

Seedling Growth of Two Honey Mesquite Varieties Under CO₂ Enrichment

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Abstract

Seedlings of 2 varieties of honey mesquite (*Prosopis glandulosa* var. *glandulosa* and *P. glandulosa* var. *torreyana*) were exposed to 2 concentrations of atmospheric carbon dioxide (CO₂) (368 and 704 μmol·mol⁻¹) in environmentally controlled glasshouses under near-optimal temperature and soil water conditions to determine if CO₂ enrichment alters above- and belowground growth responses. CO₂ enrichment substantially enhanced both above- and belowground growth variables of both varieties for all harvest dates (8, 16, and 24 days postemergence). This growth enhancement was greater for aboveground variables (21%–35%) at the first harvest, greater for belowground variables (36%–40%) at the second harvest, and similar for both above- (13%–68%) and belowground (10%–40%) variables at the last harvest. Differences in temporal growth enhancement associated with CO₂ enrichment suggest changing carbon allocation priorities, with initial carbon investment allocated primarily aboveground to develop photosynthetic machinery, and later carbon allocations predominately directed toward increased investment in roots. The absence of significant CO₂ × variety interactions at any harvest date provides evidence that CO₂ enrichment did not exaggerate growth responses between the 2 varieties. These results suggest that varietal differences in rooting and other characteristics did not modify the size advantage of the *glandulosa* over the *torreyana* variety, as the absolute differences in sizes did not change as a function of CO₂ treatment. Although CO₂ enrichment did not exaggerate growth differences between varieties in this species, it is evident that honey mesquite seedlings possess the capacity to respond markedly to CO₂ enrichment. The greater root depth of honey mesquite seedlings exposed to CO₂ enrichment confers a competitive advantage to mesquite seedlings over grass seedlings, assuming that C₃ and C₄ grass seedlings will not respond as vigorously to CO₂ enrichment. As such, this species should continue to aggressively encroach into grasslands in future CO₂-enriched environments.

Resumen

Plántulas de dos variedades de “Mesquite” (*Prosopis glandulosa* var. *glandulosa* y *P. glandulosa* var. *torreyana*) fueron expuestas a dos concentraciones de CO₂ (368 y 704 μmol·mol⁻¹) en invernaderos con ambiente controlado bajo condiciones de temperatura y humedad en suelo cercanas al óptimo a fin de determinar si el enriquecimiento de CO₂ altera la respuesta del crecimiento aéreo y radicular. El enriquecimiento de CO₂ mejoró sustancialmente las variables del crecimiento tanto aéreo como radicular en ambas variedades en todas las fechas de cosecha (8, 16, y 24 días a la postemergencia). El estímulo al crecimiento fue mayor para variables de crecimiento aéreo (21%–35%) en el primer corte, mayor para variables de crecimiento radicular (36%–40%) en el segundo corte y similar para ambos aéreo (13%–68%) y radicular (10%–40%) en el último corte. Las diferencias en la mejoría temporal del crecimiento asociadas al enriquecimiento de CO₂ sugieren un cambio en las prioridades de ubicación del carbón, con inversiones iniciales de carbón asignadas principalmente a la parte aérea a fin de desarrollar la maquinaria fotosintética y translocación posterior de carbón para el crecimiento de raíces. La ausencia de interacciones significativas de CO₂ X variedad en cualquier fecha de cosecha provee evidencia de que el enriquecimiento de CO₂ no estimula exageradamente la respuesta al crecimiento entre las dos variedades. Los resultados sugieren que las diferencias varietales en raíces y otras características no modifican la ventaja del tamaño de la *glandulosa* sobre la *torreyana*, las diferencias absolutas en tamaño no cambian en función de los tratamientos de CO₂. Aunque el enriquecimiento de CO₂ no magnificó las diferencias de crecimiento entre variedades de esta especie, es evidente que las plántulas de “Mesquite” poseen la capacidad de responder marcadamente al enriquecimiento de CO₂. La mayor profundidad de raíces de plántulas de mesquite expuestas al enriquecimiento de CO₂ le confiere una ventaja competitiva sobre plántulas de zacates, asumiendo que las plántulas de zacates C₃ y C₄, no responderán tan vigorosamente al enriquecimiento de CO₂. Por lo tanto, en el futuro, esta especie continuará expandiéndose agresivamente hacia los pastizales en ambientes enriquecidos con CO₂.

Key Words: atmospheric carbon dioxide, leguminous shrub, root growth, shrub encroachment

INTRODUCTION

Honey mesquite (*Prosopis glandulosa* Torr.) is a leguminous shrub that has displaced native herbaceous vegetation in arid and semiarid rangeland ecosystems of the southwestern United States (Archer 1989, 1990, 1995; Van Auken 2000; Ansley et al. 2001). Two varieties of honey mesquite occupy distinct habitats in this region. *Prosopis glandulosa* var. *glandulosa* occurs in mesic environments of west-central and central Texas (Burkhart 1976) as large, single-stem trees, whereas *Prosopis glandulosa* var. *torreyana* occurs in xeric environments of west Texas and southern New Mexico (Hilu et al. 1982) as short, multi-stem shrubs. Pennington et al. (1999) found that under well-watered conditions the carbon isotope composition of leaves, a surrogate for leaf water-use efficiency, differed between seedlings of honey mesquite from xeric and mesic habitats, strongly suggesting a genetic difference between types. Additionally, random amplified polymorphic DNA (RAPD) marker results classified honey mesquite trees along the southern portion of the New Mexico–Texas border as the variety *torreyana* and trees from central Texas as the variety *glandulosa* (Pennington and Tischler, personal communication, 2004).

Bond and Midgley (2000) suggested that invasion by woody plants and subsequent increase in density of these species in grass-dominated ecosystems may be attributable to a significant positive effect of atmospheric carbon dioxide (CO₂) enrichment on growth of woody plant species. The relative responsiveness of these 2 honey mesquite varieties to CO₂ enrichment may indicate which will more aggressively encroach into rangelands in the future. Previous investigations have identified species-specific (e.g., Joel et al. 2001; Goverde et al. 2002) and genotype-specific (e.g., Roumet et al. 2002) responses of plants to CO₂ enrichment. Dissimilar growth responses to CO₂ for these 2 honey mesquite varieties may be manifested in differential rates of shrub encroachment in arid and more mesic rangelands (Polley 1997; Polley et al. 1997).

Our objective in this investigation was to determine if CO₂ enrichment exaggerates variation in above- and belowground growth responses between 2 honey mesquite varieties. Because plants from mesic environments generally grow faster than those from arid environments (Chapin 1980; Lambers and Poorter 1992) and CO₂ enrichment usually increases relative growth rate (RGR) (Poorter 1993) and biomass (Bunce 1997) of species that grow rapidly, we tested the null hypothesis that CO₂ enrichment does not preferentially increase growth of honey mesquite from mesic (var. *glandulosa*) compared to xeric (var. *torreyana*) environments. We assessed above- and belowground growth responses by growing seedlings of honey mesquite in environmentally-controlled glasshouses at near-optimal temperature and soil water conditions. Because the first weeks of growth are most critical to the establishment of honey mesquite (Polley et al. 2002a), we evaluated growth responses during this time period. In addition, CO₂ effects on plant growth may be transient (Poorter 1993; Centritto et al. 1999), disappearing after seedlings are just a few weeks old; therefore, we conducted multiple harvests during the first weeks of growth to discern the time-course of CO₂ responses through measurements of RGR (Polley et al. 2002b). Understanding the basic ecological responses to CO₂ enrichment of varieties of

honey mesquite seedlings from contrasting environments may assist in assessing the invasiveness of this noxious shrub in rangelands.

MATERIALS AND METHODS

Seeds were collected in late July 2000 from 3 individual trees (genotypes) from each of 2 sites: the Temple, Texas vicinity (87.9 cm mean annual precipitation [MAP]) and near the Texas–New Mexico border off Interstate 10 (24.7 cm MAP), which is about 1 050 km to the west of the Temple site. Caution was taken to ensure that these trees were at least 2 km from any major road. As noted previously, RAPD marker results of trees in the vicinity of our collection sites (but not our trees specifically) classified honey mesquite trees near the Texas–New Mexico border as the variety *torreyana* and trees from central Texas as the variety *glandulosa* (Pennington and Tischler, personal communication, 2004).

To prevent confounding influences of seed mass on initial seedling development (Villar et al. 1998), seeds of each genotype were weighed prior to planting to ensure similar seed mass within a genotype. On 14 June 2001, 2 seeds of each genotype were planted in each of 36 pots (each pot measuring 0.05 m in diameter × 1.60 m deep), for a total of 216 pots. Pots were constructed from polyvinyl chloride pipe cut longitudinally into 2 pieces of equal size to facilitate recovery of intact root systems. The 2 halves of each pot were taped together and secured at the base with a perforated cap. Pots were filled with a sandy loam soil (Alfisol, Udic Paleustalfs; Huckabee et al. 1977) with the following properties: pH = 7.1, organic carbon content = 0.57%, 76.2% sand, 16.2% silt, 7.6% clay, field capacity = 18% on a volumetric basis. Soil in the pots was wetted to drip prior to planting by adding half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Following planting, pots with each genotype were randomly assigned to 1 of 4 glasshouse bays. Two bays were maintained at ambient CO₂ and 2 at elevated CO₂, with 9 pots per genotype in each bay (2 varieties × 3 genotypes per variety × 2 CO₂ concentrations × 2 glasshouse bays × 9 pots/genotype/bay = 216 pots). Full-strength Hoagland's solution was added daily to each pot to maintain soil water content near field capacity. Seedlings were thinned to 1 per pot following emergence.

The CO₂ concentration of air in each of 4 glasshouse bays was measured at 4-minute intervals with a Li-Cor Model LI-6262 infrared gas analyzer (Li-Cor, Inc., Lincoln, NE). The CO₂ readings were corrected for atmospheric pressure measured with a Druck Model DPI 260 pressure indicator (Druck, Inc., New Fairfield, CT). The infrared analyzer was calibrated daily against 4 CO₂ gas standards and weekly against a Li-Cor LI-610 dewpoint generator (Li-Cor). Air temperature, manually set at 30°C for both day and night, was measured in the center of each bay with fine-wire (25- μ m diameter) thermocouples. Pure CO₂ gas was injected into appropriate bays as required to maintain the elevated CO₂ concentration. The CO₂ concentration of air in the ambient and elevated CO₂ treatments averaged 368 and 704 μ mol · mol⁻¹, respectively. Photosynthetic photon flux density (PPFD) was measured on the glasshouse roof with a quantum sensor (LI-190SB; Li-Cor) and within the glasshouse

Table 1. Mean (\pm 1 SE) above- and belowground responses at 8 days postemergence of 2 varieties of mesquite seedlings exposed to ambient and elevated CO₂ concentrations (368 and 704 $\mu\text{mol} \cdot \text{mol}^{-1}$, respectively).

Variable	CO ₂		% Increase ²	Variety	
	Ambient	Elevated		<i>torreyana</i>	<i>glandulosa</i>
Aboveground					
Cotyledon area (cm ²)	3.06 (0.12)	3.06 (0.12)	0	2.89 (0.12)	3.23 (0.12) ¹
Cotyledon mass (g)	0.017 (0.001)	0.023 (0.001) ¹	35	0.019 (0.001)	0.021 (0.001)
Leaf pair 1 area (cm ²)	3.15 (0.18)	3.39 (0.18)	8	3.16 (0.18)	3.38 (0.18)
Leaf pair 1 mass (g)	0.010 (0.001)	0.013 (0.001)	30	0.011 (0.001)	0.012 (0.001)
Other leaf mass (g)	0.014 (0.001)	0.017 (0.001)	21	0.015 (0.001)	0.016 (0.001)
Stem mass (g)	0.009 (0.001)	0.012 (0.001)	33	0.009 (0.001)	0.012 (0.001)
Total leaf mass (g)	0.040 (0.002)	0.053 (0.002) ¹	33	0.044 (0.002)	0.049 (0.002)
Aboveground mass (g)	0.049 (0.003)	0.065 (0.003) ¹	33	0.053 (0.003)	0.061 (0.003) ¹
Belowground					
Root depth (cm)	83 (3)	94 (3) ¹	13	86 (3)	90 (3)
Root length (cm)	139.9 (10.0)	150.9 (9.4)	8	135.5 (10.0)	155.4 (9.4)
Root surface area (cm ²)	23.2 (1.2)	25.6 (1.1)	10	22.8 (1.2)	26.0 (1.1) ¹
Root volume (m ³)	0.31 (0.02)	0.35 (0.01) ¹	13	0.30 (0.02)	0.35 (0.01) ¹
Root mass (g)	0.017 (0.001)	0.021 (0.001) ¹	24	0.016 (0.001)	0.022 (0.001) ¹
Whole plant					
Total plant mass (g)	0.064 (0.004)	0.086 (0.004) ¹	34	0.068 (0.004)	0.083 (0.004) ¹

¹Significant at the 0.05 level between ambient and elevated CO₂ means across varieties, and between variety means across [CO₂].

²Increase (%) of elevated [CO₂] over ambient [CO₂].

bays with 1-m-long line quantum sensors (LI-191SA; Li-Cor) mounted about plant height. On average, the daily integral of PPFD inside the glasshouse bays was 73% of that measured above the glasshouse.

Seedlings were harvested at 8, 16, and 24 days postemergence from each of the glasshouse bays. Aboveground biomass was separated into stem and leaf components with the area of each leaf blade measured using a LI-3000A portable leaf area meter (Li-Cor). Soil was manually washed from roots and the depth of deepest root penetration recorded. Roots were digitally scanned at high resolution (600 dpi) using the WinRHIZO software (version 4.1c; Regent Instruments, Inc., Quebec, Canada) and a Hewlett Packard ScanJet 6100C scanner to determine root length, surface area, and root volume. Roots were not stained prior to analyses, which results in underestimations (Bouma et al. 2000), but these were minimized by using the WinRHIZO automatic threshold (i.e., Lagarde's method) for pale roots with greater sensitivity. Aboveground tissues and roots were dried at 60°C for 72 hours prior to weighing. RGRs were calculated for each genotype for the 0–8-, 8–16-, 16–24-, and 0–24-day intervals using observed total biomass (aboveground + belowground) for the latter harvest date and the genotype mean for the previous harvest date. Average seed mass was used in calculations involving day 0.

Data were analyzed by harvest date using a split-split plot design with CO₂ as the split plot and variety as the split-split plot in a balanced design (SAS Institute Inc. 1997). The 0.05 level of probability was used for all analyses. Single degree of freedom contrasts were used to compare the 3 genotypes within each variety. When needed to normalize residuals, data were

logarithmically transformed before analysis; means and standard errors are reported after back-transforming.

RESULTS

Day 8 Harvest

CO₂ enrichment increased mass of cotyledons, leaves, and total aboveground plant material by 33%–35%; aboveground plant mass was 15% greater for seedlings of the *glandulosa* than for the *torreyana* variety (Table 1). CO₂ enrichment enhanced root depth, root volume, and root mass by 13%–24%; root surface area, root volume, and root mass were 14%–38% greater for the *glandulosa* than for the *torreyana* variety. Aboveground responses generally displayed greater increases (21%–35%) with CO₂ enrichment than did belowground responses (8%–24%). Total plant mass was 34% greater with CO₂ enrichment

Table 2. Mean (\pm 1 SE) relative growth rates (RGR, $\text{g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$) of 2 varieties of mesquite seedlings exposed to ambient and elevated CO₂ concentrations (368 and 704 $\mu\text{mol} \cdot \text{mol}^{-1}$, respectively).

Growth Interval	CO ₂		% Increase ²	Variety	
	Ambient	Elevated		<i>torreyana</i>	<i>glandulosa</i>
0–8 days	0.049 (0.006)	0.082 (0.006) ¹	67	0.057 (0.007)	0.075 (0.006) ¹
8–16 days	0.121 (0.005)	0.148 (0.005) ¹	22	0.132 (0.007)	0.138 (0.005)
16–24 days	0.080 (0.008)	0.129 (0.008) ¹	61	0.104 (0.008)	0.107 (0.011)
0–24 days	0.095 (0.003)	0.112 (0.003) ¹	18	0.102 (0.004)	0.105 (0.003)

¹Significant at the 0.05 level between ambient and elevated CO₂ means across varieties, and between variety means across [CO₂].

²Increase (%) of elevated [CO₂] over ambient [CO₂].

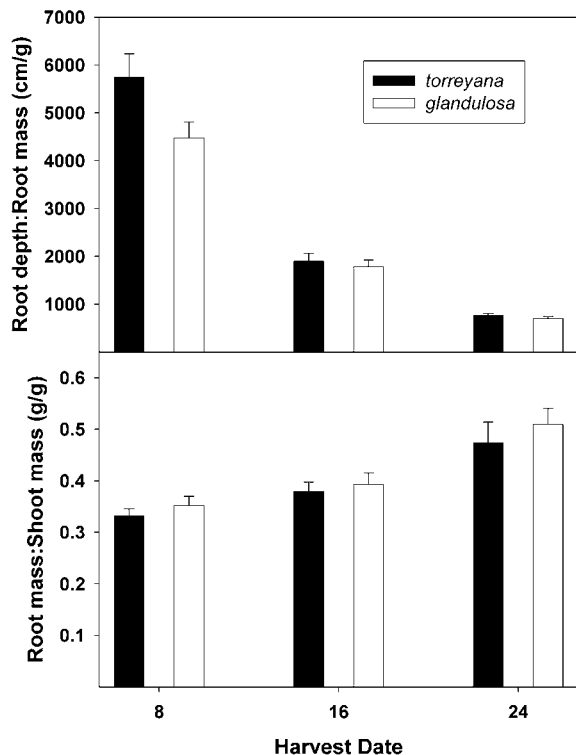


Figure 1. Mean (± 1 SE) root-depth to root-mass ratios (upper panel) and root-mass to shoot-mass ratios (lower panel) at 8, 16, and 24 days postemergence of 2 varieties of mesquite seedlings averaged over ambient and elevated CO₂ concentrations.

and 22% greater for *glandulosa* than for *torreyana* seedlings. Concurrent with this finding, RGR (Days 0–8) was 67% greater with CO₂ enrichment and 32% greater in *glandulosa* than in *torreyana* seedlings (Table 2). Root-depth to root-mass ratios, averaged over CO₂ treatments, were greater for *torreyana* than for *glandulosa* seedlings, but root-mass to shoot-mass ratios did not differ between varieties (Fig. 1). Significant interactions between CO₂ and the 2 varieties were not observed for any variable, indicating that growth responses to CO₂ enrichment were similar for seedlings of both varieties. There were no significant differences for above- or below-ground growth responses among the 3 genotypes from each variety (data not shown).

Day 16 Harvest

In contrast to the Day 8 harvest, CO₂ enrichment caused greater percentage increases in belowground variables (36%–40%) other than root depth than in aboveground variables (10%–35%) (Table 3). CO₂ enrichment increased stem, total leaf, and aboveground plant mass by 23%–35%. In general, the magnitude of increases with CO₂ enrichment for aboveground variables was less than those observed for the Day 8 harvest. Aboveground plant mass was 12% greater for *glandulosa* than for *torreyana* seedlings. CO₂ enrichment enhanced root length, root surface area, root volume, and root mass by 36%–40%. The magnitude of CO₂-induced increases for root variables was greater at the Day 16 harvest than at the Day 8 harvest. Rooting depth and root mass were 14%–20% greater for the *glandulosa* than for the *torreyana* variety. Total plant mass

increased by 27% with CO₂ enrichment and was 14% greater in *glandulosa* than in *torreyana* seedlings. RGR (Days 8–16) was 22% greater with CO₂ enrichment, but did not differ between varieties (Table 2). Root-depth to root-mass and root-mass to shoot-mass ratios, averaged over CO₂ treatments, did not differ between varieties, but were lower and higher, respectively, than in the Day 8 harvest (Fig. 1). Consistent with the Day 8 harvest, significant interactions between CO₂ and the 2 varieties were not observed for any variable, and differences were not observed for any variable among the 3 genotypes from each variety (data not shown).

Day 24 Harvest

In contrast to the 2 previous harvests, percentage increases with CO₂ enrichment were similar for both aboveground (13%–68%) and belowground (10%–40%) variables, and CO₂ enrichment increased all variables (Table 4). In general, the magnitude of increases with CO₂ enrichment was greater for aboveground variables, and similar for belowground variables, compared to the Day 16 harvest. Roots of seedlings of the *glandulosa* variety were longer and heavier, exhibited greater surface area, and extended deeper in pots compared to roots of the *torreyana* variety seedlings. Total plant mass increased by 50% with CO₂ enrichment, but did not differ between varieties. Concurrent with this, RGR (Days 16–24) was 61% greater with CO₂ enrichment and did not differ between varieties, whereas RGR over the entire experiment (Days 0–24) was 18% greater with CO₂ enrichment. Consistent with Day 16 harvest, root-depth to root-mass and root-mass to shoot-mass ratios, averaged over CO₂ treatments, did not differ between varieties (Fig. 1). Consistent with the 2 previous harvests, significant interactions between CO₂ and the 2 varieties were not observed for any variable, and differences were not observed for any growth response among the 3 genotypes from each variety (data not shown).

DISCUSSION

Both varieties of honey mesquite responded to CO₂ enrichment with enhanced growth. Seedlings of the variety *glandulosa* had greater biomass and a more developed root system compared to the variety *torreyana* at the first 2 harvests (Days 8 and 16). However, these differences were attributed to inherent variation between varieties and not to elevated CO₂. The absence of significant CO₂ × variety interactions at any harvest date provides evidence that CO₂ enrichment did not exaggerate differences in above- and belowground responses between varieties in this glasshouse study under near-optimal temperature and soil water conditions. Therefore, our null hypothesis that CO₂ enrichment does not preferentially increase seedling growth of the honey mesquite variety from mesic environments was not rejected. These results suggest that varietal differences in rooting and other characteristics did not modify the size advantage of *glandulosa* over *torreyana* varieties, as the absolute differences in sizes did not change as a function of CO₂ treatment. Our assumption that growth of plants from mesic environments is greater compared to those from arid environments was supported only over the 8 days following seedling emergence.

Table 3. Mean (\pm 1 SE) above- and belowground responses at 16-days postemergence of 2 varieties of mesquite seedlings exposed to ambient and elevated CO₂ concentrations (368 and 704 $\mu\text{mol} \cdot \text{mol}^{-1}$, respectively).

Variable	CO ₂		% Increase ²	Variety	
	Ambient	Elevated		<i>torreyana</i>	<i>glandulosa</i>
Aboveground					
Leaf pair 1 area (cm ²)	6.56 (0.33)	7.30 (0.30) ¹	11	7.31 (0.30)	6.55 (0.33)
Leaf pair 1 mass (g)	0.038 (0.002)	0.051 (0.002) ¹	34	0.046 (0.002)	0.043 (0.002)
Other leaf area (cm ²)	8.12 (0.82)	8.99 (0.75)	11	8.10 (0.75)	9.01 (0.82)
Other leaf mass (g)	0.046 (0.004)	0.051 (0.003)	11	0.045 (0.003)	0.053 (0.004)
Stem mass (g)	1.046 (0.039)	1.412 (0.057) ¹	35	1.403 (0.056)	1.048 (0.048)
Total leaf area (cm ²)	17.74 (1.14)	19.48 (1.04)	10	18.43 (1.04)	18.78 (1.13)
Total leaf mass (g)	0.107 (0.006)	0.132 (0.005) ¹	23	0.114 (0.006)	0.124 (0.006)
Aboveground mass (g)	0.146 (0.008)	0.180 (0.007) ¹	23	0.153 (0.007)	0.172 (0.008) ¹
Belowground					
Root depth (cm)	108 (5)	110 (5)	1	102 (5)	116 (5) ¹
Root length (cm)	179.5 (12.2)	251.6 (11.9) ¹	40	215.4 (11.9)	215.6 (12.2)
Root surface area (cm ²)	31.5 (1.8)	43.1 (1.7) ¹	37	36.9 (1.7)	37.7 (1.7)
Root volume (m ³)	0.46 (0.03)	0.63 (0.03) ¹	37	0.53 (0.03)	0.56 (0.03)
Root mass (g)	0.055 (0.003)	0.075 (0.003) ¹	36	0.059 (0.003)	0.071 (0.003) ¹
Whole plant					
Total plant mass (g)	0.201 (0.011)	0.255 (0.010) ¹	27	0.213 (0.009)	0.243 (0.011) ¹

¹Significant at the 0.05 level between ambient and elevated CO₂ means across varieties, and between variety means across [CO₂].

²Increase (%) of elevated [CO₂] over ambient [CO₂].

Growth enhancement of honey mesquite with CO₂ enrichment was greater for aboveground variables at the first (Day 8) harvest, greater for belowground variables at the second (Day 16) harvest, and similar for both above- and belowground

variables at the last (Day 24) harvest. These differences in temporal enhancement of plant growth associated with CO₂ enrichment suggest changing carbon allocation priorities. Initial carbon investment was allocated primarily aboveground to

Table 4. Mean (\pm 1 SE) above- and belowground responses at 24-days postemergence of 2 varieties of mesquite seedlings exposed to ambient and elevated CO₂ concentrations (368 and 704 $\mu\text{mol} \cdot \text{mol}^{-1}$, respectively).

Variable	CO ₂		% Increase ²	Variety	
	Ambient	Elevated		<i>torreyana</i>	<i>glandulosa</i>
Aboveground					
Leaf pairs	9.2 (0.5)	10.7 (0.5) ¹	16	9.4 (0.5)	10.6 (0.5)
Leaf pair 2&3 area (cm ²)	7.03 (0.36)	7.95 (0.34) ¹	13	7.51 (0.36)	7.48 (0.34)
Leaf pair 2&3 mass (g)	0.045 (0.003)	0.053 (0.002) ¹	18	0.050 (0.003)	0.048 (0.003)
Other leaf area (cm ²)	18.93 (4.10)	31.83 (3.83) ¹	68	25.77 (4.10)	24.99 (3.83)
Other leaf mass (g)	0.103 (0.021)	0.170 (0.020) ¹	65	0.139 (0.022)	0.135 (0.021)
Stem mass (g)	0.100 (0.013)	0.160 (0.012) ¹	60	0.111 (0.013)	0.151 (0.013) ¹
Total leaf area (cm ²)	37.04 (4.41)	52.87 (4.21) ¹	43	47.80 (4.60)	42.11 (4.01)
Total leaf mass (g)	0.213 (0.23)	0.311 (0.023) ¹	46	0.278 (0.024)	0.246 (0.021)
Aboveground mass (g)	0.314 (0.033)	0.480 (0.032) ¹	53	0.397 (0.034)	0.397 (0.031)
Belowground					
Root depth (cm)	114 (3)	126 (3) ¹	10	116 (4)	125 (3) ¹
Root length (cm)	364.6 (31.6)	488.3 (28.9) ¹	34	379.7 (31.0)	473.2 (29.5) ¹
Root surface area (cm ²)	67.8 (4.9)	87.8 (4.5) ¹	29	70.9 (4.8)	84.7 (4.6) ¹
Root volume (m ³)	1.03 (0.08)	1.35 (0.07) ¹	31	1.10 (0.08)	1.28 (0.07)
Root mass (g)	0.146 (0.011)	0.205 (0.011) ¹	40	0.159 (0.011)	0.191 (0.010) ¹
Whole plant					
Total plant mass (g)	0.461 (0.041)	0.691 (0.040) ¹	50	0.562 (0.043)	0.589 (0.038)

¹Significant at the 0.05 level between ambient and elevated CO₂ means across varieties, and between variety means across [CO₂].

²Increase (%) of elevated [CO₂] over ambient [CO₂].

develop photosynthetic machinery (leaves) as rapidly growing plants invest heavily in leaves (Tilman 1988; Poorter and Remkes 1990). Allocation priorities switched following the first harvest to increased carbon investment in roots for enhancing water and nutrient uptake.

The absence of variety-specific responses to CO₂ enrichment in this investigation, despite differences such as greater root-depth to root-mass ratios for *torreyana* than for *glandulosa* at the Day 8 harvest, is consistent with a previous experiment evaluating genotype-specific growth responses of *P. glandulosa* var. *glandulosa* seedlings grown from seeds collected along an east-west precipitation gradient (36–93 cm MAP) in Texas (Derner et al. 2004). These results contradict literature supporting variety- or genotype-specific responses to CO₂ enrichment for other C₃ species, including grasses (Goverde et al. 2002; Roumet et al. 1999, 2002), forbs (Andalo et al. 2001), and shrubs (Atkin et al. 1999). Although this leguminous shrub does not conform to responses exhibited by other C₃ plants, it is unclear whether this inconsistency results from inherent species characteristics, such as the ability to fix nitrogen, or from soil nutrient and water differences between investigations. For example, CO₂ enrichment increased growth more in tree seedlings of xeric than of mesic habitats when soils were dry, but when soils were wet, growth of seedlings from mesic habitats was greater (Catovsky and Bazzaz 1999). Conversely, similar growth responses to CO₂ enrichment irrespective of whether soil water was limiting or abundant have been shown in C₃ and C₄ crop monocultures in a field setting (Derner et al. 2003), and in C₄ grass seedlings in a glasshouse experiment (Derner et al. 2001).

Substantial CO₂-induced growth increases in above- and belowground variables of *Prosopis* seedlings are in agreement with previous investigations with honey mesquite (Polley et al. 1994, 1996, 1999), *Pinus* spp. (Jach and Ceulemans 1999; Runion et al. 1999), and other woody species (Cornelissen et al. 1999). Both the absolute and relative responses of aboveground, belowground, and total biomass of honey mesquite seedlings to CO₂ enrichment increased from the first to last harvest date of these seedlings, although the effects of CO₂ on growth previously have been shown to diminish over extended time periods (i.e., years) (Idso 1999; Jach and Ceulemans 1999). Observed decreases in RGR with increasing plant size are consistent with previous investigations (Jarvis and Jarvis 1964; Bazzaz et al. 1993; Cornelissen et al. 1998). The RGR for both varieties was similar over the 24-day period, resulting in similar total biomass in spite of significantly greater RGR for *glandulosa* at the Day 8 harvest. Lack of a significant RGR response by Day 24 indicates that the *torreyana* variety compensated for lower RGR at Day 8. This compensation occurred due to greater increases in aboveground growth in *torreyana* from Day 16 to Day 24. These increases overshadowed the increasing greater root mass in *glandulosa* relative to *torreyana* throughout the 24-day sample period.

A main effect of CO₂ enrichment on plant growth is increased ontogeny (e.g., Centritto et al. 1999), which is likely responsible for the rapid response to CO₂ enrichment displayed by honey mesquite in this investigation. For example, roots extended 13% deeper with CO₂ enrichment 8 days following emergence. Ecological implications of this greater root depth include access to greater soil water and nutrient supplies, which

combined with the marked CO₂-induced aboveground responses after 8 days, appears to confer a competitive advantage to honey mesquite seedlings regarding successful establishment, assuming that C₃ or C₄ grass seedlings do not respond as vigorously to CO₂ enrichment. Thus, we should expect that this species will continue aggressive encroachment into grasslands in future CO₂-enriched environments, because C₄ grasses that currently dominate these grasslands are not as responsive to elevated CO₂ as is honey mesquite (Polley et al. 1994).

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LITERATURE CITED

- ANDALO, C., I. GOLDRINGER, AND B. GODELLE. 2001. Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. *Ecology* 82:157–164.
- ANSLEY, J. R., X. B. WU, AND B. A. KRAMP. 2001. Observation: long-term increases in mesquite canopy cover in a north Texas savanna. *Journal of Range Management* 54:171–176.
- ARCHER, S. 1989. Have southern Texas savannas been converted to woodlands in recent history? *American Naturalist* 134:545–561.
- ARCHER, S. 1990. Development and stability of grass/woody mosaics in a subtropical savanna parkland, Texas, U.S.A. *Journal of Biogeography* 17:453–462.
- ARCHER, S. 1995. Tree-grass dynamics in a *Prosopis*-thornscrub savanna parkland: reconstructing the past and predicting the future. *Ecoscience* 2:83–99.
- ATKIN, O. K., M. SCHORTEMAYER, N. MCFARLANE, AND J. R. EVANS. 1999. The response of fast- and slow-growing *Acacia* species to elevated atmospheric CO₂: an analysis of the underlying components of relative growth rate. *Oecologia* 120:544–554.
- BAZZAZ, F. A., S. L. MIAO, AND P. M. WAYNE. 1993. CO₂-induced growth enhancements of co-occurring tree species decline at different rates. *Oecologia* 96:478–482.
- BOND, W. J., AND G. F. MIDGLEY. 2000. A proposed CO₂-controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* 6:865–869.
- BOUMA, T. J., K. L. NIELSEN, AND B. KOUTSTALL. 2000. Sample preparation and scanning protocol for computerized analysis of root length and diameter. *Plant and Soil* 218:185–196.
- BUNCE, J. A. 1997. Variation in growth stimulation by elevated carbon dioxide in seedlings of some C₃ crop and weeds species. *Global Change Biology* 3:61–66.
- BURKHART, A. 1976. A monograph of the genus *Prosopis*. *Journal of Arnold Arboretum* 57:450–525.
- CATOVSKY, S., AND F. A. BAZZAZ. 1999. Elevated CO₂ influences the response of two birch species to soil moisture: implications for forest community structure. *Global Change Biology* 5:507–518.
- CENTRITTO, M., H. S. J. LEE, AND P. G. JARVIS. 1999. Increased growth in elevated [CO₂]: an early, short-term response? *Global Change Biology* 5:623–633.
- CHAPIN, F. S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11:233–260.
- CORNELISSEN, J. H. C., A. L. CARNELLI, AND T. V. CALLAGHAN. 1999. Generalities in the growth, allocation and leaf quality responses to elevated CO₂ in eight woody species. *New Phytologist* 141:401–409.
- CORNELISSEN, J. H. C., P. CASTRO-DIEZ, AND A. L. CARNELLI. 1998. Variation in relative growth rate among woody species, *In*: H. Lambers, H. Poorter, M. M. I. van Vuuren [eds.]. Inherent variation of plant growth: physiological mechanisms and ecological consequences. Leiden, The Netherlands: Backhuys Publishers. p 363–392.
- DERNER, J. D., H. B. JOHNSON, B. A. KIMBALL, P. J. PINTER, JR, H. W. POLLEY, C. R.

- TISCHLER, T. W., BOUTTON, R. L., LAMORTE, G. W., WALL, N. R., ADAM, S. W., LEAVITT, M. J., OTTMAN, A. D., MATTHIAS, AND T. J. BROOKS. 2003. Above- and below-ground responses of C₃-C₄ species mixtures to elevated CO₂ and soil water availability. *Global Change Biology* 9:452–460.
- DERNER, J. D., H. W. POLLEY, H. B. JOHNSON, AND C. R. TISCHLER. 2001. Root system response of C₄ grass seedlings to CO₂ and soil water. *Plant and Soil* 231: 97–104.
- DERNER, J. D., C. R. TISCHLER, H. W. POLLEY, AND H. B. JOHNSON. 2004. Effects of elevated CO₂ on growth responses of honey mesquite (*Prosopis glandulosa* var. *glandulosa*) seedlings from sites along a precipitation gradient. In: A. L. Hild, N. L. Shaw, S. E. Meyer, E. W. Schupp, and T. Booth [eds.]. Proceedings—Seed and soil dynamics in shrubland ecosystems. Proceedings RMRS-P-31. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p 156–160.
- HILU, Y. W., S. BOYD, AND P. FELKER. 1982. Morphological diversity and taxonomy of California mesquites (*Prosopis*, Leguminosae). *Madrono* 29:237–254.
- HOAGLAND, D. R., AND D. I. ARNON. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347. 39 p.
- HUCKABEE, J. W., JR., D. R. THOMPSON, J. C. WYRICK, AND E. G. PAULAT. 1977. Soil Survey Bell County Texas. Washington, D.C.: USDA Soil Conservation Service, 77 p.
- GOVERDE, M., J. A. ARNONE III, AND A. ERHARDT. 2002. Species-specific reactions to elevated CO₂ and nutrient availability in four grass species. *Basic and Applied Ecology* 3:221–227.
- LDISO, S. B. 1999. The long-term response of trees to atmospheric CO₂ enrichment. *Global Change Biology* 5:493–495.
- JACH, M. E., AND R. CEULEMANS. 1999. Effects of elevated atmospheric CO₂ on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field. *Tree Physiology* 19: 289–300.
- JARVIS, P. G., AND M. S. JARVIS. 1964. Growth rates of woody plants. *Physiologia Plantarum* 17:654–666.
- JOEL, G., F. S. CHAPIN III, N. R. CHIARIELLO, S. S. THAYER, AND C. B. FIELD. 2001. Species-specific responses of plant communities to altered carbon and nutrient availability. *Global Change Biology* 7:435–450.
- LAMBERS, H., AND H. POORTER. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23:187–261.
- PENNINGTON, R. E., C. R. TISCHLER, H. B. JOHNSON, AND H. W. POLLEY. 1999. Genetic variation for carbon isotope composition in honey mesquite (*Prosopis glandulosa*). *Tree Physiology* 19:583–589.
- POLLEY, H. W. 1997. Invited synthesis paper: implications of rising atmospheric carbon dioxide concentration for rangelands. *Journal of Range Management* 50:562–577.
- POLLEY, H. W., H. B. JOHNSON, AND H. S. MAYEUX. 1994. Increasing CO₂: comparative responses of the C₄ grass *Schizachyrium* and grassland invader *Prosopis*. *Ecology* 75:976–988.
- POLLEY, H. W., H. B. JOHNSON, H. S. MAYEUX, C. R. TISCHLER, AND D. A. BROWN. 1996. Carbon dioxide enrichment improves growth, water relations and survival of droughted honey mesquite (*Prosopis glandulosa*) seedlings. *Tree Physiology* 16:817–823.
- POLLEY, H. W., H. B. JOHNSON, AND C. R. TISCHLER. 2002a. Woody invasion of grasslands: evidence that CO₂ enrichment indirectly promotes establishment of *Prosopis glandulosa*. *Plant Ecology* 164:85–94.
- POLLEY, H. W., H. S. MAYEUX, H. B. JOHNSON, AND C. R. TISCHLER. 1997. Viewpoint: atmospheric CO₂ soil water, and shrub/grass ratios on rangelands. *Journal of Range Management* 50:278–284.
- POLLEY, H. W., C. R. TISCHLER, H. B. JOHNSON, AND J. D. DERNER. 2002b. Growth rate and survivorship of drought: CO₂ effects on the presumed tradeoff in seedlings of five woody legumes. *Tree Physiology* 22:383–391.
- POLLEY, H. W., C. R. TISCHLER, H. B. JOHNSON, AND R. E. PENNINGTON. 1999. Growth, water relations, and survival of drought-exposed seedlings from six maternal families of honey mesquite (*Prosopis glandulosa*): responses to CO₂ enrichment. *Tree Physiology* 19:359–366.
- POORTER, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio* 104/105:77–97.
- POORTER, H., AND C. REMKES. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* 83:553–559.
- ROUMET, C., G. LAURENT, G. CANIVENC, AND J. ROY. 2002. Genotypic variation in the response of two perennial grass species to elevated carbon dioxide. *Oecologia* 133:342–348.
- ROUMET, C., G. LAURNET, AND J. ROY. 1999. Leaf structure and chemical composition as affected by elevated CO₂: genotypic responses of two perennial grasses. *New Phytologist* 143:73–81.
- RUNION, G. B., J. A. ENTRY, S. A. PRIOR, R. J. MITCHELL, AND H. H. ROGERS. 1999. Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO₂ and water stress. *Tree Physiology* 19: 329–335.
- SAS INSTITUTE, INC. 1997. SAS/STAT Software: Changes and enhancements through Release 6. 12. Cary, NC: SAS Institute, Inc. 1176 p.
- TILMAN, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton, NJ: Princeton University Press. 376 p.
- VAN AUKEN, O.W. 2000. Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics* 31:197–215.
- VILLAR, R., E. J. VENEKLAAS, P. JORDANO, AND H. LAMBERS. 1998. Relative growth rate and biomass allocation in 20 *Aegilops* (Poaceae) species. *New Phytologist* 140:425–437.