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Usefulness of remote sensing for the assessment of growth traits in individual cereal plants grown in the field

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Biomass determination usually involves destructive and tedious measurements. This study was conducted to evaluate the usefulness of the Normalized Difference Vegetation Index (NDVI) and the Simple Ratio (SR), calculated from the spectra of individual plants, for the assessment of leaf area per plant (LAP), green area per plant (GAP) and plant dry weight (W) at different growth stages. Two varieties of four cereal species (barley, bread wheat, durum wheat and triticale) were sown in a field experiment at a density of 25 plants m^{-2}. The spectra were captured on three plants per plot on eight occasions from the beginning of jointing to heading using a narrow-bandwidth visible-near-infrared portable field spectroradiometer adapted for measurements at plant level. Strong associations were found between NDVI and SR and growth traits, both indices being better estimators of GAP and W than of LAP. Exponential models fitted to NDVI data were more useful for a wide number of situations than the linear models fitted to SR data. However, SR was able to discriminate between genotypes within a species. The accuracy of the reflectance measurements was comparable to that obtained by destructive measurements of growth traits, in which differences between varieties of over 24% were needed to be statistically significant. However, differences in SR of only 18% were statistically significant ($P<0.05$). The reliability of the spectral reflectance measurements and the non-destructive nature convert this methodology into a promising tool for the assessment of growth traits in spaced individual plants.

1. Introduction

Common methods for measuring biomass in cereal plants involve destructive and tedious sampling. Remote sensing techniques based on measurements at ground level of the reflectance spectra of crop canopies at different wavelengths through the photosynthetically active radiation (PAR, 400–700 nm) and near-infrared radiation (NIR, 700–1300 nm) regions, have been largely proposed as suitable, non-destructive tools for estimating crop characteristics such as green biomass, leaf area (Elliot and Regan 1993, Aparicio *et al.* 2000, 2002), and chlorophyll content (Sims and Gamon 2002). In addition, spectral reflectance measurements can supply information on the current physiological state of a crop and can be used for assessing the incidence of a range of stresses (Peñuelas and Filella 1998).

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The most common approach consists of combining reflectance at given wavelengths according to mathematical formulas for obtaining single spectral values, or spectral reflectance indices. Among them, two vegetation indices (VI) are the most widely used not only at ground based level, but also at aircraft and satellite levels (Wiegand and Richardson 1990): the Normalized Difference Vegetation Index (NDVI) and the Simple Ratio (SR). These spectral VI involve rationing a linear combination of the reflectance in the red (R) and near-infrared (NIR) bands, and have been shown to be closely related to growth traits such as green biomass, Leaf Area Index and fraction of radiation intercepted in cereals (Vaesen *et al.* 2001, Aparicio *et al.* 2000, 2002) and other crops.

Since grain yield is closely linked to the ability of the crop to intercept radiation and photosynthesize, and the relationship between the traits determining such abilities and VI has largely been demonstrated, several studies have focused on the usefulness of spectral reflectance measurements at canopy level for yield forecasting in cereals such as wheat (Aparicio *et al.* 2000, Hansen *et al.* 2002, Royo *et al.* 2003, Gutiérrez-Rodríguez *et al.* 2004), and other crops (Ma *et al.* 2001).

The usefulness of spectral reflectance measurements for evaluating large amounts of plant material in a fast and non-destructive manner has attracted the interest of plant breeders as a suitable selection tool. In this context, it seems interesting to investigate the value of terrestrial remote sensing techniques as selection tools for segregating populations of breeding programmes, when large amounts of genotypes have to be evaluated on a single plant basis. The estimation of plant biomass, which by conventional methodologies involves destructive and laborious measurements, would be interesting not only for selection purposes, but for several applications in physiological studies with isolated plants or plants grown in pots.

Although over the last few years, high spectral resolution devices have significantly improved the sensitivity and availability of spectral reflectance measurements—as more convenient and simplified equipment has been developed for fast field diagnosis of crops—while reducing the cost, the methodology for spectral reflectance measurements in individual plants has still to be developed. Equipment designed for measurements at plot level may be less appropriate for the measurement of individual plants, especially under field conditions. In order to avoid background and neighbouring plant interference, the field-of-view of the spectroradiometer sensor should be limited to the area occupied by the plant being measured. In a previous study, Casadesús *et al.* (2000) measured reflectance spectra of barley and durum wheat seedlings grown in pots enclosing them in a tube with reflecting walls and providing an artificial source of light, obtaining strong relationships between NDVI and growth traits. However, when the size and structure allow only the target plant in the field of view of the spectroradiometer sensor, as in the case of shrub plants, by just pointing the sensor on the top of each plant canopy, good relationships between NDVI and biomass can be still achieved (Filella *et al.* 2004). Nevertheless, the use of an enclosing system has the additional advantage of allowing individual plants grown under field conditions to be sampled, independently of external factors affecting the plant reflectance such as soil background, view and solar angles, and atmospheric conditions (Huete 1987), providing potentially more accurate estimates. Moreover, a more homogeneous distribution of light may reduce the interference of the spatial structure of the plant.

The objectives of this study were to determine the suitability of the most widespread vegetation indices (NDVI and SR) calculated from the reflectance spectra, for predicting growth traits (leaf area per plant, green area per plant and

plant dry weight) of individual plants of bread wheat, durum wheat, triticale and barley grown under field conditions.

2. Materials and methods

2.1 *Plant material and experimental set-up*

Two commercial varieties from each of four small grain cereal species were used in this study: Graphic and Sunrise for barley (*Hordeum vulgare* L. spp *vulgare*), Sarina and Soissons for bread wheat (*Triticum aestivum* L.), Simeto and Vitron for durum wheat (*Triticum turgidum* L. var *durum*) and Medellin and Trujillo for triticale (*XTriticosecale* Wittmack).

A field experiment was conducted during the growing season 2003/2004 in Gimenells (Lleida, north-eastern Spain, 41°39′ N, 0°51′ E, 200 m above sea level). Genotypes were sown on 16 December 2003 following a randomized complete block design, with three replicates. Experimental plots were of 20 m^2 (6 rows 0.2 m apart). Sowing rate was adjusted to 25 viable seeds m^{-2}, in order to obtain isolated plants.

The site was medium-high in fertility with a fine, Calcixerolic Xerochrept soil. The previous crop was alfalfa. Before sowing, the experimental field was fertilized with 66 kg N ha^{-1}, 124 kg P_2O_5 ha^{-1} and 124 kg K_2O ha^{-1}. Flood irrigation was provided twice, at jointing and heading. The total amount of water received by the crop until the end of the experiment was 296 mm. Weeds and pests were chemically controlled.

2.2 *Data recording*

Spectral reflectance measurements were made on three plants per plot at eight occasions from the beginning of jointing (first node detectable stage) to heading using a narrow-bandwidth visible-near-infrared portable field spectroradiometer (FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, CO, USA). In order to avoid background effects the spectrum of each plant was sampled after covering the plant by a tube of reflecting walls and provided by an artificial source of light adapted from Casadesús *et al.* (2000). The spectroradiometer was fitted to a receptor for diffuse spectral irradiance, centred at the top of the tube (figure 1). The enclosing tube was 1 m long and 31.5 cm diameter. Its inner face was coated with a highly reflective aluminium foil. Crushed aluminium foil was placed around the base of each plant, covering the entire tube base and providing a homogeneous background. Three halogen lamps of 10 W (HALOTEC G4), placed equidistantly from each other on the top of the tube, lightened the plant inside the tube. The captured reflectance spectra ranged from 400 nm to1050 nm in wavelength with a sampling interval of 1.4 nm. One spectral reflectance measurement (20–30 s), with an average of five scans, was taken at each plant. The spectra obtained were standardized with the spectrum previously sampled in the empty tube with the soil covered with a white homogeneous reflecting surface.

Vegetation indices (VI) were calculated using relative reflectance data as follows:

$$\mathrm{NDVI} = (R_{771} - R_{680})/(R_{771} + R_{680})$$

$$\mathrm{SR} = R_{771}/R_{680}$$

where R_n is the reflectance at the wavelength (in nm) indicated by the subscript (Hall *et al.* 1990).

Immediately after each reflectance measurement, the plants were pulled-up and moved to the laboratory in hermetically closed bags. The growth stage was

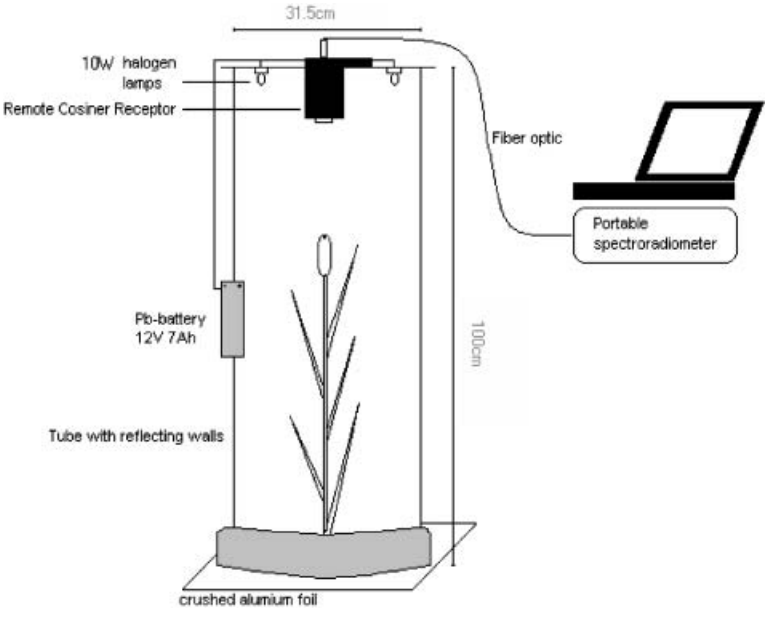

Figure 1. Layout of the equipment used for measuring spectral reflectance in isolated plants.

determined for each plant according to Zadoks' scale (Zadoks *et al.* 1974). Leaf area per plant and green area per plant were determined using a leaf-area meter (AT Dias II, Delta-T Devices, Cambridge). Leaf area per plant was calculated as the sum of the projection (one side) of leaf blades being leaf sheaths not removed from the stems. Green area per plant was calculated as: leaves projection + (stem projection $\times \pi$) + (spike projection $\times 2$). Yellow and dry tissues were not considered. Dry weight per plant was determined by weighing oven-dried plants at 80°C for 48 h. A total number of 576 plants were measured throughout the experiment.

2.3 *Statistical analysis*

Analyses of variance (ANOVA) were performed on growth traits and VI. As the four species differed in developmental stage when measurements were taken after the second sampling date (figure 2), data were grouped into five main developmental stages according to the Zadoks' scale: first node detectable (stage 31), stem elongation (from 32 to 37), flag leaf expansion (from 38 to 41), booting (from 42 to 50) and heading (from 51 to 59). Least square means for each species were compared by a non-adjusted pairwise test. TableCurve 2D (Jandel Corporation, 1994 was used to fit the best simple equation to the relationships between growth traits and VI. Dummy categorical variables were used to compare the models of bread wheat, barley and triticale with the models of durum wheat. For linear models, experimental data were fit to the following equation (Draper and Smith 1998):

$$Y = \beta_0 + \beta_1 X + D_1(\gamma_0 + \gamma_1 X) + D_2(\delta_0 + \delta_1 X) + D_3(\lambda_0 + \lambda_1 X) + \varepsilon \qquad (1)$$

where Y is the growth trait, X is SR, D_1 is the dummy variable for barley (0, 1), D_2 is the dummy variable for bread wheat (0, 1) and D_3 is the dummy variable for triticale (0, 1). Slopes of the models for barley, bread wheat and triticale were considered to be different to the slope of the model for durum wheat when γ_1, δ_1 and λ_1 were

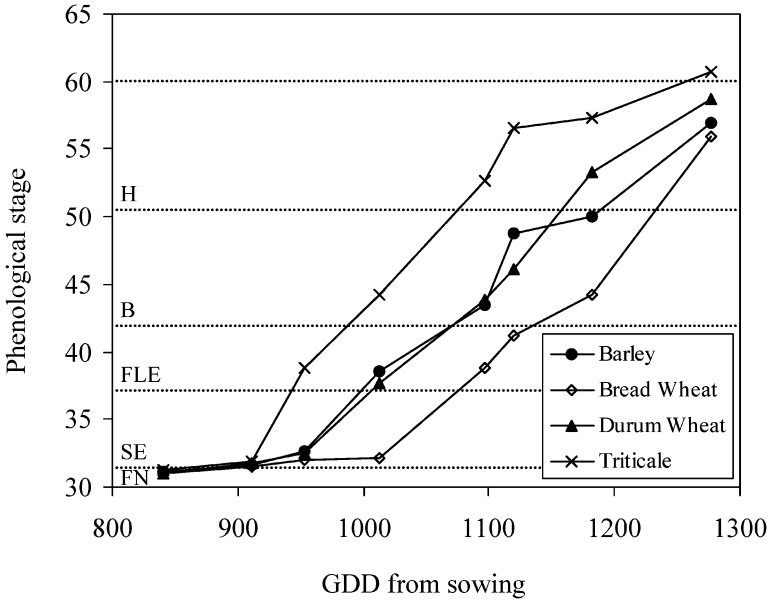

Figure 2. Growth stage according to the Zadoks' scale for each species at each sampling date. Values are means of three plants of two varieties for each of three replicates. Samples were grouped in five main developmental stages: FN, first node detectable (stage 31); SE, stem elongation (stages 32–37); FLE, flag leaf expansion (stages 38–41); B, booting (stages 42–50) and H, heading (stages 51–59).

significantly different to 0 at $P<0.05$ for a t-test. The parameters of the exponential equations were compared using the methodology proposed by Kimura (1980). Experimental data were fitted to the equation:

$$Y_{ij} = \sum_{i=1}^{4} D_i \left(\alpha_i e^{\left(\beta_i \cdot x_{ij}\right)} \right) + \varepsilon_{ij} \quad j = 1, \ldots, n_i \tag{2}$$

where D_i (i=1 to 3) were the dummy variables for each species as in the case of linear models, and D_4 corresponded to durum wheat. The maximum likelihood estimates of the unknown parameters were obtained using PROC NLIN (SAS Institute Inc. 2000). The following hypotheses: $H_0^{(1)}$: $\alpha_1=\alpha_4$ versus not equality; $H_0^{(2)}$: $\beta_1=\beta_4$ versus not equality; $H_0^{(3)}$: $\alpha_2=\alpha_4$ versus not equality; $H_0^{(4)}$: $\beta_2=\beta_4$ versus not equality; $H_0^{(5)}$: $\alpha_3=\alpha_4$ versus not equality and $H_0^{(6)}$: $\beta_3=\beta_4$ versus not equality, were tested against the corresponding alternatives, by using the chi-square approximation of the likelihood ratio test statistics $-n\ln\left(\hat{\sigma}_{\Omega}^2 \Big/ \hat{\sigma}_{\omega_i}^2\right)$, i=1, 2, 3, 4. The ω_i are the respective subsets of the parameter space Ω defined by the null hypotheses $H_0^{(i)}$, i=1,…, 4. All calculations were conducted using SAS-STAT statistical package (SAS Institute Inc. 2000).

3. Results

3.1 *Growth traits*

The four species differed in their developmental stages throughout the sampling dates (figure 2). Triticale varieties were always the earliest (at the end of the

experiment plants were at anthesis), while bread wheat varieties were the latest (at the end of the experiment most plants were at middle heading). Differences in development between the four species were mainly due to the duration of the stem elongation stage, which was much shorter in triticale than in the other species, particularly bread wheat.

The analysis of variance (table 1) showed highly significant differences in LAP, GAP and W between growth stages and species. The pattern of variation of the growth traits between species changed across development, as shown by the significance of the interaction species × growth stage. Varieties within species differed significantly in the three growth traits. Maximum LAP values were reached at booting in durum wheat, at flag leaf expansion in triticale, and at heading in barley and bread wheat (table 2). On the other hand, maximum GAP and W values were recorded at heading in all the species except triticale, which reached its maximum GAP during flag leaf expansion. Differences in LAP and GAP between species were significant beyond stem elongation (table 2), while differences in W were not significant until heading. Leaf area per plant, GAP and W were closely associated. The correlation coefficients between LAP and GAP, LAP and W, and GAP and W across species and sampling dates were $r=0.92$ ($P<0.001$), $r=0.77$ ($P<0.001$) and $r=0.92$ ($P<0.001$), respectively.

3.2 *Vegetation indices*

Differences in VI were mostly explained by phenological variations; however, the species effect and the interaction species × growth stage were also highly significant (table 1). SR detected differences between varieties, while NDVI did not. The highest values of both NDVI and SR were reached at the last sampling date for all the species except for triticale, which showed maximum SR and NDVI values at the flag leaf expansion stage, as occurred with LAP and GAP (table 2).

At the first node detectable stage SR did not differentiate between species, but NDVI did. Nevertheless, the performance of both indices was similar at further growth stages (lower part of table 2). In general terms, when comparing the four species, VI adequately tracked both the pattern of changes in growth traits and the detection of differences between them.

Table 1. Sum of squares (type III) of the analyses of variance for the growth traits and the vegetation indices NDVI and SR measured from the first node detectable stage to heading in isolated plants. LAP, leaf area per plant; GAP, green area per plant; W, dry weight per plant.

Source of variation	df	LAP ($\times 10^4$)	GAP ($\times 10^4$)	W	NDVI	SR
Growth stage	4	300 ***	2005 ***	4963 ***	1.8 ***	117 ***
Species	3	106 ***	405 ***	249 ***	0.12 ***	13 ***
Species × growth stage	12	153 ***	558 ***	702 ***	0.18 ***	29 ***
Variety (species)	4	26 *	74 *	101 *	0.04	4.2 *
Variety (species) × growth stage	16	41	147	199	0.09	9.0
Block	2	1.6	10	19	0.02	1.9
Residual	131	255	736	1181	0.66	45
Total	172					

*$P<0.05$, ***$P<0.001$

Table 2. Least square means of the growth traits and VI (NDVI and SR) measured in isolated plants at each sampling stage. Values are means of three plants for each of two varieties and three replicates.

	Growth stage	Barley	Bread wheat	Durum wheat	Triticale
Leaf area per plant (LAP, cm^2)	First node	409 a	266 a	280 a	318 a
	Stem elongation	539 a	518 a	492 a	417 a
	Flag leaf expansion	698 a	699 a	496 b	689 ab
	Booting	767 a	757 a	591 b	541 b
	Heading	802 b	1117 a	562 c	434 d
Green area per plant (GAP, cm^2)	First node	580 a	364 a	398 a	458 a
	Stem elongation	796 a	760 a	723 a	618 a
	Flag leaf expansion	1201 a	1042 a	750 b	1105 a
	Booting	1450 a	1205 b	1025 b	1004 b
	Heading	1999 a	2046 a	1095 b	937 b
Plant dry weight (W, g)	First node	3.8 a	2.7 a	3.2 a	3.9 a
	Stem elongation	5.7 a	5.9 a	6.0 a	5.2 a
	Flag leaf expansion	9.6 a	10 a	7.2 a	9.9 a
	Booting	14 a	13 a	11 a	11 a
	Heading	23 a	26 a	16 b	13 c
SR	First node	2.0 a	1.6 a	1.8 a	1.9 a
	Stem elongation	2.3 a	2.2 a	2.2 a	2.2 a
	Flag leaf expansion	3.3 a	3.0 ab	2.4 b	3.4 a
	Booting	3.6 a	3.1 ab	2.8 b	3.2 a
	Heading	5.2 a	5.6 a	3.3 b	3.3 b
NDVI	First node	0.32 a	0.22 b	0.27 ab	0.29 ab
	Stem elongation	0.38 a	0.35 a	0.36 a	0.34 a
	Flag leaf expansion	0.51 a	0.47 ab	0.40 b	0.52 a
	Booting	0.54 a	0.49 ab	0.45 b	0.50 ab
	Heading	0.65 a	0.67 a	0.52 b	0.50 b

Means within a row followed by the same letter are not significantly different according to a non-adjusted pairwise difference test at 5% probability level.

3.3 *Relationship between vegetation indices and growth traits*

Linear regression equations were the simplest ones that fitted properly to the relationships between SR and growth traits across species and growth stages (figure 3(*a*), (*b*) and (*c*)). However, the association between NDVI and growth traits was better explained by exponential equations, being the simplest equation that converged in all cases $y=a\ e^{bx}$ (figure 3(*d*), (*e*) and (*f*)). Given that in some cases points from plants bearing spikes were outliers, data from plants with and without spikes were fitted separately. The results showed that the same models worked well for both sets of plants, but the parameters of the equations changed depending on the dataset (table 3).

Highly significant associations ($P<0.001$) were also found between VI and growth traits when the four species were studied separately (table 4). The coefficients of determination of the relationships with SR ranged from $R^2=0.41$ for LAP in triticale, to $R^2=0.97$ for W in bread wheat, and for NDVI they ranged from $R^2=0.46$ for LAP in triticale, to $R^2=0.96$ for W in bread wheat. The models fit properly both when all growth stages were considered together and when only data from plants without spikes were analysed.

Dummy variables were used to compare the slopes of the regression equations fitted to the relationships between growth traits and SR in durum wheat with the

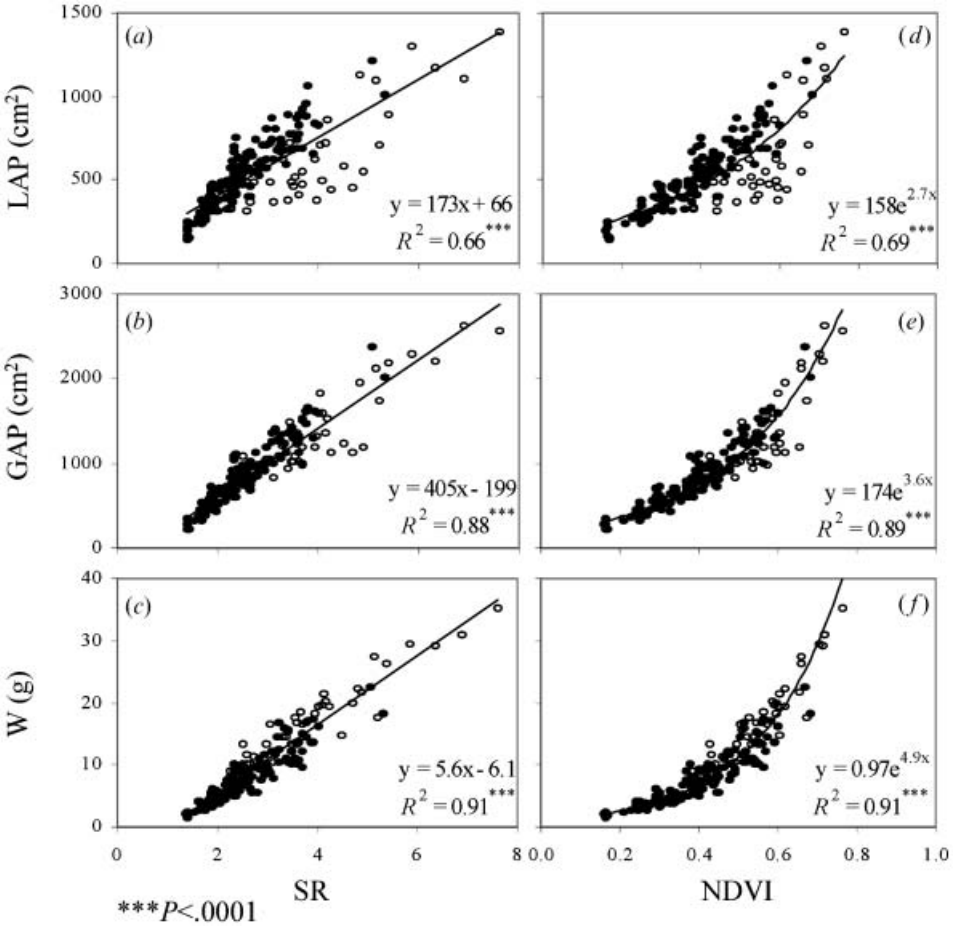

Figure 3. Relationship between VI (NDVI and SR) and growth traits for the whole experimental data involving measurements on two varieties for each of four species and eight sampling occasions (n=192). Each point represents mean data of three plants. Close circles represent samples measured before heading. Open circles refer to data obtained at heading.

slopes of the models for the other three species (table 4). The slopes of the models for the prediction of growth traits in triticale were significantly lower than those of durum wheat in all cases. However, no significant differences were found when the slopes of the models for predicting LAP and GAP in barley and bread wheat were compared to the slopes of the durum wheat models, except in the case of the model for predicting LAP in barley when calculated from jointing to booting. The slopes of the models for the prediction of W on durum wheat significantly differed to those of the other three species. The parameters of the exponential equation fitted to the relationship between NDVI and growth traits were similar in all cases to the ones of durum wheat when all growth stages were considered together. However, when the spikes were removed from the analyses, the parameters differed depending on the species (table 4).

In order to assess the capability of VI for predicting LAP, GAP and W when less variability was involved in the experimental data, the relationships between VI and

Table 3. Equations fitted to the relationships between growth traits, as dependent variables and VI as independent variables, for the data of the four species and from samples with and without spikes.

		SR		NDVI	
	n	Equation	R^2		R^2
Leaf area per plant (LAP)					
From jointing to booting	134	$y=249x-91$	0.83 ***	$y=128e^{3.4x}$	0.85 ***
Heading	39	$y=186x-107$	0.70 ***	$y=97e^{3.2x}$	0.59 ***
Green area per plant (GAP)					
From jointing to booting	134	$y=484x-374$	0.91 ***	$y=156e^{4.0x}$	0.90 ***
Heading	39	$y=383x-195$	0.83 ***	$y=174e^{3.5x}$	0.79 ***
Plant dry weight (W)					
From jointing to booting	134	$y=5.1x-5.3$	0.86 ***	$y=0.99e^{4.8x}$	0.89 ***
Heading	39	$y=4.9x-2.0$	0.89 ***	$y=2.1e^{3.6x}$	0.87 ***

*** $P<0.001$

the growth traits were determined for each species at each sampling date (table 5). Models were not fitted for flag leaf expansion since the briefness of this stage hindered capturing enough data. The results showed that most of the associations were significant ($P<0.05$). The lowest predictive values were obtained for LAP at heading in durum wheat and triticale.

3.4 *Discrimination within species*

Table 1 shows that varieties differed in growth traits and SR but not in NDVI. In order to compare the accuracy of SR to the precision of conventional biomass measurements, we assessed the magnitude of differences between varieties that made them statistically significant for growth traits and SR. For this purpose the least square means of each growth trait were compared between the two varieties of each species at each growth stage, according to a non-adjusted pairwise difference test. The results showed that the two barley varieties (Graphic and Sunrise) were the only varieties that differed in the three growth traits, but only at booting and heading (table 6). Apart from these, the only significant differences between varieties appeared for triticale in GAP at booting (data not shown). The results indicated that Graphic and Sunrise differed significantly when LAP, GAP and W differed by 289 cm^2 (or 32%), 393 cm^2 (or 24%) and 4 g (or 25%), respectively. Likewise, differences in SR between both barley varieties were not statistically significant until booting, when they were 0.73% or 18% in relative terms.

4. Discussion

4.1 *Methodological concerns*

The instrumental and methodological adaptations made in the spectroradiometer to allow the collection of spectra of individual plants in the field, offered accurate estimates of LAP, GAP and W. The advantages of this method were that measurements could be done at any time of the day, independently of the environmental conditions (sun light angle and intensity, weather conditions, etc.) and avoiding background disturbances such as soil colour. The size of the enclosing tube used for isolating the plants proved to be appropriate for the purposes of this

Table 4. Regression equations of the relationships between growth traits as dependent variables and VI as independent variables. Comparison tests evaluate similarities between barley, bread wheat and triticale irrespective of durum wheat. *t*-values correspond to the comparisons between slopes for the linear equation $y=a+bx$. χ_i^2 values correspond to the comparison between parameters of the exponential equation $y=a\,e^{bx}$.

	SR			NDVI			
	Regression equation	R^2	*t*-value of slopes comparison	Regression equation	R^2	χ_i^2 test *a*	χ_i^2 test *b*
Leaf area per plant (LAP)							
			All growth stages				
Barley	$y=150x+162$	0.65	−1.7	$y=224e^{2.1x}$	0.66	1.16	0.50
Bread wheat	$y=202x+55$	0.87	0.02	$y=191e^{2.7x}$	0.91	0.04	0.63
Triticale	$y=100x+162$	0.41	−3 ***	$y=205e^{1.7x}$	0.46	0.25	2.35
Durum wheat	$y=197x-3.07$	0.66		$y=185e^{2.4x}$	0.62		
			From jointing to booting				
Barley	$y=218x-2.57$	0.85	−2.8 **	$y=183e^{2.7x}$	0.86	6.22*	5.99*
Bread wheat	$y=283x-126$	0.87	−0.35	$y=160e^{3.1x}$	0.90	2.73	1.15
Triticale	$y=226x-102$	0.90	−2.1 *	$y=141e^{2.9x}$	0.87	0.41	1.87
Durum wheat	$y=293x-207$	0.87		$y=125e^{3.5x}$	0.81		
Green area per plant (GAP)							
			All growth stages				
Barley	$y=446x-242$	0.94	0.5	$y=205e^{3.5x}$	0.93	0.08	0.90
Bread wheat	$y=394x-126$	0.93	−1.1	$y=228e^{3.2x}$	0.94	0.26	0.00
Triticale	$y=289x-11$	0.83	−3.7 ***	$y=237e^{2.7x}$	0.87	0.43	2.67
Durum wheat	$y=429x-267$	0.86		$y=213e^{3.2x}$	0.82		
			From jointing to booting				
Barley	$y=495x-375$	0.94	−1.1	$y=196e^{3.6x}$	0.94	3.82	3.76
Bread wheat	$y=459x-276$	0.89	−2.0	$y=215e^{3.3x}$	0.90	6.38*	6.01*
Triticale	$y=404x-288$	0.93	−2.9 ***	$y=178e^{3.5x}$	0.92	1.13	3.94*
Durum wheat	$y=538x-507$	0.92		$y=147e^{4.2x}$	0.89		
Dry weight per plant (W)							
			All growth stages				
Barley	$y=5.54x-6.86$	0.91	−3.7 ***	$y=0.98e^{4.7x}$	0.89	0.05	1.14
Bread wheat	$y=5.70x-6.17$	0.97	−3.5 ***	$y=1.2e^{4.5x}$	0.96	0.84	3.15
Triticale	$y=5.23x-5.36$	0.86	−3.9 ***	$y=1.3e^{4.3x}$	0.87	1.03	3.51
Durum wheat	$y=7.22x-9.26$	0.91		$y=1.0e^{5.1x}$	0.91		
			From jointing to booting				
Barley	$y=5.15x-6.11$	0.89	−3.0 ***	$y=1.1e^{4.4x}$	0.89	0.99	5.08*
Bread wheat	$y=5.48x-5.71$	0.92	−2.2 *	$y=1.2e^{4.5x}$	0.91	2.04	3.67
Triticale	$y=4.12x-3.61$	0.89	−4.2 ***	$y=1.4e^{3.8x}$	0.89	3.14	8.05**
Durum wheat	$y=6.63x-8.20$	0.88		$y=0.87e^{5.4x}$	0.87		

All R^2 values were significant at $P<0.001$
*$P<0.05$, **$P<0.01$, ***$P<0.001$

study, as discussed later. Nevertheless, two people were needed for handling the equipment and the preparation of plants. Moreover, as the intensity of the incident light inside the tube was not comparable to the intensity of sunlight in measurements made on open plots at midday, the integration time of each scan was adjusted to 4.35 s instead of the 17 ms usually used on conventional measurements (Aparicio *et al.* 2004). Thus, between 4 and 5 minutes were needed for capturing the spectra of each plant, including the time of sample preparation, the capture of five scans and the periodical measurement of the white reference. As a result, not more than 12–15 plants could be managed per hour with this system.

Table 5. Coefficients of determination of the relationships between growth traits and VI for each species at different growth stages. The models used were $y=a+bx$ for SR and $y=a\,e^{bx}$ for NDVI.

		Barley		Bread wheat		Durum wheat		Triticale	
		SR	NDVI	SR	NDVI	SR	NDVI	SR	NDVI
Leaf area per plant (LAP)	First node	0.97^{***}	0.97^{***}	0.84^{**}	0.83^{***}	0.93^{***}	0.87^{***}	0.96^{**}	0.99^{***}
	Stem elongation	0.76^{***}	0.78^{***}	0.77^{***}	0.77^{***}	0.90^{***}	0.77^{***}	0.87^{**}	0.92^{***}
	Booting	0.73^{***}	0.74^{***}	0.76^{*}	0.77^{***}	0.79^{***}	0.74^{***}	0.91^{*}	0.90^{***}
	Heading	0.63^{*}	0.46^{***}	0.72^{*}	0.67^{***}	0.31	0.20^{**}	0.37^{**}	0.39^{***}
Green area per plant (GAP)	First node	0.91^{***}	0.86^{***}	0.80^{**}	0.77^{***}	0.91^{*}	0.85^{***}	0.96^{**}	0.99^{***}
	Stem elongation	0.78^{***}	0.90^{***}	0.76^{***}	0.81^{***}	0.89^{***}	0.84^{***}	0.86^{**}	0.98^{***}
	Booting	0.89^{***}	0.69^{***}	0.77^{*}	0.75^{***}	0.87^{***}	0.46^{***}	0.99^{***}	0.79^{***}
	Heading	0.83^{**}	0.81^{***}	0.79^{*}	0.76^{***}	0.58^{*}	0.77^{***}	0.76^{***}	0.91^{***}
Plant dry weight (W)	First node	0.93^{**}	0.93^{***}	0.85^{**}	0.84^{***}	0.92^{**}	0.89^{***}	0.87^{**}	0.94^{***}
	Stem elongation	0.63^{**}	0.66^{***}	0.83^{***}	0.82^{***}	0.86^{***}	0.68^{***}	0.81^{**}	0.81^{***}
	Booting	0.80^{***}	0.77^{***}	0.89^{**}	0.90^{***}	0.82^{***}	0.82^{***}	0.98^{**}	0.98^{***}
	Heading	0.78^{***}	0.65^{***}	0.88^{**}	0.85^{***}	0.85^{***}	0.81^{***}	0.87^{***}	0.89^{***}

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

4.2 *Relationship between vegetation indices and growth traits*

The study of the relationship between VI and growth traits across species and growth stages showed that both SR and NDVI were strongly associated to the three growth traits studied. The best models fitted to the relationships involving SR were linear, while for NDVI they were exponential (figure 3 and table 3). Coefficients of determination of such associations ranged from 0.66 to 0.91 ($P<0.001$), values similar to those reported for the relationship between SR and LAI at plot level in durum wheat during grain filling (Aparicio *et al.* 2000), and green biomass in other cereals (Hansen and Schjoerring 2003).

The linear nature of the association between SR and growth traits, already reported from studies at plot level (Peñuelas and Filella 1998, Broge and Mortensen 2002), suggests that SR adequately tracked changes on them in the range of observed values. The only exception arose for SR values below 2, for which this index overestimated LAP. The exponential relationship between NDVI and growth traits has been widely reported for canopy measurements (Carlson and Ripley 1997, Aparicio *et al.* 2000, Royo *et al.* 2003). On assessments in wheat at plot level NDVI is expected to be saturated at LAI>2 (Gamon *et al.* 1995). The decrease in the sensitivity of NDVI at high LAI values has been attributed to the attenuation of the reflectance from the underlying soil surface or lower leaf layers when the ground surface is completely obscured by the leaves (Carlson and Ripley 1997). In our experiments NDVI lost sensitivity at its uppermost values, but without becoming saturated. The reason is that in measurements of individual plants inside a tube, the size of the plant that determines the saturation of NDVI depends on the diameter of the tube (Casadesús *et al.* 2000). This indicates that in our study the diameter of the tube was appropriately chosen, since the sensitivity of the apparatus was accurate enough for plants of a wide range of sizes, but NDVI did not saturate when big plants were measured.

Although the three growth traits were strongly correlated, predictive values of SR and NDVI were higher for W and GAP than for LAP, both considering the whole

Table 6. Differences between the least square means of growth traits and SR for the two barley varieties used in the experiment.

	Growth stage	Graphic	Sunrise	*P*-value	Difference between varieties (%)
Leaf area per plant (LAP, cm^2)	First node	416	402	0.897	3.3
	Stem elongation	569	510	0.471	10
	Flag leaf expansion	726	671	0.668	7.5
	Booting	911	622	0.001	32
	Heading	1000	604	0.001	40
Green area per plant (GAP, cm^2)	First node	578	582	0.979	−0.8
	Stem elongation	827	764	0.653	7.6
	Flag leaf expansion	1240	1162	0.718	6.3
	Booting	1646	1253	0.006	24
	Heading	2413	1585	<0.0001	34
Plant dry weight (W, g)	First node	3.8	3.8	0.979	−1.6
	Stem elongation	6.2	5.1	0.551	17
	Flag leaf expansion	10	9.2	0.771	8.0
	Booting	16	12	0.028	25
	Heading	29	18	<0.0001	37
SR	First node	2.0	2.0	0.938	−1.8
	Stem elongation	2.4	2.2	0.600	7.5
	Flag leaf expansion	3.5	3.1	0.532	9.6
	Booting	4.0	3.3	0.038	18
	Heading	6.2	4.2	<0.0001	33

data or each species separately. This result contrasts with the results reported from studies at plot level, where the relationships between VI and biomass are usually weaker than the relationships between VI and LAI (Bellairs *et al.* 1996, Aparicio *et al.* 2002), being often even not significant (Serrano *et al.* 2000). Differences between the results given by both approaches could be a consequence of the effect of the reflecting walls of the tube, as they provide diffuse light through the whole plant, while on plot measurements the major fraction of reflected light comes from the upper levels of the canopy, more represented by the surface of leaves.

4.3 *Effect of plant ontogeny on the relationship between growth traits and spectral reflectance indices*

The growth stage and its interactions explained most of the variability obtained in VI and growth traits (table 1), suggesting that changes in biomass due to plant development caused much more variation than induced by the species or the variety of effects.

Vegetation indices appropriately tracked changes in GAP and W in barley, bread wheat and durum wheat throughout development, as well as changes in LAP in barley and bread wheat, where LAP increased from the first node to heading (table 2). On the contrary, the maximum LAP was reached in durum wheat at booting, decreasing afterwards, in agreement with the growth pattern described for this species when grown under Mediterranean conditions (Royo *et al.* 2004). However, NDVI and SR increased until heading in durum wheat, probably because the area of the spikes compensated for the decrease in leaf area. In the case of triticale, although maximum values of VI were recorded at the same stage as

maximum LAP, decreases in VI after flag leaf expansion were much less dramatic than those of LAP, due probably to the compensation caused by the reflectance of spikes. In consequence, estimation of LAP through VI was much more accurate before the maximum LAP than after this point, as shown in table 5. The separation of the growing period into two phases, before and after the growth trait reaches its maximum, has been recommended in rice to improve the performance of VI (Yang and Chen 2004).

4.4 *Predictive value of VI within each species*

Vegetation indices were useful to predict developmental changes in GAP, LAP and W within each species (table 4) with a similar accuracy to when the data of the four species were considered together (table 3 and figure 3). The high values of the coefficients of determination of the relationships between VI and both GAP and W in the four species ($R^2 \geqslant 0.82$, $P > 0.001$) indicate that VI were suitable in all species to track changes in both growth traits. Changes in LAP were properly assessed by VI in bread wheat either considering plants with emerged spikes or not. The comparison of the slopes of the regression equations fitted to the relationships between SR and growth traits for each species suggests that the models to predict GAP and LAP in barley, bread wheat and durum wheat were similar and different from the model for the prediction of these growth traits in triticale, which had a significantly lower slope. Differences observed between the slopes of the models to predict W would recommend fitting a different model for durum wheat to the other three cereal species. A comparison of the parameters of the exponential equations fitted to the relationships between NDVI and growth stages for each species suggests that the model useful for durum wheat was also useful for barley, bread wheat and triticale when all growth stages were considered together, but they differed when plants bearing spikes were removed from the analyses. According to the results presented in table 4, NDVI seems more widely useful than SR for the assessment of growth traits in individual plants.

The study of the relationships between VI and the growth traits for each species at each growth stage revealed that the models were useful in all the cases tested, excepting for the prediction of LAP in durum wheat by SR at heading. The reduction observed in the coefficients of determination of the relationships between VI and growth traits at heading irrespective of the preceding stages, was greater in the case of LAP than in the case of GAP and W (table 5), just confirming the disrupting effect of the emerged spikes on the assessment of LAP by spectral reflectance measurements.

4.5 *Phenotypic variability needed to discriminate between plants*

The results discussed until now refer to the suitability of VI to estimate growth traits in individual plants when a wide range of variation is present. In the previous cases this variation was a consequence of the inclusion of data from different species and/or from plants at different growth stages within the same species. Nevertheless, in spite of the theoretical interest of such analyses, from breeding and physiological points of view it becomes much more relevant to test whether our methodology was able to discriminate between plants when much less variability was present, which is the most frequent case in experimental plots. Thus, when selecting from segregated generations of a breeding programme, the plants to be screened not only belong to

the same species, but to the same cross, and even frequently they have developmental synchrony. In this context it becomes critical to ascertain how much variability must exist between plants for the VI to detect differences between them.

The ANOVA (table 1) revealed that significant differences between varieties existed in LAP, GAP and W, and that they were detected by SR but not by NDVI. In order to ascertain the origin of the differences between varieties, the mean values of LAP, GAP, W and SR were calculated for each variety at each growth stage and the two varieties of each species were compared. The results of the pairwise comparison showed that the two varieties of bread wheat, durum wheat and triticale did not differ on their growth traits, neither in VI. On the contrary, the two barley varieties, Graphic and Sunrise, differed in LAP, GAP and W at booting and heading, detected by SR. In relative terms, differences in GAP $\geqslant$ than 24% were significant, while differences of 7.6% or lower were not. Differences in LAP of 10% were not significant, while of 32% and above were significant. For W, differences should be $\geqslant$25% to be significant. SR had an accuracy not lower than that of direct measurements, since differences in SR between genotypes of 18% and above resulted in statistical significance ($P<0.05$). These results indicate that differences of 32%, 24% and 25% in LAP, GAP, and W, respectively, were precisely detected by SR, and that this index had a similar reliability as the destructive measurements to determine differences between genotypes in growth traits when determined in isolated plants.

5. Conclusions

The enclosing system used in this study to limit the field of view of the sensor offered accurate estimates of growth traits through vegetation indices. Simple ratio and NDVI properly assessed leaf area, green area and dry biomass of individual plants of the four species, mostly in measurements made before anthesis. Exponential models fitted to NDVI data were more useful for a wide number of situations than the linear models fitted to SR data. However, SR was able to discriminate between genotypes within a species. The accuracy of the method was comparable to results obtained by destructive measurements of biomass, in which differences around 24% were needed to be statistically significant. The precision of SR was even higher than this, given that differences of 18% in the SR between two varieties of the same species were statistically significant. The disadvantage of the methodology was the time required for capturing the spectra. This may constrain its use in breeding programmes, where thousands of plants have to be evaluated over short periods of time. Nevertheless, the accuracy of the method and its non-destructive nature make it a promising tool for plant growth characterization in studies dealing with a limited number of plants.

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