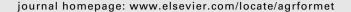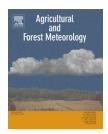# Carbon dioxide and high temperature effects on growth of young orange trees in a humid, subtropical environment ☆

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ABSTRACT

Rising atmospheric carbon dioxide (CO_2) concentration and global warming could impact growth of citrus trees. Five 2-year-old Ambersweet orange trees on Swingle citrumelo rootstocks were transplanted into soil containers in two temperature-gradient greenhouses on 9 August 1994 at Gainesville, FL, USA. Either 360 or 720 μmol (CO_2) mol^{-1} (air) was maintained in the greenhouses. Two containers were located in each of four temperature zones maintained at 1.5 °C increments between each zone with a 4.5 °C difference between zones 1 and 4. The main objective was to test the hypothesis that biomass growth ratios of CO_2-enriched to ambient CO_2-exposed young sweet orange trees would be similar to the large growth enhancements (about 2.6-fold) reported from Phoenix, AZ, USA during the first 3 years of growth of sour orange trees. One tree per container was harvested in 1995 and four trees per container were harvested in 1996. Growth parameters were different between years except leaf fresh weight and fine root biomass. Elevated CO_2 increased growth parameters except leaf growth and fine root biomass. Biomass response ratios to CO_2 (720/360) for 1995 and 1996, respectively, were 1.57 and 1.18 for shoot wood, 1.34 and 1.15 for total above-ground, 1.46 and 1.08 for tap roots, 1.67 and 1.54 for secondary roots, 1.29 and 0.95 for fine roots (NS-CO_2), 1.40 and 1.19 for total roots, 1.47 and 1.18 for total wood, and 1.37 and 1.17 for total plants. The decrease in response to CO_2 in the second year was attributed to crowding of shoot and root space. Components of shoot wood, total above ground, taproot, fine root, total root, total wood, and total plant biomass increased slightly (0.01 < P < 0.05) with increasing temperature. No $CO_2 \times$ temperature interactions were significant. The hypothesis that elevated CO_2 would cause biomass increases of about 2.6-fold compared to ambient CO_2 treatments (as found in the midlattitude desert environment of Phoenix) was not supported. May through September mean maximum daily vapor pressure deficit (VPD) was 5.54 and 2.25 kPa for Phoenix and Gainesville, respectively, a ratio of $\approx$2.5. High summertime VPD coupled with limited water flow capacity within citrus trees that lead to pronounced midday depressions in photosynthesis in ambient CO_2 is discussed as the underlying environmental cause of the large CO_2 enrichment effects in a midlattitude desert compared to the Gainesville humid subtropical climate.

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1. Introduction

The carbon dioxide (CO_2) concentration of the atmosphere has been steadily rising due mainly to burning of fossil fuels (Houghton et al., 2001). Furthermore, global warming seems to be an inevitable consequence of increasing concentrations of greenhouse gases in the atmosphere. Depending on scenarios of energy use coupled with population growth, prediction are that CO_2 concentration could increase to between 470 and 940 μmol (CO_2) mol^{-1} (air) by 2100, which could lead to a mean surface global warming of 1.4–5.8 °C (Houghton et al., 2001; Schneider, 2001).

A large number of studies have been conducted on responses of various types of crop systems to elevated CO_2 (Ainsworth et al., 2002; Ainsworth and Long, 2005). However, fewer studies have been conducted on responses of crops to both high CO_2 and temperature (such as Prasad et al., 1999, 2002, 2003). There are beneficial effects of elevated CO_2 on citrus ranging from seedling growth (Koch et al., 1983, 1986, 1987; Ferguson et al., 1986) to large tree growth and yield (Downton et al., 1987; Idso et al., 1991a; Idso and Kimball, 1992a,b,c, 1994, 1997, 2001). Very few studies have described effects of both elevated CO_2 and high temperature on citrus, either short-term (Brakke and Allen, 1995; Allen et al., 2000) or long-term (Martin et al., 1995; Vu, 1999; Vu et al., 2002).

Large biomass growth responses to CO_2 enrichment have been found in sour orange (*Citrus aurantium* L.) trees planted as seedlings outdoors in July 1987 at Phoenix, AZ, USA. After 3 years of exposure to air enriched by 300 μmol mol^{-1}, trees exposed to elevated CO_2 were found to have 2.3-fold more fine root mass (Idso and Kimball, 1992c). Above-ground data indicated that the trees had 2-fold more branches, 1.75-fold more leaves, 2.6-fold more trunk and branch volume, and 2.9-fold more trunk, branch, and fruit rind volume than trees grown at ambient CO_2 (Idso and Kimball, 1992a). After 13 years, the relative effects of CO_2 enrichment decreased somewhat from the early years (Idso and Kimball, 1997), and the aboveground wood ratio of elevated to ambient CO_2 treatments appeared to have stabilized at about 1.8 (Idso and Kimball, 2001), due in part to crowding of the trees. This elevated CO_2 enhancement of biomass growth is larger than that obtained by many other crops and natural species which tend to show a response ratio of about 1.3 (Allen et al., 1996; Poorter, 1993; Wullschleger et al., 1995, 1997).

Martin et al. (1995) reported responses of 'Eureka lemon citrus trees (*Citrus limon* L.) to elevated CO_2 concentration (constant 680 μmol mol^{-1} versus ambient 350–380 μmol mol^{-1}) and day/night temperatures of 29/21 °C and 42/32 °C. They found plant growth to be 87% greater in elevated CO_2 at this higher temperature treatment but only 21% greater in elevated CO_2 at the lower temperature treatment.

The objectives of this experiment were to determine the combined effects of elevated CO_2 concentration and a range of four elevated temperatures on young sweet orange trees grown in Gainesville, FL, USA. This study was conducted in temperature-gradient greenhouses (TGGs) beginning with budded trees grown for 29 months, and data collection focused on responses measured at the end of two seasons. We hypothesized that the early life biomass growth ratios of CO_2-enriched to ambient CO_2-exposed sweet orange trees would be similar to the large growth enhancement reported by Idso and Kimball (1992a,b,c) during the first 3 years of growth of sour orange trees. We also expected that the growth response of sweet orange trees to elevated CO_2 would be enhanced at elevated temperatures (a significant CO_2 by temperature interaction) as reported by Martin et al. (1995).

2. Materials and methods

2.1. Temperature-gradient greenhouses

Two TGGs were used in this study (Sinclair et al., 1994). One TGG was maintained at an ambient CO_2 concentration of about 360 μmol mol^{-1} and the other at a doubled elevated CO_2 concentration of about 720 μmol mol^{-1}. The 29.3 m long by 4.3 m wide arch-shape structures, with the central ridge pole at 2.2 m above ground level, were covered with a transparent greenhouse polyethylene plastic film that transmitted about 90% of the solar photosynthetic photon flux density (PPFD). Each TGG was divided into a 3.6-m long entry zone to stabilize incoming air-flow, four sequential 5.5-m long experimental zones, and a 1.8-m long flow convergence exit zone before the air was expelled by a controlled-speed greenhouse ventilation fan (Vu et al., 2002). The entry end of each TGG was covered with a fine-mesh screen with a screen-door for entry. Four temperature zones were maintained throughout the study in each TGG at baseline temperature, and at +1.5, +3.0, and +4.5 °C above baseline. The first temperature zone (baseline) was about 1.5 °C above the daily and seasonal ambient temperatures at Gainesville. Thus, the temperatures of the zones were +1.5, +3.0, +4.5, and +6.0 °C above the ambient temperatures of Gainesville. The temperature gradients were maintained by a combination of electrical resistance heaters and computer-controlled rotation speed of a 90-V DC, greenhouse ventilation fan mounted at the south end of each TGG. Radiation-shielded, aspirated thermocouples (adapted from radiation-shielded, aspirated devices obtained from Radiation and Energy Balance Systems, Inc., Seattle, WA, USA) were mounted in pairs in a single device at an intake level of 1.5 m above ground level in the middle of each of the temperature zones of the TGG. The thermocouples in zone 1 and zone 4 were used to maintain the temperature gradient to the set-point of +4.5 °C. One 1500-W, household-type, electrical resistance heater was mounted on each side of the TGG at the beginning of zones 2, 3, and 4, which provided 3000 W as required to each zone in a step-fashion. Heating was required most of the time except during bright midday periods when solar energy provided much of the energy for maintaining temperature gradients. All the heaters within an individual TGG were turned on or off simultaneously by command from a computer. The ventilation fan-speeds were also controlled by computer. When all other factors were constant, higher rates of ventilation would tend to decrease the temperature gradient, and lower rates of ventilation would allow the temperature gradient to increase. When the speed of the controlled ventilation fan exceeded a prescribed set-point value, the electrical resistance heaters were turned off. When the speed of the controlled ventilation fan fell below another

prescribed set-point value, the heaters were turned on to restore and maintain the temperature gradient.

A Supervisory Control and Data Acquisition (SCADA) system was used to measure and control temperatures in the TGGs (Allen et al., 2006). The hardware component was a Keithley Metrabyte system (Keithley Instruments, Boston, MA, USA) with a chassis containing thermocouple input boards, analog input/output boards, and digital output (on–off control) boards. The hardware was managed by FIX-DMACS programmable software (Intellution, Inc., Norwood, MA, USA).

2.2. Plant materials

Studies were conducted using 'Ambersweet' orange trees [a hybrid of 'Clementine' tangerine (*Citrus reticulata* Blanco) and 'Orlando' tangelo (*C. paradisi* Macfad. × *C. reticulata*)] that had been grafted on 'Swingle' citrumelo (*C. paradisi* Macfad. × *Poncirus trifoliata* [L.] Rafin) rootstocks. Two-year-old bare-rooted trees obtained from Hart Citrus Nurseries, Inc., Groveland, FL on the morning of 9 August 1994 were transplanted into two 0.5-m^3 rooting containers (oblong galvanized steel stock watering tanks with drainage ports) in each of the four temperature zones in both the ambient- and the elevated-CO_2 TGGs. These tanks were 0.6 m wide × 1.5 m long and 0.6 m deep, and had been previously filled with local soil (Millhopper sand, a loamy, siliceous, hyperthermic Grossarenic Paleudult). Four of these soil containers were placed in each of the four temperature zones. Two of the containers in each zone were used for the citrus experiments. Five trees each were equally spaced along the length of the containers which gave 10 trees per temperature treatment zone and 40 trees per greenhouse. On 13 August, 4.5 kg per container of commercial organic fertilizer (Hyponex cow manure plus 10% organic compost) was added and tilled in by hand-tool. The minimum analysis was 0.5–0.5–0.5 N–P–K (N–P_2O_5–K_2O) which provided a minimum of 4.5 g N per tree or approximately 200 kg/ha on a land area basis. On 23 September 1994, 0.2 kg/container of 12–10–12 (Scotts all-purpose plant food) was added, which provided 5 g N per tree or about 220 kg/ha.

At the nursery, both the roots and branches had been severely trimmed back on these bare-rooted citrus trees in preparation for transplanting. Fifty-eight extra young trees prepared for transplanting were used to measure mean size and dry weight attributes of each plant, including rootstock base diameter, scion (stem) base diameter, scion (stem) top diameter, total length, stem length, rootstock (taproot) dry weight, stem dry weight, and branch (branch nubs that remained after pruning the prepared transplants) dry weight. From these measurements, total dry weight, stem plus branch dry weight, stem wood volume, and stem wood density were calculated (Table 1).

For 1995 and 1996, a fertilization program of a February, April, June, August, and October application of 200 g/container of 7-5-6 citrus fertilizer, 50 g/container of ammonium nitrate, and 10 g/container of lime was setup. The analysis of other elements of the 7-5-6 citrus fertilizer was Mg = 1.5%, S = 6.0%, B = 0.02%, Cu = 0.07%, Fe = 1.0%, Mn = 0.23%, Zn = 0.06%, and Cl = 3.0%. The calculated amounts per tree per application was 6.15 g N, 2.0 g P_2O_5, and 2.4 g K_2O in 1995 and 7.7 g N, 2.5 g P_2O_5,

Table 1 – Mean and standard deviation (S.D.) of dimensions (diameters and lengths) and dry weights (DW) of component parts of 58, 2-year-old, bare-root citrus transplants with pruned roots and branches. Total dry weight correlation coefficients were computed for correlating total dry weight with rootstock base diameter, scion base diameter, scion top diameter, and total length of the transplant. These correlation coefficients indicated that total dry weight correlated best with scion top diameter.

Statistic	Rtstk base dia. (mm)	Scion base dia. (mm)	Scion top dia. (mm)	Total length (mm)	Stem length (mm)	Rtstk DW (g)	Stem DW (g)	Branch DW (g)	Total DW (g)	St + Br DW (g)	Stem wood vol. (mm³)	Density stem basis (kg m⁻³)
Mean	27.8	17.7	13.4	636	322	38.8	31.2	41.6	111.6	72.7	62,873	493
S.D.	3.3	2.4	2.4	26	37	20.5	10.3	22.6	50.7	31.1	16,675	50

Correlations of total dry weight with rootstock base diameter (Cor = 0.788, r^2 = 0.622), scion base diameter (Cor = 0.886, r^2 = 0.785), scion top diameter (Cor = 0.956, r^2 = 0.914), and total length of the transplant (Cor = 0.497, r^2 = 0.248).

Table 2 – ANOVA for 1995 and 1996 data of citrus trees grown at two CO_2 concentrations (720 and 360 μmol (CO_2) mol^{-1} (air) at four temperature treatments (baseline = 0.0, +1.5, +3.0, and +4.5 °C, or about 1.5, 3.0, 4.5, and 6.0 °C above Gainesville ambient temperatures). Mean growth variables on a per tree basis are Leaf Area (LAREA), Leaf Fresh Weight (LFW), Leaf Dry Weight (LDW), Stem Dry Weight (SDW), Above Ground Dry weight (AGDW = LDW + SDW), TapRoot Dry Weight (TPRDW), Secondary Root Dry Weight (SRDW), Fine Root Dry Weight (FRDW), Total Root Dry Weight (TRDW = TPRDW + SRDW + FRDW), Total Wood Dry Weight (TWDW = SDW + TRDW), and Total Plant Dry Weight (TPDW = LDW + TWDW).

Year	[CO$_2$] (μmol mol^{-1})	TEMP (°C)	LAREA (m^2)	LFW (g)	LDW (g)	SDW (g)	AGDW (g)	TPRDW (g)	SRDW (g)	FRDW (g)	TRDW (g)	TWDW (g)	TPDW (g)
1995	720	0.0	1.67	436	186	404	590	208	79	207	493	897	1083
		+1.5	1.65	452	167	452	618	141	130	314	585	1036	1203
		+3.0	3.00	702	290	675	965	220	130	381	730	1405	1694
		+4.5	2.86	717	260	541	800	209	104	321	633	1173	1433
	360	0.0	1.68	466	184	295	479	114	57	196	366	661	845
		+1.5	2.19	560	214	301	515	114	56	202	372	673	887
		+3.0	2.28	627	233	329	562	159	45	184	387	716	949
		+4.5	2.86	784	266	392	658	145	106	369	620	1012	1278
1996	720	0.0	1.28	500	395	748	1143	223	358	181	762	1510	1904
		+1.5	1.06	594	352	716	1068	218	337	191	747	1463	1815
		+3.0	1.58	725	500	927	1428	261	401	280	942	1869	2370
		+4.5	1.43	809	520	904	1424	249	381	301	930	1834	2354
	360	0.0	1.55	774	435	657	1092	192	218	221	631	1288	1722
		+1.5	1.56	610	398	734	1132	196	281	264	740	1474	1872
		+3.0	1.24	529	391	731	1122	268	235	263	766	1497	1888
		+4.5	0.91	436	371	679	1050	228	227	251	706	1385	1755
ANOVA													
YEAR			<0.01	NS	<0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01
[CO$_2$]			NS	NS	NS	<0.01	<0.01	0.01	<0.01	NS	<0.01	<0.01	<0.01
TEMP			NS	NS	NS	0.01	0.02	0.03	NS	0.05	0.02	0.01	0.02
[CO$_2$] × TEMP			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
YEAR × [CO$_2$]			NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS
YEAR × TEMP			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

and 3.0 g K_2O in 1996. Irrigation at the surface to soil field capacity was applied 2–3 times per week, and adjusted to give a slight excess of water for leaching at each irrigation.

At the end of 1995, one tree from each container (the tree nearest to the sloping-arch greenhouse wall of each container) was harvested and the following components of each tree were measured: (1) total leaf area, (2) total leaf fresh weight, and dry weights of each of the following plant components; (3) leaves, (4) above-ground woody materials, (5) tap roots, (6) secondary roots, and (7) fine roots. Taproot was judged as the large clump of below-ground material from which secondary roots sprung. Fine roots were defined as roots of less than 4 mm diameter attached to the secondary roots. The fine root mass consisted of numerous branches of even finer roots down to about 1 mm in diameter. The separation into the secondary roots and fine roots was judged visually rather than by precise callipering. Plant components were aggregated in other combinations during later analysis of the data.

All of the leaves of each tree were harvested quickly and immediately weighed to obtain total leaf fresh weight. Immediately a subsample of about 100 g was weighed and leaf area measured with a LI-3100 area meter (LI-COR, Inc., Lincoln, NB, USA). Total leaf area of each tree was inferred from the area-to-weight ratio of the leaf data sample. All leaves plus leaf subsamples were dried at 70 °C for at least 3 days to obtain total leaf dry weight. All below-ground woody components were washed thoroughly, separated into taproot, secondary root, and fine root components and air dried, and later dried further at 70 °C for a week. Plant component dry weights of (8) total above-ground biomass, (9) total root biomass, (10) total woody biomass, and (11) total plant biomass were also calculated for each tree. At the end of 1996, a similar procedure was followed to obtain the plant growth components of each of the four trees remaining in each rooting container. (The original plan had been to sample one tree per container in the first year, two per container the second year, and two per container for a third year, but the trees grew faster than anticipated and necessitated that all trees be harvested the second year.)

2.3. Statistical analysis

An analysis of variance was conducted on the yield data components using individual container as the experimental unit rather than individual tree as the experimental unit. Thus, because four trees per container were harvested at the end of the 1996 season (rather than one tree per container as at the end of the 1995 season), the mean value per tree for each container was calculated in a spreadsheet, and this mean value per tree used for 1996 in the statistical analysis. The objective of the experiment was to study the main environmental effects of CO_2 concentration and air temperature, and the potential environmental interaction of CO_2 concentration × air temperature. However, preliminary examination of the data indicated that there were major differences in the responses to CO_2 between tree harvests at the end of the 1995 growing season and the end of the 1996 growing season. Therefore, using the general linear model (PROC GLM) of SAS Institute Inc. (2003), an analysis of variance was conducted for main effects of YEAR (age of citrus tree, i.e., effect at the 1995 and 1996 end-of-season harvest), CO_2

(360 and 720 μmol mol^{-1}), air temperature, TEMP (+1.5, +3.0, +4.5 and +6.0 °C), and the interaction effect of CO_2 × TEMP, YEAR × CO_2, and YEAR × TEMP. Because of large differences in biomass data between the 1995 and the 1996 growing seasons, within each year ratios of the elevated CO_2 to ambient CO_2 treatment responses were calculated to indicate relative responses to treatments.

Statistical analyses (ANOVA) were performed on the final harvest data for 1995 and 1996. The absolute root and stem growths were computed as difference between final root dry weight and stem dry weight and corresponding root and stem initial weights (computed as mean values based on the original bare-rooted transplants). However, these biomass gain data are shown in figures only. The statistical inferences apply equally to the total dry weight harvest data or the dry weight gain data (increase of dry weight above the mean initial transplant dry weight).

3. Results

From the data of the original transplants, the mean weight of the root (taproot) was 39 ± 20 g/tree (mean ± S.D.) and the mean weight of the stem (stem plus branch nubs) was 73 ± 31 g/tree (Table 1). Even though, the weights (and concomitantly total wood volumes) were variable, the total length of the transplants was rather constant with an mean length of 636 ± 26 mm. Thus, the correlation coefficient (0.497) between length and total dry weight of 112 ± 51 g/tree was somewhat weak ($r^2 = 0.24$). Most of the variation in transplant dry weight was related to diameter of the scion near the top of the stem (correlation coefficient = 0.956, $r^2 = 0.91$). The correlation between diameter of the scion near the base of the stem and dry weight was less (correlation coefficient = 0.886, $r^2 = 0.78$) and the correlation between diameter of the base of the rootstock and dry weight was even less (correlation coefficient = 0.788, $r^2 = 0.62$).

The means of the eleven growth components measured or calculated from trees sampled at the end of 1995 and 1996 are summarized in Table 2, along with the analysis of variance. The eleven growth variables on a per tree basis are (1) Leaf Area (LAREA), (2) Leaf Fresh Weight (LFW), (3) Leaf Dry Weight (LDW), (4) Stem Dry Weight (SDW), (5) Above Ground Dry weight (AGDW = LDW + SDW), (6) TapRoot Dry Weight (TPRDW), (7) Secondary Root Dry Weight (SRDW), (8) Fine Root Dry Weight (FRDW), (9) Total Root Dry Weight (TRDW = TPRDW + SRDW + FRDW), (10) Total Wood Dry Weight (TWDW = SDW + TRDW), and (11) Total Plant Dry Weight (TPDW = LDW + TWDW, or also TPDW = LDW + SDW + TRDW).

There were no CO_2 × air temperature ([CO_2] × TEMP) interactions for any of the biomass variables, so these interactions were not considered further. The YEAR effect was significant for all biomass factors ($P < 0.01$) except LFW and FRDW (Table 2). None of the leaf variables of LAREA, LFW, or LDW was affected by CO_2 concentration ([CO_2]) or air temperature (TEMP). Also, FRDW was not affected by CO_2 concentration, but all other effects were significant ($P < 0.01$). Furthermore, temperature did not affect SRDW, but the other variables were affected by the temperature treatments ($0.05 \leq P \leq 0.01$) but not as strongly as by the CO_2 treatments.

Table 3 – Means of citrus growth variables by CO_2 concentration and ratios of 720/360 μmol mol⁻¹ responses within each year. Abbreviations of growth variables are the same as listed in Table 2.

Year	[CO_2] (μmol mol⁻¹)	LAREA (m²)	LFRWT (g)	LFDW (g)	STDW (g)	AGDW (g)	TPRDW (g)	SRDW (g)	FRDW (g)	TRDW (g)	TWDW (g)	TPDW (g)
1995	720	2.29	577	225	518	743	194	110	305	610	1128	1353
1995	360	2.25	609	224	329	553	133	66	237	436	765	989
Ratio	720/360	1.02	0.95	1.00	1.57	1.34	1.46	1.67	1.29	1.40	1.47	1.37
1996	720	1.34	657	442	824	1265	238	369	238	845	1669	2111
1996	360	1.32	587	399	700	1099	221	240	250	711	1411	1809
Ratio	720/360	1.02	1.12	1.11	1.18	1.15	1.08	1.54	0.95	1.19	1.18	1.17

Since determination of relative responses to elevated CO_2 was one of the primary objectives, ratios of 720/360 CO_2 responses for each of the eleven growth variables for 1995 were: LAREA = 1.02, LFW = 0.95, LDW = 1.00, SDW = 1.57, AGDW = 1.34, TPRDW = 1.46, SRDW = 1.67, FRDW = 1.29, TRDW = 1.40, TWDW = 1.47, and TPDW = 1.37 (Table 3). In general, these ratios of 720/360 CO_2 responses were smaller for 1996, being LAREA = 1.02, LFW = 1.12, LDW = 1.11, SDW = 1.18, AGDW = 1.15, TPRDW = 1.08, SRDW = 1.54, FRDW = 0.95, TRDW = 1.19, TWDW = 1.18, and TPDW = 1.17 (Table 3). Summarizing, the ratios of leaf dry weight stem dry weight, root dry weight and total plant dry weight were 1.00, 1.57, 1.40, and 1.37 for 1995, but were 1.11, 1.18, 1.19, and 1.17 for 1996.

The stem, root, and total dry weight gains of the average harvests of each CO_2 treatment at the end of 1995 and 1996 were calculated by subtracting the mean initial dry weight of the stems (73 g) and roots (39 g) of the companion set of the original bare-root transplants (data from Table 1). The mean dry matter of leaves and dry matter gains of stems, roots, and total plant biomass are shown in Fig. 1. The data in this figure were pooled across all temperatures because the ANOVA (Table 2) showed no $CO_2 \times$ temperature interaction effects for any of the biomass factors. The growth differences were due mainly to stems and roots, with little difference in leaf biomass obtained at the time of the end-of-season harvests.

Calculating dry matter gain by subtracting the mean weight of the stems and roots of the original transplant stocks resulted in slightly different ratios of 720/360 CO_2 responses. For the 1995 data, the ratios increased from 1.57 to 1.74 for stem biomass, from 1.40 to 1.44 for root biomass, and from 1.37 to 1.42 for total plant biomass. For 1996 data, the ratios increased much less, namely from 1.18 to 1.20 for stem
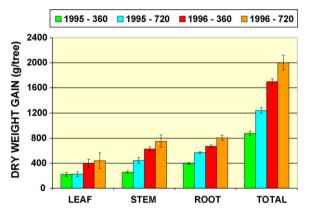

Fig. 1 – Citrus tree dry weight gains of components (leaf, stem, root, and total) for harvests at the end of the 1995 and 1996 growing seasons of transplants that were grown in temperature-gradient greenhouses at Gainesville, FL, USA exposed to either 360 (daytime-ambient) and 720 (elevated) μmol (CO_2) mol⁻¹ (air). The stem, root, and total dry weight gains were calculated by subtracting the mean initial dry weight of the stems (73 g) and roots (39 g) of a companion set of the original bare-root transplants (Table 1). Data shown were pooled across all temperatures because the ANOVA (Table 2) showed no $CO_2 \times$ temperature interaction effects for any of the biomass factors. Standard errors of the means are shown.
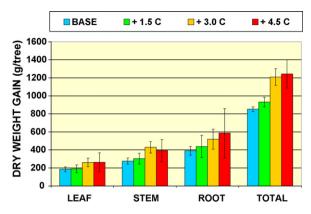
Fig. 2 – Citrus tree dry weight gains of components (leaf, stem, root, and total) for harvests at the end of the 1995 (first) growing season of transplants that were grown in temperature-gradient greenhouses exposed continuously day and night to four temperatures (baseline temperature, base +1.5 °C, base +3.0 °C, and base +4.5 °C). The baseline temperature was about 1.5 °C above ambient temperatures at Gainesville, FL, USA. The stem, root, and total dry weight gains were calculated by subtracting the mean initial dry weight of the stems (73 g) and roots (39 g) of a companion set of the original bare-root transplants (Table 1). Data shown were pooled across both CO_2 concentration treatments because the ANOVA (Table 2) showed no $CO_2 \times$ temperature interaction effects for any of the biomass factors. Standard errors of the means are shown.

biomass, from 1.19 to 1.20 for root biomass, and from 1.17 to 1.18 for total plant biomass.

Fig. 2 shows the leaf biomass responses and the biomass gain responses of stem, root, and total plant components of citrus to the temperature differences within the TGGs pooled for both levels of CO_2 at the end of the first year of growth (1995), corrected for initial mean dry weight of transplants as indicated above. Data shown were pooled across both CO_2 concentration treatments because the ANOVA (Table 2) showed no $CO_2 \times$ temperature interaction effects for any of the biomass variables. Biomass growth tended to increase with temperature, and appeared to begin to flatten out at about 4.5 °C above zone 1 baseline temperature (6.0 °C above Gainesville ambient temperatures). There was no indication of a temperature $\times$ CO_2 interaction (Table 2).

Fig. 3 presents data for the second year (1996) in the same format as shown in Fig. 2. All findings stated in the above paragraph for 1995 as illustrated for Fig. 2 are generally applicable for the 1996 data.

4. Discussion

We hypothesized that the biomass growth ratios of CO_2-enriched to ambient CO_2-exposed young citrus trees would be similar to those reported by Idso and Kimball (i.e., a large direct CO_2 fertilization effect), and that the response to elevated CO_2 would be enhanced by growth at elevated temperatures (a CO_2 by temperature interaction) as reported by Martin et al. (1995).

However, the stem, root, and total plant biomass ratios (Table 3) for the 720/360 CO_2 treatments for the first year, 1995 (1.57, 1.40, and 1.37, respectively), were much lower than the stem (trunk plus branch) growth ratio of about 2.6 reported by Idso and Kimball (1992b) and the fine root biomass ratio of 2.3 reported by Idso and Kimball (1991). Furthermore, the stem, root, and total plant biomass ratios for the 720/360 CO_2 treatments for the second year, 1996 (1.18, 1.19, and 1.17, respectively), were even lower than the first year. After 13 years, the stem (trunk plus branch) growth ratios stabilized at about 1.8 (Idso and Kimball, 2001), which is still much higher than our ratios. Our findings are consistent with the elevated-CO_2 enhancement effect on citrus plant growth of 1.21 reported by Martin et al. (1995) for 'Eureka' lemon grown for 6 months at day/night temperatures of 29/21 °C (daily mean of 25 °C).

We attribute the smaller effect of CO_2 during the second year to greater crowding of both root and shoot space. Root confinement by the walls of the 0.6-m wide container and crowding from adjacent trees (effect observed but not measured) probably led to lower effects of elevated CO_2 on root biomass (Table 3). Castle (1987) and Marler and Davies (1987) illustrated the impact of small rooting volumes on crowding the complex of taproot, secondary roots, and fine roots.

We saw a temperature enhancement (but no $CO_2 \times$ temperature interactions) on vegetative biomass production over the 4.5 °C range. The mean daily baseline temperatures in zone 1 of the TGGs were about 25.5 °C and about 30.0 °C in zone 4 during the Gainesville summer (May
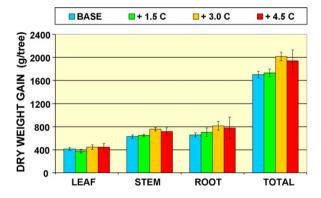

Fig. 3 – Citrus tree dry weight gains of components (leaf, stem, root, and total) for harvests at the end of the 1996 (second) growing season of transplants that were grown in temperature-gradient greenhouses exposed continuously day and night to four temperatures (baseline temperature, base + 1.5 °C, base + 3.0 °C, and base + 4.5 °C). The baseline temperature was about 1.5 °C above ambient temperatures at Gainesville, FL, USA. The stem, root, and total dry weight gains were calculated by subtracting the mean initial dry weight of the stems (73 g) and roots (39 g) of a companion set of the original bare-root transplants (Table 1). Data shown were pooled across both CO_2 concentration treatments because the ANOVA (Table 2) showed no $CO_2 \times$ temperature interaction effects for any of the biomass factors. Standard errors of the means are shown.

through September 1996) reported by Vu et al. (2002). The mean daily temperature of the high-temperature treatment of Martin et al. (1995) was 37 °C (maximum of 42 °C) and the leaf-to-air vapor pressure difference ranged from 4.3 kPa in the early morning to 6.2 kPa during midday as measured with a portable photosynthesis system. Their elevated-to-ambient CO_2 biomass enhancement of 1.87 was obtained at higher temperatures with a larger vapor pressure deficit (VPD) than in the study in Gainesville.

The hypothesis that biomass growth enhancements of CO_2-enriched 'Ambersweet' orange would be similar to sour orange reported by Idso et al. (1991a,b) and Idso and Kimball (1991,1992a,b,c) during the first 3 years of their study was not supported by our research. The likely reasons for the dissimilarity between the two studies lies in the differences between the VPD of Gainesville, FL and Phoenix, AZ coupled to plant leaf responses to the different humidity environments as governed by whole-tree water transport limitations. The summer climate of Gainesville is humid subtropical, whereas the summer climate of Phoenix is hot, arid subtropical (a midlattitude desert). We summarized mean maximum daily temperatures and VPDs for a 30-year period (1961–1990) for Phoenix and Gainesville. The May through September mean maximum daily temperature was 38.3 °C for Phoenix and 31.5 °C for Gainesville. Moreover, the May through September mean maximum daily VPD was 5.54 and 2.25 kPa for Phoenix and Gainesville, respectively, a ratio of ≈2.5.

Idso et al. (1991b) measured leaf net photosynthetic rates (net carbon dioxide exchange rates, CER) of sour orange trees at Phoenix that were exposed to either ambient or elevated CO_2 concentrations (ambient + 300 μmol mol^{-1} CO_2). Data were obtained from 07:00 to 17:00 h during summer conditions. Allen and Amthor (1995) commented on these findings. The 07:00-h mean leaf CERs were 11.1 and 7.1 μmol m^{-2} s^{-1} for elevated and ambient CO_2, respectively, an enhancement of 1.56. Mean leaf CERs of the CO_2-enriched treatment declined steadily to about 4.8 μmol m^{-2} s^{-1} by 1400 h and remained at this rate until 1700 h, whereas ambient CO_2 mean leaf CERs declined to 1.2 μmol m^{-2} s^{-1} at 1300 h and remained at this rate. The mean morning enhancement of leaf CER by elevated CO_2 was about 83% and the mean afternoon enhancement was about 284% (Idso et al., 1991b). Overall, the mean leaf CER was about 2.2-fold greater for elevated CO_2. Midday depression of citrus leaf CERs (e.g., Bielorai and Mendel, 1969; Sinclair and Allen, 1982; Brakke et al., 2003; Li et al., 2007) began early at Phoenix and continued throughout the day, and leaf CERs were depressed 4-fold more during the afternoon at ambient CO_2 than at elevated CO_2.

Sinclair and Allen (1982) found that leaf CERs of sweet orange and grapefruit were sensitive to VPD with midday depressions prevalent on days with high maximum VPD. The demarcation point appeared to be a maximum VPD of about 2.8 kPa associated with a maximum temperature of about 31 °C. They discussed evidence that citrus trees tend to stabilize maximum whole-tree transpiration rates by stomatal closure which limits whole-tree water transport and maximum leaf transpiration rate, with decreases in leaf CERs. When temperatures were lower early in the season, midday depressions in citrus CERs occurred at lower VPDs (Sinclair et al., 1983).

The concept of an upper limit for whole-tree water transport is supported by Halevy (1956) who found that transpiration rates of "Shamouti" orange leaves were no greater on hot, dry days than on mild days. Hall et al. (1975) found that leaf transpiration rates of orange trees stabilized at a maximum rate of about 1.4 mmol m^{-2} s^{-1} as VPD was increased. Furthermore, 6 days after pruning these trees, leaf maximum transpiration rate increased up to 2.5 mmol m^{-2} s^{-1} which indicates that the probable limitation of tree water transport allowed greater transpiration rate per unit leaf area when there were fewer leaves.

Meyer and Green (1981) found that transpiration rates of 'Valencia' orange trees grown in lysimeters were nearly constant from 09:30 to 15:30 h at 4.1 mmol m^{-2} (ground area) s^{-1}, whereas transpiration rates of lysimeter-grown soybean and wheat peaked with maximum rates of 13.9 and 10.8 mmol m^{-2} (ground area) s^{-1}, respectively. This is further evidence that water uptake and transport throughout citrus trees can limit transpiration rates compared to agronomic crops.

With young citrus trees growing in outdoor controlled environment chambers at a CO_2 concentration of 330 μmol mol^{-1}, Brakke and Allen (1995) increased VPD from 2.4 to 3.6 kPa for 2 h by abruptly changing the temperature/dewpoint from 29/14 to 37/22 °C. Canopy CER dropped drastically from 7.6 to 0.8 μmol m^{-2} (ground area) s^{-1}, but canopy transpiration decreased only about 17%, from 4.50 to 3.75 mmol m^{-2} (ground area) s^{-1}, which indicates that CER is reduced more than transpiration rate in response to a sudden increase of VPD and temperature. When environmental controls were switched back after 2 h, canopy CERs and transpiration rates recovered quickly. Brakke (1989) showed that canopy CERs of citrus display a midday depression when exposed to high temperature (37 °C) and high VPD (3.6 kPa) at CO_2 concentrations of 330 μmol mol^{-1}. However, exposure to 840 μmol mol^{-1} CO_2 effectively eliminated midday depression of CER and doubled the canopy CERs (Brakke, 1989; Brakke and Allen, 1995). Thus, elevated CO_2 can overcome midday depression of photosynthesis caused by moderately high VPD.

Syvertsen and Lloyd (1994) used a model to compare daily cycles of leaf stomatal conductance, CER, and transpiration rate of citrus in three climates with increasing maximum leaf-to-air vapor pressure difference (VPd), i.e., Valencia, Spain, (Mediterranean) Lake Alfred, Florida (Humid Subtropical), and Yuma Arizona (Midlattitude Desert). The VPd maxima were about 2.3, 3.3, and 6.8 kPa, respectively). Predicted stomatal conductance and leaf CER throughout the day was relatively constant for Valencia, showed a slight depression for Lake Alfred, and showed a pronounced depression for Yuma. Syvertsen and Lloyd (1994) also computed the impact of VPd throughout an annual cycle on stomatal conductance and monthly CO_2 assimilation [mol (CO_2) m^{-2} month^{-1}] at ambient CO_2 concentrations using actual VPd values compared to holding VPd to a low value of 0.5 kPa. For June through September, actual VPd in Yuma caused predicted monthly CO_2 assimilation to be less than 50% of that predicted without a VPd effect on stomata and CER.

Not all *Citrus* spp. and cultivars show the same extent of responses to VPD. In both humid and dry air, the temperature optimum for CER of leaves of Frost Eureka lemon was about

5 °C higher than for Washington Navel and Valencia orange (Kriedemann, 1968). The demarcation point of VPD for causing decreases of leaf CER appeared to be about 3.7 and 2.4 kPa for these cultivars of lemon and oranges, respectively. For Pera sweet orange at 25 °C and high PPFD, Habermann et al. (2003) found that mean leaf CER was 9.3 and 4.0 μmol m^{-2} s^{-1}, stomatal conductance was 0.296 and 0.062 mol m^{-2} s^{-1}, and transpiration rate was 3.32 and 1.67 mmol m^{-2} s^{-1} at VPDs of 1.2 and 2.5 kPa, respectively. The stomata responded to VPD and transpiration rates decreased by about 50% rather than remaining constant or decreasing slightly with high VPD as found by Halevy (1956), Sinclair and Allen (1982), and Brakke and Allen (1995). Machado et al. (2005) compared leaf CERs of 'Valencia' orange, 'Murcote' tangor, and 'Tahiti' acid lime to temperature and VPD. We extracted leaf CERs (A) of 8.1, 7.9, 5.5, and 3.5 μmol m^{-2} s^{-1} for treatment combinations of 28 °C and 1.5 kPa, 35 °C and 1.5 kPa, 28 °C and 3.5 kPa, and 35 °C and 3.5 kPa, respectively. Stomatal conductances (g$_s$) were 0.101, 0.101, 0.066, and 0.045 mol m^{-2} s^{-1} for the respective combinations of temperature and VPD. Calculated respective values of A/g$_s$ were nearly constant, namely 80, 78, 83, and 78 μmol mol^{-1}. Finally, leaf transpiration rates were 2.1, 1.7, 1.6, and 1.5 mmol m^{-2} s^{-1} for the respective combinations of temperature and VPD. These data indicate a greater influence of VPD than temperature on reductions in CER. However, in moderate shading experiments, Jifon and Syvertsen (2003) and García-Sánchez et al. (2006) concluded that the direct effect of high temperature on citrus leaf CER was more important than the secondary effects of leaf-to-air vapor pressure differences mediated via decreased stomatal conductance.

The 'Valencia' orange data of Machado et al. (2005) showed a slight reduction in transpiration rates with an increase of VPD from 1.5 to 3.5 kPa that would support the proposition of Sinclair and Allen (1982) that citrus trees restrict water flow with increasing VPD that limits total tree water loss rates at high VPD. However, the 'Murcote' tangor and the 'Tahiti' acid lime did not fit the pattern well. Possibly these plants have less severe limitations of water transport through the plant and possibly they would exhibit clear-cut water transport restrictions only at higher VPD.

5. Conclusions

Within Citrus spp., a limitation to water transport appears to be common, which, in high VPD conditions, results in stomatal closure to the point of stabilizing whole-tree water transport with concomitant reductions in leaf and canopy CERs. In nature, the highest VPD conditions are mostly related to high temperature environments with low humidity, e.g., midlattitude deserts. Leaf CERs may be decreased more than leaf transpiration rates in such high temperature, high VPD conditions. Quantum yields decrease with increasing temperature (Ehleringer and Björkman, 1977), but this tells little about mechanisms. Mechanisms for reduction in leaf CERs might involve the competition of O$_2$ with CO$_2$ for Rubisco binding sites (Ku and Edwards, 1977a,b; Ku et al., 1977; Tenhunen et al., 1979) or it might involve the deactivation of Rubisco and the slowness of re-activation at moderately high temperatures (Salvucci and Crafts-

Brandner, 2004). Elevated CO$_2$ concentration appears to readily offset the negative impacts of high VPD and high temperature on citrus canopy CERs (Brakke, 1989; Idso et al., 1991b; Brakke and Allen, 1995).

The hypothesis that biomass growth of young citrus trees will be increased by CO$_2$ enrichment of 360 μmol mol^{-1} in a humid subtropical environment similar to that observed in a midlattitude desert environment was not supported by the data. This discussion indicates that several Citrus spp. have limits to water flow that induce severe reductions in stomatal conductance and leaf net photosynthetic rates in hot, arid environments. The VPDs in Gainesville apparently were not severe enough to cause large restrictions in water flow and lead to reductions in biomass accumulation via decreased photosynthesis. Thus, in Gainesville, the biomass production enhancements by elevated CO$_2$ were very similar to the mean of other reports (e.g., Wullschleger et al., 1995, 1997). Finally, the coupled gas exchange components (Wong et al., 1979) must be interrelated with VPD and direct temperature effects on photosynthesis.

Since it appears that VPD might have a prominent effect on plant growth responses, VPD control and/or measurement (either air saturation deficit or leaf-to-air vapor pressure differences, or both) should be an essential part of environmental controls in all controlled environment research, especially as related to global climate change. Furthermore, rising global CO$_2$ concentrations might not have the same effects on plants in all environments, and it could be unwise to extend predictions of research findings of well-watered plants grown in hot, arid environments to milder, humid environments, and vice versa.

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