Estimating the Mean Leaf Inclination Angle of Wheat Canopies Using Reflected Polarized Light

Michio Shibayama¹ and Yoshiaki Watanabe²

(¹National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan; ²National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan)

Abstract : In this study, we extended previous work linking the polarization of reflected light from crop canopies with characteristics of the canopy structure, such as the leaf inclination angle. We obtained reflectance and polarized reflectance in 8 spectral bands from the canopies of two varieties of wheat, planted in plots fertilized with a basal dressing and topdressed at the jointing and booting stages. The optical measurements were carried out on 3 clear-sky days when the plants were at the stem-elongation, heading and ripening stages, respectively. On each measurement date, we assessed the leaf orientation geometry of the plants using a Plant Canopy Analyzer (LAI-2000), measured the leaf greenness (an indicator of leaf chlorophyll content) using a handheld SPAD-502 (SPAD) optical sensor, and also measured plant height. Both polarization and leaf greenness observations at the heading stage were able to distinguish the canopies that had received topdressing from those without topdressing. However, no significant correlation was observed between the polarization in the blue, green and red bands and the SPAD ($r=0.425\sim0.456$, n=12 observations, p>0.05). On the other hand, the mean leaf inclination angle (= mean tip angle: MTA) measured by the LAI-2000 was inversely correlated with the polarization in the 3 visible bands ($r=-0.85 \sim -0.88$, n=12, p<0.001). Adjusting the view zenith angle according to the solar position at the time of measurement improved the accuracy. We tested a linear regression model to predict the MTA of the two wheat varieties based on polarized reflectance in the red band centered at 660 nm $(r^2=0.73, n=12, p<0.001)$. Validation of this model obtained in the subsequent cropping season confirmed that polarization measurements were potentially useful for estimating the MTA of wheat stands in which the panicles were located below the topmost leaf layer of the canopy.

Key words : Jointing-stage topdressing, LAI-2000, Mean tip angle, Polarization, Remote sensing, SPAD-502, Wheat.

The stand geometry of crop canopies has previously been measured in order to determine the best productive structure for improving the photosynthetic ability of crops, and to breed high-yielding varieties (Wall and Kanemasu, 1990; Hirota and Nakano, 2000). Since leaf inclination angle is closely related to the plant light-intercepting capabilities, this has been one of the most important parameters for describing canopy geometry (Isoda et al., 1994; Chhina and Kler, 1997). However, it is a laborious task to measure the leaf inclination angle for growing plants in the field. A method using a clinometer (Norman and Campbell, 1989), image analysis of side-view photographs (Oka and Hinata, 1988), 3-D digitizing of foliage (Shibayama et al., 1989; Sinoquet et al., 1991; Rakocevic et al., 2000; Shibayama, 2001), and measurements of the gap fraction of foliage (Welles and Norman, 1991) have all been proposed as possible solutions. The disadvantage of these techniques, however, is that it is not possible to carry out remote sensing over a large

area of farmland in a short time. In agricultural and environmental research activities, the remote sensing of canopy structure promises efficient discrimination of crop and plant species (Shibayama and Akita, 2002). Accordingly, there has been increasing interest in the use of remote sensing to assess the geometrical characteristics of plant canopies (Vanderbilt, 1985; Shibayama, 2006) as well as the biomass, green leaf area and nutrient conditions (Huang et al., 2004). One of the remote sensing techniques available is the measurement of reflected polarized light from crop canopies. This has been related to the leaf inclination angles of several crop species (Vanderbilt et al., 1985; Rondeaux and Herman, 1991; Ghosh et al., 1993). Theoretical and practical research has shown that it is possible to use the polarization of the light reflected from plant canopies to determine the leaf inclination geometry of those canopies (Egan, 1970; Curran, 1981; Fitch et al., 1984; Shibayama, 2003, 2004).

Atmospheric disturbance should be taken into

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Abbreviations: ANOVA, analysis of variance; LAI, leaf area index; MTA, mean tip angle; NDVI, normalized difference vegetation index; Qx, polarized reflectance (%) measured in a band centered at *x* nm; *Rx*, reflectance (%) measured in a band centered at *x* nm; SPAD, leaf greenness (an indicator of leaf chlorophyll content) using a handheld SPAD-502 optical sensor; *Zs*, solar zenith angle; *Zv*, view zenith angle.

consideration in case the observations are made from space or from very high altitudes (Nadal and Bréon, 1999). As yet, there is no satellite-borne sensor available with an appropriate spatial resolution for observing fields of crops (Grant et al., 2004). However, to enable the future utilization of polarimetry from airborne optical sensors, the Japan Aerospace Exploration Agency (JAXA) has been developing imaging spectropolarimeters for the polarimetric analysis of solar rays reflected from the Earth's surface (Shingu et al., 2002), and efforts are currently underway to realize the practical application of remote sensors for monitoring crop parameters (Homma et al., 2005). The imaging spectropolarimeter provides us with a practical tool for surveying crop fields by promptly detecting changes in canopy structure. However, not enough systematic research has yet been carried out to establish a practical technique using reflected polarized light for vegetation survey. Hence, measuring polarization for crop canopies, even though the instrument used is not an imaging-type sensor, allows us to generate basic ground truth data for use in designing future sensors not only for spaceborne, but also for handheld, cherry picker-mounted, or lowaltitude airborne instruments.

In a previous study, we examined the seasonal variations of polarized reflectance (in the band centered at 660 nm) from wheat canopies with different fertilization rates and planting densities (Shibayama and Watanabe, 2006). To determine which plant canopy parameters influenced the polarization of light, the relationship between the leaf inclination angle and the polarization of reflected light was examined. In the previous study, however, leaf inclination angles were assessed using a 3-D digitizer and measurements were taken a few days apart from the radiometric observations in the first experiment, while a Plant Canopy Analyzer (LAI-2000, LICOR, Inc.) was used for the second experiment. The results obtained may, therefore, have been somewhat inaccurate. In the present study, all mean leaf inclination angles were measured using the LAI-2000 on the same day that the radiometric measurements were carried out, to accurately quantify the relationship between the polarization and the stand geometry of the canopies.

There is little variation in polarization among the visible wavelength bands due to spectral homogeneity in the inflection coefficient of the light reflected from the cuticle of the leaf epidermis (Vanderbilt et al., 1985). That is why the previous study analyzed reflectance and polarized reflectance in the band centered solely at a wavelength of 660 nm. However, our next step was to examine 8-band reflectance and polarization not only in the visible, but also in the near- and short-wave infrared ranges of the spectrum, in order to fully investigate its effects.

This paper demonstrates the relationship between the polarization of light reflected from wheat canopies and plant canopy parameters such as the leaf area index, the mean inclination angle of leaves, the plant height, and the leaf greenness (an indicator of leaf chlorophyll content). The objectives of this study were to generate a polarimetric indicator that was sensitive to the changes in the leaf orientation of the canopy brought about by the fertilization conditions, and to test this technique on different varieties in different cropping seasons.

Materials and Methods

1. Optical variables and instruments used

The reflectance (R%) is the ratio of the reflected light intensity to the incident light intensity, and the polarized reflectance (Q%) is the term used for the polarized part of R. Due to the limited measurement time available for the numerous targets to be observed from various directions, the calculated solar irradiance at the upper atmosphere was used as the incident light intensity instead of standard reference panel observations in order to calibrate the radiometer in situ (Emori and Yasuda, 1985). Hence, the term "reflectance" correctly refers to the "radiant coefficient" in this paper. However, we use the term "reflectance" instead of "radiant coefficient" since the former is more commonly used. In order to avoid the need for reference panel observations and atmospheric corrections, we collected radiometric data only on clear-sky days. The procedure for measuring reflectance and polarization was based on that of Shibayama (2004). The ratio of Q to R is the degree of polarization. In this paper, R and Q, measured in a band centered at x nm, are referred to as Rx (%) and Qx (%), respectively. The zenith angle of the viewpoint of the sensor and the solar zenith angle are abbreviated to Zv (°) and Zs (°), respectively. A portable spectropolarimeter (Donarec Co., Ltd., Machida, Tokyo) was used to measure the light intensity and the degree of polarization in the wavelength bands centered at 490, 560, 660, 830, 1150, 1250, 1650, and 2200 nm with the field of view (FOV) of the optical system set at 10° (Shibayama and Akita, 2002). For reference purposes, values of band reflectance were used to obtain the most frequently used index: normalized difference vegetation index (NDVI) (Rouse et al., 1973; Reed et al., 1994). This was expressed as:

NDVI = (R830 - R660) / (R830 + R660)(1), and one of the common band transforms:

PVR = R560 / R660 (2). Since the ratio of green band (R560) to red band (R660) reflectance shown in Eq. 2 is high for leaves with strong chlorophyll absorption, it was therefore designated as the photosynthetic vigor ratio (PVR) (Warren and Metternicht, 2005). The procedure proposed by Shibayama (2004) and tested by Shibayama and Watanabe (2006) was employed for Zv in order to adjust the values of polarized reflectance measured at a given Zs. The normal observation geometry was empirically set at $Zv=75^{\circ}$ for $Zs=40^{\circ}$. The Zv required for observation at a given Zs was derived using the following equation:

 $Zv'=35^\circ + Zs$ (3). Linear interpolation or extrapolation was utilized to estimate the optical variables at the Zv' calculated from Eq. 3 using the two values observed at the nearest two values of Zv. Extrapolation was applied in case the Zs exceeded 40° at the time of measurement (which only occurred a few times and the maximum excess angle was 2° at most). The polarized reflectance values derived from this procedure in a band centered at xnm are referred to as Qx (Zv'). Optical variables Rx and Qx observed at a value of Zv are referred to as Rx(Zv)and Qx(Zv), respectively. Likewise, the band transforms NDVI and PVR observed at a value of Zv are referred to as NDVI(Zv) and PVR(Zv).

2. Experimental wheat plants, and the plant canopy parameters measured

The experimental site was located in the study field that had been established for the Program of Field Management for High Quality Wheat Production, conducted by the National Institute of Crop Science, Yawara, Ibaraki (36° 00' N, 140° 01' E). Two varieties of wheat (Triticum aestivum L. cv. 'Norin-61' and 'Ayahikari') seeds were each planted in east-west rows at a row width of 30 cm in a 5-a field in early November 2004. A basal fertilizer dressing was applied at three rates (4, 6, and 8 g Nm⁻²), a jointing-stage topdressing was applied at two rates (0 and 2 g Nm⁻² on 11 March, 2005), and a booting-stage topdressing was applied at two rates (0 and 1 g Nm⁻² on 18 April for 'Ayahikari', and on 21 April for 'Norin-61'). Two replicates were included in the experimental design so that the whole field was divided into 48 plots of equal area $(4.5 \text{ m} \times 3 \text{ m})$ after the final topdressing. After the harvest, we carried out a yield survey on all plots. At the same time that the radiometric observations were carried out, a Plant Canopy Analyzer (LAI-2000, LI-COR, Inc., Lincoln, NE, U.S.A.) was used on targeted wheat stands to estimate the LAI and the mean tip angle (MTA) of the canopies (Welles and Norman, 1991; Yamamoto et al., 1995). The LAI-2000 measures the attenuation of diffuse sky radiation at 5 zenith angles simultaneously with a "fish-eye" optical sensor and calculates LAI and MTA as a measure of how the leaves are oriented (LICOR, 1992). Measurements made above and below the canopy are used to determine canopy light interception, from which the LAI and MTA of the foliage are then computed using a mathematical inversion of a model for radiation transfer in vegetation canopies (Lang et al., 1985; Perry

et al., 1988). Four below-the-canopy measurements were repeated at three different locations in each plot. We used the MTA as the measure of the mean leaf inclination angle or mean tilt angle of the wheat canopy since previous work had shown this to be highly correlated with the results obtained using a 3-D digitizer (Shibayama and Watanabe, 2006).

On randomly selected 3-plant hills in each plot, the plant heights (PH, cm) above ground level were manually measured and averaged. The leaf greenness (used as an indicator of leaf chlorophyll content per unit leaf area) was measured using a handheld optical sensor (SPAD-502, Konica Minolta Holdings, Inc., Tokyo, Japan). Inada (1963) found that the deepness of the green color of intact leaves, optically measured in the visible and near infrared bands, was linearly correlated with the chlorophyll content per unit leaf area. Based on these findings, the sensor used was developed in the "Soil and Plant Analyzer Development" program sponsored by the Ministry of Agriculture, Forestry and Fisheries. (The term "SPAD" originates from the abbreviation of the program title). The digital reading of the SPAD-502, commonly known as the "SPAD-value" or "SPAD", is widely used as an indicator of the chlorophyll content of a single leaf. We used the value of "SPAD" as a direct measure of leaf greenness without converting the value into chlorophyll content because the plant material being tested was restricted to a single crop species (Inada, 1985). We measured and averaged the values of SPAD recorded at the middle parts of 15 randomly selected upper leaf blades in each canopy.

Another experiment with the same experimental design was carried out using the same field in the 2005-2006 cropping season. In the second experiment, the wheat variety 'Kinunonami' (instead of 'Ayahikari'), and the variety 'Norin-61' were both sown in the first week of November. The variety 'Norin-61' was used throughout the two experiments.

3. Radiometric observations

Reflectance and polarization were observed on three days: 5 and 27 April, and 10 May, 2005. The wheat plants were at the stem-elongation stage on 5 April, at the heading stage on 27 April and at the early-ripening stage on 10 May. The observations on the first two days were carried out between 10:00 and 12:00 (JST), and from 12:30 to 14:30 on the last day. The optical sensing unit of the spectropolarimeter was set on a 1.6 m-high tripod that stood at the north end of each plot, and observations were carried out with values for Zvof 15°, 30°, 45°, 60°, and 75°. The field of view (FOV) of the optical system was 10°. The variation in the area of the viewing ellipse of the sensor (depending on Zvand distance between the sensor and the target) was not considered. Sensor-target distance did not affect on the observed polarization when the crop canopies

were well-developed (Shibayama and Akita, 2002). The azimuth direction of the view was always oriented towards the sun. The *Zs* range during the observations varied from 37° to 32° on 5 April, 32° to 22° on 27 April, and 22° to 42° on 10 May. Measurements were carried out once for each value of *Zv*.

To validate the initial model used for predicting MTA, we carried out radiometric and plant canopy parameter measurements on 28 April, 2006. Two radiometric observations for each plot were repeated at values for Zv of 45°, 60°, and 75°. In addition to the on-site measurements for the 4 variables of plant canopy parameters described above, we randomly selected 3 plants in each plot and measured the heights of the top of the panicles and the top of the leaves above the ground.

4. ANOVA tests

The radiometric variables and canopy parameters measured on 27 April and 10 May were evaluated using an analysis of variance test for a completely randomized four-factor factorial experiment carried out twice using a software package for microcomputers (SAS Institute Inc., 2001). The statistical model used was a linear combination of the factors "variety", "basal dressing", "jointing-stage topdressing", and "bootingstage topdressing" $(2 \times 3 \times 2 \times 2)$. In the analysis of the data set for 5 April, the factor "booting-stage topdressing" was excluded. The independent variables tested were the 8-band R(Zv) and Q(Zv), where the values of Zv ranged from 15° to 75° at 15° intervals, and the 8-band Q(Zv). In addition, the NDVI(15) and PVR(15) that the NDVI and PVR measured at the Zv value of 15° were included in the analysis. The Zv value of 15° was selected in place of the nadir view $(Zv=0^{\circ})$ that was excluded from the measurements.

5. Pooling of regression equations

"Pooling of regression equations" is one of the possible techniques that can be used to improve the accuracy of predictions using more than one regression model (obtained separately for different crops, varieties, districts, and/or times). Referring to Kawabata (1982), we applied the method for pooling regression equations that relates a radiometric variable such as the polarized reflectance to the plant canopy parameters, mainly the MTA. Putting the superscript "(k)" on each statistic discriminates the observation set; the pair of regression coefficients (slopes, $b_1^{(1)}$ and $b_1^{(2)}$) of two linear regression equations are then statistically tested using the following procedure. The sample sizes of the observation sets (1) and (2) are $n^{(1)}$ and $n^{(2)}$, respectively, and the sample means are $\overline{x}^{(1)}$ and $\overline{y}^{(1)}$, for (1) and $\overline{x}^{(2)}$ and $\overline{y}^{(2)}$, for (2). The sums of squares are denoted by the symbols $S_{xx}^{(1)}$, $S_{yy}^{(1)}$, $S_{xx}^{(2)}$, and $S_{yy}^{(2)}$. The residual sums of squares are denoted as $Se^{(1)}$ and $Se^{(2)}$, respectively. Then, the null hypothesis $H_0: \beta_1^{(1)}=\beta_1^{(2)}$

can be rejected if:

$$t = \frac{b_1^{(1)} - b_1^{(2)}}{s[b_1^{(1)} - b_1^{(2)}]} \tag{4}$$

is larger than "Student's" *t* with $n^{(1)}+n^{(2)}-4$ degree of freedom (= *t* ($n^{(1)}+n^{(2)}-4$, *p*), *p* : level of significance), where $s[b_1^{(1)}-b_1^{(2)}]$ is the estimate of standard error that is obtained by:

$$s[b_{I}^{(1)}-b_{I}^{(2)}] = \sqrt{(1/S_{xx}^{(1)}+1/S_{xx}^{(2)}) Ve}$$
(5).

Hypothesizing a common population variance of regression residual, the best estimate *Ve*, used in the equation above, is defined as:

$$Ve = \frac{Se^{(1)} + Se^{(2)}}{n^{(1)} + n^{(2)} - 4}$$
(6).

In case the two regression coefficients can be considered equal, b_1 denotes the common regression coefficient, which is obtained by:

$$b_{I} = \frac{b_{I}^{(1)} S_{xx}^{(1)} + b_{I}^{(2)} S_{xx}^{(2)}}{S_{xx}^{(1)} + S_{xx}^{(2)}}$$
(7).

Next, the null hypothesis $H_0: \beta_0^{(1)} = \beta_0^{(2)}$, which means the intercepts of the regression equations are the same, can be rejected if:

$$t = \frac{b_{0^{*}}^{(1)} - b_{0^{*}}^{(2)}}{\sqrt{\left\{\frac{1}{n^{(1)}} + \frac{1}{n^{(2)}} + \frac{(\overline{x}^{(1)} - \overline{x}^{(2)})^{2}}{S_{xx}^{(1)} + S_{xx}^{(2)}}\right\} Ve^{*}}}$$
(8)

exceeds $t(n^{(1)}+n^{(2)}-3, p)$, where $b_{0*}^{(1)}$ and $b_{0*}^{(2)}$ are the estimates of intercepts obtained by:

$$b_{0^*}^{(1)} = \overline{y}^{(1)} - b_I \overline{x}^{(1)}$$
, and $b_{0^*}^{(2)} = \overline{y}^{(2)} - b_I \overline{x}^{(2)}$ (9),

and the estimate of variance of the regression residual Ve* is denoted afresh as:

$$Ve *= \{S_{yy}^{(1)} + S_{yy}^{(2)} - b_I^2 (S_{xx}^{(1)} + S_{xx}^{(2)})\} / (n^{(1)} + n^{(2)} - 3)$$
(10).

Results and Discussion

1. The plant canopy parameters and the grain yields

The plant canopy parameters examined were LAI and MTA (measured with a LAI-2000 Plant Canopy Analyzer), plant height (PH), and the color of the leaves (SPAD-value) measured with a SPAD-502. Heading was observed on 21 April for 'Ayahikari', and 26 April for 'Norin-61'. To compare the seasonal changes in the plant canopy parameters, we calculated the mean, maximum and minimum values of each parameter for each variety throughout all plots (Fig. 1). The minimum LAI of 1.27 was recorded for a plot of 'Norin-61' on 5 April, and the maximum LAI of 5.74 was recorded for a plot of 'Norin-61' on 27 April. The MTA varied from 48°, recorded for a plot of 'Norin-61' on 5 April, to 66° for a plot of 'Ayahikari' on 10 May.



Fig. 1. The plant canopy parameters of leaf area index (LAI), mean tip angle (MTA), plant height (PH), and leaf greenness (SPAD) on 2 wheat varieties measured on 3 different dates.

The averages of MTA in both varieties increased by several degrees during the experimental period. Overall, this indicates that the leaves became more erect from stem-elongation to the early maturing stage (Udagawa, 1980). The LAI of 'Norin-61' and the PH of 'Ayahikari' stabilized after the heading stage. The SPAD of 'Ayahikari' was higher than that of 'Norin-61' and slightly decreased at around the heading stage.

The grain yields in 'Norin-61' plots varied from 260 g m² to 562 g m², and the mean value was 425 g m². In the plots of 'Ayahikari', grain yields were 293 g m² to 620 g m² with the mean of 479 g m². The average yield in the experimental plots was at the expected standard yield level (Sato et al., 1992a, b; Yoshida et al., 2001; Fukushima et al., 2003), and varied in response to the different rates of fertilization. Hence, the trial plots were an appropriate test subject for the remote sensing experiment.

2. The effects of fertilization conditions at the different stages of wheat development

Measurements were carried out at three developmental stages in the later growing period. We carried out a separate statistical analysis on each data set collected at each stage of development because the experimental treatments were not fully completed before the booting-stage topdressing was applied in mid-April. The wheat plants were at the stem-elongation stage on the first measurement date of 5 April, about a month after the application of the jointing-stage topdressing. The second measurement was carried out on 27 April when the panicles had emerged, just underneath the canopy surfaces. The third measurement was carried out on 10 May, at the early stage of ripening, when the panicles appeared above the canopy surfaces.

As mentioned above, the values of Zs obtained during optical measurement varied between 32° and 37° on 5 April, between 22° and 32° on 27 April, and

Zs		31°-32°			Whole data			
Date	Mean	95% confidence interval	Sample size	Mean	95% confidence interval	Sample size		
5 April	0.136ª	0.111-0.162	30	0.151ª	0.140 - 0.162	120		
27 April	0.216 ^b	0.176 - 0.256	17	0.175 ^b	0.165 - 0.185	240		
10 May	0.136ª	0.114 - 0.159	24	0.138ª	0.131 - 0.144	240		

Table 1. Means and 95% confidence intervals of Q660 measured on 3 different dates at the time when the solar zenith angle (*Zs*) varied only between 31° and 32° (left), and for the whole data set (right).

 $^{a, b}$: Different letters indicate that the means are significantly different at the 5% probability level.

between 22° and 42° on 10 May. In order to assess the effect of changes in canopy characteristics on polarization, we compared the observations made at a common Zs range: 31°-32° on the three measurement dates. In this instance, the Zs angular difference of less than 1° was ignored, and means of Q660 measured at Zs between 31° and 32° on each date were compared using Tukey-Kramer's HSD test (Kramer, 1956). The mean value for Q660 of 0.216% observed on 27 April was significantly (p < 0.05) larger than the remaining two mean values obtained on 4 April and 10 May (Table 1). Statistical analysis of all data at various values of Zs gave a similar result. The ranges of Zs recorded on 27 April and 10 May overlapped each other so much that the decrease in Q660 noted on 10 May might not have been attributable to any actual difference in Zs. On the other hand, the Zs values noted on 5 April were larger than those recorded on 27 April. One possible explanation for the decrease in polarization observed on 10 May was the 'shielding effect' due to the emerged panicles (Fitch et al., 1984; Shibayama, 2004).

Table 2 shows the results of the analysis of variance of the measured plant canopy parameters and some of the optical variables obtained on each measurement date, showing the F-obs values obtained for the factors "variety", "basal dressing", "jointing-stage topdressing", "booting-stage topdressing", and their interaction terms. The plant canopy parameters tested were LAI, MTA, PH and SPAD. As for the optical variables, we examined Q660(Zv'), NDVI(15) and PVR(15). To avoid possible type II errors in repeated statistical tests, it may be better to use 0.1% (*p*<0.001) as the level of significance. On the first measurement date of 5 April, none of the plant canopy parameters and optical variables were significantly different (p > 0.001) with regard to all of the factors of "variety", "basal dressing" and "jointing-stage topdressing". The "basal dressing" might have increased the LAI but only at the 1% significance level (0.01 > p > 0.001). Based on the results of these statistical analyses, the wheat canopies tested showed no significant difference with regard to any of the parameters recorded at the stem-elongation stage.

On the last measurement date of 10 May, the values of SPAD were significantly different (p < 0.001) with

regard to all the factors tested, including "booting-stage topdressing". The "basal dressing" had a significant effect on LAI, MTA and PH (p < 0.001). The "basal dressing" and "jointing-stage topdressing" also had a significant effect on MTA (p < 0.001) although "variety" and "booting-stage topdressing" did not have a significant effect (p > 0.05). These results suggest that the fertilization conditions and wheat variety significantly affected the plant canopy parameters measured at the ripening stage. However, the optical variables tested, Q660(Zv'), NDVI(15) and PVR(15), were hardly affected by these factors. As described above, we assume that the panicles emerging above the leaf layers of the canopy might have obstructed the incident and reflected light from the leaf layers beneath (Fitch et al., 1984; Shibayama, 2004; Shibayama and Watanabe, 2006).

At the heading stage on 27 April, F-obs values indicated that the factor "variety" had a significant effect on SPAD, and that "basal dressing" had a significant effect on LAI, PH and SPAD as well as Q660(Zv') (p<0.001). The factor "jointing-stage topdressing" only had a significant effect on SPAD (F-obs=70.4, p<0.001) and Q660(Zv') (F-obs=56.8, p<0.001)p < 0.001). Although the variance to error variance ratio (*F-obs*=9.1, 0.01 > p > 0.001) was not large enough to be conclusive, the factor "jointing-stage topdressing" might have had an effect on MTA. These results suggest that further analysis is needed to clarify the relationships among the 3 parameters, MTA, SPAD and Q660(Zv'). Based on the day-by-day statistical analysis presented above, the data of 27 April were the most suitable for further analysis in order to characterize the relationship between the optical parameters, fertilization conditions and plant canopy parameters.

3. Detailed analysis of the radiometric variables measured at the heading stage

(1) Effects of spectral band and Zv

We conducted the same statistical analyses to clarify the relationships among the radiometric variables, reflectance and polarized reflectance in the 8 wavelength bands observed at the several values of Zv at the heading stage, with regard to the factors of fertilization conditions and varieties (Fig. 2). The Table 2. Summary of the analysis of variance tests applied to the plant canopy parameters and the radiometric variables. The *F-obs* values obtained for the factors and their interaction terms are presented for the values of $F_{a,ol}$ and $F_{a,ool}$.

		Jointing stage					
Variable \setminus Factor	Variety (V)	Basal dressing (BD)	topdressing (JT)	$V \times BD$	$V \times JT$		
LAI	0.00	8.81	0.80	0.72	0.19		
MTA	0.24	1.45	2.08	1.43	0.02		
PH	0.12	1.35	0.00	1.52	0.26		
SPAD	2.46	5.45	0.96	1.13	1.48		
Q660(Zv')	0.03	1.96	0.00	0.28	2.89		
NDVI(15)	7.38	1.27	0.73	0.47	0.08		
PVR(15)	2.63	0.57	0.01	1.93	3.06		
Degrees of freedom	1	2	1	2	1		
$F_{0.01}$	8.53	6.23	8.53	6.23	8.53		
$F_{0.001}$	16.12	10.97	16.12	10.97	16.12		

Measurement date: 5 April, 2005

Measurement date: 27 April, 2005

Variable \ Factor	Variety (V)	Basal dressing (BD)	Jointing stage topdressing (JT)	Booting stage topdressing (BT)	V imes BD	$V \times IT$	V imes BT
LAI	1.39	11.22	3.44	0.78	0.07	0.05	3.34
MTA	1.75	7.12	9.06	0.18	0.00	0.06	4.22
РН	1.70	11.05	1.04	0.02	0.27	0.10	0.48
SPAD	25.74	10.82	70.36	3.38	0.75	4.10	6.03
Q660(Zv')	0.01	8.99	56.85	0.06	1.68	0.03	0.26
NDVI(15)	0.01	1.24	0.59	0.73	0.08	0.18	1.48
PVR(15)	0.00	0.29	0.09	0.53	0.03	0.67	0.14
Degrees of freedom	1	2	1	1	2	1	1
$F_{0.01}$	7.35	5.21	7.35	7.35	5.21	7.35	7.35
$F_{0.001}$	12.72	8.33	12.72	12.72	8.33	12.72	12.72

Measurement date: 10 May, 2005

Variable \ Factor	Variety (V)	Basal dressing (BD)	Jointing stage topdressing (JT)	Booting stage topdressing (BT)	V imes BD	$v \times JT$	V imes BT
LAI	0.06	13.45	7.94	0.14	0.13	0.09	4.64
MTA	1.21	9.99	26.97	3.00	0.43	2.40	6.98
РН	7.51	9.10	3.35	0.00	1.04	0.15	0.23
SPAD	21.20	9.44	45.63	32.94	0.31	5.57	13.00
Q660(Zv')	0.29	0.59	3.47	0.19	0.61	0.59	3.74
NDVI(15)	2.22	1.96	2.61	0.61	0.18	0.16	0.02
PVR(15)	0.00	5.34	4.10	0.00	0.59	0.88	0.22

Degrees of freedom, values of $F_{0.01}$ and $F_{0.001}$ are the same as those which shown at the bottom in the middle table for 27 April measurements.

factor "jointing-stage topdressing" influenced the polarized reflectance in the 3 visible bands (490, 560 and 660 nm) measured at the Zv values of 60° and 75°. At all bands except the visible wavelength range, Q2200(60°) and Q2200(75°), which are the polarized reflectance at the 2200 nm band measured at Zv

values of 60° and 75°, successfully detected the differences due to "jointing-stage topdressing". Neither reflectance nor polarized reflectance detected any difference if the Zv was 45° or below. Among the radiometric variables tested, the Q660(Zv') was the best indicator for detecting the differences due to the



Fig. 2. Graphical summary of analysis of variance tests (*Fobs* values for the 3 factors tested) for 8-band reflectance and polarized reflectance measured at 5-view zenith angles on 27 April, 2005. The variable Q660(Zv'), at the bottom of the chart, provided the largest *Fobs* (= 56.8) for the factor "jointing-stage topdressing" (See text).

jointing-stage topdressing. Estimating the polarized reflectance at the interpolated Zv derived from Zsusing Eq. 3 performed better than measurements at any fixed viewing angles. Among the 8-band polarized reflectance range adjusted for Zv, the 3 visible bands of 490, 560 and 660 nm were the best for detecting the differences due to the jointing-stage topdressing, and the 2200 nm band was the second best (Table 3). The transparency of plant tissues to near-infrared range light explained the insensitivity of polarization shown in the 830, 1150 and 1250 nm bands. At present, the optical mechanisms of variation in polarization at the 2200 nm band for differently fertilized plant canopies are not clear. Further studies including investigations on the somewhat different properties of polarization in the two short-wave infrared bands, 1650 nm and 2200 nm are required. The clear contrast between the

high scores for *F-obs* obtained using the 3 visible-bands and the short-wave infrared-band Q(Zv')s for the factor "jointing-stage topdressing", when compared with the lower scores obtained for the other factors, raises the question of which plant canopy parameters influenced the polarized reflectance observed from the canopies of the differently fertilized trial plots.

(2) Canopy parameters that influenced the polarization

In order to clarify the aforementioned problem, we applied a correlation analysis to the data set comprising the 8-band polarized reflectance in which Zv was adjusted to Zv' and the four plant canopy parameters of LAI, MTA, PH and SPAD (Table 4). Correlation analysis outputs indicated close correlation between the MTA and polarized reflectance in the bands centered at 490, 560, 660 and 2200 nm (r=-

Table 3. Summary of analysis of variance tests (*F-obs* values) for the 8-band polarized reflectance values measured on 27 April, 2005, against the experimental factors and their interaction terms shown. The polarized reflectance values were calculated using the *Zv* derived by Eq. 3 (see text).

	Variety	Basal dressing	Jointing stage topdressing	Booting stage topdressing			
Variable 📉 Factor	(V)	(BD)	(JT)	(BT)	$V \times BD$	$V \times JT$	$V \times BT$
Q490(Zv')	0.00	8.05	57.95	0.38	1.58	0.35	0.22
Q560(Zv')	0.01	8.11	52.89	0.08	0.56	0.34	0.01
Q660(Zv')	0.01	8.99	56.85	0.06	1.68	0.03	0.26
Q830(Zv')	0.50	0.11	1.10	1.58	0.97	1.54	0.12
Q1150(Zv')	0.43	0.06	1.65	1.45	1.06	1.69	0.14
Q1250(Zv')	0.97	0.24	1.47	1.29	0.95	3.02	0.07
Q1650(Zv')	0.72	3.56	12.10	0.00	0.07	3.23	0.77
Q2200(Zv')	0.01	6.39	34.99	0.17	2.03	0.24	0.03
Degrees of freedom	1	2	1	1	2	1	1
F _{0.01}	7.35	5.21	7.35	7.35	5.21	7.35	7.35
$F_{0.001}$	12.72	8.33	12.72	12.72	8.33	12.72	12.72

 $0.85 \sim -0.88$, n=12 observations, p < 0.001). The minus signs of the correlation coefficients suggested that the larger the MTA (equivalent to an increase in the area of erect leaf surfaces), the smaller the polarization of light reflected from the leaves. The high correlations between the MTA and polarized reflectance agreed with the results of a previous study (Shibayama and Watanabe, 2006). However, the SPAD readings for the *F-obs* values were also high for the jointing-stage topdressing (Table 2). Therefore, we examined the possibilities that the close relationship of the visibleband polarized reflectance and the MTA ascribed to the leaf color change. The SPAD readings had no significant correlations with the visible-band polarized reflectance (*r*=0.425~0.456, *n*=12, *p*>0.05). Moreover, no significant correlation was found between the MTA and the SPAD (r=0.355, p>0.2), and the partial correlation coefficients $(r_{xy, z})$ calculated for the visible-band polarized reflectance (x) and the MTA (y) subtracting the effect of SPAD (z) ranged from -0.830 to -0.861. Consequently, there was no obvious drop in the values of $r_{xy,z}$ by comparison with the simple correlation coefficients. This suggested the possibility that the MTA corresponded directly to the polarization. Although the jointing-stage topdressing deepened the green color of the leaf by increasing the chlorophyll content, the polarized reflected light was not associated with the leaf color but was correlated with leaf orientation. As shown in Fig. 3, the SPAD of 'Ayahikari' was higher than that of 'Norin-61' even though the overall ranges and levels of values of Q660(Zv') from the 2 varieties were similar. Polarized reflectance responded solely to the difference in fertilization conditions rather than to the difference in varieties. On the other hand, SPAD responded to

Table 4. Correlations between the plant canopy parameters and the 8-band polarized reflectance values obtained by the 27 April measurements.

	LAI	MTA	PH	SPAD
Q490(Zv')	0.690	-0.854 **	0.585	0.456
Q560(Zv')	0.711*	-0.878 **	0.622	0.448
Q660(Zv')	0.705	-0.853 **	0.605	0.425
Q830(Zv')	0.218	-0.196	0.190	0.196
Q1150(Zv')	0.203	-0.237	0.198	0.186
Q1250(Zv')	0.204	-0.263	0.214	0.317
Q1650(Zv')	0.679	-0.770*	0.652	0.378
Q2200(Zv')	0.712*	-0.834**	0.634	0.319

*, **: Significant at the 1% and 0.1% level, respectively.

both of these factors. This indicates that polarized reflectance is sensitive to the way in which MTA is affected by fertilization conditions but not to the color of the leaves. In terms of the wavelengths used, the near infrared range was less effective than the visible and short-wave infrared ranges in linking polarization and MTA. The short-wave infrared bands, especially the band centered at 2200 nm, showed a significant correlation with MTA (r=-0.834, p<0.001), however, the practical advantage of using short-wave infrared bands with or without visible-band polarization has not been examined in this study. Shibayama (2006) reported that complete 8-band polarization and reflectance measurement data captured in an artificial neural network could provide leaf orientation estimates for various crop species, including wheat, although the accuracy for each individual crop species needed to be improved. Our findings on the



Fig. 3. Effects of topdressing and variety on leaf greenness (SPAD) and polarized reflectance (*Q660(Zv'*)).

spectral characteristics of polarization suggest that some spectral bands might be better than others for maintaining accuracy while reducing measurement costs.

(3) A linear regression model for predicting MTA and validation of the results

Since the factor "booting-stage topdressing" did not significantly affect the Q660 (Zv'), we reduced the amount of data to 6 pairs of averaged MTA and Q660(Zv') values for each variety by calculating means of observations from the "booting-stage topdressing" and "no booting-stage topdressing" plots in both replicates ($n=24/(2\times 2)=6$ observations). First, we constructed a linear regression model:

$$Q660(Zv') = b_0 + b_1 \text{ MTA}$$
(11)

for each variety and applied the procedure for pooling of regression equations. The calculations produced *t*values of 0.099 with 8 degrees of freedom (d. f.), and 0.078 with 9 d. f. using Eq. 4 and Eq. 8, respectively. This indicated that both of the regression coefficients (b_l) and the intercepts (b_0) of the equations for the two varieties were not significantly different (p>0.5). Based on this result, we then pooled the models into a single regression equation:

$$Q660(Zv') = 1.44 - 0.021 \text{ MTA}$$
 (12)

which accounted for approximately 73% of the variation of the data (n=12, p<0.001). In this analysis, carried out for the two wheat varieties, a single linear regression model successfully described the relationship (Fig. 4). Consequently, we concluded that the polarized reflectance with Zv' could be used for predicting the MTA. The model performed successfully over the relatively narrow range of MTA,



Fig. 4. Scatter-plot of Q660(Zv') values versus the mean tip angles (MTA) measured for the canopies of the 2 wheat varieties tested on 27 April, 2005.

varying by only 7° at most.

The validation test for the regression model described in Eq. 12, carried out using the new data collected in 2006, indicated that the model underestimated MTA in the case of the variety 'Norin-61' but overestimated for the 'Kinunonami' canopies (Fig. 5). For the 'Norin-61' variety, the model predicted the MTA with a coefficient of determination (r^2) of 0.68 (n=6 observations, p<0.05)



Fig. 5. Scatter-plot of radiometric estimates of the mean tip angles (MTA) obtained using Eq. 4 versus MTAs measured using a Plant Canopy Analyzer (LAI-2000) in the subsequent cropping season.

and an RMSE of 2.9° compared to the Y=X (1:1). The plants of 'Norin-61' headed about 5 days before the measurement date. As for the 'Kinunonami' variety, these plants headed 8 days before the measurement date. The mean distance between the top of the panicles and the surface of the leaf layer was 0.08 cm (with a 1.15 cm 95% confidence interval, n=72observations) for 'Norin-61' and 5.4 cm (with a 0.73 cm 95% confidence interval, n=72 observations) for 'Kinunonami'. In the latter variety, the panicles were located above the leaf layer and intercepted at least part of the reflected light from the leaf layers. The reduced amount of polarized light decreased Q660(Zv'), which then led to an overestimation of MTA using Eq. 12. In contrast, the emerged panicles of the variety 'Norin-61' were located just underneath the leaf layer. We have not yet discovered the causes of the decrease in sensitivity of the model's predictions for the variety 'Norin-61'. If another measurement had been performed a few days earlier, it would have helped explain the causes. Unfortunately, however, the unusually wet local weather conditions in 2006, from the booting stage through to ripening, prevented more timely radiometric measurements.

We analyzed the 2006 data set in order to obtain a model (derived from Eq. 11) for the variety 'Norin-61' (Fig. 6). The model for 'Kinunonami' was excluded because of the poor relationship between polarization and MTA that might be caused by the emerged panicles. The same procedure was applied in order to obtain the *t*-value for the statistical test of the null hypothesis that the regression coefficient of Eq. 12



Fig. 6. Scatter-plot of Q660(Zv') values versus the mean tip angles (MTA) measured for canopies of each wheat variety on 27 April, 2005, and 28 April, 2006. The lines "a" and "b" were produced by the model: Q660(Zv')=C –0.0167 MTA, where C is 1.16 for the line "a" derived from 'Norin-61' and 'Ayahikari' measured in 2005, and 1.23 for the line "b" derived from 'Norin-61' measured in 2006. The data points for 'Kinumonami' were excluded from the regression calculations.

and that of the 2006 model for 'Norin-61' were equal. The t-value of 1.91 did not exceed t (14, 0.05) (= 2.145). This indicated that the two regression lines might be parallel. Generally speaking, it is usually recommended that the null hypothesis be accepted at a significance level of 20% or more, although accepting the null hypothesis at the 5% significance level is allowable if there is no rational reason for the different regression slopes obtained (Kawabata, 1982). Needless to say, further studies are needed to determine if the regression coefficients are equal. We tentatively assumed that the 2 equations had a common regression coefficient $(b_1 = -0.0167)$ for the two years of data. Further statistical tests indicated that the intercepts (b_0) of the 2 equations were significantly different because the *t*-value of 3.76 (> t(15, 0.01) = 2.95) was obtained for the test comparison between Eq. 12 and the 2006 model of 'Norin-61' Hence, the resultant model derived was:

Q660(Zv')=C-0.0167 MTA (13), Where, the constant, C, varied according to the measurement year (2005 or 2006). For the 2005 model of the 2 varieties, C was 1.16, and for the 2006 model of 'Norin-61' it was 1.23. The regression lines obtained



Fig. 7. Scatter-plot of the mean tip angles (MTA) estimated using the model Q660(Zv')=C -0.0167 MTA versus the MTA values measured for each wheat canopy at the heading stage in 2005 and 2006 (For details regarding the constant, C, see the caption for Fig. 6).

by using the model, showing a common slope with different intercepts, are graphically presented in Fig. 6. The correlation between the estimates obtained using the model and the measured MTA is shown on the scatter-plot in Fig. 7. The RMSE was 1.8° for the 18 observations. The accuracy level was comparable to the RMSE of 2.5° acquired by an artificial neural network which was used to capture all 8-band polarization and reflectance variables from 3 crop species (Shibayama, 2006). Although adjusting the intercept (C) in the model improved the prediction performance, the causes of the different levels of polarized reflectance remain unknown. Atmospheric effects and the stability of the instruments used, which were not included in this study, will need to be taken into account in the future and calibration carried out accordingly, just as it is for most other instruments used in the field. Assuming that the common regression coefficient is confirmed in retests, the practical calibrations required should be fairly simple. Despite the effects of the stage of plant development (the extent of panicle emergence above the canopy) and the irradiance and/or instrument conditions on performance, the simple model of polarized reflectance measured in a single visible band successfully proved the potential capabilities of MTA predictions for wheat canopies. Further investigation using varieties that are more sensitive to fertilization conditions in their MTA will be required to validate and test the usefulness of the

model in practice. Future studies will investigate the causes of the changes in the intercept produced by the model and refine the method further.

Conclusions

In this study, we conducted an experiment to measure the mean leaf inclination angle (= mean tip angle, MTA) of wheat canopies using polarized reflectance measurements. Statistical analysis revealed that the heading stage of plant development was the most appropriate time for polarization assessment. If measurements were carried out later, at the ripening stage, the panicles emerging above the canopy surface intercepted the reflected light from the leaf layers whereas, if measurements were carried out earlier, about a month before heading during the stemelongation stage, there were no detectable differences in the canopies. The visible range of the spectrum, including blue, green, and red bands, proved the best for detecting the MTA change resulting from jointing-stage topdressing. The short-wave infrared band centered at 2200 nm was the next best while the near infrared range was least effective. View zenith angles of 60° and 75° proved to be the most suitable, but adjusting the view zenith angle in accordance with the solar zenith angle at the time of measurement improved the performance remarkably. The view-angle adjusting procedure was verified successfully. Although leaf color became greener as the amount of fertilizer added increased, leaf color itself had little effect on the polarization, which corresponded more closely to leaf orientation geometry. A simple linear regression analysis produced an equation for predicting the MTA of the canopies of two wheat varieties using the viewadjusted polarized reflectance in the band centered at 660 nm. According to the validation test carried out in the subsequent cropping season on the same wheat variety, the equation used provided reasonable predictions although underestimations were noted at higher (more erect) MTAs. The cause of this degradation of sensitivity is still unknown and will need to be examined in a future study. The model did not perform as well on the other wheat variety tested and this was largely attributable to the panicles emerging above the leaf layers. These results emphasize the importance of plant growth stages when making use of optical measurements. As a result of these experiments, we produced two different models for the 2 years and 2 varieties studied. Statistical tests allowed us to assume a common regression coefficient (slope) in the models. Therefore, we tested a combined model with a single slope and a varying intercept, which performed successfully over the MTA range of 55°-65° with an RMSE of 1.8°. To further refine this technique, more study will be needed in order to improve the stability of the model and to provide a practical means for calibrating the measurement system in the field.

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* In Japanese with English abstract.

*** In Japanese. The authors of this paper translated the title.

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