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## Prediction of red clover content in mixed swards by near-infrared reflectance spectroscopy

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**Introduction** Because of the legume fixation capacity, their high protein content, digestibility and intake characteristics, more and more attention is paid to grassland clover content. In field experiments, clover content must often be determined, for example to quantify nitrogen flux or the best practices to manage such species (Stilmant *et al.*, 2004). However hand sorting of clover and grass, even if accurate, is time-consuming and has a high labour cost. In comparison, accuracy of visual estimation of clover content, directly in the field, varies according to training and experience. Near-infrared reflectance spectroscopy (NIRS) has been proposed as a method for the rapid determination of sward botanical (Petersen *et al.*, 1987; Pitman *et al.*, 1991) and morphological composition (Leconte *et al.*, 1999; Stilmant *et al.*, 2005). This paper describes the performance of a NIRS calibration developed to characterise red clover (*Trifolium pratense*) content when associated to different grass species and this at different phenological stages.

**Materials and methods** Plant material used to set up NIRS calibration was collected in three swards, located on loamy soil. In each sward red clover (cv. Merviot) was associated to different grass species: perennial ryegrass (*Lolium perenne*) cv. Merlinda, hybrid ryegrass (*Lolium hybridum*) cv. Barsilo and cocksfoot (*Dactylis glomerata*) cv. Lupré. The samples were taken in May, July, August and October by cutting the sward 7 cm above ground level. Samples were hand sorted into clover and grass fractions. These fractions were dried, weighed (G %) and then ground and remixed before being submitted to NIRS analysis (NIRSystem monochromator 5000). Spectral data, in the range of 1100–2500 nm scanned at 2 nm steps, were correlated to red clover content. Calibrations were developed according to the Partial Least Square procedure with cross validation using the ISI (Infrasoft International) software. The spectra of 647 samples were used for calibration and cross validation, while 45 samples were kept for independent validation.

**Results and conclusions** Among the 647 samples, the red clover content ranged from 0 to 100%, with a mean of 59.5%. The  $R^2$  was 0.99 in calibration (Standard Error of 2.74%) as in cross validation (Standard Error of 2.87%) and the ratio of the standard deviation of the initial sample set on the standard error in cross validation (SD/SECV) was 9.4 (Williams, 2004). The performance of this calibration is sufficiently precise and reliable to quantify red clover content in a wide range of situations. This was confirmed by the results obtained on the independent validation set (Table 1).

**Table 1** Statistics of the independent validation, including number of samples (N) and standard error of prediction (SEP)

N	Mean	SEP (%)	Bias	Slope	$R^2$
45	67.18	2.88	0.73	0.99	0.98

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