Spectral reflectance imagery and baseline analysis of anthocyanin concentration in Gossypium hirsutum L.

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SPECTRAL REFLECTANCE IMAGERY AND BASELINE ANALYSIS OF ANTHOCYANIN CONCENTRATION IN *GOSSYPIUM HIRSUTUM* L.

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agriculture and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Agronomy & Environmental Management

by

Tyson Andrew Phillips
B.S. Dakota Wesleyan University, 2000
May 2006
ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Gerald O. Myers, Associate Professor of Agronomy, for his time, patience and guidance during this project. I would also like to thank my graduate advisory committee, Drs. B. Rogers Leonard and Sandy Stewart for their advice and assistance. Without their help, I would have been overwhelmed.

I wish to thank the numerous student workers and graduate students at the Macon Ridge Research Station in Winnsboro, Louisiana, for their assistance and efforts with the collection of data. A special thanks is extended to Josh Temple, Department of Entomology graduate student, for his many extra hours and assistance with collecting and analyzing images for this study.

I extend my appreciation to the entire faculty of the Department of Agronomy for all of the little ways they assisted me along my path. A special thanks goes to Dr. Freddie Martin, Department Head of the Department of Agronomy, for giving me the chance to work on this degree. I would also like to give thanks to all of the ladies in the front office; they are a wealth of knowledge that is impossible to fully appreciate.

I would like to express my deep gratitude to all of my friends and co-workers who kept me going when I didn’t think I could. Special thanks also to Brooks, Byron and Wade who were always there with suggestions and critiques, wanted or not. To my family who pushed me to be more than I thought I could be and never let me settle for less, I have the utmost respect and admiration.

Finally, I would like to express my deepest gratitude to my wife for her unlimited patience, faith, understanding, and support for my decision to attend graduate school. Without her sacrifices, I would not be where I am today.
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Figure 2.1. Relationship of anthocyanin concentrations for 0, 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours of exposure to irradiation and seed anthocyanin concentration. ................................................................. 34
Correlations between spectral reflectance imagery and anthocyanin content have the potential to influence the interpretation of imagery data. The objectives of this study were to correlate leaf anthocyanin concentrations in cotton (Gossypium hirsutum L.) leaves with selected types of spectral reflectance imagery, to determine if the imagery could be used as a predictive tool for anthocyanin concentration, and to establish a baseline anthocyanin concentration in cotton leaf tissue. Field experiments were conducted in 2004 near Winnsboro, LA, with both an aerial-based camera and a hand-held device to investigate imagery responses to anthocyanin concentration. Multiple planting and sampling dates were used to produce variation in both anthocyanin concentration and imagery values. Anthocyanin concentration had a positive correlation with increasing sampling date and a negative correlation with planting date confirming that anthocyanin levels increase with crop maturity. The Normalized Difference Vegetation Index (NDVI) values recorded with a NTech GreenSeeker and the Green Normalized Difference Vegetation Index (GNDVI) values obtained from aerial imagery both had a negative and significant (p<0.01) correlation with anthocyanin concentration. NDVI values from the aerial imagery also had a negative relationship, but were not significant (p>0.05). While anthocyanin concentration in cotton leaves have an influence on spectral reflectance imagery, the effect appears to be minimal. Attempts to fit a predictive linear regression model to leaf anthocyanin concentration using spectral reflectance imagery were not successful. Laboratory experiments were also conducted using cotton seeds and cotton seedlings in a germination chamber under irradiation at approximately 100 µmol m⁻² s⁻¹ for varying lengths of time. Baseline anthocyanin
content for cotton seeds was determined to be approximately 2.0E-6 mol/cm². A
significant (p<0.05) difference was observed between the mean anthocyanin
concentrations of seedlings with the first true leaf exposed to 24 hours of continuous light
and seeds unexposed to light. No significant (p<0.05) differences were observed in mean
anthocyanin concentrations in seedlings with the first true leaf exposed to 12 hours of
irradiance or less. No apparent lag period between commencement of irradiance and
initiation of anthocyanin production was observed.
INTRODUCTION

Cotton (Gossypium hirsutum L.) profit margins are becoming more restricted each growing season. Over the years, technological advances have helped make management decisions more efficient and more profitable. The introduction of genetically modified cotton varieties that provide passive resistance to damage from insect feeding has reduced the amount of insecticide used to control certain insect pests. This has a dual benefit in that it reduces the cost associated with multiple applications of expensive pesticides and at the same time it reduces the environmental impact from pesticides. Equipment and technology advances in areas ranging from conservation tillage and precision spray application allow for timely, environmentally friendly, and efficient management techniques that ultimately benefit producers. Efficient and profitable crop production results from sound decisions based on an understanding and an accurate interpretation of the technology.

One technological advance that has been used with increasing frequency during the last three decades is the application of vegetative indices in cropping systems. Vegetative indices (VI), which come in a variety of forms, are equations used to interpret data acquired using spectral reflectance imagery (Zarco-Tejada et al. 2005). Normalized Difference Vegetation Index (NDVI) data has been used to determine which portions of a field are growing aggressively and which are not. Researchers have begun to examine NDVI and other spectral reflectance imagery, such as Green Normalized Difference Vegetation Index (GNDVI), as a means to explain growth variations in crops that may be too subtle to be detected by the unaided eye. However, VI may be able to provide considerable information on the condition of a crop well beyond its growth patterns.
Most plants contain pigments called anthocyanins. Anthocyanins absorb wavelengths of light between 475nm and 560nm. Depending on the specific anthocyanin, various wavelengths of light are reflected to give plants seemingly endless variations in color (Hopkins 1995). Although much is known about anthocyanin structure and function, there is more to learn about their relationship to the condition of plants. Anthocyanins, like most plant compounds, can behave in different ways depending on the specific plant material.

The type, amount, and intensity of anthocyanin levels vary greatly in response to numerous external factors. Plant responses to these factors create considerable difficulty in distinguishing the primary effects of anthocyanin production. A high proportion of studies examining anthocyanins in crop species have been performed in a laboratory setting. In a controlled environment, researchers can isolate the specific factors suspected to have an effect on anthocyanin production. By producing cotton seedlings in a controlled environment, a baseline rate of anthocyanin production can be identified. A basic understanding of the baseline rate, given certain external factors, will form a core of knowledge to understand anthocyanin response in the field where factors affecting production vary greatly and can be difficult to quantify.

The ability to determine anthocyanin concentrations in real time instead of the three days, minimum, that it takes to quantify concentrations at present could potentially be very useful. In many crop situations, timeliness is critical to management because production decisions must occur daily. By determining if anthocyanin levels are rising or falling, we may be able to intercede and correct a potential problem. Moreover, it would be useful to determine if anthocyanin concentrations have a relationship to NDVI or
GNDVI values. If anthocyanin concentrations are related to the data in aerial NDVI or GNDVI images, healthy plants may contain a specific concentration of anthocyanin. It may be necessary to factor the anthocyanin levels into NDVI and/or GNDVI images to correctly represent crop growth patterns. One objective of this research is to determine if NDVI and/or GNDVI can be used to predict anthocyanin concentrations in field grown plants.

A better understanding of the relationship between anthocyanins and spectral reflectance imagery may enhance the efficiency with which cotton is grown in the field. Information that can be used to reduce time and energy expended and to increase profit margins in a cropping situation is very valuable. Although the reasons are not yet well understood, anthocyanins exist in plants for a purpose. Whether that purpose is to provide insect resistance or to protect chlorophyll from damaging ultra-violet rays, or for some as yet unknown reason, an understanding of what anthocyanins can tell us about the plants and situations they exist in will enhance our ability to manage plants as a whole. It remains to be seen whether spectral reflectance imagery can be used to apply our increasing knowledge of anthocyanins and their role in plant production.
REVIEW OF LITERATURE

R.1 Vegetation Indices as Measures of Plant Growth

Remote sensing involves the acquisition of data through a camera by various means, usually radiation, from a distance (Campbell 1996). The types of data gathered are dependent on the type of radiation measured. Remote sensing as it is used in crop production normally collects data using reflected wavelengths of light in three channels of differing wavelengths. Images typically consist of a green channel encompassing wavelengths ranging from 530nm to 570nm, a red channel of wavelengths from 660nm to 680nm, and a near infrared channel with wavelengths of about 775nm to 825nm (MS1400 2005). Vegetation indices (VI) are models used to interpret information obtained through spectral reflectance imagery and typically fall into one of three categories. Chlorophyll indices such as Modified Chlorophyll Absorption in Reflectance Index (MCARI) are one type of VI that are used to estimate leaf chlorophyll concentration in plants (Daughtry et al. 2000). Water indices such as Normalized Difference Water Index (NDWI) and Plant Water Index (PWI) are VI that are used to estimate plant water content using a ratio of certain wavelengths reflected from a canopy of plants (Gao 1996; Zarco-Tejada et al. 2003). The most widely used VI’s in crop research are structural indices. Used to examine the health of vegetation in an area, structural VI’s include (but are not limited to) Modified Triangular Vegetation Index (MTVI), Simple Ratio Index (SR), and Normalized Difference Vegetation Index (NDVI) and are based on the reflectance of near infrared and red wavelengths (Rouse et al. 1973; Jordan 1969; Haboudane et al. 2004). Zarco-Tejada et al. (2005) completed an extensive study on VI to determine the strengths and weaknesses in identifying cotton variability.
All structural VI's studied a strong correlation with crop growth during early growth stages with the correlation becoming less strong as the growing season progressed.

Rouse et al. (1973) was the first to fully develop the concept of NDVI. NDVI is derived by deducting the percent of visible red wavelengths of light (between 660nm and 680nm) reflected from the percent of near-infrared wavelengths of light (between 775nm and 825nm) reflected by vegetation, and dividing this result by the sum of the percent near-infrared wavelengths of light reflected and the percent of visible red wavelengths of light reflected. NDVI is represented by the following formula:

\[
NDVI = \frac{(NIR - Red)}{(NIR + Red)}
\]

Because healthy vegetation absorbs most visible wavelengths of light, NDVI values for healthy vegetation have a maximum around 0.7 (Goward et al. 1985). Sparse or unhealthy vegetation reflects more visible wavelengths and absorbs near-infrared wavelengths, demonstrating NDVI values closer to zero. NDVI values can be obtained between negative one and zero. Values between -0.1 and 0.1 are usually associated with extremely sparse vegetation or bare soil. Water, snow, ice and cloud interference produce NDVI values below -0.1 (Goward et al. 1985). Another VI similar to NDVI that has attracted attention in recent years is the Green Normalized Difference Vegetation Index (GNDVI) (Gitelson et al. 1996). GNDVI is very similar to NDVI except that the formula used to compute GNDVI values uses percent visible green wavelengths of light (between 530nm and 570nm) reflected in place of the percent visible red wavelengths of light reflected used in NDVI. The formula is as follows:

\[
GNDVI = \frac{(NIR - Green)}{(NIR + Green)}
\]
Much of the work relating spectral reflectance imagery (mostly NDVI) with row crops has focused on yield differences and the application to precision application prescriptions. NDVI has repeatedly shown a correlation with in-season plant health. Jayroe et al. (2005) found that NDVI correlated very well with soil electrical conductivity data and yield maps in cotton and soybean. Visual comparisons of plant variability across fields with NDVI images showed that images were consistent recognizing field variability. Li et al. (2001) conducted a large study comparing various spectral reflectance models with several agronomic characteristics in cotton. NDVI correlated strongly with lint yield, soil and plant water content, N uptake and plant fresh biomass. The only parameter in the study that did not show a significant correlation with NDVI was leaf N content. Read et al. (2002) reported similar results. NDVI images obtained during peak bloom in cotton correlated well with plant height, leaf area index, and lint yield. Another study that examined several agronomic traits compared NDVI values obtained with a GreenSeeker Hand Held™ Optical Sensor Unit (NTech Industries, Inc.) to cotton plant height, total nodes, and elongation of the fourth internode. NDVI values were found to be correlated with all of these traits until two weeks prior to crop defoliation. Because the cotton plant is beginning to mature at this time of the season, elongation of the fourth internode was the only trait not shown to correlate with NDVI values after defoliation (Sharp et al. 2004). In contrast to the above mentioned studies, Bronson et al. (2005) found that NDVI did not correlate well with biomass, lint yield, or leaf N content. However, NDVI values did correspond to the addition of N to some plants when compared to plants that did not receive N. This suggests that even if NDVI
is unable to reflect specific values of leaf N content, it is able to detect a change in N, which may be important when attempting to determine if enough N is available to plants.

In soybeans (*Glycine max* L.), Ma et al. (2001) showed NDVI to correlate with final yield. Correlation between NDVI and yield became stronger as the growing season progressed. As the plants began to senesce, however, the correlation disappeared. In this study, soil type had an indirect effect on NDVI because of differences in plant growth characteristics. Ma et al. (2001) did not show that NDVI could be used to predict soil type variations, but did suggest that variations needed to be considered when evaluating spectral reflectance data. Soybean maturity groupings were also found by Ma et al. (2001) to impact NDVI, thus, genotypes that exhibited earlier maturity (maturing faster) were predicted to yield higher than later maturing genotypes. Plant maturity variations could be identified by taking multiple NDVI images as the different genotypes progressed through the growing season and thus relative yield potential could be assessed.

Much previous research relating NDVI to agronomic characteristics of common wheat (*Triticum aestivum* L.) has been conducted. Values of NDVI correlate with leaf area index (LAI), final grain yield, and N uptake (Serrano et al. 2000; Raun et al. 2001; Flowers et al. 2003). Serrano et al. (2000) found that N stress in common wheat negatively affected the correlation between NDVI and LAI. While leaf area was kept constant, leaf chlorosis as a result of N stress resulted in low NDVI values. NDVI may have been influenced by an increase in anthocyanin concentration in chlorotic leaves (Serrano et al. 2000). Raun et al. (2001) determined that in-season application of fertilizer could be directed by successive NDVI images taken early in the growing season
of wheat. NDVI was correlated with final yield and by combining data from two or more successive remote sensing samplings, supplemental N could be applied in-season to the areas in a field predicted to produce low yields. However, in a study conducted by Flowers et al. (2003), N rates could only be directed by in-field variations of NDVI when biomass was above 1000 kg ha\(^{-1}\). The correlation between NDVI and final yield was not significant in areas of the field with biomass values lower than this level.

In durum wheat, \textit{(Triticum turgidum} L. subsp. \textit{durum (Desf.) Husn.)}, NDVI correlates with LAI, biomass, and yield (Aparicio et al. 2000; Aparicio et al. 2002). When durum wheat is water-stressed, NDVI and final grain yield correlate best at anthesis. Conversely, when durum wheat is not well watered, NDVI and final grain yield correlate best at later stages of growth closer to crop maturation. Moreover, the correlation between NDVI and final grain yield was only significant when LAI was \(\geq 3.0\) at the time of imaging regardless of moisture status (Aparicio et al. 2000).

Research with spectral imaging in corn \textit{(Zea mays L.)} is largely limited to correlating NDVI values with final grain yield and dry biomass accumulation. NDVI does not show a strong correlation with final yield when values are used from a single image (Chang et al. 2003). However, NDVI values do correlate with final yield when models are constructed using multiple successive sampling dates and analyzed as a progression of biomass accumulation (Chang et al. 2003). One problem identified with estimating dry biomass accumulation in corn is that near the end of the growing season NDVI values tend to reach a plateau at the same time that dry biomass is accumulating rapidly (Calera and González-Piqueras 2004). To combat this problem, a derivative of
NDVI was used called the Time-Integrated value of NDVI (TINDVI). Application of a TINDVI model produces a linear relationship with dry biomass accumulation.

Few examples of using NDVI images to detect insect infestations are available in the literature. Sudbrink et al. (2003) showed that NDVI images correlated well with beet armyworm hits in cotton fields. However, plots damaged by cabbage looper larvae did not have significantly different NDVI values than plots where the larvae were controlled by insecticides.

In relation to NDVI, the use of GNDVI is a new concept, and research is lacking. Earnest and Varco, (2005) showed that GNDVI correlates well with crop growth differences resulting from variable N applications and also with leaf N concentrations. GNDVI images acquired at or soon after peak bloom in cotton correlate well with final yield data. When the agronomic trait being studied is leaf chlorophyll-a concentration as it relates to plant health, GNDVI has been shown to be more sensitive than NDVI (Gitelson et al. 1996). Shanahan et al. (2001) found that GNDVI correlates with final corn yield better than NDVI. Using maps generated with GNDVI values, the study predicted potential final yield and also identified regions in a field where a potential yield loss occurred. By identifying field variations in yield, it is possible to adjust inputs to focus on higher potential yield in sections of a field.

**R.2 Anthocyanins in Plants**

Anthocyanins are water soluble pigments that exist primarily in the vacuolar sap (Ting 1982a; Hopkins 1995). They are usually red, yellow or blue and are found throughout the plant kingdom in different concentrations and compositions (Salisbury and Ross, 1992b; Mohr and Schopfer, 1995a). The basic anthocyanin structure consists
of two benzene rings (A-ring & B-ring) separated by three carbon atoms which are arranged in various combinations depending on the type of anthocyanin. All known anthocyanins exist as glycosides with glucose attached to the A-ring (Ting 1982a).

The color exhibited by anthocyanins is dependent on a few factors. Attachment of a methyl group to the anthocyanin B-ring presents a red color while association with flavones or flavonols and/or a hydroxyl group on the B-ring provides a blue tint. The vibrancy of the color is dependent on the anthocyanin concentration and the pH of the vacuoles (Salisbury and Ross 1992b; Hopkins 1995).

Anthocyanin synthesis is dependent on many factors. One of the major components is genetics. Two plants of a single species, but differing in genetic backgrounds, may produce very different levels of anthocyanins (Downs 1964). However, genetics alone cannot account for all variations in type and amount of anthocyanin produced. Production of anthocyanins by plants has been shown to be triggered by ultra violet and visible wavelengths of light, nutrient stress, and low temperatures (Hopkins 1995; Mohr and Schopfer 1995b). Wagner and Mohr (1966) found that the potential of a plant to produce anthocyanins is always present and that specific wavelengths of light merely acted as a stimulus for anthocyanin synthesis. The length and intensity of ultra violet radiation exposure can have a significant effect on the concentration of anthocyanins in plant tissues (Mancinelli 1990; Mohr and Schopfer 1995c). Maximum levels of anthocyanin production can be triggered in response to exposure to red (approximately 650nm), far-red (approximately 675nm) and blue (approximately 475nm) wavelengths of light. Conversely, almost no production response is achieved when plants are exposed to green wavelengths around 550 nanometers (nm).
(Salisbury and Ross 1992c). Prolonged growth in darkness has also been shown to have a negative effect on total anthocyanin production once plants are irradiated. Photoreceptor precursors for light-dependent anthocyanin production reaction probably are volatile and may be produced in limited quantities until irradiance is initiated (Grill 1965).

Anthocyanins are also produced in response to plant stresses, most notably N and P deficiencies. Plants under stress from a N deficiency have been shown to accumulate anthocyanins in stems, petioles and lower leaf surfaces, particularly along veins. Anthocyanins produced in response to N deficiencies tend to exhibit a purple coloration (Ting 1982b; Salisbury and Ross 1992a). Similar effects are observed when plants are stressed by a P deficiency. However, most of the anthocyanin accumulation tends to be in the leaves and most noticeably along the leaf margins. Anthocyanins produced in response to phosphorous deficiencies typically produce a much darker purple that appears dark green on the interior of leaves and a vibrant purple on leaf margins (Ting 1982b; Salisbury and Ross 1992a; Osbourne et al. 2002). Some studies attempted to correlate anthocyanins with mineral deficiencies and have yielded inconsistent results. Osbourne et al. (2002) produced promising findings by looking at the specific wavelengths anthocyanins reflect when plants are under N and/or P deficiency stress. While N deficiency was detectable throughout the growing season, P deficiency was best identified early in the season with accuracy of detection declining with crop growth stage progression. However, anthocyanin production and concentration can be affected by other environmental factors such as pH and the time of year.
The pH of the internal plant environment appears to play a significant role in the concentrations of each type of anthocyanin (Mancinelli 1990; Mohr and Schopfer 1995c). This could cause discrepancies in anthocyanin concentrations between differing environments that are experiencing similar mineral deficiency stresses. Parks et al., (1972) was able to show that environment had a major role in the concentration and the ratio of each anthocyanin type relative to one another in individual plant species. However, it was also shown that regardless of environment, similar types of anthocyanins were produced by a single plant species. Previous research has revealed that anthocyanins are produced in response to cutout in cotton crops (Wells 1995, 1996, 2001). Anthocyanin levels were observed to increase in conjunction with a drop in chlorophyll levels in late season (July and August) cotton leaves (Wells 2001). This increase could be delayed by removing fruit from the plants and suggested that anthocyanin levels were connected with plant maturity, although the precise reason for this is yet to be determined.

Although anthocyanins exist in most higher plant species, relatively few plants have been studied. Wagner and Mohr (1966) showed that mustard seedlings grown in the dark generated very low levels of anthocyanins while mustard seedlings grown in light produced anthocyanins via phytochrome in all cells of the first subepidermal layer of cotyledons and the hypocotyl. Lange et al. (1971) found that irradiating white mustard seedlings with five minutes of red wavelengths of light had a lag phase of about three hours before anthocyanin began to accumulate. By increasing the exposure time, the total amount of anthocyanin increased, but was unable to overcome the lag phase. This is known as phytochrome-(far red type, $P_{fr}$) mediated anthocyanin production. This study
showed that a narrow band of wavelengths was required to initiate the production of anthocyanin precursors. In white mustard seedlings, three hours were needed to accumulate enough of these precursors to produce anthocyanin. The lag phase in white mustard seedlings is constant and independent of the intensity or duration of irradiance.

Turnip seedlings, similar to mustard seedlings, produce a very small amount of anthocyanin in the absence of irradiance. Another similarity is that turnip seedlings exhibit a lag phase prior to anthocyanin production when exposed to irradiation. However, in contrast to mustard seedlings, anthocyanin production in turnip seedlings is highly dependent on the intensity of irradiation. Also, the lag phase in turnip seedlings is much shorter, with production of anthocyanin commencing within about an hour (Siegelman and Hendricks 1957). A second lag phase exists in turnip seedlings. Once turnip seedlings have been exposed to irradiation and anthocyanin production has begun, reintroduction to irradiance following a period of growth in darkness results in a secondary lag phase similar to the primary lag phase (Grill and Vince 1969). This is in contrast to other experiments that determined that a second lag phase does not exist in mustard seedlings (Mohr 1966). In mustard seedlings, anthocyanin production resumes immediately after irradiation is commenced following a dark period interruption. Grill and Vince (1964) found that removal or covering of the cotyledons of turnip seedlings prevented the production of anthocyanin in irradiated hypocotyls and no anthocyanin was produced when the cotyledons alone were irradiated. Cotyledons removed from the hypocotyl produced the same amount of anthocyanin as the cotyledons and hypocotyl produced in total when the cotyledons are not removed. A light-dependent precursor may
be developed in the cotyledons of turnip seedlings and translocated to the hypocotyl where a second reaction is required for anthocyanin synthesis (Grill and Vince 1964).

Red cabbage seedlings have the distinction of being one of the only plants shown not to have a lag phase between the onset of irradiation and anthocyanin production. Although anthocyanin is produced in red cabbage seedlings at the same rate as in turnip seedlings, the total amount produced is much higher. In the absence of irradiance, red cabbage seedlings produce about five times the amount of anthocyanin above that of turnip seedlings. This trend appears to be constant even after irradiance begins (Downs 1964).

Downs and Siegelman (1962) also found that production of anthocyanin in ‘Wheatland’ variety of milo seedlings is unique. Milo seedlings grown in total darkness do not produce any detectable anthocyanin concentrations. By removal of certain plant parts it was shown that the root, the coleoptile, and the rudimentary leaves were not required for anthocyanin production (Downs 1964). Rather, anthocyanin production in young milo seedlings appeared to be dependent on whether the seed was kept attached to the shoot. If the root is removed from the seed, the root does not produce any anthocyanin when irradiated, but the shoot does. When the seed stays attached to the root and the shoot is removed, the root produces small amounts of anthocyanin. Conversely, if the seed and shoot are kept intact while the roots are removed, anthocyanin is produced at levels comparable to a complete intact milo seedling. The precursors for anthocyanin production are not isolated to a specific part of the plant for all plant species. In milo, when irradiance was doubled, no increase in anthocyanin concentration was detected. When the length of exposure to 1200 ft-c of irradiance was doubled, however,
anthocyanin concentration also doubled (Downs 1964). Moreover, at high irradiance intensities, no lag period for anthocyanin production was evident but at lower irradiance intensities, a lag period of about five hours existed. Exposure to wavelengths of light at an irradiance of 1,200 ft-c for two to sixteen hours caused an apparent saturation effect where the amount of anthocyanin detected in the milo seedling leaves was held constant (Downs 1964).

Anthocyanins appear to serve many useful functions. The primary utility of anthocyanins is for the attraction of pollinators and as a protective barrier to ultra violet (UV) radiation for light sensitive porphyrens in underlying plant tissues (Salisbury and Ross 1992b; Mazza and Miniati 1993; Hopkins 1995; Mohr and Schopfer 1995c). Anthocyanins absorb strongly in the UV-B region of the light spectrum at wavelengths between 475 nm and 560 nm and allowing wavelengths associated with both blue and red visible wavelengths of light pass through so that even when anthocyanins are present at high concentrations, underlying chlorophyll can carry out photosynthesis with minimal interference (Hopkins 1995). Anthocyanin may also function to protect chlorophyll from certain wavelengths of UV but that the total flavonoid (flavonols, flavones, and anthocyanin) content is more important than the individual anthocyanin concentration. Many of the roles that anthocyanins have in plants are performed by a number of other compounds, so it is possible that anthocyanins are not essential in some roles (Gould et al. 2000).

Some evidence suggests that anthocyanins are not edible or simply non-preferred by insect pests (Salisbury and Ross 1992b; Jones 2000) although there have not been specific studies to verify either statement. Jones (2000) was able to show that red leaf
cotton, which produces very high levels of anthocyanins, had a detrimental effect on fall armyworm and beet armyworm growth and survival. It was not determined if anthocyanins specifically were the cause of this alteration. A potential connection between anthocyanins and host plant resistance in peas to the pathogens *Fusarium solani* and *Pythium ultimum* has been suggested (Muehlbauer and Nicholson 1978). However, in-depth research into the precise mode of action and the role anthocyanins play in pathogen resistance appears to be lacking.
1.1 Materials and Methods

The experiment was done on Gigger silt loam (fine-salty, mixed, thermic typic Fragiudalfs) at the Macon Ridge Research Station near Winnsboro, Louisiana. The research project used three cotton genotypes: Stoneville (STV) ‘STV5599BR’, ‘STV4793R’, and Delta and Pine Land (DP) ‘DP555BG/RR’. Seed were planted on five different planting dates including: STV4793R planted on April 16, 2004 and April 28, 2004; DP555BG/RR planted on May 10, 2004; and STV5599BR planted on May 24, 2004 and June 7, 2004.

Plants were sampled on July 27, 2004, August 10, 2004 and September 3, 2004. These dates were conditional on clear weather to obtain aerial NDVI and GNDVI readings preferably on the sampling day or one day prior to sampling. Aerial images were obtained using a DuncanTech™ multi-spectral camera mounted on a light airplane flying at an altitude of approximately 3000 ft above ground level. Each sample consisted of at least ten individual sub-sample sites within each of the five separate plots for a minimum of fifty sites on each day. Each individual sub-sample site was geo-referenced using the Global Positioning System (GPS) on handheld computers and was given a unique identifying number. At each site, a leaf from the third node below the uppermost fully expanded leaf was removed from three consecutive plants, bagged, and placed on ice. A Model 505 GreenSeeker Hand Held™ Optical Sensor Unit (NTech Industries, Inc.) was used to obtain a ground-based NDVI value at each site.

After all samples had been collected, three leaf discs were removed from each leaf using a standard single-hole punch. The three leaf discs from each sub-sample site with a
total area of approximately 21.195 mm² were placed in a 15 cm test tube with 3 mL of acidified methanol solution (10 mL HCl L⁻¹), marked with the sample identification number and agitated to completely submerge leaf discs. All test tubes were placed in racks and completely covered with aluminum foil to prevent any light penetration during anthocyanin extraction. Test tube racks were then placed in a refrigerator at 4°C for 48 hours. After this period, the test tubes were removed and the contents transferred to a cuvette and placed in a spectrophotometer (Spectronic Helios Gamma, Thermo Electron, UK). Absorption readings were taken at 532 nm (peak absorbance for anthocyanin) and also at 653 nm (peak absorbance of chlorophyll degradation products) and the results recorded. The sample was then removed and the cuvette rinsed with de-ionized water for reuse before proceeding to the next sample. After all samples had been processed through the spectrophotometer, anthocyanin levels were calculated after Wells (1995) as follows:

\[
\text{Anthocyanin} = \text{Absorption}_{532\text{nm}} - 0.25(\text{Absorption}_{653\text{nm}})
\]

The optical density values were then divided by the molecular extinction coefficient of cyanidin (2.45x10⁴) then divided by the area of the three leaf discs to transform these values into concentrations of mols of anthocyanin per cm² (Siegelman and Hendricks 1957).

Aerial NDVI and GNDVI photographs were converted into approximately one-meter resolution pixilated maps using ArcView GIS 3.3 (ESRI 2002) and the geo-referenced data points were superimposed on top of these maps. NDVI and GNDVI values within a radius of one meter from the geo-referenced data points were averaged to develop a single sample data point. NDVI and GNDVI values from aerial photographs
were compared to the corresponding anthocyanin concentration using PROC CORR in the SAS system (version 9). PROC CORR was also used to determine the strength of the correlation between ground-based NDVI values from the GreenSeeker unit and anthocyanin concentration. In addition to correlations, a linear regression model was generated using PROC REG in the SAS system version 9.12 (SAS Institute, Cary, NC) to determine if NDVI or GNDVI values could be used to estimate anthocyanin concentrations.

1.2 Results and Discussion

1.2.1 Correlations

A negative, but highly significant correlation was detected between sample date and both ground and aerial NDVI values (p=0.0005 and p<0.0001 respectively) based on Pearson correlation coefficients (Table 1.1). However, a negative and highly significant (p<0.0001) correlation was found between sample date and aerial based GNDVI values. NDVI and GNDVI values represent healthy growing plants. Sampling dates were near the end of the growing season and each successive sampling date was closer to cotton plant senescence. As samples are taken later in the growing season, NDVI and GNDVI values probably decreased. Conversely, a slight positive and highly significant (p=0.0004) correlation exists between sampling date and leaf anthocyanin concentration (Figure 1.1). This relationship is plausible because anthocyanin levels recorded at later sampling dates as plants senesce are expected to be higher than in less mature plants.

The correlation between planting date and NDVI, GNDVI, and leaf anthocyanin concentration is nearly the inverse of the relationship between these variables and sampling date (Table 1.1). Planting date has a slight positive but highly significant
Table 1.1. Pearson correlation coefficients with corresponding p-values.

<table>
<thead>
<tr>
<th></th>
<th>Plant Date</th>
<th>Ground NDVI</th>
<th>Aerial NDVI</th>
<th>Aerial GNDVI</th>
<th>Anthocyanin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Date</td>
<td>0.0000</td>
<td>-0.3083</td>
<td>-0.4111</td>
<td>-0.6750</td>
<td>0.2635</td>
</tr>
<tr>
<td>Ground NDVI</td>
<td>1.0000</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
</tr>
<tr>
<td>Aerial NDVI</td>
<td></td>
<td>0.3771</td>
<td>0.2093</td>
<td>0.2175</td>
<td>-0.2726</td>
</tr>
<tr>
<td>Aerial GNDVI</td>
<td></td>
<td></td>
<td>0.2093</td>
<td>0.0039</td>
<td>0.0003</td>
</tr>
<tr>
<td>Ground NDVI</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>0.0061</td>
</tr>
<tr>
<td>Aerial NDVI</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>-0.1290</td>
</tr>
<tr>
<td>Aerial GNDVI</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>0.0898</td>
</tr>
<tr>
<td>Anthocyanin Concentration</td>
<td>0.8023</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>-0.2273</td>
</tr>
</tbody>
</table>

Figure 1.1. Anthocyanin concentration in cotton leaves on three dates of sampling.
correlation with ground based NDVI values (p<0.0001), aerial based NDVI values (p=0.0056), and aerial based GNDVI values (p=0.0039). In contrast, planting date has a slight negative but highly significant (p=0.0004) correlation with leaf anthocyanin concentration. Anthocyanin concentration would normally be expected to decrease as planting date increased. Cotton plants planted earlier would be more mature, in both age and development, than cotton planted at a later date and therefore would likely have higher concentrations of anthocyanin in their leaves and would therefore appear less green as the season progressed. During the end of March 2004 and beginning of April 2004, however, low rainfall was recorded at the Winnsboro test area (Figure 1.2). In contrast, the month of June 2004 was one of the top five wettest months recorded in Louisiana. Therefore, cotton planted during the drier period (April 28, 2004; May 10, 2004; and May 24, 2004) did not develop very rapidly during the first couple of weeks after planting while the later planted cotton (June 7, 2004) had sufficient moisture and was able to develop rapidly. Because cotton planted on the earliest planting date (April 16, 2004) had available soil moisture when it was planted, it was able to develop normally before the soil dried. These environmental conditions help to explain why mean anthocyanin concentrations are highest in the cotton planted on the first and last planting dates (Figure 1.3).

Ground based NDVI values show a positive and highly significant (p<0.001) correlation compared to aerial based NDVI values (Table 1.1). A positive and significant relationship is expected in this situation because both tools are measuring NDVI. The
Figure 1.2. Daily rainfall near Winnsboro, LA during the 2004 planting season. Arrows indicate the dates of planting.

Figure 1.3. Anthocyanin concentration in cotton leaves on five dates of planting.
The coefficient value of 0.5296 is most likely the result of the ground based unit having its own light source as opposed to the aerial based unit which uses reflected solar radiation that can vary in intensity as a light source. Somewhat unexpectedly, ground based NDVI values had a positive and highly significant (p<0.0001) correlation with aerial based GNDVI values. Correlation between these two values is likely due to the fact that both use percent near-infrared wavelengths reflected from the leaf surface in their computation. Although NDVI uses percent red wavelengths reflected and GNDVI uses percent green wavelengths reflected, the data indicate that percent near-infrared wavelengths reflected in both formulas is sufficient for a significant relationship.

The correlation between aerial based NDVI values and leaf anthocyanin concentration was negative and significant at p<0.1. The degree of this correlation was an objective in this study. When leaf anthocyanin concentrations increase, aerial based spectral reflectance imagery should detect the corresponding decrease in greenness of the plant material. The data suggest that the effect of increasing anthocyanin concentration on aerial based NDVI values is minimal. When the relationship between leaf anthocyanin concentration and ground based NDVI values is examined, the correlation (-0.2438) is still weak and significant (p=0.0061) but is a slightly stronger negative correlation than with aerial based NDVI values (-0.1290). Examination of the data reveal that most of the anthocyanin concentrations lie along a line with a negative slope, but the amount of scatter above the line reduces the strength of the correlation (Figure 1.4). A comparison of aerial based GNDVI values and leaf anthocyanin concentration indicates a similar negative (-0.2273) and highly significant (p=0.0026) relationship. As with the
Figure 1.4. Relationship between anthocyanin concentration and the Normalized Difference Vegetation Index recorded by the GreenSeeker hand-held unit on selected sampling dates.
correlation between anthocyanin concentration and ground based NDVI values, the relationship between anthocyanin concentration and aerial based GNDVI values is reduced by the amount of scatter (Figure 1.5). A possible explanation is that there may be yellow-colored anthocyanin pigments present in cotton plants and the spectral reflectance imagery is recording the yellow wavelength when capturing data in the green range of wavelengths for the GNDVI values making this relationship stronger than expected.

1.2.2 Linear Regression

An attempt to develop a regression model to predict leaf anthocyanin concentration using spectral reflectance imagery was not successful. R-square values reported in Table 1.2 indicate that a representative model can not be constructed from the data. The best regression model has an r-square value of 0.0819 and uses ground based NDVI values, aerial based NDVI values and aerial based GNDVI values to predict leaf anthocyanin concentration. No other quantifiable variables in the data can be added to the formula. These data suggest that predicting leaf anthocyanin concentration with spectral reflectance imagery using the conditions of this test is not valid.

1.3 Summary and Conclusions

The data indicate that leaf anthocyanin concentrations likely do not have a strong influence on aerial imagery and NDVI values. Conversely, although the correlations are weak, ground based NDVI values and aerial based GNDVI values appear to be at least partially skewed by the presence of leaf anthocyanins. Caution should be exercised when interpreting these types of imagery.
Figure 1.5. Relationship between anthocyanin concentration and the Green Normalized Difference Vegetation Index recorded by the aerial unit on selected sampling dates.

Table 1.2. Proportion of anthocyanin concentration predicted by select spectral reflectance variables.

<table>
<thead>
<tr>
<th>Variables in Regression Model</th>
<th>R-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground based NDVI</td>
<td>0.0594</td>
</tr>
<tr>
<td>Aerial based NDVI</td>
<td>0.0518</td>
</tr>
<tr>
<td>Aerial based GNDVI</td>
<td>0.0215</td>
</tr>
<tr>
<td>Ground based NDVI + Aerial based GNDVI</td>
<td>0.0739</td>
</tr>
<tr>
<td>Ground based NDVI + Aerial based NDVI</td>
<td>0.0598</td>
</tr>
<tr>
<td>Aerial based GNDVI + Aerial based NDVI</td>
<td>0.0544</td>
</tr>
<tr>
<td>Ground based NDVI + Aerial based GNDVI + Aerial based NDVI</td>
<td>0.0819</td>
</tr>
</tbody>
</table>
The data suggests that leaf anthocyanin concentrations depend on crop maturity at the time of sampling and indicates that planting date and varietal influences may also have some effect. No two varieties were planted on the same day, therefore it is impossible to determine the amount of influence that variety has on leaf anthocyanin concentration. The significant correlations between aerial based NDVI and aerial based GNDVI (0.8023, p<0.0001), aerial based NDVI and ground based NDVI (0.5296, p<0.0001), and aerial based GNDVI and ground based NDVI (0.5092, p<0.0001) suggest that they all effectively measure plant growth. Conversely, weak correlations between leaf anthocyanin concentration and the three spectral reflectance imagery units indicate that while anthocyanin may have a small effect on the imagery, the imagery is a poor indicator of anthocyanin concentration. This effect is observed even more strongly when the data are subjected to a regression model. Low r-square values suggest that spectral reflectance imagery is a poor tool for determining leaf anthocyanin concentration in cotton.
SECTION II
BASELINE ANALYSIS

2.1 Materials and Methods

To determine basal levels of anthocyanin concentration in cotton seedlings, a growth chamber experiment was conducted. Approximately 100 cm³ of Garden Soil (0.15-0.05-0.10, Miracle-Gro Lawn Products, Inc.) was added to one-half of the cells in eleven twenty-four-cell trays (HIKO V-150, Stuewe & Sons, Inc., Corvallis, OR). One seed was planted in each cell at a depth of one-half inch and the soil was lightly packed. The cotton cultivar ‘Deltapine 493’ (Delta and Pine Land Company, Scott, MS) was used in this study. The trays were placed into a germination chamber held at a constant temperature of 28°C and exposed to twelve hours of irradiation daily from 8:00 am to 8:00 pm. Irradiation was provided by a bank of three cool-white fluorescent bulbs directly above each seed tray. Each bank of bulbs emitted approximately 100 µmol m⁻² s⁻¹ of irradiation measured 5 cm above the soil surface. Twelve hour irradiation treatments were continued until the plants had germinated and the cotyledons had fully expanded. As soon as the first true leaf began to emerge on at least six of the twelve seedlings in each tray all irradiation was discontinued. The seedlings continued to develop in total darkness until the first true leaf was approximately 2.5 cm in diameter on at least four of the twelve seedlings in each tray. Seedlings with first true leaf diameters larger than 3 cm or smaller than 2 cm were removed to ensure that all treatments were at nearly identical development stages. One of the trays was removed and placed into a growth chamber with no irradiation and held at a constant temperature of 28°C. Removal of this tray marked time zero and all other irradiation treatments were then based on this time. A total of eleven treatments were used in this study: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.
hours of exposure to irradiation. The lights in the germination chamber were turned on to expose the remaining ten trays to the additional irradiation treatments. Another tray was removed every hour and placed into the dark growth chamber until all eleven trays had been exposed to the required amount of irradiation and moved back to darkness. Twenty four hours after time zero, the first tray was removed from the growth chamber. Three leaf discs with a total area of approximately 21.195 mm² were removed from each leaf using a standard paper hole punch. The three discs were placed into a 15 cm test tube along with 3 mL of acidified methanol solution (10 mL HCl L⁻¹) and stirred slightly to completely submerge the leaf discs. Each test tube was wrapped individually with aluminum foil to prevent any light penetration while the solution extracted the anthocyanin. This process was repeated for each tray of seedlings resulting in a minimum of four tubes per tray. All of the test tubes were placed in a test tube rack and held in a refrigerator for 48 hours in darkness at 4°C until extraction of anthocyanins. The test tubes were then removed, the liquid contents of one test tube was poured into a cuvette, and placed into a spectrophotometer (Spectronic Helios Gamma, Thermo Electron, UK). Absorption readings were taken at 532 nm and 653 nm and the cuvette was removed and rinsed with de-ionized water for reuse. This process was then repeated for all refrigerated samples. Values for the optical density of anthocyanin were calculated after Wells (1995) as follows:

\[
\text{Anthocyanin} = \text{Absorption}_{532\text{nm}} - 0.25(\text{Absorption}_{653\text{nm}})
\]

The optical density values were then divided by the molecular extinction coefficient of cyanidin, 2.45x10⁴, then divided by the area of the three leaf discs to transform these values into concentrations of mols of anthocyanin per cm² (Siegelman and Hendricks}
Data was then subjected to PROC GLM in the SAS system version 9.12 (SAS Institute, Cary, NC) to compare hourly anthocyanin concentration means.

This experiment was then repeated in its entirety using the following ten treatments: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 12, and 24 hours of exposure to irradiation. Also during this second experiment, the seed coat from ten seeds of the cotton cultivar ‘Deltapine 493’ was removed after being submerged in distilled water for 24 hours. All internal seed components were chopped using a sterile scalpel and the contents of each individual seed were placed in a 15 cm test tube along with 3 mL of acidified methanol solution (10mL HCl L⁻¹) and agitated to completely submerge the chopped seed parts. Test tubes from this experiment were refrigerated and anthocyanin determined using the same process as with leaf discs.

2.2 Results and Discussion

No significant differences (p=0.05) in anthocyanin concentration were observed between any of the irradiation treatments (Table 2.1). These findings indicate that a lag period prior to anthocyanin production in the first true leaf of cotton does not exist. Conversely, anthocyanin concentrations for seedlings with the first true leaf exposed to 24 hours of continuous irradiation were significantly (p<0.0001) different than the anthocyanin concentration of the cotton seeds themselves (Table 2.2). Some anthocyanin, between 2.06E-6 and 4.05E-6 mol/cm², is produced regardless of exposure to irradiation (Table 2.1). The fact that some production takes place in the absence of irradiation is consistent with studies on other crop species (Siegelman and Hendricks 1957; Downs 1964; Grill and Vince 1964, 1969; Lange et al. 1971; Mancinelli 1990). However, cotton seedlings will not produce a true leaf unless the cotyledons are exposed
Table 2.1. Anthocyanin concentration for 0, 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours of exposure to irradiation along with seed anthocyanin concentration.

<table>
<thead>
<tr>
<th>Irradiation Exposure (hrs)</th>
<th>Mean Anthocyanin Concentration ± S.E. (mol/cm²)</th>
<th>Tukey Grouping(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed (0 hours of exposure)</td>
<td>2.060E-6±7.083E-7</td>
<td>A</td>
</tr>
<tr>
<td>0</td>
<td>4.050E-6±7.920E-7</td>
<td>AB</td>
</tr>
<tr>
<td>0.25</td>
<td>6.174E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>0.50</td>
<td>3.680E-6±1.120E-6</td>
<td>AB</td>
</tr>
<tr>
<td>0.75</td>
<td>3.822E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>1</td>
<td>3.828E-6±6.754E-7</td>
<td>AB</td>
</tr>
<tr>
<td>1.25</td>
<td>6.563E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>1.50</td>
<td>6.220E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>1.75</td>
<td>5.943E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>2</td>
<td>4.552E-6±1.120E-6</td>
<td>AB</td>
</tr>
<tr>
<td>3</td>
<td>5.723E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>4</td>
<td>4.724E-6±1.120E-6</td>
<td>AB</td>
</tr>
<tr>
<td>5</td>
<td>5.466E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>6</td>
<td>4.522E-6±1.001E-6</td>
<td>AB</td>
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<td>7</td>
<td>5.289E-6±9.145E-7</td>
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<td>8</td>
<td>4.870E-6±1.120E-6</td>
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<td>9</td>
<td>5.009E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>10</td>
<td>4.579E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>12</td>
<td>5.439E-6±9.145E-7</td>
<td>AB</td>
</tr>
<tr>
<td>24</td>
<td>6.838E-6±6.754E-7</td>
<td>B</td>
</tr>
</tbody>
</table>

\(^1\)Means followed by a common letter do not differ significantly at the 5% protection level
Table 2.2. Significance of comparisons between anthocyanin concentration of seeds and 24 hours of exposure to irradiation against all other treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seed 24</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>0.25</td>
<td>*</td>
</tr>
<tr>
<td>0.50</td>
<td>NS</td>
</tr>
<tr>
<td>0.75</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>1.25</td>
<td>NS</td>
</tr>
<tr>
<td>1.50</td>
<td>NS</td>
</tr>
<tr>
<td>1.75</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
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<td>4</td>
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<td>8</td>
<td>NS</td>
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<tr>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>24</td>
<td>**</td>
</tr>
</tbody>
</table>

* Means differed significantly at the 10% protection level
** Means differed significantly at the 5% protection level
NS Means did not differ significantly at the 10% protection level
to irradiation. Attempts to grow seedlings in the absence of irradiation result in elongated stems and seedling desiccation prior to development of the first true leaf as the seedling uses up available food in the seed. In this study, seedling cotyledons were exposed to 12 hours of irradiation, at an irradiance of approximately 100 µmol m\(^{-2}\) s\(^{-1}\), each day until the first true leaf began to emerge. Given that seedlings in the cotyledon stage require exposure to light and no significant (p>0.05) difference exists between the anthocyanin concentration in cotton seeds and the concentration in the first true leaf grown in the absence of irradiation, it is difficult to distinguish when and where anthocyanin production begins.

A significant difference in anthocyanin concentration observed between cotton seeds and seedlings with the first true leaf exposed to 24 hours of continuous irradiation suggests that anthocyanin concentration increases as the length of irradiance exposure increases when the level of irradiance is held constant. Although mean anthocyanin concentrations for all irradiation treatments were not significantly (p>0.05) different, anthocyanin accumulation appears to increase as the length of irradiation increases (Figure 2.1). For turnip seedlings (Grill and Vince 1964), it is possible that light dependent precursors are developed in the cotyledons when exposed to irradiation and then translocated into the first true leaf as it develops. Conversely, because no significant difference in anthocyanin concentration exists between seeds and seedlings exposed to 12 hours of irradiation or less, it may be that the cotyledons have little association with anthocyanin production in the first true leaf as Downs (1964) found in milo seedlings. The lack of separation for anthocyanin concentrations between treatments of 12 hours of
Figure 2.1. Relationship of anthocyanin concentrations for 0, 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours of exposure to irradiation and seed anthocyanin concentration.
irradiance or less may also be due to level of irradiance used in this study. All of the treatments were exposed to a constant irradiance level of approximately 100 µmol m\(^{-2}\) s\(^{-1}\) which is well below the maximum irradiance of direct sunlight in Louisiana during the summer months (Table 2.3). The relatively low irradiance level used in this study was not enough to cause a significant increase in the accumulation of anthocyanins until seedlings had been irradiated for a full 24 period. This is supported by the comparison of maximum and minimum values associated with this study and the values reported in the first chapter of this document (Table 2.3). Data collected from the field from cotton 50 to 140 days after planting indicate that, when irradiated with direct sunlight for extended periods of time, leaf anthocyanin concentrations reach well above the maximum observed anthocyanin concentration of seedlings exposed to light at 100 µmol m\(^{-2}\) s\(^{-1}\). However, it is difficult to determine whether this is the saturation point of cotton at this particular irradiance or rather the maximum anthocyanin concentration of a young first true leaf. The lack of separation of concentration means of seedlings irradiated for 0-24 hours makes it impossible to determine if concentrations are steadily increasing or approaching a saturation point.

2.3 Summary and Conclusions

While some similarities can be drawn to other studies examining seedling anthocyanin production, there are a few unique characteristics of cotton plants. Many species of plants have been shown to have a lag period after the onset of irradiance before anthocyanin production begins. Cotton appears to be similar to red cabbage seedlings (Downs 1964) in that it does not have a lag period before anthocyanin production.
Table 2.3. Range of cotton leaf anthocyanin concentrations recorded from field experiments and growth chamber study.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Method of Irradiance</th>
<th>Maximum Irradiance (µmol m(^{-2}) s(^{-1}))</th>
<th>Length of Irradiance</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Field</td>
<td>Direct Sunlight</td>
<td>2000</td>
<td>50-140 Days</td>
<td>1.46E-05</td>
<td>2.87E-06</td>
</tr>
<tr>
<td>Growth Chamber</td>
<td>Cool-white Fluorescent</td>
<td>100</td>
<td>0-24 Hours</td>
<td>6.84E-06</td>
<td>2.06E-06</td>
</tr>
</tbody>
</table>
Cotton also appears to respond differently to light exposure compared to most other species of plants. Even at low levels of irradiation intensity, other studies indicate that anthocyanin is produced at a constant rate (Siegelman and Hendricks 1957; Downs 1964; Grill and Vince 1964, 1969; Mohr 1966). This does not appear to be the case in cotton. This trend may be unique only to the first true leaf of the cotton plant. Because cotton does not have any formed leaves in its seeds, the first true leaf of a cotton plant is the major source of energy in a young seedling. Energy may be shifted from anthocyanin production to other systems of the leaf, such as chlorophyll production, or the leaf may not have enough energy to produce any more anthocyanin. Another possibility is that at such a young growth stage, a cotton seedling does not have a need for whatever advantage anthocyanin provides beyond the concentration of anthocyanin that the saturation level represents.

The baseline analysis is necessary to understand the mechanisms that control anthocyanin production in cotton. The absence of an initial lag period suggests that precursors for anthocyanin production are readily available even before the cotton seed germinates. The lack of an initial lag period also suggests that anthocyanin production in cotton is not initiated by exposure to irradiance. However, an accumulation of anthocyanins above a baseline level between 2.06E10\(^{-6}\) and 6.56E10\(^{-6}\) appears to be dependent on continuous irradiation. While this is consistent with most anthocyanin producing plants, it is by no means a given and can not be assumed without further study. The presence of a maximum concentration of anthocyanin in the first true leaf that is lower than maximum concentrations observed in the field may also reveal hints about anthocyanin production in cotton. A finite amount of precursor may be available for
activation to anthocyanin production in the first true leaf. A restricted amount of material suggests that one or more of the precursors needed for anthocyanin production are used by other systems, such as chlorophyll production, in the first true leaf.
REFERENCES


ESRI ArcView 3.3. 2002. Environmental Systems Research Institute, Inc.. Redlands, CA.


VITA

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He attended elementary school in Bruce, South Dakota, before moving to Pigeon, Michigan where he received his high school education at Laker High in Elkton, Michigan, in 1995. Tyson then attended Dakota Wesleyan University where he received his Bachelor of Science degree in biology in 2000. For the next two years, he worked as a research technician for Monsanto Company in Arkansas. He entered the Graduate School at Louisiana State University in September of 2002 under the direction of Dr. Gerald O. Myers in cotton breeding. He is now a candidate for the degree of Master of Science in the Department of Agronomy.