AIR TEMPERATURE MODIFIES THE SIZE-ENHANCING EFFECTS OF ATMOSPHERIC CO₂ ENRICHMENT ON SOUR ORANGE TREE LEAVES

S. B. TISO,* B. A. KIMBALL* and D. L. HENDRIX

*U.S. Water Conservation Laboratory, 4301 E. Broadway, Phoenix, AZ 85040, U.S.A. and
†Western Cotton Research Laboratory, 6135 E. Broadway, Phoenix, AZ 85040, U.S.A.

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Inno S. B. KIMBALL, B. A. and HENDRIX, D. L. Air temperature modifies the size-enhancing effects of atmospheric CO₂ enrichment on sour orange tree leaves. ENVIRONMENTAL AND EXPERIMENTAL BOTANY 33, 293-299. 1993. - Every other month for a period of 2 years, leaf area and dry weight measurements were made on the foliage of sour orange trees growing in ambient air and in air enriched with an extra 300 p.p.m. CO₂. Leaf-starch content measurements were made at approximately 2-month intervals for a period of 1 year. Data demonstrated that all three plant parameters were significantly increased by atmospheric CO₂ enrichment, except in the coldest portion of the year. A plot of the ratio of CO₂-enriched leaf dry weight to ambient-treatment leaf dry weight against the mean air temperature of the preceding month revealed this relationship with temperature to be linear. The relationship shows atmospheric CO₂ enrichment to have a negligible effect on leaf dry weight at a mean air temperature of approximately 15°C. As a mean air temperature of 25°C, however, it shows individual CO₂-enriched leaves of our experiment to weigh 60%, more than their ambient-treatment counterparts. This phenomenon helps to explain the vastly different effects of atmospheric CO₂ enrichment that have been reported for a number of diverse ecosystems.

INTRODUCTION

HUNDREDS of experiments conducted over the past few decades have shown atmospheric CO₂ enrichment to have a number of beneficial impacts on plant growth and development. In a study of the hardy willow, Salix spp., MASLAعنا, for example, Inno et al. identified 29 different plant properties that benefited by a doubling of the atmospheric CO₂ concentration. In spite of this wealth of experimental evidence, however, many scientists feel that we still do not know how earth’s biosphere will ultimately react to the atmosphere’s steadies rising CO₂ concentration.

The primary reason for this concern is that only a small number of CO₂-enrichment experiments have utilized plants growing out-of-doors with their roots in the undisturbed and non-confining soil of the natural environment. This protocol, having recently been demonstrated to be essential for determining the real-world response of earth’s vegetation to the rising CO₂ concentration of the planet’s atmosphere, has not, yet even in those experiments where this requirement has been met, seemingly conflicting results have been obtained. In the harsh climate of the Arctic tundra, for example, tussok tundra has shown almost no response to atmospheric CO₂ enrichment.

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In the more temperate climate of a subarcticus of the Chesapeake Bay in Maryland, however, the
productivity of C3 plants, in a mesophyll salt marsh has risen nearly 90% in response to a doubling of the atmospheric CO2 concentration. 2, 24 And in the intense heat of the Arizona desert, aeropaquely fertilized and irrigated sour orange trees have nearly tripled their growth in response to an 85% enhancement of the atmospheric CO2 concentration. 12,13,22

One explanation for the diversity of these observations is that temperature may be influencing the expression of potential biological benefits of atmospheric CO2 enrichment, suppressing them when the weather is cold and enhancing them when it is hot. This hypothesis was independently advanced by Deno et al. 11 and Mortensen; 12 it has subsequently been found to apply to a number of different plants, as noted in the reviews of Long 22 and Hugan et al. 11 Few of these studies have dealt with trees, however, and as woody plants fully account for three-quarters of all terrestrial photosynthesis, 23 it is of great importance to know how they may be affected by this phenomenon. Indeed, one of the major uncertainties presently confronting the scientific community centers on the ability of trees to enhance their carbon sequestering capacity in response to the rising CO2 concentration of the atmosphere. 1,11,12,23,24

If trees did so, for example, they could significantly slow the rate of rise of atmospheric CO2 and mitigate the severity of CO2-induced global warming. 25 Consequently, the primary purpose of this study was to determine the effects of air temperature on the growth characteristics of sour orange trees exposed to ambient and elevated concentrations of atmospheric CO2.

MATERIALS AND METHODS

In prior work on tree response to atmospheric CO2 enrichment at our laboratory, we have focused on trunk, branch, and root growth. 22,24 This approach is not well suited to our present purpose, however, because of the difficulties associated with adequately sampling the large branch and root systems of the trees which currently stand about 5 m tall as regular time intervals. Hence, we herein concentrate our efforts on leaves.

Leaf measurements were made throughout the third and fourth years (1990 and 1991) of a long-term CO2-enrichment study of sour orange (Citrus 

aurantium L.) trees. Various aspects of the parent experimental program have been described previously. 25,26 In brief, eight small seedlings were planted directly into the ground at Phoenix, Arizona in July of 1987 and surrounded in pairs with transparent walls of clear plastic film. Through perforated plastic tubes suspended just above the surface of the soil, ambient air has been continuously supplied to half of these open-top enclosures at a rate sufficient to provide for four complete chamber air exchanges per minute, while the other enclosures have been similarly supplied with air enriched with an extra 300 ppm CO2.

The experimental trees have been subjected to these two treatments protocols since mid-November of 1987. In addition, they have been irrigated at intervals deemed appropriate to prevent the development of significant water stresses, and they have been supplied with a commercial citrus tree fertilizer (Arizona’s Best Citrus Food; 13F, 14N, 10P, 13P, K) at intervals recommended for optimum growth and fruit production (spring, summer, fall).

Every other month throughout the 2-year period of our study, 17 leaves were removed from each of the north, south, east and west quadrants of each tree. The total area of the 68 leaves thus removed from each tree at each sampling date was determined by means of a LI-COR LI-3100 Area Meter. Thereafter, the leaves were dried in an oven and weighed. Area and dry weight averages were then determined for each of the two chamber replications of each CO2 treatment, and final treatment means and associated standard deviations were derived from these results.

Throughout the last year of the study, mid-afternoon (1500 hr local time) measurements of leaf starch content were also made approximately every 2 months. Six leaf discs of 0.765 cm diameter were removed from leaves from the cardinal sides of the trees. These discs were placed into vials containing 5 ml cold 80°, ethanol (v/v), stored on ice, and transported to the laboratory for later analysis. Leaf samples were extracted (without grinding) in hot (80°C), 80°, ethanol (v/v) to remove soluble sugars. An aliquot (1 ml) of 0.2 M KOH was added to the tubes containing
the ethanol/inaldehyde residue. The tubes were then heated in a boiling water bath for 1 h. Following this treatment, the tubes were cooled and the KOH neutralized with 1 M acetic acid. A small aliquot (0.5 ml) of 1 M Tris buffer (pH 7.2) was added to each tube and the pH of the contents was lowered to between 6.6 and 7.3 with acetic acid. An aliquot of α-amylase from Bacillus licheniformis was added and the tubes were incubated for 30 min at 85°C in a shaking water bath. The pH of the tubes was then lowered to approximately 3.0 by adding additional acetic acid and the contents treated with amylase-glucose isomerase to convert the gelatinized starch to glucose. Known amounts of amylpectin, treated identically, served as standards in the starch analysis. Starch in the samples was measured using an enzymatic analysis technique in which glucose from the digested starch was reacted with glucose-6-phosphate dehydrogenase and the chromophore 3,3'dioctadecylxanthyline violet 1-NIT for 15 min at 37°C on a microplate. The resulting color was measured at 492 nm with a standard microplate reader. Standards and samples used in all assays were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Again, treatment mean and standard deviations were derived from the two chamber replications of each CO₂ concentration.

RESULTS AND DISCUSSION

Atmospheric CO₂ enrichment increased leaf starch content and leaf dry weight per unit area from March through to November (Fig. 1). Dividing the CO₂-enriched treatment results by the corresponding ambient treatment results illustrates the relative responses of these parameters.

![Graph showing influence of atmospheric CO₂ enrichment on leaf starch content and leaf dry weight per unit area](image)

**Fig. 1. Influence of atmospheric CO₂ enrichment (+ 300 µl/l CO₂) on leaf starch content per leaf area (upper panel) and leaf dry weight per leaf area (lower panel) of four orange trees. Error bars represent standard deviations of the two replications of each treatment about their means.**
to atmospheric CO₂ enrichment (Fig. 2). The similarities of the seasonal trends of these parameters provides strong support for the conclusion that leaf productivity and photosynthetic output are stimulated by atmospheric CO₂ enrichment throughout the major portion of the year. It also clearly demonstrates, however, that these benefits are considerably reduced in winter, and that they may even totally disappear during the coldest months of the year (January).

In addition to leaf dry weight per unit area being increased by atmospheric CO₂ enrichment over the greater portion of the year, mean leaf size increases also enhanced, as shown in the upper panel of Fig. 3. And multiplying the mean area per leaf by the mean leaf dry weight per area—data for 2 years of which are presented in the middle panel of Fig. 3—results in an even more dramatic enhancement of mean dry weight per leaf produced by atmospheric CO₂ enrichment (Fig. 3, lower panel).

To explicitly identify the temperature dependency of mean dry weight per leaf, we have plotted the 12 data points of the lower panel of Fig. 3 against the mean regional air temperatures of the individual months immediately preceding their acquisition (measured at the Phoenix National Weather Service Forecast office), obtaining the results depicted in Fig. 4. As with similar analyses of this nature,1,2,9 a linear equation adequately describes the relationship, which suggests that at a mean air temperature of about 4 °C there would probably be no effect of atmospheric CO₂ enrichment on the individual leaf dry weights of orange trees. At a mean air temperature of 32°C, however, a 300 μl/l enhancement of the atmospheric CO₂ concentration boosts the mean dry weight of each CO₂-enriched leaf by a full 46%.
This finding helps to resolve the dilemma presented by the disparate experimental results described in the Introduction. In light of the phenomenon portrayed in Fig. 4, for example, it is not surprising that plants in the Alaskan tundra would not respond significantly to atmospheric CO₂ enrichment, nor that plants in the Arizona desert would respond so much more dramatically than those in the more moderate climatic regime of coastal Maryland. Although other factors are undoubtedly involved as well, temperature variability is clearly a potent factor in determining the ultimate consequences of atmospheric CO₂ enrichment for earth's plant life.

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REFERENCES