

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Theses, Dissertations, and Student Research in
Agronomy and Horticulture

Agronomy and Horticulture Department

12-4-2015

Evaluation of Genetic Gain for Dynamic Leaf Traits in Maize Using Field Spectroscopy

Jonathan Luetchens

University of Nebraska-Lincoln

Follow this and additional works at: <http://digitalcommons.unl.edu/agronhortdiss>



Part of the [Plant Breeding and Genetics Commons](#)

Luetchens, Jonathan, "Evaluation of Genetic Gain for Dynamic Leaf Traits in Maize Using Field Spectroscopy" (2015). *Theses, Dissertations, and Student Research in Agronomy and Horticulture*. 97.

<http://digitalcommons.unl.edu/agronhortdiss/97>

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Theses, Dissertations, and Student Research in Agronomy and Horticulture by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Evaluation of Genetic Gain for Dynamic Leaf Traits in Maize Using Field Spectroscopy

By
Jonathan Paul Luetchens

A Thesis

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professors Aaron Lorenz and Timothy Arkebauer

Lincoln, Nebraska

December, 2015

Evaluation of Genetic Gain for Dynamic Leaf Traits in Maize Using Field Spectroscopy

Jonathan Paul Luetchens, MS
University of Nebraska, 2015

Advisors: Aaron Lorenz and Timothy Arkebauer

Rapid introduction of cheap and precise genotyping technology has created a void between genotypes and phenotypes in maize breeding. While detailed genetic information is easily accessible, the data are lacking robust phenotypes to be used in mapping studies like genome-wide association. As a result, high-throughput phenotyping tools are necessary to rigorously characterize specific traits. In this study, agronomic traits and an active spectrometer system were used to monitor 36 era hybrids – popular commercial maize hybrids grown from 1936 to 2012 – to discover how various traits have changed over time. In conjunction with increased grain yield of 76 kg/ha per year, modern hybrids displayed a decreased anthesis silking interval, as well as decreased stalk lodging, root lodging, plant height, ear height, and early vegetative biomass, and increased staygreen. In addition, modern hybrids displayed increased leaf chlorophyll and water contents. The 760/730 vegetation index, designed to study plant health and nitrogen uptake using the red edge region of the electromagnetic spectrum, correlated strongly to total leaf chlorophyll content ($R^2 = 0.64$) and also displayed higher values in modern hybrids at numerous points throughout the growing season. By understanding these morphological and physiological trends of maize hybrids over time, breeders can continue to select for traits that are known to enhance yield. Moreover, this research shows that high-throughput phenotyping tools that estimate chlorophyll content can be implemented into a breeding program because the technology can detect superior cultivars.

Acknowledgements

Having always wanted to participate in plant breeding research, but without experience, I'm grateful for the opportunity to study at the University of Nebraska. I'd like to thank many people for supporting me, trusting me, and enabling me to finish this project. First, I'm grateful for my advisor, Dr. Aaron Lorenz, for not only bringing me onto his team, but also for the responsibilities he gave me as a research technician. In addition, he spent numerous hours helping me to develop this thesis project – all the while continuing to encourage and challenge me. He should be given an award for being able to utilize and transform my novice statistical and research skills into a comprehensive project. I'm thankful for the funding from the UNL Life Sciences Competition and the Nebraska Corn Board.

Next, I'd like to thank my committee members for taking the time to work with me and for providing extra expertise: Dr. Timothy Arkebauer, Dr. Yufeng Ge, and Dr. Roger Elmore. A special thanks to Dr. Timothy Arkebauer for becoming my committee chair when Dr. Lorenz moved to Minnesota. Also, thank you to Dr. Tom Hoegemeyer for helping us to select proper hybrids for this experiment and to Mike Livingston for training me to use lab equipment. The people at the Center for Advanced Land Management Information Technologies, specifically Bryan Leavitt, were extremely helpful in setting me up to use their backpack spectrometer system.

I couldn't have finished all of my field and laboratory work without the help of undergraduate research assistants and fellow Lorenz Lab members. I will be ever grateful for the hard work put in by Ryan Luetkenhaus, Melissa Shadoin, Nathan Bruning, and Travis Orrell. Lab members contributing to this work were Dnyaneshwar Kadam, Erin

Gilbert, Amrit Singh, Nonoy Bandillo, Diego Jarquin, and Josiel Nascimento. It was truly a joy to work alongside, get to know, and socialize with these great people.

Finally, I want to thank all of my friends and family for their support, especially my wife Katie. She was constantly encouraging during this busy time of my life. In all, and utmost, I thank the one true, just, and compassionate God for giving my life purpose, meaning, hope, and joy in Jesus Christ.

Table of Contents

Abstract	ii
Acknowledgements	iii
Table of Contents	v
List of Figures	vii
List of Tables	vii
Appendix	viii
Overview of Work	1

Chapter 1 Literature Review

Introduction	3
Demand for Maize Agriculture	3
Stress Tolerant Traits	6
Selecting Secondary Traits in Nebraska	11
Era Advances	14
Phenomics	16
Vegetation Indices	20
Figures	25
Tables	26
Literature Cited	27

Chapter 2 Correlations between Vegetation Indices and Leaf Traits

Introduction	32
Materials and Methods	33
Results	37
Discussion	40
Conclusions	43
Figures	44
Tables	48
Literature Cited	54

Chapter 3 Genetic Gain in Popular Midwest United States Maize Hybrids

Introduction	57
--------------------	----

Materials and Methods.....	59
Results.....	65
Discussion.....	68
Conclusions.....	74
Figures.....	75
Tables.....	81
Literature Cited.....	84
Appendix.....	87
Literature Cited.....	92

List of Figures

Chapter 1

Figure 1.1.	Typical reflectance signature of a healthy maize leaf.....	25
-------------	------------------------------------------------------------	----

Chapter 2

Figure 2.1.	Rainfall and temperature patterns during the 2014 and 2015 growing seasons	44
Figure 2.2.	Variation in relative water content and chlorophyll content laboratory measurements.....	45
Figure 2.3.	Correlation between the 760/730 index and 2014 relative water content laboratory measurements	46
Figure 2.4.	Correlation between the 760/730 index and 2015 chlorophyll content laboratory measurements	47

Chapter 3

Figure 3.1.	Anthesis silking interval regressed over year of hybrid release	75
Figure 3.2.	Chlorophyll content regressed over year of hybrid release	76
Figure 3.3.	Relative water content regressed over year of hybrid release.....	77
Figure 3.4.	Grain yield regressed over year of hybrid release	78
Figure 3.5.	760/730 index values regressed over year of hybrid release	79
Figure 3.6.	Comparison of 760/730 genetic gain at various developmental stages	80

List of Tables

Chapter 1

Table 1.1.	Summary of important vegetation indices	26
------------	-----------------------------------------------	----

Chapter 2

Table 2.1.	Set of era hybrids used in this study	48
Table 2.2.	Mean temperature and rainfall accumulation per month during the 2014 and 2015 growing seasons compared to climate normals.....	49

Table 2.3.	ANOVA results for relative water content and chlorophyll content laboratory measurements	50
Table 2.4.	Best correlations between vegetation indices and relative water content and chlorophyll content laboratory measurements	51
Table 2.5.	ANOVA results for the 760/730 and CI_{RE} vegetation indices.....	52
Table 2.6.	Comparing the effectiveness of chlorophyll content laboratory measurements and 760/730 index values to differentiate hybrids	53

Chapter 3

Table 3.1.	ANOVA results for detecting differences among hybrids and eras for various traits.....	81
Table 3.2.	Genetic gain of various traits	82
Table 3.3.	Genetic gain of 760/730 index values at multiple developmental stages ..	83

Appendix

Supplementary Figure 1.	Multiple regression analysis for predicting aboveground dry biomass during vegetative stages	87
Supplementary Figure 2.	Multiple regression analysis for predicting aboveground dry biomass during reproductive stages.....	88
Supplementary Table 1.	List of all vegetation indices calculated in this study	89

Overview of Work

Retrospective analyses are essential for breeders to understand how plant growth and development has changed over time. When comparing modern maize hybrids to popular cultivars used throughout the 20th century, distinguishable traits between the hybrids provide a glimpse of how productivity has increased. Now, with phenotyping technology that can quickly assess plant health, one can monitor the various hybrids throughout the growing season.

As a result, this research utilizes a set of era hybrids – hybrids used readily by farmers from 1936 to 2012 – to study physiological differences over time. The hybrids were grown in well-watered conditions and they were phenotyped routinely with visual scores, laboratory assays, and a hyperspectral reflectance sensor. Leaf samples were destructively harvested to measure chlorophyll and water content in the lab. Chlorophyll content and water content are basic leaf characteristics that inform about a plant's health and productivity. However, the destructive samples require many man hours and it is unrealistic to use this protocol to monitor large research plots throughout the growing season. Rather, data collection with a spectrometer has numerous advantages. Such sensors are nondestructive and high-throughput – one research plot can be measured in a few hours with two people, and data can be collected throughout the growing season on these large populations. Also, the sensors can capture data outside the boundaries of the visible spectrum, detecting differences not visible to the human eye and without the need of a laboratory. Finally, the sensors use calibrated reflectance values so comparisons can be made across the season and across years.

Along with these, basic agronomic traits were noted. Grain yield, senescence, root and stalk lodging, aboveground biomass, canopy temperature, and flowering notes were gathered to further characterize the hybrids.

Because this research utilizes a set of era hybrids and new phenotyping technology, two primary objectives exist. One objective is to determine how well vegetation indices, calculated from the reflectance data, correlate to the destructively sampled leaf traits (chlorophyll and water content). The second objective is to observe how all of the traits have changed from older to newer hybrids. In other words, the goal is to determine how maize has changed with selection for yield.

The first objective was realized by harvesting leaf samples for laboratory analysis and using the sensor to record reflectance data on the same days. In turn, the measurements could be correlated to determine if the sensor was actually monitoring leaf chlorophyll and water content. Next, the second objective was accomplished in two distinct ways. One, the general agronomic traits provided an overview of how maize gross morphology has changed over time. Two, the sensor measurements provided details about the unique leaf characteristics of modern hybrids compared to historic cultivars throughout the growing season. In all, information about changes in maize physiology that accompany the increase in grain yield over time will prove useful to the breeding community.

Chapter 1

Literature Review

Introduction

Maize agriculture is vital for the wellbeing of humans across the globe. Maize is a preferred staple in developing countries and it is necessary for the production of milk, eggs, and meat in developed nations (Shiferaw et al., 2011). The demand for maize has outpaced wheat and rice and it will double by 2050 (Shiferaw et al., 2011). However, the climate will not make this task of increasing production easier. Drought is already prevalent in many maize-growing regions, and it is only expected to become more severe in the future (Harrison et al., 2014). For breeders, the goal is to develop stress tolerant, productive cultivars. Grain yields have increased consistently since the advent of hybrid maize because of improved stress tolerance (Duvick et al., 2004). Newer hybrids are able to maintain production as they are planted at higher densities (Duvick et al., 2004). These trends must continue to meet the projected maize demands.

Currently, breeders have access to powerful genotyping methods, and high-throughput phenotyping tools are in development (Campos et al., 2004 and Montes et al., 2007). These precision phenotyping techniques need to be utilized to make the genomic information even more powerful. The phenotypes delivered by this new technology may provide the next push in breeding that continues to increase maize productivity.

Demand for Maize Agriculture

The worldwide utilization, and therefore demand, of maize is incredibly high. Maize is grown in many different regions and climates, and there is a wide variety of germplasm to make this possible. While maize has a number of uses in each distinct

region of the world, one thing remains – maize is a critically important crop for the livelihoods of people everywhere.

The United States of America is extremely dependent on maize production. Until 2006 there was an excess of inexpensive maize in the USA (Klopfenstein et al., 2013). As a result, livestock operations grew in size. However, with the advent of the ethanol industry in 2006, the demand and price of maize increased (Klopfenstein et al., 2013). In the 1960s, as much as 75 percent of the maize was fed to livestock (Benson & Gibson, 2002). Now, the primary uses of maize are split between feed, fuel, and food. The National Corn Growers Association reported the following uses of maize in 2014: Feed (38.8%), Distiller's Dried Grain with Solubles (7.6%), Fuel Ethanol (30.5%), Exports (12.9%), High-Fructose Corn Syrup (3.6%), Sweeteners (2.1%), Starch (1.8%), Cereal/Other (1.5%), Beverages (1%), and Seed (0.2%) (Bowling & Novak, 2015).

Worldwide, considering over 4.5 billion people in 94 developing countries, maize, wheat, and rice provide at least 30% of the food calories (Shiferaw et al., 2011). Maize alone provides over 20% of food calories in certain parts of Africa and Central America (maize is the preferred staple for 900 million poor consumers) (Shiferaw et al., 2011). Maize is obviously an important part of global food security.

Pressure continues to mount on these poor nations as other countries develop rapidly. Economic growth in various parts of Asia, the Middle East, and Latin America allow people to purchase milk, eggs, and meat (Shiferaw et al., 2011). As a result, additional maize is demanded and the price increases. While this might benefit some farmers, it is detrimental to poor consumers and poor nations that desire to import the grain.

Shiferaw et al. (2011) analyzed trends in global maize production and its effect on land use. Land used for maize production is currently around 150 million hectares, which is a 50% increase from 1961 because of the growing popularity of maize. Much of the land usage increase was a result of newly cultivated areas in developing countries; farmers typically choose maize because it is much higher yielding than wheat or rice, and they don't have much land to utilize. The global demand for maize has dramatically increased since 1961 – from 189 million metric tons to 771 million metric tons, which is a much higher demand than wheat or rice – this is primarily from the increasing popularity of maize in developing nations. As the population continues to grow, as people make more money, move to the city, and eat more meat, the demand for maize will double by 2050 (Shiferaw et al., 2011).

Finally, while most of these problems seem to affect the developing world, the United States is still of extreme importance. Wu and Guclu (2013) performed an analysis of the global maize trade from 2000-2009. The United States is by far the largest exporter of maize worldwide; many nations depend on our maize production to meet their needs. Because the United States is such a large exporter, many nations solely rely on the USA for their maize. Therefore, if production decreased in the United States, many nations would be without maize – there would not be enough worldwide production to supply everyone's needs. For example, Canada and Mexico import over 99% of their maize from the USA; in addition, four out of the top five importers of maize rely heavily on the USA – Japan (90% from the USA), Taiwan (80%), Egypt (40-75%), and South Korea (85%). Mexico was impacted in 2005 when the United States reduced exports in order to fuel the new ethanol industry. As a result, prices soared and riots broke out in Mexico because the

poor could not afford corn tortillas anymore (Wu & Guclu, 2013). In addition, the drought of 2012 showed how a decreased supply of maize could affect the world. As expected, the world maize price increased, and trade and consumption decreased (Chung et al., 2014). Decreased consumption enhances food insecurity, and it especially hurt the poor countries in the Caribbean, northern Africa, and western Asia because of their dependency on imports (Chung et al., 2014). Obviously, maize production in the United States is critical for food security around the globe.

Stress Tolerant Traits

In order to increase maize production in light of highly variable climatic conditions, breeding efforts must develop cultivars which have high yield potentials in both stressed and optimal environments. Certain physiological characteristics can enhance productivity in both situations.

Naturally, during drought, plants have reduced leaf area and seed number in order to preserve a few viable seeds (Tardieu et al., 2014). Reducing leaf area in turn reduces the transpiration rate and water is saved for the reproductive stage (Tardieu et al., 2014). However, this conservative strategy would be completely outperformed under mild drought scenarios because it stops accumulating biomass (Tardieu et al., 2014). Therefore, different traits/physiology can lead to drought tolerance in different situations – it all depends on the drought scenario (Tardieu, 2012, Harrison et al., 2014, and Tardieu et al., 2014). In order to make strides in drought tolerance in a certain area, one must ask whether an allele/trait confers a positive effect on yield in the majority of years for that specific location (Tardieu, 2012).

Yue et al (2005) describe three mechanisms toward drought resistance that have unique genetic bases: drought escape, drought avoidance, and drought tolerance. The drought escape mechanism is simply a shortened life cycle. Drought avoidance culminates in the least reduction of leaf hydration, while drought tolerance expresses itself as maintained plant life even when tissues are dehydrated (Blum et al., 1982 and Yue et al., 2005).

Typically, drought tolerant traits enable plants to survive severe droughts. In the most extreme case, resurrection plants can become totally dehydrated and then recover upon rehydration. This is made possible by constitutive traits like a high antioxidant capacity, high sugar levels, and expression of late embryogenesis abundant and heat shock proteins (Gechev et al., 2012). While many other pathways accompany these constitutive traits to provide desiccation tolerance, it is still uncertain whether resurrection plant genes can be used to provide drought tolerance in agronomic crops.

On a more practical note, drought tolerant crops are generally good at remobilizing stem water-soluble carbohydrates, accumulating molecular protectants, maintaining cell-membrane stability, and detoxifying cells (Tuberosa, 2012 and Yue et al., 2005). Redox molecules can act as signals for the cell to detoxify itself to prevent irreversible damage to photosystems, and the ability of a membrane to maintain its integrity during dehydration and rehydration is imperative (Chaves & Oliveira, 2004). Even though these features allow plants to maintain functionality in a dehydrated state, it results in decreased carbon assimilation and overall productivity through stomatal closure, reduced leaf growth, and leaf rolling (Chaves & Oliveira, 2004 and Tardieu, 2012).

On the other hand, drought avoidance is characterized by enhanced water uptake and maintained turgor pressure in the cells (Tuberosa, 2012 and Yue et al., 2005). In other words, the plant is avoiding the drought because it still has high leaf water potential. Primary traits influencing drought avoidance are deep roots and osmotic adjustment. Root exploration allows necessary water to be acquired, and the accumulation of solutes in cells maintains turgor pressure (Tuberosa, 2012). Leaf-canopy temperature is a reliable indicator of the drought avoidance mechanism; if a plant closes its stomata because of low leaf water potential then there is a reduction in evaporative cooling (Blum et al., 1982).

As decreased leaf growth is the first effect of water stress on plants, one can decipher between avoidance and tolerance mechanisms based on the variability in this process (Tardieu, 2012). Leaf relative water content (LRWC) has a strong positive correlation with dry matter and height, indicating that maintained water content leads to maintained growth (Mohammady-D. & Hasannejad, 2006). As early as 1990, correlations were made between growth and yield; Sinclair, et al. (1990) showed very strong positive correlations between biomass and yield in maize under water stress. Maintained growth should be the result of plants utilizing the drought avoidance mechanism.

However, increasing total biomass should not be the goal while breeding for drought tolerance (Edmeades et al., 1999). Instead, partitioning should be directed to the developing ear, increasing the harvest index (HI) (Edmeades et al., 1999). Maintaining growth leads to higher yield potential under mild drought conditions; maintaining leaf growth shares genetic determinism with reproductive growth, so leaf growth is correlated with ear growth rate, a short anthesis silking interval (ASI), and a reduced abortion rate

(Tardieu, 2012). The ASI is the time between pollen shed and silk emergence. While maintaining growth seems suitable for most agriculture environments, it holds the risk of failing during a severe drought.

A new drought resistant hybrid from Monsanto, which was genetically engineered to express cold shock protein B, exhibits a drought tolerant phenotype. The hybrid had an average yield increase of 6% in water limiting conditions, but that was associated with higher soil water content at 0.5 meter depth, increased ear growth, increased harvest index, and decreased leaf area, leaf dry weight, and sap flow rate (Nemali et al., 2015). Somehow, the hybrid was able to conserve water (more water in the soil and decreased leaf growth and sap flow rate) while still partitioning enough assimilates to the ear to out-yield the other hybrid, which had the same genetic background just without the transgene (Nemali et al., 2015). The unique phenotype expressed can most likely be attributed to the environment it was created for – an extreme drought. The experiment was conducted in California and water was withheld from the V10 to R3 stages. This resulted in severe stress; additional irrigation was required to rescue the crop from failure during some years (Nemali et al., 2015). This extreme environment enabled the drought tolerant mechanism of decreasing leaf area and conserving water to be successful.

However, drought avoidance mechanisms are the norm in the industry. Progress achieved by breeders has mainly been in the area of constitutive traits affecting dehydration avoidance (Tuberosa, 2012). In Texas, compared to a conventional hybrid, a drought-resistant cultivar extracted the same amount of water or less from the soil, but extracted more water from deeper soil layers (Hao et al., 2015). The resistant cultivar had a higher yield attributed to its increased biomass, harvest index, and kernel weight (Hao

et al., 2015). In addition, a CIMMYT study showed that delayed senescence was the most important factor for hybrid grain yield under water stress (Cairns et al., 2012).

Dupont Pioneer Optimum AQUAmax hybrids display drought avoidance traits by maintaining leaf water potential. In a large on-farm study, the AQUAmax hybrids were 6.5% higher yielding in water stressed conditions and 1.9% higher yielding in optimal conditions compared to other popular hybrids (Gaffney et al., 2015). Therefore, drought avoidance mechanisms are capable of not only improving yield under mild drought scenarios, but also maintaining yield in optimal conditions. Progress needs to continue for drought avoidance in target environments that frequently experience mild droughts.

Luckily, large genetic variability does exist for growth under water deficit (Tardieu et al., 2014). Some plants refrain from growing in relatively wet soil while others continue growing until soil-available water is near its minimum (Tardieu et al., 2014). Multiple studies have even found QTLs for leaf elongation rate under different temperatures, vapor pressures, and soil water statuses, and could predict how each line would respond in unique environments (Reymond et al., 2003 and Sadok et al., 2007). Primarily, these differences in growth rates are linked to hydraulic processes in the plant (cell turgor, osmotic adjustment, cell wall extensibility, water potential, conductance, etc.), and they are responsible for sink strength (a kernel's capacity to store photosynthates). Meanwhile, photosynthesis operates through a separate process and determines the source strength (amount of photosynthates available). These interdependent processes, controlled by water content (growth potential) and chlorophyll content (photosynthetic potential), must work efficiently to enhance grain yield (Tardieu et al., 2014).

Selecting Secondary Traits in Nebraska

In Nebraska, a breeder should select for traits, like the AQUAmax hybrids, that confer increased productivity in both water limited and optimal environments. According to the University of Nebraska-Lincoln website, Lincoln receives approximately 28.93 inches of rain per year, and the wettest months are May and June with 4.29 and 4.34 inches, respectively. While drought stresses are sporadic, eastern Nebraska can typically expect moist springs and intermittent rainfall throughout the growing season; late terminal water stresses are most frequent. As a result, traits can be selected that encourage maintained growth and production – drought avoidance.

Obviously, grain yield is the primary trait of interest. However, if other secondary traits are selected with grain yield, selection efficiencies can increase (Chapman & Edmeades, 1999). One study, under nitrogen stress, showed an increase in selection efficiency of 20% by using secondary traits (Chapman & Edmeades, 1999). More recently, Dr. Samuel Trachsel of CIMMYT (International Maize and Wheat Improvement Center) found that selecting for high NDVI (Normalized Difference Vegetation Index) and low canopy temperature can increase grain yields in maize under heat and drought stress (personal communication, February 6, 2015). According to Chapman and Edmeades (1999), ideal secondary traits should be, “associated with grain yield under drought, carry no yield penalty under favorable conditions, be heritable, cheap and rapid to measure, stable over the measurement period, and be able to be observed at or before flowering so that undesirable parents are not crossed.”

As many of the traits have already been mentioned, this section will simply summarize secondary traits that could be selected for in Nebraska:

- Increased fertile ears per plant (Chapman & Edmeades, 1999).
- Reduced barrenness (Campos et al., 2004).
- Increased grains per fertile ear (Chapman & Edmeades, 1999).
- Increased kernel number per plant (Campos et al., 2004).
- Increased grain number per square meter (Chapman & Edmeades, 1999).
- Maintained 1000 grain weight (Chapman & Edmeades, 1999).
- Reduced ASI (Chapman & Edmeades, 1999).
- Quick silk emergence (Campos et al., 2004).
- Decreased days to 50% anthesis (Chapman & Edmeades, 1999 and Lopes et al., 2011).
- Decreased primary tassel branch number (Chapman & Edmeades, 1999).
- Increased ear growth rate (Campos et al., 2004 and Barker et al., 2005).
- Maintained photosynthesis (Tardieu, 2012).
- Maintained stomatal conductance (transpiration rate) (Lopes et al., 2011 and Tardieu, 2012).
- Maintained plant growth (Campos et al., 2004, Lopes et al., 2011, and Tardieu, 2012).
- Reduced senesced leaf area (staygreen) (Campos et al., 2004 and Chapman & Edmeades, 1999).
- Increased chlorophyll concentration (Campos et al., 2004).
- Decreased leaf rolling (Campos et al., 2004).
- Decreased canopy temperature (Campos et al., 2004).
- Increased rooting depth (Tardieu, 2012).

- Maintained leaf relative water content (Mohammady-D. & Hasannejad, 2006).
- Increased harvest index (Edmeades et al., 1999).

Many of these traits are self-explanatory as to why they promote maintained growth and productivity under mild drought scenarios. All of the kernel and ear traits (ears per plant, kernels per ear, etc.) are directly correlated with yield (Chapman & Edmeades, 1999). In addition, these traits are also highly correlated with ASI under drought (Chapman & Edmeades, 1999). This proves how important it is for Nebraska hybrids to grow and partition photoassimilates to the developing ear in order for the silks to emerge quickly, the ASI to decrease, and fertilization to occur.

Maintained 1000 grain weight and days to 50% anthesis (or maturity) are less important characteristics. Grain yield is determined more by kernel number than kernel weight; therefore, the focus should be on increasing kernel number and maintaining kernel weight (Chapman & Edmeades, 1999). Then, days to 50% anthesis is generally reduced when selections are made under drought because the crop is trying to escape the late-season stress (Lopes et al., 2011). However, a happy medium must be in place because during low stress years, a late-maturing hybrid will be the most productive (Tardieu, 2012).

Energy generation through photosynthesis must be increased. This is accomplished by maintaining carbon uptake (stomatal conductance) and by maintaining chlorophyll concentration (Tardieu, 2012). Maintained plant growth depends primarily on the water status of the plant (Tardieu et al., 2014). Therefore, increased rooting depth and leaf relative water content as well as decreased leaf rolling and canopy temperature would all benefit this cause (Campos et al., 2004, Lopes et al., 2011, and Tardieu, 2012).

Leaves roll because they are experiencing water deficit, and canopy temperature is decreased when sufficient water uptake allows the plant to continue to transpire (Campos et al., 2004). Finally, while plant growth should be promoted, assimilates should always be favorably partitioned to the ear to promote fertility, high yield, and a high harvest index (Edmeades et al., 1999).

Era Advances

Because maize breeding has been around for at least a century, remarkable gains have been made in the crop's performance. The current inbred-hybrid breeding method, used to exploit the extra vigor in the F1 generation, was designed by George Harrison Shull and Edward Murray East in 1908 (Duvick, 2001). Initially double-cross hybrids were used, but as the companies improved inbred lines, they were capable of selling single cross hybrids in the 1960s (Duvick, 2001). Interestingly, there has been no improvement in heterosis over time (the difference between the hybrid and the mid-parent value) and the molecular mechanisms underlying heterosis are still a mystery (Duvick, 2001). As a result, average grain yield in the United States has risen from 1 megagram per hectare in 1930 to nearly 10 megagrams per hectare in 2011 (Smith et al., 2014).

Pioneer Hi-Bred International has released a summary of how their cultivars have changed after years of pedigree breeding to improve inbred lines, and they attribute the increases in grain yield to improved efficiency of grain production and improved stress tolerance (Duvick et al., 2004). Now, maize hybrids are planted at much higher densities than in the 1930s. Actually, today's hybrids do not yield more per plant than the old hybrids; instead, they are able to maintain that yield while being planted much closer

together. In addition, the new hybrids outperform the old hybrids in low- and high-yielding environments. When a series of hybrids sold by Pioneer Hi-Bred International from 1930 to 2000 were planted under drought, linear gains in grain yield over time were similar to gains in normal conditions (Duvick et al., 2004).

Changes in secondary traits have enabled new hybrids to become more productive in stressful environments. Several changes have occurred, including a shorter ASI, more ears per 100 plants, increased staygreen, reduced stalk and root lodging, less European corn borer damage, and increased tolerance to northern corn leaf blight (Duvick et al., 2004). In addition, traits like smaller tassels, increased grain starch, more upright leaves, and fewer tillers allowed the plants to be more efficient in transporting assimilates to the grain. Assimilates were delivered to the developing ear rather than to extra vegetative growth in the tassel or tillers (Duvick et al., 2004).

Tollenaar & Wu (1999) found that yield improvements can be attributed to greater stress tolerance. They showed that new hybrids outperform old hybrids in all scenarios: high plant density, weed interference, low night temperatures during grain-filling, low soil moisture, and low soil nitrogen. The new hybrids were advantageous because they captured and used resources more efficiently. Primarily, new hybrids intercepted sufficient solar radiation from increased leaf angles and staygreen characteristics, and their roots could acquire enough water and nitrogen to maintain a larger source/sink ratio (Tollenaar & Wu, 1999). Valentinuz and Tollenaar (2004) noticed that newer hybrids had a larger leaf area index at flowering and that old hybrids senesced 3.4 and 2.1 times faster than newer hybrids during two separate halves of grain-filling. This prolonged period of photosynthesis during grain fill and the efficient partitioning to the kernels has enabled

new hybrids to accumulate greater biomass at high plant densities (Tollenaar & Lee, 2006).

Using hydroponics, Sanguineti et al. (2006) noticed that newer hybrid seedlings had significantly reduced sizes and weights (of roots and shoots) compared to old hybrids. They attributed this to the increase in fertilizer and plant density experienced by new hybrids. As a result, new hybrids would not need vigorous roots to capture nutrients, and they could delay competition (Sanguineti et al., 2006). On the other hand, Hammer et al. (2009) noted a change in root architecture as the most likely cause for increased grain yields in maize. In their model, as long as water was available at depth, narrow, deep, and steep roots proved to be more important than changes in canopy architecture for increased biomass and yield at high density.

Absciscic acid (ABA) is a plant growth regulator that encourages survivability rather than productivity – increased concentrations lead to stomatal closure, leaf shedding, and tip kernel abortion. Yields are said to increase when ABA leaf concentration is reduced in mild-drought scenarios. New hybrid seedlings had less ABA in their leaves 24 hours after water stress compared to old varieties (Sanguineti et al., 2006). Therefore, selection may have favored those genotypes that reduce ABA production and/or signaling (Sanguineti et al., 2006).

Phenomics

In order to create hybrids that bear these beneficial traits, breeders need to integrate phenotyping and genotyping technologies in conjunction with crop modeling programs (Cooper et al., 2014). While each of these aspects is crucial, and while phenotyping involves many variables (experimental design, managed environments,

understanding soil variability, etc.), this review will focus on the acquisition of measurements by new phenotyping technology.

The phenome refers to the phenotype as a whole; therefore, phenomics is the gathering of high-dimensional phenotypic data of an organism (Houle et al., 2010). Many authors suggest that large-scale phenotyping is the “natural complement to genome sequencing as a route to rapid advances in biology,” so the field of phenomics must be pushed to the forefront (Houle et al., 2010).

While there has been an exponential increase in genotyping technologies and a similar decrease in cost per data point, the ability to measure important phenotypes has not kept pace; phenotyping large experiments for multiple traits remains laborious and expensive (Campos et al., 2004 and Montes et al., 2007). As QTL mapping, genome wide association studies, and genomic selection have become mainstays, a lack of accurate, rapid, precise, thorough, reproducible, and descriptive phenotypes limits the discovery power of these genomic technologies. Instead, high throughput phenotyping would allow plant characteristics to be captured in detail, and they would provide reliable estimates of important traits (Campos et al., 2004).

For a high throughput phenotyping technology to be successful, it must be able to take many measurements rapidly (Cooper et al., 2014). In a commercial breeding program, multiple breeders have multiple breeding cycles to evaluate every season – from new inbred evaluations, early testing, to final pre-commercial evaluations (Cooper et al., 2014). As a result, the number of genotypes to be phenotyped for the traits of interest will be in the tens of thousands (Cooper et al., 2014). In addition, these phenotypes need to be analyzed in the field. Oftentimes traits analyzed in the laboratory

do not correlate well with how the plants behave season-long in the field (Passioura, 2012). Next, dynamic traits such as drought tolerance need to be monitored throughout the season (Montes et al., 2007). High throughput phenotyping that can take many measurements throughout the growing season can capture the genes that are active at different phases of plant development (Montes et al., 2007).

Two technologies that are promising are near-infrared spectroscopy on harvesters and spectral reflectance of the plant canopy (Montes et al., 2007). With near-infrared spectroscopy on harvesters, the plant material can be analyzed as it is harvested. Reflectance values corresponding to one plot are summarized in the near-infrared spectrum. By using calibration models with known references, the spectrum can elucidate many physical and chemical characteristics of the harvested material. This spectroscopy technique reduces manpower and expenditure for determining significant traits, while producing representative measurements with smaller sampling errors. In maize, this technique has provided accurate details of grain dry matter, starch, and crude protein, and it has the potential to determine other quality components like amino acids, fatty acids, and vitamins (Montes et al., 2007).

Canopy spectral reflectance is promising because radiation that is reflected off of a leaf can provide information about the status of that leaf (Peñuelas & Filella, 1998). The unique reflectance signatures are a result of leaf surface properties, internal structure, and concentrations and distributions of biochemical components. A typical reflectance pattern shows low reflectance in the visible spectrum (400-700 nm) because of absorption by photosynthetic pigments. Meanwhile, since there are no molecules which absorb near-infrared radiation (700-1300 nm), the high reflectance values in that region primarily

represent cell structure. Finally, the middle infrared region (1300-3000 nm) is best linked to water content (Peñuelas & Filella, 1998). Figure 1.1 displays a typical reflectance curve of a healthy maize leaf.

Canopy spectral reflectance is a non-invasive technique that allows for high temporal resolution measurements of dynamic traits (Montes et al., 2007). Sensors capture the reflectance from the canopy and a spectrum is produced as a result; these sensors can be mounted on tractors, unmanned aerial vehicles (UAVs), wands, and even satellites (Montes et al., 2007 and Thenkabail et al., 2014). Physical and chemical characteristics of the plot can be inferred (following correlation studies), like canopy architecture, water status, and nitrogen concentration.

Two kinds of canopy spectral reflectance sensors exist: active and passive (Montes et al., 2007). Active sensors have their own light source; radiation is generated within the sensor which is directed toward the canopy. In turn, it also measures the proportion of incoming energy that was reflected off of the canopy. Active sensors can be used at any time during the day or night because they are not dependent on radiation from the sun. In addition, active sensors are less sensitive to environmental conditions and are useful for multi-location trials (Montes et al., 2007). On the other hand, passive sensors utilize the electromagnetic energy from the sun to measure reflectance. Sensors observe the total radiation from the sun and the proportion of that radiation that is reflected off of the plant canopy. Passive sensors can measure reflectance from a wide range of wavelengths, but they are influenced by environmental conditions (Montes et al., 2007).

Hyperspectral data provides the best coverage of the electromagnetic spectrum; 100s to 1000s of narrow bands can provide reflectance information across the spectrum

(Thenkabail et al., 2014). To this point, much research has been conducted with hand-held spectrometers like the FieldSpec (Analytical Spectral Devices, Inc.) which operates from 400 to 2500 nm and has small bandwidths of 1 to 10 nm (Thenkabail et al., 2014). Devices like these are favored because they are easy to use and they avoid challenges like cloud cover and high costs of airborne systems. However, to become more high-throughput, the use of airborne systems and satellites are also being developed. The main obstacles to overcome with the use of this airborne technology are the background noise and atmospheric effects (Thenkabail et al., 2014).

Hyperspectral data as a whole has other concerns. First, one has to mine large volumes of data to find useful information and valuable bands (Thenkabail et al., 2014). Second, many of the bands are redundant; typically, bands that are next to each other are almost perfectly correlated (680 nm and 690 nm bands have an R-squared of >0.99). While the research is nowhere near complete, Thenkabail et al. (2014) have identified 15 to 20 unique, non-redundant bands which can provide useful descriptions of vegetation.

Vegetation Indices

When a small number of spectral bands can be utilized for analysis, the data is much more manageable. Equations that use these optimal spectral bands have been designed that can describe certain characteristics of the vegetation – these calculations are called vegetation indices (Thenkabail et al., 2014). Vegetation indices are more powerful than analyzing individual bands by themselves (Bannari et al., 1995).

The power of the vegetation indices stems from the inverse relationship of the near infrared and red regions of the electromagnetic spectrum. A healthy plant will reflect high amounts of radiation in the near infrared region and low amounts of radiation in the

red region. As a result, the ratio of near infrared reflectance over red reflectance is the baseline for distinguishing plants based on their health status, or color. In fact, the first vegetation index created, the simple ratio, used this exact equation ($SR = \frac{NIR}{red} \text{ or } \frac{R745}{R675}$) (Birth & McVey, 1968). With their simple ratio, Birth and McVey (1968) found a correlation of 0.984 between their index and a visual score of turf color. Most vegetation indices take advantage of this inverse relationship. Now, the most popular vegetation index, the normalized difference vegetation index (NDVI), simply provides a standardized score (with results between 0 and 1) for easier comparisons.

These indices have the potential to observe many important characteristics of a crop, ranging from biomass and leaf area index (LAI), to chlorophyll and carotenoid concentrations, to the extent of stresses (Thenkabail et al., 2014). Because many vegetation indices currently exist, a number of different indices will be briefly explained and summarized in Table 1.1.

First, Thenkabail et al (2014) list their top indices which monitor four generic features of vegetation.

1. HBSI – Hyperspectral Biomass and Structural Index
2. HBCI – Hyperspectral Biochemical Index
3. HREI – Hyperspectral Red-Edge Index
4. HWTI – Hyperspectral Water and Moisture Index

HBSI is used to study biomass, LAI, plant height, and grain yield; HBCI is used to study pigments like carotenoids, anthocyanins, and chlorophyll as well as nitrogen; HREI is used to study plant stress and drought; HWTI is used to study plant water and moisture (Thenkabail et al., 2014).

Chlorophyll content is a necessary trait to characterize, and there are many spectral indices that try to do so. Main et al (2011) compare 73 different chlorophyll indices to actual leaf chlorophyll content (mg/m^2) using leaf level hyperspectral data collected from multiple species. When combining the datasets from multiple species (maize, tomato, cabbage, and savanna tree), Main et al (2011) found that the red-edge position linear extrapolation (REP_LE) and modified red-edge inflection point (mREIP) indices had the highest predictive power. When only looking at the maize data, the modified NDVI (mND_{705}) and mREIP indices were the best at predicting total chlorophyll content (Main et al., 2011). From the University of Nebraska-Lincoln, Anatoly Gitelson and co-workers (2003) created a robust index to quantify chlorophyll content in vegetation. Their chlorophyll index_{red edge} (CI_{RE}) had a coefficient of determination of 0.95 when correlated with chlorophyll content in maize and soybean leaves.

NDVI is the most commonly referred to index. Generally, it is known to correlate with leaf greenness, crop cover, and crop productivity (Hazratkulova et al., 2012). In one study, NDVI correlated to grain yield in winter wheat; higher yielding lines maintained NDVI throughout the season (negative correlation between grain yield and reductions in NDVI), even through periods of stress (Hazratkulova et al., 2012). Meanwhile, low-yielding lines experienced a more rapid decline in NDVI (Hazratkulova et al., 2012). Teal et al (2006) found an R-squared relationship of 0.77 between NDVI at the V8 stage and grain yield of maize.

For understanding plant water content, Winterhalter et al (2011) found the best indices that correlate to canopy water mass (CWM). CWM is measured by destructively

sampling, drying, and weighing the maize plants to see how much total water is in the biomass – the units are kilograms per square meter. Coefficients of determination for three indices were 0.72; these indices are CWMI I, NIR/NIR, and 760/730, respectively (Winterhalter et al., 2011). Additionally, the 760/730 index correlated strongly with canopy temperature. In their study, the drought tolerant cultivars had elevated chlorophyll content and water mass and low canopy temperatures compared to susceptible cultivars, and the respective indices had tight correlations (Winterhalter et al., 2011). With improved water status, the plants are able to maintain chlorophyll production and transpiration. In wheat, two water indices have shown great correlations with grain yield (Gutierrez et al., 2010). These normalized water indices, NWI-1 and NWI-3, have correlated with relative water content, leaf water potential, and stomatal conductance in the plants (Gutierrez et al., 2010).

Finally, the photochemical reflectance index (PRI) correlates with the state of the xanthophyll cycle pigments and tells about the efficiency of photosynthesis and the degree of stress the plant is experiencing (Gamon et al., 1992). With excess light, the absorbed radiation exceeds the amount that can be used in the photosynthetic reactions, and the xanthophyll cycle pigment violaxanthin is deepoxidized to zeaxanthin. Therefore, if zeaxanthin levels are elevated, the plants are said to be less light use efficient (LUE) – they are not utilizing the available radiation in photosynthesis, typically due to stress (Gamon et al., 1992).

While many more indices exist, those in Table 1.1 have been cited as correlating strongly with measured traits. Obviously, as high-throughput phenotyping becomes a mainstay in agriculture, robust indices that hold true for a characteristic across

experiments need to be identified. Breeders and physiologists need to collaborate in order to maximize the potential of high-throughput phenotyping to pinpoint ideal traits that need to be selected.

Figures

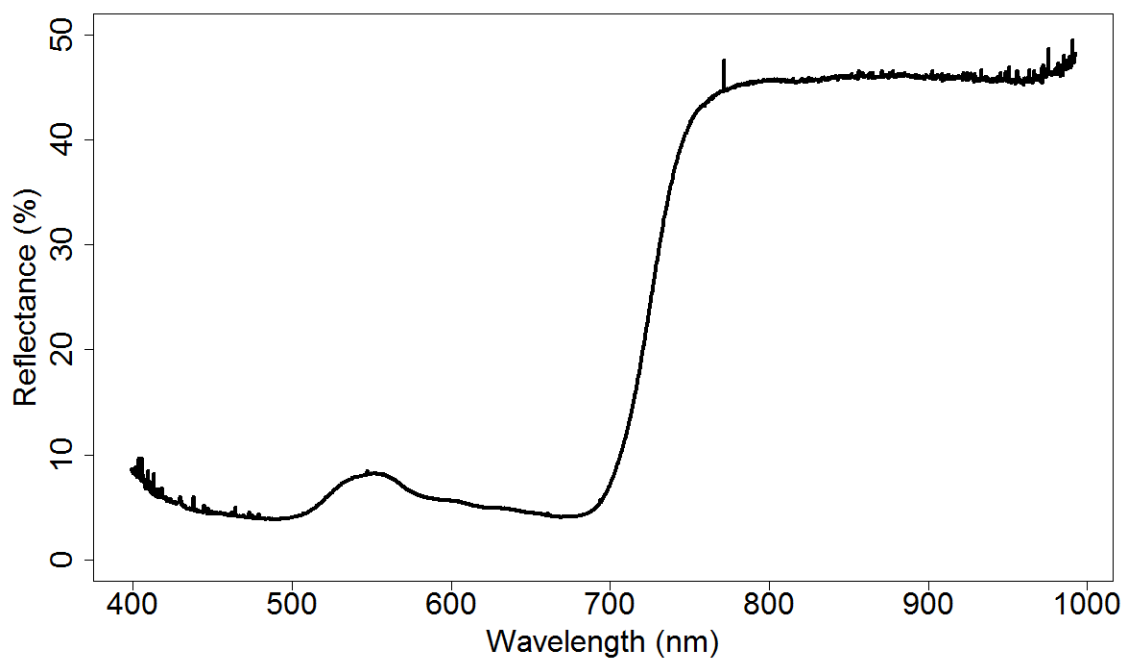


Figure 1.1. Typical reflectance signature of a healthy maize leaf in the visible and near infrared regions of the electromagnetic spectrum (400 to 1000 nm). Acquired on July 28, 2014 with a spectrometer (USB2000+ VIS-NIR, Ocean Optics, Dunedin, FL).

Tables

Table 1.1. Summary of important vegetation indices, their equations, and the traits that are monitored. In the equations, an R followed by a number is the reflectance value at that specific wavelength in nanometers. Subscript numbers designate the mean reflectance value across the listed wavelengths.

Index	Equation	Trait	Reference
HBSI1	$(R_{855}-R_{682})/(R_{855}+R_{682})$	Biomass	(Thenkabail et al., 2014)
HBCI8	$(R_{550}-R_{515})/(R_{550}+R_{515})$	Pigments	(Thenkabail et al., 2014)
HWMI17	$(R_{855}-R_{970})/(R_{855}+R_{970})$	Water Content	(Thenkabail et al., 2014)
mND ₇₀₅	$(R_{750}-R_{705})/(R_{750}+R_{705}-2*R_{445})$	Chlorophyll Content	(Main et al., 2011)
CI _{RE}	$(R_{750-800}/R_{710-730}) - 1$	Chlorophyll Content	(Gitelson et al., 2003)
NDVI	$(R_{800}-R_{670})/(R_{800}+R_{670})$	Productivity, Greenness, Cover	(Main et al., 2011)
CWMI I	R_{850}/R_{725}	Canopy Water Mass (CWM)	(Winterhalter et al., 2011)
NIR/NIR	R_{780}/R_{740}	CWM	(Winterhalter et al., 2011)
760/730	R_{760}/R_{730}	CWM and Canopy Temperature	(Winterhalter et al., 2011)
NWI-1	$(R_{970}-R_{900})/(R_{970}+R_{900})$	Water Status	(Gutierrez et al., 2010)
NWI-3	$(R_{970}-R_{880})/(R_{970}+R_{880})$	Water Status	(Gutierrez et al., 2010)
PRI	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Light Use Efficiency (LUE)	(Gamon et al., 1992)

Literature Cited

- Bannari, A., Morin, D., Bonn, F., & Huete, A. R. (1995). A review of vegetation indices. *Remote Sensing Reviews*, 13(1-2), 95–120.
- Barker, T., Campos, H., Cooper, M., Dolan, D., Edmeades, G., Habben, J., Schusler, J., Wright, D., & Zinselmeier, C. (2005). Improving drought tolerance in maize. *Plant Breeding Reviews*, 25, 173–253.
- Benson, G., & Gibson, L. (2002). Origin, history and uses of corn. Retrieved August 18, 2015, from http://agron-www.agron.iastate.edu/Courses/agron212/readings/corn_history.htm
- Birth, G. S., & McVey, G. R. (1968). Measuring the color of growing turf with a reflectance spectrophotometer. *Agronomy Journal*, 60(6), 640–643.
- Blum, A., Mayer, J., & Gozlan, G. (1982). Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. *Field Crops Research*, 5, 137–146. [http://doi.org/10.1016/0378-4290\(82\)90014-4](http://doi.org/10.1016/0378-4290(82)90014-4)
- Bowling, C., & Novak, C. (2015). *World of Corn 2015*. National Corn Growers Association. Retrieved from <http://www.worldofcorn.com/pdf/WOC-2015.pdf>
- Cairns, J. E., Sanchez, C., Vargas, M., Ordoñez, R., & Araus, J. L. (2012). Dissecting maize productivity: ideotypes associated with grain yield under drought stress and well-watered conditions. *Journal of Integrative Plant Biology*, 54(12), 1007–1020. <http://doi.org/10.1111/j.1744-7909.2012.01156.x>
- Campos, H., Cooper, M., Habben, J. E., Edmeades, G. O., & Schussler, J. R. (2004). Improving drought tolerance in maize: a view from industry. *Field Crops Research*, 90(1), 19–34. doi:10.1016/j.fcr.2004.07.003
- Chapman, S. C., & Edmeades, G. O. (1999). Selection improves drought tolerance in tropical maize populations: II. Direct and correlated responses among secondary traits. *Crop Science*, 39(5), 1315–1324.
- Chaves, M. M., & Oliveira, M. M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany*, 55(407), 2365–2384. <http://doi.org/10.1093/jxb/erh269>
- Chung, U., Gbegbelegbe, S., Shiferaw, B., Robertson, R., Yun, J. I., Tesfaye, K., Hoogenboom, G., & Sonder, K. (2014). Modeling the effect of a heat wave on maize production in the USA and its implications on food security in the developing world. *Weather and Climate Extremes*, 5-6, 67–77. <http://doi.org/10.1016/j.wace.2014.07.002>

- Cooper, M., Gho, C., Leafgren, R., Tang, T., & Messina, C. (2014). Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. *Journal of Experimental Botany*, 65(21), 6191–6204. doi:10.1093/jxb/eru064
- Duvick, D. N. (2001). Biotechnology in the 1930s: the development of hybrid maize. *Nature Reviews Genetics*, 2(1), 69–74.
- Duvick, D. N., Smith, J. S. C., & Cooper, M. (2004). Long-term selection in a commercial hybrid maize breeding program. *Plant Breeding Reviews*, 24(2), 109–151.
- Edmeades, G. O., Bolaños, J., Chapman, S. C., Lafitte, H. R., & Banziger, M. (1999). Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. *Crop Science*, 39(5), 1306–1315.
- Gaffney, J., Schussler, J., Löffler, C., Cai, W., Paszkiewicz, S., Messina, C., Groeteke, J., Keaschall, J., & Cooper, M. (2015). Industry-scale evaluation of maize hybrids selected for increased yield in drought-stress conditions of the US corn belt. *Crop Science*, 55(4), 1608. <http://doi.org/10.2135/cropsci2014.09.0654>
- Gamon, J., Peñuelas, J., & Field, C. (1992). A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sensing of Environment*, 41, 35–44.
- Gechev, T. S., Dinakar, C., Benina, M., Toneva, V., & Bartels, D. (2012). Molecular mechanisms of desiccation tolerance in resurrection plants. *Cellular and Molecular Life Sciences*, 69(19), 3175–3186. <http://doi.org/10.1007/s00018-012-1088-0>
- Gitelson, A., Gritz, Y., & Merzlyak, M. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, 160, 271–282.
- Gutierrez, M., Reynolds, M. P., Raun, W. R., Stone, M. L., & Klatt, A. R. (2010). Spectral water indices for assessing yield in elite bread wheat genotypes under well-irrigated, water-stressed, and high-temperature conditions. *Crop Science*, 50(1), 197–214.
- Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., & Cooper, M. (2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. corn belt? *Crop Science*, 49(1), 299. <http://doi.org/10.2135/cropsci2008.03.0152>
- Hao, B., Xue, Q., Marek, T. H., Jessup, K. E., Hou, X., Xu, W., Bynum, E.D., & Bean, B. W. (2015). Soil water extraction, water use, and grain yield by drought-tolerant

- maize on the Texas High Plains. *Agricultural Water Management*, 155, 11–21. <http://doi.org/10.1016/j.agwat.2015.03.007>
- Harrison, M. T., Tardieu, F., Dong, Z., Messina, C. D., & Hammer, G. L. (2014). Characterizing drought stress and trait influence on maize yield under current and future conditions. *Global Change Biology*, 20(3), 867–878. doi:10.1111/gcb.12381
- Hazratkulova, S., Sharma, R. C., Alikulov, S., Islomov, S., Yuldashev, T., Ziyaev, Z., Khalikulov, Z., Ziyadullaev, Z., & Turok, J. (2012). Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. *Plant Breeding*, 131(6), 716–721. doi:10.1111/pbr.12003
- Houle, D., Govindaraju, D., & Omholt, S. (2010). Phenomics: the next challenge. *Nature Reviews Genetics*, 11, 855–866.
- Klopfenstein, T. J., Erickson, G. E., & Berger, L. L. (2013). Maize is a critically important source of food, feed, energy and forage in the USA. *Field Crops Research*, 153, 5–11. doi:10.1016/j.fcr.2012.11.006
- Lopes, M. S., Araus, J. L., van Heerden, P. D. R., & Foyer, C. H. (2011). Enhancing drought tolerance in C4 crops. *Journal of Experimental Botany*, 62(9), 3135–3153. doi:10.1093/jxb/err105
- Main, R., Cho, M. A., Mathieu, R., O’Kennedy, M. M., Ramoelo, A., & Koch, S. (2011). An investigation into robust spectral indices for leaf chlorophyll estimation. *ISPRS Journal of Photogrammetry and Remote Sensing*, 66(6), 751–761. doi:10.1016/j.isprsjprs.2011.08.001
- Mohammady-D., S., & Hasannejad, R. (2006). Effect of water-stress on some water related traits and their relationships with height and dry matter in maize early maturing inbred lines. *Pakistan Journal of Biological Sciences*, 9(15), 2852–2857.
- Montes, J. M., Melchinger, A. E., & Reif, J. C. (2007). Novel throughput phenotyping platforms in plant genetic studies. *Trends in Plant Science*, 12(10), 433–436.
- Nemali, K. S., Bonin, C., Dohleman, F. G., Stephens, M., Reeves, W. R., Nelson, D. E., Castiglioni, P., Whitsel, J. E., Sammons, B., Silady, R. A., Anstrom, D., Sharp, R. E., Patharkar, O. R., Clay, D., Coffin, M., Nemeth, M. A., Leibman, M. E., Luethy, M., & Lawson, M. (2015). Physiological responses related to increased grain yield under drought in the first biotechnology-derived drought-tolerant maize: Physiological responses in MON 87460 under drought. *Plant, Cell & Environment*, 38(9), 1866–1880. <http://doi.org/10.1111/pce.12446>

- Passioura, J. B. (2012). Phenotyping for drought tolerance in grain crops: when is it useful to breeders? *Functional Plant Biology*, 39(11), 851. doi:10.1071/FP12079
- Peñuelas, J., & Filella, I. (1998). Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science*, 3(4), 151–156.
- Reymond, M., Muller, B., Leonardi, A., Charcosset, A., & Tardieu, F. (2003). Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiology*, 131(2), 664–675. doi:10.1104/pp.013839
- Sadok, W., Naudin, P., Boussuge, B., Muller, B., Welcker, C., & Tardieu, F. (2007). Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. *Plant, Cell & Environment*, 30(2), 135–146.
- Sanguineti, M. C., Duvick, D. N., Smith, S., Landi, P., & Tuberosa, R. (2006). Effects of long-term selection on seedling traits and ABA accumulation in commercial maize hybrids. *Maydica*, 51(2), 329.
- Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, 3(3), 307–327. doi:10.1007/s12571-011-0140-5
- Sinclair, T. R., Bennett, J. M., & Muchow, R. C. (1990). Relative sensitivity of grain yield and biomass accumulation to drought in field-grown maize. *Crop Science*, 30(3), 690–693.
- Smith, S., Cooper, M., Gogerty, J., Löffler, C., Borchering, D., Wright, K., Smith, S., Diers, B., Specht, J., & Carver, B. (2014). Maize. In *CSSA Special Publications*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Retrieved from <https://dl.sciencesocieties.org/publications/books/abstracts/cssaspecialpubl/yieldgainsinmaj/125>
- Tardieu, F., Parent, B., Caldeira, C. F., & Welcker, C. (2014). Genetic and physiological controls of growth under water deficit. *Plant Physiology*, 164(4), 1628–1635. doi:10.1104/pp.113.233353
- Tardieu, F. (2012). Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *Journal of Experimental Botany*, 63(1), 25–31. doi:10.1093/jxb/err269

- Teal, R. K., Tubana, B., Girma, K., Freeman, K. W., Arnall, D. B., Walsh, O., & Raun, W. R. (2006). In-season prediction of corn grain yield potential using normalized difference vegetation index. *Agronomy Journal*, 98(6), 1488–1494.
- Thenkabail, P. S., Gumma, M. K., Teluguntla, P., & Mohammed, I. A. (2014). Hyperspectral remote sensing of vegetation and agricultural crops. *Photogrammetric Engineering & Remote Sensing*, 80(8), 697–709.
- Tollenaar, M., & Wu, J. (1999). Yield improvement in temperate maize is attributable to greater stress tolerance. *Crop Science*, 39(6), 1597–1604.
- Tollenaar, M., & Lee, E. A. (2006). Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. *Maydica*, 51(2), 399.
- Trachsel, S. (2015, February 6). Selection for NDVI and canopy temperature can increase GY under high temperatures and combined heat and drought stress.
- Tuberosa, R. (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology*, 3. <http://doi.org/10.3389/fphys.2012.00347>
- University of Nebraska-Lincoln. (n.d.). Monthly Climate Normals. Retrieved from <http://snr.unl.edu/lincolnweather/data/monthly-normals.asp>
- Valentinuz, O. R., & Tollenaar, M. (2004). Vertical profile of leaf senescence during the grain-filling period in older and newer maize hybrids. *Crop Science*, 44(3), 827–834.
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011). High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage. *European Journal of Agronomy*, 35(1), 22–32. doi:10.1016/j.eja.2011.03.004
- Wu, F., & Guclu, H. (2013). Global maize trade and food security: Implications from a social network model: Global maize trade and food security. *Risk Analysis*, 33(12), 2168–2178. doi:10.1111/risa.12064
- Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y., & Zhang, Q. (2005). Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics*, 172(2), 1213–1228. <http://doi.org/10.1534/genetics.105.045062>

Chapter 2

Correlations between Vegetation Indices and Leaf Traits

INTRODUCTION

Chlorophyll pigments are essential for converting radiation energy from the sun into stored chemical energy (Gitelson et al., 2003). The amount of radiation that can be used by the plant is directly proportional to the chlorophyll content. As a result, chlorophyll content has shown strong correlations to photosynthetic potential and primary production (Gitelson et al., 2003 and Peng et al., 2011). As primary production increases, or the rate at which the crop can capture and store chemical energy, the yield potential increases – the crop simply has to partition the photosynthates to the grain. In addition, chlorophyll provides an estimation of nitrogen status as most of the leaf nitrogen is located in these pigments (Filella et al., 1995). Leaf chlorophyll is also related to plant stress and senescence (Gitelson et al., 2003). Because chlorophyll is the source of energy for the plant, it is essential for monitoring plant health and productivity.

In conjunction with chlorophyll content, leaf water content is of extreme importance. Changes in water content, affecting total water potential, osmotic potential, and turgor pressure, in turn affect physiological processes (Zygielbaum et al., 2012). For example, turgor pressure is not only necessary to maintain cell structure integrity, but also to open stomata (Zygielbaum et al., 2009). Only when stomata are open can carbon dioxide be incorporated into the plant. This carbon dioxide is used in the Calvin cycle to create products for sucrose and starch synthesis (Zygielbaum et al., 2012). Therefore, no matter the amount of chlorophyll present, without sufficient turgor pressure the plant will not be able to utilize the light energy to create carbohydrates.

Because chlorophyll content and water content can be used to monitor plant health and productivity, many techniques have been developed to measure these traits. Apart from using destructive leaf samples in laboratory assays, spectrometers (mounted on platforms ranging from backpacks, tractors, and airplanes to satellites) have been used to estimate these traits based on reflectance spectra. However, because of the popularity of this field, an abundance of vegetation indices exist. The Index DataBase (Henrich et al., n.d.) is an online resource that records all vegetation indices used to date. Currently, there are 112 unique indices that supposedly detect chlorophyll concentration. While all indices have been proven in one way or another, the sheer number of possible calculations generates confusion.

The goal of this project is to identify those indices which correlate best to chlorophyll content and relative water content in maize leaves. Additionally, the indices will be analyzed to determine if they are more or less robust in detecting differences among hybrids grown in optimal conditions. Indices with these characteristics will become valuable assets to the breeding community.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the University of Nebraska-Lincoln East Campus (40.8° N and 96.7° W) in Lincoln, Nebraska. Trials were performed during the summers of 2014 and 2015. Thirty-six popular commercial era hybrids (released from 1936 to 2012), two irrigation treatments, and six replications were arranged as a randomized complete block design. Each of the 216 plots consisted of 2 rows with 0.76

m spacing between rows and a plot length of 6.1 m. Stands were thinned to an average density of 60,000 plants per hectare.

The maize hybrids were attained from two different sources and were assigned to eras as follows: Era 1 = 1936-1958, Era 2 = 1963-1969, Era 3 = 1970-1975, Era 4 = 1982-1988, Era 5 = 1991-1999, Era 6 = 2008-2012 (Table 2.1). The hybrids were acquired from DuPont Pioneer or inbred lines were retrieved from the North Central Regional Plant Introduction Station (NCRPIS) and hybrids were made by hand pollination at the University of Nebraska-Lincoln. All hybrids exhibited similar maturities and developmental stages were synchronous in the field.

The two water treatments, irrigated (well-watered, WW) and rain fed (water stressed, WS), were placed in blocks side-by-side in the field. The WW treatment received drip tape irrigation on an as needed basis. Sixteen mm diameter and 15 mm wall thickness drip tape offered a .32 gallon per hour flow rate. Plots were planted on May 15 and May 22 in 2014 and 2015, respectively. Pre- and post-emergent herbicides along with manual weeding minimized the effects of stress from weeds.

Hyperspectral Reflectance

Leaf level reflectance data was collected with a spectrometer (USB2000+ VIS-NIR, Ocean Optics, Dunedin, FL) on a near weekly basis beginning at V8. Measurements were always taken in the early afternoon (between 1200 and 1500 h CDT); approximately three hours were required to sample all plots. The active sensor system was mounted on a backpack. The spectrometer was connected to a halogen lamp light source and a “leaf clip” with a dual branch flexible fiber optic. The leaf clip covered the leaf to reduce environmental factors and had a field of view of 0.4 cm in diameter.

Two marked plants were measured in each plot. Prior to ear formation, scans were taken on the newest, fully expanded leaf. After ear formation, scans were taken on the ear leaf. The spectrometer analyzed reflected radiation at 2022 unique spectral bands with a detection range from 349 to 1028 nm and a bandwidth of approximately 1.5 nm. A number of spectral indices were calculated for use in analysis (Supplementary Table 1).

Chlorophyll Content

In tandem with reflectance measurements (on the same leaf), 0.9 cm diameter leaf discs were acquired to measure chlorophyll concentrations in the lab using the method of Warren (2008). Leaf discs were kept in a -80° Celsius freezer until they were lyophilized. A 5 mm ball bearing was added to the tissue; the samples were ground to a fine powder by shaking in a vibratory ball mill (TissueLyser II, Qiagen, Inc., Valencia, CA). Methanol was added; after being mixed and centrifuged the supernatant was used for analysis. A BioTek Synergy 2 microplate reader (BioTek Instruments, Inc., Winooski, VT) measured the absorbance of the chlorophyll extract dissolved in a methanol solution. To determine the pathlength of the microplate reader, a number of the solutions were also measured in a DU 730 spectrophotometer (Beckman Coulter, Inc., Indianapolis, IN). Total chlorophyll content is used for all analysis (Chl *a* + *b*, µg/mL).

Microplate samples were corrected to a 1-cm pathlength absorbance:

$$A_{652, 1 \text{ cm}} = (A_{652, \text{microplate}} - \text{blank}) / \text{pathlength}$$

$$A_{665, 1 \text{ cm}} = (A_{665, \text{microplate}} - \text{blank}) / \text{pathlength}$$

Chlorophyll concentrations were calculated from these corrected absorbance values:

$$\text{Chl } a \text{ (}\mu\text{g/mL)} = -8.0962 A_{652, 1 \text{ cm}} + 16.5169 A_{665, 1 \text{ cm}}$$

$$\text{Chl } b \text{ (}\mu\text{g/mL)} = 27.4405 A_{652, 1 \text{ cm}} - 12.1688 A_{665, 1 \text{ cm}}$$

Relative Water Content

Scissors were used to cut portions of the same leaves monitored by the spectrometer approximately 2x8 cm in size for relative water content determination. Two samples per plot were cut and immediately placed in labeled and sealed Ziploc bags in a cooler. As soon as possible, the samples were taken to the lab and weighed to the nearest milligram on an analytical balance (Denver Instrument, Bohemia, NY). De-ionized water was added to each bag to hydrate the leaves. The leaves were left overnight at room temperature because there were too many samples for the fridge; they were removed from the bags and weighed the next day to achieve the turgid weight (after surface moisture was removed). Finally, the samples were placed in the dryer for 24 hours at 65 degrees Celsius – after which the dry weight was measured. In all, approximately 20 man hours were required to complete the process for all plots. The following calculation was used to determine relative water content:

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] * 100, \text{ where}$$

FW = fresh weight

DW = dry weight

TW = turgid weight

Statistical Analysis

Analysis of variance (ANOVA) and linear regression models were used to find differences between all hybrids for various traits and to find correlations between traits. PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC) was used for the ANOVA analysis. Traits were treated as the response variable, hybrid as a fixed effect, and replication as a random effect. Coefficients of variation were determined by dividing the

square root of the model mean square error by the overall mean. Repeatability was calculated by dividing the hybrid variation by the sum of hybrid variation and total error variation divided by the number of replications.

Since leaf reflectance and leaf samples were collected on the same leaf, correlations were made on a plot by plot basis. Both sampling dates were combined within a year, but the years were analyzed separately. The `cor()` function was utilized in R (The R Foundation) to correlate leaf chlorophyll and relative water content to all calculated indices (Indices listed in Supplementary Table 1). Pearson correlation coefficients were squared to present coefficients of determination.

RESULTS

Environmental Conditions

Rainfall distributions were unique in the 2014 and 2015 growing seasons. Although total rainfall between May and September were similar for both years (66.3 cm in 2014 and 75.3 cm in 2015), the timing of the rainfall events created a water stressed environment in 2014, but not 2015 (Figure 2.1). In 2014, the month of July only received 1.3 cm of rainfall. On the other hand, 2015 received above average rainfall in the spring, followed by average accumulations throughout the growing season (Table 2.2). As a result, the WS treatment in 2014 was discarded from all analysis except the correlations, while both 2015 treatments were combined for analysis as well-watered replications. The WS treatment in 2014 was located near the field edge and became highly variable at the onset of water stress. Repeatability of measurements was extremely low – the same hybrid produced varied results in each replication. Because of this, ANOVA models could only find differences among all hybrids for a few traits. The increased spatial

variability of the field and the increased variability in traits that could not be explained by hybrids made analysis of the WS treatment unprofitable.

Differentiating Hybrids Based on Leaf Traits

An ANOVA analysis was conducted separately for each sampling date of chlorophyll content and relative water content (RWC) because of significant hybrid x sampling date interaction effects. For every destructive leaf sampling date, chlorophyll content could significantly detect differences among all era hybrids (Table 2.3). On the other hand, relative water content could not find differences among all hybrids in 2014, but it could in 2015. Repeatability values averaged 0.68 for all chlorophyll measurements, but only 0.27 for relative water content. In 2014, the hybrids were unable to account for the extra variation in relative water content. Figure 2.2 displays the variability in the leaf traits.

Correlating Indices and Leaf Traits

Correlations were made between a large number of vegetation indices and RWC and chlorophyll content to determine which spectral indices predict these traits best (Supplementary Table 1 lists all indices calculated). All data (including all treatments) on a plot by plot basis were used for the correlations; sampling dates were combined in each respective year for the analysis to see if the index could successfully track changes in trait values across the growing season. Linear models are desired when predicting traits as quadratic models result in saturated indices. Therefore, only linear models were used (quadratic models did not show significant improvements). Table 2.4 displays the best correlating index to each trait in each year and other indices within one standard error of the top index.

With water stress apparent in 2014, the best index relating to RWC was Carter6 ($R^2 = 0.481$). However, there is obviously not one superior index as 41 unique indices are within one standard error of each other. In 2015, the lack of variability in RWC led to poor correlations (Figure 2.2).

As for chlorophyll content, the 760/730 index predicts chlorophyll concentrations best in both years (Average $R^2 = 0.643$). The CI_{RE} , CWMI, and MTCI indices also appear in the top tier for both years. Interestingly, the 760/730 and CI_{RE} indices also appear in the large group of indices correlating with RWC in 2014. Figures 2.3 and 2.4 show the 760/730 index's relationship with RWC in 2014 and chlorophyll content in 2015, respectively.

Using Vegetation Indices to Differentiate Hybrids

As the chlorophyll and water content were able to differentiate hybrids (based on the ANOVA results in Table 2.2), the 760/730 and CI_{RE} indices were also capable of finding differences among all hybrids on those same dates (Table 2.5). The only non-significant results on August 14, 2014 were a result of missing values because the spectrometer over-heated while collecting data.

Although chlorophyll and water content laboratory assays were only conducted on these sampling dates to correlate with vegetation indices, the spectrometer was used throughout the growing season. In 2014, the spectrometer was used on a near-weekly basis from V10 to R6 for a total of ten sampling dates. In 2015, the spectrometer was used weekly from V8 to R6 for a total of 13 sampling dates.

In both years, the spectrometer was capable of finding significant differences among all hybrids at every sampling date except two. One, August 14, 2014 (R5 stage)

was an outlier because of missing values; two, June 30, 2014, was very near to the .05 significance level (760/730 Index P-value = .0596).

DISCUSSION

Correlations

Previous studies have also correlated reflectance indices to leaf chlorophyll and water content. Specifically, the 760/730 and CI_{RE} indices have been correlated to these traits. Misteale and Schmidhalter (2010) first used the 760/730 index to monitor above-ground biomass and nitrogen uptake in wheat. They found strong correlations between the index and shoot dry biomass ($R^2 = 0.86$) and nitrogen uptake ($R^2 = 0.92$). Subsequently, Winterhalter et al (2011a, 2011b) used the 760/730 index to monitor traits in tropical maize. Again, the index correlated strongly to nitrogen uptake ($R^2 = 0.74$) (Winterhalter et al., 2011a). In addition, the index was shown to relate strongly to canopy water mass and canopy temperature, with R-squared values of 0.72 and 0.68, respectively (Winterhalter et al., 2011b).

The CI_{RE} index was developed by Gitelson et al (2003) to provide a robust indicator of plant chlorophyll content. The CI_{RE} index displayed an R-squared value of 0.96 when compared to the total chlorophyll content of beech, chestnut, maple, and wild vine leaves (Gitelson et al., 2003). When compared to soybean and maize leaves, the index maintained a strong relationship ($R^2 = 0.95$) (Gitelson et al., 2005).

Unsurprisingly, the 760/730 index and CI_{RE} index have similar equations; both utilize the red edge region of the electromagnetic spectrum ($760/730 = (R_{760}/R_{730})$ and $CI_{RE} = (R_{750-800}/R_{710-730}) - 1$). The red edge has been used for a variety of applications: nitrogen status (Li et al., 2014), insect defoliation levels (Adelabu et al., 2014), response

to phenanthrene stress (Zhu et al., 2014), general stress detection (Das et al., 2014 and Eitel et al., 2011), and aboveground biomass (Ren et al., 2011).

The red edge region is used widely because it monitors the overall health status of plants. As noted in the introduction, chlorophyll content is an important trait underlying plant health and productivity. As a result, even though indices may be looking directly at chlorophyll content, the applications vary. This research suggests that the 760/730 index is a robust predictor of leaf chlorophyll content, but secondarily, the index can monitor water content – also supported by Winterhalter et al (2011b). In addition, this research shows that chlorophyll content and water content respond together – stressed or less productive plants will have less water and chlorophyll in their leaves. Sanchez et al (1983) found that water stress reduced chlorophyll content, stomatal conductance, and photosynthetic rates in maize. Romano et al (2011) also found that canopy temperatures correlate well with NDVI and SPAD meter readings. In this study, not only does the same index correlate well with both RWC and chlorophyll content, but RWC and chlorophyll content correlate well with each other ($R^2 = 0.34$). Although RWC can be said to act similarly to chlorophyll content, the 760/730 index does not provide an exact estimate of RWC *per se*. Instead, spectrometers that can take advantage of the middle infrared region of the electromagnetic spectrum are more accurate at predicting RWC (Gao, 1996).

Differentiating Hybrids

The 760/730 index is capable of monitoring maize chlorophyll content; however, with the goal of plant improvement, cultivars must be able to be distinguished for the results to be valuable. Currently, the primary usage of vegetation indices is to monitor the

health status of uniform cultivars across fields with different fertility or irrigation treatments (Clay et al., 2006, Li et al., 2014, and Zaman-Allah et al., 2015). Rodrigues et al (2015) epitomize this trend by utilizing sensing technology to assess low and high yield areas in a wheat field.

While these methodologies are well-studied, the power of sensor technology to differentiate many unique cultivars under optimal growing conditions is less well-known. Adebayo et al (2014) at CIMMYT used NDVI to correlate with grain yield and to find differences among all of their 96 test-cross hybrids. Our study shows that laboratory measurements of chlorophyll content and spectrometer measurements are capable of distinguishing hybrids at every sampling date (excluding August 14, 2014 for the sensor). In fact, the spectrometer is as accurate, if not slightly more accurate, at detecting these differences (Table 2.6). Across all dates, the 760/730 index maintained a smaller coefficient of variation – the spectrometer provides more precise measurements. In 2014, the chlorophyll laboratory assay had a greater repeatability, R-squared value, and number of LSD groups. Repeatability describes how similar measurements are for the same hybrid taken at different times, the R-squared tells of the amount of variation in the model explained by the different hybrids, and the number of LSD groups is the unique number of significantly different hybrid groups that could be distinguished based on Tukey's LSD. In 2015, the 760/730 index was superior in all categories at both sampling dates. This is promising because in 2015 there were twice as many replications in optimal conditions. Therefore, the spectrometer can be used to distinguish hybrids in a large study (216 plots) quicker, more efficiently, and more accurately than destructive laboratory assays.

The RWC measurements were able to distinguish hybrids in 2015, but not 2014. This is surprising because much more variability existed in 2014 (Figure 2.2). However, the low repeatability values in 2014 (Table 2.3) suggest that samples for the same hybrids were dissimilar across replications. Because we only used the values from the WW treatment, this is most likely due to poor laboratory measurements. After being cut, leaf samples were exposed to the ambient weather conditions for a longer period of time in 2014. This could explain the poor results obtained for RWC that year.

CONCLUSIONS

Chlorophyll content is a primary trait of interest because it is the underlying driver of plant health and productivity. We found that the 760/730 index correlates well with total leaf chlorophyll content for both growing seasons in WW treatments (Average $R^2 = 0.643$). Indirectly, the index also correlates well with relative water content ($R^2 = 0.431$). It is obvious that these traits respond similarly; therefore, the 760/730 index is a robust indicator of plant health and productivity.

In addition, the spectrometer offers precise, repeatable measurements that can be used to distinguish cultivars. The spectrometer can be easily implemented to quickly measure fields throughout the growing season. In this study, with 36 era hybrids, as many as 9 significantly different groups of hybrids could be distinguished with the 760/730 index. Multiple reviews suggest the implementation of this technology for plant improvement (Araus & Cairns, 2014, Houle et al., 2010, and White et al., 2012). This experiment reveals that spectral reflectance technology can in fact be used in breeding programs to differentiate cultivars grown in well-watered environments.

Figures

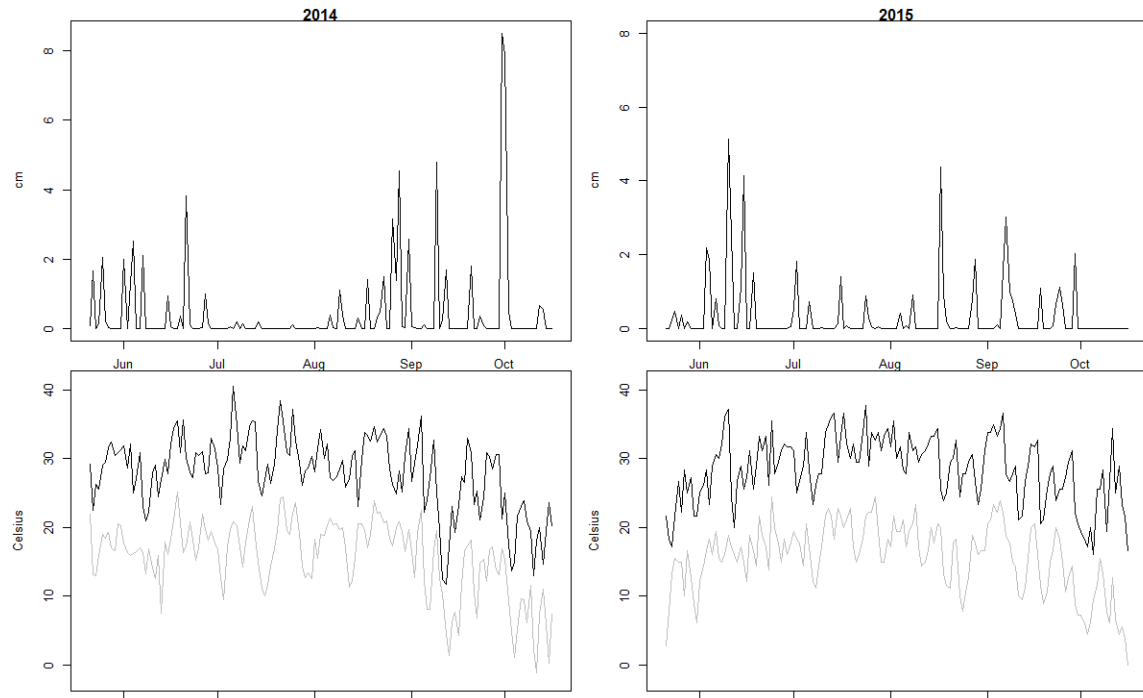


Figure 2.1. Rainfall and temperature patterns for the 2014 and 2015 growing seasons. Top panels display rainfall accumulation per day in centimeters. Daily high (black line) and low (gray line) temperatures are displayed in the bottom panels.

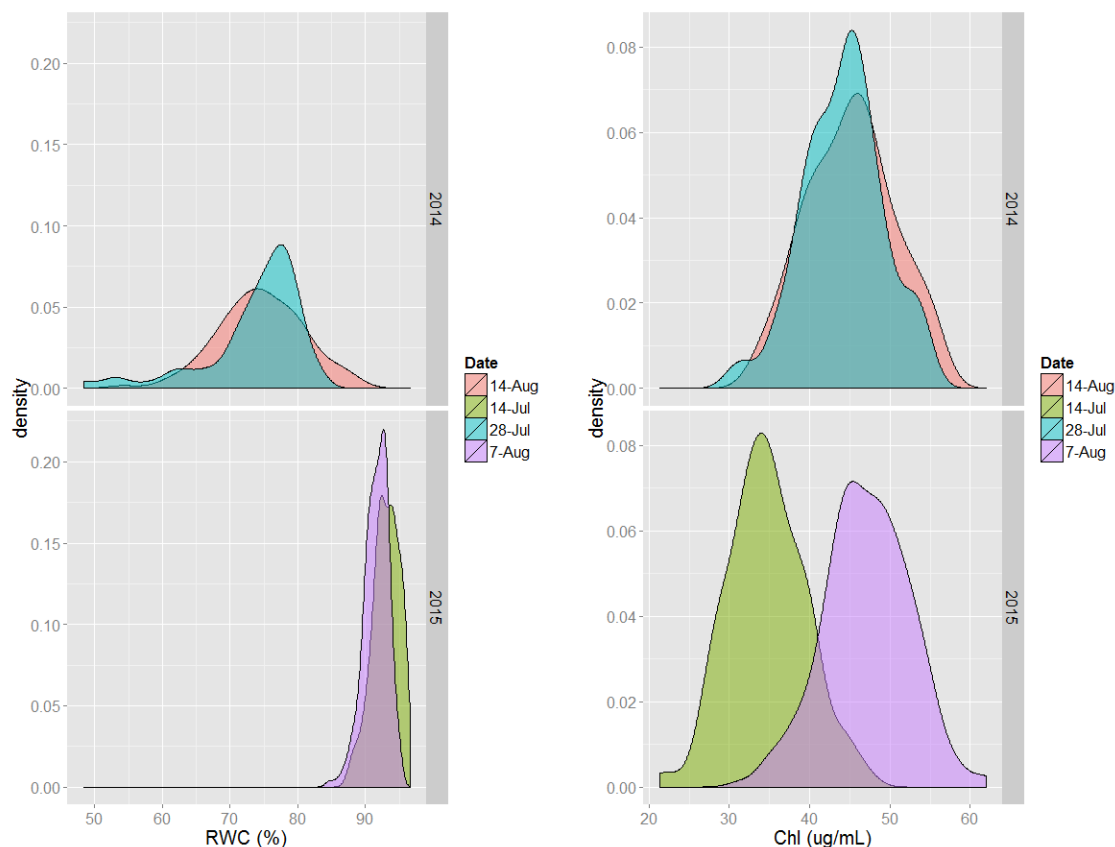


Figure 2.2. Variation in RWC and chlorophyll content for all sampling dates in both years. In 2014, samples were collected on July 28 and August 14 at the R3 and R5 developmental stages, respectively (only WW treatment). Samples were collected on July 14 (V16) and August 7 (R3) in 2015 (all replications). Chlorophyll content was significantly greater in the early reproductive stage compared to the vegetative stage in 2015. Excess variability in RWC in 2014 was attributable to increased plant stress and imperfect laboratory measurements.

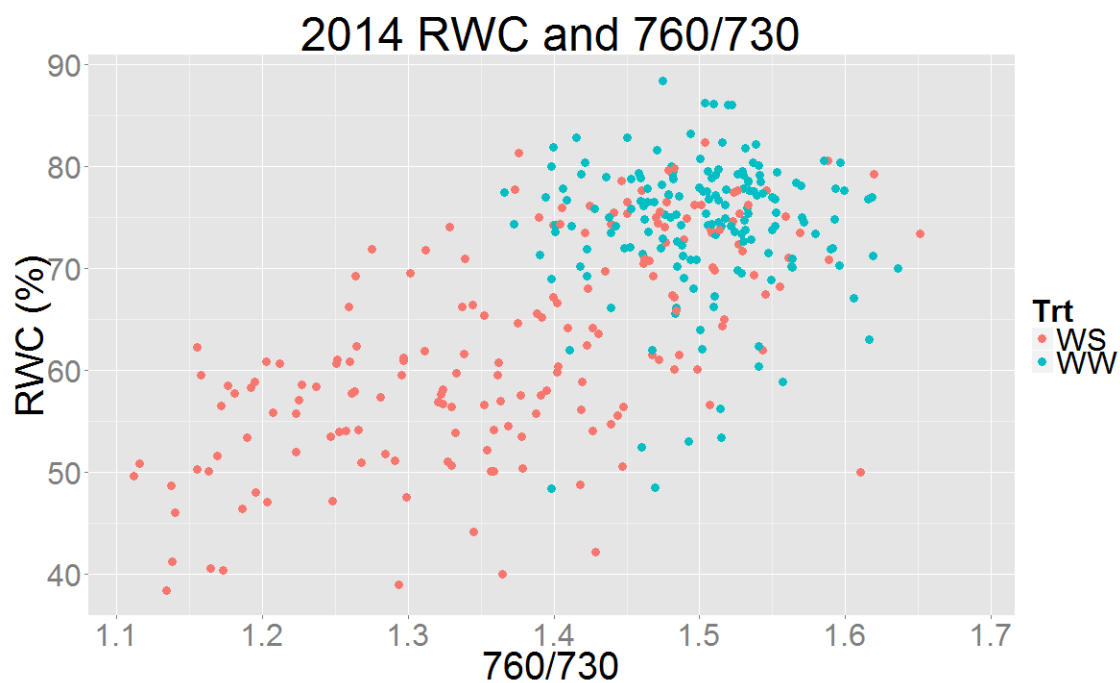


Figure 2.3. Relationship between relative water content and 760/730 index values in 2014 ($R^2 = 0.43$). The WS treatment was included to capture extra variation in RWC.

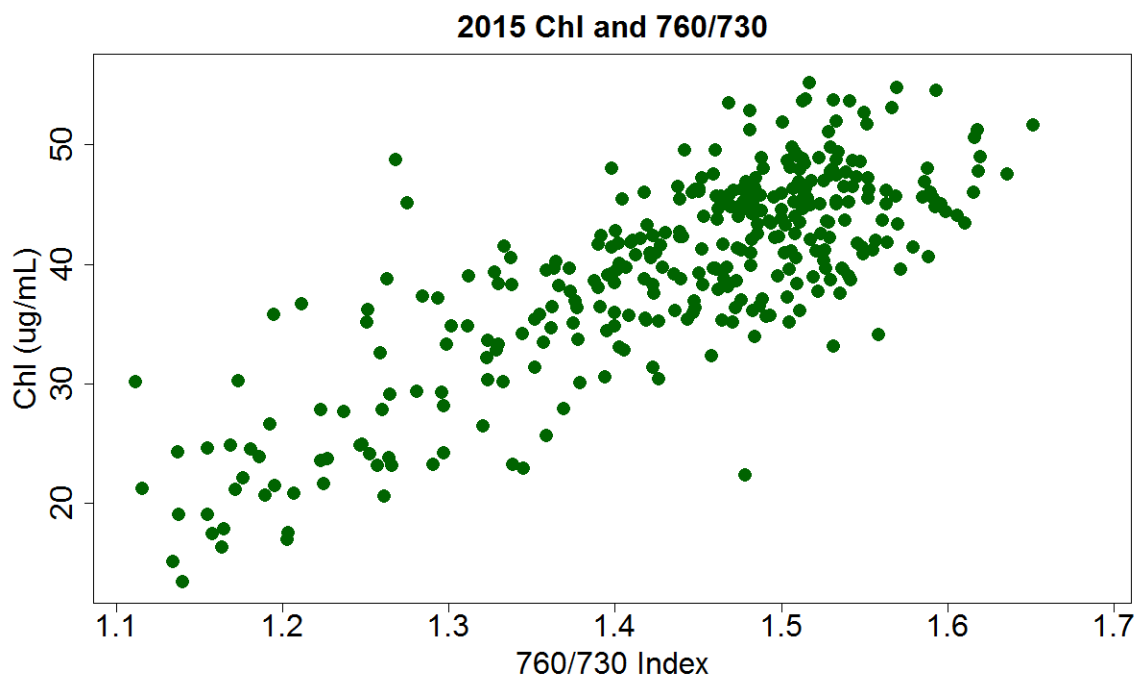


Figure 2.4. Relationship between chlorophyll content and 760/730 index values in 2015 ($R^2 = 0.65$). Correlations were made on a plot by plot basis for both sampling dates.

Tables

Table 2.1. Hybrids used in this study. Hybrids were grouped into six distinct eras based on decade of year of release (YOR). The first era spans multiple decades due to the limited number of genotypes from 1936 to 1958. Era hybrids acquired from Pioneer or current hybrids obtained from other companies are listed by their commercial hybrid number. Hybrids created by hand pollination at the University of Nebraska-Lincoln (UNL) show the female and male parentage.

Source	Pedigree	YOR	Era
Pioneer	307HYB	1936	1
UNL	WF9/38-11//Hy/L317	1948	1
UNL	NS 0	1948	1
UNL	W9/Hy/L289/I205	1950	1
Pioneer	329HYB	1954	1
UNL	W64A/OH43	1954	1
UNL	B37/B14//C103/Oh43	1958	1
UNL	B14A/B57	1963	2
UNL	N501D	1964	2
UNL	B37/OH43	1965	2
UNL	B37/B14//Mo17	1965	2
Pioneer	3390	1967	2
Pioneer	3334	1969	2
UNL	N7A/Mo17	1970	3
Pioneer	3366	1972	3
UNL	NS[RFS_NB]3_8	1972	3
UNL	B73/Mo17	1974	3
Pioneer	3541	1975	3
UNL	B73/LH39	1982	4
UNL	B73/LH51	1983	4
UNL	LH132/LH51	1985	4
UNL	LH156/MBS2333	1988	4
UNL	LH132/LH59	1988	4
Pioneer	3379	1988	4
UNL	LH192 /LH82	1991	5
Pioneer	3394	1991	5
Pioneer	33A14	1997	5
Pioneer	33P67	1999	5
Mycogen	2A555	2007	6
Pioneer	33D49	2008	6
Golden Harvest	H-7949	2010	6
Hoegemeyer	7630RR	2011	6
Pioneer	P0876HR	2012	6
Pioneer	PO987HR	2012	6
Hoegemeyer	7644 Hx/LL/RR	2012	6
NK	N45P-4011	2012	6

Table 2.2. Mean temperatures and total rainfall per month during the 2014 and 2015 growing seasons in Lincoln, NE as compared to local climate normals.

Year	May	June	July	August	September
Temperature (Celsius)					
2014	18.4	23.3	23.9	24.5	18.7
2015	16.4	23.0	25.1	23.2	21.9
Normal	16.8	22.5	25.3	24.1	18.9
Precipitation (Centimeters)					
2014	13.4	15.0	1.3	19.2	17.5
2015	27.7	19.5	6.1	9.6	12.5
Normal	10.9	11.0	8.6	8.8	7.7

Table 2.3. ANOVA results for relative water content (RWC) and chlorophyll content (Chl) laboratory measurements. All replications were utilized in 2015; only the WW treatment was used in 2014.

	2014	(7-28) R3		(8-14) R5		2015	(7-14) V16		(8-7) R3	
Source	df	Chl	RWC	Chl	RWC	df	Chl	RWC	Chl	RWC
		MS	MS	MS	MS		MS	MS	MS	MS
		µg/mL	%	µg/mL	%		µg/mL	%	µg/mL	%
Hybrid	35	51.09***	30.89	47.49***	40.87	35	46.76***	6.31**	83.55***	6.1***
Rep	2	6.35	1273***	224.04***	388.24***	5	94.01***	18.79***	63.05**	19.13***
Error	70	12.46	28.34	15.22	28.78	175	16.07	3.05	15.53	2.25
Mean		43.31	62.87	47.06	73.11		35.83	93.28	46.62	91.33
CV		0.082	0.076	0.086	0.077		0.109	0.019	0.086	0.016
Repeatability		0.759	0.018	0.557	0.065		0.608	0.453	0.803	0.554

MS = Mean Square, CV = Coefficient of Variation

‘***’ = significant at the <0.001 level; ‘**’ = <0.01; ‘*’ = <0.05; ‘.’ = <0.10

Table 2.4. Coefficients of determination for the relationship between chlorophyll content (Chl) and relative water content (RWC) with various vegetation indices. All indices within one standard error of the best index are listed. Correlations were made on a plot by plot basis combining both sampling dates in each year.

2014				2015			
Chl		RWC		Chl		RWC	
Index	R ²	Index	R ²	Index	R ²	Index	R ²
760/730	0.639	Carter6	0.481	760/730	0.647	ZTM4	0.141
HREI15	0.636	RARSb	0.472	CWMI1	0.629	PRI4	0.126
Datt	0.636	Green.NDVI	0.470	Gitelson2	0.628	HBCI8	0.122
Maccioni	0.633	HBCI12	0.470	NIR.NIR	0.618	GLI	0.121
ND	0.632	HREI16	0.469	CI _{RE}	0.617	ZTM5	0.119
CI _{RE}	0.626	LABR	0.468	MTCI	0.606	HBCI9	0.117
NDRE	0.626	Gitelson	0.463	Git2	0.603		
Carter4	0.623	R701	0.463	Git3	0.603		
TCARI	0.618	Carter4	0.462				
HREI16	0.616	NDVI2n	0.461				
TCARI.OSAVI	0.612	OSAVI2	0.461				
HBCI12	0.611	Carter2	0.460				
Green.NDVI	0.610	Carter3	0.460				
CWMI1	0.607	TCARI	0.460				
Carter6	0.605	ND	0.459				
CWMI2	0.604	PSNDb	0.458				
NDVI2n	0.600	TCARI.OSAVI	0.457				
OSAVI2	0.600	TCARI2.OSAVI2	0.456				
MTCI	0.598	CI2	0.446				
Git3	0.596	SR3	0.441				
Git2	0.596	NIR.green	0.439				
Vogelmann2	0.596	CG	0.439				
LABR	0.595	Maccioni	0.437				
R701	0.594	HBSI2	0.437				
Vogelmann	0.593	RNIR.CRI550	0.436				
		Datt	0.436				
		HBSI1	0.434				
		HREI15	0.434				
		760/730	0.431				
		NDVIc	0.431				
		CI _{RE}	0.431				
		NDRE	0.430				
		Git5	0.430				
		Git6	0.430				
		mSR2	0.430				
		NDVI	0.430				
		OSAVI	0.429				
		NDVIw	0.429				
		PSNDb	0.429				
		Datt2	0.429				
		Git4	0.428				

Table 2.5. ANOVA results for the 760/730 and CI_{RE} vegetation indices. All replications were utilized in 2015; only the WW treatment was used in 2014. The August 14, 2014 measurement date shows poor results because of missing data.

	2014	(7-28) R3		(8-14) R5		2015	(7-14) V16		(8-7) R3	
Source	df	760/730	CI _{RE}	760/730	CI _{RE}	df	760/730	CI _{RE}	760/730	CI _{RE}
		MS	MS	MS	MS		MS	MS	MS	MS
Hybrid	35	0.0049***	0.0256**	0.0042	0.0249	35	0.0048***	0.0257***	0.0088***	0.053***
Rep	2	0.0004	0.0029	0.0056	0.0534	5	0.0008	0.0034	0.0047**	0.036**
Error	70	0.002	0.011	0.003	0.019	175	0.0013	0.007	0.0014	0.009
Mean		1.51	1.11	1.38	0.786		1.38	0.835	1.42	0.911
CV		0.028	0.095	0.039	0.167		0.026	0.099	0.026	0.098
Repeatability		0.607	0.58	0.505	0.444		0.732	0.732	0.832	0.816

MS = Mean Square, CV = Coefficient of Variation

‘***’ = significant at the <0.001 level; ‘**’ = <0.01; ‘*’ = <0.05; ‘.’ = <0.10

Table 2.6. Comparing chlorophyll content (Chl) laboratory measurements and 760/730 vegetation index ANOVA results among all hybrids. Coefficients of variation (CV), repeatability, and coefficients of determination (R^2) were used to display the phenotypes' power to detect differences. Tukey's LSD was used to organize hybrids into statistically different groups. The August 14, 2014 measurement was excluded because of missing data.

2014	(7-28) R3		2015	(7-14) V16		(8-7) R3	Average	
	Chl	760/730		Chl	760/730		Chl	760/730
CV	0.082	0.028		0.109	0.026	0.086	0.026	0.092
Repeatability	0.759	0.607		0.608	0.732	0.803	0.832	0.723
LSD Groups	5	3		3	4	8	9	5.333
R^2	0.67	0.55		0.33	0.42	0.5	0.54	0.500

Literature Cited

- Adebayo, M. A., Menkir, A., Blay, E., Gracen, V., Danquah, E., & Hearne, S. (2014). Genetic analysis of drought tolerance in adapted \times exotic crosses of maize inbred lines under managed stress conditions. *Euphytica*, 196(2), 261–270. <http://doi.org/10.1007/s10681-013-1029-5>
- Adelabu, S., Mutanga, O., & Adam, E. (2014). Evaluating the impact of red-edge band from Rapideye image for classifying insect defoliation levels. *ISPRS Journal of Photogrammetry and Remote Sensing*, 95, 34–41. <http://doi.org/10.1016/j.isprsjprs.2014.05.013>
- Araus, J. L., & Cairns, J. E. (2014). Field high-throughput phenotyping: the new crop breeding frontier. *Trends in Plant Science*, 19(1), 52–61. <http://doi.org/10.1016/j.tplants.2013.09.008>
- Clay, D. E., Kim, K.-I., Chang, J., Clay, S. A., & Dalsted, K. (2006). Characterizing water and nitrogen stress in corn using remote sensing. *Agronomy Journal*, 98(3), 579. <http://doi.org/10.2134/agronj2005.0204>
- Das, P. K., Choudhary, K. K., Laxman, B., Kameswara Rao, S. V. C., & Seshasai, M. V. R. (2014). A modified linear extrapolation approach towards red edge position detection and stress monitoring of wheat crop using hyperspectral data. *International Journal of Remote Sensing*, 35(4), 1432–1449. <http://doi.org/10.1080/01431161.2013.877616>
- Eitel, J. U. H., Vierling, L. A., Litvak, M. E., Long, D. S., Schulthess, U., Ager, A. A., Krofcheck, D. J., & Stoscheck, L. (2011). Broadband, red-edge information from satellites improves early stress detection in a New Mexico conifer woodland. *Remote Sensing of Environment*, 115(12), 3640–3646. <http://doi.org/10.1016/j.rse.2011.09.002>
- Filella, I., Serrano, L., Serra, J., & Penuelas, J. (1995). Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop Science*, 35(5), 1400–1405.
- Gao, B. (1996). NDWI – A normalized difference water index for remote sensing of vegetation liquid water from space. *Remote Sensing of Environment*, 58(3), 257–266.
- Gitelson, A., Gritz, Y., & Merzlyak, M. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, 160, 271–282.
- Gitelson, A. A., Vina, A., Ciganda, V., Rundquist, D. C., & Arkebauer, T. J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research*

- Letters*, 32(8). Retrieved from <http://onlinelibrary.wiley.com/doi/10.1029/2005GL022688/full>
- Henrich, V., Krauss, G., Gotze, C., & Sandow, C. (n.d.). IDB - Index DataBase. Retrieved November 21, 2015, from <http://www.indexdatabase.de/>
- Houle, D., Govindaraju, D., & Omholt, S. (2010). Phenomics: the next challenge. *Nature Reviews Genetics*, 11, 855–866.
- Li, F., Miao, Y., Feng, G., Yuan, F., Yue, S., Gao, X., Liu, Y., Liu, B., Ustin, S., & Chen, X. (2014). Improving estimation of summer maize nitrogen status with red edge-based spectral vegetation indices. *Field Crops Research*, 157, 111–123. <http://doi.org/10.1016/j.fcr.2013.12.018>
- Mistele, B., & Schmidhalter, U. (2010). A comparison of spectral reflectance and laser-induced chlorophyll fluorescence measurements to detect differences in aerial dry weight and nitrogen uptake of wheat. In *10th International Conference of Precision Agriculture. Denver, Colorado*. Retrieved from <http://www.pe.wzw.tum.de/publikationen/pdf/sd695.pdf>
- Peng, Y., Gitelson, A. A., Keydan, G., Rundquist, D. C., & Moses, W. (2011). Remote estimation of gross primary production in maize and support for a new paradigm based on total crop chlorophyll content. *Remote Sensing of Environment*, 115(4), 978–989.
- Ren, H., Zhou, G., & Zhang, X. (2011). Estimation of green aboveground biomass of desert steppe in Inner Mongolia based on red-edge reflectance curve area method. *Biosystems Engineering*, 109(4), 385–395. <http://doi.org/10.1016/j.biosystemseng.2011.05.004>
- Rodrigues, F. A., Ortiz-Monasterio, I., Zarco-Tejada, P. J., Schulthess, U., & Gérard, B. (2015). High resolution remote and proximal sensing to assess low and high yield areas in a wheat field. In *Precision agriculture '15* (pp. 38–50). Wageningen Academic Publishers. Retrieved from http://www.researchgate.net/profile/Francelino_Rodrigues_Jr/publication/281641273_High_resolution_remote_and_proximal_sensing_to_assess_low_and_high_yield_areas_in_a_wheat_field/links/55f1f4e408aef559dc49314e.pdf
- Romano, G., Zia, S., Spreer, W., Sanchez, C., Cairns, J., Araus, J. L., & Müller, J. (2011). Use of thermography for high throughput phenotyping of tropical maize adaptation in water stress. *Computers and Electronics in Agriculture*, 79(1), 67–74. <http://doi.org/10.1016/j.compag.2011.08.011>
- Sanchez, R. A., Hall, A. J., Trapani, N., & Cohen de Hunau, R. (1983). Effects of water stress on the chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. *Photosynthesis Research*, 4(1), 35–47.

- Warren, C. R. (2008). Rapid measurement of chlorophylls with a microplate reader. *Journal of Plant Nutrition*, 31(7), 1321–1332. <http://doi.org/10.1080/01904160802135092>
- White, J. W., Andrade-Sanchez, P., Gore, M. A., Bronson, K. F., Coffelt, T. A., Conley, M. M., Feldmann, K. A., French, A. N., Heun, J. T., Hunsaker, D. J., Jenks, M. A., Kimball, B. A., Roth, R. L., Strand, R. J., Thorp, K. R., Wall, G. W., & Wang, G. (2012). Field-based phenomics for plant genetics research. *Field Crops Research*, 133, 101–112. <http://doi.org/10.1016/j.fcr.2012.04.003>
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011a). High-throughput sensing of aerial biomass and above-ground nitrogen uptake in the vegetative stage of well-watered and drought stressed tropical maize hybrids. *Crop Science*, 51(2), 479–489.
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011b). High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage. *European Journal of Agronomy*, 35(1), 22–32. <http://doi.org/10.1016/j.eja.2011.03.004>
- Zaman-Allah, M., Vergara, O., Araus, J. L., Tarekegne, A., Magorokosho, C., Zarco-Tejada, P. J., Hornero, A., Alba, A. H., Das, B., Craufurd, P., Olsen, M., Prasanna, B. M., & Cairns, J. (2015). Unmanned aerial platform-based multi-spectral imaging for field phenotyping of maize. *Plant Methods*, 11(35), 1–10.
- Zhu, L., Chen, Z., Wang, J., Ding, J., Yu, Y., Li, J., Xiao, N., Jiang, L., Zheng, Y., & Rimmington, G. M. (2014). Monitoring plant response to phenanthrene using the red edge of canopy hyperspectral reflectance. *Marine Pollution Bulletin*, 86(1-2), 332–341. <http://doi.org/10.1016/j.marpolbul.2014.06.046>
- Zygielbaum, A. I., Gitelson, A. A., Arkebauer, T. J., & Rundquist, D. C. (2009). Non-destructive detection of water stress and estimation of relative water content in maize. *Geophysical Research Letters*, 36(12). <http://doi.org/10.1029/2009GL038906>
- Zygielbaum, A. I., Arkebauer, T. J., Walter-Shea, E. A., & Scoby, D. L. (2012). Detection and measurement of vegetation photoprotection stress response using PAR reflectance. *Israel Journal of Plant Sciences*, 60(1), 37–47. <http://doi.org/10.1560/IJPS.60.1-2.37>

Chapter 3

Genetic Gain in Popular Midwest United States Maize Hybrids

INTRODUCTION

Retrospective analyses are necessary to understand how breeding has transformed a crop. Through its long history, maize has experienced physiological and morphological changes in conjunction with increases in grain yield. Knowing this, breeders can continue selecting for the traits that correspond to improved productivity and attempt to discern the most limiting factors to continued yield increases.

Much research has been conducted with era hybrids to determine specific traits contributing to increased productivity (Duvick et al., 2004, Duvick, 2005, Russell, 1991, Smith et al., 2014, Tollenaar, 1989, & Tollenaar and Lee, 2006). Duvick (2005) summarizes the changes in maize gross morphology over the years. Briefly, plant and ear heights have slightly decreased while leaves have become more upright. Tassel weights and branch numbers have decreased, but no significant trends have been found in leaf number or leaf area index. Delayed leaf senescence (staygreen) is improved in modern hybrids, the number of tillers is reduced, and the anthesis silking interval (ASI) has shortened. New hybrids have a longer grain-fill period, less grain protein, and less root and stalk lodging, but no change in harvest index (HI) (Duvick, 2005). Tollenaar and Wu (1999) note that modern hybrids outperform older varieties in all environments – these environments range from high weed competition, low night temperatures, and low soil moisture to low soil nitrogen.

From these results, yield gains in maize have generally been attributed to more efficient capture and utilization of resources, and greater stress tolerance (Duvick et al.,

2004 & Tollenaar and Wu, 1999). Because new hybrids have small tassels and few tillers, more resources are available to the developing ear. The upright, long-lived leaves improve radiation capture, provide increased assimilate supply, and promote root exploration (Duvick et al., 2004 & Tollenaar and Wu, 1999). Meanwhile, physiology leading to a short ASI creates new hybrids with fewer barren plants; they can endure environmental stresses and high planting densities and remain productive (Duvick et al., 2004).

These gross morphological traits provide great insights into the genetic gain of corn over time; however, specific leaf level physiology such as chlorophyll content and water content could help explain the underlying causes to improved productivity. Chlorophyll is necessary to harvest light energy to create ATP and NADPH. Meanwhile, sufficient water content allows for the incorporation of carbon dioxide through stomata. The light-harvested compounds, carbon dioxide, and water molecules are used in the Calvin cycle to generate sugars used in vegetative and reproductive growth (Taiz and Zeiger, 2010). In addition, chlorophyll content has been associated with gross primary production, nitrogen status, and plant stress (Gitelson et al., 2003). Yield potential increases in conjunction with chlorophyll and water content.

Hyperspectral reflectance technology is capable of monitoring plant chlorophyll and water content; many vegetation indices have been created (Henrich et al., n.d. & Main et al., 2011). In this situation, the 760/730 index (Winterhalter et al., 2011a & 2011b) proved to be useful as a proxy for chlorophyll content, and indirectly relative water content (Chapter 2).

Therefore, in order to learn from the past in order to make contributions for the future, this research utilizes high-throughput phenotyping to assess how leaf characteristics have changed in maize over time. In addition, as the sensor was utilized throughout the growing season, this research identifies specific growth stages when differences between old and new hybrids are exaggerated. By understanding how maize has become more productive over time, the same, novel phenotyping procedures can be used to assist breeders in selecting cultivars in the present.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the University of Nebraska-Lincoln East Campus (40.8° N and 96.7° W) in Lincoln, Nebraska. Trials were performed during the summers of 2014 and 2015. Thirty-six popular commercial era hybrids (released from 1936 to 2012), two irrigation treatments, and six replications were arranged as a randomized complete block design. Each of the 216 plots consisted of 2 rows with 0.76 m spacing between rows and a plot length of 6.1 m. Stands were thinned to an average density of 60,000 plants per hectare.

The maize hybrids were attained from two different sources and were assigned to eras as follows: Era 1 = 1936-1958, Era 2 = 1963-1969, Era 3 = 1970-1975, Era 4 = 1982-1988, Era 5 = 1991-1999, Era 6 = 2008-2012 (Table 2.1). The hybrids were acquired from DuPont Pioneer or inbred lines were retrieved from the North Central Regional Plant Introduction Station (NCRPIS) and hybrids were made by hand pollination at the University of Nebraska-Lincoln. All hybrids exhibited similar maturities and developmental stages were synchronous in the field.

The two water treatments, irrigated (well-watered, WW) and rain fed (water stressed, WS), were placed in blocks side-by-side in the field. The WW treatment received drip tape irrigation on an as needed basis. Sixteen mm diameter and 15 mm wall thickness drip tape offered a .32 gallon per hour flow rate. Plots were planted on May 15 and May 22 in 2014 and 2015, respectively. Pre- and post-emergent herbicides along with manual weeding minimized the effects of stress from weeds.

Hyperspectral Reflectance

Leaf level reflectance data was collected with a spectrometer (USB2000+ VIS-NIR, Ocean Optics, Dunedin, FL) on a near weekly basis beginning at V8. Measurements were always taken in the early afternoon (between 1200 and 1500 h CDT); approximately three hours were required to sample all plots. The active sensor system was mounted on a backpack. The spectrometer was connected to a halogen lamp light source and a “leaf clip” with a dual branch flexible fiber optic. The leaf clip covered the leaf to reduce environmental factors and had a field of view of 0.4 cm in diameter.

Two marked plants were measured in each plot. Prior to ear formation, scans were taken on the newest, fully expanded leaf. After ear formation, scans were taken on the ear leaf. The spectrometer analyzed reflected radiation at 2022 unique spectral bands with a detection range from 349 to 1028 nm and a bandwidth of approximately 1.5 nm. A number of spectral indices were calculated for use in analysis (Supplementary Table 1).

Chlorophyll Content

In tandem with reflectance measurements (on the same leaf), 0.9 cm diameter leaf discs were acquired to measure chlorophyll concentrations in the lab using the method of Warren (2008). Leaf discs were kept in a -80° Celsius freezer until they were lyophilized.

A 5 mm ball bearing was added to the tissue; the samples were ground to a fine powder by shaking in a vibratory ball mill (TissueLyser II, Qiagen, Inc., Valencia, CA).

Methanol was added; after being mixed and centrifuged the supernatant was used for analysis. A BioTek Synergy 2 microplate reader (BioTek Instruments, Inc., Winooski, VT) measured the absorbance of the chlorophyll extract dissolved in a methanol solution. To determine the pathlength of the microplate reader, a number of the solutions were also measured in a DU 730 spectrophotometer (Beckman Coulter, Inc., Indianapolis, IN).

Total chlorophyll content is used for all analysis (Chl $a + b$, $\mu\text{g/mL}$).

Microplate samples were corrected to a 1-cm pathlength absorbance:

$$A_{652, 1 \text{ cm}} = (A_{652, \text{microplate}} - \text{blank}) / \text{pathlength}$$

$$A_{665, 1 \text{ cm}} = (A_{665, \text{microplate}} - \text{blank}) / \text{pathlength}$$

Chlorophyll concentrations were calculated from these corrected absorbance values:

$$\text{Chl } a \text{ } (\mu\text{g/mL}) = -8.0962 A_{652, 1 \text{ cm}} + 16.5169 A_{665, 1 \text{ cm}}$$

$$\text{Chl } b \text{ } (\mu\text{g/mL}) = 27.4405 A_{652, 1 \text{ cm}} - 12.1688 A_{665, 1 \text{ cm}}$$

Canopy Temperature and Relative Water Content

Canopy temperatures were recorded with an Extech Instruments 421307 infrared thermometer (FLIR Commercial Systems, Nashua, NH) weekly from R1 to R3 stages.

Measurements were taken in the early afternoon, and two temperature readings were recorded per plot.

Scissors were used to cut portions of the same leaves monitored by the spectrometer approximately 2x8 cm in size for relative water content determination. Two samples per plot were cut and immediately placed in labeled and sealed Ziploc bags in a cooler. As soon as possible, the samples were taken to the lab and weighed to the nearest

milligram on an analytical balance (Denver Instrument, Bohemia, NY). De-ionized water was added to each bag to hydrate the leaves. The leaves were left overnight at room temperature because there were too many samples for the fridge; they were removed from the bags and weighed the next day to achieve the turgid weight (after surface moisture was removed). Finally, the samples were placed in the dryer for 24 hours at 65 degrees Celsius – after which the dry weight was measured. In all, approximately 20 man hours were required to complete the process for all plots. The following calculation was used to determine relative water content:

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] * 100, \text{ where}$$

FW = fresh weight

DW = dry weight

TW = turgid weight

Visual Scores

Leaf rolling visual scores were recorded on a weekly basis from pollination to physiological maturity during the early afternoon (between 1200 and 1500 CDT). A ranking system of 1 to 5 was utilized: 1 = no rolling, 2 = only leaf edges are beginning to curl, 3 = a v-shaped leaf, 4 = the leaf rolling hides the top of the leaf, and 5 = the leaves look like onion leaves.

Leaf senescence (or staygreen) scores were recorded weekly beginning at the onset of senescence. A ranking system of 0 to 10 was utilized: 0 refers to 0% dead leaf area, 1 refers to 10% dead leaf area, up to 10, which is 100% dead leaf area.

Days to 50% anthesis and silking were also recorded.

Biomass Estimation

A subset of 24 hybrids was used to calibrate nondestructive measurements to aboveground dry biomass. Plant height, in centimeters, was measured to the collar of the newest, fully expanded leaf, or to the flag leaf. The total number of leaves was counted. The newest, fully expanded leaf or the leaf above the ear was measured for length and width in centimeters; width was measured near the center (lengthwise) of the leaf. The stalk diameter was measured in centimeters with calipers between the topmost nodal roots and the next stalk node. Finally, if an ear was present, the diameter of the lower third of the ear was measured in centimeters with calipers and the length of the ear was measured from the node to the tip of the cob.

Two plants were measured in this fashion, and then the fresh weights of 2, 4, and 10 plants were recorded. Two plants were shredded and placed in the dryer at 60 degrees Celsius for 72 hours to determine sample dry weights. This process was conducted in 2014 at the V11 and R3 growth stages in order to generate biomass estimation models for plants with or without an ear. Multiple regression models were generated with the dry weight of 10 plants as the response variable and with all other measurements as explanatory variables (Supplementary Figures 1 and 2). The explanatory variables were the means of each of the nondestructive measurements for two plants, respectively:

$$DW10 \sim mH2 + mLW2 + mLL2 + mLN2 + mSD2 + mED2 + mEL2$$

DW10 = dry weight of ten plants

mH2 = mean of plant height from two plants

mLW2 = mean of leaf width from two plants

mLL2 = mean of leaf length from two plants

mLN2 = mean of leaf number from two plants

mSD2 = mean of stalk diameter from two plants

mED2 = mean of ear diameter from two plants

mEL2 = mean of ear length from two plants

The same nondestructive measurements taken on the biomass calibration plots were taken throughout the experiment at three different stages: vegetative, early reproductive, and physiological maturity. As a result, the equations of best fit determined by the calibration plots were used to generate a plot biomass estimate for each of the hybrids.

Grain Yield

The two-row plots were harvested with a plot combine (8-XP, Kincaid Equipment Mfg., Haven, KS). Yield data such as grain weight and moisture were collected with HarvestMaster's Single Plot High Capacity GrainGage (Juniper Systems, Logan, UT). Calculations were used to standardize grain weight at 15.5% moisture and to convert the yield to units of megagrams per hectare.

Statistical Analysis

Analysis of variance (ANOVA) and linear regression models were used to find differences between all hybrids for various traits and to find the genetic gain of traits. Differences were found among all hybrids and eras using the aov() function in R (The R Foundation). Trait values were treated as the response variable, hybrid or era and replication were fit as fixed effects. The drop1() function was used to change the analysis to a Type III Sums of Squares rather than a Type I. LS-means were calculated for all traits based on hybrid year of release. Trait values were treated as the response variable while hybrid year of release and replication were fit as fixed effects in the lsmeans() function.

The LS-means were used in the `lm()` function to analyze genetic gain. Trait values were the response variable and hybrid year of release was the numeric independent variable. Slopes acquired from this analysis were compared with an analysis of covariance (ANCOVA) to determine if the rate of change in index values across years of hybrid release were different throughout the growing season. Using the `aov()` function to identify interaction effects, the index values were modeled as the dependent variable with maize developmental stage as a factor and hybrid year of release as the covariate.

RESULTS

Environmental Conditions

Rainfall distributions were unique in the 2014 and 2015 growing seasons. Although total rainfall between May and September were similar for both years (66.3 cm in 2014 and 75.3 cm in 2015), the timing of the rainfall events created a water-stressed environment in 2014, but not 2015 (Figure 2.1). In 2014, the month of July only received 1.3 cm of rainfall. On the other hand, 2015 received above average rainfall in the spring, followed by average accumulations throughout the growing season (Table 2.2). As a result, the WS treatment in 2014 was discarded from all analysis while both 2015 treatments were combined for analysis as well-watered replications. The WS treatment in 2014 was located near the field edge and became highly variable at the onset of water stress. Repeatability of measurements was extremely low – the same hybrid produced varied results in each replication. Because of this, ANOVA models could only find differences among all hybrids for a few traits. The increased spatial variability of the field and the increased variability in traits that could not be explained by hybrids made analysis of the WS treatment unprofitable.

Differences in Traits

Prior to analyzing genetic gain, ANOVAs for all traits were conducted to determine if hybrids could be differentiated. In addition to differences among all hybrids, ANOVAs to find differences among the six eras were computed as well. Those traits which showed significant differences among all hybrids and eras are candidates for genetic gain analysis. Traits were analyzed separately for each year because of significant hybrid x year interaction effects for most traits.

Variation among individual hybrids and eras was found for most traits. Table 3.1 summarizes these differences for all of the traits collected. All gross morphological traits could differentiate hybrids and eras – from pollen date, ASI, biomass, and growth rate to lodging, plant and ear height, and grain yield. However, all leaf level traits could not differentiate hybrids and eras. In both years canopy temperature was unresponsive among hybrids, and in 2014 RWC didn't show differences. Also in 2014 slight leaf rolling occurred during a hot week and the visual scores could differentiate hybrids and eras. In 2015 RWC was able to differentiate hybrids and eras on both sampling dates. Finally, both years showed significant differences among hybrids and eras for chlorophyll content and senescence.

Genetic Gain of Agronomic Traits

For each of the traits that showed significant differences among all hybrids and all eras, linear regression models were used to identify genetic gain, or how the traits have changed over time (other models were not fit because traits generally displayed linear relationships). Table 3.2 presents the results of the linear regression models of each trait

over hybrid year of release. Years were analyzed separately because of significant hybrid year of release x year interactions, except for grain yield.

Although days to anthesis have not significantly changed over time, the anthesis silking interval has continuously become shorter at a rate of 0.065 days per year ($R^2 = 0.63$, Figure 3.1). Aboveground dry biomass failed to separate hybrids based on year of release except for the V13 sampling in 2014. There, modern hybrids display a smaller biomass; dry matter was reduced by 0.656 grams per year of hybrid release ($R^2 = 0.21$). Corresponding to that, the growth rate of modern hybrids during early vegetative stages was less than that of old hybrids. In 2014, the growth rate between the V6 and V13 stages declined at a rate of 0.039 grams/day over year of hybrid release ($R^2 = 0.19$). Both plant height and ear height have decreased in hybrids over time as well. Plant height decreased at a rate of 0.23 cm per year ($R^2 = 0.23$) and ear height at 0.36 cm per year ($R^2 = 0.46$). Modern hybrids are less prone to root and stalk lodging. Stalk lodging has decreased by 0.1% per year ($R^2 = 0.43$) and root lodging has also decreased at a rate of 0.6% per year ($R^2 = 0.5$).

Leaf characteristics have also changed over time. Most significantly, new hybrids maintain higher chlorophyll concentrations and relative water contents in their cells. Chlorophyll contents have increased at a rate of 0.1 $\mu\text{g/mL}$ per year ($R^2 = 0.3$, Figure 3.2) and water contents at 0.02% per year ($R^2 = 0.38$, Figure 3.3). Also, new hybrids senesce at slower rates; senescence scores decreased at a rate of 0.04 of a ranking per year ($R^2 = 0.31$). Finally, combining both experimental years, grain yield has increased at a rate of 76 kg/ha per year ($R^2 = 0.71$, Figure 3.4).

Genetic Gain of the 760/730 Vegetation Index

Previously it was shown that the 760/730 index captures differences between all hybrids throughout the growing season and correlates well with leaf traits, providing a general indicator of plant health (Chapter 2). The index also expresses significant genetic gain at multiple stages throughout the growing season. Higher index values are associated with new hybrids, while low values are consistently partnered with old hybrids. Unlike the other traits, the 760/730 index did not have significant hybrid year of release x year interaction effects. As a result, measurements taken at the same growth stage from both years were combined for analysis. Table 3.3 shows the linear regression, or genetic gain results for the 760/730 index taken at various points throughout the growing season.

The maximum slope and coefficient of determination was found at the R1 growth stage ($b=.001$, $R^2 = 0.49$, Figure 3.5). The next highest slopes occur at the V13, V17, R3, R4, and R5 growth stages; however, the best coefficients of determination are at the V13, V17, and R1 growth stages ($R^2 > 0.44$). As a result, at those late vegetative and early reproductive growth stages, a hybrid's year of release explains more of the variation in the 760/730 index than at other points in the growing season.

Despite the differences in the coefficients of determination, an analysis of covariance showed that there was no significant interaction effect between the slopes of the 760/730 index at different growth stages (Figure 3.6). In other words, removing the interaction effect between the different lines did not significantly affect the fit of the model. Rather, significant main effects existed between growth stages. The 760/730 index was significantly greater in the R1 to R5 growth stages compared to the V10 to V17 and R6 stages.

DISCUSSION

Differences in Traits

The only trait that did not show any differences among all hybrids or eras at any measurement date was canopy temperature. This is unsurprising as all measurements were collected in well-watered environments and ambient air temperature was not recorded for each plot. Typically, air temperature is subtracted from canopy temperature to differentiate cultivars in water-stressed scenarios. However, Romano et al (2011) did find differences among all genotypes in well-watered conditions using an infrared camera. Newer infrared technology than what was used in this project may have more precision to differentiate genotypes in any environment.

Genetic Gain of Agronomic Traits

Of the traits that showed significant differences among all hybrids and eras, aboveground dry biomass did not show any trend over time except at early vegetative stages. At those stages, new hybrids were consistently smaller than old hybrids. Using hydroponics, Sanguineti et al (2006) also found that new hybrid seedlings have significantly smaller roots and shoots compared to older hybrids. In contrast, they noted that the height of field grown era hybrids did not differ at the V4 stage – although total biomass was not recorded. Changes in farm management strategies are plausible culprits of this decreased early biomass. Increased nitrogen fertilizer usage and planting densities may have resulted in plants that don't need to search for nutrients and that delay competition from adjacent plants (Sanguineti et al., 2006). In agreement with Duvick et al (2004), the weight of mature plants has not changed over time, but plant height and ear height has decreased slightly by 0.1 cm and 0.3 cm per year in their set of era hybrids released from 1934 to 2001, respectively ($R^2 = 0.11, 0.40$). This research shows similar,

but slightly steeper and better fit slopes for plant height and ear height changes in a set of era hybrids released from 1936 to 2012. Plant height and ear height have decreased by 0.23 and 0.36 cm per year ($R^2 = 0.23, 0.46$). Breeders have purposefully selected for stable plant height as farmers dislike overly tall plants, but it is unknown how this and decreased ear height has directly affected grain yield (Duvick et al., 2004). It may be another result of increased plant density over time. At higher densities, old hybrids maintain plant size, but lose yield because of an increased proportion of barren plants (Duvick, 2005). Meanwhile, new hybrids manage to maintain their harvest index in densely planted populations (Duvick, 2005).

The effects of root and stalk lodging on grain yield are more obvious. Downed plants are not only more difficult to machine harvest, but also are not given the chance to fully utilize the grain-filling period. Similar to Duvick et al (2004), this research shows that new hybrids are more resistant to both root and stalk lodging. While there was minimal root lodging in 2014, a wind storm in 2015 made differences in root lodging apparent among eras.

For flowering traits researchers agree that days to anthesis have not significantly changed over time, but the anthesis silking interval (ASI) has consistently decreased (Meghji et al., 1984 and Duvick et al., 2004). The ASI is extremely important for hybrid yield potential under stress – maintained silk exertion and ear growth are necessary to reduce the number of barren plants (Bolanos & Edmeades, 1996).

Interestingly, while this research showed no significant effect of hybrid year of release on leaf rolling, Barker et al (2005) showed that newer hybrids tend to roll their leaves to a greater extent. Because leaf rolling is not a sign of maintained growth and

production under mild drought scenarios it is generally assigned a negative connotation (Tardieu, 2012).

Another trait that has shown consistent improvement over time is staygreen, or delayed leaf senescence. Duvick et al (2004) showed an increase in the staygreen score (rank from 1 to 9) at a rate of 0.06 per year ($R^2 = .75$). This experiment showed a less significant trend of a decrease in senescence ranks at a rate of 0.04 per year ($R^2 = 0.31$). Yield potential is increased as new hybrids are less prone to premature death and have extended grain-fill periods (Cavaliere & Smith, 1985).

Changes in leaf chlorophyll and relative water contents in maize era hybrids have not been reported previously in the literature. This study shows that significant trends do exist for both traits. Over time, chlorophyll content has increased at a rate of 0.1 $\mu\text{g/mL}$ per year ($R^2 = 0.3$) and RWC at 0.02% per year ($R^2 = 0.38$). Ying et al. (2000) actually discovered that the photosynthetic rate at saturating irradiance of an old hybrid was greater than two new hybrids. However, other results show that leaf photosynthetic rates from a larger group of era hybrids did not show any differences (Tollenaar & Lee, 2002). While it might be expected that leaves with increased chlorophyll contents would exhibit increased rates of ATP and NADPH production from electron transport, this cannot be concluded. New hybrids undoubtedly have a photosynthetic advantage as they maintain a larger leaf area index when planted at high densities; however, on a per plant basis, leaf area index has remained stable over the years (Tollenaar & Lee, 2002). This study, consisting of uniform plant populations and individual leaf chlorophyll estimations, hints that chlorophyll contents have increased concomitantly with grain yield over time.

Although previous era studies have not looked directly at chlorophyll content, researchers have examined nitrogen uptake and partitioning. Chen et al. (2015) found that newer hybrids partition more dry matter to the leaf instead of the stem at silking, that new hybrids retrieve more nitrogen from the soil post-silking, and that new hybrids retained higher leaf dry matter and nitrogen content at maturity. On top of that, newer hybrids have higher nitrogen contents in the whole plant at silking; therefore, a greater source strength at the onset of grain filling (Ciampitti & Vyn, 2013). Because remobilization of nitrogen from vegetative organs to the ear is delayed and post-silking nitrogen uptake is prolonged in new hybrids, leaf photosynthesis duration can be expanded (Chen et al., 2015). Old hybrids typically accumulate as much nitrogen as possible at silking and then transfer most of this nitrogen to the grain throughout the reproductive stages (Chen et al., 2015). On the other hand, recent hybrids require this post-silking nitrogen uptake because remobilized nitrogen from the vegetative organs at silking will not fulfill the ear nitrogen demand (Chen et al., 2015). Therefore, the greater nitrogen status in the leaves of newer hybrids coincides with the increased chlorophyll contents found in this study.

Barker et al. (2005) found a significant downward trend in canopy temperature over hybrid year of release in water stressed scenarios. This implies that newer hybrids are able to access soil water and/or maintain leaf relative water content better in comparison to old hybrids. Our study shows that even in optimal environments new hybrids retain more water in their leaves. The importance of chlorophyll and water content was elucidated in Chapter 2, from that introduction and this discussion it is clear that new hybrids are productive because of their ability to capture and utilize additional carbon and nitrogen. Sufficient water status allows for continual transpiration and

incorporation of carbon dioxide. Increases in chlorophyll and nitrogen contents allow prolonged photosynthesis which creates the chemical energy necessary to produce useful carbohydrates for continued root and ear development.

Finally, Duvick et al (2004) note that the average annual genetic gain for grain yield is 77 kg/ha in optimum growing conditions. This study shows, using the lsmeans for each year of release, that the annual genetic gain of grain yield is 76 kg/ha ($R^2 = 0.71$).

Genetic Gain of the 760/730 Vegetation Index

The 760/730 index has been correlated to nitrogen uptake and canopy water content (Winterhalter et al., 2011a and 2011b). In this study, the index shows strongest correlations to total leaf chlorophyll content (Chapter 2). This phenotype is advantageous because it can monitor plants accurately and efficiently throughout the growing season.

First, per raw index values, total chlorophyll content is greatest in the leaves during early reproductive stages as compared to vegetative stages and physiological maturity. Second, mimicking the genetic gain of chlorophyll content, index values for new hybrids are consistently higher than those for old hybrids throughout the growing season. Third, while there are no differences between the slopes of these regression lines at different growth stages, the R1 growth stage can explain the most variation in the index values by hybrid year of release ($R^2 = 0.49$).

These results verify that the chlorophyll content, nitrogen status, and even relative water contents are improved in modern hybrids. Increases in these traits have been associated with gains in grain yield over time. More specifically, because gains in chlorophyll content have changed with breeding over time, this research validates that the

many new phenotyping tools that utilize reflectance technology to estimate chlorophyll have merit to be used in selection programs to continue improving maize.

CONCLUSIONS

In agreement with Duvick et al. (2004), modern hybrids exhibit many unique characteristics that are associated with increases in productivity over time. For example, new hybrids exhibit increased grain yield with a decreased ASI, decreased stalk lodging, root lodging, plant height, ear height, early vegetative biomass, and senescence. In addition, increases in leaf chlorophyll concentrations and water contents were discovered in new hybrids. Hyperspectral reflectance indices confirmed these changes in leaf traits over time, and the differences were optimized surrounding flowering.

By understanding the morphological and physiological trends of maize hybrids over time, breeders can continue to select for these traits that enhance yield. Moreover, this research shows that high throughput phenotyping tools that estimate chlorophyll content can be implemented into a breeding program. Research needs to be conducted to reveal molecular mechanisms behind these changes in leaf characteristics over time.

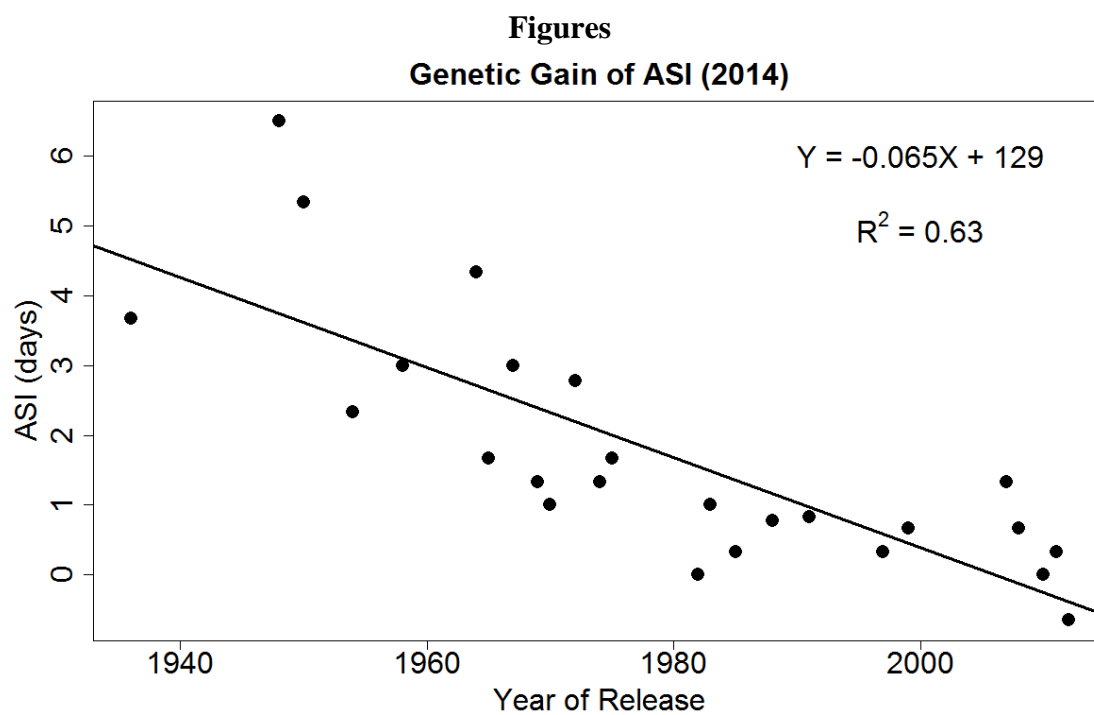


Figure 3.1. LS-means for anthesis silking interval (ASI) regressed over year of hybrid release from the 2014 WW treatment.

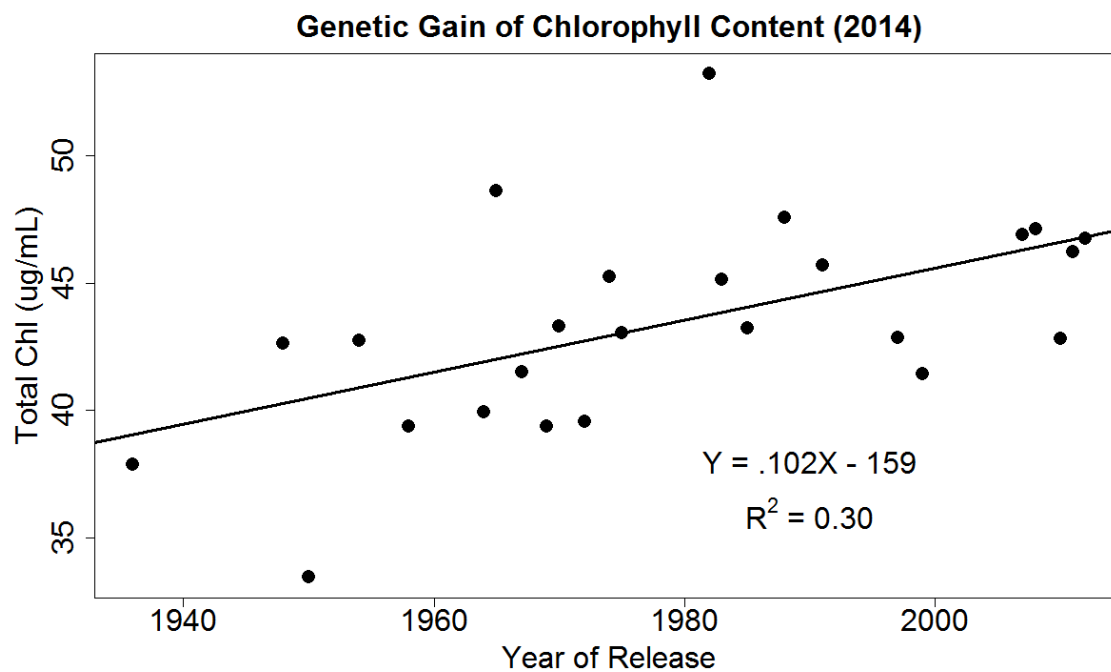


Figure 3.2. LS-means for chlorophyll content regressed over year of hybrid release from the July 28, 2014 sampling date.

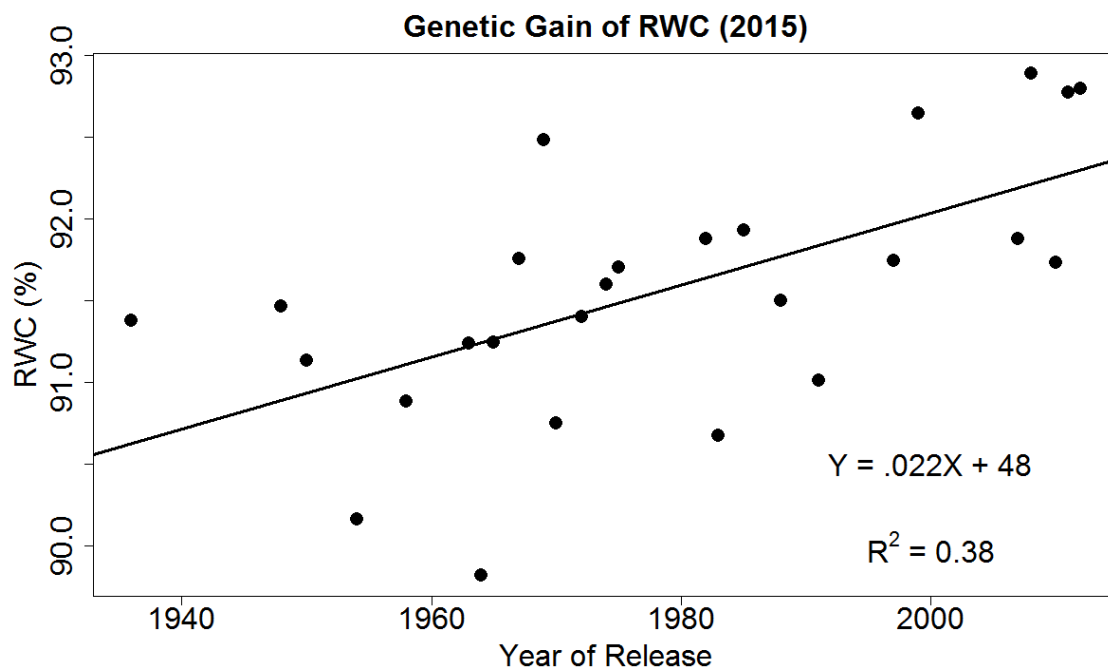


Figure 3.3. LS-means for relative water content (RWC) regressed over year of hybrid release from the August 7, 2015 sampling date.

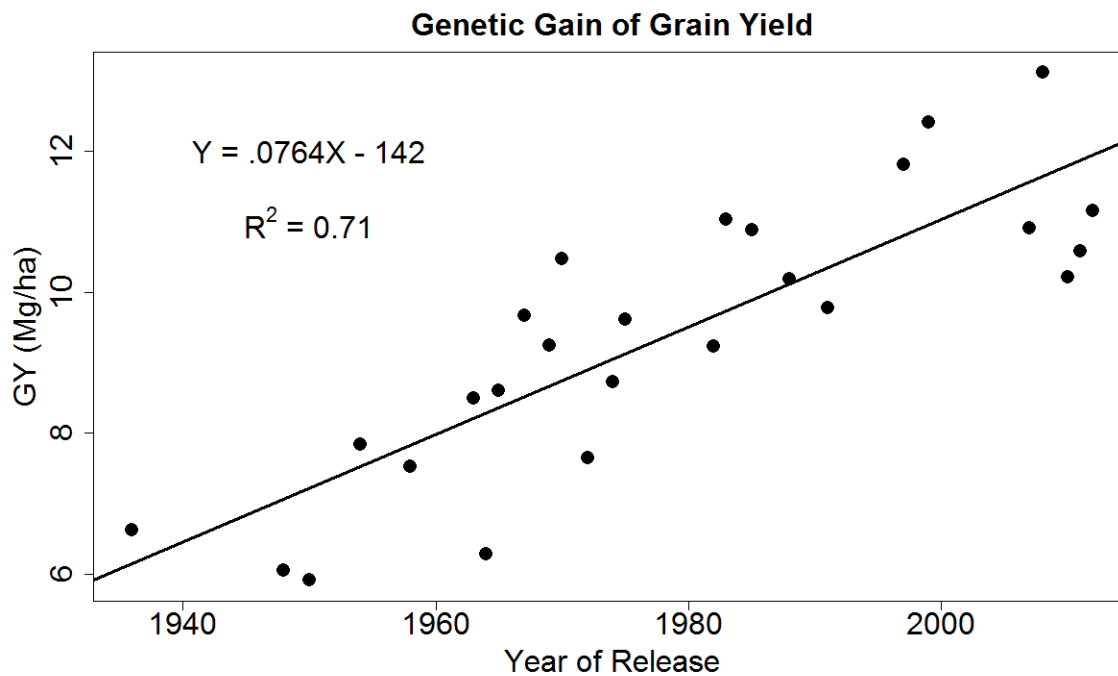


Figure 3.4. LS-means for grain yield (Mg/ha) regressed over year of hybrid release from both years (2014: only WW; 2015: all replications).

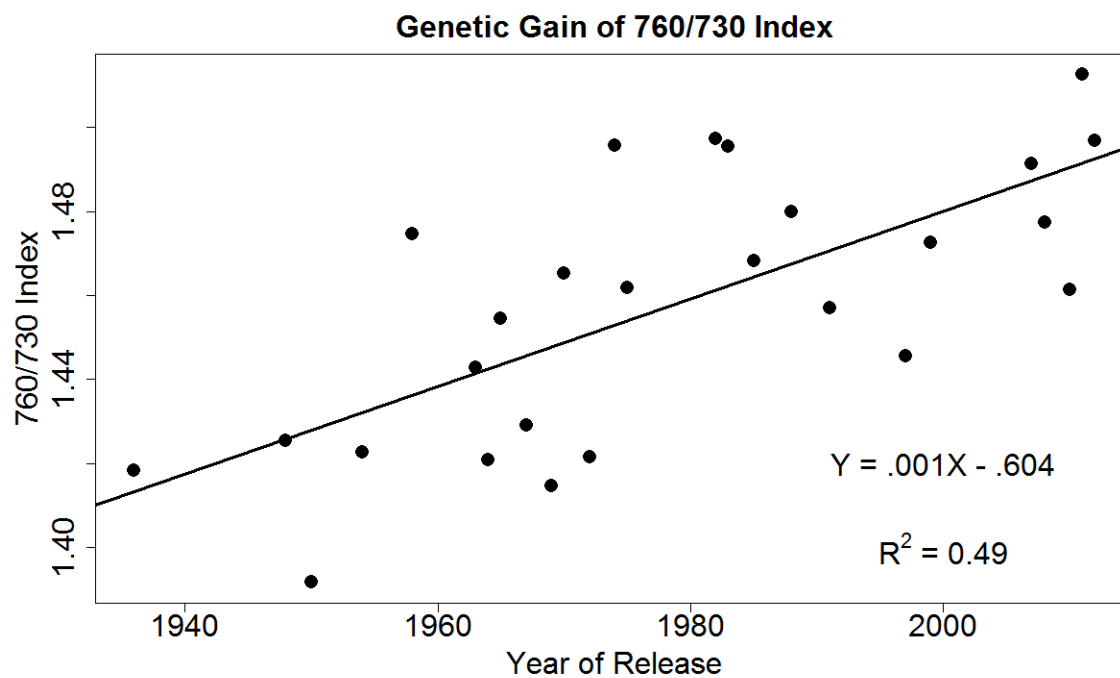


Figure 3.5. LS-means of 760/730 index values regressed over year of hybrid release from both years at the R1 developmental stage (2014: only WW; 2015: all replications).

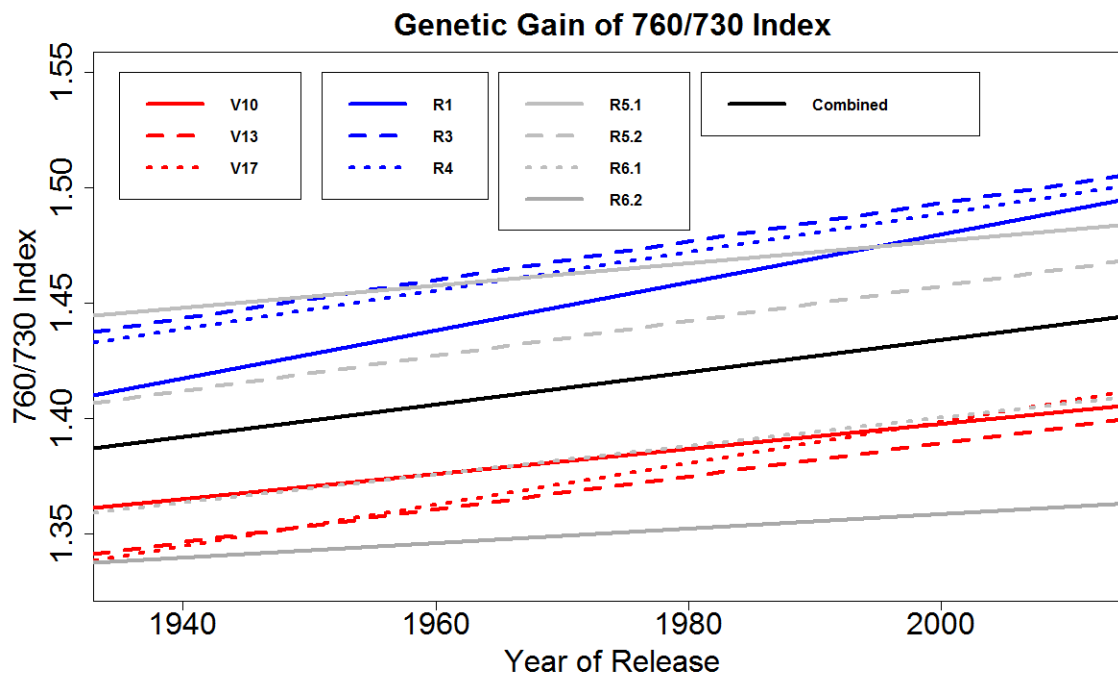


Figure 3.6. Slopes from the LS-means of 760/730 index values regressed over year of hybrid release from both years at all measured developmental stages (2014: only WW; 2015: all replications). Red lines represent late vegetative stages, blue lines represent early reproductive stages, gray lines represent late reproductive stages, and the black line represents the average slope across all developmental stages ($b = 0.0007$, $R^2 = 0.76$).

Tables

Table 3.1. ANOVA results for detecting significant differences between all 36 hybrids or between the 6 eras for various traits for each year. Blank spaces are a result of traits not being sampled that year. Developmental stages are listed behind the trait in most cases.

	2014		2015	
	Hybrid	Era	Hybrid	Era
Pollen Date	***	**	***	***
ASI	***	***	***	***
V6 Biomass	***	NS		
V13 Biomass	***	***	***	***
R2 Biomass	***	**	***	.
Black Biomass	***	***	***	***
GR1	***	***		
GR2	***	***	***	NS
GR3	***	***	***	*
Grain Yield	***	***	***	***
Stalk Lodge (%)	***	***	***	***
Root Lodge (%)			***	***
Plant Height	***	***	***	***
Ear Height	***	***	***	***
CT.R1			NS	NS
CT.R3			NS	.
CT.R4	NS	NS	NS	NS
CT.R5	NS	NS	NS	NS
CT.R5.2	NS	NS		
CT.R6	NS	NS		
LR.R4	***	*		
LR.R5	NS	NS		
RWC.V16			**	*
RWC.R3	NS	NS	***	***
RWC.R5	NS	NS		
Chl.V16			***	*
Chl.R3	***	***	***	***
Chl.R5	***	*		
Sen.R5			***	***
Sen.R6	***	***	***	***
Sen.R6.2	***	**	***	***
Sen.R6.3			***	***

‘***’ = significant at the <0.001 level; ‘**’ = <0.01; ‘*’ = <0.05; ‘.’ = <0.10

ASI = anthesis silking interval; GR = growth rate (GR1 = dry matter accumulation in grams per day between the V6 and V13 stages, GR2 = between V13 and R2, GR3 = between R2 and R6); CT = canopy temperature; LR = leaf rolling; RWC = relative water content; Chl = chlorophyll content; Sen = senescence score

Table 3.2. Linear regression results from the LS-means of various traits over year of hybrid release. Slopes (b), coefficients of determination (R^2), and significance levels are presented. Blank spaces are a result of non-significant ANOVAs among all hybrids and eras. Developmental stages are listed behind the trait.

	2014			2015		
	b	R ²		b	R ²	
Pollen Date	-0.026	0.078	NS	-0.021	0.077	NS
ASI	-0.065	0.633	***	-0.053	0.530	***
V13 Biomass	-0.656	0.212	*	-0.474	0.103	NS
R2 Biomass	3.034	0.089	NS	-0.434	0.005	NS
R6 Biomass	-1.910	0.057	NS	-1.950	0.095	NS
Growth Rate 1	-0.039	0.191	*			
Growth Rate 2	0.241	0.121	NS			
Growth Rate 3	-0.081	0.085	NS	-0.043	0.122	.
Grain Yield	0.076	0.678	***	0.077	0.684	***
Stalk Lodge (%)	-0.130	0.428	***	-0.049	0.318	**
Root Lodge (%)				-0.634	0.496	***
Plant Height	-0.233	0.233	*	-0.133	0.063	NS
Ear Height	-0.362	0.457	***	-0.261	0.220	*
LR.R4	-0.005	0.039	NS			
RWC.V16				-0.001	0.001	NS
RWC.R3				0.022	0.376	***
Chl.V16				0.030	0.066	NS
Chl.R3	0.102	0.297	**	0.085	0.273	**
Chl.R5	0.052	0.092	NS			
Sen.R5				-0.013	0.146	.
Sen.R6	-0.041	0.312	**	-0.018	0.119	.
Sen.R6.2	-0.030	0.119	.	-0.023	0.227	*
Sen.R6.3				-0.011	0.089	NS
Combined GY	0.076	0.709	***			

‘***’ = significant at the <0.001 level; ‘**’ = <0.01; ‘*’ = <0.05; ‘.’ = <0.10

ASI = anthesis silking interval; LR = leaf rolling; RWC = relative water content; Chl = chlorophyll content; Sen = senescence score; Combined GY = LS-means for grain yield from both experimental years regressed over hybrid year of release

Table 3.3. Linear regression results from the LS-means of 760/730 index values over year of hybrid release for various developmental stages. LS-means are from both experimental years when the sensor was used at the same stage. Slopes (b), coefficients of determination (R^2), and significance levels are presented.

Stage	b	R^2
V10	0.0005	0.270 **
V13	0.0007	0.444 ***
V17	0.0009	0.453 ***
R1	0.0010	0.489 ***
R3	0.0008	0.257 **
R4	0.0008	0.276 **
R5	0.0005	0.145 .
R5	0.0008	0.282 **
R6	0.0006	0.146 .
R6	0.0003	0.031 NS

‘***’ = significant at the <0.001 level; ‘**’ = <0.01; ‘*’ = <0.05; ‘.’ = <0.10

Literature Cited

- Barker, T., Campos, H., Cooper, M., Dolan, D., Edmeades, G., Habben, J., Schussler, J., Wright, D., & Zinselmeier, C. (2005). Improving drought tolerance in maize. *Plant Breeding Reviews*, 25, 173–253.
- Bolanos, J., & Edmeades, G. O. (1996). The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Research*, 48(1), 65–80.
- Cavaliere, A. J., & Smith, O. S. (1985). Grain filling and field drying of a set of maize hybrids released from 1930 to 1982. *Crop Science*, 25(5), 856–860.
- Chen, K., Kumudini, S. V., Tollenaar, M., & Vyn, T. J. (2015). Plant biomass and nitrogen partitioning changes between silking and maturity in newer versus older maize hybrids. *Field Crops Research*, 183, 315–328.
<http://doi.org/10.1016/j.fcr.2015.08.013>
- Ciampitti, I. A., & Vyn, T. J. (2013). Grain nitrogen source changes over time in maize: A review. *Crop Science*, 53(2), 366. <http://doi.org/10.2135/cropsci2012.07.0439>
- Duvick, D. N., Smith, J. S. C., & Cooper, M. (2004). Long-term selection in a commercial hybrid maize breeding program. *Plant Breeding Reviews*, 24(2), 109–151.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy*, 86, 83–145.
- Gitelson, A., Gritz, Y., & Merzlyak, M. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, 160, 271–282.
- Henrich, V., Krauss, G., Gotze, C., & Sandow, C. (n.d.). IDB - Index DataBase. Retrieved November 21, 2015, from <http://www.indexdatabase.de/>
- Main, R., Cho, M. A., Mathieu, R., O’Kennedy, M. M., Ramoelo, A., & Koch, S. (2011). An investigation into robust spectral indices for leaf chlorophyll estimation. *ISPRS Journal of Photogrammetry and Remote Sensing*, 66(6), 751–761.
<http://doi.org/10.1016/j.isprsjprs.2011.08.001>
- Meghji, M. R., Dudley, J. W., Lambert, R. J., & Sprague, G. F. (1984). Inbreeding depression, inbred and hybrid grain yields, and other traits of maize genotypes representing three eras. *Crop Science*, 24(3), 545–549.
- Romano, G., Zia, S., Spreer, W., Sanchez, C., Cairns, J., Araus, J. L., & Müller, J. (2011). Use of thermography for high throughput phenotyping of tropical maize

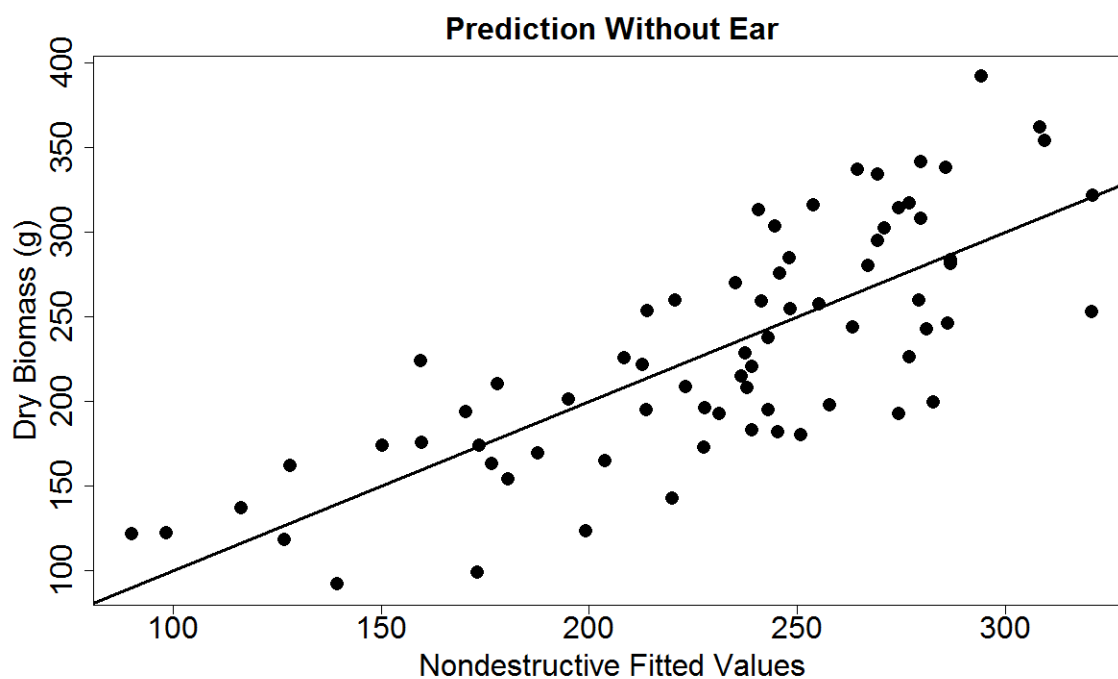
- adaptation in water stress. *Computers and Electronics in Agriculture*, 79(1), 67–74. <http://doi.org/10.1016/j.compag.2011.08.011>
- Russell, W. A. (1991). Genetic improvement of maize yields. *Advances in Agronomy*, 46, 245–298.
- Sanguineti, M. C., Duvick, D. N., Smith, S., Landi, P., & Tuberosa, R. (2006). Effects of long-term selection on seedling traits and ABA accumulation in commercial maize hybrids. *Maydica*, 51(2), 329.
- Smith, S., Cooper, M., Gogerty, J., Löffler, C., Borcharding, D., Wright, K., Smith, S., Dier, B., Specht, J., Carver, B. (2014). Maize. In *CSSA Special Publications*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Retrieved from <https://dl.sciencesocieties.org/publications/books/abstracts/cssaspecialpubl/yieldgainsinmaj/125>
- Taiz, L., & Zeiger, E. (2010). *Plant Physiology* (5th ed.). Sunderland, MA: Sinauer Associates, Inc.
- Tardieu, F. (2012). Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *Journal of Experimental Botany*, 63(1), 25–31. <http://doi.org/10.1093/jxb/err269>
- Tollenaar, M. (1989). Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. *Crop Science*, 29(6), 1365–1371.
- Tollenaar, M., & Wu, J. (1999). Yield improvement in temperate maize is attributable to greater stress tolerance. *Crop Science*, 39(6), 1597–1604.
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field Crops Research*, 75(2), 161–169.
- Tollenaar, M., & Lee, E. A. (2006). Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. *Maydica*, 51(2), 399.
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011a). High-throughput sensing of aerial biomass and above-ground nitrogen uptake in the vegetative stage of well-watered and drought stressed tropical maize hybrids. *Crop Science*, 51(2), 479–489.
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011b). High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage.

European Journal of Agronomy, 35(1), 22–32.
<http://doi.org/10.1016/j.eja.2011.03.004>

Ying, J., Lee, E. A., & Tollenaar, M. (2000). Response of maize leaf photosynthesis to low temperature during the grain-filling period. *Field Crops Research*, 68(2), 87–96.

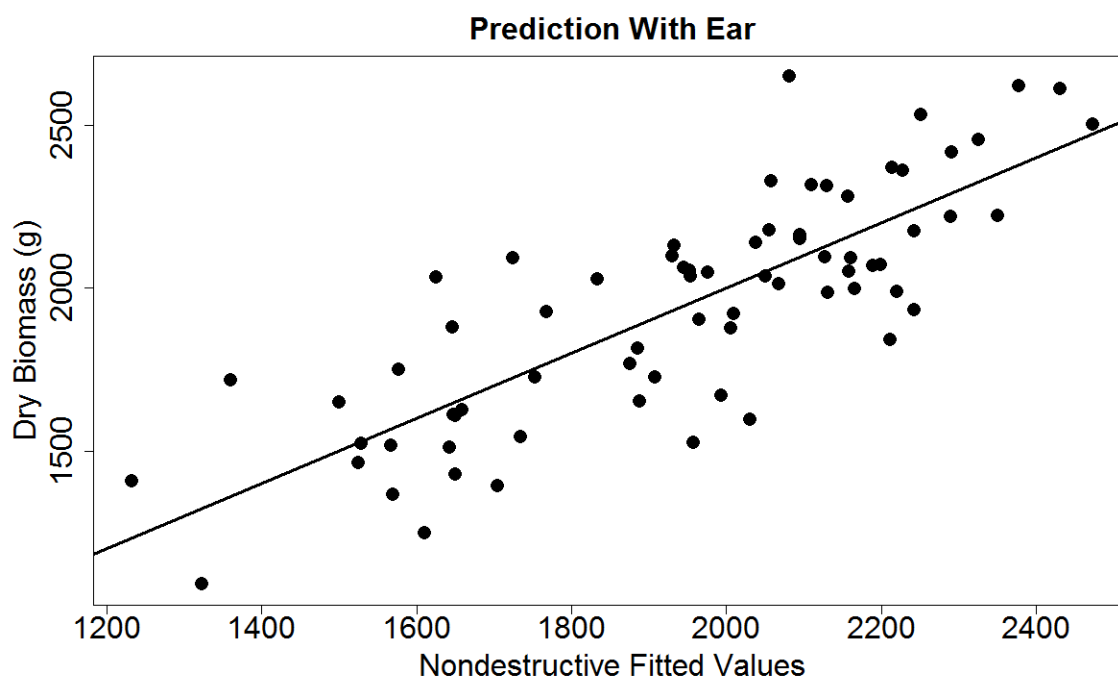
Appendix

Supplementary Figure 1



Multiple regression results of nondestructive measurements against aboveground dry biomass at vegetative stage ($R^2 = 0.59$). Nondestructive measurements consisted of plant height, leaf width, leaf length, leaf number, and stalk diameter. Correlations were made on a plot by plot basis using the average measurements of two plants to estimate the actual dry biomass of ten plants.

Supplementary Figure 2



Multiple regression results of nondestructive measurements against aboveground dry biomass at reproductive stage ($R^2 = 0.65$). Nondestructive measurements consisted of plant height, leaf width, leaf length, leaf number, stalk diameter, ear diameter, and ear length. Correlations were made on a plot by plot basis using the average measurements of two plants to estimate the actual dry biomass of ten plants.

Supplementary Table 1

Index	Equation	Trait	Reference
BGI1	$(R400/R550)$	Pigments	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2005)
BGI2	$(R450/R550)$	Pigments	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2005)
BRI1	$(R400/R690)$	Pigments	(Zarco-Tejada et al., 2005)
BRI2	$(R450/R690)$	Pigments	(Zarco-Tejada et al., 2005)
CAR	$(R515/R570)$	Carotenoids	(Rodrigues et al., 2014 & Hernández-Clemente et al., 2012)
CARI	$(R700 * (\sqrt{((R700-R550)/150) * 670 + R670 + ((R550 - (R700 - R550)/150) * 550))^2})) / ((R670 * (((R700 - R550)/150)^2 + 1)^{0.5}))$	Chlorophyll	(Main et al., 2011)
Cars	R470	Carotenoids	(Blackburn, 1998)
Carter6	R550	Chlorophyll	(Main et al., 2011 & Carter, 1994)
CG	$(R780/R550) - 1$	Total Chl	(Gitelson et al., 2006)
CI	$(R675 * R690) / R683^2$	Chlorophyll a	(Main et al., 2011 & Zarco-Tejada et al., 2003)
CI2	$(R760/R700) - 1$	Chlorophyll	(Gitelson et al., 2003)
Datt	$(R850 - R710) / (R850 - R680)$	Chlorophyll	(Main et al., 2011 & Datt, 1999)
Datt2	R850/R710	Chlorophyll	(Main et al., 2011 & Datt, 1999)
Datt4	$R672 / (R550 * R708)$	Chlorophyll	(Main et al., 2011 & Datt, 1998)
Datt5	R672/R550	Chl b	(Main et al., 2011 & Datt, 1998)
Datt6	$R860 / (R550 * R708)$	Chlorophyll	(Main et al., 2011 & Datt, 1998)
DD	$(R749 - R720) - (R701 - R672)$	Total Chl	(Main et al., 2011 & le Maire et al., 2004)
DDn	$2 * (R710 - R660 - R760)$	Total Chl	(Main et al., 2011 & le Maire et al., 2008)
EVI	$2.5 * ((R800 - R770) / (R800 + 6 * R670 - 7.5 * R400 + 1))$	Biomass	(Huete et al., 2002 & Rodrigues et al., 2014)
GI	R554/R677	Canopy Chl	(Main et al., 2011 & Zarco-Tejada et al., 2005)
Gitelson	1/R700	Total Chl	(Main et al., 2011 & Gitelson et al., 1999)
Cl _{RE}	$(R_{750-800}) / (R_{710-730}) - 1$	Total Chl	(Main et al., 2011 & Gitelson et al., 2003)
GLI	$(2 * R560 - R660 - R485) / (2 * R560 + R660 + R485)$	Total Chl	(Hunt et al., 2011)
GRE	$322 * ((R790/R715) - 1) + 27$	Chlorophyll	(Gitelson et al., 2003)
Green.NDVI	$(R800 - R550) / (R800 + R550)$	Chl a	(Main et al., 2011 & Gitelson et al., 1996)
HBCI10	$(R720 - R550) / (R720 + R550)$	Pigments	(Thenkabail et al., 2014)
HBCI11	$(R550 - R375) / (R550 + R375)$	Pigments	(Thenkabail et al., 2014)
HBCI12	$(R855 - R550) / (R855 + R550)$	Pigments	(Thenkabail et al., 2014)
HBCI13	$(R550 - R682) / (R550 + R682)$	Pigments	(Thenkabail et al., 2014)
HBCI8	$(R550 - R515) / (R550 + R515)$	Pigments	(Thenkabail et al., 2014)
HBCI9	$(R550 - R490) / (R550 + R490)$	Pigments	(Thenkabail et al., 2014)
HBSI1	$(R855 - R682) / (R855 + R682)$	Biomass	(Thenkabail et al., 2014)
HBSI2	$(R910 - R682) / (R910 + R682)$	Biomass	(Thenkabail et al., 2014)
LABR	$(R820 - R701) / (R820 + R701)$	Nitrogen	(Carter, 1998)
Maccioni	$(R780 - R710) / (R780 - R680)$	Total Chl	(Main et al., 2011 & Maccioni et al., 2001)
MCARI	$((R700 - R670) - 0.2 * (R700 - R550)) * (R700 / R670)$	Canopy Chl	(Rodrigues et al., 2014, Main et al., 2011, & Daughtry et al., 2000)
MCARI.MTVI2	MCARI/MTVI2	Total Chl	(Hunt et al., 2011)
MCARI.OSAVI	MCARI/OSAVI	Canopy Chl	(Rodrigues et al., 2014, Main et al., 2011, & Daughtry et al., 2000)
MCARI1	$1.2 * (2.5 * (R800 - R670) - 1.3 * (R800 - R550))$	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2004)
MCARI2	$(1.5 * (2.5 * (R800 - R670) - 1.3 * (R800 - R550))) / (\sqrt{(2 * R800 + 1)^2 - (6 * R800 - 5 * \sqrt{R680}) - 0.5}))$	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2004)
MCARI2n	$((R750 - R705) - 0.2 * (R750 - R550)) * (R750 / R705)$	Canopy Chl	(Main et al., 2011 & Wu et al., 2008)
MCARI2n.OSAVI2	MCARI2n/OSAVI2	Canopy Chl	(Main et al., 2011 & Wu et al., 2008)
mND705	$(R750 - R705) / (R750 + R705 - 2R445)$	Total Chl	(Main et al., 2011 & Sims and Gamon, 2002)
mNDVI	$(R800 - R680) / (R800 + R680 - 2 * R445)$	Total Chl	(Main et al., 2011 & Sims and Gamon, 2002)

Supplementary Table 1 Continued

MSAVI	$0.5 * (2 * R800 + 1 - \sqrt{(2 * R800 + 1)^2 - 8 * (R800 - R670)})$	Canopy Chl	(Main et al., 2011 & Qi et al., 1994)
mSR	$(R800 - R445) / (R680 - R445)$	Total Chl	(Main et al., 2011 & Sims and Gamon, 2002)
mSR3	$((R800 / R670) - 1) / (((R800 / R670)^{0.5} + 1)$	Canopy Chl	(Rodrigues et al., 2014 & Chen, 1996)
mSR2	$(R750 / R705) - 1 / \sqrt{(R750 / R705) + 1}$	Canopy Chl	(Main et al., 2011 & Chen, 1996)
mSR705	$(R750 - R445) / (R705 - R445)$	Total Chl	(Main et al., 2011 & Sims and Gamon, 2002)
MTCI	$(R754 - R709) / (R709 - R681)$	Canopy Chl	(Main et al., 2011 & Dash and Curran, 2004)
MTVI1	$1.2 * (1.2 * (R800 - R550) - 2.5 * (R670 - R550))$	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2004)
	$(1.5 * (1.2 * (R800 - R550) - 2.5 * (R670 - R550))) / (\sqrt{(2 * R800 + 1)^2 -$		
MTVI2	$(6 * R800 - 5 * \sqrt{(R670) - 0.5)})$	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2004)
ND	$(R925 - R710) / (R925 + R710)$	Canopy Chl	(le Maire et al., 2008)
NDRE	$(R790 - R720) / (R790 + R720)$	Nitrogen, Chl	(Winterhalter et al., 2011 & Rodriguez et al., 2006)
NDVI	$(R800 - R670) / (R800 + R670)$	Canopy Chl	(Main et al., 2011 & Tucker, 1979)
NDVI2n	$(R750 - R705) / (R750 + R705)$	Chl a	(Main et al., 2011 & Gitelson and Merzlyak, 1994)
NDVI3n	$(R682 - R553) / (R682 + R553)$	Canopy Chl	(Main et al., 2011 & Gandia et al., 2004)
NDVIc	$(R895 - R675) / (R895 + R675)$	Canopy Chl	(Colombo et al., 2008)
NDVIw	$(R800 - R680) / (R800 + R680)$	Nitrogen	(Winterhalter et al., 2011 & Misteale and Schmidhalter, 2008)
NIR.green	R780/R550	Nitrogen	(Winterhalter et al., 2011 & Misteale and Schmidhalter, 2008)
NIR.NIR	R780/R740	Nitrogen	(Winterhalter et al., 2011 & Misteale and Schmidhalter, 2008)
NIR.red	R780/R700	Nitrogen	(Winterhalter et al., 2011 & Misteale and Schmidhalter, 2008)
NPCI	$(R680 - R430) / (R680 + R430)$	Chlorophyll	(Main et al., 2011 & Peñuelas et al., 1994)
NPI	$(R415 - R435) / (R435 + R415)$	Chlorophyll	(Peñuelas et al., 1995)
OCAR	R630/R680	Chl & RWC	(Winterhalter et al., 2011 & Schlemmer et al., 2005)
OSAVI	$((1 + 0.16) * (R800 - R670)) / ((R800 + R670 + 0.16))$	Canopy Chl	(Rodrigues et al., 2014 & Rondeaux et al., 1996)
OSAVI2	$(1 + 0.16) * (R750 - R705) / (R750 + R705 + 0.16)$	Canopy Chl	(Main et al., 2011 & Wu et al., 2008)
PRICI	$((R531 - R570) / (R531 + R570)) * ((R760 / R700) - 1)$	Carotenoid	(Rodrigues et al., 2014 & Garrity et al., 2011)
PSNDa	$(R800 - R680) / (R800 + R680)$	Chl a	(Blackburn, 1998)
PSNDb	$(R800 - R635) / (R800 + R635)$	Chl b	(Blackburn, 1998)
PSNDc	$((R800 - R470) / (R800 + R470))$	Carotenoids	(Rodrigues et al., 2014 & Blackburn, 1998)
PSRI	$(R678 - R500) / R750$	Carotenoid/Chl ratio	(Rodrigues et al., 2014 & Merzlyak et al., 1999)
PSSRa	$(R800 / R680)$	Chl a	(Rodrigues et al., 2014 & Blackburn, 1998)
PSSRb	$(R800 / R635)$	Chl b	(Rodrigues et al., 2014 & Blackburn, 1998)
PSSRc	$(R800 / R470)$	Carotenoids	(Rodrigues et al., 2014 & Blackburn, 1998)
RARSa	$(R675 / R700) / (Ref675 / Ref700)$	Chl a	(Chappelle et al., 1992)
RARSb	$(R675 / R650 * R700) * (Ref650 * Ref700 / Ref675)$	Chl b	(Chappelle et al., 1992)
RARSc	$(R760 / R500) / (Ref760 / Ref500)$	Carotenoids	(Chappelle et al., 1992)
RDVI	$(R800 - R670) / (\sqrt{(R800 + R670)})$	Biomass	(Roujean and Breon, 1995 & Rodrigues et al., 2014)
REP.Li	$700 + 40 * ((R670 + R780 / 2) / (R740 - R700))$	Total Chl	(Main et al., 2011)
RGI	$(R690 / R550)$	Pigments	(Zarco-Tejada et al., 2005)
RNIR.CRI550	$(1 / R510) - (1 / R550) * R770$	Pigments	(Rodrigues et al., 2014)
RNIR.CRI700	$(1 / R510) - (1 / R700) * R770$	Pigments	(Rodrigues et al., 2014)
SARVI2	$2.5 * (R800 - R670) / (1 + R800 + (6 * R670) - (7.5 * R475))$	Canopy Chl	(Huete et al., 1997)
SARVI2m	$2.5 * ((R800 - R670) / (R800 - (6 * R670) - (7.5 * R475) + 1))$	Canopy Chl	(Main et al., 2011)
SIPI	$(R800 - R445) / (R800 - R680)$	Pigments	(Main et al., 2011 & Blackburn, 1998)
SPVI	$0.4 * (3.7 * (R800 - R670) - 1.2 * \text{abs}(R550 - R670))$	Canopy Chl	(Main et al., 2011 & Vincini et al., 2006)
SR	R800/R675	Canopy Chl	(Jordan, 1969)
SR1	R750/R700	Total Chl	(Main et al., 2011 & Gitelson and Merzlyak, 1997)
SR2	R752/R690	Total Chl	(Main et al., 2011)

Supplementary Table 1 Continued

SR3	R750/R550	Total Chl	(Main et al., 2011 & Gitelson and Merzlyak, 1997)
SR4	R700/R670	Total Chl	(Main et al., 2011)
SR5	R675/R700	Chl a	(Main et al., 2011)
SR6	R750/R710	Total Chl	(Main et al., 2011)
SRc	R895/R675	Canopy Chl	(Colombo et al., 2008)
SRPI	R430/R680	Chl a	(Main et al., 2011 & Peñuelas et al., 1995)
SRw	R900/R680	Canopy Chl	(Winterhalter et al., 2011) & (Aparicio et al., 2002)
TCARI	$3*((R700-R670) - 0.2*(R700-R550)*(R700/R670))$	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2002)
TCARI.OSAVI	TCARI.OSAVI	Canopy Chl	(Rodrigues et al., 2014 & Haboudane et al., 2002)
TCARI2	$3*((R750-R705) - 0.2*(R750-R550)*(R750/R705))$	Canopy Chl	(Main et al., 2011 & Wu et al., 2008)
TCARI2.OSAVI2	TCARI2.OSAVI2	Canopy Chl	(Main et al., 2011 & Wu et al., 2008)
TGI	$(-.5*((R660-R485)*(R680-R530) - (R660-R560)*(R640-R510)))$	Total Chl	(Hunt et al., 2011)
TVI	$0.5*(120*(R750-R550) - 200*(R670-R550))$	Canopy Chl	(Main et al., 2011 & Broge and Leblanc, 2000)
Vog2	$(R734-R747)/(R715+R726)$	Total Chl	(Zarco-Tejada et al., 2001)
Vog3	$(R734-R747)/(R715+R720)$	Total Chl	(Zarco-Tejada et al., 2001)
Vogelmann	R740/R720	Total Chl	(Main et al., 2011 & Vogelmann et al., 1993)
Vogelmann2	$((R734-R747)/n)/((R715-R726)/n)$	Total Chl	(Main et al., 2011 & Vogelmann et al., 1993)
YCAR	R600/R680	RWC, Chl	(Winterhalter et al., 2011 & Schlemmer et al., 2005)
ZTM3	(R750/R670)	Total Chl	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2001)
ZTM4	(R710/R700)	Total Chl	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2001)
ZTM5	(R710/R670)	Total Chl	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2001)
Carter	R695/R420	Stress	(Main et al., 2011 & Carter, 1994)
Carter2	R695/R760	Stress	(Main et al., 2011 & Carter, 1994)
Carter3	R605/R760	Stress	(Main et al., 2011 & Carter, 1994)
Carter4	R710/R760	Stress	(Main et al., 2011 & Carter, 1994)
Carter5	R695/R670	Stress	(Main et al., 2011 & Carter, 1994)
HREI15	$(R855-R720)/(R855+R720)$	Stress	(Thenkabail et al., 2014)
HREI16	$(R910-R705)/(R910+R705)$	Stress	(Thenkabail et al., 2014)
PRI	$(R531-R570)/(R531+R570)$	LUE	(Gamon et al., 1992)
PRI2	$((R570-R530)/(R570+R530))$	LUE	(Rodrigues et al., 2014 & Gamon et al., 1992)
PRI3	$((R550-R531)/(R550+R531))$	LUE	(Gamon et al., 1992)
SR7	R440/R690	Stress	(Main et al., 2011)
760/730	R760/R730	Nitrogen, CWM	(Winterhalter et al., 2011 & Misteke and Schmidhalter, 2010)
CWMI1	R850/R725	CWM	(Winterhalter et al., 2011)
CWMI2	R890/R715	CWM	(Winterhalter et al., 2011)
CWMI3	R980/R715	CWM	(Winterhalter et al., 2011)
HWMI17	$(R855-R970)/(R855+R970)$	Water Content	(Thenkabail et al., 2014)
NWI1	$(R970-R900)/(R970+R900)$	Water Status	(Gutierrez et al., 2010)
NWI3	$(R970-R880)/(R970+R880)$	Water Status	(Gutierrez et al., 2010)
PRI4	$((R512-R531)/(R512+R531))$	Stomatal Conductance	(Rodrigues et al., 2014 & Hernández-Clemente et al., 2011)
	$((R570-R531)/(R570+R531))/(((R800-R670)/(\sqrt{(R800+R670)})))*R700/R670$	Stomatal Conductance	(Rodrigues et al., 2014 & Zarco-Tejada et al., 2013)
PRIn	R701/R820	Stomatal Conductance	(Carter, 1998)
R701	R900/R970	Plant Water Content	(Winterhalter et al., 2011 & Peñuelas et al., 1997)
WBI	$(R900/R970)/((R800-R680)/(R800+R680))$	Plant Water Content	(Winterhalter et al., 2011 & Peñuelas et al., 1997)
WBLNDVI	(R520/R720)	Relative Water Content	(Zygielbaum et al., 2009)
ZRWC			

List of all vegetation indices used in this study. Indices are identified by their acronyms; the traits they measure and their calculations are provided. The letter R followed by a number is the reflectance value at that specific wavelength in nanometers. Chl = chlorophyll; RWC = relative water content; LUE = light use efficiency; CWM = canopy water mass

Literature Cited

- Aparicio, N., Villegas, D., Araus, J. L., Casadesus, J., & Royo, C. (2002). Relationship between growth traits and spectral vegetation indices in durum wheat. *Crop Science*, 42(5), 1547–1555.
- Blackburn, G. A. (1998). Spectral indices for estimating photosynthetic pigment concentrations: A test using senescent tree leaves. *International Journal of Remote Sensing*, 19(4), 657–675. <http://doi.org/10.1080/014311698215919>
- Broge, N. H., & Leblanc, E. (2000). Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. *Remote Sensing of Environment*, 76(2), 156–172.
- Carter, G. A. (1994). Ratios of leaf reflectance in narrow wavebands as indicators of plant stress. *International Journal of Remote Sensing*, 15(3), 697–703.
- Carter, G. A. (1998). Reflectance wavebands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. *Remote Sensing of Environment*, 63(1), 61–72.
- Chappelle, E. W., Kim, M. S., & McMurtrey III, J. E. (1992). Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sensing of Environment*, 39, 239–247.
- Chen, J. M. (1996). Evaluation of vegetation indices and a modified simple ratio for boreal applications. *Canadian Journal of Remote Sensing*, 22(3), 229–242.
- Colombo, R., Meroni, M., Marchesi, A., Busetto, L., Rossini, M., Giardino, C., & Panigada, C. (2008). Estimation of leaf and canopy water content in poplar plantations by means of hyperspectral indices and inverse modeling. *Remote Sensing of Environment*, 112(4), 1820–1834. <http://doi.org/10.1016/j.rse.2007.09.005>
- Dash, J., & Curran, P. J. (2004). The MERIS terrestrial chlorophyll index. *International Journal of Remote Sensing*, 25(23), 5403–5413. <http://doi.org/10.1080/0143116042000274015>
- Datt, B. (1998). Remote sensing of chlorophyll a, chlorophyll b, chlorophyll a+b, and total carotenoid content in eucalyptus leaves. *Remote Sensing of Environment*, 66(2), 111–121.

- Datt, B. (1999). Visible/near infrared reflectance and chlorophyll content in Eucalyptus leaves. *International Journal of Remote Sensing*, 20(14), 2741–2759. <http://doi.org/10.1080/014311699211778>
- Daughtry, C. S. T., Walthall, C. L., Kim, M. S., De Colstoun, E. B., & McMurtrey, J. E. (2000). Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment*, 74(2), 229–239.
- Gamon, J., Penuelas, J., & Field, C. (1992). A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sensing of Environment*, 41, 35–44.
- Gandia, S., Fernández, G., García, J. C., & Moreno, J. (2004). Retrieval of vegetation biophysical variables from CHRIS/PROBA data in the SPARC campaign. *ESA SP*, 578, 40–48.
- Garrity, S. R., Eitel, J. U. H., & Vierling, L. A. (2011). Disentangling the relationships between plant pigments and the photochemical reflectance index reveals a new approach for remote estimation of carotenoid content. *Remote Sensing of Environment*, 115(2), 628–635. <http://doi.org/10.1016/j.rse.2010.10.007>
- Gitelson, A., & Merzlyak, M. N. (1994). Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and maple leaves. *Journal of Photochemistry and Photobiology B: Biology*, 22(3), 247–252.
- Gitelson, A. A., Kaufman, Y. J., & Merzlyak, M. N. (1996). Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Remote Sensing of Environment*, 58(3), 289–298.
- Gitelson, A. A., & Merzlyak, M. N. (1997). Remote estimation of chlorophyll content in higher plant leaves. *International Journal of Remote Sensing*, 18(12), 2691–2697. <http://doi.org/10.1080/014311697217558>
- Gitelson, A. A., Buschmann, C., & Lichtenthaler, H. K. (1999). The chlorophyll fluorescence ratio F735/F700 as an accurate measure of the chlorophyll content in plants. *Remote Sensing of Environment*, 69, 296–302. [http://doi.org/10.1016/S0034-4257\(99\)00023-1](http://doi.org/10.1016/S0034-4257(99)00023-1)
- Gitelson, A., Gritz, Y., & Merzlyak, M. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, 160, 271–282.
- Gitelson, A. A., Keydan, G. P., & Merzlyak, M. N. (2006). Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. *Geophysical Research Letters*, 33(11). <http://doi.org/10.1029/2006GL026457>

- Gutierrez, M., Reynolds, M. P., Raun, W. R., Stone, M. L., & Klatt, A. R. (2010). Spectral water indices for assessing yield in elite bread wheat genotypes under well-irrigated, water-stressed, and high-temperature conditions. *Crop Science*, 50(1), 197–214.
- Haboudane, D., Miller, J. R., Tremblay, N., Zarco-Tejada, P. J., & Dextraze, L. (2002). Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. *Remote Sensing of Environment*, 81(2), 416–426.
- Haboudane, D., Miller, J. R., Pattey, E., Zarco-Tejada, P. J., & Strachan, I. B. (2004). Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. *Remote Sensing of Environment*, 90(3), 337–352.
<http://doi.org/10.1016/j.rse.2003.12.013>
- Hernández-Clemente, R., Navarro-Cerrillo, R. M., Suárez, L., Morales, F., & Zarco-Tejada, P. J. (2011). Assessing structural effects on PRI for stress detection in conifer forests. *Remote Sensing of Environment*, 115(9), 2360–2375.
<http://doi.org/10.1016/j.rse.2011.04.036>
- Hernández-Clemente, R., Navarro-Cerrillo, R. M., & Zarco-Tejada, P. J. (2012). Carotenoid content estimation in a heterogeneous conifer forest using narrow-band indices and PROSPECT+DART simulations. *Remote Sensing of Environment*, 127, 298–315. <http://doi.org/10.1016/j.rse.2012.09.014>
- Huete, A. R., Liu, H. Q., Batchily, K., & van Leeuwen, W. (1997). A Comparison of Vegetation Indices over a Global Set of TM Images for EOS-MODIS. *Remote Sensing of Environment*, 59, 440–451.
- Huete, A., Didan, K., Miura, T., Rodriguez, E. P., Gao, X., & Ferreira, L. G. (2002). Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment*, 83(1), 195–213.
- Hunt, E. R., Daughtry, C. S. T., Eitel, J. U. H., & Long, D. S. (2011). Remote sensing leaf chlorophyll content using a visible band index. *Agronomy Journal*, 103(4), 1090. <http://doi.org/10.2134/agronj2010.0395>
- Jordan, C. F. (1969). Derivation of leaf-area index from quality of light on the forest floor. *Ecology*, 50(4), 663. <http://doi.org/10.2307/1936256>
- le Maire, G., François, C., & Dufrêne, E. (2004). Towards universal broad leaf chlorophyll indices using PROSPECT simulated database and hyperspectral reflectance measurements. *Remote Sensing of Environment*, 89(1), 1–28.
<http://doi.org/10.1016/j.rse.2003.09.004>

- le Maire, G., Francois, C., Soudani, K., Berveiller, D., Pontailier, J., Bréda, N., Genet, H., Davi, H., Dufrêne, E. (2008). Calibration and validation of hyperspectral indices for the estimation of broadleaved forest leaf chlorophyll content, leaf mass per area, leaf area index and leaf canopy biomass. *Remote Sensing of Environment*, 112(10), 3846–3864. <http://doi.org/10.1016/j.rse.2008.06.005>
- Maccioni, A., Agati, G., & Mazzinghi, P. (2001). New vegetation indices for remote measurement of chlorophylls based on leaf directional reflectance spectra. *Journal of Photochemistry and Photobiology B: Biology*, 61(1), 52–61.
- Main, R., Cho, M. A., Mathieu, R., O’Kennedy, M. M., Ramoelo, A., & Koch, S. (2011). An investigation into robust spectral indices for leaf chlorophyll estimation. *ISPRS Journal of Photogrammetry and Remote Sensing*, 66(6), 751–761. doi:10.1016/j.isprsjprs.2011.08.001
- Merzlyak, M. N., Gitelson, A. A., Chivkunova, O. B., & Rakitin, V. Y. (1999). Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. *Physiologia Plantarum*, 106, 135–141.
- Mistele, B., & Schmidhalter, U. (2008). Spectral measurements of the total aerial N and biomass dry weight in maize using a quadrilateral-view optic. *Field Crops Research*, 106(1), 94–103. <http://doi.org/10.1016/j.fcr.2007.11.002>
- Mistele, B., & Schmidhalter, U. (2010). A comparison of spectral reflectance and laser-induced chlorophyll fluorescence measurements to detect differences in aerial dry weight and nitrogen uptake of wheat. In *10th International Conference of Precision Agriculture. Denver, Colorado*. Retrieved from <http://www.pe.wzw.tum.de/publikationen/pdf/sd695.pdf>
- Peñuelas, J., Gamon, J. A., Fredeen, A. L., Merino, J., & Field, C. B. (1994). Reflectance indices associated with physiological changes in nitrogen-and water-limited sunflower leaves. *Remote Sensing of Environment*, 48(2), 135–146.
- Peñuelas, J., Filella, I., Lloret, P., Muñoz, F., & Vilajeliu, M. (1995). Reflectance assessment of mite effects on apple trees. *International Journal of Remote Sensing*, 16(14), 2727–2733.
- Peñuelas, J., Piñol, J., Ogaya, R., & Filella, I. (1997). Estimation of plant water concentration by the reflectance water index WI (R900/R970). *International Journal of Remote Sensing*, 18(13), 2869–2875.
- Qi, J., Chehbouni, A., Huete, A. R., Kerr, Y. H., & Sorooshian, S. (1994). A modified soil adjusted vegetation index. *Remote Sensing of Environment*, 48, 119–126.

- Rodrigues, F. A., Ortiz-Monasterio, I., Zarco-Tejada, P. J., Ammar, K., & Gérard, B. (2014). Using precision agriculture and remote sensing techniques to improve genotype selection in a breeding program. Retrieved from <http://quantalab.ias.csic.es/pdf/Rodrigues%20Jr.%20et%20al.%202014.pdf>
- Rodriguez, D., Fitzgerald, G. J., Belford, R., & Christensen, L. K. (2006). Detection of nitrogen deficiency in wheat from spectral reflectance indices and basic crop eco-physiological concepts. *Australian Journal of Agricultural Research*, 57(7), 781. <http://doi.org/10.1071/AR05361>
- Rondeaux, G., Steven, M., & Baret, F. (1996). Optimization of soil-adjusted vegetation indices. *Remote sensing of environment*, 55(2), 95-107.
- Roujean, J. L., & Breon, F. (1995). Estimating PAR absorbed by vegetation from bidirectional reflectance measurements. *Remote Sensing of Environment*, 51(3), 375–384. [http://dx.doi.org/10.1016/0034-4257\(94\)00114-3](http://dx.doi.org/10.1016/0034-4257(94)00114-3)
- Schlemmer, M. R., Francis, D. D., Shanahan, J. F., & Schepers, J. S. (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agronomy Journal*, 97(1), 106–112.
- Sims, D. A., & Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, 81(2), 337–354.
- Thenkabail, P. S., Gumma, M. K., Teluguntla, P., & Mohammed, I. A. (2014). Hyperspectral remote sensing of vegetation and agricultural crops. *Photogrammetric Engineering & Remote Sensing*, 80(8), 697–709.
- Tucker, C. J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing of Environment*, 8(2), 127–150.
- Vincini, M., Frazzi, E., & D'Alessio, P. (2006). Angular dependence of maize and sugar beet VIs from directional CHRIS/Proba data. In *Proc. 4th ESA CHRIS PROBA Workshop* (pp. 19–21). Retrieved from http://www.researchgate.net/profile/Ermes_Frazzi/publication/228413259_Angular_dependence_of_maize_and_sugar_beet_VIs_from_directional_CHRISProba_data/links/0046352d50c18b3fe6000000.pdf
- Vogelmann, J. E., Rock, B. N., & Moss, D. M. (1993). Red edge spectral measurements from sugar maple leaves. *International Journal of Remote Sensing*, 14(8), 1563–1575.
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011). High throughput phenotyping of canopy water mass and canopy temperature in well-watered and

- drought stressed tropical maize hybrids in the vegetative stage. *European Journal of Agronomy*, 35(1), 22–32. <http://doi.org/10.1016/j.eja.2011.03.004>
- Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011). High-throughput sensing of aerial biomass and above-ground nitrogen uptake in the vegetative stage of well-watered and drought stressed tropical maize hybrids. *Crop Science*, 51(2), 479–489.
- Wu, C., Niu, Z., Tang, Q., & Huang, W. (2008). Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. *Agricultural and Forest Meteorology*, 148(8-9), 1230–1241. <http://doi.org/10.1016/j.agrformet.2008.03.005>
- Zarco-Tejada, P. J., Miller, J. R., Noland, T. L., Mohammed, G. H., & Sampson, P. H. (2001). Scaling-up and model inversion methods with narrowband optical indices for chlorophyll content estimation in closed forest canopies with hyperspectral data. *Geoscience and Remote Sensing, IEEE Transactions on*, 39(7), 1491–1507.
- Zarco-Tejada, P. J., Pushnik, J. C., Dobrowski, S., & Ustin, S. L. (2003). Steady-state chlorophyll a fluorescence detection from canopy derivative reflectance and double-peak red-edge effects. *Remote Sensing of Environment*, 84(2), 283–294.
- Zarco-Tejada, P., Berjon, A., Lopez-Lozano, R., Miller, J., Martin, P., Cachorro, V., Gonzalex, M. R., de Frutos, A. (2005). Assessing vineyard condition with hyperspectral indices: Leaf and canopy reflectance simulation in a row-structured discontinuous canopy. *Remote Sensing of Environment*, 99(3), 271–287. <http://doi.org/10.1016/j.rse.2005.09.002>
- Zarco-Tejada, P. J., González-Dugo, V., Williams, L. E., Suárez, L., Berni, J. A. J., Goldhamer, D., & Fereres, E. (2013). A PRI-based water stress index combining structural and chlorophyll effects: Assessment using diurnal narrow-band airborne imagery and the CWSI thermal index. *Remote Sensing of Environment*, 138, 38–50. <http://doi.org/10.1016/j.rse.2013.07>
- Zygielbaum, A. I., Gitelson, A. A., Arkebauer, T. J., & Rundquist, D. C. (2009). Non-destructive detection of water stress and estimation of relative water content in maize. *Geophysical Research Letters*, 36(12). <http://doi.org/10.1029/2009GL03>