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Row Spacing and Nitrogen: Effect on Alfalfa-Bermudagrass Quality Components

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ABSTRACT

Interseeding legumes into grass sods increases herbage quality. Interseeding alfalfa (*Medicago sativa* L.) into bermudagrass [*Cynodon dactylon* (L.) Pers.] has given yields comparable to grass fertilized with high rates of N. Our objective was to compare forage quality attributes of N-fertilized bermudagrass with alfalfa-bermudagrass mixtures. N rates of 0, 112, 224, and 448 kg ha^{-1} were applied to bermudagrass monoculture and to alfalfa interseeded into bermudagrass at 20-, 40-, and 60-cm row spacings. Experiments were conducted on a Cecil sandy clay loam (clayey, kaolinitic, thermic Typic Kanhapludult) and a Norfolk sandy loam (fine-loamy, siliceous, thermic Typic Kandiudult) site. Hand-harvested herbage samples were separated into botanical components. Crude protein, acid-detergent fiber and neutral-detergent fiber were measured using a combination of wet-lab and near infrared reflectance spectroscopy (NIRS) procedures. Nitrogen increased the crude protein in bermudagrass monoculture by 11 to 61 g kg^{-1}. The crude protein response of bermudagrass in mixtures to N was slight to nonsignificant. Increasing row spacing of alfalfa reduced grass crude protein by 9 to 23 g kg^{-1} and had no effect on alfalfa crude protein. Fiber fractions decreased slightly in grass with added N, but fiber in alfalfa was not influenced by any treatment. Yield of crude protein increased with N, particularly in bermudagrass monoculture, but interseeding alfalfa without N produced crude protein yields that usually exceeded those of bermudagrass monoculture at the 448 kg ha^{-1} N rate. Interseeded alfalfa, even at wide row spacings, appears to produce enough biological N to replace 448 kg fertilizer N ha^{-1} or more in the production of herbage protein in bermudagrass.

BERMUDAGRASS forms a dependable perennial base for many pasture and hay production enterprises in the southeastern USA. There are many well-adapted ecotypes of common bermudagrass (BG), as well as improved hybrid varieties. All of these cultivars share the common trait of relatively low forage quality for grasses of tropical origin (Moore and Mott, 1973), especially under mid- to late-summer weather conditions (Akin, 1989). The forage quality deficit derives from high fiber concentration (Mertens et al., 1987) and relatively low crude protein concentration in the absence of high rates of fertilizer N. Alfalfa and other legumes exhibit lower concentrations of fiber fractions and higher protein and digestible energy than grasses (Sheaffer et al., 1990), and have been used to increase forage quality and animal performance on grass-based pastures (Burns and Standaert, 1985). Alfalfa can be established and will persist when interseeded in BG sods (Brown and Byrd, 1990; and Stringer et al., 1994). Yields of interseeded mixtures were equivalent to BG monoculture fertilized with 200 kg ha^{-1} or more of N. The growing season was lengthened by earlier growth initiation in spring and more active growth in autumn. Bermudagrass persistence was favored by alfalfa row spacings narrower than 60 cm. No information is available on the forage quality components of alfalfa and BG when grown together. Our objective was to examine the effects of alfalfa row spacing and N fertilization on concentration and yield of forage quality components of BG and interseeded alfalfa.

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MATERIALS AND METHODS

Experiment establishment and maintenance are detailed in Stringer et al. (1994) and are summarized here. 'Cimarron' alfalfa was interseeded into a 'Tifton 44' BG sod at two locations, Pendleton on the Piedmont and Edisto on the Coastal Plain of South Carolina. Seedings were made in mid-October, after clipping the sod to five cm. Stand-type treatments included BG monoculture and alfalfa sod-seeded in 20-, 40-, and 60-cm row spacings. A no-tillage drill was used, and no pesticides were used for establishment. Each stand-type treatment was fertilized with 0, 112, 224, and 448 kg ha^{-1} of N as NH_4NO_3 in four split applications per harvest season (complete factorial). The experimental design was a randomized complete block. Fertility and pH were maintained with P, K, and B fertilizer and dolomitic lime, as indicated by soil test.

The experiments were harvested at 35-d intervals, resulting in six cuts per year, except for the first harvest season at Edisto, when only four cuts were made. At each harvest, 0.34-m^2 quadrats were hand-clipped and manually separated to determine botanical composition. Botanical fractions were dried at 65°C for 5 d, weighed and ground through a cyclone mill with a 1-mm screen for chemical analysis. All samples were scanned with a Pacific Instruments Model 6250 near-infrared reflectance spectroscopy system, and spectra stored on computer disk. A 10% subset was selected from each species sample population using the SUBSET procedure from the NIRS software package (Shenk, 1985). These subset samples were submitted for wet-lab analysis for total N using the micro-Kjeldahl procedure, and neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (Goering and Van Soest, 1970). Kjeldahl N was multiplied by 6.25 to calculate percentage crude protein (CP). The spectra and corresponding wet-lab values were used in stepwise multiple regression (CAL procedure) to develop separate calibration equations for the alfalfa and BG components, with 25% being held out for equation validation (Shenk, 1985). These calibration equations were applied to the remainder of the sample populations to estimate CP, ADF and NDF concentration. Quality component concentrations of mixtures were calculated as the sum of the proportion of each botanical component times its quality component concentration.

Concentration of quality constituents and CP yield data were subjected to analysis of variance (ANOVA) using the GLM procedure (SAS Inst., Cary, NC). Nitrogen rate responses were evaluated as orthogonal polynomial single degree of freedom contrasts within each stand type. Locations and years are each analyzed separately, due to cutting schedule changes necessitated by widely varying weather patterns. All comparisons are made at the 0.05 level of probability unless otherwise

Abbreviations: ADF, acid-detergent fiber; BG, bermudagrass; CP, crude protein; NDF, neutral-detergent fiber; NIRS, near infrared reflectance spectroscopy.

Table 1. Crude protein concentration of alfalfa and bermudagrass herbage averaged over all harvests, as influenced by N fertilization and alfalfa row spacing in bermudagrass sods (Edisto, 1987–1988).

	Crude protein concentration													
	Bermudagrass					Alfalfa				Mixture				
	Grass alone	Alfalfa row spacing			N mean†	Alfalfa row spacing			N mean	Grass alone	Alfalfa row spacing			N mean
N rate		20 cm	40 cm	60 cm		20 cm	40 cm	60 cm			20 cm	40 cm	60 cm	
	g kg^{-1}													
1987 (four cuts)														
0	102	146	139	130	138c‡	191	178	182	184	102	181	165	159	168c
112	119	153	149	140	146b	195	185	182	187	119	186	170	161	172bc
224	137	156	158	145	153b	190	185	188	188	137	184	172	169	175b
448	146	170	160	153	160a	198	198	183	193	146	194	188	170	184a
Stand type mean	125c‡	155a	151a	142b		194a	187ab	184b		125d	183a	159c	165b	
Contrasts§	L**	NS	L*	L*		NS	NS	NS		L**	NS	NS	NS	
1988 (six cuts)														
0	98	161	138	141	144c	224	211	209	214	98	214	191	190	198
112	119	165	156	148	154b	212	207	211	210	119	204	194	189	196
224	144	167	170	162	166a	218	212	220	217	144	213	198	202	204
448	159	179	176	164	171a	217	218	210	215	159	212	208	194	205
Stand type mean	130c	167a	160ab	154b		218	212	213		130c	210a	198b	194b	
Contrasts§	L*	NS	L**	L*		NS	NS	NS		L**	NS	L*	NS	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† N means calculated on interseeded data only.
‡ Within years and variables, means followed by the same letter are not significantly different at the 0.05 level of probability.
§ Significant stand type × N rate interactions were evaluated by comparing N rate responses of different stand types, using orthogonal polynomial contrasts. L, linear contrast.

stated. Detailed yield and quality data were collected for two years at each location and plantings were observed under hay harvest for three additional years (no data reported).

RESULTS

Crude protein concentration in BG monoculture herbage increased linearly with increased N fertilization in each year and at each location (Tables 1 and 2). Across locations, BG monoculture fertilized with 448 kg ha^{-1} N ranged from 11 to 61 g kg^{-1} higher in CP than the 0 N check. At Edisto, adding N to interseeded plots increased CP concentration in BG fractions, with increases of 22 to 27 g kg^{-1} at the high N rate over the 0 N interseeded plots. Corresponding differences at Pendleton were not significant. Interseeding alfalfa increased the CP concentration of the BG fraction. Increasing row spacing caused small decreases in CP concentration of the grass fraction, except for the second harvest season at Pendleton (Tables 1 and 2). The grass fraction CP ranged from 9 to 23 g kg^{-1} lower at 60-cm row spacing than at 20-cm spacings. The CP concentration of alfalfa fractions was not affected by N fertilization or row spacing.

Mixed herbage CP of interseeded swards increased with N fertilization only in the first season at Edisto, where the mean mixture CP was 16 g kg^{-1} higher at

Table 2. Crude protein concentration of alfalfa and bermudagrass herbage averaged over six cuts per year, as influenced by N fertilization and alfalfa row spacing in bermudagrass sods (Pendleton, 1988–1989).

	Crude protein concentration													
	Bermudagrass					Alfalfa				Mixture				
	Grass alone	Alfalfa row spacing			N mean†	Alfalfa row spacing			N mean	Grass alone	Alfalfa row spacing			N mean
N rate		20 cm	40 cm	60 cm		20 cm	40 cm	60 cm			20 cm	40 cm	60 cm	
	g kg^{-1}													
1988 (six cuts)														
0	133	157	158	148	154	227	226	229	228	133	215	207	194	206
112	141	160	156	150	155	228	224	227	226	141	213	205	194	204
224	140	160	156	153	156	226	232	225	228	140	211	211	194	206
448	144	162	159	155	158	225	227	234	229	144	218	210	203	210
Stand type mean	140c‡	160a	157a	151b		227	227	229		139c	214a	208a	196b	
	L**	NS	NS	NS		NS	NS	NS		L**	NS	NS	NS	
1989 (six cuts)														
0	115	151	147	141	145	188	189	195	191	115	184	184	185	184
112	118	149	150	145	147	185	187	189	187	118	182	181	185	183
224	125	160	156	150	153	186	187	185	187	125	183	184	183	183
448	148	162	155	153	155	184	186	191	186	148	178	181	185	181
Stand type mean	126b	155a	152a	148a		186	187	191		126b	181a	183a	185a	
	L*	NS	NS	NS		NS	NS	NS		L**	NS	NS	NS	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† N means calculated on interseeded data only.
‡ Within years and variables, means followed by the same letter are not significantly different at the 0.05 level of probability.
§ Significant stand type × N rate interactions were evaluated by comparing N rate responses of different stand types, using orthogonal polynomial contrasts. L, linear contrast.

Table 3. Fiber content of bermudagrass herbage averaged over all harvests, as influenced by N fertilization and alfalfa row spacing in bermudagrass sods (Edisto, 1987–1988).

	Acid-detergent fiber					Neutral-detergent fiber				
	Grass	Alfalfa row spacing				Grass	Alfalfa row spacing			
N rate	alone	20 cm	40 cm	60 cm	N mean†	alone	20 cm	40 cm	60 cm	N mean
	g kg^{-1}									
1987 (four cuts)										
0	328	314	313	313	313a‡	715	653	658	661	658a
112	315	310	308	312	310ab	684	648	653	662	655a
224	311	305	303	309	306bc	671	631	652	659	650a
448	302	298	304	302	302c	670	618	648	649	642a
Stand type mean	314a‡	307b	307b	309b		686a	640b	653b	658b	
N rate response	L*				L*	L*				NS
1988 (six cuts)										
0	321	300	311	308	308a	748	711	736	738	730a
112	313	306	268	299	299ab	737	709	719	713	714b
224	299	305	289	292	295b	715	717	698	707	704bc
448	288	299	290	292	292b	703	701	691	711	702c
Stand type mean	305a*	303a	296a	298a		725a	715ab	711b	709b	
N rate response§	L*				L*	L*				L*

* Significant at the 0.05 probability level.
† N means calculated on interseeded data only.
‡ Within years, row and column means followed by the same letter are not significantly different at the 0.05 level of probability.
§ N rate response evaluated with orthogonal polynomial single degree of freedom contrasts.

448 kg ha^{-1} N than where no N was applied to interseeded stands. Increasing row spacing decreased mixture CP concentration, except at Pendleton in the second harvest season. The mixture CP concentration ranged from 16 to 18 g kg^{-1} higher in 20- than in 60-cm plots. The mean CP concentration of interseeded herbage with no N fertilization was higher than BG monoculture at the highest N rate in the second harvest season at Edisto and the first season at Pendleton and not different in other instances (compared via single degree of freedom contrasts).

Fiber fraction concentrations of alfalfa were not affected by treatments or interactions in either location or year, and so are not presented. Mean values for alfalfa ADF and NDF were 320 and 423 g kg^{-1}, respectively. Nitrogen fertilization caused a linear decrease in the ADF content of BG monoculture in both locations and seasons, from 13 to 33 g kg^{-1} lower in the high N rate than in the 0 N check (Tables 3 and 4). In the first growing season at both locations, ADF of BG monoculture was slightly higher than for the grass component of interseeded stands. Nitrogen fertilization of interseeded stands decreased ADF concentration of the grass component by 11 to 16 g kg^{-1} at Edisto, but not at Pendleton. Row spacing had no effect on ADF concentration of the BG component, and there were no N × stand type interactions. Neutral-detergent fiber in BG monoculture decreased linearly with N fertilization, from 24 to 45 g kg^{-1} lower at 448 kg ha^{-1} N than at the 0 N check (Tables 3 and 4). In interseeded plots, NDF in the BG component decreased linearly with N fertilization in the second season at Edisto and the first season at Pendleton. In most instances, the mean NDF concentration in in-

Table 4. Fiber content of bermudagrass herbage averaged over all harvests, as influenced by N fertilization and alfalfa row spacing in bermudagrass sods (Pendleton, 1988–1989).

	Acid-detergent fiber					Neutral-detergent fiber				
	Grass	Alfalfa row spacing				Grass	Alfalfa row spacing			
N rate	alone	20 cm	40 cm	60 cm	N mean†	alone	20 cm	40 cm	60 cm	N mean
	g kg^{-1}									
1988 (six cuts)										
0	326	296	300	307	302	701	670	662	681	671a
112	310	293	299	300	298	681	666	662	671	666ab
224	309	296	303	298	299	689	661	662	666	664ab
448	310	292	306	304	302	677	657	657	663	660b
Stand type mean	314a‡	294b	301b	302b		687a	663bc	661c	670b	
N rate response§	L*				NS	L*				L*
1989 (six cuts)										
0	335	334	337	328	332	722	676	686	682	688
112	334	333	338	331	334	681	675	689	677	685
224	334	328	333	332	331	676	676	686	682	683
448	322	333	336	332	334	686	675	689	678	681
Stand type mean	331	332	336	331		708a	678b	687b	684b	
N rate response	L*				NS	L*				NS

* Significant at the 0.05 probability level.
† N means calculated on interseeded data only.
‡ Within years, row and column means followed by the same letter are not significantly different at the 0.05 level of probability.
§ N rate response evaluated with orthogonal polynomial single degree of freedom contrasts.

Table 5. Total yield of crude protein by bermudagrass and alfalfa-bermudagrass mixtures as influenced by N rate and alfalfa row spacing (Edisto, 1987-1988).

N rate	Grass alone	20 cm	40 cm	60 cm	N means†
	Total crude protein yield				
		Alfalfa row spacing			
	kg ha^{-1}				
1987 (four cuts)					
0	294	1483	1353	1082	1305b‡
112	353	1532	1366	1161	1353b
224	446	1522	1291	1248	1353b
448	811	1705	1543	1402	1550a
Stand type means	454d‡	1560a	1388b	1223c	
N rate response§	L**	Q*	NS	L*	
1988 (six cuts)					
0	127	1938	1730	1198	1622b
112	260	2358	1473	1267	1697b
224	432	2133	1570	1391	1699b
448	1096	2524	2149	2060	2244a
Stand type means	437.6c	2238.4a	1730.4b	1478.8b	
N rate response	L**	Q*	NS	L*	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† N means calculated on interseeded data only.
‡ Within years, row or column means followed by the same letter are not significantly different at the 0.05 level of probability.
§ N rate response evaluated using orthogonal polynomial contrasts.

terseeded BG herbage was lower than in the BG monoculture treatments.

Herbage crude protein yield of BG monoculture increased linearly with N fertilization at both locations and seasons (Tables 5 and 6). The maximum CP yields ranged from 811 to 2469 kg ha^{-1}, and the mean yield at 448 kg ha^{-1} of N ranged from 1.02 to 7.63 times higher than corresponding yields at the 0 N check. The increase in CP yield was 2.27 kg per kg of applied N. In the interseeded stands, increasing alfalfa row spacing from 20 to 60 cm decreased CP yields in every instance, the size of the decrease ranging from 279 to 759 kg CP ha^{-1}, or a decrease of 11 to 34%. Adding N to interseeded plots increased yield of CP in each instance except the second season at Pendleton. Increase in yield of CP with added N in interseeded plots averaged only 0.83 kg CP kg^{-1} N. The mean CP yield of interseeded plots with no N fertilization was higher than BG monoculture at the highest N rate in every instance except the second harvest season at Pendleton (compared via single degree of freedom contrasts).

Table 6. Total yield of crude protein by bermudagrass and alfalfa-bermudagrass mixtures as influenced by N rate and alfalfa row spacing (Pendleton, 1988-1989).

N rate	Grass alone	20 cm	40 cm	60 cm	N means†
	Total crude protein yield				
		Alfalfa row spacing			
	kg ha^{-1}				
1988 (six cuts)					
0	796	2187	1995	1581	1921c‡
112	1151	2176	2001	1832	2003bc
224	1299	2232	2103	1920	2084ab
448	1609	2407	2073	2046	2175a
Stand type means	1214d‡	2250a	2043b	1845c	
N rate response§	L**	L*	NS	L**	
1989 (six cuts)					
0	702	2713	2422	2189	2441
112	1172	2653	2342	2207	2401
224	1647	2339	2425	2339	2377
448	2469	2638	2425	2495	2514
Stand type means	1498c	2586a	2411ab	2307b	
N rate response	L**	NS	NS	L**	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† N means calculated on interseeded data only.
‡ Within years, row or column means followed by the same letter are not significantly different at the 0.05 level of probability.
§ N rate response evaluated using orthogonal polynomial contrasts.

DISCUSSION

Yield responses of BG to N fertilization corresponding to the composition data reported here were linear increases (Stringer et al., 1994). Linear increases in CP concentration of BG monoculture in response to N were similar to those reported by Burton and Jackson (1962) for 'Coastal' BG. Thom et al. (1990) reported curvilinear responses for Tifton 44 in Kentucky in the transition zone for BG. The presence of alfalfa without N fertilizer increased the CP of companion BG to a level comparable with BG monoculture with 112 to 224 kg ha^{-1} of N, which is similar to the response of orchardgrass (*Dactylis glomerata* L.) to white clover (*Trifolium repens* L.) reported by Wagner (1954). The lack of alfalfa CP response to N fertilization is consistent with reports of reduced nodulation (Trimble et al., 1987) and reduced biological N_2 fixation (Hannaway and Schuler, 1993) when ample available soil N is present. Crude protein concentration of mixed forage reflects the predominance of alfalfa in the harvested forage (Stringer et al., 1994) and the high CP concentration in alfalfa (Tables 1 and 2). Adding alfalfa to BG sods increased the mixture CP concentration to a level 36% higher than BG monoculture fertilized with 448 kg ha^{-1}. Yield of CP showed an even greater advantage for alfalfa-BG over N-fertilized BG, particularly at Edisto. The large advantage of alfalfa-BG at Edisto may have resulted from less retention of applied N in the root zone in the sandy soils there.

Nitrogen fertilization decreased ADF and NDF in BG, particularly at Edisto. Others have reported fiber concentration increases (Thom et al., 1990), or no change (Burton, 1954). Bermudagrass growing with alfalfa was usually lower in ADF and NDF than BG monoculture. This may be due to shading by the relatively taller alfalfa. Kephart and Buxton (1993) found that reducing incident light with shade cloth reduced the fiber content of cool-season grasses. Neither N fertilization nor row spacing had significant impact on the concentration of fiber fractions in alfalfa.

Growing alfalfa in mixtures with BG has produced yields equal to or higher than BG with high applications of N (Stringer et al., 1994). The advantage from interseeding alfalfa into BG sods is even greater when examined from the standpoint of yield of quality components. Interseeding alfalfa in 60-cm or wider row spacings is the most reasonable practice, as it allows BG to persist. Alfalfa interseeded in 60-cm rows with no fertilizer N yielded slightly more crude protein than BG fertilized with 448 kg ha^{-1} of N, at a conservative savings of \$247 ha^{-1} per year. This annual savings will more than cover the higher pest management costs [mainly to control alfalfa weevil, *Hypera postica* (Gyllenhal)] associated with alfalfa in the mixture. Economic enterprise budgets for alfalfa (Harwell et al., 1992) suggest a 4-yr amortized establishment cost for alfalfa of $165 ha^{-1} per year. Because of the low seeding rates (6 to 8 kg ha^{-1} in 60-cm rows) and reduced tillage used to interseed alfalfa, these establishment costs are probably inflated. We have stands of alfalfa–BG that have persisted under hay management for four or more years. Under good management, interseeding alfalfa into BG may result in considerable quality and economic advantages over BG monocultures.

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