

# Effects of Elevated Atmospheric Carbon Dioxide on the Growth and Linear Furanocoumarin Content of Celery

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The effects of elevated atmospheric carbon dioxide on the growth and development of celery (*Apium graveolens*) were examined to determine if anticipated global increases in CO<sub>2</sub> will affect the nutritional quality and secondary chemistry of celery. The size (fresh and dry mass), nitrogen and carbon composition, and concentrations of linear furanocoumarins of celery grown under ambient (363 μL L<sup>-1</sup>) and elevated (718 μL L<sup>-1</sup>) carbon dioxide were analyzed. Growth under elevated CO<sub>2</sub> resulted in larger petioles, reduced nitrogen content, and higher C:N ratios in both leaves and petioles. However, CO<sub>2</sub> treatment did not affect plant water content or carbon content. Moreover, in contrast to the carbon–nutrient balance hypothesis, the increased C:N ratios of plants grown under elevated CO<sub>2</sub> were not associated with increased concentrations of potentially harmful linear furanocoumarins. Levels of linear furanocoumarins in the petioles of plants from each treatment did not exceed concentrations reported to cause acute or chronic contact dermatitis.

**Keywords:** *Apium graveolens*; elevated carbon dioxide; carbon–nutrient balance hypothesis; linear furanocoumarins; psoralen; bergapten; xanthotoxin

## INTRODUCTION

Concerns about increases in atmospheric carbon dioxide (CO<sub>2</sub>) levels from anthropogenic sources have prompted considerable research on the effects of elevated CO<sub>2</sub> on natural systems [e.g. Kareiva et al. (1993)]. One central area of concern to agriculturalists and ecologists is the effect that increasing atmospheric CO<sub>2</sub> will have on the growth and nutritional quality of plants. Because terrestrial plants use atmospheric CO<sub>2</sub> as their sole source of carbon for photosynthesis, changes in atmospheric CO<sub>2</sub> can have dramatic effects on the photosynthetic rates of plants (Bazzaz, 1990; Fajer et al., 1991) and, consequently, on plant growth and resource allocation (Kimball, 1983). C<sub>3</sub> photosynthetic plants commonly respond to increased CO<sub>2</sub> by increasing growth (Kimball, 1983; Kimball et al., 1993; Julkunen-Tiitto et al., 1993), although this response is far from universal (Bowes, 1991; Lindroth et al., 1993).

One hypothesis appropriate for predicting the phenotypic effects of increased CO<sub>2</sub> on plants is the carbon–nutrient balance hypothesis (Bryant et al., 1983; Tuomi et al., 1988). This hypothesis predicts that, as the carbon to nitrogen ratio of plants increases under elevated atmospheric CO<sub>2</sub>, a greater proportion of the plant's carbohydrate resources will be allocated to secondary metabolism, resulting in the production of greater amounts of carbon-based secondary chemicals.

Celery, *Apium graveolens* L. var. Dulce, is a C<sub>3</sub> photosynthetic metabolism plant that produces enlarged, succulent petioles and contains a number of carbon-based secondary compounds (Quiros, 1993). Prominent among these secondary compounds are the

linear furanocoumarins, psoralen, bergapten (5-methoxypsoralen), and xanthotoxin (8-methoxypsoralen). These linear furanocoumarins are constitutive components of celery and also can be induced by various environmental stresses such as pathogens, cold temperatures (Beier and Oertli, 1983), and acidic atmospheric pollution (Dercks et al., 1990). At high enough concentrations, these compounds can cause acute (Austad and Kavli, 1983) and chronic dermatitis in humans (Seligman et al., 1987).

We investigated the effect of elevated atmospheric CO<sub>2</sub> on the growth and linear furanocoumarin content of celery. Growth of young celery plants has been shown to increase under elevated CO<sub>2</sub> (Tremblay et al., 1988). Therefore, we were most interested in whether elevated CO<sub>2</sub> increases the carbon to nitrogen ratio of *A. graveolens* and, if so, whether such an increase is associated with higher levels of the three major linear furanocoumarins present in *A. graveolens*, psoralen, bergapten, and xanthotoxin.

## MATERIALS AND METHODS

Young celery plants (cv. Conquistador), each approximately 8 weeks old and 15 cm tall, were obtained from West Coast Nursery (Oxnard, CA) on July 12, planted immediately into individual pots (15-cm diameter) containing Hyponex (Marysville, OH) potting soil, and placed in the greenhouse at the University of Michigan Biological Station in Pellston, MI. Two days later, 60 randomly chosen experimental plants were assigned to three treatments: 12 plants to each of two chambers maintained at ambient CO<sub>2</sub>, 12 plants to each of two chambers maintained at elevated CO<sub>2</sub>, and 12 plants as unchambered controls (which were placed just outside each chamber and compared with those grown in ambient CO<sub>2</sub> chambers to test for chamber effects). All plants were watered daily and fertilized twice each week with 500 mL of 15N–12.9P–12.5K fertilizer (3 g L<sup>-1</sup>) (Stern's Miracle Grow, Port Washington, NY). On July 14, when test plants were first placed in chambers, an additional set of the transplants (*n* = 15) was removed from pots and shipped overnight on ice to the University of California, Riverside, to determine initial

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carbon, nitrogen, water and linear furanocoumarin contents of plants. These transplants typically had two mature petioles.

The 0.5-m<sup>3</sup> open-topped chambers were similar to those described by Drake et al. (1989). To simulate natural conditions as much as possible, chambers were located in a field adjacent to the greenhouse. Light and temperature within the chambers were within 2% of the values recorded outside the chambers. Two chambers were maintained at ambient CO<sub>2</sub> (363 ± 6 μL L<sup>-1</sup>,  $x \pm SD$ ), and two were maintained at elevated CO<sub>2</sub> (718 ± 7 μL L<sup>-1</sup>). CO<sub>2</sub> content was elevated by dispensing 100% CO<sub>2</sub> into the inlet port of an outlet blower connected by dryer hose to the base of each elevated chamber. CO<sub>2</sub> levels were monitored continuously by pumping air from each chamber to a microcomputer-controlled valve manifold that directed the gas stream past an infrared gas analyzer. Levels detected by the gas analyzer were recorded to disc approximately every 5 min. CO<sub>2</sub> flow to each elevated chamber was adjusted via a manual flowmeter.

Seventy days after placement in chambers, experimental plants were removed from their pots and sent on ice by overnight mail to the University of California, Riverside, for determination of final carbon, nitrogen, water, and linear furanocoumarin contents. Upon arrival, above-ground tissue was divided into petioles and foliage, which were weighed separately.

The first inner petiole and foliage of each experimental plant were selected for linear furanocoumarin analysis (Diawara et al., 1995). We selected this petiole and its associated foliage because they were the first to develop completely during the CO<sub>2</sub> treatments and would constitute part of the marketable produce. The baseline linear furanocoumarin content of transplants was determined by analyzing one of the two mature, healthy petioles, and its foliage.

Celery plants were analyzed for three of the major linear furanocoumarins that have been isolated from *Apium* spp.: psoralen, bergapten, and xanthotoxin (Beier and Oertli, 1983; Trumble et al., 1990). These three linear furanocoumarins were extracted and concentrations determined using high-performance liquid chromatography (HPLC) methods previously described (Trumble et al., 1992; Diawara et al., 1996). Sample leaves and petioles were separated and stored at -65 °C until the time of chemical analysis. Sample tubes were spiked with 5 μg of a synthetic internal standard, 7-benzyl-oxy-coumarin (synthesized from commercially available 7-hydroxycoumarin, Aldrich, Milwaukee, WI). Plant samples were homogenized in distilled H<sub>2</sub>O and extracted with toluene. The crude extract was partially purified by passage through an Extract Clean solid-phase extraction cartridge tube (Alltech, Deerfield, IL) and eluted with acetone/chloroform (95:5, v/v). The purified extracts were concentrated to dryness and then dissolved in 250 μL of hexane. HPLC analyses were carried out with a Beckman 126 HPLC pump and Beckman 166 diode array detector with a Gold data system (Beckman Instruments, Fullerton, CA). The pump and detector were interfaced with a Beckman 507 autosampler. Peaks were monitored and quantified at 290 nm. This wavelength was chosen because all three linear furanocoumarins and the internal standard have strong and similar absorbances at this wavelength, and interference from other compounds was minimized (Diawara et al., 1992). Relative response factors at this wavelength were as follows: internal standard, 1.00; psoralen, 2.93; 5-methoxy-psoralen, 0.79; 8-methoxypsoralen, 1.78. An Alltech Econosil silica column (25 cm × 4.6 mm, 5-μm particle size) with a guard column (10 × 4.6 mm) filled with the same packing material was used and eluted isocratically with hexane/tetrahydrofuran (81:19, v/v). Concentrations of linear furanocoumarins are expressed as their proportion of the fresh (wet) mass of the samples. Extraction efficiencies have been calculated at 1 and 20 μg/g, as follows: psoralen, 90.7 ± 5.7% and 95.6 ± 0.5%; 5-methoxypsoralen, 87.7 ± 5.4% and 96.9 ± 1.0%; 8-methoxypsoralen, 90.2 ± 6.6% and 95.2 ± 3.0% (Diawara et al. 1992). Calibration curves for the three compounds are linear over the region of interest with  $r^2$  values > 0.99. Minimum detectability has been conservatively estimated as 0.005 μg/g for each compound.

**Table 1. Baseline Characteristics of Celery Transplants Prior to the Initiation of Carbon Dioxide Treatments<sup>a</sup>**

component	mean CI
total wet mass (g)	7.26 ± 0.82
Foliage	
wet mass (g)	2.83 ± 0.37
nitrogen <sup>b</sup> (%)	4.13 ± 0.09
carbon (%)	36.33 ± 0.43
carbon:nitrogen ratio	8.80 ± 0.12
water content (%)	88.62 ± 0.38
psoralen <sup>c</sup> (μg g <sup>-1</sup> )	0.00
bergapten (μg g <sup>-1</sup> )	1.17 ± 0.26
xanthotoxin (μg g <sup>-1</sup> )	0.68 ± 0.30
furanocoumarin total (μg g <sup>-1</sup> )	1.84 ± 0.47
Petioles	
wet mass (g)	4.43 ± 0.48
nitrogen (%)	3.12 ± 0.16
carbon (%)	30.84 ± 0.48
carbon:nitrogen ratio	10.01 ± 0.61
water content (%)	93.21 ± 1.14
psoralen (μg g <sup>-1</sup> )	0.00
bergapten (μg g <sup>-1</sup> )	0.81 ± 0.45
xanthotoxin (μg g <sup>-1</sup> )	0.99 ± 0.60
furanocoumarin total (μg g <sup>-1</sup> )	1.86 ± 1.00

<sup>a</sup> Plants were separated into foliage and petiole components for analysis. Values are means ± 95% confidence intervals. <sup>b</sup> Percentage of dry mass. <sup>c</sup> μg g<sup>-1</sup> of wet mass.

The remainder of the petioles and foliage was dried separately in an oven (65 °C) for at least 1 week and reweighed to determine water content. Dried petiole and leaf samples were ground to a fine powder and assayed individually for carbon and nitrogen content with a CHN analyzer (Pella, 1990a,b). Carbon and nitrogen contents are expressed as proportions of the dry mass.

All data were examined for normality prior to analysis. No transformations were necessary to normalize data. Descriptive statistics were calculated for plants harvested prior to carbon dioxide treatments. Atmospheric treatment effects on various plant characteristics were analyzed by one-way analysis of variance (Sokal and Rohlf, 1995). Chambers within each CO<sub>2</sub> treatment did not differ. Chamber effects were determined by comparing the external control with the ambient treatment. Pearson product moment correlations were calculated for specific variables.

## RESULTS

**Baseline Results.** Among transplants, carbon and nitrogen contents were higher in leaves than in petioles (Table 1). However, linear furanocoumarin content of these transplants was extremely low. For instance, psoralen was not detected in either the foliage or petioles of these young plants.

Total linear furanocoumarin content was not correlated with carbon to nitrogen (C:N) ratio in leaves ( $r = 0.05$ ,  $P = 0.89$ ) or in petioles ( $r = 0.28$ ,  $P = 0.38$ ). Moreover, no significant correlation was observed between linear furanocoumarin concentrations in the petioles and foliage ( $r = -0.32$ ,  $P = 0.26$ ).

**CO<sub>2</sub> Treatments.** Elevated CO<sub>2</sub> significantly increased the growth of celery. Overall, the wet (fresh) mass of plants grown in elevated CO<sub>2</sub> chambers was approximately 8% greater than those grown in the ambient CO<sub>2</sub> chambers, due primarily to a 9% increase in petiole biomass (Table 2). Water and carbon contents of both leaves and petioles were unaffected by elevated CO<sub>2</sub>. However, nitrogen content declined significantly in both tissues, resulting in increased C:N ratios under elevated CO<sub>2</sub>.

**Table 2. Characteristics of Celery Plants Grown Outside Environmental Chambers (Control), in Environmental Chambers with Ambient Carbon Dioxide (Ambient), and in Environmental Chambers with Elevated Carbon Dioxide (Elevated)<sup>a</sup>**

component	chamber effect <sup>b</sup>	control	ambient	elevated	treatment effect <sup>c</sup>
total wet mass (g)	*	33.69 ± 2.20	49.77 ± 2.46	53.85 ± 2.90	*
Foliage					
wet mass (g)	*	12.25 ± 0.65	15.15 ± 0.82	15.67 ± 0.98	ns
nitrogen <sup>b</sup> (%)	ns	2.61 ± 0.18	2.47 ± 0.09	2.18 ± 0.11	*
carbon (%)	*	35.40 ± 1.23	33.38 ± 0.97	33.76 ± 0.85	ns
carbon:nitrogen ratio	ns	13.74 ± 0.84	13.60 ± 0.53	15.68 ± 0.75	*
water content (%)	*	84.86 ± 0.79	86.61 ± 0.56	86.04 ± 0.47	ns
psoralen <sup>e</sup> (μg g <sup>-1</sup> )	ns	0.00	0.08 ± 0.07	0.16 ± 0.11	ns
bergapten (μg g <sup>-1</sup> )	*	4.01 ± 1.12	8.85 ± 3.58	10.49 ± 2.05	ns
xanthotoxin (μg g <sup>-1</sup> )	ns	5.72 ± 1.34	8.63 ± 3.70	7.74 ± 2.30	ns
furanocoumarin total (μg g <sup>-1</sup> )	*	9.73 ± 2.24	17.56 ± 7.16	18.39 ± 4.33	ns
Petioles					
wet mass (g)	*	15.37 ± 0.90	25.27 ± 1.56	27.54 ± 2.08	*
nitrogen (%)	ns	1.90 ± 0.15	1.79 ± 0.12	1.62 ± 0.13	*
carbon (%)	ns	32.76 ± 1.11	32.88 ± 0.89	33.65 ± 1.06	ns
carbon:nitrogen ratio	ns	17.57 ± 1.21	18.98 ± 1.65	21.69 ± 2.14	*
water content (%)	*	92.48 ± 0.82	93.68 ± 0.57	93.49 ± 0.68	ns
psoralen (μg g <sup>-1</sup> )	ns	0.00	0.02 ± 0.03	0.01 ± 0.01	ns
bergapten (μg g <sup>-1</sup> )	ns	1.64 ± 0.56	2.35 ± 0.91	3.15 ± 0.47	ns
xanthotoxin (μg g <sup>-1</sup> )	ns	0.75 ± 0.28	1.47 ± 1.04	0.50 ± 0.16	ns
furanocoumarin total (μg g <sup>-1</sup> )	ns	2.39 ± 0.82	3.84 ± 1.79	3.66 ± 0.61	ns

<sup>a</sup> Values are means ± 95% confidence intervals of the mean. <sup>b</sup> Comparison between external controls and ambient carbon dioxide treatment. ns, not significantly different ( $P > 0.05$ , ANOVA); \*, significantly different ( $P < 0.05$ , ANOVA). <sup>c</sup> Between ambient carbon dioxide treatment and elevated carbon dioxide treatment. ns, not significantly different ( $P > 0.05$ , ANOVA); \*, significantly different ( $P < 0.05$ , ANOVA). <sup>d</sup> Percentage of dry mass. <sup>e</sup> μg g<sup>-1</sup> of wet mass.

The concentrations of psoralen, bergapten, xanthotoxin, and total linear furanocoumarins did not differ between celery plants grown in elevated versus ambient CO<sub>2</sub> chambers (Table 2). At both CO<sub>2</sub> levels, psoralen was present at very low concentrations in both foliage and petioles. Bergapten was the predominant linear furanocoumarin in both foliage and petioles, where it comprised 50–86% of the total linear furanocoumarin content. Total linear furanocoumarin concentration was not correlated with C:N ratio in foliage ( $r = 0.02$ ,  $P = 0.87$ ) or petioles ( $r = 0.01$ ,  $P = 0.99$ ). Moreover, total linear furanocoumarin concentration in the leaves was not correlated with concentrations in petioles for either ambient CO<sub>2</sub>-grown plants ( $r = -0.03$ ,  $P = 0.89$ ) or elevated CO<sub>2</sub>-grown plants ( $r = 0.22$ ,  $P = 0.31$ ).

Overall, as celery plants matured, nitrogen contents decreased and concentrations of linear furanocoumarins increased dramatically (compare Tables 1 and 2). However, total carbon content did not differ as greatly between 8-week old transplants and 18-week-old plants. Carbon content of foliage was lower in the older plants, but carbon content of petioles was slightly higher in these plants compared with the 8-week-old transplants.

**Chamber Effects.** Plants grown outside chambers revealed significant chamber effects. Relative to plants grown in the ambient atmosphere chambers, unchambered control plants were smaller, because of lower biomass of both foliage and petioles (Table 2). Foliar and petiole water contents were also lower in unchambered controls. Despite higher foliar carbon content, unchambered control plants had a lower total linear furanocoumarin content than did plants grown in the adjacent ambient CO<sub>2</sub> chambers. This difference in total linear furanocoumarin content is attributable to the lower concentration of bergapten in the foliage of unchambered control plants (Table 2). There was no significant chamber effect on the concentration of psoralen or xanthotoxin.

## DISCUSSION

According to the carbon–nutrient balance hypothesis (Bryant et al., 1983; Tuomi et al., 1988), environmental conditions that alter carbon availability play a pivotal role in determining resource allocation patterns in plants; in particular, increased C:N ratios are predicted to result in greater allocation of carbon to carbon-based secondary compounds. On the basis of this hypothesis, we expected that growth of celery under elevated CO<sub>2</sub> would result in increased concentrations of linear furanocoumarins, a class of carbon-based allelochemicals. However, although celery plants were larger under elevated CO<sub>2</sub>, they did not contain more carbon by dry weight, nor did they contain greater concentrations of linear furanocoumarins. A common physiological response of plants to elevated CO<sub>2</sub> is reduced nitrogen content, expressed as a percent of plant dry weight, generally due to increased carbon assimilation [e.g. Garbutt et al. (1990), Lincoln et al. (1993), Baxter et al. (1994), Epron et al. (1996), and Karowe et al. (1997)]. However, we did not observe this nitrogen dilution effect (Strain and Cure, 1985). Rather, we observed a reduction in nitrogen content without a concomitant increase in carbon content. This lack of an effect on carbon content may result from a negative feedback mechanism that, over the long term, keeps photosynthesis at low levels (Bowes, 1991). Our observation that plant water content was unaffected by CO<sub>2</sub> treatment is consistent with some previous studies [e.g. Idso et al. (1988)] but not with others [e.g. Osbrink et al. (1987) and Karowe and Spencer (1997)].

Our finding that linear furanocoumarin content of celery did not conform to the predictions of the carbon–nutrient balance hypothesis is not unique; previous studies addressing the effect of CO<sub>2</sub> enhancement on production of carbon-based secondary compounds have yielded mixed results. For instance, under elevated

CO<sub>2</sub>, total volatile leaf mono- and sesquiterpenes in peppermint did not change (Lincoln and Couvet, 1989); several additional studies have also reported no increase in carbon-based secondary compounds [e.g. Johnson and Lincoln (1990, 1991) and Fajer et al. (1992)]. In contrast, other studies have observed significant increases (Lindroth et al., 1993; Julkunen-Tiitto et al., 1993; Lavola and Julkunen-Tiitto, 1994), while Fajer et al. (1992) observed the unusual result that concentrations of the iridoid glycosides aucubin and catalpol in plantain decreased significantly under elevated CO<sub>2</sub>. The species-specific nature of plant responses to elevated CO<sub>2</sub> is best illustrated by multispecies studies. For instance, Penuelas et al. (1996) observed an increase in total phenolic concentration of wheat, no change in orange, and a decrease in total phenolic concentration of pine. In the most comprehensive study to date, Lindroth et al. (1993) showed that the effects of elevated CO<sub>2</sub> on carbon-based allelochemicals differed among three tree species and, moreover, varied among types of phenolics within species. Elevated CO<sub>2</sub> resulted in significant increases in foliar concentrations of salicortin (a phenolic glycoside) in trembling aspen and of ellagitannin and condensed tannin in sugar maple. However, elevated CO<sub>2</sub> also resulted in a significant decrease in foliar ellagitannin in red oak but in no significant change in gallotannin levels in maple or oak or in condensed tannin levels in aspen or oak. Clearly, broad generalizations about responses of carbon-based allelochemicals to elevated CO<sub>2</sub> are not warranted at this time.

Finally, this study addresses a major concern of celery producers, namely, maintaining concentrations of linear furanocoumarins below levels that may cause chronic or acute dermatitis in workers or consumers. It is still unclear how growing conditions modulate linear furanocoumarin production in celery leaves and petioles. Linear furanocoumarin content varies with plant maturity (Trumble et al., 1992; Diawara et al., 1993), and their production can be induced by various environmental stresses such as ultraviolet light, cold temperatures, and pathogen infection (Beier and Oertli, 1983) and some forms of air pollution (Dercks et al., 1990). However, the concentrations of linear furanocoumarins in celery grown under elevated CO<sub>2</sub> were not significantly greater than concentrations in celery grown under ambient CO<sub>2</sub>. Concentrations of linear furanocoumarins in the petioles, which are the marketable portion of the produce, did not exceed levels reported to cause either acute or chronic dermatitis (Austad and Kavli, 1983; Seligman et al., 1987). Because we sampled the petioles and foliage that were exposed for the longest time to CO<sub>2</sub> treatments, linear furanocoumarin levels in younger, more interior portions of the plant would, in all probability, be even lower (Diawara et al., 1995).

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