A Leaf Layer Spectral Model for Estimating Protein Content of Wheat Grains

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Abstract. The spectral signatures of crop canopies in the field provide much information relating morphological or quality characteristics of crops to their optical properties. This experiment was conducted using two winter-wheat (*Triticum aestivum*) cultivars, Jingdong8 (with erect leaves) and Zhongyou9507 (with horizontal leaves). We analysiced the relation between the direction spectral characteristics and the laeves nitrogen content(LNC). The result showed that the spectral information observed at the 0° angle mainly provided information on the upper canopy and the lower layer had little impact on their spectra. However, the spectral information observed at 30° and 60° angles reflected the whole canopy information and the status of the lower layer of the canopy had great effects on their spectra. Variance analysis indicated that the ear layer of canopy and the topmost leaf blade made greater contributions to CDS. The predicted grain protein content (GPC) model by leaf layers spectra using 0° view angle was the best with root mean squares (RMSE) of 0.7500 for Jingdong8 and 0.6461 for Zhongyou9507. The coefficients of determination, R^2 between measured and estimated grain protein contents were 0.7467 and 0.7599. Thus, grain protein may be reliably predicted from the leaf layer spectral model.

Keywords: wheat canopy, leaf distribution, direction spectra, view angle, model.

1 Introduction

The spectral characteristics of a crop canopy are determined not only by biophysical and biochemical features and also plant structural attributes. Leaf optical properties are a main factor for canopy spectral.

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Crop canopy spectral characteristics represent important information needed to guide crop management. Crop composition and structure are difficult to assess by traditional spectral measurement with vertical canopy direction. The direction spectrum includes a great deal of crop canopy information. Multiangle data significantly improved the accuracy of recovering forest parameters when inverting 3-D optical models (Kimes et al., 2002) but forest vertical structure can not be captured accurately using only the 4 spectral bands in the nadir view or all view angles with a single spectral band (Kimes et al.,2006). Directional radiances in or near the principal plane of the sun provides information that leads to more accurate prediction of canopy structure parameters than from other azimuth planes (Gobron et al., 2000). Canopy emissivity increased with increasing view angle due to the greater proportion of vegetation observed at off-nadir view angles; when the proportion of leaves was lower than that of soil, canopy emissivity grew with increasing view angle (Sobrino et al., 2005).

It should be noted that these models assume a Lambertian behaviour for soil and vegetation surfaces. Although vegetation surfaces show a near Lambertian behaviour, bare soil surfaces do not and the angular variation on emissivity can not be neglected. Lidars, multiangle radiometers, radars and imaging spectrometers have been identified as systems that can capture information in the vertical dimension. This requires a capability to remotely measure the vertical and spatial distribution of forest structural parameters that are needed for more accurate models of energy, carbon, and water flux over regional, continental, and global scales. Thus, we examined the utility of hyperspectral data for the quantitative characterization of vertical wheat structure.

Most remote sensing systems provided an image of the horizontal scope, but could not provide the vertical information on biochemical distribution in a crop canopy, thereby reducing the accuracy of measurement. Multi-angle data can increase the precision of forest parameters (Kimes,et al., 2006).

The distribution of tissues in a crop canopy has certain characteristics - biochemical distribution is different because of transfer of matter during the growth stage. Leaves in a wheat canopy are composed of under, middle, upper layer and ear layers (Wang et al.,2004). The spectral characteristic differed among canopy leaves because of the different reflection and scatter, so their effect on canopy spectra was different (Wang et al., 2004).

Various biochemical (foliar lignin and nitrogen) and biophysical factors influencing canopy reflectance signatures have been studied in previous works. Information on biochemical parameters is important and the multi-angle spectra which provide information on different directions can facilitate a more exact prediction of biochemical parameters. To date, there are no studies on the relative importance of vertical distribution of wheat leaves that determines canopy reflectance across the shortwave (350–2500nm) spectrum. The contribution of each leaf layer relative to all other factors has also not been adequately determined. Yet, it is the interaction of these factors, including their potential covariance or unique behavior, that must be understood if advances in remote sensing are to be achieved.

In this study, the leaf slice method was used to characterize a wheat canopy, evaluate the spectral response of leaf vertical distribution in the canopy, determine the characteristics of spectral curve for different leaf layers and develop a methodology for predicting the biochemistry of the canopy.

2 Materials and Methods

2.1 Preliminary Experiment

A field experiment was conducted in the Experimental Station of the Institute of Crop Science of the Chinese Academy of Agricultural Sciences, Beijing (39°57'55" N, 116°19'46" E) in the 2005-2006 growing season. The soil was a silt clay loam containing 1.16% organic matter, 42.6 mg kg-1alkali-hydrolysable N, 26.5 mg kg-1 available phosphorus and 139.4 mg kg-1 available potassium.

Two winter wheat cultivars were used: Jingdong8, an erect leaf plant-type and Zhongyou9507, a lax leaf plant-type. Four N fertilizer (urea, 46%N,) treatments were set up with four randomized replications of each cultivar: N0, no N fertilization, N1, 150 kg hm-2 pure N fertilization, N2, 300 kg hm-2 (rationally fertilized), N3, 450 kg hm-2 (excessively fertilized). The N rates were applied in three splits at pre-sowing (50% of the total amount), reviving stage (25% of the total amount) and jointing stage (25% of the total amount). All the treatments were fertilized with the same amounts of P (P2O5, 144 kg hm-2) and K (K2O, 75 kg hm-2) at pre-sowing.

The leaf slice method of wheat canopy: whole (plant) wheat samples 0.5m long and 0.8m wide were chosen; based on the vertical distribution of wheat canopy, the samples were measured off the whole wheat canopy (WWC), ear layer of canopy (ELC), inverse first leaves layer of canopy (ILLC-1), inverse second leaves layer of canopy(ILLC-2), inverse third leaves layers of canopy (ILLC-3) and inverse fourth leaves layer of canopy (ILLC-4). Canopy layers were severed with a scissors from the ear layer to lower layer (Fig. 3).

2.2 Measured Traits and Methods

All canopy spectral measurements were taken from a height of 1.3 m above ground (the height of the wheat was 90 cm at maturity), under clear sky conditions between 10:00 and 14:00 (Beijing local time), using an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO,USA) fitted with a 258 field of view fiber optics, operating in the 350–2500 nm spectral region with a sampling interval of 1.4 nm between 350 and 1050 nm and 2 nm between 1050 and 2500 nm and with spectral resolutions of 3 nm at 700 nm and10 nm at 1400 nm. A 40 cm ×40 cm BaSO4 calibration panel was used for calculation of reflectance. The spectra were measured with view angles of 0, 30,60,90,120, 150, and 180° to the line vertical to the wheat row using the Simple multi-angle spectral measurement equipment (Fig.1) after every layer was removed (N2 treatment). The model spectra were measured with a view angle of 0°

using other treatments at ear layer (EL), upper leaves layer (ULL) and lower leaves layer(LLL)(Fig.2).

Leaf samples from EL,ULL,LLL were taken almost synchronously with the spectral measurements. Measurements were conducted at jointing, heading, anthesis, milking and waxing stages. These samples were oven-dried at 70℃ and nitrogen content was determined by the Kjeldahl technique (Bremneretal.,1981) using a B-339 DistillationUnit (BUCHIAnalyticalLtd, Flawil, Switzerland). Wheat grain protein content estimated from the formula : Pro% =6.25 × Nitr (%).

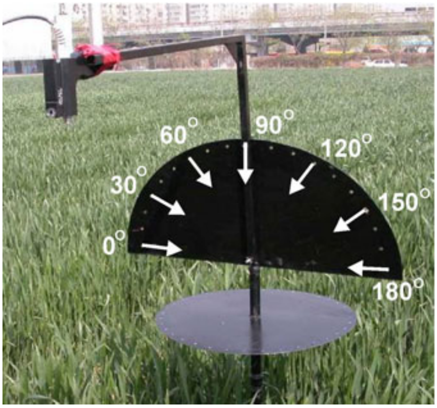

Fig. 1. Simple multi-angle spectral measurement equipment

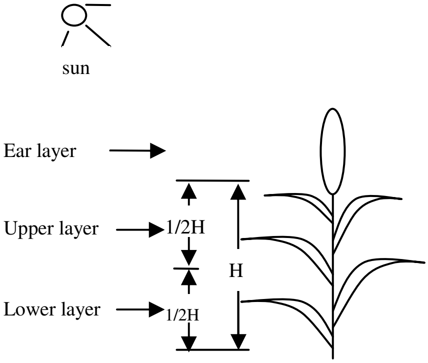

Fig. 2. Sketch of measured method by layer

2.3 Data Analysis

The hyperspectral data were analyzed using the Matlab6.5 software and quantitative data were analyzed using an analysis of variance (ANOVA) procedure.

3 Results

3.1 The Spectral Curves Following Removal of Different Leaf Layers

The lower leaves of the canopy changed the spectral reflectivity (Fig.3) with different view angles at 350-700nm, 800-1300nm and 1400-1800nm. This is important for pigment content within the visible wave band (350-700nm). The reflectivity of near infrared (800-1300nm) is influenced by canopy characteristics. The wavebands (1400-1800nm) provide information on the water content. In this paper, we analyzed the characteristics of visible (350-700nm) and near infrared (800-1300nm) wavebands.

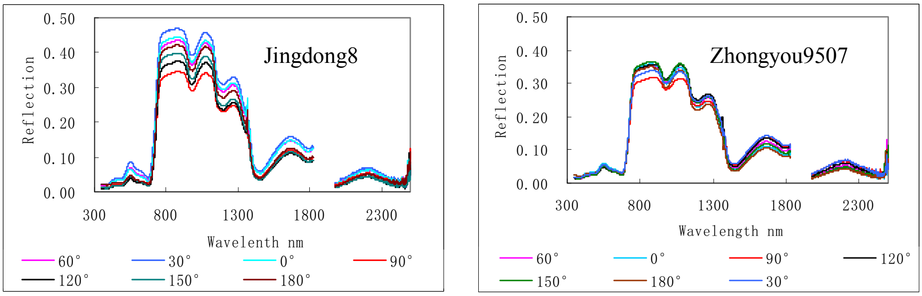

Fig. 3. Spectra of removing the forth leaf layer at different angles for Jingdong8 and Zhongyou9507 wheat

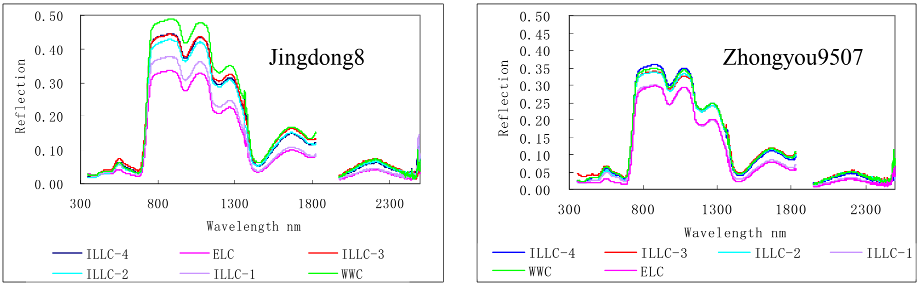

Fig. 4. Spectra of removing different leaf layers at 60°angles for Jingdong8 and Zhongyou9507

Comparing the direction spectral characteristic of the two wheat canopies, the spectral response of Jingdong8 with different view angles was more significant than that of Zhongyou9507 at visible and near infrared wavebands; the max reflectivity of Jingdong8 was 31% higher than that of Zhongyou9507 at 350-700nm and 22.4% higher at 800-1300nm.

The spectral curve of doing away with different leaves at the 60° view angle showed that the leaf influenced the curve of visible and near infrared wavebands (Fig.4). The change for Jingdong8 was more significant than for Zhongyou9507; the spectral reflectivity of Jingdong8 was 11.2% higher than that of Zhongyou9507 at 350-700nm and 26.8% higher at 800-1300nm.

To further analyze the relation between canopy spectra and leaves, we selected six spectral reflectivities at 450, 550, 670, 980, 1090, 1200nm.

3.2 Analysis of Spectral Reflectivity of Leaf Layers at Different View Angles

As shown in Fig.5, for Jingdong8, the spectral reflectivity of the whole canopy was similar with the leaf layer removal treatment, ILLC-4; doing away with ELC and ILLC-1 reduced the reflectivity. The changes in reflectivity at 550nm were more

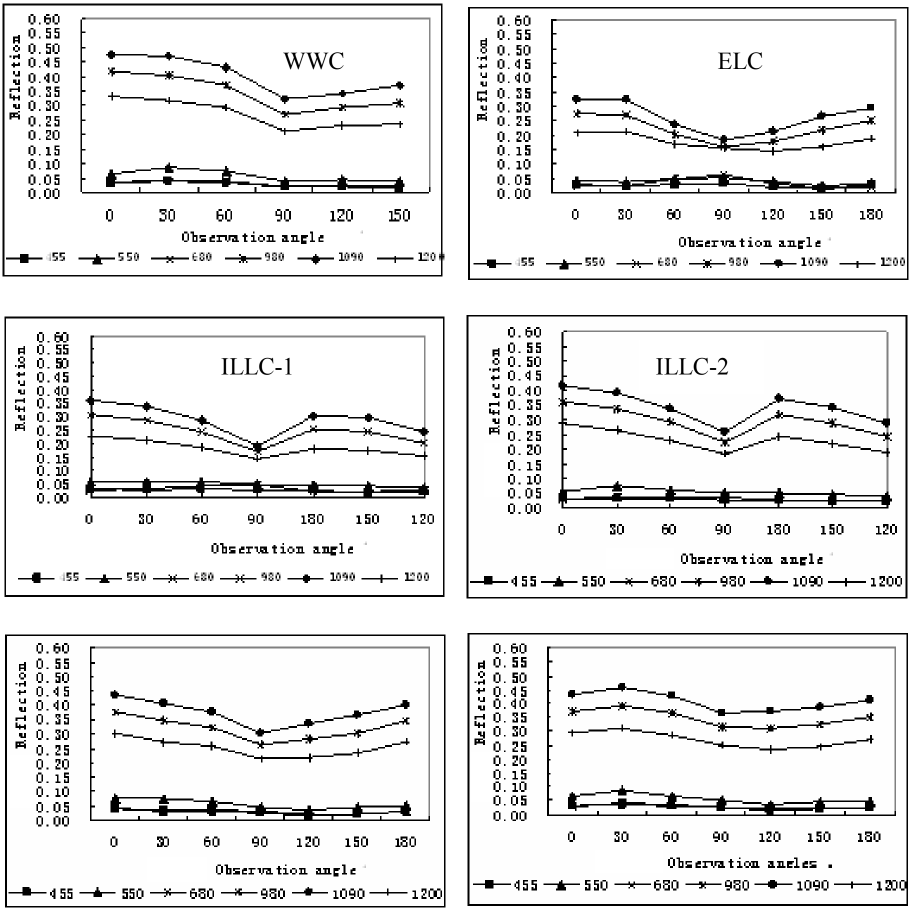

Fig. 5. Spectral characteristics following removal of different leaf layers in Jingdong8

obvious than at 450 and 670nm. In visible wave bands, changes were evident for 980, 1090, 1200nm in near infrared wave bands. At different view angles, the reflectivity of the 90° view angle was the lowest and with increasing distance from the vertical measurement, the reflectivity increased, especially in visible wave bands than in near infrared. The reflectivity changes due to ILLC-1 and ELC were more obvious than those of other leaf layers.

The reflectivity changes due to LLC-3 at 1090nm were 8.8% at 0° view angle and 48.6% for ILLC-1. At 90° view angle, the changes were 5.6 and 40.7%; at 30°, they were 12.9 and 27.7% and at 60° they were 12.2 and 34%, respectively. The change was less pronounced at the 180, 150 and 120° view angles. Compared to the 90° view angle, the reflectivity at 30 and 60° had more information on the lower leaves, which were important for reflectivity of the canopy. The reflectivity at 0° contained very important information on upper leaves.

The canopy spectral reflectivity of Zhongyou9507 was lower than that of Jingdong8 (Fig.6) at1090nm; the reflectivity due to ILLC-3 at 0° view angle changed 4.4%, while that due to ILLC-1 changed14.7%; at 90° view angle, the changes were 5.9 and 46.8%; at 30°, they were 16.9 and 44.4% and at 60° they were 16.8 and 41.2%. Compared with the traditional 90° view angle, the lower leaves were important for canopy spectra at 30 and 60° view angles; the upper leaves were important at 0° view angle although the upper leaves of Zhongyou9507 had less influence on canopy spectra than those of Jingdong8.

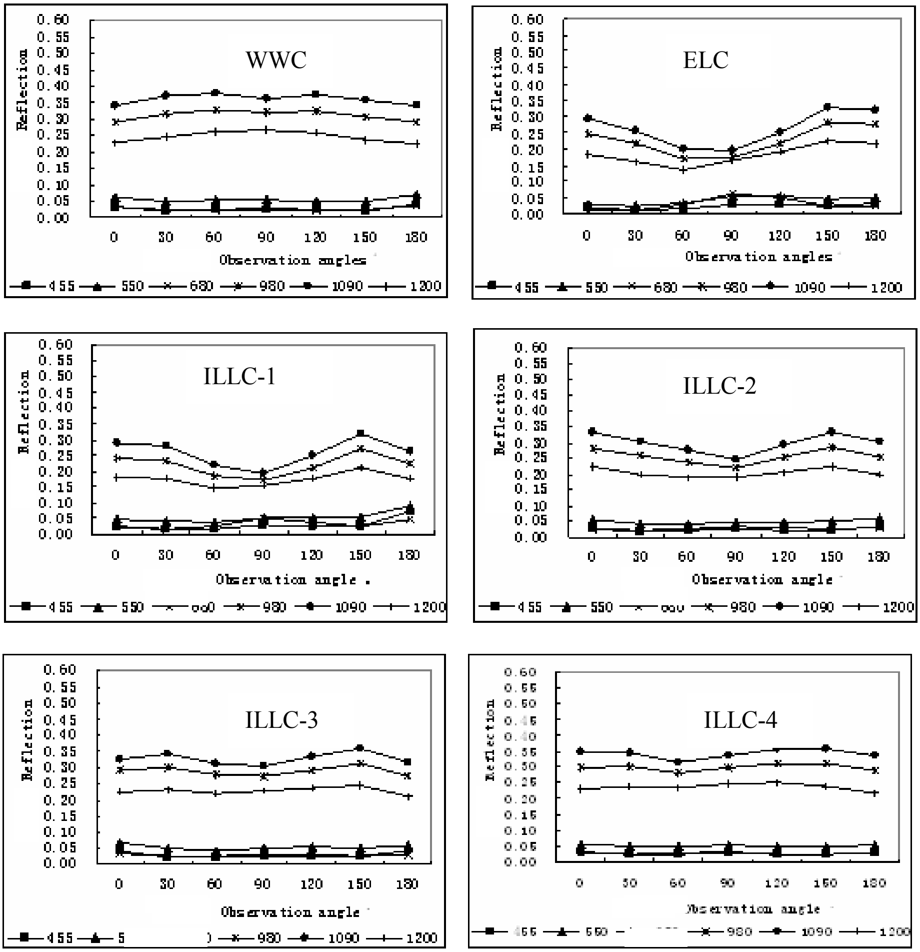

Fig. 6. Spectral characteristics following removal of different leaf layers in Zhongyou9507

3.3 Analysis of Variance (ANOVA) of Spectral Reflectivity for Different Leaf Layers

The ANOVA of mean reflectivities across six wavelengths showed that the reflectivity of Jingdong8 was lower than that of Zhongyou9507 (Table 1). For Jingdong8, the response of canopy spectra due to ILLC-1 and ELC were significant at the 0° view angle; at 30° as for 60°, the effects of ELC, ILLC-1, ILLC-2 and ILLC-3 were significant; at 90°, the ELC and ILLC-1 were important and at 120, 150 and 180° view angles, the ELC and ILLC-1 were significant. The ELC and ILLC-1 were important for canopy spectra and the influence of lower leaves were less. For the view angles of 0°, 90°, 120°, 150°, 180°), the under leaves were less influential; at 30 and 60°, the response of lower leaves were significant. The canopy spectral responses of different leaves for Zhongyou9507 differed from that of Jingdong8; at 0 and 90° view angles, the ELC and ILLC-1 effects were significant at 30 and 60°, the ELC, ILLC-1, ILLC-2 effects were significant.

At the same view angle, the ELC and ILLC-1 were more important than the lower leaves.

The spectral response of lower leaves was related to the view angle. With vertical and horizontal measurements, the influence of lower leaves were less than that of other view angles.

Table 1. Canopy spectral characteristic of different leaf layers of Jingdong8 and Zhongyou9507

Angles°	Treatments	Average value					
		Jingdong8			Zhongyou9507		
0	WWC	0.2254	a	A	0.1657	a	A
	ILLC-3	0.2110	a	A	0.1643	a	A
	ILLC-4	0.2046	a	AB	0.1662	a	A
	ILLC-2	0.1974	ab	AB	0.1573	a	A
	ILLC-1	0.1690	bc	BC	0.1354	b	B
	ELC	0.1508	c	C	0.1308	b	B
30	WWC	0.2265	a	A	0.1713	a	A
	ILLC-4	0.2196	ab	A	0.1646	a	AB
	ILLC-3	0.1951	bc	AB	0.1619	ab	AB
	ILLC-2	0.1886	c	AB	0.1398	bc	BC
	ILLC-1	0.1591	d	BC	0.1276	cd	C
	ELC	0.1487	d	C	0.1143	d	C
60	WWC	0.2064	a	A	0.1784	a	A
	ILLC-4	0.2021	a	A	0.1553	ab	A
	ILLC-3	0.1816	ab	AB	0.1503	ab	AB
	ILLC-2	0.1645	bc	ABC	0.1323	bc	ABC
	ILLC-1	0.1412	bc	BC	0.1052	c	BC
	ELC	0.1225	c	C	0.0974	c	C

Table 1. (*continued*)

Angles°	Treatments	Average value					
		Jingdong8			Zhongyou9507		
90	WWC	0.1588	a	A	0.1766	a	A
	ILLC-4	0.1726	a	AB	0.1657	a	AB
	ILLC-3	0.1451	ab	AB	0.1532	abc	AB
	ILLC-2	0.1292	ab	AB	0.1276	abcd	AB
	ELC	0.1081	b	B	0.1137	bcd	B
	ILLC-1	0.1040	b	B	0.1084	d	B
120	WWC	0.1645	a	A	0.1765	a	A
	ILLC-4	0.1642	a	A	0.1692	ab	AB
	ILLC-3	0.1511	ab	AB	0.1616	abc	AB
	ILLC-2	0.1341	abc	AB	0.1432	bcd	AB
	ILLC-1	0.1139	bc	AB	0.1255	d	AB
	ELC	0.1051	c	B	0.1325	cd	B
150	ILLC-4	0.1734	a	A	0.1679	ab	A
	ILLC-3	0.1637	ab	AB	0.1692	a	A
	ILLC-2	0.1547	abc	AB	0.1568	bc	A
	WWC	0.1454	abc	ABC	0.1672	ab	AB
	ILLC-1	0.1325	cd	BC	0.1515	c	AB
	ELC	0.1166	d	C	0.1555	c	B
180	WWC	0.2254	a	A	0.1678	a	A
	ILLC-4	0.1879	ab	AB	0.1599	a	A
	ILLC-3	0.1883	ab	ABC	0.1555	a	A
	ILLC-2	0.1725	bc	BC	0.1464	a	A
	ILLC-1	0.1388	c	BC	0.1461	b	B
	ELC	0.1360	c	C	0.1537	b	B

Note: Means followed by different lower case letters differ significantly at $P< 0.05$; those followed by different upper case letters differ significantly at $P<0.01$.

3.4 Correlation of Spectral Parameters of Leaf Layers with Leaf Nitrogen Content (LNC)

The correlation between canopy spectral parameters, spectral grads and corresponding nitrogen content in different leaf layers (Table 2) was significant between △RVI[670, 890] and leaf layer grads at the flowering stage for Jingdong8 and milky stage for Zhongyou9507.

Table 2. Correlation coefficients between nitrogen contents at different layers and spectral characteristics (SC)

Growth stage	Cultivar	nitrogen content	LSCP					
			△680nm	△*NDVI* [857,1210]	△*PRI* (570,531)	△*PPR* (550,540)	△*OSAVI*	△*RVI* [670,890]
Anrhesis	Jingdong8	*ULNC*	-0.2058	0.6835*	0.1929	-0.4270	-0.6850*	0.6900*
		LLNC	-0.2016	0.6437*	0.2082	-0.3300	-0.5669	0.5361
		△*LNC*	-0.2779	0.6431*	0.5443	-0.5240	-0.3256	0.7710**
	Zhongyou 9507	*ULNC*	0.0627	-0.2781	0.0508	-0.6561*	-0.0322	0.6171*
		LLNC	0.5923	-0.0223	0.2141	-0.5069	0.1373	0.5710
		△*LNC*	-0.2234	-0.4238	-0.6304*	-0.4990	-0.1748	-0.6335*
Milky ripe	Jingdong8	*ULNC*	0.3290	0.5788	0.6957*	-0.5719	0.4431	0.5831
		LLNC	0.3656	0.5690	0.4514	-0.5913	0.4368	0.6097*
		△*LNC*	0.5610	0.3882	-0.4454	-0.3284	-0.5557	0.6627*
	Zhongyou 9507	*ULNC*	0.2041	0.1279	0.1432	-0.5355	0.2822	0.4570
		LLNC	0.4674	-0.4111	-0.5394	0.6751*	-0.2721	0.5118
		△*LNC*	-0.1453	-0.5269	-0.6252*	-0.4591	-0.1190	-0.7888**

* and ** : significant correlations at 0.05 and 0.01 probability levels, respectively. ULNC: upper layer nitrogen content; LLNC: lower layer nitrogen content; △LNC: the difference between ULNC and LLNC. LSCP: spectral characteristics of leaf layers. NDVI: normalized difference vegetation index; PRI: pigment ratio index; PPR: plant pigment ratio; OSAVI: optical soil adjusted index; RVI: ratio of vegetation index.

3.5 Model Relating Grads of Spectral Parameter and Those of Nitrogen Content across Leaf Layers

The following models were developed from the siginificant correlations between grads of spectral parameter and those of nitrogen content in leaf layers:

Flowering stage of Jingdong8:

$$\triangle LNC\ (\%) = 35.2842\triangle RVI+0.3822$$

$$R^2=0.8267^{**},\ n=24 \qquad (1)$$

Milking stage of Zhongyou9507:

$$\triangle LNC\ (\%) = -8.0692\triangle RVI+1.5506$$

$$R^2=0.8099^{**},\ n=24 \qquad (2)$$

3.6 Leaf Layer Spectral Model for Protein Content in Wheat Grains

A prediction model for GPC based on spectra of leaf layers was developed as follows:

a) Jingdong8 at the flowering stage:

$$GPC\ (\%) = 12.0168\triangle RVI + 15.6975$$

$$R^2=0.7727^{**},\ n=24 \qquad (3)$$

b) Zhongyou9507 at the milk stage:

$GPC\ (\%) = -8.9028 \triangle RVI + 18.7397$

$R^2 = 0.7838^{**}$, n=24 (4)

The conventional spectral model using 90°view angle was also developed as follows:

a) Jingdong8 at the flowering stage:

GPC (%) = 11.98RVI +16.71

$R^2 = 0.6537^{**}$, n=24 (5)

b) Zhongyou9507 at the milk stage:

GPC (%) = 19.21RVI +13.59

$R^2 = 0.6659^{**}$, n=24 (6)

The determinant coefficients, R^2 of models (5) and (6) were 18 and 17% higher than those of models (3) and (4), hence, the predicted *GPC* model of leaf layer spectral was better.

3.7 Validation of the Predicted GPC Model of Leaf Layer Spectra

The root mean square error (RMSE) was used to test the reliability and estimation accuracy of these models for grain protein content. The RMSE of Jingdong8 was 0.7500 while that of Zhongyou9507 was 0.6461. The determinant coefficients, R2 between measured and estimated grain protein contents were 0.7467 and 0.7599, respectively (Fig. 7). These result indicated that prediction of grain protein using the leaf layer spectral model is reliable.

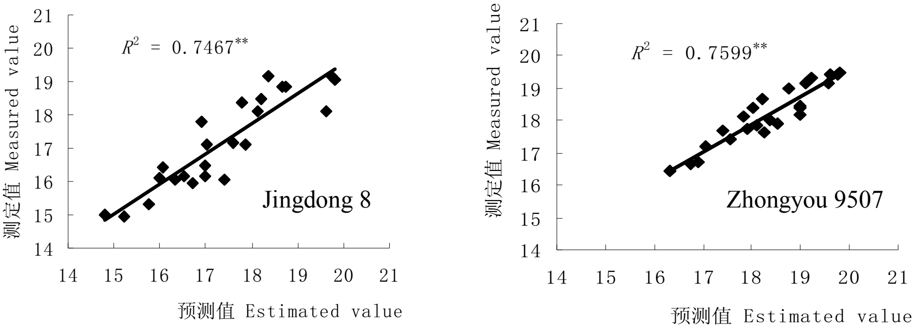

Fig. 7. Estimated and measured grain protein contents (GPC) in Jingdong 8 and Zhongyou 9507

4 Conclusions

The crop canopy has a three-dimensional structure whose characteristics are determined by species composition and the three-dimensional distribution of leaves. The biochemical content has some regulatory role in the distribution of the canopy space. The horizontal radiation balances are likely to occur if the internal variability of derived surface products, the typical distances that photons may travel horizontally within such 3-D media extend to spatial scales that are similar to or larger than those of the measuring sensor In order to maintain the energy balance of the overal forest domain, lacal canopy volumes with rather large positive net horizontal fluxes must also exist,thus underscoring the importance of properly locating local flux measurement equipment(Jean-Luc Widlowski, 2006). The more accurate modeling of energy are required for remotely measure the vertical and spatial distribution of forest structure. The potential of using a multi-angle spectral sensor to predict forest vertical structure were researched, the results showed the multi-angle spectral provided a relatively direct measure of vertical structure(D.S.Kimes 2006). The anisotropic scattering of both the vegetation canopy and the background is taken into consideration in a two-layer model of the bidirectional reflectance of homogeneous vegetation canopies(S.Liangrocapart, 2002).

In this study, two wheat varieties differing in plant-type were studied using the leaf layer slice method by analyzing the spectral changes for different leaf layers at different view angles in the plane of vertical wheat line. We noted that:

(1) The spectral characteristics varied in 300-700nm, 800-1300nm and 1400-1800nm wave bands because of removal of leaf layers and differences in view angle. The change in the visible wave bands was less than that in near infrared wave bands and that in Jingdong8 was less than that in Zhongyou9507.

(2) Compared with the traditional 90° view angle, the spectral information of under leaves were more at 30 and 60° than other view angles; for upper leaves the information was more at 0° view angle. The spectral influence of upper leaves of Zhongyou9507 at 0° view angle was less than that of Jingdong8. The upper leaves of the lax-leafed wheat captured much radiation because of their horizontal orientation, the proportion of upward leaves was bigger than that of downwards leaves. In contrast, the radiation characteristics were complicated in the erect leaf plant-type.

(3) The predicted *GPC* model by leaf layer spectra using 0° view angle were feasible, the *RMSE* was 0.7500 for Jingdong8 and 0.6461 for Zhongyou9507. The determinant coefficients, R^2 between measured and estimated grain protein contents were 0.7467 and 0.7599. Thus, the leaf layer model would be reliable for predicting grain protein content.

In precision agriculture, it is important to accurately measure the state of a crop's growth using remote sensing techniques. In this study, we analyzed the canopy spectral characteristics of two wheat types and observed that canopy spectra were influenced by leaf layers and view angles; the spectral response of under leaves varied with different view angles. A quantitative study of different leaf layers and their spectra is needed for precise crop management. Since results were obtained only in Peking region, more experiments should done in other regions.

Acknowledgments

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