SHORT COMMUNICATION

Variety of responses of plant phenolic concentration to CO₂ enrichment

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Abstract

A wide range of responses to elevated CO₂ was found for leaf total phenolic concentration of one grass species (wheat) growing in a Free-Air CO₂ Enrichment (FACE) system and two woody species (orange and pine trees) growing in Open-Top Chambers (OTC). The total phenolic concentration of wheat flag leaves grown at elevated [CO₂] was increased for most of the grain-filling stages studied; there was no significant change in phenolic concentration of CO₂-enriched orange tree leaves and CO₂-enriched pine tree needles had reduced total phenolic concentration. There was an inverse relationship between the increase in leaf total phenolic concentration and increase in biomass of these pine trees. Different rates of increase in growth (carbon sink) produced by different environmental conditions or different resource availabilities apart from CO₂ itself must be considered in order to understand the response of carbon-based-secondary-compounds to elevated CO₂.

Key words: CO₂, leaf phenolics, leaf condensed tannins, growth, nutrient, wheat, orange trees, pine trees.

Introduction

The current and future anthropogenic increase of atmospheric CO₂ is expected to produce changes in vegetation (Peñuelas and Matamala, 1990, 1993; Peñuelas and Azcón-Bieto, 1992; Rozema et al., 1993; Kimball et al., 1993). Among these changes, plant tissue quality is predicted to be modified by variations in the concentration of primary and secondary compounds. The study of this question is ecologically important because of the effects of plant compounds on plant-herbivore interactions (Feeley, 1976; Estiarte et al., 1994) and on decomposition rates (Horner et al., 1988).

The increase in atmospheric CO₂ increases photosynthesis and growth of C₃ plants at least at temperatures above 18 °C (Kimball et al., 1993). As a consequence of a relative increase in carbon concentration, plant tissue nitrogen concentration decreases (Peñuelas and Matamala, 1990), and the C/N ratio increases, leading some authors (Strain and Bazzaz, 1983; Lambers, 1993) to suggest that atmospheric CO₂ increases might produce changes in the concentration of carbon-based secondary compounds (CBSC) in a manner predicted by the carbon-nutrient balance hypothesis (CNB) (Bryant et al., 1983). This hypothesis states that environmental conditions that produce carbon excess (or deficiency) increase (or decrease) the amount of CBSC (e.g. phenolics) in plant tissues. Nitrogen supply, light availability and water stress have been identified as factors that affect CBSC concentration in this way (reviewed in Fajer et al., 1992, Estiarte et al., 1994) because of their different effects on growth and photosynthesis. However, the experiments conducted with CO₂-enrichment and reported in the literature have
been unable to support the hypothesis (Lincoln, 1993). The responses reported include increases, no change, and decreases in the concentration of different CBSCs (Lincoln and Couvet, 1989; Ruffy et al., 1989; Johnson and Lincoln, 1990; Fajer et al., 1991, 1992; Cipollini et al., 1993; Julkunen-Tiitto et al., 1993; Lindroth et al., 1993; Lavola and Julkunen-Tiitto, 1994).

Data are presented on the phenolic concentration of three species, two tree species and one grass species, growing in different CO₂ enrichment conditions: Open-Top Chambers (OTC) and Free-Air CO₂ Enrichment (FACE). Thus, several species and experimental systems were surveyed to examine CO₂-enrichment effects on CBSC, and to investigate the carbon-nutrient balance (CNB) and the growth-differentiation balance (GDB) (Herms and Mattson, 1992) hypotheses to explain the different results obtained under enriched CO₂ atmospheres.

Materials and methods

Experimental growth conditions (CO₂ enrichment)

A wheat crop (Triticum aestivum L. cv. Yecora rojo, a spring wheat) was grown in flat beds in an open field at Maricopa, Arizona, USA. The soil was Tris clay loam (fine-loamy, mixed (calcareous) hyperthermic Typic Torrifuvent). The wheat was planted on 15 December 1992, and emerged on 1 January 1993. Anthesis took place during the last week of March and wheat was harvested during the third week of May 1993. It was sown in rows spaced 25 cm apart at a density of 130 plants m⁻². The crop was irrigated using a subsurface drip system with tubes spaced 0.5 m apart at a depth of about 0.2 m. Emissors were spaced every 0.5 m along each tube.

In a similar way to previous experiments (Mauney et al., 1994), 20 m diameter plots of the wheat field were subjected to 550 μmol mol⁻¹ of CO₂ from a Free-Air CO₂ Enrichment (FACE) system. Unlike previous experiments, however, the CO₂ enrichment was supplied for 24 h d⁻¹ from emergence until harvest. There were also control plots at ambient levels of CO₂ (about 370 μmol mol⁻¹ during daytime). Four replicates of both FACE and CONTROL were conducted.

The wheat plots were split into semi-circular subplots, half of which were well-watered using the drip irrigation system by frequent replacement of the water lost by potential evapotranspiration (WET treatment) and half of which received half as much water as the wet plots (DRY treatment). Thus, there were a total of 16 plots (two levels of CO₂, two levels of water supply, four replicates). Nutrients were supplied at ample rates (276 kg N ha⁻¹ and 44 kg P ha⁻¹).

Eight sour orange (Citrus aurantium L.) trees have been grown from the seedling stage in soil in four clear-plastic-wall open-top chambers since 1987 in Phoenix, Arizona (Idso and Kimball, 1992). Two chambers (with two trees each) have been exposed to air enriched with CO₂ by 300 μmol mol⁻¹ above ambient (380 μmol mol⁻¹ during the day and much higher at night). The other two chambers (with two trees each) have been exposed to ambient 380 μmol mol⁻¹ CO₂. Water and nutrients have been supplied at ample rates.

Eight 40 cm tall Eldrida pine (Pinus eldarica L.) seedlings were planted in a nearby field in the same station of Phoenix, Arizona, in early March 1991 surrounded in pairs by clear-plastic-wall open-top chambers. One chamber received ambient air that averaged 408 μmol mol⁻¹ CO₂ during the day over the following 2 years, while the three other chambers received CO₂ concentrations that averaged 554, 680 and 812 μmol mol⁻¹. Water and nutrients were also supplied at ample rates (Idso and Kimball, 1994).

Leaf sampling

Fully developed flag leaves of wheat were collected on days 103, 112, 118, 125, and 140 during the 1993 growth cycle. Six different leaves from each one of the 16 plots (two levels of CO₂, two levels of water supply, four replicates) were sampled. Samples were stored in dry ice until arrival at the laboratory (a few hours). Samples were kept in the freezer at −20°C and analysed within 36 h of collection.

Several fully developed leaves (0.7–1 g dry weight) from the sidebranches were sampled from sour orange trees on 10 December 1992 and 21 May 1993 and from pine trees on 12 December 1992. They were immediately extracted for phenolics.

Dry matter content of different species was evaluated for subsamples of leaves after drying them at 60°C until constant weight.

Phenolic concentration

For each sample, the leaves were cut in small pieces. This material (0.7–1.0 g dry weight) was ground by hand using a mortar and pestle with liquid nitrogen and extracted with 20 ml of 70% aqueous methanol for 1.5 h at ambient temperature. The extract was filtered and filled to 25 ml. One-fifth dilutions of the extract were assayed for total phenolics by colorimetry using the Folin–Ciocalteu method (Singleton and Rossi, 1965). Values were expressed in gallic acid equivalents. The extract was also analysed for condensed tannins using the vanillin method of Broadhurst and Jones (1978). The values were expressed as catechin equivalents.

Statistical analyses

All statistical analyses were performed using SYSTAT 5.2 (SYSTAT, Inc., Evanston, IL). Analysis of variance was used to examine temporal and treatment-related variation in wheat phenolic concentration by using CO₂, water and time as factors in a strip-split plot model. CO₂ was used as the factor likely to affect total phenolic concentration of orange and pine trees. Regression and correlation analysis for phenolics, condensed tannins, plant growth, and CO₂ concentrations was also conducted.

Results

Total phenolic concentration of wheat flag leaves from elevated CO₂ in the FACE experiment was significantly higher (P < 0.01) than in ambient CO₂ for most of the grain-filling stages studied (from DOY 110 to DOY 130; Fig. 1a). Water availability and DOY (day of the year) also had clear influences on the phenolic concentration (P < 0.01 for both factors). There were no significant interactions except that of water treatment and DOY (P < 0.05). Throughout the sampling period, the increase in phenolic concentration of plants in the elevated treatment ranged between 6% and 17% for the wet treatment and between −2 (at the end of growth cycle) and 12% in
the dry treatment, as it is apparent when graphed as the enriched CO₂/ambient CO₂ ratio (Fig. 1b).

CO₂-enriched orange trees in the open-top chambers showed no significant change in total leaf phenolic concentration in either winter or spring (Fig. 2a). Pine tree needles had lower total phenolic (Fig. 2b) and condensed tannin (Fig. 2c) concentrations as CO₂ increased (Fig. 2).

FACE treatment increased above-ground wheat biomass about 20% until DOY 120, when accelerated senescence in the FACE plots led to a final biomass and yield increase of about 8% in the wet plots. The 20% increase was maintained in the dry plots (Pinter et al., 1993). CO₂-enriched atmospheres (670 μmol mol⁻¹) in the open-top chambers produced orange tree growth 2.7 times greater than that of the ambient trees (Idso and Kimball, 1993). The biomass of Eldarica pine trees growing in CO₂-enriched open top chambers (554, 680 and 810 μmol mol⁻¹) also increased by 76, 208, and 275% relative to control plants (Idso and Kimball, 1994). Figure 3 shows an inverse linear relationship between the percentage of increase in leaf total phenolic concentration and the percentage of increase in biomass under elevated CO₂ concentrations for these pine trees.

Discussion

All the range of possible phenolic concentration responses to CO₂ enrichment were obtained: decrease in pine trees, no change in orange trees, and increase in well-watered and water-stressed wheat. In the orange tree leaves, the parallel increase in non-structural carbohydrates and in dry weight per area (20%) reported by Idso et al. (1993) shows that when considering structural dry weight, phen-
olic concentration slightly increased. In wheat leaves, the CO₂ effect on increasing phenolic concentration took place even though there was a parallel increase in non-structural carbohydrates at elevated CO₂ (about 25% in wet treatment; Nie et al., 1995) that otherwise would have diluted phenolics. In all the three studied species there was an increase in the total amount of phenolics per plant because significant growth increases were produced for all three species with increasing atmospheric CO₂.

The interspecific variation found in the pattern of response of leaf phenolic concentration falls within the wide range of responses reported for carbon-based secondary compounds (CBSC) with atmospheric CO₂ enrichment. Increases (Cipollini et al., 1993; Lindroth et al., 1993; Ruffy et al., 1993; Lavola and Julkunen-Titto, 1994), no change (Lincoln and Couvet, 1989; Johnson and Lincoln, 1990; Fajer et al., 1992; Lindroth et al., 1993; Ruffy et al., 1993), and decreases (Fajer et al., 1992; Lindroth et al., 1993) have been reported in the literature. Therefore, to date, there is little evidence for a consistent effect of atmospheric CO₂ on the concentration of secondary compounds in higher plants (Lambers, 1993).

In these experiments plants were well fertilized and were grown in the field, so there was no restriction in root growth. However, as is common under CO₂ elevation, especially under conditions of nutrient limitation (Strain and Cure, 1985; Peñuelas and Matamala, 1990), there was a reduction in plant tissue nitrogen concentration (15% in orange trees or 20% in wheat at DOY 120 coinciding with the maximum differences in phenolic concentrations or 5% in DOY 100 when the minimum phenolic differences were found; Dr T Sinclair, personal communication). This has been one of the central reasons for predicting an increase of CBSCs under CO₂ enrichment (Cipollini et al., 1993; Lambers, 1993) following the CNB hypothesis (Bryant et al., 1983). Nevertheless, several different responses reported in the literature about CO₂ enrichment effects on CBSC are in apparent contradiction with the CNB hypothesis: for example, there are reports of larger CBSC increases with high nutrient availabilities than under low nutrient availabilities (Fajer et al., 1992; Julkunen-Titto et al., 1993).

The results show a possible inverse trend between phenolic concentration and growth under elevated CO₂. When the increase in growth was small (wheat), there were phenolic concentration increases, while when increase in growth was large (pine), phenolic concentration decreased (Fig. 3). Thus, elevated CO₂ promoted carbon assimilation (dry matter production), but when carbon was allocated relatively more to plant growth, there was less carbon allocated to secondary compounds. A similar pattern is found in data from literature reports on the response of CBSC to CO₂ for different genotypes, treatments, species, CBSC, or CO₂ concentrations (Johnson and Lincoln, 1990; Fajer et al., 1992; Cipollini et al., 1993; Julkunen-Titto et al., 1993; Lindroth et al., 1993; Lavola and Julkunen-Titto, 1994). All these results fit the growth-differentiation balance hypothesis (Herrms and Mattson, 1993; Tuomi, 1992) that states that growth is dominant under more favourable conditions, and differentiation (with increased synthesis of CBSC) dominates under less favourable conditions.

In summary, the results of this study show the variable effect of elevated CO₂ on phenolic concentration and suggest a possible inverse relationship between increases in growth produced by CO₂ enrichment and leaf phenolic concentration. Hence, different rates of increase in growth (carbon sink) produced by different environmental conditions or different resource availabilities apart from CO₂ itself must be considered to understand CBSC response to elevated CO₂.

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