

# Effects of nutrient and CO<sub>2</sub> availability on tolerance to herbivory in *Brassica rapa*

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**Abstract** The ability of plants to recover from herbivore damage and maintain their fitness depends on physiological mechanisms that are affected by the availability of resources such as carbon and soil nutrients. In this study, we explored the effects of increased carbon and nutrient availability on the response of rapid cycling *Brassica rapa* to damage by the generalist herbivore, *Trichoplusia ni* (Noctuidae), in a greenhouse experiment. Using fruit mass as an estimate of plant fitness, we tested three physiological models, which predict either an increase or a decrease of tolerance to herbivory with increasing resource availability. We used leaf demography to examine some plausible mechanisms through which resource availability may affect tolerance. Our results contradict all models, and, rather, they support a more complicated view of the plasticity of resource uptake and allocation than the ones considered by the models tested. Fruit mass was negatively affected by herbivore damage only under elevated CO<sub>2</sub>, and only for certain harvest dates. Increased CO<sub>2</sub> had no effect on the number of leaf births, but it decreased leaf longevity and the total number of leaves on a plant.

Nutrient addition increased the number of leaf births, leaf longevity and the total number of leaves on a plant. We conclude that a shortening of the life span of the plants, brought about by elevated CO<sub>2</sub>, was responsible for a higher susceptibility of plants to herbivore damage under high CO<sub>2</sub> concentration.

**Keywords** Compensation · Elevated CO<sub>2</sub> · Insect damage · Leaf demography · Growth rate model · Continuum of responses model · Resource limitation model

## Introduction

The removal of plant tissue by herbivores is generally thought to have a negative effect on plant fitness (Harper 1977; Belsky 1986; but see Marquis 1992). However, a series of factors may influence both the magnitude and direction of the fitness effects of herbivory, including the intensity, timing and spatial pattern of the damage, the type of structures damaged, the frequency of attack, plant developmental stage, and the availability of resources such as light, nutrients, and water (Whitham et al. 1991). Some studies that have found positive or negligible effects of herbivory have been criticized for measuring growth rather than a more direct measure of fitness (e.g., seed production), or for using mechanical damage to simulate herbivory, a procedure that does not necessarily elicit the same responses in

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plants as real herbivore damage (Belsky 1986; Baldwin 1990; Karban and Baldwin 1997; see also Tiffin and Inouye 2000; Tiffin 2000; Lehtilä 2003; Inouye and Tiffin 2003). Nevertheless, careful studies have demonstrated that plants do not always suffer a negative impact due to herbivory in terms of variables that are convincingly good measures of plant fitness, either through female or male function (Dyer et al. 1993; Gronemeyer et al. 1997; Lennartsson et al. 1998; Paige 1999; Paige et al. 2001; Avila-Sakar et al. 2003). Therefore, recent studies of plant defence consider not only traits that decrease the frequency of herbivore attack (resistance), but also the ability of plants to reduce the detrimental effects of herbivory on their fitness (tolerance) (Trumble et al. 1993; Strauss and Agrawal 1999; Pilson 2000; Juenger and Lennartsson 2000; Tiffin 2000; Stowe et al. 2000; Strauss et al. 2003; Leimu and Koricheva 2006).

The main traits or physiological mechanisms proposed to confer plants with tolerance to herbivory are: increased net photosynthetic rate after damage, high relative growth rates, increased branching due to the release from apical dominance, availability of ample carbon stored in roots for future reproduction, the ability to shunt carbon from storage in roots to shoots after damage, and plant architecture (Strauss and Agrawal 1999; Tiffin 2000; Haukioja and Koricheva 2000; Stowe et al. 2000). While the availability of resources such as nutrients, light, water and carbon clearly influences the aforementioned physiological mechanisms, it is still not clear whether plants are more or less tolerant to herbivory when resources are readily available (Strauss and Agrawal 1999; Hawkes and Sullivan 2001; Wise and Abrahamson 2005).

Hilbert et al. (1981) argue that the relative growth rate (RGR) of a plant can be used as a measure that summarizes the physiological mechanisms that allow plants to compensate for herbivore damage, and they developed a mathematical model that shows that it is easier to compensate (or overcompensate) for herbivore damage for plants growing at low RGRs than for plants growing at high RGRs at the time of defoliation. This model has been called the growth rate model (GRM) (Hicks and Turkington 2000). In terms of resource availability, plants growing under conditions that reduce their RGR (e.g., low nutrient or carbon availability) should be more tolerant to

herbivory than plants growing under conditions that favour a high growth rate.

In contrast to the GRM, the compensatory continuum hypothesis (CCH) (Maschinski and Whitham 1989; Whitham et al. 1991; Hicks and Turkington 2000), predicts that plants will be more likely to compensate for tissue lost to herbivores under conditions of high resource availability (water, nutrients and light). According to the CCH, a scarcity of the resources needed by the plant to construct new tissues would make it difficult for plants to recover from damage. Therefore, plants growing under higher resource availability should be more tolerant than those growing under conditions of low resource availability.

Clearly, the predictions of these two models are contradictory. Moreover, both models fail to address the possibility that different kinds of resources may affect tolerance of herbivory differently, and that herbivore damage may affect the acquisition of one resource more than other. The recently proposed Limiting Resource Model (LRM), addresses these points for the case of two resources, and shows that plants may display greater, equal or lower tolerance in response to increased resource levels depending on which of seven possible scenarios occurs (Wise and Abrahamson 2005). According to the LRM, as the abundance of one resource increases, plant performance may become limited by a second resource. However, since herbivory may or may not affect the relative availability/uptake of both, either, or none of the resources, the predictions of the LRM require some basic knowledge of the responses of plants to elevated levels of the resources in question and the effects of herbivory on the availability of such resources.

Changes in resource availability affect many different aspects of plant development in addition to resource uptake and growth rate. Increased nitrogen availability, for example, not only enhances growth, but also it prolongs juvenility and delays flowering and senescence (Addicott 1968; Smart 1994). Elevated CO<sub>2</sub> also enhances growth, but may have the opposite effect to increased nitrogen on the rate of development, hastening both flowering and senescence (St. Omer and Horvath 1983). Such effects, though, seem to vary from one species to another, precluding generalisations at this point (Farnsworth and Bazzaz 1995; Thomas et al. 1999). Differences

among resources in their impact on development may well affect the capacity of the plant to compensate for damage. Previous studies have found that compensation for herbivore damage is dependent upon the amount of time that elapses between the damage and the time at which plant fitness is assessed (usually, the end of the growing season) (Whitham et al. 1991; Tiffin 2000; Boege 2005). Since compensation for herbivore damage is often dependent upon the production of new vegetative tissue (e.g., new leaves to replace those removed by a herbivore), plants which mature more slowly and remain in the vegetative state for a longer period of time, may be in better position to compensate for damage provided the growing season is of sufficient length. On the other hand, plants that mature more rapidly and switch to reproductive growth early in the season may be less able to compensate for herbivore damage.

The main goal of this study was to explore the effects of resource availability on tolerance to herbivory in *Brassica rapa*. In particular, we asked (1) whether an increase in carbon or nutrient availability increased or decreased tolerance, and (2) whether any of the proposed models for the influence of resource availability on tolerance to herbivory (GRM, CCH or LRM) appropriately explained the response of *Brassica rapa* to these resource treatments. We examined the time course of fruit maturation in detail and used leaf demography to describe the temporal pattern of growth and senescence since we anticipated an important effect of resource availability and herbivory on the rate of plant development. In addition to increasing our understanding of the mechanisms underlying the responses of plants to herbivory, this study should provide some insight into the expected changes in plant responses to herbivore damage under atmospheric CO<sub>2</sub> concentrations predicted by global change models.

## Methods

### Plant material

In order to use life-time seed production as a measure of individual fitness, we used the “standard” rapid cycling variety of *Brassica rapa* L. (wild mustard, Brassicaceae; Wisconsin Fast Plants, Carolina

Biological Supplies, Inc., Madison), which is characterized by rapid onset of flowering and seed maturation. *Brassica rapa* is an annual plant, originally from Eurasia, but naturalized worldwide as a crop or weed (Pilson 2000). In North America, it is attacked by a wide variety of herbivores, including flea beetles (*Phyllotreta* spp.) and weevils (*Ceutorhynchus* spp.), and several generalist herbivores such as *Plutella xylostella* (Pilson 2000; Siemens et al. 2002).

Plants for this experiment were grown from one of several full-sib families generated through manual pollination for a larger experiment. We randomly chose one family with higher pod production in order to have enough seeds for the experiment. While the use of a single family precluded us from estimating tolerance to herbivory as the family-by-damage treatment interaction (or the area under the curve for each family, see Pilson 2000), it increased the genetic uniformity of the experimental material, thus allowing us to examine the plasticity of the response to herbivory in a set of closely related genotypes grown under contrasting conditions of carbon dioxide and nutrient availability.

### Experimental design

We grew a total of 160 plants under high and low carbon dioxide treatments, and under high and low nutrient treatments in a split plot design with two replicates of each CO<sub>2</sub> level (40 plants × 2 fertilizer levels × 2 CO<sub>2</sub> levels). On July 19, 2004, we planted seeds individually in 10 cm pots filled with a soil mix of peat moss, vermiculite, and perlite (Promix30 VPW30, ASB-Greenworld Ltd., Pointe Sain, New Brunswick). We placed 40 pots in each of four controlled environment greenhouse rooms. Two of the rooms were set at normal, atmospheric CO<sub>2</sub> concentration (~375 ppm), and two, at high CO<sub>2</sub> concentration (700 ppm). Temperature was controlled to follow outside conditions. Twenty plants in each greenhouse were supplied once per week with 0.5 l of 20:20:20 N:P:K fertilizer (Plant Products Company Ltd., Brampton, Ontario) at 100 ppm for the duration of the experiment. This was the high fertilizer treatment. The other half of the plants received no additional fertilizer and this was the low fertilizer treatment. To avoid positional effects, we rotated the plants within greenhouse rooms once a

week and among rooms along with CO<sub>2</sub> levels on a monthly basis. Plants were watered when the soil felt dry to the touch.

On July 29, we subjected half of the plants in each fertilizer × CO<sub>2</sub> combination (nested in greenhouse room) to herbivory by third or fourth instar larvae of the generalist herbivore *Trichoplusia ni* (Lepidoptera: Noctuidae). We placed one larva on each plant in the herbivory treatment and let it feed on the leaves until approximately 15% of the original leaf area was removed, as assessed by visual estimation. The length of time the larvae fed was recorded for each plant. The actual amount of leaf area removed was later measured (see below). Larvae were contained on the plants by means of a cage made out of a plastic cup and bridal veil that allowed air exchange. During the feeding trial, the control plants were also covered with cages. We performed the feeding trial working on the two rooms of one replicate of the CO<sub>2</sub> treatment before moving to the next replicate. The first replicate was done during the morning and the second replicate was done in the afternoon of the same day. Each feeding trial lasted for approximately 2 h.

Leaf area measurements were taken using a transparent dotted grid with dots 2 mm apart. This procedure took about 1 h for all 80 plants (herbivory and control) in each replicate. Measurements were recorded as the number of dots covered by the leaf area. A regression between the measurements obtained with this technique and those obtained using a LI-3100 leaf area metre (Li-Cor, Inc. Nebraska) was highly significant ( $F = 1816.03$ ,  $P < 0.0001$ ,  $R^2 = 0.93$ ). Both experimental and control plants were measured before and after feeding. In a pilot experiment, leaf expansion after 6 h was significantly greater than zero. Therefore, after the feeding trial, plants in the herbivory treatment were measured before the controls to reduce any possible underestimation of the area removed due to leaf expansion during the approximately three hours that the feeding and measuring of each replicate took. We tested whether the amounts and proportion of leaf area removed by herbivores differed among treatments before proceeding with further analyses.

*Trichoplusia ni* eggs were obtained from the insect rearing facility of the Southern Crop Protection and Food Research Centre of Agriculture and Agri-Food Canada (London, Ontario) and reared on artificial diet

(Adkisson et al. 1960; Webb and Shelton 1988). No food was provided to the larvae for 8 h before being placed on the experimental plants.

### Seed collection

The flowers of the mature plants were pollinated by two bumble bee colonies that were moved between the four greenhouses daily, giving each greenhouse a colony every second day. Colonies were obtained from Koppert Biological Systems Canada Limited (Scarborough, Ontario). Bee colonies became inactive the second week of October due to the cold temperature. At this time very few new flowers were being produced. Once pods began to mature (indicated by green pods turning yellow) they were collected daily and placed in labelled coin envelopes. The experiment was terminated 105 days after planting. All remaining pods were collected at this point, regardless of maturity. By this time, only 11 plants had pods, and for these plants, the remaining pods typically amounted to less than 10% of the total number of pods produced. Pods were weighed and counted to give a measure of total reproductive output.

### Leaf demography

Due to time constraints, leaf demography was monitored only on half of the plants. From each greenhouse 10 high fertilizer and 10 low fertilizer plants were randomly chosen, half of each in the damaged and control herbivory treatments. Once a week the plants were observed and new leaves were marked with tags made by looping colour-coded wire around the petiole of the leaf or stem above the leaf. A different colour tag was used for each week. A leaf was considered “born” once it had reached 1.5 cm in length, as this was considered a reasonable size to support a tag. A leaf was considered dead when it finished turning yellow (no remaining green area). At this point the tag was removed from the leaf. The dates of birth and death were recorded for all leaves.

### Statistical analysis

We used a split plot design with CO<sub>2</sub> level as the between-chamber factor, and two replicates of each CO<sub>2</sub> level, for a total of four phytotron chambers. The

chamber factor was entered as a random effect since CO<sub>2</sub> treatments were assigned randomly to chambers (chamber was nested within CO<sub>2</sub> level). All other effects were considered fixed. All possible interactions were entered in the models, but they are only reported when significant.

We examined the effects of herbivore damage, nutrient and CO<sub>2</sub> availability on daily cumulative fruit mass, as well as the number of leaves on the plant (a measure of size and vigour), and leaf births at different times during the growth period by means of multivariate analysis of variance for repeated measures (GLM procedure of SAS, SAS Institute 1988; see also von Ende 1993). Fruit mass was square root transformed prior to analysis to improve normality. While we had weekly values for the number of leaves on each plant, we pooled the data for weeks 8–14 since many of the plants had already lost all of their leaves after week 8. Leaf number data were log-transformed to homogenize variances and improve normality. Leaf birth data for weeks 5–9 were also pooled, following the same rationale. We analysed leaf longevity using a log-rank survival test (LIFE-TEST procedure of SAS). A separate analysis was performed on the first four leaf cohorts. Cohorts 5–9 were pooled for the analysis. This is a right-censored analysis, and therefore, survival can be underestimated. GLM was also used to test whether nutrient and CO<sub>2</sub> treatments affected the feeding rate of larvae (amount of tissue removed per larvae per unit time), and to confirm that the damage treatment was equal for plants in both nutrient and CO<sub>2</sub> levels in terms of the proportion of leaf area removed by *T. ni* larvae. Since only half of the plants were subjected to the herbivory treatment, the sample size for these analyses was 80. For all other analyses, the sample size was 158 since two of the plants died before the end of the experiment.

## Results

### Feeding rate and leaf area removed

We did not find any effects of CO<sub>2</sub> or fertilizer treatment either on the feeding rate of *T. ni* larvae or on the proportion of leaf area they removed. The damaged plants in all four treatment combinations of nutrient and CO<sub>2</sub> levels had  $15.0 \pm 0.9\%$  (mean  $\pm$  SE)

of their leaf area removed. Due to normal growth during the time of the feeding trial, the undamaged plants increased their total leaf area between 1.5 and 2.5%, with a mean of  $1.8 \pm 0.4\%$ . In this analysis, we also detected a significant block effect (nested in CO<sub>2</sub>), indicating that the replicate glasshouse rooms did differ sufficiently in environment to impact plant growth, and confirming the necessity of using a split-plot design to analyse treatment effects. Although significant block effects were found in most of the analyses below, no further mention of them will be made as they were not the focus of this study.

### Fruit mass

The repeated-measures analysis of variance on cumulative fruit mass detected significant between-subjects effects of CO<sub>2</sub> and damage, and within-subjects effects of time, time  $\times$  CO<sub>2</sub>, time  $\times$  fertilizer, time  $\times$  fertilizer  $\times$  CO<sub>2</sub>, and time  $\times$  damage  $\times$  fertilizer  $\times$  CO<sub>2</sub> (Table 1). Put simply, the pattern of daily accumulation of fruit mass varied depending on the particular combination of damage, fertilizer, and CO<sub>2</sub> (Fig. 1).

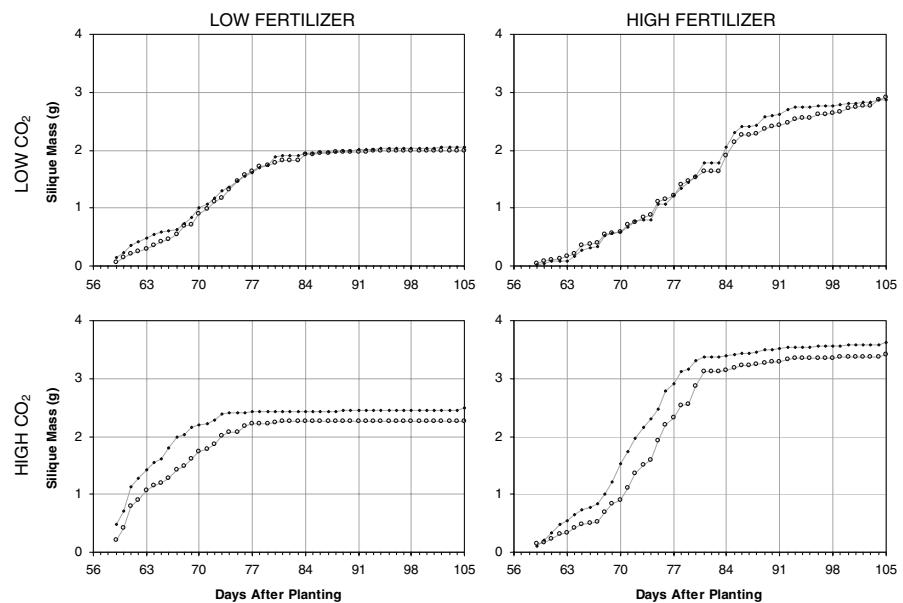
Plants in the high fertilizer treatment attained a greater final fruit mass, although it took them longer to reach their maximum fruit mass, than plants grown under low fertilizer conditions (Fig. 1). Averaged across the other treatments, plants in the low fertilizer treatment reached 90% of their final fruit mass on day 74 compared to day 84 in the high fertilizer treatment, a difference of 10 days. Elevated CO<sub>2</sub> also increased fruit mass, but the magnitude of this increase (9.8%) was much smaller than that observed for high fertilizer (21.5%). In contrast to the effect of fertilizer, plants grown under high CO<sub>2</sub> concentrations accumulated fruit mass faster than those in the low CO<sub>2</sub> treatment (Fig. 1). Plants achieved 90% of their final fruit mass on day 83 in the low CO<sub>2</sub> treatment compared to day 75 in the high CO<sub>2</sub> treatment, a difference of 8 days.

In general, damage by herbivores reduced fruit mass, but this effect was highly variable depending upon time of sampling and resource treatment (Fig. 1). Under low CO<sub>2</sub>, the curves of fruit mass accumulation of damaged and undamaged plants overlapped almost completely, except for a period of about 6 days at the beginning of the reproductive period in the low fertilizer treatment, and also during

**Table 1** Repeated-measures multivariate analysis of variance for fruit mass

Source	MS	F	df	P
<i>Between subjects</i>				
CO <sub>2</sub>	155.30	77.35	1	0.0127
CO <sub>2</sub> × block (error term for CO <sub>2</sub> )	2.00	0.96	2	0.3854
Damage	10.03	4.79	1	0.0301
Fertilizer	0.83	0.40	1	0.5295
Damage × fertilizer	0.05	0.02	1	0.8760
Damage × CO <sub>2</sub>	5.09	2.43	1	0.1209
Fertilizer × CO <sub>2</sub>	0.56	0.27	1	0.6049
Damage × fertilizer × CO <sub>2</sub>	0.44	0.21	1	0.6466
Error	2.09		148	
Source	Wilks' lambda	F	df	P
<i>Within subjects</i>				
Time	0.0387	61.2	43, 106	<0.0001
Time × CO <sub>2</sub>	0.4163	3.46	43, 106	<0.0001
Time × CO <sub>2</sub> × block	0.2467	2.5	86, 212	<0.0001
Time × damage	0.7286	0.92	43, 106	0.6155
Time × fertilizer	0.2716	6.61	43, 106	<0.0001
Time × damage × fertilizer	0.7178	0.97	43, 106	0.5339
Time × damage × CO <sub>2</sub>	0.6623	1.26	43, 106	0.1734
Time × fertilizer × CO <sub>2</sub>	0.4202	3.4	43, 106	<0.0001
Time × damage × fertilizer × CO <sub>2</sub>	0.5791	1.79	43, 106	0.0084

**Fig. 1** Fruit mass accumulation during the course of the experiment (means per fertilizer and CO<sub>2</sub> treatment in this and following figures). Legend: dots: control plants; open circles: damaged plants. Straight lines joining consecutive data points have been added as a visual aid to follow the behaviour of plants in each treatment in this and following figures



approximately 12 days at the end of the reproductive period in the high fertilizer treatment. Prior to reproductive maturity, damaged plants grown at

elevated CO<sub>2</sub> generally had lower fruit mass than undamaged plants. For example on day 71, herbivory resulted in an 18% decrease in fruit yield at high CO<sub>2</sub>

(averaged across fertilizer levels) compared to a 0.4% increase in fruit yield at low  $\text{CO}_2$  ( $P = 0.0165$  for  $\text{CO}_2 \times$  damage interaction in the univariate ANOVA for that date). However, at reproductive maturity, there were no significant effects of damage on fruit yield in any of the resource treatments. For example, by day 85 when plants had more or less reached their maximum fruit yield, herbivory resulted in a 3.7% decrease in fruit yield (averaged across all resource treatments) ( $P = 0.1466$  for damage in the univariate ANOVA for that date). Fertilizer had relatively little effect on the damage resulting from herbivory, but it did affect the pattern described above by decreasing the negative effect of herbivory that was observed early in the experiment. The reduction in fruit mass due to damage that was observed at elevated  $\text{CO}_2$  was, on average, slightly less at the high fertilizer level (Fig. 1, see the time  $\times$  damage  $\times$  fertilizer  $\times$   $\text{CO}_2$  interaction in Table 1). However, the only day on which this fertilizer  $\times$  damage interaction was significant at the 0.05 level (as determined by the univariate ANOVA) was day 59 ( $P = 0.0022$ ). On this particular day, the effect was quite large with damage causing a 45.6% decrease in fruit mass at low nutrient levels compared to an almost imperceptible increase at the high fertilizer level. It should be noted, however, that levels of reproductive output this early in the experiment were quite low.

In contrast to the fertilizer and  $\text{CO}_2$  treatments, herbivory had relatively little effect on time of fruit maturation. Plants achieved 90% of their final fruit

mass on day 78 in the control treatment versus day 80 in the damaged treatment, a difference of only 2 days.

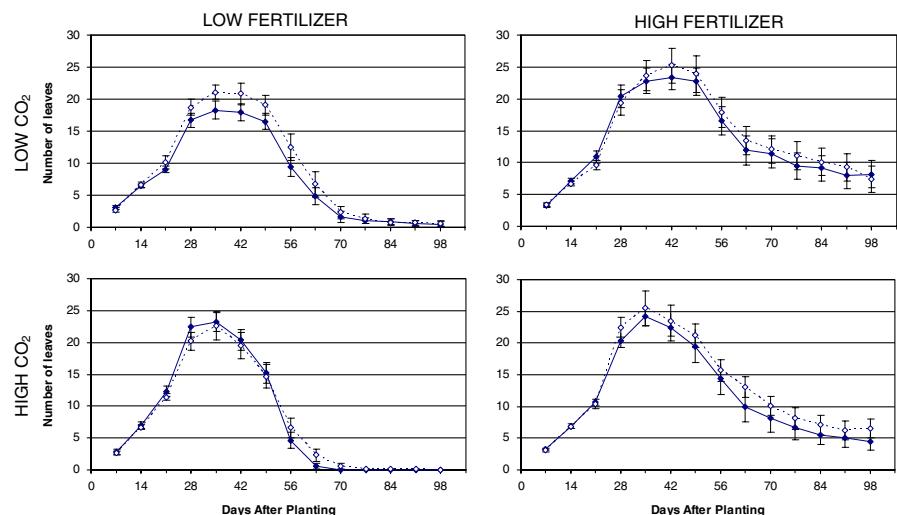
#### Number of leaves

We found a significant between-subjects effect of fertilizer ( $F_{1, 70} = 29.21, P < 0.0001$ ), as well as significant within-subjects effects of time (*Wilks' lambda* = 0.0227,  $F_{7, 64} = 393.12, P < 0.0001$ ), time  $\times$   $\text{CO}_2$  (*Wilks' lambda* = 0.6461,  $F_{7, 64} = 5.01, P = 0.0001$ ), and time  $\times$  fertilizer (*Wilks' lambda* = 0.4381,  $F_{7, 64} = 11.73, P < 0.0001$ ), but no time  $\times$  damage effects were detected. Plants grown under high fertilizer had a few more leaves than those in low fertilizer, especially at the end of the growing season (days 56–98; Fig. 2). High  $\text{CO}_2$  level accelerated the rate of leaf loss (e.g., within low fertilizer conditions, plants grown at high  $\text{CO}_2$  had about five fewer leaves by day 56 than those under low  $\text{CO}_2$ , Fig. 2).

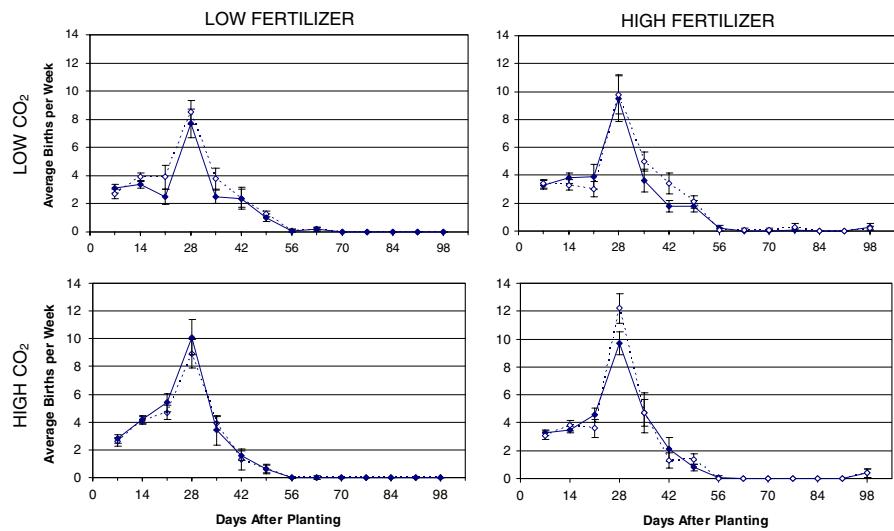
#### Leaf births

The number of leaf births was low throughout the growing season, except for week 4, when there was a peak of leaf production (Fig. 3). We found a significant between-subjects effect of fertilizer on the number of leaf births ( $F_{1, 69} = 5.51, P = 0.0217$ ), and significant within-subjects effects of time (*Wilks' lambda* = 0.1845,  $F_{4, 66} = 72.93, P < 0.0001$ ), time  $\times$   $\text{CO}_2$  (*Wilks' lambda* = 0.8075,  $F_{4, 66} = 3.93,$

**Fig. 2** Mean number of leaves present on the plants each week. Error bars represent  $\pm 1$  SE. Legend as in Fig. 1



**Fig. 3** Mean number of leaf births per week. Error bars represent  $\pm 1$  SE. Legend as in Fig. 1



$P = 0.0063$ ), and time  $\times$  fertilizer (*Wilks' lambda* = 0.8564,  $F_{4, 66} = 2.77$ ,  $P < 0.0345$ ). The effects of added fertilizer and CO<sub>2</sub> on leaf production were positive but small in magnitude and varied throughout the experiment: Plants in the high fertilizer treatment had more leaf births than those in the low fertilizer treatment (evident for weeks 1, and pooled data of weeks 5–9, marginal for week 4, univariate ANOVAS not shown). Similarly, plants grown under elevated CO<sub>2</sub> had more leaf births, especially during week 3. No effects of the damage treatment were detected (Fig. 3).

#### Leaf longevity

The tests of equality over strata (defined by the levels of CO<sub>2</sub>, fertilizer and damage treatment) were highly significant for all leaf cohorts analysed (Table 2). This indicates that at least two of the eight survival curves examined in each cohort differed significantly

from each other. In order to examine the effects of damage, CO<sub>2</sub> and fertilizer on leaf longevity, we performed the following eight pair-wise comparisons of the mean longevity, and considered the difference significant if the 95% confidence intervals of the means did not overlap: between damage treatments within each combination of CO<sub>2</sub> and fertilizer levels (four comparisons), between CO<sub>2</sub> levels within each fertilizer level (two comparisons within undamaged plants only), and between fertilizer levels within each CO<sub>2</sub> treatment (two comparisons within undamaged plants only).

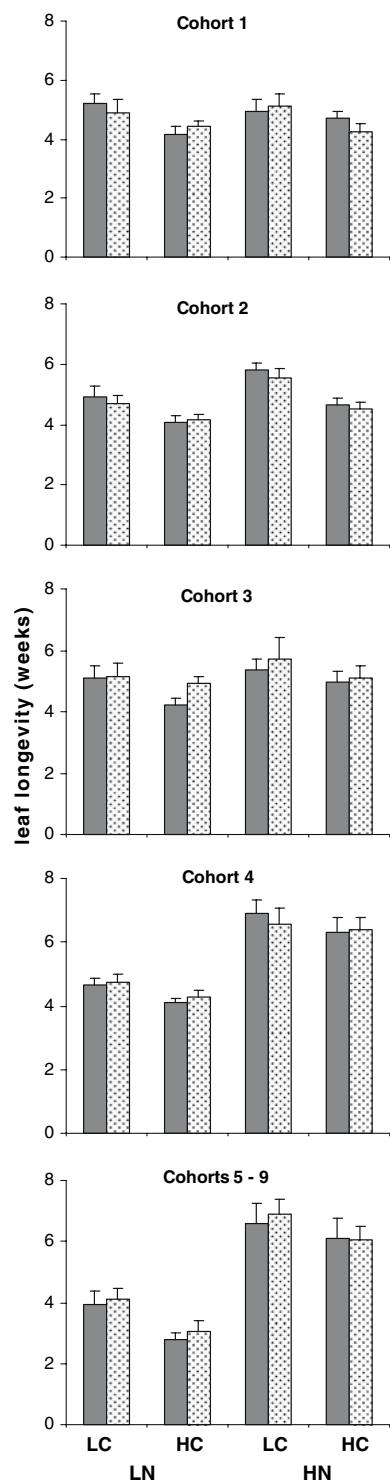
In all cohorts, leaf longevity decreased significantly (senescence accelerated) under high CO<sub>2</sub> for those plants grown at low fertilizer level (Fig. 4). The same trend is evident at high fertilizer level, but it is only significant in cohort 2. Nutrient addition increased leaf longevity at both high and low CO<sub>2</sub> levels in cohorts 4 and 5, and also in cohort 2, but only under low CO<sub>2</sub>. Herbivore damage did not affect leaf longevity, except in plants of cohort 3 in the low fertilizer—high CO<sub>2</sub> treatment. For these plants, damage seemed to counteract the negative effects of the high CO<sub>2</sub> level on leaf longevity, and restore it to the levels of undamaged plants in the low CO<sub>2</sub> treatment (Fig. 4).

#### Discussion

Using cumulative fruit mass as an estimate of plant fitness, our results show that an increase in CO<sub>2</sub>

**Table 2** Log-rank tests of equality over strata for leaf longevity of five cohort groups of leaves during the experiment

Cohort	Chi-square	df	P
1	40.43	7	<0.0001
2	111.27	7	<0.0001
3	55.26	7	<0.0001
4	266.02	7	<0.0001
5–9	266.46	7	<0.0001



**Fig. 4** Mean leaf longevity. Solid bars: control; hatched bars: damaged plants. Error bars are 95% CI. Legend: L: low; H: high; C: CO<sub>2</sub>; N: fertilizer

availability has the potential to decrease tolerance to herbivory: a negative effect of insect damage was detected on plants grown under high CO<sub>2</sub> early in their reproductive phase. This result was not an artifact of differential feeding rates or total leaf area removed between CO<sub>2</sub> treatments, as both of these variables were found to be similar across treatments. Tolerance to herbivory in the closely related *Arabidopsis thaliana* also decreased at high CO<sub>2</sub> levels in an experiment where plants were subjected to 20% leaf area removal by the specialist herbivore of Brassicaceae, *Plutella xylostella* (Bidart-Bouzat 2004; Bidart-Bouzat et al. 2004). In contrast, saplings of some hardwood tree species have been reported to display greater tolerance under elevated CO<sub>2</sub> levels (Knepp et al. 2005), but this response was not consistent over time. In general, it is expected that woody plants should be more tolerant of herbivory than herbs (Haukioja and Koricheva 2000). It should be noted that measuring tolerance to herbivory in terms of reduced vegetative growth, as it is in most studies of woody species, is fundamentally different from measuring tolerance in terms of reduced reproduction. When tolerance is measured in terms of reduced reproduction, the timing of the switch from vegetative to reproductive growth in relation to the herbivory event becomes critical as sufficient time for the recovery of lost resources is required. If the initial switch to reproductive growth is relatively early, and if the switch is abrupt (i.e., little vegetative growth after reproduction is initiated), then the plant will have little opportunity to compensate for the lost resources.

The fact that plants grown at high CO<sub>2</sub> reached their highest number of leaves earlier, had a more accelerated senescence of leaves, and accumulated fruit mass faster than those in low CO<sub>2</sub> (Figs. 1, 2), suggests that the switch from vegetative to reproductive growth was more abrupt in plants with an ample supply of CO<sub>2</sub> than those under low CO<sub>2</sub> conditions. Under the accelerated growth pattern at elevated CO<sub>2</sub>, the loss of leaf area to herbivores became an important disadvantage to the plants, decreasing the capacity of the plants to compensate for herbivory in the short term. However in the longer term, plants at elevated CO<sub>2</sub> were able to compensate for the tissue lost to herbivory; by the end of the experiment damage had no effect on fruit mass in any of the

treatments. While the switch to reproduction at elevated CO<sub>2</sub> was more abrupt than at low CO<sub>2</sub>, in neither case was it an absolute switch: vegetative growth continued for some time after reproduction was initiated at both high and low CO<sub>2</sub> (cauline leaves are produced along with the inflorescence). As a consequence, plants at elevated CO<sub>2</sub> were eventually able to compensate for the lost tissue; it just took longer for compensation to occur.

Earlier flower initiation and reduced plant longevity at elevated CO<sub>2</sub> have been reported for a number of other species (St Omer and Hovath 1983). Does this mean that all plants will display a reduced capacity to tolerate herbivory at elevated CO<sub>2</sub> when tolerance is assessed in terms of reproductive output? Earlier flowering at elevated CO<sub>2</sub> is not a universal response, some species show no effect, and in a few cases, a delay in the onset of reproduction at elevated CO<sub>2</sub> has been observed (Paez et al. 1980; Reekie and Bazzaz 1991; Farnsworth and Bazzaz 1995; Wand et al. 1996; Navas et al. 1997; Leishman et al. 1999). It is also important to note that the relationship between reproductive phenology and total seed output varies by species (Farnsworth and Bazzaz 1995; Kellogg et al. 1999). Thus, there is a need for thorough studies of the mechanisms underlying these responses before coming to any firm conclusions regarding the effect of elevated CO<sub>2</sub> on herbivory tolerance.

In contrast to the effect of CO<sub>2</sub>, nutrient addition had relatively little effect on tolerance to herbivory. Plants grown under high fertilizer attained greater final fruit mass, but this effect did not differ to any great extent between damaged and undamaged plants. The effect of fertilizer on the response to damage was restricted to modifying the impact of CO<sub>2</sub> level; the difference between damaged and undamaged plants at elevated CO<sub>2</sub> was slightly less at the high than at the low fertilizer level (i.e., high fertilizer increased tolerance in the short term). Based upon the above analysis of the effect of reproductive timing on herbivory tolerance, this fertilizer effect is to be expected as the high fertilizer treatment did prolong vegetative growth and slow the rate at which the fruit matured. However, the effect of fertilizer on tolerance was relatively small compared to the effect of CO<sub>2</sub> even though the effect of fertilizer on phenology was at least as important as the effect of CO<sub>2</sub> on phenology.

One possible explanation for the relatively weak effect of fertilizer on tolerance is the very positive effect it had on growth and eventually, fruit mass. It has been argued that the beneficial effects of nutrient supplementation on undamaged plants make it more difficult for damaged plants to attain similar levels of fruit mass (Rautio et al. 2005). Final plant size is simply the product of initial size and average relative growth rate. Therefore, the absolute difference in final size of two plants that differ in initial size will always be greater when the plants grow at a more rapid rate. Since herbivory essentially reduces the initial size of the plants and fertilizer increases the growth rate, it stands to reason that in the absence of compensatory mechanisms, fertilized plants will exhibit a greater effect of herbivory on final size, and consequently reproductive output. In other words, undamaged plants with nutrient supplementation are faster growing targets for the damaged plants to catch up to, and this limits the capacity of the plant to compensate for the tissue lost to herbivory. So fertilizer supplementation, on one hand, makes it more difficult for damaged plants to catch up with undamaged plants, but on the other, it delays the switch to reproductive phase and allows for more time to recover from herbivory.

Previous studies have shown that herbivory has the potential to delay senescence and rejuvenate vegetative growth (Hodgkinson 1974; Harper 1977; Meyer 1998; Tiffin 2000; McIntire and Hik 2002). If such were the case, the rejuvenating effects of herbivory could counteract the accelerated senescence brought about by high CO<sub>2</sub> (Hättenschwiler and Schafellner 1999; see below) and so reduce its negative impact on tolerance. However, in our study, insect damage had little effect on senescence. The only effect of insect damage was an increase of leaf longevity seen in cohort 3 under low fertilizer—high CO<sub>2</sub> conditions and its effect on the time course of fruit maturation was minimal relative to the effects of CO<sub>2</sub>.

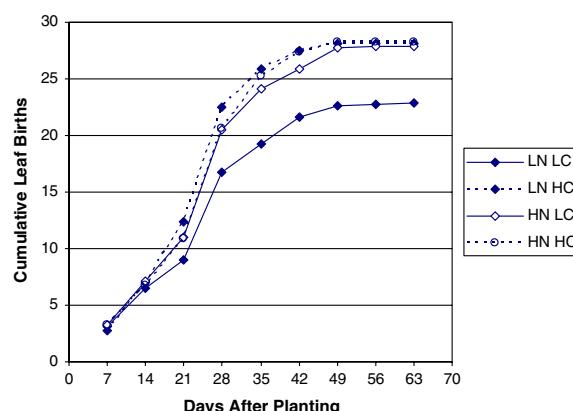
One of the most important outcomes of our study is that whether or not the plants were tolerant of herbivory was highly dependent upon when the plants were sampled. For example, plants sampled on day 59 were tolerant at high nutrient levels, but not at low levels, plants sampled on day 71 were tolerant at low CO<sub>2</sub> but not at high CO<sub>2</sub>, and by day 85, all plants were tolerant of herbivory regardless of resource treatment. It might be argued that the only relevant

time to measure herbivory tolerance is when all fruit are fully mature. However, in natural populations the end of the growing season is often determined by meteorological conditions such as an early frost or a severe drought (Oyama et al. 1993; Jordan and Smith 1995; Ehrman and Cocks 1996), rather than by the endogenous senescence of the plant.

Although the contrasting effects of CO<sub>2</sub> and nutrient availability on herbivory tolerance can be interpreted in light of their opposing effects on phenology as discussed above, correlation does not necessarily mean causation. Therefore, it is important to consider other possible explanations for these results. In this regard we will consider the three models that have been proposed in the literature to explain the relationship between resource availability and herbivory tolerance. According to the GRM, plants growing slowly should be more tolerant to herbivory than those growing fast (Hilbert et al. 1981). Using the cumulative number of leaf births as a measure of growth, we observed that those plants in the three treatment combinations in which fertilizer, carbon or both resources were added grew faster than plants under low fertilizer and low CO<sub>2</sub> conditions (Fig. 5). Therefore, according to the GRM, the control plants (low fertilizer—low CO<sub>2</sub>) should have been more tolerant than those in the other three treatment combinations. The fact that plants under the high fertilizer—low CO<sub>2</sub> treatment, which were growing faster, were just as tolerant as the controls

contradicts the GRM. According to the CCH, plants growing under an ample supply of resources should be more tolerant than those growing under more limiting conditions (Whitham et al. 1991). Clearly, the control plants were the ones growing under the most limiting conditions of all treatments, and therefore they should have been the least tolerant. Again, our results do not conform to this prediction: control plants were tolerant to herbivore damage, while plants with the most ample supply of nutrients and carbon (high fertilizer—high CO<sub>2</sub> treatment) were equally or less tolerant, depending upon when tolerance was assessed. Similarly, the predictions of the LRM (Wise and Abrahamson 2005) were not met in our experiment. Our results show that fruit production was limited by both nutrient and carbon availability. The LRM predicts that increased carbon availability should increase herbivore tolerance since the herbivore of interest removes leaf material and therefore has a negative impact on carbon uptake. On the other hand, the LRM predicts increased nutrient availability will lower herbivore tolerance since removal of leaf material decreases the uptake of an alternate resource (i.e., carbon). Neither of these outcomes was observed in our experiment.

Evidently, our ability to predict the circumstances under which tolerance to herbivory should increase or decrease is compromised by an incomplete understanding of the mechanisms through which resource availability affects the physiology of growth, reproduction and defensive responses. We concur with authors who advocate the continued cross-talk between molecular, genetic, physiological and ecological studies in order to gain a better understanding of the ecological factors that may alter plant responses to natural enemies (Baldwin and Preston 1999; Baldwin et al. 2001; Wise and Abrahamson 2005). In particular, we feel that a better understanding of how both resource treatments and herbivory affect reproductive timing and plant development will be critical in this endeavour.



**Fig. 5** Mean cumulative leaf births during the first 9 weeks of growth (few leaves were produced afterwards; (see Fig. 3). The slopes of the curves depict the mean relative growth rates of the plants in each treatment combination. Only the curves for control plants are shown. Legend: Treatment codes as in Fig. 4

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