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Using leaf spectral reflectance to monitor the effects of shading on nicotine content in tobacco leaves

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ABSTRACT

The objectives of this study were to determine the relationship between tobacco leaf nicotine content and leaf spectral reflectance, to identify specific regions of the light spectrum that could be used to detect nicotine content, and to develop spectral indices and quantitative models for quick and accurate estimation of nicotine content in flue-cured tobacco leaves under different shade conditions. The flue-cured tobacco cultivar Yunyan 87 was subjected to shade treatments in the field extending from the root elongation stage through the vigorous growth and maturing stages. Three shade treatments of 85% (S1), 65% (S2), and 45% (S3) of full solar radiation (S0, control) were applied and the nicotine, pigment, and nitrogen content and spectral reflectance of tobacco leaves were measured over a time course. Normalized difference vegetation indices (NDVI) and spectral ratio (SR) indices based on leaf reflectance spectra from 350 to 2500 nm and correlations with nicotine content were determined. Shading significantly increased the nicotine content during the maturing stage. Significant differences in reflectance were measured for different shade treatments during the vigorous growth stage particularly at 350–700 nm in the visible range and 750–1000 nm in the near-infrared range. There was a significant correlation between nicotine content and nitrogen and pigment content. The regions of the spectrum that gave the best indication of nicotine content in flue-cured tobacco leaves were 420–750 nm in the visible range in combination with 1400–1800 nm and 2000–2400 nm in the short-wave infrared range. Optimal spectral indices for SR (R450, R500) and NDVI (R2150, R610) were derived from measurements in these ranges. We also established linear models based on the spectral indices derived from field data gathered in 2012 for NDVI (R2150, R610), SR (450, 500), stepwise multiple linear regression (SMLR), and a back-propagation (BP) neural network with R^2 values of 0.796, 0.810, 0.842, and 0.968, respectively. The linear models were validated using an independent data set from 2011 with RMSE values of 0.784, 0.958, 0.883, and 0.109 for NDVI (R2150, R610), SR (450, 500), SMLR, and the BP neural network, respectively. The results indicated that hyperspectral remote sensing can be used for quick and accurate monitoring of the leaf nicotine content and shading status of flue-cured tobacco crops.

1. Introduction

Since the mid-1970s, interactions between air pollution and solar radiation have contributed to climate change. One consequence of the interaction is an increase in aerosols which can cause dimming or shading of solar radiation. Brown clouds were shown to contribute to global dimming by causing a large reduction in sunlight, with the largest reduction (40%) occurring in the visible and ultraviolet (UV) wavelengths (Ramanathan and Feng, 2009). Global radiation has been reduced by as much as 2% on average per decade over large regions of Africa, Asia, Europe, and North America (Stanhill and Cohen, 2001). Global dimming has become a major challenge for crop production in many areas of the world (Li et al., 2010; Mu et al., 2010).

Plants have the ability to adapt to different light regimes through changes in external and internal properties of leaves and alterations to canopy structure (Boardman, 1977; Bjorkman and Demmig-Adams, 1994). Small decreases in direct radiation, if accompanied by increases in the fraction of diffuse radiation, will cause substantial increases in leaf conductance and photosynthesis (Healey et al., 1998). Crops grown under shade conditions will show increases in leaf area and the rate of dry matter production (Blackman and Wilson, 1951). Similar results were reported for trees, maize, soybean, peanut, and thuja (Lloyd et al., 1995; Wang et al., 1994).

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Agronomic benefits may result from a reduction in radiation under semi-arid conditions (Cohen et al., 1999). In general, shading can lead to marked changes in plant morphology and architecture and may affect the rate of plant development (Stuefer and Huber, 1998).

Measurement of the physiological parameters of a crop can be used to detect and study stress caused by low light conditions (Cruz, 1997), provide guidance for precise agriculture practices and efficient field management (Chameides et al., 1999; Landau et al., 1998), and allow the prediction of crop yield and quality. Traditional methods of physiological monitoring often rely on sampling in the field followed by analysis in the laboratory (Christophe et al., 2006; Mu et al., 2010). Although this approach is reliable, it does not satisfy the requirement for real-time, rapid, and non-destructive analysis of crop physiology. To overcome this limitation, remote sensing technology has been developed as a powerful tool for in situ measurements that give quick and accurate data on chemical composition with no damage to the crop (Chen et al., 2010; Comar et al., 2012; Datt, 1999; Teng et al., 2010). The link between crop spectral reflectance and abiotic and biotic stress has been well established (Osborne et al., 2002; Penuelas et al., 1994). Numerous studies have focused on monitoring crop growth (Zhao et al., 2003), yield (Serrano et al., 2000), and the content of biochemical components (Kokaly and Clark, 1999) by remote sensing.

Flue-cured tobacco (*Nicotiana tabacum* L.) is one of the most economically important crops in China, which has the largest planted area and total yield of tobacco in the world. There are many potential uses for tobacco in food and medical applications. Nicotine, leaf protein, amino acids, solanesol, pectin, and rutin isolated from tobacco leaves all have high value in the fields of food and medicine.

Nicotine is the primary alkaloid in the leaves of most *Nicotiana* species (Doolittle et al., 1995). Nicotine concentration is a key indicator of tobacco quality (Liu et al., 2008) and contributes significantly to smoking properties. Nicotine is harmful to humans and the environment (Novotny and Zhao, 1999), but is readily available and approved by the United States Food and Drug Administration for prescription use in humans (Domino et al., 2000). Nicotine is available in a range of products aimed at individuals trying to stop smoking and is also used in insecticides (Corkery et al., 2010). There is a close relationship between nicotine and the content of nitrogen and pigments in tobacco (Howes, 1974; Liu et al., 2005; Rizvi et al., 1989). Rapid monitoring of changes in nicotine content caused by shading of tobacco leaves could aid in implementing good agricultural practices. The objectives of this study were (1) to test the response of flue-cured tobacco to different degrees of shading; (2) to determine the wavelengths that specifically reflect nicotine content in flue-cured tobacco and to define new spectral indices; (3) to quantify the relationship between nicotine content and the spectral ratio (SR) index and the normalized difference vegetation index (NDVI); and (4) to identify reliable regression models for estimating the nicotine content of flue-cured tobacco.

2. Materials and methods

2.1. Experimental design

The experiment was conducted under open field conditions in Fangcheng City (112°54′E 33°15′N), Henan Province, China during 2011 and 2012 using the flue-cured tobacco cultivar Yunyan87. The soil was classified as a yellow loam soil (Alfisol in U.S. taxonomy) with 0.72 g kg^{-1} total N, 55.01 mg kg^{-1} alkali-hydrolysable nitrogen, 135.21 mg kg^{-1} available potassium, 18.0 mg kg^{-1} available phosphate, and 11.45 g kg^{-1} organic matter (0–0.25 m soil depth). The top of the tobacco canopy was covered with one, two, or three layers of white polyethylene netting from planting until harvest to provide three shading treatments with approximately 85% (S1), 65% (S2), and 45% (S3) of full solar radiation. No shading was set as the control (S0). The experiment was a randomized complete block design using a factorial arrangement of treatments with three replications.

The netting was placed on 2.8 m tall arcuate iron brackets with a bottom width of 6 m and a length of 6 m. The brackets were positioned in a north-to-south direction in random order, and the bottom 1 m of the south and north openings were open to allow for ventilation. Tobacco plants were transplanted on 26 April 2011 and 25 April 2012 with a spacing of 1.2 m × 0.6 m. Standard local cultivation and management methods for producing high-quality tobacco were used. Temperature and humidity values were recorded every half an hour by an automatic hygrothermograph (WYSTH000T11-2-0.5, Wangyunshan, China) and are shown in Table 1.

2.2. Measurements and methods

2.2.1. Leaf spectral measurements

Leaf reflectance was measured every 15 days until harvest beginning 30 days after transplant to the field using a Field Spec Pro FR spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) equipped with a leaf clip. Reflectance between 350 nm and 1000 nm was recorded with a sampling interval of 1.40 nm and a resolution of 3 nm. Reflectance between 1000 nm and 2500 nm was recorded with a sampling interval of 2 nm and a resolution of 10 nm. Measurements were taken under clear sky conditions between 10:00 and 14:00 Beijing local time. Use of the leaf clip allowed measurements to be made in a confined environment with a stable simulated light source to decrease error in the spectral data. Three leaves from each plant were measured and three plants were measured per treatment. For each leaf sample, reflectance was measured at five locations: the leaf tip and the upper left, upper right, lower left, and lower right leaf surface. For each location, five reflectance curves were made and the average reflectance of the total of 75 reflectance measurements was used as the final value for spectral reflectance of a given leaf. A standard white reference panel was used for calibration before each test and the scan time was 0.2 s for each curve.

2.2.2. Plant collection and measurements

Samples were collected from the plants measured for reflectance in the field using a 0.4 cm diameter hole punch. Samples weighed approximately 0.2 g each. After transfer to the laboratory, selected samples were placed in a 95% ethanol solution and left to stand for 24 h in the dark. After the dark treatment, the leaves were white-green in color. Leaf pigments (chlorophyll a, chlorophyll b and carotenoid) densities were measured using a colorimetric spectrophotometer (Jasco mod. 560-V, Jasco, Tokyo, Japan). The concentration of extracted pigments was calculated from the absorbance values at 665, 649, and 470 nm (Lichtenthaler, 1987).

Green leaves were separated and dried at 105 °C for 30 min and then at 60 °C until they reached a constant weight. Dried leaf samples were ground finely enough to pass through a 0.3 mm screen and leaf nitrogen content was determined by the micro-Kjeldahl method (Zhang and Qu, 2003).

The nicotine concentration in samples was determined using a high-performance liquid chromatography system (Waters 2690, Japan) equipped with a Nova-pak C-18 column (5 μm, 3.9 mm × 150 mm) and an UV detector operating at a wavelength of 260 nm. The column was eluted with a mixture of acetonitrile:distilled water:triethylamine (20:80:0.2, v/v/v) at a flow rate of 0.7 ml min^{-1} at room temperature (25 °C). Quantitative data were obtained by comparing the peak areas of the query