

# In vitro ruminal undegradable proteins of alfalfa cultivars

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Tremblay, G. F., Michaud, R., Bélanger, G., McRae, K. B. and Petit, H. V. 2000. **In vitro ruminal undegradable proteins of alfalfa cultivars.** *Can. J. Plant Sci.* **80**: 315–325. The quality of alfalfa would be greatly improved by an increase in its ruminal undegradable protein (RUP) concentration. Protein degradation rate (PDR), in vitro dry matter digestibility (IVDMD), leaf weight ratio (LWR), dry matter yield (DMY), total nitrogen (TN), in vitro RUP (expressed on both TN, RUP-TN, and dry matter basis, RUP-DM), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations were determined in 27 alfalfa cultivars. Cultivars were seeded in triplicate on 2 consecutive years and evaluated during the 2 subsequent production years with two harvests per year. Protein degradation rate and RUP-TN were determined using a ruminal inhibitor in vitro system. Data were averaged for spring growth, summer regrowth, and both harvests across 2 production years. Each of the three data sets was analyzed by ANOVA followed by a principal component analysis (PCA) on the ANOVA means. For the four-harvest data, cultivar differences were highly significant ( $P < 0.001$ ) for all variates except for PDR ( $P = 0.07$ ) and RUP-TN concentration ( $P = 0.10$ ). The first PCA axis was largely defined positively by RUP-DM, IVDMD, TN, LWR, and RUP-TN, but negatively with ADF, NDF, PDR, and DMY. The second PCA axis defined a contrast between PDR versus RUP-TN, DMY, ADF, and NDF. Five cultivars were distinctive with high or low PCA scores in all three PCA. Rangelander and Heinrichs, along with Ultra, had low PDR; the first two cultivars had low DMY whereas Ultra was a medium-yielding cultivar. In contrast, Algonquin and Oneida VR had high PDR and medium DMY. While the first principal component (PC) indicated a general trend that low PDR and high RUP were associated with low-yielding cultivars, the second PC identified specific cultivars with both low PDR and high DMY. Therefore, selection for low PDR and high DMY is feasible.

**Key words:** ruminal protein escape, dry matter digestibility, alfalfa

Tremblay, G. F., Michaud, R., Bélanger, G., McRae, K. B. et Petit, H. V. 2000. **Teneur en protéine non dégradée au niveau du rumen de différents cultivars de luzerne.** *Can. J. Plant Sci.* **80**: 315–325. La valeur nutritive de la luzerne serait grandement améliorée si son contenu en protéine non dégradée au niveau du rumen (RUP) était augmenté. Le taux de dégradation ruminale de la protéine (PDR), la digestibilité in vitro de la matière sèche (IVDMD), le rapport feuilles:plante entière (LWR), le rendement en matière sèche et les concentrations en azote total (TN), en RUP (exprimées sur une base de TN, RUP-TN, ou de matière sèche, RUP-DM), en fibre par détergent acide (ADF) et en fibre par détergent neutre (NDF) ont été mesurés chez 27 cultivars de luzerne. Les cultivars ont été semés en trois répétitions pendant deux années consécutives et évalués au cours des deux années de croissance suivantes au rythme de deux coupes par an. Le PDR et la RUP-TN ont été mesurés à l'aide d'une méthode in vitro utilisant du liquide ruminal et des inhibiteurs. Une ANOVA et une analyse en composantes principales (PCA) ont été effectuées sur la moyenne des deux premières coupes, des deux secondes coupes et des quatre coupes. Pour la moyenne des quatre coupes, les différences entre cultivars étaient hautement significatives pour toutes les variables mesurées sauf pour le PDR ( $P = 0.07$ ) et la RUP-TN ( $P = 0.10$ ). Le premier axe de la PCA était surtout défini par la RUP-DM et associé positivement avec la IVDMD, TN, LWR et la RUP-TN, mais négativement avec le ADF, NDF, PDR et le rendement en matière sèche. Le deuxième axe de la PCA a permis de définir un contraste entre le PDR d'une part et la RUP-TN, le rendement, l'ADF et le NDF d'autre part. Cinq cultivars avaient des scores PCA faibles ou élevés dans chacune des trois PCA. Les cultivars Rangelander, Heinrichs et Ultra avaient de faibles PDR; le niveau de production des deux premiers cultivars étaient faibles alors que celui d'Ultra était moyen. À l'opposé, Algonquin et Oneida VR avaient des PDR élevés et des rendements moyens. Alors que le premier axe des PCA indiquait une tendance générale à ce qu'un faible PDR et une concentration en RUP élevée soient associés aux cultivars peu productifs, le deuxième axe identifiait des cultivars ayant à la fois de faibles PDR et des rendements élevés. Une sélection génétique pourrait donc permettre d'améliorer ces deux paramètres.

**Mots clés:** Protéine non dégradée, digestibilité de la matière sèche, luzerne

**Abbreviations:** ADF, acid detergent fiber; DM, dry matter; DMY, dry matter yield; IVDMD, in vitro dry matter digestibility; LWR, leaf weight ratio; NDF, neutral detergent fiber; PC, principal component; PCA, principal component analyses; PDR, protein degradation rate; RUP, ruminal undegradable proteins; RUP-DM, RUP expressed

on a DM basis; RUP-TN, RUP expressed on a TN basis; SEE, standard error of the estimate; SEM, standard error of the mean; SEP, standard error of prediction; SEP(C), standard error of prediction corrected for the bias; TAA, total amino acids; TN, total nitrogen

Forage proteins are often poorly used by ruminants because they are extensively degraded during silage fermentation (Petit and Tremblay 1992) and in the rumen. Excessive ruminal protein degradation may be the most limiting factor of high-quality legume forages (Cadorniga and Satter 1993; Dhiman and Satter 1993; Dhiman et al. 1993; Broderick 1995). The value of these forages as a protein source could be greatly enhanced by decreasing ruminal degradability through genetics, bioengineering, or processing (Klopfenstein 1991). Gutek et al. (1976) indicated that it should be possible to lower the soluble protein content of alfalfa by consecutive cycles of recurrent selection involving well-replicated progeny tests because the heritability estimates for soluble proteins were relatively low. Broderick and Buxton (1991) observed that RUP of whole plants was 24% greater in *Medicago falcata* than in *M. sativa* germplasms. Skinner et al. (1994) observed differences in rates of protein degradation among 11 alfalfa germplasms, but the proportion of proteins digested by ficin was equivalent after 2 h of digestion. Large differences in degradability among individual plants were also observed, indicating that substantial variation for this trait exists within populations (Skinner et al. 1994). However, other studies indicated no significant differences among alfalfa lines for protein degradability (Venter and Skinner 1993), suggesting that differences previously reported (Broderick and Buxton 1991) may be due to plant factors and not to differences in the protein per se. Furthermore, there are few reports in the literature on the relationship between protein degradability and other parameters of nutritive value during regrowth. Griffin et al. (1994) reported that cultivars with high RUP concentrations tended to be low in other quality parameters and that plant selection based solely on RUP concentration would likely be counterproductive.

This study was conducted to determine if the PDR and in vitro RUP varied genetically among 27 alfalfa cultivars, and to explore the relationships among their RUP, DMY, LWR, IVDMD, TN, ADF, and NDF concentrations.

#### MATERIALS AND METHODS

Twenty-seven alfalfa cultivars were chosen to represent a wide variability in genetic background: AC Caribou, Admiral, Algonquin, Anchor, Angus, Apica, Armor, Arrow, Crown II, DK 125, Excalibur, Heinrichs, Horizon, Iroquois, LIRD-4, OAC Minto, Oneida VR, Rangelander, Saranac, Spredor 2, Ultra, Vernal, WL 222, WL 225, WL 316, 5246, and 5262. These cultivars were seeded at 15 kg ha<sup>-1</sup> in the spring of 1994 and again in 1995 at the Normandin Research Farm of Agriculture and Agri-Food Canada (lat. 48°51'N, long. 72°32'W) on a well-drained Normandin silty clay (humic Gleysol) with a soil pH in water between 6.0 and 6.4. The cultivar LIRD-4 was included only in the 1995 seeding. Individual plot size was 6 m × 1.5 m (10 rows, 6-m long with a row spacing of 15 cm). Cultivars were established in a randomized complete block design with three replications.

During the seeding years, 40 kg N ha<sup>-1</sup>, 95 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 95 kg K<sub>2</sub>O ha<sup>-1</sup> were applied at seeding, and 15 kg P<sub>2</sub>O<sub>5</sub>

ha<sup>-1</sup> and 30 kg K<sub>2</sub>O ha<sup>-1</sup> were applied in the fall. One cut was taken in the seeding years. In each of the 2 yr following seeding, 15, 110, and 219 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O were applied, respectively. All the N was applied in the spring whereas P and K were applied 40% in the spring, 30% after the first harvest, and 30% after the second. Gramoxone was applied at 4 L ha<sup>-1</sup> within 5 d after the first and second harvests of each production year.

A strip, 5-m long and 0.6-m wide, was harvested twice each year in each plot for the first 2 yr of production of each seeding to determine DMY. Harvests were taken at the same dates for all cultivars when the majority was in early flowering stage (10% bloom). Two whole-plant samples of approximately 400 g were taken from each plot at each harvest and dried at 55°C in a forced-draft oven for 3 d. For one dry sample, leaves were separated by hand from the stems to determine the LWR. Whole plants from the second sample were ground using a Wiley mill (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) fitted with a 1-mm screen. Half of each ground sample was then passed through a 1-mm screen of a cyclone mill (Ultra Centrifugal Mill Retsch, model ZM1, West Germany). Chemical and near infrared analyses of TN and IVDMD were done using samples ground with a cyclone mill, whereas those of ADF and NDF were done on Wiley-milled samples.

#### Chemical Analyses

Samples selected for NIRS calibration were analyzed in duplicate for TN, ADF, NDF and IVDMD using wet chemistry procedures. They were mineralized using a mixture of sulfuric and selenious acids as described by Isaac and Johnson (1976) and TN was measured on a QuickChem AE Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI) using the Lachat method 13-107-06-2-D based on salicylate, hypochlorite, and sodium nitroprusside. The nonsequential procedure of Goering and Van Soest (1970) was used to determine ADF and NDF concentrations using sodium sulfite and  $\alpha$ -amylase for NDF. The IVDMD analyses were done according to Tilley and Terry (1963).

All cyclone-milled samples were analyzed for in vitro RUP concentration using the wet chemical procedure. Rates of ruminal protein degradation were determined in triplicate with an inhibitor in vitro procedure (Broderick 1987) scaled down to use 5 mL of buffer and 10 mL of rumen inoculum. Forage proteins were added at 0.125 mg TN ml<sup>-1</sup> medium. The inhibitors hydrazine and chloramphenicol were incorporated into the medium to give quantitative recovery of NH<sub>3</sub> and total amino acids (TAA) produced during protein degradation. In vitro samples were analyzed for NH<sub>3</sub> and TAA using the method of Broderick and Kang (1980) adapted to the Lachat autoanalyzer. The PDR is the proportion of TN degraded for each sample after 2 h of incubation minus the proportion degraded at zero-time divided by 2 h of incubation. The proportion of TN degraded at zero time or after 2 h of incubation is the NH<sub>3</sub> and TAA concentrations at zero time or after 2 h of incubation minus NH<sub>3</sub> and TAA concentrations in blank incubations, and these proportions were both evaluated for each sample. The RUP-TN was calculat-

**Table 1. NIR statistics of calibration and validation sets for analytes in alfalfa samples**

	Total N (g kg <sup>-1</sup> DM)				IVDMD <sup>y</sup>	NDF		ADF
	First seeding		Second seeding			(g kg <sup>-1</sup> DM)		
	First <sup>z</sup>	Second	First	Second	First and second seeding			
<i>Calibration set</i>								
<i>N</i>	45	45	45	45	180	180	180	
Range	17.3–35.8	22.9–35.8	22.0–43.3	27.5–45.0	570–761	265–530	257–441	
Mean	27.9	29.4	34.8	34.1	668.0	401.7	345.4	
Wavelengths ( <i>n</i> )	3	3	6	2	7	5	6	
Mathematical treatment <sup>x</sup>	0	2	2	1	1	2	2	
R <sup>2</sup> <sup>w</sup>	0.99	0.98	0.99	0.91	0.86	0.93	0.96	
SEE <sup>v</sup>	0.69	0.76	0.66	1.74	21.3	17.5	10.3	
<i>Validation set</i>								
<i>N</i>	15	15	15	15	60	60	60	
Range	20.5–35.5	24.4–36.1	28.2–42.8	27.8–41.8	576–779	300–513	263–421	
Mean	28.2	29.4	35.6	33.8	672.7	398.1	339.5	
r <sup>2u</sup>	0.99	0.97	0.99	0.99	0.80	0.84	0.90	
SEP <sup>t</sup>	0.83	1.05	0.88	0.66	19.3	21.0	13.7	
SEP(C) <sup>s</sup>	0.86	1.08	0.90	0.68	18.6	21.0	13.8	
Bias	0.18	-0.05	-0.12	0.06	-5.6	-3.0	0.5	

<sup>z</sup> Year of production.

<sup>y</sup> Samples from the first year of production of the first seeding were analyzed using the wet chemical procedure.

<sup>x</sup> Mathematical treatment, 0 = -log (Reflectance), 1 = first derivative, 2 = second derivative.

<sup>w</sup> Multiple correlation coefficient.

<sup>v</sup> Standard error of the estimate.

<sup>t</sup> Correlation between laboratory and predicted values squared.

<sup>u</sup> Standard error of prediction.

<sup>s</sup> Standard error of prediction corrected for the bias.

ed using PDR assuming a passage rate from the rumen (*k<sub>p</sub>*) of 0.06 h<sup>-1</sup>:

$$\text{RUP-TN (g kg}^{-1} \text{ of TN)} = B [k_p / (\text{PDR} + k_p)],$$

where *B* is the proportion of undegraded proteins, assumed to be one minus the proportion degraded at zero time and corresponds to the degradable true protein fraction. Values of RUP were also expressed on a DM basis:

$$\text{RUP-DM (g kg}^{-1} \text{ of DM)} = \text{RUP-TN (g kg}^{-1} \text{ of TN)} \times \text{TN (g kg}^{-1} \text{ of DM)} \times 6.25 / 1000.$$

This inhibitor in vitro method is among other things able to identify significant differences between samples of the same feed type (Neutze et al. 1993).

Rumen contents collected for IVDMD and PDR analyses were obtained from a ruminally cannulated dry cow, which was fed daily a basal diet composed of 50% alfalfa and 50% timothy hay served ad libitum, supplemented with 500 g ground corn, 150 g soybean meal, and 100 g of a premix of minerals and vitamins (P18, Coopérative Fédérée du Québec, St-Romuald, QC). The cow was fed at 0600 and 1800 h, and rumen contents were collected between 0800 and 0830. Care and handling of the cow were conducted according to guidelines of the Canadian Council on Animal Care (1993).

### Near Infrared Analyses

All cyclone and Wiley-milled samples were dried again at 55°C for 24 h before being scanned by a Technicon

Infralyzer 500 scanning monochrometer (Bran and Luebbe, Buffalo Grove, IL). Spectra of all samples were ranked using PICKS program of IDAS-PC version 1.2 software (Bran and Luebbe Analyzing Technologies, Elmsford, NY); the 45 spectra having the highest relative significance were selected for the calibration set, and the 15 next most significant spectra were selected for the validation set. Total nitrogen was predicted after each year of production for each seeding because these values were necessary to analyze chemically samples for RUP concentration. At the end of the study, one calibration equation per nutritional parameter, based on the same spectra as those used for TN, was used to predict IVDMD, ADF, and NDF concentrations of all samples. Samples from the first year of production of the first seeding, however, were analyzed for IVDMD using the wet chemical procedure. Calibration equations were established using the Step-up program of IDAS-PC software, with up to seven wavelengths for absorbance data and with first and second derivative mathematical treatments of absorbance data smoothed over 14-nm intervals. For each nutritional parameter, the selection of calibration was based on R<sup>2</sup>, *t*, *H* statistics, and SEP with a preference for a minimal number of wavelengths. Statistics for calibration and validation sets are given in Table 1.

### Statistical Analyses

There was a total of 159 experimental units (plots); 26 and 27 cultivars in the first and second seeding years × three field replicates. The cultivar LIRD-4 was not included in the first seeding, but the analysis of variance estimated a value based on the additive model. On each plot there were four

**Table 2. Principal component scores and loadings for nine alfalfa forage traits, and the correlation matrix among variates and their correlation with the PC scores. Loadings between -0.2 and 0.2 were omitted**

Variates	PC loadings			Correlation matrix <sup>2</sup> (n = 27; units given in row titles)								
	1	2	3	RUP-DM	IVDMD	TN	LWR	RUP-TN	ADF	NDF	PDR	DMY
<i>Four-harvest means</i>												
RUP-DM (g kg <sup>-1</sup> of DM)		0.39		1.00								
IVDMD (g kg <sup>-1</sup> of DM)		0.37		0.82	1.00							
TN (g kg <sup>-1</sup> of DM)	0.36			0.89	0.81	1.00						
LWR	0.35			0.73	0.70	0.73	1.00					
RUP-TN (g kg <sup>-1</sup> of TN)	0.30	-0.56		0.83	0.58	0.49	0.51	1.00				
ADF (g kg <sup>-1</sup> of DM)	-0.36	-0.27	-0.37	-0.71	-0.75	-0.75	-0.78	-0.43	1.00			
NDF (g kg <sup>-1</sup> of DM)	-0.34	-0.27	-0.42	-0.67	-0.71	-0.71	-0.75	-0.42	0.99	1.00		
PDR (h <sup>-1</sup> )	-0.29	0.58		-0.78	-0.56	-0.43	-0.44	-0.97	0.38	0.37	1.00	
DMY (t ha <sup>-1</sup> )	-0.21	-0.33	0.82	-0.41	-0.50	-0.48	-0.51	-0.14	0.37	0.34	0.18	1.00
PC score 1				0.95	0.89	0.88	0.85	0.74	-0.87	-0.84	-0.70	-0.51
PC score 2				-0.22	0.08	0.18	0.22	-0.66	-0.33	-0.32	0.69	-0.40
PC score 3				-0.05	-0.07	-0.04	-0.01	-0.01	-0.33	-0.37	0.09	0.73
Eigenvalue (% of total)	66	16	9									
<i>Spring growth</i>												
RUP-DM (g kg <sup>-1</sup> of DM)	0.43		-0.25	1.00								
IVDMD (g kg <sup>-1</sup> of DM)		0.37	0.20	0.68	1.00							
TN (g kg <sup>-1</sup> of DM)	0.30	0.41	-0.28	0.71	0.66	1.00						
LWR	0.38			0.63	0.57	0.45	1.00					
RUP-TN (g kg <sup>-1</sup> of TN)	0.33	-0.53		0.73	0.32	0.06	0.49	1.00				
ADF (g kg <sup>-1</sup> of DM)	-0.32	-0.21	-0.59	-0.36	-0.44	-0.32	-0.41	-0.22	1.00			
NDF (g kg <sup>-1</sup> of DM)	-0.32		-0.62	-0.37	-0.39	-0.27	-0.36	-0.28	0.96	1.00		
PDR (h <sup>-1</sup> )	-0.33	0.52		-0.68	-0.33	-0.02	-0.47	-0.96	0.26	0.33	1.00	
DMY (t ha <sup>-1</sup> )		-0.42	0.27	-0.22	-0.26	-0.37	-0.41	0.06	0.17	0.10	-0.04	1.00
PC score 1				0.90	0.77	0.62	0.78	0.69	-0.66	-0.65	-0.69	-0.32
PC score 2				-0.12	0.26	0.54	0.07	-0.70	-0.27	-0.17	0.68	-0.55
PC score 3				-0.30	-0.18	-0.33	-0.19	-0.08	-0.69	-0.72	0.02	0.31
Eigenvalue (% of total)	48	19	15									
<i>Summer regrowth</i>												
RUP-DM (g kg <sup>-1</sup> of DM)		0.39		1.00								
IVDMD (g kg <sup>-1</sup> of DM)		0.37		0.86	1.00							
TN (g kg <sup>-1</sup> of DM)	0.37			0.91	0.87	1.00						
LWR	0.32			0.67	0.68	0.73	1.00					
RUP-TN (g kg <sup>-1</sup> of TN)	0.30	-0.53		0.83	0.59	0.54	0.37	1.00				
ADF (g kg <sup>-1</sup> of DM)	-0.35	-0.32		-0.73	-0.74	-0.81	-0.78	-0.41	1.00			
NDF (g kg <sup>-1</sup> of DM)	-0.34	-0.34		-0.71	-0.71	-0.79	-0.79	-0.39	0.99	1.00		
PDR (h <sup>-1</sup> )	-0.26	0.60		-0.76	-0.54	-0.46	-0.24	-0.98	0.31	0.29	1.00	
DMY (t ha <sup>-1</sup> )	-0.28			-0.60	-0.64	-0.54	-0.53	-0.46	0.48	0.48	0.41	1.00
PC score 1				0.96	0.91	0.92	0.79	0.74	-0.86	-0.85	-0.65	-0.68
PC score 2				0.20	-0.03	-0.15	-0.39	0.65	0.40	0.42	-0.73	-0.04
Eigenvalue (% of total)	68	17										

<sup>2</sup>The critical value for the 5% two-sided significance test is 0.38.

harvests over 2 production years for both seedings and data were averaged in three ways: spring growth, summer regrowth, and all four harvests. These averages summarized the repeated measurements on the same plot. For each cultivar and each parameter, there were three averages made of two values. They were analyzed by ANOVA as a randomized complete block design replicated over 2 seeding years (SAS Institute, Inc. 1985). Sources of variation were partitioned as follows: seeding years, replicates within seeding years (Error A), cultivars, seeding years × cultivars and cultivars × replicates within seeding years (residual error). Cultivar means, the SEM, and LSD (5%) range were calculated. Cultivars with values higher or lower than the LSD (5%) limits centered on mean were grouped. Correlations between variates were computed on the cultivar means from

the ANOVA for each of the three harvest averages. Principal component analyses were performed on these means using the correlation matrix method to give equal weight to all variates (Genstat 5 Committee 1993). Thus, the variates were standardized with mean zero and standard deviation of one for the PCA.

The correlation matrix between variates was calculated for each harvest average, and the correlations with the PC scores were augmented to the correlation matrix (Table 2) to assist in identifying the contribution of each variate to a PCA axis. The first PC score was used to sort the cultivar information (Table 3) but only the PC axes that had an eigenvalue of >1 are given. The variates were arranged according to their correlation with the first PC score. Significant differences between cultivars were determined

**Table 3.** The first three principal component scores and variate means for the four-harvest means of 27 cultivars seeded in 2 consecutive years all sorted according to the first principal component score<sup>z</sup>

Cultivar	PC 1 (λ <sub>1</sub> = 66%)	PC 2 (λ <sub>2</sub> = 16%)	PC 3 (λ <sub>3</sub> = 9%)	RUP-DM (g kg <sup>-1</sup> DM)	IVDMD (g kg <sup>-1</sup> DM)	TN (g kg <sup>-1</sup> DM)	LWR	RUP-TN (g kg <sup>-1</sup> TN)	ADF (g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	PDR (h <sup>-1</sup> )	DMY <sup>y</sup> (t ha <sup>-1</sup> )
Rangelander	<b>7.43</b>	-0.01	-0.95	<b>50.2</b>	<b>692</b>	<b>33.3</b>	<b>0.462</b>	<b>243</b>	<u>326</u>	<u>381</u>	<u>0.163</u>	<u>2.43</u>
Heinrichs	<b>4.63</b>	-0.55	0.39	<b>48.9</b>	<b>680</b>	<b>32.8</b>	<b>0.432</b>	<b>241</b>	<u>331</u>	<u>385</u>	<u>0.166</u>	<u>2.71</u>
Spredor 2	<b>4.53</b>	0.95	-0.32	<b>48.0</b>	<b>686</b>	<b>32.6</b>	<b>0.446</b>	236	<u>333</u>	<u>386</u>	0.169	<u>2.57</u>
Crown II	<b>1.83</b>	0.51	-0.32	<b>47.6</b>	673	<b>33.0</b>	0.423	233	345	403	0.172	2.74
Horizon	<b>1.25</b>	-0.85	0.91	<b>47.2</b>	670	32.2	0.424	236	347	402	0.170	<b>2.99</b>
Ultra	0.91	<u>-1.74</u>	0.14	46.9	668	31.7	0.415	<b>239</b>	351	406	<u>0.168</u>	2.89
Arrow	0.60	0.33	0.16	46.0	671	31.8	0.424	233	347	403	0.173	2.82
Angus	0.48	<b>1.83</b>	0.01	45.8	669	<b>32.5</b>	<b>0.428</b>	228	<u>344</u>	404	<b>0.177</b>	2.76
Apica	0.32	-0.16	0.92	46.3	669	32.1	0.413	233	348	403	0.173	<b>2.98</b>
Algonquin	0.04	<b>3.17</b>	0.68	45.1	669	<b>32.3</b>	<b>0.436</b>	<u>225</u>	<u>343</u>	<u>397</u>	<b>0.182</b>	2.80
WL 222	-0.11	-0.83	0.59	46.3	<b>674</b>	31.8	<u>0.400</u>	234	351	408	0.172	<b>2.99</b>
AC Caribou	-0.43	-0.19	-1.11	45.8	670	31.9	0.416	232	<b>357</b>	<b>419</b>	0.173	2.76
WL 225	-0.52	-0.41	0.74	44.9	672	<u>31.1</u>	0.417	233	351	408	0.172	<b>2.99</b>
Vernal	-0.54	0.42	-0.81	45.2	<b>674</b>	31.5	0.421	230	<b>357</b>	<b>414</b>	0.174	2.77
Anchor	-0.56	<u>-1.43</u>	0.70	45.5	<u>661</u>	<u>31.0</u>	0.417	236	353	407	0.169	<b>2.99</b>
Excalibur	-0.82	0.85	<u>-1.71</u>	45.5	<u>660</u>	31.7	0.420	230	<b>356</b>	<b>415</b>	0.175	<u>2.56</u>
Iroquois	-0.85	-0.53	-0.23	45.0	<u>657</u>	<u>31.1</u>	0.422	233	353	410	0.171	2.81
OAC Minto	-0.94	<u>-1.29</u>	-0.31	45.3	664	<u>31.0</u>	0.421	236	<b>359</b>	<b>418</b>	0.171	2.91
Armor	-0.96	0.47	-0.68	45.1	666	31.7	<u>0.406</u>	231	<b>355</b>	410	0.175	2.73
Saranac	-1.06	1.10	0.61	<u>44.4</u>	664	31.2	0.417	229	347	403	<b>0.177</b>	2.86
5262	-1.06	<u>-1.97</u>	<b>1.53</b>	45.9	<u>660</u>	31.5	0.413	236	<b>356</b>	<b>413</b>	0.171	<b>3.22</b>
Admiral	-1.17	0.48	0.51	<u>44.5</u>	<u>662</u>	<u>31.1</u>	<u>0.402</u>	230	347	401	0.174	2.84
DK 125	<u>-1.28</u>	-0.85	-0.82	45.4	<u>658</u>	31.2	0.421	234	<b>361</b>	<b>420</b>	0.172	2.82
WL 316	<u>-1.49</u>	-0.64	-0.19	45.5	664	31.5	<u>0.405</u>	232	<b>359</b>	<b>418</b>	0.174	2.92
5246	<u>-1.73</u>	0.34	1.13	<u>43.9</u>	663	<u>30.9</u>	0.415	230	349	408	0.175	<b>3.02</b>
LIRD-4	<u>-4.21</u>	-1.05	<u>-2.23</u>	<u>43.4</u>	<u>660</u>	<u>30.5</u>	<u>0.386</u>	229	<b>376</b>	<b>437</b>	0.174	2.74
Oneida VR	<u>-4.30</u>	<b>2.05</b>	0.66	<u>41.8</u>	<u>654</u>	<u>30.2</u>	<u>0.399</u>	<u>222</u>	354	410	<b>0.181</b>	2.90
Mean	(0.00)	(0.00)	(0.00)	45.8	668	31.7	0.419	232	350	407	0.173	2.84
SEM (n = 6, 102 df)	(1.00)	(1.00)	(1.00)	0.90	4.0	0.42	0.006	3.8	4.0	4.9	0.0034	0.086
LSD (5%) upper limit	1.21	1.21	1.21	47.1	674	32.3	0.428	238	355	412	0.177	2.96
lower limit	-1.21	-1.21	-1.21	44.5	662	31.1	0.410	227	344	400	0.168	2.72

<sup>z</sup>Extreme values higher or lower than the LSD (5%) limits centred on mean are noted in bold or underlined, respectively.

<sup>y</sup>DM yield per harvest.

by the LSD (5%) range, centered about the overall mean for each variate. For the PC scores, the LSD range was based on a (scaled) variance of 1 unit.

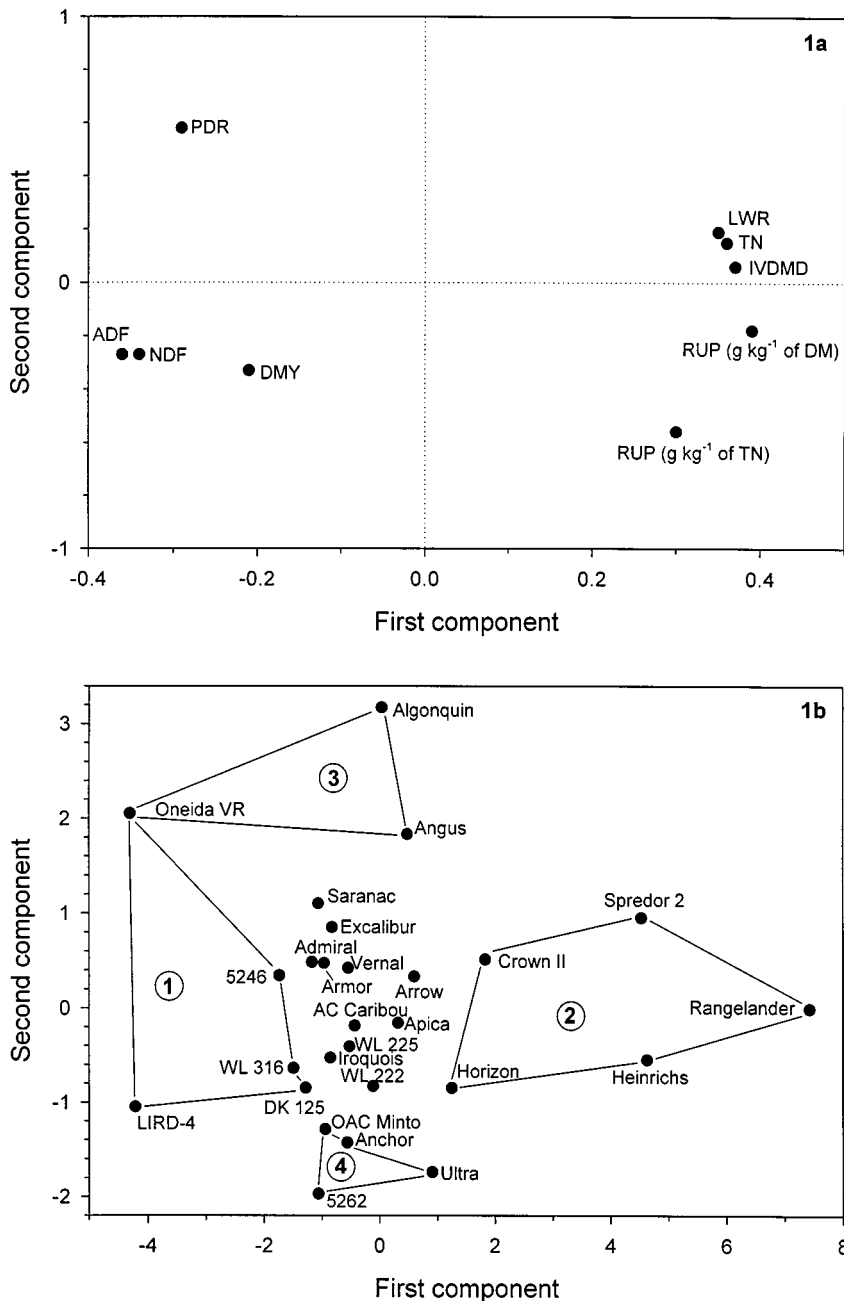
## RESULTS

### Combined Data Across Four Harvests

Cultivar differences were highly significant ( $P < 0.001$ ) for all variates except for PDR ( $P = 0.07$ ) and RUP-TN concentration ( $P = 0.10$ ). Differences between seeding years were significant except for RUP-TN, PDR and DMY, but the seeding year × cultivar interaction was not significant except for ADF and NDF concentrations. For the four-harvest average, 66% of the total covariation was explained by the first axis, 16% by the second, and 9% by the third. The PC scores for the first axis defined a contrast; RUP-DM, IVDMD, TN, LWR, and RUP-TN versus ADF, NDF, PDR, and DMY (Table 2). Variates within the same group were positively correlated; variates in opposing groups were negatively correlated. A single descriptor for this axis could be called “RUP based on DM”. Dry matter yield was negatively correlated with most of the quality parameters; RUP-

DM, IVDMD, TN, and LWR, and positively correlated with ADF and NDF (Table 2, Fig. 1a). There was also a positive correlation between LWR and TN concentration. The contribution of a variate to a PC axis can be seen from its loading or its correlation coefficient; both were used. The first PC scores were largely determined by RUP-DM ( $r = 0.95$ ) but they were also associated positively with IVDMD ( $r = 0.89$ ), TN ( $r = 0.88$ ), LWR ( $r = 0.85$ ), and RUP-TN ( $r = 0.74$ ), and negatively associated with ADF ( $r = -0.87$ ), NDF ( $r = -0.84$ ), PDR ( $r = -0.70$ ), and DMY ( $r = -0.51$ ).

On the first axis, three cultivars that have a greater proportion of *M. falcata* (Rangelander, Heinrichs, and Spredor 2) in their germplasm, along with cultivars Crown II and Horizon (group 2, Fig. 1b) had significantly higher scores (Table 3) than those of DK 125, WL 316, 5246, LIRD-4, and Oneida VR (group 1, Fig. 1b). They also had significantly higher RUP-DM values than Saranac, Admiral, 5246, LIRD-4, and Oneida VR (Table 3). Across the other variates, there were fewer significantly different cultivars (LSD 5%); i.e., for IVDMD, TN, LWR, RUP-TN, NDF, ADF, PDR, and DMY (Table 3).



**Fig. 1.** PCA diagrams of the first two principal component scores of the four-harvest averages calculated for each variate (a) and the 27 cultivars (b) seeded in 2 consecutive years and evaluated during the 2 subsequent production years with two harvests per year. The groups of cultivars 1, 2, 3, and 4 were based on partition from the LSD (5%) limits, centered about the mean.

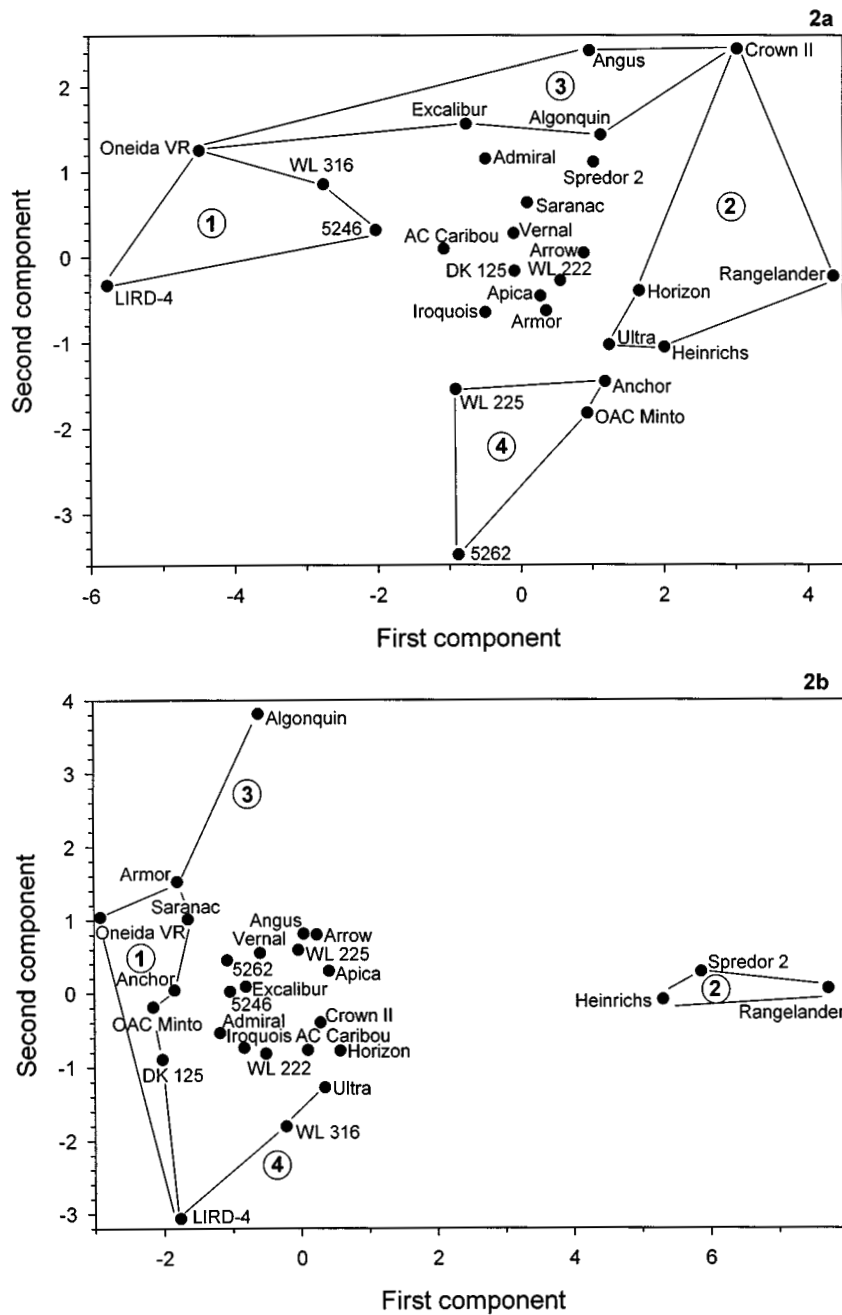
In the second axis, there was also a contrast; PDR versus RUP-TN, ADF, NDF, and DMY (Table 2, Fig. 1a). In this axis, there were some unique cultivars with extreme PC scores (Table 3); 5262, Ultra, Anchor, and OAC Minto (group 4, Fig. 1b) had high negative scores while Algonquin, Oneida VR, and Angus had large positive scores (group 3, Fig. 1b). Cultivars of the group 4 tended to have low PDR and high RUP-TN, DMY, ADF, and NDF, while cultivars from the group 3 were just the opposite (Table 3). Groups 3 and 4 were defined by the second axis and characterized by cultivars with similar yield potentials but con-

trasting PDR values with RUP-TN. A descriptor for this axis would be either “RUP on a TN basis”, or “PDR”.

The third axis was largely defined by three cultivars for DMY versus ADF and NDF concentrations. In general, ADF and NDF concentrations increase with DMY. All three cultivars LIRD-4, Excalibur, and 5262 had high ADF and NDF concentrations but Excalibur and LIRD-4 had low DMY whereas 5262 had higher DMY.

**Spring Growth**

The first PC axis explained 48% of the total covariation ( $\lambda_1$



**Fig. 2.** PCA diagrams of the first two principal component scores calculated for spring growth (a) and summer regrowth (b) of 27 cultivars seeded in 2 consecutive years and evaluated during the 2 subsequent production years with two harvests per year. The groups of cultivars 1, 2, 3, and 4 were based on partition from the LSD (5%) limits, centered about the mean.

= 48%, Table 2) with the scores being largely determined by RUP-DM ( $r = 0.90$ ), and positively associated with LWR ( $r = 0.78$ ), IVDMD ( $r = 0.77$ ), RUP-TN ( $r = 0.69$ ), and TN ( $r = 0.62$ ), and negatively associated with PDR ( $r = -0.69$ ), ADF ( $r = -0.66$ ), NDF ( $r = -0.65$ ), and DMY ( $r = -0.32$ ). On this axis, cultivars Rangelander, Crown II, Heinrichs, Horizon, and Ultra had high scores (group 2, Fig. 2a) that were significantly higher than the other extreme cultivars (5246, WL 316, Oneida VR, and LIRD-4; group 1, Fig. 2a) for at least one of the following variates: RUP-DM, LWR, IVDMD, RUP-TN, and TN. A single descriptor for this axis could be “RUP based on DM”.

In the second axis ( $\lambda_2 = 19\%$ ) there were some unique cultivars with extreme scores; Crown II, Angus, Excalibur, Algonquin, and Oneida VR (group 3, Fig. 2a) had large positive scores while 5262, OAC Minto, WL 225, and Anchor (group 4, Fig. 2a) had negative scores. Cultivars of group 4 were lower than their neighbors on the second axis in PDR and TN, and higher in RUP-TN and DMY; cultivars of group 3 were just the opposite.

The third axis defines a contrast between ADF, NDF, TN, RUP-DM versus DMY (Table 2). The cultivars AC Caribou, Excalibur, DK 125, and Vernal had low DMY and high values in at least one of following variates: ADF, NDF,

TN, and RUP-DM. Admiral, Oneida VR, and Apica had high DMY but low fiber concentrations.

### Summer Regrowth

The first PC axis explained 68% of the total covariation; the first PC scores (Table 2) were largely determined by RUP-DM ( $r = 0.96$ ), TN ( $r = 0.92$ ), and IVDMD ( $r = 0.91$ ), and also positively associated with LWR ( $r = 0.79$ ) and RUP-TN ( $r = 0.74$ ), but negatively associated with ADF ( $r = -0.86$ ), NDF ( $r = -0.85$ ), DMY ( $r = -0.68$ ), and PDR ( $r = -0.65$ ). On this axis, cultivars Rangelander, Spredor 2, and Heinrichs had high positive scores (group 2, Fig. 2b). They had significantly higher values for RUP-DM, IVDMD, TN, LWR, and RUP-TN but lower values for ADF, NDF, PDR, and DMY, based on the LSD (5%) range. They were opposite to Saranac, LIRD-4, Armor, Anchor, DK 125, OAC Minto, and Oneida VR (group 1, Fig. 2b).

The second axis ( $\lambda_2 = 17\%$ ) identified LIRD-4, WL 316, and Ultra (group 4, Fig. 2b) as distinct from Algonquin and Armor (group 3, Fig. 2b). Cultivars from group 4 had lower PDR and LWR, but higher RUP-TN, ADF, and NDF relative to those from group 3.

## DISCUSSION

### Genetic Variability for Protein Degradability

Even though our main objective was to identify cultivars with high RUP-TN, three related variates were used to characterize protein degradability. The PDR is the primary measure of protein degradability and it describes the rate at which the protein is degraded in the rumen. The RUP-TN is based on the PDR values adjusted for the degradable true protein fraction and the passage rate in the rumen, and it represents the proportion of proteins that will not be degraded after passing through the rumen. The PDR and RUP-TN values provide complementary information on protein degradability. For instance, a cultivar with a low PDR and a low degradable true protein fraction, which could be associated with a low TN concentration, would have a similar RUP-TN to a cultivar with high PDR and a high degradable true protein fraction. One objective would be to maximize the degradable true protein fraction and minimize its degradation rate. Furthermore, it has been shown that RUP-DM would be easier to predict by NIRS than RUP-TN (Tremblay et al. 1996). It was therefore useful to determine how RUP-DM was related to the other parameters. The RUP-DM represents the proportion of proteins on a DM basis that will not be degraded in the rumen. Increases in RUP-DM can therefore be due to increases in RUP-TN and/or TN concentration.

Cultivars with extreme PC scores in spring growth, summer regrowth and in the four-harvest average, reflecting low PDR and high RUP-TN, were of interest. Nine cultivars were in peripheral groups of the three graphs (Figs. 1b, 2a, and 2b): Rangelander, Heinrichs, Ultra, Algonquin, Oneida VR, Anchor, LIRD-4, OAC Minto, and WL 316. Of these nine cultivars, Rangelander, Heinrichs, and Ultra had low PDR and high RUP-TN at both spring growth and summer regrowth, and on the four-harvest average. Rangelander and

Heinrichs had less DMY than the lower LSD (5%) limit (2.43 and 2.71 t ha<sup>-1</sup>, respectively), but Ultra was a medium-yielding cultivar (2.89 t ha<sup>-1</sup>) on the four-harvest average (Table 3). Algonquin and Oneida VR had high PDR and medium DMY. The PDR of Oneida VR was high for both spring growth and summer regrowth, whereas its DMY was always near the average. Algonquin had PDR close to the LSD (5%) upper limit in spring growth (0.165 vs. 0.168 h<sup>-1</sup>) and higher PDR than this limit in the summer regrowth (0.199 vs. 0.191 h<sup>-1</sup>) and on a four-harvest basis (0.182 vs. 0.177 h<sup>-1</sup>).

The cultivars Anchor, LIRD-4, OAC Minto, and WL 316 had extreme PC scores at every harvest but not necessarily because of their PDR and not always for the same reasons. Anchor was distinctive because it had high DMY at all harvests but low PDR in spring growth and low IVDMD, TN, and RUP-DM concentrations in the summer regrowth. The cultivar OAC Minto had low TN and high fiber concentrations in both spring growth and summer regrowth. The cultivar LIRD-4 had high PDR in spring growth but low PDR in summer regrowth; it always had, however, low LWR and TN concentration, and high fiber concentrations. Finally, WL 316 had high PDR in spring growth and low PDR in summer regrowth, but it always had low LWR and high fiber concentrations.

Among the cultivars that were not simultaneously in peripheral groups in the three graphs, Spredor 2, a cultivar with a great proportion of *M. falcata* in its germplasm, had low PDR and DMY on a four-harvest basis, but its PDR was medium in spring growth and low in summer regrowth. Horizon, which had a high PC 1 score for the four-harvest average, was a high-producing cultivar with medium PDR in spring growth and a medium-producing cultivar with low PDR in summer regrowth. Horizon, Crown II, and Spredor 2 were in the second group of cultivars because they had a high RUP-DM concentration, which was mainly due to their high TN concentrations (Fig. 1b). Differences among cultivars for protein degradability were not always consistent across harvests, indicating that differing growth habits and environmental effects may play an important role in determining the relative degradability of alfalfa cultivars, as previously reported (Griffin et al. 1994). Fall dormancy of the 27 cultivars varied between 1 and 4 (Barnes et al. 1991), but this variation can barely explain the present results because they do not include fall harvests.

In the second seeding, we observed that cultivars with a greater proportion of *M. falcata* (Heinrichs, Rangelander, and Spredor 2) were less mature than the other cultivars when harvested during the summer regrowth of the first year of production, and at both harvests of the second year of production. The *M. falcata* lines were also less mature than the *M. sativa* lines in the Broderick and Buxton experiment (1991). In the present study, whole plant samples of all cultivars were taken at the same date, but, for Heinrichs, Rangelander, and Spredor 2, additional samples were also taken 1 wk later. In vitro RUP-TN concentrations were higher at the second sampling date for both the summer regrowth of the first year of production and the spring growth of the second year of production, but they were sim-

ilar in the summer regrowth of the second production year. When averaged over the three cultivars and the three harvests, RUP-TN concentration was 10.7% higher at second than at first sampling date (270 vs. 244 g kg<sup>-1</sup> of TN). If the present analyses had been done at the same stage of maturity instead of the same sampling date, differences between the three cultivars with a greater proportion of *M. falcata* germplasm and the other cultivars would have been greater. The RUP-TN concentration of cool season grasses and perennial legumes increases with plant maturity (Balde et al. 1993; Hoffman et al. 1993; Coblenz et al. 1998). The present results also confirm those of Griffin et al. (1994) and Kohn and Allen (1995) who observed that advanced maturity results in a lower degradation of alfalfa proteins. Similarly, Elizalde et al. (1997) reported that undegraded TN, measured using the in situ procedure, increased from 163 to 244 g kg<sup>-1</sup> of TN with increased maturity of alfalfa from the vegetative to the late flowering stage.

In the present study, PDR values were lower and RUP-TN concentrations were higher (Table 3) than in the study of Broderick and Buxton (1991) who reported PDR ranging from 0.179 to 0.252 h<sup>-1</sup>, and estimated RUP concentrations ranging from 150 to 219 g kg<sup>-1</sup> of TN for 22 alfalfa entries. In both studies, however, entries with a greater proportion of *M. falcata* had slower PDR and higher RUP-TN concentration than *M. sativa* entries. In the present study, PDR and RUP concentration averaged 0.166 h<sup>-1</sup> and 240 g kg<sup>-1</sup> of TN for the three cultivars with a greater proportion of *M. falcata* as compared with 0.174 h<sup>-1</sup> and 232 g kg<sup>-1</sup> of TN for the other 24 cultivars. In vitro RUP-TN concentration was 3.5% greater for these three cultivars, as compared with the 24 others, and 7.4% greater than the averaged value observed for Algonquin and Oneida VR, which had the lowest RUP-TN values (Table 3). In the Broderick and Buxton (1991) experiment, PDR and estimated net protein escape from the rumen averaged 0.227 h<sup>-1</sup> and 171 g kg<sup>-1</sup> of TN for *M. sativa*, whereas those for *M. falcata* averaged 0.181 h<sup>-1</sup> and 212 g kg<sup>-1</sup> of TN, respectively; RUP-TN concentration was 24% greater for *M. falcata*. Broderick and Buxton (1991) reported values representing averages over two harvests during one growing season, whereas averages in the present study represent 2 seeding years evaluated during 2 yr of production with two harvests per year. Samples of the present experiment were then taken during three different growing seasons instead of one, as in the Broderick and Buxton (1991) experiment. Furthermore, Rangelander, Heinrichs, and Spredor 2 were not pure *M. falcata* cultivars; this could partly explain the differences between the two studies.

### Relationship among Variates

In the present experiment, we used PCA to study the relationship between DMY and several nutritive parameters. Because it is well accepted that the nutritive value, or more specifically digestibility, of alfalfa decreases with increases in DMY during regrowth (Lemaire and Allirand 1993; Julier and Huyghe 1997), we hypothesized that differences in digestibility among cultivars were due to differences in DMY, not to intrinsic characteristics independent of DMY. Consequently, cultivars with a low yield would tend to have

high DM digestibility, and cultivars with a high potential yield would have a low DM digestibility. Our study confirms this finding; RUP-DM, IVDMD, TN, LWR, and RUP-TN were all negatively correlated with regrowth DMY. Only TN and LWR were significantly and negatively correlated with spring DMY and thus DMY did not contribute significantly to the first PC for spring growth. Apart from this exception, loadings for spring growth, summer regrowth, and the four-harvest mean were similar.

The second axis, however, indicated that the PC scores for DMY and IVDMD for the four-harvest data were determined by the summer regrowth (Table 2; Fig. 1a). For instance, with the four-harvest data WL 322 had high DMY and IVDMD whereas Excalibur had low DMY and IVDMD (Table 3). Several studies also reported no correlation or weak correlations between digestibility and DMY when cultivars or lines were compared on a single sampling date corresponding to a normal harvesting date (Heinrichs et al. 1969; Hill and Barnes 1977; Sunberg et al. 1983; Coors et al. 1986; Julier and Huyghe 1997). Other studies, however, reported a reduced forage DMY as a result of selection for improved nutritive quality (Kephart et al. 1989).

Concentrations of ADF and NDF were positively related to regrowth DMY, and negatively related to IVDMD (Table 2; Fig. 1a). For instance, the high-yielding cultivar 5262 based on the four-harvest data had high ADF and NDF concentrations whereas the low-yielding cultivars Rangelander, Heinrichs, and Spredor 2 had low ADF and NDF concentrations (Table 3). The third component of the four-harvest data and the spring growth, however, had DMY contrasting with ADF and NDF concentrations, which suggests that several cultivars had similar DMY but contrasted ADF and NDF concentrations. For instance, even though they had similar DMY based on the four-harvest data, Angus and Algonquin had low ADF and NDF concentrations, while AC Caribou, Vernal, and DK 125 had high ADF and NDF concentrations.

The first component indicated a negative relationship between TN concentration and DMY for the four-harvest data, the spring growth, and the summer regrowth (Table 2, Fig. 1a). Differences in TN concentration among cultivars were related to their DMY. For example in the four-harvest data, the low-yielding cultivars Rangelander, Heinrichs, and Spredor 2 had high TN concentrations whereas the high-yielding cultivars WL 225, 5246, and Anchor had low TN concentrations (Table 3). Lemaire et al. (1985) also reported that TN concentration decreases with increasing DMY during regrowth. In the second axis, however, the PC scores of DMY and TN concentration were not opposed except for the spring growth (Table 2; Fig. 1a). This suggests that, for a given level of DMY, there is variability for TN concentration in alfalfa, which agrees with previously reported observations (Garza et al. 1965; Coors et al. 1986).

The LWR decreases with increases in DMY (Lemaire and Gastal 1997), and as expected, we observed a negative relationship between LWR and DMY (Table 2; Fig. 1a). Hence, low-yielding cultivars had a high LWR and high-yielding cultivars had a low LWR (Table 3). The LWR was also positively correlated with IVDMD and TN concentration, and

negatively correlated with ADF and NDF concentrations (Table 2; Fig. 1a). This suggests that selection on the basis of one of the nutritive value parameters would affect LWR, which agrees with other studies (Kephart et al. 1989).

The second component, however, indicated no relationship between DMY and LWR, which suggests that for similar DMY, there was a wide range of LWR (Table 2; Fig. 1a). Similarly, the second component indicated a weak relationship between LWR, IVDMD, ADF, and NDF concentrations. For instance, WL 322 had a low LWR but high IVDMD and cultivars Admiral, LIRD-4, and Oneida VR had a low LWR and low IVDMD (Table 3). Our results confirm that LWR plays a significant role in explaining differences in IVDMD, and TN, ADF, and NDF concentrations among cultivars. Some of the variability, however, could not be explained by the LWR. This agrees with results from comparisons of early- and late-maturing timothy cultivars (Bélanger and McQueen 1996) and of alfalfa cultivars (Julier and Huyghe 1997), and indicates that the intrinsic characteristics of leaves, stems or both can affect the nutritive value of forages.

Our results clearly showed that yield potential affected protein degradability and DM digestibility of alfalfa cultivars, and this was often related to their LWR. Consequently, the benefits of low protein degradability and high digestibility would be negated by the lower DMY. Selection based only on protein degradability or other parameters of nutritive value without consideration for DMY can result in low-yielding cultivars. In our study, the first component was primarily driven by the presence of cultivars with a great proportion of *M. falcata* (Rangelander, Heinrichs, and Spredor 2). These cultivars were characterized by a lower DMY and higher LWR than cultivars recommended in Eastern Canada. As a result, they had greater IVDMD, TN, RUP-TN and RUP-DM concentrations as well as lower ADF and NDF concentrations than the other cultivars. Broderick et al. (1993) stated that the protein degradability of leaves was less than that of stems. Our results support these findings because cultivars with greater LWR had a lower proportion of RUP.

Our study also showed, primarily through the second component of the PCA, that variability exists for protein degradability and other parameters of nutritive value for cultivars having a similar yield potential. Hence, DMY and nutritive value can be dissociated genetically, and improvements in nutritive value may be realized without reduction in DMY.

### CONCLUSIONS

The first PCA axis for the four-harvest data was largely defined by RUP-DM and associated positively with IVDMD, TN, LWR, and RUP-TN, but negatively with ADF, NDF, PDR, and DMY. The second PCA axis defined a contrast between PDR and RUP-TN, DMY, ADF, and NDF. The principal component analyses identified five distinctive cultivars that had simultaneously high or low PC scores in both spring growth and summer regrowth. Two cultivars with a great proportion of *M. falcata* in their germplasm, Rangelander and Heinrichs, along with Ultra

had low PDR. Rangelander and Heinrichs had low DMY whereas Ultra was an average-yielding cultivar. Algonquin and Oneida VR had high PDR and average DMY. While the first PC indicated that low PDR, and high RUP-DM, were generally associated with low-yielding cultivars, the second PC identified specific cultivars with both slower protein degradation and medium DMY. Therefore, genetic selection for low PDR and high DMY is feasible.

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- Balde, A. T., Vandersall, J. H., Erdman, R. A., Reeves, J. B. and Glenn, B. P. 1993.** Effect of stage of maturity of alfalfa and orchardgrass on in situ dry matter and crude protein degradability and amino acid composition. *Anim. Feed Sci. Technol.* **44**: 29–43.
- Barnes, D. K., Smith, D. M., Teuber, L. R. and Peterson, M. A. 1991.** Fall Dormancy. Page A-1 in C.C. Fox et al. eds. Standard tests to characterize alfalfa cultivars. 3rd ed. North American Alfalfa Improvement Conference, Minneapolis, MN.
- Bélanger, G. and McQueen, R. E. 1996.** Digestibility and cell wall concentration of early- and late-maturing timothy (*Phleum pratense* L.) cultivars. *Can. J. Plant Sci.* **76**: 107–112.
- Broderick, G. A. 1987.** Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. *Br. J. Nutr.* **58**: 463–475.
- Broderick, G. A. 1995.** Desirable characteristics of forage legumes for improving protein utilization in ruminants. *J. Anim. Sci.* **73**: 2760–2773.
- Broderick, G. A. and Buxton, D. R. 1991.** Genetic variation in alfalfa for ruminal protein degradability. *Can. J. Plant Sci.* **71**: 755–760.
- Broderick, G. A. and Kang, J. H. 1980.** Automated simultaneous determination of ammonia and total amino acid in ruminal fluid and in vitro media. *J. Dairy Sci.* **63**: 64–75.
- Broderick, G. A., Goh, Y. G., Smith, R. R. and Barnes, D. K. 1993.** Ruminal degradability of protein in leaves and stems from samples of alfalfa germplasm. *J. Dairy Sci.* **76** (Suppl. 1): 248.
- Cadorniga, C. and Satter, L. D. 1993.** Protein versus energy supplementation of high alfalfa silage diets for early lactation cows. *J. Dairy Sci.* **76**: 1972–1977.
- Canadian Council on Animal Care. 1993.** Guide to the care and use of experimental animals. Volume I. CCAC, Ottawa, ON.
- Coblentz, W. K., Fritz, J. O., Fick, W. H., Cochran, R. C. and Shirley, J. E. 1998.** In situ dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and eastern gamagrass at four maturities. *J. Dairy Sci.* **81**: 150:161.
- Coors, J. G., Lowe, C. C. and Murphy, R. P. 1986.** Selection for improved nutritional quality of alfalfa crops. *Crop Sci.* **26**: 843–848.
- Dhiman, T. R. and Satter, L. D. 1993.** Protein as the first-limiting nutrient for lactating dairy cows fed high proportions of good quality alfalfa silage. *J. Dairy Sci.* **76**: 1960.
- Dhiman, T. R., Cadorniga, C. and Satter, L. D. 1993.** Protein and energy supplementation of high alfalfa silage diets during early lactation. *J. Dairy Sci.* **76**: 1945.

- Elizalde, J. C., Merchen, N. R. and Faulkner, D. B. 1997.** Effects of species and stages of maturity of fresh forages on in situ dry matter and crude protein degradation. *J. Anim. Sci.* **75** (Suppl. 1): 208.
- Garza, T. R., Barnes, R. F., Mott, G. O. and Rhykerd, C. L. 1965.** Influence of light intensity, temperature, and growth period on the growth, chemical composition and digestibility of Culver and Tanverde alfalfa seedlings. *Agron. J.* **57**: 417–420.
- Genstat 5 Committee. 1993.** Genstat 5 Release 3 reference manual. Clarendon Press, Oxford, UK. 749 pp.
- Goering, H. K. and Van Soest, P. J. 1970.** Forage fiber analyses (apparatus, reagents, procedures and some applications). Agricultural Handbook No. 379. ARS-USDA, Washington, DC.
- Griffin, T. S., Cassida, K. A., Hesterman, O. B. and Rust, S. R. 1994.** Alfalfa maturity and cultivar effects on chemical and in situ estimates of protein degradability. *Crop Sci.* **34**: 1654–1661.
- Guttek, L. H., Goplen, B. P. and Howarth, R. E. 1976.** Heritability of soluble proteins in alfalfa. *Crop Sci.* **16**: 199–201.
- Heinrichs, D. H., Troelsen, J. E. and Warder, F. G. 1969.** Variation of chemical constituents and morphological characters within and between alfalfa populations. *Can. J. Plant Sci.* **49**: 293–305.
- Hill, Jr., R. R. and Barnes, R. F. 1977.** Genetic variability for chemical composition of alfalfa. II. Yield and traits associated with digestibility. *Crop Sci.* **17**: 948–952.
- Hoffman, P. C., Sievert, S. J., Shaver, R. D., Welch, D. A. and Combs, D. K. 1993.** In situ dry matter, protein, and fiber degradation of perennial forages. *J. Dairy Sci.* **76**: 2632–2643.
- Isaac, R. A. and Johnson, W. C. 1976.** Determination of total nitrogen in plant tissue, using a block digester. *J. Assoc. Anal. Chem.* **59**: 98–100.
- Julier, B. and Huyghe, C. 1997.** Effect of growth and cultivar on alfalfa digestibility in a multi-site trial. *Agronomie* **17**: 481–489.
- Kephart, K. D., Buxton, D. R. and Hill, Jr., R. R. 1989.** Morphology of alfalfa divergently selected for herbage lignin concentration. *Crop Sci.* **29**: 778–782.
- Klopfenstein, T. 1991.** Utilization of alfalfa protein by ruminant livestock. 21st Int. Alfalfa Symposium. Feb. 14–16. Rochester, MN. pp. 47–55.
- Kohn, R. A. and Allen, S. 1995.** Effect of plant maturity and preservation method on *in vitro* protein degradation of forages. *J. Dairy Sci.* **78**: 1544–1551.
- Lemaire, G. and Allirand, J. M. 1993.** Relation entre croissance et qualité de la luzerne: interaction génotype-mode d'exploitation. *Fourrages* **134**: 183–198.
- Lemaire, G. and Gastal, F. 1997.** N uptake and distribution in plant canopies. Pages 3–43 in G. Lemaire, ed. Diagnosis of the nitrogen status in crops. Springer-Verlag, Berlin, Heidelberg, Germany.
- Lemaire, G., Cruz, P., Gosse, G. and Chartier, M. 1985.** Étude des relations entre la dynamique de prélèvement d'azote et la dynamique de croissance en matière sèche d'un peuplement de luzerne (*Medicago sativa* L.). *Agronomie* **5**: 685–692.
- Neutze, S. A., Smith, R. L. and Forbes, W. A. 1993.** Application of an inhibitor in vitro method for estimating rumen degradation of feed protein. *Anim. Feed Sci. Technol.* **40**: 251–265.
- Petit, H. V. and Tremblay, G. F. 1992.** In situ degradability of fresh grass and grass conserved under different harvesting methods. *J. Dairy Sci.* **75**: 774–781.
- SAS Institute, Inc. 1985.** SAS/STAT® User's Guide. Version 6.0. SAS Institute, Inc., Cary, NC.
- Skinner, D. Z., Fritz, J. O. and Klocke, L. L. 1994.** Protein degradability in a diverse array of alfalfa germplasm sources. *Crop Sci.* **34**: 1396–1399.
- Sunberg, J. E., Murphy, R. P. and Lowe, C. C. 1983.** Selection for fiber and protein concentration in a diverse alfalfa population. *Crop Sci.* **23**: 11–14.
- Tilley, J. M. A. and Terry, R. A. 1963.** A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassl. Soc.* **18**: 104–111.
- Tremblay, G. F., Broderick, G. A. and Abrams, S. M. 1996.** Estimating ruminal protein degradability of roasted soybeans using near infrared reflectance spectroscopy. *J. Dairy Sci.* **79**: 276–282.
- Venter, J. D. and Skinner, D. Z. 1993.** Quantifying degradability of total protein from selected alfalfa lines. Central Alfalfa Improvement Conference, 20–22 June, University of Nebraska, Omaha, NE.

