SHORT COMMUNICATION

Elevated CO₂ effects on stomatal density of wheat and sour orange trees

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Abstract

No significant differences were found in stomatal densities or stomatal indices of wheat or sour orange trees grown at high CO₂ concentrations in two different CO₂ enrichment systems (Free-Air CO₂ Enrichment for wheat and Open-Top Chambers for orange trees). These results are in accordance with most of the previous results obtained in short-term experimental studies which suggest that plants do not acclimate to increasing CO₂ concentration by changing stomatal density within a single generation.

Key words: Triticum aestivum, Citrus aurantium, stomatal density, elevated atmospheric CO₂.

Introduction

Stomata regulate the rates of carbon uptake and water loss (Friend and Woodward, 1990). The light regime is the most important determinant of stomatal density, but atmospheric CO₂ concentrations have also been reported as secondary determinants (Woodward, 1987). In many cases, variations in stomatal density under different environmental conditions are a consequence of variations in the final size of epidermal cells and not of variations in the stomatal index (number of stomata related to total epidermal cell number) (Ticha, 1982).

Several papers have reported changes in stomatal parameters of fossil and herbarium plant material and correlated them to changes in atmospheric CO₂ concentration. Fossil tree leaves from the last 10 million years (Van der Burg et al., 1993) and from the last 140 000 years (Beerling et al., 1993) and herbarium leaves from the last three centuries (Woodward, 1987; Peñuelas and Matamala, 1990; Van der Burg et al., 1993) have shown a pattern of stomatal density inversely correlated with atmospheric CO₂ concentrations. However, there are also reports showing no significant differences in herbaceous plants from the last 100 years (Körner, 1988). The results obtained with plants experimentally grown under lower levels than current CO₂ atmospheric concentrations have also shown an increase in stomatal numbers (Woodward and Bazzaz, 1988). In greenhouse conditions, a lower stomatal density of leaves under elevated CO₂ has also been reported (Madsen, 1973). It is suspected that such changes are caused by a reduction in the initiation of stomata as atmospheric CO₂ increases.

Typically, however, plants grown under artificially CO₂-enriched atmospheres do not show this pattern of stomatal reduction as CO₂ increases (Thomas and Harvey, 1983; Kelly et al., 1991; Woodward and Bazzaz, 1988; Radoglou and Jarvis, 1990, 1992). It is not clear, then, whether the higher atmospheric CO₂ concentrations foreseen for the next decades will produce changes in stomatal numbers. New insights on short-term versus long-term responses, phenotypic acclimation versus genotypic adaptation and higher-than-present versus lower-than-present atmospheric CO₂ concentrations are needed.

We provide data on stomata of plants grown under two different CO₂ enrichment conditions: wheat plants in an open-field with Free-Air CO₂ Enrichment (FACE) and sour orange trees in Open-Top Chambers (OTC).

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Our aim was to study stomatal numbers under higher-than-present atmospheric CO₂ concentrations. A secondary aim was to compare the CO₂ response in FACE and OTC in order to assess possible disturbing effects of chambers on stomatal numbers.

Materials and methods

A wheat crop (Triticum aestivum L. cv. Yecora rojo, a hard red spring wheat) was grown in flat beds in an open field at Maricopa, Arizona, USA. The soil was Trix clay loam (fine-loamy, mixed (calcareous) hyperthermic Typic Torrifuvent). The wheat was planted on 15 December 1992. It emerged on 1 January 1993, and was harvested during the third week of May 1993. It was sown in rows spaced 25 cm apart at a population of 130 plants per m². The crop was irrigated using a subsurface drip system with tubes spaced 0.5 m apart at a depth of about 0.2 m. Emitters were spaced every 0.3 m along each tube.

Similar to previous cotton experiments (Mauney et al., 1994), 20 m-diameter plots of the wheat were subjected to 550 μmol mol⁻¹ of CO₂ from a free-air CO₂ enrichment (FACE) system. Unlike the cotton experiments, however, the CO₂ enrichment was supplied 24 h per day from emergence until harvest. There were also control plots at ambient CO₂ concentrations (about 370 μmol mol⁻¹ during daytime). Four replicates of both FACE and control treatments were included.

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 concentrations of CO₂, two rates of water supply, four replicates).

Eight sour orange (Citrus aurantium L.) trees rooted in the ground have been grown from the seedling stage in clear-plastic-wall open-top chambers since 1987 (Idso and Kimball, 1992). Half of the trees have been exposed to air enriched with CO₂ by 300 μmol mol⁻¹ above ambient (350-380 μmol mol⁻¹ during the daytime and much higher at night). Water and nutrients have been supplied at ample rates. The trees in the CO₂-enriched atmosphere have exhibited growth rates nearly triple those of the trees at ambient CO₂.

Fully developed flag leaves of wheat were collected on day 125 (5 May), when the wheat was nearing maturity. Three leaves per plot were collected, resulting in 12 leaves per treatment. The central part of each leaf was bathed in chloral hydrate in order to loosen the epidermis (without changing cell sizes) as described previously (Péñuelas and Matamala, 1990). The epidermis from each leaf was mounted on a slide and photographed with the help of a stereo microscope under ×75 magnification. Two fields per slide were randomly selected and photographed.

Fully developed and exposed leaves from the south side of each sour orange tree were sampled on 3 December 1992. Two leaves per tree were sampled yielding eight leaves per plant treatment. Varnish prints of the middle leaf surface were collected and photographs were taken under ×160 magnification with the help of a microscope.

Stomatal density was calculated on a leaf surface basis and stomatal index was calculated as: number of stomata/(number of stomata + number of epidermal cells) (Salisbury, 1927).

Results and discussion

No statistical differences (ANOVA) in wheat stomatal density or stomatal index were found among the four CO₂-irrigation treatments, either in the adaxial or in the abaxial epidermis (Fig. 1a, b).

However, plants grown under the dry treatments tended to have lower stomatal densities in adaxial epidermis and higher in abaxial epidermis than those from the wet treatments. Plants from the FACE treatments had slightly higher stomatal densities in adaxial epidermis and lower in abaxial epidermis than those from control treatments. In any case, FACE treatments had more influence on the dry treatments than on the wet treatments (Fig. 1a).

Similar to wheat, orange tree leaves did not show any clear effect of different concentrations of atmospheric CO₂ on stomatal density (Fig. 1c).

There were no significant changes in the number of stomata or in the ratio of the number of stomata to the total number of epidermal cells in either the orange tree or the wheat studies (Fig. 1b, d).

Fig. 1. Stomatal densities (±SE) (a, c) and stomatal indices (±SE) (b, d) of wheat flag leaves (a, b) and sour orange tree leaves (c, d) under FACE (F, 550 μmol mol⁻¹), control (C, ~360 μmol mol⁻¹), ambient (A, ~360 μmol mol⁻¹), and enriched (E, ambient + 300 μmol mol⁻¹) CO₂ concentrations and well-watered (W, wet) and dry (D) irrigation regimes. Wheat results are from abaxial and adaxial surfaces of leaves from the FACE experiment, while orange tree results are from abaxial surface of leaves from the OTC experiment.
Our results are in accordance with several other sets of experimental data from plants grown in artificially CO$_2$-enriched atmospheres. Although some studies (Oberbauer et al., 1985) have found a decrease in stomatal density and others have found some increase (Kimball et al., 1986; Gaudillère and Mousseau, 1989), almost all the reports of experiments on plants growing in artificially elevated CO$_2$ have shown no response or a very small decrease in stomatal density (Thomas and Harvey, 1983; Oberbauer et al., 1985; Woodward and Bazzaz, 1988; Radoglou and Jarvis, 1990, 1992; Kelly et al., 1991; Körner and Arnone, 1992).

Other variables such as humidity, wind, temperature or irradiance that are modified in experimental conditions in OTC’s and greenhouses could explain some of the conflicting results in the literature. Water availability affects stomatal density. Increased humidity in chambers could reduce stomatal density by increasing epidermal cell expansion (Ticha, 1982). Wind speed has also been shown to increase the number of stomata per unit area as a consequence of the reduction in leaf expansion (Grace and Russell, 1977). A reduction in stomatal density has been reported at low wind speeds in chambers as CO$_2$ concentration increases, while high wind speeds increase stomatal density as CO$_2$ increases (Retuerto and Woodward, 1993). These experimental disturbances were avoided in our FACE study. Nevertheless, we still did not find any significant differences in stomatal numbers.

Our results are also in accordance with the prediction that stomatal response to CO$_2$ concentrations would be non-linear and that any changes are likely to be smaller in the range from the current ambient CO$_2$ concentration (350 μmol mol$^{-1}$) to higher concentrations (e.g. 700 μmol mol$^{-1}$), than from the current to lower concentrations (225 μmol mol$^{-1}$) (Woodward and Bazzaz, 1988).

The absence of differences in stomatal density and in stomatal index supports the hypothesis that there are no direct effects of CO$_2$ enrichment on the initiation of stomata during ontogenesis or in epidermal cell expansion at a later stage (Radoglou and Jarvis, 1990, 1992), i.e. that there is no short-term acclimation of the plants within a single generation. However, these experiments did not test the genetic component of long-term plant adaptation to changes in CO$_2$ concentration that have been suggested by the observed changes in stomatal numbers from fossil and herbarium plant material (Woodward, 1987; Peñuelas and Matamala, 1990; Beering et al., 1993).

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