

# Elevated carbon dioxide concentrations indirectly affect plant fitness by altering plant tolerance to herbivory

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**Abstract** Global environmental changes, such as rising atmospheric CO<sub>2</sub> concentrations, have a wide range of direct effects on plant physiology, growth, and fecundity. These environmental changes also can affect plants indirectly by altering interactions with other species. Therefore, the effects of global changes on a particular species may depend on the presence and abundance of other community members. We experimentally manipulated atmospheric CO<sub>2</sub> concentration and amounts of herbivore damage (natural insect folivory and clipping to simulate browsing) to examine: (1) how herbivores mediate the effects of elevated CO<sub>2</sub> (eCO<sub>2</sub>) on the growth and fitness of *Arabidopsis thaliana*; and (2) how predicted changes in CO<sub>2</sub> concentration affect plant resistance to herbivores, which influences the amount of damage plants receive, and plant tolerance of herbivory, or the fitness consequences of damage. We found no evidence that CO<sub>2</sub> altered resistance, but plants grown in eCO<sub>2</sub> were less tolerant of herbivory—clipping reduced aboveground biomass and fruit production by 13 and 22%, respectively, when plants were reared under eCO<sub>2</sub>, but plants fully compensated for clipping in ambient CO<sub>2</sub> (aCO<sub>2</sub>) environments. Costs of tolerance in the form of reduced fitness of undamaged plants were detected in eCO<sub>2</sub>

but not aCO<sub>2</sub> environments. Increased costs could reduce selection on tolerance in eCO<sub>2</sub> environments, potentially resulting in even larger fitness effects of clipping in predicted future eCO<sub>2</sub> conditions. Thus, environmental perturbations can indirectly affect both the ecology and evolution of plant populations by altering both the intensity of species interactions as well as the fitness consequences of those interactions.

**Keywords** Browsing · Cost · Carbon dioxide · Global change · Herbivory

## Introduction

Human activities are causing widespread environmental changes, including increases in plant resource availability on global and local scales. When plants are grown in simple environments, increased resource availability increases plant growth and fitness (Bazzaz 1990; Reich et al. 2001; Poorter and Navas 2003; Niklaus and Körner 2004; Reich et al. 2006). In more complex and thus more ecologically realistic environments, however, resource availability can alter interspecific interactions making it difficult to predict reliably the effects of increased resources. For example, when grown in the absence of competition, plants typically grow larger in elevated CO<sub>2</sub> (eCO<sub>2</sub>) compared to ambient CO<sub>2</sub> (aCO<sub>2</sub>) environments. When plants are grown in competition with species that have greater positive responses to eCO<sub>2</sub>, however, the positive growth effects of eCO<sub>2</sub> are often reduced or reversed (Navas 1998; Brooker 2006). Thus, changes in resource availability can affect plant populations indirectly by altering the intensity or outcome of interspecific interactions, such as competition. Because plants in natural environments are exposed to competitors,

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herbivores, pathogens, and mutualists, understanding the indirect effects of increased resource availability is necessary for estimating the full impact that ongoing environmental changes will have on plant populations.

Elevated atmospheric CO<sub>2</sub> concentrations typically increase plant C:N ratios and can alter the concentrations of defensive compounds in plant tissues (reviewed in Bezemer and Jones 1998; Coviella and Trumble 1999; Stiling and Cornelissen 2007). Because herbivore preference and performance are often tightly linked to foliar quality (Scriber and Slansky 1981), these physiological changes may be expected to alter plant resistance to herbivores and pathogens. Numerous studies have reported that plants are more susceptible to natural enemies under eCO<sub>2</sub> concentrations, although decreased damage has been observed (reviewed in Bezemer and Jones 1998; Stiling and Cornelissen 2007). All else being equal, changes in susceptibility would be expected to reduce plant fecundity, because increased susceptibility would mean increased herbivore damage, which frequently causes reductions in plant fitness. It is unlikely, however, that all else will be equal in eCO<sub>2</sub> compared to aCO<sub>2</sub> environments. In particular, changes in resource availability may alter the effect herbivore damage has on plant growth and reproduction, i.e., tolerance (reviewed in Wise and Abrahamson 2007). If increased concentrations of CO<sub>2</sub> increase plant tolerance to herbivores, then herbivore damage will have reduced effects on plant fitness. By contrast, if tolerance is lower in eCO<sub>2</sub> environments then herbivores may have a greater effect on plant fitness and population sizes, even if susceptibility to herbivores is unchanged. In this manner, indirect effects of global change on populations can result from both changes in the likelihood or intensity of species interactions and changes in the fitness consequences of those interactions (e.g., changes in resistance and tolerance, respectively).

While the physiological mechanisms and traits underlying plant tolerance to herbivory are poorly understood, mobilizing resources from storage organs, altering allocation patterns, and altering resource acquisition are all potentially important (Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000). As a result, tolerance is expected to depend heavily on resource availability. Three hypotheses have been put forth to explain how resource availability should influence the effect herbivore damage has on plant fitness. The compensatory continuum hypothesis (CCH) predicts that plants growing in resource-rich environments have more resources available for regrowth following damage and thus should have greater tolerance than plants growing in poorer resource environments (Maschinski and Whitham 1989; see also Whitham et al. 1991; Hawkes and Sullivan 2001). The growth rate model (GRM) assumes that plants grown in stressful environments typically grow slower than their innate maximum growth rate and thus

should be more able to compensate for lost tissue (i.e., have higher tolerance) in lower resource environments (Hilbert et al. 1981; see also Alward and Joern 1993; Hawkes and Sullivan 2001). Finally, the limiting resource model (LRM) predicts that the effect of resource availability on tolerance will depend on whether the focal resource is the major limitation on plant fitness and whether herbivory affects the availability or acquisition of this resource versus alternate resources (Wise and Abrahamson 2005). To date, the LRM has most empirical support—a recent survey of published studies (Wise and Abrahamson 2007) showed that 39 of 41 studies detected evidence that resources altered the effects of folivore damage on plant growth or fecundity in ways that were consistent with the LRM. Because CO<sub>2</sub> is a limiting resource and removal of above-ground tissue by herbivores is expected to reduce CO<sub>2</sub> assimilation, the LRM predicts greater plant tolerance in eCO<sub>2</sub> compared to aCO<sub>2</sub> environments (see Box 1 in Wise and Abrahamson 2007; Marshall et al. 2008). The CCH also predicts greater tolerance in eCO<sub>2</sub> than aCO<sub>2</sub> environments, whereas the GRM predicts reduced tolerance when plants are grown in eCO<sub>2</sub> environments.

The primary objectives of this experiment were to characterize herbivore-mediated indirect effects on plant growth responses to elevated atmospheric CO<sub>2</sub> concentrations and, reciprocally, how eCO<sub>2</sub> affects plant resistance and tolerance to herbivory. Specifically, we tested the prediction that higher concentrations of CO<sub>2</sub> increase tolerance to herbivore damage and examined whether eCO<sub>2</sub> alters the amount of folivore damage plants incur (resistance). We also investigated how eCO<sub>2</sub> alters the expression of genetic variation for tolerance and the costs of tolerance—both of which may impact the evolution of tolerance. We accomplished these objectives by investigating the effects of folivory and clipping (to simulate apical meristem damage from mammalian browsing) on the growth and reproduction of 1,300 plants from 18 *Arabidopsis thaliana* accessions, grown in aCO<sub>2</sub> or eCO<sub>2</sub> environments in an existing free air carbon dioxide enrichment (FACE) experiment.

## Materials and methods

To investigate how herbivores influence the effects of rising atmospheric CO<sub>2</sub> concentrations on *Arabidopsis thaliana* growth and fitness, we used a 2 × 2 × 2 split-split-plot experimental design, in which atmospheric CO<sub>2</sub> concentration was manipulated on whole plots (hereafter “rings”), and insecticide (to manipulate insect herbivory) treatments were applied to sub-plots (hereafter “blocks”). Clipping treatments were applied to randomly selected plants from each accession growing in each CO<sub>2</sub> × herbivory treatment. FACE was used to manipulate atmospheric CO<sub>2</sub>

concentrations; concentrations in each eCO<sub>2</sub> ring were maintained at ca. 560 p.p.m. by surrounding the ring with an array of pipes that blew air enriched with CO<sub>2</sub>. aCO<sub>2</sub> (ca. 378 p.p.m.) rings were surrounded by a similar pipe structure, but the air blown into the ring was not enriched with CO<sub>2</sub>. There were three replicate rings per CO<sub>2</sub> treatment. The CO<sub>2</sub> treatments were applied during daylight hours throughout the duration of the experiment [see Reich et al. (2001) for more information on CO<sub>2</sub> manipulation].

The amount of insect herbivory was manipulated with insecticide or water control treatments applied to four blocks per ring. Insecticide treatments were maintained by spraying two blocks (54 plants in each block) per ring with the generalist insecticide Sevin (Bayer CropScience, RTP, N.C.) and the remaining two blocks with an equal amount of water as a control. While not tested on *A. thaliana*, Sevin has no detectable direct effects on plant growth or fitness in other plant taxa (Lau and Strauss 2005). Insecticide treatments were applied every 2–4 weeks throughout the growing season, depending on rainfall. Clipping treatments (control or clipped) were applied to randomly selected individuals by clipping the main stem and half of the side stems 1 cm above the base of the stem 1–2 days after flowering to simulate apical meristem damage caused by small mammals (Weinig et al. 2003). There were nine replicates per accession per clipping × insecticide × CO<sub>2</sub> treatment ( $n = 162$  plants per treatment).

#### Plant genotypes and measurements

We sowed seeds from 18 different *A. thaliana* accessions into separate flats that had been bottom-watered to saturation. *A. thaliana* accessions were originally obtained from the *Arabidopsis* Biological Resource Center, The Ohio State University and were provided to us by M. T. Brock (University of Wyoming). These accessions had been collected from across the European range of *A. thaliana*. Flats were placed in a 4°C cold room, and 2 days later the flats were moved to a greenhouse where the seeds germinated. After most seeds had germinated, we transplanted seedlings singly into 164-ml conetainers (Ray Leach Conetainers; Stuewe & Sons, Corvallis, Ore.) that had been filled with potting mix (Sunshine Mix no. 5; Sun Gro Horticulture Canada, Alberta). Two days later on 14 June 2006 we measured rosette diameter to use as a size covariate to control for differences in early growth, before CO<sub>2</sub> and insecticide treatments were applied. Inclusion of pre-treatment diameter as a covariate in analyses did not, however, affect the results, so for simplicity, we present results only from analyses that did not include initial rosette diameter. That same day, we placed each conetainer into a randomly assigned location within each block in each CO<sub>2</sub> ring (three plants per accession per block = 72 plants per accession). After

being placed in the field, plants were bottom-watered as needed, so that all plants were able to soak up similar amounts of water.

On 26 June 2006, before any plants were clipped, we measured rosette diameter and calculated the proportion of leaves with insect herbivore damage. Plants began flowering on 26 June, and we censused for flowering every other day throughout the growing season. For clipped plants, we also recorded the date on which they re-flowered. Between 7 July and 6 August, we harvested each plant as it senesced and fruits on the main stem began to dehisce. Harvesting in this way, rather than harvesting all plants on a specific date, allowed each plant to complete its entire life cycle and senesce naturally. After harvest, we measured height, and counted the number of stems and fruits produced. Above-ground biomass estimates were obtained after drying plants for at least 2 days at 60°C.

#### Statistical analyses

We used mixed model ANOVA (PROC MIXED; SAS Institute 2001) to test whether CO<sub>2</sub> influences levels of insect herbivory, plant growth, or fitness effects of herbivory. The model included CO<sub>2</sub>, insecticide treatment, clipping treatment and all interactions as fixed factors. Ring nested within CO<sub>2</sub> treatment, block nested within ring, accession, and all interactions between accession and the fixed factors were included as random factors. Significance of random factors was assessed by comparing the likelihood ratio statistic to a  $\chi^2$  distribution with 1 *df*. Insect herbivory (angular transformed proportion of leaves damaged), growth measures (rosette diameter, height, stem number, and biomass) and natural log-transformed fitness measures (fruit number) were included as response variables. Significant interactions between CO<sub>2</sub> and the insecticide or clipping treatments on fitness measures are evidence that CO<sub>2</sub> alters the fitness impacts of herbivory (i.e., tolerance). Significant accession × insecticide or accession × clipping terms are evidence that not all accessions respond similarly to damage (i.e., there is genetic variation for tolerance to herbivore damage), and significant CO<sub>2</sub> × accession × insecticide (or clipping) terms would provide evidence that CO<sub>2</sub> alters the expression of genetic variation for tolerance. Similarly, a significant accession effect on insect herbivory provides evidence for genetic variation in resistance to herbivores, and a significant CO<sub>2</sub> × accession effect would indicate that CO<sub>2</sub> alters the expression of genetic variation for resistance. Because time of flowering can be important for fitness in natural environments (e.g., Ehrenreich and Purugganan 2006), for the clipped plants we also tested for evidence of genetic variation and CO<sub>2</sub> and insecticide effects on the length of time between initial flowering and flowering after clipping damage.

To investigate how CO<sub>2</sub> affects the costs of tolerance to clipping, we calculated tolerance for each accession in each CO<sub>2</sub> treatment as the difference in mean fruit production between clipped and unclipped plants. Costs of tolerance to natural insect herbivory were not calculated because plants received relatively little herbivory and the insecticide treatments did not affect mean fitness (see “Results”). The covariance between tolerance and accession mean fitness (fruit production) in the undamaged state is indicative of a cost of tolerance. Because the predictor and response variables are not independent (fitness in the unclipped treatment is the response variable and also part of the composite predictor variable tolerance), cost estimates may be biased (Mauricio and Rausher 1997; Tiffin and Rausher 1999). To account for this artifactual covariance, we applied a correction developed by Mauricio and Rausher (1997) and modified for discrete damage data by Tiffin and Rausher (1999). As in Tiffin and Rausher (1999), we estimate confidence intervals (CIs) for the corrected cost estimates in each CO<sub>2</sub> environment by jackknifing family means.

Because tolerance may be affected by the characteristics of undamaged plants (“constitutive traits”), damaged plants (“induced traits”), or plastic changes in trait values (Strauss et al. 2003; Wise et al. 2008), we conducted three series of partial correlation analyses to identify traits contributing to variation in tolerance. We calculated the partial correlations between tolerance and the accession mean values for traits (rosette diameter, aboveground biomass, height, stem number, flowering date, reflowering time, and senescence date) measured on either clipped or unclipped plants, as well as the change in trait values after clipping, in each CO<sub>2</sub> environment. We used partial correlation analyses to identify potential mechanisms of tolerance because tolerance is calculated using values of fitness in the damaged and undamaged states and many traits are correlated with fitness. As a result, traits correlated with fitness will appear to be correlated with tolerance, even if these traits do not contribute to variation in tolerance per se. The partial correlation approach controls for variation in fitness and, therefore, will identify traits that are correlated with tolerance— independent of their relationship with fitness. We also performed similar multiple regression analyses in which tolerance was included as the dependent variable and fitness and all traits were included as independent variables. We used Akaike’s information criterion as implemented by the selection option in SAS (Proc REG) to select the best model for each class of predictor traits (either traits measured on damaged plants, traits measured on undamaged plants, or the difference in mean trait values of damaged and undamaged plants) in each CO<sub>2</sub> environment. In addition to accounting for covariances between tolerance and fitness, the multiple regression analyses also take into account the correlations between traits.

## Results

### Resistance and growth traits

We found significant among-accession variation in flowering time, rosette size, height, stem number, biomass, and fruit production (Tables 1, 2). We also found that, on average, plants in eCO<sub>2</sub> flowered 0.4 days earlier, had rosettes that were 17% larger, were 18% taller, and produced 16% more stems, 73% more biomass and 38% more fruits (Tables 1, 2). By contrast, we found no statistically significant evidence for variation among accessions in plant resistance to insect herbivore damage ( $P < 0.39$ ) or that CO<sub>2</sub> environment affected the proportion of leaves damaged by insect herbivores (lmeans  $\pm$  1SE: aCO<sub>2</sub> 0.047  $\pm$  0.012; eCO<sub>2</sub> 0.050  $\pm$  0.012; Table 2). Despite low levels of natural insect damage in all environments, the insecticide treatment significantly reduced the amount of insect folivore damage plants experienced (lmean  $\pm$  1SE insecticide = 0.024  $\pm$  0.010; control = 0.073  $\pm$  0.010). Although clipping treatments were randomly imposed, plants assigned to the unclipped/eCO<sub>2</sub> treatment experienced less insect herbivory than other clipping  $\times$  CO<sub>2</sub> treatment combinations (lmean  $\pm$  1SE: aCO<sub>2</sub>/clipped = 0.044  $\pm$  0.014; aCO<sub>2</sub>/unclipped = 0.051  $\pm$  0.014; eCO<sub>2</sub>/clipped = 0.063  $\pm$  0.013; eCO<sub>2</sub>/unclipped = 0.037  $\pm$  0.013).

### Tolerance

Although insecticide significantly reduced herbivore damage, we found no evidence that insecticide significantly

**Table 1** ANOVA of the effects of *Arabidopsis thaliana* accession, and insecticide (*Insect.*), clipping (*Clip.*), and CO<sub>2</sub> treatments on final biomass and fruit production (both ln-transformed)

	Biomass		Fruit no.	
	df	F	df	F
CO <sub>2</sub>	20	<b>35.53****</b>	21	<b>9.06**</b>
Insect.	20	0.32	21	1.00
Clip.	825	1.15	827	<b>7.55**</b>
CO <sub>2</sub> $\times$ Insect.	20	0.88	20	0.30
CO <sub>2</sub> $\times$ Clip.	825	<b>4.09*</b>	825	1.11
Insect. $\times$ Clip.	825	0.33	2,828	0.08
CO <sub>2</sub> $\times$ Insect. $\times$ Clip.	825	0.84	828	0.73
Random factors ( $\chi^2$ )				
Accession		<b>121.1****</b>		<b>169.1****</b>
Ring(CO <sub>2</sub> )		0.0		0.0
Rack(Insect. $\times$ Ring)		<b>21.0****</b>		<b>15.5****</b>

Significance of random factors was assessed with likelihood ratio tests. Results from models including initial size covariates were qualitatively similar

Values in bold indicate  $P < 0.05$

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

**Table 2** ANOVA of the effects of *A. thaliana* accession and insecticide, clipping, and CO<sub>2</sub> treatments on early season growth measures and on the amount of insect herbivory received

	Rosette diameter		Flowering date		Height		Stem #		Insect herbivory	
	df	F	df	F	Df	F	df	F	df	F
CO <sub>2</sub>	19.7	<b>10.71**</b>	3.49	<b>9.44*</b>	20	<b>38.96****</b>	20	<b>13.28**</b>	4	0.05
Insect.	19.7	2.15	836	0.25	20	3.67 <sup>+</sup>	19	1.03	16	<b>18.53****</b>
Clip.	828	0.28	835	0.00	826	<b>16.87****</b>	828	<b>46.62****</b>	835	1.22
CO <sub>2</sub> × Insect.	19.7	0.08	836	<b>7.14**</b>	20	1.38	19	0.44	16	0.87
CO <sub>2</sub> × Clip.	829	0.88	836	2.85 <sup>+</sup>	826	1.95	829	3.00 <sup>+</sup>	838	<b>4.01*</b>
Insect. × Clip.	829	1.09	834	1.12	826	0.01	829	0.23	837	0.84
Insect. × Clip. × CO <sub>2</sub>	829	0.14	835	0.05	826	0.35	829	0.09	837	<b>11.66****</b>
Random effects (χ <sup>2</sup> )										
Accession	85.1****		<b>1,342.8****</b>		<b>386.2****</b>		<b>93.8****</b>		0.7	
Ring(CO <sub>2</sub> )	0.0		0.0		0.0		0.0		0.8	
Rack(Insect. × Ring)	<b>11.4***</b>		0.0		<b>8.6**</b>		<b>5.6*</b>		<b>6.9**</b>	

Significance of random factors was assessed with likelihood ratio tests. Results from models including interactions between accession and other factors were qualitatively similar. For abbreviations, see Table 1

Values in bold indicate  $P < 0.05$

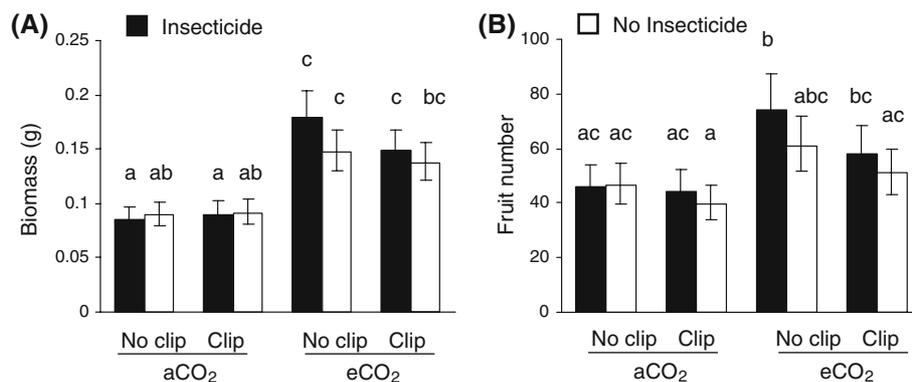
<sup>+</sup>  $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$

affected early growth, final biomass or fruit production (Table 1, 2; Fig. 1a, b). Plants grown in aCO<sub>2</sub> and sprayed with insecticide did, however, flower 0.4 days earlier than unsprayed plants whereas insecticide-treated plants grown in eCO<sub>2</sub> flowered 0.6 days later than unsprayed plants, resulting in a significant CO<sub>2</sub> × insecticide effect on flowering date (Table 2). Similar results were obtained when the actual amount or presence/absence of herbivory, rather than insecticide treatment, was used as a predictor variable in the analyses (results not shown).

Clipped plants produced significantly less biomass and fewer fruits than unclipped plants, although the effects of clipping were only statistically significant in the eCO<sub>2</sub> environment (clipping effect on fruit production, aCO<sub>2</sub>  $F_{1,824} = 1.37$ ,  $P < 0.24$ ; eCO<sub>2</sub>  $F_{1,831} = 7.58$ ,  $P < 0.006$ ;

clipping effect on biomass, aCO<sub>2</sub>  $F_{1,822} = 0.44$ ,  $P < 0.44$ ; eCO<sub>2</sub>  $F_{1,828} = 5.01$ ,  $P < 0.03$ ; see also Table 1; Fig. 1). Thus, clipping (simulated browsing) had greater impacts on growth and fitness in eCO<sub>2</sub> compared to aCO<sub>2</sub> environments, or in other words, plants were less tolerant to clipping damage when grown in eCO<sub>2</sub>.

In addition to the immediate ecological effect of CO<sub>2</sub> on tolerance to herbivory, it is possible that eCO<sub>2</sub> could influence the evolution of tolerance by altering the expression of tolerance and/or the costs (or benefits) of tolerance. Estimates of tolerance for plants of a given genotype grown in aCO<sub>2</sub> were not tightly correlated with estimates for plants grown in eCO<sub>2</sub> environments ( $r = 0.17$ ,  $P = 0.49$ ). The lack of strong correlation suggests that the accessions that are most tolerant to clipping in aCO<sub>2</sub> are not necessarily the most tolerant



**Fig. 1** Effects of clipping and insecticide treatments (shaded bars represent insecticide-treated plants, open bars represent no insecticide controls) on biomass (a) and fruit production (b) in ambient CO<sub>2</sub> (aCO<sub>2</sub>) and elevated CO<sub>2</sub> (eCO<sub>2</sub>) environments. Values shown are

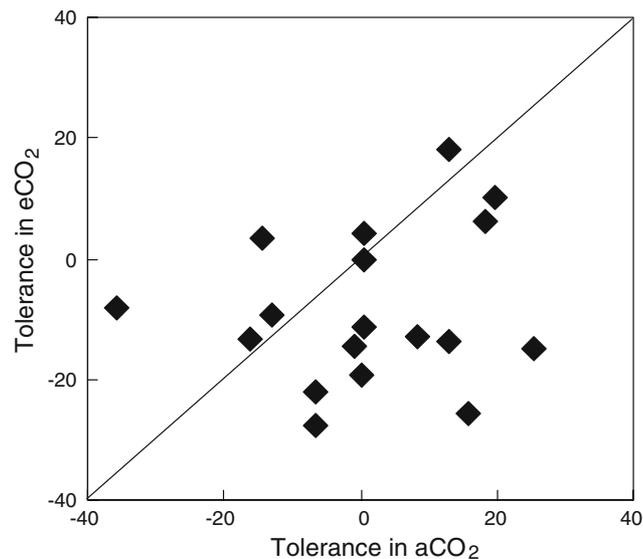
back-transformed lsmeans ± 1SE. Different letters denote significant differences among treatments, corrected for multiple comparisons within tests with a Tukey correction

under eCO<sub>2</sub>. Tolerance was, however, lower in the eCO<sub>2</sub> than the aCO<sub>2</sub> environment for the majority of accessions (Fig. 2). We detected significant costs of tolerance in the eCO<sub>2</sub> treatment, but not in aCO<sub>2</sub> (mean corrected covariance between tolerance and fitness of unclipped plants  $\pm$  95% CI: eCO<sub>2</sub>  $-73.49 \pm 12.08$ , aCO<sub>2</sub>  $1.12 \pm 13.28$ ). Moreover the 95% CIs of these corrected covariances do not overlap, indicating that the costs of tolerance were greater in the eCO<sub>2</sub> than aCO<sub>2</sub> environment. Together, these findings suggest that eCO<sub>2</sub> could alter the evolution of tolerance. These results should be interpreted with caution, however, since we failed to detect significant genetic variation in tolerance to clipping: the effects clipping had on biomass and fruit production did not appear to vary among accessions (non-significant clipping  $\times$  accession interactions on biomass  $\chi^2 = 0.0$ ,  $P > 0.5$  and fruit production  $\chi^2 = 0.9$ ,  $P > 0.4$ ).

We detected no evidence that the fitness effect of damage from one type of herbivore was affected by damage from the second (no clipping  $\times$  insecticide interactions on fitness; Table 1). However, clipped plants that were not sprayed with insecticide re-flowered 0.6 days later than clipped plants that were protected from insect herbivory with insecticide ( $F_{1,410} = 9.62$ ,  $P = 0.0021$ ).

#### Mechanisms of tolerance

Tolerance in both the aCO<sub>2</sub> and eCO<sub>2</sub> environments was correlated negatively with several traits measured on undamaged plants, including aboveground biomass, rosette



**Fig. 2** Relationship between tolerance to clipping in aCO<sub>2</sub> versus eCO<sub>2</sub> environments. Tolerance was calculated for each accession as the difference between mean fruit production of unclipped versus clipped individuals. Each accession is represented by one data point. Most accessions showed no effect or a reduction in tolerance under eCO<sub>2</sub> (points fell below the 1:1 line). For abbreviations, see Fig. 1

diameter, and number of stems as well as the plastic changes following clipping in these same traits (Table 3). The partial correlation analyses, which control for the covariance between these traits and fitness, however, revealed a significant relationship between tolerance and only a few phenotypic traits: tolerance was correlated with earlier flowering and senescence dates of undamaged plants in eCO<sub>2</sub> environments and plastic increases in biomass and rosette diameter in response to clipping in both CO<sub>2</sub> environments. The multiple regression analyses, which account for both covariances with fitness and correlations among predictor variables, corroborated the partial correlation results; tolerance was correlated with earlier senescence dates, and also with plastic increases in plant aboveground biomass and rosette diameter, at least in some environments (Table 3). Interestingly, the multiple regression analyses also indicated a significant association between tolerance and later flowering dates.

#### Discussion

Our study of *A. thaliana* growing in ambient and eCO<sub>2</sub> environments revealed little evidence that CO<sub>2</sub> altered the amount of natural insect damage plants experienced. The lack of evidence that CO<sub>2</sub> altered resistance, or the amounts of damage plants received, is somewhat surprising given that C:N ratios of plant tissues are often greater in plants grown in eCO<sub>2</sub> environments. Greater C:N often causes herbivores to increase consumption to compensate for lower plant quality, resulting in increased herbivore damage (reviewed in Lincoln et al. 1993; Bezemer and Jones 1998; Stiling and Cornelissen 2007). However, predicting the effects of eCO<sub>2</sub> on amounts of herbivore damage is not straightforward because C-based defenses also might increase under eCO<sub>2</sub>, which could reduce attack by herbivores (Lincoln et al. 1993; Bezemer and Jones 1998; Stiling and Cornelissen 2007). For example, Bidart-Bouzat et al. (2005) recently documented greater induction of glucosinolate defenses in *A. thaliana* grown in eCO<sub>2</sub> compared to plants grown in aCO<sub>2</sub>. Glucosinolates typically reduce damage from generalist herbivores but are positively related to damage from specialist herbivores such as *Phyllotreta cruciferae* (Siemens and Mitchell-Olds 1998), the most abundant herbivore in our study. Of course, the production of other chemical defenses (e.g., myrosinase) may also be influenced by CO<sub>2</sub> in ways that make the prediction of eCO<sub>2</sub> effects difficult. In fact, effects of eCO<sub>2</sub> on plant-herbivore interactions appear to vary widely, with several other studies also observing only minimal effects of eCO<sub>2</sub> on amounts of herbivory (e.g., Diaz et al. 1998; Goverde et al. 2002; Lau et al. 2007; Lau et al. 2008; see also Watt et al. 1993).

**Table 3** Correlations between tolerance and growth or phenological traits ( $r_{xy}$ ), partial correlations between tolerance and growth or phenological traits after correcting for covariances with fitness ( $r_{xy,z}$ ), and

partial regression coefficients ( $\beta$ ) from the best fit model predicting tolerance based on plant traits

Trait	aCO <sub>2</sub>			eCO <sub>2</sub>		
	$r_{xy}$	$r_{xy,z}$	$\beta$	$r_{xy}$	$r_{xy,z}$	$\beta$
Independent traits measured on undamaged plants						
Biomass	-0.63**	-0.44 <sup>+</sup>	-	-0.47 <sup>+</sup>	-0.22	-
Diameter	-0.49*	-0.25	-	-0.42 <sup>+</sup>	-0.36	-
Stem no.	-0.49*	-0.18	-	-0.42**	0.01	-
Height	-0.32	0.07	-	-0.20	0.25	-
Flowering date	-0.02	-0.32	4.05***	0.00	-0.52*	-
Senescence date	-0.10	-0.47 <sup>+</sup>	-5.90***	-0.12	-0.66**	-1.80**
Independent traits measured on damaged plants						
Biomass	0.06	-0.24	-	-0.11	-0.03	-
Diameter	-0.14	-0.33	-	-0.16	-0.12	-1.35
Stem no.	0.20	0.15	-	0.27	0.29	3.67
Height	-0.08	-0.37	2.18 <sup>+</sup>	-0.12	-0.03	-
Flowering date	-0.01	0.25	0.06	-0.04	-0.20	-
Senescence date	0.05	0.33	-	0.01	-0.15	-
Plastic changes in trait means in response to damage						
Biomass	0.86****	0.87****	13.05****	0.56*	0.57**	-
Diameter	0.49*	0.46 <sup>+</sup>	-	0.50*	0.67**	8.00***
Stem no.	0.50*	0.32	-	0.56*	0.36	-
Height	0.29	0.30	-	0.04	0.26	-
Flowering date	0.03	-0.01	3.66*	-0.21	-0.13	-
Senescence date	0.41 <sup>+</sup>	0.37	-	0.44 <sup>+</sup>	0.26	5.89**

Results are shown for independent traits measured on undamaged plants, damaged plants, or the plastic changes in trait means in response to damage. aCO<sub>2</sub> Ambient CO<sub>2</sub>, eCO<sub>2</sub> elevated CO<sub>2</sub>

<sup>+</sup>  $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$

Although fewer studies have investigated how atmospheric CO<sub>2</sub> concentrations affect the fitness consequences of herbivore damage, as opposed to effects of CO<sub>2</sub> on the amount of damage, most studies (including this one) have generally found that eCO<sub>2</sub> decreases tolerance to herbivory (e.g., Bidart-Bouzat et al. 2004; Marshall et al. 2008), although in several cases the effect is small and not statistically significant (e.g., Fajer et al. 1991; Johnson and Lincoln 2000; Wilsey 2001). Moreover, these studies have examined tolerance to many types of damage, including artificial folivory, simulated browsing, belowground nematode herbivory, and natural insect herbivory, in annual and perennial grasses and forbs grown in either growth chambers or field environments. Taken together, these results suggest that plants grown in eCO<sub>2</sub> environments will be less tolerant than plants grown in aCO<sub>2</sub> environments. Because herbivory has more negative fitness effects when plants are grown in eCO<sub>2</sub>, herbivores reduced the positive effect of eCO<sub>2</sub> on fitness. In fact, in our study, *A. thaliana* only experienced a statistically significant increase in fruit production in response to eCO<sub>2</sub> when they were not clipped

and were protected from insect herbivory with insecticide (Fig. 1). Thus, the indirect effect that results from eCO<sub>2</sub> altering the fitness consequences of herbivory largely negates the direct positive effects of eCO<sub>2</sub> on plant growth and reproduction. Such indirect effects may explain why the growth-stimulating effects of eCO<sub>2</sub> are often reduced in more complex, ecologically realistic environments [e.g., field versus growth chamber studies (Ainsworth and Long 2005)].

Our data suggest that higher concentrations of atmospheric CO<sub>2</sub> also may alter selection on *A. thaliana* tolerance to herbivory. In particular, we detected higher costs of tolerance to clipping, in the form of a negative correlation between tolerance and fitness in the absence of damage, for plants grown in the eCO<sub>2</sub> than the aCO<sub>2</sub> environment. Unless increases in atmospheric CO<sub>2</sub> concentration are accompanied by large increases in herbivory, the higher cost of tolerance is expected to weaken the strength of selection for tolerance as atmospheric CO<sub>2</sub> concentrations increase. We detected little evidence that tolerance to clipping is genetically variable, however, so the likelihood that

tolerance will respond to such changes in selection may be minimal. The lack of evidence for genetic variation may be due more to low power than a true lack of genetic variation, however, especially since genetic variation for tolerance to herbivory has been reported in many species, including *A. thaliana* (Mauricio and Rausher 1997; Weinig et al. 2003).

#### Resource availability

Reduced tolerance in eCO<sub>2</sub> environments appears to be inconsistent with the CCH and the LRM, both of which predict higher tolerance with increased availability of resources. The application of the LRM, the prevailing model of how resource availability affects tolerance, is based on identifying CO<sub>2</sub> as the focal resource limiting plant growth. This assumption is supported by the fact that plants in the eCO<sub>2</sub> environment grew significantly larger than plants in the aCO<sub>2</sub> environment. Application of the LRM also requires an understanding of how herbivore damage affects the acquisition or use of resources. Because herbivores remove photosynthetic material, herbivory should decrease the assimilation or acquisition of CO<sub>2</sub> and, therefore, the LRM would predict higher tolerance in the high-resource eCO<sub>2</sub> environment. Removal of stems should also remove C and thus is likely to increase C demand—leading also to the prediction of increased tolerance to clipping in the eCO<sub>2</sub> environment. Instead, we found that eCO<sub>2</sub> reduced tolerance to clipping damage and also tended to reduce tolerance to insect folivory (Fig. 1). It is possible, however, that plants grown in eCO<sub>2</sub> environments were larger than plants grown in aCO<sub>2</sub> not because of the direct effects of CO<sub>2</sub>, but due to indirect effects on other potentially limiting resources, such as water or nutrient availability that result from eCO<sub>2</sub> increasing water use efficiency and/or altering nutrient use efficiencies (reviewed in Körner 2003). If this is the case, predictions of the LRM are less clear. For example, if the removal of aboveground plant parts had weak effects on the acquisition of the limiting resource (as might be expected if it were water or nutrients), the LRM would predict higher, lower or equal tolerance in eCO<sub>2</sub> environments, depending on whether acquisition of alternate resources was limiting plant growth in eCO<sub>2</sub> conditions and whether herbivore damage exacerbates or reduces this resource limitation.

Regardless of whether CO<sub>2</sub> or a different resource limited plant growth in the aCO<sub>2</sub> environment, our finding of reduced tolerance in the eCO<sub>2</sub>-grown plants is consistent with predictions from the GRM, which predicts that plants should be less tolerant of damage when growing in resource-rich environments. Interestingly, the GRM appears to predict the effects that water or nutrients (i.e., resources acquired through below-ground tissues) have on

tolerance nearly as well as the LRM model, correctly predicting how these resources impact tolerance in 23 of 30 cases (Wise and Abrahamson 2007). In contrast, the CCH fails to predict the effects of water and nutrients on tolerance, but does successfully predict how aboveground resources (light) impact tolerance (ten of 11 cases) (Wise and Abrahamson 2007). If in our study eCO<sub>2</sub> had a greater impact on plant growth through indirect effects on water balance or nutrient availability, rather than direct effects on CO<sub>2</sub> supply, then the eCO<sub>2</sub> treatment would have affected below-ground resources more than above-ground resources. Because of the challenges in correctly identifying limiting resources and how herbivory alters the acquisition of these resources and applying the LRM, it may be valuable to consider multiple models of tolerance, utilizing the GRM for below-ground and the CCH for above-ground resources, rather than strive towards a single unified model.

#### Mechanisms of tolerance

The partial correlation analyses revealed evidence that genotypes that flowered and senesced earlier when undamaged and maintained larger aboveground biomass and rosette diameter in response to clipping were more tolerant of clipping damage. The multiple regression approach also indicated that tolerance was associated with later senescence dates, as well as with plastic increases in biomass (aCO<sub>2</sub>) or rosette diameter (eCO<sub>2</sub> environment) in response to damage. Interestingly, the multiple regression analysis also revealed evidence that tolerance was increased by delayed (not accelerated) flowering, at least in the aCO<sub>2</sub> environment. The apparently differing effect of flowering time detected from the partial correlation compared to regression models reflects correlations among the predictor variables. In particular, flowering time is positively and tightly correlated with senescence date ( $r = 0.94$ ,  $P < 0.0001$ ) and the effects of flowering time that are independent of senescence date are evaluated in the partial regression model only. In other words, in the partial correlation analyses, which examine correlations between traits and tolerance independent of their effect of fitness but do not account for correlations between traits, the strong positive correlation with senescence time may have overwhelmed the relationship between delayed flowering and tolerance. Phenological traits may underlie variation in tolerance because plants that flowered later may have accumulated more resources that would then be available for re-growth following damage. The associations between plastic changes in biomass or rosette diameter and tolerance also suggest that patterns of resource re-allocation in response to damage may be important to tolerating clipping. Specifically, the positive relationship between the difference in biomass between clipped and unclipped plants

and tolerance may imply that those genotypes that most effectively reallocate resources from roots to shoots are most tolerant. Such plants should have a greater (less negative) difference in biomass than genotypes that are less effective at reallocating resources or that have fewer below-ground resources to reallocate.

In contrast to the partial correlations and multiple regression analyses, standard correlation analyses revealed significant negative correlations between tolerance and rosette diameter, stem number, and biomass measured in the undamaged state, as well as plastic changes in these same traits following clipping. However, these significant Pearson correlations do not provide evidence for these traits being mechanisms of tolerance. The reason for this is that tolerance is a function of fitness, and thus any trait correlated with fitness also likely will be correlated with tolerance. Accounting for the correlation between fitness and tolerance offers an opportunity to identify traits that underlie variation in tolerance independent of their relationship with the fitness estimates used to estimate tolerance. Multiple regression in which fitness is included as an independent variable takes this one step further and also accounts for correlations between predictor variables that may obscure relationships between a particular trait and tolerance. Accordingly, we advocate the use of partial correlations and multiple regressions that include fitness as an independent variable for identifying the traits underlying tolerance to herbivory. These approaches should also be used to identify traits associated with plastic responses in general, not just tolerance to herbivore damage.

## Conclusion

The data from this study inform how changing resource availability may influence plant tolerance to herbivory. More generally our results highlight that predicting how global environmental changes affect plant populations requires an understanding of indirect effects, including effects on both the intensity and fitness consequences of species interactions. Our results show that elevated atmospheric CO<sub>2</sub> concentrations may affect plant–herbivore interactions not by altering the amount of damage plants experience, but by changing the fitness consequences of that damage, i.e., tolerance. These environmentally dependent shifts in tolerance may, in turn, have consequences for plant population dynamics, potentially reducing the beneficial impacts of increased resource availability on plant growth and reproduction.

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