

PROGENY TESTING NITROGEN FIXING TREES  
PROSOPIS (MESQUITE) AND LEUCAENA  
FOR BIOMASS PRODUCTION

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Abstract. The common mesquite of the American southwest belongs to the nitrogen fixing genus Prosopis which contains 44 species native to North and South America, Africa and the Middle East. In their native habitat the specimens range from 50 cm tall prostrate shrubs to 20 m tall trees with a DBH of 1.4 m. The tree coppices well and the wood has a low volumetric shrinkage (4%) and a specific gravity of 0.7. A Prosopis progeny trial was conducted under heat drought stress in the California Imperial Valley to select material for short rotation energy production. At 2 years of age, fifty five half-sib families ranged from 0.15 to 29 kg dry weight/tree. Clones were made of most productive individual trees. Techniques have since been developed to routinely propagate these clones and to develop efficient cultural practices for their establishment and growth without irrigation. The first clonal plots were harvested in 1986. The standing dry biomass was 3.5 Mg/ha at year 1, 17.6 Mg/ha at year 2 and 39.3 Mg/ha at year 3. Another progeny trial has been completed for the nitrogen fixing tree Leucaena. This trial examined 65 half-sib families of Leucaena leucocephala, the native L. pulverulenta and L. retusa. After 2 seasons growth the biomass of the L. leucocephala and L. pulverulenta families ranged from 650 to 3602 g/tree and 437 to 2505 g/tree respectively. The lone L. retusa accession has a biomass of 290 g/tree. Despite L. retusa's low productivity it has promise since it tolerates -17 C without damage and hybridizes with the more productive species.

Additional keywords: Semi-arid, cold tolerance, coppice, fuelwood.

Nitrogen fixing trees of the genera Prosopis and Leucaena offer considerable promise for semi-arid lands. Generally speaking Leucaena is less well adapted than Prosopis to regions with low rainfall or with freezing weather (Felker et al., 1983; Felker et al 1982). This paper summarizes progeny and clonal

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biomass production tests conducted in California and Texas for both Leucaena and Prosopis (Glumac et al., 1987; Felker et al., 1983; Felker et al., manuscript in preparation).

Leucaena has been primarily viewed as a tropical species with little potential in the United States due to lack of cold tolerance. However Leucaena pulverulenta and Leucaena retusa are native to southern Texas and west Texas respectively and exhibit considerably more cold tolerance than the more widely known Leucaena leucocephala. L. pulverulenta may reach heights over 15 m while the L. retusa seldom reaches heights of 3 m. L. pulverulenta rarely grows farther north than Kingsville Texas (27 30 N) latitude while L. retusa has native populations that exist in far west Texas near Alpine and in southern New Mexico where temperatures reach -18 C. Interspecific hybrids have been made between L. leucocephala and L. pulverulenta and L. retusa. In 1982 a collection of L. pulverulenta half-sib families was made over its naturalized range in south Texas. The productivity of these families were compared to more widely used L. leucocephala species and to a single L. retusa species.

In 1979 a major test of the biomass productivity of Prosopis and other arid adapted genera were examined in a California Imperial Valley field trial (Felker et al., 1983). This site was chosen because of the high daily July maximum temperatures (42 C). This trial compared the biomass productivity of 55 half-sib families that included the genera; Prosopis, Leucaena, Parkinsonia, Cercidium, and Olneya. The dry biomass after 2 years from seeding in the greenhouse ranged from 0.2 kg/tree for Prosopis tamarugo to 29 kg/tree for P. alba (0166). Prosopis alba and P. chilensis from south America were considerably more productive than the California native P. glandulosa var torreyana. The mean dry biomass of 23 California native Prosopis half-sib families ranged from 1.2 to 12.6 kg/tree. Individual Prosopis trees that had a basal diameter greater than 5 cm at the end of the first year were cloned by rooting of cutting techniques.

This paper reports the results of the first field trial using Prosopis clones. This trial had dual objectives of (1) comparing 2 greatly different clones for biomass productivity and (2) determining the absolute productivity for advanced Prosopis selections with good management. One clone B9V18 is a putative P. alba by P. glandulosa var torreyana naturally occurring hybrid and was thorny, and very bushy. Clone B9V18 was selected from the half-sib family P. alba 0166 that had the greatest biomass production of all the families in the California field trial. The female parent of clone 89V18 was thornless and taxonomically was a good specimen of P. alba. The other clone, B2V50 is a Prosopis alba that is nearly thornless. Although clone B2V50 is multistemmed it has a much more erect form than clone B9V18.

## METHODS

Sixty-five accessions of *L. leucocephala*, *L. pulverulenta*, and *L. retusa* were planted in March of 1983 on the research plots of Texas A&I University in Kingsville, Texas. A complete description of the origins of these accessions can be found in Glumac et al., (1987). A randomized complete block design with 4 replicates was used with the 65 accessions. Each replicate consisted of a single row of 5 trees with a 0.5 m in-row and a 1.5 m between row spacing. Thus, 20 trees were evaluated per half-sib family. Each accession was a single tree seed collection (half-sib family) with the exception of the exotics which are mentioned below.

The *L. retusa* accession came from a tree in Junction, Texas and was provided by D. Ueckert (Texas A&M Research Center, San Angelo, TX). The 38 *L. pulverulenta* accessions and 5 of the 26 *L. leucocephala* accessions were collected by Reyes and Felker from trees growing in south Texas during 1982 (Glumac et al 1987). Our accession numbers for named *L. leucocephala* cultivars are as follows; K4-1086, K8-0147 and 1090 and 1101, K28-0990, K67-0989, K72-1087, K99-1098, K341-1103, Cunningham-1089, and McCarty-1094. The *L. leucocephala* accessions were obtained from Mark Hutton (CIAT) and sources in Hawaii.

Seeds were hand scarified by nicking, inoculated with *Rhizobia* (NGR 8) from Nitragin Co., germinated in Ray Leach "supercell" dibble tubes (Canby, Oregon) containing artificial soil mix and grown in a greenhouse for 4 months. The seedlings were handplanted in the field into a raised-bed made with a sub-soiling tree planter. They were planted on a between-row spacing of 1.5 m and an in-row spacing of 0.5 m. Oryzalin (Surflan), a pre-emergent herbicide was incorporated into the soil at the time of planting at a rate of 2.8 kg ha<sup>-1</sup> and a liquid ammonium phosphate fertilizer (10-34-0) at a rate of 176 kg ha<sup>-1</sup> was sprayed into the furrow created by the tree planter. The plot was cultivated with a sweep cultivator. Pydrin at a rate of 0.6 kg a.i. ha<sup>-1</sup> was sprayed on the stems of the trees in October 1984 to control twig girdler (*Oncideres pustulata*) infestation. A solar powered electric fence was constructed around the plot to prevent browse from wildlife.

Nine months after planting, on December 25 1983, an all time record low temperature of - 12 C occurred. This killed all *Leucaena leucocephala* and *L. pulverulenta* accessions to ground level. *L. retusa* was undamaged by this freeze. Four months after this freeze all of these trees (except *L. retusa*) were harvested at ground level and weighed. Four entire *L. leucocephala* and four *L. pulverulenta* trees were cut into pieces, bagged and dried at 50°C for moisture content determinations. This biomass is listed in Table 1 as biomass

after 9 months. Many of the *L. leucocephala* were over 3 m tall at the time of this freeze.

Nearly all of these trees resprouted from the stump and were 3 to 4 m tall by the next winter. During the winter of 1984/1985 temperatures of - 9 C occurred which again killed all of the *L. leucocephala* to ground level and which caused varying degrees of stem kill to the *L. pulverulenta*. An evaluation of the extent of the freeze damage was made in May 1985 after new growth occurred and the position of stem kill was firmly established. The greatest stem dieback (distance from tip to regrowth) on each tree and height of the stem was measured. The mean ratio of stem dieback to height was calculated for each accession (1 = 100 % dieback). Also in May 1985 the number of surviving rootstocks and the biomass of the resprouts was measured with regressions as previously described (Glumac et al. 1987).

A means separation of the biomass measurements conducted with Tukey's HSD test indicated the 65 accessions could be grouped into about 8 significant groups. As the reporting of this grouping was cumbersome and not very useful in practical terms, only the standard errors of the mean biomass and freeze dieback ratios are reported.

#### Prosopis clonal biomass production trial

A completely randomized design was used in which 3 plots of *P. alba* clone B2V50 were harvested at the end of 1, 2, and 3 years growth. At the end of 3 year's growth, 3 plots of the hybrid clone *P. alba* X *P. glandulosa* var *torreyana* were also harvested. Each sample plot consisted of 25 trees on a 3m x 3m spacing for a plot size of 225 m<sup>2</sup>. The sample plots for biomass estimation were surrounded by 2 border rows. Due to the difficulty in making clones, the most productive *Prosopis* from seed i.e. *P. alba* 0166 was used for the border rows. In the second and third growing season, the biomass of the *P. alba* 0166 border row closest to the measurement plots was also determined.

Site preparation for this trial began in the fall of 1983. After small trees were uprooted, the site was mowed, sprayed with glyphosate to kill perennial grasses, mouldboard plowed and disked several times. Rooted cuttings were made as previously described (Klass et al., 1985) and transplanted into 38 cm long plant bands. The rooted cuttings were transplanted into the field on March 16, 1984 using a previously described mechanical tree planter (Felker et al, 1984) modified from a subsoiler. The trees were planted in a rectangular grid pattern to allow cultivation perpendicular to the direction of planting. Two days prior to planting, oryzalin was applied at a rate of 2.4 kg a.i./ha. No irrigation was used at any phase of the planting. After planting, a 50 cm tall chickenwire fence was placed around the planting to reduce rabbit browse.

The plots received no rain either 6 weeks prior to planting or 8 weeks after planting. Additionally temperatures of 38.8 C (102 F) and 40.5 C (105F) occurred after the planting but before the rains. In spite of the adverse conditions only 3 trees out of 225 had to be replaced (98.7% survival). No leaf loss or other sign of stress appeared on the other trees. The high survival is attributable to deep plowing during the previous rainy season and the long cardboard plant bands.

An intensive weed control program was used. These plots were mechanically cultivated using disk harrows and sweep cultivators several times a year for each of the 3 years. Cultivation was carried in the direction of the rows and across the rows. At the end of the first and second growing season the herbicide solicam was applied at 5 kg/ha and effectively controlled nutsedge, johnsongrass and bermuda grass. Three plots of 25 trees/plot were harvested December 6-7, 1984, November 26 and 27 1985, and in December 1986. For dry matter determinations, entire trees were reduced in size and dried at 70 C until equilibrium was reached.

## RESULTS

### Leucaena trial

The mean dry biomass per tree for 9 months growth after planting (April 1984 harvest of freeze killed trees) and for the second year's regrowth after the freeze (April 1985 regression estimation) is shown in Table 1. The accessions are ranked according to the highest mean for the 2nd year estimates.

The biomass of the L. leucocephala half-sib families ranged from 3602 g/tree for accession 1090 to 654 g/tree for accession 1099. The biomass of the L. pulverulenta half-sibs ranged from 2505 g/tree for accession 0999 to 437 g/tree for accession 1041. The lone L. retusa had the lowest biomass productivity of 290 g/tree. Thus there was considerable overlap in productivity between the species L. pulverulenta and L. leucocephala depending upon which family was examined.

An opposite trend was observed in freezing tolerance with all of the L. leucocephala having a freeze dieback ratio of 1.00 (100 % dieback), with L. pulverulenta ranging from 0.07 to 0.37 and with the L. retusa having a freeze dieback ratio of 0.00.

The five most productive accessions were L. leucocephala K8(2 sources), K72, K67 and a local strain from Zapata TX. The 3 most productive L. pulverulenta accessions were from Kingsville (0999) and Harlingen (1046 & 1047). Another productive accession was 1094 from a tree growing at the University of Florida. The standard errors for biomass productivity of the L. pulverulenta are generally greater than the L. leucocephala. This is presumably due to the fact that L. leucocephala is

highly self fertile and inbred while the other specie's has a greater tendency to outcross.

Table 1. A comparison of biomass production, cold tolerance and survival of Leucaena leucocephala, Leucaena pulverulenta and Leucaena retusa.

Accession	9 month growth after planting		2nd year regrowth after freeze		Freeze dieback ratio		% survival 1983-85 (n=20)
	mean	SE**	mean	SE**	mean	SE**	
	(g)		(g)				
1090 L	1998	149	3602	280	1.00	0.00	95
1071 L	2009	326	3169	474	1.00	0.00	95
1087 L	1585	185	2866	910	1.00	0.00	65*
0147 L	1928	229	2784	152	1.00	0.00	75*
0989 L	1527	282	2780	396	1.00	0.00	90
1094 L	1657	130	2645	318	1.00	0.00	100
1086 L	1494	150	2645	223	1.00	0.00	75
0999 P	1051	383	2505	1041	0.19	0.07	75
1046 P	727	338	2381	763	0.08	0.01	65*
1047 P	635	140	2219	758	0.20	0.06	80*
1044 P	1090	564	2164	766	0.18	0.03	65*
1092 L	1207	166	2119	488	1.00	0.00	100
1096 L	718	362	2117	434	1.00	0.00	75
1002 P	879	190	2020	731	0.19	0.05	75
1005 P	653	270	2020	622	0.14	0.08	50*
1073 P	1057	230	2017	236	0.09	0.02	95
1091 L	910	315	1953	649	1.00	0.00	60*
1056 P	732	321	1878	540	0.08	0.01	70
0993 L	1113	233	1868	471	1.00	0.00	60*
1060 L	1384	93	1806	298	1.00	0.00	100
1103 L	1340	211	1793	466	1.00	0.00	90
1036 P	703	259	1780	548	0.15	0.04	90
1001 P	865	84	1751	225	0.11	0.02	95
1050 P	733	382	1732	975	0.19	0.05	65
1070 L	1007	266	1728	412	1.00	0.00	75*
1067 P	895	197	1710	606	0.49	0.12	75
1088 L	1390	249	1710	307	1.00	0.00	75
1101 L	818	288	1614	330	1.00	0.00	65*
1089 L	905	185	1610	523	1.00	0.00	85
1052 L	815	278	1602	73	1.00	0.00	75*
1097 L	1009	260	1590	285	1.00	0.00	80
1069 P	875	54	1566	375	0.07	0.01	70
1078 P	715	79	1563	411	0.09	0.02	70*
1085 L	916	137	1506	217	1.00	0.00	90
1059 P	674	250	1444	556	0.25	0.07	70
1045 P	616	163	1404	318	0.18	0.03	90
1043 P	553	111	1358	303	0.22	0.04	90
1003 P	512	76	1330	201	0.22	0.04	60
1098 L	774	177	1292	374	1.00	0.00	100

Table 1. Continued

Accession	9 month growth after planting		2nd year regrowth after freeze		Freeze dieback ratio		% survival 1983-85 (n=20)
	mean $\pm$ SE**	(g)	mean $\pm$ SE**	(g)	mean $\pm$ SE**		
1035 P	851	149	1281	166	0.29	0.07	95
1074 P	684	96	1269	376	0.14	0.09	80
1065 P	540	107	1267	56	0.14	0.06	75
1100 L	493	102	1203	622	1.00	0.00	85
1081 P	556	166	1189	310	0.17	0.06	80
1004 P	529	166	1180	250	0.18	0.03	90
1038 P	704	171	1173	359	0.16	0.05	90
1079 P	541	228	1138	379	0.26	0.08	65*
0990 L	616	127	1137	377	1.00	0.00	75
1064 P	548	201	1078	239	0.27	0.00	85
1000 L	848	129	1060	337	0.18	0.02	90
1055 P	470	164	943	309	0.23	0.07	70
1080 P	376	141	920	373	0.21	0.07	80
1076 P	265	129	870	274	0.15	0.02	45*
1077 P	364	51	816	296	0.19	0.08	75
1075 P	560	254	815	277	1.00	0.00	60
1095 L	676	111	785	126	1.00	0.00	90
1066 P	608	253	707	131	0.08	0.02	80
1061 P	432	185	691	249	0.13	0.05	85
1099 L	645	246	654	333	1.00	0.00	80
1051 P	358	55	577	70	0.32	0.08	80
1037 P	359	104	569	106	0.21	0.05	65
1042 P	181	36	537	52	0.37	0.07	60
1040 P	181	47	450	97	0.24	0.07	70
1041 P	134	16	437	136	0.34	0.06	60
1084 R	***	***	290	***	0.00	0.00	5

L=L. leucocephala P=L. pulverulenta R=L. retusa. Freeze dieback ratio is stem dieback/height of stem. Survival based on number of trees still alive out of 20 on May 8, 1985.

\* Accession was located in area of block 4 where damage was incurred from the herbicide bromacil

\*\* Mean and standard errors are based on a maximum of 20 trees. The sample size for each accession can be derived by dividing the corresponding percent survival (1983-85) by 5.

\*\*\* L. retusa was not harvested and was represented by 1 tree.

#### RESULTS

The biomass productivity of clone B2V50, B9V18 and associated seed propagated border rows is presented in Table 2. Prosopis alba clone B2V50 had a standing dry weight of 3.5 Mg/ha, 17.6 Mg/ha, and 39.3 Mg/ha at the end of the first, second and third year's growth respectively. The high productivity of 21.7 Mg/hE for the third growing season is especially promising. Prosopis

alba by P. glandulosa var. torreyana clone B9V18 had a mean standing dry biomass 16.6 + 4.2 Mg/ha.

It is interesting to compare the productivity of clones B2V50 and B9V18 to the border row of P. alba 0166. As an average, clone B2V50 had 74 % greater biomass than the adjacent border row of seed propagated P. alba 0166 trees. In contrast clone B9V18 had 35 % less fresh weight than the border rows. Thus in this case a decrease in productivity was observed by cloning an individual within a family. In contrast, P. alba clone B2V50 yielded 74 % greater biomass than the best seed propagated material available to date.

**Table 2 Biomass production of clone B2V50 at 1, 2 and 3 seasons growth.**

		Mean Tree fresh wt. (kg)	Standing dry weight (Mg/ha)	Annual growth (Mg/ha)
CLONE B2V50				
Year 1	inner B2V50	7.3 ± 0.7	3.5 ± 0.3	3.5
Year 2	inner B2V50	31.4 ± 2.2	17.6 ± 1.2	14.1
	outer 0166	25.0 ± 4.7		
Year 3	inner B2V50	65.4 ± 12.6	39.3 ± 7.6	21.7
	outer 0166	37.4 ± 12.8		
CLONE B9V18				
Year 3	inner B9V18	25.1 ± 6.5	16.6 ± 4.2	
	outer 0166	38.7 ± 14.2		

#### DISCUSSION

Leucaena leucocephala has grown 3 m in height from seedlings the first year and one year coppice regrowth has been 5 m tall with a biomass production of 14 dry Mg/ha (Glumac et al, 1987). A 6 fold variation in biomass production was observed for L. leucocephala. Natural populations of Leucaena pulverulenta had 5 fold variation in growth rate and a 4 fold variation in the percent freeze dieback. Nevertheless Leucaena pulverulenta is not sufficiently cold tolerant to be adaptable to major regions of southeastern United States. However L. retusa has survived -18 C (0 F) without damage and has been reported to form interspecific hybrids with the other species in this study (Brewbaker pers. comm.) The possibility of developing a cold tolerant Leucaena species to the low fertility conifer sites of southeastern United States is truly exciting.

Major strides have been made in the last 10 years in identifying and clonally propagating superior Prosopis phenotypes for biofuel production (Felker et al 1983), salt tolerance (Rhodes and Felker 1987, and pod production (Oduol et al, 1986). However the selection process used less than 10 non-related half-sib families of Prosopis alba and only 1 half-sib family of Prosopis chilensis. Funding sources need to be identified to permit a broader evaluation of this genus to reduce inbreeding problems, to reduce the genetic vulnerability of the clones, and to identify more productive genotypes with greater cold hardiness.

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