Effects of Shade and Rhizobium Inoculation on Herbage of Black and Button Medics

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ABSTRACT

Shade tolerance and *Rhizobium* inoculation in annual, cool-season legumes may affect yield of both herbage and seed under arboreal canopies. Naturalized black (*Medicago lupulina* L.) and button (*M. orbicularis* [L.] Bartal.) medics were grown under 0, 30, 55, and 80% shade with and without specific *Rhizobium* inoculation in a two-year field trial. Soil moisture was greatest under 55 and 80% shade and in Yr 1 prior to herbage harvest. Under more stable growing conditions (Yr 1) herbage yields decreased under 80% shade. Herbage yields were undifferentiated between species in Yr 1 but were greater for button medic in Yr 2. Black medic stems were longer in Yr 1 while button medic stems were longer in Yr 2. Seed number peaked for black medic in Yr 1 under 30 and 55% shade but was undifferentiated between species or shade levels in Yr 2. Herbage crude protein concentration was greatest in Yr 2 and at 55% shade for button medic. Acid detergent fiber and lignin concentrations of both species tended to increase as shade levels increased. *Rhizobium* inoculation had no consistent effects on parameters measured. Regression analyses provided no significant model statements. These medics appear tolerant of up to 30% shade and may not require commercial *Rhizobium* inoculation in field conditions where native Rhizobia are already present.

Key words: cool season legumes, forage, quality
Abbreviations: CP, crude protein; ADF, acid detergent fiber; NS, no significant differences at $P = 0.05$.

INTRODUCTION

The planting of introduced and widely naturalized (Diggs et al. 1999) herbaceous cool-season forage legumes into pastures and native range can increase crude protein (CP), energy, and minerals in animal diets, improve animal performance, and increase total herbage production and grazing capacity during critical winter months (Muir et al. 2001). In addition, introduction of supplemental forage legumes as a wildlife management tool may be an efficient and effective technique for improving wildlife habitat, increasing wildlife populations, and enhancing reproductive efficiency.

Forage legumes have also been studied for uses in agroforestry and silvicultural systems. The inclusion of legumes in alley cropping production systems has been shown to contribute to nutrient recycling, reduction in soil nutrient leaching losses, stimulation of higher soil faunal activities, reduction in soil erosion, improved soil fertility, and sustained levels of crop production (Kang 1997). Silvopastoral systems have many benefits over open pastures including greater nutrient cycling, increased soil organic matter (OM) content, reduced erosion, shade for livestock, and greater herbage availability during the dry season (Bazill 1987).

When infected with the effective *Rhizobium* bacteria species, legumes and bacteria form a symbiotic association that enables them to fix atmospheric nitrogen (N_2) (Recourt et al. 1991; van Kessel and Hartley 2000) and transform it into plant-available nitrogen (NH_4^+) (Salisbury and Ross 1992; Graham and Vance 2000). Inoculating legumes with species-specific *Rhizobium* increases the success of legume establishment (Java et al. 1995), root nodulation, herbage yields, and herbage N yield (Zhu et al. 1998). However, the *Rhizobium* inoculation requirements of many cool-season forage legumes are unknown. Research has shown that species exhibiting the C_3 photosynthetic pathway, such as forage legumes, are usually more adaptable to shade than species with the C_4 photosynthetic pathway (Kephart et al. 1992). There is, however, limited research on the shade tolerance of many annual cool-season forage legumes, especially members of the genus *Medicago*. The objectives of this study were to evaluate the shade tolerance and the efficacy of specific *Rhizobium* inoculation of black medic (*Medicago lupulina* L.) and button medic (*M. orbicularis* [L.] Bartal.) in field conditions.

MATERIALS AND METHODS

This research was conducted from 2000-2001 (Yr 1) and repeated in 2001-2002 (Yr 2) at the Texas Agricultural Experiment Station (TAES) in Stephenville, Texas USA (32° 13' N / 98° 10' W at 401 m elevation). Soil at the site was a Windthorst fine sandy loam (fine, mixed, thermic Udic Paleustalf) and no amendments or sterilization were applied. November to April (effective growing period for the trial) rainfall was 493 mm in Yr 1 and 366 mm in Yr 2 (Fig. 1).

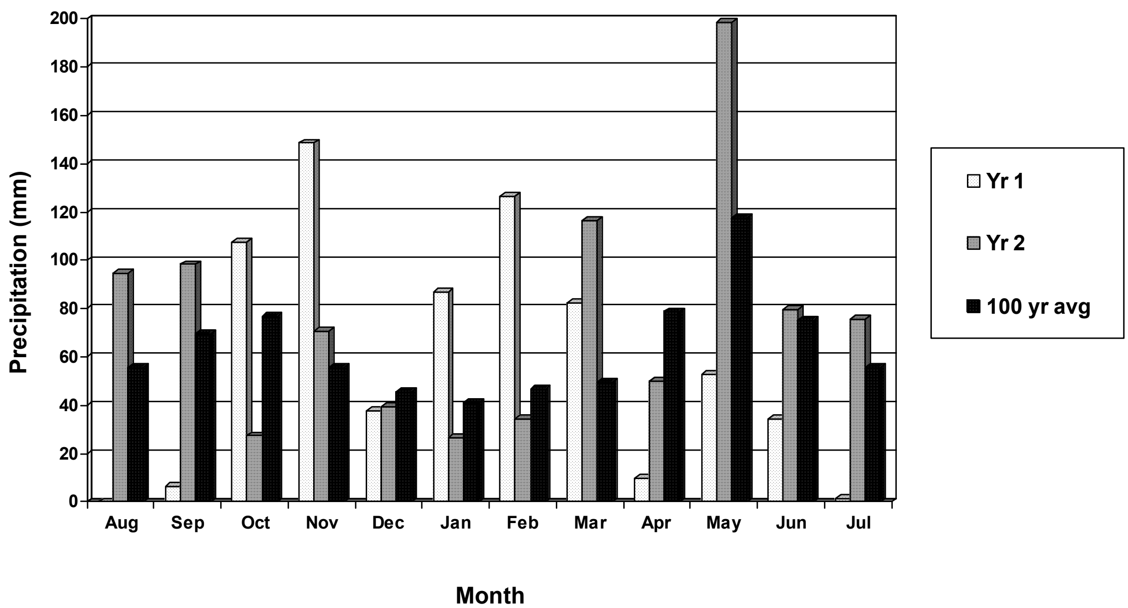

Figure 1. Total monthly precipitation for 2001, 2002, and the 100 yr average for Stephenville, TX.

The study area was treated with clethodim [(E)-2[1-[[(3-chloro-2-propenyl)oxy] imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] at 1122 ml ha^{-1} in September of each trial year to remove vegetation prior to seedling transplantation. The plots were disked in October 2000 but were only manually weeded in preparation for transplanting in Yr 2. Established plots were manually weeded throughout both study periods to reduce competition from other vegetation. Carbaryl [1-naphthyl N-methylcarbamate] was applied in February 2002 at 3553 ml ha^{-1} to control the damage by alfalfa weevil larvae (*Hypera postica* Gyllenhal). In June 2002, fluazifop-P-butyl [butyl (*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate] was applied at 1122 ml ha^{-1} to control invasive grasses, especially grassbur (*Cenchrus incertus* Curtis).

Seeds of locally collected black and button medics were scarified and planted in peat pellets (Jiffy pot #7, Jiffy Products, Batavia, IL) in early to mid-September of each study year. Seedlings were established in the greenhouse and manually thinned to contain one seedling per peat pellet. Seedlings were transplanted into the field plots each year in November. Seedlings that were transplanted into the inoculated treatments were inoculated with Urbana Powdered Peat inoculant for medics (Urbana Laboratories, St. Joseph, MO) by dipping the peat pellet in an aqueous solution of the inoculant just prior to transplanting.

The study area was divided into four blocks; each contained four shade treatments (0, 30, 55, and 80% shade) organized randomly within the block. The artificial shade environment was created by covering the top and sides of steel frames (1.68 m L x 1.20 m W x 1.03 m H) with 30, 55, and 80% UV resistant black polypropylene PAK woven shade cloth (PAK Unlimited, Inc., Cornelia, GA). Each block contained three frames; the 0% shade plots did not contain frames.

The experimental research design for this study was a randomized split-split block comprised of degree of shade, species, and *Rhizobium* inoculation treatment. Each plot was randomly divided into species subplots consisting of two rows of four black medic plants and two rows of four button medic plants. Each species sub-plot was further randomly divided in half into inoculation sub-sub-plots (one row of four inoculated plants and one row of four uninoculated plants per species). Plants were spaced 20 cm apart within each row and 35 cm apart between rows in order to allow for plant spread, reduce plant overlap and mutual shading, and insure desired number (sixteen plants total) and spacing arrangement within plots.

Photosynthetic photon flux density (PPFD) measurements (μmol s^{-1} m^{-2}) were recorded as descriptors of shade treatments. Measurements were taken with a LI-COR® LI-190SA quantum sensor mounted to a LI-COR® LAI-2000 plant canopy analyzer (LI-COR, Inc., Lincoln, NE). Soil samples, taken to a depth of 15 cm, were collected at approximately the same time each month from each plot to measure gravimetric soil moisture (%) concentration (Gardner 1965). At approximately the mid-point of each growing season, soil from each plot was analyzed for pH (Thomas 1996), %OM (Walkley-Black Method as described by Nelson and Sommers, 1996), and NO_3^- concentration (mg NO_3^- kg^{-1} soil) (copperized cadmium reduction method as described by Mulvaney 1996).

Plant harvest was initiated each year when plants achieved approximate early bloom stage of maturity. A maximum of 2 plants from each sub-sub-plot were harvested each year for laboratory analysis of CP (utilizing a modification of the aluminum block digestion procedure of Gallaher et al. 1975), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations (utilizing the methods described by Van Soest and Robertson 1980). The longest stem of each harvested plant was measured to estimate maximum stem length (cm) per treatment. Plants were clipped to approximately 3 cm from the crown and dried in a forced-air oven at 55°C for 48 h. Dried plants were weighed to estimate herbage dry matter (DM) yield (g $plant^{-1}$), and ground through a sheer mill fitted with a 1 mm screen. Seeds from plants not harvested for herbage quality and yield analyses were collected and counted to estimate average seed number (seed $plant^{-1}$) and average seed yield (g $plant^{-1}$) for each treatment.

Measured variables were submitted to analysis of variance (ANOVA) by general linear model to identify differences across and within treatments and years. For the soil variables, ANOVA was used to detect differences between shade treatments and years, as well as interactions. For herbage quality and production variables, ANOVA was used to detect differences for shade, species, inoculation, years, and interactions. A least significant difference (LSD) test was utilized to separate means among entries whenever appropriate ($P<0.05$). Linear and quadratic regression analyses were performed by species for DM yield, stem length, seed number, CP, ADF, and ADL against shade level.

RESULTS AND DISCUSSION

Soil

Shade affected soil moisture ($P=0.002$; $LSD_{0.05}=0.57$) although there was a year x month interaction ($P<0.0001$; $LSD_{0.05}=0.90$) (Table 1). Soil under the 55 and 80% shade cloth contained greater soil moisture (14.10 and 14.37%, respectively) than the 0 and 30% treatments (12.57 and 13.11%, respectively). Soil moisture was greater in the cooler months of January and March than in the warmer months of June and July for Yr 1. There were also differences in monthly soil moisture across years, with January, March, and April of Yr 1 having greater soil moisture levels than Yr 2, and June and July of Yr 2 having greater soil moisture levels than Yr 1.

Table 1. Soil moisture as affected by shade level ($P=0.002$) and year x month interaction ($P<0.0001$) at Stephenville, TX.

Shade (%)	**Soil Moisture (%)**		
0	12.57 b†		
30	13.11 b		
55	14.10 a		
80	14.37 a		
Month	**Yr 1**	**Yr 2**	***P***
	-------Soil Moisture (%)-------		
Jan	18.77 b	14.43 a	* ‡
Mar	19.98 a	13.64 ab	*
Apr	16.28 c	13.32 b	*
Jun	9.43 d	13.45 b	*
Jul	6.95 e	9.16 c	*

† Means in the same column followed by same letter are undifferentiated at 0.05 probability level.
‡ "*" Difference between years at 0.05 probability level.

There were no differences (P>0.05) in soil NO_3^- or OM concentrations related to shade treatments or years. There was a difference (P=0.03; $LSD_{0.05}$=0.193) in pH between years, with the mean pH for Yr 1 (6.073) being slightly lower than Yr 2 (6.308), likely due to spatial variability of pH in the plots (data not shown).

Herbage Dry Matter Yield and Stem Length

Dry Matter Yield: Year x Shade Interaction

Dry matter yield was affected by a year x shade interaction (P=0.02; $LSD_{0.05}$=0.84). In Yr 1, DM yield was lower at the 80% shade level (0.46 g DM plant^{-1}) than at the 0, 30, and 55% shade levels (2.27, 2.17, and 1.84 g DM plant^{-1}, respectively) (Table 2). The greater soil moisture level at 80% shade (Table 1) did not appear to compensate for lower sunlight in terms of DM production. However, in Yr 2, DM yield was lower at the 55% shade level (0.43 g DM plant^{-1}) than at the 0 and 30% shade levels (1.58 and 1.43, respectively). When comparing DM yields across years, there was a difference (P=0.002) only at the 55% shade level, with Yr 1 DM yields greater than Yr 2 (1.84 g DM plant^{-1} and 0.43 g DM plant^{-1}, respectively). Plants under 0, 30, and 80% shade were undifferentiated across years. Reductions in DM yields of cool-season legumes under high levels of shade and no differences in yield under moderate levels of shade were also reported by Lin et al. (1999).

Table 2. Herbage dry matter (DM) yield as affected by year x shade interaction (P=0.02) at Stephenville, TX pooled for two legume species.

Shade (%)	**Yr 1**	**Yr 2**	***P***
	-------g DM plant^{-1}------		
0	2.27 a†	1.58 a	NS ‡
30	2.17 a	1.43 a	NS
55	1.84 a	0.43 b	*
80	0.46 b	1.26 ab	NS

† Means in the same column followed by the same letter are undifferentiated at the 0.05 probability level.
‡ "NS" No difference between years at 0.05 probability level;
"*" Difference between years at 0.05 probability level.

Dry Matter Yield: Year x Species Interaction

Species DM yields responded to shade differently each year (year x species interaction P=0.004) (Fig. 2) and there were no differences in DM yield between black and button medics for Yr 1 (P>0.05). However, the DM yields were different (P<0.0001) between the two species for Yr 2 (0.50 g DM plant^{-1} black medic and 1.85 g DM plant^{-1} button medic). There was no difference in DM yields for button medic between years (P>0.05). However, DM yield of black medic was greater (P<0.0001) in Yr 1 than Yr 2

(1.80 and 0.50 g DM plant^{-1}, respectively). The average DM yield for Yr 1 was greater (*P*=0.03) than Yr 2 (1.60 and 1.17 g DM plant^{-1}, respectively).

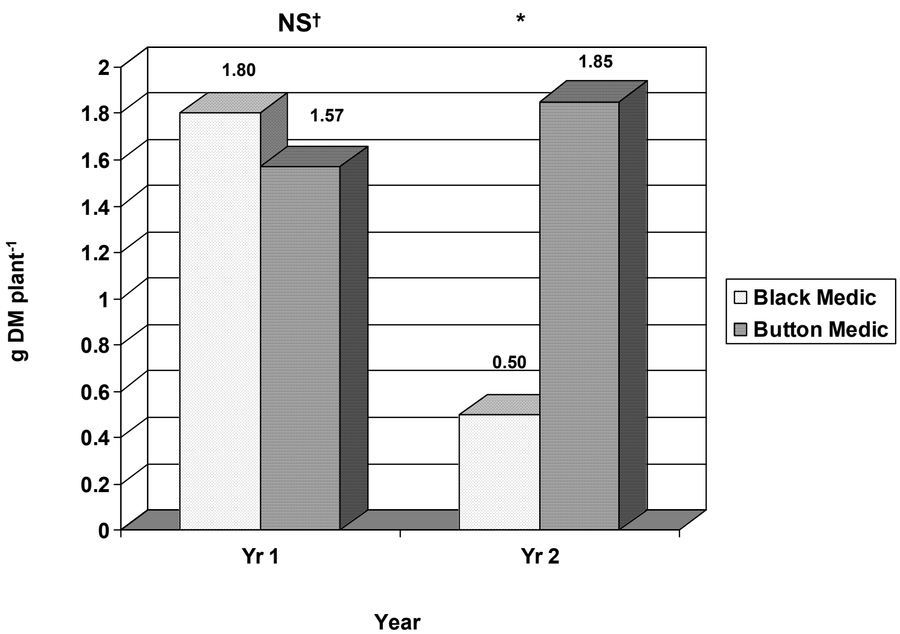

Figure 2. Herbage dry matter (DM) yields as affected by a medic species x year interaction (*P*=0.004) pooled over four levels of shade († No difference between species at 0.05 probability level; * Difference between species at 0.05 probability level).

There was considerable plant damage in Yr 2 from alfalfa weevil larvae, and carbaryl was sprayed in mid-February as a control measure. Average temperatures in November, December, and January of the Yr 2 trial were warmer than both Yr 1 and the 20 yr average (data not shown), which, along with possible population buildup from Yr 1, may have contributed to more favorable growing conditions for alfalfa weevil larvae that resulted in reduced black medic DM yields in Yr 2. Average temperatures in November, December, and January of Yr 1 were cooler than the 20 yr average, which possibly inhibited weevil egg-hatching, thus minimizing plant damage and DM yield loss in Yr 1. Black medic appears to be either less tolerant than button medic to alfalfa weevil larvae damage or preferred over button medic as a host plant to the larvae. There were also greater soil moisture levels in Yr 1 than in Yr 2 for the months prior to harvest. This reduction in soil moisture in Yr 2, alone or compounded with insect damage, could also have contributed to the greater DM yields in Yr 1 as compared to Yr 2. Foulds (1978) determined that black medic DM production was reduced under low soil moisture levels (5% moisture) although levels this low were not recorded in the present study. Button medic may be more tolerant to reduced soil moisture than black medic.

Inoculation was not a factor in DM yield (*P*>0.05). Zhu et al. (1998) reported similar results regarding annual medic inoculation in a field study. They concluded that the native soil rhizobia were as effective as the commercial inoculum in root nodulation and symbiotic N_2-fixation. In this field plot, naturalized medics were locally abundant and indigenous *Rhizobium* population in the soil appeared to have been as effective as local populations in combination with commercial inoculum in root nodulation and symbiotic N_2-fixation. In soils without local *Rhizobium* populations, these results may be different.

<u>Stem Length: Year x Species Interaction</u>

Species stem length changed with year (year x species interaction, *P*=0.001) (Fig. 3) since there was no difference in button medic stem length across years (*P*>0.05). Stems of black medic were longer (*P*<0.0001) in Yr 1 (26.89 cm) than Yr 2 (14.30 cm). Stem lengths also differed for species within years as shown in Fig. 3. In Yr 1, black medic stems (26.89 cm) were longer (*P*=0.01) than button medic stems (19.63 cm) while in Yr 2 stems of button medic (20.65 cm) were longer (*P*=0.01) than black medic (14.30 cm).

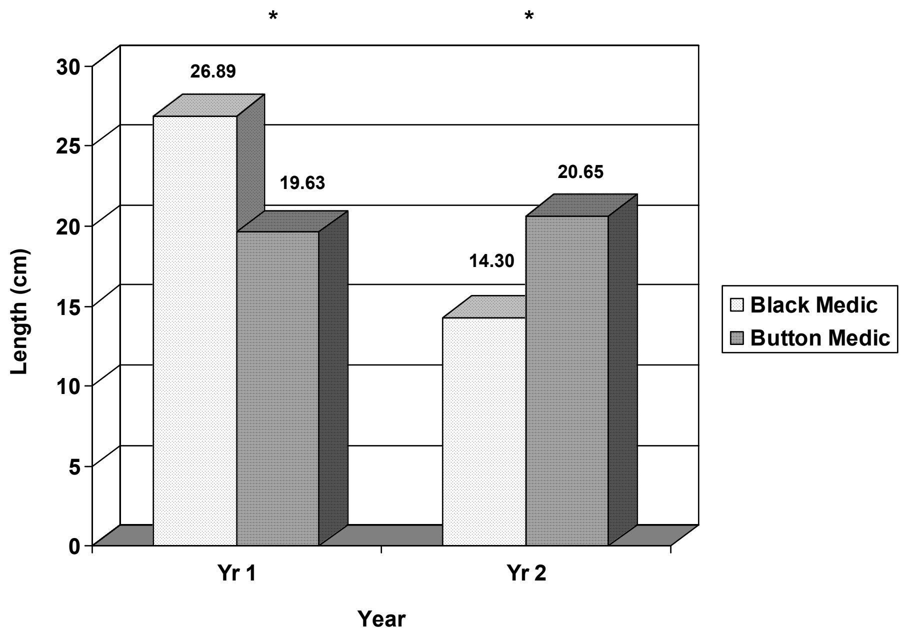

Figure 3. Stem length as affected by a medic species x year interaction (*P*=0.001) pooled over four shade levels (*Difference between species at 0.05 probability level).

The damage inflicted by alfalfa weevil larvae on plants in Yr 2 could account for the decrease in black medic stem length from Yr 1 to Yr 2 because black medic appeared to be more susceptible to insect damage than button medic. There were also greater soil moisture levels in Yr 1 than in Yr 2 for the months prior to harvest (Table 1) and this reduction in soil moisture in Yr 2, alone or compounded by insect damage, could also have contributed to the longer stems in Yr 1 as compared to Yr 2. Foulds (1978) determined that black medic herbage yield was reduced under low soil moisture conditions, which could explain the reduction of stem length in Yr 2. Button medic may be more tolerant to reduced soil moisture than black medic.

Seeds

<u>Seed Number: Year x Shade x Species Interaction</u>

There was a year x shade x species interaction (*P*=0.001; $LSD_{0.05}$=457) for total number of seeds produced $plant^{-1}$ (Table 3). In Yr 1, there were no surviving seed-producing button medic plants in the 0 or 80% shade treatments. The lower temperatures in Yr 1, possibly compounded with the reduced irradiation in the 80% shade treatment, may have contributed to the greater mortality of button medics.

Table 3. Seed number of black and button medic as affected by year x shade x species interaction (*P*=0.001).

Year	Shade (%)	Black medic	Button medic	*P*
		---------number plant^{-1}-------		
1	0	1672 b†	-- ‡	--
	30	2365 a	478 a	* §
	55	2027 ab	131 a	*
	80	280 c	--	--
2	0	198 a	218 a	NS
	30	266 a	127 a	NS
	55	77 a	25 a	NS
	80	0 a	11 a	NS

† Within year, means in the same column followed by the same letter are undifferentiated at 0.05 probability level.
‡ No surviving seed-producing plants for treatment data collection.
§ "*" Difference between species at 0.05 probability level;
"NS" No difference between species at 0.05 probability level.

Button medic seed number was unaffected by shade levels for either year, and was not different between years ($P>0.05$). It was also undifferentiated at the 30 and 55% shade levels evaluated. In both years, button medic produced mature seeds later than black medic, possibly accounting for the lower seed number of button medic as compared to black medic as a result of greater soil moisture in March and April of Yr 1.

Black medic produced more seeds at 30 and 55% shade than button medic in Yr 1 but seed was unaffected by shade levels in Yr 2. In Yr 1, the greatest seed production occurred under 30 and 55% shade, with black medic producing more seeds ($P<0.0001$) at these levels than button medic. Black medic seed number at 55% shade (2027 seeds plant^{-1}) was not different from 0 and 30% shade. There were no differences in the number of seeds produced in Yr 2 between species or shade levels.

In the absence of severe insect pest damage, black medic has the potential to produce more seeds than button medic at moderate shade levels than in full sun environments. From their study on flowering of crimson clover, Butler et al. (2002) determined that high temperature inhibited flowering. The effect of shade cloth in moderating temperatures under the frames may have created a more favorable environment for black medic flowering and seed production in Yr 1. Moomaw (1995) found that black medic planted under soybean canopies produced viable seeds, a characteristic desired in potential cover crops. Rumbaugh and Johnson (1986) also found that black medic was a prolific seed producer capable of quickly developing a soil seed bank.

The difference in soil moisture between years could have contributed to differences in black medic seed number. Soil moisture concentrations were greater in January, March, and April of Yr 1 compared to Yr 2, while soils in June and July of Yr 2 exhibited greater soil moisture levels than in Yr 1. Foulds (1978) reported that the number of flowers per inflorescence and total seed production of black medic were reduced under low soil moisture conditions. The lower soil moisture concentration in January, March, and April of Yr 2 could have accounted for reduced black medic seed number. Likewise, the lower soil moisture concentration in June and July of Yr 1 could also have accounted for the reduced seed yield of button medic because it flowers later than black medic. The greater soil moisture concentration at 80% shade (Table 1) did not appear to compensate for lack of sunlight in terms of black medic seed number for Yr 1.

Seed Yield: Year x Shade x Species Interaction

Shade affected seed yield, reported as total weight (g) of seeds produced $plant^{-1}$, differently for each species (year x shade x species interaction P=0.0004; $LSD_{0.05}$=0.787) (Table 4). In Yr 1, there were no surviving seed-producing button medic plants in the 0 or 80% shade treatments. The lower temperatures in Yr 1, possibly compounded by extreme light interception in the 80% shade treatment, may have contributed to the greater mortality of button medics. Seed yield of button medic did not differ between years (P>0.05) and were also undifferentiated at the 30 and 55% shade levels. Seed yields for button medic in Yr 1 at 30 and 55% shade were lower than for black medic.

Table 4. Total seed yield of black and button medic at Stephenville, TX as affected by year x shade x species interaction (P=0.0004).

Year	**Shade (%)**	**Black medic**	**Button medic**	***P***
		------------g $plant^{-1}$-----------		
1	0	3.00 b†	-- ‡	--
	30	3.85 a	1.14 a	* §
	55	3.23 ab	0.61 a	*
	80	0.40 c	--	--
2	0	0.28 a	0.54 a	NS
	30	0.42 a	0.29 a	NS
	55	0.15 a	0.06 a	NS
	80	0.00 a	0.02 a	NS

† Within year, means in the same column followed by the same letter are undifferentiated at 0.05 probability level.
‡ No surviving seed-producing plants for treatment data collection.
§ "*" Difference between species at 0.05 probability level;
"NS" No difference between species at 0.05 probability level.

Black medic can potentially produce greater seed yields per plant than button medic at moderate shade levels when insect damage is not severe. As in seed number, the differences in soil moisture between Yr 1 and Yr 2 could also explain the differences in seed yields. Foulds (1978) reported that the number of flowers per inflorescence and total seed production of black medic were reduced under low soil moisture conditions. The lower soil moisture concentration in January, March, and April of Yr 2 could have accounted for the reduced seed yield of black medic and possibly button medic. Likewise, the lower soil moisture concentration in June and July of Yr 1 could also have accounted for the reduced seed yield of button medic. The greater soil moisture levels under 80% shade (Table 1) did not appear to compensate for lack of sunlight in terms of black medic seed weight for Yr 1 whereas moderate shade levels appeared to enhance the seed yield of black medic. Moderate, but not extreme, shade appears to favor seed production in black medic when moisture becomes limiting.

Seed Yield: Year x Species x Inoculation Interaction

Total seed yield was also affected by a year x species x inoculation interaction (P=0.04) (Table 5). There were no differences in total seed weights in Yr 2 between species or across inoculation treatments (P>0.05). In Yr 1, uninoculated button medic plants produced more seeds by weight than inoculated button medics (1.35 and 0.24 g $plant^{-1}$, respectively). Black medic seed yields were unaffected by inoculation in Yr 1 (P>0.05) but were greater than button medic for both inoculated and uninoculated treatments in that year. Although uninoculated button medics in Yr 1 (Table 5) produced more seeds by weight than inoculated button medics, inoculation was not a factor in total numbers of seeds produced.

Table 5. Total seed yield of black and button medics as affected by year x species x inoculation interaction (*P*=0.04).

Year	**Inoculation**	**Black medic**	**Button medic**	
		----------- g $plant^{-1}$---------		*P*
1	+ †	2.46	0.24	* ‡
	-	2.78	1.35	*
		NS §	*	
2	+	0.33	0.23	NS
	-	0.11	0.22	NS
		NS	NS	

† "+" Plants inoculated with *Rhizobium*; "-" Plants not inoculated with *Rhizobium*.
‡ Within year, means in the same row are compared.
"*" Difference at 0.05 probability level; "NS" No difference at 0.05 probability level.
§ Within year, means in the same column are compared.

Bhalu et al. (1995), Rani and Kodandaramaiah (1997), and Singh et al. (1998) observed increases in seed yields with inoculation treatments in field studies on blackgram, soybean, and pigeonpea, respectively. Materon and Zibilske (2001) determined that nodule initiation was determined by the *Rhizobium* strain that was initially exposed to the host plant. In the present study site, naturalized medics were locally abundant and appeared to be well established. The commercial inoculum might have been redundant or less effective for legume nodulation and symbiotic N_2-fixation compared to indigenous soil *Rhizobium*. Commercial inoculation did not appear to be a requirement for black or button medic seed production in this study area.

Herbage Crude Protein

Year

Herbage CP concentrations changed with year (*P*=0.03), with Yr 2 (21.4%) having 10% greater CP than Yr 1 (19.5%). Extremes in temperature, moisture, and pH negatively affect soil rhizobial populations. The numbers of rhizobia that are symbiotic with medics, *Sinorhizobium meliloti*, were found to be numerous in alkaline soils (pH 7-8) but rare or absent in soils more acidic than pH 6 (Hirsch 1996). The pH of the soil in Yr 1 could have been too acidic for high survival of rhizobia, and may have resulted in reduced nodule formation, N_2-fixation, and herbage CP concentration (Ibekwe et al. 1997). Lower soil moisture levels in January, March, and April of Yr 2 may also have contributed to the increase in CP concentration that year, although there are inconsistent reports in the literature on the effects of soil moisture on CP concentration of forage legumes. Carter and Sheaffer (1983) and Halim et al. (1989) reported that soil water deficits had no effects on CP concentration in alfalfa while, in contrast, Walgenbach et al. (1981) and Petit et al. (1992) reported that, under reduced soil moisture conditions, alfalfa exhibited increased concentrations of N fractions. Peterson et al. (1992) reported that the effects of drought conditions on CP concentrations of perennial forage legumes were not consistent. Whatever the reason in the CP differences among years, these were minute and would not likely change herbage nutritive value to herbivores.

Shade x Species Interaction

Shade affected the CP concentration differently in each species (shade x species interaction *P*=0.03; $LSD_{0.05}$=1.52) (Table 6). Black medic CP concentration was unaffected by shade level while button medic CP concentration was greatest at 55% shade (21.96% CP), was undifferentiated at 0 and 30% shade (19.07 and 19.79% CP, respectively), and was lowest at 80% shade (16.48% CP). When comparing CP across species, black medic produced greater concentrations of CP than button medic at 0 and 80% shade (*P*=0.01 and *P*=0.001, respectively). The CP concentrations of black and button medics were not different at 30 and 55% shade.

Table 6. Black and button medics herbage acid detergent fiber (ADF) concentration as affected by year x shade x species x inoculation interaction (*P*=0.0004) and crude protein (CP) concentration as affected by shade x species interaction (*P*=0.03) and pooled over two study years at Stephenville, TX.

Year	**Inoculation**	**Shade (%)**	**Black medic**	**Button medic**	***P***
			--------- % ADF ----------		
1	+†	0	21.6 c	‡ 21.3 b	NS§
		30	23.7 ab	21.7 b	NS
		55	23.1 bc	23.7 a	NS
		80	25.2 a	-- ∂ --	
	-	0	21.9 c	22.3 a	NS
		30	23.9 b	23.0 a	NS
		55	24.4 b	23.5 a	NS
		80	28.4 a	-- ∂ --	
2	+	0	22.9 b	20.5 b	NS
		30	24.4 ab	20.7 b	*
		55	-- ∂	21.5 b	--
		80	25.6 a	27.7 a	NS
	-	0	25.4 a	18.5 b	*
		30	19.2 c	21.0 a	NS
		55	-- ∂	21.2 a	--
		80	23.6 b	22.3 a	NS
	Pooled		----------- % CP -----------		
		0	22.1 a	19.1 b	*
1 & 2	+ & -	30	21.4 a	19.8 b	NS
		55	21.4 a	22.0 a	NS
		80	20.8 a	16.5 c	*

†= "+" Plants inoculated with *Rhizobium*; "-" Plants not inoculated with *Rhizobium*.
‡= Within year, inoculation treatment, and species, means in the same column followed by the same letter are undifferentiated at 0.05 probability level.
§= "NS" No difference between species at 0.05 probability level; "*" Difference between species at 0.05 probability level.
∂= Insufficient plant material for analysis.

Inoculation had no effect on CP concentration of either black or button medics (*P*>0.05). Based on results obtained from a field study in Minnesota, Zhu et al. (1998) reported that the herbage N production of black medic and two other medic species were not affected by inoculation. They concluded that the indigenous soil rhizobia were effective in root nodulation of these medics. In the present study, the indigenous *Rhizobium* in the soil appeared to have been either as effective as the commercial inoculum in root nodulation and symbiotic N_2-fixation or similarly negatively affected by low soil pH.

Many studies have reported that shade does not affect CP concentration in cool-season legumes. Watson et al. (1984) reported that shade did not affect the total N or CP concentrations of hairy vetch or several *Trifolium* species studied in Mississippi. Lin et al. (2001) found that CP concentrations of 'Cody' alfalfa and white clover were not affected by shade treatments in Missouri. In contrast, several authors studying tropical legumes have reported increased herbage CP concentrations with increasing shade levels (Wong et al. 1985; Muir and Pitman 1989; Lin et al. 2001). In this study, the CP concentration of black medic was not affected by shade while button medic CP was affected by shade. It appears that the CP concentration of button medic can be increased with shade levels up to 55% but, at 0 and 80% shade, black medic produced greater CP concentrations than button medic.

Baltensperger and Smith (1984) reported that black medic had similar CP concentrations (12.44%) to the other forages studied in New Mexico. Zhu et al. (1996) reported that black medic consistently produced high CP concentrations (239 g kg^{-1} DM) when compared to other medic species. Reported CP requirements for growing and finishing beef cattle range from 6.5 to 18.4% (NRC, 1996) while the American Farm Bureau Federation recommended general CP concentrations of 8% for maintenance of older ruminants, 12% for young animals at 50% of their mature weight, 12-14% for nursing cows and sheep, and 16-18% for lactating dairy cows, ewes, and goats (Ball et al. 2001). The Texas Agricultural Extension Service defined poor quality forage for beef cattle production as less than 6% CP concentration, medium quality forage as 7-11% CP concentration, and high quality forage as 12-14% CP concentration (Hammack and Gill 2000.) At moderate shade levels, both black and button medics appear to produce sufficient amounts of CP for livestock maintenance and production.

Acid Detergent Fiber

Year x Shade x Species x Inoculation Interaction

Herbage ADF concentrations were affected (P=0.0004; $LSD_{0.05}$=1.70) by a year x shade x species x inoculation interaction. Between species, there were no differences in ADF concentrations except at inoculated 30% shade and at uninoculated 0% shade in Yr 2 (Table 6). In both cases, button medic had lower ADF concentrations than black medic. When LS mean separation for inoculation was calculated, there was a 4% increase in ADF with inoculation (0.93% ADF) (P=0.03). When LSD mean separation for years was calculated, there was a 7% increase in ADF in Yr 1 (0.72% ADF) (P=0.001). In general, ADF concentration for both species, regardless of inoculation or year, increased as shade levels increased. The low DM yields at 80% (Yr 1) and 55% shade (Yr 2) (Table 2) resulted in insufficient plant material for ADF analysis because the majority of the plant material harvested at these shade levels was used for CP analysis.

Lin et al. (2001) reported that ADF concentration of cool-season legumes in Missouri was either unaffected or increased with 50 and 80% shade treatments. They attributed the reduced digestibility to decreased non-structural carbohydrate contents and increased cell-wall contents at greater shade levels. Blair et al. (1983) also attributed a decrease in digestibility with shade level to a decrease in the content of readily digestible cell solubles and an increase in fibrous cell-wall fractions. The explanation for the increase in ADF with inoculation was not known. Elsheikh and Elzidany (1997) reported that *Rhizobium* inoculation had no significant effects on the crude fiber or ash contents of faba bean seeds.

The higher soil moisture levels in January, March, and April of Yr 1 may have also affected the increase in ADF concentration in Yr 1, although the literature on the effects of soil moisture level on ADF was inconsistent. Seguin et al. (2002) reported that, as soil moisture decreased, ADF concentrations increased. Conversely, Peterson et al. (1992) and Petit et al. (1992) reported that ADF decreased as soil moisture levels decreased. The decrease in ADF in Yr 2 may have resulted from a decrease in soil moisture in that year.

Lignin

Year x Shade x Species x Inoculation Interaction

Herbage lignin concentration was affected (P=0.02; $LSD_{0.05}$=0.39) by year x shade x species x inoculation interaction (Table 7). There were no differences in lignin concentration between species except at uninoculated 0% shade in Yr 2, in which the lignin concentration of black medic was higher (P=0.0001) than button medic (5.12 and 3.70% lignin, respectively). When LSD mean separation for inoculation was calculated, there were no differences between inoculation treatments (P>0.05). When a LS means separation for years was calculated, there were no differences between years for lignin (P>0.05).

Table 7. Black and button medic herbage lignin concentration as affected by year x shade x species x inoculation interaction (*P*=0.02) at Stephenville, TX.

Year	**Inoculation**	**Shade (%)**	**Black medic**	**Button medic**	***P***
			------------ %Lignin -----------		
1	+ †	0	4.29 b ‡	4.18 b	NS§
		30	4.52 b	4.79 a	NS
		55	4.33 b	4.95 a	NS
		80	4.93 a	-- ∂	--
	-	0	4.30 c	4.11 b	NS
		30	4.71 b	4.81 a	NS
		55	4.83 b	4.92 a	NS
		80	5.68 a	-- ∂	--
2	+	0	4.63 b	4.11 c	NS
		30	4.69 b	4.64 b	NS
		55	-- ∂	4.89 b	--
		80	5.84 a	5.72 a	NS
	-	0	5.12 a	3.70 c	*
		30	4.28 b	4.38 b	NS
		55	-- ∂	4.95 a	--
		80	4.64 b	5.23 a	NS

†= "+" Plants inoculated with *Rhizobium*; "-" Plants not inoculated with *Rhizobium*.
‡= Within year, inoculation treatment, and species, means in the same column followed by the same letter are undifferentiated at 0.05 probability level.

§= "NS" No difference between species at 0.05 probability level; "*" Difference between species at 0.05 probability level.
∂= Insufficient plant material for analysis.

In general, lignin concentration for both species, regardless of inoculation or year, increased as shade levels increased. This trend was similar to the trend for ADF concentration and shade level. Forage quality, as estimated by ADF and lignin, tended to decrease as shade levels increased, regardless of inoculation or year. The low DM yields at 80% (Yr 1) and 55% shade (Yr 2) (Table 2) resulted in insufficient plant material for lignin analysis. The majority of the plant material harvested at these shade levels was used for CP analysis and there was not sufficient plant material remaining to analyze lignin.

The effects of shade on plant morphology and digestibility have been reported in many studies. Buxton (1989) suggested that shading induced stem elongation, achieved through lignification, so that leaves were oriented higher in order to be exposed to and to capture more sunlight for photosynthesis. Babu and Nagarajan (1993) also described higher shoot height, internodal elongation, and lower leaf area index as competitive responses of plants grown under shade. Lin et al. (2001) also reported decreased leaf: stem ratios at 50% shade for 'Cody' and 'Vernal' alfalfa.

Visual observation of plants growing in the field in this study indicated that plants growing under 80% shade exhibited a more upright growth habit, while plants in full sun were more decumbent. As shade levels increased, upright growth habit of plants became more prominent. However, medic stem length was not affected by shade. The increase in lignin of both black and button medics at greater shade levels was possibly a result of lower leaf area indices, and reduced leaf: stem ratios, although these characteristics were not measured directly.

Linear and Quadratic Regression Analyses

Regression analyses were not significant for any of the models tested. This indicated that levels of shade did not have correlating effects on any of the variables measured during the trial.

CONCLUSIONS

Irregular soil moisture levels and insect predation in Yr 2 may have contributed to the reduction in black medic DM yield, stem length, and seed numbers. These results are consistent with the findings of Foulds (1978) on the effects of water deficit on black medic. Dry matter yield, stem length, and seed numbers of button medic were not different between years, indicating that button medic may be more tolerant to differences in soil moisture level and to insect damage than black medic. Additional research on the soil moisture requirements and tolerance to insect damage of local medics appears to be warranted. In the absence of severe insect damage and with greater soil moisture (Yr 1), black medic produced equivalent DM yields, longer stems, and more seeds than button medic.

The increase in available soil moisture at 55 and 80% shade may have partially compensated for the reduced solar irradiation at these shade levels in terms of overall DM yield and black medic seed numbers (Yr 1). In Yr 1, black medic produced more seeds under moderate shade levels than button medic, indicating that the overall seed production of black medic may be increased with up to 55% shade. The seed production of button medic was not affected by shade.

Shade, however, did affect the CP concentration of button medic, with CP concentrations greatest at 55% shade (not different from black medic). By contrast, black medic CP concentration was not affected by shade and produced greater CP concentrations than button medic at the shade extremes (0 and 80% shade). While black medic can maintain high herbage CP concentrations at all shade levels, button medic appears to benefit from moderate shade and can produce similar CP concentrations to black medic at moderate shade levels.

In general, digestibility, as estimated by ADF and lignin, decreased as shade levels increased. There were few differences in ADF and lignin concentrations between black and button medics.

Rhizobium inoculation generally had no effect on yield or quality of black or button medics. In areas such as this study area, where annual medics are naturalized, occur frequently, and soil rhizobia populations are well established, the need for commercial inoculation does not appear to be essential in obtaining comparable DM and seed yields and herbage CP concentrations in black and button medics. Nevertheless, application of commercial *Rhizobium* inoculum should be recommended as a precautionary measure. Additional research on local medics and their commercial *Rhizobium* requirements appears to be warranted.

Moderate shade levels (up to 30% shade) did not appear to negatively affect either black or button medics. When growing conditions were favorable (Yr 1), overall DM yield was not negatively affected for either species until shade levels reached 80%. Seed numbers of black medic and CP concentration of button medic may be increased with up to 55% shade.

When soil moisture is adequate and the damage from insects is not severe, both black and button medics have the potential to produce high quality forage and abundant quantities of seeds under moderate shade environments such as those present in agroforestry, silvicultural, and silvopastoral systems. The apparent shade tolerance of both black and button medics indicates their potential value for use as understory forage crops in agroforestry, silvicultural, and silvopastoral systems, where multiple commodity yields are desired. In grazing situations where the goal is improved forage nutritive value and animal performance, addition of forage legumes such as black and button medics into pastures could help livestock producers achieve these goals.

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