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RELATING LIGHT REFLECTANCE OF A LEAF TO LIGHT ABSORBANCE BY FOLIAR CHLOROPHYLL AS A PRELIMINARY APPROACH TO DETECTION OF FOREST CONDITION BY REMOTE SENSING

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ABSTRACT

A trial for estimating leaf chlorophyll content based on leaf reflectance was conducted for three major plant species in Daegu, South Korea. To estimate the chlorophyll content in a leaf, we measured the leaf elongation and related the leaf reflectance to its absorbance. Leaves of the target species were found to start elongating around the middle of April and continued elongation until the middle of May, indicating that April and May in South Korea are the proper period to detect differences in leaf size. Reflectance increased progressively from the first survey date (April 14) to the last survey date (May 16); however, the reflectance became saturated on May 10. There was the possibility to detect within-species and/or among-species variations with temporal changes in leaf reflectance, especially at NIR (near infrared) ranges during the leaf-growing period. Absorbance by a leaf and its trend during the leaf-growing period tended to be contrary to leaf reflectance, and gradually increased with leaf elongation. The chlorophyll content was shown to increase gradually and continuously, regardless of trends in leaf reflectance. Correlation maps combining the NIR range with wavelengths other than blue for NDVI (normalized difference vegetation index) were shown to explain the chlorophyll content quite well.

Key words: Leaf reflectance, Leaf absorbance, Chlorophyll content, Vegetation index, Correlation map

INTRODUCTION

Remote sensing has been widely applied to monitor surface conditions worldwide using sensors onboard satellites to detect the reflectance and scattering derived from a variety of surface conditions, including land uses, textures, roughness, and greenness. Multispectral images have long been used to quantify the reflectance characteristics of target plants in a few relatively wide wavelength bands, often representing blue, green, red, and near infrared (NIR) wavelengths (Lillesand *et al*., 2004). Improvements in computer and imaging technology have made such systems more affordable, while the ability to schedule aerial image acquisitions has allowed researchers to collect ground data simultaneously with images. In an effort to exploit the improved spatial and spectral resolution of airborne hyperspectral imaging systems, a number of researchers have investigated their use in precise analyses (e.g., Kim *et al*., 2001; Yang *et al*., 2002). A recent development in analysis using aerial hyperspectral imaging systems has enabled quantification of the reflectance in a large number of relatively narrow wavelength bands (Jang *et al*., 2006, 2009; Sudduth *et al*., 2015).

The reflectance and absorbance of a leaf differ because pigments in a leaf prefer to use sunlight at a specific wavelength to synthesize organic nutrients via photosynthesis (Ding *et al*., 2009). Although almost all visible light can be used for photosynthesis and about 80% of the visible light arriving at a leaf is absorbed, chlorophyll absorbs only a portion of this light; therefore, there are peaks located in the red (600 to 700 nm) and blue (400 to 500 nm) ranges of the spectrum (Sims and Gamon, 2002).

Many researchers have attempted to estimate chlorophyll content using light reflectance and/or the vegetation index (VI) (ratio combining information contained in two spectral bands to derive vegetation greenness and canopy characteristics) because measurement of spectral reflectance provides a rapid, nondestructive method for pigment estimation (Thomas and Gausman, 1977; Sims and Gamon, 2002). A variety of trials have been conducted to estimate chlorophyll content. Wu *et al*. (2008) attempted to estimate chlorophyll content from hyperspectral vegetation indices, which included the normalized difference vegetation index (NDVI), modified simple ratio (MSR) index and modified chlorophyll absorption ratio index (MCARI). Zarco-Tejada *et al*. (2004) used two types of high-spatial hyperspectral remote sensing systems, a Reflective Optics System Imaging Spectrometer (ROSIS) and a Digital Airborne Imaging Spectrometer (DAIS), to investigate leaf biochemical constituents in open crop canopies. Moreover, hyperspectral remote sensing technology has been applied to monitor water quality by estimating chlorophyll and/or turbidity within a lake or a river (Sudduth *et al*., 2015). However, many investigators prefer indirect methods in which already existing models are used to estimate the chlorophyll content instead of directly calibrating it by quantifying the amount (Richardson *et al*., 2002; Maire *et al*., 2008).

One representative case in which the indirect method is employed is when only optical remote sensing is used to estimate vegetation conditions. In this process, only the reflectance derived from the digital number on a pixel is applied to estimate the condition of the vegetation; accordingly, there can be errors that should be corrected to enable exact quantification. Therefore, if a model that employs leaf reflectance as an independent variable is developed to relate the chlorophyll-based factors, it may be possible to estimate the leaf extract absorbance, chlorophyll content, and growth conditions for vegetation using only reflectance, which can be obtained from a satellite image. In this study, several approaches were applied to three major plant species in Daegu, South Korea to reveal the relationship between leaf reflectance and leaf extract absorbance, which is the key indicator utilized for precise estimation of the chlorophyll content using the leaf reflectance.

MATERIALS AND METHODS

Study sites and target species: This study was conducted at six urban forests in Daegu, South Korea, which is located at 35°36′N to 36°01′N and 128°21′ to 128°46′ E (Fig. 1). These forests, which are now used as parks, have been isolated by damage and reduction derived from the enlargement of industrial complexes and residential areas to accommodate the 2.5 million citizens of Daegu. Two categories of forests were studied (Fig. 1), those within urban areas, such as Beomeo (Fig. 1a), Daebul (Fig. 1b), Galsan (Fig. 1c) and Yeonam (Fig. 1d) parks, and natural forests located in peripheral areas of the city, such as Daeduk (Fig. 1e) and Palgong (Fig. 1f) mountains. We selected three dominant plant species, Chinese cork oak (*Quercusvariabilis*; Qv), sawtooth oak (*Quercusacutissima*; Qa), and false acacia (*Robiniapseudoacacia*; Rp), as target species. These plants are found in all of the study forests and commonly present in urban forests and forest edges in South Korea (Song *et al*., 2005; Beon and Bartsch, 2003). The target species were surveyed in April and May, which is when leaves of deciduous trees rapidly reach their maximum size after buds break. This season is also the critical period for detection of differences among various plant species because the type of pigments and their volume within a unit leaf in this period differ and are detectable in all species before becoming saturated (Sims and Gamon, 2002). This phenomenon can be a good method for discrimination of individual species.

Surveying and analyzing the leaf elongation and light reflectance on a leaf of the target species: Because there are usually differences in leaf elongation among plant species during the growing season, leaf buds of the target species were visually checked to determine if the buds had started to open and extend leaves. As trees started to sprout their leaves, five pieces of leaves (the first unfolded on each stem) were collected from an individual tree in each forest during every sampling event. The lengths of the major axes of individual leaves were measured using a ruler to compare phenological leaf growth among plant species. This was done because leaf growth has often been studied during the linear phase of elongation, which provides an easy system for analysis of the responses of leaf growth to environmental conditions (Passioura and Gardner, 1990; Salah and Tardieu, 1997).

The leaf reflectance and light absorbance within a unit leaf area were calibrated for five different leaves of each species at each site. Leaves were sampled twice a week from April to late May in 2011, which covered most of the leaf-growing period of the target plants during spring. As soon as a leaf was collected from a tree, light reflectance was calculated using a hand-held spectro-radiometer (GER3700; Spectra Vista Corporation, USA). This instrument recorded upwelling radiance from the leaf in the wavelength range of 400 to 800 nm at a 1 nm interval. Light reflected from the target entered the spectrometer through a fiber optic cable with a 1° field of view foreoptic attachment that was pointed at the leaf surface approximately 90° from the principal vertical plane of the sun at a 40° angle from nadir. This protocol, which is similar to those suggested by Mobley (1999) and Sudduth *et al*. (2015), was used to minimize the effects of sun glint on the data collection process. In this orientation, the 1° foreoptic collected light from an ellipse of approximately 4 cm^2 on the leaf surface. Data from the reference panel were compared to verify that radiance did not change appreciably during the measurement. During post-processing, radiance values on each leaf were divided by the preceding reference panel data to obtain the apparent reflectance of the leaf surface as a function of wavelength. Leaf reflectance was calculated five times using different leaves from a tree, and the average was used for the leaf reflectance of the target species in each forest. Reflectance was measured in 80 wavelength bands from 400 nm to 800 nm, where each band covered 5 nm.

Calibration of leaf extract absorbance by the chlorophyll in leaves: To calibrate the leaf extract absorbance by foliar chlorophyll, a traditional destructive method (Arnon, 1949) was adopted to extract the chlorophyll from the leaves. Briefly, leaves selected for measuring the leaf reflectance were placed in a cold bag until they were thoroughly washed, after which they were put in moisture-proof zipper bags and placed in a refrigerator for several hours. The turgid leaves were then torn and ground by hand into small shreds, after which 3 grams of a leaf blade were blended with 99.5% acetone solution in a conical tube and refrigerated overnight. The samples were subsequently centrifuged for 20 minutes at 25,000 rpm. The supernatant in the tube was then decanted into a 10mm tube, after which leaf absorbance was determined by measuring the sunlight penetrating the aqueous acetone chlorophyll extracts in the tube using a UVD-3200 spectrophotometer (Labomed, Inc., CA, USA). Corresponding to the leaf reflectance data, the absorbance of the chlorophyll extracts was determined on the same 80 wavelength bands from 400 nm to 800 nm. The leaf extract absorbance obtained from the spectrophotometer was then applied to calculate the chlorophyll content using the following Arnon's (1949) equations:

$Chl_a = 0.0127\ A_{663} – 0.00269\ A_{645}$

$Chl_b = 0.0029\ A_{663} – 0.00468\ A_{645}$

$Chl_t = 0.0202\ A_{663} + 0.00802\ A_{645}$,

where, Chl_a is the content of chlorophyll a, Chl_b is the content of chlorophyll b, Chl_t is the total chlorophyll, A_{663} is the absorbance at 663 nm, and A_{645} is the absorbance at 645 nm.

Chlorophyll content derived from the leaf extract absorbance of the extracts was related to the leaf reflectance as captured by the normalized difference vegetation index (NDVI = (NIR – red) / (NIR + red)) (Rouse *et al*., 1974). NDVIs were calculated with all possible pairwise combinations of wavelength bands for each species and then correlated to the chlorophyll content. The correlation coefficients between the pairwise combinations of bands and the chlorophyll content for each species were mapped using Surfer program v. 10 (Golden Software, USA) and used to determine the best band combination for estimation of the chlorophyll content for each species.

Piecewise linear regression for relating leaf reflectance to extract leaf extract absorbance: As mentioned earlier (Ding *et al*., 2009), leaves are known to reflect sunlight with a trend contrary to the leaf absorbance, and there is a nonlinear relationship between leaf reflectance and leaf extract absorbance rather than a linear relationship. Nonlinear relationships between the response and explanatory variables can sometimes be successfully modelled using a linear model that has different slopes for certain ranges of the covariable. In this model, the regression of Y on X follows a particular linear relationship in some range of X, but a different linear relationship elsewhere. This regression is a general class in statistical analysis in which the independent variable, X, is segmented according to its value and the regression analysis is performed separately for these segments. The boundaries between segments are called breakpoints. If the resulting regression equations do not show a discontinuity at the breakpoints, they must be analyzed using a method of switching regression known as piecewise regression.

Generally, the equation commonly used for piecewise regression is expressed as:

$Y = f_1(X),\ X \le J_1$

$Y = f_2(X),\ J_1 \le X \le J_2$

$\vdots$

$Y = f_n(X),\ X \ge J_n$,

where, Y is the dependent variable, X is the independent variable, and J_1 to J_n are the breakpoints.

The relationship between leaf reflectance and leaf extract absorbance in this study was expressed by piecewise linear regression (PLR) with only one breakpoint. IBM SPSS (Statistical Package for the Social Sciences) Statistics (v. 22) was used to explain the relationship between leaf reflectance (explanatory variable) and leaf extract absorbance (response variable).

RESULTS

Leaf emergence and its elongation for three species during the growing season: Leaves of the target species started elongating around the middle of April and continued to do so through the middle of May (Fig. 2), although there was a difference in the date of bud break between urban areas and peripheral areas around Daegu. Specifically, all three species located in urban area broke buds together around April 14, while the same species showed leaf emergence several days later in the surrounding areas. Two *Quercus* species (Qv and Qa) showed leaf emergence approximately 4 days earlier in urban areas rather than peripheral areas; however, Rp in the urban space showed leaf emergence 15 days earlier than in the peripheral region.

The time of leaf emergence in the peripheral area showed obvious differences among three species, while species in the urban areas did not show great differences in the time of leaf emergence. For all three species in urban areas, leaves emerged before April 15, while in peripheral areas the leaves of the two *Quercus* species emerged around April 20, while Rp leaf emergence occurred around May 2. All three species continued to undergo leaf elongation until May 10, when the process of elongation slowed and the temporal curvature of elongation plateaued.

Trends in light reflectance and leaf extract absorbance depending on leaf elongation for three dominant species: Leaf reflectance was relatively low in the blue and red ranges, which were used for photosynthesis, while higher reflectance was observed for the green and NIR ranges, which are not used in high amounts. The leaf reflectance during the leaf-elongation period did not reach over 20% in the visible wavelength (400 to 700 nm), and it did not have a wide standard deviation. However, the NIR had relatively higher reflectance than the visible wavelengths, while having a wider range in its variation (Fig. 3). Reflectance progressively increased from the first survey date (April 14) to the last survey date (May 16); however, the reflectance was saturated from May 9. There was an obvious variation among the average reflectances during the leaf-growing period (from April 14 to May 16) in the NIR range, while little variation was observed in the visible range. The reflectance in NIR was around 30% in the middle of April, while it plateaued at over 40% after the first ten days of May. This inclination was much clearer in the longer-wavelength NIR range, where all of the target species showed a gap of greater than 15% between the maximum reflectance (May 16) and the minimum reflectance (April 25 for Rp; in April for others) (Fig. 4; Table 1).

Leaf extract absorbance and its trend during the leaf-growing period contrasted with leaf reflectance, gradually increasing with leaf elongation. The absorbance increased in the blue and red ranges, which are the spectra absorbed by a leaf, while they decreased in the green and NIR ranges, in which light is liable to be reflected by a leaf (Fig. 5). There were two peaks shown in blue and red regions, one is in the shorter-wavelength blue range (400 nm to 450 nm) exceeding 1.0 abs in absorbance and another in the mid-wavelength red range (650 nm to 670 nm) showing slightly lower absorbance (> 0.5 abs). The absorbance was obviously higher on May 16, especially in the mid-wavelength red range, where the maximum absorbance was 2.02 for Qv, 2.02 for Qa, and 1.71 for Rp, while the absorbance at other wavelengths was below 0.1. These findings indicate that the variation within a species can be discriminated based on differences in absorbance in the mid-wavelength red range. Moreover, leaves showed little absorption of NIR over 700 nm.

Relationship between light reflectance and light absorbance during the leaf growing season: Regressions estimating light absorbance using light reflectance had a higher coefficient of determination in earlier stages of leaf growth. As shown in Table 3, the light reflectance data taken on April 18 and 21 explain the leaf extract absorbance well (R^2>0.9). However, the coefficients of determination in each PLR decreased as leaves grew and were finally<0.8 in the PLR models as the reflectance became saturated (around May 10). The models comparing the leaf reflectance (X) to the leaf absorbance (Y) using PLR had one breakpoint under 0.08 (0.03 min; 0.08 max) (Table 3). When the reflectance range was lower than each breakpoint, there was a linear regression with a steep negative slope between the leaf reflectance (X) and the leaf absorbance (Y), while the regression line at values higher than the breakpoint had a negative relationship with a gentle slope (Fig. 6; Table 3). There were not many changes in leaf absorbance at over 15% reflectance (0.15 on X) (Fig. 6); therefore, the variation in absorbance was found at less than 15% leaf reflectance.

Correlation of Normalized Difference Vegetation Indices derived from light reflectance with absorbance-derived chlorophyll content: NDVIs were calculated using all possible pairwise combinations of wavelength bands, and correlation maps (Fig. 7) correlating each NDVI to the absorbance-derived chlorophyll content were created. Traditionally, correlation of vegetation indices to chlorophyll content is known to increase as red and NIR light are combined (Gitelson *et al*., 2003). This study also showed that the combination of NIR with red resulted in a higher correlation with NDVI for all three species. The shorter wavelength red range (600 to 650 nm) combined with NIR for NDVI explained the chlorophyll content quite well. As shown in Fig. 7, the correlation maps also revealed that the combination of NIR with any wavelength besides blue explained the chlorophyll content quite well. Moreover, the combination of wavelengths of 550 nm to 650 nm and 400 nm to 550 nm resulted in higher correction coefficients for estimation of the chlorophyll content.

Conversely, several combinations showed lower correlation coefficients with chlorophyll content for some species. Combining blue with NIR did not explain the chlorophyll content well. Blue wavelengths combined with those in the green range (500 to 550 nm) or red range (650 to 700 nm) were also not good combinations for estimation of chlorophyll content (Fig. 7). There was not a good correlation for the combination of blue wavelengths with longer green wavelengths (550 to 600 nm) or shorter red wavelengths (600 to 650 nm) for Qa (Fig. 7b).

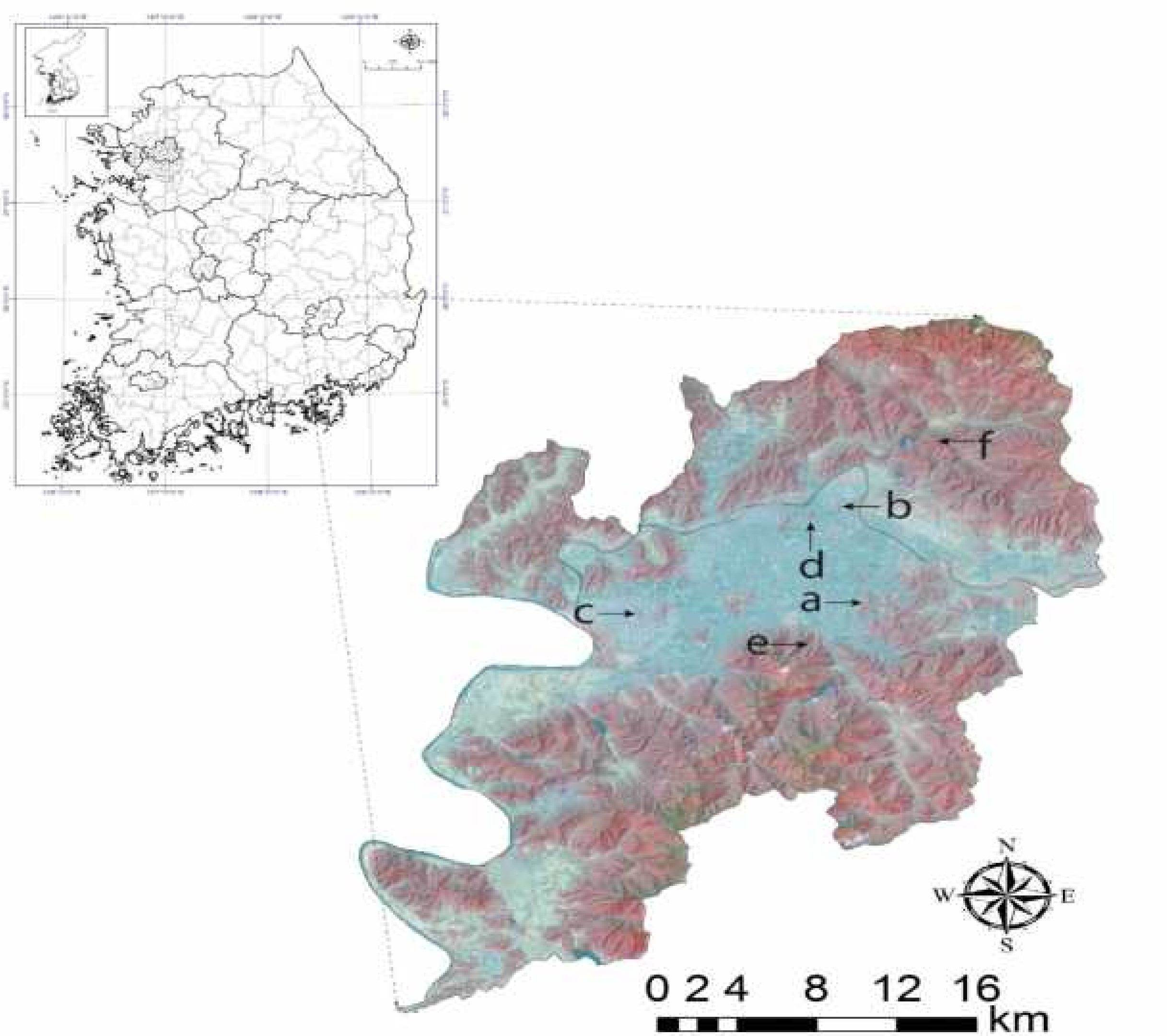
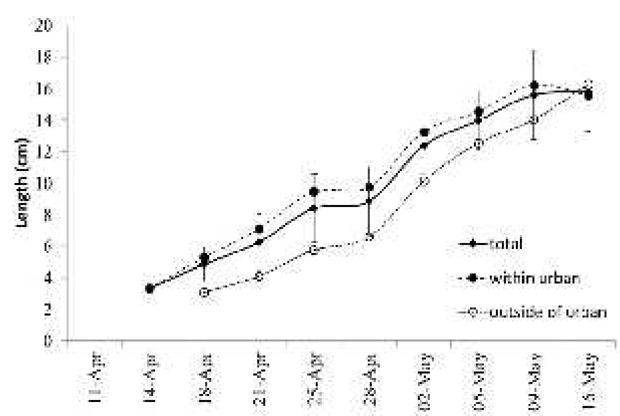
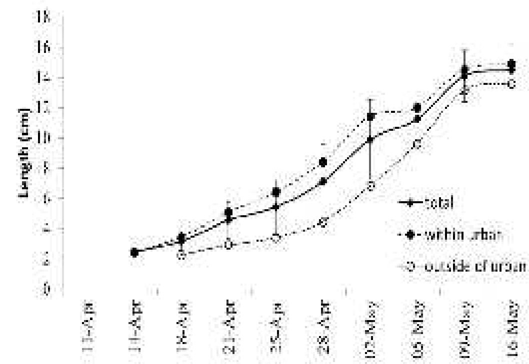


Fig. 1. Six urban forests used as study sites in Daegu, South Korea; a: Beomeo Park, b: Daebul Park, c: Galsan Park, d: Yeonam Park, e: Daeduk Mountain, f: Palgong Mountain.

a. **Leaf elongation of Qv**

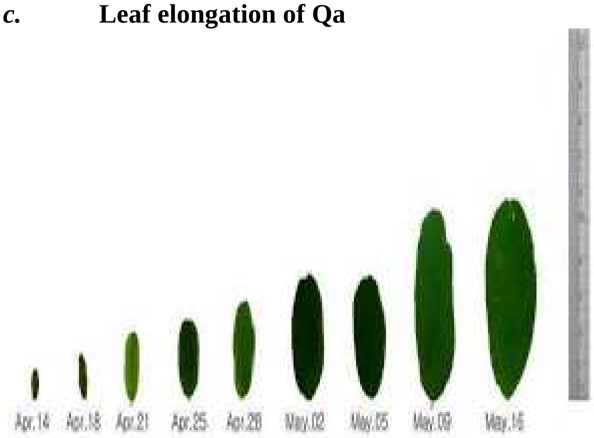


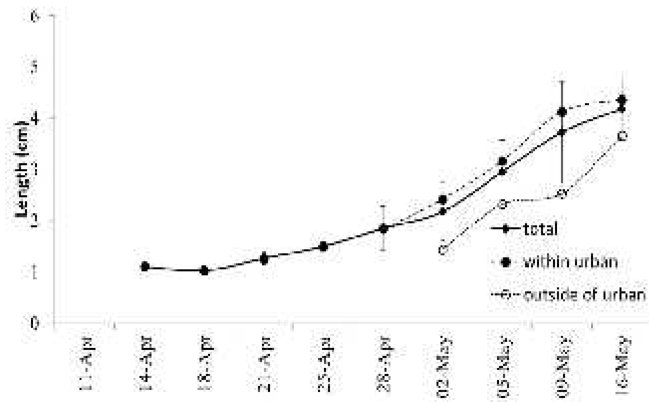
b. **Average leaf emergence and its elongation in Qv**



c. **Leaf elongation of Qa**

d. **Average leaf emergence and its elongation in Qa.**





e. **Leaf elongation of Rp**

f. **Average leaf emergence and its elongation in Rp.**

Fig. 2. Trends in leaf elongation of the three major trees during the leaf-growing period.

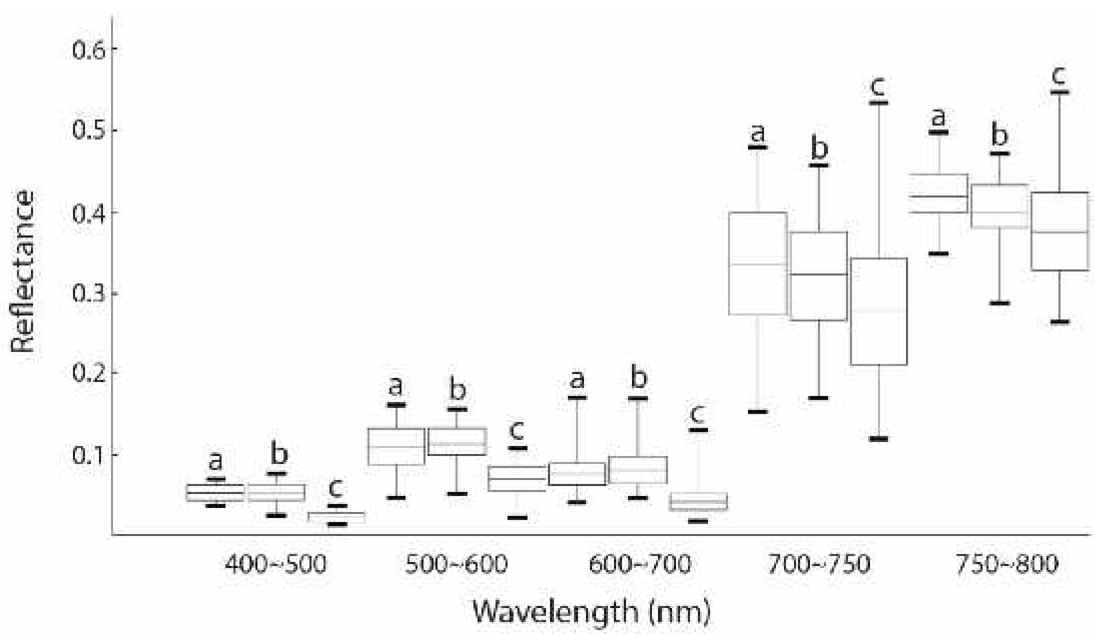
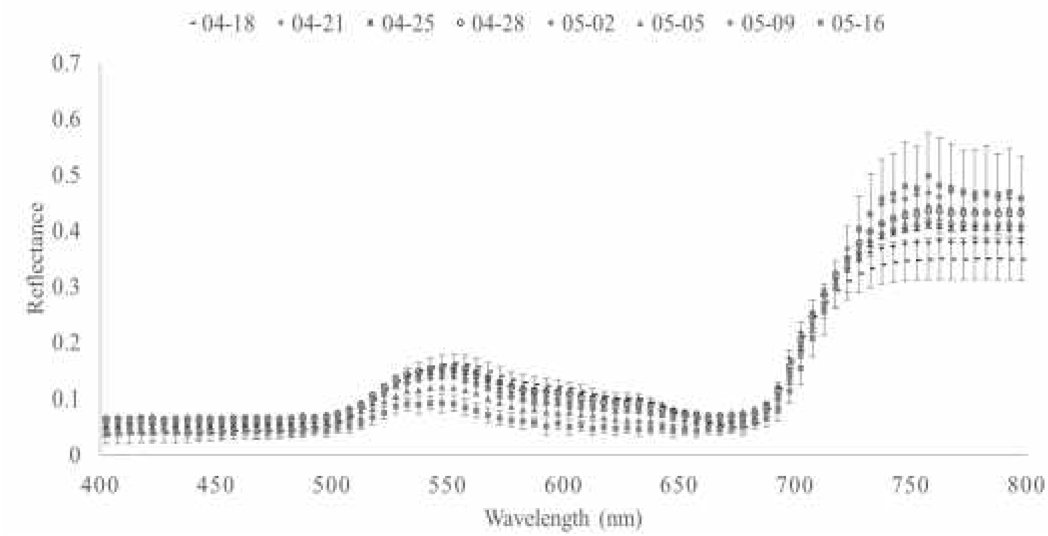
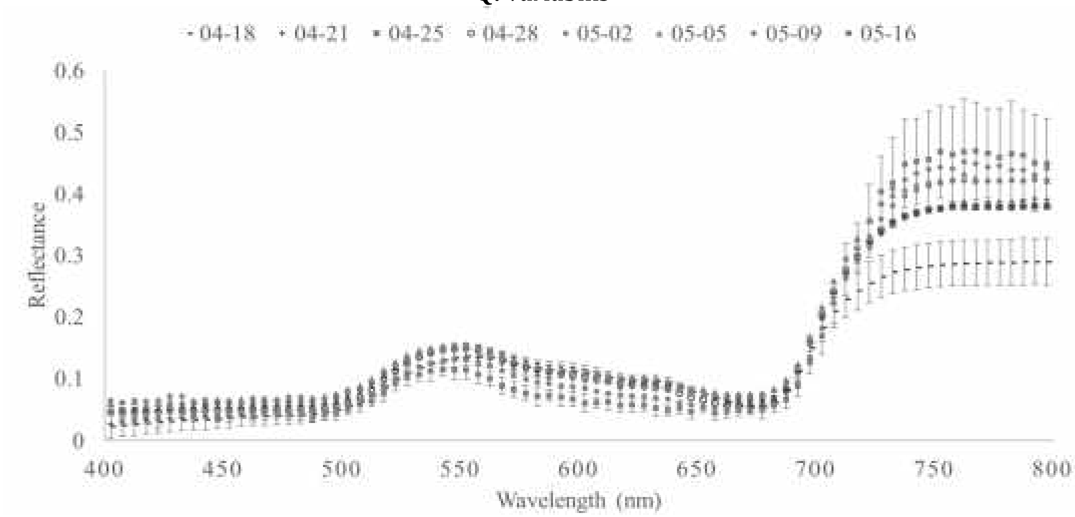


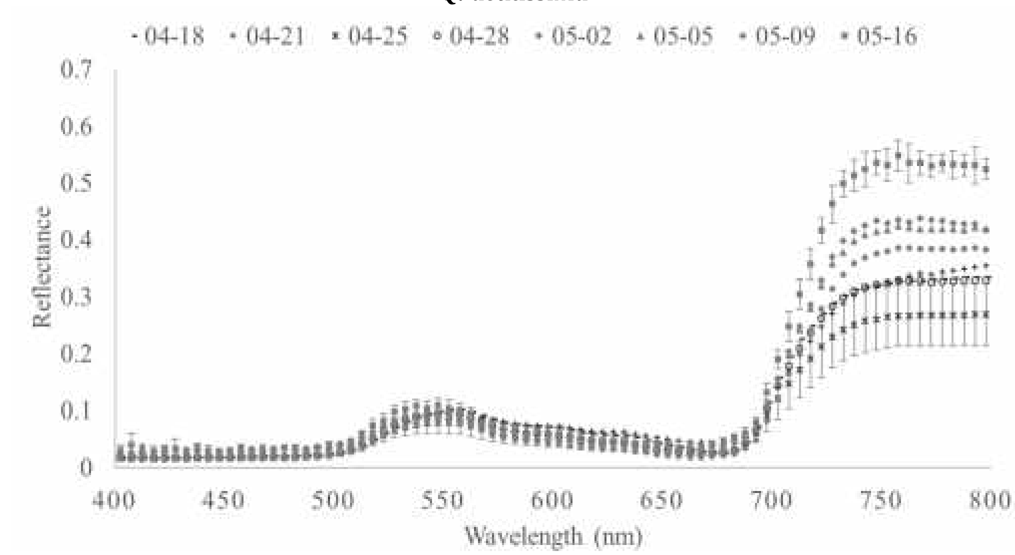
Fig. 3. Variations in leaf reflectance among the three major trees; the max, min, mean, and standard deviation of each range (blue, green, red, and NIR, respectively) are shown (a: *Quercus variabilis*, b: *Quercus acutissima*, c: *Robinia pseudoacacia*).



Q. variabilis

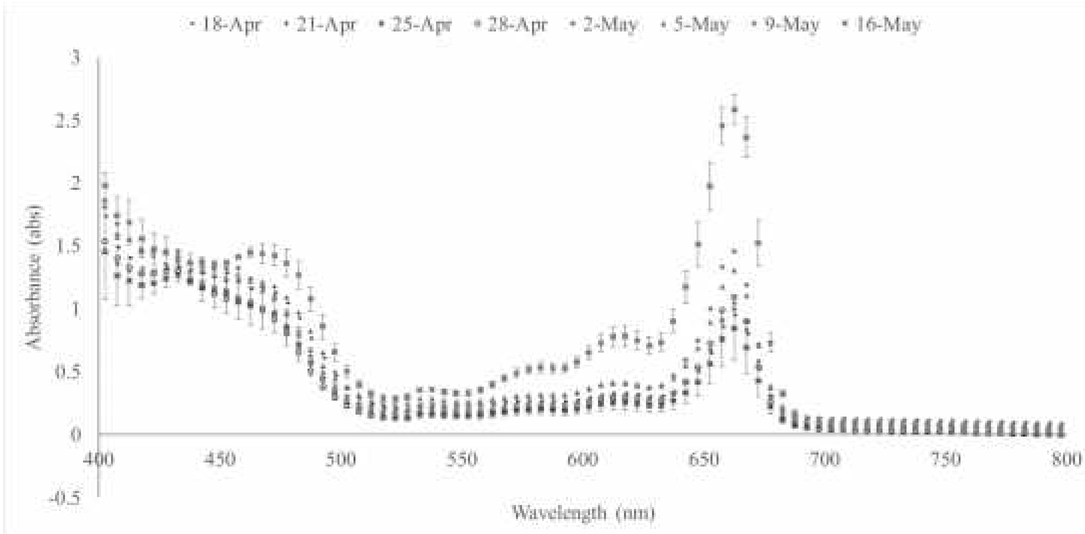


Q. acutissima

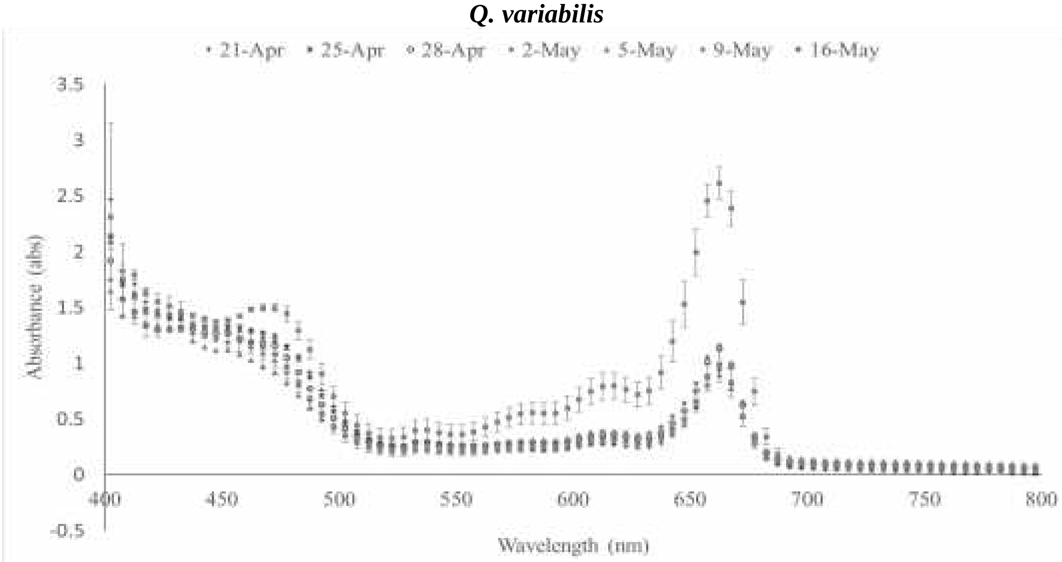


R. pseudoacasia

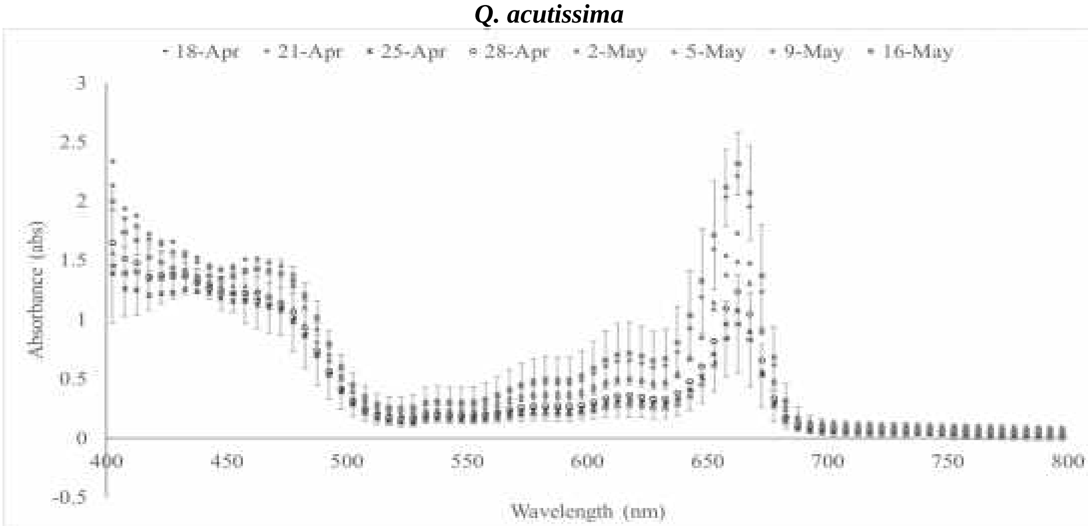
Fig. 4. Leaf reflectance and its trend for the three major trees during the leaf-growing period.



Q. variabilis



Q. acutissima



R. pseudoacasia

Fig. 5. Absorbance and its trend for the three major trees during the leaf-growing period.

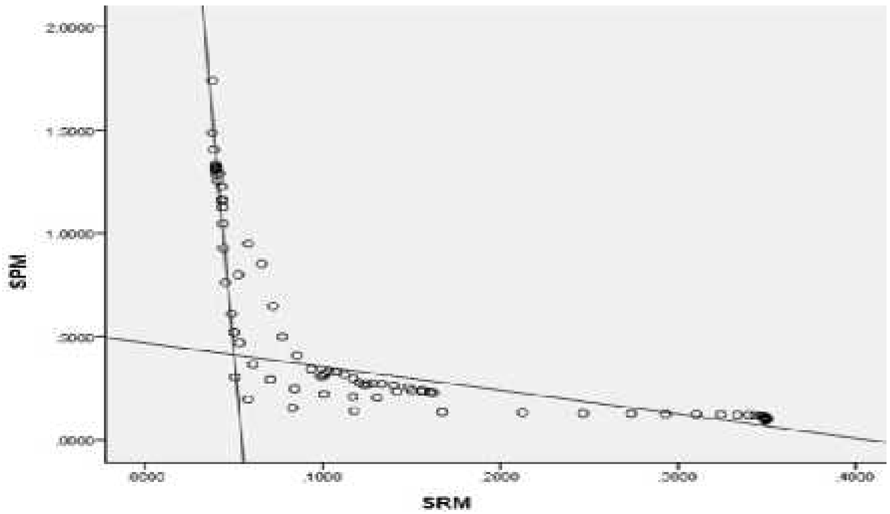


Fig. 6. Piecewise linear regression relating leaf reflectance to extract absorbance (April 18, 2011).

Fig. 7a. Correlation map for *Quercus variabilis*

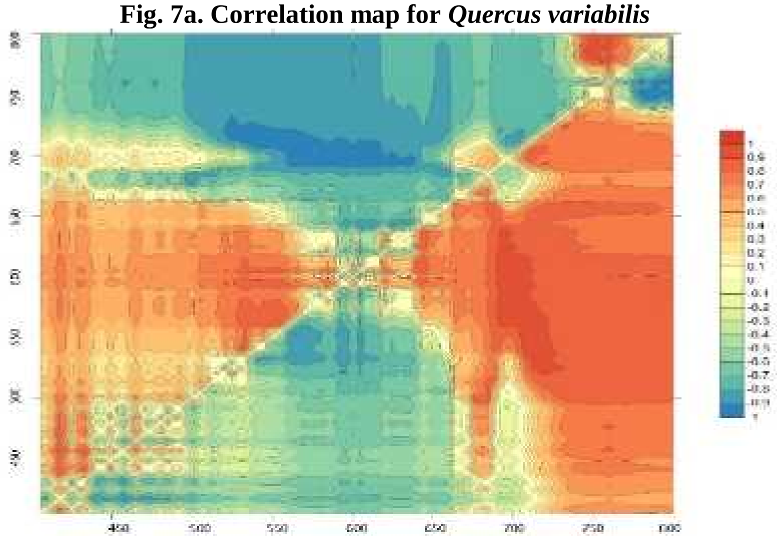


Fig. 7b. Correlation map for *Quercus acutissima.*

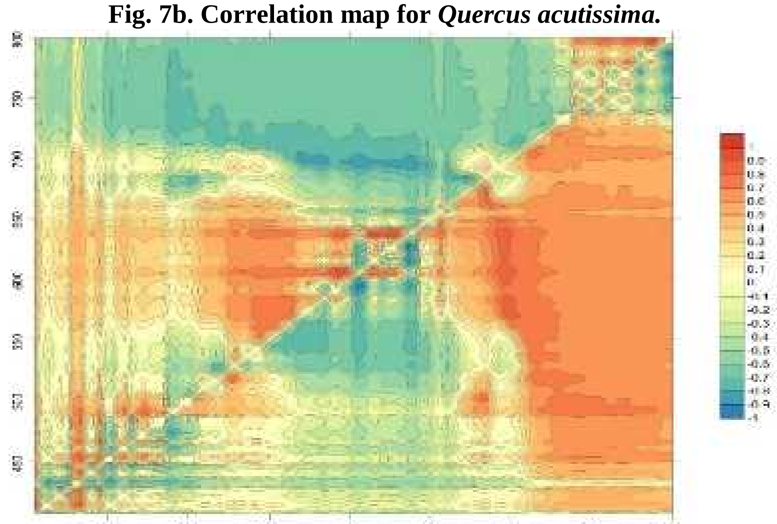


Fig. 7c.Correlation map for *Robinia pseudoacacia*

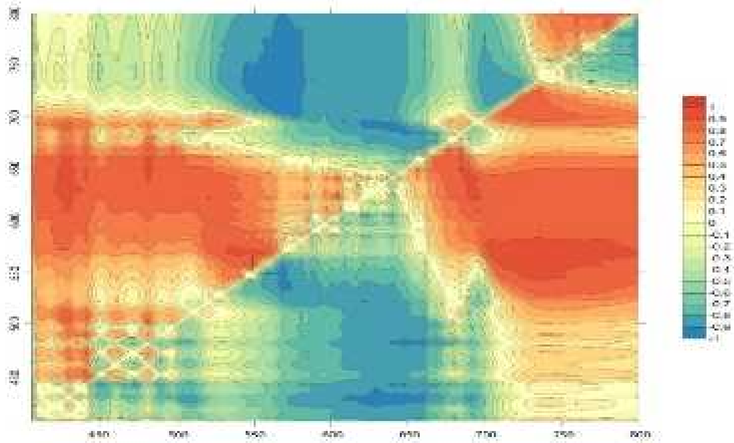
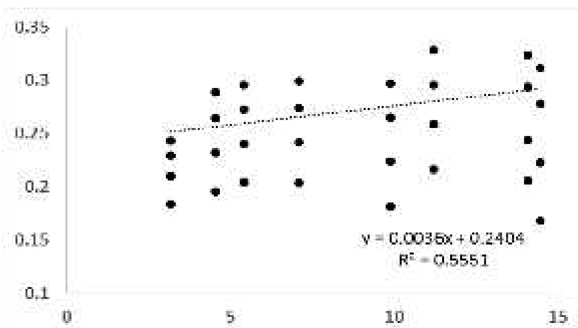
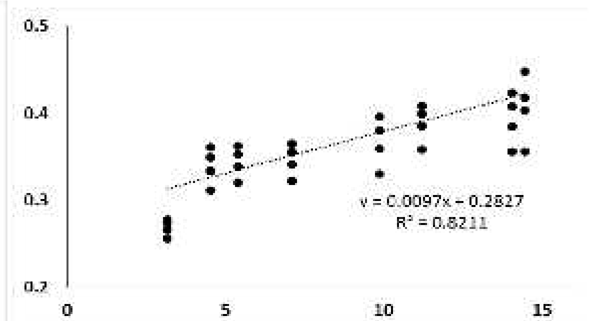


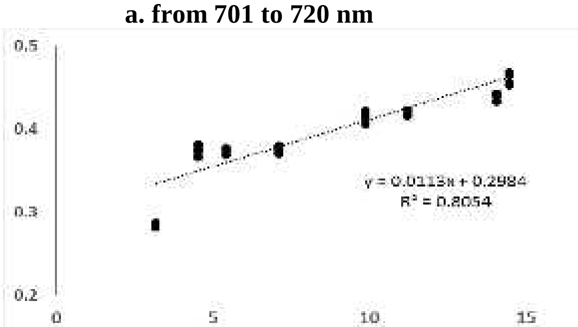
Fig. 7. Correlation coefficients obtained between chlorophyll content and Normalized Difference Vegetation Index (NDVI) using all possible combinations of the 20 available leaf reflectance wavelengths.



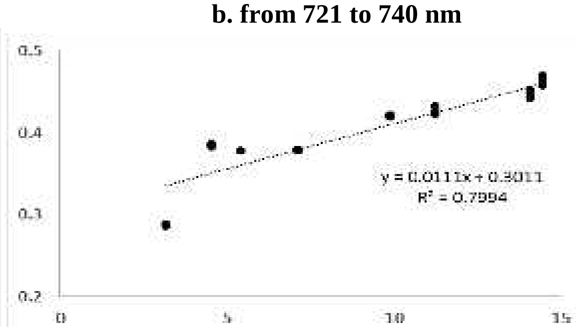
a. from 701 to 720 nm



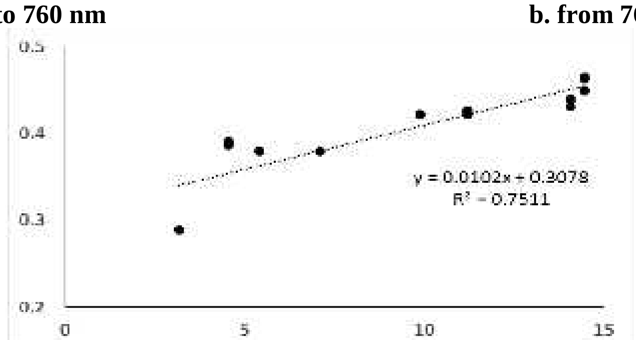
b. from 721 to 740 nm



a. from 741 to 760 nm



b. from 761 to 780 nm



e. from 781 to 800 nm

Fig. 8. The relationship of leaf elongation to leaf reflectance for *Quercus acutissima* (X-axis: leaf length (cm), Y-axis: leaf reflectance) based on the range of wavelength (a: 701–720nm; b: 721–740 nm; c: 741–760 nm; d: 761–780 nm; 781–800 nm).

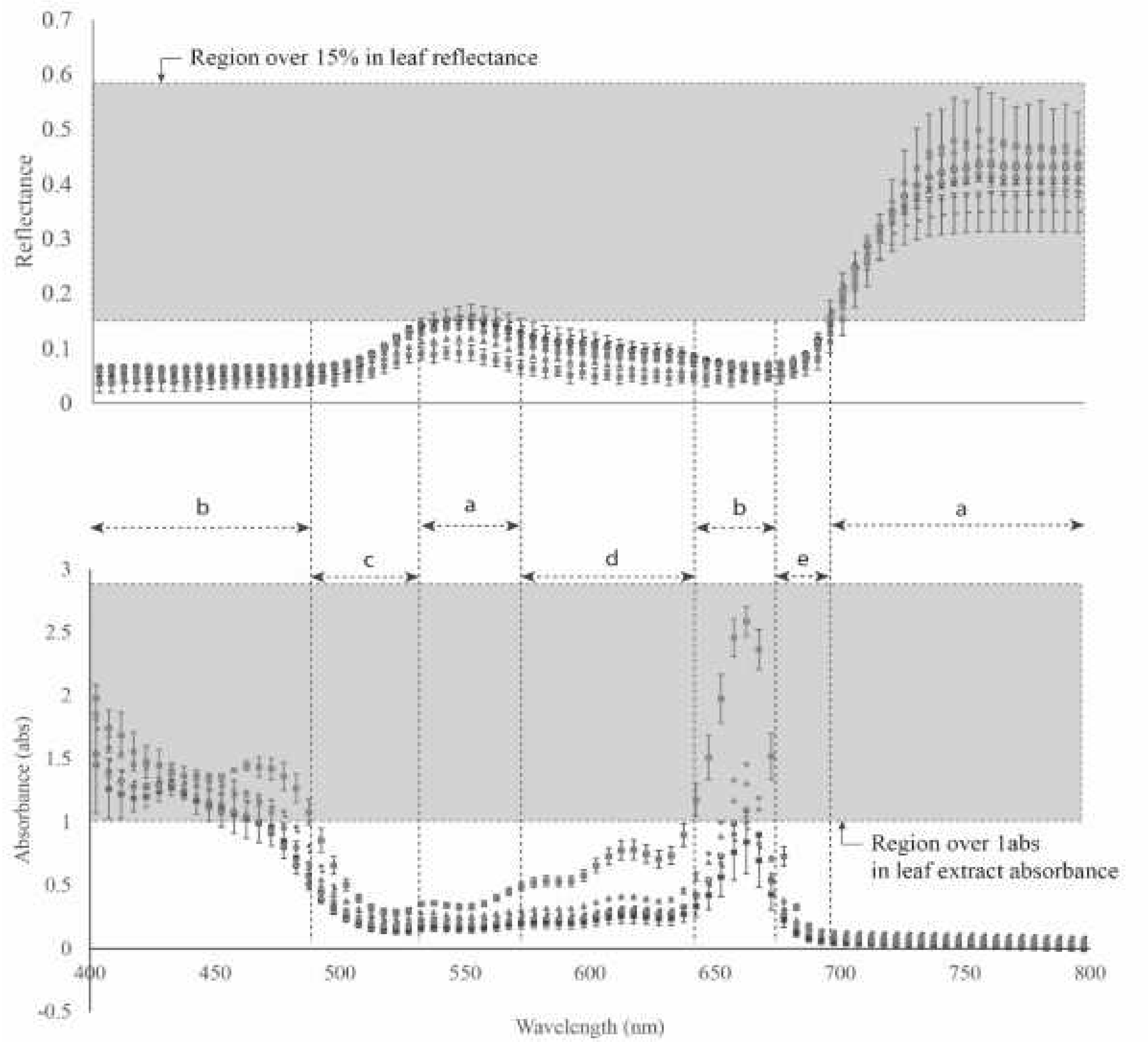


Fig. 9. An example of the relationship between leaf reflectance and leaf extract absorbance for *Quercus variabilis*.

Table 1. Leaf reflectance in five wavelength bands for three major plant species.

Species		Wavelength (nm)				
		Blue (400–500)	**Green (500–600)**	**Red (600–700)**	**S-NIR (700–750)**	**L-NIR (750–800)**
Qv	Mean	0.053	0.109	0.078	0.334	0.419
	Stdev	0.010	0.029	0.024	0.080	0.039
	Max	0.071	0.162	0.171	0.479	0.498
	Min	0.038	0.048	0.042	0.154	0.348
Qa	Mean	0.050	0.113	0.083	0.320	0.398
	Stdev	0.012	0.026	0.024	0.075	0.051
	Max	0.075	0.156	0.168	0.456	0.470
	Min	0.023	0.050	0.045	0.169	0.285
Rp	Mean	0.024	0.070	0.046	0.287	0.379
	Stdev	0.006	0.020	0.019	0.098	0.077
	Max	0.039	0.108	0.131	0.535	0.548
	Min	0.015	0.023	0.018	0.120	0.264

S-NIR: shorter wavelength NIR; L-NIR: longer wavelength NIR

Table 2. Leaf extract absorbance in five wavelength bands for three major plant species.

Species		Wavelength (nm)				
		Blue (400–500)	Green (500–600)	Red (600–700)	S-NIR (700–750)	L-NIR (750–800)
Qv	Mean	1.167	0.251	0.510	0.076	0.055
	Stdev	0.341	0.084	0.439	0.030	0.030
	Max	1.976	0.576	2.587	0.129	0.111
	Min	0.291	0.131	0.045	0.022	0.000
Qa	Mean	1.257	0.305	0.511	0.091	0.069
	Stdev	0.352	0.084	0.424	0.028	0.029
	Max	2.467	0.616	2.610	0.141	0.119
	Min	0.427	0.202	0.057	0.033	0.010
Rp	Mean	1.249	0.268	0.594	0.076	0.056
	Stdev	0.336	0.091	0.480	0.026	0.026
	Max	2.334	0.525	2.314	0.134	0.111
	Min	0.391	0.120	0.053	0.033	0.010

S-NIR: shorter wavelength NIR; L-NIR: longer wavelength NIR

Table 3. Equations obtained by piecewise linear regression of SPM on SRM for major plant species

Quercus variabilis

Date	Reflectance<Threshold	Break Point	ReflectanceñThreshold	R^2
4/18	SPM[*]=5.242-96.666×SRM[**]	0.05	SPM=0.467-1.149×SRM	0.926
4/21	SPM=6.438-100.876×SRM	0.06	SPM=0.457-1.19×SRM	0.908
4/25	SPM=6.467-103.861×SRM	0.06	SPM=0.273-0.634×SRM	0.879
4/28	SPM=4.761-56.926×SRM	0.08	SPM=0.254-0.589×SRM	0.834
5/02	SPM=6.759-92.984×SRM	0.07	SPM=0.287-0.533×SRM	0.901
5/05	SPM=4.017-62.149×SRM	0.06	SPM=0.322-0.568 ×SRM	0.891
5/09	SPM=5.813-69.668×SRM	0.08	SPM=0.279-0.491×SRM	0.869
5/16	SPM=3.973-60.777×SRM	0.06	SPM=0.371-0.744×SRM	0.728

Quercus acutissima

Date	Reflectance<Threshold	Break Point	ReflectanceñThreshold	R^2
4/18	SPM=2.64-35.502×SRM	0.06	SPM=0.625-1.928×SRM	0.909
4/21	SPM=3.267-48.809×SRM	0.06	SPM=0.403-1.08×SRM	0.933
4/25	SPM=5.254-69.975×SRM	0.07	SPM=0.428-1.03×SRM	0.939
4/28	SPM=5.111-78.898×SRM	0.06	SPM=0.44-1.053×SRM	0.887
5/02	SPM=11.318-219.131×SRM	0.05	SPM=0.407-0.91×SRM	0.881
5/05	SPM=4.901-57.168×SRM	0.08	SPM=0.383-0.694×SRM	0.913
5/09	SPM=5.445-63.829×SRM	0.08	SPM=0.398-0.745×SRM	0.863
5/16	SPM=3.108-38.407×SRM	0.07	SPM=0.492-1.027×SRM	0.734

Robinia pseudoacasca

Date	Reflectance<Threshold	Break Point	ReflectanceñThreshold	R^2
4/18	SPM=2.604-76.532×SRM	0.03	SPM=0.333-0.837×SRM	0.881
4/21	SPM=2.588-47.428×SRM	0.05	SPM=0.254-0.744×SRM	0.907
4/25	SPM=2.246-65.699×SRM	0.03	SPM=0.309-1.139×SRM	0.867
4/28	SPM=2.614-76.975×SRM	0.03	SPM=0.333-0.951×SRM	0.865
5/02	SPM=2.867-86.451×SRM	0.03	SPM=0.294-0.66×SRM	0.853
5/05	SPM=2.687-58.44×SRM	0.04	SPM=0.378-0.699×SRM	0.860
5/09	SPM=3.196-57.207×SRM	0.05	SPM=0.372-0.729×SRM	0.831
5/16	SPM=2.684-39.259×SRM	0.06	SPM=0.367-0.635×SRM	0.790

[*]SPM: spectrophotometer
[**]SRM: spectroradiometer

DISCUSSION

Meaning of leaf elongation and its variation during the growing season: Fig. 2 shows the leaf elongation during the growing season and indicates the critical period at which leaves show remarkable growth during spring in South Korea. Temporal changes in leaf size were somewhat related to the trend of leaf reflectance and could therefore be estimated using the band-derived reflectance from satellite imagery, although the reflectance is canopy reflectance, not leaf reflectance. April and May in almost all of South Korea were recognized as the proper period for detection of differences in leaf size, with the period before leaf elongation plateaued (i.e., before May 9) being critical for discrimination of the variation within and/or among species. The curve of leaf elongation was also linked to leaf reflectance, in which the trends of NIR reflectance for each species were shown to explain the trends in leaf elongation during the leaf-growing period especially well (Fig. 8). With the exception of shorter-wavelength NIR (701–720 nm), most of the wavelength ranges showed higher R^2 values in models relating the leaf elongation to the leaf reflectance.

Changes in leaf reflectance depending on leaf elongation: Leaf reflectance is well known as an indirect indicator for investigation of leaf conditions. Differences in reflectance at every leaf-growing stage can be good references to differentiate one species from another. A large variation in the NIR range during the growing season was evident in Fig. 3 and 4, implying that the variation appearing within or among species can be applied to distinguish one species from another. However, it may be difficult to determine the critical threshold to obtain a representative reflectance for a species from compound reflectance among multiple species. As shown in Fig. 3 and 4, the reflectance ranges of three species overlapped each other. Leaf reflectance for each species was also found to become saturated (Fig. 3 and 4) much earlier than leaf elongation (Fig. 2) during the leaf-growing period, indicating that differentiation of species from one another based on reflectance is very complicated. Nevertheless, the reflectance of a species can be one of potentially eligible factors for discrimination from others. We also found that it was possible to detect the temporal changes of leaf reflectance at NIR ranges within and/or among species during the leaf-growing period. Evaluation of the standard deviation of the leaf reflectance shown in figure 4 revealed that there was no overlap between the range of maximum reflectance (in April 18) and the range of minimum reflectance (in May 16). Based on this finding, we propose that it is necessary to investigate the potential for estimating variation in leaf reflectance on two or three critical dates (e.g., April 20, April 30 and/or May 10 in South Korea) during the leaf-growing period. Data obtained during this period can then be used to investigate other factors such as leaf elongation and leaf extract absorbance.

Leaf absorbance: Leaf absorbance is a critical indicator for measurement of the content of pigment (chlorophyll, carotenoid, and anthocyanin) in a leaf. There are several methods to extract a pigment from a leaf, and multiple equations to estimate the content of a pigment. As shown in Fig. 5, the absorbance of leaves increases gradually and continuously according to leaf elongation, indicating that the chlorophyll content increases similarly to the absorbance. The results shown in Fig. 5 differ somewhat from the trend of light reflectance of a leaf (Fig. 4) during the leaf-growing period. Based on the peaks in the longer wavelength red range (650 to 670 nm), the chlorophyll content gradually and continuously increases, regardless of trends in leaf reflectance, which is rapidly saturated.

Relationship between leaf reflectance and leaf absorbance: The equations for estimating pigments in a leaf using absorbance have been widely applied and are now generalized (Sims and Gamon, 2002; Roelofsen *et al*., 2014). The negative relationship between leaf reflectance and leaf absorbance is well known. However, based on the data shown in Fig. 4, 5 and 6, we can rearrange the relationship between leaf reflectance and leaf extract absorbance (Fig. 9). Specifically, if the leaf reflectance exceeds about 15%, there is little increase in leaf absorption (Fig. 9a), while there is not much decrease in leaf reflectance when the leaf extract absorbance was over 1 abs (Fig. 9b). These findings indicate that excessive reflectance and absorbance could not explain the variations in each other well. Therefore, the shorter green range (Fig. 9c), shorter red range (Fig. 9d), and longer red range (Fig. 9e) were finally determined to be the proper wavelengths for evaluation because they showed obvious variations together between leaf reflectance and leaf extract absorbance.

The combination of red and NIR wavelengths, which have been shown to reflect vegetation undergoing growth, was also applied to estimate the chlorophyll content. The shorter red wavelengths (600 to 650 nm), which showed the greatest absorption by leaves (Fig. 4 and 5), were combined with NIR and used to estimate the chlorophyll content (Fig. 7). Correlation maps demonstrated that the NIR wavelengths played an important role in generation of the proper combination for correlation with the chlorophyll content, indicating that combination with NIR on the correlation map was likely to explain the content of chlorophyll in a leaf well. However, combination with blue and NIR wavelengths did not explain the chlorophyll content well.

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