Effects of Selenium Application on Heat Tolerance in Peanut Seedlings

Chad Michael Benton

Research Project Report submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Agricultural and Life Sciences

Dr. Maria Balota
Dr. Anton Baudoin
Dr. Ames Herbert

December 3rd, 2012
Suffolk, Virginia

Keywords: Peanut Seedling, Selenium, Heat Stress

Studying the Effects of Selenium Application on Heat Tolerance in Peanut Seedlings

Chad Michael Benton

ABSTRACT

Peanut production faces many challenges in the world today, and one such issue is heat stress. One of the implications of heat stress on the plants is oxidative stress, which is detrimental to plant health and physiological function. A method to combat oxidative stress could be through the use of antioxidants such as Selenium (Se), which is thought to increase growth and development of plant material.

This experiment evaluated tolerance to heat stress in four-week-old seedlings from a set of diverse peanut genotypes. Additionally, the antioxidant activity of Selenium was examined to determine if a) it allowed for increased heat tolerance, and b) if it was associated with specific genotypes. At the conclusion of the treatments, SPAD chlorophyll measurements and fresh-weights were taken, with dry-weights being calculated after seedlings were oven-dried at 80˚C for 48 hours. The experiment concluded that Se did not produce a significant change in tolerance to heat stress. Genotype had a significant effect on SPAD chlorophyll readings and dry-weight. Additionally, temperature did not have a significant effect on dry-weight, but did on SPAD chlorophyll readings and fresh-weight. The effect of temperature on SPAD readings is of interest as the above optimal temperature of 39˚C produced higher relative chlorophyll contents than the 26˚C normal temperature. Due to this result, seedling total leaf area (LA) was further measured to determine if a potential trend could exist. Even though not statistically significant, LA was slightly smaller for seedlings at 39˚C treatment compared to the 26˚C treatment.

In summary, the experiment did not discover genotypes that allowed for a significant improvement in tolerance to above-optimal temperatures or validate Se as an additive for increased tolerance to heat stress and improved seedling growth. However, it did demonstrate the ability of peanut seedlings to withstand an increase in temperature at an early stage without significant detrimental impacts.

Acknowledgments

I would like to begin by thanking my Academic Advisor and Advisory Committee Chair, Dr. Maria Balota, for all her direction and guidance over the past three years. She challenged me from our first conversation, and has introduced me to many new and rewarding challenges through the course of this research project. I would also like to thank Dr. Anton Baudoin and Dr. Ames Herbert for their time and involvement on my Advisory Committee and helping me navigate through this program of study. Performing this program at a distance could potentially be a challenge, but has not been the case due to these individuals at Virginia Tech.

I am indebted to BASF Plant Science as a whole for the opportunity to further my education. In particular I would like to thank Dr. Paula Olhoft and Dr. Hee-Sook Song for allowing me to pursue this program and elevate my understanding in our field of Agricultural Biotechnology. Much gratitude and appreciation goes to Dr. Jill Stevenson-Paulik for gaining approval to perform this research at BASF Plant Science, and for the resources to carry it to completion. Without her efforts, this particular experiment could not have happened in RTP.

Finally, I would like to thank my wife for her complete support and encouragement throughout my time with Virginia Tech. While both taking courses and welcoming our first child into the world in October 2011, it has been an exciting journey to share together.

Table of Contents

Abstract i

Acknowledgments ii

Table of Contents iii

List of Figures iv

Lists of Tables v

1. Introduction 1
    1.1 Peanut Crop Overview…….....1
    1.2 Variety, Cultural, and Climate Characteristics …….....3
    1.3 Research Overview and Scope 4

2. Literature Review 5
    2.1 Diverse Genotypes …….....5
    2.2 Heat Stress …….....6
    2.3 Oxidative Stress …….....10
    2.4 Selenium Interaction …….....12

3. Materials and Methods …….....15
    3.1.1 Growth Conditions Experiment 15
    3.1.2 Growth Conditions Experiment Results and Discussion ….....16
    3.2 Full Implementation Screen Materials and Methods ……..…18
    3.3 Additional Methods for Replicate Four….....21

4. Results……...……22
    4.1 SPAD…….....22
    4.2 Dry Weight …….....24
    4.3 Fresh Weight …….....25
    4.4 Leaf Area ……27

5. Discussion and Conclusion ……..29

6. Final Thoughts and Reflection …..……32
    6.1 Limitations of Research ……..32
    6.2 Future Implications of Research ……..……..32
    6.3 Personal Reflection ….……33

7. Citations ……35

List of Figures

Figure 1.1.1 Peanut production areas in the United States…….……1

Figure 1.1.2 Distribution of peanut yield for peanut growing regions in the U.S.A. ……2

Figure 3.1.1 Seedlings two weeks after planting ……..17

Figure 3.1.2 Seedlings four weeks after planting …….17

Figure 3.2.1 Plants at day of treatment …….19

Figure 3.2.2 Plants in track sprayer ……..19

Figure 3.2.3 The last two leaflets on the youngest fully expanded leaf ……..20

Figure 3.3.1 Freshly harvested seedlings prepared for LA (leaf area) …….21

Figure 3.3.2 Freshly harvested leaf material being measured (for leaf area) ……..21

Figure 4.1.1 SPAD Measurements for 26˚C control and 26˚C Se ……23

Figure 4.1.2 SPAD Measurements for 39˚C control and 39˚C Se ……23

Figure 4.2.1 Dry Weight Measurements for 26˚C control and 26˚C Se treated ……...24

Figure 4.2.2 Dry Weight Measurements for 39˚C control and 39˚C Se treated ……...25

Figure 4.3.1 Fresh Weight Measurements for 26˚C control and 26˚C Se ……26

Figure 4.3.2 Fresh Weight Measurements for 39˚C control and 39˚C Se ……26

Figure 4.4.1 Leaf Area Measurements for 26˚C control and 26˚C Se ……..27

Figure 4.4.2 Leaf Area Measurements for 39˚C control and 39˚C Se treated ……..28

Figure 4.4.3 Comparison of Leaf Area measurements of Control 26˚C and Control …...28

Figure 5.1.1 14 DAT seedling at 26˚C (left) and 39 ˚C (right) …….30

List of Tables

Table 1.1.1 Worldwide peanut production ……1

Table 3.1.1 Soil and Container Development Data……...16

Table 3.2.1 Genotype Name and Unique ID ……18

Table 4.1.1 Analysis of Variance Data for SPAD ……22

Table 4.2.1 Analysis of Variance Data for Dry Weight Measurements ……...24

Table 4.3.1 Analysis of Variance Data for Fresh Weight Measurements ……25

Table 4.4.1 Analysis of Variance and Least Squares Means Data for Leaf Area …….27

1. Introduction

1.1 Peanut Crop Overview

Peanuts are a very important crop today both in the United States and worldwide. "World peanut production totals approximately 29 million metric tons per year, with the U.S. being the world's third largest producer, after China and India. Worldwide peanut exports are approximately 1.25 million metric tons [American Peanut Council, 2011]." Table 1.1.1 displays the worldwide peanut production figures and the key countries of production [Soyatech, 2012]. The U.S. produces nearly 1.9 million metric tons of peanuts on roughly 1.44 million acres. Of this total, an average of 250,000 to 300,000 metric tons of peanut are exported each year with over eighty percent going to Canada, Mexico, and Europe [American Peanut Council, 2011].

Table 1.1.1 Worldwide peanut production

Worldwide Peanut Production (million metric tons)		
#1	China:	13,420,000
#2	India:	7,700,000
#3	United State	1,880,000
#4	Nigeria:	1,510,000
#5	Indonesia:	1,130,000
#6	Burma:	710,000
#8	Chad:	450,000
#9	Senegal:	450,000
#10	Ghana:	440,000
#11	Argentina:	420,000
#12	Vietnam:	400,000
#13	Sudan:	370,000
#14	Congo, Dem	360,000
#15	Burkina Faso	320,000
#16	Guinea:	250,000
#17	Brazil:	220,000
#18	Egypt:	190,000
#19	Mali:	160,000
#20	Mexico:	90,000
	Total:	30,470,000

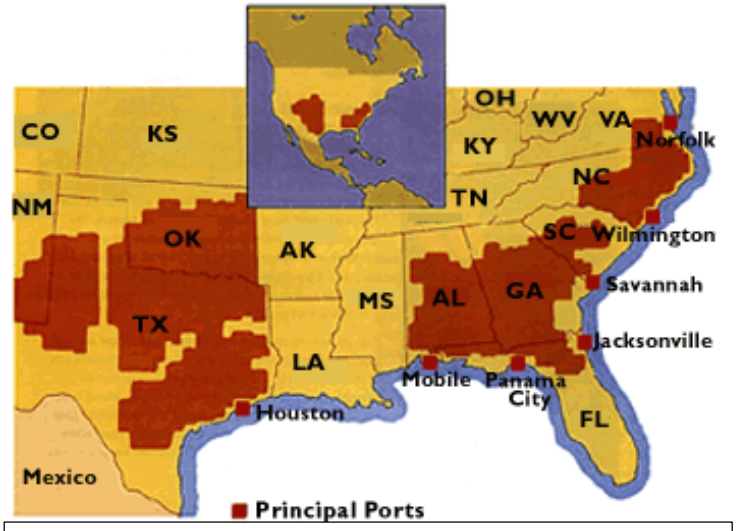


Figure 1.1.1 Peanut Production Areas in the United States

Figure 1.1.1 [SoyaTech, 2012] displays the main peanut production areas in the Unites States, and also shows key ports

near these areas allowing for ease of transport [Soyatech, 2012]. Seven states account for approximately 99% of all peanuts grown in the U.S with Georgia leading the way with 41%, followed by Texas at 24%, Alabama at 10%, North Carolina at 9%, Florida at 6%, Virginia at 5%, and Oklahoma with 5% [American Peanut Council, 2011]. This can also be visualized from the USDA chart indicating the pounds per acre by county in the U.S. during 2010 [USDA NASS 2012]. Figure 1.1.2 illustrates the high yields that are obtained in southern Georgia and Alabama, as well as in Texas compared to the other production areas. Yield differences can be due to the differences in varieties and market types that are grown in each region.

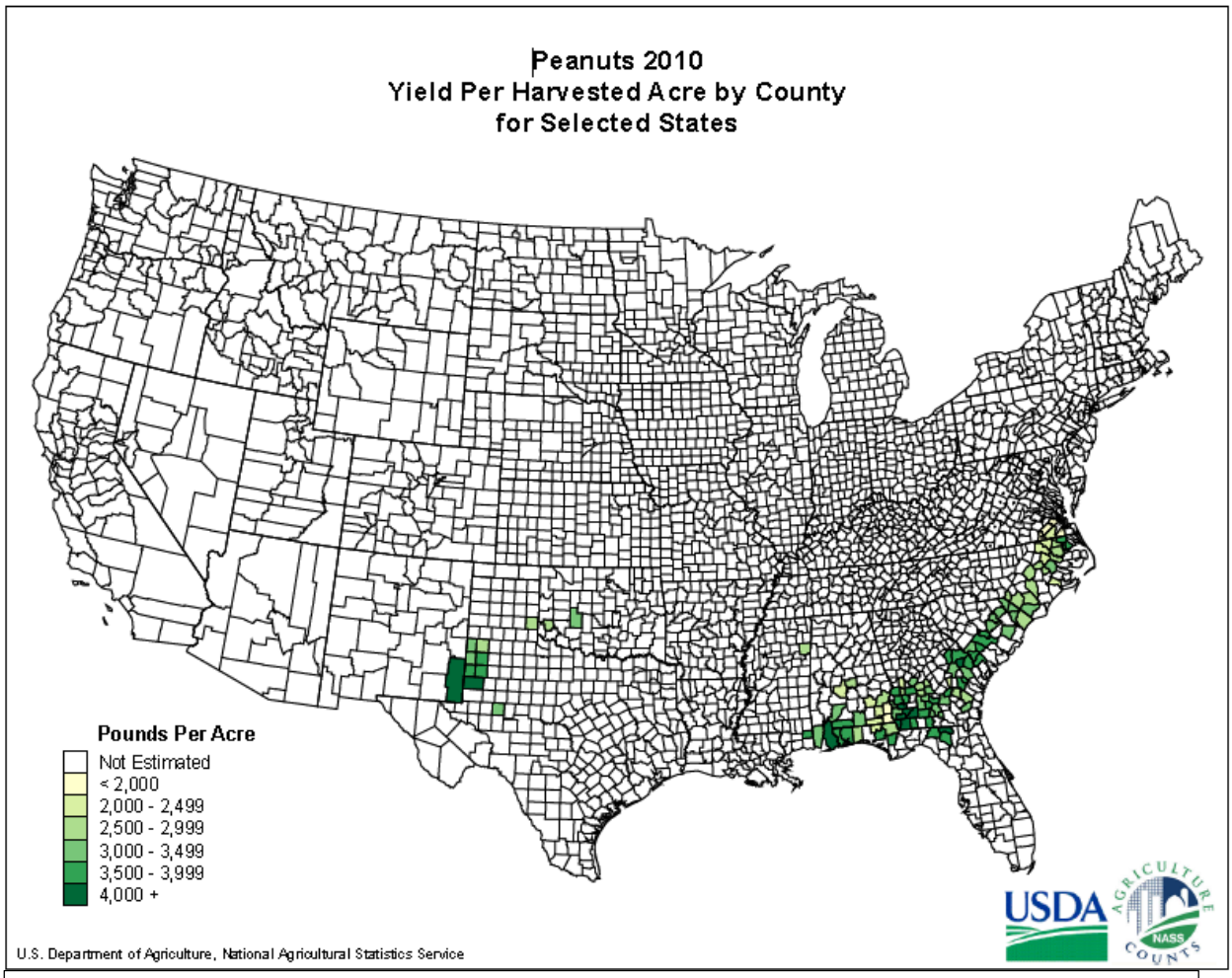


Figure 1.1.2 Distribution of peanut yield for peanut growing regions in the U.S.A.

Peanut production is currently on the rise. In 2012, 1.42 million acres of peanut were planted, which is up twenty-five percent from 2011. A record high acreage was planned in South Carolina for 2012 and, if realized, the planted area in Florida and Mississippi would be the highest since 1951 and 1943, respectively. This increase is being driven by higher peanut prices and low supply [NASS, 2012].

1.2 Variety, Cultural, and Climate Characteristics

In the U.S. there are four major types of peanuts used in production: Runner, Virginia, Spanish, and Valencia. Runner type is the most common and used primarily for peanut butter. Virginia type is large-kerneled and marketed as snack peanuts and in-shell products. Spanish type has smaller and rounder kernels than other types, and is used for snacks, peanut butter, and confections. Valencia type has longer pods and contains three to five kernels in each shell. These are mostly grown for in-the-shell uses such as roasting and boiling [American Peanut Council, 2011]. In the U.S., the runner-type is the predominant market type, while the virginia-type cultivars are traditionally grown in the Virginia and Carolinas, known as VC region [Balota et al., 2010].

"U.S. peanuts are planted after the last frost in April or May when the soil temperatures reach 20˚C. Pre-planting tillage ensures a well-prepared seedbed. Seeds are planted one to one and a half inches deep, one every two to four inches in the Southeast and Southwest, and four to six inches in the Virginia-Carolina area, in rows about three feet apart. A climate with 200 frost-free days (175 for Spanish varieties) is required for a good crop. Warm weather, adequate moisture, soil fertility, and sandy soils result in emergence after 10-14 days after planting [American Peanut Council, 2011.]"

In the 2012 Virginia Peanut Production Guide, it is noted that the base temperature required for peanut to germinate, grow, and produce yield is approximately 13˚C. The optimal growth temperature is between 25˚C and 30˚C, with growth being significantly slower below 15.5˚C and above 35˚C. As the plants mature, the optimum temperatures for flowering and fruit-setting are between 28˚C and 33˚C [Virginia Cooperative Extension, 2012].

"Photosynthesis and vegetative growth are well adapted to high temperature… [and] the optimum mean diurnal temperature for these processes is between 30 and 35˚C. In contrast, reproductive processes [are] extremely sensitive to high air temperature [Craufurd et al., 2003]." The pods and kernels develop and mature underground so tolerance to high soil temperature is also required. High soil temperatures of approximately 38˚C also reduced dry matter accumulation, flower production, pod count, and seed mass [Craufurd et al., 2003].

1.3 Research Overview and Scope

This research experiment was carried out at BASF Plant Science, in Research Triangle Park, NC. For this experiment, a diverse set of peanut germplasm was utilized to analyze the impact of heat stress on young seedlings. In addition, selenium (Se) was applied via foliar spray to determine its effect on growth habits when the plants were subjected to optimal and supra-optimal temperature regimen.

The objectives of this experiment were to: a) analyze peanut genotypes for a differential response to heat stress during early growth, determining tolerant and susceptible lines, and b) validate the efficacy of Se in providing tolerance to heat and improving plant performance under heat stress.

2. Literature Review

2.1 Diverse Genotypes

Utilizing diverse genotypes is one of many methods to determine varying germplasm performance when subjected to experimental treatments. This approach allows for the opportunity to study the phenotype and then potentially associate the gene involved in phenotypic expression. Once discovered, it allows for multiple pathways of incorporating the trait of interest into other lines through traditional breeding or from transgenic approaches. The key to using this method is to ensure that the germplasm used is diverse enough to be able to utilize such an approach.

One issue to overcome has been identifying and verifying the diversity, and then producing populations that are logistically feasible to study in an experiment. For example, Holbrook and Dong [2005] worked to create a "mini-core" population by examining a core collection of 831 U.S. peanut accessions that had been shown to improve the efficiency of identifying genes of interest in the complete germplasm population [Holbrook and Dong, 2005]. In the 2005 study, they sought to narrow down the 831 lines into a set that still maintained the diversity and ability to identify genes of interest. By examining attributes such as growth habit, size, leaf color, maturity, pod shape, pod weight, seed weight, and leaf spot among many others they compared the mean scores of the mini-core to that of the total core population. By achieving comparable scores for the traits mentioned above, they were able to shrink the sample size down from 831 to 112 and still maintained the ability to discover genes while using fewer germplasm resources in the process [Holbrook and Dong, 2005].

Aside from the U.S. core population, another study by Holbrook et al. [2011] studied the impact that molecular genetic research can have on new cultivar development. They noted that "Molecular breeding in peanut has lagged that of many crops in part due to a lack of investment, but also because of low levels of molecular polymorphism among cultivated varieties… [Furthermore] Genomic research might also be used to enhance the amount of genetic diversity available for application in conventional breeding [Holbrook et al., 2011]." They discussed the use of wild diploid *Arachis* populations for new cultivar development as many have high levels of resistance to pests and diseases. The key issue with *Arachis* is the ploidy level, as cultivated peanut is a tetraploid. The authors note that introgression is tough using natural breeding techniques but solutions are being developed [Holbrook et al., 2011]. Overall, this would introduce another set of diverse germplasm for future research.

For this particular experiment, it is important to note that genotypes across several crops are known to differ in heat resistance [Craufurd et al., 2003]. The ability to produce and assess the genotypic variation is a critical component to the utilization of the diverse genotypes for trait development and introgression. However, it can be equally important to ensure that no genotypic variation exists when choosing particular genotypes for alternative traits.

2.2 Heat Stress

Considering the warm climates that peanuts are grown in, the ability to withstand heat stress can be a very important trait. In parts of the world such as the semi-arid regions of India and Africa, water and high temperature stresses are constraints of concern for farmers. Predicted increases of average temperature of 1.4-6˚C as well as increases in

variability of temperature can add to these concerns [Craufurd et al., 2003]. These increased temperatures are likely to cause serious damage to growth and yield of C3 crops, which include peanuts [Soliman et al., 2012]. Due to the potential increase in heat stress, heat-tolerant genotypes will be needed to sustain production in these environments [Craufurd et al., 2003].

Chauhan and Senboku [1997] stated that "to meet the growing demand for food…crops are increasingly grown outside their traditional area of adaptation and outside their natural growing seasons. [Due to this] daily or seasonal temperature becomes a major factor limiting crop production [Chauhan and Senboku, 1997]." They mentioned the importance of discovering heat-tolerant genotypes to be used for future breeding efforts. In their study they looked at six different genotypes of peanut, and two of those genotypes were used for hardening studies as well. Hardening is a process of exposing seed or plant material to sub- or above-optimal conditions in order to prepare them for an environmental change. "It has also been shown that hardening treatments improve the heat tolerance of wheat, cucumber, and soybean [Chauhan and Senboku, 1997]." For their peanut study, a temperature of 30˚C was used for non-hardening while combinations of 30˚C and 37˚C were used for hardening at one month after sowing. In addition, leaf discs were taken at thirty days after sowing for determinations of chlorophyll fluorescence and electrolyte leakage. Electrolyte leakage is a measure of the thermostability of cellular membranes before and after exposure to stress. First, leaf discs are placed in distilled water for 24 hours. Then the electric conductivity of the solution is measured with a conductivity meter before and after the heat treatment. Chlorophyll fluorescence is a measure of light efficiency for photosynthesis through the

use of a fluorimeter. For the fluorescence measurements, the leaf discs were stressed at 49˚C, 51˚C, 53˚C, or 55˚C for 5 minutes in a water bath to induce heat injury and were then placed in a dark room for 30 minutes at room temperature prior to being measured [Chauhan and Senboku, 1997]. The results showed a differential response between genotypes due to hardening treatments, as well as differences in chlorophyll fluorescence. Of interest is that the chlorophyll concentrations of all genotypes except one increased when plants were hardened at 37˚C. Also, there was a positive linear correlation between chlorophyll fluorescence and chlorophyll concentration after a heat stress treatment of 49˚C, which was the lowest heat treatment they performed [Chauhan and Senboku, 1997].

Though not analyzed in this experiment, research has also looked into the impact of soil temperature on peanut performance. As noted in the introduction, increased soil temperatures can have an impact on peanut production. A 2003 study utilized growth chambers to analyze ten peanut genotypes varying in heat tolerance/susceptibility. All plants were grown at 28˚C day and 22˚C night temperatures from sowing through pod formation at 45 days after sowing. From this time-point through 90 days after sowing the plants were divided into two treatments: ambient soil temperature and ambient plus 10˚C. At harvest, the plants were separated into roots, leaves, stems, pegs and pods. The roots were washed to remove the potting medium and dry weights were obtained on roots, leaves, stems, pegs, pods, and kernels after oven drying at 60˚C for four days. Total dry matter, pod harvest index, and root-to-shoot ratio were calculated form the weights. The authors discovered that increasing the soil temperature from 28˚C to 38˚C during the day reduced dry matter accumulation between 20 and 28%. However, high soil temperature

had no significant effect on root-to-shoot ratio. The high soil temperature also had no effect on the ratio of pegs forming pods, and the ratio of pods with and without kernels was similar at both temperatures. However, pod size was larger under the optimal 28˚C temperature. Also of interest is that there were significant differences between genotypes in response to the high soil temperature [Craufurd et al., 2003].

This study also analyzed the effect of increased air temperature across twelve genotypes. The plants were maintained at the same 28/22˚C conditions from plant to either six days before flowering or to first flowering. At the appropriate time-point, three plants of each genotype were kept under optimal temperature or moved to the high temperature treatment of 38˚C. The high temperature was imposed for six days and afterwards the plants were moved back to the optimal temperature. Of note is that high air temperature increased the number of flowers that opened but had no effect on the number of pegs and pods that formed. The authors discovered that fruit-set was decreased from 71% to 58% by high temperature at both growth stages, six days before and at first flowering [Craufurd et al., 2003].

Up to this point, the studies have been focused on more mature plant stages, which means longer time needed to complete the screen. As a study by Selvaraj et al. [2010] points out, "As field screening for heat tolerance can be inconsistent and seasonally-limited, it is important to develop a reliable protocol under controlled conditions that allows simultaneous screening of multiple genotypes [Selvaraj et al., 2010]." The researchers devised a plan to examine leaf discs subjected to high heat stress conditions for acquired thermotolerance. SPAD chlorophyll readings were taken to measure chlorophyll accumulation during the temperature treatments, and served as an internal control. SPAD

stands for Special Products Analysis Division (of Minolta) and has become a common name for the units of measure obtained through the use of their chlorophyll concentration meter, which measure leaf light transmission at several wavelengths (Scottech, 2012). Seeds from 16 genotypes from the mini-core population were chosen for their diversity and tolerance/susceptibility to heat from a previous experiment by Kottapalli et al. [2006]. Three to five seeds per genotype were germinated in 0.1X Hoagland's solution for seven to ten days at 28˚C. After this time period, a preincubation treatment was performed for four hours at 38-40˚C prior to the heat challenge of 48-50˚C for thirty minutes. The leaf discs were then examined over an 18-hour evaluation period. The 50˚C challenge resulted in a complete loss of chlorophyll accumulation but 48˚C was not fully lethal [Selvaraj et al., 2010]. The authors also were able to determine that seed weight had no significant effect on acquired thermotolerance of the seedlings. Of note is that this study determined that chlorophyll accumulation decreased in the acquired thermotolerance treatments for all genotypes compared to the control, and noted previous studies showing thirty minutes at 48˚C or above resulted in more than 95% inhibition of chlorophyll accumulation [Selvaraj et al., 2010].

2.3 Oxidative Stress

"It is now well established that virtually all abiotic stresses induce or involve oxidative stress to some degree and the ability of plants to control oxidant levels is highly correlated with stress tolerance [Hasanuzzaman et al., 2010]." This is an excellent summation of the role that oxidative stress plays in physiological aspects of plants. Oxidative stress is further described as a "condition when the generation of reactive oxygen species (ROS) in a system exceeds the system's ability to neutralize and

eliminate them. If not regulated properly, excess ROS can damage cellular lipids, proteins or DNA, thus inhibiting signal transduction pathways, and, in general, normal cellular function [Hasanuzzaman et al., 2010.]" In line with this injury, heat stress is one such stressor that can lead to oxidative damage. The high temperature stress can promote the accumulation of ROS in the chloroplasts, especially when the antioxidant activity needed to detoxify ROS is low [Djanaguiraman et al., 2010.] It is noted that heat stress can also result in injury to membranes, pigments, proteins, and nucleic acids leading to impaired growth and development due to oxidative stress and the ROS that is produced [Kumar et al., 2012].

A study by Soliman et al. [2012] on *Lolium perenne* noted a linear relationship between accumulated hydrogen peroxide in leaves and functional damage measured by chlorophyll fluorescence during high temperature treatments. A sensitive variety of *L. perenne* accumulated larger quantities of hydrogen peroxide than a tolerant cultivar did. The authors concluded that the functional damage due to high temperature was caused by oxidative stress [Soliman et al., 2012]. Soliman et al [2012] also made a point of interest stating that a plant requires both low hydrogen peroxide production and high antioxidant activity to be able to obtain greater heat tolerance. Furthermore, they noted that a significant correlation was found between structural leaf properties such as leaf thickness and dry matter content and hydrogen peroxide concentration. This could play a key part in tolerance to oxidative stress [Soliman et al., 2012]. This brings back the importance of screening diverse genotypes in order to discover the differential response, if any, between the genotypes and if they have unique physiological traits such as thicker leaves or higher concentration of dry matter.

Overall, the ability of plants to minimize the impact of the oxidative stress is directly impacted by antioxidants, which will vary based on the species and genotype being produced [Kumar et al., 2012]. One such potential antioxidant and aid in detoxifying ROS will be discussed in the next section.

2.4 Selenium Interaction

Selenium (Se) is noted as an "essential micronutrient needed in antioxidation and hormone balance in human and animal cells [and has] immunostimulating, cardio protective and anti-carcinogenic activity in man and animals [Djanaguiraman et al., 2005]." Selenium is an integral part of the enzyme gluthione peroxidase, which is a seleno enzyme preventing oxidative damage to body tissue. Research has demonstrated that Se is not only able to promote growth and development, but to increase resistance and antioxidant capacity of plants [Hasanuzaman et al., 2010]. One such study has been conducted to determine if a spray application of Se can increase antioxidant enzyme activity and alleviate oxidative stress in high temperature environments [Djanaguiraman et al., 2010]. Though not an essential nutrient in higher plants, studies are showing that Se can protect plants from abiotic stresses and can "increase tolerance of plants exposed to lower temperature, drought stress and aluminum toxicity [Djanaguiraman et al., 2010]."

A study by Hartikainen et al. [2000] on ryegrass found that Se displayed dual effects on metabolism and growth. Selenium did, in fact, serve as an antioxidant at low concentrations. However, at high concentrations Se served as a pro-oxidant and at rates of greater than or equal to 10 mg/kg resulted in drastic yield losses [Hartikainen et al.,

2000]. Thus the application concentration and method is of importance to the potential efficacy.

The 2005 study by Djanaguiraman et al. [2005] examined Se ability to act as an antioxidant in soybeans during senescence and counteract related oxidative stress during this time period. A previous study indicated that application of up to 50 ppm Se in soybean increased yield by preventing chlorophyll degradation and maintaining longer leaf area duration [Djanaguiraman et al., 2005]. In this case, Se as sodium selenate was sprayed seventy-eight days after sowing, when the plants were at pod filling stage and the leaves were starting to senesce. Growth attributes such as plant height, number of leaves, total dry matter production, leaf area, and chlorophyll content were measured on the plants. Soybean leaf samples were taken at two and twelve days after treatment with Se. The authors discovered that Se application "significantly promoted the shoot growth in terms of shoot length, number of leaves per plant, total leaf area per plant and total dry matter production [Djanaguiraman et al., 2005]." Another point of interest is that leaf and grains varied significantly in their ability to accumulate Se. In both sprayed and control treatments, chlorophyll content decreased between 2 and 12 days after treatment with Se. However, total chlorophyll decreased 39.5% in the control compared to 24.3% in the Se treated plants when comparing twelve days after treatment plants to two days after treatment plants [Djanaguiraman et al., 2005].

In a follow-up study, Djanaguiraman et al. [2010] studied the effects on a foliar application of 75 mg/L selenium on sorghum when subjected to heat stress. The plants were sown in pots and allowed to grown for sixty-three days prior to treatment in a 16-hour photoperiod in a greenhouse prior to being placed into growth chambers to control

the temperature treatments. The plants were divided into four treatments consisting of two temperature regimes (optimum or high) and two Se treatments (treated or untreated). After the treatment, the plants were maintained for an additional forty-five days. The authors looked at physiological traits such as SPAD chlorophyll readings, leaf temperature, chlorophyll fluorescence and gas exchange. The measurements were taken on attached, fully expanded flag leaves every seven days for twenty-eight days. Furthermore, plant height was recorded at maturity and dry weights were measured as well. They discovered Se application significantly increased photosynthetic rate, stomatal conductance, and transpiration rate compared the unsprayed control. Across all treatments, chlorophyll content measured as SPAD chlorophyll units decreased from day seven to twenty-eight in the measurement period. Of interest, is that temperature and Se application did not influence plant height nor did it affect chlorophyll content. The heat stress did have a significant effect on yield formation determined by leaf dry weight, dry matter production, seed size, and seed filling weight. The Se foliar application significantly increased total dry matter production, seed size, and filled seed weight. The authors concluded that Se alleviated oxidative stress in sorghum by enhancing antioxidant defense mechanisms [Djanaguiraman et al., 2010]. This was noted by the significant increase of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase after Se treatment. These enzymes are involved in scavenging of reactive oxygen species that can accumulate under heat stress conditions [Djanaguiraman et al., 2010].

3. Materials and Methods

3.1.1 Growth Conditions Experiment

A developmental assay was first conducted to determine the optimal growth conditions for the experiment. The purpose was to establish the baseline growth habits in a growth chamber (Conviron TC30, Winnipeg, Canada) for the emergence and seedlings stages. The *A. hypogaea* variety Perry was utilized in this assay comparing two container and three soil types. The seeds were obtained from Dr. Maria Balota and were pre-treated with Trilex Star (Bayer Crop Science LP, Research Triangle Park, NC) to reduce incidence of seed diseases. Trilex Star is a peanut seed treatment that contains active ingredients of 40% captan, 2% trifloxystrobin, 13.6% thiophanate-methyl, and 0.8% metalaxyl (Bayer Crop Science LP, 2005). Environmental conditions were 26 ˚C day/night, 20 hour photoperiod, and 65% relative humidity. The plants were well watered in all treatments with no fertilizer applications.

The first container used was a square top pot with dimensions of four inches wide by five inches deep. The second was a two and a half inch circular container that had a depth of ten inches. Both containers can be seen in Figure 3.1.1. In addition, three different potting mixes were examined for use in the experiment. The first (Mix) was a 3:2:1 combination of RediEarth Potting Mix, NC Dark Sand, and Contractor's Light Color Sand. The second (CLS) was Contractor's Light Color sand only, and the third (MM360) was MetroMix360 Potting Medium. It is important to note that the MM360 came with a pre-charge of fertilizer, while the other soils did not have added nutrients. A total of three pots were seeded for each soil type, with three replications for each container type. The plants were allowed to grow for six weeks and were then destructively harvested.

The roots and shoots were separated and a fresh-weight (FW) was taken for each portion. The tissue samples were then placed into a mesh bag and stored in a dryer oven at 80˚C for 48 hours for the drying process. A dry-weight (DW) was taken for each sample at the end of the 48 hours. The MM360 and Mix soils were difficult to separate from the root material, thus this could have had an impact on the root weight measurements. The plants were also examined for root nodules at this time.

3.1.2 Growth Conditions Experiment Results and Decision

Table 3.1.1 Soil and Container Development Data

Soil and Container Evaluation Trial							
Peanut Variety: Perry			FW Taken on 11/20/11		DW Taken on 11/22/11		
Container	**Soil**	**Rep**	**Shoot FW (g)**	**Shoots DW (g)**	**Root FW**	**Root DW**	**Nodules**
Cone	Mix	1	17.20	4.37	7.98	0.98	No
Cone	Mix	2	13.76	2.93	6.73	0.71	No
Pot	Mix	1	21.42	5.90	9.99	1.66	No
Pot	Mix	2	21.64	5.75	2.35	2.34	No
Pot	Mix	3	22.53	5.00	4.00	1.39	No
Pot	MM360	1	27.08	6.90	23.31	3.24	Yes-1
Cone	Sand	1	5.84	2.13	4.27	1.04	No
Cone	Sand	2	5.20	1.42	3.45	0.52	Yes-3
Cone	Sand	3	7.97	2.77	3.70	0.42	Yes-4
Pot	Sand	1	12.01	3.50	5.80	0.24	Yes-10
Pot	Sand	2	10.17	3.26	3.85	0.85	Yes-18

Table 3.1.1 displays the results from the growth conditions experiment. The containers were single-seeded in this growth conditions experiment, and germination was not at one hundred percent for all treatments. This could have been due to the different water holding capacity of the soils and my lack of experience with peanut germination and growth conditions. With this in mind, the chart displays the fact that not all treatments had three replications as intended, and the MM360 allowed for germination in only 1 pot.

The root measurements are shaded in green to reflect the inability to fully clean the Mix and MM360 completely off of the roots. Fungal infection was noted on one of the MM360 container replicates. The first set of photos [Figure 3.1.1] is two weeks after seeding, while the second set of photos [Figure 3.1.2] is four weeks after seeding. This allows for a visual representation of the size of the plants at Se application and harvest.

Figure 3.1.1 Seedlings two weeks after planting

Figure 3.1.2 Seedlings four weeks after planting

The plants in the contractors sand alone appeared to be stunted compared to the other treatments, and displayed minor leaf stress symptoms that could be attributed to nutrient deficiency. The shoot freshweights were lower than the Mix and the MM360 as well. After reviewing the germination percentages, fresh weights, and overall plant health it was decided to move forward with the Mix soil in the square pot for the full implementation screen.

3.2 Full Implementation Screen Materials and Methods

Table 3.2.1 Genotype Name and Unique ID

Unique ID	Line
31977	N05024J
31978	N05006
31979	Georgia Green
31980	GA06G
31981	Gregory
31982	Georgia 08V
31983	Florida07
31984	Florun107
31985	Florunner
31986	VA98R
31987	Perry
31988	N04074FCT
31989	GA09B
31990	AP-4
31991	Mississippi Giant
31992	Titan
31993	CHAMPS
31994	Phillips
31995	GAGreener
31996	Middleton
31997	NC-V11
31998	Bailey
31999	Spain
32000	Streeton
32001	N05008
32002	VT024051
32003	Florida Fancy
32004	Sugg
32005	SPT06-07
32006	Tifguard

For the implementation screen, the number of genotypes was increased to thirty. Each line was given a unique identifier to use for the experiments instead of the line designation. Table 3.2.1 displays all of the genotypes that were used as well as their corresponding unique-identification number. The seeds were treated with Trilex Star (Bayer Crop Science, RTP NC) on January 5th, 2012 at a rate of one teaspoon per 100 seeds in the bag as advised by Dr. Balota. Trilex Star is a peanut seed treatment from Bayer Crop Science and is a combination of Captan, Trifloxystrobin, Thiophanate-methyl, and Metalaxyl. The seeds were stored at 4˚C with 40% relative humidity when not in use to maintain viability. The seeds were pulled out of cold storage and allowed to acclimate at room temperature for 48 hours prior to planting.

As noted in section 3.1, the square pots and 3:2:1 RediEarth, NC Dark Sand, and Contractor's Light Sand Mix (Mix) were used in the implementation screen. A total of four pots were seeded for each line, one pot per treatment, and each pot was double-seeded to ensure that enough plants were available for the treatment. The genotypes were arranged in a randomized complete block design, within the chosen treatment. The pots were then maintained in the Conviron chamber under well watered conditions. At ten days after seeding, plants were thinned out to have only one plant per pot. All plants started out in the same Conviron chamber with growth conditions of 26˚C day and 20˚C night with a photoperiod of 18 hours and relative humidity of 65 percent. The Conviron TC30 is a two-shelf chamber with fluorescent tube lights. Once seeded, the plants were kept in the chamber for 14 days (shown in Figure 3.2.1) and then half of them were subjected to the chemical treatment of 75 mg L^{-1} Se as selenium selenate dissolved in water at a spray volume of 15 gallons per acre as advised by Dr. Balota. An 8002E Tee-Jet fan tip nozzle was used for the spray application on an indoor track

Figure 3.2.1 Plants at Day of Treatment

Figure 3.2.2 Plants in Track Sprayer

sprayer (Figure 3.2.2). The nozzle height for calibration and application was 18 inches above canopy height. The plants remained in the drying chamber until completely dry prior to leaving the spray room to prevent cross-contamination of the Se with plants that did not receive the application. After the spray, the plants were placed back into the same Conviron TC30 chamber for 48 hours post application. After the 48 hours the plants were divided into their specified temperature treatment. One set of sprayed and unsprayed plants was moved into a Conviron PGR16 chamber at the same optimal temperature settings (26˚C/20˚C), while the other set of sprayed and unsprayed plants was moved into an identical Conviron PGR16 set at the same 18-hour photoperiod and 65% relative humidity, but temperatures were set to 39˚C day and 32˚C night. Both chambers had fluorescent tubes and incandescent bulbs allowing for better heat control with light intensities averaging between 1550-1690 Lux at canopy level. The plants were maintained under well-watered conditions for two weeks after the start of the temperature treatment. The entire experiment from seeding to harvest ran for thirty days per replicate, with only one replicate receiving the temperature treatment at a time. Four total replications were performed.

At the end of the two week temperature treatment, a SPAD reading was conducted using a Konica Minolta 502 plus (Ramsey, NJ) SPAD chlorophyll meter. A measurement was taken on the last two leaves along the mid-rib on the youngest fully expanded leaf as shown in Figure 3.2.3. The two measurements on the same plant were then averaged. The SPAD chlorophyll meter measures absorbance of wavelengths in the visible spectrum when light passes through the leaf, and serves as a relative measure of the concentration of chlorophylls *a* and *b*. After the measurement, a destructive harvest was performed to allow for above-ground biomass data collection. The stems were cut at soil level and the shoot portion was weighed to obtain fresh weight. After this, plants were placed into a waxed paper bag and stored at 80˚C for 48 hours. After this, each shoot was weighed again to obtain a dry weight.

Figure 3.2.3 The last two leaflets on the youngest fully expanded leaf

3.3 Additional Methods for Replicate Four

Figure 3.3.1 Freshly harvested seedlings prepared for LA

Due to results that will be discussed later, a slight change was made in the data collection for the fourth and final replicate. Seeding growth stages, spray conditions, and treatments, temperature and Se, were maintained the same as in the previous three replicates. However, in the destructive harvest stage a new step was included. After the SPAD chlorophyll readings and the fresh-weight were recorded on the above ground shoot section, the leaf material was physically separated from the stems and leaf area (LA) was measured with a LI-COR LI-3100 Area Meter. These results were reported in cm^2. The material was then combined back into the same wax bag with the stem and dried at 80˚C in the oven. A combined dry-weight was then measured for the leaf and stem tissue.

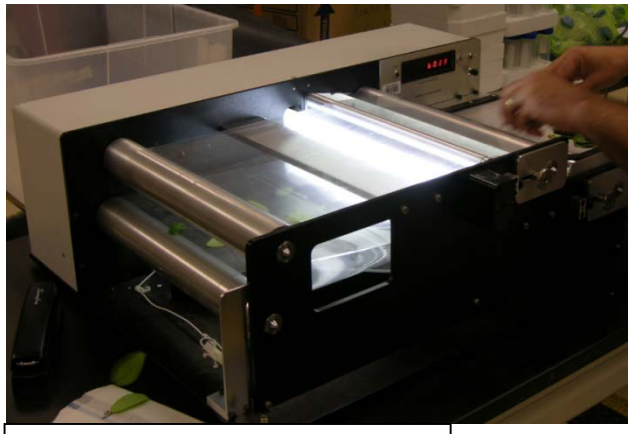


Figure 3.3.2 Freshly harvested leaf material being measured

4. Results

The data from all four replicates were combined for the analysis of variance (ANOVA) using the general linear model (GLM) procedure in SYSTAT 10.2 (SYSTAT Software, 2002). Although thirty lines were planted, Georgia Green and Georgia 08V did not germinate and were removed from the comparisons, leaving 28 genotypes, and 27 degrees of freedom for the genotype effect. As shown in the figures displaying the individual phenotypes there was a high level of variation among genotypes for SPAD readings and dry weights (Table 4.1.1 and Table 4.2.1) and among temperature regimes for SPAD chlorophyll readings and fresh weights (Table 4.1.1 and Table 4.3.1).

4.1 SPAD

Table 4.1.1 Analysis of Variance Data for SPAD

Analysis of Variance for SPAD Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	2,376.470	27	88.017	2.415	0.000*
TEMPERATURE	7,009.687	1	7,009.687	192.344	0.000*
SE_TREATMENT	0.458	1	0.458	0.013	0.911
GENOTYPE$*TEMPERATURE	873.446	27	32.350	0.888	0.630
GENOTYPE$*SE_TREATMENT	477.030	27	17.668	0.485	0.987
TEMPERATURE*SE_TREATMENT	30.150	1	30.150	0.827	0.364
GENOTYPE$*TEMPERATURE*SE_TREATMENT	356.307	27	13.197	0.362	0.999
Error	12,172.124	334	36.443		

For the SPAD chlorophyll measurements, both genotype and temperature had a significant effect ($p < 0.003$). The Se treatment did not show a significant effect on the chlorophyll content measured in SPAD units. Interactions of genotype × temperature, genotype × Se treatment, and genotype × temperature × Se treatment were not significant.

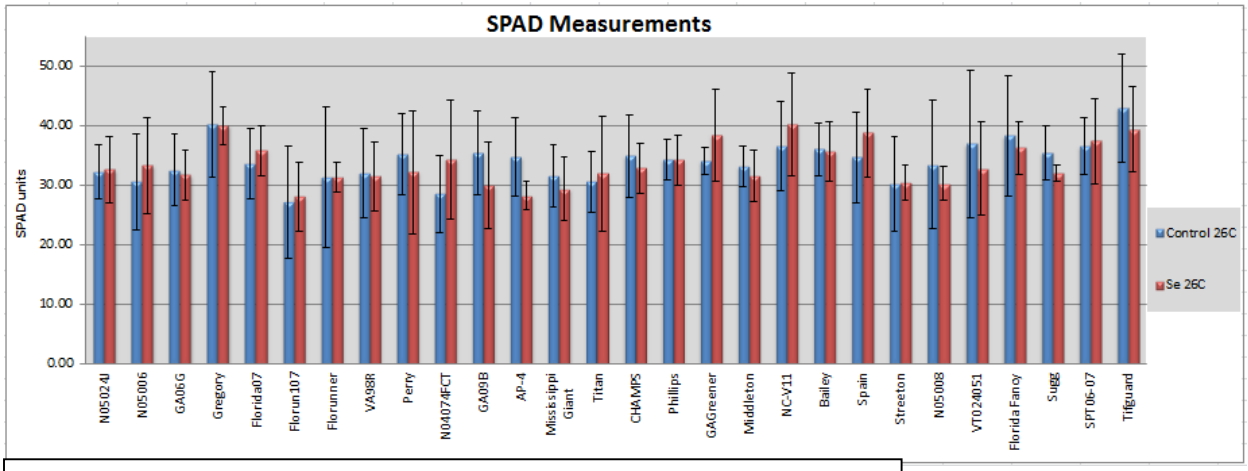


Figure 4.1.1 SPAD Measurements for 26˚C control and 26˚C Se

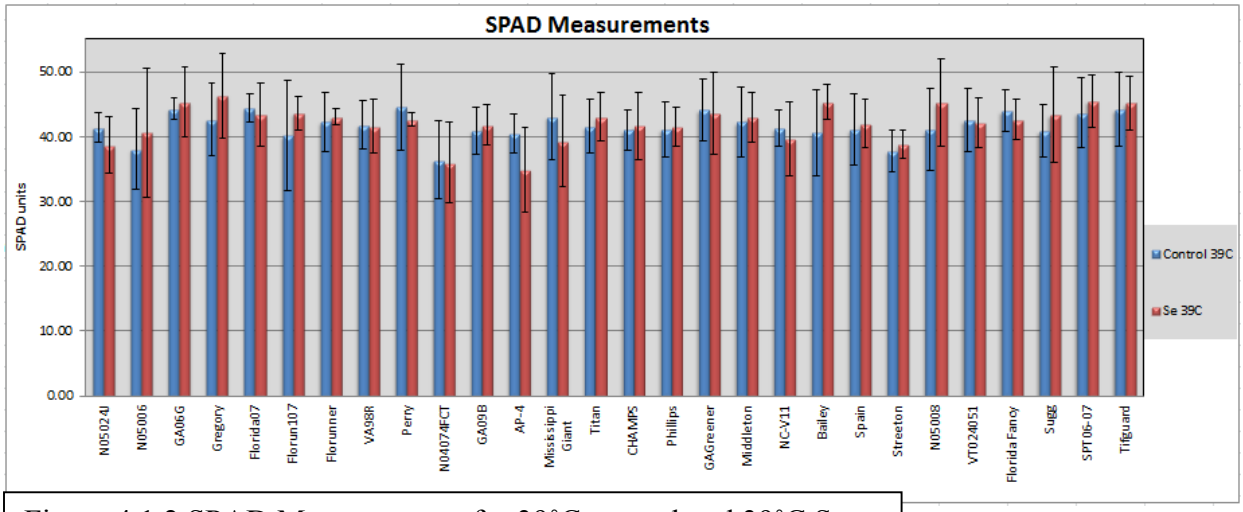


Figure 4.1.2 SPAD Measurements for 39˚C control and 39˚C Se

The average SPAD value for 26˚C was 34.12, with a minimum average value of 27.27 (Florunner), and a maximum average value of 43.08 (Tifguard). The average SPAD value for 39˚C was 41.71, with a minimum average value of 36.43 (N04074FCT), and a maximum average value of 44.55 (Perry). Even though the genotype × temperature interaction was not significant, some genotypes behaved differently relative to the others when temperature was increased. For example, N04074FCT had 28.58 average SPAD value at 26˚C close to the average of all genotypes, but was the lowest with a 36.43 SPAD value when exposed to 39˚C. This is in agreement with previous observations that N04074FCT has less SPAD chlorophyll under field conditions and when temperature usually exceeds 30˚C (Balota et al., 2012).

4.2 Dry-Weight

Table 4.2.1 Analysis of Variance Data for Dry Weight Measurements

Analysis of Variance for DW					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
GENOTYPE$	46.615	27	1.726	5.823	0.000*
TEMPERATURE	0.064	1	0.064	0.216	0.643
SE_TREATMENT	0.174	1	0.174	0.588	0.444
GENOTYPE$*TEMPERATURE	3.548	27	0.131	0.443	0.994
GENOTYPE$*SE_TREATMENT	3.333	27	0.123	0.416	0.996
TEMPERATURE*SE_TREATMENT	0.087	1	0.087	0.293	0.589

Analysis of Variance for DW					
Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$*TEMPERATURE*SE_TREATMENT	4.246	27	0.157	0.530	0.975
Error	99.033	334	0.297		

Only genotype had a significant effect on seedling dry-weight, which means that certain genotypes were inherently smaller than others. This is an expected result as most of the runner-type peanuts are smaller than the virginia-type as well. Neither temperature nor Se application had an effect on the dry-weight of the seedlings. Interactions of genotype × temperature, genotype × Se treatment, and genotype × temperature x Se treatment were also not significant.

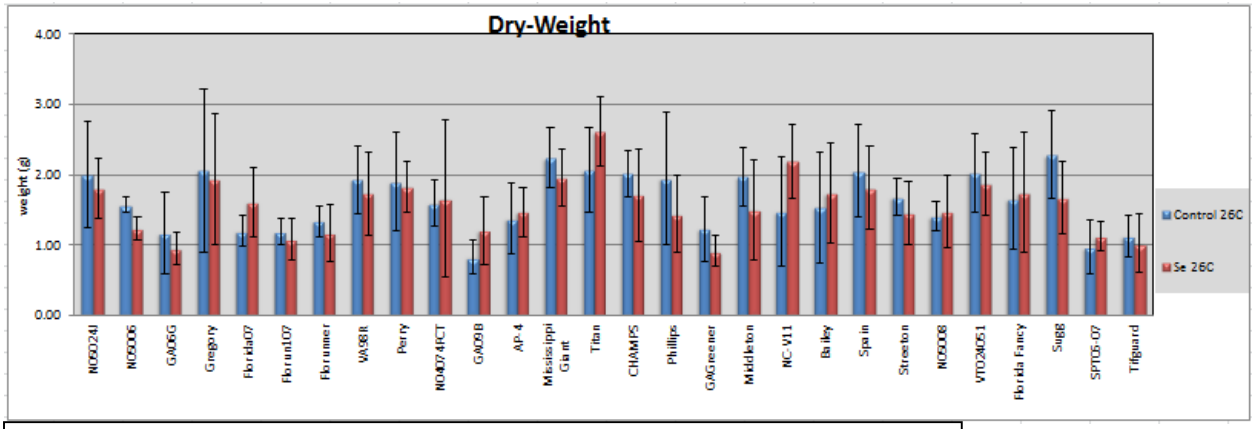


Figure 4.2.1 Dry Weight Measurements for 26˚C control and 26˚C Se

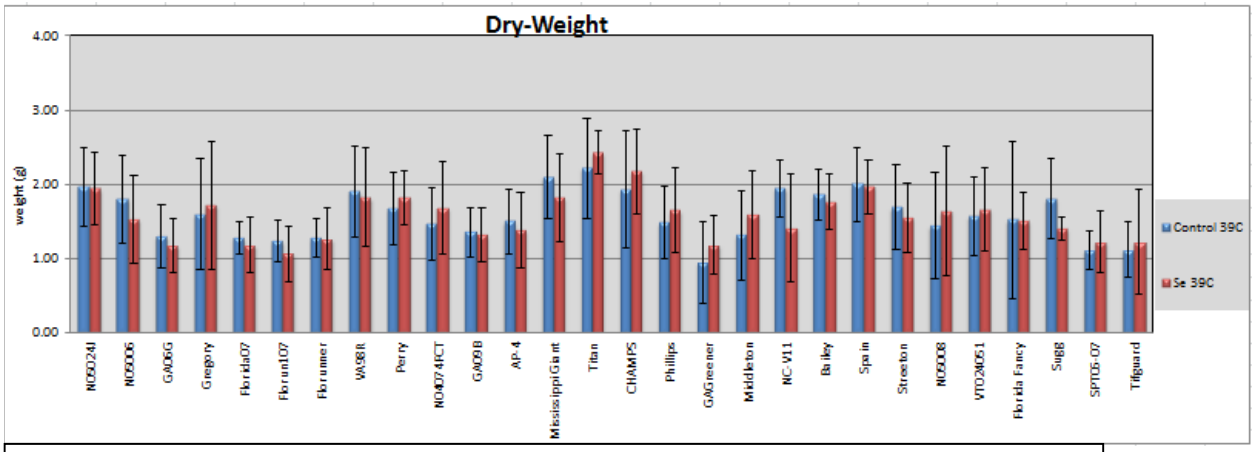


Figure 4.2.2 Dry Weight Measurements for 39˚C control and 39˚C Se treated

The average dry weight for 26˚C was 1.64g, with a minimum average weight of 0.83g (GA09B), and a maximum average weight of 2.28g (Sugg). The average dry weight for 39˚C was 1.59g, with a minimum average weight of 0.95g (GAGreener), and a maximum average weight of 2.22g (Titan).

4.3 Fresh-Weight

Table 4.3.1 Analysis of Variance Data for Fresh Weight Measurements

Analysis of Variance for FW					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
GENOTYPE$	3,125.536	27	115.761	1.071	0.372
TEMPERATURE	451.774	1	451.774	4.182	0.042
SE_TREATMENT	137.024	1	137.024	1.268	0.261
GENOTYPE$*TEMPERATURE	2,351.530	27	87.094	0.806	0.744
GENOTYPE$*SE_TREATMENT	2,525.168	27	93.525	0.866	0.662
TEMPERATURE*SE_TREATMENT	112.893	1	112.893	1.045	0.307
GENOTYPE$*TEMPERATURE*SE_TREATMENT	2,787.852	27	103.254	0.956	0.531
Error	36,084.683	334	108.038		

Of interest in the fresh-weight results, is that after the first three replicates, both genotype and temperature were significant ($p<0.003$). However, after including the fourth replicate, no significant effect due to genotype was observed. This displays the high level of variability between the genotypes, and further reiterates the lack of differences seen in biomass from both temperature and Se application.

The average fresh weight for 26˚C was 8.74g, with a minimum average weight of 5.48g (GA09B), and a maximum average weight of 12.27g (Mississippi Giant). The average fresh weight for 39˚C was 9.91g, with a minimum average weight of 6.56g (GAGreener), and a maximum average weight of 13.87g (Titan).

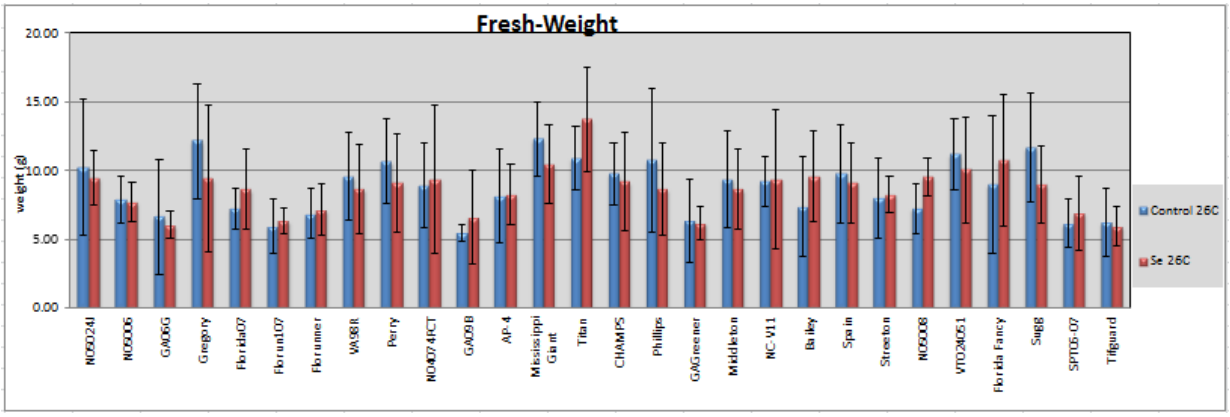


Figure 4.3.1 Fresh Weight Measurements for 26˚C control and 26˚C Se

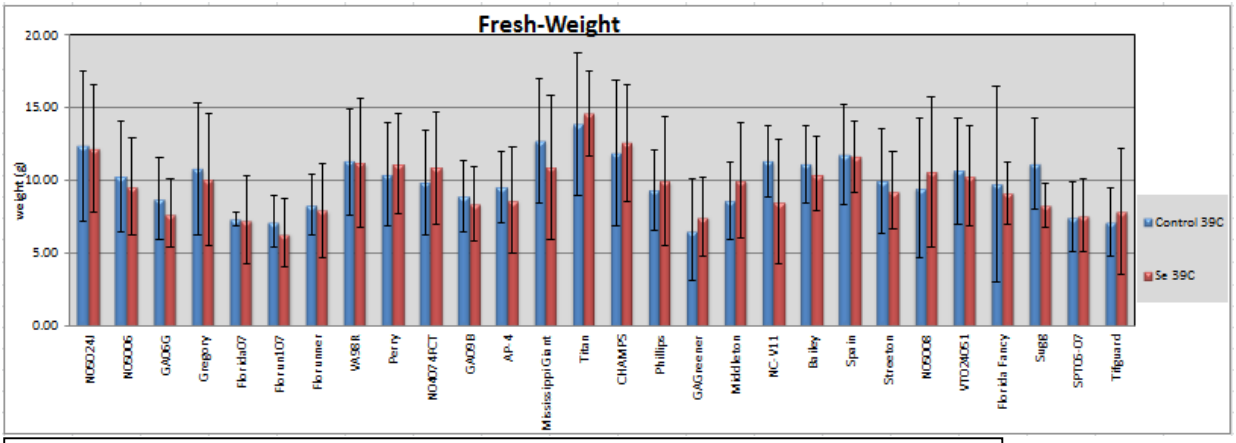


Figure 4.3.2 Fresh Weight Measurements for 39˚C control and 39˚C Se

4.4 Leaf-Area

Table 4.4.1 Analysis of Variance and Least Squares Means Data for Leaf Area

Analysis of Variance for LA					
Source	Type III SS	df	Mean Squares	F-ratio	p-value
TEMPERATURE	2,647.762	1	2,647.762	0.851	0.358
SE_TREATMENT	11.997	1	11.997	0.004	0.951
TEMPERATURE*SE_TREATMENT	1,756.705	1	1,756.705	0.565	0.454
Error	329,766.807	106	3,111.008		

Least Squares Means				
Factor	Level	LS Mean	Standard Error	N
TEMPERATURE	26	252.173	7.453	56.000
TEMPERATURE	39	242.359	7.590	54.000

As noted in section 3.3 only the fourth replicate utilized the leaf area measurement. Being only one replicate we cannot use the data for conclusive significance testing, but could look at a potential trend. It appears that the plants subjected to the 39 ˚C had slightly smaller LA on average of all genotypes compared to those subjected to the 26 ˚C temperature. Figures 4.4.1 and 4.4.2 show the treatments and Figure 4.4.2 compares only the unsprayed (no Se) plants from the 26 ˚C (in blue), and unsprayed 39 ˚C (in green) temperature regimes. Though not the case for every genotype, the average trend was that 26 ˚C plants had slightly larger LA compared to 39 ˚C.

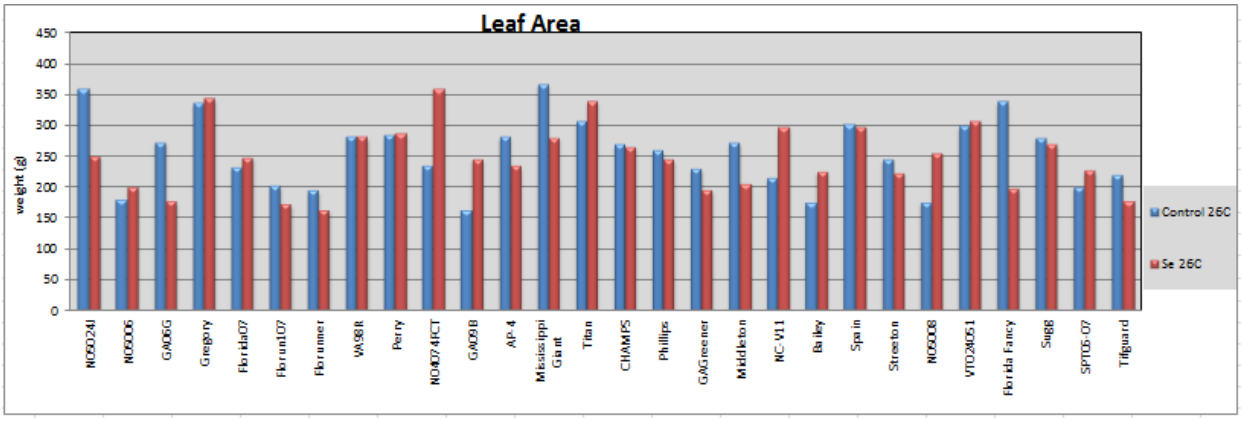


Figure 4.4.1 Leaf Area Measurements for 26˚C control and 26˚C Se

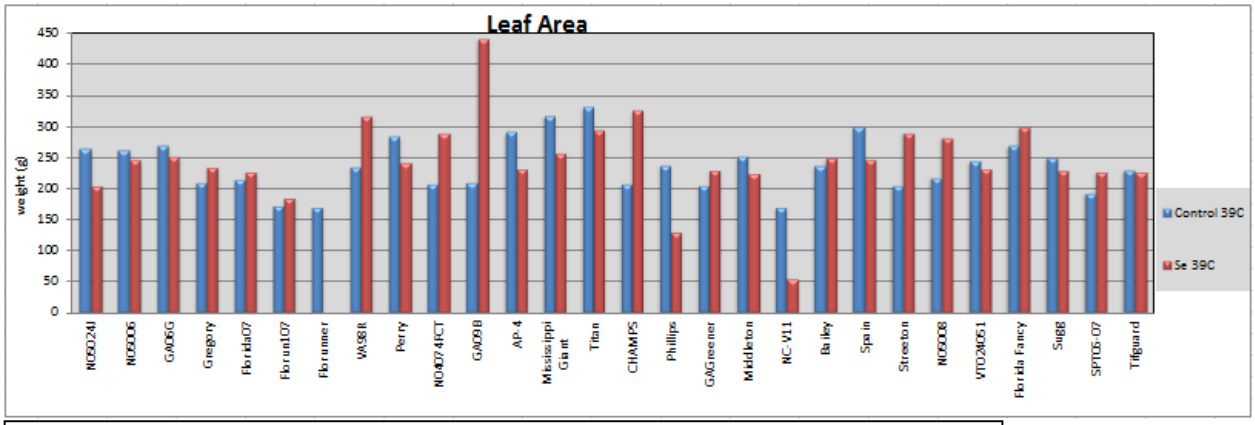


Figure 4.4.2 Leaf Area Measurements for 39˚C control and 39˚C Se

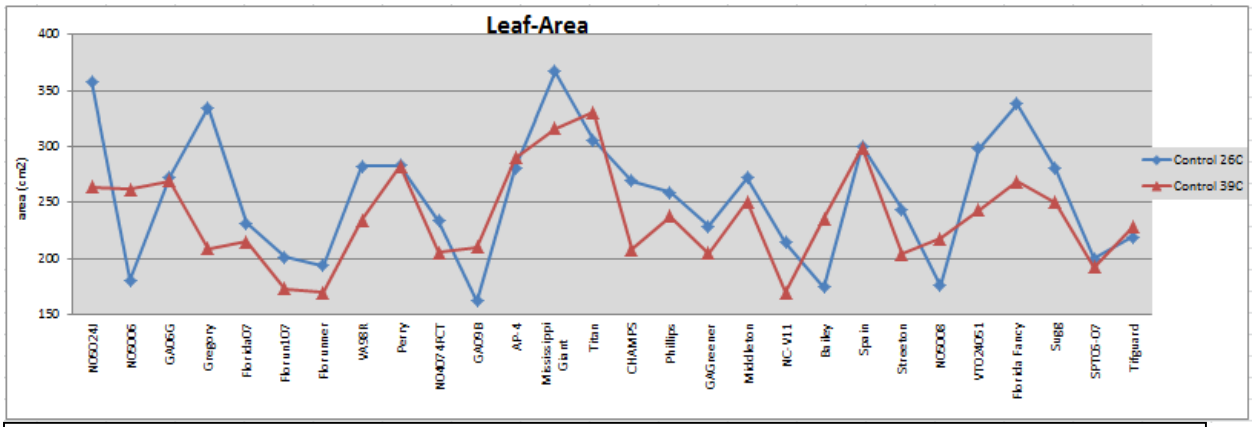


Figure 4.4.3 Comparison of Leaf Area measurements of Control 26˚C and Control 39˚C

5. Discussion and Conclusion

The results for seedling biomass in this experiment were not as expected. As shown above, only temperature had a significant effect on fresh-weight of the plants, and only genotype had a significant effect on the dry-weight. This was surprising as significant genotype x temperature interaction for seedling biomass was expected. These results would conclude that the lines utilized do not exhibit a strong differential response for biomass, where some of the lines are resistant and others are susceptible to the above-optimal temperature regime. Another reason could be that 39 ˚C is not high enough to cause stress responses in peanut. The majority of studies cited here used much higher temperatures to induce physiological changes in peanut.

Instead, only having genotype as a significant parameter for dry-weight could be explained by the different inherent biomasses of the lines used. For example, some of the genotypes have naturally larger biomass depending on the peanut type as outlined in the introduction section.

Notably missing from above is the presence of a Se interaction effect with genotype and temperature. In both the optimal and high temperature regimes there was no significant effect from the Se application. This was also against the hypothesis that an application of Se to the seedlings would allow for improved tolerance to the high temperature treatment. This finding is not consistent with the Djanaguiraman et al., [2005] study on soybean that showed a promotion of plant growth attributes. Furthermore, the results were not consistent with findings from Djanaguiraman et al. [2010] showing that a Se foliar application significantly increased total dry matter production in sorghum. However, the

Se treatment did not affect chlorophyll content in the Djanaguiraman et al. [2010] experiment.

Figure 5.1.1 14 DAT seedling at 26˚C (left) and 39 ˚C (right)

Continuing on with the SPAD chlorophyll measurements in this peanut experiment, both temperature and genotype displayed significant results for the SPAD readings. The high temperature treatment of 39˚C (on the right in Figure 5.1.1) actually produced higher chlorophyll content compared to that of the optimal standard temperature regiment of 26˚C (on the left in Figure 5.1.1). The data is consistent with Chauhan and Senboku [1997] where they noted an increase in chlorophyll content in leaf discs that were subjected to hardening through exposure to 37˚C. Potential reasons for this could be in that the genotypes have differences in leaf area and thus the accumulation of chlorophyll could be different across the genotypes. Another hypothesis is that the increased temperature actually caused the leaf area to decrease, thus packing the chlorophylls closer together in a smaller area. This could explain why the SPAD chlorophyll readings were higher at the higher temperature of 39˚C. Selvaraj et al. [2010] did witness a complete

loss of chlorophyll accumulation but he treated the leaf discs at much higher temperatures of 48 and 50˚C.

The idea of decreased leaf area is the rationale behind examining the leaf area in the fourth replicate. However, with only one replicate being performed on this measurement it does not render itself available to significance testing, but rather a potential trend can be seen. In the graphs in section 4.4 above it appears that across the majority of the genotypes that leaf area was indeed smaller in the plants at 39˚C compared to 26˚C. Again, more replications on leaf area are necessary for better understanding of the implications of this measurement. It could be that the seedling vegetative stage is better suited to withstand higher temperatures, especially if drought conditions are not a limiting factor. This would be in stark comparison to the impact that heat stress plays on plants that are in the reproductive phase. Further replication is necessary for better understanding of the leaf area aspect.

6. Final Thoughts and Reflection

6.1 Limitations of Research

The key limiting factors of this research experiment were time and space availability. BASF Plant Science was very gracious and allowed the use of three Conviron growth chambers throughout the course of the experiment. The nature of the experiment testing at the increase temperatures made it imperative to utilize the chambers, and thus space was at a premium since it could not be performed in a greenhouse environment. BASF Plant Science was also accommodating as they allowed open access to equipment and resources ranging from soil and pots, to the use of the indoor track sprayer and data instrumentation. However, the availability of the growth chambers had to be synchronized with the ongoing research pipeline at BASF Plant Science, and therefore the short life-cycle screen was imperative in order to allow for replication. Fortunately a window of opportunity was available in the pipeline to allow for four replications on the seedling to young growth stage, but not to flower.

6.2 Future Implications and Research

As the experiment drew to a close the learning aspect for me personally became more and more apparent. The measurements of chlorophyll content and plant biomass were nice data to collect, but with complex interactions such as heat stress and Se application this is just the beginning. In my opinion, it would be very beneficial to carry out such an experiment at multiple growth stages, and to be able to compare the seedling stage directly to flowering and pod formation stages to determine the correlation of this particular chamber screen to the more mature life-cycle stages. This would further enable researchers to determine if the seedling responds the same as the mature plants, and if so

much time could be saved in future research. Furthermore, additional measurements and capabilities such as chlorophyll fluorescence would be highly beneficial.

This being said, a more in depth evaluation of root phenotypes such as biomass and architecture would be interesting to determine if a root-to-shoot correlation could be seen in this controlled screen. Though Se showed no significant advantage for the above ground biomass, the impact on the below ground (if any) would in my opinion be beneficial.

Furthermore, it would be interesting to study the most effective loading of the Se compound, and determine the most effective adjuvant or loading package to better deliver the Se to the plant material. Plant uptake could have very well played a role in the efficacy of the Se to prevent oxidative stress in this experiment since a technical powder of sodium selenate was used rather than a commercial type formulation. If the compound was available in a pre-mixed formulation, this may allow for better uptake from the plant and that the compound will be fully dissolved upon application.

Another point to note is that these plants were well watered, but received no additional fertilization treatment during the course of the experiment (a total of 30 days from seeding to destructive harvest). A follow-up experiment with the addition of a fertilization step could be of interest to determine if this would significantly alter the differences between SPAD chlorophyll measurements among the 26˚C and 39 ˚C temperature treatments. The 39 ˚C high temperature regime should be reconsidered. It is possible that the leaf temperature may have been lower than the air temperature in the growth chamber, due to evaporational cooling under well-watered, well-ventilated conditions. This temperature could be too low to induce temperature stress in peanuts.

Even though 39 ˚C is expected in the field at seedling stage, temperature is measured in shade and at 2 m above the ground. In the sun on clear days and close to heated soil, seedlings can experience much higher temperatures than 39˚C at least for part of the day.

6.3 Personal Reflection

Above all, this experiment has successfully taken me as a scientist out of my comfort zone in herbicide tolerance, trait discovery and introgression, and breeding activities. In my role in other projects, I have spent the majority of my time collecting samples and not following them downstream or knowing the whole story. This project allowed me to take a deeper look and investigation on a different set of phenotypes in heat stress and Se interaction. Peanut was personally a new crop, and allowed for time to dedicate towards growing in the agriculture field as a whole by examining growth habits and practices in a previously unexplored plant. The time spent researching literature and previous trials displays just how far I have to go in my career, but also will strengthen my research and personal confidence moving forward. Thank you for this opportunity.

7. Citations

American Peanut Council. "Fast Facts about Peanuts." American Peanut Council 2011. 19 July 2012. Available at: http://www.peanutsusa.com/MainMenu/About-Peanuts/Fast-Facts.html.

American Peanut Council. "Export Promotion." American Peanut Council 2011. 19 July 2012. Available at: http://www.peanutsusa.com/MainMenu/Export-Promotion.

Balota, M., T.G. Isleib, and J.W. Chapin. "Description and Performance of the Virginia-Market-Type Peanut Cultivars." Publication 432-201. Virginia Cooperative Extension 2010. Available at: http://www.peanut.ncsu.edu/PDFFiles/005094/Description_and_Performance_of_the_Virginia-Market-Type_Peanut_Cultivars.pdf.

Balota, M., T.G. Isleib, and S. Tallury. 2012. Variability for drought related traits of virginia type peanut cultivars and advanced breeding lines. Crop Sci. 52(6) (in press).

Bayer Crop Science. Trilex Star Fungicide Label. Bayer Crop Science LP, 2005. Available at: http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5COK%5Cpesticide%5CProduct%20Label%5C264%5C264-998%5C264-998_Trilex_Star_Peanut_Seed_Treatment_Fungicide_5_22_2006_4_48_50_PM.pdf

Chauhan, Y.S., and T. Senboku. Evaluation of groundnut genotypes for heat tolerance. Association of Applied Biologists 1997; 131:481-489.

Craufurd, P.Q., P.V.V. Prasad, V.G. Kakani, T.R. Wheeler, and S.N. Nigam. Heat tolerance in groundnut. Field Crops Research 2003; 80: 63-67.

Craufurd, P.Q., P.V. V. Prasad, and R. J. Summerfield. Dry Matter Production and Rate of Change of Harvest Index at High Temperature in Peanut. Crop Science 2002; 42:146-151.

Djanaguiraman, M., D.D. Devi, A.K. Shanker, J.A. Sheeba, and U. Bangarusamy. Selenium- an Antioxidative Protectant in Soybean During Senescence. Plant and Soil 2005; 272: 77-86.

Djanaguiraman, M., P.V.V. Prasad, and M. Seppanen. Selenium Protects Sorghum Leaves from Oxidative Damage Under High Temperature Stress by Enhancing Antioxidant Defense System. Plant Physiology and Biochemistry 2010; 48: 999-1007.

Hartikainen, H., T. Xue, and V. Piironen. Selenium as an Anti-Oxidant and Pro-Oxidant in Ryegrass. Plan and Soil 2000; 225: 193-200.

Hasanuzzaman, M., M.A. Hossain, and M. Fujita. Selenium in Higher Plants: Physiological Role, Antioxidant Metabolism and Abiotic Stress Tolerance. Journal of Plant Sciences 2010; 5(4):354-375.

Holbrook, C., and W. Dong. Development and Evaluation of a Mini core Collection for the U.S. Peanut Germplasm Collection. Crop Science2006; 45:1540-1544.

Holbrook, C., P. Ozias-Akins, Y. Chu, and B. Guo. Impact of Molecular Genetic Research on Peanut Cultivar Development. Agronomy 2011; 1:3-17.

Kottapalli KR, G.S. Michael, G.B. Burow, J.L. Ayers, J.J. Burke, N. Puppala, and M.D. Burow. Towards Development of Markers for Heat Stress Tolerance in Peanut. In: Southwest Consortium on Plant Genetics and Water Resources. 21st Annual Symposium, Las Cruces, New Mexico, 2006; August 24–26.

Kumar, S., D. Gupta, and H. Nayyar. Comparative Response of Maize and Rice Genotypes to Heat Stress: Status of Oxidative Stress and Antioxidants. Acta Physiologiae Plantarum 2012; 34: 75-86.

Scottech. Chlorophyll Concentration Meter. Scottech 2012. Available at: http://www.scottech.net/faq/apogee/chlorphyll_concentration_meter/

Selvaraj, M.G., G. Burow, J.J. Burke, V. Belamkar, N. Puppala, and M.D. Burow. Heat Stress Screening of Peanut for Acquired Thermotolerance. Plant Growth Regulation 2011; 65:83-91.

Soliman, W.S., M. Fujimori, K. Tase, and S. Sugiyama. Heat Tolerance and Suppression of Oxidative Stress: Comparative Analysis of 25 Cultivars of the C3 Grass *Lolium perenne*. Environmental and Experimental Botany 2012; 78:10-17.

Soyatech. "Peanut Facts." Soyatech 2012. 22 July 2012. Available at: http://www.soyatech.com/peanut_facts.htm.

SYSTAT Software, 2002. The Systat statistics system for windows. Release 10.2. SYSTAT Software, Richmond, CA.

Virginia Cooperative Extension. "2012 Virginia Peanut Production Guide." Publication PPWS-3. Virginia Cooperative Extension 2012.

USDA National Agricultural Statistics Service. Prospective Plantings. USDA NASS 2012. 9 August 2012. ISSN:1949-159X.

USDA National Agricultural Statistics Service. Yield per Harvested Acre by County. USDA NASS 2012. 9 August 2012. Available at http://www.nass.usda.gov/Charts_and_Maps/A_to_Z/in-peanuts.asp.

VirginiaTech
Invent the Future

Effects of Selenium Application on Heat Tolerance in Peanut Seedlings

Chad Benton
December 3, 2012
Suffolk, VA

PPWS
Plant Pathology Physiology and Weed Science

VirginiaTech
Invent the Future

Introduction

- **The U.S. is the worlds third largest peanut producer with ~ 1.9 million metric tons.**
  - In 2012, 1.42 million acres were planted, which is up 25% from 2011 [USDA NASS, 2012].
- **To meet the growing demand for food, crops are increasingly grown outside their traditional areas [Chauhan and Senboku, 1997].**
  - Predicted increases of average temperature of 1.4-6 °C as well as variability of temperature could play an impact on future production [Craufurd et al., 2003].

VirginiaTech Invent the Future

Background

- **Diverse Genotypes**
  - Provides a method of gene discovery in plant research.
    - Important to ensure enough genetic diversity is present to allow for a differential response.
  - Holbrook and Dong [2005] set aside a "mini-core" population by analyzing traits such as growth habit, size, leaf color, maturity, pod shape, pod weight, seed weight, and leaf spot.
    - Future research could go into enhancing genetic diversity through use of wild *Arachis* populations- however ploidy level an issue [Holbrook, 2011].

VirginiaTech Invent the Future

Background

- **Heat Stress**
  - Many studies have examined the impact of heat stress on mature plant stages.
    - Craufurd et al. [2003] determined that fruit-set was decreased from 71% to 58% when a treatment of 38˚C was imposed six days before and at first flowering.
  - Heat Stress can lead to oxidative stress through accumulation of reactive oxygen species.
    - This is detrimental to overall plant health and function.
  - Selvaraj et al. [2010] noted that a reliable protocol was needed to look at multiple genotypes under controlled conditions.

VirginiaTech Invent the Future

Background

- **Selenium (Se) as an antioxidant**
  - Antioxidants play a direct role in minimizing the impact of oxidative stress.
  - Se is a micronutrient for plants, but does play a role in antioxidation in human and animal cells.
    - Studies are showing that Se can protect plants from abiotic stresses [Djanaguiraman et al., 2010].
    - In soybean, Se promoted shoot growth, number of leaves per plant, total leaf area, and total dry matter production [Djanaguiraman et al., 2005].
    - High concentrations of Se can actually act as a pro-oxidant and cause yield loss [Hartikainen et al., 2000].

VirginiaTech Invent the Future

Objectives

- **Analyze peanut genotypes for a differential response to heat stress during early growth.**
  - Determine potential heat tolerant or susceptible genotypes.
- **Validate the efficacy of Se in providing tolerance to heat and improving plant performance under heat stress.**
  - Also determine if genotype specific.

VirginiaTech Invent the Future

Materials and Methods

- **28 genotypes were utilized in this experiment.**
  - Seeds were treated with Trilex Star ™ as a preventative for fungal issues.
- **Seeds were double-seeded into 4"x4"x5" pots containing a 3:2:1 mix of RediEarth Potting Mix, NC Dark Sand, and Contractor's Light Color Sand.**
  - Pots were thinned to 1 plant per pot at 10DAS.
- **Pots were arranged in a randomized complete block design within the chosen treatment.**
  - Total of 4 pots per genotype were seeded.
- **Four total replicates were performed.**

VirginiaTech Invent the Future

Materials and Methods

- **Plants were germinated and maintained under well-watered conditions at 26 °C day and 20 °C night with a photoperiod of 18 hours. The relative humidity was maintained at 65%. Light intensity of ~1600Lux.**
- **At 14DAS half of the plants were subjected to the chemical treatment of 75 mg L^{-1} Se (as Selenium Selenate) at a spray volume of 15 gallons per acre.**
  - An 8002E Tee-Jet fan tip nozzle was used with a nozzle height of 18 inches above canopy.

VirginiaTech
Invent the Future

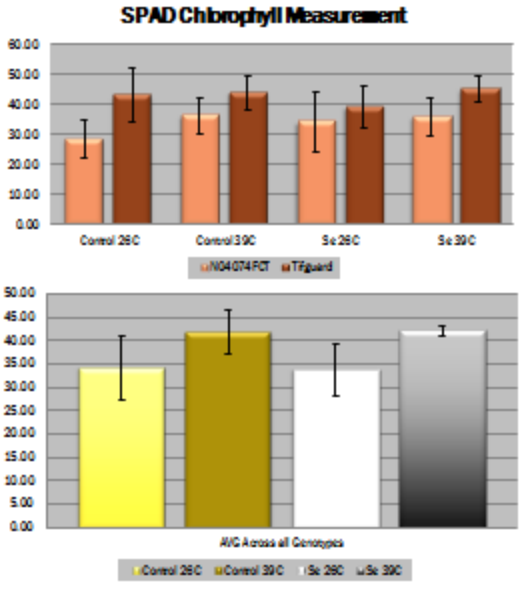
Materials and Methods

- 48 hours after spray, plants were separated into treatments of either 26 ˚C day and 20 ˚C night or 39 ˚C day and 32 ˚C night in Conviron PGR16 growth chambers. RH of 65%.
- Plants were maintained for 14 days after start of treatment prior to a destructive harvest and measurements consisting of:
  - SPAD chlorophyll measurement taken on the last two leaflets on the youngest fully expanded leaf.
  - Shoot Freshweight(g)
  - Shoot Dryweight(g) taken 48 hours after 80 ˚ C drying treatment.

VirginiaTech
Invent the Future

Results- SPAD

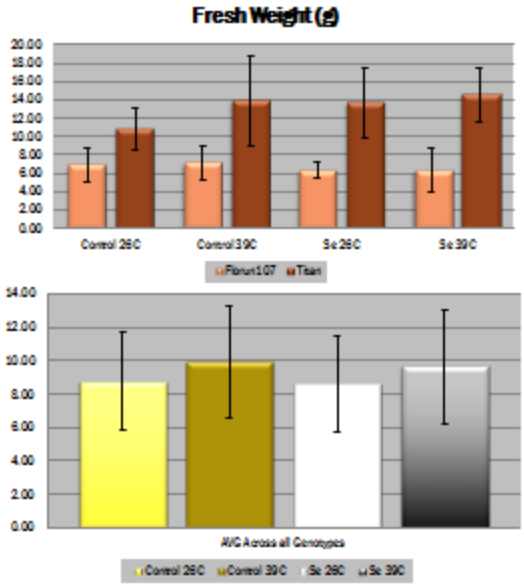
- Genotype and temperature had a significant effect ($p<0.003$).
- N04074FCT had the lowest reading at 39 ˚C, which was in agreement with previous observations of less SPAD chlorophyll under field conditions when temperature exceeded 30 ˚C compared to other lines [Balota et al., 2012].



VirginiaTech
Invent the Future

Results- Fresh Weight

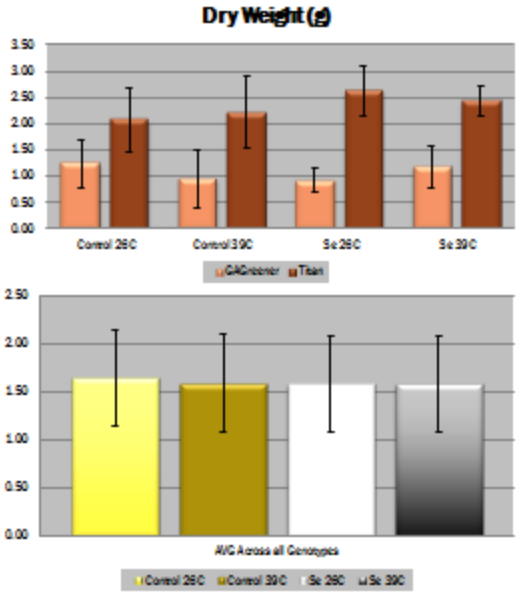
- After three replicates, both genotype and temperature were significant ($p<0.003$), but after including the fourth replicate no significant effect was observed.
- However, much like the SPAD readings a difference was seen between genotypes, it was just not statistically significant.
- The AVG FW for 26˚C was 8.74g, while the average fresh weight for 39˚C was 9.91g.



VirginiaTech
Invent the Future

Results- Dry Weight

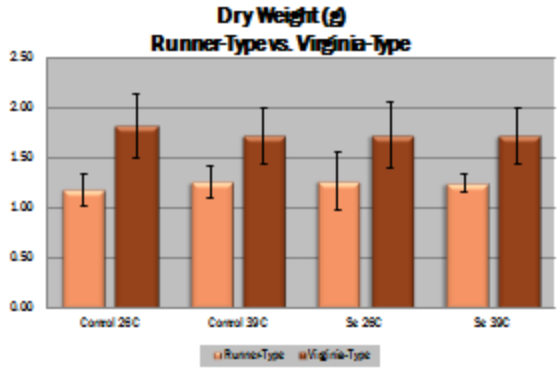
- Only genotype had a significant effect on seedling dry weight ($p<0.003$).
- This is an expected result due to inherent size differences among peanut genotypes.
- The average dry weight for 26˚C was 1.64g, while the average dry weight for 39˚C was 1.59g.



VirginiaTech Invent the Future

Results- Dry Weight

- **Virginia-Type versus Runner-Type:**
  - As noted previously, the genotypic effect was not surprising as runner-type tend to be smaller than the virginia-type.
    - The values were relatively stable across all treatments when averaged across all four replicates.
  - A total of 8 runner-type and 20 virginia-type genotypes were used in this experiment.



VirginiaTech Invent the Future

Conclusions

- No significant genotype x temperature interaction were discovered.
  - These results would conclude that the lines utilized do not exhibit a strong differential response for seedling biomass at above-optimal temperatures.
  - Furthermore, it could be that 39˚C is not high enough to cause stress to seedlings.
- No significant Se interaction effects were noted with genotype or temperature.
  - This was against the hypothesis that application of an antioxidant such as Se would allow for improved tolerance to high temperature treatment.

Conclusions

- Of interest, SPAD chlorophyll concentration measurements were higher in the 39 ˚C than the 26 ˚C
  - This finding is consistent with Chauhan and Senboku [1997] where they noted an increase in chlorophyll content in leaf discs that were subjected to hardening through exposure to 37 ˚C.
- The increased concentration could be due to decreased leaf area in the 39 ˚C plants.
  - In the fourth replicate, leaf area was taken and a trend was visible, but more research and replications are necessary for a better conclusion.

Future Directions

- Follow-up studies on impact of root structure and shoot-root ratio.
- Light intensity.
- Uptake studies for most effective loading of the antioxidant material.
- Look at impact of heat stress and Se at more mature growth stages.
- Range of temperatures used.
- Drought and fertilization studies.

VirginiaTech Invent the Future

Acknowledgements

- Dr. Maria Balota – Virginia Tech
- Dr. Anton Baudoin – Virginia Tech
- Dr. Ames Herbert – Virginia Tech
- Mr. Doug Redd – Virginia Tech
- Dr. Paula Olhoft – BASF Plant Science
- Dr. Jill Stevenson-Paulik – BASF Plant Science
- Dr. Steve Crafts-Brandner – BASF Plant Science
- Dr. Hee-Sook Song – BASF Plant Science
- Mr. Devin Gillis – BASF Plant Science

PPWS
Plant Pathology Physiology and Weed Science

VirginiaTech Invent the Future

Questions?

Thank You!

PPWS
Plant Pathology Physiology and Weed Science