Elevated CO_2 Increases Constitutive Phenolics and Trichomes, but Decreases Inducibility of Phenolics in *Brassica rapa* (Brassicaceae)

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Abstract Increasing global atmospheric CO_2 has been shown to affect important plant traits, including constitutive levels of defensive compounds. However, little is known about the effects of elevated CO_2 on the inducibility of chemical defenses or on plant mechanical defenses. We grew *Brassica rapa* (oilseed rape) under ambient and elevated CO_2 to determine the effects of elevated CO_2 on constitutive levels and inducibility of carbon-based phenolic compounds, and on constitutive trichome densities. Trichome density increased by 57% under elevated CO_2. Constitutive levels of simple, complex, and total phenolics also increased under elevated CO_2, but inducibility of each decreased. Induction of simple phenolics occurred only under ambient CO_2. Although induction of complex and total phenolics occurred under both ambient and elevated CO_2, the damage-induced increases were 64% and 75% smaller, respectively, under elevated CO_2. Constitutive phenolic levels were positively correlated with leaf C:N ratio, and inducibility was positively correlated with leaf N and negatively correlated with leaf C:N ratio, as would be expected if inducibility were constrained by nitrogen availability under elevated CO_2. We conclude that *B. rapa* is likely to exhibit higher constitutive levels of both chemical and mechanical defenses in the future, but is also likely to be less able to respond to herbivore damage by inducing phenolic defenses. To our knowledge, this is only the second study to report a negative effect of elevated CO_2 on the inducibility of any plant defense.

Introduction

Largely as a result of human activities, the concentration of carbon dioxide (CO_2) in Earth's atmosphere has risen from the pre-industrial level of 280 ppm to ~390 ppm today, and continues to increase at an accelerating rate (Conway and Tans, 2011). Because atmospheric CO_2 is expected to reach 700 ppm during this century and remain elevated for several centuries (Alley et al., 2007), plant responses to elevated CO_2 will be an important determinant of many ecological interactions into the foreseeable future.

Typical responses of plants to elevated CO_2 include increased photosynthesis and, therefore, increased biomass, decreased percent nitrogen, and increased carbon:nitrogen ratios, and altered investment in chemical defenses (Stiling and Cornelissen, 2007). The response of plant chemical defenses to elevated CO_2 is likely to be a particularly important determinant of plant-herbivore interactions in the future. Phenolic compounds are one of the most common types of chemical defense, and have been shown or implied to reduce consumption of plant tissue (Haukioja et al., 2002), and growth and survivorship of insect herbivores (Karowe, 1989; Haviola et al., 2007). Growth under elevated CO_2 increases phenolic levels in many tree species, including aspen, birch, oak, maple, eucalyptus, spruce, and pine (Tuchman et al., 2003; Lindroth, 2010).

Few studies have addressed the effect of elevated CO_2 on phenolics in herbaceous plants (Zvereva and Kozlov, 2006),

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and existing studies suggest that the response is less predictable than for trees. For instance, phenolics did not change under elevated CO_2 in hairy bittercress, common groundsel, or corn spurrey (Hartley et al., 2000), increased in alfalfa (Agrell et al., 2004) and ginger (Ghasemzadeh et al., 2010), increased or did not change in soybean (O'Neill et al., 2010) and increased or decreased in birdsfoot trefoil (Bazin et al., 2002; Goverde et al., 2004).

When damaged, many plant species increase levels of defensive compounds. Such chemical induction is thought to be an integral component of adaptive anti-herbivore chemistry (Karban and Baldwin, 1997). Phenolics are among the most inducible compounds in response to damage (Kaplan et al., 2008). Unfortunately, little is known about the effect of elevated CO_2 on the inducibility of plant defenses. Two opposing hypotheses seem reasonable to us: 1) that increased carbon availability will result in increased inducibility, due to increased substrate availability, or 2) that decreased nitrogen content will result in decreased inducibility, due to decreased availability of nitrogen-rich RNA and enzymes required for rapid induction.

While scores of studies have addressed the effect of elevated CO_2 on constitutive chemical defenses, to our knowledge only seven studies have addressed the effect of elevated CO_2 on the inducibility of plant defenses. Induction of phenolics was not affected by elevated CO_2 in aspen (Lindroth and Kinney, 1998) or oak (Rossi et al., 2004), nor was induction of flavonoids in soybean (O'Neill et al., 2010), phenolics and cyanide glycosides in birdsfoot trefoil (Bazin et al., 2002), or saponins and flavonoids in alfalfa, and terpenoid aldehydes in cotton (Agrell et al., 2004). To our knowledge, only two studies have detected an effect of elevated CO_2 on defense induction in response to damage. Bidart-Bouzat et al. (2005) observed an increase in induction of glucosinolates in *Arabidopsis thaliana* under elevated CO_2, and Zavala et al. (2008) observed a decrease in inducibility of soybean protease inhibitors under elevated CO_2.

In addition to chemical defenses, most plants possess mechanical defenses. Trichomes ("leaf hairs"), which are present in virtually every plant family (Marks, 1997), are the most common mechanical defense. Like phenolics, trichomes can reduce oviposition, consumption, growth, and survivorship of insect herbivores (Simmons and Gurr, 2005; Plett et al., 2010). Despite being identified by Pritchard et al. (1999) over a decade ago as a high priority subject, we are aware of only a handful of studies that address the effect of elevated CO_2 on trichomes. Existing studies report either a decrease (Bidart-Bouzat et al., 2005; Lake and Wade, 2009) or no change (Lau et al., 2008; Riikonen et al., 2010; Vannette and Hunter, 2011) in trichome density under elevated CO_2.

Brassica rapa (oilseed rape or canola) possesses both phenolic and trichome defenses and belongs to a genus that

has been the subject of much research on plant-herbivore interactions. In addition, *B. rapa* is ranked behind only soybean as the most important source of vegetable oil in the world (Raymer, 2002). In this study, we asked whether growth under elevated CO_2 affects constitutive levels and inducibility of phenolics, and density of trichomes in leaves of *B. rapa*.

Methods and Materials

Plant Growth Brassica rapa seeds were germinated in mid-June in the greenhouse at the University of Michigan Biological Station (UMBS) and transplanted 2 day later to 6-in pots containing topsoil. Plants were watered daily or as necessary, and were inspected periodically for insect herbivory; only undamaged plants were used. Plants were fertilized every 2 week with 100 ml full strength Peter's 20-20-20 to avoid nutrient stress. Three days after transplanting, 3 plants were randomly placed into each of 12 0.5-m^3 open-topped chambers maintained at ambient CO_2 (379±10 ppm) and each of 12 chambers maintained at elevated CO_2 (744± 12 ppm). Chambers were arranged into blocks, with each block containing one ambient and one adjacent elevated chamber. Within blocks, CO_2 treatment was randomly assigned to chambers. The chambers were located in a field near the UMBS greenhouse and were designed to maintain natural temperature and light conditions as much as possible (Karowe, 2007). CO_2 content was elevated by dispensing 100% CO_2 into the inlet port of an outlet blower connected by a dryer hose to the base of each elevated chamber. CO_2 levels were monitored by an infrared gas analyzer approximately every 25 min and CO_2 flow was manually adjusted by a flowmeter when needed.

All surviving plants ($N=27$ at each CO_2 level, including at least 2 from each chamber) were harvested after 40–44 day in the chambers. Constitutive and induced phenolic levels were quantified from opposite halves of the second fully expanded leaf. A razor blade was used to remove one randomly chosen half of the leaf, leaving the midrib intact. At the same time, a garlic press was used to damage 9 cm^2 in the middle of the 1st, 3rd, and 4th fully expanded leaves. This method allowed us to standardize the amount of damage across plants. Twenty-four hours later, the remaining half of the 2nd leaf was harvested. Immediately after removal, each leaf half was flash frozen in liquid nitrogen, stored at −80°C for 1 week, and freeze-dried. We believe that, relative to the usual approach of using different plants for constitutive and induced levels, this paired design is a more powerful method for detecting induction.

Total Phenolics After each leaf half was ground to a fine powder, 20 mg of tissue were washed x 3 with 1 ml diethyl

ether containing 1% (v/v) acetic acid to remove chlorophyll. After each wash, samples were centrifuged at 4,600 g for 10 min and the ether was removed. The resulting decolorized pellet was resuspended in 0.5 ml of 70% acetone to extract total phenolics, sonicated on ice for 30 min, and centrifuged at 4,600 g for 10 min. This was repeated x 3, and the 3 supernatants were combined and brought to a total volume of 1.5 ml using 70% acetone. Total phenolics were quantified using the Folin-Denis method. To each reaction tube, 0.5 ml of supernatant, 1 ml Folin-Denis reagent (75 mM sodium tungstate, 1.3 mM phosphomolybdic acid, 2.8% phosphoric acid), and 1 ml 2 N sodium carbonate were added. Samples were incubated for 1 hr at room temperature for color development. Sample absorbency was determined at 725 nm against a blank of deionized water (FAO/IAEA 2000). All samples were run in duplicate. Total phenolic levels were quantified by comparison to a standard curve prepared from tannic acid.

Simple Phenolics To determine levels of simple phenolics, tannins were removed by adding 100 mg of polyvinyl polypyrrolidone, which binds complex phenolics such as condensed dimers and tannins, to a 100×12 mm test tube containing 1.0 ml deionized water and 0.5 ml of the total phenolic extract (FAO/IAEA 2000, modified by M. Grant). After 15 min at 4°C, the extract was centrifuged at 4,600 g for 10 min, and the supernatant, presumably containing only simple phenols (phenolic acids, alcohols and amines, flavonoids, anthocyanogens, catechins, and flavanols), was collected. The Folin-Denis method was used to determine relative concentration of simple phenolics. Complex phenolics were calculated as the difference between total and simple phenolics measured from the same extract.

Carbon and nitrogen contents were determined for each ground leaf sample using a Perkin Elmer CHN Elemental Analyzer (Waltham, MA, USA).

Trichomes From a different set of plants than those used for C, N, and phenolic analyses ($N=115$ plants at ambient CO_2 and 119 plants at elevated CO_2), trichomes on the lower leaf surface from two 0.9 cm^2 disks removed from the distal tip of the second fully expanded leaf were counted under a dissecting microscope. Trichome counts from the two disks per leaf were averaged.

Statistical Analysis The experimental design was a split-plot with repeated measures, with CO_2 as whole plots, and damage as the repeated measure, since carbon, nitrogen, C:N ratio, and phenolics were measured on opposite (pre- and post-damage) halves of the same leaf (Littell et al., 1998). Variables were analyzed with a *Type III ANOVA* (PROC MIXED) with CO_2 level and damage as fixed effects, and block and the CO_2*block interaction as random

effects (SAS Institute, 2000). To further determine whether phenolics were inducible at each CO_2 level, paired *t*-tests were used to compare phenolic levels in the half of a leaf sampled immediately before damage and the opposite half of the same leaf sampled 24 hr after damage. To determine whether CO_2 affected inducibility, the magnitude of change in phenolic levels was compared between CO_2 levels by independent samples *t*-tests. Normality of inducibility values was verified by *Kolmogorov-Smirnov* tests and equality of variances was verified by F_{max} tests.

To determine whether leaf percent N influenced constitutive phenolic levels and/or magnitude of phenolic induction, these two variables were regressed against leaf N and C:N ratio.

Trichome density was measured only on undamaged plants. Since variances were unequal and data were not normal, mean trichome density was compared between CO_2 levels by a *Mann–Whitney U* test. The proportion of leaves with at least one trichome was compared between CO_2 levels by a *chi-square* test.

Results

Nutritional Quality Under elevated CO_2, nitrogen content of *B. rapa* leaves decreased significantly (by 16%, from 2.70 to 2.29% of dry weight, means for constitutive and induced combined) and C:N ratio increased significantly (by 20%, from 15.47 to 18.11) (Table 1). Carbon content averaged 40.17% and was not affected by CO_2 level. No measured component of leaf nutritional quality was affected by damage, or showed a significant CO_2*Damage interaction.

Constitutive Phenolics When both constitutive and induced levels were considered together, growth of *B. rapa* under elevated CO_2 did not result in a significant change in levels of simple, complex, or total phenolics (in each case, $P>0.2$ for the overall CO_2 effect; Table 1). However, when only constitutive levels were considered, elevated CO_2 resulted in a 17% increase in complex phenolics ($t=2.83$, $P=0.007$) and a 14% increase in total phenolics ($t=2.76$, $P=0.008$). In contrast, constitutive levels of simple phenolics were not affected by growth of *B. rapa* under elevated CO_2 ($t=1.65$, $P=0.10$).

Inducibility of Phenolics When all plants were considered together, damage resulted in significant increases in complex (by 20%) and total phenolics (by 14%), but did not affect levels of simple phenolics (see the Damage effect in Table 1). ANOVA analysis detected an effect of CO_2 on inducibility, indicated by the significant CO_2*Damage interaction for each phenolic variable in Table 1. Paired *t*-tests at each CO_2 level revealed that simple, complex, and total

Table 1 Constitutive and induced levels (as a percent of leaf dry weight; mean ± SD) of nitrogen, carbon, C:N ratio, and simple, complex, and total phenolics in *Brassica rapa* leaves grown under ambient and elevated CO_2

Variable	CO_2 level	Damage	Mean ± SD [a]	Effect	Significance[b]
Nitrogen	Ambient	Constitutive	2.67±0.47	CO_2	**<0.001**
		Induced	2.73±0.58	Damage	0.42
	Elevated	Constitutive	2.25±0.38	CO_2*Damage	0.90
		Induced	2.33±0.43		
Carbon	Ambient	Constitutive	39.99±1.42	CO_2	0.33
		Induced	40.04±1.40	Damage	0.74
	Elevated	Constitutive	40.29±1.03	CO_2*Damage	0.95
		Induced	40.37±1.04		
C:N ratio[c]	Ambient	Constitutive	15.50±3.20	CO_2	**<0.001**
		Induced	15.44±3.91	Damage	0.55
	Elevated	Constitutive	18.35±3.06	CO_2*Damage	0.81
		Induced	17.87±3.27		
Simple phenolics	Ambient[**d]	Constitutive	0.35±0.04	CO_2	0.83
		Induced	0.39±0.05	Damage	0.40
	Elevated	Constitutive	0.37±0.06	CO_2*Damage	**0.028**
		Induced	0.34±0.10		
Complex phenolics	Ambient[***]	Constitutive	0.58±0.10	CO_2	0.26
		Induced	0.77±0.14	Damage	**<0.001**
	Elevated[**]	Constitutive	0.68±0.15	CO_2*Damage	**0.015**
		Induced	0.75±0.14		
Total phenolics	Ambient[***]	Constitutive	0.94±0.12	CO_2	0.36
		Induced	1.15±0.17	Damage	**<0.001**
	Elevated[*]	Constitutive	1.05±0.16	CO_2*Damage	**0.008**
		Induced	1.11±0.19		

[a] Sample size is 27 for each CO_2 × Damage combination

[b] *P*-values from *ANOVA* analyses; significant effects are in bold

[c] Data were normalized by log transformation prior to analysis

[d] Induction was significant at this CO_2 level by a paired *t*-test; *P*<0.05 (*), *P*<0.01 (**), or *P*<0.001 (***); see Fig. 1

phenolics were all more inducible under ambient CO_2 than under elevated CO_2. In response to damage, simple phenolics increased by 11% under ambient CO_2 (*paired t*=3.03, *P*=0.006), but did not change under elevated CO_2 (*paired t*=1.31, *P*>0.2; Fig. 1); this difference in inducibility was highly significant (*t*=5.51, *P*<0.001). Induction of complex phenolics occurred at both CO_2 levels, but was stronger for plants grown under ambient CO_2 than for plants grown under elevated CO_2. Complex phenolics increased by 33% under ambient CO_2 (*paired t*=9.39, *P*<0.001) but by only 12% under elevated CO_2 (*paired t*=3.67, *P*=0.002); this difference in inducibility was also highly significant (*t*=4.10, *P*<0.001). Similarly, total phenolics increased by 24% under ambient CO_2 (*paired t*=9.90, *P*<0.001) but by only 6% under elevated CO_2 (*paired t*=2.11, *P*=0.045). Like that of simple and complex phenolics, inducibility of total phenolics was greater at ambient CO_2 (*t*=4.47, *P*<0.001; Fig. 1).

Correlations between Nitrogen, C:N, and Phenolics CO_2 level affected the strength of correlation between measures of leaf nutritional status (N and C:N) and phenolics. For plants grown under ambient CO_2, neither N nor C:N was significantly correlated with constitutive level or inducibility of

simple, complex, or total phenolics. However, for plants grown under elevated CO_2, constitutive levels of simple phenolics were positively correlated with leaf C:N ratio (R=0.55,
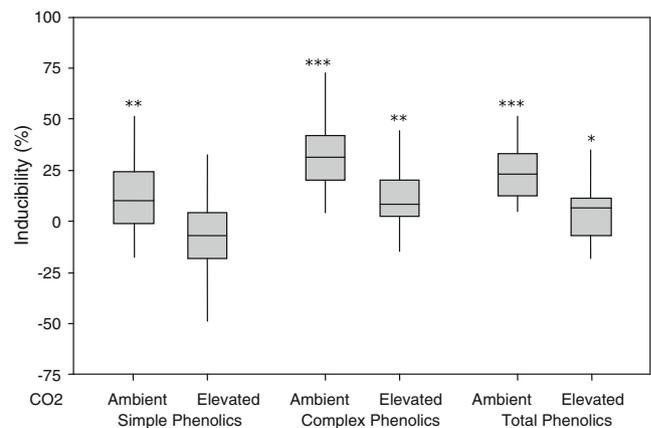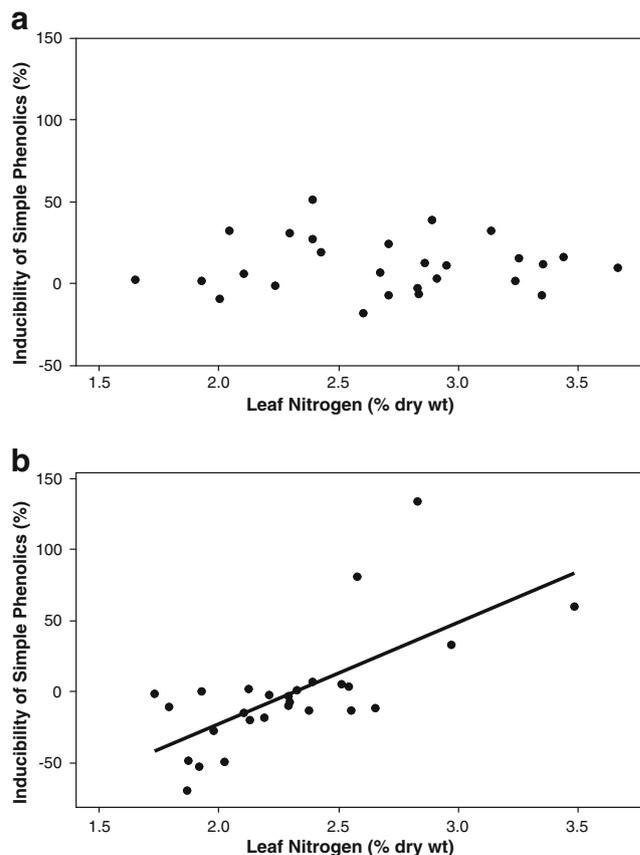

Fig. 1 Inducibility (% change during 24 hr after damage) of simple, complex, and total phenolics in *Brassica rapa* leaves[a]. [a]*Shaded* areas include the first through third quartiles, *horizontal lines* indicate medians, and *vertical lines* indicate the range. *Asterisks* denote significant induction at a given CO_2 level (* *P*<0.05, ** *P*<0.01, *** *P*<0.001). In all cases, inducibility was significantly greater at ambient CO_2 than at elevated CO_2

$d.f. = 26$, $P=0.003$; Fig. 2) and negatively correlated with leaf nitrogen ($R=-0.53$, $d.f. = 26$, $P=0.005$). In contrast, the inducibility of simple phenolics was positively correlated with leaf nitrogen ($R=0.54$, $d.f. = 26$, $P=0.004$; Fig. 3) and negatively correlated with leaf C:N ratio ($R=-0.46$, $d.f. = 26$, $P=0.015$). When all plants were considered together (but not at either CO_2 level alone), the inducibility of complex phenolics was not significantly positively correlated with leaf nitrogen ($R=0.24$, $d.f. = 53$, $P=0.084$; Fig. 4) nor significantly negatively correlated with leaf C:N ratio ($R=-0.23$, $d.f. = 53$, $P=0.095$). When inducibility was expressed as an absolute rather than percent change, the inducibility of complex phenolics was positively correlated with leaf nitrogen ($R=0.33$, $d.f. = 53$, $P=0.014$) and negatively correlated with leaf C:N ratio ($R=-0.34$, $d.f. = 53$, $P=0.013$). In no case was leaf carbon content significantly correlated with any phenolic measure.

Trichomes Growth of *B. rapa* under elevated CO_2 resulted in a significant increase in constitutive trichome density (*Mann–Whitney U*=12,362, $d.f. = 1$, $P=0.015$). Under ambient CO_2, there were 28.2 ± 5.6 trichomes/cm^2 of leaf area, and this value increased by 57% to 44.3 ± 6.4 trichomes/cm^2
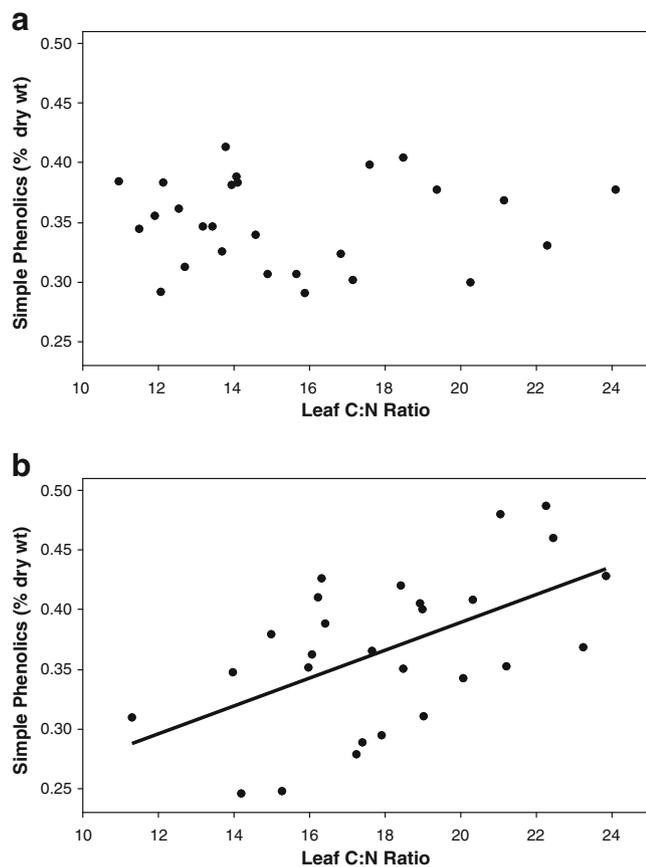
Fig. 2 Relationship between C:N ratio of *Brassica rapa* leaves and constitutive levels of simple phenolics (as a% of leaf dry weight) for plants grown at (a) ambient CO_2 ($R=0.01$, $P>0.9$) and (b) elevated CO_2 ($R=0.68$, $P=< 0.001$)

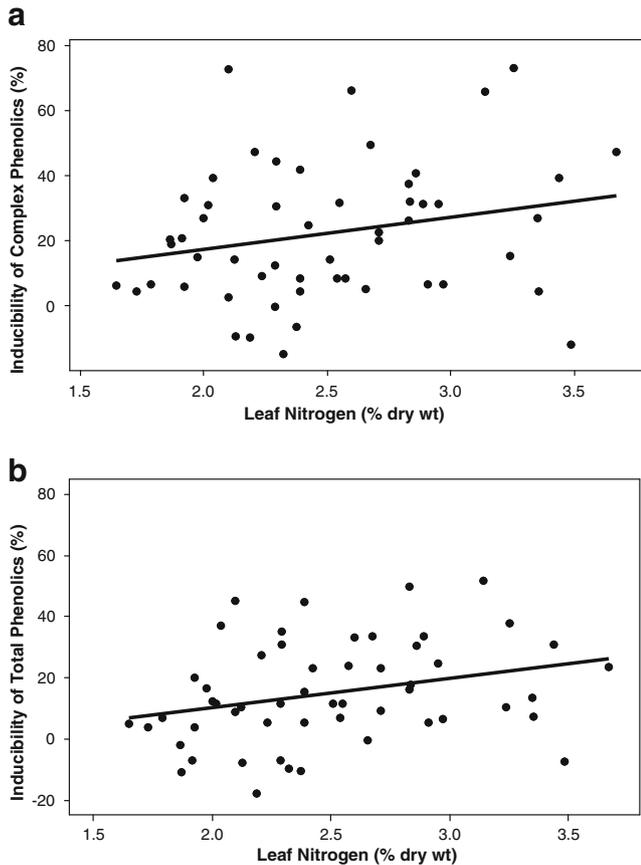

Fig. 3 Relationship between nitrogen content of *Brassica rapa* leaves and inducibility (% change during 24 hr after damage) of simple phenolics for plants grown at (a) ambient CO_2 ($R=0.01$, $P>0.9$) and (b) elevated CO_2 ($R=0.54$, $P=0.004$)

under elevated CO_2 (Fig. 5). In addition, a significantly lower proportion of leaf discs contained zero trichomes under elevated CO_2 (46.2%) than under ambient CO_2 (61.7%) ($\chi^2=5.67$, $d.f. = 1$, $P=0.017$). Trichome density was also nearly significantly more variable at ambient CO_2 than at elevated CO_2 (*Levene's statistic* = 3.66, $P=0.057$; Fig. 5)

Discussion

The results of this study indicate that rising atmospheric CO_2 can affect both chemical and mechanical defenses of *Brassica rapa*. To our knowledge, this is only the second study to demonstrate that inducibility of a chemical defense is diminished under elevated CO_2.

The observed 16% decrease in nitrogen and 20% increase in C:N ratio of *B. rapa* leaves under elevated CO_2 are similar to the mean changes reported for non-legume C_3 plants (Cotrufo et al., 1998), herbaceous plants (Zvereva and Kozlov, 2006), and other *Brassica* species (Karowe et al., 1997). Given the observed increase in *B. rapa* C:N ratio under elevated CO_2, it is reasonable to expect that constitutive levels

Fig. 4 Relationship between nitrogen content of *Brassica rapa* leaves and inducibility (% change during 24 hr after damage) of (**a**) complex phenolics ($R=0.24$, $P=0.084$) and (**b**) total phenolics for plants grown at both CO_2 levels ($R=0.29$, $P=0.033$)

of carbon-based phenolics would also be higher (Gayler et al., 2008). Indeed, constitutive levels increased significantly under elevated CO_2 for complex (17%) and total phenolics (14%). These increases are larger than the mean increases of 1% and 5% reported for phenolics in shrubs and forbs, respectively, similar to the 10–14% increases in trees, but smaller than the 29% increase reported for tannins in dicots (Peñuelas et al., 1997; Stiling and Cornelissen, 2007). It is likely that increased constitutive phenolic levels observed in this study will result in more effective defense in *B. rapa*, since phenolic compounds can reduce growth (Ossipov et al., 2001; Haukioja et al., 2002) and survivorship (Karowe, 1989; Haviola et al., 2007) of insect herbivores.

Given the observed increase in constitutive phenolics in *B. rapa* under elevated CO_2, greater inducibility of phenolics under elevated CO_2 might also be expected. However, we observed the opposite: phenolics were less inducible under elevated CO_2. Induction of simple phenolics occurred only for plants grown under ambient CO_2. In addition, although induction of complex and total phenolics occurred under both ambient and elevated CO_2, the damage-induced increases were 64% and 75% smaller, respectively, under

elevated CO_2. Therefore, the results of this study suggest that rising atmospheric CO_2 will decrease the ability of *B. rapa* to respond to herbivore damage by rapidly increasing levels of phenolic defenses.

We hypothesize that, in *B. rapa*, decreased leaf nitrogen levels under elevated CO_2 constrain the rapid mobilization of nitrogen required to produce additional jasmonic acid elicitor, mRNA and enzymes required for induction of phenolics, but do not constrain the long-term accumulation of higher levels of constitutive phenolics. In the present study, constitutive phenolic levels under elevated CO_2 were positively correlated with leaf C:N ratio, as would be expected if carbon, rather than nitrogen, availability was the primary determinant of constitutive phenolic levels. In contrast, inducibility of both simple and complex phenolics were positively correlated with leaf N (and negatively correlated with leaf C:N ratio), as would be expected if nitrogen availability were an important determinant of inducibility. Consistent with this hypothesis, Lou and Baldwin (2004) observed that, when grown under low nitrogen, tobacco plants showed decreased induction of chemical defenses, including the phenolics rutin and chlorogenic acid. Moreover, fewer than half of the genes upregulated by herbivore attack under high nitrogen were upregulated under low nitrogen.

Despite the importance of induction to plant defense strategy (Karban and Baldwin, 1997), surprisingly few studies have addressed the effect of elevated CO_2 on the inducibility of chemical defenses in response to herbivory. Most existing studies have not detected an effect of elevated CO_2 on inducibility. For instance, growth under elevated CO_2 did not affect the inducibility of phenolic glycosides or condensed tannin in aspen (Lindroth and Kinney, 1998; Roth et
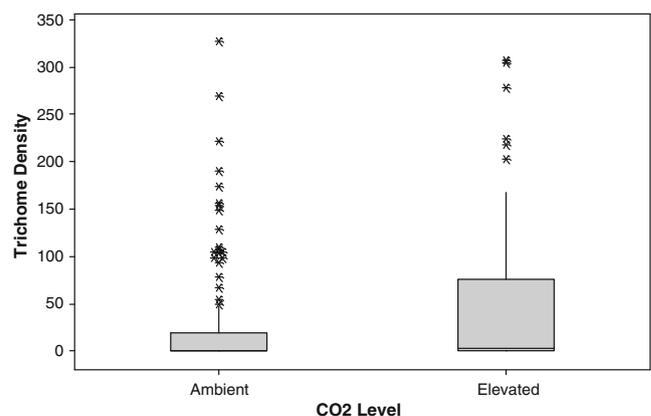
Fig. 5 Number of trichomes per cm^2 of leaf tissue (mean ± SE) in *Brassica rapa* grown under ambient and elevated CO_2[a]. [a]*Shaded* areas include the first through third quartiles, *horizontal lines* indicate medians (median = 0 for ambient CO_2), *vertical lines* indicate the range, and *asterisks* indicate outliers. Sample size is 115 at each CO_2 level. Trichome density was significantly higher at elevated CO_2 ($P=0.015$)

al., 1998), tannins in myrtle oak (Rossi et al., 2004), or saponins in alfalfa and triterpenoid aldehydes and gossypol in cotton (Agrell et al., 2004). The only two studies to detect a significant effect report contrasting responses: glucosinolates in *Arabidopsis thaliana* induced only under elevated CO_2 (Bidart-Bouzat et al., 2005), while elevated CO_2 reduced induction of soybean cysteine protease inhibitors by 31% (Zavala et al., 2008).

It is possible that the methodology used in this study enhanced the ability to detect an effect of elevated CO_2 on inducibility. In all previous studies of which we are aware, constitutive and induced levels were compared between separate leaves (Rossi et al., 2004) or separate plants (Lindroth and Kinney, 1998; Roth et al., 1998; Agrell et al., 2004; Bidart-Bouzat et al., 2005; O'Neill et al., 2010). These approaches require the assumption that constitutive defense levels are similar between leaves or plants; to the extent that this assumption is violated (Boege and Marquis, 2005), power to detect a difference in inducibility will be reduced. In contrast, the present study compared constitutive and induced levels within a single leaf, and thus only requires the more reasonable assumption that constitutive defense levels were similar between opposite halves of the same leaf.

It should be noted that mechanical damage such as that used in this study can result in patterns of induction that differ from those produced by herbivore damage (Reymond et al., 2000), since herbivore saliva contains elicitors (Musser et al., 2006). However, in general, there is broad similarity in the transcriptional responses to mechanical and herbivore damage (reviewed in Howe and Jander, 2008). For instance, in Sitka spruce, transcriptome changes in response to mechanical wounding overlapped considerably with those in response to beetle and caterpillar herbivory (Ralph et al., 2006). Moreover, differences in response to mechanical vs. herbivore damage may be greater for volatile compounds than for the within-leaf phenolic defenses we assayed here (Howe and Jander 2008). Nonetheless, given that we applied neither herbivore oral secretions nor jasmonic acid to the site of mechanical damage, we cannot say with certainty whether the patterns of defense induction we observed would occur in response to actual herbivory.

We also observed a significant 57% increase in trichome density for *B. rapa* grown under elevated CO_2, and a 41% increase in the proportion of leaf discs that contained at least one trichome. It is likely that an increase of 57% in trichome density would result in enhanced antiherbivore defense, since slower growth and higher mortality of the two most important crucifer specialists, *Pieris rapae* and *Phyllotreta cruciferae*, were associated with a 43% increase in trichome density in a related species, *Brassica nigra* (Traw and Dawson, 2002). Higher trichome density may also increase *B. rapa* resistance to pathogens (Lake and Wade, 2009).

We are not aware of any other study demonstrating an increase in trichome density that is clearly attributable to elevated CO_2. Paoletti et al. (2007) report increased trichome density in *Quercus ilex* growing near a natural CO_2 spring, but plants also were exposed to elevated levels of H_2S and SO_2. In contrast to our results, trichome density decreased under elevated CO_2 in *Arabidopsis thaliana* (Bidart-Bouzat et al., 2005; Lake and Wade, 2009), and did not change in birch (Riikonen et al., 2010), milkweed (Vannette and Hunter, 2011), or round headed bush clover (Lau et al., 2008). Given this variation in response, trichomes should remain a high priority subject for elevated CO_2 studies.

In summary, our results suggest that *Brassica rapa* will possess increased constitutive levels of both phenolic compounds and trichomes as atmospheric CO_2 continues to rise, which are likely to increase resistance to herbivores, but that phenolics will be less responsive to herbivore attack, which is likely to decrease resistance to herbivores. Whether these changes together will be advantageous, neutral, or disadvantageous will depend on the relative importance of chemical vs. mechanical defenses and, particularly, of constitutive vs. induced chemical defenses for *B. rapa*.

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