, 1997; Herrick and Thomas, 2003), decreased after clipping in plants grown at both nutrient levels. This result suggests that following clipping, the increased N necessary for increased amounts of the photosynthetic enzyme Rubisco (Chapin, 1980) was not allocated to leaves. Instead, N may have been allocated to reproduction (pollen and seed production; Sage, 1994), which would have been accelerated in defoliated plants (e.g., Mabry and Wayne, 1997; Gassmann, 2004).

Even though there was no photosynthetic upregulation in response to simulated herbivory, preclipping F_v/F_m was positively associated with tolerance, suggesting that intrinsic differences in photosynthetic efficiency among genotypes are associated with the ability to tolerate herbivore damage. As a result, herbivores could select for higher photosynthesis in *A. barbata* populations. This result is similar to that observed by Gassmann (2004) in which high photosynthetic genotypes of the C_4 annual *Amaranthus hybridus* also had higher tolerance. Nevertheless, we observed a positive association between photosynthetic efficiency and
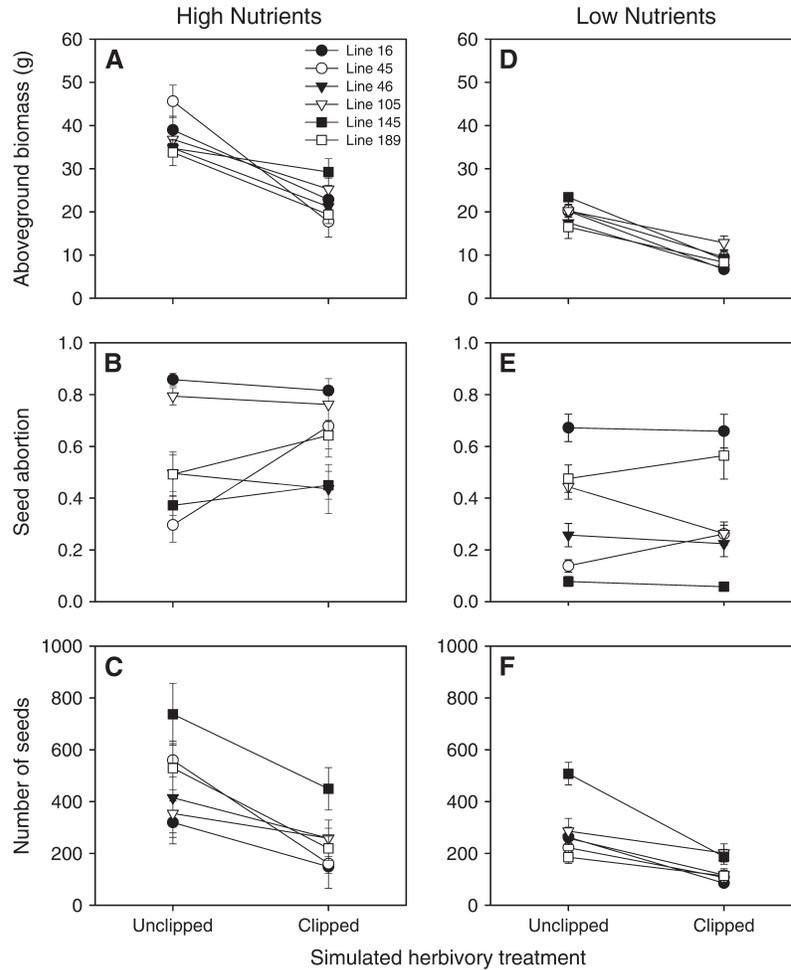
Fig. 2.   Reaction norms for mean (±1 SE) aboveground biomass (A, D), seed abortion (B, E), and number of seeds (C, F) for each *Avena barbata* recombinant inbred genotype in response to clipping at high (left column) and low nutrient (right column) levels.

tolerance only under high nutrients. We also note that there was no statistical difference in slopes between environments, though our ability to detect these differences was low. Regardless of any potential environment specific differences, our results suggest that constitutive photosynthetic efficiency may be more strongly associated with tolerance than induced photosynthetic responses following herbivory in *A. barbata* (cf. Strauss et al., 2003).

Our observation that tolerance was similar in both nutrient treatments differed from expectations based on the limiting resource model (LRM; Wise and Abrahamson, 2005) and the growth rate model (GRM; Hilbert et al., 1981), which predicts that tolerance should be stronger in the low nutrient treatment. Our results also differ from the compensatory continuum hypothesis (CCH; Maschinski and Whitham, 1989), which predicts that tolerance following herbivory should be greater in resource rich environments. In addition, our results differ from most of the previous studies of tolerance in contrasting nutrient environments, in which tolerance was greater under low nutrients (as reviewed in Wise and Abrahamson, 2007).

There are several potential explanations for why our results differed from predictions and previous studies. One explanation is that hypotheses about the effect of resources on tolerance assume that the same amount of tissue was removed in all resource environments (Hawkes and Sullivan, 2001; Wise and Abrahamson, 2007).

This assumption was violated in our study because more tissue was removed from the high than low nutrient treatment ($F_{1,54}$ = 6.36, P = 0.015) by simulated herbivory. However, removing more tissue from plants in the high nutrient treatment should have caused stronger negative effects on tolerance in this environment, increasing the likelihood of supporting the predictions of the LRM and GRM. A second explanation is that our results are not representative because experiments were done in the relatively nutrient-rich and benign conditions of the greenhouse. As a result, even plants from the low nutrient treatment had higher fitness than that observed in the field (Latta et al., 2007). Though higher greenhouse fitness may have altered the magnitude of tolerance in the greenhouse relative to the field, we note that plant growth and fitness were still stimulated by nutrient addition in our experiment, suggesting that the relative difference in tolerance (or lack thereof) between nutrient treatments was ecologically realistic. Specific to the predictions from the LRM, a third explanation is that our assumption that herbivore damage exacerbates carbon limitation in the high nutrient treatment was false. The LRM predicts equal tolerance across resource environments when the alternate resource does not limit growth (Wise and Abrahamson, 2007). However, simulated herbivore damage caused a reduction in aboveground biomass in all RILs, suggesting that leaf removal did limit carbon fixation.
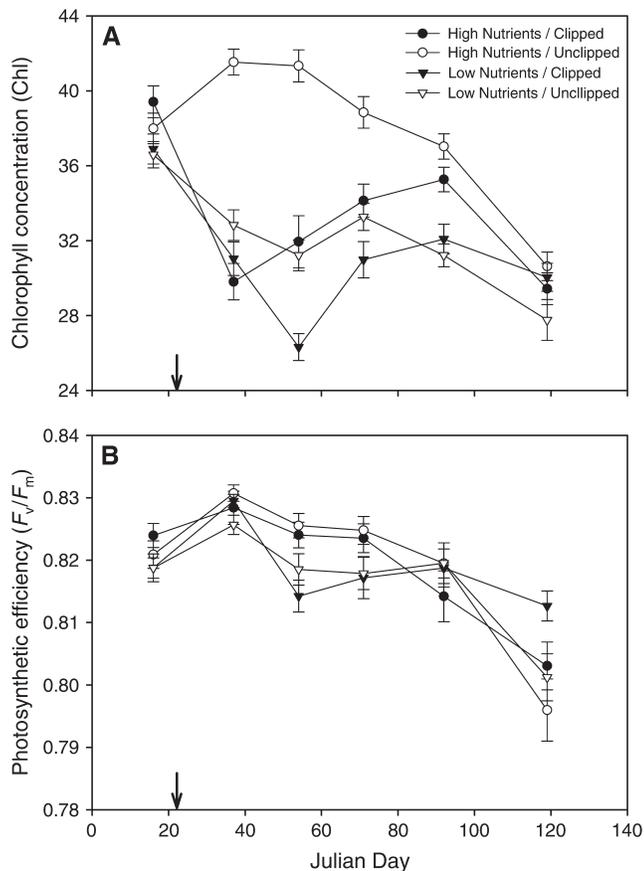
Fig. 3. Mean (±1 SE) chlorophyll concentration (Chl) (A) and photosynthetic efficiency (F_v/F_m) (B) for *Avena barbata* in each clipping by nutrient level treatment from January 16 to April 29, 2004. The arrows indicate the date of the clipping treatment (January 22).

Our work suggests that the variables used to evaluate tolerance can complicate experimental tests of predictions about the effects of resources on tolerance. If, for example, we had used aboveground biomass as a measure of tolerance in our study, our results would have supported the CCH (Maschinski and Whitham, 1989), which predicts that tolerance should be greater in resource rich environments. However, if tolerance can be uncoupled from biomass regrowth at high nutrients, as was observed in our study, growth measures may not reflect real differences in tolerance between environments. Because there are still relatively few nutrient manipulation experiments in which reproductive fitness is used to estimate tolerance (reviewed in Tiffin, 2000 and Wise and Abrahamson, 2007), a consensus on the effects of nutrient availability on tolerance still awaits the completion of additional studies.

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TABLE 2.   Regression equations describing the relationship between tolerance and its mechanisms (biomass regrowth, chlorophyll [Chl], photosynthetic efficiency [F_v/F_m], and seed abortion responses to clipping), and tolerance and preclipping Chl and F_v/F_m for genotypes of *Avena barbata*. Equations follow the form: $y = mx + b$. $N = 6$ for each regression analysis. Analysis of covariance (ANCOVA) was used to determine if slopes differed statistically between nutrient treatments. Statistically significant regressions and slope differences between treatments (at $P < 0.05$) are in boldface type.

		Regression variables					ANCOVA	
y	*x*	Nutrient	*m* (SE)	*b*	r^2	*P*	*F*	*P*
Tolerance	Biomass regrowth response	High	0.848 (0.34)	0	0.60	0.07	1.07	0.33
		Low	1.30 (0.16)	−0.10	0.94	**0.001**		
Tolerance	Chl response	High	−0.21 (1.59)	0.67	0	0.90	0.033	0.86
		Low	−0.53 (0.90)	0.99	0.08	0.59		
Tolerance	F_v/F_m response	High	−1.97 (8.29)	2.49	0.01	0.82	0.018	0.90
		Low	−4.54 (16.5)	5.05	0.02	0.80		
Tolerance	Seed abortion response	High	−0.24 (0.10)	0.83	0.61	0.07	1.389	0.27
		Low	−0.03 (0.16)	0.52	0.01	0.86		
Tolerance	Preclip Chl	High	0.027 (0.02)	−0.55	0.26	0.30	4.35	0.07
		Low	−0.039 (0.02)	1.96	0.47	0.13		
Tolerance	Preclip F_v/F_m	High	21.56 (7.61)	−17.21	0.67	**0.047**	0.61	0.46
		Low	0.849 (29.3)	−0.21	0	0.98		

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