Three new methods indicate that CO₂ concentration affects plant respiration in the range relevant to global change

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Abstract

Short-term responses of plant dark respiration to carbon dioxide concentration ([CO₂]) in the range anticipated in the atmosphere with global change remain controversial, primarily because it is difficult to convincingly eliminate the many possible sources of experimental error in measurements of carbon dioxide or oxygen exchange rates. Plant dark respiration is a major component of the carbon balance of many ecosystems. In seedlings without senescent tissue, the rate of loss of dry mass during darkness indicates the rate of respiration. This method of measuring respiration was used to test for [CO₂] effects on respiration in seedlings of three species with relatively large seeds. The time it took respiration to exhaust substrates and cause seedling death in darkness was used as an indicator of respiration rate in four other species with smaller seeds. The third method was measuring rates of CO₂ exchange in excised petioles sealed in a cuvette submerged in water to prevent leaks. Petioles were utilized as the plant tissue type with the most reliable rates of respiration, for excised tissue. The rate of loss of dry mass in the dark decreased with increasing [CO₂] in the range of 200–800 μmol mol⁻¹ in all three large-seeded species. The seedling survival time in the dark increased with [CO₂] in the same concentration range in all four of the smaller-seeded species. Respiration rates of excised petioles of several species also decreased over this [CO₂] range. The data provide new evidence that the rate of dark respiration in plant tissue often decreases with increasing [CO₂] in the 200–800 μmol mol⁻¹ range.

Keywords: Amaranthus hybridus; Amaranthus hyochondriacus × hybridus; atmospheric CO₂; Datura stramonium; dry mass loss; Glycine max; Gossypium hirsutum; Helianthus annuus; Medicago sativa; respiration; Zea mays.

Introduction

Because plant dark respiration fuels many vital plant processes, and is also a major term in the carbon budget of the biosphere, a response of respiration rate of plants to changes in atmospheric carbon dioxide concentration could be of considerable importance. The concentration of CO₂ in the atmosphere has been between 180 and 280 μmol mol⁻¹ for most of the last 600 000 years (The Royal Society 2020), until it began to increase in the last half of the 1800s. It is currently over 400 μmol mol⁻¹ and could increase to ~800 μmol mol⁻¹ by the end of the current century (IPCC 2014). Long-term exposure to elevated CO₂ concentrations ([CO₂]) lowered the rate of respiration in several species, probably at least partly by reducing tissue protein content and consequently the respiratory costs of tissue construction and maintenance (Yin 2013). However, the existence of direct responses of respiration to [CO₂] in the relevant range of atmospheric concentrations (e.g. 200–800 μmol mol⁻¹) remains controversial. This is because it is difficult to convincingly eliminate all potential sources of error in measurements of CO₂ or O₂ exchange. Some of the known
potential errors include the limited sensitivity of gas analysers, changes in analyser calibration with background concentration, interference in gas analysis by water vapour, adsorption of gases onto cuvette and tubing surfaces and leakage through gaskets, and through leaves and the seals between leaves and gaskets in clamp-on systems (Jahnke 2001). Lower rates of leaf respiration at higher than lower measurement [CO 2] have still been found despite many precautions taken to minimize the known errors in gas exchange analysis (e.g. Ayub et al. 2014, supplementary data; Haworth et al. 2016). Comparisons of rates of respiration measured after minimizing the known errors with independent measures of the rates of processes dependent on respiration provided stronger evidence of short-term reduction in respiration with elevated [CO 2] (Bunce 2004). While Davey et al. (2004) did not detect short-term effects of [CO 2] on rates of O 2 exchange, such effects have more recently been found by others (e.g. Trimborn et al. 2014; Asensio et al. 2015). In this paper a measurement of whole-plant respiration not dependent upon gas exchange analysis was used to test whether respiration varied with short-term changes in [CO 2] in three species, and a quantitative indicator of whole-plant respiratory response to [CO 2] was developed for four other species which were not suitable for this direct measurement of whole-plant respiration.

CO 2 efflux from excised leaf petioles sealed in an air-tight container was also measured over a range of [CO 2] by gas exchange analysis using infrared CO 2 and H 2 O analysers. Petioles were utilized because they were the plant tissue type with the most reliable rates of respiration of excised tissue (see Materials and Methods). By all three of these new methods, increasing [CO 2] in the range relevant to climate change reduced CO2 efflux rates in several plant species. These methods were designed to provide unambiguous indication of whether dark respiration responded to [CO 2], not to represent realistic experiments regarding the projected rise in atmospheric [CO 2]. However, plant respiration is a major component of the carbon balance of many ecosystems, so responses of plant respiration to changes in atmospheric [CO 2] would be important to the global carbon balance (Drake et al. 1999).

Materials and Methods

All plants in these experiments were initially grown in indoor controlled environment chambers with 23 °C air temperature, 12 h of light per day at 1000 μmol m−2 s−1 photosynthetic photon flux density (PPFD), 60% relative humidity and 400 ± 20 (SD) μmol mol−1 [CO 2], as controlled by injection of either CO 2 or CO 2-free air based on continuous measurements of [CO 2] with a WMA4 CO 2 analyser (PP Systems, Amesbury, MA, USA). Plants were grown in pots filled with vermiculite and watered daily either with deionized water or with a complete nutrient solution, depending upon the experiment.

Experiment 1: rate of loss of dry mass

Whole-plant loss of dry mass in darkness at different [CO 2] was determined in sunflower (Helianthus annuus cv. Gray Stripe), cotton (Gossypium hirsutum cv. Delta Pine 555) and maize (Zea mays cv. Silver Queen) seedlings. Seeds of relatively uniform fresh mass were planted one per 10 x 10 x 20 cm square plastic pot filled with vermiculite. Pots were watered daily with deionized water. Approximately 1 day before seedlings emerged from the vermiculite half of the plants were harvested to determine dry mass, including roots, and half were placed in a darkened controlled environment chamber at 28 °C, and the [CO 2] was controlled at 200, 400 or 800 ± 20 (SD) μmol mol−1 for 24 h. The plants were then harvested to determine total dry mass. Seedlings were harvested by gently rinsing in water to remove all vermiculite and the seed coat (if still attached), placed into paper envelopes and dried in a forced air oven at 65 °C. The mean change in dry mass over 24 h was calculated based on the mean dry mass of 20 seedlings harvested before and after exposure to darkness at different [CO 2] for 24 h, and expressed as percent loss in dry mass. The dry masses compared were roughly 100 mg, measured to the nearest 0.1 mg, and the losses in dry mass were on the order of 10 mg. For all three species, dry mass loss was determined for four batches of seedlings for each [CO 2] treatment. [CO 2] treatments were compared for mean relative losses separately for each species using ANOVA. ANOVA was used to test effects of [CO 2] separately for each species, because there was no hypothesis to be tested concerning comparisons of responses among species. One-way ANOVA was implemented using GraphPad Prism 9.0, with means separated by Tukey's Multiple Comparison Test.

Experiment 2: dark seedling survival

Survival of seedlings in darkness at different [CO 2] was determined for four species, Amaranthus hypochondriacus × hybrida cultivar Plainsman, A. retroflexus, Datura stramonium and Medicago sativa, cultivar ARC. The weedy species were from 'local' populations. Seeds of one species were planted in vermiculite in two 25-cm-diameter pots at 400 μmol mol−1 [CO 2] and grown with the normal 12-h light–dark cycle for 3 days after most seedlings had emerged. Pots were watered with deionized water. Late emerging seedlings were removed. Emerged seedlings in each pot were then counted, and one pot was placed in darkness in a chamber at 23 °C and 400 μmol mol−1 [CO 2], and the other in another chamber in darkness at 23 °C, at either 200 or 800 μmol mol−1 [CO 2]. Pots were watered daily with deionized water carefully avoiding disrupting any seedlings. The number of seedlings surviving, that is, still upright, in each pot was recorded every 24 h until most seedlings had died and fallen over. The number of surviving seedlings decreased approximately linearly with time in every case after an initial time lag, and linear regression was used to estimate the time of 50% seedling survival in each pair of pots (Fig. 1).

The number of seedlings initially in each pot was 30–40 for the two Amaranthus species, 15–25 in M. sativa and 10–15 in D. stramonium. [CO 2] treatment effects on the times to half-survival were analysed separately for each species by pairwise t-tests, comparing 200 with 400 μmol mol−1 [CO 2], and 400 with 800 μmol mol−1 [CO 2]. There were four replications in each paired t-test for all species. The paired t-tests were conducted using GraphPad Prism 9.0.

Experiment 3: respiration rate of excised petioles

Plants of sunflower (H. annuus cv. Gray Stripe), cotton (G. hirsutum Delta Pine 555), amaranth (A. hypochondriacus × hybridus, cultivar Plainsman) and soybean (Glycine max cv. Kent) were grown one per pot in 15-cm-diameter pots filled with vermiculite and watered daily with a complete nutrient solution. The nutrient solution was about 5:1 Na 2−:NH 4+ . Plants were grown under the standard conditions given earlier for about 4 weeks, when some main stem leaves had become fully expanded. CO2 exchange rates of excised petioles of mature leaves were determined in darkness. All petioles contained chlorophyll, but photosynthetic rates were
not assessed. These experiments used petioles so that stable rates of CO₂ exchange of excised tissue could be determined. Rates of respiration of excised leaves or roots were not sufficiently stable over time, presumably because of desiccation. It was feared that stem tissue might contain large amounts of dissolved CO₂, which might obscure respiration rates. In many herbaceous plants and deciduous trees, petiole dry mass may equal or exceed leaf dry mass. In the species studied here, as in many species, petioles are green and photosynthetically active, although presumably they have a smaller ratio of photosynthetic to structural tissue than the leaf lamina. In G. max leaf lamina dark respiration rates per unit of dry mass were nearly equal to the rates obtained here for petioles (Bunce 2005).

Petioles were excised during the dark period, cut to lengths of about 8 cm with a razor, and left in darkness for about 10 min before being placed in a cuvette for measurement of CO₂ efflux rate. Net rates of CO₂ efflux from each petiole were determined in darkness at 200, 400 and 800 μmol mol⁻¹ [CO₂], with random starting [CO₂], and measured again at the initial measurement concentration to check for stability of the efflux rate over time. The measurement cuvette was an acrylic cylindrical tube, 2.2 cm internal diameter, and 9.5 cm in length, with O-ring sealed end caps connected with O-rings to tubing to and from the gas analyser. The cuvette containing the excised petiole and the connections to the gas lines were placed below the surface of water in a water bath kept at 23 °C. Rates of CO₂ efflux were measured with a CIRAS-3 portable photosynthesis system, using an air flow rate of 150 cm³ min⁻¹. Tests were made of the sensitivity of the CO₂ differential to differentials in water vapour, by scrubbing reference and/or analysis lines of water vapour, and by placing a few drops of deionized water in the empty cuvette. The instrument corrections for water vapour were found to be accurate. Measurements of respiration at the three [CO₂] were made on four or five petioles from different plants for each species. After gas exchange measurements, petiole sections were dried at 65 °C, and rates of dark respiration were expressed as μg CO₂ per gram of tissue dry mass per minute. Effects of [CO₂] on respiration were compared separately for each species using one-way ANOVA, with means separated by Tukey’s Multiple Comparison Test. No statistical comparisons were made across species, because there was no experimental hypothesis concerning species differences.

Results

Rate of loss of dry mass

The rate of loss of dry mass decreased with increasing [CO₂] in all three species (Fig. 2). The overall response pattern was quite similar in all species, although only cotton had significant decreases at each step of increasing [CO₂]. In all species, differences between the 200 and 800 μmol mol⁻¹ CO₂ treatment were significant at P = 0.05, by ANOVA. The F-ratio was 5.07 in maize, 10.93 in cotton and 12.67 in sunflower, with 2 and 9 degrees of freedom for the F-test in each species. See Supporting Information for primary data.

Dark seedling survival

Survival of seedlings in darkness was prolonged by exposure to higher [CO₂] during the darkness in all four species examined (Fig. 3). In all species except D. stramonium, survival time was increased significantly at each step of increasing [CO₂], using paired t-tests, at P = 0.05. Values of the ‘t’ statistic between
200 and 400 μmol mol\(^{-1}\) were 5.22 in *A. hypochondriacus*, 5.62 in *A. retroflexus*, 1.73 in *D. stramonium* and 4.91 in *M. sativa*, and between 400 and 800 μmol mol\(^{-1}\); \(t\) values were 5.62 in *A. hypochondriacus*, 5.62 in *A. retroflexus*, 3.41 in *D. stramonium* and 4.94 in *M. sativa*. The overall pattern of response was very similar in all four species. See Supporting Information for primary data.

**Respiration rate of excised petioles**

In all four species, respiration rates of excised petioles decreased with increasing [CO\(_2\)] (Fig. 4). The overall pattern was a consistent decrease at each step increase in [CO\(_2\)], although significant differences did not occur at all steps in [CO\(_2\)] in all species. In all species, differences between the 200 and 800 μmol mol\(^{-1}\) [CO\(_2\)] treatments were significant at \(P = 0.05\), by one-way ANOVA. The F-ratio was 9.13 in amaranth, with 2 and 12 degrees of freedom, \(F_{2,12} = 4.88\) in cotton, \(F_{2,12} = 14.3\) in soybean and \(F_{2,12} = 6.27\) in sunflower. See Supporting Information for primary data.

**Discussion**

While inhibition of plant dark respiration by high [CO\(_2\)] was detected at least a hundred years ago (Kidd 1916), more recent interest in the phenomenon was stimulated by observations of rapidly reversible decreases in respiration rate with increases in [CO\(_2\)] in the range anticipated in the atmosphere (Gale 1982; Reuveni and Gale 1985; Bunce 1990; El Ken et al. 1991, Baker et al. 2000). At about the same time, others found no increase and sometimes a decrease in respiration when plants were exposed to elevated [CO\(_2\)] (Jahnke 2001).

High [CO\(_2\)] could be perceived as a means to reduce nitrate assimilation and both CO\(_2\) and O\(_2\) exchange rates in darkness. Reduced leaf respiration and nitrate reduction in darkness at elevated [CO\(_2\)] were also reported (Davey et al. 2004; Asensio et al. 2015). The observations presented here indicating reduced respiration at elevated [CO\(_2\)] are consistent with recent experiments indicating [CO\(_2\)] effects on O\(_2\) exchange in darkness in some species (Gomez-Casanovas et al. 2007; Trimborn et al. 2014; Asensio et al. 2015). Asensio et al. (2015) reported that elevated [CO\(_2\)] could reduce nitrate assimilation and both CO\(_2\) and O\(_2\) exchange rates in darkness. Reduced leaf respiration and nitrate reduction in darkness at elevated [CO\(_2\)] were also reported (Davey et al. 2004). Tcherkez et al. (2008) found that decarboxylation rates in darkness were decreased with increasing [CO\(_2\)] from 140 to 1000 μmol mol\(^{-1}\), using gas chromatography. The experiments reported here utilized young seedlings, which may have higher rates of respiration than older tissue. However, in general whole-plant respiration decreases as a fraction of the daily carbon balance as plants age and relative growth rates decline, so [CO\(_2\)] effects on respiration could be at least as important as plant carbon balance in older plants as in seedlings, although much more difficult to detect. While clearly it is not always the case elevated [CO\(_2\)] in darkness reduces respiration, the results presented here lend credence to reports such as Haworth et al. (2016) that very careful leaf gas exchange measurements indicate that elevated [CO\(_2\)] treatments can reduce leaf dark respiration.

**Supporting Information**

The supporting information consists of three tables, containing the individual data points behind the mean values presented in Figs 2–4, respectively. The data in Fig. 1 are individual data points.

**Data Availability**

All data for this manuscript are available as Supporting Information.
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