EFFECTS OF ATMOSPHERIC CO₂ ENRICHMENT ON CHLOROPHYLL AND NITROGEN CONCENTRATIONS OF SOUR ORANGE TREE LEAVES

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Idso S. B., Kimball B. A. and Hendrix D. L. Effects of atmospheric CO₂ enrichment on chlorophyll and nitrogen concentrations of sour orange tree leaves. Environmental and Experimental Botany 36, 323-331, 1996.—Since 18 November 1987, eight sour orange (Citrus aurantium L.) trees have been maintained under well-watered and fertilized conditions within four clear-plastic-wall open-top enclosures, two of which have been continuously supplied with ambient air of approximately 400 μl l⁻¹ CO₂ and two of which have been supplied with air enriched to approximately 700 μl l⁻¹ CO₂. At weekly intervals throughout years 4-7 of this long-term experiment, we measured chlorophyll a contents of 60 leaves on each of the trees with a hand-held chlorophyll meter that was specifically calibrated for our study. At bi-monthly intervals, we also measured the areas, dry weights and nitrogen contents of 48 leaves from each tree. Expressed on a per-unit-leaf-area basis, leaves from the CO₂-enriched trees contained 4.8% less chlorophyll and nitrogen than leaves from the trees exposed to ambient air. Because of their greater leaf numbers, however, the CO₂-enriched trees contained 75% more total chlorophyll and nitrogen than the ambient-treatment trees; the total productivity of the CO₂-enriched trees was 175% greater. Consequently, although per-unit-leaf-area chlorophyll and nitrogen contents were slightly lowered by atmospheric CO₂ enrichment in our experiment, their use efficiencies were greatly enhanced.

Keywords: Citrus aurantium, sour orange tree, carbon dioxide, CO₂, leaf chlorophyll content, leaf nitrogen content.

INTRODUCTION

Nitrogen supply can markedly affect plant productivity, partly as a result of its impact on the production of nitrogen-containing components of the leaf photosynthetic machinery. Hence, it has been suggested that the reduced chlorophyll concentrations often found in the leaves of plants grown in air enriched with carbon dioxide may negatively impact their photosynthetic efficiencies, resulting in reduced plant growth. In reviewing a number of studies of the effects of atmospheric CO₂ enrichment on leaf chlorophyll content, however, Arp found that it was only when rooting volume was 6.1 or less that CO₂-enriched plants contained less chlorophyll per unit leaf area than plants grown in normal air; for larger rooting volumes and open-field conditions, the unit-leaf-area chlorophyll con-

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tent of CO₂-enriched plants was always equal to or greater than, that of plants exposed to the ambient atmosphere. In a free-air CO₂ enrichment study of cotton plants growing under natural field conditions, Pinter et al. [5] also observed slightly higher per-unit-leaf-area chlorophyll contents in plants exposed to elevated CO₂. Consequently, to further test the hypothesis that plants unaffected by below-ground space restrictions will not experience reductions in per-unit-leaf-area chlorophyll content when exposed to elevated CO₂, we conducted a study of the chlorophyll and nitrogen contents of the leaves of sour orange trees rooted in the ground and growing out-of-doors at Phoenix, Arizona in an on-going long-term CO₂-enrichment experiment.

MATERIALS AND METHODS

The sour orange tree (Citrus aurantium L.) study was begun in July 1987, when eight 30-cm-tall seedlings were planted directly into the ground at Phoenix, Arizona within four clear-plastic-wall open-top enclosures. On 18 November of that year, two of these enclosures had the CO₂ content of the air within them increased to 300 µl l⁻¹ above that of the air within the other two enclosures, which averaged about 400 µl l⁻¹ over the course of the study, which slight increase above the global mean is presumably due to the entrainment of some of the excess CO₂ that escapes from the tops of the two CO₂-enriched enclosures. This differential treatment is maintained by the CO₂ measurement-supply system described by Ise et al. [16] and Kimball et al. [17] which brings either ambient or CO₂-enriched air to the enclosures through perforated plastic tubes that lie upon the soil beneath the trees. Kitting through the trees' foliage to escape from the tops of the enclosures, these air streams exit the chambers at a rate of four enclosure volumes per minute.

Throughout the experiments the trees have been irrigated and fertilized at frequencies believed to be sufficient to preclude the development of water and nutrient stresses. We also enlarged the enclosures each year to prevent the development of above-ground physical restraints. The walls have been replaced annually with new clear plastic to allow the transmission of as much light as possible to the trees.

After 3 full years of differential CO₂ exposure, we began our study of leaf chlorophyll concentrations in March 1991. From that point in time, at approximately weekly intervals for a period of 4 years, we measured the chlorophyll concentrations of 30 leaves distributed across the north side of each tree and 30 leaves distributed across the south side of each tree. These measurements were made with a hand-held Minolta SPD 502 chlorophyll meter (Minolta Corporation, Ramsey, NJ) which measures the differential attenuation of light in wavebands centered near 550 and 665 nm. This device has been found to be an excellent indicator of leaf chlorophyll concentrations when properly calibrated [18,19,20,21] a procedure that we performed twice, once on 22 March 1991 and again on 29 May 1991. On each of these dates, six SPAD meter readings were taken from each of a total of 40 leaves—20 from ambient-treatment trees and 20 from CO₂-enriched trees—after which six dishes, 0.95 cm in diameter, were removed from each of the leaves and placed in 3 ml of 80% acetone containing 200 µg l⁻¹ butylated hydroxytoluene for extraction for several weeks in complete darkness at ~10°C. At the end of this period, aliquots were removed and chlorophyll a, chlorophyll b, and accessory pigments (carotenoids and xanthophylls) were determined according to the procedures of Lichtenthaler and Wellburn [15] after which least-squares regression techniques were used to develop predictive relations between pigment concentrations and SPAD meter readings (General Linear Models (GLM); Statistical Analysis Systems Institute, Inc. 1984). These equations were then used to transform our weekly SPAD meter readings into weekly chlorophyll concentrations.

Also during this 4 year period, at 2 month intervals, we removed 60 leaves from each tree (34 from the north-facing side and 34 from the south-facing side), measured their total area with a LiCor Model 3100 leaf area meter (Li-COR, Lincoln, NE), and dried and weighed them, after which we sent the leaf tissue from each tree to a local laboratory (LAS Laboratories, Phoenix, AZ) for leaf nitrogen (N) content determination. At these latter assessments were made on a dry weight basis, we used our dry weight and leaf area measurements to convert them to a per-unit-leaf-area basis for direct comparison with our per-unit-leaf-area chlorophyll measurements.
RESULTS AND DISCUSSION

Figure 1 presents the results of our calibration procedure for chlorophyll $a$, where it can be seen that 96% of the variation in chlorophyll $a$ is described by a simple linear regression against SPAD meter reading. Similar relationships for chlorophyll $b$ ($r^2=0.910$) and carotenoids and chlorophyll $a$ were also well defined.
Fig. 2. Four years of leaf chlorophyll a contents derived from weekly SPAD meter readings of ambient and CO₂-enriched sour orange tree foliage.
Results of the 4 years of chlorophyll a assessments are presented in Fig. 2. Initially, each year's first flush of foliage is low in chlorophyll; however, concentrations rise rapidly over the next 3-4 months to attain a broad maximum that persists throughout the summer and into the early fall. A slow decline then begins that we monitored until the next year's first flush of foliage appears, whereupon we switched to measuring the new year's leaves.

Throughout the 4 years of our measurements, leaves from the ambient-treatment trees had higher chlorophyll concentrations than leaves from the CO₂-enriched trees, except in June 1991 and July 1993, when the ambient-treatment concentrations were closely approached by those of the CO₂-enriched leaves. Averaged across the entire 4-year period, the 90,000-plus individual leaf chlorophyll a measurements that comprise our complete data set produced a mean CO₂-enriched/ambient leaf chlorophyll a content ratio of 0.532.

![Graph showing the relationship between CO₂-enriched leaf chlorophyll a content and ambient leaf chlorophyll a content.]

Fig. 3. CO₂-enriched leaf chlorophyll a concentration vs. ambient-treatment leaf chlorophyll a concentration.
Although this 4.8% decrease in the chlorophyll a content of the CO₂-enriched foliage is small, and contrary to what we had expected on the basis of the findings of Pinter et al.\(^5\) and the analysis of Arp,\(^7\) we believe it is real for two reasons: first, because of the sheer magnitude of the number of SPAD meter readings we made and, second, because of the results of our leaf N measurements. Specifically, at the times of our many leaf N content determinations, the mean value of the CO₂-enriched/ambient leaf chlorophyll a content ratio was 0.955, which was identical to the value of the contemporaneous mean CO₂-enriched/ambient leaf N content ratio. This essentially perfect correspondence of two completely different and independently measured, though highly coupled,\(^{10}\) plant properties leads us to conclude that the 300 μl l\(^{-1}\) atmospheric CO₂ enrichment of our non-root-restricted sour orange trees truly decreased both their chlorophyll a and N contents per unit leaf area by approximately 4.8% over the 4-year period of our chlorophyll and nitrogen measurement program.

Another perspective on the subject is provided.

Fig. 4. CO₂-enriched leaf nitrogen concentration at ambient-treatment leaf nitrogen concentration.
by plotting the chlorophyll \( a \) concentrations of the CO\(_2\)-enriched foliage against the chlorophyll \( a \) concentrations of the ambient-treatment leaves. Results of this procedure are presented in Fig. 3, while results of the analogous procedure for leaf N are plotted in Fig. 4. As can be seen from these graphs, the resultant relationships run nearly parallel to the 1:1 lines we have included in the figure. Consequently, percentage reductions in the chlorophyll \( a \) and leaf N concentrations of the CO\(_2\)-enriched foli-
age become smaller and smaller as leaf chlorophyll and N concentrations rise. Based on the relationships of Figs 3 and 4, we calculated this effect and plotted it for both parameters over the ranges of our ambient-treatment leaf chlorophyll a and N content measurements in Fig. 5. Once again, the results for leaf N are very similar to those for chlorophyll a, becoming identical at the highest values of each parameter encountered in our experiment.

Although the CO₂-induced decrease in leaf chlorophyll and nitrogen that we measured are clearly real, they should not be construed to be negative or undesirable responses, for we have continued to observe a 175% enhancement in the total productivity of the CO₂-enriched trees from the start of the second year of the study to our most recent total growth assessment at the 6.4-year point of the experiment. Indeed, the small (4.8%) decrease in leaf chlorophyll a and N contents we have documented pale in comparison with the large (175%) CO₂-induced increase in whole plant productivity. Hence, as others have also found, the use efficiency of nitrogen and chlorophyll in our study were significantly enhanced by atmospheric CO₂ enrichment.

Finally, based on the empirical relationship developed by Idso and Kimball, relating the number of leaves per tree to trunk cross-sectional area, and the results of the 2-year study of relative leaf sizes conducted by Idso et al., we calculate that the total chlorophyll a and nitrogen contained in all the leaves of the CO₂-enriched trees averaged 75% more than the total chlorophyll a and nitrogen contained in all the leaves of the ambient-treatment trees over the 4-year period of our study. It is interesting to note that this percentage enhancement is identical to the percentage increase in atmospheric CO₂ concentration experienced by the CO₂-enriched trees. With only two CO₂ treatments, however, we cannot say whether this exact correspondence is anything more than coincidence.

In conclusion, our data do not support the hypothesis that plants uncumbered by belowground space restrictions will not experience reductions in per-unit-leaf-area chlorophyll content when the CO₂ concentration of the air is increased. However, they show the observed reductions to be so small in comparison with the CO₂-induced increase in whole plant productivity, that they are essentially inconsequential.

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