

Nitrogen Fixation Estimates for Some Native and Introduced Legumes, Forbs, and Shrubs

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Abstract

Seedling plants of 16 legume, forb, and shrub species were compared with 2 alfalfa cultivars (*Medicago sativa*) for growth and survival in a nitrogen-free sand culture in the greenhouse. All seed accessions were inoculated with the same inoculum mixture. After 11 weeks all surviving plants were harvested and evaluated for top and root growth, percent nitrogen in roots and tops, and acetylene reduction rate of the root mass. All nonlegumes and several legumes died before the experiment was terminated at 11 weeks. Severe chlorosis and subsequent death appeared to be caused primarily from nitrogen deficiency. Six of the legume species—sainfoin (*Onobrychis viciaefolia*), black medic (*Medicago lupulina*), *Medicago media*, *Phaseolus metcalfei*, *Vicia calcarata*, and mesquite (*Prosopis juliflora*)—lived for 11 weeks and produced sufficient top and root growth and accumulated total nitrogen, indicating nitrogen was fixed in the root nodules. There were no differences among accessions for acetylene reduction rates in this experiment. Black medic produced as much or more top and root growth than the other species and merits additional study.

Materials and Methods

Seed was obtained by collections made from the Jornada Exper-

imental Range in southern New Mexico by Dr. Robert P. Gibbens, from other parts of New Mexico by the authors, and from the Plant Materials Center at Los Lunas. The species evaluated and the source of seed are indicated in Table 1.

Accessions were seeded into plastic pots 20 cm in diameter containing washed mason sand. The experiment was a completely randomized design with 5 pots per entry. No nitrogen fertilizer was added during the 11-week growth period. Plants were watered on alternate days with 250 ml of N-free Bryan's Modified Crone's Nutrient Stock Solution.

All seed planted was inoculated with a mixture of commercial inoculum containing the alfalfa, clover, bean, lupine, sainfoin, and vetch groups plus 4 lots of soil associated with several of the native legumes. Plants in each pot were thinned to 5 plants per pot. A total of 5 pots or 25 plants were grown to evaluate each accession.

Following the 11-week growth period, plants were brought to the laboratory and carefully removed from the sand. The top-growth was removed 6 mm above the soil surface and weights recorded as fresh top growth.

Immediately after top growth removal, roots were weighed and acetylene reduction values were determined by a method outlined by Hardy et al. (1972). Procedures followed were as outlined by Duhigg et al. (1978).

Nitrogen in tops and roots was determined by grinding in a Wiley Mill through a 40-mesh screen and analyzing for N by the microkjeldahl process.

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Table 1. Species evaluated and source of seed for accessions³ of forage plants grown in N free medium in greenhouse, 1978.

Entry No.	Common name ¹	Species	Source
1.	Sainfoin	<i>Onobrychis viciaefolia</i> Scop.	New Mexico State Univ.
2.	Alfalfa	<i>Medicago sativa</i> L. (Mesilla)	New Mexico State Univ.
3.	Alfalfala	<i>Medicago sativa</i> L. (A-12357)	Plant Materials Center
4.	Black medic	<i>Medicago lupulina</i> L.	Plant Materials Center
5.	—	<i>Medicago media</i>	Plant Materials Center
6.	—	<i>Phaseolus metcalfei</i> Woot. & Standl.	Plant Materials Center
7.	Deer-vetch	<i>Lotus Wrightii</i> (Gray) Greene	Plant Materials Center
8.	—	<i>Vicia americana</i> Muhl.	Plant Materials Center
9.	—	<i>Vicia calcarata</i>	Plant Materials Center
10.	Mesquite	<i>Prosopis juliflora</i> (Swartz)	New Mexico State Univ.
11.	Prairie clover	<i>Petalostemum purpureum</i> (Vent) Rydb.	Plant Materials Center
12.	—	<i>Astragalus mortoni</i>	Plant Materials Center
13.	Fourwing saltbush	<i>Atriplex canescens</i> (Pursh) Nutt.	New Mexico State Univ.
14.	Australian saltbush	<i>Atriplex semibaccata</i> R. Br.	New Mexico State Univ.
15.	Winterfat	<i>Eurotia lanata</i> (Pursh) Noq.	Jornada Range
16.	Leatherweed croton	<i>Croton corymbulosus</i> Engalm.	Jornada Range
17.	Two-leaf senna	<i>Cassia bauhinioides</i> Gray.	Jornada Range
18.	Dwarf dalea	<i>Dalea nana</i> Torr.	Jornada Range

¹All entries except 13, 14, 15, and 16 are legumes.

Results and Discussion

Only 8 of the 18 entries remained alive throughout the 11-week growing period (Table 2). However, seed of all entries germinated satisfactorily to produce a good stand except for that of *Vicia americana* Muhl., which did not germinate enough seed for thinning and evaluation. Deer-vetch, the first entry to show chlorosis, died about 5 weeks after planting. Prairie clover, (*Astragalus mortoni*), Australian saltbush, and winterfat became chlorotic at 6 weeks after planting and died. Two-leaf senna and dwarf dalea died at about 8 weeks from an apparent lack of nitrogen.

Table 2. Mean, top dry weight, root dry weight, percent protein in tops and roots for pots (5 plants) of selected seed accessions of forage plants grown in N-free medium in greenhouse, 1978.¹

Species	Top dry wt. g	Top nitrogen %	Root dry wt. g	Root nitrogen %
1. Sainfoin	.12	2.49	.23	1.31
2. Alfalfa (Mesilla)	.23	1.93	.33	1.51
3. Alfalfa (A-12357)	.12	2.08	.31	1.09
4. Black medic	.30	1.99	.35	0.61
5. <i>Medicago media</i>	.14	2.13	.16	1.25
6. <i>Phaseolus metcalfei</i>	.10	1.29	.33	1.49
9. <i>Vicia calcarata</i>	.27	1.77	.13	1.49
10. Mesquite	.06	1.93	.15	1.50
LSD .01	.07	0.20	.11	0.25
C.V.	27%	7%	30%	13%

¹Five pots with 5 plants per pot were measured for all entries except entry no. 2 which had a pot with only 3 plants and entry no. 9 which had only 4 pots—one with 2 plants.

Of the nonlegumes that did not survive the full 11 weeks without nitrogen fertilizer, four-wing saltbush and leatherweed croton lived longest; they survived for 6 to 7 weeks.

Mesilla alfalfa, black medic, and *Vicia calcarata* had the greatest mean dry top growth (Table 2). Mesquite and *Phaseolus metcalfei* had the greatest root to shoot (top growth) ratio.

All accessions had similar percent nitrogen in the top growth. On the other hand black medic had the lowest percent nitrogen in the roots (Table 2). The acetylene reduction rate as an estimate of nitrogen fixation by the roots is reported in Table 3. However, the standard deviations and coefficients of variation indicate that differences among entries cannot be shown with these data.

Another measure of nitrogen fixation is the amount of nitrogen recovered in the plant parts (Table 4). Black medic, and *Vicia calcarata* had high amounts of total N in the top growth. Mesilla

alfalfa and *P. metcalfei* had the most total N in the roots and Mesilla alfalfa and black medic had high total N on a total plant basis.

Table 4. Mean total nitrogen for pots (5 plants) of harvested plants for selected accessions of forage legumes grown in N-free medium in greenhouse, 1978.¹

Species	Total N Tops mg	Total N Roots mg	Total N Tops & Roots mg
1. Sainfoin	14.5	15.5	30.0
2. Alfalfa (Mesilla)	20.6	24.0	44.6
3. Alfalfa (A-12357)	12.5	16.9	29.4
4. Black medic	29.4	10.4	39.8
5. <i>Medicago media</i>	15.0	10.2	25.2
6. <i>Phaseolus metcalfei</i>	6.8	24.7	31.5
9. <i>Vicia calcarata</i>	21.6	8.5	30.1
10. Mesquite	5.8	11.2	17.0
LSD .01	6.86	8.56	13.72
C.V.	30%	38%	30%

¹Five pots with 5 plants per pot were measured for all entries except entry no. 2 which had a pot with only 3 plants and entry no. 9 which had only 4 pots—one with only 3 plants.

In conclusion, 6 legume species and alfalfa lived for the full period of the experiment (11 weeks) in a nitrogen-free sand medium and produced significant top and root growth, indicating nitrogen was fixed in the root nodules. The total nitrogen recovered in the plant parts also indicated nitrogen was fixed.

Black medic produced large amounts of top and root growth and, therefore, merits additional study relative to nitrogen fixation and its potential for revegetation.

Literature Cited

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Table 3. Mean, standard deviation, range, and coefficient of variation of acetylene reduction rates on eight seed accessions of selected legumes grown in N-free medium, 1978.

	No. of plants	Acetylene reduction rate - Nanomoles C ₂ H ₂ /plant/hour			
		mean	s	Range	c.v.
1. Sainfoin	25	4.83	5.35	0.0-19.7	110.67
2. Alfalfa (Mesilla)	23	14.00	19.40	0.0-56.2	138.58
3. Alfalfa (A-12357)	25	1.68	3.02	0.0 10.5	179.64
4. Black medic	25	4.96	15.46	0.0-74.0	311.67
5. <i>Medicago media</i>	25	1.61	1.63	0.0-5.4	101.85
6. <i>Phaseolus metcalfei</i>	25	5.34	13.56	0.0-64.6	14.52
9. <i>Vicia calcarata</i>	18	7.78	7.30	0.0-16.2	93.83
10. Mesquite	25	0.90	0.9	0.0-4.1	98.55