



United States
Department of
Agriculture

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Forest
Products
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Research
Paper
FPL-RP-648



Long-Term Effects of Elevated Carbon Dioxide on Sour Orange Tree Specific Gravity and Anatomy

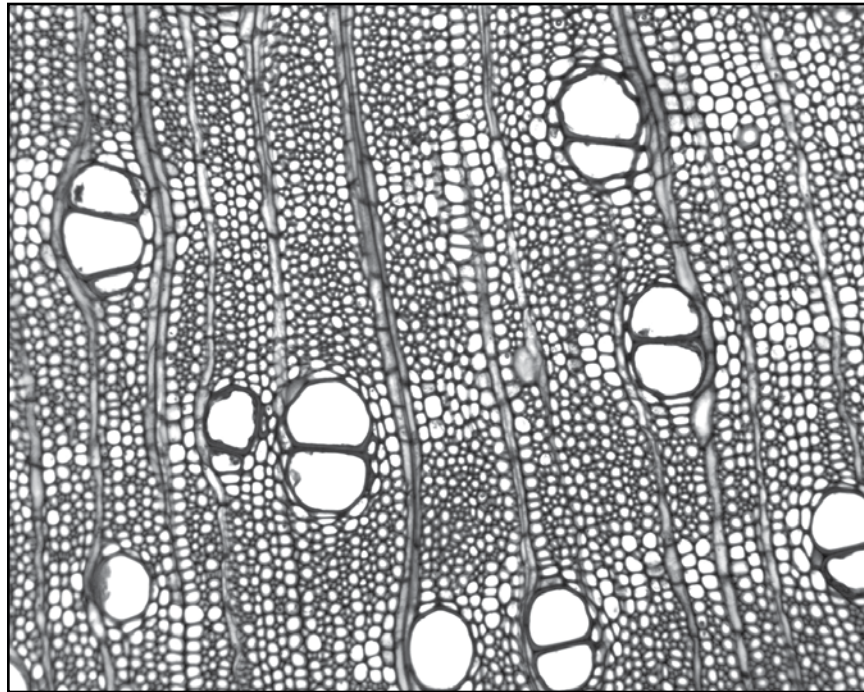
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Abstract

Exposure to elevated levels of atmospheric CO₂ for a period of 17 years resulted in small but statistically significant decreases in wood basic specific gravity and number of rays per millimeter. Other anatomical characteristics (percentages of tissues, number of vessels per square millimeter, vessel diameters, and fiber wall thickness) were unaffected by treatment. Differences due to distance from pith were important, but cardinal direction (north, south, east, west) was not.

Keywords: carbon dioxide, specific gravity, wood anatomy, vessels, rays, axial parenchyma, fibers

Acknowledgments

The authors thank Ernie Ives for preparing microscope slides; Mary Crow for taking wood anatomical measurements; and Regis B. Miller, Henry Spelter, and Mark Knaebe for their reviews of the manuscript. This research was supported by CSREER NRI Competitive Grant ARZR 2004 02088.

February 2008

Wiemann, Michael C.; Kretschmann, David; Rudie, Alan; Kimball, Bruce A.; Idso, Sherwood B. 2008. Long-term effects of elevated carbon dioxide on sour orange tree specific gravity and anatomy. Research Paper FPL-RP-648. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 5 p.

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Long-Term Effects of Elevated Carbon Dioxide on Sour Orange Tree Specific Gravity and Anatomy

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Introduction

A previous paper (Kretschmann et al. 2007) reported the effects of elevated CO₂ on specific gravity, modulus of elasticity, and microfibril angle of sour orange wood, analyzing earlywood and latewood separately. This paper covers the effects of CO₂ treatments on wood anatomy and specific gravity, but the samples were larger and contained both earlywood and latewood, which are not treated separately.

Materials and Methods

Kimball et al. (2007) and Kretschmann et al. (2007) describe in detail the background, greenhouse gas experiment, harvesting protocol, and collection of material for measurement of wood properties. In summary, eight sour orange trees (*Citrus aurantium* L.) were planted as seedlings in four plastic-walled chambers (two trees per chamber) in Phoenix, Arizona. The chambers, initially 2.6 by 5.3 by 2.0 m high, were periodically enlarged as the plants grew, until their final dimensions of 5.1 by 6.3 by 9.0 m high. Two of the chambers were enriched with CO₂ to a concentration of 300 µmol/mol above the concentration in the two control (ambient) chambers, which had a CO₂ concentration of 370 µmol/mol. The CO₂ enrichment was continuous from November 1987 until January 2005, after which the trees were marked, measured, harvested, and weighed. The harvesting consisted of a branch-by-branch removal of the above-ground biomass, followed by excavation of the root system. Prior to felling, the four cardinal directions—north, south, east, or west (N, S, E, or W)—were marked on each tree trunk. A 10-cm trunk segment from 50 cm above ground level, marked with the cardinal directions, was cut from each tree, sealed in a plastic bag, and shipped to the USDA Forest Service, Forest Products Laboratory (FPL). Along each cardinal direction radii were measured and growth rings were counted and marked from the pith to the bark.

Approximately 2-cm-thick disks were cut from the 10-cm trunk segments. Sectioning blocks, approximately 1-cm

cubes, were removed from each disk centered on the fourth, eighth, and fifteenth growth rings, which were notched for identification. Each block was marked with a code number that included tree number (1–8), cardinal direction (N, S, E, W) and ring number (4, 8, 15). Thus, growth ring number 4 from the north side of tree number 1 was marked 1N4. Trees 1, 2, 7, and 8 were CO₂-enriched, and trees 3, 4, 5, and 6 were the controls.

Microscope slides containing thin cross sections were prepared from the cubes by a company specializing in wood microslides. The sections were cut 20 µm thick and stained with a 1% solution of safranin in 50% ethanol. The cross sections were observed and measured using a projecting microscope, digitizing tablet, and software. A rectangular area with dimensions of 1.00 mm (radial direction) by 0.76 mm (tangential direction) was oriented and projected onto the tablet, with a projected magnification of 213×. The cross-sectional areas composed of vessels, rays, and axial parenchyma, the number of vessels, the tangential and radial diameters of complete vessels, and the number of rays were measured on the projected 213- by 162-mm cross sections. Each cross section was measured twice: The slide was positioned randomly so that the field of view was completely filled by wood cross section, the measurements were taken, then the slide was moved to a different area for the second set of measurements.

The areas occupied by vessels, rays, and axial parenchyma were calculated from the projected 0.76-mm² cross sections. The percentages occupied by vessels, rays, and axial parenchyma were calculated from these areas, and the percentage occupied by fibers was calculated by subtraction from 100%. The number of rays per millimeter was calculated from the 0.76-mm tangential dimension, and the number of vessels per square millimeter was calculated from the 0.76-mm² area.

Double fiber wall thicknesses of the tangential walls were measured on the cross sections with a calibrated ocular

micrometer. The measurements were made using a binocular microscope with 12.5× oculars and a 63× objective, for a total magnification of 788×. Ten measurements were made on each slide, and average fiber wall thicknesses were calculated by dividing the sum of the 10 measurements by 20.

The blocks remaining after the cross sections were prepared were used for measurement of basic specific gravity (SG). Green volumes were determined by water displacement of the swollen blocks, which were then dried in an oven at 103°C until constant weight. The SG of each block was then calculated as its oven-dried weight divided by weight of water displaced by the green block (that is, green volume × density of water).

Statistical significance of differences in anatomical characteristics and specific gravity due to treatment (CO₂ exposure level), chamber, tree, cardinal direction, and ring number were determined using SAS 9.1 General Linear Model *F*-tests. When significant differences were found, Tukey groupings were computed to determine where the differences among the means occur.

Results and Discussion

The mean diameter-inside-bark was 21 cm for the CO₂-enhanced trunk segments and 18 cm for those maintained at ambient atmospheric conditions. No statistically significant differences were found for chamber or tree number, so these data were pooled. Statistical significance values of class combinations are shown in Table 1. Treatment affected only number of rays per millimeter and SG (0.05 level). Distance from pith (ring) was a significant variable for percentages of rays, axial parenchyma, and fibers (0.01 level); frequency of vessels (0.001 level) and rays (0.01 level); vessel radial and tangential diameters (0.001 level); and SG (0.001 level). Direction (N, S, E, W) was not significant. Only two interactions were significant: CO₂*ring with vessel radial diameter (0.05 level) and direction*ring with percentage of fibers (0.01 level, Table 1). The significance of these interactions is probably due only to ring, because direction and vessel diameter were not significant with any other variables (Table 1).

Mean values and Tukey comparisons (0.05 level) are given in Table 2 (treatment) and Table 3 (ring). Treatment (Table 2) decreased SG and number of rays per millimeter, although both the differences were small (2% and 6%, respectively). Whether the slight specific gravity decrease is consistent with the finding of Kimball et al. (2007) (who reported mean stem diameters of 24.4 and 21.0 cm, respectively, for the CO₂-enriched and ambient trees, with mean trunk biomasses of 110.0 and 80.4 kg, respectively, for these trees) depends upon certain assumptions. Assuming the height growth was concomitant with diameter growth, volumes of enriched tree trunks would have increased more than did their dry biomass, resulting in somewhat lower SGs. On the other hand, Kimball et al. (2007) also reported

mean dry weight trunk densities of 703 and 697 kg/m³ for enriched and control, respectively, values that were not significantly different, but their densities include both wood and bark and so are not directly comparable to our wood SGs. Finally, our finding of a slight but significant decrease in specific gravity due to CO₂ enhancement is not the same as that of Kretschmann et al. (2007) who found no effect. However, they measured earlywood and latewood separately and used test volume rather than green volume, so the results are also not directly comparable.

The decrease in number of rays per millimeter due to enrichment is harder to explain, especially because overall ray volume was higher (but not significantly) for the treated trees (Table 2). However, Harlow (1927) and Fegel (1941) reported that an increase in number of rays did not necessarily result in an increase in ray volume. Taylor (1969) found that, due to its compactness, ray tissue had a higher specific gravity than wood as a whole. Therefore, smaller, more numerous rays may be more compact and therefore contribute more to specific gravity than larger rays. Because we looked only at wood cross sections, we did not measure ray width or height, so we can only surmise that rays were smaller in the ambient trees and that their compactness might contribute to the somewhat higher specific gravity.

Ring number affected all measured characteristics except for vessel cross section and fiber wall thickness (Table 3). Ring 4 had a lower percentage of fibers, more vessels per square millimeter, and vessels with smaller radial and tangential diameters than did rings 8 and 15. Ring 15 had a lower percentage of rays, higher percentage of axial parenchyma, and larger diameter vessels than did rings 4 or 8. Ring 4 had a lower SG (0.59) than did rings 8 and 15 (0.66) (Table 3). The stabilization of wood specific gravity with increase in distance from the pith was also found by Kretschmann et al. (2007) on this material, although in that study earlywood specific gravity continued to increase slightly between rings 8 and 15.

The effects of ring number and treatment on SG and anatomical characteristics are summarized in Table 4. Note that CO₂ enrichment had no effect on SG during early growth period (the SG of ring 4 was 0.59 in both treatment and control), but SG of CO₂-enriched trees was lower than SG of ambient trees as growth progressed. Figure 1 shows the relationship between SG and the other significant effect of treatment, number of rays per millimeter, for each wood sample and for the group means, with ring numbers and treatments designated by different symbols. The means clearly indicate that, within rings, higher SG wood (ambient trees) had more rays per millimeter, although this difference was not statistically significant for ring 4. The relationship between ray number and SG is weak and ring-dependent. The samples with the most rays (>9.5 rays per millimeter) had high-SG wood, but the wood with the fewest (<5.5 rays per millimeter) also had high-SG wood (Figure 1).

Conclusions

Exposure to elevated CO₂ resulted in a slight but significant decrease in wood SG. Within rings, treatment resulted in a decrease in number of rays per millimeter, although overall ray volume was not affected. Other wood anatomical characteristics were unaffected by CO₂ treatment. At tree ages 8 and 15 years, the reduced number of rays per millimeter was associated with a statistically significant SG decrease, but this was not the case when the tree was only 4 years old. Surprisingly, fiber percentage and wall thickness were not factors explaining SG differences.

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Table 1—Statistical significance of various class combinations^a

	Percentage of cross-sectional area				Frequency		Vessel diameter (mm)		Fiber wall thickness (mm)	Basic SG
	Vessels	Rays	Axial parenchyma	Fibers	Vessels/mm ²	Rays/mm	Radial	Tangential		
CO ₂ (treatment)	ns	ns	ns	ns	ns	*	ns	ns	ns	*
Ring	ns	**	**	**	***	**	***	***	ns	***
Direction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO ₂ *Ring	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
CO ₂ *Direction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Direction*Ring	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
CO ₂ *Direction*Ring	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

^a Levels of significance are labeled ns, *, **, and ***, representing the non-significant, 0.05, 0.01, and 0.001 levels, respectively.

Table 2—Mean values for CO₂-enriched and ambient exposures for all samples and the CO₂/ambient ratios^a

	Percentage of cross-sectional area				Frequency		Vessel diameter (mm)		Fiber wall thickness (mm)	Basic SG
	Vessels	Rays	Axial parenchyma	Fibers	Vessels/mm ²	Rays/mm	Radial	Tangential		
No. of measurements per cell	96	96	96	96	96	96	96	96	480	48
CO ₂	5.5	22.4	16.6	55.5	17.6	7.1	0.062	0.061	0.00243	0.632
Ambient	5.7	22.3	16.9	55	17.6	7.6	0.062	0.059	0.00247	0.643
Ratio, CO ₂ /ambient	0.96	1.00	0.98	1.01	1.00	0.94*	1.00	1.02	0.98	0.98*

^a Data pooled for chamber, tree, ring, and cardinal direction. For each column, ratios with asterisks are significantly different at the 0.05 level.

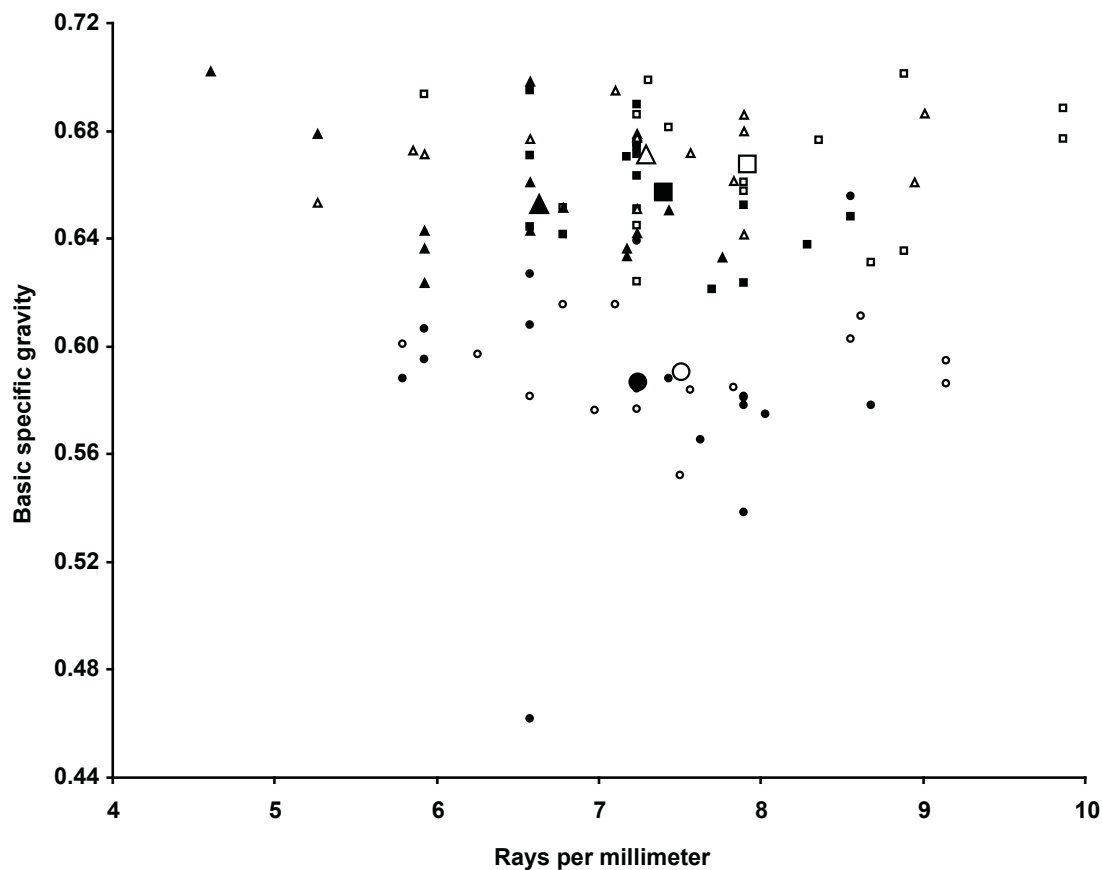
Table 3—Mean values for each ring for all samples^a

	Percentage of cross-sectional area				Frequency		Vessel diameter (mm)		Fiber wall thickness (mm)	Basic SG
	Vessels	Rays	Axial parenchyma	Fibers	Vessels/mm ²	Rays/mm	Radial	Tangential		
No. of measurements per cell	64	64	64	64	64	64	64	64	320	32
Ring 4	6.0 A	23.6 A	17.1 A	53.3 A	23.1 A	7.4 AB	0.058 A	0.054 A	0.00243 A	0.589 A
Ring 8	5.2 A	23.1 A	14.6 B	57.1 B	15.0 B	7.7 A	0.061 B	0.059 B	0.00247 A	0.662 B
Ring 15	5.6 A	20.3 B	18.6 C	55.4 B	14.8 B	7.0 B	0.066 C	0.067 C	0.00246 A	0.662 B

^a Data pooled for chamber, tree, cardinal direction, and CO₂ treatment. For each column, means sharing the same letter are not significantly different at the 0.05 level.

Table 4—Mean values for CO₂-enriched (bold), ambient (roman), and CO₂/ambient ratios (italics) for each ring^a

	Percentage of cross-sectional area				Frequency		Vessel diameter (mm)		Fiber wall thickness (mm)	Basic SG
	Vessels	Rays	Axial parenchyma	Fibers	Vessels/mm ²	Rays/mm	Radial	Tangential		
No. of measurements per cell	32	32	32	32	32	32	32	32	160	16
Ring 4	6.1	23.9	17.0	53.3	24.2	7.2	0.057	0.054	0.00239	0.587
	5.9	23.3	17.5	53.4	22.0	7.5	0.059	0.054	0.00246	0.590
	<i>1.03</i>	<i>1.03</i>	<i>0.97</i>	<i>1.00</i>	<i>1.10</i>	<i>0.96</i>	<i>0.97</i>	<i>1.00</i>	<i>0.97</i>	<i>0.99</i>
Ring 8	4.6	23.1	14.4	57.9	14.0	7.4	0.059	0.058	0.00252	0.657
	5.7	23.1	14.7	56.4	16.0	7.9	0.063	0.060	0.00242	0.668
	<i>0.81</i>	<i>1.00</i>	<i>0.98</i>	<i>1.03</i>	<i>0.88</i>	<i>0.94</i>	<i>0.94</i>	<i>0.97</i>	<i>1.04</i>	<i>0.98</i>
Ring 15	5.7	20.1	18.6	55.5	14.7	6.6	0.068	0.070	0.00238	0.653
	5.5	20.5	18.6	55.3	14.9	7.3	0.064	0.064	0.00254	0.671
	<i>1.04</i>	<i>0.98</i>	<i>1.00</i>	<i>1.00</i>	<i>0.99</i>	<i>0.90</i>	<i>1.06</i>	<i>1.09</i>	<i>0.94</i>	<i>0.97</i>

^a Data pooled for chamber, tree, and cardinal direction.**Figure 1—Relationships among ring number, rays per millimeter, and basic specific gravity. Circles: ring 4; squares: ring 8; triangles: ring 15. Closed symbols represent CO₂-enriched trees, open symbols represent ambient trees. Small symbols represent individual samples, large symbols represent group means.**