Using hyperspectral remote sensing to quantify the effects of urbanization on foliar pigment content in *Quercus rubra* seedlings

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Abstract

Prolonged changes in environmental conditions, such as those associated with urbanization, have the potential to greatly alter the growth rates and physiological processes of terrestrial vegetation. In this study, hyperspectral remote sensing techniques and traditional spectrophotometric methods were used to investigate how the pigment content of *Quercus rubra* seedling foliage differed between an urban environment (Central Park, New York City) and a suburban environment (Lamont-Doherty Earth Observatory; Palisades, NY) between May 2007 and October 2007. The Chlorophyll Index (CHL), the Carotenoid Reflectance Index (CRI), and the Photochemical Reflectance Index (PRI), which estimate chlorophyll, carotenoid, and light use efficiency (LUE), respectively, were calculated from the reflectance data and compared to concentrations determined through traditional spectrophotmetric analysis. CHL exhibited a strong relationship with chlorophyll concentration (r^2=0.74) and can therefore be reliably used to estimate chlorophyll concentrations in *Quercus rubra.* CHL also showed a strong negative relationship with Car:Chl when May data points were removed (r^2 = 0.71); these results suggest that CHL can be used to estimate Car:Chl in mature *Quercus rubra* leaves, but not young leaves. CRI was not sensitive to carotenoids concentrations (r^2 = 0.086), likely due to the carotenoids spectral signal being ‘masked’ by the much stronger signal of chlorophylls. PRI exhibited a negative relationship with Car:Chl (r^2 = 0.42). More research needs to be done with these indices to determine what leaf physiological factors may be influencing them and also to test their application across-species. The direct spectrophotometric measurements and vegetation indices showed that the Central Park (urban) seedlings had significantly higher chlorophyll content and lower carotenoid:chlorophyll ratios than the Lamont-Doherty Earth Observatory (suburban) seedlings. These results indicate that urbanization may be positively affecting light use efficiency in the urban seedlings or that it is limiting light use efficiency in the suburban plants. Furthermore, the remote sensing data shows great potential for further application to quantifying urban and rural differences in foliar form and function at the canopy scale via air or spaceborne remote sensing.

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Introduction

Prolonged changes in environmental conditions, such as those associated with urbanization and anthropogenic climate change, have the potential to greatly alter the physiological and biochemical processes of terrestrial vegetation. Vegetated systems remove carbon dioxide (CO_2) from the atmosphere and help to regulate global climate conditions. Some studies suggest that CO_2 fertilization, in which increased atmospheric CO_2 levels promote increased rates of photosynthesis, may be occurring; this would provide an explanation for the increasing CO_2 sink (reviewed in Battle and others 2000). However, the extent to which vegetated systems will be able to counteract increasing anthropogenic CO_2 release has been widely debated (Field 2001). Other studies have suggested that increased atmospheric CO_2 alone could not have caused changes in Net Primary Productivity (NPP) large enough to account for the total “missing” carbon sink (Thompson and others 1996). It has therefore been suggested that in addition to CO_2 levels, there are other factors associated with urbanization that influence rates of photosynthesis and NPP. In order to identify these other factors, this study examines how vegetation is affected by the changing environmental conditions associated with urbanization by focusing on small-scale mechanisms that ultimately determine measures such as biomass and Net Primary Productivity.

Urban Environmental Conditions and their Possible Effects on Vegetation

Carreiro and Tripler (2005) suggest that studying forests along urban-to-rural gradients can provide important insight into the ecosystem impacts of climate change because they represent forests that have already begun to respond to factors associated with global climate change.The urban environment is similar to the predicted future global environment in its increased temperature and higher levels of pollutants such as carbon dioxide (CO_2), ozone (O_3), and certain forms of inorganic nitrogen. Because of these similarities, studies have suggested that urban centers may serve as appropriate models for future global climate conditions (Idso and others 2002; Ziska and others 2003). The expansion of urban centers is expected to cause changes in the productivity and composition of ecosystems worldwide (IPCC Working Group II, 2001), although the nature and extent of these changes are not well understood and are poorly represented by current analytical models (Carreiro and Tripler 2005). Carreiro and Tripler (2005) argue that studying vegetation along urban-rural gradients may provide insight into complex interactions that are not well represented in current models of urban impacts on surrounding ecosystem processes.

Urban environments are generally warmer than surrounding areas due to thermal absorption properties of urban land cover, combustion activities that produce heat, and low soil moisture (Oke 1995). This ‘urban heat island effect’ may explain the 8 day longer growing season experienced by urban plants compared to rural plants in eastern deciduous broadleaf forests (White and others 2002). Other studies suggest that elevated urban temperatures increase the Net Primary Productivity of urban vegetation, especially in cold regions of the United States (Imhoff and others 2004). However, it is unclear how warmer temperatures and longer growing seasons affect NPP in urban areas when other environmental factors are considered. For instance, White and others (2002) found that despite the longer growing season of urban plants, they experienced reductions in peak vegetation activity and thus likely had lower overall productivity. In addition to higher average temperature, cities also have lower diurnal temperature ranges (DTRs), which may decrease a plant's susceptibility to temperature stresses.

Atmospheric carbon dioxide (CO_2) concentrations are elevated in urban environments (Pataki and others 2003). As plant growth depends on availability of CO_2, higher levels of CO_2 would be expected to promote plant growth and increases in NPP. Higher CO_2 concentrations also result in lower stomatal conductance, which helps to protect plants from atmospheric pollutants, particulate matter, tropospheric O_3, and water stress (Volin and others 1998). Many studies have found that elevated CO_2 enhances vegetation growth (DeLucia and others 1996; Norby 1996; Bazazz and Miao 1993; Zak and others 2000); however, Korner (2000) suggests observed short-term effects of CO_2 enrichment on plant biomass accumulation may not hold over long time periods as vegetation may adjust to elevated CO_2.

Inorganic nitrogen sources such as atmospheric NO_x gases and NH_{4+} are higher in urban areas (Galloway and others 2002). However, rates of N deposition from these sources is just beginning to be studied and thus its effect on plant growth and NPP is still highly uncertain (Carreiro and Tripler 2005). Lovett and others (2000) found that over the course of the growing season, N deposition from the atmosphere was 17 times greater in New York City oak forests than in suburban and rural ones located on an urban-to-rural gradient. Steep changes in urban-to-rural N deposition were also shown in the Louisville, Kentucky metropolitan region (Carreiro and Tripler 2005). Because nitrogen is essential for many plant functions, including pigment production, increased N fertilization in urban areas would likely promote overall NPP, although further research is needed to quantify its effect.

Tropospheric ozone (O_3) is produced in urban and industrial areas, but is often carried great distances from its source and may be reduced in immediate urban centers due to NO_x scavenging reactions (Gregg and others 2003). Vegetated systems far from industrial areas may therefore be affected by tropospheric O_3, which damages plant tissue at moderate levels (60-170ppb; Smith 1990) and may inhibit plant growth at levels as low as 50ppb (Mackenzie and El-Ashry 1989). Ozone concentrations may therefore have dramatic effects on plant growth around urban centers. This was shown by Gregg and others (2003), who found that the biomass of Eastern Cottonwood (*Populus deltoides*) clones grown in New York City was twice that of those grown in a nearby rural environment due to higher tropospheric ozone concentrations at the rural sites.

The net effect of urbanization on plant growth and carbon assimilation is still highly uncertain. Although many of the environmental factors have been studied and modeled independently and/or in controlled environments, little is known about how the effects of such factors will interact with each other to influence plant physiological and biochemical processes. Computer models are currently being developed to investigate these complex interactions, but these models are limited by current data availability about individual environmental effects, and thus exhibit the need for more empirical studies of biophysical responses to urbanization (Carreiro and Tripler 2005). Data about the effects of urban conditions on plants are not only limited, but they have also proven to be contradictory; this may be due to differential responses among studied tree species or to differential effects of particular atmospheric interactions in various cities. This uncertainty further reflects the need for more empirical studies about the nature of these relationships and the influences of various underlying physiological interactions.

Importance of Foliar Pigment Content

Pigments are vital to the functioning of photosynthetic mechanisms and provide valuable information about physiological processes within leaves. Pigments absorb light in the visible portion of the spectrum between 400 and 700 nm. Chlorophylls are the dominant light-harvesting pigments of leaves, and have strong absorption peaks in the red and blue regions of the spectrum. This absorbed light energy is then transferred to the photosynthetic apparatus. Chlorophyll concentration (Chl) indicates the photosynthetic potential of a leaf. It has also been used as an indicator of plant nutrient status because chlorophylls contain a large portion of a leaf's nitrogen (Filella and others 1995).

Carotenoids are accessory pigments with a yellow-orange color and have several functions within leaves. They contribute to light-harvesting, absorbing most strongly in the blue region of the spectrum. Notably, the absorption peak of carotenoids overlaps with one of the absorption peaks of chlorophylls. Carotenoids have also been shown to play a structural role in photosynthetic membranes (Young and Britton 1990).

Most importantly to this study, carotenoids involved in the xanthophylls cycle serve a photoprotective role by quenching chlorophyll excited states (Demmig-Adams and Adams 1996). When leaves become stressed, chlorophylls cannot manage all of the incoming solar radiation, resulting in excess absorbed light energy and singlet oxygen production, which is harmful to the photosynthetic apparatus. Carotenoids involved in the xanthophylls cycle act to dissipate this excess energy so that the photosynthetic apparatus of a leaf is not damaged (Demmig-Adams and Adams 1996). A plant or leaf facing environmental stress will therefore experience chlorophyll breakdown and carotenoid retention or production, resulting in a higher carotenoid:chlorophyll (Car:Chl) ratios than a non-stressed plant. Higher Car:Chl ratios result in lower photosynthetic light use efficiency (LUE), which is a measure of carbon assimilation per unit leaf area.

Pigment concentrations and their ratios can serve as indicators of many physiological conditions and can assess photosynthetic activity. Overall, chlorophyll concentration indicates the photosynthetic potential of a leaf, but LUE is affected by the presence of other pigments, especially carotenoids (Car). Car:Chl ratios increase both during times of water or nutrient stress as well as during senescence, when rates of carotenoid breakdown are much lower than those of chlorophyll breakdown.

The response of pigment production to urbanization can provide great insight about how LUE, biomass accumulation, NPP, and overall growth of vegetation will respond to changing environmental conditions. However, as with these other measures, pigment production is influenced by a wide array of environmental conditions and the net effect of these variable is unknown. Certain urban environmental conditions are likely to promote overall pigment production. The higher carbon dioxide concentrations found in urban environments would likely be beneficial for pigment production, as they are generally correlated with higher NPP (Delucia and others 1996). The lower diurnal temperature range generally characteristic of cities may reduce the risk of temperature stress on urban plants, allowing them to be more efficient pigment producers. Higher atmospheric nitrogen deposition in cities may also prove important in determining pigment content because nitrogen is a necessary component in the process of pigment production. This idea has been supported by Evans (1989), who found that chlorophyll concentrations are correlated with nitrogen content in C3 plants.

On the other hand, other factors associated with urban environments would be expected to limit pigment production. Urban areas tend to have higher levels of atmospheric particulates, which causes stomatal closure. Urban environments also contain higher concentrations of air pollutants such as $CuSO_4$ and SO_2, which have been found to suppress rates of photosynthesis (Geiser and Neitlich 2006).

Remote Sensing as a Tool for Studying Biological Systems

Acquiring pigment concentration data through the traditional method of extraction and spectrophotometric analysis is an expensive and time-consuming process. It requires sample destruction, therefore limiting temporal studies that attempt to measure changes in pigments over time (Sims and Gamon 2002). Furthermore, these traditional methods, although reliable, cannot be easily applied to landscape and ecosystem level analyses and thus limit the scale of vegetation health studies (Sims and Gamon 2002). Spectral remote sensing techniques that measure reflectance, on the other hand, have the potential to provide accurate information about physiological characteristics while foregoing the problems associated with traditional studies (Gamon and Qiu 1999). Spectrometers are instruments that can be used to measure reflectance across a spectrum, and now have the technology to do so in narrow (1 nm) spectral bands. These hyperspectral remote sensing techniques allow for collection of more precise and accurate information.

Measured leaf reflectance includes direct reflectance from external surface structures as well as reflectance of photons scattered by the internal structure, air spaces, and air-water interfaces of leaves (Ustin and others 2004). In general, leaf reflectance in the visible portion of the spectrum is low because absorption by photosynthetic pigments is high in this region (Penuelas and Filella 1998). Reflectance in the near-infrared region between 700 and 1300 nm is mainly influenced by leaf structural properties, while the middle infrared region between 1300 and 3000 nm is generally altered by changes in water content (Penuelas & Filella 1998). Because of scattering effects and inter-species differences in internal leaf structure, the ability to use reflectance to obtain accurate information about leaf properties has proven to be complicated (Gamon and Surfus 1999). Assessing pigment content in particular is difficult because pigments in intact leaves are bound within leaf structures (Gamon and Surfus 1999). However, developments in these methods and the indices used to measure various properties have made spectroscopic methods more accurate (Gamon and Surfus 1999).

Spectral vegetation indices (VIs) are empirically derived equations that use reflectance data to provide information about various physiological properties based on their specific effects on reflectance spectra. Vegetation indices have been designed to measure pigment and water content, chemical composition, biomass, plant physiological and phonological status, nitrogen content, and many other important physiological measures. Although initially designed for leaf-level studies, many VIs are now being tested or developed for application to air and spaceborne remote sensing, which is further complicated by atmospheric scattering properties. Scaling up to landscape and ecosystem level analyses has great potential for large-scale studies, including urban-to-rural gradient studies.

For pigment analysis, VIs focus on the premise that different concentrations of a certain pigment will affect the shape of a leaf-level reflectance spectrum at a certain wavelength or range of wavelengths. Although some VIs are now widely used and generally accepted as being accurate, many were developed using data from only one or a small group of species and have not been widely tested (Sims and Gamon 2002). Differences in leaf structure, including thickness, density, air-water interfaces, cuticle thickness, and pubescence, may significantly alter the relationships between reflectance and pigment content (Sims and Gamon 2002). Because these morphological and physiological characteristics vary between species, VIs must be tested across species to verify their accuracy and reliability (Sims and Gamon 2002).

Overarching Goals of Study

There are two overarching goals of this project. First, this study aimed to better understand how foliar pigment content of *Quercus rubra* (Red Oak) seedlings differs between Central Park (located in the center of New York City) and Lamont-Doherty Earth Observatory (located in a suburban area 38 km north). As urbanization is expected to increase, and as environmental factors are expected to change globally, it is important that we understand how terrestrial vegetation will respond. By studying pigment content in an urban and a suburban area, we hoped to gain valuable insight into how environmental factors associated with urbanization affect pigment production and photosynthetic light use efficiency (LUE). *Quercus rubra* was chosen as an appropriate species because it is dominant in hardwood forests of the northeastern United States and has proved to be a significant contributor to the carbon sink (Field 2001).

Second, this study assessed how accurately foliar pigment content can be detected for *Quercus rubra* seedlings in the New York metropolitan area using spectral remote sensing techniques and three pigment-specific vegetation indices: the Chlorophyll Index (CHL), the Carotenoid Reflectance Index (CRI), and the Photochemical Reflectance Index (PRI), which estimate chlorophyll content, carotenoids content, and LUE, respectively. This information was also used to produce empirically derived relationships that can be used in future studies.

It was hypothesized that the urban Central Park (CP) seedlings would have higher foliar chlorophyll and carotenoid concentrations than the suburban Lamont-Doherty Earth Observatory seedlings. It was also expected that the carotenoid:chlorophyll ratios would be lower in the Central Park seedlings than in the Lamont-Doherty Earth Observatory (LDEO) seedlings. The chlorophyll index (CHL) was expected to be higher in the Central Park seedlings. Similarly, CRI was expected to be greater for the Central Park seedlings, as carotenoid levels generally increase with chlorophyll levels. The Photochemical Reflectance Index (PRI), which is positively correlated with light-use efficiency (LUE) and negatively correlated with carotenoid:chlorophyll ratios, was expected to be lower in Central Park plants.

Methods and Explanations of Vegetation Indices

Site Location and Experimental Design

The sites chosen for this study were Central Park located in the center of New York City, and Lamont-Doherty Earth Observatory (LDEO), which is located in a suburban area about 38 km north of the Central Park site (Figure 1). Monthly measurements were taken on fifteen pot-grown *Quercus rubra* seedlings, all grown from the same set of acorns, between May and October 2007. In order to control for differences in soil composition, all pots contained the same soil, which was fertilized to ensure that seedlings did not face nutrient limitation due to soil deficiencies. All seedlings were placed in open areas with full access to sunlight.

Figure 1: Map of the Central Park (CP) and Lamont-Doherty Earth Observatory (LDEO) sites. The LDEO site is found in a suburban area about 38 km north of the urban Central Park site.

Pigment content was assessed in two ways: (1) by directly measuring pigment concentrations through extraction and spectrophotometric methods and (2) through ground-level hyperspectral remote sensing and the use of pigment-specific Vegetation Indices (VIs). Each month, one upper canopy leaf was removed from each tree, wrapped in a moist paper towel and Ziploc bag, and placed on ice in a cooler. The leaf samples were then immediately brought back to the lab and placed in a freezer. Reflectance measurements were taken within two hours of collection and spectrophotometric measurements were later made using the same leaf samples.

Pigment Quantification through Extraction and Spectrophotometry

On the same day leaves were collected and reflectance measurements were taken, three 9 mm diameter disks were punched from each leaf, immediately enclosed together in aluminum foil packets and frozen in liquid nitrogen, and then stored at -70°C for pigment analysis. Foliar pigments from the three leaf disks were extracted into a 100% acetone solution using a mortar and pestle and in a dimly lit environment. Small amounts of washed sea sand and liquid nitrogen were added to make grinding easier. $MgCO_3$ was also added to act as a buffer in the acetone solution to prevent acidification (Lichtenthaler 1987). The supernatant was extracted from the mortar using a pipette and transferred to a test tube. This process was repeated twice so that the total volume of solution in the test tube was 6 ml (3 additions of 2 ml acetone). The test tube was sealed with Parafilm and placed on ice in an enclosed, insulated dark container. After preparation of four different solutions, each test tube was shaken and its contents were transferred to a microcentrifuge tube. The solutions were centrifuged for two minutes at 3000 rpm. The supernatant from each microcentrifuge tube was then transferred to a semi-micro UV glass cuvette. A Perkin Elmer Lambda 3 spectrophotometer was used to measure absorbance at a spectral resolution of 1 nm from 400 nm to 800 nm. From these values, absorption spectra were produced (Figure 1) and concentration values were determined based on the definitions described below.

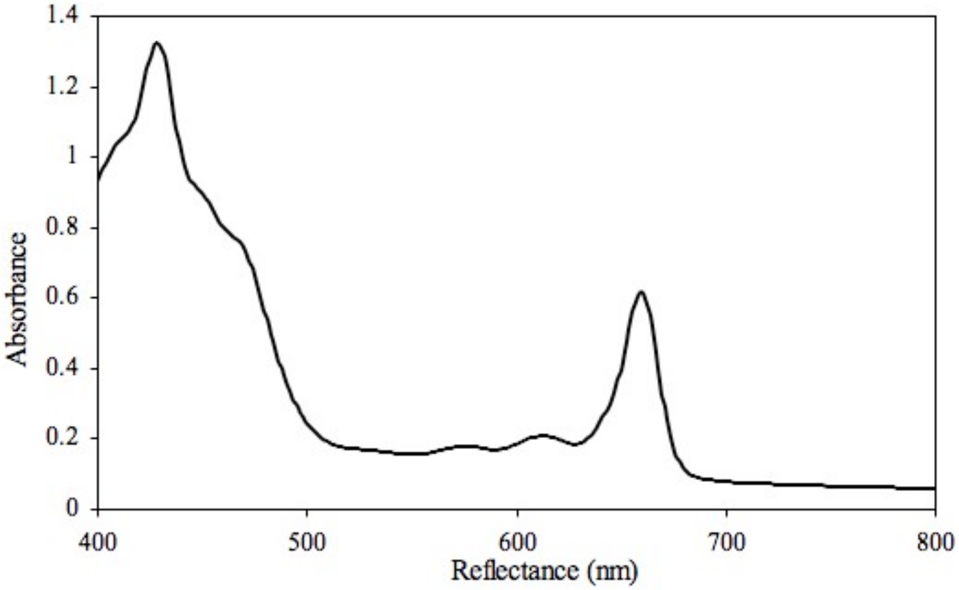


Figure 2: The absorption spectrum (400-800nm) of the pigment solution obtained from a Central Park seedling leaf in August 2007.

The chlorophyll and carotenoid concentrations, referred to as Chl and Car respectively and measured in micromoles per milliliter (µmol ml^{-1}), of the diluted pigment solutions were calculated from the absorption data using the following definitions from Lichtenthaler (1987) and Lichtenthaler and Buschmann (2001):

$$[\mathrm{ChlA}] = 11.24 \times (A_{662} - A_{710}) - 2.04 \times (A_{645} - A_{710})$$
$$[\mathrm{ChlB}] = \quad 20.13 \times (\mathrm{A}_{645} - \mathrm{A}_{710}) - 4.19 \times (\mathrm{A}_{662} - \mathrm{A}_{710})$$
$$[\mathrm{ChlAB}] = [\mathrm{ChlA}] + [\mathrm{ChlB}]$$
$$\text{Bulk Carotenoids} = \frac{1000 \times (A_{470} - A_{710}) - (1.90 \times Chl\ A) - (63.14 \times Chl\ B)}{214}$$

where A_X refers to the absorbance of the solution at wavelength X nm.

The concentration values for each solution were then converted to units of micrograms of pigment per square centimeter (µg cm^{-2}) of leaf by using the following formula (Lichtenthaler & Buschmann 2001):

$$[\text{pigment}] = \frac{\mu mol\ ml^{-1} \times ml\ of\ \ solution}{leaf\ \ area\ \ from\ which\ solution\ was\ created} = \frac{\mu mol\ ml^{-1} \times 6\ ml}{3 \times [\pi(0.9cm)^2]}$$

Thus, the final concentration value is a measure of the amount of each pigment per unit area of leaf (µg cm^{-2}).

Estimating Pigment Content with Spectral Remote Sensing and Vegetation Indices (VIs)

An Analytical Spectral Devices (ASD) FieldSpec® spectrometer was used to collect leaf-level reflectance values at a spectral resolution of 1 nm and between wavelengths 350-1050 nm. Prior to reflectance measurement, the instrument was calibrated using a white standard. The spectrometer has a 25° field-of-view (FOV) fiber optic and was used with an integrating sphere. Three spectral reflectance measurements were taken from different areas on each leaf. From the spectral reflectance data produced (Figure 2), four different vegetation indices (VIs) were calculated.

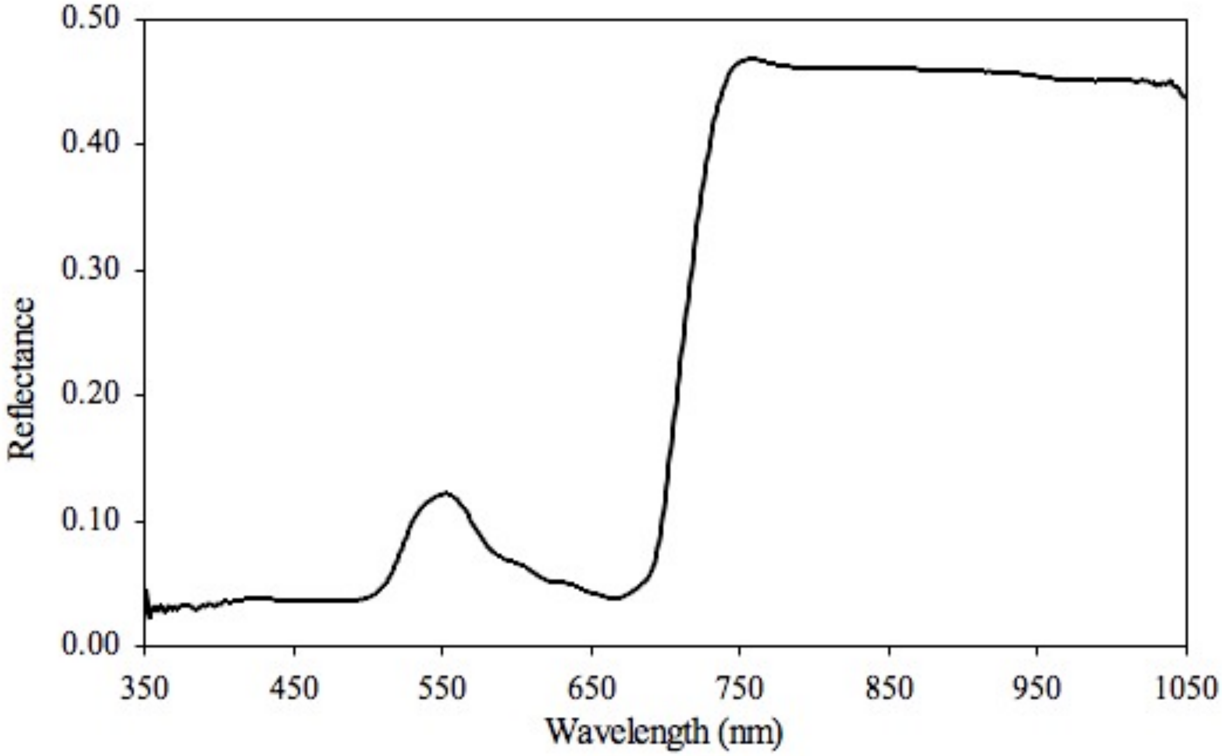


Figure 3: Foliar reflectance spectrum (350-1050nm) for Central Park seedling in August 2007. Note that where absorbance is high for a leaf, reflectance is low, and vice versa.

Chlorophyll Index (CHL)

The Chlorophyll Index (CHL) is a narrow band reflectance index used to estimate a leaf's chlorophyll content. Chlorophyll's region of maximum absorbance in the red region of the spectrum is between 660 nm and 680 nm. Using spectral reflectance values in this region as an indicator of chlorophyll content has proven to be unsuccessful because reflectance values reach a plateau and cause measurements to be insensitive to very high levels of chlorophyll [CITE]. Therefore, the Chlorophyll Index (CHL) is based on reflectance at 700 nm, which is just outside of chlorophyll's region of maximum absorbance. At 700 nm, absorbance is not saturated as easily, and thus higher chlorophyll contents are able to be distinguished (Buschman and Nagel 1993; Datt 1998, 1999). CHL values generally range from 0 to 60 for leaf-level analyses (Boelman and others, manuscript in preparation).

Carotenoid Reflectance Index (CRI)

The Carotenoid Reflectance Index (CRI) was created by Gitelson and others (2002) to estimate foliar carotenoid content, which is much more difficult than estimating chlorophyll content for two main reasons. First, carotenoids are always present in much lower concentrations than are chlorophylls, regardless of the relative ratios. This results in the overall signal of carotenoids being much weaker than that of chlorophylls. Second, the carotenoid absorption peak overlaps with that of chlorophyll, and thus distinguishing between the two absorption peaks can be difficult (Sims and Gamon 2002, Gitelson and others 2001, 2002; Merzlyak and others 1999). In other words, chlorophyll's strong absorption peak "masks" the weaker absorption peak of carotenoids. Reflectance measurements are sensitive to carotenoid content between 500 and 520 nm, although chlorophyll has a strong signal in this region as well (Gitelson and Merzlyak 1997). CRI attempts to adjust for the effects of chlorophyll's high abundance and strong absorption by subtracting the chlorophyll contribution to the reflectance signal at a wavelength where reflectance is affected by both pigments. In other words, the carotenoids contribution to reflectance can be determined by using a reflectance value influenced by both carotenoids and chlorophyll (R_{510}) and then subtracting the contribution of chlorophyll (R_{550}).

Photochemical Reflectance Index (PRI)

Gamon and others (1990) designed PRI, initially called the physiological reflectance index, to be indicative of xanthophylls cycling. Panuelas and others (1995) then altered this original form of the index when they found a strong relationship between a modified form of the index and ΔF/Fm', an indicator of light use efficiency (Gamon and others 1997). The name of the index was then changed to the photochemical reflectance index in order to represent the positive correlation between PRI and light use efficiency (Gamon and others 1997) and has since been widely used as a measure of LUE, a primary factory influencing NPP (Penuelas and others 1995, Gamon and others 1997, Gamon and Surfus 1999, Sims and Gamon 2002). PRI is normalized difference index Sims and Gamon (2002) found that PRI is also negatively correlated with carotenoid:chlorophyll concentration ratios (Car:Chl). High PRI values therefore correspond to lower car:chl concentration ratios. Both photosynthetic LUE and Car:Chl can provide valuable information about leaf stress and have generally found to be correlated with each other as well as PRI, where PRI is positively correlated with LUE and negatively correlated with Car:Chl (Gamon and Surfus 1999; Stylinski and others 2002; Gamon and others 1992; Sims and Gamon 2002; Ustin and others 2004; Martin and others 2007)

Calculations

Calculations were based on the following Vegetation Index (VI) definitions, where R_X = reflectance at X nm (i.e R_{700} = reflectance at 700 nm)

Index Name	Measures	Formula	References
Chlorophyll Index (CHL)	Chlorophyll content	$1/R_{700}$	Gitelson and Merzlyak 1997, Gitelson and Merzlyak 1994
Carotenoid Reflectance Index (CRI)	Carotenoid content	$1/R_{510} - 1/R_{550}$	Gitelson and others 2002
Photochemical Reflectance Index (PRI)	Photosynthetic light use efficiency	$(R_{531} - R_{570}) / (R_{531}+R_{570})$	Gamon, Serrano, and Surfus 1997; Gamon and others 1990

Table 1: Information about Vegetation Indices used in this study.

Statistical Analysis

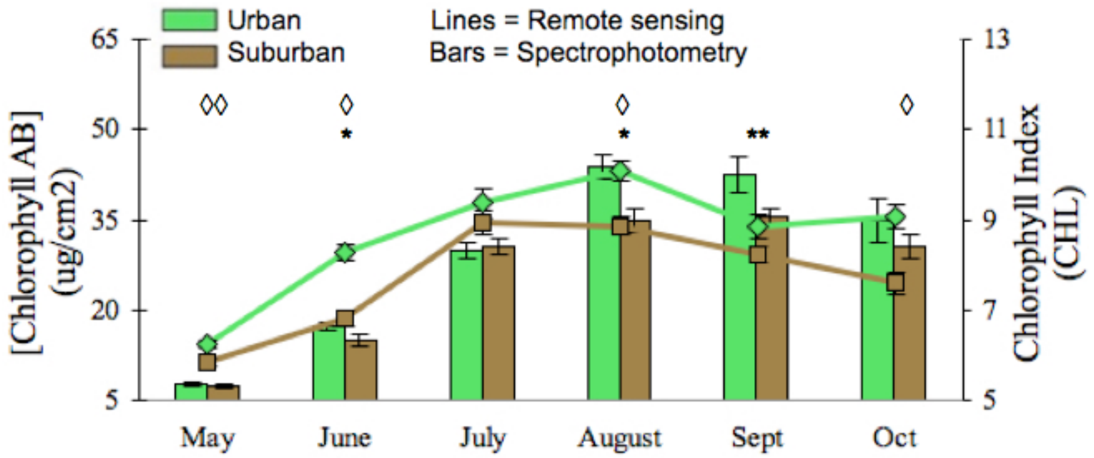
Univariate analysis of variance (ANOVA) was used to test for between-site differences in measured variables during each individual month (SPSS for Windows, Chicago: SPSS). General Linear Model Repeated Measures ANOVA was used to test for between-site differences in each variable over the entire 6-month study period (SPSS for Windows, Chicago: SPSS). Linear regressions were used to determine relationships between physiological variables and their respective vegetation indices. All results were considered significant at $p \leq 0.05$, although values of $p \leq 0.1$ were also noted.

Results

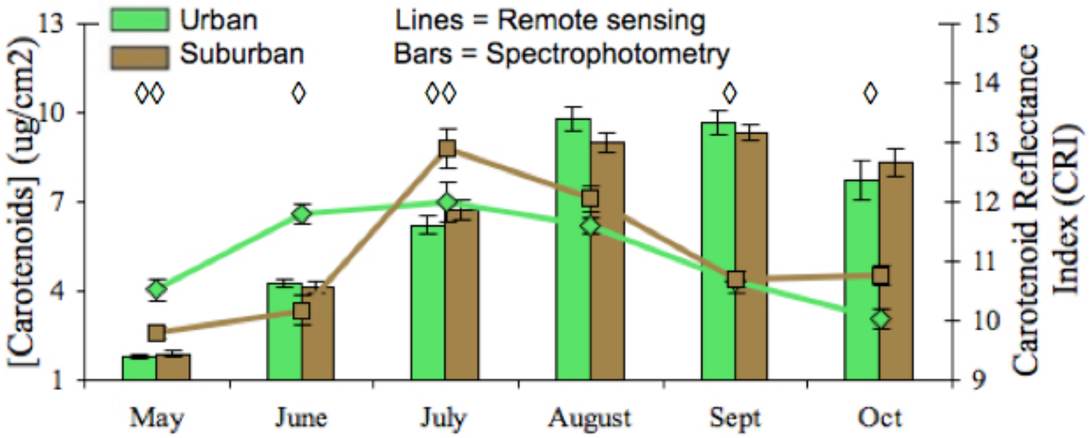
During the six-month study period, average chlorophyll concentrations increased during the first 2-3 months at each site, peaking in August at Central Park and September at LDEO (Figure 4a). The spectrophotometric measurements show that the Central Park seedlings had significantly higher foliar chlorophyll levels than the LDEO seedlings during June and August; the Chlorophyll Index (CHL) also indicates significant between-site differences in chlorophyll content during June and August, as well as October (Figure 4a, Table 2). A repeated measures ANOVA also showed significant differences in chlorophyll concentration ($p = 0.03$) and CHL values ($p=0.04$) for the entire 6-month study period (Table 3). Average total chlorophyll concentrations and CHL values showed a significant positive relationship ($r^2 = 0.74$, $p \leq 0.05$; Figure 5a). [Note: Reflectance spectra for 4 individual leaves from the September Central Park samples only were removed from analysis; they were clear outliers and were the result of error during reflectance measurement.]

The concentrations of foliar carotenoids showed a temporal pattern similar to chlorophyll concentrations at both sites, where carotenoids increased until August at Central Park and September at LDEO and then began to decrease (Figure 4b). These spectrophotometrically measured carotenoid concentrations, although showing seasonal patterns, were not significantly different *between* sites during any of the months studied (Table 2) or over the entire study period ($p = 0.92$, Table 3). CRI, however, suggests that foliar carotenoid content was significantly higher in the Central Park seedlings during June and significantly lower in the Central Park seedlings during September and October (Figure 4b, Table 2). The coefficient of determination for the relationship between carotenoid concentrations and Carotenoid Reflectance Index (CRI) values was low, at $r^2 = 0.086$ ($p \leq 0.05$; Figure 5b).

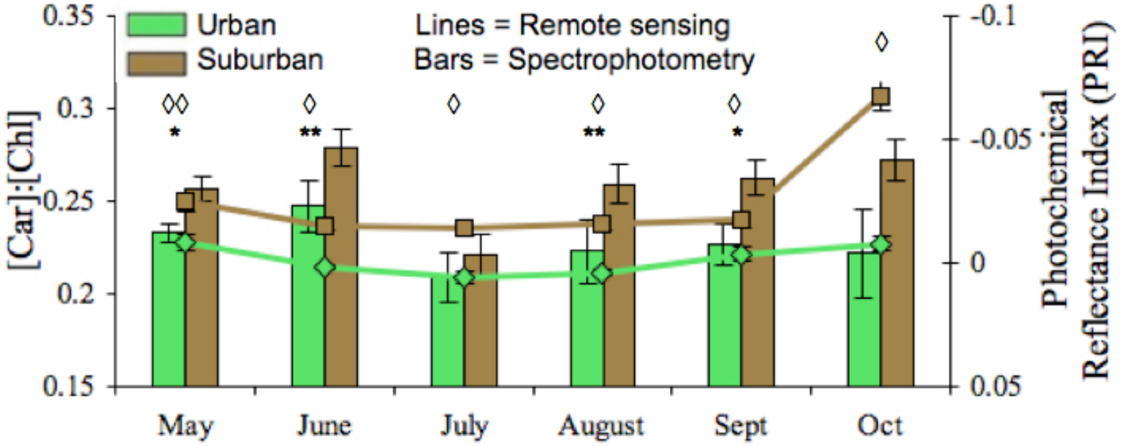
Carotenoid:Chlorophyll (Car:Chl) ratios were highest at both sites in June and October, with similar levels of about 0.25 at Central Park and 0.28 at LDEO (Figure 4c). The lowest Car:Chl ratio occurred at both sites during the month of July (Figure 4c). There were significantly higher foliar Car:Chl ratios in the LDEO seedlings during May and September (Figure 4c, Table 2). PRI, which is negatively correlated with Car:Chl ratio (note the inverted axis in Figure 4c), was significantly lower in LDEO during all months studied except May (Figure 4c, Table 2). The between-site differences over the entire 6-month period were significant for both Car:Chl ($p = 0.01$) as well as PRI ($p = 0.00$), as shown in Table 3. The relationship between Car:Chl and PRI had an r^2 of 0.42 ($p \leq 0.05$; Figure 5c). The LDEO seedlings experienced a smaller decline than the Central Park seedlings in both chlorophyll and carotenoid concentrations relative to peak concentrations of each pigment (Figure 4a,b). There was a large decrease in the average PRI level of the LDEO seedlings between September and October (Figure 4c) that corresponds to a possible outlier in the relationship plot (Figure 5c); however, when the outlying point was removed from the plot, the r^2 only increased to 0.44 ($p \leq 0.05$; Figure 6a).



a. Chlorophyll Index (CHL)

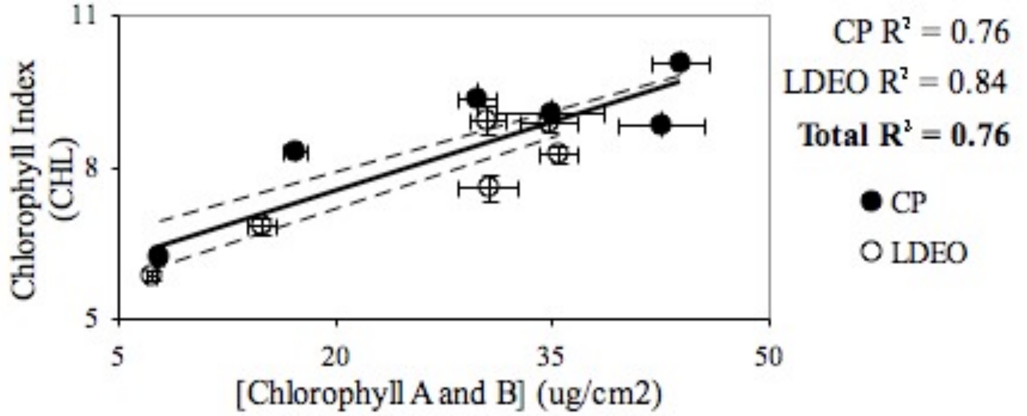


b. Carotenoid Reflectance Index (CRI)

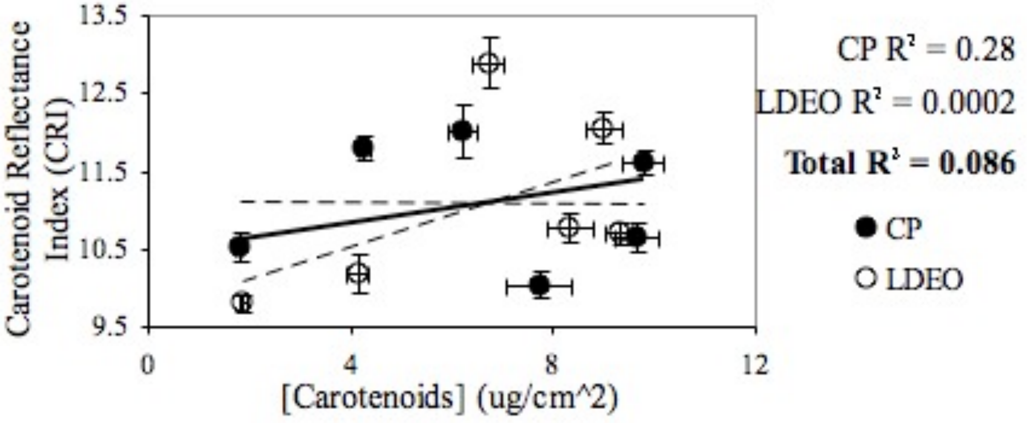


c. Photochemical Reflectance Index (PRI)

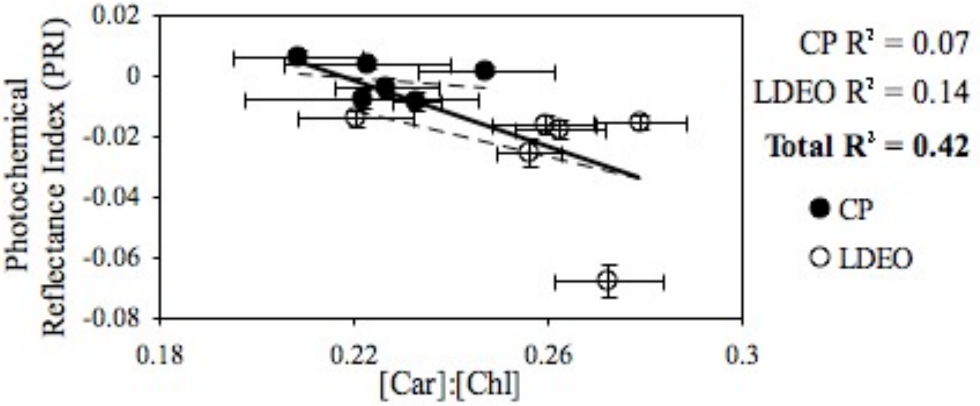
Figure 4. Conventionally measured values and respective spectral vegetation index values for Central Park (CP) and Lamont-Doherty Earth Observatory (LDEO) sites from May 2007 through October 2007. All values represent the mean +/- standard error. Between-site differences are marked with asterisks (*) for traditionally measured concentrations and with diamonds (◊) for spectral index values. One symbol denotes significance at $p \leq 0.05$ (*,◊) and two symbols represents $p \leq 0.1$ (**,◊◊).



a. Chlorophyll Index (CHL) vs. total chlorophyll concentration [Chlorophyll A and B]



b. Carotenoid Reflectance Index (CRI) vs. carotenoids concentration [Carotenoids]



c. Photochemical Reflectance Index (PRI) vs.[Carotenoids]:[Chlorophyll A and B]

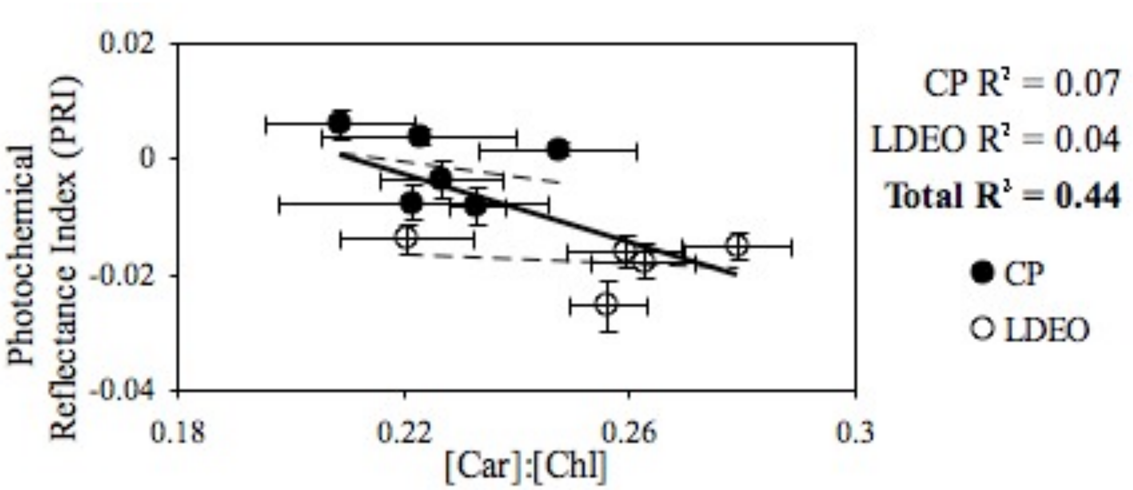
Figure 5. Relationships between vegetation index values calculated from reflectance data and concentrations determined using traditional spectrophotometric methods. Values represent the average of measurements from all seedlings at each site +/- standard error. All correlations were significant at $p \leq 0.05$.

	P value					
	May	*June*	*July*	*August*	*September*	*October*
[Chl AB]	0.39	**0.05***	0.56	**0.00***	*0.06***	0.33
[Car]	0.55	0.37	0.14	0.17	0.33	0.32
Car:Chl	**0.01***	*0.06***	0.59	0.10**	**0.04***	0.20
CHL	*0.09***	**0.00***	0.66	**0.02***	0.50	**0.05***
CRI	*0.06***	**0.00***	*0.08***	0.21	0.37	**0.00***
PRI	**0.07***	**0.00***	**0.00***	**0.00***	**0.02***	**0.00***

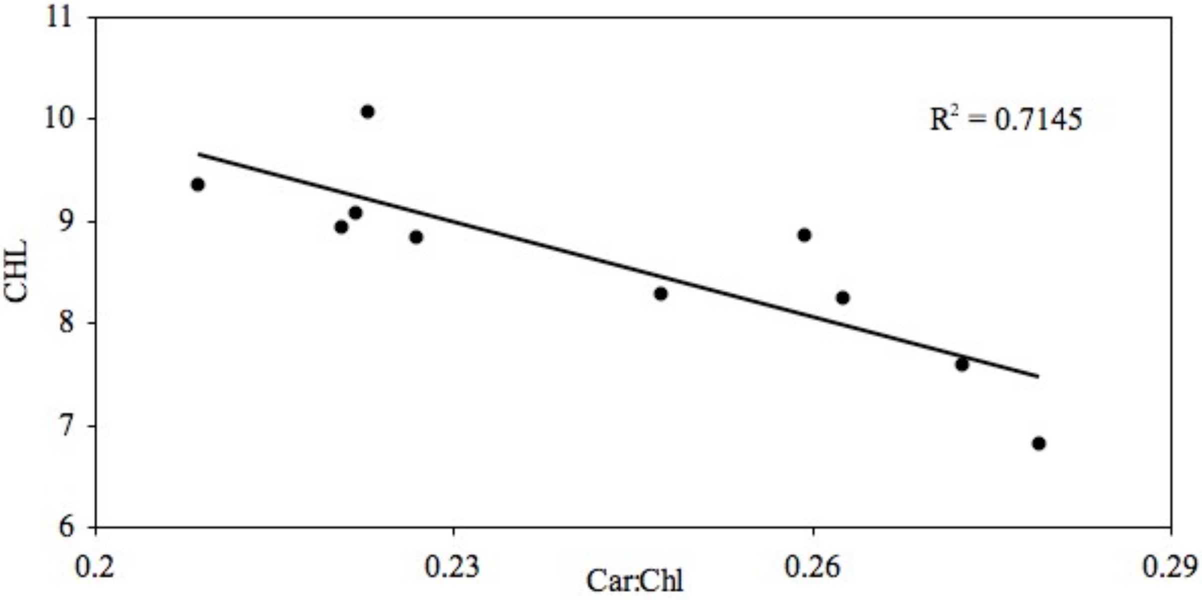
Table 2: Results of univariate ANOVA to test for between-site differences in measured variables during each individual month. ***p ≤ 0.05**, **p ≤ 0.1.

Measure	*P value*
[Chl AB]	**0.03***
[Car]	0.92
Car:Chl	**0.01***
CHL	**0.04***
CRI	0.09**
PRI	**0.00***

Table 3: Results of General Linear Model Repeated Measures ANOVA to test for significant between-site differences in pigment concentrations over the entire 6-month study period. ***p ≤ 0.05**, **p ≤ 0.1.



a) Relationship between the Photochemical Reflectance Index (PRI) and Car:Chl with outlying October LDEO data point removed.



b) Relationship between the Chlorophyll Index (CHL) and Carotenoid:Chlorophyll ratio with May (young leaf) data removed.

Figure 6: Relationships when specified data is omitted. Correlation is significant at $p \leq 0.05$**.**

Discussion

Differences in measured physiological variables over time and between sites

Seasonal Patterns

The overall bell-shaped pattern exhibited by chlorophyll and carotenoid concentrations at both sites (Figure 4a,b) coincides with the expected trends associated with increasing pigment pools followed by the initiation of leaf senescence, which results in pigment breakdown (Merzlyak and Gitelson 1995; Demmig-Adams and others 1996; Biswall 1995). Although Car:Chl ratios were expected to increase toward the end of the season because chlorophylls generally break down at greater rates than do carotenoids, this trend was only minimally represented in the results of this study. During the latter half of the season, chlorophyll and carotenoid concentrations decreased but Car:Chl remained relatively stable at both sites, indicating that Car and Chl were decreasing at similar relative rates (Figure 4c). Car:Chl likely did not increase significantly toward the end of the season because most of the leaves were still green when the October measurements were taken and the elevated Car:Chl ratios associated with senescence generally occur when leaves have visibly changed color (see Gitelson and others 2002). It should be noted that there was a slight increase in Car:Chl for the LDEO seedlings between August (Car:Chl = 0.26) and October (Car:Chl = 0.27), suggesting that the LDEO seedlings may have been experiencing pigment changes associated with senescence earlier than the Central Park seedlings. Although most LDEO seedlings leaves were still green at the time measurements were taken, field notes indicate that a few of the LDEO seedlings' leaves had already begun to change color.

The Car:Chl values were highest at both sites during the month of June (Figure 4c). Because this is not a general trend associated with pigment pools at different developmental stages, it may be suggested that these elevated Car:Chl levels are indicative of some unkown environmental stress. Stress due to soil nutrient deficiencies was probably not the cause, as all pots contained fertilized soil. The low water content and Water Index (WI) values in June suggest that the seedlings may have been experiencing water stress (see Fig 4-A in Appendix). However, the seedlings at both sites were watered regularly and, according to NOAA precipitation data, there was a large rainstorm the day before samples were collected during the month June (see Appendix H). Therefore, the low water content of leaves at both sites may be a symptom, rather than the cause, of some other unknown environmental stressor(s). There was evidence of slight insect consumption of leaf matter at both sites during June, as well as the appearance of small brown spots on a few of the LDEO leaves. The elevated Car:Chl levels may have been caused by one or both of these factors, or may be due to an environmental factor not measured in this study.

Car:Chl ratios were lowest at both sites during July, suggesting that leaves use light most efficiently during this time even though chlorophyll concentrations and total pigment pools were largest during August at Central Park and September at LDEO. In other words, although photosynthetic potential was higher in August at Central Park and in September at LDEO, the leaves of seedlings at both sites were using incoming solar radiation more efficiently during July.

Between-Site Differences

The Central Park seedlings had significantly higher ($p \leq 0.05$) foliar chlorophyll concentrations during June and August (Figure 4a, Table 2). The between-site difference during September was significant at a level of $p=0.06$ (Table 2). These results suggest that the leaves of the Central Park (urban) seedlings had higher photosynthetic potential than the leaves of the LDEO (suburban) seedlings. The results of the repeated measures ANOVA indicate that there was also an overall significant difference between the two chlorophyll concentration datasets over the course of the six-month study ($p = 0.03$; Table 3). Furthermore, the chlorophyll peak at Central Park (August, 43.9 $\mu g/cm^2$) was higher than the chlorophyll peak at LDEO (September, 35.5 $\mu g/cm^2$). It can therefore be concluded that the Central Park seedlings had significantly higher chlorophyll levels, and thus photosynthetic potential/capacity, than the LDEO seedlings. Although the data show that there were significantly higher foliar chlorophyll concentrations in the Central Park seedlings, there were no significant between-site differences in carotenoid concentrations during any individual months or over the entire season ($p = 0.92$).

The Car:Chl values of the LDEO (suburban) seedlings were significantly *higher* ($p \leq 0.05$) than those of the Central Park (urban) during two of the six months studied (May and September). During June and August, the LDEO seedlings had lower Car:Chl ratios at a significance level of $p \leq 0.1$. For the entire season, the LDEO seedlings had overall higher Car:Chl ratios than the CP seedlings at a between-subjects significant level of $p=0.01$ (Table 3). Because Car:Chl is generally considered to be indicative of photoprotection and light-use efficiency where lower Car:Chl ratios correspond to higher LUE (Gamon and others 1990, 1997), these results indicate that the suburban LDEO seedlings had lower LUE than the urban Central Park seedlings. This conclusion is further supported by the significant differences in PRI, which has been shown to be indicative of LUE (Gamon and Surfus 1999, Gamon and others 1992, Asner and others 1994). Although measured in this study against Car:Chl, it is more commonly used to estimate LUE (see next section). Overall, the results of the Car:Chl and PRI measurements suggest that environmental factors associated with the Central Park seedlings' urban environment *positively* affected their pigment production, or alternatively, that the suburban environment in which the LDEO seedlings were located limited pigment their foliar pigment production.

Although identifying the specific environmental factors causing these between-site pigment differences is beyond the scope of this study, a few such factors can be ruled out. For instance, seed origin, watering regimen, and soil conditions were held constant across sites, so it can be concluded that these factors were not responsible for the between-site pigment differences. Possible beneficial environmental factors that may have influenced Car:Chl and LUE in the urban Central Park seedlings include *higher*: CO_2 concentrations (Pataki and others 2003), temperatures (White and others 2002), nitrogen deposition (Lovett and others 2000), and *lower*: diurnal temperature range (DTR). The results of this study suggest that these positive factors outweigh possible negative environmental factors, including higher particulate matter, air pollutants such as $CuSO_4$ and SO_2, and tropospheric O_3 concentrations (see Gregg and others 2002).

Relationships between Vegetation Indices and Pigment Concentrations

Chlorophyll Index (CHL)

The Chlorophyll Index (CHL) showed a strong relationship ($r^2 = 0.74$) with chlorophyll concentration (Chl). CHL trends were similar to those of Chl, both in terms of seasonal variability and between-site differences (Figure 4a). The September CHL value for the Central Park seedlings was lower than would be expected based on the spectrophotometric measurements and did not show a significant between-site difference with the LDEO CHL value even though spectrophotometric methods yielded a between-site difference at a significance level of $p = 0.06$. This is likely due to errors that occurred during spectral measurement. The September Central Park reflectance data had showed several outlying spectra that were much higher than the others for that particular dataset; these spectra corresponded to four individual leaves and were subsequently removed from analysis as outliers. This removal of outliers resulted in a higher September Central Park CHL value than was seen before; however, the CHL value is still lower than is indicated by the spectrophotometric data. This observation suggests that other reflectance spectra were affected by experimental error but could not be identified as clear outliers and therefore influenced the final September Central Park CHL value. Note that if the Central Park September data are removed altogether from the relationship plot, the r^2 value increases from 0.74 to 0.79.

Notably, CHL also showed a strong relationship with Car:Chl when the May data was excluded from analysis ($r^2 = 0.71$, Figure 5b); this supports suggestions that chlorophyll content can accurately indicate stress levels because of the tendency for stressed plants to lose chlorophyll (Carter and Knapp 2001; Hendry and others 1987). Leaf reflectance has been shown to increase between 690 and 720 nm as a general response to various environmental stressors (Carter and Knapp 2001); because CHL uses reflectance values at 710 nm, it is sensitive to such a response. When the May data was included in the relationship plot, the r^2 value declined to 0.32 (Figure E-2 in Appendix). These results suggest that CHL is not an appropriate measure of Car:Chl in young *Quercus rubra* leaves, but can be reasonably used to estimate Car:Chl ratios in mature leaves.

Carotenoid Reflectance Index (CRI)

The Carotenoid Reflectance Index (CRI) was not sensitive to foliar carotenoid concentrations in *Quercus rubra* seedlings, showing an overall r^2 of only 0.086. The seasonal and between-site differences in traditionally measured carotenoid concentrations were relatively small (Figure 4b), and CRI may not be sensitive enough to detect such small variations in carotenoid content. CRI was more sensitive to chlorophyll concentrations ($r^2 = 0.12$) and Chlab + Car ($r^2 = 0.11$) than carotenoids alone (see Appendix E). These results support the notion that detecting the signal of carotenoids is difficult due to the strong signal and higher concentrations of chlorophylls (Gitelson and others 2002). The inability of CRI to detect carotenoids may also have been affected by species-specific physiological and structural characteristics. CRI is a relatively new index, created in 2002**,** and its effectiveness across species has not been widely documented (Gitelson and others 2002). Overall, these results suggest that more research is needed to define a widely applicable index that can accurately detect the spectral signal of carotenoids without chlorophyll interference.

Photochemical Reflectance Index (PRI)

The Photochemical Reflectance Index (PRI) demonstrated a negative relationship with foliar Car:Chl (r^2 = 42). PRI is generally considered to be indirectly related to Car:Chl where higher PRI values correspond to lower Car:Chl ratios (Sims and Gamon 2002; Ustin and others 2004). Previous studies have found strong negative relationships between leaf PRI and Car:Chl ratio, and thus provide empirical evidence of this indirect relationship (Stylinski and others 2002; Martin and others 2007). The data in this study, however, show only a moderately strong relationship between PRI and Car:Chl (Figure 5c). PRI is most commonly used as a measure of photosynthetic Light Use Efficiency (LUE) and thus may have been more accurately representative of LUE, which was not measured in this study.

Although relatively insensitive to seasonal differences in Car:Chl, PRI did appear to be sensitive to between-site differences in Car:Chl (Figure 4c). This suggests that PRI can be used to estimate between-site differences in Car:Chl when leaves are at the same phenological or developmental state, but that it does not necessarily represent seasonal trends well. Importantly, seasonal changes in Car:Chl were relatively minor for both sites in this study, and therefore PRI may simply not be sensitive to small variations in Car:Chl in *Quercus rubra* leaves.

Conclusions and Implications

With respect to the relationships between the VIs and their traditionally measured pigment concentrations, CHL showed the strongest relationship (r^2 = 0.74) with its respective physiological measurement (chlorophyll concentration). These results indicate that CHL can be used as a relatively accurate estimator of chlorophyll concentrations in *Quercus rubra*. CHL also showed a strong relationship with Car:Chl (r^2 = 0.71) when May data were removed; this supports studies suggesting that measures of chlorophyll content can be accurately used to estimate Car:Chl and thus also stress in mature *Q. rubra* leaves (see Carter and Knapp 2001). CRI was shown to be insensitive to carotenoid concentrations, most likely because (1) its signal was ‘masked’ by that of chlorophylls and (2) the influence of morphological/structural leaf properties on reflectance made detection of small differences in carotenoid content difficult. Although CRI was designed to factor out the strong signal of chlorophylls, this study shows that it does not do so effectively in *Q rubra*. Finally, although Car:Chl and LUE are generally related, and PRI is often used to measure LUE, the relationship between Car:Chl and PRI was not particularly strong in this study (r^2 = 42). This may be because (1) PRI is more closely related to LUE than Car:Chl, (2) the index is not strongly sensitive to small variations in Car:Chl, (3) PRI is influenced by *Quercus rubra* leaf structural properties, or (4) some combination of these factors.

The results of this study indicate that more work needs to be done to create vegetation indices that are consistent across species, ecosystems, and phenological variations (see review in Ustin and others 2004; Sims and Gamon 2002). Leaf reflectance is affected by a wide variety of leaf structural properties that often show substantial intra- and inter-species variation (Ustin and others 2004). This can make detection of small differences in physiological measures difficult, as was likely the case with CRI and PRI.

Chlorophyll concentrations, as well as CHL, indicated that the Central Park seedlings had significantly higher photosynthetic capacity than the LDEO seedlings. Both Car:Chl and PRI values suggest that the urban Central Park seedlings also had significantly higher LUE over the course of the entire season. These results have important implications as they suggest that urbanization may be positively affecting LUE in urban plants or that it is limiting LUE in suburban plants. In order to gain more insight what factors may be causing this difference, additional studies will need to be performed. Specifically, the collection of detailed environmental data is essential to drawing correlations between specific environmental factors and effects on pigment production. This preliminary study is part of a larger study that will be investigating the effects of urbanization on many small-scale physiological and biochemical mechanisms, in addition to pigment content, that are related to photosynthesis. This larger study will four sites along an urban-rural transect and will include measurements of certain environmental factors, fluorescence, specific leaf area (SLA), water content, and foliar carbon, hydrogen, and nitrogen content. The results of these measurements will hopefully provide further insight into how the photosynthetic apparatus of urban plants functions compared to that of suburban plants. Furthermore, if differences are found, these results will help to clarify which environmental factors are most influential. This study will ultimately contribute to an expanding body of knowledge about the effects of urbanization on terrestrial vegetation.

Recommendations for Further Research

Further studies should focus on testing the accuracy of spectral vegetation indices across a broad range of species, geographical areas, and phenological stages. Many studies have shown that VIs have the potential for wide application (see Ustin and others 2004; Sims and Gamon 2002), but studies such as this one show the need for further work in making VIs more reliable. With more time, the spectral data in this study could have been used to provide insight into how carotenoids concentrations could be more accurately measured through leaf-level remote sensing techniques. It is important that such spectral data is analyzed to detect specific wavelength combinations that are sensitive specifically to carotenoids without interference from chlorophyll reflectance signals. A useful way to gain insight into how various pigments affect reflectance data is to subject plants to various experimental treatments that have significant effects on pigment concentrations and then measure how reflectance spectra respond.

Research into the effects of urbanization on vegetation should focus on identifying specific beneficial/detrimental environmental factors. If pigment production, NPP, and growth in suburban and rural environments is indeed lower than that in urban environments due to detrimental environmental factors in the suburban/rural environment, it is important to study ecosystem responses to such factors. This is especially important in the New York Metropolitan Area, where the entire area depends on native-forest ecosystems to provide clean and affordable drinking water to millions of people.

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Appendix A: Leaf Water Content

The Water Index (WI, also called the Water Band Index) and an index that measures Equivalent Water Thickness (EWT) were used to estimate water content; however, the data obtained from directly determining water content exhibited some questionability. Furthermore, we were missing the dry weight information for October due to a fire in the lab, as well as for the Central Park seedlings for June; this made the dataset against which we were to measure the indices incomplete. For these two reasons, an assessment of water indices was not included in the final paper. This appendix includes much of the work done to measure water content, and also outlines the reasons why an analysis of water content was excluded from the final paper.

Assessment of Physiological Measurements

Initially, a measure of percent water content was used to provide a reliable variable against which the water indices could be compared. The percent water (% H_2O) of each leaf was calculated using the fresh weight (FW) and dry weight (DW) of leaf disks removed from each leaf. Fresh weights were measured directly after spectral remove sensing measurements were taken and dry weights were measured after the leaves had been dried in an a 70° oven for at least 72 hours. Calculations were based on the following definition:

$$\%H_2O = \frac{wet\ weight - dry\ weight}{wet\ weight} \times 100$$

However, we decided that the equation used to measure water should be per area, and we therefore switched to using a separate measurement known as Equivalent Water Thickness (EWT), which is different from the index (with the same name) that is used to estimate it. This measure was called water content in this paper to distinguish it form the EWT index. Equivalent Water Thickness is a commonly used measure of water content and is generally measured using disk weight. This per area measure of water content of each leaf was calculated as follows:

$$\text{Water Content} = [(\text{wet weight} - \text{dry weight})/\text{area}] * 10$$

During data collection, we were supposed to measure wet and dry weights of the punched leaf (entire leaf – 4 disks) as well as the wet and dry weights of an individual disk. However, for October we are missing all dry weights and for August and September we had mistakenly took the dry weight of the punched leaf + 1 disk instead of doing them separately. Thus, for most months, we had the option of using measurements for the entire punched leaf, as we had measured the total leaf area using a leaf area meter and also had the wet and dry weight of the whole leaves. We also could have calculated water content for single disks, using the wet and dry weight of the disks and dividing by the disk area. For August and September, we were able to fix our error by adding the wet weight of each leaf (with punches removed) and the wet weight of a single disk and then subtracting the dry weight of the punched leaf + 1 disk and dividing by the area of the punched leaf + area of 1 disk.

All of these measurements should have yielded similar water content values regardless of whether we used wet weight, dry weight, and area of a punched leaf, single disk, or punched leaf+single disk. However, the disk-based calculations yielded much higher water content values. We could produce almost an entire dataset of water content values by using the leaf+single disk for each month. However, disk weight calculations are often used for hyperspectral index studies involving water content (see Colombo and others 2007). The relationship between the two different set of calculations was tested (Figure A-1), but they did not yield a strong relationship.

All water content data presented in the appendices is based on the leaf area+single disk calculations unless otherwise specified. However, the accuracy of this water content data is somewhat uncertain since it does not agree with the single disk measurements that were available.

	Area (w. disks removed)	**Single Disk**	**Area + single disk**
May Cp	0.091	0.143	**0.093**
May LDEO	0.096	0.157	**0.097**
June LDEO	0.078	0.150	**0.079**
July CP	0.104	0.182	**0.104**
July LDEO	0.096	0.177	**0.097**
August CP			**0.088**
August LDEO			**0.081**
Sept CP			**0.080**
Sept LDEO			**0.078**

Table A-1

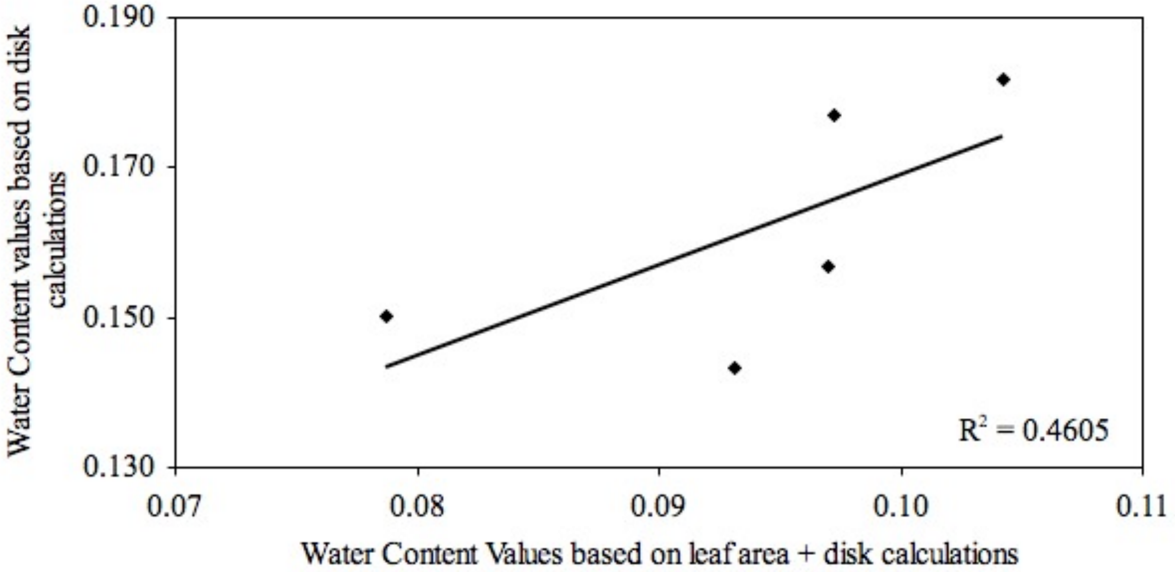


Figure A-1

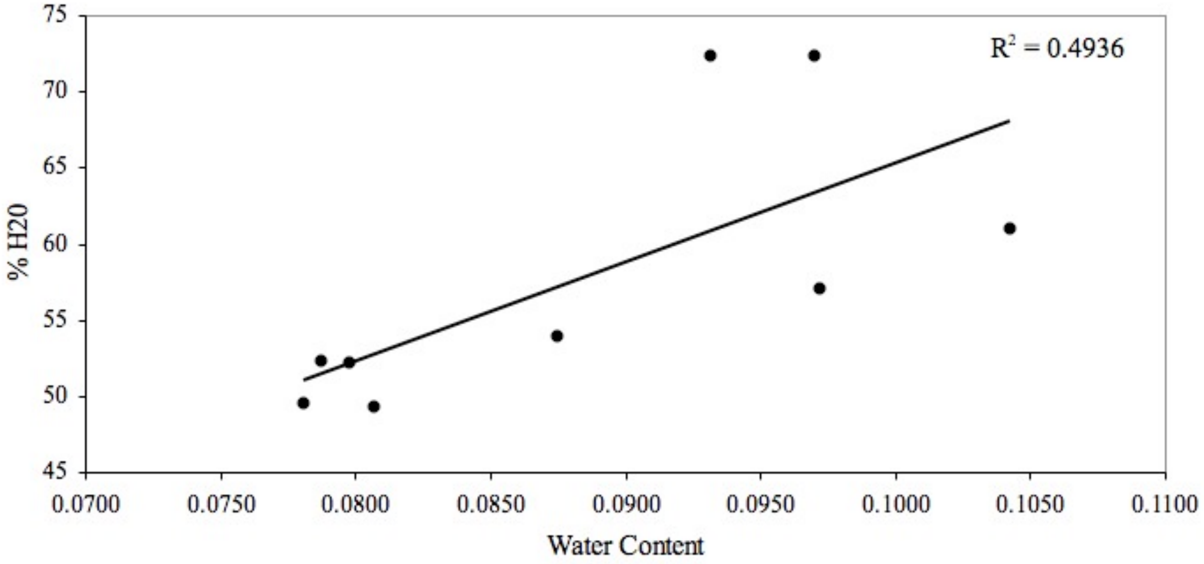


Figure A-3

The directly measured water content was compared to two different indices designed to estimate leaf water content: (1) Water Index (WI, also called the Water Band Index or WBI) and (2) Equivalent Water Thickness Index (EWT). WI was first developed by Penuelas and others (1993) to measure plant water deficiency. The original formula defined the Water Index as the ratio R970/R900, although Penuelas and others (1997) later inversed the ratio, resulting in its current form that is used to measure water content rather than water deficiency. EWT is an index that was designed for canopy and landscape level airborne remote sensing, not for leaf-level analysis (Green and others 1993).

WI = R_{900}/R_{970}

EWT = R_{867} through R_{1049}

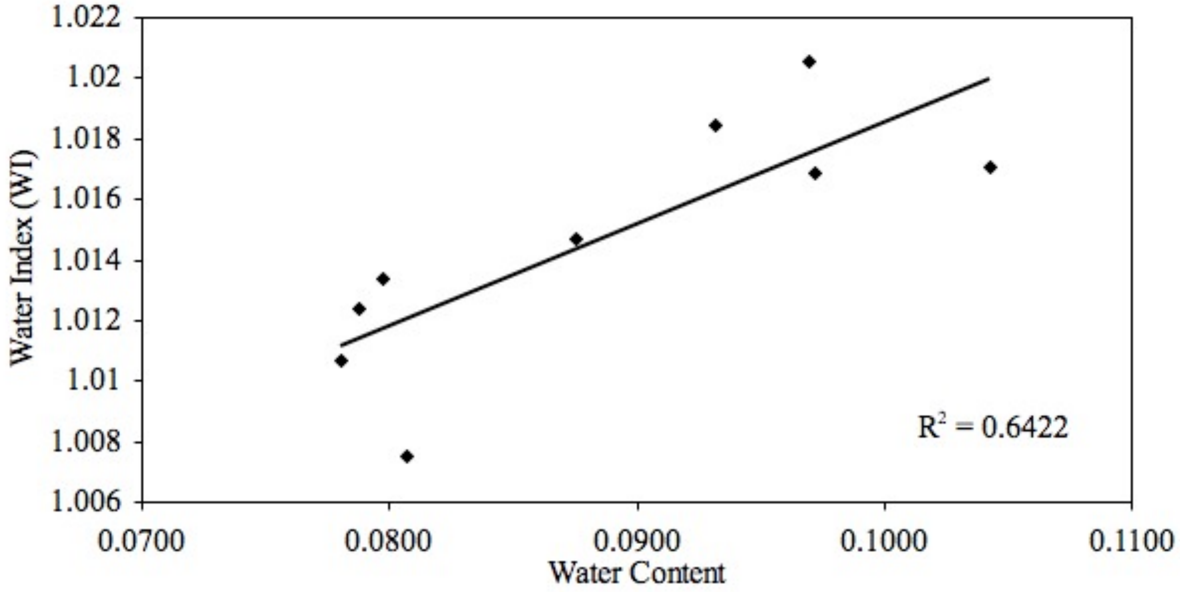


Figure A-2

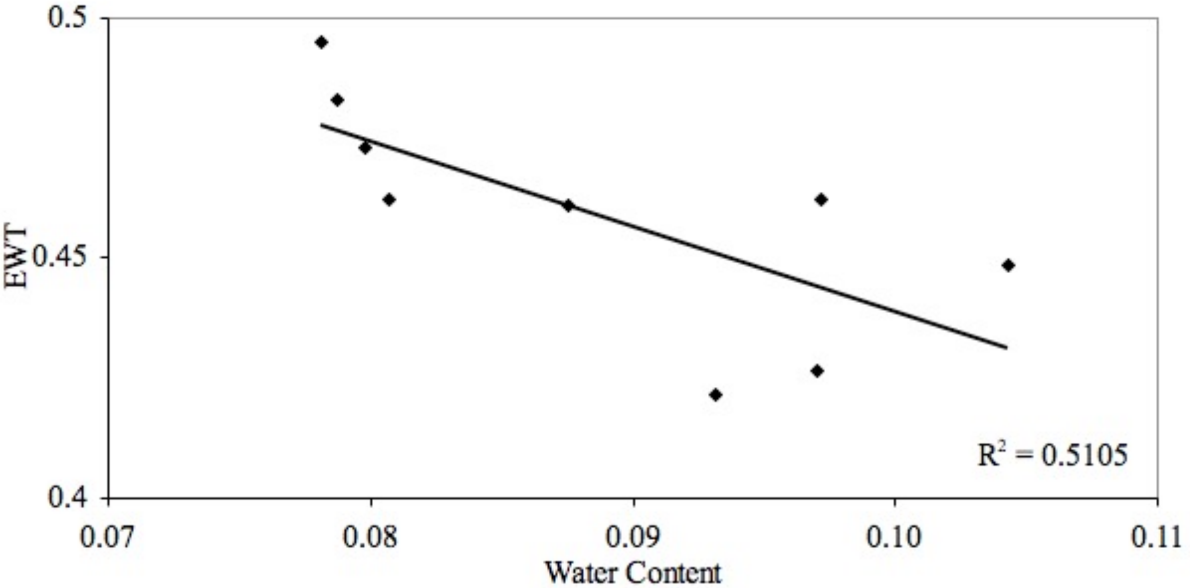
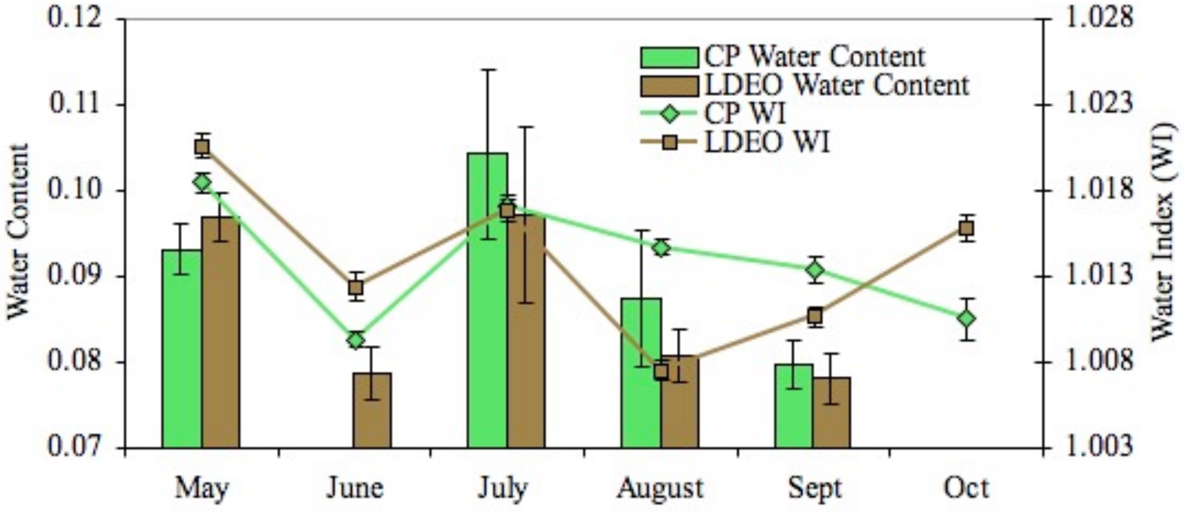


Figure A-3



0.12
0.11
0.10
0.09
0.08
0.07
Water Content
1.028
1.023
1.018
1.013
1.008
1.003
Water Index (WI)
CP Water Content
LDEO Water Content
CP WI
LDEO WI
May
June
July
August
Sept
Oct

Figure A-4

Appendix B: Specific Leaf Area

Specific Leaf Area (SLA) is a measure of leaf area per unit dry mass and is often used to quantify variation in leaf structure (Reich and others 1998). It is expressed in cm_2/g and calculated as follows:

$$SLA = \text{leaf area} / \text{leaf dry weight}$$
$$\text{or } SLA = \text{disk area} / \text{disk dry weight}$$

Because we were using either whole leaf or disk dry weight, the same problems were encountered while calculating SLA as when calculating water content. This data, however, may still provide important insight into how leaf structure influences spectral reflectance and VIs and was thus still included in the appendix.

Leaves with lower SLA are denser or thicker than leaves with lower SLA (Reich and others 1998). SLA should also be related to foliar nitrogen and photosynthetic pigment content, where leaves with lower SLA have higher N and pigment concentrations per area because of thicker mesophyll tissue (Martin and others 2007).

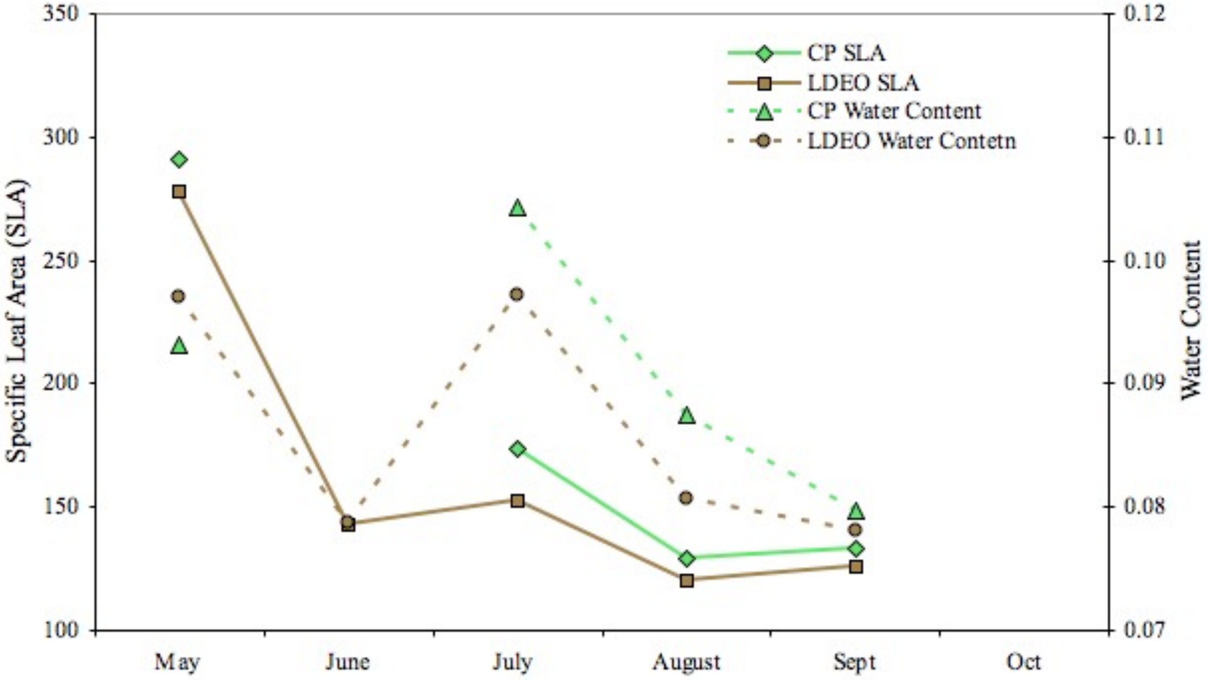


Figure B-1

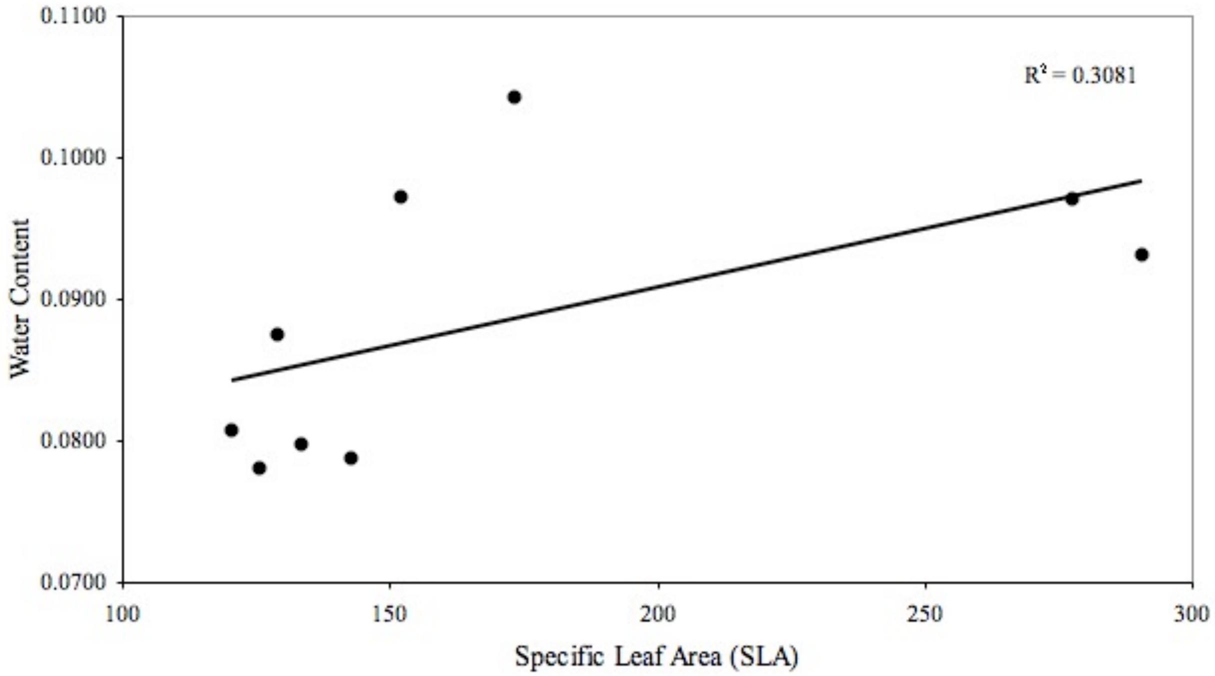

0.1100
0.1000
0.0900
0.0800
0.0700
Water Content
100
150
200
250
300
Specific Leaf Area (SLA)
R² = 0.3081

Figure B-2

Appendix C: Effects of Water Content on VIs

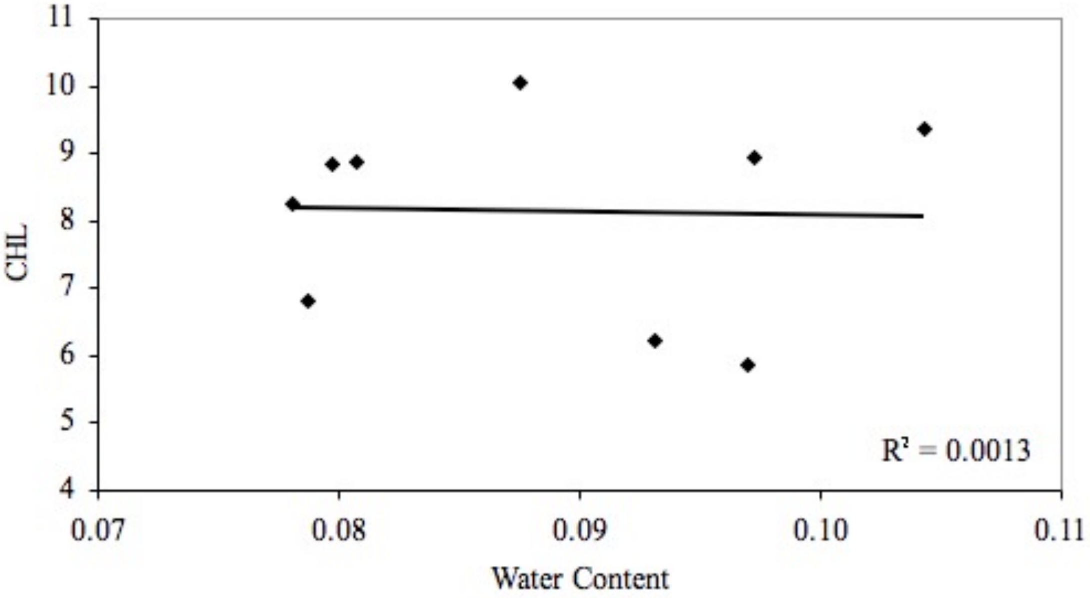


Figure C-1

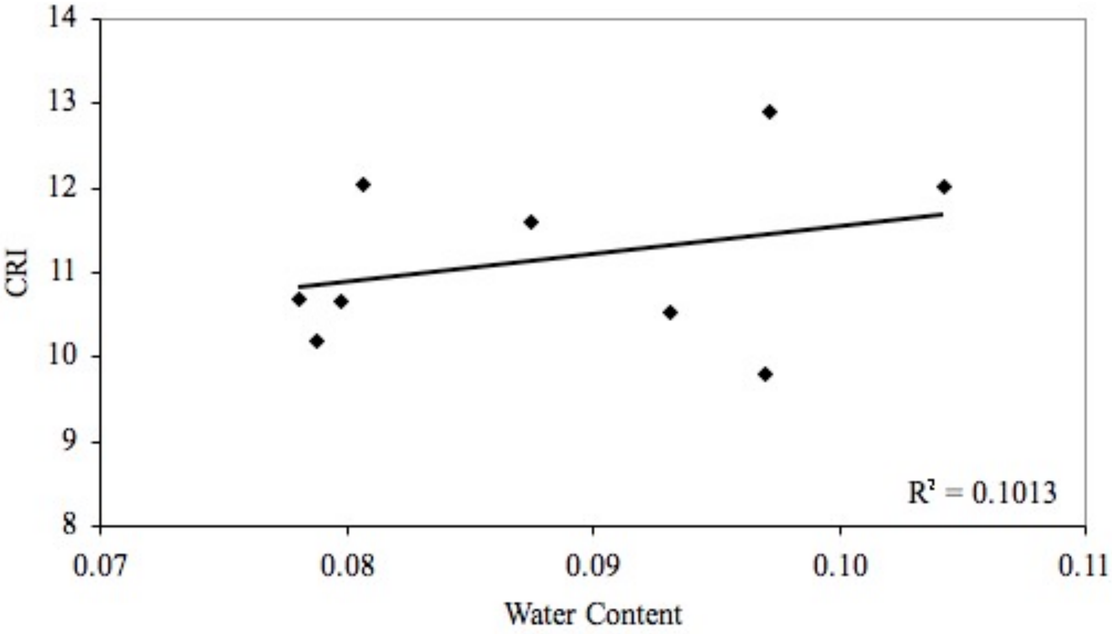


Figure C-2

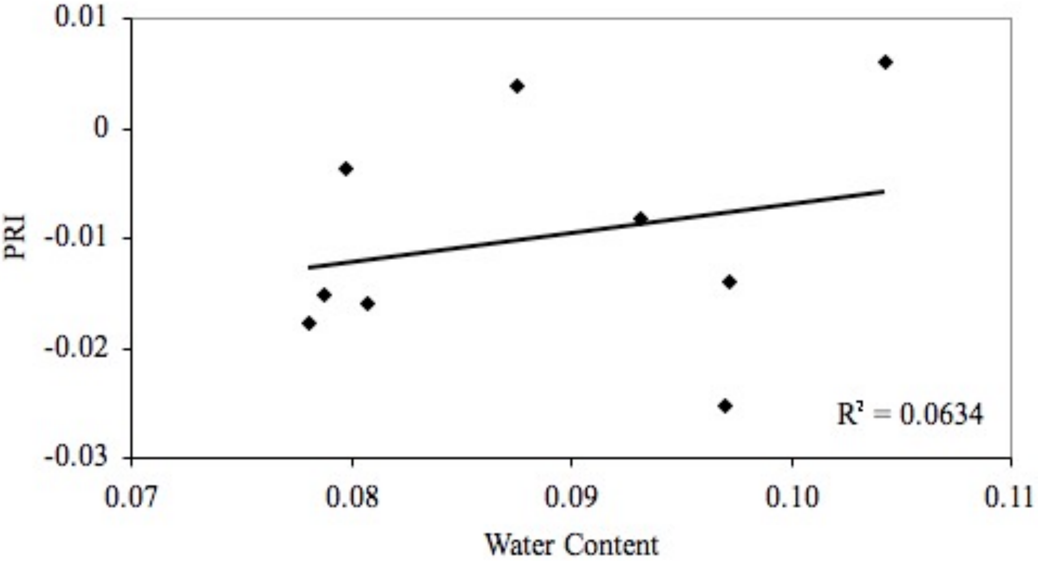


Figure C-3: Note that October LDEO PRI value has been removed as an outlier.

Appendix D: Effects of Leaf Structure (SLA) on VIs

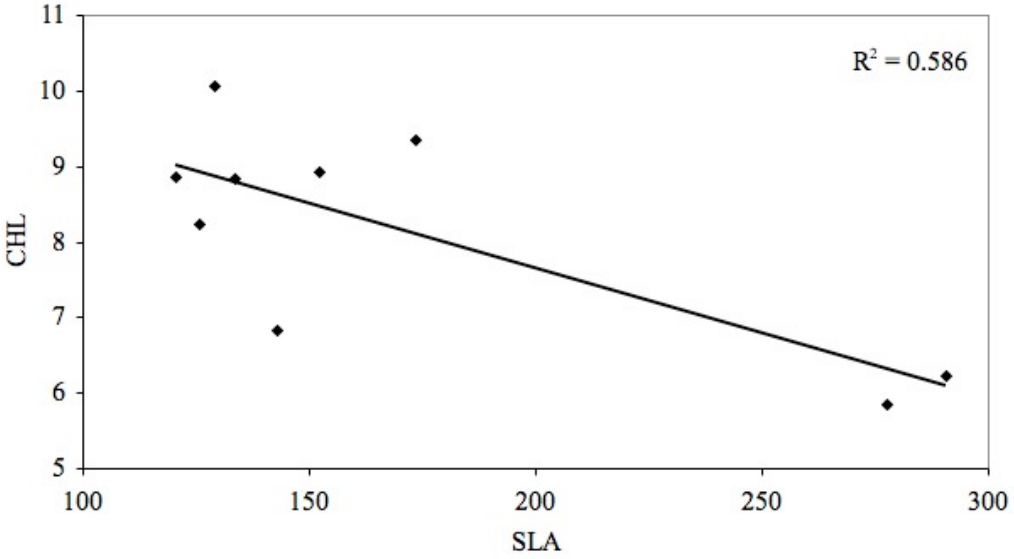


Figure D-1

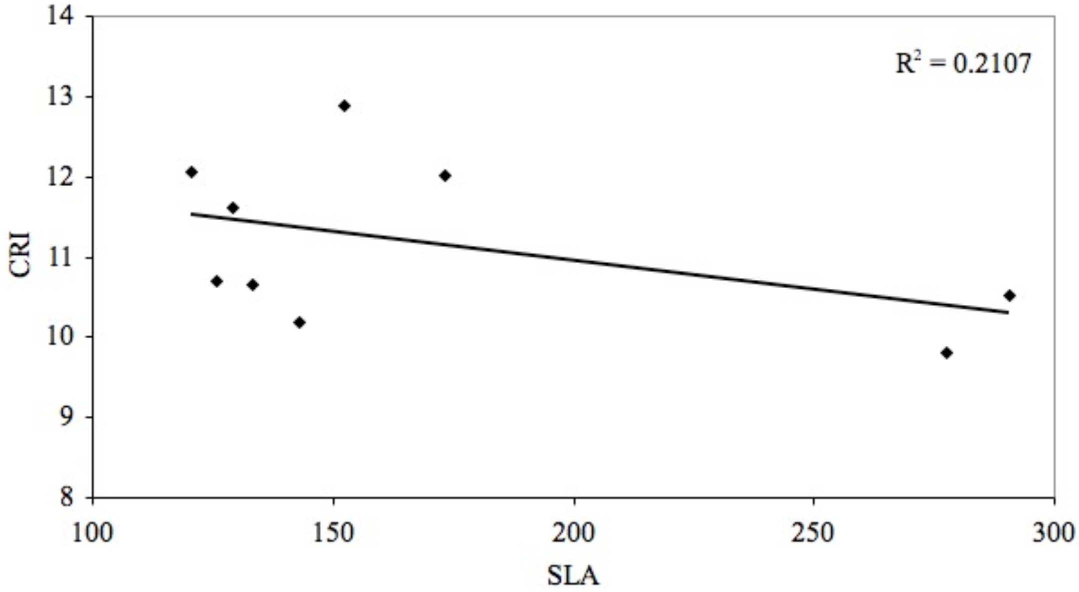


Figure D-2

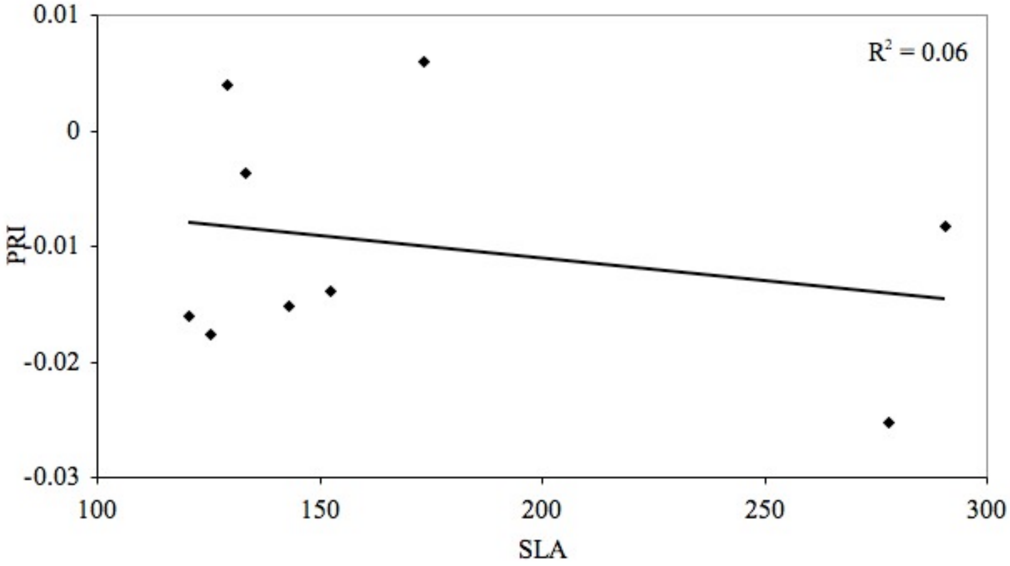


Figure D-3

Appendix E: Other Relationships involving VIs and Physiological Measurements

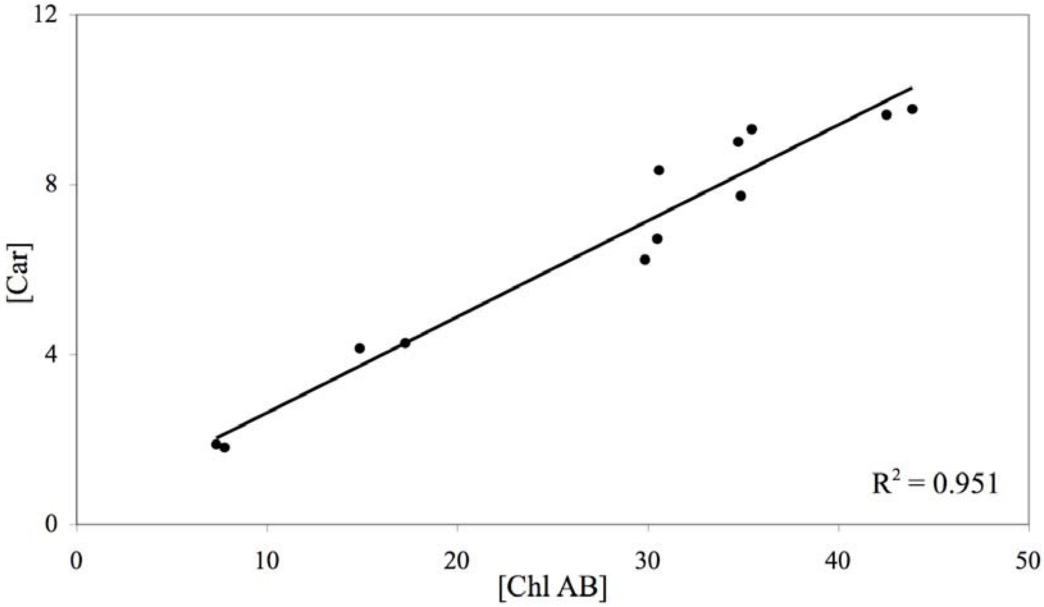


Figure E-1

CHL relationships

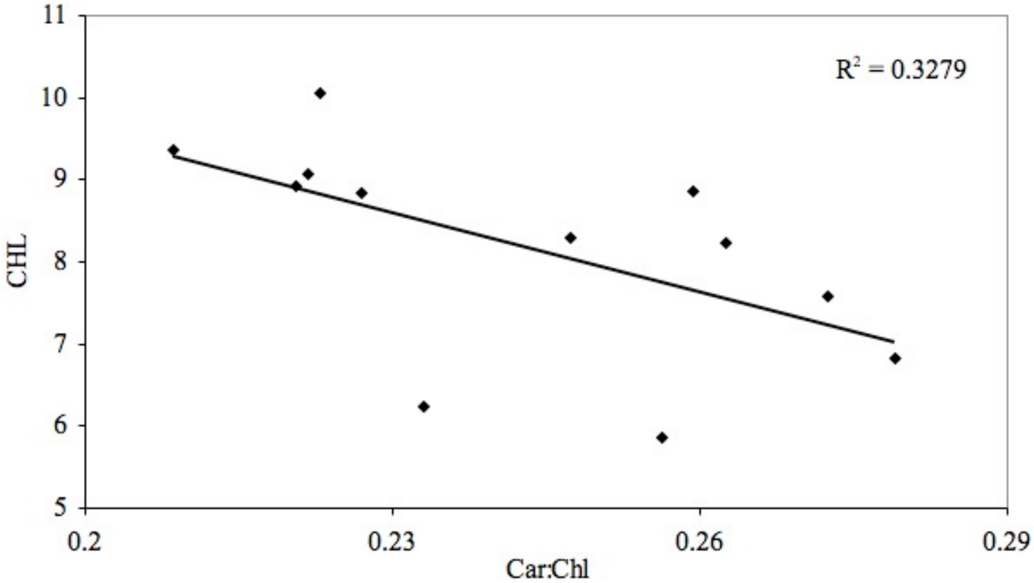


Figure E-2

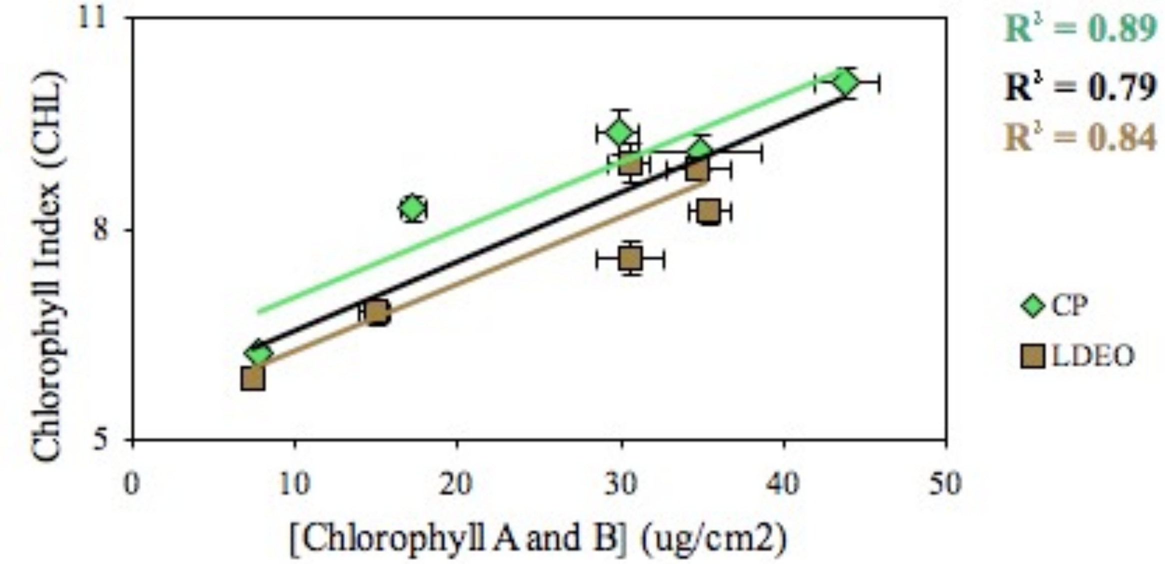


Figure E-3: September values removed.

CRI Relationships

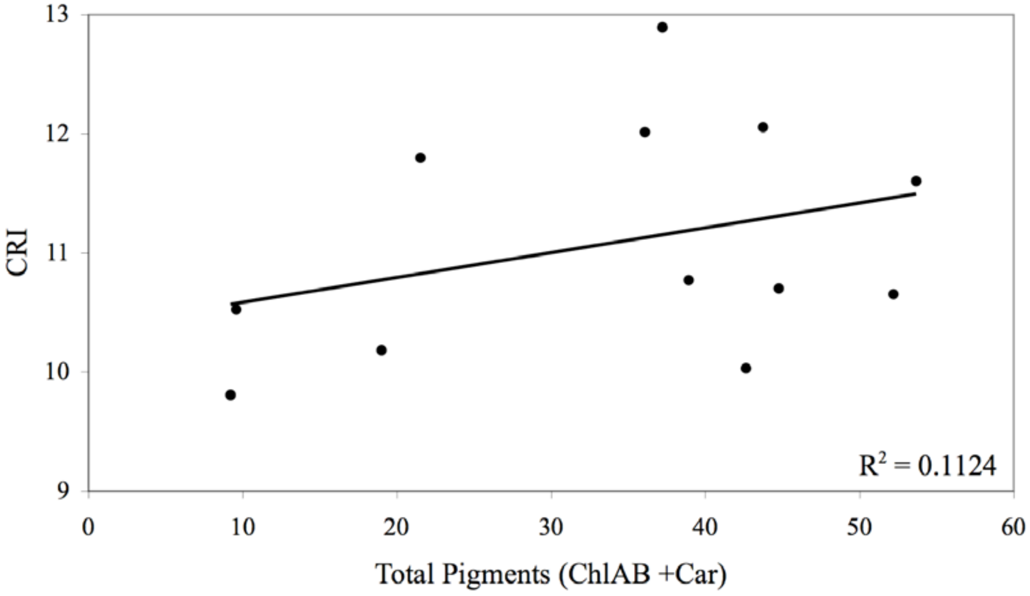


Figure E-4

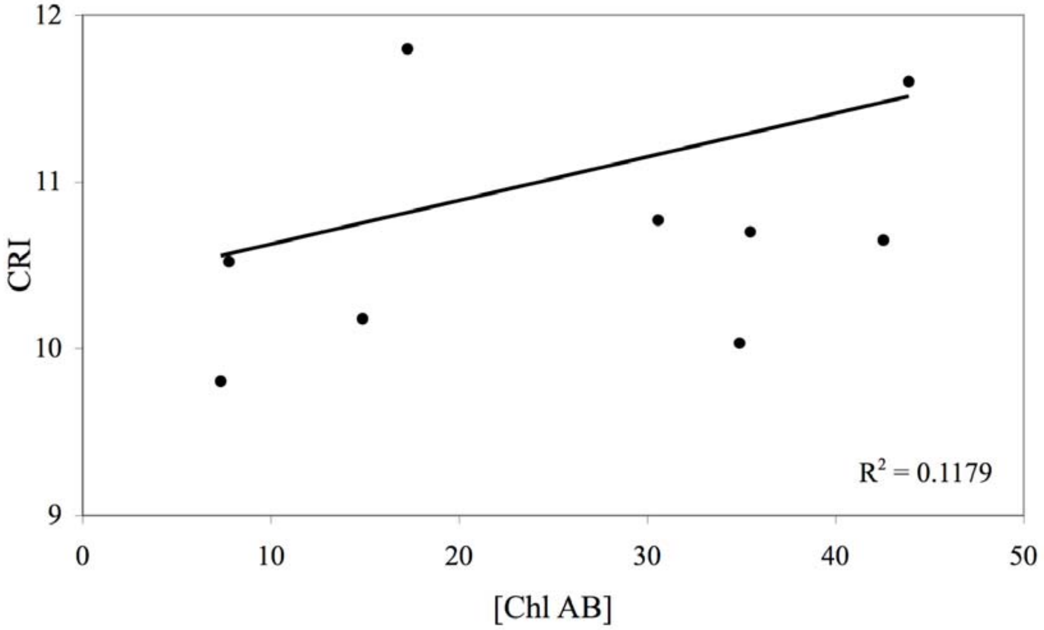


Figure E-5

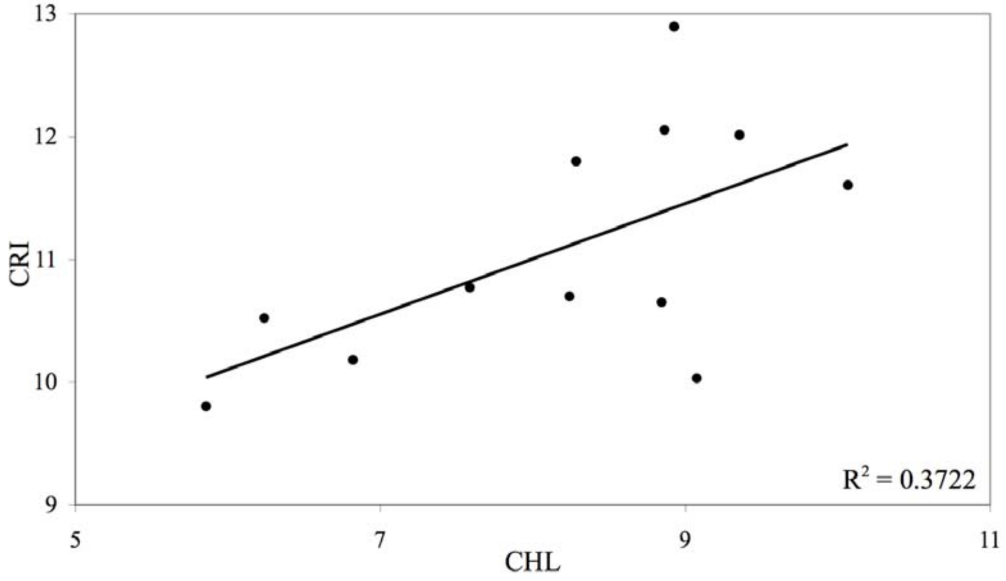


Figure E-6

PRI Relationships

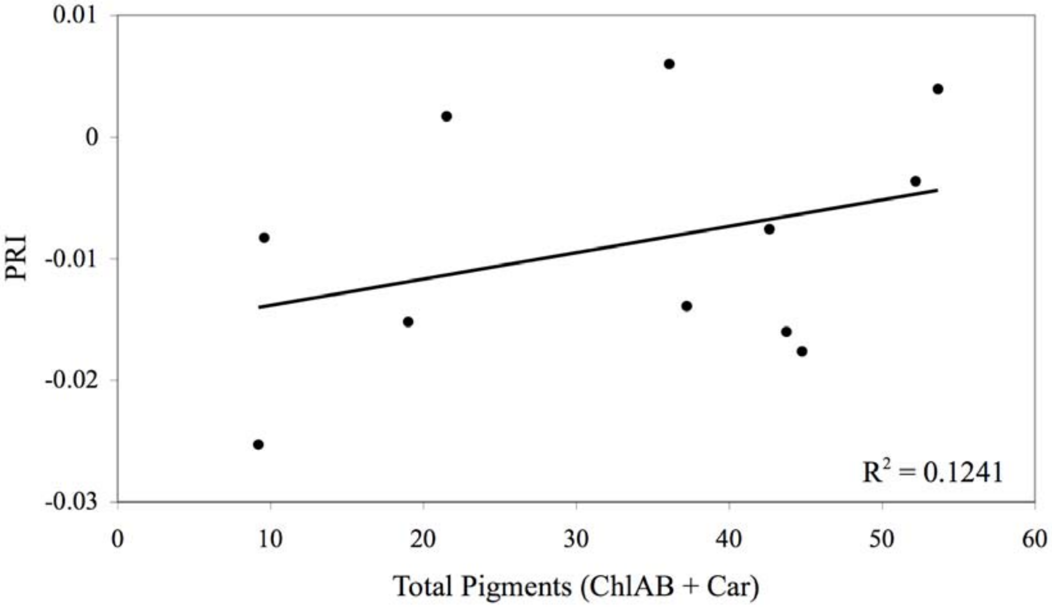


Figure E-7

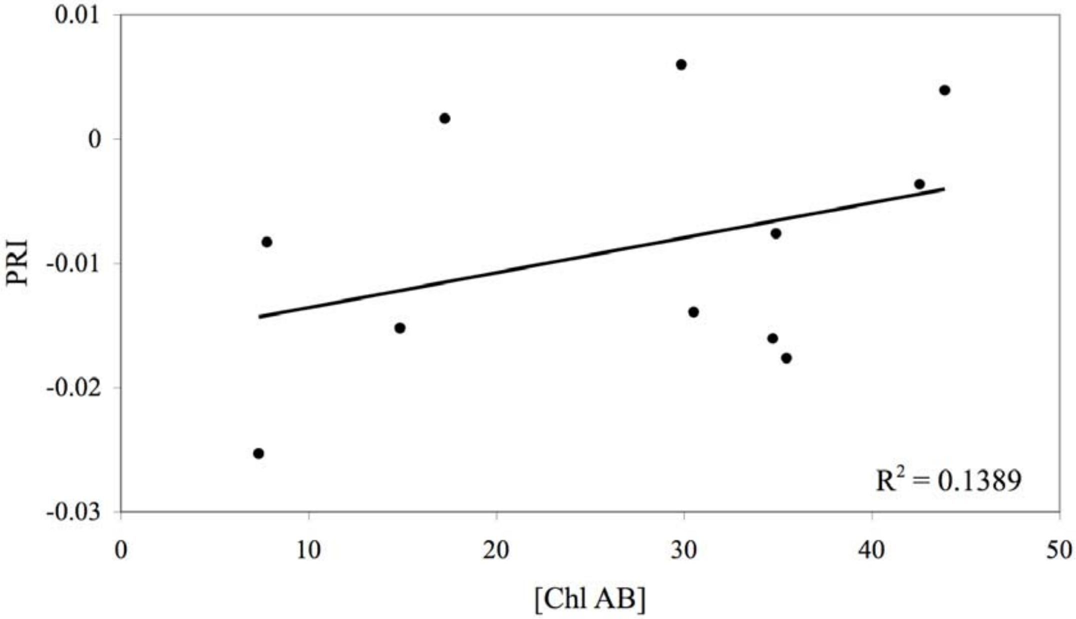


Figure E-8

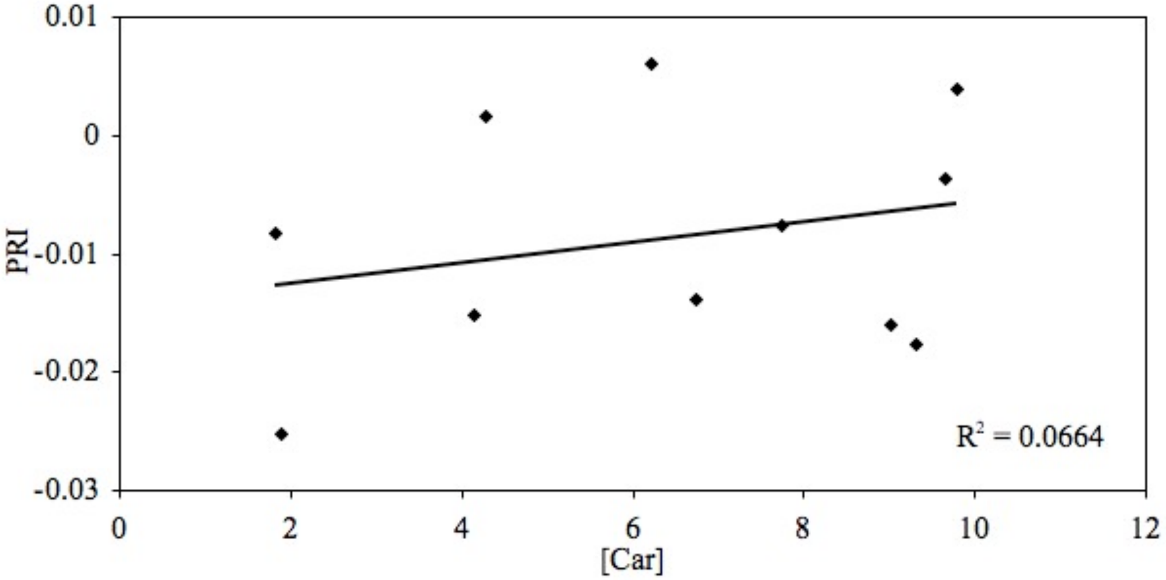


Figure E-9

Appendix F: Anthocyanin Reflectance Index (ARI)

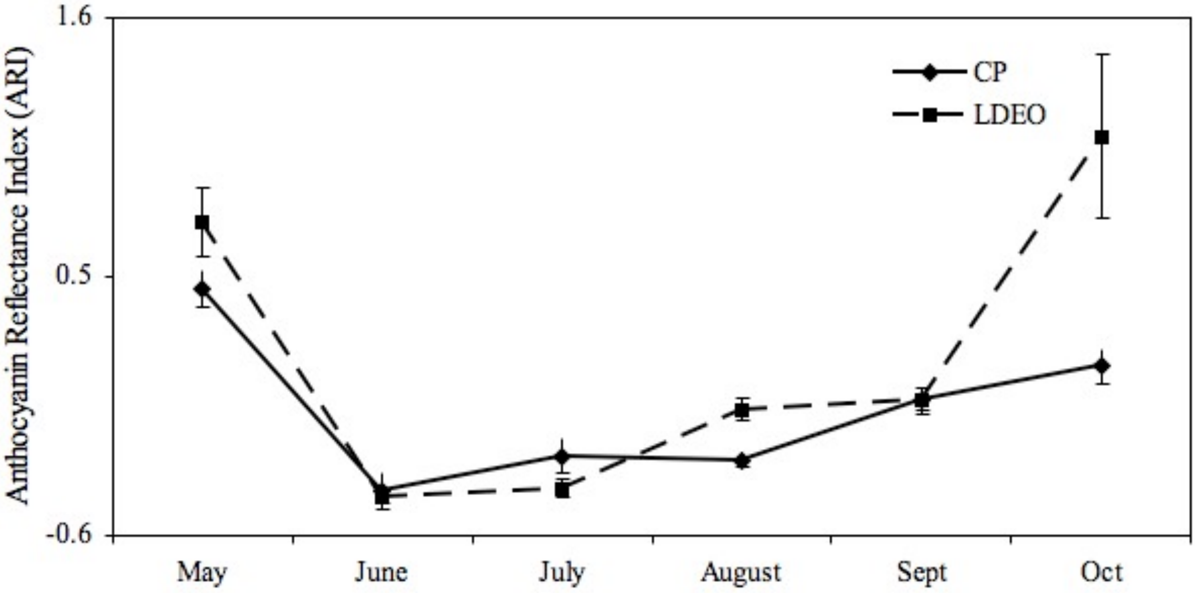


Figure F-1

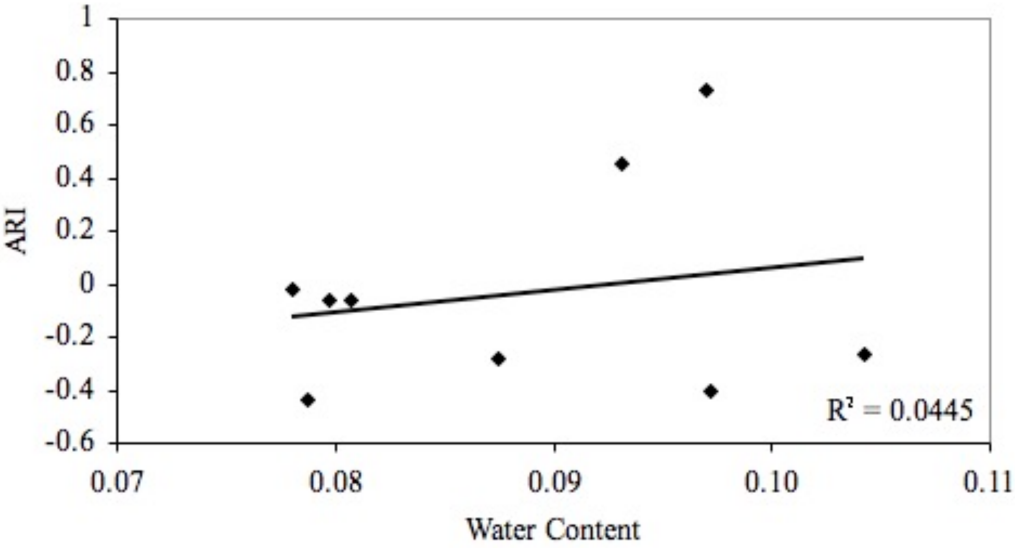


Figure F-2

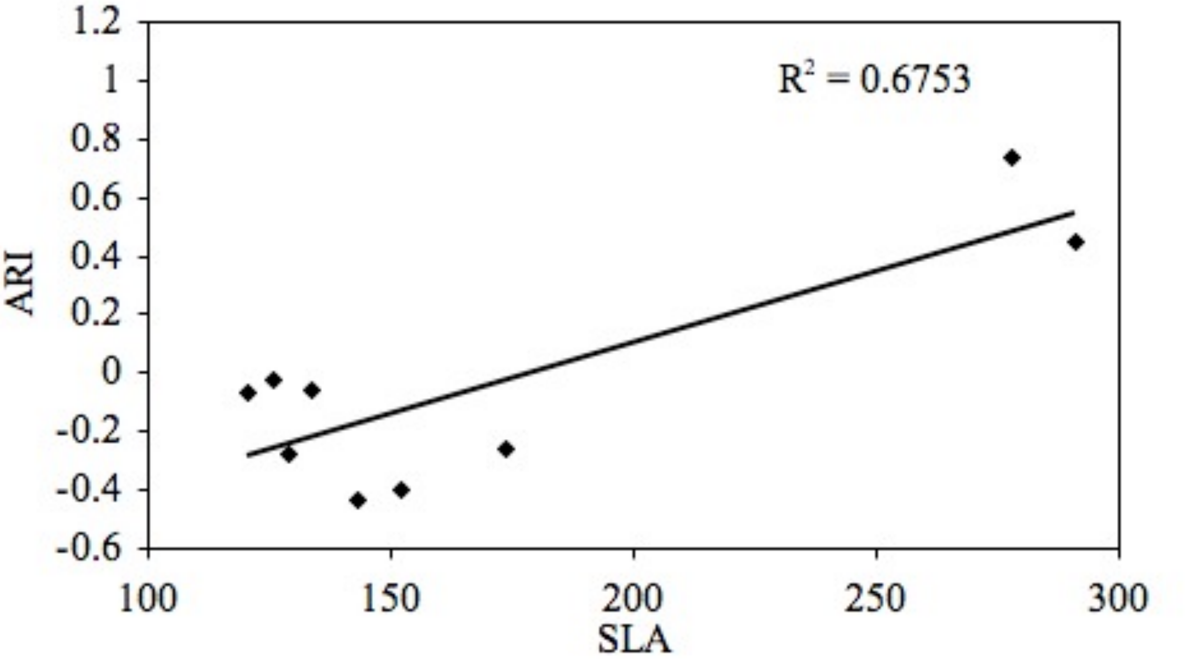


Figure F-3

Appendix G: Variation of Carotenoid Reflectance Index (CRI_2)

CRI_2 is a variation of the Carotenoid Reflectance Index and was created by Gitelson and others (2002) in the same paper as the more commonly used CRI. The relationship between CRI2 and Car was tested to see if it was stronger than CRI v. Car.

$CRI_2 = (1/R_{550}) – (1/R_{700})$

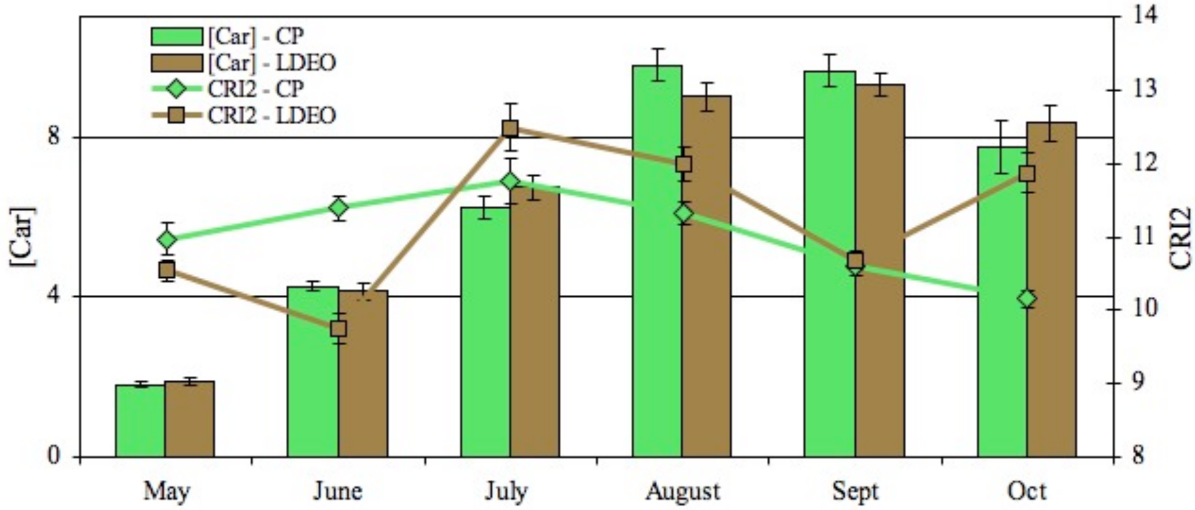


Figure G-1

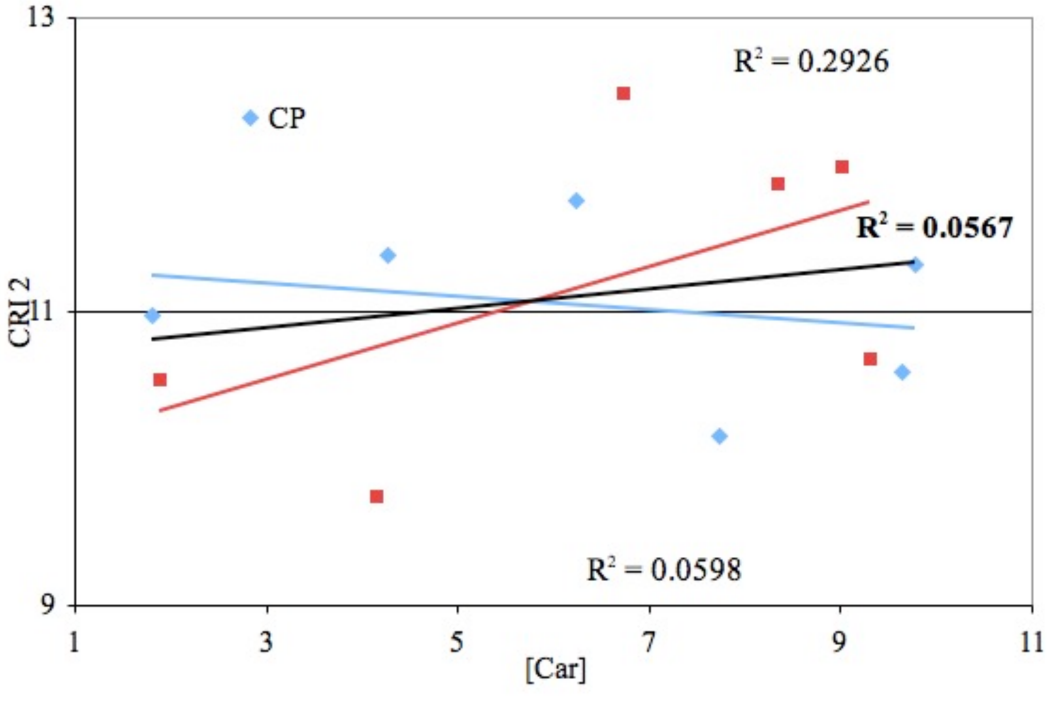


Figure G-2

Appendix H: Data Collection Dates and Precipitation Data (From NOAA, Central Park site)

Data collection dates: 5/17, 6/13 (CP) and 6/14 (LDEO), 7/30, 8.31, 9/27, 10/28

Month	Date (monthday)	Rainfall (mm)	Monthly Total (mm)
May	502	10.7	**47.8**
	511	4.3	
	512	0.5	
	513	4.6	
	517	21.1	
	520	5.3	
	521	0.5	
	528	0.8	
June	604	67.8	**168.7**
	605	31.5	
	609	0.3	
	612	0.8	
	613	30	
	617	1	
	620	10.9	
	620	2.5	
	621	1	
	622	4.6	
	628	17.8	
	629	0.5	
July	701	0.3	**175.5**
	705	32	
	706	3.6	
	712	25.7	
	716	0.3	
	718	23.9	
	719	16.5	
	720	4.8	
	723	1.8	
	724	56.9	
	730	9.7	

Month	Date (monthday)	Rainfall (mm)	Monthly Total (mm)
August	804	29.5	**182.9**
	808	63.5	
	810	8.9	
	811	23.6	
	813	1	
	817	0.3	
	818	18.8	
	820	3	
	821	8.1	
	822	26.2	
September	910	1.3	**44**
	911	5.6	
	912	25.1	
	915	2	
	923	7.9	
	928	1.3	
	929	0.8	
October	1003	0.5	**118.4**
	1004	0.3	
	1010	16.5	
	1011	0.8	
	1012	39.6	
	1019	0.3	
	1020	10.2	
	1024	0.8	
	1025	6.4	
	1026	0.8	
	1027	33.3	
	1028	8.9	

Table H-1