Limitations on Growth and Net Gas Exchange of Diploid and Tetraploid *Citrus* Rootstock Cultivars Grown at Elevated CO$_2$

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ABSTRACT. Diploid (2x) and autotetraploid (4x) *Citrus* L. rootstock cultivars were grown at elevated CO$_2$ to obtain insights into limitations on growth and net gas exchange that have been associated with tetraploidy. Well-nourished 2x and 4x seedlings of 'Volkamer' lemon (Volk, *C. volkameriana* Ten & Pasq.), 'Troyer' citrange [Troy, *C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] and 'Cleopatra' mandarin (Cleo, *C. reticulata* Blanco.), were grown in greenhouses at either ambient or twice ambient CO$_2$ for 4 months. Plant growth, water relations, mineral nutrition, and net gas exchange characteristics of leaves were measured. Most 4x plants were smaller and had lower rates of whole plant transpiration but shorter fibrous roots than 2x plants. Fibrous roots of 4x were thicker than 2x roots as indicated by a lower specific root length (SRL) in 4x than in 2x roots. Root hydraulic conductivity was correlated to total plant growth but there were no effects of CO$_2$ or ploidy on root conductivity. Tetraploid leaves had lower N concentrations than 2x leaves when expressed on a dry weight basis but these differences disappeared when N concentration was expressed on an leaf area basis because 4x leaves had more leaf dry weight per area (LDW/a) than 2x leaves. Plant growth was greater and SRL was lower at elevated CO$_2$ than at ambient CO$_2$. LDW concentrations of N, P, and K were lower at elevated CO$_2$ than at ambient apparently due to a growth dilution effect. LDW/a, net CO$_2$ assimilation (A$_{CO2}$), and leaf water use efficiency were greater at elevated CO$_2$ than at ambient. Overall, there was no effect of ploidy on A$_{CO2}$ but 4x Volk and Troy had lower rates of A$_{CO2}$ than their 2x at elevated CO$_2$. Net gas exchange of tetraploid leaves was less responsive to elevated CO$_2$ than 2x leaves. The low SRL of tetraploids was correlated with low whole plant transpiration rates and low leaf area-based N concentrations, which may be operative in determining the growth characteristics associated with tetraploidy.

Enrichment of atmospheric CO$_2$ above that of ambient air results in increased net CO$_2$ assimilation (A$_{CO2}$), plant growth (Bowes, 1997; Roden and Ball, 1996), and mineral nutrient requirements of most C$_3$ plants (Epron et al., 1996; Lawlor and Mitchell, 1991). Elevated CO$_2$ can result in increased root development but may not result in increased root to shoot dry weight (DW) ratios (R/S, Rogers et al., 1996). Elevated CO$_2$ also decreases stomatal conductance and increases water use efficiency (Brakke and Allen, 1995; Saxe et al., 1998). Thus, determining plant responses to elevated CO$_2$ may be useful for obtaining insights into water, mineral nutrient, and carbon limitations on plant growth.

Genotypic differences in growth, water relations (Syvertsen and Graham, 1985), and mineral nutrient acquisition among citrus rootstocks (Wutscher, 1989) can affect yield of citrus trees (Castle et al., 1993). Since citrus trees are strongly carbon source-limited (Goldschmidt and Koch, 1996; Syvertsen and Lloyd, 1994), the CO$_2$-enhanced growth of citrus seedlings (Koch et al.,

1986) and trees (Idso et al., 1996) is relatively great compared to other C$_3$ species. Enhanced growth of sour orange trees at elevated CO$_2$ resulted in reduced leaf N apparently due to either growth dilution (Idso et al., 1996) or to preferential reallocation of N preferentially to roots (Saxe et al., 1998).

Most citrus rootstock seedlings are clonal (nucellar) in origin, but spontaneous tetraploids (4x) can arise from a single diploid (2x) progenitor (Lee, 1988; Lee et al., 1990). Tetraploid seedlings usually have thicker, less elongated roots and larger, thicker leaves (Barrett and Hutchison, 1978). Thicker 4x leaves of some species can have higher A$_{CO2}$ rates than 2x leaves due to the greater leaf tissue per unit area in 4x leaves (Warner and Edwards, 1993). However, the thicker mesophyll anatomy of 4x citrus leaves may result in increased internal diffusive resistances and lower A$_{CO2}$ in 4x than in 2x leaves (Romero-Aranda et al., 1997). Growing leaves at elevated CO$_2$ could overcome the greater limitations of CO$_2$ supply to the mesophyll in 4x leaves than in 2x leaves and their differences in A$_{CO2}$ should diminish.

Use of tetraploids as rootstocks can result in smaller, more desirable citrus trees (Grosser et al., 1995; Lee et al., 1990) but the underlying mechanism of tree size control is not understood. Tree size control is an important objective for rootstock improvement as pest control, harvest, and frost protection efficiencies increase with smaller trees. Citrus rootstock cultivars with relatively thick

roots, or a low specific root length (SRL = root length to DW ratio), have a lower hydraulic conductivity (Eissenstat, 1992; Graham and Syvertsen, 1985) and tend to produce leaves with lower mineral nutrient contents than species with high SRL (Syvertsen and Graham, 1985). If growth limitations associated with 4x rootstocks are due to diminished uptake of water and nutrients by tetraploid roots with low SRL, then root conductivity and mineral nutrition of 4x seedlings may be lower than 2x. To test this hypothesis (H_o) and also the H_o above concerning the limitations of CO_2 diffusion in thick 4x leaves, we grew three 2x and their autotetraploid 4x *Citrus* rootstock cultivars at elevated CO_2 and measured plant growth, water relations, mineral nutrition, and net gas exchange of leaves.

Materials and Methods

PLANT MATERIAL AND GROWTH CONDITIONS. Seeds of three common 2x (2n = 2x = 18) rootstock cultivars, 'Volkamer' lemon (Volk., *C. volkameriana*), 'Troyer' citrange (Troy, *C. sinensis* x *Poncirus trifoliata*), and 'Cleopatra' mandarin (Cleo, *Citrus reticulata*), along with their three autotetraploids (2n = 4x = 36) were used. The autotetraploid seed came from spontaneous nucellar somatic tetraploid plants and were confirmed by chromosome microscopy (Lee et al., 1990). Seeds were germinated in a greenhouse in autoclaved, native Candler fine sandy soil with <1% organic matter (Alva and Syvertsen, 1991), and 3.8 mg·kg^{-1} of available P as determined by double-acid extraction (Mehlich, 1953).

Uniform 1-month-old seedlings (about three- to five-leaf stage) were transplanted individually into 2.3 L containers filled with autoclaved soil. Seedlings were grown from 2 Feb. to 1 June 1997 in either of two identical air-conditioned greenhouses made of clear double-walled polycarbonate. One greenhouse was supplied continuously with additional CO_2 which was monitored with an infrared gas analyzer (ADC-2000, Akofi Inc., Buffalo, N.Y.) to maintain an elevated minimum diurnal CO_2 concentration at about twice ambient (720 ± 10 µL·L^{-1}). The other well-ventilated greenhouse maintained ambient CO_2 concentration which was about 360 µL·L^{-1} during the daytime. Maximum photosynthetically active radiation (*PAR*) (LI-170; LI-COR, Inc., Lincoln, Nebr.) measured above the plants was 1700 µmol·m^{-2}·s^{-1}, with natural photoperiods. Average day/night temperatures were 36/21 °C and relative humidity was maintained above 40% during the day by misting with water below the benches in the greenhouse. Atmospheric vapor pressure deficit approached 0 at night and was kept between 2 to 4 kPa during the day. Plants were watered and fertilized three times per week with 200 mL of a complete nutrient solution with N at 120 mg·L^{-1} (= 72 mg·week^{-1}) from a commercial water soluble fertilizer (20N–8.7P–16.6K, Peters, St. Louis, Mo.) with minor elements. Since even fast growing citrus seedlings do not require N at more than 53 mg·L^{-1}·week^{-1} (Lea-Cox and Syvertsen, 1996), 72 mg·week^{-1} was considered to be more than adequate. The 200 mL volume was enough nutrient solution to leach from the bottom of all containers. The positions of the cultivars within each greenhouse were completely randomized every week.

GAS EXCHANGE AND WATER RELATIONS. All gas exchange measurements were made over a 6-week period between 24 Apr. and 1 June 1997 using a single tagged mature leaf from the middle of the shoot for six replicate seedlings in each treatment. Net assimilation of CO_2 (A_{CO2}), leaf transpiration rates (E_l), and calculated stomatal conductances (g_s) were determined with a portable photosynthesis system (LI-6200; LI-COR) using a well-

stirred 0.25-L measurement cuvette. This set of gas exchange values was evaluated for each measurement leaf on 2 or 3 separate d and these values were averaged to represent a single set gas exchange values for each plant. Average gas exchange rates of such leaves have been shown to be representative of whole seedlings (Syvertsen and Graham, 1999). During all measurements, a constant *PAR* was supplied from a light emitting diode (LED) light source (Quantum Devices, Inc., Barneveld, Wis.) (Tennessen et al., 1994) which was attached to the cuvette and set at 850 µmol·m^{-2}·s^{-1}. Leaf temperature was 29 ± 1 °C and vapor pressure difference between the leaf and air was 2.6 ± 3 kPa. Concentration of CO_2 within the measurement cuvette was 365 ± 10 µL·L^{-1} when gas exchange measurements were made in the ambient CO_2 greenhouse and 740 ± 20 µL·L^{-1} when measured in the elevated CO_2 greenhouse. All gas exchange measurements were made from 0900 to 1200 HR when environmental conditions best fit the above measurement conditions. Leaf transpiration rates were used to calculate instantaneous leaf water use efficiency (WUE) as A_{CO2}/E_l.

Whole plant transpiration (E_{WP}) was measured as the weight loss from each pot during 6 to 7 h daytime periods and averaged over four selected clear days between 16 and 23 May. Three replicate pots with similar evaporative surfaces but without a plant were also weighed to correct for soil water evaporation in each greenhouse. Plants were harvested within 1 week after E_{wp} was measured. Total leaf area per plant was measured (LI-3000; LI-COR) and used to calculate E_{wp} in units of mg·m^{-2}·s^{-1}.

After gas exchange and E_{wp} measurements, hydraulic conductivity of the intact root systems was measured between 12 May and 1 June. Plants were well watered, drained, and brought into the laboratory. Shoots were severed about 4 cm above the soil line for all plants and the entire pot sealed in a pressure pot with the cut stump protruding (Syvertsen and Graham, 1985). Stem xylem sap exudation rates were measured at 0.5 MPa of pressure until rates were constant which required 15 to 30 min. Roots were gently washed free of sand and the total length (RL) of fibrous roots <2 mm in diameter was determined using a grid line intercept method (Tennant, 1975). Root conductivity was expressed in units of µg·MPa^{-1}·m^{-1}·s^{-1}.

Plants were separated into fibrous roots, woody roots, stems, and leaves. Plant tissues were dried at 70 °C for 3 d and weighed. The leaves used for gas exchange measurements, plus the adjacent two leaves above and below the measurement leaves (five leaves total), were used to evaluate average leaf dry weight per area (LDW/a). Dried leaves were milled to a powder for mineral nutrient analyses. Tissue P and K concentrations were determined with an inductively coupled plasma atomic emission spectrometer (ICPES) after the tissue had been ashed (500 °C, 5 h) and suspended in 1 M HCl. Leaf N, and total carbon were determined using an NA-1500 C–N analyzer (Fisson Inst. Inc., Dearborn, Mich.). Mineral nutrient concentrations in leaf tissues were expressed on a percentage dry weight basis and also on a leaf area basis (mmol·m^{-2}).

Data analyses. Data were analyzed using analysis of variance (ANOVA) procedures and means separated by Duncan's multiple range test (DMRT) (SAS Institute Inc., Cary, N.C.) with three cultivars × two ploidy levels × two CO_2 levels and six replicate plants in each treatment. When significant three-way interactions occurred, a two ploidy × two CO_2 factorial ANOVA was run within each cultivar (Milliken and Johnson, 1984). In these cases, the two-way interactions between ploidy and CO_2 levels were almost always nonsignificant which allowed unam-

Table 1. Effects of cultivar (C), ploidy (2x, 4x) and atmospheric CO_2 concentration (1 = ambient or 2= twice ambient) on mean (n = 6) total plant dry weight (TPDW), total root dry weight (RDW), root to shoot (R/S) dry weight ratio, fibrous root length (RL), specific root length (SRL), whole plant transpiration rate (E_{WP}), and root hydraulic conductivity (RHC) of *Citrus* rootstock seedlings.

C	Ploidy	CO_2	TPDW (g)	RDW (g)	R/S	RL (m)	SRL (m·g^{-1})	E_{wp} (mg·m^{-2}·s^{-1})	RHC (μg·MPa^{-1}·m^{-1}·s^{-1})
Volk	2x	1	21.5 b A^z	4.5 b A	0.26 a C	31.1 a B	14.1 a B	22.7 a B	28.2^{NS} A
		2	30.2 a	5.7 a	0.23 b	35.5 a	13.2 a	8.4 c	23.6
	4x	1	21.2 b	4.1 bc	0.24 ab	24.6 b	13.0 a	19.4 b	28.0
		2	18.6 b	3.4 c	0.23 b	17.5 c	11.1 b	4.3 d	39.4
Troy	2x	1	15.8 ab B	4.0 bc A	0.34 b A	32.5 b A	16.8 a A	30.6 a A	8.2 ab B
		2	21.1 a	5.3 ab	0.34 b	42.6 a	16.4 a	16.7 b	15.8 a
	4x	1	11.5 b	3.4 c	0.44 a	21.1 c	13.9 b	17.9 b	4.1 b
		2	21.0 a	5.9 a	0.42 a	31.6 b	12.3 c	18.7 b	11.0 ab
Cleo	2x	1	10.6^{NS} C	2.1 b B	0.26 b B	20.5 a C	17.1 a B	13.6 a C	12.0^{NS} B
		2	12.6	2.4 ab	0.24 b	19.3 a	14.0 b	4.9 c	11.9
	4x	1	8.8	1.8 b	0.26 b	11.2 b	11.8 c	8.3 b	6.6
		2	12.7	2.9 a	0.32 a	12.1 b	9.1 d	5.2 bc	8.2
C			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Ploidy			0.004	0.06	0.0002	0.0001	0.0001	0.0001	NS
CO_2			0.0001	0.0001	NS	0.06	0.0001	NS	NS
C × ploidy			NS	0.01	0.004	NS	0.001	NS	NS
C × CO_2			NS	0.01	NS	0.001	NS	0.0001	NS
Ploidy × CO_2			NS	NS	NS	NS	NS	0.001	NS
C × Ploidy × CO_2			0.001	0.004	NS	NS	NS	0.002	NS

^{z}Significant differences among cultivars (from three-way ANOVA and Duncan's multiple range test) are indicated by upper case letters ($P < 0.05$). Significant differences within cultivars (from two-way ANOVA) are indicated by lower case letters ($P < 0.05$); means followed by no letter are not significantly different from each other (NS, $P > 0.07$).

biguous interpretations of the significance of main effects using DMRT. Regression and correlation analyses were performed to test for significant relationships between selected dependent variables.

Results

GROWTH CHARACTERISTICS AND WATER RELATIONS. There were significant amounts of variation attributable to cultivar type in all dependent variables tested which contributed to the significant three-way treatment interactions in growth measurements and water relations characteristics (Table 1). Seedlings of Volk had the largest total plant dry weight (TPDW) whereas Cleo were the smallest with less total root dry weight (RDW) than the other two cultivars. Troy had the highest root to shoot DW ratios (R/S), greatest fibrous RL, thinnest roots (i.e., highest specific root length, SRL) and highest rates of whole plant transpiration (E_{WP}) compared to the other cultivars. Cleo seedlings had the lowest E_{WP}. Volk seedlings had the highest root conductivity as root conductivity was positively correlated to TPDW ($r = 0.39$, $P < 0.001$). There was no correlation between root conductivity and E_{WP} ($r = 0.07$).

Tetraploid seedlings tended to be smaller than 2x but in Volk, TPDW and RDW of 4x were significantly greater than 2x (Table 1). There was no effect of ploidy on TPDW at ambient CO_2. Tetraploid Troy and 4x Cleo grown at elevated CO_2 allocated relatively more growth to roots than to shoots and thus, had higher R/S ratios than their 2x. None of the root systems appeared visibly potbound regardless of treatment. Tetraploids had less fibrous root length (RL) and lower SRL than diploids. Tetraploid seedlings also had lower E_{WP} than diploids except in 4x Troy and Cleo grown at elevated CO_2. Whole plant transpiration rates were positively related to TPDW within tetraploids ($r = 0.42$, $P < 0.01$)
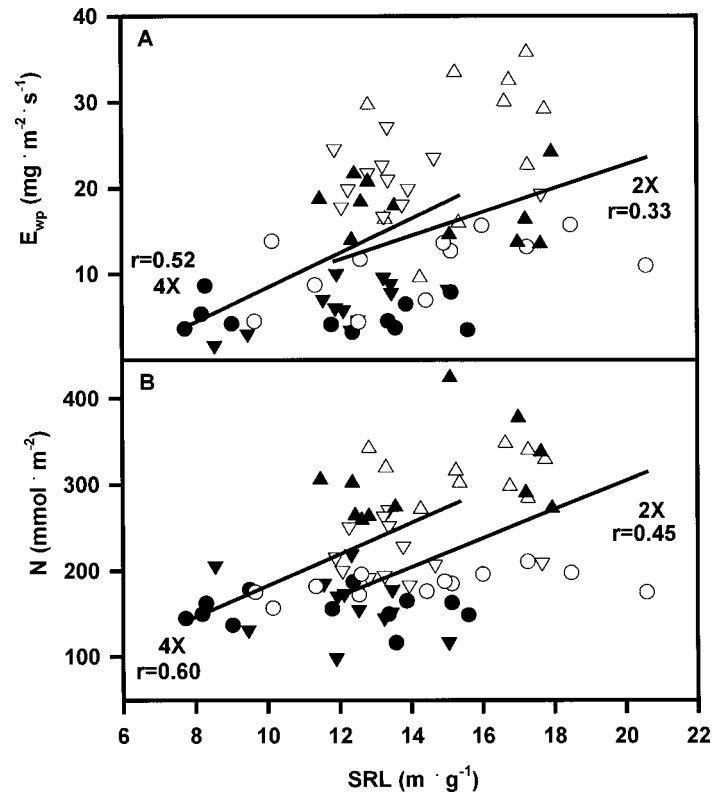
Fig. 1. Effects of ploidy (2x, 4x) and atmospheric CO_2 concentration (ambient = open symbols, twice ambient = closed symbols) on correlations (r) between (**A**) whole plant transpiration (E_{WP}) and (**B**) leaf nitrogen concentration (N) and specific root length (SRL) of *Citrus* rootstock seedlings. Each symbol represents one plant; Cleo = ○ ●, Volk = ▽ ▼, and Troy = △ ▲. The critical value for correlation coefficients (r) for n = 36 at $P < 0.05$ is 0.32.

Table 2. Effects of cultivar (C), ploidy (2x, 4x) and atmospheric CO_2 concentration (1 = ambient, 2 = twice ambient) on mean (n = 6) leaf mineral nutrient concentrations, expressed as percentage dry weight (%) or on a leaf area basis (mmol·m^{-2}), and leaf dry weight area (LDW/a) of *Citrus* rootstock seedlings.

C	Ploidy	CO_2	C	N	P	K	C/N	LDW/a	C	N	P	K
			(%)					(g·m^{-2})	(mol·m^{-2})	(mmol·m^{-2})		
Volk	2x	1	43.1 a B[z]	3.5 a B	0.16 a B	1.5 a B	16.1 b B	95 c A	3.41 c A	213 ab B	4.9 b C	45.9 a B
		2	42.5 b	1.4 c	0.12 b	0.6 c	36.9 a	144 b	5.09 b	144 c	5.5 b	25.5 b
	4x	1	42.7 b	2.2 b	0.15 a	1.0 b	22.4 b	144 b	5.13 b	232 a	6.8 a	48.2 a
		2	42.0 c	1.6 c	0.13 b	0.9 b	32.8 a	163 a	5.68 a	178 bc	6.6 a	44.8 a
Troy	2x	1	43.0[NS] A	4.3 a A	0.20 a A	1.8 b A	11.6 c C	103 b B	3.70 b B	319[NS] A	6.7[NS] A	58.1 b A
		2	43.1	3.3 b	0.16 b	1.0 c	15.2 b	138 a	4.96 a	329	6.9	42.8 c
	4x	1	43.6	4.2 a	0.22 a	2.3 a	12.0 c	102 b	3.69 b	309	7.0	76.1 a
		2	42.6	2.9 b	0.16 b	1.1 c	17.1 a	134 a	4.74 a	279	7.1	48.4 c
Cleo	2x	1	43.2 ab A	2.5 a C	0.19 a B	1.1 a C	20.3 d A	108 d A	3.89 d A	192 a C	6.8 a B	36.6 b C
		2	42.7 c	1.5 c	0.15 b	0.8 b	33.8 b	144 b	5.10 b	155 b	6.8 a	34.7 bc
	4x	1	43.3 a	1.8 b	0.16 b	1.0 a	27.8 c	135 c	4.88 c	176 a	6.9 a	44.7 a
		2	42.9 bc	1.3 c	0.10 c	0.6 c	38.8 a	166 a	5.95 a	154 b	5.4 b	31.5 c
C			0.0008	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0006	0.0001
Ploidy			NS	0.0001	0.01	NS	0.01	0.0001	0.0001	NS	0.05	0.0001
CO_2			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	NS	0.0001
C × ploidy			0.03	NS	0.0003	0.0001	NS	0.0001	0.0001	0.01	0.0001	0.03
C × CO_2			NS	0.02	NS	0.0001	0.0001	NS	NS	0.02	NS	0.004
Ploidy × CO_2			NS	0.002	NS	NS	NS	0.004	0.002	NS	0.02	NS
C × ploidy × CO_2			NS	0.001	NS	0.0001	NS	0.03	0.03	NS	NS	0.0002

[z]Significant differences among cultivars (from three-way ANOVA and Duncan's multiple range test) are indicated by upper case letters ($P < 0.05$). Significant differences within cultivars (from two-way ANOVA) are indicated by lower case letters ($P < 0.05$); means followed by no letter are not significantly different from each other (NS, $P > 0.07$).

but there was no correlation between TPDW and E_{WP} in diploids ($r = -0.02$). There was no significant effect ploidy on root conductivity.

Elevated CO_2 resulted in increased TPDW but not significantly so in Cleo or in 4x Volk (Table 1). Shoot DW (data not presented) increased with RDW at elevated CO_2 such that there was no consistent effect of elevated CO_2 on R/S ratio. Total RDW and fibrous RL were generally higher, whereas SRL was consistently lower in plants grown in elevated CO_2 than in those from ambient CO_2. There was no correlation ($r = -0.12$) between root conductivity and SRL. Although elevated CO_2 tended to increase root conductivity in Troy, elevated CO_2 decreased E_{WP} in all three cultivars. Overall, E_{WP} was correlated with SRL and their relationship was similar across both ploidy levels (Fig. 1A).

Leaf mineral nutrition. Again, cultivar differences accounted for significant amounts of variation in all mineral nutrient concentrations tested regardless of unit of expression (Table 2). Leaf C concentration, expressed as a percentage of LDW, was lower in Volk than in the other cultivars. Leaf N, P, and K DW concentrations were highest in Volk whereas leaf N and K were lowest in Cleo compared to the other cultivars. Leaf C/N ratio was highest in Cleo and lowest in Troy. The trifoliate leaves of Troy also had lower LDW/a and leaf C concentration, expressed on a leaf area basis, than the other cultivars. Due to the relatively important contribution of C compounds to LDW, LDW/a was strongly correlated to leaf C ($r = 0.99$) and to leaf C/N ratio ($r = 0.72$, $P < 0.0001$). Troy had the highest leaf area-based concentrations of N, P, and K whereas leaves of Cleo had the lowest levels of N and K regardless of units of expression.

Leaf dry weight C and K were not affected by ploidy but 4x leaves of Volk and Cleo had lower DW concentrations of N than 2x leaves at ambient CO_2 (Table 2). Cleo 4x leaves had lower dry weight P and K than 2x leaves at elevated CO_2. Leaf C/N ratio was higher in 4x Troy and Cleo than in their 2x leaves. Leaf DW/a and leaf area-based C also were greater in 4x Volk and Cleo than in 2x leaves. Leaf DW/a was increased by tetraploidy such that there was no effect of tetraploidy on leaf N concentration when expressed on a leaf area basis. Volk 4x had higher area-based P than 2x at both levels of CO_2 and higher area-based K than 2x at elevated CO_2. At ambient CO_2, leaf area K was also higher in 4x Troy and Cleo than in their 2x leaves.

Elevated CO_2 actually resulted in decreases in leaf C concentration on a DW basis in both Volk and Cleo even though leaf area concentration of C increased consistently at elevated CO_2 (Table 2). Elevated CO_2 resulted in decreased leaf P on a percent DW basis also decreased leaf N and K regardless of units of expression. Leaf dry weight N concentrations in Volk and Cleo were reduced to levels considered to be deficient(<2.0%) in bearing trees (Tucker et al., 1995). Leaf C/N increased markedly in response to elevated CO_2. Leaf DW/a were increased consistently by elevated CO_2 such that there was no effect of CO_2 on leaf area P.

Leaf area-based N concentration was correlated with SRL across both levels of CO_2 (Fig. 1B). Since leaf area N was not affected by ploidy, the different intercepts (*t* test) of the 2x and 4x relationships of leaf area N vs. SRL, can be attributed to the lower SRL of 2x than 4x seedlings. Leaf area N was also closely related to E_{WP} ($r = 0.67$, $P < 0.0001$).

Gas exchange characteristics. Net A_{CO2} of the measurement leaves was correlated ($r = 0.37$, $P < 0.05$) with TPDW indicating that the largest plants had the highest A_{CO2}. Troy had higher rates of net gas exchange than Volk and Cleo (Table 3). Overall, there was no significant effect of ploidy level on A_{CO2}. At ambient CO_2, however, 4x Cleo had higher A_{CO2} than 2x Cleo. Tetraploid leaves had lower stomatal conductances (g_s) and lower E_l than 2x in Volk but g_s and E_l were higher in 4x Cleo than in 2x. Thus, there was

Table 3. Effects of cultivar (C), ploidy (2x, 4x) and atmospheric CO_2 concentration (1 = ambient, 2 = twice ambient) on mean (n = 6) net assimilation of CO_2 (A_{CO2}), stomatal conductance (g_s), leaf transpiration (E_l), and leaf water use efficiency (WUE) of *Citrus* rootstock seedlings.

C	Ploidy	CO_2	A_{CO2} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	g_s ($mmol \cdot m^{-2} \cdot s^{-1}$)	E_l	WUE ($mmol \cdot mol^{-1}$)
Volk	2x	1	9.17 b B[z]	130 a B	3.0 a B	3.0 b B
		2	13.18 a	96 b	2.3 b	5.6 a
	4x	1	7.36 b	92 b	2.5 b	2.9 b
		2	9.65 b	60 c	1.5 c	6.4 a
Troy	2x	1	10.33 c A	130 b A	3.6[NS] A	2.9 c A
		2	25.31 a	148 b	3.7	7.0 a
	4x	1	11.70 c	186 a	4.0	3.0 c
		2	20.17 b	148 b	3.5	6.0 b
Cleo	2x	1	6.26 b B	76 b B	2.3 b B	2.8 c B
		2	8.55 a	50 c	1.3 c	6.6 a
	4x	1	9.54 a	142 a	3.3 a	2.9 c
		2	10.82 a	87 b	2.2 b	5.0 b
C			0.0001	0.0001	0.0001	0.02
Ploidy			NS	0.005	NS	0.04
CO_2			0.0001	0.0001	0.0001	0.0001
C × ploidy			0.001	0.0001	0.0001	0.004
C × CO_2			0.0001	0.01	0.0004	NS
Ploidy × CO_2			0.005	0.003	0.01	0.01
C × ploidy × CO_2			NS	NS	NS	0.0003

[z]Significant differences among cultivars (from three-way ANOVA and Duncan's multiple range test) are indicated by upper case letters ($P < 0.05$). Significant differences within cultivars (from two-way ANOVA) are indicated by lower case letters ($P < 0.05$); means followed by no letter are not significantly different from each other (NS, $P > 0.07$).

also no significant effect of ploidy on E_l.

Elevated CO_2 resulted in increased A_{CO2} in all 2x plants but among the 4x plants, elevated CO_2 increased A_{CO2} only in Troy (Table 3). At elevated CO_2, 4x Volk and Troy had lower rates of A_{CO2} than their 2x. Elevated CO_2 decreased g_s and E_l so that WUE was consistently higher at elevated CO_2 than at ambient CO_2. Single leaf transpiration rates were correlated strongly with E_{WP} ($r = 0.61$, $P < 0.0001$). Net A_{CO2} was more strongly related to leaf area-based N in elevated CO_2 than at ambient CO_2 regardless of ploidy level (Fig. 2). Leaf area N reached low enough levels (<175 mmol$\cdot$m^{-2}) to limit A_{CO2} at elevated CO_2 concentrations in both Volk and Cleo.

Discussion

Using 2x and autotetraploid 'Femminello' lemon and 'Valencia' orange shoots which were grafted onto a common rootstock, Romero-Aranda et al. (1997) described lower A_{CO2} in 4x than in 2x leaves. This reduction was attributed to greater biophysical resistances to diffusion of CO_2 in thicker 4x leaves than 2x leaves resulting in the decreased A_{CO2} per unit leaf area (Syvertsen et al., 1995). We, therefore, hypothesized that increased CO_2 supply would result in smaller differences between A_{CO2} in 4x and 2x leaves. In these seedlings grown at ambient CO_2, however, there were no differences in A_{CO2} of Volk and Troy attributable to ploidy and 4x Cleo actually had higher A_{CO2} than 2x. Elevated CO_2 did result in higher A_{CO2} of 4x Troy and Cleo than their 2x relatives at ambient CO_2 but not in Volk. At elevated CO_2, however, 4x Volk and Troy leaves had lower A_{CO2} than their 2x and there was no difference between A_{CO2} of 2x and 4x Cleo leaves. Thus, CO_2 diffusion was apparently no more limiting to A_{CO2} in thicker 4x leaves than in 2x leaves.

Under the well-watered, well-nourished growth conditions of this study, LDW/a can be a good indicator of leaf thickness

(Romero-Aranda et al., 1997 but see Witkowski and Lamont 1991). It is possible that the increase in leaf thickness (i.e., increase in LDW/a) that occurred at elevated CO_2, may have resulted in increased diffusion resistances and negated the poten-
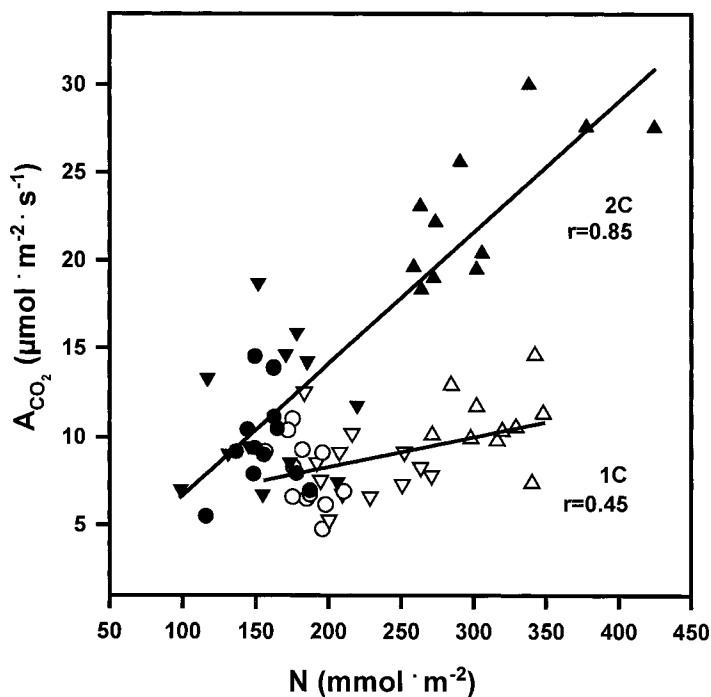

Fig. 2. Effect of atmospheric CO_2 concentration (ambient = 1C, open symbols; twice ambient = 2C, closed symbols) on correlations (r) between net assimilation of CO_2 (A_{CO2}) and leaf nitrogen concentration (N) of diploid and tetraploid citrus rootstock seedlings combined. Each symbol represents one plant; Cleo = ○ ●, Volk = ▽ ▼, and Troy = △ ▲. The critical value for correlation coefficients (r) for n = 36 at $P < 0.05$ is 0.32.

tial effects of increased CO_2 supply on A_{CO2}. The already thicker 4x leaves of Volk and Cleo were not as responsive to elevated CO_2 as were their 2x leaves. The ability of diffusion resistance to limit A_{CO2} (Syvertsen et al., 1995) may still have been important at elevated CO_2. In addition, the lack of any growth increase in both 4x Volk and Cleo in response to elevated CO_2, implied that there was a limitation on growth of 4x seedlings that could not be overcome by growing plants at the elevated CO_2.

Although all seedlings received uniformly high rates of fertilization, 4x leaves grown at ambient levels of CO_2 had lower dry weight concentrations of N than 2x leaves. Tetraploid Volk and Cleo also had lower dry weight concentrations of K than 2x leaves. Leaf area-based K, however, was higher in thicker 4x Volk and Troy leaves than in 2x leaves. Since leaf N expressed on an leaf area concentration basis was unaffected by ploidy, the greater LDW/a of tetraploid leaves must have compensated for the lower dry weight concentrations of N and K in 4x leaves. The similarities in leaf area-based N status were reflected in similar rates of A_{CO2} in 2x and 4x leaves. Our previous studies using shoots grafted on the same rootstock (Romero-Aranda et al., 1997), also showed no increase of leaf area N in thicker 4x leaves over that of 2x leaves. Thus, the dilution of leaf dry weight N in 4x tissue occurred in both seedlings and grafted trees. The fact that growth at elevated CO_2 resulted in decreases in both leaf N and K concentrations regardless of unit of expression, is especially noteworthy since elevated CO_2 also increased LDW/a. Similar to field trees grown at elevated CO_2 (Idso et al., 1996), this decrease must have been a growth dilution phenomenon which occurred in the larger, elevated CO_2 plants in spite of the uniformly high fertilizer application rates. Nutrient limitations were physiologically important since low leaf N levels apparently limited A_{CO2} in both tetraploid Volk and Cleo even at elevated CO_2.

Leaf starch concentration per unit leaf area has been correlated with both leaf C/N and LDW/a in citrus grown at elevated CO_2 (Syvertsen and Graham, 1999). Although we did not measure leaf starch, leaf C expressed on an area basis, C/N, and LDW/a were all increased as a result of tetraploidy and elevated CO_2 in both Volk and Cleo. Since high leaf starch concentrations can lead to feedback inhibition of A_{CO2} (Goldschmidt and Koch, 1996), it is possible that the higher concentrations of starch in 4x Volk and Cleo leaves may have diminished their A_{CO2} response to elevated CO_2. Troy had the lowest levels of leaf area-based C and the greatest increase in A_{CO2} in response to elevated CO_2 compared to the other two cultivars. Leaf C and LDW/a were not increased by tetraploidy in Troy, however, and 4x Troy had lower A_{CO2} than 2x leaves at elevated CO_2. Thus, at least in Troy, lower A_{CO2} in 4x than in 2x was apparently not due to higher CO_2 diffusion resistances in thicker leaves nor to an accumulation of C per unit leaf area.

Leaf C/N was negatively related ($r = -0.47$, $P < 0.05$) to whole-plant R/S ratio regardless of tetraploidy or CO_2 level during growth. Leaf C/N, therefore, could have been used as an indicator of relative growth of roots and shoots (Gleeson 1993). Elevated CO_2 can enhance root growth more than shoot growth and limitations in pot size are known to modify plant growth and A_{CO2} responses to elevated CO_2 (Hogan et al., 1991). In this study, R/S was not affected by elevated CO_2. Troy not only had the longest RL, highest R/S, greatest E_{WP}, and highest rates of leaf gas exchange, but Troy also had the highest accumulation of N, P, and K in leaves compared to the other two cultivars. Thus, there was no evidence that root confinement limited growth, net gas exchange or root function.

Root conductivities were quite variable and were not increased by elevated CO_2. Although we hypothesized that the thicker roots and lower SRL of tetraploids would result in lower rates of root conductivity than 2x, there was no significant effect of tetraploidy on root conductivity. These root conductivity data, therefore, did not support the genotypic differences among citrus seedlings that have been associated with low SRL (Eissenstat, 1992) and high conductivity (Graham and Syvertsen, 1985). Overall, the largest seedlings had the greatest root conductivity but there was no correlation between root conductivity, measured under standardized pressure pot conditions, and daily E_{WP} averaged over 4 d. Largest 4x plants had the highest rates of E_{WP} but TPDW was not related to E_{WP} in 2x probably because of the confounding effects of elevated CO_2 which increased growth while decreasing stomatal conductance and plant water use.

Net gas exchange of a single leaf, or plant water loss at a single point in time, may not always be good indicators of growth and water use history of a plant. There are large differences in net gas exchange characteristics of citrus leaves that occur as leaves expand and mature (Syvertsen and Graham, 1990). In order to avoid leaf age affects, we evaluated net gas exchange of a single representative leaf and found that A_{CO2} was positively correlated to total plant DW. In addition, E_l of the measurement leaf was positively related to E_{wp}. Thus, the largest plants had the highest rates of net gas exchange supporting the idea that the measured rates of selected leaves were representative of whole seedlings.

Do growth and leaf nutrient responses of 4x seedlings at elevated CO_2 implicate the root system's inability to acquire or transport water and mineral nutrients even when grown under well-watered, high fertility conditions? Tetraploids allocated relatively more growth below ground than 2x but this increase in root weight occurred in shorter 4x fibrous roots that had lower SRL than 2x roots. Low SRL was correlated with low leaf N and low rates of water use especially in 4x seedlings which grew less than 2x. Thus, the low SRL of tetraploids resulted in limited water uptake, N acquisition, and growth responses to elevated CO_2. Such root characteristics may be responsible for the smaller, more compact trees on 4x rootstocks than on more typical 2x rootstocks.

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