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EFFECTS OF ATMOSPHERIC CO_2 ENRICHMENT AND FOLIAR METHANOL APPLICATION ON NET PHOTOSYNTHESIS OF SOUR ORANGE TREE (*CITRUS AURANTIUM*; RUTACEAE) LEAVES[1]

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Foliar spray applications of 40% aqueous methanol were made to sunlit leaves of sour orange trees that had been grown continuously in clear-plastic-wall open-top enclosures maintained out-of-doors at Phoenix, Arizona, for over 5.5 years in ambient air of approximately 400 μmol mol^{-1} CO_2 and in air enriched with CO_2 to a concentration of approximately 700 μmol mol^{-1}. No unambiguous effects of the methanol applications were detected in net photosynthesis measurements made on foliage in either of the two CO_2 treatments. The 75% increase in CO_2, however, raised the upper-limiting leaf temperature for positive net photosynthesis by approximately 7 C, which resulted in a 75% enhancement in net photosynthesis at a leaf temperature of 31 C, a 100% enhancement at a leaf temperature of 35 C, and a 200% enhancement at 42 C.

Nonomura and Benson (1992) claim that foliar sprays of aqueous methanol increase the growth of C_3 plants exposed to high temperatures and full sunlight by 50% to 100% by inhibiting photorespiration. If true, this phenomenon would have implications of far-ranging significance. Hence, we felt it important to attempt to provide independent verification of possible effects of methanol on photosynthetic rates under conditions that are known to favor high photorespiration rates. Moreover, as atmospheric CO_2 enrichment is known to have an analogous effect, i.e., to increase net photosynthesis by suppressing photorespiration (Long, 1991), we decided to study both phenomena concurrently to compare their effectiveness and to see if they produce any synergisms when acting in concert.

MATERIALS AND METHODS

Plant material—Nonomura and Benson (1992) reported substantial increases in the yields of all of the C_3 plants that they treated with methanol. We chose to work with one of the same species they had studied: *Citrus aurantium* L., the common sour orange tree. Nonomura and Benson also reported that methanol treatment increased the growth of the colonial alga *Botryococcus braunii* by only 15% in ambient air but by nearly 100% when enriched with CO_2. In light of this strong methanol/CO_2 interaction, we decided to test for methanol effects on four trees we had continuously exposed to ambient air of approximately 400 μmol CO_2 per mol air (μmol mol^{-1}) and four trees we had exposed to CO_2-enriched air of approximately 700 μmol mol^{-1} CO_2 for a period of 5.5 years (Idso and Kimball, 1993).

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Experimental conditions—The eight sour orange trees were rooted in the ground and grown within clear-plastic-wall open-top enclosures during the same time of year and in the same general area as the plants of Nonomura and Benson's study: "during the summer on irrigated farm fields in the desert Southwest, Maricopa County, Arizona." As with their plants, our trees were also "given sufficient fertilizers to maintain normal growth." Specifically, each tree was given 0.45, 0.45, 0.90, and 1.13 kg of Arizona Best Citrus Food (N,P,K=13,10,4) with flood irrigations of the enclosures on 5 April, 5 and 25 May, and 15 June 1993, respectively. We also restricted our measurements, as did Nonomura and Benson, to leaves exposed to full sunlight located in the outer portions of the trees' crowns.

Methanol applications—Nonomura and Benson (1992) typically utilized methanol concentrations of 10%–50%. Consequently, we sprayed several branches of several different *Citrus* species with methanol concentrations ranging from 20% to 60% until the solutions dripped profusely from the foliage, repeating the procedure at intervals of 2–3 days for a period of 2 weeks. Observing no visible ill effects at any of these concentrations, we decided to use a concentration of 40% methanol and 0.1% Triton X-100 (the same surfactant and percentage used by Nonomura and Benson) in our primary experiment.

Experimental protocol—Since Nonomura and Benson (1992) implicated the inhibition of photorespiration as the primary reason for the growth-stimulating effect of methanol application, we decided to measure the net photosynthetic rates of methanol-treated and nontreated sour orange tree foliage in ambient and CO_2-enriched air over a range of naturally occurring high air temperatures that would normally render photorespiration a serious detriment to growth (Long, 1991). We thus tagged a number of leaves on the trees growing in each CO_2 treatment, identifying them as targeted for methanol application or to serve as controls. Then, on 13 May 1993, and on 11

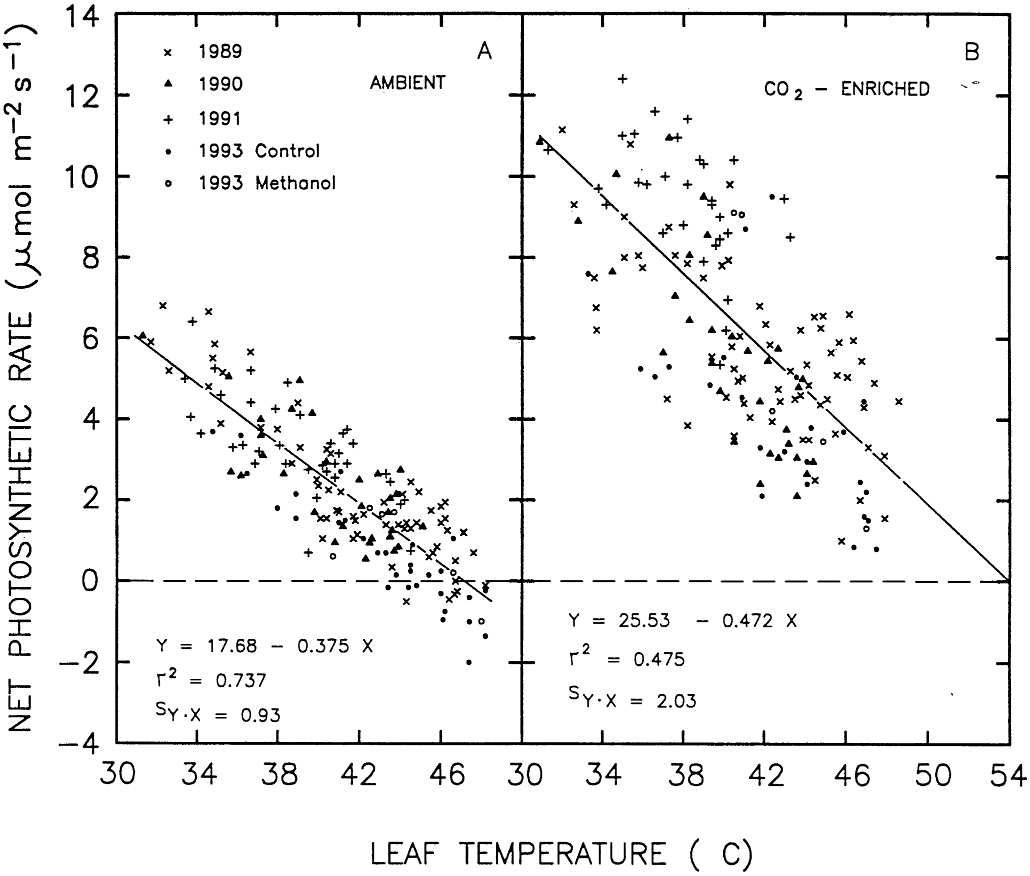

Fig. 1. Net photosynthetic rate vs. leaf temperature of foliage of sour orange trees grown since 18 November 1987 in clear-plastic-wall open-top enclosures maintained out-of-doors at Phoenix, Arizona in ambient air of approximately 400 μmol mol^{-1} CO_2 and at an elevated CO_2 concentration of approximately 700 μmol mol^{-1}.

other cloudless days stretching to 24 June 1993, we measured the net photosynthetic rates of these tagged leaves over various 2-hour portions of the day between 0900 and 1500 hours local time, when for this season of the year solar radiation was not a limiting factor for the photosynthetic process (Idso, Wall, and Kimball, 1993), but air temperatures were sometimes high enough to reduce it to zero or less (Idso, Kimball, and Allen 1991).

On 27 June 1993, between 0700 and 0900 hours local time, the leaves targeted for methanol application were sprayed with a 40% solution. Each leaf was sprayed separately on both surfaces until solution dripped profusely from the leaf. Net photosynthesis measurements were then made on 30 June and 1 and 2 July. The same leaves were sprayed a second time on 3 July; and additional net photosynthesis measurements were made on 6, 8, and 9 July. Last of all, net photosynthesis measurements that had been made on the same sour orange trees in 1989, 1990, and 1991 were retrieved from a data repository for inclusion in some of the analyses.

Photosynthesis measurements—Net photosynthetic rates of individual leaves were measured with an LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, NE). All measurements were made on fully expanded outer-canopy leaves held in a horizontal position within the measuring system's leaf cuvette, which was exposed to the direct rays of the sun. In 1989, 1990, and 1991, single 30-second measurements were made on each leaf thus sampled. In 1993, a series of three 20-second measurements were made on each leaf before freeing it from the cuvette. Results reported herein for 1989, 1990, and 1991 are averages of 12 such individual measurements made on 12 different leaves. Results reported for 1993 are averages of 18 measurements made on six leaves.

RESULTS

Net photosynthesis vs. leaf temperature—Figure 1 displays all of the 12-point net photosynthesis measurements made during the summers of 1989, 1990, and 1991 and the 18-point measurements made in 1993 as functions of concurrently measured leaf temperatures derived from the thermocouple that presses against the underside of the leaf during the time of the net photosynthesis measurement. The linear regression results superimposed upon these data were derived from nonmethanol-treated leaves only. Their negative slopes confirm that all of the data were collected at temperatures above the optimum for net photosynthesis in sour orange tree foliage, where effects of photorespiration and residual or "dark" respiration are most pronounced.

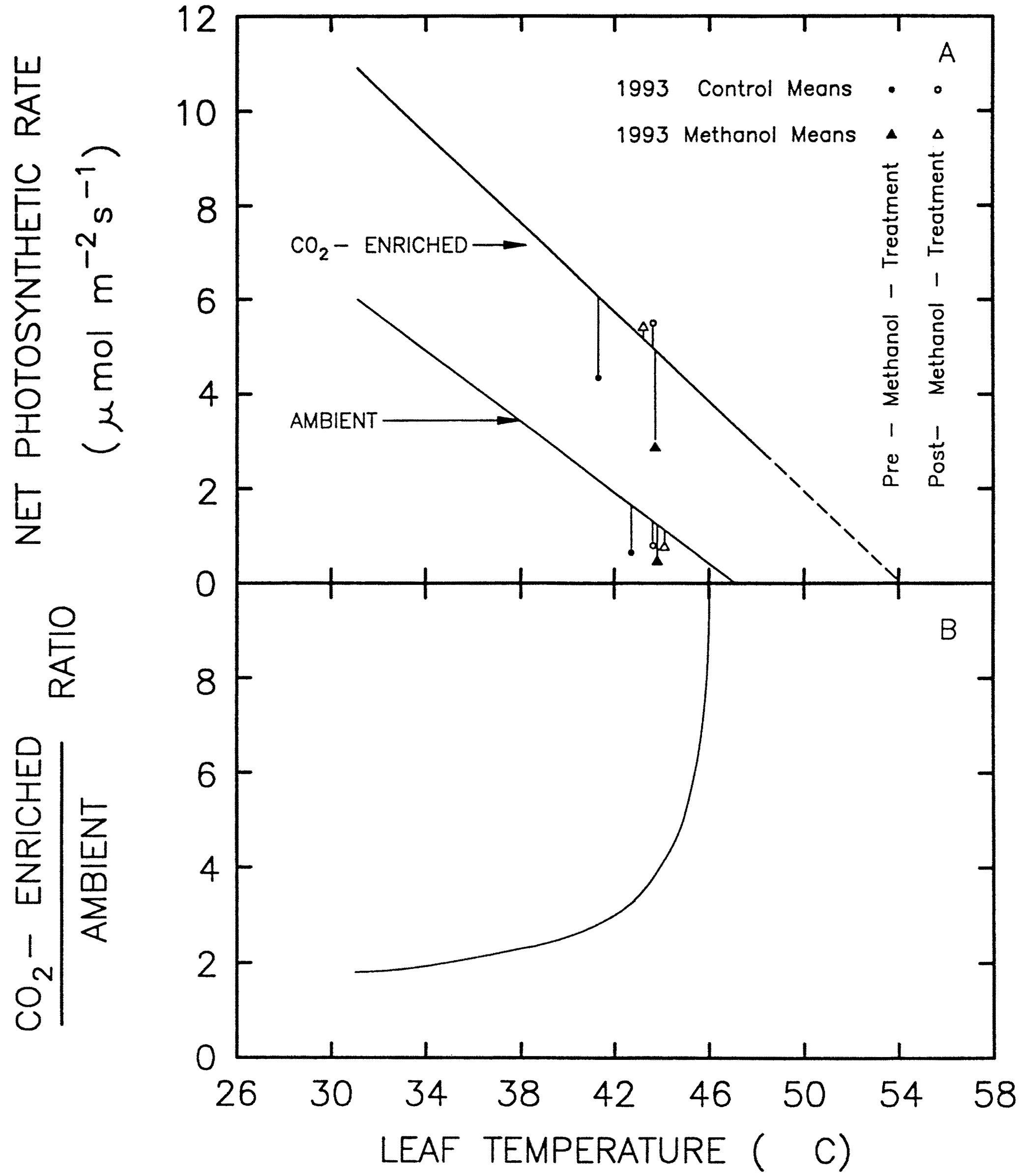

Fig. 2. (A) The linear relationships between net photosynthesis and leaf temperature derived from the data of Fig. 1, along with the mean pre- and postmethanol-treatment results for the summer of 1993. (B) The ratio of CO_2-enriched leaf net photosynthetic rate to ambient-treatment leaf net photosynthetic rate derived from the two relationships of Fig. 2A and plotted as a function of leaf temperature.

Mean Co_2 and methanol effects—The upper panel of Fig. 2 displays the linear regression lines of Fig. 1 that describe the effects of leaf temperature on the net photosynthetic rates of leaves in ambient air of approximately 400 μmol mol^{-1} CO_2 and in CO_2-enriched air of approximately 700 μmol mol^{-1} CO_2. Also plotted in this panel are the mean results obtained for control and methanol-sprayed leaves both before and after the start of methanol applications in 1993. The lower panel of Fig. 2 displays the ratio obtained by dividing the net photosynthetic rates predicted by the CO_2-enriched relationship of the upper panel by the net photosynthetic rates predicted by the ambient relationship of that panel for equivalent leaf temperatures.

DISCUSSION

CO_2 effects—Exposure to air enriched to 700 as compared to 400 μmol mol^{-1} CO_2 markedly increased the net fixation of carbon dioxide. As shown in Fig. 2B, the enhancement was approximately 75% at a leaf temperature of 31 C, 100% near 35 C, and 200% at 42 C. At higher leaf temperatures the net photosynthetic rate of the foliage growing in ambient air dropped to zero at 47 C and became negative thereafter. In the CO_2-enriched foliage, by contrast, the net fixation of carbon dioxide was still substantial at 47 C. The dashed-line extension of the linear regression line for these trees suggests that their mean rate of net photosynthesis may not drop to zero until leaf temper-

TABLE 1. Mean net photosynthetic rates and their standard deviations (μmol m^{-2} s^{-1}) of control and methanol-sprayed foliage of sour orange trees growing in ambient air and in air enriched with an extra 300 μmol mol^{-1} of CO_2.

	Ambient foliage		CO_2-enriched foliage	
	Control	Methanol	Control	Methanol
Before date of spraying	0.70 ± 0.45	0.54 ± 0.37	4.38 ± 0.58	2.84 ± 0.52
After date of spraying	0.78 ± 0.60	0.82 ± 0.46	5.48 ± 1.51	5.43 ± 1.56
After/before	1.11	1.52	1.25	1.91
$(After/before)_{Methanol}$ / $(After/before_{Control}$	1.37		1.53	

atures reach 54 C, approximately 7 C above the upper-limiting temperature for positive net photosynthesis in trees growing in ambient air.

These results are consistent with those of numerous other studies that have shown the *relative* or *percentage* increase in plant growth as a result of atmospheric CO_2 enrichment to rise in response to an increase in leaf or air temperature, as documented in the review of Idso and Idso (1994). They are also in harmony with the results of several experiments that have revealed sizable CO_2-induced upward shifts in the optimum temperature for the net fixation of CO_2 (Idso and Idso, 1994). Both of these phenomena, as elegantly demonstrated by Long (1991), arise from the fact that photorespiration typically increases at higher air temperatures under current atmospheric CO_2 concentrations but is significantly suppressed by atmospheric CO_2 enrichment. From theoretical considerations alone, Long (1991) calculated that a 300 μmol mol^{-1} increase in atmospheric CO_2 should raise the optimum temperatures of most C_3 plants by about 5 C. This conclusion is supported by the review of Idso and Idso (1994), who report a mean optimum temperature rise of 5.9 ± 1.3 C for comparable CO_2 concentration increases in seven different species studied by Bjorkman, Badger, and Armond (1978), Nilsen et al. (1983), Jurik, Weber, and Gates (1984), Seeman, Berry, and Downton (1984), Harley, Tenhunen, and Lange (1986), Stuhlfauth and Fock (1990), and McMurtrie et al. (1992).

Methanol effects—In the case of the orange trees growing in ambient air, the mean net photosynthetic rates of the pretreatment control and methanol-targeted leaves were, respectively, 1.0 and 0.7 μmol m^{-2} s^{-1} below the mean rates predicted by the linear relationship between net photosynthesis and leaf temperature in ambient foliage (Fig. 2A). After spray applications of methanol to the targeted foliage, these same populations of leaves had mean rates of net photosynthesis that were 0.5 and 0.3 μmol m^{-2} s^{-1} below their predicted mean rates. When adjusted for the small differences in temperature that existed between the pre- and postmethanol-treatment periods (before and after 27 June 1993), the net photosynthetic rate of the control foliage rose by 0.5 μmol m^{-2} s^{-1} while that of the methanol-treated foliage rose by 0.4 μmol m^{-2} s^{-1}. These observations suggest that the methanol treatment had no effect on the net photosynthetic rates of the leaves of the sour orange trees growing in ambient air, even when photorespiration was high enough to reduce net photosynthetic rates to negative values (Fig. 1).

The effect of methanol treatment on CO_2-enriched orange tree leaves was essentially the same. Prior to the methanol applications, the average rate of net photosynthesis in the control leaves was 1.7 μmol m^{-2} s^{-1} below that predicted by the mean trend line giving net photosynthetic rate as a function of leaf temperature, while the average rate of the methanol-targeted leaves was 2.0 μmol m^{-2} s^{-1} below the predicted rate. Subsequent to 27 June 1993, these values rose to 0.6 and 0.3 μmol m^{-2} s^{-1} above their respective predicted rates, for net increases of 2.3 μmol m^{-2} s^{-1} in both instances. Hence, as with the trees growing in ambient air, the application of aqueous methanol to leaves of sour orange trees growing in air enriched with an extra 300 μmol mol^{-1} of CO_2 had no measurable effect on the rates of net photosynthesis of the sprayed leaves when analyzed in this manner.

It must be acknowledged, however, that the different foliage temperature trends for which we adjusted the net photosynthetic data in the preceeding analysis may themselves have been wholly or partly a consequence of the presence or absence of methanol applications to the tree leaves. Assuming such an influence, we can directly compare the mean net photosynthetic rates of the different treatments without any adjustments for changes in temperature, as shown in Table 1.

For the trees growing in ambient air, this direct comparison suggests that the spray applications of methanol increased leaf net photosynthetic rates by approximately 37%; but the overlapping standard deviations reveal this result to be of no significance. For the trees growing in CO_2-enriched air, however, the pre- and post-methanol-treatment leaves did have significantly different mean net photosynthetic rates, indicative of a 53% increase in net photosynthesis due to the application of methanol. But as all of the data obtained in this part of the study fall within the range of scatter defined by the larger population of 1989, 1990, and 1991 data, even this result must be considered to be within the bounds of natural variability.

Based on the results of all of our experimental data, we thus conclude that enriching the air with CO_2 significantly enhances rates of net photosynthesis in sour orange tree leaves at high temperatures, when photorespiration is typically a major factor in liberating recently fixed CO_2. However, we find no compelling evidence for an analogous enhancement of net photosynthesis attributable to the application of aqueous methanol to the foliage.

LITERATURE CITED

BJORKMAN, O., M. BADGER, AND P. A. ARMOND. 1978. Thermal acclimation of photosynthesis: effect of growth temperature on photosynthetic characteristics and components of the photosynthetic apparatus in *Nerium oleander*. *Carnegie Institution of Washington Yearbook* 77: 262–276.

HARLEY, P. C., J. D. TENHUNEN, AND O. L. LANGE. 1986. Use of an analytical model to study the limitations on net photosynthesis in *Arbutus unedo* under field conditions. *Oecologia* 70: 393–401.

IDSO, K. E., AND S. B. IDSO. 1994. Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past ten years' research. *Agricultural and Forest Meteorology* 69: 153–203.

IDSO, S. B., AND B. A. KIMBALL. 1993. Tree growth in carbon dioxide enriched air and its implications for global carbon cycling and maximum levels of atmospheric CO_2. *Global Biogeochemical Cycles* 7: 537–555.

———, ———, and S. G. Allen. 1991. Net photosynthesis of sour orange trees maintained in atmospheres of ambient and elevated CO_2 concentration. *Agricultural and Forest Meteorology* 54: 95–101.

———, G. W. WALL, AND B. A. KIMBALL. 1993. Interactive effects of atmospheric CO_2 enrichment and light intensity reductions on net photosynthesis of sour orange tree leaves. *Environmental and Experimental Botany* 33: 367–375.

JURIK, T. W., J. A. WEBER, AND D. M. GATES. 1984. Short-term effects of CO_2 on gas exchange of leaves of bigtooth aspen (*Populus grandidentata*) in the field. *Plant Physiology* 75: 1022–1026.

LONG, S. P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? *Plant, Cell and Environment* 14: 729–739.

MCMURTRIE, R. E., H. N. COMINS, M. U. F. KIRSCHBAUM, AND Y.-P. WANG. 1992. Modifying existing forest growth models to take account of effects of elevated CO_2. *Australian Journal of Botany* 40: 657–677.

NILSEN, S., K. HOVLAND, C. DONS, AND S. P. SLETTEN. 1983. Effect of CO_2 enrichment on photosynthesis, growth and yield of tomato. *Scientia Horticulutrae* 20: 1–14.

NONOMURA, A. M., AND A. A. BENSON. 1992. The path of carbon in photosynthesis: improved crop yields with methanol. *Proceedings of the National Academy of Sciences, USA* 89: 9794–9798.

SEEMANN, J. R., J. A. BERRY, AND J. S. DOWNTON. 1984. Photosynthetic response and adaptation to high temperature in desert plants. A comparison of gas exchange and fluorescence methods for studies of thermal tolerance. *Plant Physiology* 75: 364–368.

STUHLFAUTH, T., AND H. P. FOCK. 1990. Effect of whole season CO_2 enrichment on the cultivation of a medicinal plant, *Digitalis lanata. Journal of Agronomy and Crop Science* 164: 168–173.