

# Agronomic and Acetylene Reduction Evaluation of Three Annual Medics

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## Abstract

Little research has been reported on nitrogen fixation of annual medics such as black medic (*Medicago lupulina*), barrel medic (*M. truncatula*) var. Jemalong, and California burclover (*M. polymorpha*). A greenhouse study was initiated with these 3 medic species to estimate their nitrogen fixation potentials. Considerable variations existed for all indices used to evaluate nitrogen fixation. Barrel medic, var. Jemalong, produced moderate acetylene reduction rates and had the highest top growth weights. This variety also maintained the greatest plant vigor throughout the observation period. High top growth weight, root fresh weight, nodule count, and root score were all associated with high acetylene reduction rates. Plant vigor and color scores were less associated with acetylene reduction rates. Assuming acetylene reduction is a true measure of nitrogen fixation, this experiment indicates the annual medics fix significant amounts of nitrogen.

Plant productivity under range and field crop situations are often limited by a lack of nitrogen. Marginal returns have prohibited the extensive use of nitrogen fertilizers in range programs. It has been shown that a legume grass mixture can increase the total yield of plant material, increase the protein percent, and produce higher daily live weight gains than a purer grass pasture (Anthony and Harris 1976, Kaiser and Cmarik 1976, Van Keuren and Heine-mann 1958).

Annual species of *Medicago* have been used as pasture plants and as a means of increasing soil fertility and pasture productivity (Crawford 1970). Within the genus *Medicago* 30 annual species have been identified (Heyn 1963). Their seed germinate in the autumn, plants grow during the fall, winter, and early spring, and mature early in the summer. The annual medics originated from areas around the Mediterranean basin, and some are now found worldwide.

Black medic can be found growing from Canada to the Gulf of Mexico (Piper 1942). It grows wild in New Mexico and Arizona and has been found growing up to 8,000 ft. elevation. Black medic's ability to colonize diverse habitats and climates has been attributed to several factors. It has been reported to have extensive genetic variability within populations with wide ecological amplitudes for germination of seeds and rapid plant growth (Sidu 1972). Associated with rapid plant growth is the continuous production of seed throughout the growing period.

Barrel medic and strand medic (*M. littoralis*) are now being grown in southern Australia. Their use is primarily to increase pasture productivity and soil fertility (Crawford 1970). In southern New Mexico both species have exhibited good spring forage and seed yields (Smith 1981).

Use of winter growing reseeding annual medics could eliminate expensive reseeding costs, give earlier spring grazing, and add nitrogen and humus to the soil.

Until the early 60's, little work was done by plant breeders in producing plant species for increased nitrogen fixation. Work with medics since the 40's has been focused upon their use as a pasture, hay, and green-manure crop (Blastes and Stokes 1946). Most work on nitrogen fixation has been with alfalfa and soybeans. Little research has been done on the ability of medics to fix atmospheric nitrogen.

In 1981 Johnson and Rumbaugh reported that black medic was profusely nodulated and did fix atmospheric nitrogen under range conditions.

The use of the acetylene reduction technique has been used to estimate the amount of nitrogen being fixed at a given time by a plant. Many workers have used nodule mass, nodule number, and nodule size as indicators of nitrogen fixation rates (Duhigg 1977, Wilson 1957). Nodulation, however, does not always indicate effective nitrogen fixation (Duhigg et al. 1978).

Nodule color has also been used as an index of nitrogen-fixation. Hardy et al. (1973) found that pink color was associated with active nitrogen-fixation. White, green, and brown nodules were ineffective.

Seetin and Barnes (1977) found morphological traits associated with acetylene reduction values to be many fibrous roots, many nodules, and high top and root weights.

The objectives of this study were to evaluate the acetylene-reduction potentials and determine the agronomic characteristics of several selected medics under greenhouse conditions.

## Materials and Methods

Three annual species of the genus *Medicago*, black medic, California burclover, and barrel medic, were grown and evaluated for N-fixation potential. Acetylene reduction rates, plant vigor scores, color scores, fresh and air dry top weights, fresh root weights, nodule count, and root scores were used as indices of N-fixation as outlined by Seetin and Barnes (1977) and Duhigg et al. (1977).

Seed of 3 accessions of black medic, 1 of California burclover and 1 variety of barrel medic var. Jemalong were planted in pots containing 5 kg of mason sand. Before planting, all seeds were inoculated with a commercial inoculum, *Rizobium meliloti*. Plants were watered on alternate days with 250 ml of N-free Bryan's Modified Crone's Nutrient Stock Solution. Approximately 3 weeks after emergence, plants were thinned to 1 plant per pot, and grown for 12 weeks. Plants were maintained in the greenhouse during winter months (replications 1 and 2), and in a shadehouse during summer months (replications 3 and 4).

During the 12-week period, plant vigor was scored visually on a scale of 1-9, with 1 indicating low vigor and 9 high vigor. Plant color was also scored visually, with 1 indicating yellow color and 9 green.

Following the 12-week growth period, plants were brought into the lab and carefully removed from the sand. Nodules were counted. Root scores were visually estimated by a scale of 1-5, with 1 having a small root system, and 5 having a large root system.

Top growth was removed 8 mm above the crown, and its weight

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**Table 1. Mean, range, standard deviation, and coefficient of variation for seedling vigor, plant vigor and color scores on five seed accessions of "medics"—greenhouse 1978-79.**

		Seedling vigor score	Plant vigor score—4 wks	Plant vigor score—9 wks	Color score
Entry No. 1 Black medic Mont.	Mean	4.79	4.37	4.85	5.57
	s	1.45	2.06	1.72	1.95
	Range	1.0-7.0	1.0-9.0	1.0-9.0	1.0-9.0
	C.V.	30.29	47.09	35.35	34.95
Entry No. 2 Black medic N.M.	Mean	4.03	4.20	5.36	6.08
	s	1.51	2.42	2.09	2.11
	Range	1.0-7.0	1.0-9.0	1.0-9.0	1.0-9.0
	C.V.	37.46	57.64	39.01	34.74
Entry No. 3 Black medic Calif.	Mean	5.07	4.92	5.29	5.99
	s	1.68	1.97	2.01	1.75
	Range	1.0-7.0	1.0-9.0	1.0-9.0	1.0-9.0
	C.V.	33.07	39.97	37.93	29.20
Entry No. 4 Barrel medic Var. Jemalong	Mean	6.62	6.08	6.96	7.07
	s	1.62	1.80	1.55	1.54
	Range	1.0-9.0	2.0-9.0	2.0-9.0	2.0-9.0
	C.V.	24.41	29.57	22.26	21.73
Entry No. 5 Burclover Calif.	Mean	5.87	2.92	3.54	3.08
	s	1.64	1.34	2.18	1.79
	Range	1.0-8.0	1.0-6.0	1.0-8.0	1.0-7.0
	C.V.	27.94	45.73	61.48	58.12

was recorded as fresh top growth. The tops were then dried in a forced air oven for 72 hours and weighed again.

Immediately after top growth removal, roots were weighed and acetylene reduction values were determined by methods outlined by Hardy et al. (1973). Procedures were followed as outlined by Duhigg et al. (1978). A total of 100 plants from each accession were evaluated in 4 replications for acetylene reduction rates, and analyzed as a randomized complete block design.

### Results and Discussion

Considerable variation existed for all indices used to evaluate nitrogen fixation. Significant differences were found in all indices except seedling vigor, at the 0.05 and/or 0.01% levels.

**Table 2. Mean, range, standard deviation and coefficient of variation for fresh top growth weight, air dry top growth weight, fresh root growth weight, nodule count, and root score for five seed accessions of "medics"—greenhouse 1978-79.**

		Top fresh weight	Top dry weight	Root fresh weight	Nodule count	Root score
Entry No. 1 Black medic Mont.	Mean	g 2.05	g 0.63	g 7.57	26.92	3.36
	s	1.66	0.63	6.88	10.06	1.11
	Range	0.1-8.7	0.0-2.8	0.5-44.1	3.0-47.0	1.0-5.0
	C.V.	80.80	100.95	90.85	38.26	33.05
Entry No. 2 Black medic N.M.	Mean	2.99	0.90	8.73	31.29	3.47
	s	2.89	0.73	6.84	13.02	1.23
	Range	0.1-11.8	0.0-2.98	0.5-32.0	0.5-50.0	1.0-5.0
	C.V.					
Entry No. 3 Black medic Calif.	Mean	2.83	0.85	12.83	32.18	3.98
	s	1.76	0.62	10.54	12.31	1.03
	Range	0.3-7.0	0.0-3.0	1.9-44.7	5.0-75.0	1.0-5.0
	C.V.	61.77	73.19	82.19	38.24	26.00
Entry No. 4 Barrel medic Var. Jemalong	Mean	4.83	1.48	11.52	31.50	4.22
	s	2.77	1.01	7.60	10.10	0.94
	Range	0.3-11.7	0.0-6.7	0.9-44.6	2.0-53.0	1.0-5.0
	C.V.	57.29	68.29	65.99	32.06	22.34
Entry No. 5 Burclover Calif.	Mean	0.62	0.23	1.86	10.52	1.70
	s	0.81	0.83	2.13	8.22	0.85
	Range	0.1-4.1	0.0-2.4	0.1-11.6	0.0-35.0	1.0-3.0
	C.V.	130.88	362.48	114.64	78.11	50.31

**Table 3. Mean, range, standard deviation, and coefficient of variation of acetylene reduction rates for five seed accessions of "medics" (*Medicago lupulina*, *M. truncatula*, and *M. polymorpha*) grown in a N-free growth medium-greenhouse 1978-79.**

Entry	Number of plants	Acetylene reduction (moles/plant/hour)			
		Mean	s	Range	C.V.
1. Black medic Mont.	89	2.21	2.21	0.0-11.2	93
2. Black medic NMSU	89	3.18	2.79	0.0-14.8	87
3. Black medic Calif.	87	3.80	3.15	0.0-15.1	83
4. Barrel medic var. Jemalong	96	2.76	2.52	0.0-12.3	91
5. California burclover Calif.	63	0.21	0.51	0.0-3.8	24

Barrel medic var. Jemalong exhibited the highest mean plant vigor and color scores of all 5 accessions (Table 1). This species showed good early vigor and maintained good vigor throughout the 12-week evaluation period. It was observed that plants that had good color at the ninth week had higher top growth weights.

California burclover had high seedling vigor scores but declined in plant vigor through the 12-week period. It also showed poor plant color at 9 weeks.

The three entries of black medic were similar in plant vigor and color scores. Entry three showed a trend to higher earlier vigor but entry two exhibited a trend to higher vigor and color scores at the ninth week.

Top growth of seedling alfalfa grown in nil-nitrogen sand cultures had been shown to be highly predictive of nitrogen fixation (Hoffman 1978, Seetin 1976, Seetin and Burns 1977). This relationship held true with the annual medics (Table 2). With the 5 seed accessions, plants that were high in individual top growth weights tended to be higher in acetylene reduction (Tables 2 and 3). Barrel medic top growth, which ranged from no measurable weight to 6.7 g, oven dried, had a high mean dry weight of 1.48 g. The weight of 3 entries of black medic ranged from no measurable top growth weight to 3.0 (Table 2). Although the 3 accessions of black medic were smaller in top weights than barrel medic, acetylene reduction values were high with entries two and three. California burclover was consistently low in top growth weights and low in acetylene reduction.

**Table 4.** Pearson's correlation coefficients of 10 attributes of annual medics grown in the greenhouse—1979.

	Plant vigor 1	Plant vigor 2	Color	Top fresh weight	Top dry weight	Root fresh weight	Nodule count	Root score	Acetylene reduction
Seedling vigor	0.082	0.175**	0.004	0.192**	0.154**	0.099*	0.033	0.114*	0.014
Plant vigor 1		0.635**	0.638**	0.575**	0.463**	0.367**	0.446**	0.444**	0.340**
Plant vigor 2			0.653**	0.698**	0.555**	0.493**	0.594**	0.593**	0.437**
Color				0.638**	0.504**	0.424**	0.579**	0.616**	0.393**
Top fresh weight					0.813**	0.697**	0.651**	0.699**	0.663**
Top dry weight						0.540**	0.532**	0.607**	0.516**
Root fresh weight							0.667**	0.724**	0.693**
Nodule count								0.730**	0.660**
Root score									0.584**

\* \*\*Significant at the 0.05 and 0.01% levels, respectively.

Seetin and Barnes (1977) found that root fresh weight was the best predictor of top growth. Within seed accessions, plants having high root weights were higher in fresh top growth weights (Table 2). Barrel medic had a high mean root weight of 11.52 g and a high mean top growth weight of 4.83 g. It was observed that within the black medic accessions, plants with high root weights tended to have high top weights. California burclover root weights were low which was expected because of low top growth.

Nodule count and root score were used as a final index to nitrogen fixation (Table 2). Nodule counts were similar between entries of black medic and barrel medic, however, California burclover had lower nodule counts.

Barrel medic maintained the highest vigor scores, color scores, and top weights had an intermediate mean acetylene reduction value of 2.76 micromoles (Table 3). Being a true annual, barrel medic may have used more of its nitrogen production to complete its growth cycle, resulting in higher vigor and lower acetylene reduction values.

Within the black medic accessions entry 3 had the highest mean acetylene reduction value of 2.80 micromoles. Entries one and two were 2.21 and 3.18 micromoles per plant per hour respectively.

California burclover which had declined in overall plant vigor at 4 weeks had the lowest mean acetylene reduction value of 0.21 micromoles. Brown and white nodules were observed at 4 and 12 weeks and may account for the low nodulation and low acetylene reduction value on this entry.

Top fresh weight and top dry weight were highly correlated with acetylene reduction and root fresh weights (Table 4). These data indicate that a rough estimate of acetylene reduction potential can be obtained from fresh and dry weight values for medic seedlings, when grown under nil-nitrogen conditions.

Root fresh weights and nodule counts were highly correlated with acetylene reduction. Although these indices may be used for estimating nitrogen fixation, possible death of the plant may result during observation and transplanting and therefore present problems in a breeding program. Plant vigor and color scores had lower correlation values with acetylene reduction rates and, therefore, appear to be of little predictive value.

This study indicates that two species of annual medics, black medic and barrel medic (var. Jemalong), fix significant amounts of nitrogen under greenhouse conditions as estimated by the acetylene reduction process. Further study is needed to determine the variability within and among these annual medic accessions.

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