Carbon Dioxide Enrichment Does Not Reduce Leaf Longevity or Alter Accumulation of Carbon Reserves in the Woodland Spring Ephemeral *Erythronium americanum*

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Received: 29 May 2008 Returned for revision: 21 July 2008 Accepted: 29 July 2008 Published electronically: 29 August 2008

- **Background and Aims** Woodland spring ephemerals exhibit a relatively short epigeous growth period prior to canopy closure. However, it has been suggested that leaf senescence is induced by a reduction in the carbohydrate sink demand, rather than by changes in light availability. To ascertain whether a potentially higher net carbon (C) assimilation rate could shorten leaf lifespan due to an accelerated rate of storage, *Erythronium americanum* plants were grown under ambient (400 ppm) and elevated (1100 ppm) CO₂ concentrations.
- **Methods** During this growth-chamber experiment, plant biomass, bulb starch concentration and cell size, leaf phenology, gas exchange rates and nutrient concentrations were monitored.
- **Key Results** Plants grown at 1100 ppm CO₂ had greater net C assimilation rates than those grown at 400 ppm CO₂. However, plant size, final bulb mass, bulb filling rate and timing of leaf senescence did not differ.
- **Conclusions** *Erythronium americanum* fixed more C under elevated than under ambient CO₂ conditions, but produced plants of similar size. The similar bulb growth rates under both CO₂ concentrations suggest that the bulb filling rate is dependant on bulb cell elongation rate, rather than on C availability. Elevated CO₂ stimulated leaf and bulb respiratory rates; this might reduce feed-back inhibition of photosynthesis and avoid inducing premature leaf senescence.

Key words: Source–sink relations, assimilation rates, growth rates, CO₂ enrichment, respiration, spring ephemeral, leaf senescence, bulbous plant, carbohydrate storage, *Erythronium americanum*.

INTRODUCTION

Spring ephemerals are a common element of the herb layer in deciduous temperate North American forests. Most of these species are clonal and perennial (Whigham, 2004), and are characterized by a relatively short epigeous growth period (40–60 d) between snowmelt and canopy closure (Lapointe, 2001). During this period, these plants grow, and may flower and set seed; concurrently, the perennial organ is renewed and stocked with nutrients and carbohydrates. Thereafter, the shoots senesce and below-ground parts become dormant until the onset of suitable environmental signals that trigger regrowth.

Spring ephemerals exhibit some of the characteristics typically associated with sun plants (Sparling, 1964, 1967; Taylor and Percy, 1976) and their shoots begin to senesce while the canopy is closing (Vézina and Grandtner, 1965). Therefore, reduced incident light intensity was thought to be one of the main signals responsible for triggering leaf senescence. However, plants grown under constant light conditions exhibit similar leaf life duration as under natural conditions (Lapointe and Lerat, 2006). It has been proposed that once the perennial organ is renewed and filled with carbohydrates, sink activity would decrease and induce leaf senescence (Lapointe, 2001). Sink-limited growth has been reported in a number of species during specific parts of their life cycle (Hocking and Steer, 1994; Schubert and Feuerle, 1997; Körner, 2003; Badri *et al.*, 2007; Fischer, 2007). Sink limitation can reduce photosynthetic rates by

feed-back inhibition, but it can also accelerate leaf senescence (Paul and Foyer, 2001).

The present study was conducted to test further the hypothesis that growth in spring ephemerals is sink rather than source limited, despite their very short leaf lifespan. *Erythronium americanum*, a common spring ephemeral in North American deciduous forests, is an attractive species to study whole plant carbon (C) exchange due to the fact that non-flowering individuals are composed of a single leaf and a single bulb and that the root system develops during the cold stratification period. Non-flowering plants of *E. americanum*, were grown in two growth chambers under ambient (400 ppm) or elevated (1100 ppm) CO₂ concentrations [CO₂]. The experiment was repeated twice (2004 and 2005). The effects of [CO₂] on bulb starch content and concentration, gas exchange rates, and plant growth were investigated. A stimulation of the net photosynthetic rate and a subsequently greater C exportation rate to the bulb was anticipated. However, this was not expected to alter bulb growth (i.e. cell division and elongation) or total sink demands. Therefore, bulbs might be filled more rapidly and leaf senescence advanced. Changes in leaf nitrogen (N) content over time were also monitored to characterize potential CO₂ acclimation of the photosynthetic apparatus, and as an early sign of leaf senescence. The validation of the sink-limited hypothesis would suggest that the growth capacity of the below-ground part must be taken into account when predicting the response of spring perennial herbs to changes in [CO₂], as well as to global climate change.

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MATERIALS AND METHODS

Experimental design

Bulbs of *Erythronium americanum* Ker Gawl. were collected from a maple forest (46°48'N, 71°23'W) near Québec City (Canada) in September 2003 and 2004. Bulbs weighing between 0.30 g and 0.35 g were planted individually in 10-cm plastic pots containing 350 mL of Turface (Applied Industrial Materials, Corp., Buffalo Grove, IL, USA) and cold (4 °C) stratified for 5 months. At that size, *E. americanum* bulbs produced a single leaf and no flower. The pots were then randomly transferred to two growth chambers [model M18(SI); Environment Growth Chambers, Chagrin Falls, OH, USA]. These provided a day/night temperature of 18/14 °C, a light intensity of 290–310 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, a light/dark regime of 14/10 h and a relative humidity of 75 %. The [CO₂] differed between chambers, with one providing approx. 400 $\mu\text{mol mol}^{-1}$ CO₂ (ambient, also called control conditions) and the other 1100 $\mu\text{mol mol}^{-1}$ CO₂ (elevated) as monitored by a CO₂ transmitter (Vaisala GMW20, Helsinki, Finland) located in each chamber. To take into account any potential growth chamber effect (Potvin and Tardif, 1988), the [CO₂] in the two chambers was inverted when the experiment was repeated. An 18/14 °C temperature regime was chosen because at this temperature the maximum growth rate of *E. americanum* is not reached (Lapointe and Lerat, 2006) and the potential effect of an elevated [CO₂] on bulb growth might be detected. To ensure significant differences in photosynthetic activity, a near saturating [CO₂] was chosen for the elevated [CO₂] treatment. Furthermore, some long-term climate change models predict future [CO₂] higher than 1000 ppm (Hasselmann *et al.*, 2003). Plants were watered daily and fertilized weekly (150 mL of 10 % Hoagland's solution, for a total of 32 mg of N added per pot for the whole season).

Growth measurements

Plants were harvested at six different growth stages (seven plants per harvest in 2004; six plants per harvest in 2005): (1) before transfer of the plants in the growth chambers; (2) and (3) during the growth period; (4) when the first visual indication of leaf senescence appeared; (5) halfway through leaf senescence; and (6) following complete leaf senescence. Leaf area (up to leaf yellowing) was measured using a leaf area meter (model 3100 Li-Cor Inc., Lincoln, NE, USA), and the fresh and dry (70 °C for 48 h) biomass of the leaf, the bulb and the roots was determined. The length of the growth phase was determined as the period between complete leaf unfolding and the onset of leaf yellowing. Leaf longevity was determined as the period between leaf unfolding and complete leaf senescence.

Leaf gas-exchange measurements

Gas exchange measurements were done using a portable LCA-4 infrared gas analyser (ADC Bioscientific Ltd,

Hoddesdon, UK). Net leaf assimilation rates (A) were measured on the single leaf of 12 plants in each of the two treatments. In 2004, measurements were taken in the afternoon on the same plants at different stages up until leaf senescence. In 2005, measurements were only taken during the 2 weeks following leaf unfolding. In 2005, leaf respiratory rates were measured 12 d and 13 d after leaf unfolding, and bulb respiratory rates were measured 13 d and 18 d after leaf unfolding. The respiration measurements were taken after a short dark exposure and just prior to harvesting. All measurements were done under growth chamber conditions (light, temperature, vapour pressure deficit, and [CO₂]), except for respiration rates that were recorded under dark conditions within the growth chambers.

Determination of cell size and number

Five bulbs per treatment per harvest date were fixed in a solution of FAA (formaldehyde–acetic acid–alcohol; Sass, 1958). Longitudinal thin sections from each bulb were mounted on microscope slides, stained with 0.01 % (w/v) toluidine blue and observed under a light microscope (Olympus, USA). Digital photographs were taken, cell numbers were counted on the diagonal of the thin section, and cell area was measured using the 3D-Doctor software (Able Software Corp., Lexington, MA, USA).

Starch quantification

The starch concentration was determined for six (in 2005) or seven (in 2004) bulbs per treatment per harvest. Harvests were done at the same time as those for biomass measurements. Starch was extracted using the method outlined by Castonguay *et al.* (1993). Briefly, bulbs were dried at 70 °C, weighed, incubated (65 °C) for 20 min in a solution of methanol, chloroform and water (12 : 5 : 3), ground, and centrifuged at 4 °C. The starch within the pellet fraction was then gelatinized in boiling distilled water (90 min) and hydrolysed at 55 °C (60 min) using *Rhizopus amyloglucosidase* (Sigma-Aldrich, St Louis, MO, USA). Reducing sugars (the products of starch hydrolysis) were quantified colorimetrically at 415 nm, using *p*-hydroxybenzoic acid hydrazide (Blakeney and Mutton, 1980). The starch concentration was determined by comparison with a standard curve. Starch content was estimated as the value of the starch concentration of each sample multiplied by the dry biomass of the bulb.

Nutrient analysis

The N analyses were done on the ground leaf tissues of the plants used for bulb starch analyses. Samples were paired and pooled to provide sufficient material for the analyses. Bulb nutrient concentration was also measured in 2004 on individual bulbs harvested at the same phenological stages as for plant biomass. The N concentrations were determined colorimetrically after digestion with sulfuric acid, selenic acid and hydrogen peroxide (Nkonge and Ballance, 1982). Nutrient content was then calculated by multiplying nutrient concentration by either leaf or bulb mass.

Statistical analysis

As the growth of *E. americanum* is influenced, in part, by conditions experienced during the previous growing season, the experiments for the two years were analysed separately using a completely randomized design in which each plant was considered as a replicate. The analyses of phenological variables of the leaf (from leaf unfolding to leaf yellowing) were performed using *t*-tests. Two-way ANOVAs, with time and [CO₂] as the main factors, were used to compare bulb, leaf and root dry biomass, leaf area, and starch and nutrient contents and concentrations. Respiratory rates were compared using two-way ANOVAs with time and [CO₂] as the main factors. Photosynthetic rates were compared using *t*-tests, because the photosynthetic measurements were not always recorded on the same days for both treatments. As gas exchange measurements were taken at different times during the afternoon and early evening, linear regressions were performed to investigate whether assimilation rates changed during the day and whether this could influence the comparison of the two treatments. Bulb cell size and number were compared using the Wilcoxon rank sum test. All statistical analyses were performed using the SAS 9.1 version (SAS Institute, Cary, NC, USA). Data were transformed when they did not meet the criteria of normality and homogeneity of the variance of the residues. An $\alpha = 0.05$ was used for the significance tests and, to complete the analysis, *a posteriori* multiple comparisons tests were performed using Fisher's LSD.

RESULTS

Leaf longevity

Elevated [CO₂] did not significantly affect the duration of the green-leaf period of the plants (Fig. 1). In 2004, the yellowing of the distal part of the leaf occurred after 23.9 ± 1.3 d of growth under ambient [CO₂], and after 21.5 ± 1.8 d under elevated [CO₂] ($t = -1.07$; $P = 0.292$). In 2005, plants grown under elevated [CO₂] began to yellow after 19.2 ± 0.9 d and control plants after 19.9 ± 1.0 d ($t = -0.51$; $P = 0.615$).

By contrast, [CO₂] affected total leaf longevity. In 2004, the longevity of leaves of plants grown under elevated [CO₂] was 4 d longer (40.3 ± 1.1 d) than that of the controls (36.1 ± 1.3 d; $t = 2.47$; $P = 0.019$). In 2005, no significant difference was found between treatments ($t = 1.80$; $P = 0.091$); the leaves of plants grown under ambient [CO₂] were fully senesced after 30.9 ± 0.9 d and those of plants grown under elevated [CO₂] after 33.5 ± 1.1 d.

Plant growth

Increased [CO₂] did not significantly influence leaf area, leaf dry biomass, or root dry biomass (root biomass was only measured in 2004; Fig. 2). Leaf dry biomass increased with time in 2004 ($F_{3,46} = 3.00$; $P = 0.040$) and in 2005 ($F_{2,26} = 7.75$; $P = 0.002$), as did leaf area in 2004

FIG. 1. Changes in *Erythronium americanum* bulb biomass during the epigeous growth period, in plants grown at ambient and elevated [CO₂] in (A) 2004 and (B) 2005. Means $\pm$ s.e. are shown ($n = 7$ in 2004 and $n = 6$ in 2005). The arrows indicate the onset of leaf yellowing.

($F_{3,46} = 13.17$; $P < 0.001$). The root dry weight in 2004 and the leaf area in 2005 remained unchanged during the growing season.

In 2004 there was a significant interaction between time and treatment ($F_{5,71} = 2.59$; $P = 0.033$) for bulb biomass, suggesting that the growth rate differed between the two treatments (Fig. 1A). During the first 2 weeks following unfolding of the leaf, the plants exhibited the same increase in biomass. However, differences appeared between day 12 and the beginning of leaf yellowing, when control plants accumulated more biomass than those grown under elevated [CO₂]. By contrast, although no significant difference in bulb biomass was observed in control plants between the onset of leaf yellowing and complete leaf senescence, at elevated [CO₂] the bulb continued to grow during leaf yellowing. Nevertheless, there was no significant difference between treatments for the final harvest at the onset of dormancy.

In 2005 (Fig. 1B), the same trends were observed, but time was the only factor that significantly influenced the growth of the bulb ($F_{5,54} = 199.29$; $P < 0.001$). The biomass of the bulb was stable during the first 4 d following unfolding of the leaf, after which it increased until leaf yellowing. A statistically significant increase in average bulb dry biomass occurred between half yellowing of the leaf and complete leaf senescence in both treatments.

FIG. 2. Changes in *Erythronium americanum* total leaf biomass (A, D), total root biomass (C), and total leaf area (B, E) from complete leaf unfolding (day 4) up to the onset of leaf yellowing, for plants grown at ambient and elevated [CO₂] in 2004 (A–C) and 2005 (D, E). Means $\pm$ s.e. are shown ($n = 7$ in 2004 and $n = 6$ in 2005).

Gas exchange measurements

In both repetitions, net assimilation rates were higher in plants grown under elevated [CO₂] than in controls (Fig. 3; 2004: $t = 6.95$, $P < 0.001$; 2005: $t = 3.94$, $P = 0.007$). In 2004, the mean assimilation rate was $6.0 \pm 0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ in plants grown under ambient [CO₂] and $11.4 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ in plants grown under elevated [CO₂], while in 2005 the rates were $16.0 \pm 0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ in plants grown under ambient [CO₂] and $24.2 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in plants grown under elevated [CO₂]. There were no significant changes in net assimilation rates throughout the day for plants grown under ambient [CO₂] ($P \geq 0.75$; data not shown) and a slight increase in net assimilation rates for plants grown under elevated [CO₂] (2004: $r^2 = 0.15$, $P = 0.008$; 2005: $r^2 = 0.04$, $P = 0.06$). In 2005, net assimilation rates were

much higher than in 2004 within each treatment (Fig. 3); however, total leaf area in 2005 was much smaller than in 2004 (Fig. 2). As a result, the total amount of C fixed per day was quite similar between years within each treatment, and was higher under elevated [CO₂] than under ambient [CO₂] each year (data not shown).

Bulb respiratory rates were considerably greater when plants were grown under elevated [CO₂] than under ambient [CO₂] (Table 1; $F_{1,28} = 14.91$, $P < 0.001$), whereas the mean values at days 13 and 18 were not significantly different ($F_{1,28} = 0.52$, $P = 0.475$). The mean leaf respiratory rate was significantly affected by both treatment ($F_{1,26} = 49.49$, $P < 0.001$) and time ($F_{1,26} = 75.17$, $P < 0.001$). Leaf respiratory rates were higher under elevated [CO₂] than under ambient [CO₂], and higher on day 12 than on day 13.

FIG. 3. Changes in the net photosynthetic rate of *Erythronium americanum* expressed per leaf area from day 4 after leaf unfolding to the onset of leaf yellowing in (A) 2004 and (B) during the first 2 weeks after leaf unfolding in 2005, for plants grown at ambient and elevated [CO₂]. Means $\pm$ s.e. are shown ($n = 1-12$).

Bulb cell size and number

Cell number determined on a cross-section of the bulb did not differ significantly between treatments ($P = 0.67$): 3876 ± 137 cells under ambient [CO₂] and 3365 ± 439 cells under elevated [CO₂]. Similarly, cell size did not differ between the two treatments ($P = 0.89$): $5195 \pm 313 \mu\text{m}^2$ for the ambient [CO₂] and $4603 \pm 822 \mu\text{m}^2$ for the elevated [CO₂] treatment.

TABLE 1. Bulb and leaf respiratory rates (mean $\pm$ s.e.) under ambient and elevated CO₂ growth conditions measured twice during the growth season in 2005

Day after leaf unfolding	Respiratory rates (nmol CO ₂ g f. wt ⁻¹ s ⁻¹)	
	Under ambient CO ₂ concentration	Under elevated CO ₂ concentration
Bulb		
Day 13	5.16 ± 0.50	6.50 ± 0.51
Day 18	4.41 ± 0.34	6.59 ± 0.47
Leaf		
Day 12	5.06 ± 0.28	7.39 ± 0.30
Day 13	3.00 ± 0.28	4.60 ± 0.19

Bulb starch storage

In 2004, there was a significant interaction between time and treatment for bulb starch concentration ($F_{5,70} = 2.77$; $P = 0.024$) and starch content ($F_{5,70} = 55.01$; $P < 0.001$) (Fig. 4A, B). The maximal starch concentration was rapidly reached (by the second harvest). A sudden drop in the bulb starch concentration of control plants was observed between half yellowing of the leaf and complete senescence. However, over most of the growth period the control plants exhibited slightly higher starch concentration than plants grown at elevated [CO₂]. The curve of the increase in bulb starch content was very similar to that of the increase in bulb biomass (Fig. 1A). Differences between the two treatments appeared about 14 d after leaf unfolding. At leaf yellowing, starch content was greater in bulbs grown under ambient condition, but this difference disappeared during leaf senescence.

In 2005, time was the only variable that influenced changes in bulb starch concentration ($F_{5,53} = 36.56$; $P < 0.001$) and starch content ($F_{5,53} = 83.94$; $P < 0.001$; Fig. 4C, D). Starch started to accumulate very early in the growth phase and the final starch concentration was reached between 5 d and 10 d following leaf unfolding. The starch content started to increase after day 4 and continued until the leaf was half yellowed. The ANOVA did not indicate a significant difference in starch content between the two treatments; however, as in 2004, at the onset of leaf yellowing the bulbs of control plants had a higher starch content than the bulbs of plants grown at elevated [CO₂].

In an independent experiment where *E. americanum* plants were also grown at both elevated and ambient [CO₂], starch final concentration reached again 800 mg g^{-1} d. wt, i.e. 80 % of bulb dry mass (A. Gandin, Université Laval, Québec, unpubl. res.). Soluble sugars were also quantified. Sucrose concentrations in the bulb at final harvest reached on average 35 mg g^{-1} d. wt, while hexose concentrations reached 70 mg g^{-1} d. wt under ambient [CO₂] and 80 mg g^{-1} d. wt under elevated [CO₂] (A. Gandin, Université Laval, Québec, unpubl. res.), confirming that starch is the main carbohydrate reserve form in this species.

Nutrient status

There was no treatment effect on foliar N concentrations (2004: $F_{1,30} = 0.00$, $P = 0.954$; 2005: $F_{1,30} = 3.14$, $P = 0.087$) (Fig. 5A, D). The concentration of N in the leaf tissues decreased slowly and steadily with time (2004: $F_{4,30} = 33.06$, $P < 0.001$; 2005: $F_{4,30} = 113.52$, $P < 0.001$), and the shape of the curves suggests that the mobilization was not fastened once the leaves started to senesce. In 2004, the foliar N content was significantly affected by an interaction between time and treatment (Fig. 5B; $F_{4,30} = 4.44$, $P = 0.006$). The N content was similar during the first days of growth, but as the growing season progressed it was slightly greater in plants grown under ambient [CO₂]. In 2005, time was the only variable that influenced the foliar N content ($F_{4,30} = 81.50$, $P < 0.001$)

FIG. 4. Changes in starch concentration (A, C) and starch content (B, D) of *Erythronium americanum* bulbs during the epigeous growth period. Data are from plants grown at ambient and elevated [CO₂] in (A, B) 2004 and (C, D) 2005. Means $\pm$ s.e. are shown ($n = 7$ in 2004 and $n = 6$ in 2005). The arrows indicate the onset of leaf yellowing.

and no significant content changes were observed before leaf yellowing (Fig. 5E).

In the bulb, N content was affected by an interaction between time and treatment (Fig. 5C; $F_{5,36} = 8.47$, $P < 0.001$). Bulb N content increased with time, as the bulb was increasing in biomass (Fig. 1A). During the final stage of leaf senescence, there was, however, a fast increase in bulb N content most probably related to the translocation process associated with leaf senescence.

DISCUSSION

The results reported in this study demonstrate that *E. americanum* did not benefit from an increase in [CO₂]. Net photosynthetic rates were much higher in plants grown under elevated [CO₂] than in those grown under ambient [CO₂]. However, in contrast to a large number of species that exhibit anatomical or physiological changes due to enhanced C fixation rates at elevated [CO₂] (Kimball, 1983; Ceulemans and Mousseau, 1994; Jablonski *et al.*, 2002; Poorter and Navas, 2003), no positive effects were observed on *E. americanum* growth and development. Leaf growth was not significantly affected by the treatment, as shown by the leaf area and leaf dry biomass values at maturity. These findings contrast with the results of several other studies that show that C₃ plants generally exhibit greater shoot production in response to elevated [CO₂] (Ceulemans and Mousseau, 1994; Taylor *et al.*, 1994; Pritchard *et al.*, 1999). Nevertheless, the results of the present study are consistent

with the view that enhanced [CO₂] tends to be less favourable to non-leguminous plants (Ainsworth and Long, 2005) and to wild plant species (Hunt *et al.*, 1991; Wolfenden and Diggle, 1995; Jablonski *et al.*, 2002), than to leguminous plants and trees. No difference was observed on foliar N concentration between the two treatments, suggesting that the leaves did not acclimate to elevated [CO₂]. However, more detailed gas exchange studies under both ambient and elevated [CO₂] would be needed to fully address the question of leaf acclimation to elevated [CO₂] in this species.

Elevated [CO₂] did not increase the growth of the below-ground parts. By contrast, enhanced final biomasses of roots (Rogers *et al.*, 1992; Jach *et al.*, 2000) and perennial organs (Daymond *et al.*, 1997; Miglietta *et al.*, 1998; Sicher and Bunce, 1999; Chen and Setter, 2003) has been widely reported in the literature. The lack of an enhanced growth response of *E. americanum* roots is likely due to the fact that root growth is initiated in early autumn and mainly occurs during the cold season (Brundrett and Kendrick, 1988). Daymond *et al.* (1997) observed that onion bulbs increased in size at high [CO₂] and Chen and Setter (2003) observed that potato tuber size also increased under elevated [CO₂], the latter being due to increased cell number rather than cell volume. That no change was observed in *E. americanum* cell size at elevated [CO₂], and given that the final bulb biomass was not different between the two treatments, suggests that elevated [CO₂] does not influence bulb cell development in this species. Plants are generally able (when nutrients are not limiting) to modulate the growth of their organs in response to

FIG. 5. Changes in *Erythronium americanum* foliar N concentration (A, D), foliar N content (B, E) and bulb N content (C) for plants grown at ambient and elevated [CO₂] in (A–C) 2004 and (D, E) 2005. Means $\pm$ s.e. ($n = 4$) are shown from leaf unfolding to half leaf senescence (harvests 1–5), except for bulb N content where data from completely senesced plants are also presented. The arrows indicate the onset of leaf yellowing.

their photosynthetic status. However, the lack of such a growth response under elevated [CO₂] may occur when plants are sink-limited (Paul and Foyer, 2001; Woodward, 2002). Sink limitation under high [CO₂] has been shown in onion plants where similar rates of biomass accumulation have been reported under ambient and high [CO₂] from the initiation of bulbing through to bulb maturity (Daymond *et al.*, 1997), and in potato plants before tuber initiation (Conn and Cochran, 2006).

In onion (Daymond *et al.*, 1997) and potato (Conn and Cochran, 2006), the sink-limited developmental stage was characterized by a temporary adjustment of the net photosynthetic rate. A similar adjustment was not so obvious in *E. americanum*, except perhaps towards the end of the mature phase during the second repetition of the experiment. However, the present results clearly indicate that elevated [CO₂] neither accelerated bulb growth, nor bulb

starch storage. As a result, elevated [CO₂] had little impact on leaf phenology. Leaf yellowing was synchronous in both treatments and in both years, and complete leaf senescence occurred at the same time in both treatments in 2005; however, it occurred 4 d later in the elevated [CO₂] treatment in 2004 (Fig. 1). As the lifespan of the leaf was not reduced under higher [CO₂], our initial hypothesis is rejected.

Since no impact of the [CO₂] treatment on leaf area, leaf biomass or leaf phenology were monitored, total C assimilation per plant for the whole season was higher under the elevated [CO₂] than under control conditions. Higher total C fixed did not result in increased bulb growth and C storage; therefore the extra C must have been used for other functions. *Erythronium americanum* plants subjected to elevated [CO₂] exhibited increased respiratory rates of both the leaf and the bulb. However, plant growth was

not enhanced, and since the N content of bulbs or leaves was not, or only slightly, different between the two treatments, there is no indication that plant-tissue maintenance was significantly greater under this treatment. As a result, the respiratory C loss under elevated [CO₂] suggests a decreased respiration efficiency, a phenomenon that generally occurs when the electron transport chain is not coupled with proton pumping (Lambers *et al.*, 1998).

It is possible that elevated [CO₂] enhanced the activity of alternative respiratory pathways. One of which might be the cyanide-insensitive respiratory pathway, which is known to be present in numerous species (Azcón-Bieto *et al.*, 1983), such as tulip (Kannevorf and van der Plas, 1994) and iris (Marissen *et al.*, 1991). The biological functions of non-phosphorylating pathways, as well as their regulation are poorly understood but several studies have demonstrated their implication in response to environmental changes (Breidenbach *et al.*, 1997; Gonzalez-Meler *et al.*, 2004; Sieger *et al.*, 2005). Increased respiratory loss supports the hypothesis that growth of *E. americanum* is more limited by bulb growth capacity, and thus sink activity, than by C uptake (Lapointe, 2001). This species was not capable of increasing the strength of its sinks under elevated [CO₂] in response to greater CO₂ availability. As a result, increased respiratory loss, via alternative pathways, appears to be an effective means of avoiding starch accumulation in the leaf (Lambers, 1982; Azcón-Bieto *et al.*, 1983) and subsequent feed-back inhibition of photosynthesis (Paul and Foyer, 2001; Rolland *et al.*, 2002), which could possibly induce earlier leaf senescence and smaller final bulb size. Cytochromic and alternative respiratory pathways are being monitored in *E. americanum* under different growth conditions to determine if the alternative respiratory pathway is stimulated under sink-limited conditions.

In summary, the results of the present experiment indicate that a substantial increase in CO₂ has no effect on the growth and final size of *E. americanum*, in contrast to data reported in most other plant species. The plants exhibited a general lack of plasticity in response to CO₂ availability. Leaf morphology did not change with [CO₂]. But more important, the growth of the bulb, which is the plant's main C sink, and its final biomass, starch content and concentration did not increase in response to increased CO₂ availability. *Erythronium americanum* bulb growth thus appears to be more limited by cell growth capacity than by C availability, at least under high light levels and warm temperatures. Despite the lack of change in growth rate, photosynthetic activity under elevated [CO₂] remained higher than under ambient conditions and no reduction in N concentration was observed. Finally, the higher leaf and bulb respiratory rates exhibited by the plants grown at higher [CO₂] might explain the fate of the surplus C. These findings corroborate the hypothesis of a growth regulation by sink strength in this species.

ACKNOWLEDGEMENTS

We thank Christophe Gouraud, Laëtitia Huillet, Olivier Larouche and Claude Fortin for their technical help and A. P. Coughlan for reviewing an earlier version of the

manuscript. This study was supported by a grant to L. Lapointe from the National Sciences and Engineering Research Council of Canada.

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