Forage Quality of Perennial Glandular-haired and Eglandular <u>Medicago</u> Populations

bу

ANDREW WILLIAM LENSSEN

B.S., Cornell University, 1980

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY Manhattan, Kansas

1987

Approved by:

Major Professor

Page PART I. In Vitro Dry Matter Disappearance and Crude Protein Contents of Leaf and Stem Fractions Total Cell Wall and Fiber Contents of Leaf and PART II.

LIST OF TABLES

			Pag
		PART I In <u>Vitro</u> Dry Matter Disappearance and Crude Protein Contents of Leaf and Stem Fractions	
Table	1.	Harvest dates of 12 <u>Medicago</u> populations from five cuttings over two years	18
Table	2.	In vitro dry matter disappearance of stem and leaf components from four harvests of 12 Medicago populations	19
Table	3.	Correlation coefficients of stem versus leaf components across 12 <u>Hedicago</u> populations for in <u>vitro</u> dry matter disappearance and crude protein content	r
Table	ц.	Crude protein of stem and leaf components from four harvests of 12 Medicago populations	21
	,	PART II Total Cell Wall and Fiber Contents of Leaf and Stem Fractions	
Table	1.	Neutral detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations	
Table	2.	Correlation coefficients of stem versus leaf for five fiber components across 12 <u>Medicago</u> populations for five fiber components	
Table	3 •	Acid detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations	44
Table	4.	Hemicellulose and lignin contents of stem and leaf components from 12 Medicago populations across four harvests	45
Table	5.	Hemicellulose and lignin contents of stem and leaf components from four harvests across 12 Medicago populations	46
Table	6.	Cellulose contents of stem and leaf components from four harvests of 12 Medicago populations.	47

LIST OF APPENDIX TABLES

		Page
Table 1.	Hemicellulose contents of stem and leaf components from four harvests of 12 Medicago populations	67
Table 2.	Lignin contents of stem and leaf components from four harvests of 12 Medicago populations	68
Table 3.	Correlation coefficients of stem and leaf in vitro dry matter disappearance with their respective fiber components across 12 Medicago populations, by cutting	69
Table 4.	Correlation coefficients of leaf and stem crude proteins with their respective fiber components from 12 <u>Medicago</u> populations, by cutting	70
Table 5.	Analysis of variance mean squares for leaf components from three harvests of 12 Medicago populations	71
Table 6.	Analysis of variance mean squares for stem components from three harvests of 12 Medicago populations	72
Table 7.	In vitro dry matter disappearance of 12 Medicago populations with five time periods, July 1985 harvest	73
Table 8.	In vitro dry matter disappearance of 12 Medicago populations with five time periods, August 1985 harvest	74
Table 9.	In vitro dry matter disappearance of 12 Medicago populations with five time periods, September 1985 harvest	75
Table 10.	In vitro dry matter disappearance of 7 Medicago populations with five time periods, October 1985 harvest	76
Table 11.	In <u>vitro</u> dry matter disappearance of 12 <u>Medicago</u> populations with five time periods, May 1986 harvest	77
Table 12.	Leaf percentage of above ground dry matter from four harvests of 12 Medicago population	s 78

Table	13.	Crude protein contents of 12 Medicago populations from five harvests	79
Table	14.	Yield of 12 Medicago populations from four harvests	80

Forage Quality of Perennial Glandular-haired and Eglandular

<u>Medicago</u> Populations. I. <u>In Vitro</u> Dry Matter Disappearance and

Crude Protein Contents of Leaf and Stem Fractions 1

A.W. Lenssen, E.L. Sorensen, G.L. Posler, and L.H. Harbers²

¹ Cooperative investigations of the Kansas Agric. Exp. Stn.,
Dep. of Agronomy and Animal Sciences and Industry, and ARS,
USDA, Kansas State Univ., Manhattan, KS 66506. Contribution No.
87-182-J from the Kansas Agricultural Experiment Station.
Received _______.

²Graduate research assistant, USDA-ARS research agronomist, professor, Dep. of Agronomy, and professor, Dep. of Animal Sciences and Industry, Kansas State Univ., Manhattan, KS 66506.

³These plants were derived from PI 346919, a plant introduction from Russia. It was labeled Medicago glutinosa. Later, Gunn et al. (1978) identified it as a mixture of the following M. sativa L. subspecies: sativa. praefalcata. glomerata, and X yaria.

ABSTRACT

Resistance available in current alfalfa (Medicago sativa L.) cultivars is inadequate to control the alfalfa weevil [Hypera postica (Gyllenhal)] or the potato leafhopper [Empoasca fabae (Harris)], the two most injurious arthropod pests of alfalfa in North America. Resistance to both insects has been documented in other Medicago species having erect, glandular hairs and these hairs have been transferred to alfalfa. The effects of glandular hairs and their exudates on forage quality of alfalfa are unknown. We established a field trial in 1985 to determine the effects of erect glandular hairs and their exudates on forage quality of some perennial Medicagos. Glandular and eglandular plant populations were selected from the diploids M. glandulosa David and M. prostrata Jacq. and tetraploids M. glutinosa Bieb.3, M. sativa (MS4n) x M. glutinosa, and MS4n x M. prostrata. Eglandular M. sativa 'Riley' and diploid M. sativa subsp. caerulea (Less. ex Ledeb.) Schmalh. were included as controls. Foliar diseases and insects were controlled. Leaves were separated from stems for three harvests in 1985 and one harvest in 1986. In vitro dry matter disappearance (IVDMD) and crude protein (CP) were determined on each component. The presence of glandular hairs on the wild Medicago

species (glandulosa, prostrata, and glutinosa) did not significantly affect the IVDMD or CP contents of leaves or stems. Stem digestibilities and crude protein contents of the diploid populations exceeded those of the alfalfa cultivar Riley.

Additional index words: M. sativa, M. glandulosa, M. prostrata, M. glutinosa, M. sativa subsp. caerulea, host-plant resistance, erect glandular hairs, Hypera postica, Empoasca fabae, in vitro dry matter disappearance, crude protein.

Alfalfa (Medicago sativa L.) is the premier forage crop for production of dairy and beef products throughout both temperate and semiarid climates. The most destructive arthropod pests of alfalfa in North America are the alfalfa weevil [Hypera postica (Gyllenhal)] and the potato leafhopper [Empoasca fabae (Harris)]. Little host-plant resistance has been found among M.sativa germplasms for these pests.

Trichomes have been recognized in many plant species as a defense mechanism (Levin, 1973). Erect glandular hairs in Medicago provide host-plant resistance by antixenotic or antibiotic effects, which include changes in adult mortality (Brewer et al., 1986a), ovipositional behavior (Brewer et al., 1986b), and larval entrapment (Shade et al., 1975). Many Medicago glandular exudates have not been fully isolated and identified. However, Triebe et al. (1981) concluded that glandular exudates from M. scutellata (L.) Mill. would not present a toxicological problem for forage-consuming livestock.

Kreitner and Sorensen (1979) detailed the structures of procumbent and erect glandular trichomes present on several annual and perennial Medicago species and reported that interspecific crosses had successfully transferred erect glandular hairs into M. sativa L. populations. Further

research has shown that lignification of vascular and interfascicular regions in the stems of some glandular-haired Medicago species may be more extensive than in currently available cultivars (Brewer et al., 1986b). Alfalfa breeders are interested in conferring resistance to alfalfa weevil and potato leafhopper by the incorporation of erect glandular hairs, but no information is available regarding the effects of erect glandular hairs and their exudates on the nutritive value of resultant herbage.

The primary goal of our study was to determine whether erect glandular hairs and their exudates influence the digestibility and crude protein content of diverse, perennial <u>Medicago</u> populations. The second objective was to compare the forage quality of the diploid species, <u>M. prostrata</u> and <u>M. glandulosa</u>, populations to cultivated tetraploid alfalfa.

MATERIALS AND METHODS

Populations of plants with (+) and without (-) erect glandular hairs were selected from perennial diploids M. prostrata Jacq. and KS94 and tetraploids KS108, KS159, and KS160. Glandular-haired plants of KS94 and KS108 were selected from populations of the 6th and 5th cycles, respectively, of recurrent phenotypic selection for erect glandular hairs. Essentially eglandular plants were selected from the 1st cycles of selection for erect glandular hairs. KS94GH6 (Sorensen et al., 1986), M. glandulosa David (perennial diploid), and KS108GH5, M. glutinosa Bieb. (P.I. 346919 perennial tetraploid) (Sorensen et al., 1985) have been previously described in detail. KS159 is an M. sativa x M. prostrata tetraploid hybrid backcrossed three times to M. sativa populations. KS160 is an M. sativa x M. glutinosa hybrid backcrossed three generations to M. sativa populations. Selected (+) and (-) populations were from first and fourth cycles of selection for erect glandular hairs, respectively. Eglandular diploid M. sativa subsp. caerulea (Less. ex Ledeb.) Schmalh. (P.I. 172984) and tetraploid M. sativa 'Riley' (Sorensen et al., 1978) were included as controls.

Seeds of all populations were scarified, treated with a commercial Rhizobium inoculant, and planted in

flats in a greenhouse. Seedlings selected for (+) or (-) were transplanted to peat pots and later transplanted into a field nursery on 19-21 May 1985. Plants were spaced 30 and 45 cm within and between rows, respectively. Experimental design was a randomized complete block with four replications of 20 plants per replicate. Diseases and insects were controlled to eliminate confounding effects on forage quality (Willis et al., 1969). Irrigation water was supplied as necessary to prevent moisture stress.

Tetraploid populations were harvested four times in 1985, but diploids were harvested only three times because growth was insufficient for an October harvest. All populations were harvested once in May 1986 (Table 1).

Freshly harvested materials were taken immediately to the laboratory. Twenty-five stems with 3 to 4 buds per stem were randomly selected from each treatment replication for separation of leaves from stems. Fifty to 75 stems of the M. prostrata populations were required to provide sufficient dry matter for laboratory analyses. M. prostrata materials used for analyses were at the full bloom stage of maturity because of their indeterminate flowering habit. Leaf separations were not done on materials from the July harvest. October-harvested

materials used for laboratory analyses were at the one bud-per-stem stage of maturity. After gently rinsing with water to remove soil, leaves were separated from stems. Leaflets and petioles comprised the leaf fraction, whereas the stem fraction included primary stems and axillary branches. Buds were discarded. Samples were oven dried at 60°C and leaf and stem fractions were ground to pass a 1 mm screen with a cyclone mill. In vitro dry matter disappearance (IVDMD) of stems and leaves was determined using a modified Tilley and Terry technique (1963). Nitrogen was determined colorimetrically following a H₂SO₄/H₂O₂ digestion. Crude protein of stems and leaves was determined as percent nitrogen * 6.25.

Analysis of variance procedures, followed by protected least significant difference tests for mean separations, were used on all data (Snedecor and Cochran, 1980). Arcsine-square root transformations were made on all percentage data. Since conclusions were not different for transformed and untransformed data, results presented are untransformed.

RESULTS

In Vitro Dry Matter Disappearance

The presence of glandular hairs on the wild Medicago species (glandulosa, glutinosa, prostrata) did not affect the digestibility of leaves and stems. The IVDMD of glandular-haired and eglandular populations from each species was similar for each of the four cuttings demonstrating that the relationship was unaffected by seasonal variations in environmental conditions (Table 2). Likewise, when the glandular hairs were transferred from M. prostrata and M. glutinosa to M. sativa (KS159 and KS160, respectively) they again had little effect on digestibility of the forage. KS160 (+) and (-) leaf digestibility comparisons for September and October were significant but data for the two months were inversely related to digestibility (Table 2).

The population x cutting interaction was significant for IVDMD. Stem digestibilities of the diploid populations, including eglandular diploid M. sativa, were significantly higher than those for the tetraploid M. sativa cultivar, Riley. The differences were dramatic during the warm season (August and September harvests) but narrowed during the cool spring weather (May harvest). The digestibility of diploids was altered little by

seasonal effects, whereas IVDMD of Riley increased during cool weather (Table 2). The IVDMD of the wild tetraploid, M. glutinosa. (KS108) trended higher than did Riley but the differences were generally nonsignificant. The hybrids (KS159 and KS160), which represented the third backcross to M. sativa, generally were comparable to Riley in digestibility (Table 2).

In contrast to stems, the leaf digestibility of diploids M. glandulosa, M. prostrata, and M. sativa were lower than those of tetraploid M. sativa (Table 2).

The overall correlation between leaf and stem digestibilities was low, but positive and significant (Table 3).

Crude Protein

Crude protein contents of stems from the glandular-haired and eglandular populations selected from three species and two hybrids varied significantly among entries for all cuttings.

However, of the (+) and (-) comparisons, only KS160 for the September and October harvests differed (Table 4). The crude protein content of the glandular-haired population exceeded that of the eglandular group for each of these harvests.

Crude protein contents of stems from the

diploids M. prostrata, M. glandulosa, and M. sativa and tetraploid M. glutinosa (KS108) (wild species) generally exceeded that of tetraploid M. sativa (Table 4).

Crude protein content of the leaves from the diploid wild species M. prostrata and M. glandulosa, was generally lower and from diploid M. sativa was higher than that from tetraploid M. sativa.

For leaves, four of the 18 (+) and (-) comparisons were significantly different (Table 4) and the (+) and (-) populations were each superior in two of these four comparisons.

The correlation between leaf and stem crude protein content was positive for each harvest and highly significant for the overall comparison (Table 3).

DISCUSSION

Several studies, including those by Brewer et al. (1986a). Danielson et al. (1986a). Levin (1973). and Shade et al. (1975), have documented the value of erect glandular hairs in self defense of plants against insects. However, the effects of erect glandular hairs on quality of forage have been questioned. In our study, the presence of erect glandular hairs on the diploids M. glandulosa and M. prostrata and tetraploid M. glutinosa did not affect the digestibility or crude protein content of the forage. Also, Triebe et al. (1981) found no toxic compounds in the exudate from the erect glandular hairs. Since the glandular hairs were easily transferred from the diploid wild species to \underline{M} . sativa via 2X x 4X crosses (Sorensen et al., 1980), it should be possible to develop hay-type cultivars adapted to the varied climates where alfalfa is grown.

Coors et al. (1986) successfully improved the nutritive value of alfalfa by utilizing M. sativa x M. sativa subsp. caerulea hybrids. We found that stems from the diploid species, including eglandular M. sativa, were more digestible and frequently had a higher crude protein content than the alfalfa cultivar Riley. The higher digestibility of the

diploid stems may be due to a larger cortex and consequently smaller pith in these diploids than in hay-type cultivars. Suksayretrup (1986) found that the cortex/radius ratio was larger in M. prostrata than in the diploid M. sativa she studied. We noted this in plants from other diploid species and in the glandular haired tetraploid, M. glutinosa. Suksayretrup (1986) also found increased lignification of vascular bundles and lignification of the interfascicular areas which resulted in a lignified cylinder surrounding the pith. This would suggest increased fiber content of the plants. Lenssen et al. (manuscript in preparation), however, failed to note increased fiber content in the diploid species. Brewer et al. (1986b) speculated that the lignified cylinder prevented oviposition into the pith area by the potato leafhopper and, thus, provided very high resistance to this insect. A similar situation may exist for resistance to the alfalfa weevil (Danielson et al., 1986b).

The possibility of increasing stem quality of alfalfa and concomitantly developing high resistance to some very destructive insect pests presents unique opportunities for plant breeders to improve alfalfa.

REFERENCES

- Brewer, G.J., E.K. Horber, and E.L. Sorensen. 1986a.

 Potato leafhopper (Homoptera: Cicadellidae)

 antixenosis and antibiosis in <u>Medicaso</u> species. J.

 Econ. Entomol. 79:421-425.
 - , E.L. Sorensen, E. Horber, and G.L.

 Kreitner. 1986b. Alfalfa stem anatomy and potato
 leafhopper (Homoptera: Cicadellidae) resistance. J.

 Econ Entomol. 79:1249-1253.
- Coors, J.G., C.C. Lowe, and R.P. Murphy. 1986. Selection for improved nutritional quality of alfalfa forage. Crop Sci. 26:843-848.
- Danielson, S.D., G.R. Manglitz, and E.L. Sorensen.

 1986a. Development of alfalfa weevil (Coleoptera:
 Curculionidae) larvae when reared on perennial
 glandular-haired <u>Medicago</u> species in the
 greenhouse. Environ. Entomol. 15:396-398.

_____, ____, and _____.

- 1986b. Resistance of perennial glandular-haired

 Medicago species to oviposition by alfalfa weevils.

 (Coleoptera: Curculionidae). Environ. Entomol. 15:
 accepted, in press.
- Gunn, C.R., W.H. Skrdla, and H.C. Spencer. 1978.
 Classification of <u>Medicago gativa</u> L. using legume characters and flower colors. U.S. Dep. Agric.
 Tech. Bull. 1574.

- Kreitner, G.L., and E.L. Sorensen. 1979. Glandular trichomes on <u>Medicago</u> species. Crop Sci. 19:380-384.
- Levin, D.A. 1973. The role of trichomes in plant defense. O. Rev. Biol. 48:3-15.
- Shade, R.E., T.E. Thompson, and W.R. Campbell. 1975. An alfalfa weevil larval resistance mechanism detected in Medicago. J. Econ. Entomol. 68:399-404.
- Snedecor, G.W., and W.G. Cochran. 1980. Statistical Methods. Seventh Ed. The Iowa State University Press. Ames, Iowa.
- Sorensen, E.L., E.K. Horber, and D.L. Stuteville. 1985. Registration of KS108GH5 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 25:1132.
- Registration of KS9%GH6 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 26:1088.

_____, _____, and _____. 1986.

- Sorensen, E.L., N. Sangduen, and G.H. Liang. 1981.

 Transfer of glandular hairs from diploid Medicago
 prostrata to tetraploid M. sativa. p.53, In: Report
 of the Twenty-seventh Alfalfa Improvement
 Conference, ARM-NC19.
 - , D.L. Stuteville, and E.K. Horber. 1978.

 Registration of Riley alfalfa. Crop Sci. 18:911.

- Suksayretrup, K. 1986. Xerophytism in Medicago. PhD

 Dissertation. Kansas State University, Manhattan,
 Kansas.
- Tilley J.M.A., and R.A. Terry. 1963. A two stage technique for the <u>in vitro</u> digestion of forage crops. J. Brit. Grassl. Sci. 18:104-118.
- Triebe, D.C., C.E. Meloan, and E.L. Sorensen. 1981. The chemical identification of the glandular-hair exudate from <u>Medicago scutellata</u>. p.52, In: Report of the Twenty-seventh Alfalfa Improvement Conference, ARM-NC19.
- Willis, W.G., D.L. Stuteville, and E.L. Sorensen. 1969.

 Effects of leaf and stem diseases on yield and
 quality of alfalfa forage. Crop Sci. 9:637-640.

List of Tables

- Table 1. Harvest dates of 12 <u>Medicago</u> populations from five cuttings over two years.
- Table 2. In <u>vitro</u> dry matter disappearance of stem and leaf components from four harvests of 12 <u>Medicago</u> populations.
- Table 3. Correlation coefficients across 12 Medicago
 populations of stem versus leaf comparisons
 for in vitro dry matter disappearance and
 crude protein.
- Table 4. Crude protein of stem and leaf components from four harvests of 12 Medicago populations.

Table 1. Harvest dates of 12 Medicago populations during two years.

	Year						
	1 98 5						
Population	July August		September	October	Мау		
MP(+)+	7-9	8 - 1	9-10	-	5-16		
MP(-)	7-9	8 - 1	9-10		5-16		
KS94(+)	7 - 9	8 - 2	9-14	-	5-15		
KS94(=)	7 - 9	8 - 9	9-15		5-15		
MS2n	7-9	8 - 1 0	9-29	10-5	5-15		
Riley	7-9	8 - 1	9-5		5-13		
KS108(+)	7 - 9	8 – 1	9-3	10-3	5-13		
KS108(-)	7 - 9	8 – 1	9-4	10-2	5-13		
(S159(+)	7-9	8 – 1	9-5	10-5	5-14		
(S159(-)	7-9	8 – 1	9-1	10-13	5-14		
KS160(+)	7 - 9	8 - 1	9 = 2	10-1	5-12		
KS160(-)	7 - 9	8 - 1	8 = 3 1	10-13	5-12		

† MP = M. prostrata, KS9% = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa. (+) = presence or erect glandular hairs, (-) = absence of erect glandular hairs.

Table 2. In <u>vitro</u> dry matter disappearance of stem and leaf components from four harvests of 12 <u>Medicago</u> populations.

	Stem				Leaf			
Population	Aug	Sept	0ct	Мау	Aug	Sept	0 c t	May
MP(+) [†] MP(-)	59.1 59.9	58.6 58.9		- dg/kg 52.3 53.7	DM 73.4 72.6	73.9		74.7
KS94(+) KS94(-)	60.9 60.4	57.5 57.0	-	59.7 60.4	73.4 72.2	74.6 73.7	-	74.8 74.9
MS2n Riley	61.8 54.4	62.0 51.2	64.9	60.8 57.4	74.7 75.3	73.6 74.9	76.6	75.1 77.3
KS108(+) KS108(-)	60.3 58.2	53.2 52.3	65.1 66.3	60.7 58.9	76.0 75.4	74.5 74.0	77.0 77.3	78.8 77.2
KS159(+) KS159(=)	56.2 56.9	50.6 51.8	63.8 63.3	57.0 58.9	75.8 75.1	74.5 74.6	77.3 76.6	76.2 77.
KS160(+) KS160(-)	58.7 58.7	51.5 53.0	63.5 60.9	60.9 59.6	75.7 75.2	73.7 74.7	76.9 75.9	77.7 76.8
LSD(.05) r ² \$CV	2.21 .72 2.21	2.39 .87 3.04	1.87 .74 1.96	.85	1.28 .78 1.19	0.79 .51 0.74	.55	1.0

TMP = M.orostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M.sativa, KS108 = M.glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa. (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs.

Table 3. Correlation coefficients of stem versus leaf components across 12 Medicago populations for in <u>vitro</u> dry matter disappearance and crude protein.

Parameter	August	September	October r values	Мау	Combined
IVDMD	20	30 *	·51**	.41**	·37***
n	48		28	48	172
C P	.32 *	-12	· 47*	· 25	.52 ***
n	48	48		48	172

^{*, **, ***} significant at the .05, .01, and .001 levels of probability, respectively.

Table 4. Crude protein contents of stem and leaf components from four harvests of 12 Medicago populations.

Populatio	n	Stem				Leaf			
	Aug	Sept	Oct	Мау	Aug	Sept	Oct	Мау	
MP(+) MP(-)	13.9	12.2	- : -	- dg/kg 10.4 10.5	DM 26.6 25.7	23.0		22.2	
KS94(+) KS94(-)	12.0	11.1	-	9.9 10.2	24.6	22.1	-	21.8	
MS2n Riley	12.7	12.6	12.1	11.8	26.9 28.5	29.3 25.9	31.7	28.0 25.5	
KS108(+) KS108(-)	13.9	12.7	15.4	10.1	29.7 28.6	27.0 28.3	32.5 32.1	24.0 24.9	
KS159(+) KS159(-)	11.8	10.5	11.7	9.9 10.0	29.9 28.9	29.0 28.7	32.1 28.0	27.4 26.1	
KS160(+) KS160(-)	12.6 13.0	12.4	14.3	10.1	28.8	28.9 27.2	32.5	24.6 27.1	
LSD(.05) r ² \$CV	1.57 .50 8.59	1.55 .53 9.57	1.69 .75 8.70	0.78 .70 5.29	2.26 .71 5.65	1.75 .89 4.67	1.92 .68 4.12	1.91	

TMP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa. (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs. Forage Quality of Perennial Glandular-haired and Eglandular

Medicago Populations. II. Total Cell Wall and Fiber Contents of

Leaf and Stem Fractions.

A.W. Lenssen, E.L. Sorensen, G.L. Posler, and L.H. Harbers.²

Cooperative investigations of the Kansas Agric. Exp. Stn.,

Deps. of Agronomy and Animal Sciences and Industry, and ARS,

USDA, Kansas State Univ., Manhattan, KS 66506. Contribution

no._____ from the Kansas Agric. Exp. Stn. Received _____.

2Graduate research assistant, USDA research agronomist, and

professor, Dep. of Agronomy, and professor, Dep. of Animal

Sciences and Industry, Kansas State Univ., Manhattan, KS 66506.

Abstract

Host-plant resistance in alfalfa (Medicago sativa L.) is insufficient for control of the alfalfa weevil [Hypera postica (Gyllenhal)] or the potato leafhopper [Empoasca fabae (Harris)], two of the most important insect pests of alfalfa. Some wild Medicago species, which have erect glandular hairs, possess adequate host-plant resistance for control of both pests, and the hairs have been transferred into alfalfa populations. We established a field trial in 1985 to determine the effects of erect glandular hairs on forage quality of several perennial Medicago species. Glandular and eglandular plant populations were selected from the diploids M. prostrata Jacq. and M. glandulosa David, and tetraploids M. glutinosa Bieb.3, M. sativa x M. glutinosa, and M. sativa x M. prostrata. Eglandular M. sativa 'Riley' and M. sativa subsp. caerulea (Less. ex Ledeb.) Schmalh. were included as controls. Foliar diseases and insects were controlled. Leaves and stems were 3These plants were derived from PI 346919, a plant introduction from Russia. It was labeled Medicago glutinosa. Later, Gunn et al. (1978) identified it as a mixture of the following M. sativa L. subspecies: sativa, praefalcata, glomerata, and X varia.

separated for three harvests in 1985 and one in 1986. Neutral and acid detergent fibers, hemicellulose, lignin, and cellulose contents were determined for leaf and stem components. The presence of erect glandular hairs did not significantly affect concentrations of neutral or acid detergent fibers, hemicellulose, lignin, or cellulose of leaves or stems within the species or hybrids tested. Neutral and acid detergent fibers and cellulose contents were generally lower in stems and higher in leaves of diploids than in corresponding parts of the tetraploid alfalfa cultivar Riley. Additional index words: M. sativa, M. glandulosa, M. prostrata, M. glutinosa, M. sativa subsp. caerulea, host-plant resistance, Hypera postica, Empoasca fabae, neutral detergent fiber, acid detergent fiber, hemicellulose, lignin, cellulose.

The production of nutritious, high yielding forage for ruminant livestock consumption has become increasingly important for optimizing meat and milk production and to enhance cost efficiency. Alfalfa (Medicago sativa L.) is the forage crop of choice in nearly all areas where it can be successfully grown and conserved. Two arthropod pests, the alfalfa weevil [Hypera postica (Gyllenhal)] and the potato leafhopper [Empoasca fabae (Harris)] decrease yield and quality of alfalfa forage by phytophagy (weevil) (App and Manglitz, 1972) or plugging of phloem cells. which hinders carbohydrate translocation (leafhopper) (Smith and Poos, 1931). Alfalfa germplasms possessing adequate resistance to control either pest have not been found. Erect glandular-haired Medicago species provide adequate host-plant resistance to both pests (Brewer et al., 1986a; Danielson et al., 1986a. 1986b: Shade et al., 1975) and hairs have been successfully transferred into alfalfa populations (Sorensen et al., 1981).

Cell walls and their components can limit forage intake and utilization by ruminant livestock.

Lenssen et al. (1987) reported that the presence of erect glandular hairs did not affect in vitro dry matter disappearance or crude protein content of leaves or stems of some wild Medicago populations and

hybrids, however, questions remain regarding their possible effects on other aspects of forage quality. Brewer et al. (1986b) documented the occurrence of early interfascicular lignification in several wild species and hypothesized that a concomitant increase in mechanical strength prevented oviposition into the stems of resistant Medicago sp. by potato leafhopper adults. They found that the steles of the resistant clones retained their integrity after 18 hours of ruminant digestion while stems of susceptible clones had largely disappeared, except for the cuticle and vascular bundles.

Suksayretrup (1986) found M. prostrata Jacq. leaves had thicker cell walls and cuticles than did diploid alfalfa while these characteristics in their hybrids were intermediate to those of the parents. Plant breeders and livestock producers likely would not be willing to sacrifice leaf or stem quality solely to gain host-plant resistance to either the potato leafhopper or the alfalfa weevil.

The purpose of our study was to determine whether the presence of erect glandular hairs affects the total cell wall, as estimated by neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HC), lignin (ADL), or cellulose (CEL) content of leaves or stems of several Medicago

populations and hybrids. The possibility that the glandular haired hybrid populations, M. sativa x M. prostrata and M. sativa x M. glutinosa Bieb., might have leaf or stem cell wall concentrations different from alfalfa requires investigation. No information is available for cell wall and fiber component concentrations of stems and leaves of the wild species M. prostrata, M. glandulosa David, and M. glutinosa.

MATERIALS AND METHODS

The plant populations used in this study were previously described by Lenssen et al. (1987). Populations with (+) and without (-) erect glandular hairs were selected from perennial diploids M. prostrata Jacq. and KS94, and tetraploids KS108, KS159, and KS160. Glandular-haired plants of KS94 and KS108 were selected from populations of the 6th and 5th cycles, respectively, of recurrent phenotypic selection for erect glandular hairs. Essentially eglandular plants were selected from the 1st cycles of selection for erect glandular hairs. KS94GH6 (Sorensen et al., 1986), M. glandulosa David (perennial diploid), and KS108GH5, M. glutinosa Bieb. (P.I. 346919 perennial tetraploid) (Sorensen et al., 1985) have been previously described in detail. KS159 is an M. sativa x M. prostrata tetraploid hybrid backcrossed three times to M. sativa populations. KS160 is an M. sativa x M. glutinosa hybrid backcrossed three generations to M. sativa populations. Selected (+) and (-) hybrid populations were from first and fourth cycles of selection for erect glandular hairs respectively. Eglandular diploid M. sativa subsp. caerulea (Less. ex Ledeb.) Schmalh. (P.I. 172984) and tetraploid M. sativa

'Riley' (Sorensen et al., 1978) were included as

Seeds of all populations were scarified, treated with a commercial Rhizobium inoculant, and planted in flats in a greenhouse. Seedlings selected for (+) and (-) were transplanted to peat pots, and later transplanted into a field nursery, 19-21 May 1985. Plants were spaced 30 and 45 cm within and between rows respectively. Experimental design was a randomized complete block with four replications of 20 plants per replicate. Diseases and insects were controlled to eliminate possible confounding effects on forage quality (Willis et al., 1969). Irrigation water was supplied as necessary to prevent moisture stress.

Tetraploid populations were harvested in July, August, September, and October 1985. Diploids were harvested only three times because growth was insufficient for an October harvest. All populations were harvested in May 1986 (Lenssen et al., 1987).

Harvest and leaf-stem separation procedures were detailed by Lenssen et al. (1987). NDF and ADF were determined as per Goering and Van Soest (1970), except hexane washes were deleted for ADF. ADL was calculated as weight loss following permanganate extraction of ADF residues. CEL was calculated as

weight loss of ADL residues resulting from 3 hours of 500°C dry heat in a muffle furnace. HC values were determined as NDF minus ADF. All fiber analyses on leaves and stems were performed for two of the four field replicates.

Analysis of variance procedures using the statistical analysis systems general linear models procedure (SAS 1982) were followed by protected least significant difference tests for mean separations. Population x cutting interactions were tested using data from August, September, and May harvests. Arcsine-square root transformations were made on all percent data. Since conclusions were the same for transformed and original data, we presented the original data.

RESHLTS

Neutral Detergent Fiber

The presence of erect glandular hairs on the wild Medicago species (prostrata, glandulosa, and glutinosa) did not affect the NDF content of leaves or stems (Table 1). Of the hybrid (+) and (-) comparisions, only those of KS160 stems for August and leaves for September were significant. The population x cutting interaction was significant for stem and leaf NDF.

All diploid entries had lower stem NDF than did the cultivated tetraploid Riley for the warm season harvests (August and September) but did not vary from Riley for the cool season harvest (May). The hybrid populations (KS159 and KS160) trended with Riley, except KS160 (+) had less NDF than Riley did for the August harvest.

Unlike stems, leaves of the diploid species were nearly always higher in NDF than the tetraploid entries across the varied seasonal environments (Table 1).

Correlation coefficients of stem versus leaf NDF were strongly negative for the warm season harvests (August and September), yet were positive for the cool season harvests (October and May) (Table 2). The negative correlations for the August and

September harvests were related to the diploid entries having lower stem and higher leaf NDF contents than did the tetraploid entries.

Acid Detergent Fiber

The only significant differences found between corresponding (+) and (-) entries were for stems of KS160, and in both cases the (+) entry had lower ADF that did the (-) (Table 3). The population x cutting interaction was significant for stem and leaf ADF. All diploid populations had significantly less stem ADF than Riley did for the August and September cuttings. Tetraploid entries generally had less leaf ADF than diploid entries did for all harvests.

Correlation coefficients for leaf and stem ADF were negative and significant for the August and September harvests and positive and significant for the October harvest (Table 2). The negative correlations for the August and September harvests resulted from the diploid entries having low stem and high leaf ADF contents.

Hemicellulose

The presence of erect glandular hairs on different Medicago populations did not significantly affect stem or leaf HC contents (Table 4).

Differences among populations over cuttings for stem

or leaf HC contents were significant. The wild diploid M. prostrata had the highest leaf and stem HC contents for the August, September, and May cuttings, while Riley was among the lowest. Leaf and stem HC contents were highest for the cool season (May) harvest (Table 5). The population x cutting interactions were nonsignificant for leaves and stems. The correlation coefficients for stem and leaf hemicellulose were low and positive for all four harvests (Table 2).

Lignin

ADL contents of leaves and stems were unaffected by the presence of erect glandular hairs.

Differences among the entries over cuttings were significant for leaf and stem fractions (Table 4).

Population x cutting interactions for leaves and stems were nonsignificant.

Although differences were seldom statistically significant, diploid entries generally had higher leaf and lower stem ADL than did Riley alfalfa (Table 4). Leaf and stem ADL contents were greater for the August and May cuttings than for the September or October harvests (Table 5).

Correlation coefficients within cuttings for stem versus leaf ADL were not significant. However, for combined harvests the correlation was low and positive (Table 2).

Cellulose

The presence of erect glandular hairs did not affect stem or leaf CEL contents (Table 6). CEL content of stems was lower for diploids than for tetraploid entries for the August and September harvests. Leaves of the diploid entries had higher CEL contents than did Riley alfalfa. The population x cutting interaction was significant for both components.

Correlation coefficients of stem versus leaf CEL contents were significant and negative for the August, September, and May harvests (Table 2), because the diploid entries generally had high leaf and low stem CEL contents.

DISCUSSION

Increased levels of NDF, ADF, and ADL have been implicated in reduced intake of feed by ruminant livestock. with resultant decreases in either milk or meat production efficiencies (Van Soest, 1982). Brewer et al. (1986b) noted increased lignification of interfascicular regions in stems of clones from glandular-haired Medicago sp. with increasing resistance to oviposition by the potato leafhopper. This suggests that total cell walls (estimated as NDF), lignin, cellulose, or hemicellulose may have been increased during selection for erect glandular hairs. Our data indicate, however, that the presence of erect glandular hairs has no effect on these constituents in stems or leaves from the wild species M. prostrata, M. glandulosa, and M. glutinosa, or the hybrids M. sativa x M. prostrata and M. sativa x M. glutinosa.

Suksayretrup (1986) found that even though lignification was more extensive in the interfascicular regions of M. prostrata than M. sativa stems, M. prostrata had a higher cortex:radius ratio, which may contribute to increased digestibility. We observed this trend toward higher digestibility in other diploid species in our study.

Lees (1984) documented differences in cell wall and cuticle thickness of leaves among bloating and nonbloating legume species. He found that nonbloating species, birdsfoot trefoil (Lotus corniculatus L.), cicer milkvetch (Astragalus sicer L.), and sainfoin (Onobrychis viciifolia Scop.), had thicker epidermal cell walls and cuticles than did the bloat-causing species M. sativa, arrowleaf clover (Trifolium vesiculosum Savi), red clover (Trifolium pratense L.), and white clover (Trifolium repens L.). However, Buxton and Hornstein (1986) reported similar cell wall contents for leaves of birdsfoot trefoil and alfalfa.

Plant hairs are known to increase resistance to moisture stress (Uphof, 1962). Suksayretrup (1986) utilized M. prostrata, M. sativa (2n = 2x = 16) and their F₁ hybrid for the characterization of anatomical and morphological features related to xerophytism. She found that M. prostrata leaves had thicker cuticles and epidermal cell walls than did M. sativa, with hybrids intermediate in nearly all parameters.

The incorporation of erect glandular hairs into adapted alfalfa cultivars could have significant economic value to both producers and consumers of alfalfa products because increased pest resistance and drought tolerance would allow decreased use of

pesticides and irrigation water. Furthermore, the possible use of wide crosses among glandular-haired Medicago populations and cultivated alfalfa to alter anatomical or morphological features could decrease the hazards of frothy bloat and allow greatly increased usage of alfalfa for grazing.

REFERENCES

- App, B.A., and G.R. Manglitz. 1972. Insects and related pests. p. 527-554, In: C.H. Hansen (ed.), Alfalfa science and technology. Agronomy Society of America, Madison, Wisconsin.
- Brewer, G.J., E.K. Horber, and E.L. Sorensen. 1986a.

 Potato leafhopper (Homoptera: Cicadellidae)

 antixenosis and antibiosis in Medicago species. J.

 Econ. Entomol. 79:421-425.
- Kreitner. 1986b. Alfalfa stem anatomy and potato
 leafhopper (Homoptera: Cicadellicae) resistance. J.
 Econ. Entomol. 79:1249-1253.
- Buxton, D.R., and J.S. Hornstein. 1986. Cell-wall concentration and components in stratified canopies of alfalfa, birdsfoot trefoil, and red clover. Crop Sci. 26:180-184.
- Danielson, S.D., G.R. Manglitz, and E.L. Sorensen.

 1986a. Development of alfalfa weevil (Coleoptera:
 Curculionidae) larvae when reared on perennial
 glandular-haired Medicago species in the
 greenhouse. Environ. Entomol. 15:396-398.

____, and ____

1986b. Resistance of perennial glandular-haired

Medicago species to oviposition by alfalfa weevils

(Coleoptera: Curculionidae). Environ. Entomol.

15: (in press).

- Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). USDA Agric. Handb. 379. U.S. Government Printing Office. Washington. DC.
- Lees, G.L. 1984. Cuticle and cell wall thickness:
 relation to mechanical strength of whole leaves and
 isolated cells from some forage legumes. Crop
 Sci. 24:1077-1081.
- Lenssen, A.W., E.L. Sorensen, G.L. Posler, and L.H.
 Harbers. 1987. Forage quality of perennial
 glandular-haired and eglandular <u>Medicago</u>
 populations. I. <u>In vitro</u> dry matter
 disappearance and crude protein contents of leaf
 and stem fractions. Crop Sci. submitted for
 publication.
- SAS Institute. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.
- Shade, R.E., T.E. Thompson, and W.R. Campbell. 1975. An alfalfa weevil larval resistance mechanism detected in <u>Medicago</u>. J. Econ. Entomol. 68:399-404.
- Smith, F.F., and F.W. Poos. 1931. The feeding habits of some leafhoppers of the genus <u>Empoasoa</u>. J. Agr. Res. 43(3):267-285.
- Sorensen, E.L., N. Sangduen, and G.H. Liang. 1981.

 Transfer of glandular hairs from diploid <u>Medicago</u>

prostrata to tetraploid M. sativa. p. 53, In:

Report of the Twenty-seventh Alfalfa Improvement

Conference, ARM-NC19.

______, D.L. Stuteville, and E.K. Horber. 1978.

Registration of Riley alfalfa. Crop Sci. 18:911.

Registration of KS940H6 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 26:1088.

- Suksayretrup, K. 1986. Xerophytism in Medicago. PhD

 Dissertation. Kansas State University, Manhattan,
 Kansas.
- Uphof, J.C.Th. 1962. Plant hairs. p. 1-206. In: W.

 Zimmermann and P.C. Ozenda (eds.) Encyclopedia of
 plant anatomy. Vol. 4, Part 5. Gebruder
 Borntraeger, Berlin.
- Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books, Corvallis, Oregon.
- Willis, W.G., D.L. Stuteville, and E.L. Sorensen. 1969.

 Effects of leaf and stem diseases on yield and
 quality of alfalfa forage. Crop Sci. 9:637-640.

List of Tables

- Table 1. Neutral detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations.
- Table 2. Correlation coefficients of stem versus leaf

 comparisions across 12 <u>Medicago</u> populations for five
 fiber components
- Table 3. Acid detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations.
- Table 4. Hemicellulose and lignin contents of stem and leaf components from 12 Medicago populations across four harvests.
- Table 5. Hemicellulose and lignin contents of stem and leaf
 components from four harvests across 12 Medicago
 populations.
- Table 6. Cellulose contents of stem and leaf components from four harvests of 12 Medicago populations.

Table 1. Neutral detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations.

			Stem					Leaf		
			1985		1986			1985		1986
Entry	Aug		Sept	Oct	May	Aug		Sept	Oct	May
					- dg/kg					-
4P(+)+	44.8	de [‡]	44.0	-	56.4	24.1	a	21.8	-	25.
1P(-)	42.7	е	43.9	-	57.8	23.1	ab	22.7	-	26.
(\$94(+)	46.4	o d	45.3	_	53.8	22.2	ab	19.7	-	25.
KS94(+)	47.3		46.0	-	54.8	23.4		19.8	-	25.
4S2n	46.5	o d	41.1	_	52.7	20.9	bс	20.0	-	21.
Riley	51.2		51.8	41.4	55.0	18.8	c d	17.8	15.5	17
KS108(+)	47.5	o d	49.9	42.3	52.9	19.5	c d	17.8	16.3	18
KS108(-)	48.4		52.0	40.8	55.4	18.3	d	17.8	14.9	18
KS159(+)	50.9	a	51.8	43.2	57.9	19.0	c d	16.8	15.1	18
KS159(-)	51.0		52.4	42.0	56.4	19.6	c d	16.8	15.2	18
KS160(+)	47.7	0	51.9	43.2	52.4	18.1	d	19.5	15.7	19
KS160(-)	50.3		51.5	46.4	55.2	18.7	c d	17.4	17.1	19
LSD (.05)			2.7	NS	2.9			1.1	NS	1.
r2	0.92		0.95	0.78	0.80	0.91		0.97	0.76	0.
\$CV	2.08		2.54	3.24	2.38	4.95		2.57	4.04	3 •

† MP = M. prostrata, KS9% = M. glanduloga, MS2n = M. gativa subsp. caerulea, Riley = M. gativa, KS108 = M. glutinoga, KS159 = M. sativa x M. prostrata, KS160 = M. gativa x M. glutinoga; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs.

‡Means within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

Table 2. Correlation coefficients of stem versus leaf comparisions across 12 <u>Medicago</u> populations for five fiber components.

Parameter		1985	1986		
	August	September	October	Мау	Combined
NDF+	52*	83***	.77**	.17	.34**
n	22	2 4	1 4	2 4	8 4
ADF	49*	79***	.64*	24	.01
n	22	24	1 4	2 4	8 4
HC	.52*	.46 *	.17	.34	.68***
n	22	24	14	24	8 4
ADL	.07	33	.13	10	.35***
n	22	2 4	1 4	24	8 4
CEL	69***	65***	.37	40*	.04
n	22	2 4	1 4	24	8 4

 $\overline{\text{TNDF}}$ = neutral detergent fiber, ADF = acid detergent fiber, HC=

hemicellulose, ADL = lignin, CEL = cellulose.

 $\bullet, \bullet \bullet, \bullet \bullet$ significant at the .05, .01, and .001 levels of probability, respectively.

Table 3. Acid detergent fiber contents of stem and leaf components from four harvests of 12 Medicago populations.

		Stem				Leaf		
Entry	1985		5 1986			1986		
	Aug	Sept	Oct	May	Aug	Sept	Oct	Мау
MP(+)†	35.9 de‡	34.7		- dg/kg 43.1	DM 18.3 ab	15.9		17.
MP(+)	34.4 e	33.2	-	43.8	16.9 abc		-	18.
(594(+)	38.6 cd	36.4	-	42.0	17.2 abc		-	17.
(S94(-)	39.6 cd	37.1	-	40.9	19.1 a	15.0	-	18.
MS2n	39.2 cd	35.5	-	40.9	16.3 bcd		-	15
Riley	44.1 a	43.4	35.3	44.1	15.0 de	.13.0	12.8	12.
KS108(+)	40.2 cd	42.0	35.0	42.7	15.0 de	13.9	12.7	12
KS108(-)	41.5 bc	43.6	33.2	44.1	14.1 e	13.5	11.3	12
(S159(+)	43.7 a	43.2	35.9	46.9	15.3 cde	11.6	12.5	14
(8159(-)	43.6 a	44.2	34.3	45.7	15.2 de	11.9	12.2	12
KS160(+)	40.7 c	43.6	35.6	42.1	14.3 e	13.7	13.7	13
(S160(-)	43.5 a	43.6	38.2	44.5	14.9 de	12.9	13.6	13
SD (.05)		1.8	NS	2.0		NS	1.6	1.
r2	0.93	0.98	0.81	0.87	0.88	0.82	0.90	0.
S C V	2.76	2.00	3.05	2.07	5.43	4.89	5.34	4.

T MP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa

subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect

‡Means within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

glandular hairs.

Table 4. Hemicellulose and lignin contents of stem and leaf components from 12 Medicago populations across four harvests.

	Hemicell	ulose	Lignin		
Population	Stem	Leaf	Stem	Leaf	
		- dg/kg	DM		
MP(+) [†]	10.1a‡	5.9ab	11.3bcde	3.6ab	
MP(-)	10.8a	6.5a	12.1abc	3.7ab	
KS94(+)	9.2b	5.5bc	10.0f	3.3abc	
KS94(-)	8.9bc	5.0bcd	10.6def	3.8a	
MS2n	8.5bcd	4.6cd	10.2ef	3.2bc	
Riley	8.1d	4.1d	12.4ab	2.9cd	
KS108(+)	8.2cd	4.7cd	11.3cde	2.7de	
KS108(-)	8.5bcd	4.5d	12.0abc	2.6e	
KS159(+)	8.6bcd	4.1d	12.4ab	2.8cd	
KS159(-)	8.5bcd	4.5d	12.3abc	2.9cd	
KS160(+)	8.3cd	4.3d	11.6abcd	2.9cd	
KS160(-)	8.4bcd	4.4d	12.6a	3.0cd	
Mean	8.9	4.9	11.9	3.1	

TMP = M. prostrata, KS94 = M. glandulosa, MS2n = M. Sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs.

Theans within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

Table 5. Hemicellulose and lignin contents of stem and leaf components from four harvests across 12 <u>Medicago</u> populations.

	Hemice	llulose	Lignin			
Harvest	Stem	Leaf	Stem	Leaf		
1985		dg/k	g DM			
August	7.3c	4.5c	12.7a	3.5a		
September	8.6b	5.1b	10.9b	2.50		
October	7.4c	3.5d	10.10	3.0b		
1986						
May	11.4a	6.2a	13.1a	3.4a		
Mean	8.9	4.9	11.9	3.1		

Theans within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

Table 6. Cellulose contents of stem and leaf components from four harvests of 12 Medicago populations.

			Stem				Leaf	
		1985		1986		1985		1986
Entry	Aug	Sept	Oct	May	Aug	Sept	Oct	Мау
+	1			- dg/kg				-
MP(+) [†] MP(-)	25.7 d‡ 27.3 cd	24.4	Ξ	28.4	14.1 a 13.5 abc	12.7	-	13.2
KS94(+) KS94(-)	27.6 c 27.6 c	26.9 27.3	-	30.1 31.1	13.0 abcd 13.7 ab	11.3	-	12.9
MS2n Riley	27.4 c 30.3 a	26.4 31.1	24.4	29.3 29.3	12.8 bcde 11.9 def		9.6	11.8
KS108(+) KS108(-)	28.2 bc 29.2 ab	30.6 31.8	24.5 23.4	30.6 31.3	11.9 cdef 11.2 f		10.4	9 . 2
KS159(+) KS159(-)	30.0 a 30.1 a	31.8 32.3	25.0 23.9	33.1 32.1	11.2 f 11.5 f	10.7	9.7 9.1	10.0
KS160(+) KS160(-)	29.1 ab 30.0 a	32.7 32.3	25.2 26.2	30.2 31.7	11.2 f 11.6 ef	11.4	9.6 10.2	9.1
LSD (.05) r2 \$CV	0.92 2.07	1.4 0.98 2.13	1.4 0.85 2.29	1.9 0.84 2.78	0.88 4.55	NS 0.71 5.67	NS 0.88 3.53	1.2 0.9 4.9

TMP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa

glandular hairs.

subsp. caerulea, Riley = M. satiya, KS108 = M. glutinosa, KS159 =

M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect

[†]Means within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

LITERATURE REVIEW

Alfalfa (Medicago sativa L.) is the premier forage crop for production of dairy and meat products throughout temperate and semiarid climates. The potential for high vields of nutritious feed has resulted in the production of alfalfa on approximately 11.000.000 ha in the United States (Barnes and Sheaffer. 1985). Numerous biotic and abiotic factors, such as temperature or moisture stress, insects, nematodes, and diseases, limit yield and/or quality of alfalfa forage. The two most destructive arthropod pests of alfalfa in North America are the alfalfa weevil [Hypera postica (Gyllenhal)] and the potato leafhopper [Empoasca fabae (Harris)], which limit yield and quality by phytophagy (weevil) (App and Manglitz, 1972) or plugging of phloem cells which hinders carbohydrate translocation (leafhopper) (Smith and Poos, 1931). Although alfalfa cultivars have been released that have moderate tolerance to both pests, little hostplant resistance has been found among M. sativa germplasms for controlling the potato leafhopper or the alfalfa weevil (App and Manglitz, 1972; Miller and Melton, 1984).

Many species within the genus Medicago contain trichomes (Grossheim, 1945). The two major types of

trichomes found within Medicago are simple and glandular hairs. Alfalfa germsplasms do not have erect glandular hairs, although simple and procumbent glandular hairs are found on many cultivars (Grossheim, 1945). Erect glandular hairs are found on many annual and perennial Medicago species. Glandular hairs vary in stature from procumbent to erect, and have single or multicellular stalks. All glandular trichomes contain exudate-secreting cells which are most often located distally from the base of the stalk.

Kreitner and Sorensen (1983) documented ontogenic gland development and early secretion of erect glandular trichomes of M. scutellata (L.)

Mill.. They described the formation of a six to seven-celled filament from an epidermal initial, and the subsequent production of a multicellular, globose head.

Kitch et al. (1985) postulated that the narrowsense heritability for erect glandular hairs in a
hybrid Medicago population was 0.55. Knox and Dodge
(1985) reported that hypericin, a glandular exudate
from hairy St. John's wort (Hypericum hirsutum L.),
produced photosensitivity in grazing ruminants. The
photosensitization resulted from disruption of lipid
membranes by singlet oxygen generated from hypericin.

Although exudates from glandular hairs of Medicago ssp. have not been thoroughly elucidated, Triebe et al. (1981) determined that exudates from M. scutellata would not present a toxicological problem for forage-consuming livestock.

Kreitner and Sorensen (1979) and Sorensen et al. (1981) reported the successful transfer of erect glandular hairs into alfalfa germplasms.

Trichomes long have been known to increase resistance to moisture stress (Uphof, 1962; Ehleringer, 1984) and provide a defense mechanism against insect pests (Levin, 1973). Mechanisms associated with host-plant resistance due to the presence of trichomes are antixenotic or antibiotic, and include changes in adult mortality (Johnson et al., 1980c), ovipositional behavior (Brewer et al., 1986b), and larval entrapment (Shade et al., 1975).

The seed chalcid, (<u>Bruchophagus roddi</u> Guss.) greatly reduces seed yields of alfalfa (Sorenson, 1930). The seed chalcid decreases seed yield by effecting ovule abortion by ovipositing (Brewer et al., 1983) or larval consumption of developing seeds (Sorenson, 1930). Little host-plant resistance has been found within cultivated alfalfa germplasms to prevent oviposition or subsequent larval development in developing seeds. Small and Brooks (1982, 1984)

postulated that increases in seed pod coiling resulted in decreased oviposition by the chalcid. Although cultivated alfalfa does have tightly coiled seed pods, seed yield reductions approaching 80% have been attributed to chalcid infestation (Sorenson, 1930). Brewer et al. (1983) found that density and length of erect glandular hairs on seed pods of Medicago species were negatively correlated with seed chalcid infestations. They also tested alfalfa plants with high and low densities of simple hairs on the seed pods and found that the percent of seed infested decreased with increasing hair density. However, the reduction in infestation was significantly greater for clones containing high densities of erect glandular hairs than for those with high densities of simple hairs.

The alfalfa weevil is an important phytophagous pest of alfalfa, for which adequate host-plant resistance has not been found. VanDenburgh et al. (1966) documented the effects of stem diameter on alfalfa weevil oviposition. They found that larger diameter stems were generally preferred to smaller stems in free-preference tests. Campbell and Dudley (1965) reported some M. falcata L. clones were resistant to weevil oviposition and that the resistance was correlated to a decrease in stem pith

or an increase in stem mechanical strength. Two subsequently released cultivars, 'Team' and 'Weevlohek', which have small percentages of M. falcata germplasm, are tolerant of moderate levels of infestation (Miller and Melton, 1984). The tolerance results from a quicker initiation of axillary branching, but is inadequate to prevent substantial yield reductions at high levels of infestation.

Shade et al. (1970) found a relationship between the rate of alfalfa weevil larval development and host plant height. They reported that the rate of larval development was greater on taller, more rapidly growing plants, and they speculated that the nutritive value of these plants was greater.

Adequate host-plant resistance to control the alfalfa weevil has been documented in several annual Medicago species that possess erect glandular hairs. These species suffered less feeding damage than the three M. sativa cutivars tested in free-choice tests (Johnson et al., 1980a), and were less preferred for oviposition in free- and no-choice tests (Johnson et al., 1980b). They concluded that olfactory, visual, and chemotactic or mechanical cues resulted in a decreased number of contacts with resistant plants (Johnson et al., 1980c, 1981).

Danielson et al. (1986a) compared the

development of alfalfa weevil larvae on annual and perennial glandular-haired Medicago sp. and M. sativa clones. They reported that most larvae did not survive to diapause on M. prostrata Jacq. or M. glandulosa David clones. Danielson et al. (1986b) also reported that the glandular-haired Medicago species prostrata and glandulosa suffered significantly less oviposition than M. sativa clones.

The potato leafhopper is a frequent pest of alfalfa in the eastern half of the United States, causing losses in yield and carotene content of forage (Barnes and Sheaffer, 1985). Brewer et al. (1986a) reported antixenotic (nonpreference) and antibiotic (larval entrapment) mechanisms were operative in resistant, perennial, glandular-haired Medicago species. Brewer et al. (1986b) later reported that the resistant clones had an early lignification of interfascicular tissue. They found that the steles of the resistant clones retained their integrity after 18 hours of ruminant digestion while stems of susceptible clones had largely disappeared, except for the cuticle and vascular bundles. They hypothesized that the lignification increased mechanical strength of the stem, decreasing oviposition and providing a high degree of resistance to the leafhopper. However, lignin concentrations

were not quantified for the clones they studied.

Data are available that correlate plant morphological characteristics with ruminant utilization of forages. Woodman and Evans (1935) stated "the leaf-stem ratio is the factor which determines the nutritive value of lucerne." They concluded that alfalfa leaves were more nutritious than stems. Numerous reports have since been published that stress the importance of the percentage of leaves in proportion to the total dry matter present. Terry and Tilley (1964), Mowat et al. (1965), and Buxton et al. (1985) reported that alfalfa leaf in vitro dry matter disappearance (IVDMD) was always higher than that of stems and that differences increased with maturity.

Heinrichs and Troelsen (1965) reported that plant types, as classified by morphological characteristics, were not reliable indicators of chemical constituents in leaf or stem fractions of a segregating M. sativa x M. falcata population. They classified plants into 10 subpopulations based on color, leaf shape, stem diameter, growth habit, internode length, pubescence, and creeping-rooted habit. Mowat et al. (1966) concluded that stem IVDMD was not correlated with stem diameter, whereas the correlation was negative for smooth bromegrass

(Bromus inermis Leyss.). Suginobu and Christie (1973b) concluded that leafiness was the only useful morphological character allowing directed selection for increased IVDMD of Medicago populations.

The wide variation often found between leaf and stem IVDMD of alfalfa, as well as other species, has frequently been attributed to anatomical or chemical differences. Akin and Robinson (1982) reported that lignification of vascular bundles in leaves and stems and interfascicular parenchyma of stems was the primary deterrent to complete digestion of arrowleaf clover (Trifolium vesiculosum Savi) and crimson clover (Trifolium incarnatum L.). Cherney and Marten (1982a, 1982b) reported that the percentages of lignified area in leaf blades and sheaths of barley (Hordeum vulgare L.) and oats (Avena sativa L.) partially explained observed differences in leaf digestibilities. Oat leaves were less digestible and had a greater percentage of lignified area in cross section than did barley. Mowat et al. (1969) reported that cell wall digestibility was negatively correlated with lignification. They found that cell wall digestibility of grasses decreased linearly with increased lignin, while legumes, alfalfa and Russian comfrey (Symphtum officinale L.), had higher digestibilities than their lignin contents would have

indicated. Allinson and Osbourn (1970) and Jung and Vogel (1986) documented the relationships between maturity, lignin contents, and IVDMD. In both studies, lignin contents increased with maturity, while both lignin and maturity were negatively correlated to IVDMD. Brazle and Harbers (1977) reported that lignified vascular tissues of leaves and stems of alfalfa were not degraded in the rumen or lower gastrointestinal tract of mature <u>Bos taurus</u> steers. They also stated, however, that presence of lignified alfalfa tissues did not decrease the rate of digestion of unlignified tissues.

Suksayretrup (1986) reported that M. prostrata
leaves had thicker cell walls and cuticles than did
the diploid M. sativa she studied. The F1 hybrids
had intermediate values compared to those of the
parents. She also reported that the cortex:radius
ratio was larger in M. prostrata than in the diploid
alfalfa. This indicates that stems of M. prostrata
might differ from those of alfalfa for IVDMD due to a
greater amount of unlignified corticular parenchyma.
Lees (1984) documented differences in cell wall and
cuticle thickness of leaves among bloating and
nonbloating legume species. He found that
nonbloating species, birdsfoot trefoil (Lotus
corniculatus L.), cicer milkvetch (Astragalus cicer

L.), and sainfoin (<u>Onobrychis viciifolia</u> Scop.), had thicker epidermal cell walls and cuticles than did the bloat-causing species M. sativa, arrowleaf clover, red clover (<u>Trifolium pratense</u> L.), and white clover (<u>Trifolium repens</u> L.). However, Buxton and Hornstein (1986) reported similar cell wall contents for leaves of birdsfoot trefoil and alfalfa.

The nutritive values of Medicago species, other than alfalfa, have not been thoroughly documented. Grossheim (1945) stated in 1919 that M. coerulea (Less) Ldb. was "probably destined to play an important role in nonirrigated agriculture" in the USSR. He reported that crude protein (CP) concentration was only 13.4% on a dry matter basis, while cellulose content was 32%. Sumberg et al. (1983) utilized M. sativa x M. caerulea (Less ex. Ledeb.) Schmahl. and X M. falcata crosses in a recurrent selection scheme based on acid detergent fiber (ADF) and CP contents. They reported that the selected synthetic populations averaged nearly 2 units lower in ADF and 1.5 units higher in CP. Coors et al. (1986) reselected within the populations developed by Sumberg et al. and obtained additional gains in forage quality due to increased CP and decreased ADF contents. Allinson et al. (1969) reported mean M. coerulea 6-hour IVDMD was higher

than that of clones from 5 of the 6 \underline{M} . sativa cultivars entered.

Allinson et al. (1969) also reported mean 6-hour IVDMD values for M. glutinosa Bieb. and M. falcata clones. They found M. glutinosa clones averaged lower than clones from 6 alfalfa cultivars. The $\underline{\mathsf{M}}$. falcata clones averaged nearly 4 units less for 6hour IVDMD than M. glutinosa clones. However, when populations were further divided into groups based on high or low nutritive value, the M. falcata clones selected for high nutritive value were not significantly different from clones selected for high nutritive value from the cultivar Vernal. The high nutritive value M. falcata population had higher cell wall, lignin, and hemicellulose concentrations than the alfalfa cultivars DuPuits and Vernal. Heinrichs et al. (1969) compared the forage quality of M. sativa. M. media, and M. falcata populations. They reported that M. falcata had the lowest leaf CP, longest time to flowering following harvest, highest leaf fiber, smallest trifoliolate-leaf area, and the lowest phosphorous and sulfur concentrations of leaves and stems.

Suginobu and Christie (1973) also compared the forage quality of M. sativa, M. msdia, and M. falcata populations, and reported that M. falcata leaves from

the first harvest were among the lowest in IVDMD of the 11 populations tested, while those from a later harvest were not significantly different from most other entries. They emphasized that the range in leaf IVDMD was small for both harvests. M. falcata stems had the lowest IVDMD for the first harvest but were not significantly different from any entry for the second cutting. Whole plant IVDMD of the M. falcata population was significantly lower than all entries for the first cutting. However, it was among the highest in IVDMD for the second harvest, largely because of a much higher percentage of leaves in the total dry matter harvested. Grossheim (1945) stated that the forage attributes, winter hardiness, and xeromorphic adaptations of M. falcata were sufficient to warrant a hybridization program with other Medicago species. Grossheim also quoted Marshall Bieberstein, who, in 1819 stated that M. glutinosa "deserves attention as forage for cattle and stands up admirably to the climate of the Ukraine."

Sorensen et al. (1981) reported the successful hybridization of M. prostrata x M. sativa. The hybrids were fertile and possessed erect glandular hairs. Sorensen et al. (1985, 1986) subsequently released M. falcata glandulosa and M. glutinosa germplasms with erect glandular hairs and multiple

pest resistance, for use by alfalfa breeders to confer resistance to the alfalfa weevil and the potato leafhopper. The results of Brewer et al. (1986b) and Suksayretrup (1986) evince the need to examine the forage quality of the released germplasms and hybrid populations prior to their wide-spread incorporation into alfalfa cultivars. Additionally, the comparision of forage quality parameters of M. glutinosa, M. prostrata, and M. falcata glandulosa with cultivated alfalfa is of interest to plant breeders considering hybridization programs with subsequent selection strategies based on forage quality, xerophytism, or multiple pest resistance.

Literature Cited

- Akin, D.E., and E.L. Robinson. 1982. Structure of leaves and stems of arrowleaf and orimson clovers as related to in <u>vitro</u> digestibility. Crop Soi. 22:24-29.
- Allinson, D.W., F.C. Elliott, and M.B. Tesar. 1969.

 Variations in nutritive value among species of the Medicago genus as measured by laboratory techniques.

 Crop Sci. 9:634-636.
- _____, and D.F. Osbourn. 1970. The cellulose-lignin complex in forages and its relationship to forage nutritive value. J. Agric. Sci., Camb. 74:23-36.
- App, B.A., and G.R. Manglitz. 1972. Insects and related pests. p. 527-554, In: C.H. Hansen (ed.), Alfalfa science and technology. Agronomy Society of America, Madison. Wisconsin.
- Barnes, D.K., and C.C. Sheaffer. 1985. Alfalfa. p.89-97, In: M.E. Heath, R.F. Barnes, and D.S. Metcalfe (eds.), Forages. the science of grassland agriculture. The Iowa State University Press, Ames, Iowa.
- Brazle, F.K., and L.H. Harbers. 1977. Digestion of alfalfa hay observed by scanning electron microscopy. J. Anim. Sci. 46:506-512.
- Brewer, G.J., E.L. Sorensen, and E.K. Horber. 1983. Trichomes and field resistance of <u>Medicago</u> species to the alfalfa seed chalcid (Hymenoptera: Eurytomidae). Environ. Entomol. 12:247-251.
- Brewer, G.J., E.K. Horber, and E.L. Sorensen. 1986a. Potato leafhopper (Homoptera: Cicadellidae) antixenosis and antibiosis in <u>Medicago</u> species. J. Econ. Entomol. 79:421-425.
- _______, E.L. Sorensen, E.K. Horber, and G.L. Kreitner. 1986b. Alfalfa stem anatomy and potato leafhopper (Homoptera: Cicadellidae) resistance. J. Econ. Entomol. 79:1249:1253.
- Buxton, D.R., J.S. Hornstein, W.F. Wedin, and G.C. Marten. 1985. Forage quality in stratified canopies of alfalfa, birdsfoot trefoil, and red clover. Crop Sci. 25:273-279.
- ______ and _____ 1986. Cell-wall concentration and components in stratified canopies of alfalfa, birdsfoot trefoil, and red clover. Crop Sci. 26:180-184.

- Campbell, W.V., and J.W. Dudley. 1965. Differences among <u>Medicago</u> species in resistance to oviposition by the alfalfa weevil. J. Econ. Entomol. 58:245-248.
- Cherney, J.H., and G.C. Marten. 1982a. Small grain crop forage potential: I. biological and chemical determinants of quality, and yield. Crop Sci. 22:227-231.
- , and . 1982b. Small grain crop forage potential: II. interrelationships among biological, chemical, morphological, and anatomical determinants of quality. Crop Sci. 22:240-245,
- Coors, J.G., C.C. Lowe, and R.P. Murphy. 1986. Selection for improved nutritional quality of alfalfa forage. Crop Sci. 26:843-848.
- Danielson, S.D., G.R. Manglitz, and E.L. Sorensen. 1986a. Development of alfalfa weevil (Coleoptera: Curculionidae) larvae when reared on perennial glandular-haired <u>Medicaro</u> species in the greenhouse. Environ. Entomol. 15:396-398.
- Resistance, of perennial glandular-haired <u>Medicago</u> species to oviposition by alfalfa weevils (Coleoptera: Curculionidae). Environ. Entomol. 15:in press.
- Ehleringer, J. 1984. Ecology and ecophysiology of leaf pubescence in north american desert plants. p.113-132, In: E. Rodriguez, P.L. Healey, and I Mehta (eds.), Biology and chemistry of plant trichomes. Plenum Press, New York, New York.
- Grossheim, A.A. 1945. <u>Medicago</u>. p.99-134, In: V.L. Komarov (ed.), Flora of the USSR. Vol. XI. Israel Program for Scientific Translations (1971), Keter Press, Jerusalem.
- Heinrichs, D.H., and J.E. Troelsen. 1965. Variability of chemical constituents in an alfalfa population. Can. J. Plant Soi. 45;405-412.
- Variation of chemical constituents and morphological characters within and between alfalfa populations. Can J. Plant Sci. 49:293-305.
- Johnson, K.J.R., E.L. Sorensen, and E.K. Horber. 1980a. Resistance in glandular-haired annual <u>Medicago</u> species to feeding by adult alfalfa weevils (Hypera postica). Environ. Entomol. 9:133-136.

Behavior of adult alfalfa weevils (Hypera postica) on resistant and susceptible Medicago species in freechoice preference tests. Environ. Entomol. 10:580:585.

- Jung, H.G., and K.P. Vogel. 1986. Influence of lignin on digestibility of forage cell wall material. J. Anim. Soi. 62:1703-1712.
- Kitch, L.W., R.E. Shade, W.E. Nyquist, and J.D. Axtell. 1985. Inheritance of density of erect glandular trichomes in the genus Medicago. Crop Soi. 25:607-610.
- Knox, J.P., and A.D. Dodge. 1985. Isolation and activity of the photodynamic pigment hypericin. Plant Cell Environ. 8:19-25.
- Kreitner, G.L., and E.L. Sorensen. 1979. Glandular trichomes on Medicago species. Crop Sci. 19:380-384.
- Lees, G.L. 1984. Cuticle and cell wall thickness: relation to mechanical strength of whole leaves and isolated cells from some forage legumes. Crop Sci. 24:1077-1081.
- Levin, D.A. 1973. The role of trichomes in plant defense. Q. Rev. Biol. 48:3-15.
- Mowat, D.N., R.S. Fulkerson, and E.E. Gamble. 1966. Relationship between stem diameter and in <u>vitro</u> digestibility of forages. Can. J. Flant Sci. 47:423-426.
 - , , W.E. Tossell, and J.E. Winch.

 1965. The <u>in witro</u> digestibility and protein content of leaf and stem portions of forages. Can. J. Plant Sci.
 45:321-331.

- ______, Kwain, M.L. and J.E. Winch. 1969. Lignification and in <u>vitro</u> cell wall digestibility of plant parts. Can. J. Plant Sci. 49:499-504.
- _____, J.D. Axtell, and M.C. Wilson. 1971. A relationship between plant height of alfalfa and the rate of alfalfa weevil larval development. J. Econ. Entomol. 64:437-438.
- Shade, R.E., T.E. Thompson, and W.R. Campbell. 1975. An alfalfa weevil resistance mechanism detected in Medicago. J. Econ. Entomol 68:399-404.
- Small, E., and B.S. Brookes. 1982. Coiling of alfalfa pods in relation to resistance against seed chalcids. Can. J. Plant Soi. 62:131-135.
- _______, and _______. 1984. Coiling of alfalfa pods in relation to resistance against seed chalcids: additional observations. Can. J. Plant Sci. 64:659-665.
- Smith, F.F., and F.W. Poos. 1931. The feeding habits of some leafhoppers of the genus <u>Empoasoa</u>. J. Agr. Res. 43(3):267-285.
- Sorensen, E.L., E.K. Horber, and D.L. Stuteville. 1985. Registration of KS108GH5 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 25:1132.
 - Registration of KS94GH6 glandular-haired alfalfa germplasm with multiple pest resistance. Crop Sci. 26:1088.
- , N. Sangduen, and G.H. Liang. 1981.
 Transfer of glandular hairs from diploid Medicago prostrata to tetraploid M. sativa. p. 53, In: Report of the Twenty-seventh Alfalfa Improvement Conference, ARM-NC19.
- Sorenson, C.L. 1930. The alfalfa-seed chalcis-fly in Utah 1926-29, inclusive. Utah Agric. Exp. Stn. Bull. 218. Utah State Agric. Coll., Logan, Utah.
- Suginobu, K., and B.R. Christie. 1973a. Relationships between in <u>vitro</u> digestibility and morphological characters in alfalfa (<u>Medicago sativa</u> L.) I. genetic variability of I.V.D. Japan. J. Breed. 23:79-85.

- , and ______. 1973b. Relationships between in vitro digestibility and morphological characters in alfalfa (Medicago sativa L.) II. selection for I.V.D. on the basis of correlation between I.V.D. and other agronomic characters. Japan. J. Breed. 23:131-138.
- Suksayretrup, K. 1986. Kerophytism in <u>Medicago</u>. PhD Dissertation. Kansas State Univ., Manhattan. Kansas.
- Sumberg, J.E., R.P. Murphy, and C.C. Lowe. 1983. Selection for fiber and protein concentration in a diverse alfalfa population. Crop Sci. 23:11-14
- Terry, R.A., and J.M.A. Tilley. 1964. The digestibility of leaves and stems of perennial ryegrams, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an in witro procedure. J. Brit. Grassl. Soc. 19:361-372.
- Triebe, D.C., C.E. Meloan, and E.L. Sorensen. 1981. The chemical identification of the glandular-hair exudate from <u>Medicago scutellata</u>. p.52, In: Report of the Twenty-seventh Alfalfa Improvement Conference, ARM-NC19.
- Uptof, J.C.Th. 1962. Plant hairs. p.1-206, In: W.
 Zimmermann and P.C. Ozenda (eds.) Encyclopedia of
 plant anatomy. Vol. 4, Part 5. Gebruder Borntraeger,
 Berlin.
- VanDenburgh, R.S., R.L. Norwood, C.C. Blickenstaff, and C.H. Hanson. 1966. Factors affecting resistance of alfalfa cones to adult feeding and oviposition of the alfalfa weevil in the laboratory. J. Econ. Entomol. 59:1193-1198.
- Woodman, H.E., and R.E. Evans. 1935. Nutritive value of lucerne IV. the leaf-stem ratio. J. Agric. Sci., Camb. 25:578-597.

APPENDIX

Table 1. Hemicellulose contents of stem and leaf components from four harvests of 12 <u>Medicago</u> populations.

		Stem				Leaf		
		1985		1986		1985		1986
Entry	Aug	Sept	Oct	May	Aug	Sept	Oct	May
				dg/				
MP(+)+	8.9	9.4	-	13.3	5.8	5 . 9	-	7 - 5
MP(-)	8.3	10.8	-	14.0	6.2	7.1	-	7 - 3
KS94(+)	7.8	8.9	-	11.8	4.9	5.6	-	7.2
KS94(-)	7 - 7	8.9	-	11.2	4.2	4.8	-	7 - 3
MS2n	7.2	7.6	_	11.8	4.6	4.8	-	5 - 7
Riley	7.1	8.4	6.0	10.9	3 - 7	4.7	2.7	5.4
KS108(+)	7.3	8.0	7 - 3	10.2	4.5	3.9	3.6	6.7
KS108(+)	6.9	8.3	7.6	11.2	4.3	4.3	3.6	5.7
KS159(+)	7.2	9.0	7 + 3	11.0	3 · 7	5.1 4.9	2.6	4.9 5.6
KS159(-)	7.4	8.2	7 - 7	10.7	4.4	4.9	3.0	5.0
KS160(+)	7.0	8.4	7 - 7	10.3	3.7	5.7	2.0	5.8
KS160(-)	6.8	7.9	8.2	10.7	3.8	4.5	3 - 5	5.7
LSD (.05)	NS	NS	NS	1.8	NS	NS	NS	1.5
r2	0.73	0.72	0.82	0.80	0.82	0.81	0.73	0.79
≴CV	6.57	8.94	7.03	7.33	13.99	15.38	19.00	11.0

† MF = M. prostrata, KS94 = M. glandulosa, MS2n = M. gativa
subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 =
M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa. (+) =
presence of erect glandular hairs, (-) = absence of erect
glandular hairs.

Table 2. Lignin contents of stem and leaf components from four harvests of 12 Medicago populations.

			Stem			L	eaf	
		1985		1986		1985		1986
Entry	Aug	Sept	Oct	Мау	Aug	Sept	Oct	Мау
MP(+) [†] MP(-)	10.4 rt 12.9 abcd	10.0	:	- dg/kg 14.2 14.8	DM 3.8 bc 5.0 a	2.9	-	4.1
(S94(+) (S94(-)	10.9 ef 11.7 def	9.3 9.4	-	11.8	3.8 bc 4.2 ab	2.3	-	3 - 9
MS2n Riley	11.9 cde 13.6 ab	8.5 12.6	10.2	12.1	3.3 cde 3.4 cde		2.8	3 · 5
KS108(+) KS108(-)	12.3 bcde 13.1 abcd		9 · 3 9 · 3	12.3 13.3	2.9 de 2.9 e	2.8	2.7	2.
KS159(+) KS159(-)	13.5 ab 13.2 abc	11.8	10.2	14.1	3.5 cd 3.4 cde	1.9	2.7	3 - 3
KS160(+) KS160(-)	13.0 abcd 13.8 a	11.2 11.9	9.9 11.3	12.2	2.9 de 3.1 de	2.4	3.2	3.
LSD (.05) r2 \$CV	0.93 5.27	1.0 0.95 4.21	NS 0.77 5.44	1.2 0.86 4.12	0.89 8.95	NS 0.60 21.8	NS 0.78 19.0	

T MP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caeruloa, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa. (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs.

†Means within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

Table 3. Correlation coefficients of stem and leaf in witro dry matter disappearance with their respective fiber components across 12 <u>Medicago</u> populations, by cutting.

		Stem			Leaf	
Parame	ter Aug	Sept	May	Aug	Sept	May
NDFT	85***	98***	83***	86***	.06	84***
n	22	24	24	23	24	24
ADF	81***	96***	46*	82***	09	91***
n	22	24	24	23	24	24
нс	.33	. 26	71***	58**	.21	40
n	22	24	24	23	24	24
ADL	44 *	92***	91***	80***	01	85***
n	22	24	24	23	24	24
CEL	79***	92***	18	73***	. 18	86***
n	22	24	24	23	24	24

TNDF = neutral detergent fiber, ADF = acid detergent fiber, HC = hemicellulose, ADL = lignin, CEL = cellulose.

*,*** significant at the .05, .01, .001 levels of probability, respectively.

Table 4. Correlation coeffficients of leaf and stem crude proteins with their respective fiber components from 12 Medicago pooulations. by cutting.

Paramete	r	Stem			Leaf	
	Aug	Sept	May	Aug	Sept	May
NDFT	58 **	15	33	65 ***	63 ***	55**
n	22	24	24	23	24	24
ADF	59**	12	52**	51*	46*	42*
n	22	24	24	23	24	24
нс	.45*	07	. 18	63**	38	70**
n	22	24	24	23	24	24
ADL	17	09	24	49*	16	24
n	22	24	2 4	23	24	. 24
CEL	52*	10	46*	58**	36	43*
n	22	24	24	23	24	24

TNDF = neutral detergent fiber, ADF = acid detergent fiber, HC = hemicellulose, ADL = lignin, CEL = cellulose.

*,**,*** significant at the .05, .01, .001 levels of probability, respectively.

Table 5. Analysis of variance mean squares for leaf components from three harvests of 12 Medicago populations.

populacione					
Source	NDF	ADF	HC	ADL	CEL
TRT	32.1***	17.0***	3.2**	0.9***	6.9***
BLOCK	0.1	3.5	3.0	2.8***	2.3*
HARV	29.5***	25.2***	16.9***	7.1***	6.1***
TRT * HARV	2.4***	2.2**	0.6	0.3	1.2**
r2	.95	.92	. 74	.82	.90
	4.27	5.78	18.0	14.9	5.24
% C V	4.27	5.10	10.0	, , , ,	

*,**,*** significant at the .05, .01, .001 levels of probability, respectively.

Table 6. Analysis of variance mean squares for stem components from three harvests of 12 Medicago populations.

Source	NDF	ADF	HEMI	ADL	CEL
TRT	26.1***	38.5***	3.0***	5.0***	19.8***
BLOCK	0.7	0.2	0.2	8.5*	1.6
HARV	356.8***	90.4***	95.9***	34.4***	26.3***
TRT * HARV	10.1***	7.8***	0.4	1.2	3.4***
r2	.96	.95	.93	.80	.96
≴ C V	2.46	2.77	8.21	8.95	2.35

^{*,**,***} significant at the .05, .01, .001 levels of probability, respectively.

Table 7. In vitro dry matter disappearance of 12 Medicago populations with five time periods, July 1985 harvest.

		Tim	e, in hour	3	
Entry	3	6	12	2 4	48
MP(+)† MP(-)	45.3 43.3	52.3 51.0	- dg/kg DM 59.7 58.5	63.5 62.2	68.8
KS94(+) KS94(-)	40.4 41.8	48.7 50.0	57·3 56·2	61.1	66.2 65.2
MS2n Riley	40.1 40.1	48.4 49.8	57.1 58.5	61.4	66.6
KS108(+) KS108(-)	41.4	50.0 51.3	58.8 58.9	63.4 63.9	68.3 67.8
KS159(+) KS159(-)	43.2 41.6	51.9 50.0	61.5 58.0	64.6 62.6	69-5
KS160(+) KS160(-)	41.9 40.0	49.5 50.4	58.4 58.3	63.3 63.7	67.5
LSD(0.05)	2.5 4.1	1.9	2.4	1.6	1.1

TMF = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

Table 8. In vitro dry matter disappearance of 12 Medicago populations with five time periods, August 1985 harvest.

		T.				
		1.	ime, in ho	urs		
3		6	12	24	48	
			dg/kg DM			_
41.6	a.‡	47.1			bcd 67.8	at
40.7	ab	46.0	56.7 b	ocd 62.1	d 66.6	bo
38.4	cd	44.7	56.3	d 62.3	cd 66.3	b
39.0	bed	44.8	56.5	ed 62.6	bed 66.5	Ъ
37.0	c d	42.3	54.9	60.3	e 65.4	С
		45.8	57.8 a	bc 63.4	a-d 67.2	a
41.1	a	46.9	59.0 a	65.0	a 68.7	a
40.7	ab	45.4	58.3 a	abc 63.5	a-d 66.9	Ъ
39.8	abc	46.2	58.6 8	ab 64.1	ab 67.3	a
		46.6	58.7 a	ab 63.8	abc 67.4	a
39.0	bed	46.1	57.8 8	abc 63.0	bcd 66.9	b
		45.3			bcd 66.7	b
3.5		3.9	2.5	1.8	1.7	
	41.6 40.7 38.4 39.0 37.0 38.5 41.1 40.7 39.8 40.8	41.6 a + 40.7 ab 38.4 cd 39.0 bcd 37.0 cd 38.5 cd 41.1 a 40.7 ab 39.8 abc 40.8 ab 39.0 bcd 39.0 bcd	11.6 a + 17.1 14.6 a + 47.1 10.7 a b 46.0 38.4 cd 44.7 39.0 bcd 44.8 37.0 cd 42.3 38.5 cd 45.8 41.1 a 46.9 40.7 a b 45.4 39.8 a bc 46.2 39.8 a bc 46.6 39.0 bcd 46.1 38.0 cd 45.3	38.4 cd 44.7 39.0 bcd 44.8 56.7 b 37.0 cd 42.3 38.5 cd 45.8 41.1 a 46.9 40.7 ab 45.4 58.7 a 56.7 b 56.7 b 56.7 b 56.7 c 56.3 c 57.8 a 57.8 a 40.7 ab 45.4 58.3 a 40.7 ab 45.4 58.3 a 39.8 bcd 46.2 58.6 a 39.0 bcd 46.1 39.0 bcd 46.1 38.0 cd 45.3		

FMF = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

 \hat{T}_{Means} within a column followed by the same letter are not significantly different using a protected LSD at p<0.05.

Table 9. In <u>vitro</u> dry matter disappearance of 12 <u>Medicago</u> populations with five time periods, September 1985 harvest.

	Time, in hours								
Entry	3	6	12	24	48				
			dg/kg DM						
MP(+) [†]	42.2	50.3	59.3	64.2	68.7				
MP(-)	40.2	49.0	58.5	63.4	69.3				
KS94(+)	42.6	50.5	60.9	65.5	68.9				
KS94(-)	38.3	47.2	57.0	61.9	65.6				
MS2n	40.2	48.2	57.6	63.2	67.0				
Riley	41.0	47.5	56.4	61.5	65.3				
KS108(+)	42.7	48.2	58.4	63.5	66.4				
KS108(-)	40.1	45.0	55.4	61.6	64.5				
KS159(+)	40.5	46.9	55.4	60.8	. 64.5				
KS159(-)	42.3	49.1	58.2	62.9	66.3				
KS160(+)	39.8	45.7	54.4	60.5	63.8				
KS160(-)	40.4	46.9	56.1	62.4	65.2				
LSD(0.05)	2.0	2.1	1.5	1.7	1.1				
%CV	2.9	2.6	1.6	1.6	1.3				

TMP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. casrulea, Riley = M. sativa, KS108 = M. slutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

Table 10. In vitro dry matter disappearance of 7 Medicago populations with five time periods, October 1985 harvest.

	Time, in hours						
Entry	3	6	12	24	48		
Riley +	46.3	54.8	- dg/kg DM - 63.1	69.4	72.5		
KS108(+) KS108(-)	47.4 48.2	55.1 57.0	64.2 64.6	69.9 70.4	73.0 73.4		
KS159(+) KS159(-)	48.7 46.6	56.0 53.9	63.4 62.7	69.7 68.6	72.2 71.8		
KS160(+) KS160(-)	46.3 44.2	54.2 50.7	62.4 60.1	68.0 66.8	71.0 70.0		
LSD(0.05)	2.3	1.9	1.7	1.6	1.7		

TRiley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x
M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence
of erect glandular hairs, (-) = absence of erect glandular hairs

Table 11. In vitro dry matter disappearance of 12 Medicaso populations with five time periods, May 1986 harvest.

		Ti	me, in hour	3	
Entry	3	6	12	24	48
+			dg/kg DM -		63.0
MP(+) [†] MP(-)	37.0 36.3	42.7 43.1	52.2 52.6	59.5 59.6	62.9
KS94(+)	35.2	40.8	52.1	61.0	65.5
KS94(-)	34.3	40.9	51.7	60.8	65.6
MS2n	35.2	41.8	52.4	62.3	66.0
Riley	36.5	44.5	54.1	62.6	66.1
KS108(+)	40.5	46.7	57.7	65.6	69.4
KS108(-)	38.8	44.1	55.0	63.1	67.2
KS159(+)	36.2	42.0	52.3	61.2	65.2
KS159(-)	37.0	43.3	53.3	61.9	65.6
KS160(+)	39.4	45.9	56.7	64.1	67.6
KS160(-)	39.3	45.3	55.4	63.7	67.0
LSD(0.05)	2.2	2.0	2.1	2.1	1.8
\$CV	4.2	3 + 2	2.8	2.3	1.5

TMF = M. prostrata, KS94 = M. glandulosa, MS2n = M. gativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

Table 12. Leaf percentage of above-ground dry matter from four harvests of 12 Medicago populations.

		Har	vest		
Entry	Aug	Sept	Oct	May	
		5	DM		
MP(+) T	60.7	54.6	-	41.8	
MP(-)	59.2	55.7	-	46.8	
	53.0	58.1	_	44.1	
KS94(+)			_	43.4	
KS94(-)	56.9	51.3	-	43.4	
MS2n	48.5	50.4	_	39.0	
	55.3	52.8	58.8	48.9	
Riley	22.2	32.0	3011		
KS108(+)	58.0	56.3	60.5	53.3	
KS108(-)	57.2	52.6	59.6	51.1	
20100(-)	311-				
KS159(+)	57.8	52.4	60.4	49.0	
KS159(-)	59.1	54.1	60.6	48.8	
K2123(-)	59.1	3441			
KS160(+)	55.0	55.3	59.0	55.2	
KS160(-)	55.5	56.0	63.6	50.0	
12100(-)	55.5	,,,,			
LSD(0.05)	3.5	3.1	1.9	3.8	
\$CV	4.3	4.0	2.1	5.5	

TMP = M. prostrata, KS9% = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

Table 13. Crude protein contents of 12 Medicago populations from five harvests.

	Harvest								
			1985			1986			
Entry	July	Aug		Sept	Oct	Мау			
				\$					
4P(+)†	21.6	23.2	a Ŧ	19.2	-	18.6			
P(-)	20.0	21.7	ab	17.5	-	18.5			
(S94(+)	18.7	18.9	С	19.2	_	17.8			
(894(-)	18.7	20.7		18.1	-	18.4			
fS2n	19.8	19.3	c	20.9	-	20.0			
Riley	20.1	22.0	ab	20.0	26.6	18.6			
KS108(+)	20.2	23.3	a	21.9	28.1	18.9			
KS108(-)	19.8	21.5	ab	20.1	27.4	20.0			
(8159(+)	22.9	22.0	a b	19.9	26.2	20.6			
(S159(-)	20.4	22.0	a b	22.6	22.9	19.5			
(S160(+)	19.9	22.0	ab	22.0	26.6	20.6			
KS160(-)	21.0	21.7		20.6	25.5	20.5			
SD(0.05)	0.9	-		1.6	1.7	1.5			
S C V	5 + 5	5.4		5.5	4.3	5.3			

TMP = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulea, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs

significantly different using a protected LSD at p<0.05.

Table 14. Yield of 12 Medicago populations from four harvests.

Entry	Harvest			
	August	Sept	Oct	Мау
		g DM/	plant	
MP(+) [†]	1.7	4.1	-	4.9
MP(-)	1.5	4.0	-	4.5
KS94(+)	7.1	8.2	-	14.6
KS94(-)	4.7	16.8	-	16.5
MS2n	12.3	14.5	-	24.0
Riley	10.4	24.5	12.5	25.6
KS108(+)	6.3	14.2	6.8	19.0
KS108(-)	8.4	19.4	9.1	21.9
KS159(+)	7.5	19.2	9.9	20.0
KS159(-)	7 - 7	14.7	13.2	20.8
KS160(+)	6.1	13.2	7.5	13.7
KS160(-)	9.2	14.9	13.2	18.3
LSD(0.05)	2.5	4.9	3.3	4.5
% C V	25.5	24.4	21.3	18.4

THF = M. prostrata, KS94 = M. glandulosa, MS2n = M. sativa subsp. caerulsa, Riley = M. sativa, KS108 = M. glutinosa, KS159 = M. sativa x M. prostrata, KS160 = M. sativa x M. glutinosa; (+) = presence of erect glandular hairs, (-) = absence of erect glandular hairs to my loving wife, Sue Blodgett

Forage Quality of Perennial Glandular-haired and Eglandular Medicago Populations

bу

ANDREW WILLIAM LENSSEN

B.S., Cornell University, 1980

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY Manhattan, Kansas

Resistance available in current alfalfa (Medicago sativa L.) cultivars is inadequate to control the alfalfa weevil [Hypera postica (Gyllenhal)] or the potato leafhopper [Empoasca fabae (Harris)], the two most injurious arthropod pests of alfalfa in North America. Resistance to both insects has been documented in other Medicago species having erect, glandular hairs and these hairs have been transferred to alfalfa. The effects of glandular hairs and their exudates on forage quality of alfalfa are unknown. We established a field trial in 1985 to determine the effects of erect glandular hairs and their exudates on forage quality of several perennial Medicagos. Glandular and eglandular plant populations were selected from the diploids M. prostrata Jacq. and M. glandulosa David and tetraploids M. glutinosa Bieb., and M. sativa (MS4n) x M. glutinosa, and MS4n x M. prostrata. Eglandular M. sativa 'Riley' and diploid M. sativa subsp. caerulea (Less ex. Ledeb.) Schmalh. were included as controls. Foliar diseases and insects were controlled. Leaves were separated from stems for three harvests in 1985 and one in 1986. In vitro dry matter disappearance (IVDMD), crude protein (CP), neutral (NDF) and acid (ADF) detergent fibers, hemicellulose (HC), lignin (ADL), and cellulose (CEL) were determined on each component. The presence of glandular hairs on the wild Medicago species (glandulosa, prostrata, and glutinosa) did not

significantly affect the IVDMD, CP, NDF, ADF, HC, ADL, or CEL concentrations of leaves or stems. The forage quality of the hybrid populations was also unaffected by the presence of erect glandular hairs and their exudates.

Stems of the diploid entries, M. prostrata, M. glandulosa, and M. gativa subsp. gasrulea, generally had higher concentrations of IVDMD, and lower concentrations of NDF, ADF, ADL, and CEL than did MS4n. Conversely, leaves of the diploid entries had lower concentrations of IVDMD, and higher concentrations of NDF, ADF, ADL, and CEL than did MS4n. Data from this study indicate that plant breeders may utilize erect glandular hairs to improve pest resistance in alfalfa without affecting forage quality, as measured in the laboratory.