Detrimental effects of fluctuating high CO₂ concentrations on peppers

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Abstract

Plants of pepper (Capsicum annuum L.) were grown in controlled environment chambers at ambient (360 μmol mol⁻¹) and fluctuating pulse-enriched CO₂ concentrations (700 μmol mol⁻¹ daily average, ranging from 500 to 3500 μmol mol⁻¹ = ECO₂) under two water regimes. A decrease in plant growth and yield together with frequent visual injuries was found in plants growing under ECO₂. Root/shoot ratio was greater, chlorophyll concentration and respiration rates were lower, and stomatal conductance and relative importance of alternative pathway respiration were higher under ECO₂. The negative effects of ECO₂ were more intense under high water availability. The symptoms produced by ECO₂ were similar to those of resource limitation, and were alleviated with increased nutrient supply. Constant elevated CO₂ concentrations (700 μmol mol⁻¹) increased pepper production and did not produce any of the injuries described for this erratic ECO₂ treatment. Thus, it is probably the erratic nature of the CO₂ concentration and not the gas itself that was causing the injury.

Key words: Capsicum annuum L.; chlorophyll; dry mass; fruit; plant height; respiration rate; root/shoot ratio; specific leaf mass; stomatal conductance; water supply.

Introduction

CO₂ enrichment increases the yield of many horticultural species (Mortensen 1987). Detrimental effects of high CO₂ concentrations on plants have only occasionally been reported. Leaf injuries including chlorosis, necrosis, and curling of leaves are sometimes observed with CO₂ enrichment (Ehret and Jolliffe 1985, Tripp et al.

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Growth reductions without visible injuries have also been observed at high CO₂ concentrations. Under high CO₂, chlorosis of leaves of cucumber (Wittwer 1967), cotton (Hesketh et al. 1971), and soybean (Hesketh et al. 1971, Chang 1975) was correlated with elevated leaf starch levels (Hesketh et al. 1971) and reduced photophosphorylation and Hill reaction activities (Chang 1975). Madsen (1974) observed characteristic leaf rolling and necrosis after CO₂ enrichment in tomatoes, and these effects were also associated with high starch accumulation. Van Berkel (1984) indicated that leaf injury induced by high CO₂ may be widespread among plant species and that susceptibility seems to vary among cultivars. However, little is known about the mechanism of CO₂ induced injury or environmental influence. A better understanding of injuries which occur under high CO₂ concentrations and of their causes is thus needed.

The objectives of this study were to investigate the effects of fluctuating elevated CO₂ concentrations on the development of leaf injury, and to evaluate growth and development related to the syndrome.

Materials and methods

Seeds of peppers, Capsicum annuum L. (pure line B6) were planted in pots and grown in chambers (Weiss, Lindenstruth, Germany) with 14/10 h light (incandescent and fluorescent lamps, 300 μmol m⁻² s⁻¹)/dark cycles, 70% relative humidity, and 25/20 °C for four months. Plants were grown in 2 500 cm³ containers in a substrate consisting of 1:1 (v:v) peat and sand. Plants were grown under atmospheres with fluctuating elevated CO₂ (ECO₂) and ambient CO₂ (ACO₂). Fig. 1 shows a typical pattern of CO₂ concentrations inside the ECO₂ chamber throughout a day of the growth cycle (the daily average was 700 ± 31 μmol mol⁻¹). As plants enlarged, CO₂ depletion was more rapid and additions more frequent. The variation in CO₂ was

![CO₂ Concentration Graph](image)

**Fig. 1.** Evolution of CO₂ concentration throughout a day in the ECO₂ (fluctuating Elevated CO₂) chamber.
reproduced each day by using the ADC infrared sensors (WA-470C Carbon Dioxide Transducer; Analytical Development Co., Hoddeston, England) and Eurotherm controllers (Eurotherm International, Barcelona, Spain) that closed electrovalves only after the CO₂ threshold (1500 μmol mol⁻¹) was overcome. It was a simulation of a CO₂ fumigation control not working with precision or of a CO₂ fumigation by pulses, as could occur in poorly operated commercial greenhouses. The CO₂ concentration in the ACO₂ chamber ranged between 340 and 380 μmol mol⁻¹. The compressed CO₂ used for CO₂ enrichment (Abello, Barcelona, 99.995 % purity) was monitored by gas chromatography for hydrocarbon contaminants. No ethylene or other hydrocarbons were detected either before or after entering the treatment chamber. Within each CO₂ treatment, two levels of irrigation were established (16 plants per treatment). Water-stressed plants were fertirrigated only when soil matric potential (measured with soil tensiometers) reached -0.04 MPa ("low water" treatment, LW) and control plants when it reached -0.01 MPa ("high water" treatment, HW). Irrigation was conducted with a solution containing [mol m⁻³] 4.88 NO₃⁻, 0.63 H₂PO₄⁻, 2.21 SO₄²⁻, 2.76 K⁺, 4.35 Ca²⁺, 1.01 Mg²⁺, 1.36 NH₄⁺, 8.75 Fe²⁺, 9.09 Mn, 1.82 Zn, 7.87 Cu, 4.62 B, 1.04 Mo, 25 Mg. During the last month, KNO₃ was added to the solution until [NO₃⁻] reached 10 mol m⁻³. The experiment was repeated three times and the allocation of treatments to chambers was rearranged. The air exchange of the growth chamber atmospheres was shorter than 10 min.

Fresh and dry masses (roots, stems, leaves and fruits) of 8 plants from each treatment were measured at the middle and at the end of the crop growth cycle. Leaf area of these plants was measured with a leaf area meter LI-COR 3000 (LI-COR, Lincoln, NE, USA). Plant components were then dried at 60 °C to constant mass. Specific leaf mass (SLM) and root/shoot ratio were also determined.

Transpiration rates (E), stomatal conductance (gs), and leaf temperature (Tl) were measured with a LI-COR model LI-1600 steady-state porometer (LI-COR, Lincoln, NE, USA) in 6 top canopy fully expanded leaves of each treatment every 3 or 4 d. Oxygen uptake rates (respiration rate, R0) of 3 mm diameter disks from different fully expanded leaves every time were measured in the dark at 25 °C, using a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK), in rapidly stirred, air saturated 20 mM MES, 0.2 mM CaCl₂ buffer (pH 5.7). KCN was added to the cuvette from a stock aqueous solution (0.4 M) with a microsyringe to get a final concentration of 1 mM and thus measure cyanide insensitive alternative pathway.

A SPAD chlorophyll (Chl) meter from Minolta (model 502) was used to measure leaf Chl concentrations (Monje and Bugbee 1991). The relative units were previously calibrated spectrophotometrically measuring leaf Chl concentrations by shaking fresh leaf discs in N,N-dimethylformamide (Porra et al. 1989).

All statistical analyses were performed using Systat 5.2 (Systat, Evanston, IL, USA). We used analysis of variance ANOVA for analysis of temporal and treatment-related variation by using CO₂, water and time as factors of a split plot model.
Results

Our experimental treatments produced severe ECO2 negative effects, and moderate water limitations in LW treatments at the end of the growth cycle study. ECO2 and water-limiting conditions reduced shoot biomass (stems, leaves, and fruits) (Fig. 2). LW treatment reduced root dry mass, but ECO2 did not affect it significantly (Fig. 2). The decrease in total biomass produced by ECO2 (50 %) was larger than the decrease produced by water limitation (40 %). Negative effects of ECO2 were more intense under high availability of water.

Fig. 2. Total, leaf, stem, root and fruit dry masses and leaf area of pepper plants exposed to different CO2 and irrigation treatments. Data correspond to two different harvests throughout the growth cycle. ECO2 = Elevated poorly regulated CO2 concentrations (500-3500 μmol mol⁻¹), ACO2 = Ambient CO2 concentrations (330-380 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.

ECO2-stressed peppers showed higher root dry mass/shoot dry mass (R/S) ratios, no difference in specific leaf mass, and lower Chl concentration than ACO2 pepper
plants at the end of the growth cycle (Fig. 3). ECO₂- and water-stressed plants were shorter and had fewer and smaller leaves than ACO₂ plants across the growth cycle (Fig. 4).

Eighty days after germination, ECO₂ plants already exhibited leaf injury symptoms. The injury was first visible as dark points followed by interveinal chlorosis. Complete chlorosis was followed by tip necrosis. Some leaves also curled. Finally, margins and patches on the leaf also became necrotic and the leaf abscissed. These injury symptoms were never visible in plants maintained at ACO₂. Water deficiency did not seem to alter symptom expression of ECO₂ injury, so symptoms were similar in all treatments. The intensity of these injury symptoms was much lower in new growth after KNO₃ was added to the nutrient solution in the last month of the growth experiment.

![Graph showing root/shoot ratio, chlorophyll concentration, stomatal conductance, and specific leaf mass in pepper plants exposed to different CO₂ and irrigation treatments. Data correspond to two different harvests throughout the growth cycle. ECO₂ = Elevated poorly regulated CO₂ concentrations (500-3500 μmol mol⁻¹), ACO₂ = Ambient CO₂ concentrations (330-380 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.](image)

Fig. 3. Root/shoot ratio, chlorophyll concentration, stomatal conductance, and specific leaf mass in pepper plants exposed to different CO₂ and irrigation treatments. Data correspond to two different harvests throughout the growth cycle. ECO₂ = Elevated poorly regulated CO₂ concentrations (500-3500 μmol mol⁻¹), ACO₂ = Ambient CO₂ concentrations (330-380 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.

$g_s$ tended to be higher in ECO₂ than in ACO₂ plants (Fig. 3). $R_D$ of leaves, measured as O₂ release, were smaller under ECO₂ for both water treatments, but the decrease was especially important for HW plants because rates of LW plants were already small (Fig. 5). However, there were no significant decreases in alternative pathway capacity. Thus, the relative importance (in total respiration) of alternative pathway increased under ECO₂ (Fig. 5).
Discussion

We found clear detrimental effects of fluctuating high CO₂ concentrations. There were significant decreases in production after plants developed chlorosis and leaf curling. The plants with injury had lower $R_D$, and a higher alternative pathway/cytochrome pathway activities ratio, in accordance with their much lower growth (Lambers 1985).

![Graph showing plant height, leaf number per plant, and average leaf area for different CO₂ and irrigation treatments.](image)

**Fig. 4.** Plant height, leaf number per plant, and average leaf area in pepper plants exposed to different CO₂ and irrigation treatments. Data correspond to four different harvests throughout the growth cycle. ECO₂ = Elevated poorly regulated CO₂ concentrations (500-3500 μmol mol⁻¹), ACO₂ = Ambient CO₂ concentrations (330-380 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.

CO₂ is generally not considered to be a harmful gas to plants or animals at concentrations under 5000 μmol mol⁻¹ (Peñuelas 1993). However, harmful effects can arise as a result of other trace gases such as ethylene which may be present during CO₂ enrichment treatments. They did not seem to be the cause of the leaf injury in our study. According to gas chromatographic measurements, the compressed gas cylinders supplying CO₂ were not contaminated with any other trace
gas. The supplementary gas was diluted approximately 1000-fold as it entered the growth chambers and the potential build-up of contaminant gases was limited by the short turnover time (<10 min on average) of the growth chamber atmospheres. Leaf injury only occurred in those chambers that received supplementary CO₂. Thus the injury was directly or indirectly due to long term plant exposure to elevated and fluctuating CO₂ concentration. Constant elevated CO₂ concentrations (700 μmol mol⁻¹) increased pepper production and yield (Peñuelas et al. 1995) and did not produce any of the injuries described for this fluctuating CO₂ treatment. Thus, it seems that it is the erratic nature of the CO₂ concentration and not the gas itself that was causing the injury. Presumably, the mechanism for the decreased biomass production in ECO₂ plants has to do with photosynthesis acclimating to high levels of CO₂ (Peet et al. 1986); photosynthesis would then be below that of non-enriched plants at low CO₂ levels. Willits and Peet (1989) have reported little or no gain in tomato yield when fractional enrichment time was less than half the light period.

![Graph showing respiration rates of cytochromic and alternative pathways in pepper plants exposed to different CO₂ fertilization treatments. Data correspond to 94 d after germination. ECO₂ = Elevated poorly regulated CO₂ concentrations (500-3500 μmol mol⁻¹), ACO₂ = Ambient CO₂ concentrations (350 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.](image)

Fig. 5. Respiration rates of cytochromic (left, Vᵣ) and alternative (right, Vₐₐₙ) pathways in pepper plants exposed to different CO₂ and fertilization treatments. Data correspond to 94 d after germination. ECO₂ = Elevated poorly regulated CO₂ concentrations (500-3500 μmol mol⁻¹), ACO₂ = Ambient CO₂ concentrations (350 μmol mol⁻¹), LW = Low Water treatment, HW = High Water treatment (described in Materials and methods). SE bars are also presented.

Although abnormalities have previously been observed in plants grown in CO₂ enriched atmospheres, the existence of high CO₂ induced leaf injury has not been widely recognized. Several factors may have contributed to this. Injuries are not always observed in plants grown at elevated CO₂ may be because levels of CO₂ are insufficiently high to cause injury. For example, Ehret and Jolliffe (1985) did not find injury symptoms in beans at CO₂ concentrations below 1200 μmol mol⁻¹. Many CO₂ enrichment investigations have been limited to lower concentrations. However, plants have been grown at CO₂ concentrations as high as 50 000 μmol mol⁻¹ without visible damage (Hickleton and Jolliffe 1980). Variability in susceptibility to injury has been observed within and between plant species (Van Berkel 1984). Where abnormalities have been reported, symptoms have varied widely. The symptoms we observed, dark points, leaf rolling wilting, interveinal chlorosis, and necrosis, had all already been reported (Madsen 1974, Ehret and Jolliffe 1985, Tripp et al. 1991a,b).
Particular environmental conditions of, e.g., irradiance, temperature, watering or photoperiod may be required for the expression of injury in CO₂ enriched plants.

The mode of action by which CO₂ enrichment promotes leaf injury remains unclear. Increases in leaf dry mass are usually a prominent early response after enrichment (Kimball and Idso 1983). Ehret and Jolliffe (1985) found that once a threshold SLM had been reached, virtually all the additional leaf dry matter increase could be attributed to starch accumulation. Large starch granules formed in high CO₂ leaves may impair photosynthesis by distorting chloroplast structure or by interfering with radiant energy absorption or CO₂ transfer (Madsen 1975, Cave et al. 1981, Wulff and Strain 1982). However, we did not find any increase in the SLM of ECO₂ plants nor any relationship between SLM and foliar deformation. This observation is in agreement with Tripp et al. (1991b) who did not find a significant relationship between foliar starch concentration and deformation severity during an entire season of tomato growth.

Lower E produces higher leaf temperatures (Peñuelas et al. 1992). The injurious effects of high CO₂ concentrations have sometimes been explained by too high leaf temperatures at high irradiances. In our study, leaves that developed injury did not exhibit decreases in gₛ indicative of reduced E. Leaf gₛ tended to increase, something unique in studies of plant response to elevated CO₂ (Fig. 3). Thus, this explanation of ECO₂ detrimental effects is not supported by our results.

ECO₂ plants contained less Chl, indicative of lower N content (Peñuelas et al. 1994). ECO₂ plants increased their relative biomass allocation to the structures involved in nutrient and water uptake, i.e. roots, and their root/shoot ratio increased (Fig. 3). ECO₂- and water-stressed plants also had fewer leaves and these leaves were smaller (Fig. 4), thus decreasing the rate of resource loss. The symptoms were alleviated with increased nutrient supply after adding KNO₃. Both N and K reductions could explain part of the detrimental effects. Thus poorly regulated pulses of high CO₂ concentrations produced a syndrome of source limitation that was not present at ambient CO₂ levels. High CO₂ concentrations produce decreases in N and elemental content (Peñuelas and Matamala 1990, 1993). Tripp et al. (1991a) found a strong positive relationship between foliar deformation and fruit yield in tomatoes and they associated increased deformation with reduced K.

The prevention or alleviation of a weak control system of CO₂ fumigation will avoid such leaf injuries and possible photosynthesis acclimation to high levels of CO₂. Adequate source supply could also be of value in plant production in commercial greenhouses submitted to high poorly regulated CO₂ concentrations, to fractional CO₂ fumigation time, or to pulses of CO₂.

References


