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ORIGINAL PAPER

Genotypes of *Brassica rapa* respond differently to plant-induced variation in air CO_2 concentration in growth chambers with standard and enhanced venting

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Abstract Growth chambers allow measurement of phenotypic differences among genotypes under controlled environment conditions. However, unintended variation in growth chamber air CO_2 concentration ($[CO_2]$) may affect the expression of diverse phenotypic traits, and genotypes may differ in their response to variation in $[CO_2]$. We monitored $[CO_2]$ and quantified phenotypic responses of 22 *Brassica rapa* genotypes in growth chambers with either standard or enhanced venting. $[CO_2]$ in chambers with standard venting dropped to 280 μmol mol^{-1} during the period of maximum canopy development, ~80 μmol mol^{-1} lower than in chambers with enhanced venting. The stable carbon isotope ratio of CO_2 in chamber air ($\delta^{13}C_{air}$) was negatively correlated with $[CO_2]$, suggesting that photosynthesis caused observed $[CO_2]$ decreases. Significant genotype × chamber-venting interactions were detected for 12 of 20 traits, likely due to differences in the extent to which $[CO_2]$ changed in relation to genotypes' phenology or differential sensitivity of genotypes to low $[CO_2]$. One trait, ^{13}C discrimination ($\delta^{13}C$), was particularly influenced by unaccounted-for fluctuations in $\delta^{13}C_{air}$ and $[CO_2]$. Observed responses to $[CO_2]$ suggest that genetic variance components estimated in poorly vented growth chambers may be influenced by the expression of genes involved in CO_2 stress responses; genotypic values estimated in these chambers may likewise be misleading such that some mapped quantitative trait loci may regulate responses to CO_2 stress rather than a response to the environmental factor of interest. These results underscore the importance of monitoring, and where possible, controlling $[CO_2]$.

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Introduction

Growth chambers are widely used integral tools in plant biology because they allow researchers to control and manipulate environmental conditions such as air temperature, humidity, light intensity, and photoperiod. Controlled growth settings are commonly used by molecular geneticists interested in annotating gene function. For instance, to understand genetic components of the photoperiod pathway for flowering time (e.g., Corbesier and Coupland 2005; Onouchi and Coupland 1998), day length may be manipulated while other factors that might affect flowering time, such as temperature, are held constant. Quantitative geneticists interested in the genetic architecture of complex traits may also use growth chambers to manipulate specific environmental factors and evaluate how trait (co)variances

or mapped quantitative trait loci (QTL) vary across environments (e.g., Andaya and Mackill 2003a). A clear expectation is that growth chambers minimize environmental "noise" and thereby provide a means to precisely estimate genotypic values as well as a trait's genetic variance and evolutionary potential in a specific environment. Although growth chamber experiments likely reduce many sources of environmental variation, spatial heterogeneity nevertheless exists within growth chambers (Potvin et al. 1990), the confounding effects of which are often avoided by rotation of experimental plants throughout the growth chamber. Temporal heterogeneity may also exist within growth chambers in the concentration of gases such as CO_2 ($[CO_2]$).

Several studies have shown that large fluctuations in $[CO_2]$ may occur in growth chambers due to photosynthetic gas exchange by experimental plants (e.g., Peet and Krizek 1997; Tibbitts and Krizek 1978). If the standard ventilation in a growth chamber does not provide a sufficient rate of air exchanges with outside air, then photosynthesis by plants in a growth chamber may reduce daily $[CO_2]$ to sub-ambient levels (Peet and Krizek 1997; Wheeler 1992). For example, photosynthesis from cotton plants in a large growth chamber reduced daily $[CO_2]$ from 350 μmol mol^{-1} (ambient conditions) to 150 μmol mol^{-1}, and corn plants in a similar chamber depleted daily $[CO_2]$ to 50 μmol mol^{-1} (Patterson and Hite 1975). *Populus* plants in a walk-in growth chamber depleted daily $[CO_2]$ from 400 to 280 μmol mol^{-1} (Bernier et al. 1994).

Sub-ambient $[CO_2]$ in poorly vented growth chambers should limit CO_2 availability, decreasing photosynthesis and/or increasing photorespiration (Farquhar and Sharkey 1982; Sharkey 1988). This decrease in photosynthesis can significantly influence plant growth and reproduction. For example, three species of C3 annual plants grown over a declining gradient of sub-ambient $[CO_2]$ demonstrated a linear decline in whole-plant biomass and water use efficiency as measured from stable carbon isotope ratios (Polley et al. 1993). Six invasive species had reduced biomass and leaf area at sub-ambient conditions (280 μmol mol^{-1}) in comparison to plants grown at current ambient conditions of 380 μmol mol^{-1} (Ziska 2003). Furthermore, in comparison to plants grown at modern ambient $[CO_2]$, *Abutilon theophrastii* plants grown at below-Pleistocene minimum $[CO_2]$ had reduced ability to assimilate nitrogen, reducing Rubisco capacity and photosynthetic rate, which in turn reduced biomass, specific leaf mass, relative growth rates, and reproduction (Dippery et al. 1995; Tissue et al. 1995).

Although multiple studies have shown that gas exchange by experimental plants in poorly vented growth chambers can cause draw-downs of CO_2 and that low CO_2 can affect phenotypic traits of plants, most quantitative genetics and QTL mapping studies carried out in growth chambers do not measure or control $[CO_2]$ (e.g., Andaya and Mackill 2003a, b; Degenkolbe et al. 2009; Funatsuki et al. 2004; Lacey 1996; Lou et al. 2007; Sato et al. 2003; Tasma et al. 2001; Welcker et al. 2007), although measurement and control of $[CO_2]$ may not always be experimentally feasible. Of greatest concern to quantitative-genetic or QTL studies carried out in growth chambers is that either different genotypes will mature in different $[CO_2]$ environments due to ongoing gas exchange or individual genotypes may react differently to changes in $[CO_2]$. Specifically, early-flowering genotypes may experience relatively high $[CO_2]$ and may be correspondingly robust, while slower developing genotypes may experience reduced $[CO_2]$ and be correspondingly less robust, particularly for traits that are expressed later in ontogeny, such as fruit set. It is also likely that genotypic variation in CO_2 sensitivity exists. In a study of six *Arabidopsis* genotypes grown in sub-ambient, ambient, and superambient $[CO_2]$, significant genotype × $[CO_2]$ interactions were detected for traits such as days to first flower, silique number per plant, seed number per silique, and seed number per plant (Ward and Strain 1997). If either unintentional reductions in $[CO_2]$ occur as a result of photosynthetic gas exchange by experimental plants in a poorly vented growth chamber and/or genotypes differ in their responsiveness to changes in $[CO_2]$, then researchers may obtain misleading estimates of trait (co)variances. QTL mapped using genotypic values from the entire mapping population may likewise reflect some combination of genes of interest to the researcher and genes of considerably less interest that are involved in a low-CO_2 stress response.

Another environmental factor that may be influenced by gas exchange from experimental plants in growth chambers is the carbon isotope ratio of the air ($\delta^{13}C_{air}$). During photosynthesis, Rubisco discriminates against ^{13}C (Farquhar et al. 1982; Oleary 1988), thereby increasing the concentration of ^{13}C relative to ambient air. In enclosed environments such as growth chambers with inadequate venting, the magnitude of the effect of photosynthesis by experimental plants on $\delta^{13}C_{air}$ may thus be compounded. Fluctuations in $\delta^{13}C_{air}$ in inadequately vented chambers may affect the measurement of two commonly used measures of plant physiology: the carbon isotope composition of the leaf ($\delta^{13}C_{leaf}$) and discrimination of ^{13}C ($\Delta^{13}C$). In C_3 plants, $\delta^{13}C_{leaf}$ is a function of (1) the composition of the $\delta^{13}C_{air}$ and (2) C_i/C_a, the ratio of partial pressures of intercellular CO_2 (C_i) and ambient CO_2 (C_a), which is a measure of the photosynthetic capacity and stomatal conductance of the plant (Farquhar et al. 1982, 1989). If $\delta^{13}C_{air}$ varies across an experiment and the leaves sampled for $\delta^{13}C$ analysis develop at different times, then $\delta^{13}C_{leaf}$ estimates will be biased according to the $\delta^{13}C_{air}$ conditions at the time of leaf development. Even if leaves were

collected at a single time point, leaf expansion rates may vary such that individual genotypes effectively sample distinct δ^{13} environments. Discrimination of ^{13}C ($\Delta^{13}C$) is calculated by correcting the $\delta^{13}C_{leaf}$ by the $\delta^{13}C_{air}$ (Farquhar et al. 1989), and many studies use a standard value of −8‰ (e.g., Farquhar et al. 1989; Hall et al. 2005; Masle et al. 2005; Rytter 2005; Takai et al. 2006; Thumma et al. 2001) or an average value for the estimate of $\delta^{13}C_{air}$ (Hausmann et al. 2005). However, if $\delta^{13}C_{air}$ varies across an experiment due to ongoing gas exchange by experimental plants, then the application of a constant atmospheric value or a chamber average of $\delta^{13}C_{air}$ to calculate $\Delta^{13}C$ may bias estimates according to when the leaves developed or were sampled. This bias will cause the photosynthesis and water use physiologies to be misinterpreted (i.e., incorrect C_i/C_a), even if $\delta^{13}C_{air}$ is constant but is not −8‰.

To our knowledge, no studies have evaluated the phenotypic responses of multiple genotypes to temporal fluctuations in $[CO_2]$ that occur in growth chambers with standard (as supplied from manufacturer) versus enhanced ventilation (as provided by extra intake fans and exhaust vents). In this study, we grew 22 genotypes of *Brassica rapa* in growth chambers with standard and enhanced ventilation to (1) determine whether differences in venting differentially affect $[CO_2]$ and $\delta^{13}C$ in the chamber air; (2) quantify variation in physiological, morphological, and phenological traits for the genotypes growing in each of the chambers; (3) investigate the influence of progressive changes in $\delta^{13}C_{air}$ on estimates of $\delta^{13}C_{leaf}$ and $\Delta^{13}C$; and (4) determine whether significant genotype × chamber-venting interactions exist for measured traits.

Materials and methods

Research species, seed source, and growth conditions

The study species, *B. rapa* L., is an annual oilseed and vegetable crop species native to Eurasia (Prakash and Hinata 1980). The genotypes used in this study were recombinant inbred lines (RILs) derived from a cross between two highly inbred genotypes. To create these lines, a rapid cycling genotype (IMB211) and an inbred annual yellow Sarson seed oil genotype (R500) were crossed, the F1 generation was selfed, and the progeny was advanced by single seed descent to the S6 generation to form 150 RILs; a detailed description of these RILs is provided in Dechaine et al. (2007). In addition to the two parental genotypes, 20 RILs from the original 150 were randomly selected for use in this study.

Eight replicates of each of the 22 genotypes were grown from seed in each of two growth chambers (PGC-9/2 with Percival Advanced Intellus Environmental Controller, Percival Scientific, Perry, Indiana, USA). A total of 176 plants were grown in each chamber; at this density, when the plants were largest (e.g., during flowering), less than half of the usable growth space of the chamber was occupied. One growth chamber contained the standard venting provided by the growth chamber manufacturer, comprising one intake fan and two exhaust vents, allowing for 10 air exchanges per hour (Don Duncan, Percival Scientific, pers. comm.). Additional ventilation was installed into the second growth chamber; two additional exhaust vents and an additional intake fan (15CFM, 115 V, 2"wheel, 3160 RPM Shaded Pole Blower, Dayton Parts LLC, Harrisburg, PA) doubled the number of air exchanges in the chamber to 20 per hour (Don Duncan, Percival Scientific, pers. comm.).

Three seeds were planted in 360 cm^3 pots filled with metromix 200 soil (Sun-Gro Horticulture, Vancouver, BC, Canada) and 1 ml Osmocote 18-6-12 fertilizer (Scotts Miracle Grow, Marysville, OH, USA). After planting, the seeds were lightly covered with vermiculite. The planting design was identical for both growth chambers, with the same randomization of genotypes in the two chambers. Following planting, pots were placed immediately into the two growth chambers with light/dark periods set to 14 h/10 h and light periods with a photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}, the temperature maintained at a constant 24°C, and the vapor pressure deficit (VPD) maintained between 0.90 and 1.65 (45–70% relative humidity). After emergence, seedlings were thinned to one plant closest to the center of the pot. Plants were watered regularly to maintain moist soil conditions.

Trait measurements

Plants in the chambers were checked daily for bolting (i.e., differentiation of buds from the apical meristem), and the number of days required to bolt was recorded for each plant. We measured photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) for each plant at bolting on one mature, fully expanded leaf using one of two steady-state gas exchange systems (LI-COR-6400; LI-COR Biosciences Inc., Lincoln, NE, USA) using standard techniques (Long and Bernacchi 2003). One machine was equipped with a red/blue light emitting diode (LED) and the other was equipped with a leaf chamber fluorimeter with a red/blue LED. Measurements were taken at least 3 h after subjective dawn in the chamber, between 1100 and 1600 hours mountain standard time (MST). All measurements were taken at an irradiance of 2,000 μmol m^{-2} s^{-1} (preliminary light response curves showed no decrease in photosynthesis at this irradiance compared with

measurements taken at 500 μmol m^{-2} s^{-1}), with CO_2 concentration maintained at 400 μmol mol^{-1}, leaf temperature maintained at ambient conditions (24°C), and the leaf-to-air VPD maintained at 1.3–1.7 kPa. Measurements were taken when the readings stabilized after approximately 5–10 min. All measurements were computed on a total leaf area basis (i.e., total leaf area in the chamber). For plants that were measured using the fluorimeter, we measured fluorescence in light (F'_v/F'_m, or maximum photosystem II efficiency in light). The measured leaf was then removed, scanned, dried in an oven at 65°C for at least 72 h and weighed. Leaf area of the scanned leaves was measured using ImageJ version 1.38X (Rasband 1997–2007). Specific leaf area (SLA) was calculated by dividing the leaf area by the dried leaf mass.

Flowers were collected from each plant when the anthers on the third and fourth flowers of the main stem dehisced and began to shed pollen. At the time of flower collection, the date of flowering was recorded and the third and fourth flowers were removed and placed immediately in 75% ethanol for subsequent dissection. Because many plants aborted flowers (i.e., the flower buds senesced prior to opening), we recorded the number of aborted flowers and collected the first two viable flowers when the third and fourth flowers were not viable. The preserved flowers were later dissected under a dissecting microscope (Nikon SMZ800, Nikon Corporation, Tokyo, Japan) and digitally photographed. Floral organ sizes were measured from the digital image using ImageJ; measured traits include petal length, petal width, long stamen length, short stamen length, and pistil length. For all floral organ size traits, the measurements of the two flowers from each individual were averaged for data analysis.

At the time of flower collection, all above-ground biomass for each plant was harvested, dried in an oven for at least 72 h at 65°C, and weighed. Because previous pilot data revealed that total below-ground biomass was highly correlated with taproot biomass (using PROC CORR in SAS ver. 9.1, $n = 134$, $r = 0.738$, $P < 0.001$), we used taproot biomass as a proxy for total below-ground biomass. The taproot of each plant was removed, cleaned, dried for at least 72 h at 65°C and weighed.

Measurements of leaf $\delta^{13}C$, $\delta^{13}C$ of chamber air, and chamber air [CO_2]

We selected the two parental genotypes, the four earliest bolting, and the four latest bolting RILs (see Fig. 1 for selected genotypes) for analysis of carbon isotope ($\delta^{13}C$) composition and percent nitrogen content (%N). Oven-dried leaves collected at bolting were ground and analyzed using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a continuous-flow inlet isotope ratio mass spectrometer (CF-IRMS; Delta-plus XP, Thermo Scientific, Waltham, MA, USA). $\delta^{13}C$ values are reported in parts per thousand relative to Vienna Peedee Belemnite (VPDB). The precision of repeated measurements of laboratory standards was <0.1‰.

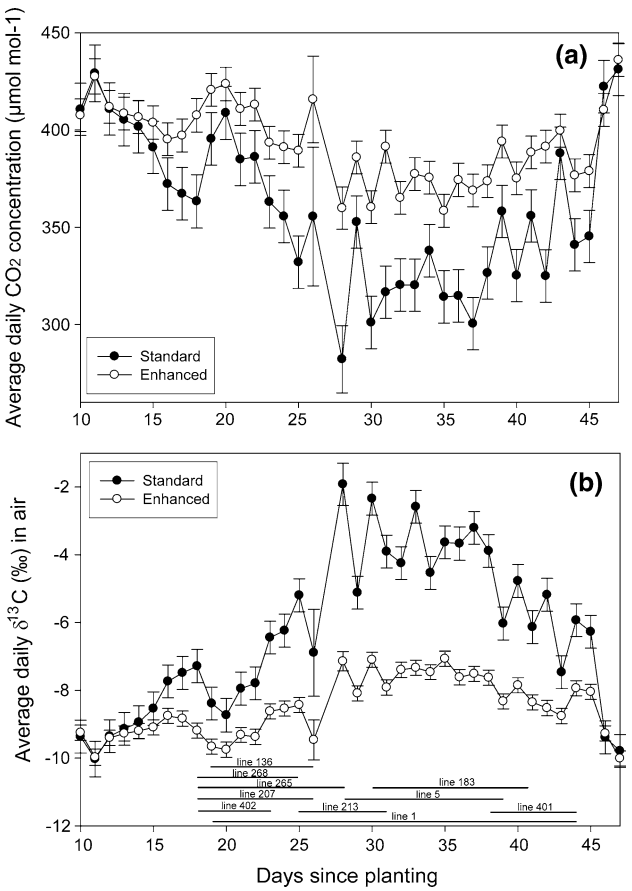

Fig. 1 CO_2 and $\delta^{13}C_{air}$ levels in growth chambers with standard (as supplied by manufacturer) or enhanced (by adding additional fans and vent holes) ventilation. **a** Average daytime CO_2 (μmol mol^{-1}), and **b** $\delta^{13}C_{air}$, with the range in bolting dates for the 8 RILs and 2 parental genotypes used for $\Delta^{13}C$ analysis (5 early flowering and 5 late flowering) indicated at the *bottom* to illustrate the differences in CO_2 and $\delta^{13}C_{air}$ (‰) conditions that genotypes experienced at bolting. Because bolting date did not vary between chambers (i.e., no chamber effect), the bolting dates for each genotype are taken from the range of dates observed in both chambers

We used an automated air sampling system to monitor daytime [CO_2] of the air inside each chamber at 30-min intervals over the entire experiment. The automated air sampling system, modified from the design described by Schauer et al. (2003), used an IRGA (infra-red gas analyzer; LI-820, LI-COR Biosciences Inc., Lincoln, NE, USA) for [CO_2] measurement and a multi-position valve to collect air into 100 ml flasks. Daytime [CO_2] measurements as determined by the IRGA at 30-min intervals were averaged to obtain a daily mean [CO_2] for each chamber.

We also periodically collected air samples from chambers for analysis of the stable carbon isotope ratio value of the chamber air CO_2 ($\delta^{13}C_{air}$). Air samples were collected in flasks three times during the photoperiod (0800, 1200, and 1600 hours) once each week during the experiment. Flask air was transferred to septa-capped vials using a stainless steel vacuum-manifold system and air CO_2 was then analyzed for $\delta^{13}C$ using a GasBench II coupled to a Delta-plus XP CF-IRMS system. The $\delta^{13}C_{air}$ values were corrected to the VPDB standard using CO_2-in-air laboratory working standards calibrated with CO_2-in-air standards obtained from NOAA-CMDL. Repeated measurements with CO_2-in-air laboratory working standards had a precision of <0.1‰. All stable isotope analyses were performed at the University of Wyoming Stable Isotope Facility.

Leaf carbon isotope discrimination ($\Delta^{13}C$) was calculated as (Farquhar et al. 1989):

$$\Delta^{13}C\,(‰) = (\delta^{13}C_{air} - \delta^{13}C_{leaf})/1 + (\delta^{13}C_{air}/1{,}000)$$

To obtain daily estimates of $\delta^{13}C_{air}$, we carried out a simple linear regression (using SAS PROC GLM in SAS ver. 9.1) to investigate the relationship between $\delta^{13}C_{air}$ values from the flask collection and the corresponding $[CO_2]$ from the IRGA at the time of flask collection. To provide a linear relationship, we regressed $\delta^{13}C_{air}$ on $[CO_2]^{-1}$. The regression equation resulting from this analysis, $y = 6422.7x - 25.007$ where $x = [CO_2]^{-1}$, was used to convert the 30-min interval CO_2 measurements to corresponding $\delta^{13}C_{air}$ values in each chamber. We then averaged the daytime estimates of $\delta^{13}C_{air}$ for each day of the experiment.

We calculated $\Delta^{13}C$ for all individuals of the 10 genotypes using three separate values for $\delta^{13}C_{air}$: (1) the background atmospheric value of −8‰, reflecting the commonly applied correction value used in many current studies (Farquhar et al. 1989; Masle et al. 2005), hereafter referred to as "common $\Delta^{13}C$"; (2) a separate value for each growth chamber, calculated as the average of all daily $\delta^{13}C_{air}$ estimates in each chamber across all days of the experiment (Hausmann et al. 2005), hereafter referred to as "chamber $\Delta^{13}C$"; and (3) because we measured $\delta^{13}C$ from each plant at bolting and genotypes bolted at different times (see "Results"), we matched a $\delta^{13}C_{air}$ value for each bolting date in each chamber by taking the average of the previous week's (i.e., the time since the leaf emerged) daily $\delta^{13}C_{air}$ values (Comstock et al. 2005), hereafter referred to as "matched $\Delta^{13}C$".

Analyses of response variables

We used ANOVA to evaluate the fixed effects of chamber type and the random effects of genotype and the genotype × chamber interaction on all measured traits (using REML in PROC MIXED, SAS ver. 9.1). Prior to analysis, the following response variables were transformed to meet the assumptions of ANOVA: days to produce a viable flower, taproot biomass, SLA, pistil length, and "matched $\Delta^{13}C$" were log10 transformed; "common $\Delta^{13}C$" and chlorophyll fluorescence were squared; above-ground biomass was square root transformed; and number of days to bolt was inverse transformed. Because we used two LI-COR 6400 machines to measure A, g_s, and E, we included machine ID as an additional random factor in the analyses of these traits.

To generate genotypic means, we estimated best linear unbiased predictors (BLUPs) of each trait in both chambers. Values of transformed traits were back-transformed for presentation in figures. These genotypic estimates were also used to estimate correlations between physiological traits (using SAS PROC CORR in SAS ver 9.1). In particular, we were interested in separating the potential intra-leaf controls on photosynthesis by correlating gas exchange measurements of photosynthesis (A) with stomatal conductance (g_s) and either the light-dependent reactions (estimated from chlorophyll fluorescence in light F'_v/F'_m) or the light-independent reactions (integrated with other components of photosynthesis through $\Delta^{13}C$ estimates).

Corroboration from different growth chambers and experiments

Finally, to determine whether the results from the current study are reproducible across experiments and growth chambers, we compared the results of this study to those of other experiments that were conducted using a subset of *B. rapa* RILs and the same data collection techniques, but using different growth chambers of the same model to control for chamber differences other than $[CO_2]$. For the unvented treatment, the experimental design was identical to the standard chamber in the current study, except a slightly different set of genetic lines was used and only eight of the 20 traits were measured. For the enhanced treatment, data were collected from plants grown in several enhanced growth chambers; genetic lines, growth conditions, and traits were the same as in the present study. We carried out the same statistical analyses on the replicated data set as we did for the present data (see "Analyses of response variables", i.e., testing effects of genotype, chamber type, and genotype × chamber interactions) for the following eight traits that were common to all of the studies: days to bolting, days to produce a viable flower, number of aborted flowers, above-ground biomass, below-ground biomass, A, g_s, and SLA.

Results

CO_2 concentration in growth chambers with standard and enhanced air exchange rates

Average daily [CO_2] levels were similar (400–450 μmol mol^{-1}) between the two chamber types at the start of the experiment, declined as the plants grew, and increased again near the end of the experiment after most of the plants had flowered and were removed (Fig. 1a). However, [CO_2] reached much lower levels in the chamber with the manufacturer's standard venting; from days 15 to 45, [CO_2] in the chamber with standard venting was consistently 25–80 μmol mol^{-1} lower than in the chamber with enhanced venting. [CO_2] in the standard chamber reached values as low as 282 μmol mol^{-1} (day 28), while the lowest [CO_2] in the enhanced chamber was 359 μmol mol^{-1} (also on day 28).

Average daily $\delta^{13}C_{air}$ values in the standard and enhanced chambers (Fig. 1b) also varied throughout the experiment. $\delta^{13}C_{air}$ values were similar in both chambers at the start of the experiment (−9 to −10‰), became less negative during bolting and flowering, and then returned to values around −10‰ at the end of the experiment. $\delta^{13}C_{air}$ in the chamber with standard venting reached values as high as −2‰, while $\delta^{13}C_{air}$ in the chamber with enhanced venting increased to only −8‰. The relationship between $\delta^{13}C_{air}$ and $[CO_2]^{-1}$ was linear and strongly positively correlated ($R^2 = 0.893$, $P < 0.001$).

The range in bolting dates for four early-flowering, four late-flowering, and the two parental genotypes in relation to [CO_2] and $\delta^{13}C_{air}$ in the two chambers are shown in Fig. 1b. Because bolting date did not differ between the two chambers (see "Phenotypic traits"; Table 1), these dates were taken from the range in bolting dates for each genotype as observed in both chambers. Genotypes experienced different [CO_2] prior to and during bolting because they differed in bolting date and because the chamber with the standard venting experienced large reductions in [CO_2]. For example, in the standard chamber, early-flowering genotypes experienced [CO_2] and $\delta^{13}C_{air}$ values at bolting around 375 μmol mol^{-1} and −8‰, respectively, while the late-bolting genotypes experienced conditions at bolting around 325 μmol mol^{-1} and −4‰, respectively. In contrast, early- and late-bolting plants in the chamber with enhanced ventilation experienced relatively similar [CO_2] over their growth and flowering periods. In the enhanced chamber, the early-bolting genotypes (136, 268, 265, 207, and 402) experienced [CO_2] and $\delta^{13}C_{air}$ values at bolting around 400 μmol mol^{-1} and −9.5‰, respectively, while the late-flowering genotypes (1, 5, 213, 183, and 401) experienced air with [CO_2] around 375 μmol mol^{-1} and $\delta^{13}C_{air}$ values of −8‰. These results demonstrate that

Table 1 Test statistics and significance of tests for effects of growth chamber venting, genotype, and their interaction on days to bolting and flowering, floral traits, and biomass traits of *Brassica rapa*

	Days to bolting	Days to produce a viable flower	Number of flowers aborted	Average long stamen	Average short stamen	Average pistil length	Average petal length	Average petal width	Above-ground biomass	Below-ground biomass
Fixed factors (F)										
Chamber	0.21	6.23*	17.57***	64.59****	91.98****	9.08**	286.91****	197.09****	19.79***	3.17†
Random factors (z)										
Genotype	3.15***	2.87**	0.35	2.62**	2.81**	3.12***	3.07**	2.97**	3.14***	3.14***
Genotype × chamber	1.15	2.08*	2.58**	2.58**	2.17*	0.42	0	1.58†	1.29†	1.19

† $P < 0.10$
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
**** $P < 0.0001$

Table 2 Test statistics and significance of tests for effects of growth chamber venting, genotype, and their interaction on physiological traits in *Brassica rapa*

	A	g_s	*E*	F'_v/F'_m	SLA
Fixed factors (*F*)					
Chamber	3.20†	3.54†	4.36*	0.04	0.03
Random factors (*z*)					
IRGA ID	0.69	0.70	0.70	Not in model	Not in model
Genotype	2.90**	1.46†	1.47†	2.80**	3.05**
Genotype × chamber	1.48†	1.04	1.16	1.72*	1.63*

A photosynthetic rate, g_s stomatal conductance, *E* transpiration rate, F'_v/F'_m fluorescence in light, *SLA* specific leaf area

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

inadequate venting of growth chambers and plant photosynthetic CO_2 uptake causes significant variation in growth conditions over the course of an experiment, causing genotypes with differing rates of development to experience different growth conditions.

Phenotypic traits

Significant chamber effects were detected for most traits (Tables 1, 2, 3; phenotypic means by chamber are presented in Table 4). Number of days to produce a viable flower, number of aborted flowers, *E*, above-ground biomass, and %N were all significantly smaller in the enhanced chamber compared to the standard chamber ($P < 0.05$; Tables 1, 2, 4), and g_s was marginally smaller in the enhanced chamber in comparison to the standard chamber ($P < 0.10$; Tables 2, 4). Conversely, all floral length traits including average long stamen length, average short stamen length, average pistil length, average petal length, and average petal width were significantly larger ($P < 0.05$; Tables 1, 4) in the enhanced chamber in comparison to the standard chamber. Below-ground biomass and *A* were also marginally larger ($P < 0.10$; Tables 1, 2, 4) in the enhanced chamber. These significant chamber effects indicate that addition of ventilation to a chamber, which consequently increases $[CO_2]$, has a significant effect on a wide variety of phenotypic traits. In addition, temporal variation in CO_2 likely affects estimates of genotypic means and trait (co)variances.

Genotypic means of traits for 10 genotypes (e.g., those genotypes selected for isotope analyses as indicated in Fig. 1) and their upper and lower 95% confidence intervals (CI) are given in Table S1. A significant genotype effect ($P < 0.05$ level; Tables 1, 2, 3) was detected for most traits investigated in this study, including bolting and flowering time, all floral organ lengths, above- and below-ground biomass, SLA, *A*, and %N. Two traits, g_s and *E*, demonstrated marginally significant genotype effects (at the $P < 0.10$ level; Tables 2, 3). These results indicate that the genotypes used in this study are segregating for allelic variation for these traits.

Significant genotype × chamber interactions were detected for six traits (at the $P = 0.05$ level; Tables 1, 2, S1; Fig. 2), including days to produce a viable flower, number of aborted flowers, average length of the long stamens, average length of the short stamens, F'_v/F'_m, and SLA. Three additional traits, above-ground biomass, petal width, and *A*, demonstrated marginally significant genotype × chamber interactions (at the $P = 0.06$ level; Tables 1, 2; Fig. 2). Because conditions in growth chambers were nearly identical except for the amount of venting and attendant $[CO_2]$ fluctuations, these significant genotype × chamber interactions indicate that genotypes respond differently to variation in $[CO_2]$ and that this genotype-specific variation in CO_2 starvation response affects estimates of genotypic means and trait (co)variances.

Results of correlations between genotypic means of physiological traits are presented in Table S2. Contrary to expectations, the suite of photosynthetic traits (*A*, g_s, SLA, "matched $\Delta^{13}C$", F'_v/F'_m, and %N) were generally not well correlated with each other (Table S2). The only strong correlation found was between *A* and F'_v/F'_m ($r = 0.77244$, $P = 0.0088$; Table S2), but further inspection of this relationship revealed that this correlation was driven by two outlying genotypes; when these genotypes were removed, the correlation became non-significant (data not shown).

Table 3 Test statistics and significance of tests for effects of growth chamber venting, genotype, and their interaction on $\delta^{13}C_{leaf}$, $\Delta^{13}C$ and N percentage values in *Brassica rapa*

	$\delta^{13}C_{leaf}$	"Matched $\Delta^{13}C$"	"Chamber $\Delta^{13}C$"	"Common $\Delta^{13}C$"	%N
Fixed factors (*F*)					
Chamber	29.15***	7.15*	0.22	29.52****	0.55
Random factors (*z*)					
Genotype	1.44†	1.96*	1.70*	1.46†	2.04*
Genotype × Chamber	1.87*	0.34	1.70*	1.91*	1.08

"Matched $\Delta^{13}C$", "chamber $\Delta^{13}C$", and "common $\Delta^{13}C$" reflect the three different $\delta^{13}C_{air}$ values; "common $\Delta^{13}C$" was corrected using a standardized $\delta^{13}C_{air}$ of −8‰; "chamber $\Delta^{13}C$" was corrected by averaging all observed $\delta^{13}C_{air}$ values in each chamber from throughout the experiment, resulting in a single unique correction value for each chamber; and "matched $\Delta^{13}C$" was corrected by calculating a unique $\delta^{13}C_{air}$ for each bolting date in each chamber by averaging the observed chamber $\delta^{13}C_{air}$ for the week prior to each bolting date

† $P < 0.10$

* $P < 0.05$

*** $P < 0.001$

**** $P < 0.0001$

Table 4 Average estimates (best linear unbiased predictors) of each phenotypic trait and upper and lower 95% confidence intervals in the standard and enhanced growth chambers

Trait	Standard chamber	Enhanced chamber
Days to bolting	25.038 (23.031–27.435)	24.882 (22.894–27.241)
Days to produce a viable flower*	38.788 (36.467–41.257)	37.008 (34.794–39.364)
Number of aborted flowers	6.319 (4.182–8.456)	0.301 (−1.83–2.431)
Average long stamen length (mm)**	5.438 (5.023–5.853)	6.704 (6.291–7.116)
Average short stamen length (mm)**	4.026 (3.679–4.373)	5.115 (4.771–5.460)
Average pistil length (mm)***	5.484 (5.082–5.92)	5.737 (5.317–6.189)
Average petal length (mm)**	6.1625 (5.7643–6.5608)	7.65 (7.2551–8.0449)
Average petal width (mm)**	2.526 (2.293–2.759)	3.392 (3.161–3.624)
Above-ground biomass (g)***	0.7827 (0.574–1.0237)	0.6249 (0.4402–0.8419)
Below-ground biomass (g)***	0.029 (0.046–0.018)	0.025 (0.04–0.016)
SLA ($cm^2\ g^{-1}$)	357.93 (329.23–389.22)	354.24 (325.84–385.12)
A ($\mu mol\ m^{-2}\ s^{-1}$)†	17.849 (16.403–19.295)	18.641 (17.195–20.088)
g_s ($mol\ m^{-2}\ s^{-1}$)†	0.577 (0.524–0.63)	0.532 (0.479–0.585)
E ($mmol\ m^{-2}\ s^{-1}$)*	10.135 (9.421–10.849)	9.473 (8.759–10.188)
F'_v/F'_m	0.409 (0.39–0.427)	0.408 (0.388–0.426)
$\delta^{13}C^{\dagger}_{leaf}$	−31.148 (−31.885–30.411)	−32.9108 (−33.647–32.173)
"Matched $\Delta^{13}C$"	24.808 (24.344–25.287)	24.513 (24.055–24.986)
"Chamber $\Delta^{13}C$"	24.452 (23.748–25.157)	24.339 (23.635–25.044)
"Common $\Delta^{13}C$"	23.427 (22.676–24.155)	25.124 (24.425–25.804)
%N	6.246 (5.231–7.261)	6.116 (5.101–7.131)

A photosynthetic rate, g_s stomatal conductance, *E* transpiration rate, F'_v/F'_m fluorescence in light, *SLA* specific leaf area

Indicates significant chamber effects at the $^{\dagger}P < 0.10$, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ levels

Results of $\delta^{13}C_{leaf}$ and $\Delta^{13}C$ analyses

Significant chamber effects were detected using $\delta^{13}C_{leaf}$, "matched $\Delta^{13}C$", and "common $\Delta^{13}C$" (Table 3; Fig. 3); the direction of the effects was similar for $\delta^{13}C_{leaf}$ and "matched $\Delta^{13}C$", but reversed using "common $\Delta^{13}C$". Specifically, using $\delta^{13}C_{leaf}$ and "matched $\Delta^{13}C$", the values in the standard chamber were significantly larger than in the enhanced chamber, and the reverse was found using "common $\Delta^{13}C$" (Table 3; Fig. 3). Significant genotype effects were detected using all three $\Delta^{13}C$ correction approaches, and a marginally significant genotype effect was detected for $\delta^{13}C_{leaf}$ (Table 3; Fig. 3). Significant interactions were found using $\delta^{13}C_{leaf}$ and the "chamber $\Delta^{13}C$" and "common $\Delta^{13}C$" corrections, but the interaction using the "matched $\Delta^{13}C$" correction was not significant (Table 3; Fig. 3). These results indicate that fluctuations in $\delta^{13}C_{air}$ and $\delta^{13}C_{air}$ correction values have an

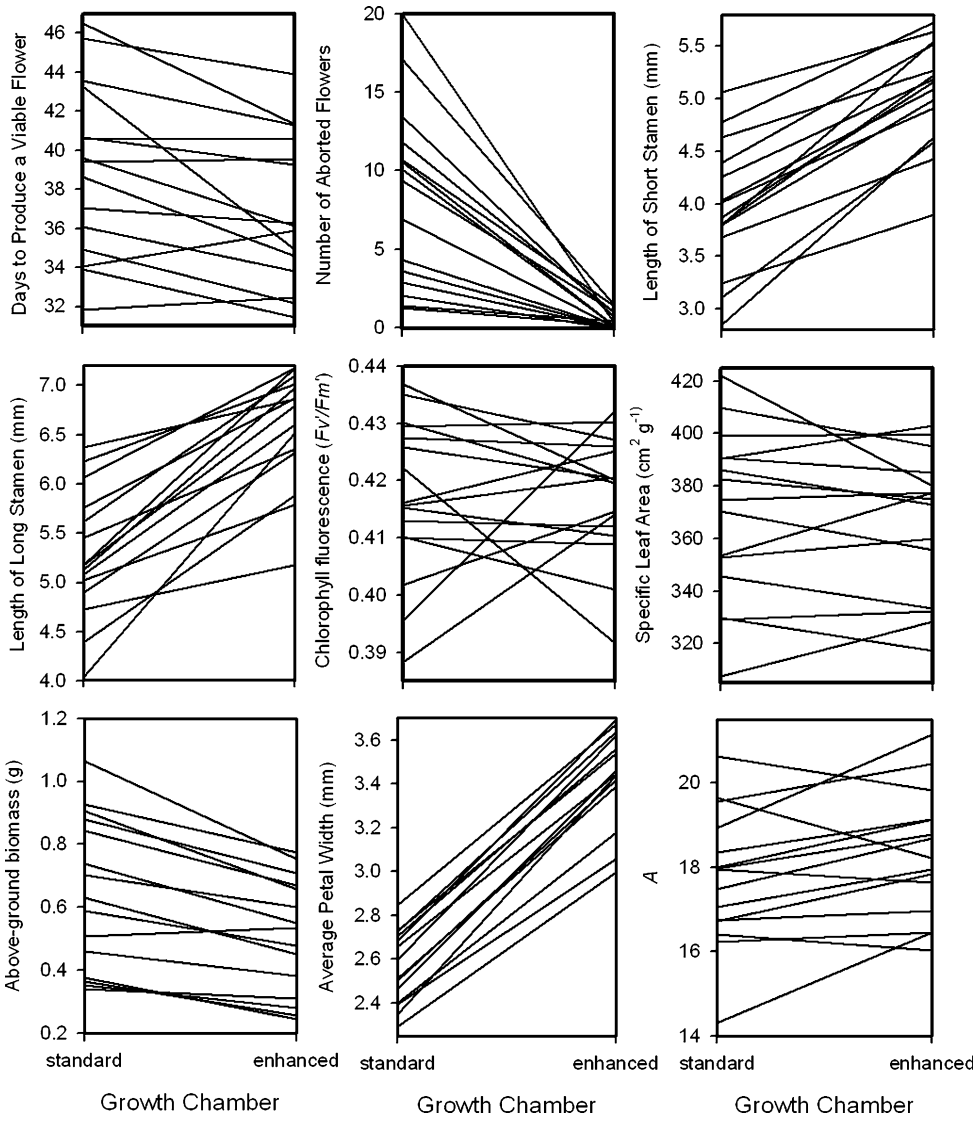

Fig. 2 Norms of reaction for traits that demonstrated significant ($P < 0.05$) or marginally significant ($P \leq 0.06$) genotype × chamber interactions in growth chambers with standard and enhanced venting; significance values are presented in Tables 1 and 2. *Each line* represents the mean trait values for one genotype of *B. rapa* in the two growth chambers. For each trait, seven genotypes that had outlying means or means that closely overlapped with another genotype were removed

important effect on the interpretation of $\delta^{13}C_{leaf}$ and $\Delta^{13}C$ estimates.

Corroboration from different growth chamber experiments

Effects of chamber type were similar (i.e., the same direction of effects with equivalent or higher significance values) in both experiments for the following six traits: days to bolting, days to produce a viable flower, number of aborted flowers, above-ground biomass, SLA, and g_s (data not shown). *A* and below-ground biomass, which demonstrated only marginally significant effects of chamber type in the current experiment, did not demonstrate significant chamber effects in the replicate experiment (data not shown). The genotype × chamber-type interactions in the replicate experiment were of similar or much greater significance than in the current study for all traits (days to bolting, days to produce a viable flower, above-ground biomass, below-ground biomass, *A*, and SLA), except number of aborted flowers, which showed a significant interaction effect in the current experiment (Table 1) but not in the replicate (data not shown).

Discussion

Air CO_2 concentration in growth chambers with standard and enhanced venting

The results of this study demonstrate that the standard ventilation in these growth chambers is not sufficient to prevent photosynthesis-induced, biologically relevant draw-downs of CO_2, even for an experiment that occupied only half of the growth capacity of a growth chamber, which is well within the capacity commonly used in

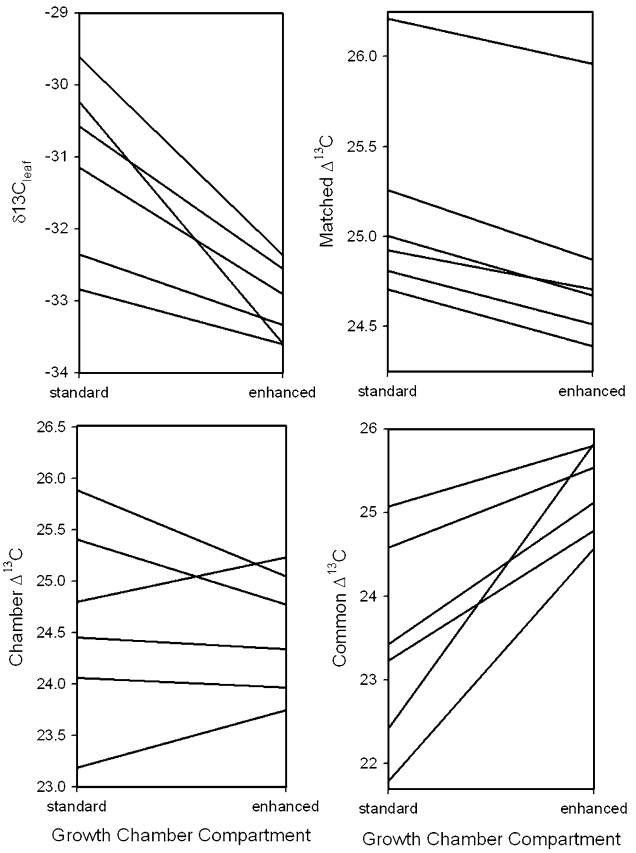

Fig. 3 The difference in norms of reaction for $\Delta^{13}C$ in growth chambers with standard or enhanced venting using the three different $\delta^{13}C_{air}$ correction values (see Table 3 for an explanation of the correction values and significance of statistical tests)

genetics studies. [CO_2] in the growth chamber with the manufacturer's standard ventilation (10 air exchanges per hour) dropped from ambient conditions of 400 to 280 μmol mol^{-1} (a 30% reduction); the last time ambient conditions were at 280 μmol mol^{-1}, was before the industrial revolution, at least 250 years ago (Etheridge et al. 1996; Neftel et al. 1982). Plant photosynthesis was likely responsible for draw-downs of [CO_2] in the standard chamber because the stable carbon isotope ratio of CO_2 in chamber air ($\delta^{13}C_{air}$) was negatively correlated with [CO_2] (Farquhar and Lloyd 1993). Plant photosynthesis also appeared to result in draw-downs of [CO_2] in the chamber with enhanced ventilation, but these reductions in [CO_2] were minimized by increasing the number of air exchanges to 20 per hour; [CO_2] in the enhanced chamber thus only dropped from 400 μmol mol^{-1} to slightly sub-ambient levels at 360 μmol mol^{-1} (a 10% reduction).

Because there is an inverse relationship between [CO_2] and photorespiration (Sharkey 1988) and a positive relationship between [CO_2] and photosynthesis (Sage 1995; Sage and Coleman 2001), plants in both chambers are likely to have experienced increased rates of photorespiration and reduced photosynthesis relative to ambient conditions. At the lowest [CO_2] in the standard chamber, the ratio of photorespiration to carboxylation would be ~0.50, whereas it would be ~0.40 in the enhanced chamber and ~0.35 at current ambient conditions (Sharkey 1988). This is likely to have caused reductions in biomass, reproduction, etc. However, because the reduction in [CO_2] was minimized in the enhanced chamber due to the addition of ventilation, the amount of photorespiration and its effects on traits should also be minimized in comparison to the standard chamber. Recent work with *B. rapa* has shown that photorespiration and photoinhibition is transient on the time scale of hours at light levels of 600 μmol m^{-2} s^{-1} of light, but is significant at that light level during drought (Jiao et al. 2004).

Effects of chamber venting on phenotypic trait estimates

Our results suggest that differences in CO_2 dynamics between the two growth chambers affected the phenotypic expression of many traits, and reproductive and physiological traits were particularly affected by differences in chamber [CO_2]. For reproductive traits, plants in the standard chamber generally had smaller floral organs, flowers were aborted more frequently, and more time was necessary to produce viable flowers in comparison to plants in the enhanced chamber (Table 4). Further, there were significant interactions between genotype and environment for many floral traits such as stamen elongation, petal development, the number of aborted flowers, and the amount of time to produce a viable flower. These results are in agreement with another study that demonstrated that constant low [CO_2] increases the number of days to flowering and that genotypes differed in flowering time in response to different levels of [CO_2] (e.g., Ward and Strain 1997).

Several physiological traits also exhibited significant genotype × environment interactions in response to differences in chamber CO_2 dynamics, including SLA, F'_v/F'_m, and *A*. The constellation of these trait interactions between genotype and environment supports the idea that these responses to low [CO_2] were due to more light-dependent reaction feedback (electron transport-related phenomena; significant interaction in F'_v/F'_m; Table 3; Fig. 3) than light-independent (e.g., integrated as carbon isotope ratios, no interaction in "matched $\Delta^{13}C$"; Table 3; Fig. 3). Some studies find a tight coupling between these two major components of photosynthesis due to correlation between photosynthetic electron transport and reductate production with elevated CO_2 (e.g., Bloom et al. 2002). However, recent studies suggest that this connection can become decoupled in certain plant types and environmental

conditions as quantified by two parameters of the Farquhar model (Farquhar et al. 1980), electron transport (J_{max}), and carboxylation rate (V_{cmax}). Medlyn et al. (2002) demonstrated that some cool-climate crop species were significantly deviated from the common correlation between J_{max} and V_{cmax}. The lack of strong correlation among F'_v/F'_m, $\Delta^{13}C$, N, g_s and A (Table S2) suggests that the photosynthetic electron transport and reductate production can become decoupled across genotypes within this species. We propose that future work on the mapping of these traits using QTL techniques in *B. rapa* can focus on individual traits of A that are either part of the light-dependent or -independent components of A, thereby allowing more fine-scale analysis of the underlying loci.

Effects of $\delta^{13}C_{air}$ correction values on $\Delta^{13}C$ estimates

Another unintended effect of poor venting in growth chambers is that ^{13}C discrimination by the plants in this study caused fluctuations in $\delta^{13}C_{air}$ (Fig. 1). Because $\delta^{13}C_{leaf}$ is partially a function of $\delta^{13}C_{air}$ and $\Delta^{13}C$ is calculated by correcting $\delta^{13}C_{leaf}$ for $\delta^{13}C_{air}$, our goals were to determine how fluctuations in $\delta^{13}C_{air}$ may influence $\delta^{13}C_{leaf}$ estimates and how using different $\delta^{13}C_{air}$ values may influence the calculation and interpretation of $\Delta^{13}C$ in growth chambers. When we used $\delta^{13}C_{leaf}$, "common $\Delta^{13}C$", or "chamber $\Delta^{13}C$", significant genotype × chamber interactions were detected. When we used "matched $\Delta^{13}C$" (matched to the phenology of each leaf by averaging the previous week's daily $\delta^{13}C_{air}$ values), we did not detect a genotype × chamber interaction. Several studies carried out in open environments that did not experience fluctuations of $\delta^{13}C_{air}$ or $[CO_2]$ demonstrated that genotypes maintain constancy in rank order for $\Delta^{13}C$ across environments (Ehleringer 1993; Ismail and Hall 1993). Because the "matched $\Delta^{13}C$" results also maintained constancy in rank order, we believe that the matched method reflects the most accurate estimate of ^{13}C discrimination in this study. Thus, for studies carried out in enclosed environments such as growth chambers or greenhouses, progressive changes in $\delta^{13}C_{air}$ (such as those revealed in this study) that are unaccounted for will systematically bias estimates of $\delta^{13}C_{leaf}$ and $\Delta^{13}C$ according to growth phenology (or day of collection). This may also induce shuffling of the rank order of genotypes' $\Delta^{13}C$ values, like those found using "common $\Delta^{13}C$" and "chamber $\Delta^{13}C$". These results are important because small changes in carbon isotope discrimination estimates can have large effects on the interpretation of the physiology of the plant, such as the interpretation of intrinsic and leaf water use efficiency and stress (Farquhar et al. 1982, 1988; Seibt et al. 2008). In enclosed environments such as the present study, the simple solution is to take the growth phenology of sampled leaves into account and to carefully measure and account for variation in $\delta^{13}C_{air}$.

In the physiological literature, it has long been recognized that temporal and spatial variation may exist in $\delta^{13}C_{air}$, even across small spatial scales in natural environments; e.g., several studies have demonstrated vertical gradients in $\delta^{13}C_{air}$ and $[CO_2]$ within a canopy (Berry et al. 1997; Broadmeadow et al. 1992; Sternberg et al. 1989). Such variation in $\delta^{13}C_{air}$ has been shown to alter $\delta^{13}C_{leaf}$ by up to several parts per thousand (Sternberg et al. 1989) and may change the interpretation of the climate to physiology connection (Panek and Waring 1995). The standard practice in the physiological and ecological literature is to account for spatial and temporal variation in $\delta^{13}C_{air}$ using measured values of $\delta^{13}C_{air}$ to calculate $\Delta^{13}C$. However, this is not the case in quantitative genetics literature; of a handful of quantitative genetics and QTL mapping studies that have measured $\delta^{13}C_{leaf}$ and/or $\Delta^{13}C$ traits in a greenhouse or growth chambers, only four measured or acknowledged that there may be temporal fluctuations in $\delta^{13}C_{air}$ (Comstock et al. 2005; Hausmann et al. 2005; Juenger et al. 2005; McKay et al. 2008), and only one (Comstock et al. 2005) accounted for these fluctuations using the "matched $\Delta^{13}C$" method to calculate carbon isotope discrimination. We are unsure if fluctuations in $\delta^{13}C_{air}$ in previous studies have influenced QTL mapping results, but future QTL mapping studies can avoid potential confounding affects by accounting for growth phenology and fluctuations in $\delta^{13}C_{air}$ in calculations of $\Delta^{13}C$.

Possible causes of genotype × chamber interactions

The significant genotype × chamber interactions detected for many phenotypic traits may have been caused by one or several of the following phenomena: (1) differences in the extent to which $[CO_2]$ changed in the two chambers in relation to genotypes' phenology, (2) the differential sensitivity of some genotypes to low $[CO_2]$, or (3) differences in the chambers that are unrelated to $[CO_2]$. In the chamber equipped with the manufacturer's standard ventilation, $[CO_2]$ rapidly decreased once the first few genotypes began floral initiation, resulting in differing $[CO_2]$ for early- versus late-flowering genotypes. Because genotypes reached a common developmental stage (i.e., bolting time) at different time intervals and measurements were taken at this common developmental stage, many traits were measured across a gradient of $[CO_2]$ in the standard chamber. This gradient may have strongly affected the expression of traits that are sensitive to $[CO_2]$. In contrast, because temporal $[CO_2]$ fluctuations were minimized in the enhanced chamber by additional ventilation, temporal variation in $[CO_2]$ and attendant effects on plant phenotypes would have been correspondingly minimized.

In short, early-flowering genotypes experienced similar conditions across the two chamber types, while late-flowering genotypes likely experienced different [CO_2], presumably contributing to the observed genotype × chamber interactions.

Another possible source of genotype × chamber interactions is that genotypes may differ in their sensitivity to low CO_2. For example, when genotypes of *Arabidopsis* were grown at constant low, ambient, and high [CO_2], significant genotype × [CO_2] interactions were detected for many traits (Ward and Strain 1997), indicating that some genotypes must be more sensitive to low [CO_2] than others. In this study, it is possible that certain genotypes may be more vigorous than others at normal CO_2, but have a reduced ability to photosynthesize at low [CO_2]. Such physiological sensitivity may have affected the expression of many phenotypic traits. However, we are unable to separate the influences of temporal variation in [CO_2] from genotypic sensitivity to low CO_2. To determine the relative effects of these phenomena in *B. rapa*, one could repeat the present study but maintain [CO_2] at constant low and ambient levels to remove the influence of temporal fluctuations in [CO_2].

A further explanation for the observed genotype × chamber interactions is that an unknown source of variation exists between the growth chambers and genotypes are sensitive to this unknown variation. Studies have shown that variation may exist in conditions among growth chambers, even between identically programmed chambers of the same model from the same manufacturer (Potvin et al. 1990). However, when we examined phenotypic traits of plants growing in different chambers but with the same venting treatments as in the current study, we detected almost identical venting effects and genotype × chamber-venting interactions, suggesting that the variation among chambers was caused by differences in venting and [CO_2], and not due to some other source of unmeasured variation. Furthermore, apart from the marked influence in [CO_2], the addition of ventilation had little effect on all other growth conditions (e.g., humidity or temperature), suggesting that differences in genotypic trait variances between the two chamber types are due to differential responses to CO_2 dynamics and not due to variation in other environmental factors.

Conclusions

Regardless of whether temporal variation in [CO_2], genotypic sensitivity to low [CO_2], or both are responsible for the genotypes' differing responses observed in this study, lack of adequate growth chamber venting and attendant fluctuations in CO_2 introduced significant unintended variation among genotypes in the expression of floral and physiological traits. This unintended variation among chambers is likely due to the increased expression of genes that are involved in the response to low CO_2 or decreased expression of genes that are normally active at ambient [CO2] in the standard chamber. Thus, instead of quantifying the genetic variation for a trait of interest (e.g., floral organ size), some combination of genes for the focal trait and for response to low CO_2 was likely responsible for the phenotypic variation estimated in the standard chamber. This suggests that QTL mapping studies carried out in growth chambers with large fluctuations in CO_2 may map some genomic regions harboring loci underlying the traits of interest, but may also map regions with genes involved in response to CO_2 stress. This pitfall could be avoided by carefully monitoring and maintaining [CO_2] at ambient or nearly ambient conditions by either enhanced venting or full control over [CO_2].

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References

Andaya VC, Mackill DJ (2003a) Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J Exp Bot 54:2579–2585

Andaya VC, Mackill DJ (2003b) QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a *japonica* × *indica* cross. Theor Appl Genet 106:1084–1090

Bernier PY, Stewart JD, Hogan GD (1994) Quantifying the uncontrolled CO_2 dynamics of growth chambers. J Exp Bot 45:1143–1146

Berry SC, Varney GT, Flanagan LB (1997) Leaf delta C^{13} in *Pinus resinosa* trees and understory plants: variation associated with light and CO_2 gradients. Oecologia 109:499–506

Bloom AJ, Smart DR, Nguyen DT, Searles PS (2002) Nitrogen assimilation and growth of wheat under elevated carbon dioxide. Proc Natl Acad Sci USA 99:1730–1735

Broadmeadow MSJ, Griffiths H, Maxwell C, Borland AM (1992) The carbon isotope ratio of plant organic material reflects temporal and spatial variations in CO_2 within tropical forest formations in Trinidad. Oecologia 89:435–441

Brouillette LC, Rosenthal DM, Rieseberg LH, Lexer C, Malmberg RL, Donovan LA (2007) Genetic architecture of leaf ecophysiological traits in *Helianthus*. J Hered 98:142–146

Comstock JP, McCouch SR, Martin BC, Tauer CG, Vision TJ, Xu YB, Pausch RC (2005) The effects of resource availability and environmental conditions on genetic rankings for carbon isotope discrimination during growth in tomato and rice. Funct Plant Biol 32:1089–1105

Corbesier L, Coupland G (2005) Photoperiodic flowering of *Arabidopsis*: integrating genetic and physiological approaches to characterization of the floral stimulus. Plant Cell Environ 28:54–66

Dechaine JM, Johnston JA, Brock MT, Weinig C (2007) Constraints on the evolution of adaptive plasticity: costs of plasticity to

density are expressed in segregating progenies. New Phytol 176:874–882

Degenkolbe T, Do PT, Zuther E, Repsilber D, Walther D, Hincha DK, Kohl KI (2009) Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. Plant Mol Biol 69:133–153

Dippery JK, Tissue DT, Thomas RB, Strain BR (1995) Effects of low and elevated CO_2 on C_3 and C_4 annuals. I. Growth and biomass allocation. Oecologia 101:13–20

Ehleringer JR (1993) Carbon isotope variation in *Encelia farinosa*: implications for growth, competition, and drought survival. Oecologia 95:340–346

Etheridge DM, Steele LP, Langenfelds RL, Francey RJ, Barnola J-M, Morgan VI (1996) Natural and anthropogenic changes in atmospheric CO_2 over the last 1000 years from air in Antarctic ice and firn. J Geophys Res 101:4115–4128

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Hall AE, Farquhar GD (eds) Stable isotopes in plant carbon–water relations. Academic Press, San Diego, pp 47–70

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33:317–345

Farquhar GD, Caemmerer SV, Berry JA (1980) A biochemical-model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90

Farquhar GD, Oleary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Farquhar GD, Hubick KT, Condon AG, Richards RA (1988) Carbon isotope fractionation and plant water-use efficiency. In: Rundel PW, Ehleringer JR, Nogy KA (eds) Stable isotopes in ecological research. Springer, New York, pp 21–40

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Funatsuki H, Matsuba S, Kawaguchi K, Murakami T, Sato Y (2004) Methods for evaluation of soybean chilling tolerance at the reproductive stage under artificial climatic conditions. Plant Breed 123:558–563

Hall NM, Griffiths H, Corlett JA, Jones HG, Lynn J, King GJ (2005) Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. Plant Breed 124:557–564

Hausmann NJ, Juenger TE, Sen S, Stowe KA, Dawson TE, Simms EL (2005) Quantitative trait loci affecting δC^{13} and response to differential water availability in *Arabidopsis thaliana*. Evol Int J Org Evol 59:81–96

Ismail AM, Hall AE (1993) Carbon isotope discrimination and gas exchange of *Cowpea* accessions and hybrids. Crop Sci 33:788–793

Jiao SX, Emmanuel H, Guikema JA (2004) High light stress inducing photoinhibition and protein degradation of photosystem I in *Brassica rapa*. Plant Sci 167:733–741

Juenger TE, McKay JK, Hausmann N, Keurentjes JJB, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH (2005) Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: δC^{13}, stomatal conductance and transpiration efficiency. Plant Cell Environ 28:697–708

Lacey EP (1996) Parental effects in *Plantago lanceolata* L. 1. A growth chamber experiment to examine pre- and postzygotic temperature effects. Evol Int J Org Evol 50:865–878

Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J Exp Bot 54:2393–2401

Lou QJ, Chen L, Sun ZX, Xing YZ, Li J, Xu XY, Mei HW, Luo LJ (2007) A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.). Euphytica 158:87–94

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature 436:866–870

McKay JK, Richards JH, Nemali KS, Sen S, Mitchell-Olds T, Boles S, Stahl EA, Wayne T, Juenger TE (2008) Genetics of drought adaptation in *Arabidopsis thaliana*. II. QTL analysis of a new mapping population, Kas-1 × Tsu-1. Evol Int J Org Evol 62:3014–3026

Medlyn BE, Dreyer E, Ellsworth D, Forstreuter M, Harley PC, Kirschbaum MUF, Le Roux X, Montpied P, Strassemeyer J, Walcroft A et al (2002) Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant Cell Environ 25:1167–1179

Neftel A, Oeschger H, Schwander J, Stauffer B, Zumbrunn R (1982) Ice core sample measurements give atmospheric CO_2 content during the past 40,000 year. Nature 295:220–223

Oleary MH (1988) Carbon isotopes in photosynthesis. Bioscience 38:328–336

Onouchi H, Coupland G (1998) The regulation of flowering time of Arabidopsis in response to daylength. J Plant Res 111:271–275

Panek JA, Waring RH (1995) Carbon-isotope variation in Douglas-fir foliage: improving the delta C13-climate relationship. Tree Physiol 15:657–663

Patterson DT, Hite JL (1975) A CO_2 monitoring and control system for plant growth chambers. Ohio J Sci 75:190–193

Peet MM, Krizek DT (1997) Carbon dioxide. In: Langhans RW, Tibbitts TW (eds) Plant growth chamber handbook. North Central Regional Research Committee Publication Number 340, Ames, IA, pp 65–79

Polley HW, Johnson HB, Marino BD, Mayeux HS (1993) Increase in C_3 plant water-use efficiency and biomass over glacial to present CO_2 concentrations. Nature 361:61–64

Potvin C, Lechowicz MJ, Bell G, Schoen D (1990) Spatial, temporal, and species-specific patterns of heterogeneity in growth chamber experiments. Can J Bot 68:499–504

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop *Brassicas*, a review. Opera Bot 55:1–57

Rasband WS (1997–2007) ImageJ. National Institutes of Health, Bethesda, MD

Rytter RM (2005) Water use efficiency, carbon isotope discrimination and biomass production of two sugar beet varieties under well-watered and dry conditions. J Agron Crop Sci 191:426–438

Sage RF (1995) Was low atmospheric CO_2 during the Pleistocene a limiting factor for the origin of agriculture? Global Change Biol 1:93–106

Sage RF, Coleman JR (2001) Effects of low atmospheric CO_2 on plants: more than a thing of the past. Trends Plant Sci 6:18–24

Sato T, Ueda T, Fukuta Y, Kumagai T, Yano M (2003) Mapping of quantitative trait loci associated with ultraviolet-B resistance in rice (*Oryza sativa* L.). Theor Appl Genet 107:1003–1008

Schauer AJ, Lai C-T, Bowling DR, Ehleringer JR (2003) An automated sampler for collection of atmospheric trace gas samples for stable isotope analyses. Agric For Meteorol 118:113–124

Seibt U, Rajabi A, Griffiths H, Berry J (2008) Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155:441–454

Sharkey TD (1988) Estimating the rate of photorespiration in leaves. Physiol Plant 73:147–152

Sternberg LDL, Mulkey SS, Wright SJ (1989) Ecological interpretation of leaf carbon isotope ratios-influence of respired carbon dioxide. Ecology 70:1317–1324

Takai T, Fukuta Y, Sugimoto A, Shiraiwa T, Horie T (2006) Mapping of QTLs controlling carbon isotope discrimination in the photosynthetic system using recombinant inbred lines derived

from a cross between two different rice (*Oryza sativa* L.) cultivars. Plant Product Sci 9:271–280

Tasma IM, Lorenzen LL, Green DE, Shoemaker RC (2001) Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. Mol Breed 8:25–35

Thumma BR, Naidu BP, Chandra A, Cameron DF, Bahnisch LM, Liu CJ (2001) Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis. J Exp Bot 52:203–214

Tibbitts TW, Krizek DT (1978) Carbon dioxide. In: Langhans R (ed) Growth chamber manual: environment control for plants. Cornell University Press, Ithaca, NY

Tissue DT, Griffin KL, Thomas RB, Strain BR (1995) Effects of low and elevated CO_2 on C_3 and C_4 annuals. II. Photosynthesis and leaf biochemistry. Oecologia 101:21–28

Ward JK, Strain BR (1997) Effects of low and elevated CO_2 partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. Plant Cell Environ 20:254–260

Welcker C, Boussuge B, Bencivenni C, Ribaut JM, Tardieu F (2007) Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of Anthesis-Silking Interval to water deficit. J Exp Bot 58:339–349

Wheeler RM (1992) Gas-exchange measurements using a large, closed plant growth chamber. HortScience 27:777–780

Ziska LH (2003) Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. J Exp Bot 54:395–404