

Environment and seedling age influence mesquite response to epicotyl removal

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Abstract

Herbivory by small mammals is a major factor controlling survival of honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*) seedlings. Clipping below the cotyledons is lethal; removal of the epicotyl may not be lethal but can severely limit seedling growth. Seedlings of other woody species sometimes compensate for epicotyl removal by prolonging the life of cotyledons. Also, projected future increases in atmospheric CO₂ concentration could influence survival and growth after epicotyl removal. Objectives of this study were to determine effects of epicotyl removal at various seedling ages, atmospheric CO₂ concentrations, and soil fertility, on (1) seedling survival, (2) cotyledonary leaf longevity, and (3) shoot and root growth of young seedlings. Mesquite seedlings were grown at 350, 700, and 1,000 μL liter⁻¹ atmospheric CO₂ concentration in nutrient poor and nutrient rich soils. All ages of seedlings survived epicotyl removal. Cotyledonary leaf fresh mass and chlorophyll content were higher in plants where epicotyls were clipped. Root and shoot mass of both clipped and unclipped plants generally increased at higher CO₂ concentrations when mineral nutrition was adequate, but responded less to CO₂ when soil fertility was low. Responses to epicotyl clipping in mesquite seedlings are complex, being strongly influenced by soil fertility, atmospheric CO₂ concentration, seedling age at clipping, and interactions between these factors.

Key Words: chlorophyll content, cotyledonary leaves, herbivory, *Prosopis glandulosa* Torr. var. *glandulosa*, mineral nutrition, CO₂ enrichment.

Honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*) has increased in density over vast areas of rangeland throughout the Southwestern United States (Buffington and Herbel 1965). Seed germinate across a wide range of conditions, so recruitment is high when conditions are conducive to establish (Scifres and Brock 1969). Seedlings grow rapidly and have rapid rates of taproot extension (Mooney et al. 1977). Collectively these traits permit mesquite to increase in abundance when a seed source is available, even on well-managed rangeland (Gibbens et al. 1992).

Despite the success of mesquite, germinating seedlings apparently face several obstacles to successful establishment. The seedling may be exposed to livestock trampling or herbivory by small mammals or insects. Because mesquite is an "epigeous" legume (emergence occurs because of elongation of the

Resumen

Los hábitos herbívoros de pequeños mamíferos es uno de los factores más importantes en la supervivencia de las plántulas de "mesquite" (*Prosopis glandulosa* Torr. Var. *glandulosa*). Podar las plántulas por debajo de los cotiledones es letal para ellas. La remoción del epicotiledón no es necesariamente fatal, pero puede limitar seriamente el desarrollo de las plántulas. Las plántulas de 8 especies leñosas compensan la remoción del epicotiledón, prolongando la vida de los cotiledones. Además, los incrementos esperados en concentración de CO₂ atmosférico, podrían influir en la supervivencia y desarrollo de las plántulas después de la remoción del epicotiledón. Los objetivos de este estudio fueron la determinación del efecto de remoción del epicotiledón en varias edades de las plántulas, en varias concentraciones de CO₂ atmosféricos y en varias fertilidades del suelo con respecto a 1) la supervivencia de las plántulas, 2) la longevidad de la hoja cotiledoneal y 3) el desarrollo de las raíces y la parte aérea de las plántulas. Plántulas de "mesquite" fueron desarrolladas en concentraciones de CO₂ atmosférico de 350, 700, y 1,000 μL L⁻¹ y en suelos de alta y baja fertilidad. Las plántulas de todas las edades sobrevivieron la remoción del epicotiledón. El peso fresco de las hojas cotiledoneales y el contenido de clorofila fueron mayores en plántulas donde el epicotiledón fue cortado. El peso de las raíces y de la parte aérea tanto en plantas podadas como en las no podadas, generalmente aumentó en altas concentraciones de CO₂ atmosférico cuando la nutrición mineral fue adecuada, pero las plántulas respondieron menos a la concentración de CO₂ cuando la fertilidad del suelo fue baja. Las respuestas de las plántulas de "mesquite" a la poda del epicotiledón son complejas. Estas son fuertemente influenciadas por la fertilidad del suelo, la concentración de CO₂ atmosférico, la edad de la plántula en el momento de la poda, y las interacciones entre los anteriores factores.

hypocotyl), "...the plant can be eliminated by a single cut below the cotyledons..." (Silcock 1980). Less severe pruning may not be lethal. Scifres and Hahn (1971), for example, found that 60% of 7-day-old mesquite seedlings survived after epicotyl removal. Gibbens et al. (1992) reported that 79 of 100 seedlings that germinated on the Jornada Experimental Range in July 1989 were killed by lagomorph browsing. Of the seedlings that survived to the following May, all had been bitten off near the cotyledonary node, and shoot growth occurred from buds present in the axil of the cotyledonary leaf petiole. Paulsen (1950) also indicated that

browsing by rodents was the major cause of mesquite seedling mortality.

Woolhouse (1967) reported that removal of portions of a plant above senescing leaves (including cotyledonary leaves) can reverse or delay senescence of those leaves. Neither Scifres and Hahn (1971) nor Gibbens et al. (1992) determined if clipping the epicotyl of young mesquite seedlings modified the longevity of cotyledonary leaves.

Predicted increases in atmospheric CO₂ could modify competitive relationships between C₃ invaders such as mesquite and resident C₄ grasses by preferentially stimulating growth in C₃ species (Mayeux et al. 1991, Johnson et al. 1993). Seedling response to CO₂, however, may depend strongly on the availability of mineral nutrients (Bazzaz and Miao 1993) as well as cotyledon size (Radoglou and Jarvis 1993). Radoglou and Jarvis (1993), for example, found that *Vicia faba* L. showed no response to elevated CO₂ during 45 days after germination, while positive responses were noted in the smaller-seeded *Phaseolus vulgaris* L. by 28 days after emergence (Radoglou and Jarvis 1992). This difference in the timing of response to CO₂ was attributed to differences in amounts of storage materials in cotyledons.

The predicted doubling of atmospheric CO₂ by the middle of the next century (Trabalka et al. 1985) could lead to a significant increase in density of mesquite if it stimulates seedling growth and survival following herbivory. Any effect of higher CO₂, however, likely will depend on other environmental factors. The objectives of this study were to determine effects of CO₂ concentration, soil fertility, and seedling age at epicotyl removal, on shoot and root growth, cotyledonary leaf longevity, and seedling survival of honey mesquite.

Materials and Methods

Seeds for these experiments were harvested from a single, isolated mesquite tree located approximately 15 miles south of Temple, Tex. in August 1989. We utilized half-sib seed for the following reasons. Firstly, we have observed that mean seed mass varies by as much as 41% between different maternal trees, and in many cases, final cotyledonary leaf area is correlated with seed mass (C.R. Tischler and R.E. Pennington, unpublished observations). Secondly, numbers of leaflets on the first true leaf often vary between half-sib families. By utilizing half-sib seed, we therefore eliminated these sources of experimental error from our protocol. Obviously, genetic differences in leaf area (as would have been experienced had we used a composite seed sample) would have strongly influenced early seedling growth, irrespective of atmospheric CO₂ concentration. Furthermore, in separate comparative studies, seedlings of this particular genotype appeared typical of "normal" mesquite seedlings. Seed pods were refrigerated until April 1994, when seeds were separated from the pods and scarified by removing a small section of the testa with a razor blade. All seedling growth experiments were conducted in air-conditioned glasshouses at Temple, Tex. Temperatures were maintained at approximately 25° C day and 18° C night. Three adjacent glasshouse bays were employed, in which CO₂ concentrations were constantly maintained near "target" concentrations of ambient (365), 700, or 1,000 mL liter⁻¹ by automatic injection. Actual values averaged over the course of the described experiments were 360.6, 679.5, and 1,038.2 mL liter⁻¹. Mean daytime air temperature for the 3 greenhouse bays were 26.2, 26.0, and

26.2° C, respectively, for the period of the experiments. Photosynthetically active radiation (PAR) was monitored by sensors placed approximately 1.5 m above plant level in each of the bays. Daily mean integrals of light intercepted in the 3 bays were 23.88, 23.73, and 23.40 mol quanta m⁻² day⁻¹. Mean daytime vapor pressure deficit averaged 1.13, 1.09, and 0.98 KPa, respectively, for the 3 greenhouse bays. Soil was an alluvial silty clay loam from a deeply buried stratum near the Colorado River at Austin, Tex. It is not identified according to normal soil classification schemes. This soil was chosen because of its extremely low nitrogen content (0.041%).

Experiment 1

The purpose of this experiment was to determine relationships between epicotyl removal, CO₂ concentration, and plant response in a nutrient-limited situation. Mesquite seedlings were grown in containers made from 5.1 cm diameter polyvinyl chloride (PVC) pipe cut longitudinally with a band saw. The lower 1 cm of the pipe was not cut, but a radial cut was made to meet the longitudinal cut on one side. This left an intact collar on 1 pipe half, which was glued into a threaded adapter fitting. A pipe cap (with drilled drainage holes covered with plastic screen) was screwed onto the threaded adapter. The other half of the PVC pipe was attached to its matching surface with duct tape, to create a container approximately 4 cm in diameter and 67 cm in length. At harvest, the tape and 1 of the longitudinal halves was removed, exposing an intact soil column.

In late April 1994, 27 containers were placed in each of the 3 controlled-CO₂ glasshouses. A commercial *Rhizobium* inoculum was added to each container, and 2 scarified seeds were planted per container. Soil was wetted to drip with distilled water initially and throughout the experiment (no fertilizer was added to the nutrient-poor soil). Within 3 days of emergence, extra seedlings were destroyed, leaving only one seedling per container. At 7 and 12 days after emergence, epicotyls of 9 plants selected at random in each CO₂ treatment were clipped about 1 mm above the point of attachment of the cotyledonary petioles. Care was taken not to damage the buds in the axils of the cotyledons. An additional 9 plants served as an unclipped control for the 7-day clipping treatment. All plants were harvested 2 weeks after clipping. Number of nodules, fresh mass of roots, shoots, cotyledons, and regrowth (clipping treatments), and cotyledonary leaf chlorophyll content were measured and recorded.

Chlorophyll was extracted with warm dimethyl sulfoxide and determined as described by Tischler and Voigt (1983), using equations presented by Hiscox and Israelstam (1979). Because cotyledons from which chlorophyll had been extracted could not be used for dry mass determinations, only fresh masses of seedling components were measured.

Experiment 2

The purpose of Experiment 2 was the same as Experiment 1, except that plant response to epicotyl removal was tested when mineral nutrition was adequate. All containers were watered with full strength Hoagland's solution initially and as needed to maintain soil moisture. No inoculum was added to the soil. Seedlings emerged on 25 and 26 May 1994. Clipping treatments and harvest procedures followed those of Experiment 1. An additional 9 PVC containers per glasshouse served as controls for the 12-day clipping treatment.

Experiment 3.

Because all seedlings survived the clipping treatments in Experiment 1, Experiment 3 was performed to determine the effects of clipping at younger seedling age and was conducted at the same time as Experiment 2. Epicotyls were clipped at 2, 4, and 6 days after emergence. To conserve space, seedlings were grown singly in 4- X 21-cm plastic cones. As in Experiment 2, cones were watered with full strength Hoagland's solution. At harvest (14 days after clipping), we determined regrowth dry mass.

Although the CO₂ greenhouse environments were not replicated, we assume that the within-greenhouse variances were similar to among-greenhouse variances. Temperature, PAR, and vapor pressure deficit were very similar between the greenhouses (as previously mentioned). Frequent watering insured that water stress was not a confounding factor in any of the experiments. Tubes or cones were frequently rearranged within each greenhouse bay, and samples were randomly picked from the population of containers. Where applicable, data were analyzed with 2 way ANOVAs, with CO₂ concentrations and clipping as main factors (CO₂ concentration and age in Experiment 3). Mean separation tests were used to establish differences between treatment means. For the chlorophyll and cotyledon mass data in Experiment 2, the GLM procedure of SAS was utilized because of unequal numbers of replications. The log(1+x) transformation was applied to nodule number data before analysis to correct for nonhomogeneous variances. Each plant within a treatment group was considered a replication.

Results

Experiment 1.

The overall ANOVA for the 7-day-post-emergence portion of the experiment indicated significant effects of clipping on root, shoot, and cotyledon weight and chlorophyll content of cotyledons. CO₂ effects were not significant, and the clipping by CO₂ interaction was significant only for root mass. Comparisons of seedlings clipped 7-days-post emergence with control seedlings after 21 days of growth in unfertilized soil are presented in Table 1. Numbers of nodules tended to be lower in clipped than control seedlings at each CO₂ concentration, although not greatly so (p = 0.096, 0.16, and 0.123, in order of increasing CO₂). Shoot fresh mass was significantly lower in clipped plants. Root fresh mass was significantly lower in clipped seedlings only at 1,000 mL

liter⁻¹ CO₂. Mass of regrowth and total shoot mass (all above-ground structures) did not differ between the CO₂ treatments. Cotyledon fresh mass among clipped plants was significantly higher at 1,000 than 365 mL liter⁻¹ CO₂. At each CO₂ concentration, chlorophyll contents of cotyledons were significantly greater in clipped than unclipped plants (p = 0.0001, 0.0001, and 0.0003 in order of decreasing CO₂). In all atmospheres, cotyledons of clipped plants were heavier than those of unclipped plants, with the difference being significant at 700 uL liter⁻¹ (p= 0.0007). Few differences were observed among the CO₂ treatments in unfertilized seedlings clipped 12 days after emergence (not shown). However, shoot fresh mass of clipped plants grown at 1000 mL liter⁻¹ was significantly greater (309 mg) than for plants grown near the current CO₂ concentration (252 mg). Chlorophyll contents of cotyledonary leaves did not differ between the 3 CO₂ levels, but within each CO₂ level, chlorophyll contents were lower in seedlings clipped at 12 than at 7 days after emergence (not shown).

Experiment 2, 7 day clipping

Clipping at 7 days post-emergence significantly affected all parameters measured on fertilized seedlings. The CO₂ effects were significant for all parameters except chlorophyll content of cotyledons. A clipping by CO₂ interaction was significant for root biomass (p =0.0291). Root and shoot fresh masses were significantly higher in control than clipped seedlings, except that shoot fresh mass did not differ with clipping treatment at 365 mL liter⁻¹ CO₂ (p=0.054) (Table 2). Cotyledon fresh mass was significantly greater in clipped than control seedlings. Cotyledon chlorophyll content was significantly higher in clipped than control plants grown at 1,000 and 700 mL liter⁻¹ CO₂, but did not differ with clipping at 365 mL liter⁻¹ CO₂ (p=0.081).

Shoot and root masses of control plants and shoot mass of clipped plants were significantly higher at 1000 mL liter⁻¹ CO₂ than the lower concentrations. Regrowth mass demonstrated no clear relationship to CO₂ concentration.

Experiment 2, 12 day clipping.

Clipping effects were significant for all factors measured on fertilized seedlings. CO₂ effects were significant for all factors except chlorophyll content of cotyledons. Treatment by CO₂ effects were significant only for regrowth mass (p = 0.0017) of fertilized seedlings clipped 12 days after emergence (Table 3). Shoot and root fresh masses were significantly higher in control

Table 1. Effects of epicotyl clipping at 7 days after emergence and elevated atmospheric CO₂ concentration on number of nodules; root, shoot, cotyledon and regrowth fresh mass, and chlorophyll content per cotyledon pair of unfertilized mesquite plants at 21 days after emergence.

CO ₂ conc. (μL liter ⁻¹)	Clipping	No. of nodules	Fresh mass			Mass regrowth	Chl. content
			Root	Shoot	Coty.		
			----- (mg) -----				--- (μg) ---
365	Clipped	5.9 a ¹ A	370a A ²	265a A	144a B	78 A	117a A
365	Control	14.1a A	380a A	382a A	122b A		30b A
700	Clipped	11.3a A	362a A	261b A	155a AB	65 A	133a A
700	Control	18.3a A	299a A	318a A	116b A		31b A
1000	Clipped	4.2a A	283b A	255b A	165a A	56 A	136a A
1000	Control	12.8a A	425a A	394a A	128b A		27b A

¹Values within a CO₂ treatment not followed by the same lower case letter are significantly different at the 5% level.

²Values within a clipping treatment not followed by the same upper case letter are significantly different at the 5% level.

Table 2. Effects of epicotyl clipping at 7 days after emergence and elevated atmospheric CO₂ concentrations on root, shoot, cotyledon and regrowth fresh mass, and chlorophyll content per cotyledon pair of fertilized mesquite plants at 21 days after emergence.

CO ₂ conc. (μL liter ⁻¹)	Clipping	Fresh mass			Mass regrowth	Chl content
		Root	Shoot	Coty.		
365	Clipped	342b ¹ A ²	388a B	171a B	186 A	106a A
365	Control	527a B	517a B	119b A		48a A
700	Clipped	327b A	385b B	177a B	145 A	84a A
700	Control	601a B	551a B	142b A		27b A
1000	Clipped	410b A	454b A	211a A	161 A	113a A
1000	Control	884a A	668a A	146b A		36b A

¹Values within a CO₂ treatment not followed by the same lower case letter are significantly different at the 5% level.

²Values within a clipping treatment not followed by the same upper case letter are significantly different at the 5% level.

than clipped treatments in 5 of 6 clipped versus unclipped comparisons. Cotyledon fresh mass and chlorophyll contents were greater for clipped than unclipped seedlings at all 3 CO₂ levels.

Within a clipping treatment, CO₂ enrichment increased (often significantly) masses of tissues measured. Shoot fresh mass of clipped plants, for example, increased with higher CO₂ concentrations. Root fresh mass was greater in clipped plants grown at 1000 than 365 mL liter⁻¹ CO₂. For clipped seedlings, cotyledon fresh mass was lower for plants at 365 than 700 and 1000 mL liter⁻¹ CO₂ level. Mass of regrowth was significantly higher at 1000 mL liter⁻¹ CO₂ than at the 2 lower concentrations.

A comparison of chlorophyll contents listed in Tables 2 and 3 demonstrates that contents were much lower in seedlings clipped at 12 than 7 after emergence. In addition, some cotyledonary leaves abscised from the control seedlings (5, 5, and 4, respectively, from low to high CO₂) in the 12-day-clipping treatment.

Experiment 3

In this experiment, age, CO₂, and age by CO₂ interactions were all significant (p = 0.0001, 0.0001, and 0.0011). Regrowth of plants clipped 4 and 6 days after emergence was greater at 700 and 1,000 than 365 mL liter⁻¹ CO₂ (Table 4). Clipping at these early dates did not reduce survival; all clipped plants survived.

Table 3. Effects of epicotyl clipping at 12 days after emergence and atmospheric CO₂ concentration on root, shoot, cotyledon and regrowth fresh mass; and chlorophyll content per cotyledon pair of fertilized mesquite plants at 26 days after emergence.

CO ₂ conc. (μL liter ⁻¹)	Clipping	Fresh mass			Mass regrowth	Chl content
		Root	Shoot	Coty.		
365	Clipped	315b ¹ B ²	236b C	119b B	68 B	64a A
365	Control	545a A	426a A	47b A		11b A
700	Clipped	368b AB	300b B	155a A	61 B	69a A
700	Control	755a A	502a A	50b A		17b A
1000	Clipped	449b A	364b A	165a A	121 A	77a A
1000	Control	767a A	526a A	65b A		14b A

¹Values within a CO₂ treatment not followed by the same lower case letter are significantly different at the 5% level.

²Values within a clipping treatment not followed by the same upper case letter are significantly different at the 5% level.

Table 4. Regrowth biomass of mesquite seedlings with epicotyls clipped at 2, 4, and 6 days after emergence. Regrowth was measured 14 days after clipping.

Age at clipping -(days)-	CO ₂ conc. (μL liter ⁻¹)	Regrowth dry mass -- mg --
2	365	29a ¹ A ²
2	700	39a B
2	1000	39a B
4	365	30b A
4	700	76a A
4	1000	76a A
6	365	38b A
6	700	64a A
6	1000	71a A

¹Values within a clipping age not followed by the same lower case letter are significantly different at the 5% level.

²Values within a CO₂ treatment not followed by the same upper case letter are significantly different at the 5% level.

Discussion

Epicotyl removal had a significant effect on carbon economy of mesquite seedlings, and significantly lowered shoot mass in 8 of 9 cases and root mass in 5 of 9 comparisons. In contrast, epicotyl removal increased cotyledonary leaf mass, chlorophyll content, and persistence. These results agree with the suggestion of Woolhouse (1967) that epicotyl removal modifies the function and longevity of cotyledons. Bevers (1976) indicated that in epigeal seedlings, cotyledons are important both as photosynthetic and storage organs. In each experiment, cotyledonary fresh mass in clipped plants increased at elevated CO₂, suggesting that CO₂ enhanced cotyledonary leaf function. Apparently in mesquite seedlings, cotyledon longevity is relatively plastic and responds to both the specific requirements of the seedling (clipping) and environmental conditions (CO₂).

The plasticity of cotyledon persistence, however, may be time-dependent. For fertilized plants (Experiment 2), the fresh mass and chlorophyll content of cotyledons were lower in seedlings clipped at 12 than 7 days after emergence (for all CO₂ treatments). Cotyledons likely were beginning to senesce at 12 days, and irreversible changes had already occurred that precluded their full recovery. This hypothesis would help explain why, in every case, regrowth mass was lower in plants clipped at 12 than 7 days (compare Tables 2 and 3). Intuitively, regrowth would be expected to be greater in plants clipped later in development. If, however, senescence had started to occur in the plants clipped at 12 days, a metabolic cost would be incurred in reversing this process, and our data indicate that this reversal was not complete.

Mineral nutrition greatly influenced the response of seedlings to CO₂. In the nutrient poor-soil (Experiment 1), the only positive response to increasing CO₂ that was statistically significant was an increase in cotyledonary leaf mass of plants clipped 7 days after emergence. With nutrient addition to the same soil (Experiment 2), however, seedlings in the 1,000 mL liter⁻¹ CO₂ atmosphere were significantly higher than those in the ambient atmosphere for root mass (control plants), shoot mass (clipped and control plants), and cotyledon mass (clipped plants). Similar trends were evident for plants in the 12-day clipping treatment. Only shoot fresh mass of clipped plants was significantly greater in the 1,000 than 365 mL liter⁻¹ CO₂ treatment in unfertilized soil (Experiment 1). With fertilizer, however, increased regrowth

mass, cotyledon mass, shoot mass, and root mass of clipped plants were observed (Experiment 2). Although not statistically significant, both root and shoot mass of fertilized control plants at 26 days after emergence (Experiment 2) increased with increasing CO₂ concentration without exception. Two other significant conclusions can be drawn. The first is that mesquite seedlings can survive epicotyl removal as early as 2 days after emergence. Scifres and Hahn (1971) observed 40% mortality when they clipped 6-day-old mesquite seedlings, but we observed no mortality in any clipping treatments. One possible explanation is that our culture conditions were optimum (high light levels and warm temperatures) for seedlings to survive this trauma. A second conclusion is that by 3 weeks after emergence, tap root length of almost all seedlings exceeded the 67-cm depth of soil in our culture tubes, confirming the potentially rapid rates at which mesquite can access water and other soil resources at depth (Brown and Archer 1989).

In summary, our results indicate that responses to epicotyl removal in young mesquite seedlings are complex, being strongly influenced by soil fertility, atmospheric CO₂ concentration, seedling age at clipping, and interactions among these factors. Epicotyl removal is not necessarily lethal even to very young seedlings. The great amount of variability we observed between replicate plants agrees with the observations of Simpson (1977) concerning variability present even in half-sib families of mesquite.

Results from these studies indicate that future increases in atmospheric CO₂ concentration could stimulate growth of mesquite seedlings (whether modified by herbivory or not), especially when soil fertility is adequate. Although soil fertility can be low on many rangelands, mesquite is also problematic in managed pastures (for example, fertilized Coastal bermudagrass [*Cynodon dactylon*] pastures) (Tischler, unpublished observations). In ecosystems such as these, effects of increasing CO₂ may be more pronounced. However, even in nutrient depleted rangelands, the possibility exists that increasing CO₂ will stimulate nitrogen fixation by mesquite and other legumes (Polley et al. 1994, 1997) resulting in feedbacks whose effects may not yet be appreciated.

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