FORAGE & GRAZING LANDS

Near-Infrared Spectroscopy on Chopper to Measure Maize Forage Quality Parameters Online

Roland Welle,* Willi Greten, Bernhard Rietmann, Scott Alley, Georges Sinnaeve, and Pierre Dardenne

ABSTRACT

Improving maize (*Zea mays* L.) forage yield and quality is a major goal for corn breeders in northern Europe. The objective of this research was to measure maize forage dry matter (DM) content and quality parameters with near-infrared spectroscopy (NIRS) directly on a harvesting machine. Coupling NIRS with harvesting eliminates time-consuming sample preparation and laboratory-based analyses. Calibrations were developed with 281 samples from the 1998-1999 growing seasons using a diode array spectrometer mounted on a forage chopper. Standard errors of cross-validation (SECVs) were 11.8 g kg^{-1} for DM, 23.6 g kg^{-1} for starch, 19.2 g kg^{-1} for in vitro digestibility, and 13.8 g kg^{-1} for soluble sugars. An independent validation study with 159 samples from the 2000 harvest resulted in standard errors of prediction (SEP) of 12.5 g kg^{-1} for DM, 22.1 g kg^{-1} for starch, 19.8 g kg^{-1} for in vitro digestibility, and 16.7 g kg^{-1} for soluble sugars. The results indicate that NIRS on chopper (NOC) can determine DM accurately, rank hybrids for starch plus sugars, and group for in vitro digestibility. Dry matter NIRS determinations were more precise than the reference method, enabling improved selection for forage yield, the key factor in product development. Instrument standardization could be achieved through spectral matching and including spectra from different instruments into the calibration database. Approximately 10 000 and 16 000 plots were analyzed by the fully automated NOC system during 2000 and 2001. The dramatic increase in the number of plots analyzed expedites development of new maize forage products.

Most maize in northern Europe is harvested for forage and is a major source of dairy cattle feed in cooler climates. Forage quality affects the nutritional value of the feed and is thus important for ruminant production. Therefore, improving quality and agronomic parameters are goals of many maize forage breeding programs (De la Roza et al., 1998; Berardo et al., 1999). At a typical maize forage breeding station, more than 10 000 yield plots are harvested during just a few weeks. Each harvested plot is weighed and subsamples are taken on the chopper. Using these subsamples, DM content is determined at the station, the dried material sent to the laboratory, ground successively to 4-mm and 1-mm particle sizes, and subjected to NIRS to measure quality parameters. Near-infrared spectroscopy can rapidly measure multiple traits in food and agricultural commodities (Shenk and Westerhaus, 1993) as well as in dried ground forage (Ronsin and Féménias, 1990; Dardenne et al., 1993; De la Roza et al., 1998). However, the handling, drying, and grinding steps are tedious and very time consuming, limiting the number of forage yield plots that can be analyzed. Using NOC to measure DM content and consequently forage yield as well as forage quality in parallel to plot harvesting would have a major impact on developing maize hybrids in northern Europe.

Analysis of undried agricultural products with limited potential for a breeding program was reported in the past (Dardenne et al., 1991; Lecomte et al., 1996). Performing NIRS on freshly harvested material in conjunction with harvesting has been shown to be technically feasible, but was restricted to calibration development of DM content with a single instrument (Dardenne and Féménias, 1999).

The objectives of the study we report herein were to (i) develop and independently validate NOC calibrations for contents of DM, starch, and soluble sugars, as well as in vitro digestibility of organic matter; (ii) standardize different NIRS instruments; and (iii) apply NOC for routine yield trials.

MATERIALS AND METHODS

NIRS instruments and choppers

Two Jaguar model 690 forage choppers (Claas GmbH, Harsefeld, Germany) were configured to harvest standard forage research plots. Material from each chopped plot was transported into a weighing bin. A 1-kg subsample was taken from each plot by an auger for reference chemical analyses. After weighing, the main samples were deposited onto a quickly moving conveyer belt. The material passed under overhead rollers that produced an even surface. A spectrometer was mounted behind the rollers 12 cm above the sample surface. Two spectrometers were used. Instrument 1 was a DA7000 diode array spectrometer (Perten Instruments GmbH, Hamburg, Germany) with a wavelength range of 400 to 1700 nm. This instrument was equipped with two arrays: a silicon detector for wavelengths in the visible region (400 to 950 nm) and

R. Welle, Pioneer Hi-Bred Northern Europe GmbH, Res. & Product Dev., Analytical Biochemistry, 24 rue du moulin, 68740 Nambsheim, France; W. Greten and B. Rietmann, Pioneer Hi-Bred Northern Europe GmbH, Res. & Product Dev., Pentruper Str 11, 48268 Greven, Germany; Scott Alley, Pioneer Hi-Bred International, Technology Integration Services, 6900NW 62nd Avenue, Johnston, IA 50131, USA; G. Sinnaeve and P. Dardenne, Centre de Recherches Agronomiques, Dép. Qualité, Chaussée de Namur 24, 5030 Gembloux, Belgium.
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Abbreviations: DM, dry matter; IVDC, in vitro digestibility cellulase; MPLS, modified partial least squares; NIRS, near-infrared spectroscopy; NOC, near-infrared spectroscopy on chopper; PLS, partial least squares; RMS, root mean square; RMSC, corrected root mean square; SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of prediction.

an Indium Gallium Arsenite detector for wavelengths in the near infrared region (950 to 1700 nm). The two detectors together provided data from 261 discrete wavelengths. Instrument 2 was also a DA7000 diode array but possessed only the near infrared array that collected signals from 151 wavelengths between 950 to 1700 nm. With both instruments, 600 individual subscans were collected and averaged with proprietary software during each 1-s acquisition period. Between 10 and 20 averaged spectra were acquired for each plot, depending on plot weight. The forage material per plot weighed between 30 and 50 kg.

Calibration Development

Field plots were planted in 1998 and 1999 near the Pioneer Hi-Bred research station in Greven, Germany, specially to develop the calibration. In both years, ≈100 maize hybrids from Pioneer Hi-Bred and other seed companies with maturities typical for northern Europe were planted in duplicate. Hybrids varied in DM, starch content, and in vitro digestibility cellulase (IVDC), ensuring a broad set of phenotypes. Each plot was 5.2 m long and two rows wide with 0.75 m between rows. In both years, duplicate plots were harvested at the beginning and end of the harvest season to increase variability among samples and environmental parameters such as temperature and humidity during the measurements. Spectra were recorded for each plot using Instrument 1.

Mathematical procedures on the spectral information were performed with ISI software 4 (Infrasoft International, Port Mathilda, PA, USA). A subset of 281 samples was chosen from the 400 forage plots from the 1998-1999 harvests by the *Select* (Shenk and Westerhaus, 1992) algorithm of ISI, to develop NOC calibrations with following settings: 1100 to 1660 nm by 5 nm, four cross-validation segments, 2.5 as T residual limit (Shenk and Westerhaus, 1992), 10.0 as global H outlier limit (Mahalanobis distance; Shenk and Westerhaus, 1992), 1 outlier elimination pass. The linear regression method was based on a normal partial least squares (PLS) algorithm (Shenk and Westerhaus, 1995a; Naes et al., 2002) or on a modified PLS algorithm (MPLS) where the X and Y residuals are standardized at each iteration. The best combinations found among many different pretreatments, derivatives, and regression algorithms to minimize SECV were *detrend-0,0,5-PLS* for DM, *none-1,5,5-MPLS* for starch, *none-1,5,5-PLS* for soluble sugars, and *none-1,10,5-PLS* for IVDC. *Detrend* removes the spectral trend based on a second-degree polynomial fit between wavelengths and absorbencies (Barnes et al., 1989). *None* means that the raw spectra are used for the subsequent transformation. In each case, the first number is the degree of the derivative, the second the gap between data points for the subtraction, and the third the number of data points for smoothing.

The 281 samples scanned on the chopper were dried, ground, and scanned at 2-nm steps between 1100 and 2500 nm in the reflection mode on a NIRSystems model 5000 monochromator (NIRSystems, Inc., Silver Spring, MD, USA). The resulting calibrations, representing the traditional NIRS, served as comparison benchmark to the NOC-derived regression models.

Wet Chemistry

One-kilogram subsamples of each plot were dried on flat bed dryers for 24 h at 55°C to constant residual moisture to determine DM content. The samples were ground successively to 4- and 1-mm particle sizes and standard procedures were used to measure starch (International Organization for Standardization, 1997), soluble sugars (Yemm and Willis, 1954), and in vitro digestibility (DeBoever et al., 1988).

Repeatability Analysis

Sixty early and eighty mid-early maturing hybrids were grown in 2000 in two yield trials in Cloppenburg, Germany. The plots were arranged with alpha lattice design with four replicates. Each plot was 6 m long and 4 rows wide with 0.75 m between rows. The middle two rows were harvested to determine forage yield. All NOC spectra were recorded with Instrument 1. Dry matter content was determined by the reference method for a 1-kg subsample of each plot. Precision of DM determinations by NOC and the reference method were compared using analysis of variance. The genotypic variance component (% v) was estimated using the Restricted Maximum Likelihood method (Patterson and Thompson, 1971; Patterson, 1997). This value is the proportion of genotypic variance to the total variance. The residual variance is the variance not explained by the factors in the model; that is, genotype, environment, and interaction of genotype with the environment.

Validation

Plant material, instrumentation, chemical procedures, processing, and acquisition were the same as described previously, except these samples were planted in 2000 and were independent of the calibration set. Two hundred plots were harvested in Greven and spectra recorded by NOC using Instrument 1. A set of 159 samples was chosen with the *Select* program. This sample set served to validate calibrations for DM, starch, IVDC, and soluble sugars developed on Instrument 1 using samples from the 1998-1999 harvests.

Standardization

Spectra used for calibration and validation were obtained with a single diode array spectrometer. A potential problem arises when additional instruments are deployed, as their outputs do not match. To transfer calibration models developed on one instrument to other spectrometers, the additional instruments need to be standardized. Two data sets were used to compare and standardize instruments. The first set consisted of 79 fresh and unground samples with maximal moisture differences, measured at the same time on both instruments 1 and 2 placed side by side in the laboratory. These samples were used to develop a standardization file, a mathematical matrix that corrected spectral differences between the two instruments. Spectra from the two spectrometers were matched using the *Clone* algorithm in the ISI program which calculates a quadratic model to align wavelengths but uses linear regression for photometric adjustments at each wavelength (Shenk and Westerhaus, 1995b; Shenk, 1990; Bouveresse et al., 1994). For the second set, 200 plots were grown in 2000 at the Pioneer Hi-Bred research station in Nambsheim, France. Instrumentation, lab wet chemistry, processing, and acquisition were the same as described previously, except plant material of higher maturities was grown in the warmer climate of Nambsheim. These plots were harvested by NOC using Instrument 2. A subset of 160 samples was chosen by the *Select* program as a validation file for the 1998-1999 harvests calibrations which were developed solely with Instrument 1 spectra.

To test the impact of combining spectra from two instruments in one database, the 160 samples were split randomly into two groups. Eighty samples were used to update the original 281-sample database from the 1998-1999 harvests and 80 were used to create a validation file. The process of merging

Table 1. Reference chemistry data and calibration statistics for forage traits measured either fresh by near-infrared spectroscopy (NIRS) on chopper using Instrument 1 or dried and ground by the NIRSsystems 5000 instrument for 281 forage samples from the 1998-1999 harvests.

	Reference chemistry				NIRS on chopper calibrations				NIRS systems 5000 calibrations‡			
Constituent	Min	Max	Mean	SD†	SEC‡	R^2C§	SECV¶	R^2V#	SEC	R^2C	SECV	R^2V
	g kg^{-1}				g kg^{-1}				g kg^{-1}		g kg^{-1}	
Dry matter	207	499	335	57	11.6	0.96	11.8	0.96				
Starch	39	430	309	67	21.8	0.89	23.6	0.88	8.9	0.98	9.8	0.98
IVDC††	609	764	705	31	18.0	0.63	19.2	0.58	9.9	0.90	11.5	0.86
Soluble sugars	22	222	78	35	12.7	0.87	13.8	0.84	3.4	0.99	4.4	0.98

† SD = standard deviation of the data set.
‡SEC = standard error of calibration.
§ R^2C = coefficient of determination of calibration.
¶ SECV = standard error of cross-validation.
# R^2V = coefficient of determination of cross-validation.
†† IVDC = in vitro digestibility cellulase.

spectra from two instruments into one database was called calibration update.

RESULTS AND DISCUSSION

Calibration and Validation

Wet chemistry reference values were highly variable among the calibration samples from the 1998-1999 harvests, especially those for DM and starch (Table 1). Such variability was well suited for developing robust NOC calibrations. Statistical values of the NOC calibrations were compared with those obtained with the same samples that were dried, ground, and scanned with the NIRS5000 monochromator (Table 1). Standard errors of calibration (SEC) and cross-validation were higher with NOC than with the monochromator. The lower accuracy with NOC was also reflected by the coefficients of determination of the calibration and cross-validation. Reduced ability to estimate properties of unchopped high-moisture maize forage was due to its very heterogeneous nature and high moisture content, making this a challenging matrix for near infrared measurement. Regardless, calibration statistics indicate that NOC can be used to analyze fresh forage maize harvested from routine experimental plots. The technique works best for quantification of DM, followed by starch and soluble sugars. Utilizing NOC to measure IVDC more precisely requires an improved calibration.

The calibrations derived on Instrument 1 from the 1998-1999 samples were applied on samples harvested in 2000 with the same instrument (Table 2, Fig. 1). Dry matter, starch, and soluble sugars were determined with good accuracy and precision as indicated by high coefficients of determination and low SEP values. No bias was observed. The SEP values were equivalent to the respective calibration SECV values (Table 1), indicating that the calibrations are robust. The SEP for IVDC was also low, as was the coefficient of determination. The latter can be explained partly by the low variability for this trait in the dataset. A second, independent NOC validation dataset for DM of 671 samples originating from routine yield trials in 2000, harvested in Cloppenburg, Germany, confirmed the validation statistics for DM (data not shown). Even with this larger dataset, the SEP of 12.2 g kg^{-1} and R^2V of 0.91 were as good as with the smaller dataset. Dry matter determinations by NOC were thus very stable, with an SEP of ≈12 g kg^{-1}. The validation statistics confirm the calibration results and corroborate that NOC can measure maize forage quantity and quality online. Hybrids can be classified rapidly according to yield and forage quality. The lower accuracy of NOC compared with dry NIRS is offset by the ability to collect data faster from many more plots.

The SEPs for starch, IVDC, and soluble sugars were observed with a wavelength range of 1100 to 1660 nm. Scanning between 950 and 1660 nm gave equivalent results. Incorporating wavelengths below 950 nm did not improve prediction accuracy. Spectral information between 400 and 550 nm obscured that from the NIR region (data not shown). This indicated that only the NIR region is important for this application. Spectra above 1660 nm were not collected due to the potential for temperature-induced noise (Perten Instruments GmbH, 1997, personal communication).

Repeatability Analysis

Improved ability with NOC to select maize genotypes with high yield potential was demonstrated with samples from forage yield trials (Table 3). Unexpectedly, the residual variance was greater for reference DM values than for the NOC values in two trials with hybrids from different maturity groups. Near-infrared spectroscopy on chopper predictions from the 2000 calibration were more precise than from 1999 because the former incorporated spectral information acquired from forage harvested in an additional year. The genotypic variance

Table 2. Near-infrared spectroscopy on chopper validation statistics of 159 samples measured during harvest 2000, predicted with calibrations developed from the 1998-1999 harvests. Calibration and validation samples were measured on Instrument 1.

Constituent	Mean	SD†	SEP‡	SEPC§	R^2P¶
	g kg^{-1}				
Dry matter	308	58	12.5	12.0	0.96
Starch	259	71	22.1	22.2	0.90
IVDC#	712	25	19.8	19.8	0.39
Soluble sugars	114	47	16.7	16.0	0.88

† SD = standard deviation of the data set.
‡ SEP = standard error of prediction.
§ SEPC = standard error of prediction after bias correction.
¶ R^2P = coefficient of determination of prediction.
# IVDC = in vitro digestibility cellulase.

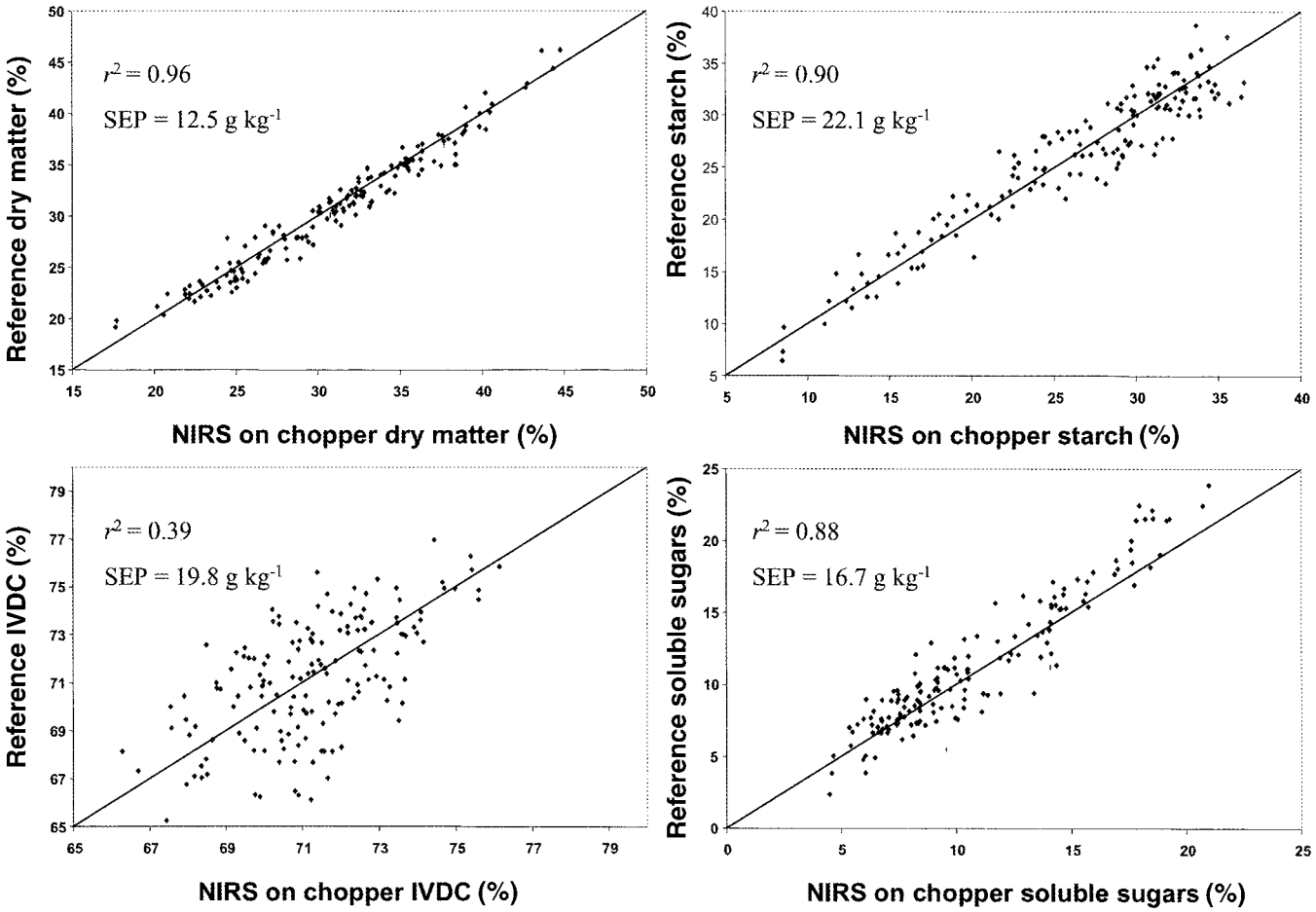

Fig. 1. Relationships between forage traits estimated by near-infrared spectroscopy (NIRS) on chopper using Instrument 1 and by reference methods. Samples (159) measured in 2000 were predicted with the 1998-1999 calibrations. IVDC = in vitro digestibility cellulase; SEP = standard error of prediction.

component (% v) was consistently higher with NOC compared with the reference method. The effectiveness of making selections due to genetic differences is related directly to the degree of genotypic variance. The high residual variance (3.93) of the reference method in the early hybrid yield trial was due to an outlier not present when NOC was used. Eliminating this outlier resulted in a residual variance of 2.24, still more than two-fold higher than the value of 1.04 obtained with the 1999 calibration. Variability associated with subsampling, weighing, drying, and transporting material under different temperature conditions and for different durations are characteristic of the reference method but mostly avoided by NOC. Thus, it can be explained why the secondary NOC method was more precise than the reference method.

Table 3. Residual and genetic variance for dry matter in early and mid-early forage yield trials measured by the reference method (oven drying) and near-infrared spectroscopy (NIRS) on chopper (n = 4).

	Early		Mid-early	
Method	Residual variance†	v‡	Residual variance	v‡
		%		%
Oven drying	3.93	0.75	2.17	0.80
NOC99§	1.04	0.92	2.06	0.82
NOC00¶	0.90	0.93	1.31	0.88

† The residual variance is the variance not explained by the factors in the model.
‡ The genotypic variance component v is the proportion of genotypic variance to the total variance.
§ NOC99 = NIRS on chopper calibration model derived with samples from the 1998-1999 harvests.
¶ NOC00 = NIRS on chopper calibration model derived with samples from the 1998-1999-2000 harvests.

Standardization

The ability to standardize instruments was evaluated by scanning 79 fresh forage samples on two different diode array spectrometers placed side by side. The instruments produced different spectra with the same samples (Fig. 2A). The average absorption spectrum produced by Instrument 1 displayed a wavelength shift and an inconsistent absorption bias compared with that from Instrument 2. The *Clone* program matched the two instruments spectrally (Fig. 2B). The transformation eliminated the wavelength shift between the average spectra and absorption differences were reduced by more than two orders of magnitude.

The *Clone* algorithm produced a standardization file containing the mathematical matrix to match the two instruments. This correction matrix was applied to 160

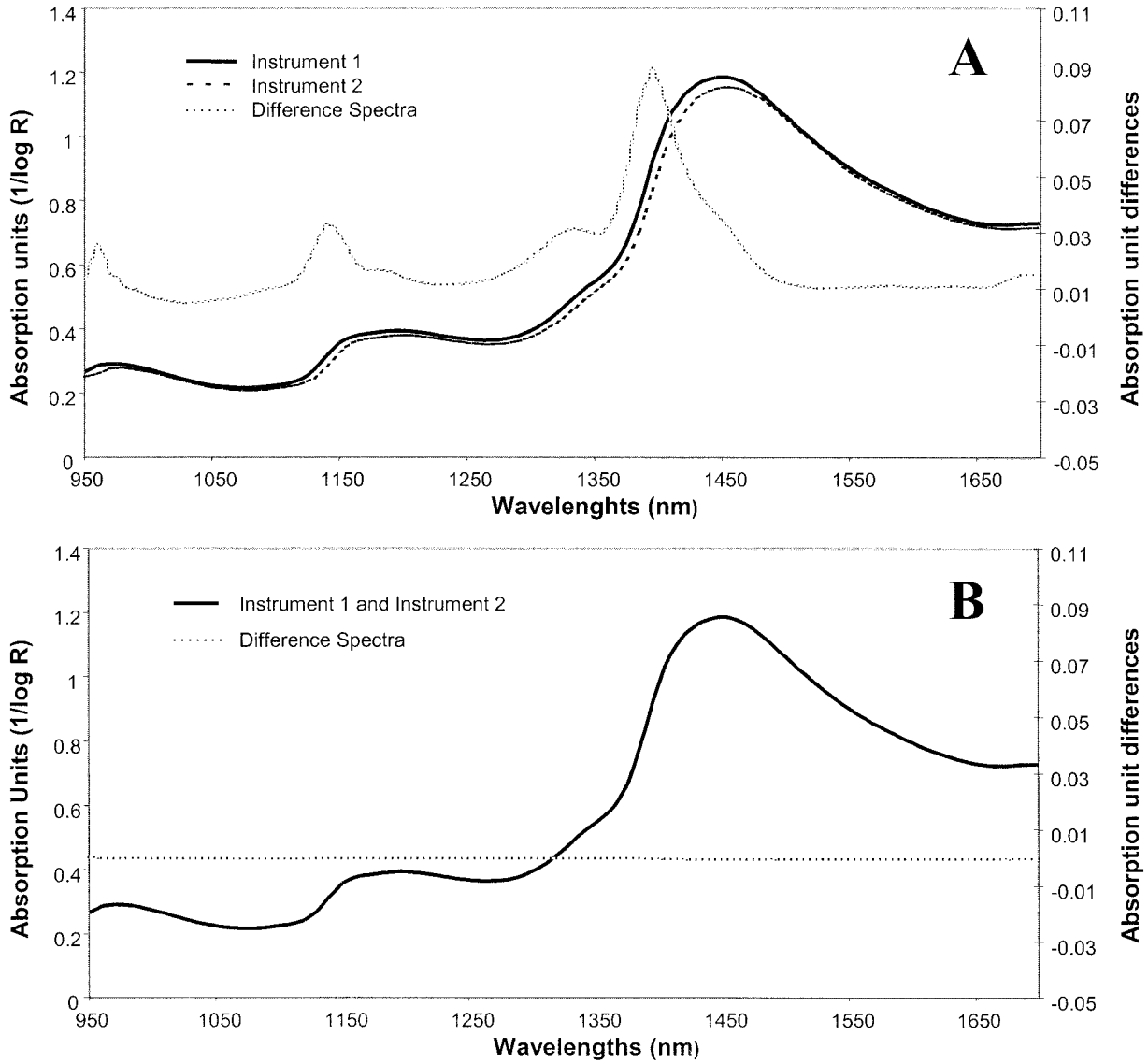

Fig. 2. Average absorption spectra of 79 forage samples measured on Instruments 1 and 2 and the difference between the two averaged spectra before (A) and after (B) spectral matching by the *Clone* program.

samples measured with NOC using Instrument 2, and the samples were projected on the principal components computed with 281 NOC spectra collected with Instrument 1 from the 1998-1999 harvests. The two distinct sample populations converged after instrument correction (Fig. 3). Although the *Clone* program aligned spectra from the two instruments, a perfect match was improbable since the two sample sets contained different hybrids grown at different locations and harvested in different years with different choppers. The standardization file reduced the Mahalanobis distance from 12.9 to 3.4, indicating that spectral differences between the two instruments were reduced significantly. The resulting excellent correlation between the two instruments is evident in an R^2 of 0.9979 at 1600 nm and similar R^2 values at 1400 nm, 1500 nm, and 1700 nm. Improved root mean square (RMS) values, bias, and bias corrected root mean square values (RMSC) between 30 samples measured by the two instruments placed side by side also demonstrated the utility of *Clone* for instrument standardization (Table 4). Values for RMSC of 500 to 800 μLog are obtained typically with NIRSystems model 5000 instruments after standardization, measuring dried products as powders in sealed cells (Dardenne, 2001). Thus, an RMSC of 1605 μLog obtained with NOC after spectral matching is a good result for unsealed, fresh, unground maize forage samples.

Calibration update was a second instrument standardization procedure utilized. Eighty samples measured on Instrument 2 were incorporated into the Instrument 1 1998-1999 harvests database. The remaining 80 samples collected with Instrument 2 were used to compute the SEP with the composite database that contained spectra collected with both spectrometers. Validation statistics (Table 5) demonstrate that standardizing spectra was required to predict quality parameters of Instrument 2 samples. Dry matter, soluble sugar, and starch values generated with unstandardized Instrument 2 spectra us-

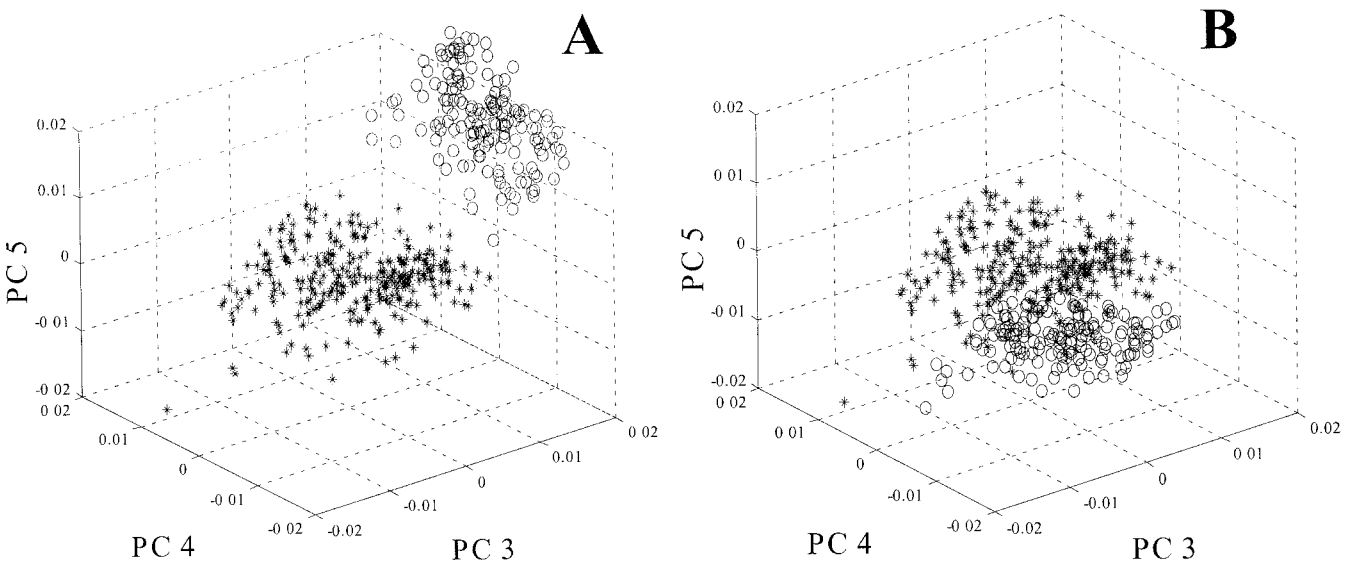

Fig. 3. Projection of 160 samples measured with near-infrared spectroscopy on chopper (NOC) using Instrument 2 in the space of three principal components (PCs) of the NOC calibration set of 281 spectra from Instrument 1 before (A) and after (B) spectral matching by the *Clone* program.

ing the Instrument 1 calibration had approximately twice the prediction errors than those generated with Instrument 1 spectra by the Instrument 1 calibration (Table 1). Unstandardized Instrument 2 spectra did not produce meaningful values for IVDC at all. Spectral matching alone corrected the DM predictions and improved significantly those of soluble sugars and IVDC, but actually increased SEP for starch. Acceptable SEP values for all constituents could only be found when spectrally matched Instrument 2 samples were used to update the Instrument 1 calibration (Table 5). In this case, the SEP values approached the SECV values found during the calibration process (Table 1) and approached the SEP values observed in the validation study of Instrument 1 (Table 2). Instrument standardization worked best for DM followed by soluble sugars, starch, and then IVDC. In summary, the two diode array spectrometers could be standardized, allowing NOC to be used at more than one location at the same time. Including 80 spectrally matched samples from Instrument 2 to the Instrument 1 1998-1999 calibration database was important for improving the validation results, but it did not change SEC or SECV (data not shown).

Although calibration updating is very important for instrument standardization, it is possible that not all spectrometers in a network have to be incorporated into a single master database depending on the spectral variability of the individual diode arrays. We are implementing this technology at two additional maize breeding stations in France. Preliminary findings with the four diode array spectrometers suggest that it is necessary to have all four instruments included in the master calibration file, although not with equal sample numbers (data not shown).

Table 4. Effect of spectral matching using *Clone* on a set of 30 samples measured on the two instruments in parallel in the laboratory.

	R^2	RMS†	Bias	RMSC‡
			µlog 1/R	
Before matching	0.9975	26 209	19 810	16 872
After matching	1.0	3 494	299	1 605

† RMS = root mean squares of differences.
‡ RMSC = root mean squares of differences after bias correction.

CONCLUSION

We demonstrated for the first time that a diode array NIR spectrometer installed on a chopper could analyze fresh maize forage quality online during routine harvesting. Whereas accuracy was not as great as that obtained with dried, ground forage, the results are useful to breeding programs. Dry matter was determined more precisely than starch or soluble sugars, and improvements will be required to measure IVDC. The results showed that NOC was more repeatable than the drying oven reference method for DM determination. With NOC, the whole plot is scanned, reducing significantly subsampling error associated with offline methods. Instrument differences preclude using calibration models developed on one instrument with another without correcting mathematically major spectral differences. Final calibration models must contain spectra obtained from multiple spectrometers.

Pioneer Hi-Bred used the described calibration models to predict quality traits of maize samples from ≈10 000 experimental plots harvested in 2000 at Greven. Ninety-nine percent of the NIR spectra could be used for predictions, and 162 spectra were outliers with Mahalanobis distances greater than 3. The predictions were used to calculate forage yield and characterize nutritional quality for breeding advancement decisions. In 2001, ≈16 000 plots were harvested at both stations, Greven and Nambsheim, with two choppers equipped with standardized NIR instruments. Again, 99% of the NIR spectra recorded were usable. Each plot was harvested and measured within 30 s, allowing the chopper to proceed through the fields at a required rate of 2 plots min^{-1}, the speed customarily obtained, without

Table 5. Near-infrared spectroscopy (NIRS) on chopper validation statistics of instrument standardization for corn forage traits. Samples from the 2000 harvest measured with Instrument 2 were predicted with the 1998-1999 Instrument 1 calibration either directly (original spectra), after applying the *Clone* procedure (after spectral matching), or after applying the *Clone* procedure plus updating the 1998-1999 calibration with 80 Instrument 2 samples. In the latter case, the remaining 80 samples were used for validation.

	Original spectra (n = 160)			After spectral matching (n = 160)†			After spectral matching and calibration update (n = 80)†		
Constituent	SEP†	SEPC‡	R^2P§	SEP	SEPC	R^2P	SEP	SEPC	R^2P
	g kg^{-1}			g kg^{-1}			g kg^{-1}		
Dry matter	25.8	14.4	0.95	15.1	14.2	0.94	13.4	13.4	0.95
Starch	43.7	34.2	0.79	56.8	30.7	0.79	25	23.3	0.85
IVDC¶	182	38.4	0.07	50.6	24.9	0.58	2.28	22.9	0.63
Soluble sugars	50.5	19.4	0.66	38.8	30.8	0.21	1.66	16.4	0.73

† SEP = standard error of prediction.
‡ SEPC = standard error of prediction after bias correction.
§ R^2P = coefficient of determination of validation.
¶ IVDC = in vitro digestibility cellulase.

NIRS. Sample throughput with NOC can accommodate any reasonably sized forage quality breeding effort.

Near-infrared spectroscopy on chopper allows for (i) huge savings of manpower and costs by avoiding subsampling, drying, grinding, and traditional laboratory NIRS analyses, (ii) superior selection for forage yield and quality by including on a larger scale early topcrosses during inbreeding, and (iii) more efficient planning and use of winter nurseries due to faster analyses.

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REFERENCES

Barnes, R., M. Dhanoa, and J. Lister. 1989. Standard normal variate transformation and detrending of near infrared diffuse reflectance spectra. Appl. Spectrosc. 39:772–777.

Berardo, N., F. Previtali, and M. Bertolini. 1999. Near infrared reflectance spectroscopy for selecting high amylomaize lines in breeding programmes. p. 651–654. *In* A. Davies and R. Giangiacomo (ed.) Proc. of the 9th Int. Conf. on NIRS, Verona, Italy. 13–18 Sept. 1999. Nir Publ., Chichester, UK.

Bouveresse, E., D. Massart, and P. Dardenne. 1994. Calibration transfer across near-infrared spectrometric instruments using Shenk's algorithm: Effects of different standardization samples. Anal. Chim. Acta 297:405–416.

Dardenne, P. 2001. Calibration transfer in near infrared spectroscopy. NIR News 13(4):3–7.

Dardenne, P., J. Andrieu, Y. Barrière, R. Biston, C. Demarquilly, N. Féménias, M. Lila, P. Maupetit, F. Rivière, and T. Ronsin. 1993. Composition and nutritive value of whole maize plants fed fresh to sheep. II. Prediction of in vivo organic matter digestibility. Ann. Zootech. 42:251–270.

Dardenne, P., and N. Féménias. 1999. Diode array near infrared instrument to analyze fresh forages on a harvest machine. p. 121–124. *In* A. Davies and R. Giangiacomo (ed.) Proc. of the 9th Int. Conf. on NIRS. Verona, Italy. 13–18 Sept. 2003. Nir Publ., Chichester, UK.

Dardenne, P., G. Sinnaeve, R. Biston, and P. Lecomte. 1991. Evaluation of NIT for predicting fresh forage quality. p. 277–283. *In* I. Murray and I. Cowe (ed.) Proc. of the 4th Int. Conf. on NIRS, Aberdeen, Scotland. 19–23 Aug. 1991. VCH Publ., New York.

De Boever, J., B. Cottyn, J. Andries, F. Buysse, and J. Vanacker. 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Technol. 19: 247–260.

De la Roza, B., A. Martinez, and B. Santos. 1998. The estimation of crude protein and dry matter digestibility of maize and grass silages by near infrared spectroscopy. J. Near Infrared Spectrosc. 6: 145–151.

International Organization for Standardization. 1997. Native starch: Determination of starch content. Ewers polarimetric method. Standard 10520. ISO, Geneva, Switzerland.

Lecomte, P., P. Dardenne, and C. Clément. 1996. La spectrométrie dans le proche infrarouge, outil d'évaluation rapide de la qualité et de la production du fourrage frais. Fourrages 148:379–387.

Naes, T., T. Isaksson, T. Fearn, and T. Davies. 2002. A user friendly guide to multivariate calibration and classification. Nir Publ., Chichester, UK.

Patterson, H.D. 1997. Analysis of series of variety trials. p. 139–161. *In* R.A. Kempton and and N.P. Fox (ed.) Statistical methods for plant variety evaluation. Chapman and Hall, London.

Patterson, H.D., and R. Thompson. 1971. Recovery of inter-block information when block sizes are unequal. Biometrica 58:545–554.

Ronsin, T., and N. Féménias. 1990. Use of NIRS determination of quality in a silage maize breeding program. p. 505–508. *In* R. Biston and N. Bartiaux (ed.) Proc. of the 3rd Int. Conf. on NIRS, Brussels, Belgium. 25–29 June 1990. Agric. Res. Centre, Gembloux, Belgium.

Shenk, J. 1990. Standardizing NIRS instruments. p. 649–654. *In* R. Biston and N. Bartiaux (ed.) Proc. of the 3rd Int. Conf. on NIRS, Brussels, Belgium. 25–29 June 1990. Agric. Res. Centre, Gembloux, Belgium.

Shenk, J., and M. Westerhaus. 1992. Winisi II Manual. Infrasoft Int., Port Matilda, PA.

Shenk, J., and M. Westerhaus. 1993. Analysis of agriculture and food products by near infrared reflectance spectroscopy. Infrasoft Int., Port Matilda, PA.

Shenk, J., and M. Westerhaus. 1995a. Calibration the ISI way. p. 199–202. *In* A. Davies and P. Williams (ed.) Proc. of the 7th Int. Conf. on NIRS, Montreal, QC, Canada. August 6–11. Nir Publ., Chichester, UK.

Shenk, J., and M. Westerhaus. 1995b. Comparison of standardization techniques. p. 112–115. *In* A. Davies and P. Williams (ed.) Proc. of the 7th Int. Conf. on NIRS. Montreal, QC, Canada. 6–11 Aug. 1995. Nir Publ., Chichester, UK.

Yemm, E.W., and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508–514.