Prediction by near infrared spectroscopy of the content of white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) in fresh or dry mixtures made up from pure botanical samples

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Introduction

The contribution of different species to the forage dry mass is a relevant attribute of sward condition.[1] This trait, usually estimated by hand separation, is a tedious, time- and labour-consuming procedure.[2] Near infrared (NIR) spectroscopy has the potential for predicting botanical composition of swards, but most of the work has been carried out taking the spectra of dry samples. The aim of this work was to evaluate the feasibility of developing NIR regression models for predicting the content of white clover and perennial ryegrass in fresh or dry mixtures prepared from pure samples of both species.

Materials and methods

Samples of pure stands of white clover and perennial ryegrass were taken weekly for five weeks at Valdivia, tenth region of Chile, from November 21 to December 19, 2000. Fresh forage was chopped to *ca* 1 cm and manually homogenised. Mixtures were prepared which contained from 0 to 100% of each species, at increments of 10% on a fresh basis, resulting in 55 mixed samples (11 mixtures, 5 cuts).

Spectra were taken in reflectance (400–2500 nm) from fresh samples located in polyethylene bags, inserted in a large rectangular forage cell, placed in a transport module attached to a scanning monochromator (6500, NIRSystems Inc, Silver Spring, MD, USA). Then samples were dried (65ºC, 48 h) and ground (Wiley mill, 1 mm screen) and spectra taken in small ring cells (5 cm diameter, 1 cm deep, with a quartz window of 35 mm diameter) placed in a spinning sample module.

The percentage of each species (as contribution to dry weight) in any sample (fresh or dry) was considered as the variable to be predicted.

Calibration models (modified partial least squares) were developed with WinISI II software (Foss NIRSystems) testing different math treatments (differentiation order, subtraction gap, smooth interval), with or without scatter correction of the spectra (*SNV* + Detrend). Equations were tested and selected according to the standard error (*SECV*) and coefficient of explained variance (1-*VR*) of the cross-validation process.

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Results and discussion

The spectra for all fresh and dry samples showed differences in absorption bands along the visible and NIR regions. In order to explore species-related absorption bands, spectra from 100% white clover and 100% perennial ryegrass samples of the five cuts, were plotted, either in the fresh [Figure 1(a)] or dry [Figure 1(b)] state. Species-related absorption bands are suggested for both groups, as can be seen at 1450 nm in Figure 1(a), where all clover samples are placed above the ryegrass samples which are located below and in a less scattered fashion (insert). This could be caused by O–H stretching associated to water or starch, which can be expected in higher concentrations as energy storage in legumes in contrast to grasses, which are more adapted to store nonstarch, nonstructural sugars as energy reserves. In Figure 1(b), the insert enhances different absorption bands for clover and ryegrass at 2180 nm, probably caused by differences in protein content.

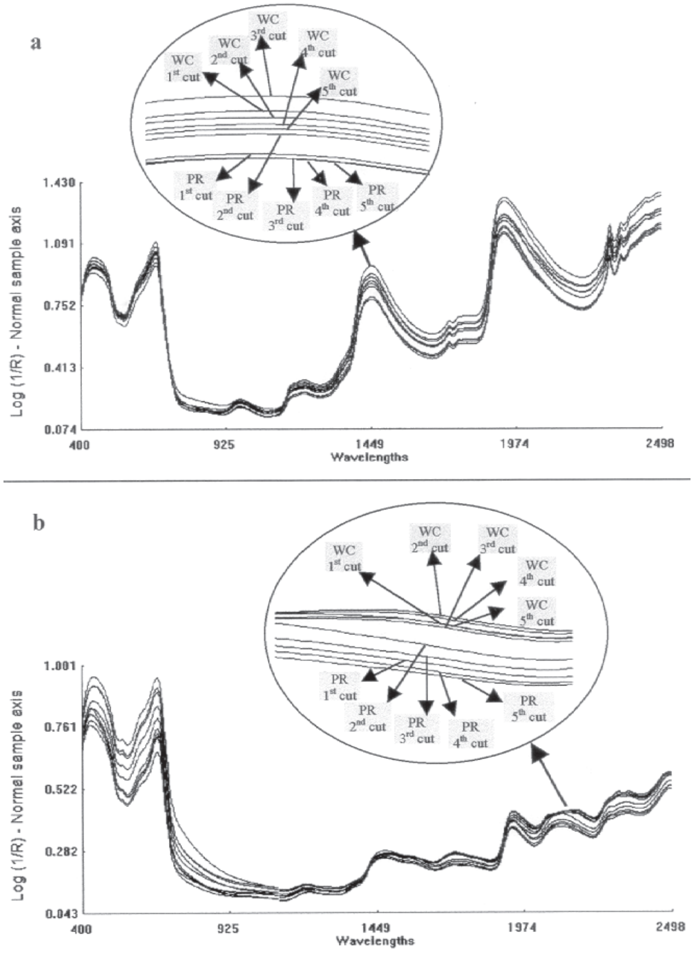

Figure 1. Spectra of (a) fresh and (b) dry samples of pure white clover (WC) and pure perennial ryegrass (PR).

Table 1. Selected equations with different math treatments, ranked by derivative order.

Variable	Terms	*SECV*	1-*VR*	s.d. $SECV^{-1}$	Math	Scatter
Fresh clover or ryegrass	6	6.92	0.953	4.64	0–0–5	Snv+det
	6	6.09	0.964	5.27	1–15–15	snv+det
	7	6.05	0.964	5.32	2–10–10	none
	5	5.87	0.966	5.48	3–10–10	snv+det
Dry clover	6	5.30	0.972	5.99	0–0–5	Snv+det
	7	3.61	0.986	8.40	1–5–5	none
	6	3.73	0.985	8.12	2–10–10	none
	6	3.34	0.988	9.07	3–10–10	none
Dry ryegrass	5	5.30	0.972	6.00	0–0–5	Snv+det
	7	3.60	0.986	8.43	1–5–5	none
	6	3.72	0.985	8.15	2–10–10	none
	6	3.36	0.988	9.02	3–10–10	none

SECV: standard error of cross-validation
1-*VR*: coefficient of determination (proportion of explained variance) of cross-validation
s.d.: standard deviation of reference data
Math: mathematical treatment where first number is subtraction order, second is subtraction gap in data points and the third is smooth interval in data points
Scatter: standard normal variate (*SNV*) and detrend (det), treatments were either applied to correct light scattering and spectra curvature, or not applied (none)

The best equations for predicting the percentage of each species on fresh or dry samples (Table 1, Figure 2) show 1-*VR* values over 0.95 and *SECV* that, although not small, if contrasted to mean reference values (*ca* 50%), represent a small fraction of the standard deviation (s.d.) of the reference data. It is generally accepted that if the ratio of s.d. to standard error of performance (*SEP*) is higher than three,

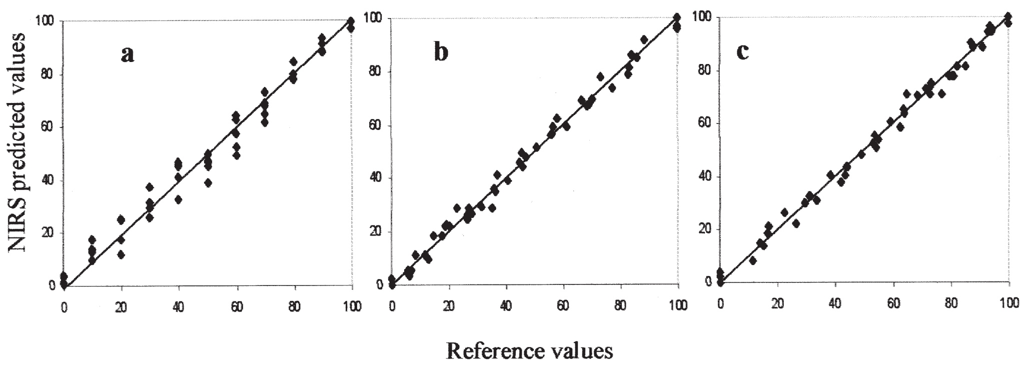

Figure 2. Actual against NIR predicted content of (a) content of clover (or ryegrass) for fresh samples (math treatment: 1–15–15–1, SNV + Detrend), (b) content of clover for dry samples (math treatment: 1–5–5–1, None) and (c) ryegrass content in dry samples (math treatment 1–5–5–1, None). See text for details.

an equation is considered to be acceptable.[3] If this criterion is assumed valid for *SECV* as well, then the equations developed can be useful for predicting botanical composition.

Best equations for fresh clover and fresh ryegrass were identical, which results from mixing complementary percentages of only two species on a fresh basis when making up the samples. In this way, reference data present increments of 10 percentage units in one species as the other decreases by the same amount, explaining identical means and s.d. An important intercorrelation can, thus, be expected. In this way the spectral "signal" for 90% clover could also be interpreted as a signal for 10% ryegrass and so on. Work is in progress to evaluate more complex and "real world" mixtures.

Not surprisingly, a better agreement is expected with dry ground samples (1-*VR* of 0.98 to 0.99) than with fresh chopped samples (1-*VR* of 0.96), as in the last case more factors could be affecting the spectra. Among them, water content, heterogeneity of plant tissues (leaves, stems, flowers), higher particle size, air pockets in the sample cup, etc.

As for math treatments, appropriate equations ranged from first to third order derivative and scatter correction (*SNV* and detrend) in general improved prediction accuracy.

Conclusions

Best equations were developed with a first or third derivative for fresh samples and with a second or third derivative for dry samples, with comparable results among math treatments for the superior equations.

In mixtures of white clover and perennial ryegrass, sward condition, in terms of botanical composition, can be successfully predicted by NIR, providing suitable calibrations are developed.

This could be accomplished in less time and with lower but still good accuracy, when using fresh chopped, instead of dry and ground samples.

Acknowledgments

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References

1. S.W. Coleman, F.E. Barton and R.D. Meyer, *Crop Sci.* **25,** 835 (1985).
2. J.A. Shaffer, G.A. Jung, J.S. Shenk and S.M. Abrams, *Agronomy J.* **82,** 669 (1990).
3. C.A. Kennedy, J.A. Shelford and P.C. Williams, in *Near Infrared Spectroscopy: The Future Waves*, Ed by A.M.C. Davies and Phil Williams. NIR Publications, Chichester, UK, pp. 524–530 (1996).