The use of NIR in predicting nutritive value of Mediterranean tree and shrub foliage

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To upgrade grazing management recommendations in the Mediterranean area, one needs to assess the nutritive value of woody foliages including their changes over time and with location. Using a wide range of native and cultivated foliages, our objective was to evaluate the potential of near infrared (NIR) reflectance spectroscopy to determine foliage chemistry and *in vitro* digestibility for ruminants. The samples, representative of small ruminant eating bites, were divided into the different plant parts. Samples were carefully conditioned, being air-dried at 60°C; drying times were individually varied to ensure complete dehydration without excessive heating. Samples were analysed for organic matter (OM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and *in vitro* digestibility with a pepsin–cellulase method developed for forages (IVDMD). NIR scans were made with an NIRSystems 5000 instrument and data analysis was performed using ISI software. Partial least squares (PLS) regression equations were developed for IVDMD and each constituent. Nine mathematical treatments, with and without scatter correction, were compared. The database of woody foliage spectra and a reference fodder database were compared in two ways: the first involved a comparison of the spectral variation in each collection while the second measured the Mahalanobis distance of each spectrum in one database from the average spectrum in the other. In the case of N, a broad-based calibration was compared with others derived from various sample sub-sets; these latter were formed either according to sample type or following a proximity analysis of five spectral groups by principal component analysis. NIR predictions of IVDMD were applied to describe the effect of phenological changes in the edible parts of *Quercus pubescens* Willd. The foliage base is very heterogeneous and wider than the reference fodder base. The fodder base variation does not include the foliage samples. The lowest standard errors of calibration (SEC) and cross-validation (SECV) are comparable to literature results on forest leaves, but better for lignin (SECV of 1.5 compared to SEC values of 2.5–2.9). SECV for IVDMD is satisfactory at almost 2.0 because this value is similar to precision data normally reported for *in vitro* digestibility estimations on narrower sample sets than the current one. The broad-based calibration for N gave similar or lower standard errors to those obtained using sample sub-sets. One can show that IVDMD values for *Quercus pubescens* leaves decrease from 65 to 15% with increasing maturity; leaf IVDMD values are approximately 15% higher than the previous year's stem value from May until October. NIR spectroscopy appears to be an adequate technique for the prediction of the nutritive value of Mediterranean foliages from trees and shrubs, with a reliability similar to that obtained from classical fodder analysis procedures. This study shows that broad-based calibrations with PLS regression could be made on extremely diverse sets of data (IVDMD ranging from 28 to 94%), grouping distinct edible plant parts within the same data base.

Keywords: Grazing management, fodder trees, leaf chemistry, herbivory, digestibility, NIR spectroscopy, PLS, Mahalanobis distance.

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Introduction

In the Mediterranean area, farmers graze their herds on a diversity of pastures, including wooded rangelands. At certain periods, some of them expect the rangeland diet to meet up to 3/4 of the total energy needs of high requirement ruminants such as lactating goats.[1,2] The shepherds therefore design their grazing route by combining native and cultivated pastures, and fenced paddocks often include a wide diversity of fodder resources.[3] To upgrade pasture management recommendations, one needs to assess the nutritive value of native foliages including their changes with time and location.[4] Assessing the nutritive diversity of native tree and shrub foliages by the conventional wet chemical analysis of samples is time-consuming and too expensive when very large sample numbers are involved. In addition, some results may be declared uncertain due to the particular composition of woody foliages as compared with cultivated forages for which standard analytical methods were developed.[5–8] Fibre assays are confounded by the possible insolubility of proteins caused by their interaction with phenolic compounds.[9] In the last ten years, simultaneously with studies that tried to improve wet chemical analysis for lignified products, NIR reflectance spectroscopy has been tested as a tool to analyse diverse pastoral resources including woody foliages.[10–12] Within the same period, remote sensing and ecological studies used this technique to predict leaf canopy chemistry.[13–17] Using a wide range of native and cultivated Mediterranean tree and shrub foliages, our objective was to evaluate the potential of NIR reflectance spectroscopy to determine foliage chemistry and *in vitro* digestibility for ruminants.

Material and methods

The database

Samples were collected from typical wooded rangeland and fodder tree plantations in the French Mediterranean area. The database comprises 25 species, representing the diversity of regional ligneous fodder resources (botanical names[18]): *Arbutus unedo* L., *Amorpha fruticosa*, *Buxus sempervirens* L., *Calycotome spinosa* L., *Cistus albidus* L., *Cistus monspeliensis* L., *Cistus salviifolius* L., *Cytisus villosus* Pourr., *Colutea arborescens* L., *Coronilla emerus* L., *Erica arborea* L., *Erica scoparia* L., *Hedera helix* L., *Juniperus communis* L., *Juniperus oxycedrus* L., *Morus alba* L., *Phillyrea angustifolia* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Pistacia terebinthus* L., *Quercus ilex* L., *Quercus pubescens* Willd., *Rhamnus alaternus* L., *Robinia pseudoacacia* L., *Ruscus aculeatus* L. Most species were sampled at different periods in the year, when they were actually being browsed by animals. The samples were taken from plant parts selected as components of different goat eating bites. These eating bites were previously defined after focused observations on intake behaviour.[19] The database comprises 222 samples, including leaves and stems only, divided before analysis into the successive growth parts

Chemical analysis

Directly after cutting, all samples were placed in a refrigerated container at +10°C before being frozen at –20°C. They were then air dried in a ventilated oven at 60°C. Drying kinetics were monitored for each sample, and the drying times were calculated to obtain 93% dry material. Drying times were much shorter than the conventional "to constant weight" durations. We had previously confirmed that this technique avoids excessive heating of foliages and denaturation of soluble compounds.[1] All samples were then ground in a cyclone mill through a mesh of 1 mm aperture diameter. For reasons of cost and ground material availability, not all the samples were analysed for all constituents. The size of each sample group is indicated in parentheses as follows: moisture (222) (103°C for 24 h); organic matter (222) (550°C for 24 h); nitrogen (222) (micro-Kjeldahl analysis); neutral detergent fibre (98), acid detergent fibre (122), acid detergent lignin (122) and *in vitro* dry matter digestibility (83). Fibre estimations were performed using the Fibertec procedure[20] while IVDMD measurements were made using a pepsin–cellulase method developed for forages.[21]

NIR analysis

All samples were scanned with a NIR reflectance monochromator (NIRSystems 5000). Each sample was packed into a sample cell having a quartz-glass cover. Reflectance measurements of monochromatic light were made from 1100 to 2500 nm at 2 nm intervals to produce a spectrum with 700 data points. The band-pass value used was 10 nm while wavelength accuracy is 0.5 nm. Reflectance (R) readings were converted to absorbance (A) values using the following equation: $A = \log(1/R)$. Data analysis was conducted using ISI software.[22]

Statistical methods

Partial Least Squares (PLS) regressions were developed for OM, N, NDF, ADF, ADL and IVDMD. For each of these analytes, calibrations were generated using nine different mathematical treatments; these corresponded to raw spectral data followed by first and second derivatives. Derivatives were made using a constant smoothing segment of five data points but with subtraction gaps of 5, 10, 15 and 20 data points.[23] For all these mathematical treatments, results obtained both with and without spectral detrending were compared. This technique is commonly used as a scatter correction method.[24] PLS regression is recognised as a very powerful tool to develop models from spectroscopic data[25] and the advantages over classical Multiple Linear Regression (MLR) have been shown.[26] PLS, by reducing the large set of raw spectral data into a small number of orthogonal factors, is particularly efficient when the data are significantly inter-correlated. Due to the limited number of samples in this study, it was not possible to select an independent set of samples for validation of the PLS equations. Thus, standard error of prediction (SEP) was estimated by cross-validation which is preferable to limiting the number of samples in the training set. The approach used was as follows: for each constituent, 75% of the samples were chosen and used for the development of a prediction equation containing one PLS factor. The performance of this equation was then evaluated on the remaining 25% of samples to produce a standard error of prediction. This exercise was repeated three further times using a different batch of samples for calibration development and evaluation; thus, four equations were produced for a one factor equation for each constituent. The standard error of cross-validation (SECV) for each constituent was then derived from the above four SEP values by calculating the square root of the mean of the SEP squared values. This exercise was repeated for models containing 2,3,4,...n factors and for each a SECV value was obtained. The best model was determined to be that with the lowest SECV and the final calibration equation was developed on the entire sample collection using the number of factors in the calibration with the lowest SECV. Cross-validation determines the optimum number of factors and minimises overfitting. The results displayed in the figures are the predicted values from cross-validation and are not the values of the calibration process. The standard error of cross-validation gives a more realistic estimate of accuracy than the standard error of calibration (SEC). The woody foliage database and a reference forage database[27] were compared, in terms both of the variation in their spectral information and their Mahalanobis distance from the average spectrum in the opposite file. Calibrations for the different constituents and *in vitro* digestibility were carried out on the whole 222 sample set (broad-based equations). The number of times the outlier elimination sequence was invoked was fixed to 1. This means that the computer program only attempts to remove outliers once before completing the final calibration. The critical "T" outlier value was fixed at 2.5. As the spectral structure of the data was checked previously by principal component analysis (PCA), the critical "H" outlier value was set at 50 to retain maximum spectral variability. For nitrogen content, calibrations were made on various sample sub-sets. These sub-sets were formed either according to sample nature or origin (deciduous vs evergreen foliages; calcareous vs acid soils; trees vs shrubs) or using the ISI "3D-Symmetry" option to produce a proximity analysis of five spectral groups.

Annual changes in oak foliage digestibility

Twelve samplings of *Quercus pubescens* Willd. were taken from April 1991 to May 1992 in an area near Avignon, France. Each sampling consisted of

20 leafy branches with representative proportions of edible plant parts collected along a 20 m transect. The edible plant parts consisted of the current year's growth of leaves (L0), the three most recently emerged stems (S0, S-1, S-2) and acorns (A). The IVDMD prediction equation from the 222 sample data base was applied to the plant parts. The plant parts were collected at a stage corresponding precisely to the opening of the buds; thus, the age of each sample was known to within a few days. Using the set of the current year's growth of leaves and stems, a calibration equation was developed to predict the age of plant parts.

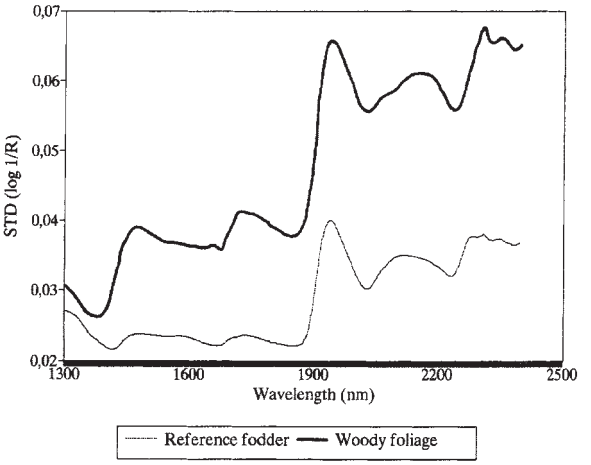

Figure 1. Standard deviation spectra of reference fodder base set and for woody foliage base set.

Results and discussion

Foliages compared with a fodder reference

The foliage base is very heterogeneous and wider than the reference forage base. Figure 1 shows the standard deviation spectra of the raw spectra of the foliage and forage databases. It is apparent that the variation in foliage spectra is greater than that in the forage spectral collection. The mean Mahalanobis distance of each foliage spectrum from the average forage spectrum is equal to 26 and vice versa; the mean Mahalanobis distance of each forage spectrum from the average foliage spectrum is equal to 3. This indicates that the foliage spectral variation includes much of the spectral variation of the forage samples but not the converse, though the reference forage samples themselves come from many species of grasses and mixtures cut at different stages and grown in several locations and years.

Calibration equations

Broad-based calibration

The 222 sample database is characterised by an extremely wide range in concentration of the components analysed (Table 1). The range of nitrogen and lignin values is wider than that found in previous studies with foliages.[13,14,16] Since not all the samples were analysed for each constituent, which might have result in an unbalanced spectral broad-base, a matrix of Malhanobis distances was made to compare the spectral variation in the different constituent-based data sets (Table 2). It is apparent that the two largest sets (OM and N) include all the others since the average *H* values are close to or lower than 1; thus, the reference sample sets for OM and N may be used also to predict successfully the other constituents. On the other hand, spectral variation in the OM and N sets is wider than that in the NDF, ADF, ADL and IVDMD sets; this means that calibrations derived using the latter sample sets will not be suitable to predict several samples included in the OM and N sets. In the case of nitrogen, the lowest standard errors of calibration (SEC) and cross validation (SECV) obtained in this work are comparable to literature data (SEC approximately equal to 0.1); results for lignin are better than those previously re-

Table 1. Wet chemical range (%) for each constituent within the calibration sample set.

Variable	*n*	Mean	STD	Range
OM	222	93.8	3.2	82.6–98.3
N	222	1.84	0.86	0.39–4.17
NDF	98	52.0	13.0	85.0–23.8
ADF	122	31.5	10.7	12.7–54.1
ADL	122	13.9	6.2	3.0–26.9
IVDMD	83	64.0	19.8	28.2–94.0

Table 2. Mahalanobis distance metrics between constituent sets.

		Predicted value					
		OM	N	NDF	ADF	ADL	IVDMD
Reference data	nb data=	(204)	(206)	(90)	(110)	(108)	(75)
	OM	*24*[3]	1.052[1]	0.800	0.799	0.812	0.982
			3[2]	2	2	2	2
	N	1.002	*24*	0.717	0.719	0.727	0.871
		1		0	0	0	0
	NDF	8.380	8.391	*15*	1.704	1.754	1.191
		88	86		11	12	1
	ADF	4.278	4.266	0.902	*18*	1.059	1.070
		64	64	1		5	1
	ADL	4.310	4.304	0.907	1.044	*18*	1.073
		70	69	2	5		2
	IVDMD	4.356	4.340	0.891	1.022	1.083	*15*
		65	65	0	3	6	

[1]Hbar: Average distance of the predicted set versus average spectrum of the reference set.
[2]Number of samples which have *H* distance greater than 3.
[3]Number of principal components used to compute the *H* distance.

ported, with a SECV of 1.48 compared to SEC values of 2.5–2.9 in previously cited work (Table 3). Reduced prediction errors were obtained for all constituents when PLS regression was preceded by a scatter correction procedure. Calibration *R*-squared values ranged from 0.96 to 0.99. With respect to IVDMD, the SECV of approximately 2.0 is satisfactory and close in magnitude to IVDML standard errors[27,28] determined on narrower sets than the current one. For OM and N, the SEC and SECV are close in value while for the other parameters, the differences between SEC and SECV are higher. This arises from the number of samples used in the cross-validation procedure; approximately 150 samples were used to make the OM and N sub-calibrations while corresponding figures for the other sub-calibrations ranged from 60 to 85. Graphical comparisons between reference (wet chemistry) values and NIR predicted values for each constituent are shown in Figure 2. Despite the skewed distribution of OM and N values, the pre diction equations are all effective.

Calibration on sub-sets for nitrogen content

Calibration equations for 11 sub-sets generated give standard errors worse than, or similar to, the broad-based calibration (Table 4). *R*-squared values are lower in all cases but one, because of the smaller range of values and number of samples. The SECV level depends on the number of factors used in the model. With very few samples, the SECV limits the number of factors and thus the accuracy; this would certainly be improved if the number of samples in each sub-set was similar to the broad-based calibration.

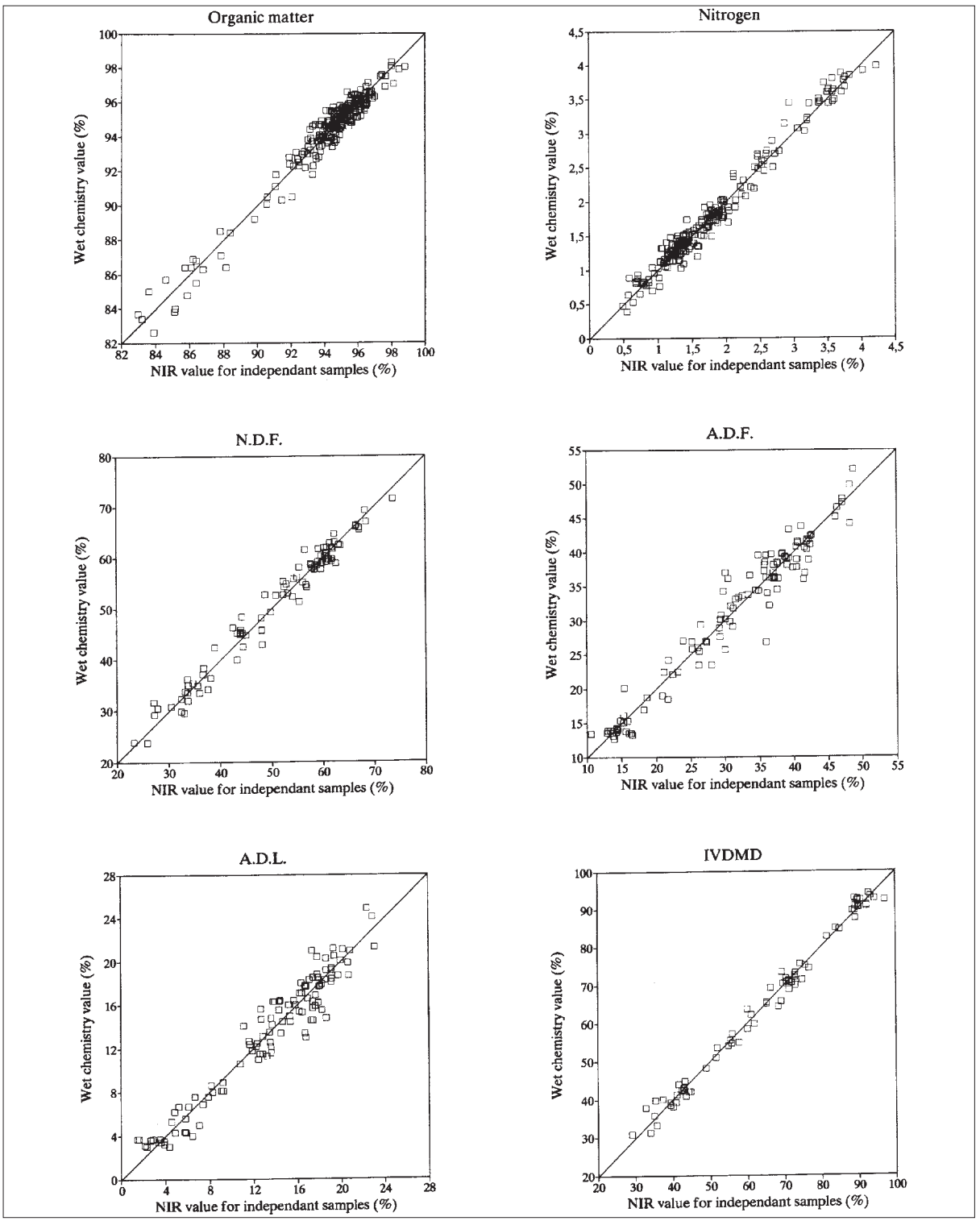

Figure 2. Relationship between NIR predicted value for independent samples and wet chemistry value (% of dry matter).

Table 3. Modified partial least squares regression. Equation calibration statistics for OM, N, NDF, ADF, ADL and IVD without outliers.

		With scatter correction			Without scatter correction			
Variable	*n*	SEC	R^2	SECV	SEC	R^2	SECV	Math treatment[a]
OM	204	0.54	0.97	0.63	0.59	0.96	0.66	1 5 5
N	206	0.11	0.98	0.14	0.13	0.98	0.15	1 5 5
NDF	90	1.36	0.99	2.06	1.37	0.99	2.11	1 5 5
ADF	110	1.85	0.97	2.36	1.83	0.97	2.50	1 10 5
ADL	108	1.04	0.97	1.48	1.1	0.96	1.66	2 5 5
IVDMD	75	1.51	0.99	2.10	1.61	0.99	2.68	2 20 5

[a]Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the length in data points over which the derivative was taken and the third the segment length over which the function was smoothed.

Application to oak foliage annual digestibility changes

The broad-base equation for IVDMD was applied to the 46 collected plant parts. Since oak species are well represented in the calibration database, all the leaf and stem samples, although not the acorns, are well predicted with the conventional limit of 3.0 used for the *H*-statistic. As maturation progresses, leaf IVDMD values decrease from 65 to 15% dry matter; leaf IVDMD values are approximately 15% higher than values for last year's stem samples over the period May to October 1991 (Figure 3). The two older growth stems have a regular and very similar variation around 30% IVDMD. This type of information, with a high frequency of sampling on individualised edible plant parts, should be linked with the intake models of small ruminant browsing on oak foliages.[1] From these models, one can estimate that the nutritive value of this oak during the summer period (mid-June to September) varies from 55 to 52% OM digestibility. The calibration on age of the current year's leaves and stems led to poor quality results, mainly because of the mixing of the two types of plant organs and probably because of the insufficent number of data. The SEC and SECV are, respectively, 20.7 and 41.8 days for a 384 days range, with an *R*-squared value of 0.88 (best math treatment is 2–10–10) (Figure 4). These figures are twice the re-

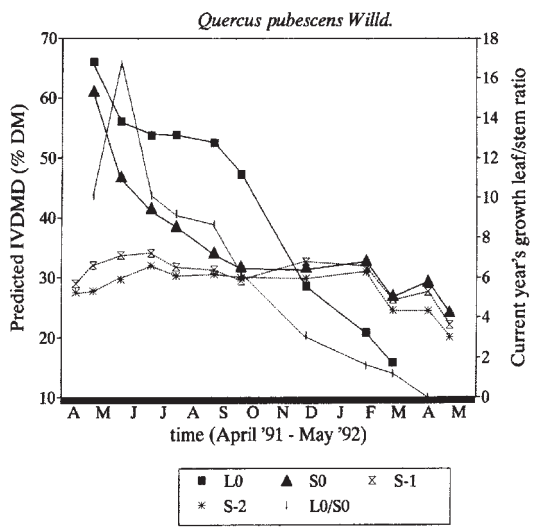

Figure 3. Variation over time in the *in vitro* pepsine–cellulase digestibility for the edible plant parts of *Quercus pubescens* Willd. L0/S0 is the current year's growth leaf-to-stem ratio (% of the dry matter).

Table 4. Modified partial least squares regression. Equation calibration statistics for N from distinct sub-sets of data. Math treatment: 1 5 5 (see Table 3).

Data sets	*n*	Mean	STD	Range	SEC	R^2	SECV
Deciduous foliages	64	2.11	0.81	0.4–4.2	0.24	0.91	0.33
Evergreen foliages	158	1.73	0.87	0.5–4.0	0.27	0.90	0.31
Calcareous soils	152	1.63	0.60	0.4–3.8	0.22	0.87	0.25
Acid soils	70	2.29	1.14	0.5–4.2	0.39	0.92	0.39
Trees	72	1.46	0.49	0.6–4.0	0.16	0.89	0.34
Shrubs	150	2.01	0.91	0.4–4.2	0.26	0.92	0.29
Set A	21	1.72	0.19	1.3–2.0	0.07	0.87	0.12
Set B	22	3.58	0.23	3.0–4.0	0.10	0.83	0.13
Set C	74	1.48	0.49	0.6–3.8	0.16	0.90	0.23
Set D	22	1.25	0.59	0.6–3.5	0.46	0.38	0.51
Set E	19	2.29	0.61	1.2–3.7	0.02	1.00	0.17

spective figures quoted in results obtained on rice and wheat plants.[29]

Conclusion

The compositional prediction of foliages from Mediterranean trees and shrubs by NIR reflectance spectroscopy is adequate for nutritive value estimations. NIR analysis was able to identify which samples had deteriorated during the conditioning process. It is a highly valuable tool for international networks that conduct surveys on the quality of woody fodder and have to compare samples from various origins.[30] This study shows that broad-based calibrations can be made on extremely diverse sets of data, including leguminous shrubs of high nutritive value, such as *Citisus* and *Robinia*, and very coarse *Ericacea* species, grouping leaves and stems within the same database. The calibration for lignified fibre fractions appears quite satisfactory con-

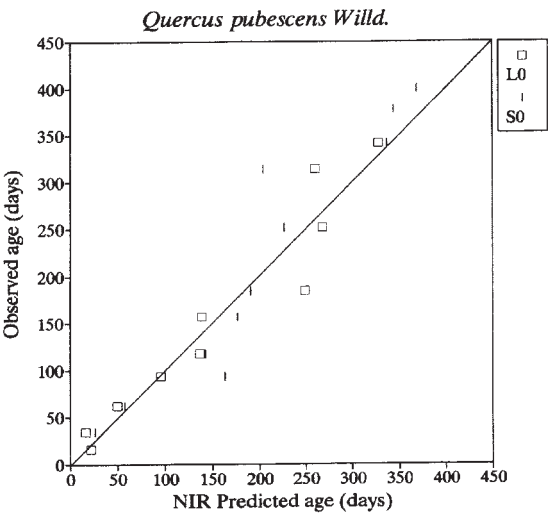

Figure 4. Relationship between NIR predicted values and observed values for the age of *Quercus pubescens* Willd. current year's growth leaves (L0) and stems (S0).

firming the appropriateness of our specific oven-drying technique for woody foliages. Prediction of pepsin–cellulase *in vitro* digestibility could be reliably used for these foliages, as is the case with more conventional green forages and herbage, to determine the effect of maturation on the nutritive value of plant parts.

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