Altered leaf and root emissions from onion (Allium cepa L.) grown under elevated CO\textsubscript{2} conditions

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Abstract

Elevated atmospheric CO\textsubscript{2} concentrations ([CO\textsubscript{2}]) have been hypothesized to increase photosynthesis rates and volatile organic compound (VOC) emissions; however, field measurements from a select group of conifer and angiosperm trees have shown that VOC emissions are in fact not affected or reduced by elevated CO\textsubscript{2} levels. To broaden the understanding of how different plant species respond to elevated atmospheric [CO\textsubscript{2}], air-flow-through, glass chambers were designed and utilized to measure photosynthesis and emissions from onion (Allium cepa cv. Purplette) under controlled environmental conditions. Here we report on VOC release and root exudation while monitoring photosynthesis from whole plants grown under ambient (400 \textmu mol mol\textsuperscript{-1}) and elevated (1000 \textmu mol mol\textsuperscript{-1}) CO\textsubscript{2}. A 22\% increase in photosynthesis in the elevated CO\textsubscript{2} plants and a 17-fold and 38-fold increase in the VOC hydrocarbons 2-undecanone and 2-tridecanone, respectively, were observed in 30-day-old onion seedlings compared to plants grown under ambient CO\textsubscript{2} conditions. In contrast TOC from root exudates decreased significantly with elevated CO\textsubscript{2} conditions. Plants harvested at 30 days had on average over 40\% greater biomass when grown at elevated CO\textsubscript{2} levels. The demonstration that VOC emissions increase in plants grown under elevated [CO\textsubscript{2}] and higher photosynthesis rates points to a fundamental difference in how carbon partitioning alters in herbaceous species such as onion versus the previously studied tree species in response to elevated concentration of atmospheric CO\textsubscript{2}.

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1. Introduction

A major goal in plant biology is to assess the responses to atmospheric increases in [CO\textsubscript{2}] that have been on a steady climb from 280 \textmu mol mol\textsuperscript{-1} in the early 1920s to current levels near 370 \textmu mol mol\textsuperscript{-1}, and are predicted to double within the next century (Cure and Acock, 1986; Conway et al., 1994). Because the emission of volatile organic compounds (VOCs) from coniferous as well as mixed angiosperm forests exerts dominant control over regional oxidative chemistry of the atmosphere in many terrestrial ecosystems, it is...
ecosystems, interest has focused on the impact of VOC emissions, especially that of isoprenoids and monoterpenes as it relates to biosphere–atmosphere interactions (Wuebbles et al., 1989; Fehsenfeld et al., 1992; Monson et al., 1995; Lerdau et al., 1995; Constable et al., 1999). Certain tree species that have been studied maintain or lower terpene production and emissions with increased CO2 availability. This is divergent from resource allocation models, such as the carbon-nutrient balance hypothesis (Hamilton et al., 2001) and the growth–differentiation balance hypothesis (Herms and Mattson, 1992) that predict that carbon in excess of that required for immediate growth will be used in the production of carbon-based defense compounds. For example, monoterpane stored or released from needles of Ponderosa pine did not change significantly when the trees were exposed to 700 ppm CO2 for 2.5 years compared to trees grown at 350 ppm CO2 (Constable et al., 1999). In an intact Populus deltoides plantation under increased CO2 concentrations (800 and 1200 ppm), reduced isoprene production by 21 and 41% were measured while above-ground biomass accumulation was enhanced by 60 and 82%, respectively (Rosenstiel et al., 2003). In Douglas fir, elevated CO2 treatment resulted in greater than a 50% reduction in monoterpene emissions (Snow et al., 2003). Tree studies conducted within a forest setting are ideally suited to probe the effect of elevated CO2 on plant growth and VOC emissions in natural-growing conditions; however, fast growing agriforest species such as Populus (temperate) and Eucalyptus (tropical) are exceptional in their high emission rates of reactive hydrocarbons and may respond differently than other crops when grown under elevated CO2 conditions (Rosenstiel et al., 2003).

Owing to the varied mix of low-molecular-weight hydrocarbons and sulfur derivatives emitted from onion (Allium cepa), this herbaceous species was selected as a model system for assessing the role of atmospheric CO2 in the induction of VOCs. Onion is an important agricultural crop high in antioxidant flavonoids, as well as sulfur volatiles enzymatically generated with tissue damage (Block et al., 1992). Sulfur volatiles are the source of the distinctive pungency and flavor as well as the lachrymator factor found in the Allium family (Imai et al., 2002). The odd-chain ketones 2-undecanone and 2-tridecanone are also emitted from onion. Although the function of these methyl ketones has not been reported in onion, these metabolites are present in tomato trichomes and confer insect resistance against a major agricultural pest, spider mites (Fery and Kennedy, 1987; Chatzivasileiadis and Sabelis, 1997; Chatzivasileiadis et al., 1999). In this study, we tested the hypothesis that onion plants grown under elevated levels of atmospheric [CO2] augment photosynthesis on a leaf area basis and VOC emissions and TOC exudation on a whole plant and weight basis.

2. Materials and methods

2.1. Plant culture

Onion seeds (A. cepa cv. ‘Purplette’) were sown into individual pieces of Oasis® solid support media and placed into glass tubes (11 × 5 cm-diameter) that were open at one end. By covering the Oasis with aluminum foil, bacterial growth was minimized. A single hole was placed into the aluminum foil to allow for seedling emergence. The glass tubes were immediately placed into cylindrical flow-through individual growth chambers (IGCs) (40 × 7 cm-diameter). Environmental conditions within the IGCs that were monitored included: temperature 25/21 °C, relative humidity 75%, airflow 600 ml min⁻¹, and photosynthetic photon flux density 350 µmol m⁻² s⁻¹. Light was provided by metal halide and high-pressure sodium lamps located 50 cm above the top of the chambers with a photoperiod of 16/8 h light/dark. Seedlings were given deionized water for 7 days after planting and then switched to Hydro-Sol (Scotts, Marysville, OH) nutrient solution, adjusted to pH 6.8. The Hydro-Sol nutrient solution was exchanged every 24 h and carefully adjusted within the chambers so that roots were not immersed but instead the solution wicked up into the Oasis media.

2.2. Gassing and volatile collection

The plants were treated with 400 (ambient) or 1000 µmol mol⁻¹ (elevated) [CO2] from planting until the termination of the experiment at 30 days. To adjust the concentration of CO2 introduced into the
IGCs, charcoal-purified air was first passed through a cylindrical filter (36 × 3 cm-diameter, 400 cc volume; Alltech Associates Inc., Deerfield, IL) filled with soda-lime (Sigma Chemical Co.; St Louis, MO) to remove all CO2 and then remixed with purified CO2 (Airgas; Corpus Christi, TX) to obtain the desired CO2 concentration (monitored with a LI-6400 portable photosynthesis system). Carbon dioxide concentrations were recorded and monitored to insure a constant gassing level. Volatiles were collected in IGCs for 24 h intervals on Super-Q (Alltech Associates Inc., Deerfield, IL) polymer filters; filters were located downstream from the IGCs after the air had passed over the contained plant. Filters were subsequently extracted with 150 ml dichloromethane (Fisher Scientific, Buffalo Grove, IL). Nonylacetate was synthesized and added as an internal standard used for quantification.

2.3. Volatile identification and quantification

Volatiles were analyzed by capillary gas chromatography (GC) (Hewlett-Packard 6890) on a 15 m × 0.25 mm (i.d.) crosslinked-methylsiloxane column with a 0.1 µm film thickness. The injector temperature was 230°C and a detector temperature of 250°C. The column was held at 40°C for 0.5 min, increased 12°C/min⁻¹ to 180°C, and then increased 40°C/min⁻¹ to 220°C and held for 2 min. Helium was used as a carrier gas at a linear flow velocity of 35 cm s⁻¹. Mass spectral (MS) analysis was conducted and the plant components were compared with commercially available standards and spectra from the National Institute of Standards and Technology database. GC–MS data was collected on an HP5973 quadrupole instrument equipped with an HP OV101 capillary column. GC–FID retention times of authentic standards and the plant-collected components were compared on both HP DB-5 (slightly polar) and HP OV101 (non-polar) capillary columns. All solvents and gasses used were of GC-grade purity.

2.4. Gas exchange measurements

Photosynthetic measurements were made using LI-COR 6400 portable photosynthesis systems (LI-COR Biosciences, Lincoln, NE). Two separate airlines provided either ambient or elevated CO2 conditions with four IGCs connected in parallel to each of these airlines. An automated solenoid switching system (Analytical Research Systems Inc., Gainesville, FL) allowed for the cycling of air flow out of each of four chambers to the LI-COR unit for 5 min intervals; the remainder of each 20 min cycle, air was pulled by vacuum through Super Q filters for the collection of VOCs (see Section 2.2). This automated switching system allowed a single LI-COR unit to be used to measure photosynthetic rates from four replicate chambers with the same CO2 set point. A fraction of the reference air set at either 400 or 1000 µmol mol⁻¹ [CO2], without passing over a plant, was continually run through the LI-6400 while a branch of that reference air passed through an IGC before entering the sample side of the CO2 analyzer. Photosynthetic readings were recorded once a minute.

2.5. Total organic carbon (TOC) sampling and data analysis

Nutrient media was drawn up by syringe from the bottom of the glass cylinders contained within the IGCs and stored up to six months at −20°C. For TOC analysis, thawed samples were acidified to pH < 2 with H2SO4 and 1 ml aliquots were injected onto a Shimadzu TOC-5050A Total Organic Carbon Analyzer. Catalytic combustion of the organic carbon was measured as carbon dioxide by infrared gas analyzer. The amount of CO2 generated assumed to be directly proportional to the amount of carbonaceous material in the sample and was quantified based on a six-point calibration curve using potassium hydrogen phthalate as the standard.

2.6. Experimental design and data analysis

Eight IGCs were arranged in a randomized design with half the chambers exposed to ambient and half to elevated CO2. Four chambers from each set were continuously monitored for VOC emissions and photosynthetic rates (a schematic of the VOC/photosynthesis monitoring at one [CO2] is shown in Fig. 1). Data were analyzed by the GLM procedure in the SAS statistical program and means separated using Duncan’s Multiple Range Test at the 5% level.
3. Results

3.1. Gas exchange

To correlate photosynthesis rates with TOC and VOC emissions under defined CO\textsubscript{2} conditions, IGC were constructed and plumbed for multiple chemical monitoring while plants were growing (Fig. 1). Photosynthesis was significantly and consistently higher in onions exposed to elevated [CO\textsubscript{2}] when compared to the ambient treatment on a plant basis during Days 25–30 (P < 0.05), with average daily carbon assimilation approximately 20% higher for plants exposed to elevated CO\textsubscript{2} (Fig. 2A). Average daily photosynthesis on Day 30 was 22% higher for plants grown at elevated CO\textsubscript{2} when measured on a leaf surface area basis (Table 1).

3.2. Whole plant volatile analysis

To determine if onion plants exposed to elevated CO\textsubscript{2} during plant development had modified VOC emissions, the more abundantly released volatile components (Fig. 3) were analyzed and quantified. Chemical identification of 2-tridecanone and 2-undecanone was based on comparisons of gas chromatography retention time and mass spectral fragmentation patterns of plant collected samples (Fig. 4) with those of authentic synthetic standards. Although these methyl ketones have not been reported before as

### Table 1

<table>
<thead>
<tr>
<th>Measurement</th>
<th>400 μmol mol(^{-1}) [CO\textsubscript{2}]</th>
<th>1000 μmol mol(^{-1}) [CO\textsubscript{2}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis (μmol m(^{-2}) s(^{-1}))</td>
<td>2.9 ± 0.1 a</td>
<td>3.54 ± 0.2 b</td>
</tr>
<tr>
<td>2-tridecanone (ng g(^{-1}) FW)</td>
<td>5.0 ± 0.7 a</td>
<td>88.0 ± 16 b</td>
</tr>
<tr>
<td>2-undecanone (ng g(^{-1}) FW)</td>
<td>16 ± 5.0 a</td>
<td>64.0 ± 12 b</td>
</tr>
<tr>
<td>TOC (μg g(^{-1}) FW(^{-1}))</td>
<td>9.0 ± 1.0 a</td>
<td>5.7 ± 1.8 b</td>
</tr>
</tbody>
</table>

Values are the means ± S.E. (n = 4). Significant differences (P < 0.05) are identified by different letters within a row.

* Total organic carbon.
being released from onion leaves, MS data for these
assigned compounds positively matched spectra from
a MS database (National Institute of Standards and
Technology). Statistically significant differences \( P \leq 0.05 \) between ambient and elevated VOC emissions
were observed from Days 28–30 for 2-undecanone
and Days 26–30 for 2-tridecanone on a plant basis
(Fig. 2B and C). Average daily VOC emissions with
elevated \( \text{CO}_2 \) for 2-undecanone and 2-tridecanone
was 17-fold and 38-fold greater, respectively, on a
gram fresh weight basis on Day 30 compared to plants
exposed to ambient \( \text{CO}_2 \) (Table 1). To assure that
these hydrocarbons were onion derived components,
voltalives were also collected from chambers contain-
ing Oasis and Hydro-Sol solution but devoid of plant
material; these samples did not contain detectable
levels of the two methyl ketones.

3.3. Root exudation

To determine if onion plants exposed to elevated
\( \text{CO}_2 \) during plant development produced modified re-
lease of organic material by the roots, total organic
carbon measurements were made from the nutrient so-
lution. There were significantly less TOCs released
from the roots into the media for the elevated \([\text{CO}_2]\)
treatment than ambient levels (Table 1).

3.4. Plant growth and development

To examine the influence of gassing conditions on
overall growth, several plant parameters were mea-
sured. Plant height, number of leaves, and number of
roots showed no differences among the treatments;
however, the total biomass measured as a sum of the
Fig. 4. Electron ionization mass spectra of (A) 2-undecanone and (B) 2-tridecanone with m/z indicating the mass to charge ratio. The molecular ion is marked as M⁺ and numbers above individual bars indicate the molecular weight of the fragment.

shoot and root weight showed a 22% increase of total biomass than the ambient treatment (Table 2). The lack of significance for the shoot weight between the elevated and ambient treatments may be due to the large amount of variability in shoot weight.

4. Discussion

Onion plants exposed to elevated [CO₂] concentrations for a 30-day period produced a greater amount of total biomass, higher rates of photosynthesis, and higher levels of 2-undecanone and 2-tridecanone when compared to plants grown at ambient [CO₂]. The results of this study support the hypothesis that whole onion plant VOC emissions are linked to onion leaf carbon balance. These data agree with resource acquisition and allocation models that predict that carbon based VOC emissions from plants fluctuate with the availability of reduced carbon (Lerdau et al., 1995; Monson et al., 1995). Carbon in excess of that required for immediate growth is predicted but not always observed to be allocated to carbon-based defenses (Lerdau et al., 1994; Constable et al., 1999). For example, in coniferous tissues monoterpene pool sizes have been observed to decrease (Williams et al., 1994; Litvak et al., 2002) or not change (Roth and Lindroth, 1994) as well as increase (Heyworth et al., 1998), in response to plant growth at elevated CO₂. Possible explanations for such changes in carbon pools include increased rates of metabolic degradation, decreased rates of synthesis or increased rates of volatilization. In Douglas fir, even when demand for defense metabolites is stimulated by mechanical wounding of needles, monoterpene synthesis, and accumulation did not increase (Litvak et al., 2002). At least for certain plant species, terpene production and storage may be controlled more by previous genetic programming than a current availability of carbon or energy resources.

This study is the first report that increased carbon in the form of atmospheric CO₂ results in increases in onion VOC emissions. The class of VOCs released from onion, odd chain length methyl ketones are not commonly observed in the profile of VOCs released from plants (Paré and Tumlinson, 1999). The observed
increase in carbon emissions is more in line with resource acquisition and allocation models (Lerdau et al., 1995; Monson et al., 1995) than monoterpene emission from several tree species that have been studied with elevated CO2 (Constable et al., 1999; Litvak et al., 2002; Snow et al., 2003). Although onion methyl ketone emissions are unlikely to have a marked effect on atmospheric chemistry, increased emissions may have ecological consequences since these odd numbered carbon ketones do exhibit increased resistance to some insect pests (Fery and Kennedy, 1987; Chatzivasileiadis et al., 1999). Our data supports the theory that plants grown under elevated [CO2] will accumulate excess carbon and that at least a portion of this excess carbon is funneled into an increased production of VOCs. This increase in onion VOC points to a fundamental difference in how carbon partitioning is altered in a herbaceous species under elevated concentration of atmospheric CO2 compared to several tree species previously investigated.

Heat studies have shown significant increases in VOC emissions with higher temperatures (Lerdau et al., 1994, 1995). Constable et al. (1999) estimated the effect of temperature on monoterpene emissions by using a model of Guenther et al. (1999) and hypothesized an increase in local temperature of 4°C can increase monoterpene emissions by up to 50%. In the current study, plants grown at ambient and elevated [CO2] were grown at the same temperature cycle (24/21°C) in a controlled growth facility; therefore, temperature was eliminated as a factor in generating elevated methyl ketone emissions.

Decreased TOC levels present in the media under elevated CO2 suggested that the root exudates were not significant pathways for releasing excess reduced carbon from the plant. However, since the nutrient solution was not sterilized nor were antibiotics added to the solution, microbial metabolism may play a significant role in the level of TOC’s present in the solutions analyzed. Since soil micro flora is an integral part of root as well as plant growth and development (Ryu et al., 2003), removal of rhizobacteria may not simulate natural plant growth conditions. The effect of elevated CO2 on flavor-defining sulfur-components rich in onion is currently being examined. We are particularly interested in examining how carbon is partitioned among stored products that are exclusive hydrocarbon-based versus mixed hydrocarbon metabolites containing sulfur or other heteroatoms in response to elevated CO2 concentrations.

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