SEED GROWTH AND PHOTOSYNTHESIS DYNAMICS OF SOYBEAN EXPERIENCING DROUGHT STRESS

By

PATRICK JAMES CURL

Bachelor of Science in Agronomy

Oklahoma State University

Stillwater, Oklahoma

2014

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2017

SEED GROWTH AND PHOTOSYNTHESIS

DYNAMICS OF SOYBEAN

EXPERIENCING DROUGHT STRESS

Thesis Approved:

Tyson E. Ochsner

Thesis Adviser

Sergio M. Abit

Committee Member

Vijaya Gopal Kakani

Committee Member

ACKNOWLEDGEMENTS

This project was made fruitful at the hands of many contributors and friends, especially those in the Soil Physics Research Group. Thank you to my parents for their continued support and guidance throughout my academic endeavors and beyond. All of this was made possible as a result of the opportunities and accommodations made by the faculty of the Department of Plant and Soil Sciences. The assistance, guidance and mentorship of Dr. Abit and Dr. Kakani forever broadened my experience in the areas of teaching and research. I am very thankful and indebted to the mentorship provided by my major advisor, Dr. Tyson Ochsner. Thank you for your willingness to invest in me as both a student and a person and to lead by example in both the professional and personal life, it has made an impact that will far outlast my graduate experience.

Name: Patrick James Curl

Date of Degree: MAY, 2017

Title of Study: SEED GROWTH AND PHOTOSYNTHESIS DYNAMICS OF SOYBEAN EXPERIENCING DROUGHT STRESS

Major Field: PLANT AND SOIL SCIENCE

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] yield is sensitive to drought stress during critical reproductive growth stages. This study was conducted to determine both the ability of the soybean to recover after drought stress has subsided and to determine if a specific soil moisture matric potential could be identified as a reference for plant stress, and thus serve as a practical irrigation guide. Soybeans were subjected to drought stress during pod-fill (R5) in a growth chamber study and measurements of the growth of individual beans were collected non-destructively. In a complementary field study with rain-fed and irrigated soybean plots, measurements of photosynthetic CO_2 assimilation, stomatal conductance, leaf fluorescence, and leaf relative water content were recorded near Braman, OK, on an Ashport silt loam (fine-silty, mixed, superactive, thermic Fluventic Haplustolls). In the growth chamber study, a four-day drought with soil matric potential not dropping below -200 kPa, was sufficient to abort 10.5% of initiated beans, reduce the final mass of individual beans by 20%, and depress the growth rate of beans by 25% after the drought stress had been removed for 5 days. The transition point from positive growth to negative growth occurred when soil matric potentials dropped below -60 kPa. Plant metrics from the field trial confirm that drought stress conditions occurring when soil matric potentials fall below -60 kPa can negatively influence the growth and development of soybean. This susceptibility of soybean to relatively mild drought stress, supports the need for improved soil moisture monitoring when irrigation capabilities are present. Direct monitoring of the soil matric potential would improve the accuracy of estimating the plant water status indirectly via the water status of the soil, and irrigation scheduling utilizing a -60 kPa base level would help to avoid yield losses due to drought stress.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

Soybean *(Glycine max)* is a global staple crop with diverse uses for peoples and industries worldwide. While by-products of soybean can be incorporated into products from ink to upholstery, most soybeans are utilized as a food source for livestock and human consumption (Janick, 1974; NCSPA, 2014). This is a direct result of the high nutritional value of soybeans which contain ~35-38% protein, omega-3 fatty acids, and a multitude of essential micronutrients (United). Such utility and nutrition has led to an ever-increasing demand for soybeans. In response, the world soybean production elevated to over 319.7 million tons in 2014-2015 growing seasons (FAO, 2016). For the 2015 production year, the United States alone accounted for 107 million tons of that global production (FAO, 2016). While soybean traditionally favors a semitropical environment, advances in genetics and agronomy have allowed soybean production to proliferate across the United States into regions less conducive to soybean growth (Miller et al., 2002).

Oklahoma lies in a region where environmental conditions can be challenging for soybean production, and only 151 thousand hectares of the total 33.1 million harvested hectares of production in the United States could be attributed to Oklahoma during 2015 season (USDA, 2016a; USDA, 2016b). Even more disconcerting for the production of soybean in Oklahoma than the production area is the resulting yield. Oklahoma returned an average yield of 2,004 kg ha^{-1}

during the 2013-15 growing seasons while the national average reached 3,127 kg ha^{-1} during the same period (USDA, 2016a; USDA, 2016b). This ranks Oklahoma last among soybean producing states in yield per hectare. Still, for regions of northern and eastern Oklahoma, soybean production is vital as a primary cash crop and as an option in crop rotations.

A leading factor in the limited production of soybean throughout Oklahoma and similar environments is the probability and frequency of drought and heat induced stress. Soybean planting occurs during a wide window from mid spring through early summer and it has a growth cycle where maturity develops during the fall from September to November. During this growing season, it is likely that the environment will present conditions unfavorable for soybean growth. As a temperate legume native to southern Siberia and eastern China (Harlan, 1995; Janick, 1974), soybeans are adapted to thrive under conditions where daytime temperatures do not exceed 30°C and water is supplied at regular intervals to total between 380-700 mm for the duration of the growing season (Dogan et al., 2007; Gibson and Mullen, 1996; Kranz, 2012). Given the sometimes extreme and erratic nature of the Oklahoma weather patterns, it is common for the temperature to exceeded optimum conditions and for irregularities in rainfall to manifest as drought.

Drought is the major driving factor in the yield and production discrepancy between Oklahoma and the more prolific soybean producing regions. As with all plants, available water is necessary as it governs four general functions. As outlined by Kramer (Kramer, 1963), 1) water is the major constituent of physiologically active tissue, 2) it is a reagent in photosynthesis and in hydrolytic processes, 3) serves as the solvent in which salts, sugars, and other solutes move from cell to cell or organ to organ, 4) and is essential for the maintenance of the turgidity necessary for cell enlargement and growth. Together, the success or failure of these processes manifests outwardly through the quality and quantity of plant growth (Kramer, 1995). In soybean, this is reflected in the ability of the plant to grow vegetatively, flower, pollinate, and set large beans with high concentrations of the oil and protein that determine quality. Depending on the soybean growth stage during which the plant is experiencing drought stress, any one of these essential plant functions could be negatively impacted as a result of the cascading effects of water stress (Liu et al., 2003).

Since most interest in the production of soybean is focused on increasing seed yield, much work has centered around determining the soybean growth stage during which drought is most damaging to the final yield. With vegetative growth encompassing a large portion of the soybean life cycle, it is important to understand if droughts during this early stage are highly influential in final yield. Work conducted by the USDA-ARS determined that mild water deficits during vegetative growth can have a significant negative impact on soybean biomass production, reducing leaf production and expansion (Muchow et al., 1986). While such stress could influence yield if the reduction in vegetative growth continued throughout the early reproductive phases; stress strictly during the vegetative period is not the most critical time in the life cycle as vegetative growth can continue through the early reproductive stages (Board and Harville, 1996; Casteel, 2009). Early irrigation studies even found that there was very little benefit in yield for irrigation applied before the reproductive stages began (Grissom et al., 1955; Spooner, 1961).

Flowering has long been considered a period susceptible to drought stress as it governs the initial number of pods on a plant and is a key contributor to final yield (Board and Tan, 1995). Work by Liu noted that, “severe drought stress soon after anthesis decreased pod set by 40% and resulted in a 50% seed reduction”. Other studies by Westgate and Peterson in addition to Andriani, all found that water deficits early in the reproductive stages where flowering is prominent (R1-R3) resulted in a decreased number of viable pods in contrast to those plants which did not experience the water deficit (Andriani et al., 1991; Liu et al., 2003; Westgate and Peterson, 1993).

In many cereal crops, pollination can be a very sensitive stage to drought stress, but in soybeans this process is not considered to be sensitive in regards to pollen function. A study on drought stress influence on pollen sterility determined that “flower abortion cause by a preanthesis water deficit is not attributed to an impairment of pollen, but was probably due to impairment of ovule function” (Kokubun et al., 2001). Lastly, the period of seed filling is arguably the most stress sensitive stage in soybean growth and development. Sionit and Kramer tested the effects of water stress during different stages of growth and determined that stress that occurred during pod formation or pod filling resulted in greater yield reduction than when stress occurs at earlier stages (Sionit and Kramer, 1977). In a three year study with ten variations in drought timing, it was determined that water availability during the pod-fill stage is most critical in achieving maximum yield (Doss et al., 1974). Brevedan and Egli suggest that a completely water stress free environment must exist during the period of pod-fill in order for soybeans to reach maximum yield (Brevedan and Egli, 2003).

From the foregoing research, it is apparent that much care should be taken to ensure a water stress free environment during rapid seed development and pod-fill (R5-R6). There are two positions from which producers must address this problem, those with irrigation capabilities and those without. For those areas with irrigation capabilities, it is necessary to have insight into the progression of the drought stress as a measure of both soil water status and plant water status. To make informed irrigation applications, one must know the level of water deficit at which the soybean plant begins to experience stress as a reference point for the current water conditions. Given both the ability to accurately measure the water status of the soil and the availability of credible thresholds levels for the onset of plant stress, growers would have the information necessary to apply water at the correct time and rate before stress is induced.

Two problems currently exist in accurately supplementing water to soybeans via irrigation. The first develops from the potential differences between the water status of the soil and the water status of the soybean plant, which is dictated by the combination of plant, soil and environmental interactions. Thus, it is not always appropriate to assume a direct correlation between the soil water status and plant water status (Kramer, 1995). This theory originated from observations of a well-watered plant experiencing temporary wilting early in the mornings and then again when transpiration is highest during midday (Kramer, 1995; Maximov and Yapp, 1929). From this we can see that a seemingly constant soil water status is at times sufficient and others insufficient in allowing the plant to transpire water at a rate that meets the atmospheric demand. This would suggest that when monitoring the water status of a sensitive crop, one should directly measure the water status of the plants and not the soil. Although existing technologies can be used to measure the plant water status, none are as easily employed in a continuous field setting as current systems designed to measure soil moisture.

This creates the second issue in that a large percentage of soil moisture measurements are based on a ratio between the volume of water and the volume of soil, this is insightful, but not a true measure of the soil moisture conditions that the plant is experiencing (Kramer, 1995). To understand the true moisture environment, either the soil physical and hydraulic properties should be determined in conjunction with the volumetric water content values, or the matric potential of the soil should be measured directly. This value could then be compared against reference critical values associated with the onset of stress within the plant. Previously, these critical values or thresholds are considered to occur when roughly 50% of the maximum plant available water remains in the rooting zone (Richard G. Allen, 1998). Having additional knowledge of these thresholds would serve as a guideline for irrigation applications across soil types and soil properties.

For many growers, irrigation is not available and only agronomic decisions can be made to prepare a plant for potential drought stress. Historically, producers have attempted to assist soybeans in escaping potential droughts via refinement of plant population, timing of planting and selection of a soybean variety with a specific growth habit. The refinement of plant populations is utilized to reduce levels of interplant competition for limited water resources. The time of planting and stem growth habit selection are utilized to position the timing of soybean reproduction during a period that is either before or after the period of the growing season with the greatest historical potential for drought. The difference in stem growth habits amongst soybeans, determinate or indeterminate, each offer unique opportunities and risks when combating potential drought conditions. Soybeans that have a determinate habit cease vegetative growth when the terminal bud flowers (Woodworth, 1932). This is beneficial if the determinate variety completes the sensitive reproductive stages before the onset of drought or withstands the drought and completes the reproductive stages after the drought has subsided. It is a liability however, if the drought stress occurs near pod-fill (R5) as inflorescence has ceased since the terminal bud has flowered and the plant is now allocating resources to the development of existing pods (Gai et al., 1984). For an indeterminate soybean, vegetative growth and reproductive growth occur sequentially as the terminal bud continues to grow while flowers initiate outward on axillary racemes (Carlson and Lersten, 2004; Woodworth, 1932). This allows the reproductive phases to extend over a longer period at the whole plant level. This creates an environment where droughts can arise during the reproductive stages, but the delay between the initiation of subsequent growth stages between nodes may allow for an extended period of pod production on the apical nodes of the plant (Egli and Bruening, 2006).

Irrespective of drought escape measures, many soybean acres are exposed to droughts of varying severity during this sensitive period. During a non-lethal drought, soybean plants can still suffer yield loss which is apparent to growers in the form of visible pod and bean abortion, reduced seed size and foliar damage. In this situation, research is needed to determine the drought's impact on soybean yield potential and the soybean's ability to recover if conditions improve and water deficit conditions reside. Our research attempts to address these issues through a drought trial in a controlled growth environment coupled with a large-scale field trial for growth chamber data validation.

Two objectives were thus developed in an effort to evaluate drought during pod-fill (R5). The first objective was to observe and quantify the loss and recovery of bean growth during drought stress and throughout the recovery period. The second focuses on drought preventive measures as our objective was to determine the matric potential where drought stress signals begin to manifest physiologically in the soybean plant.

CHAPTER II

METHODOLOGY

2.1 Growth Chamber Experiment

2.1.1 Settings and Soils

This study was conducted in a controlled environment growth chamber (Percival Modular Control Systems, Boone, IA). The climate was maintained at one static environment with variation only occurring in night and day temperatures and lengths. Daytime temperature was maintained at 27.8°C while the night temperature was reduced to 20.0°C. Day length was adjusted to mimic the light conditions observed from May – October in northern Oklahoma with light ranges starting at fourteen hours of sunlight and diminishing to eleven hours by maturity. Carbon dioxide and humidity fluctuated with CO_2 ranging from 500 – 700 ppm and humidity from 20 – 40%. A preliminary experiment was conducted to determine the specific lighting conditions necessary to grow a photoperiod sensitive plant anatomically correct. We determined that an alternating placement of metal halide and high pressure sodium bulbs would be utilized as the main energy source, with additional 440 nm (blue) LED lights placed in between to reduce stem elongation. The soil medium was Ashport silt Loam extracted from the site of the field trial near Braman, OK (36◦ 56’ 44’’ N, 97◦ 23’ 25’’ W).

2.1.2 Plant Propagation

Soybeans were selected to be representative of a widely used variety for northern Oklahoma and southern Kansas, the variety Asgrow 45X6 was also selected for its indeterminate growth habit. Prior to planting, seed was inoculated using the product Vault HP which contains living strains of *Bradyrhizobium japonicum*. The inoculated beans were planted into a pre-watered silt loam soil (35.8% sand, 17.2% silt and 47.0% clay) in 1.5 liter pots. At V5, fifth trifoliate, selected plants were transplanted into 10 liter pots containing the same soil. At this time, Decagon soil moisture sensors (5TE) were inserted horizontally into the base of the established root ball. These plants remained well watered from VC-R5 (emergence to pod-fill).

2.1.3 Experimental Design

Five plants were selected at the time of transplanting and soil probe insertion. The five plants were ordered randomly within the chamber to mitigate any bias in the micro environment within the climate control system. One plant was chosen at random to serve as the well-watered control. For the other four plants, short-term water stress was induced via a four-day dry down period. The dry down was initiated at early R5, beginning pod-fill. After four days the plants were experiencing severe wilting, with matric potentials approaching -200 kPa. Re-watering to levels between field capacity and saturation ensued, with conditions remaining well-watered until plant maturity.

2.1.4 Soil Moisture Measurements

Volumetric water content of the soil was measured via Decagon 5TE probes (Decagon Devices, Pullman, WA) with data recorded via a Decagon Em 50 ECH$_2$O data logger. Sensors measured moisture in 20 minute intervals with commands and data review occurring in ECH$_2$O Utility software program. To convert the gathered volumetric water content measurements, a soil moisture retention curve was developed utilizing the Rosetta pedotransfer function (Schaap et al.,

2001) within HYDRUS/1D to estimate the soil hydraulic properties. Measurements of the basic physical properties of the soil were needed to complete this estimate, and thus soil samples were taken directly from the soybean pots to measure texture, bulk density, volumetric water content at field capacity (-33 kPa) and volumetric water content at permanent wilting point (-1500 kPa). Soil texture was determined utilizing the hydrometer method as defined by Gee (Gee, 2002). Determination of field capacity was made using Tempe cells while a pressure plate was used to determine the water content at permanent wilting point (Dane, 2002). These parameters were used in the van Genuchten water retention curve equation to obtain corresponding tension values (Van Genuchten, 1980).

2.1.5 Bean Measurements

All of the ~450 beans initiated at the start of R5, beginning pod fill, were tagged and labeled for sampling. First, pots were labeled based on their water status (1 well-watered & 2-5 stressed). Tagging started at the base (node 1) of the soybean plant with a numbered tag tied around the node locations on the main stem. Second, the pods within each node were numbered directly via permanent marker. To determine the individual bean in that pod, the bean located nearest to the petiole was considered number one with progression towards the distal end. To measure the size of the bean non-destructively, digital calipers with a resolution of 0.01 mm was used to measure the thickness of the bean within the pod (Figure 1). Careful practice was taken amongst individuals recording the measurements to develop a uniform understanding of the firmness at which to measure each bean. The same lab technician typically measured each plant in an additional effort to reduce sampling error. With the soybeans entering R5, all beans were measured and the drought stress was initiated. Thickness of all beans were measured daily for twenty-five consecutive days. At plant maturity, soybeans were hand harvested and final thickness and bean mass measurements were recorded. For data analysis, thickness of the bean was considered as an effective diameter, and the beans were considered to be spheres.

2.1.6 Statistical Analysis

Data were analyzed using Graphpad Prism Version 7.0 (Graphpad Software Inc., La Jolla, CA). Significance was tested for the resulting accumulated volumes via a one-sample t-test. Since no replication was available for the well-watered treatment at the whole plant level, the accumulated volume for the well-watered control was considered a hypothetical value and results were compared at α=0.05. A one-sample t-test, α=0.001, was conducted to compare the individual masses of the finished beans between the control and drought stressed plants (Table 1). A sigmoid function was used to derive the relationship between bean growth rates and the volumetric water content.

2.2 Field Experiment

2.2.1 Locations and Soils

The field trial was established for a single growing season (2015) at a location near Braman, OK (36◦ 56’ 44’’ N, 97◦ 23’ 25’’ W). The location was selected due to its irrigation capabilities with access to both center pivot irrigation and rain-fed production. The soil was an Ashport silt loam 0-1% Slopes (fine-silty, mixed, superactive, thermic Fluventic Haplustolls) with a bulk density of 1.24 g/cm^3.

2.2.2 Experimental Design

Since irrigation location could not be randomized and soil moisture measurements are sensitive to soil variation, the trial utilized a split plot design. The plot was split in half east to west, in ~53 meter long blocks, by the soil moisture regime (irrigated or rain-fed) and was split in half north to south by planting population (247,000 seeds ha^{-1} or 494,000 seed ha^{-1}) in ~12.2 meter wide blocks. The plot design was established to create four zones of varying degrees of water stress. Based on the combination of water availability plus planting population, the following zones were created in increasing order of potential drought stress: irrigated population 247,000 seeds ha^{-1}, irrigated population 494,000 seeds ha^{-1}, rain-fed 247,000 seeds ha^{-1}, and rain-fed 494,000 seeds ha^{-1}. Within each zone, five locations were selected at random to establish locations for continued plant and soil sampling.

2.2.3 Field Methodology

The soybean variety utilized (Asgrow 3832) was an indeterminate group 3.8 maturity. Before planting, the seed was treated with Vault HP to supplement the bacterial strain *Bradyrhizobium jacpanicum* and aid in root nodule development. Soybeans were planted via a White 9816 plate planter into no-till corn residue on 0.762 m row spacing. Two gallons per acre of liquid started, Agroliquid Progerminator (9% nitrogen 24% phosphorus 3% potassium), were applied in furrow. A pre-emergent application of 2.5 oz. Zidua, (0.13 lb. pyroxasulfone) in addition to a post-emergent application of 24 oz. Roundup Powermax (1.03 lb. glyphosate salt) were used for weed control. A fungicide application of 8 oz. Priaxor (0.087 lb. fluxapyroxad and 0.174 lb. pyraclostrobin) was applied aerially at R5, one day prior to sampling. Harvest was manual as an area of two row meters was collected from all 20 sampling location. Soybeans were later threshed via a small plot combine.

2.2.4 Soil and Plant Measurements

Soil moisture was recorded at each of the 20 sampling locations weekly. Readings were taken to a depth of 20 cm ~~,~~ utilizing a Hydro Sense II portable moisture probe (Campbell Scientific Inc., Logan, UT). The instrument has a typical accuracy of ±3% with a volumetric water content resolution of <0.05%. Triplicate soil moisture measurements were made at the base of the plants being sampled at each location, if soil conditions allowed for probe insertion.

Measures of photosynthetic CO_2 assimilation, stomatal conductance and fluorescence (Fv'/Fm') were collected using a LI-6400XT portable photosynthesis system (LI-COR, Lincoln, NE). A single reading was taken from the top-most fully expanded leaf at each of the 20 sampling locations. The sampled leaf was removed for further analysis of leaf relative water content. Collected leaves were chilled until the current field weight could be determined. Leaves were then soaked to determine turgid weight and dried for 24 hours at 80°C to determine the dry weight. Protocols for this procedure follow the methods described by Barr and Weatherly, but deviate in that an entire leaflet was utilized instead of leaf disks (Barr and Weatherley, 1962). Leaf relative water content (RWC) was calculated utilizing the following equation:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100$$

FW = sample fresh weight

TW = sample turgid weight

DW = sample dry weight

To determine the physical properties of the soil, 7.6 cm. diameter core samples were taken to a depth of 50 cm. using a hydraulic probe. Two cores were taken from both the irrigated and rain-fed plots with the top 20 cm. being analyzed to develop a soil water retention curve. Laboratory methods for this analysis follow those outlined in the measurement section for the growth chamber experiment (Section 2.1.4).

2.2.5 Statistical Analysis

Data were analyzed using Graphpad Prism Version 7.0 (Graphpad Software Inc., La Jolla, CA). Significance was tested for the adjusted grain yields and seed size utilizing one-way ANOVA at α=0.01 to analyze the influence of drought in the rain-fed treatment as compared to the irrigated. To determine significance amongst treatments for volumetric water content, photosynthetic CO_2 assimilation, stomatal conductance, fluorescence (Fv′/Fm′) and leaf relative water content, the rain-fed trails which experienced the water stress were compared to the irrigated treatments using two-way ANOVA at α=0.05 .

CHAPTER III

RESULTS AND DISCUSSION

3.1. Growth Chamber

3.1.1. Soybean Development

3.1.1.1. Bean Abortion

3.1.1.1.1 Abortion Timing

Beginning with the first day of the drying phase, all plants with developing drought stress experienced a dramatic decline in the percentage of soybeans experiencing positive growth (Figure 2). During the same four-day period, nearly 100% of the soybeans in the well-watered control experienced positive growth (Figure 2), with an average growth rate of 7.02 mm^3 day^{-1} (data not shown). With continued growth during this period, no bean abortions occurred for the well-watered control. In contrast, the period of stress forced the abortion of 39 beans (10.5% of total) amongst the plants experiencing the drought stress and the reduction of plant available water (Figure 3). Of these 39 abortions, 33 of the initiated embryos finished with less than 1 mm^3 volume while 6 others accumulated less than 10 mm^3 volume. Previous work supports the absence of abortions in the check and the presence of abortions in the treatments as individual seeds must experience multiple days of assimilate deprivation before abortion of the bean will occur (Egli, 2010; Weibold, 1990). Currently, uncertainty exists about the actual length of assimilate deprivation necessary to result in the act of abortion or termination (Egli, 2010). Though determining the exact duration of stress required to induce abortion was not the direct objective of this study, our data indicates that a four-day drought stress period was sufficient to abort a portion of the soybeans (Figure 3). Determining the exact time of termination of a bean proves difficult as minute accumulations of assimilate could be occurring at a volume below the volume of water loss, resulting in a net loss of volume but a continued carbohydrate gain. Alternatively, after the plants have been re-watered, there may be a time lag in the recovery process such that assimilate flow to the beans may not resume immediately. If this occurs, the actual length of time in which beans could survive with no inputs of assimilate could be longer than the four-day drought stress period that our plants endured. Work by Boyer and Westgate offer similar reasoning for the difficulty in determining ovary abortion in corn (Boyer and Westgate, 2004).

While other research has asserted that though pod abortion is largely a phenomenon that occurs early in the reproductive stages of soybean development (R1-R5), pod abortion can occur 10-12 days after R5 (Board and Tan, 1995; Liu et al., 2003; Westgate and Peterson, 1993). Our study supports the occurrence of abortion after R5 for individual beans within a pod. Even though the abortion of entire pods remained possible during this period, the majority of abortions in our study resulted from the loss of a single bean within a pod. Understanding these abortions is critical in analyzing the soybean plant's ability to cope with drought stress at the sensitive stage of R5. Since yield is the product of the number of seeds produced and the relative size of those seeds, losing beans through late R5 abortions can lower yield potential (Board and Tan, 1995).

3.1.1.1.2 Abortion Location

While it is common to observe pod or bean abortion throughout the entirety of the plant, abortion is typically most notable in the extremities such as branches, or the most apical or basal locations on the main stem (Frederick et al., 2001; Liu et al., 2003). Frederick observed that an earlier drought stress, between flowering and early seed fill, is especially damaging to the vegetative growth of lateral branches (Frederick et al., 2001). Thus, combining the sensitivity of drought on the early branch vegetative growth with the sensitivity reported during the reproductive stages, branch yield as a component of the whole plant is highly dependent on water stress conditions (Norsworthy and Shipe, 2005). For our study, classification of the location of the beans was not dependent on main stem or branch location, instead our classification scheme categorized beans based on their point of attachment to a node on the main stem. We documented a negative correlation between abortion and nodal location as the magnitude of abortion increased with decreasing node height (Figure 3). The bottom third of the drought stressed plants experienced 61.5% of the total abortions while the middle third contained 35.9%, and top third 2.6% . When corrected for the number of beans located within each nodal region; 16.6% of the beans within the lower third aborted, 10.4% within the middle third and 1.1% in the upper third.

The soybean variety utilized in our study has an indeterminate growth habit, so one would have hypothesized that this late season drought stress would result in the reverse effect. We would expect more abortion in the upper portion of the plant, as the upper nodes have smaller pods with less mature seed and the beans in the lower nodes are growing rapidly making them a stronger sink and thus less likely to abort (Duthion and Pigeaire, 1991; Egli, 2010; Westgate and Peterson, 1993). There are two likely explanations as to why the soybean plants in our study experienced the negative correlation between abortion and node location. First, the timing of the drought could have aborted late stage flowers and infant soybean pods in the upper nodes which are more susceptible to abortion than the more developed pods at lower nodal locations (Egli, 2010; Egli and Bruening, 2006; Heitholt et al., 1986). This would result in a potentially greater assimilate availability in the upper nodes as fewer soybeans are competing for assimilate because of the early reproductive abortions. This has been observed by Gent, who depodded branches of soybean and noticed that the remaining beans had lower abortion rates and faster growth due to greater assimilate availability (Gent, 1982). Second, during intense prolonged drought, tissue damage and degradation due to age can severely reduce the photosynthetic capacity of the leaves lower in the canopy, and these leaves are directly associated with the basal nodes that experienced abortion (Boyer, 1976; Field, 1987). Both hypotheses have their origin in the mechanics of carbon partitioning.

Throughout the period of drought stress and the subsequent recovery, there is an intra-plant competition for the available carbon resources (Wardlaw, 1990). Fundamental to this competition is the phyllotaxis of source leaves with respect to the reproductive and vegetative sinks. Within the soybean canopy, the vertical growth and initiation of new nodes creates a continuously evolving hierarchy of source leaves with changing assimilatory capacity. Initially, the most productive source leaves are associated with the lower nodes. As development proceeds, the most productive source leaves are attached to ever-higher nodes and thus, a large proportion of assimilate is available to those sinks in the immediate vicinity (Rawson and Hofstra, 1969).

This evolution of the location of the maximum assimilate producing region within the soybean plant likely contributes to the variable distribution of abortion. As witnessed, the lower third of the soybean plants experienced greater abortion relative to the nodes in more elevated locations (Figure 3). At this stage in the plants' lifecycle (R5-R6) many of the main source leaves are attached to the main stem and branches associated with the more elevated nodes. As the older leaves directly associated with the lower nodes progress, they lose photosynthetic capacity and become more shaded as the canopy density intensifies, thus resulting in less readily available assimilate for those beans located on the lower nodes (Boyer, 1976; Field, 1987; Wardlaw, 1990). This variation in the location and strength of the sources is important as it plays a role in "regulating the pattern of carbon portioning" to the pods throughout the plant (Wardlaw, 1990). While assimilate is still being produced in the younger source leaves above, this localized reduction of assimilate could ultimately starve beans of assimilate thus limiting the growth and development of the beans on the lower nodes. If the drought stress is creating a concurrent assimilate shortage in the upper regions of the plant, we would expect there would be less assimilate transfer and thus, the localized shortage could be more pronounced leading to abortion if continued (Egli, 2010). A comparable observation was made in the allocation of carbon between kernels in a wheat head in which the kernels closest to the awn producing the photosynthate received 10-30 times more ^{14}C-labled photosynthate than did kernels of comparative size located on the opposite side of the grain head (Cook and Evans, 1983). Similarly in soybean, photosynthate movement from the leaves to the pods was favored to the sinks directly attached to the source or within adjacent nodes of one to two node locations away from the source (Blomquist and Kust, 1971; Stephenson and Wilson, 1977; Thrower, 1962).

In addition to the availability of assimilate, the strength of which a soybean is able to extract assimilate from the vascular system is influential in determining carbon partitioning amongst the competing sinks. The magnitude of a sink's strength to attract assimilate depends on the actual physical size of the sink and the relative growth rate that the organ is experiencing (Marcelis, 1996; Starck and Ubysz, 1974; Wardlaw, 1990). Together, these features determine the ability of the organ to "effectively lower the concentration of photosynthate in the sieve elements servicing the sinks and thus establish a favorable concentration gradient between the sink and the source" which then dictates the priority for assimilates amongst competing sinks (Wardlaw, 1990). With the onset of the drought stress at R5, our trial lacks the ability to accurately describe the relative growth rate of the newly initiated embryos because of their small size. What can be stated is that of the beans that ultimately aborted, all held volumes in the lower 20th percentile of the entire bean population. The largest bean that aborted had an initial volume of 11.74 mm^3 when competing beans had volumes ranging from 0.51 to 180.27 mm^3 (Figure 2 and 3). The average aborted bean had a volume of 3.43 mm^3 while the average initial volume of the non-aborted beans was 39.91 mm^3. In a study by Duthion and Pigeaire, images of bean lengths were taken through the pod wall over a period of 5.7 weeks. With this data, they were able to denote the maximum length which a soybean was able to achieve during its growth if it ultimately aborted before maturity. They found that 95% of aborted beans never reached a length of above 10 mm (Duthion and Pigeaire, 1991). By using a conversion factor of 1:2 (Shahbazi et al., 2011) for converting the measured bean thickness of our data set to the length measurement used in the Duthion Pigeaire study, we found no abortions to occur in beans with lengths greater than 6 mm long.

3.1.1.2 Growth Recovery

3.1.1.2.1 Whole Plant Recovery

While the onset of the drought stress produces a rapid decline in the number of growing beans, the period of recovery necessary to resume growth was just as rapid (Figure 2). This suggests that the vascular system in the plant was not extensively harmed during the short drought. This allowed water uptake and distribution to regain functionality upon re-watering, which is critical for the recovery process (Boyer, 1971; Grace, 1993). Even though the plants were able to rehydrate and bean growth resumed, sampling continued in an effort to determine the degree of growth recovery. Figure 2 displays this sudden recovery with the percentage of beans growing nearly reaching that of the well-watered control, but then slightly declining below control levels for the remainder of the filling period. In a similar short-term drought study conducted at the University of Kentucky, similar results for the recovery of drought stressed plants were observed through measurements of carbon exchange rate. After experiencing small periods of water stress and rescue applications of water during the R6 growth stage, it was noted that the," carbon exchange rate rapidly increased to near control levels in early stress-relief treatment, but it was always less than the control for the rest of filling" (Brevedan and Egli, 2003). Visualization of bean volume accumulation, post stress initiation, further supported the hypothesis that the stress was not a single event, but that it could depress plant growth indefinitely (Figure 4).

To quantify the residual influence of drought stress during the recovery, the average daily rate of growth was calculated for a five-day period. The five-day window began ten days after the first bean measurement at early R5 and ended with ten days of sampling remaining. This period removes both the early portion of the onset and recovery from drought, plus the end of the trial where the plants are close to reaching R7, beginning maturity, in an effort to reduce error generated by potential ripening of the control plant. During this timeframe, beans which experienced drought stress averaged a growth rate of 4.35 mm^3 day^{-1} while the well-watered control beans gained an average 5.8 mm^3 day^{-1} (Figure 5). This equates to a 25 % reduction in growth rate for the stressed plants lasting at minimum ten days after the drought stress had subsided. This is made more alarming by the fact that aborted beans were not included in this calculation. Thus, the plant had already lost yield potential through abortion and it was then losing yield through reduced growth rates. The exact mechanisms involved were not the target of this growth chamber study. However, results from our field study and from Brevedan and Egli 2003, support the hypothesis that reduced photosynthetic levels may have been a significant contributor to the reduced recovery growth rates. Observations of lower leaf senescence in the water stressed plants, supports the hypothesis that composite assimilate production was reduced in the drought stress treatments.

The accumulated volume, total mass of beans and average mass of beans (Figure 6 and Table 2) encompass the accrued physiological effects that the single period of stress had throughout the growing season. The accumulated volume for all of the beans on the stressed plants were significantly lower (p-value 0.05) than the volume amassed by beans on the well-watered check (Figure 6). Upon maturity, analysis of the mass of these beans indicated that the individual beans were only ~80% the mass of the well-watered check with this difference being significant to p-value 0.001. The total mass of beans per plant returned similar results with the drought-stressed plants only yielding ~83% of the mass of the check (Table 2). While some of this loss is attributed to the actual period of stress where the beans experienced reduced growth to volume loss, a significant portion of this loss resulted from the discrepancy in the growth which occurred during the pod-fill stage when the plants had ample plant available water.

3.1.1.2.2 Nodal Recovery

In contrast to the nodal variation observed during the period of stress. Growth and development during the recovery phase seems indiscriminate of the nodal location. This is apparent in the relative uniformity of the bean growth rates (Figure 5), average mass per finished bean and total bean mass (Table 1). The average growth rates during the five-day window between ten days after the first bean measurement at early R5 and ten days prior to the end of sampling, did not follow the same pattern of injury as did the bean abortion rates. Growth rates for this period were greatest in the top nodal region (nodes 7+) and lowest in the middle region (nodes 4-6) (Figure 5). This pattern is also expressed in the well-watered control. The difference in the growth rates experienced by the drought stressed plants in relation to the growth rate experienced by the well-watered check is the widest in the top nodes (7+) and the lower third (nodes 1-3). In the drought stressed plants, the additional mass per finished bean (Table 1) of the beans located in nodes 7+ is likely the result of a slightly extended growing period relative to the control (Figure 2C), and low sink demand due to a low pod density. The extended growth can be seen several days beyond the point where the control plant was entering R7, beginning maturity (Figure 2C). At this time, the control is starting to decrease its' water content, losing volume, while the beans on the upper nodes of the stressed plants continue to accumulate carbohydrates.

When analyzed by nodal location, the total bean mass produced by nodal groups again does not follow the same pattern as the abortion rates. The plants were able to balance production with each region bearing ~29% to ~36% of the total plant production and average bean size not varying greater than ~7% between node regions (Table 1). This balance is beneficial to the overall yield of a soybean plant that has experienced a period of drought stress. After losing yield potential early by aborting the soybeans for which the plant was unable to supply assimilate, the plant has maintained beans which it must fill to reach its new maximum yield potential. For optimal bean growth to occur, the plant must be able to distribute the produced assimilate across the plant to sinks where localized production has been diminished. Since the plant has the capability to transport the carbon throughout the plant (Gent, 1982), improved rates of growth during the recovery will be dictated by the plant's ability to maintain the largest and healthiest photosynthetic area possible.

3.1.2 Soil Moisture

Through concurrent measurements of soil moisture during the growth chamber trial, we found the transition from positive growth to negative growth rates occurred at a volumetric water content of 18.3% (Figure 7). This corresponds to a soil moisture matric potential of -60 kPa (Figure 8). Previous work has shown the magnitude of soil moisture deficit necessary to induce stress on soybeans not in terms of matric potential but in terms of percent available water and fraction of transpirable soil water (FTSW).

In a similar study where drought stress was introduced earlier in the lifecycle, near anthesis, pod fresh weight started to decrease at FTSW = 0.43 ± 0.02 and pod set started to decrease at FTSW = 0.30 ± 0.01 (Liu et al., 2004). Our data supports a heightened sensitivity to a decline in plant available water as the initial reduction in positive growth occurred near 19% volumetric water content or FTSW = 0.47. The transition from positive growth to loss of volume of beans for the entire plant occurred near FTSW = 0.43, consistent with the results of Liu et al. (2004). Sinclair found similar sensitivities of soybeans to water stress, noting that transpiration rates would begin to decline as FTSW approached 0.3, it was also determined that reactions within the nitrogen fixation process declined when FTSW was below 0.5 (Sinclair, 1986). While FTSW is a useful measurement in visualization of the plant and soil water status, one must carefully note the boundaries considered upon calculation of the FTSW as slight shifts in the upper bounds (field capacity) can shift the FTSW value of a volumetric soil moisture value.

Similarly, the terms readily available water (RAW) and total available water (TAW) can be utilized to describe the plants' ability to uptake water at the roots in response to a given transpirational demand and given soil medium (Richard G. Allen, 1998). Figure 7 illustrates how closely the onset of growth reduction and the utilization of the RAW correspond. Based on literature from the FAO, the percentage of TAW that was deemed at a tension low enough to be accessed by the plant at a rate at or exceeding the transpirational demand routinely occurs when TAW is 50% of maximum (Richard G. Allen, 1998). Since this value is in respect to the transpirational demand by the plant, it is subject to change with environmental conditions (Kramer, 1963). Thus, in conditions that create a greater atmospheric demand for water the percentage of TAW that is considered RAW would be reduced, shifting the lower limit for RAW on Figure 7 to the right (Kramer, 1995; Richard G. Allen, 1998). The opposite is true during periods where there is a low atmospheric demand, under those conditions less water is required to be taken up by the plant roots allowing the plant to meet the transpirational demand at a lower soil water content without experiencing drought stress. With day and night conditions being static in the growth chamber, we would not expect the lower limit of RAW to deviate between days except for the fact that as the soybeans grow the larger canopy transpires greater volumes of water. This is often compensated for through root development and extraction of water from a larger volume of the soil profile (Richard G. Allen, 1998).

The dependency of the stress threshold on the environmental conditions also applies to the matric potential by which the water is held by the soil (Figure 8). Given that the data from the growth chamber was generated in that static environment, the critical tension value would shift with increasing or decreasing transpirational demand under field conditions. To maximize soybean yields where irrigation capabilities are present, our data suggests it is critical to apply irrigation before water tension drops below -60 kPa while being mindful of additional environmental stressors that may shift this critical value. Slight increases in the tension by which the water is held by the soil can significantly affect plant functions and result in a suboptimal level of bean growth and development. This data suggests that for growers with irrigation potential, irrigation planning should be determined via direct analysis of soil moisture tension. Measures of volumetric water content cannot provide the necessary information of the soil moisture status unless a thorough analysis of the soil physical properties are conducted to provide the corresponding moisture release curve.

3.2 Field Trial

3.2.1 Plant and Soil Measurements

Field trial results showed an increase in seed size and yield for plots irrigated versus rain-fed with differences significant at $\alpha=0.01$ (Table 2). Periods of drought stress occurring after full pod (R4) negatively affected the ability of the soybean to set, maintain, and fill beans under the rain-fed treatments. Figure 9 illustrates periods of stress that occurred after sampling began at R4 with convergence of the estimated crop evapotranspiration and cumulative precipitation on the week of 9/6. Weekly monitoring of the plant water status and soil water status (from R4 through the end of R6) supports conclusions drawn about the critical thresholds of soil moisture stress determined during the growth chamber study. Based on the critical matric potential value determined to induce stress under the static conditions in the growth chamber trial, -60 kPa, we determined, via the soil water retention curve, that volumetric water content of <21% should induce water stress in the field if the evaporative demand is similar to that in the growth chamber

(Figure 8). Soil moisture level, for the field trial revealed that the rain-fed trails experienced similar levels of soil moisture stress for roughly three weeks during their reproductive stages (Figure 10A).

This water stress caused progressively more pronounced impacts on the soybean physiological process over time. During the second week of sampling (8/30), the photosynthetic CO_2 assimilations rates were reduced in the rain-fed treatments and the relative water content of the sampled leaves was reduced in the rain-fed treatment with the lower population (Figure 10B & 10D). In the third week of data collection (9/6) and the second straight week in which the plants experience soil water contents below the determined water content threshold, significant reductions occurred in the levels of photosynthetic carbon assimilation, leaf relative water content, and leaf conductance for the rain-fed treatments relative to the irrigated treatments at $\alpha=0.05$ (Figure 10A,B,C & D). By the fourth week of data collection, (9/13), after three weeks of soil volumetric water content below the critical threshold, measurements of fluorescence were also significantly reduced in the rain-fed treatments relative to the irrigated treatments (Figure 10A & 10D).

The gradual progression of symptomology occurring in the plant after the onset of water stress, can be contributed to at least three factors. First, with a soil volumetric water content measurement depth of 20 cm, the plants are able to extract water being held at lower tensions (closer to zero) at deeper depths within the rooting zone (Richard G. Allen, 1998). Second, osmotic adjustment, which can contribute to the lowering of the osmotic potential in the soybean cells can assist in maintaining the physiological process in the early stages of drought as solute accumulation aids in the maintenance of turgor pressure (Morgan, 1992; Morgan, 1984). Lastly, the relative water content of a leaf for a given soil water deficit can vary widely based on genotype and full turgidity may not be necessary for maximal photosynthetic levels to occur (Boyer, 1976; James et al., 2008).

Interestingly, the large disparity in the soil volumetric water content of treatments during the second week (8/30) is less apparent in the related plant measurements for the same week (Figure 10A & 10D). This suggests plant mechanisms that are relatively resilient during the early onset of drought. Several studies have found that leguminous plants utilize a feed-forward mechanism of drought sensing in the root cap, coupled to stomatal regulation as a means to control the internal water balance of the plant (Bates and Hall, 1981). Via a dry down experiment similar to the one we conducted, Liu found that stomatal conductance was reduced at a very high water availability, FTSW = 0.64 (Liu et al., 2005). This is far more sensitive than the crops of wheat and sunflowers in which signaling occurred when only 40% of plant available water remained (Gollan et al., 1986; Schulze, 1993). Stomatal regulation became clear in the second week of water stress in our study (9/6) as lower leaf conductance levels coincide with much lower photosynthetic levels and reduced leaf water content in the rain-fed plots. Thus, there is a reduction in carbon dioxide entering the plant, which results in the lack of available carbon for assimilation in the mesophyll cells (Chaves et al., 2009; Flexas et al., 2004).

Throughout the following weeks, this trend of reduced conductance and photosynthetic CO_2 assimilation continued (Figure 10B & 10C). Differences between treatments in the leaf relative water content and Fv'/Fm' become significant in the final two weeks of data collection, which occurred mostly during the R6 growth stage. Tardieu and Simonneau recorded a similar findings in the timing of plant drought response as "most down regulation of photosynthesis occurred before any change in RWC could be detected" (Tardieu and Simonneau, 1998).With the drought stress below the determined soil moisture threshold for the rain-fed treatments from week two (8/30) through week four (9/13), the stressed soybean plants exhibited accelerated maturity and potential chlorophyll damage based on the fluorescence values in figure 10E, likely as a result of photoinhibition (Krause, 1988; Souza et al., 2004). These processes, in conjunction with the progressing growth stage of the plant (R6), hinder the plant recovery when improved soil moisture conditions occurred at the end of the season.

The trends in plant functions and parameters that arose as a result of the levels and durations of induced soil moisture stress help to confirm the soil moisture threshold determined via the growth chamber study. One such parameter that is crucial in relating these experiments is the association seen between the levels of soil moisture and the relative water content of the leaf. By plotting these values for the first three weeks of the sampling period where no major leaf damage or senescence had occurred, an apparent trend arises in the decline of water in the leaf when soil water contents drops below the threshold value (Figure 11). Visual observation of the sampled leaves reflect morphological reactions similar to the results seen in a study by James et al. where well-watered soybeans had RWC values about 90%, and turgor loss did not occur until leaves reached a RWC near 70% (James et al., 2008). As drought continued, it took leaf RWC 50% or below to reach levels near lethal (James et al., 2008). This critical lower level is supported by our data as we witnessed neither the occurrence of 50% RWC nor plant death at the levels near 60%, which were observed (Figure 11). Research by Sinclair and Ludlow confirm this lethal value as they observed plant death when the RWC of the youngest fully expanded leaf dropped below 50 ± 0.6% (Sinclair and Ludlow, 1986). Although the exact point of decline in leaf water content cannot be determined precisely from the data, the decline appears to be consistent with the estimate value of 21% soil water content, or -60 kPa. Based on this field trail study, we can confirm that soil moisture conditions at or below -60kPa will likely initiate drought stress within the soybean; response in the plant will vary based on genotype and environmental conditions.

3.2.2 Priaxor Treatment

During an experimental trial day, one week prior to the start of sampling, the photosynthetic measurements recorded rates considerably higher than typical carbon assimilation rates gathered in the weeks that followed (Table 3). The day prior to sampling, the fungicide Priaxor (active ingredients fluxapyroxad and pyraclostrobin) was applied to the crop. The manufacturer, BASF, has reported this fungicide to have plant health benefits, one of which is increasing photosynthetic assimilation rates. One hypothesis is that the fungicide acts to promote the carboxylation activity of rubisco (ribulose 1,5-bisphosphate). This method of accelerated photosynthesis has been proposed as an explanation for the ability of soybean to increase photosynthetic rates in unshaded leaves when neighboring leaves have been intentionally shaded and their assimilate production levels decline (Peet and Kramer, 1980). Further research could determine the utility or efficacy of such an application in deterring potential flower and bean abortions. It may also be beneficial in improving growth and development after a period of stress via an upregulation in photosynthesis to produce additional assimilates.

CHAPTER IV

CONCLUSION

Soil water stored at a relatively high matric potential of -60 kPa is held tightly enough to the soil that environmental conditions could generate a demand greater than the root uptake potential for soybean. Such deficits will lead to the impairment of necessary physiological processes if allowed to persist. Abortion rates of 10.5% occurred for initiated beans on plants stressed by the four-day drought. At maturity, beans on the stressed plants were 20% smaller than beans growing on the well-watered treatment. Growth rates exhibited by once stressed beans averaged 25% less growth per day after stressed conditions had been alleviated for 5 days, confirming theories of chronic damage due to past stress events. The cumulative effects of a short-term drought at R5 resulted in a 20% decrease in the plant yield compared to the control, despite ideal moisture conditions from mid R5 to maturity.

In light of soybean sensitivity to droughts of short duration and limited intensity, it is necessary to pursue agronomic practices that aid in the preservation of soil moisture, continue efforts in breeding for improved drought tolerance mechanisms, and to promote the aggressive monitoring of the soil moisture status in fields where irrigation capabilities are present. While the most precise water status monitoring would occur at the plant level, current practices and the most utilized technology for monitoring the water status of a crop is via analysis of volumetric water content. These measurements of volumetric water content are not informative without knowledge of the soil hydrological properties and are thus inadequate for providing an accurate measure of the water status of the plant. Current technology for monitoring the plant water status is not conducive for growers to implement in soybean production, and the nature of the soybean to adjust various processes at the onset of a soil water deficit can delay visibility of approaching water deficit conditions. Thus, soil moisture monitoring is still necessary. Our research supports the use of soil moisture sensors such as tensiometers that directly measure soil water matric potential, so that the hydraulic properties of the soil are accounted for in determining the moisture content as a tension value. Best soybean production practices would employ use of these sensors at varying depths within the soybean rooting profile with irrigation applied prior to the integrated tension value reaching -60 kPa.

Further analysis of this critical matric potential value across soil types and additional soybean genotypes would contribute additional support to this value as a base irrigation threshold. After recording reduced bean growth rates post drought recovery, it would be beneficial to analyze the growth rates after varying intensities and duration of drought. Lastly, exploration of any efforts to manipulate photosynthetic rates post drought stress could prove as a means to assist bean growth during the remaining period of pod-fill and result in improved soybean yield recovery.

REFERENCES

Andriani J., Andrade F., Suero E., Dardanelli J. (1991) Water deficits during reproductive growth of soybeans. I. Their effects on dry matter accumulation, seed yield and its components. Agronomie 11:737-746.

Barr H., Weatherley P. (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15:413-428.

Bates L., Hall A. (1981) Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. Oecologia 50:62-65.

Blomquist R., Kust C. (1971) Translocation pattern of soybeans as affected by growth substances and maturity. Crop science 11:390-393.

Board J., Tan Q. (1995) Assimilatory capacity effects on soybean yield components and pod number. Crop science 35:846-851.

Board J.E., Harville B.G. (1996) Growth dynamics during the vegetative period affects yield of narrow-row, late-planted soybean. Agronomy Journal 88:567-572.

Boyer J. (1971) Recovery of photosynthesis in sunflower after a period of low leaf water potential. Plant Physiology 47:816-820.

Boyer J. (1976) Water deficits and photosynthesis. Water deficits and plant growth 4:153-190.

Boyer J.S., Westgate M.E. (2004) Grain yields with limited water. Journal of Experimental Botany 55:2385-2394. DOI: 10.1093/jxb/erh219.

Brevedan R.E., Egli D.B. (2003) Short Periods of Water Stress during Seed Filling, Leaf Senescence, and Yield of Soybean Published with the approval of the Director of the Kentucky Agric. Exp. Stn. as paper No. 03-06-001. Crop Science 43:2083-2088. DOI: 10.2135/cropsci2003.2083.

Carlson J.B., Lersten N.R. (2004) Reproductive morphology. Soybeans: improvement, production, and uses:59-95.

Casteel S. (2009) Soybean Physiology, How well do you know soybeans? Purdue University, www.soybeanstation.org.

Chaves M., Flexas J., Pinheiro C. (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of botany 103:551-560.

Cook M., Evans L. (1983) The roles of sink size and location in the partitioning of assimilates in wheat ears. Functional Plant Biology 10:313-327.

Dane J.H.H., Jan. W. (2002) The Soil Solution Phase - Laboratory, in: W. A. Dick (Ed.), Methods of Soil Analysis - Part 4 Physical Methods, Soil Science Society of America, Madison, Wisconsin. pp. 684-390.

Dogan E., Kirnak H., Copur O. (2007) Deficit irrigations during soybean reproductive stages and CROPGRO-soybean simulations under semi-arid climatic conditions. Field Crops Research 103:154-159.

Doss B., Pearson R., Rogers H.T. (1974) Effect of soil water stress at various growth stages on soybean yield. Agronomy journal 66:297-299.

Duthion C., Pigeaire A. (1991) Seed lengths corresponding to the final stage in seed abortion of three grain legumes. Crop Science 31:1579-1583.

Egli D.B. (2010) SOYPOD: A Model of Fruit Set in Soybean All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Agronomy Journal 102:39-47. DOI: 10.2134/agronj2009.0222.

Egli D.B., Bruening W. (2006) Temporal profiles of pod production and pod set in soybean. European Journal of Agronomy 24:11-18.

FAO. (2016) Food Outlook, Biannual Report on Global Food Markets, Food and Agricultural Organization of the United Nations. pp. 35-36.

Field C.B. (1987) Leaf-Age Effects on Stomatal Conductance Christopher B. Field. Stomatal function:367.

Flexas J., Bota J., Loreto F., Cornic G., Sharkey T. (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biology 6:269-279.

Frederick J.R., Camp C.R., Bauer P.J. (2001) Drought-stress effects on branch and mainstem seed yield and yield components of determinate soybean. Crop science 41:759-763.

Gai J., Palmer R.G., Fehr W.R. (1984) Bloom and Pod Set in Determinate and Indeterminate Soybeans Grown in China1. Agronomy Journal 76:979-984. DOI: 10.2134/agronj1984.00021962007600060026x.

Gee G.W.O., Dani. (2002) The Solid Phase - Particle-Size Analysis, in: W. A. Dick (Ed.), Methods of Soil Analysis - Part 4 Physical Methods, Soil Science Society of America, Madison, Wisconsin. pp. 278-282.

Gent M.P. (1982) Effect of defoliation and depodding on long distance translocation and yield in Y-shaped soybean plants. Crop Science 22:245-250.

Gibson L., Mullen R. (1996) Influence of day and night temperature on soybean seed yield. Crop Science 36:98-104.

Gollan T., Richards R., Rawson H., Passioura J., Johnson D., Munns R. (1986) Soil water status affects the stomata1. Functional Plant Biology 13:459-464.

Grace J. (1993) Consequences of xylem cavitation for plant water deficits, in: J. A. C. G. Smith, H. (Ed.), Water Deficits - plant responses from cell to community, Environmental Plant Biology. pp. 109-128.

Grissom P.H., Hogg P.G., Raney W.A. (1955) Crop response to irrigation in the Yazoo-Mississippi Delta.

Harlan J.R. (1995) The Living Fields - Our Agricultural Heritage Cambridge University Press

Heitholt J.J., Egli D., Leggett J. (1986) Characteristics of reproductive abortion in soybean. Crop Science 26:589-595.

James A.T., Lawn R.J., Cooper M. (2008) Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild Glycine spp. for epidermal conductance, osmotic potential, and relative water content. Australian Journal of Agricultural Research 59:656-669. DOI: https://doi.org/10.1071/AR07159.

Janick J.S., Robert W. Woods, Frank W. Ruttan, Vernon W. (1974) Plant Science - An Introduction to World Crops. 2 ed. W. H. Freeman and Company

Kokubun M., Shimada S., Takahashi M. (2001) Flower abortion caused by preanthesis water deficit is not attributed to impairment of pollen in soybean. Crop Science 41:1517-1521.

Kramer P.J. (1963) Water stress and plant growth. Agronomy Journal 55:31-35.

Kramer P.J.B., John. S. (1995) Water Relations of Plants and Soils. 2nd ed. Academic Press Inc.

Kranz W.L.S., James E. (2012) Irrigating Soybean, University of Nebraska - Lincoln Extension, Institute of Agriculture and Natural Resources, University of Nebraska - Lincoln.

Krause G.H. (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiologia Plantarum 74:566-574.

Liu F., Andersen M.N., Jacobsen S.-E., Jensen C.R. (2005) Stomatal control and water use efficiency of soybean (Glycine max L. Merr.) during progressive soil drying. Environmental and Experimental Botany 54:33-40.

Liu F., Andersen M.N., Jensen C.R. (2003) Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. Functional Plant Biology 30:271-280. DOI: http://dx.doi.org/10.1071/FP02185.

Liu F., Andersen M.N., Jensen C.R. (2004) Root signal controls pod growth in drought-stressed soybean during the critical, abortion-sensitive phase of pod development. Field Crops Research 85:159-166. DOI: http://dx.doi.org/10.1016/S0378-4290(03)00164-3.

Marcelis L. (1996) Sink strength as a determinant of dry matter partitioning in the whole plant. Journal of Experimental Botany:1281-1291.

Maximov N.A., Yapp R.H. (1929) The plant in relation to water: a study of the physiological basis of drought resistance Allen & Unwin.

Miller P.R., McConkey B.G., Clayton G.W., Brandt S.A., Staricka J.A., Johnston A.M., Lafond G.P., Schatz B.G., Baltensperger D.D., Neill K.E. (2002) Pulse crop adaptation in the northern Great Plains. Agronomy Journal 94:261-272.

Morgan J. (1992) Adaptation to water deficits in three grain legume species. Mechanisms of turgor maintenance. Field Crops Research 29:91-106.

Morgan J.M. (1984) Osmoregulation and water stress in higher plants. Annual review of plant physiology 35:299-319.

Muchow R., Sinclair T., Bennett J., Hammond L. (1986) Response of leaf growth, leaf nitrogen, and stomatal conductance to water deficits during vegetative growth of field-grown soybean. Crop science 26:1190-1195.

NCSPA. (2014) Uses of Soybeans, Uses of Soybeans, North Carolina Soybean Producers Association INC.

Norsworthy J.K., Shipe E.R. (2005) Effect of row spacing and soybean genotype on mainstem and branch yield. Agronomy journal 97:919-923.
Peet M., Kramer P. (1980) Effects of decreasing source/sink ratio in soybeans on photosynthesis, photorespiration, transpiration and yield. Plant, Cell & Environment 3:201-206.
Rawson H.M., Hofstra G. (1969) Translocation and remobilization of 14C assimilated at different stages by each leaf of the wheat plant. Australian Journal of Biological Sciences 22:321-332.
Richard G. Allen L.S.P., Dirk Raes, Martin Smith. (1998) Crop evapotranspiration - Guidelines for computing crop water requirements in: N. R. M. a. E. Department (Ed.), FAO Irrigation and Drainage.
Schaap M.G., Leij F.J., Van Genuchten M.T. (2001) ROSETTA: a computer program for estimating soil hydraulic parameters with hierarchical pedotransfer functions. Journal of hydrology 251:163-176.
Schulze E.D. (1993) Soil water deficits and atmospheric humidity as environmental signals. , in: J. A. C. G. Smith, H. (Ed.), Water Deficits - plant responses from cell to community. pp. 136-141.
Shahbazi F., Analooei M., Saffar A. (2011) Mechanical damage to pinto bean seeds as affected by moisture content, impact velocity and seed orientation. Int. J. Food. Eng 7.
Sinclair T., Ludlow M. (1986) Influence of Soil Water Supply on the Plant Water Balance of Four Tropical Grain Legumes. Functional Plant Biology 13:329-341. DOI: https://doi.org/10.1071/PP9860329.
Sinclair T.R. (1986) Water and nitrogen limitations in soybean grain production I. Model development. Field Crops Research 15:125-141. DOI: http://dx.doi.org/10.1016/0378-4290(86)90082-1.
Sionit N., Kramer P.J. (1977) Effect of water stress during different stages of growth of soybean. Agronomy Journal 69:274-278.
Souza R., Machado E., Silva J., Lagôa A., Silveira J. (2004) Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. Environmental and experimental botany 51:45-56.
Spooner A.E. (1961) Effects of irrigation timing and length of flooding periods on soybean yields.
Starck Z., Ubysz L. (1974) Effect of limited supply of assimilates on the relationships between their sources and acceptors. Acta Societatis Botanicorum Poloniae 43:427-445.
Stephenson R., Wilson G. (1977) Patterns of assimilate distribution in soybeans at maturity. I. The influence of reproductive development stage and leaf position. Crop and Pasture Science 28:203-209.
Tardieu F., Simonneau T. (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. Journal of experimental botany 49:419-432.
Thrower S. (1962) Translocation of labelled assimilates in the soybean II. The pattern of translocation in intact and defoliated plants. Australian Journal of Biological Sciences 15:629-649.

United S.B. Soybean Nutritional Information, Soy Connection.
USDA. (2016a) Acreage Report, National Agricultural Statistics Service Agricultural Statistics Board of the United States Department of Agriculture.
USDA. (2016b) Oklahoma Soybean County Estimates, United States Department of Agriculture National Agricultural Statistics Service Southern Plains Region.
Van Genuchten M.T. (1980) A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil science society of America journal 44:892-898.
Wardlaw I.F. (1990) Tansley Review No. 27 The control of carbon partitioning in plants. New Phytologist 116:341-381. DOI: 10.1111/j.1469-8137.1990.tb00524.x.
Weibold W.J. (1990) Rescue of soybean flowers destined to abscise. Agronomy Journal 82:85-88.
Westgate M., Peterson C. (1993) Flower and pod development in water-deficient soybeans (Glycine max L. Merr.). Journal of Experimental Botany 44:109-117.
Woodworth C.M. (1932) Genetics and breeding in the improvement of the soybean. Bulletin (University of Illinois (Urbana-Champaign campus). Agricultural Experiment Station); no. 384.

APPENDICES

Table 1. Mass of soybeans at maturity in growth chamber experiment. Values marked (*) are significantly different from the control at p-values < 0.001.

	Check	Plant Replicates				
	WW	P1X 0.171	P2X 0.151	P3X 0.171	P4X 0.132	Rep. Avg.
			Average Mass per Finished Bean (grams)			
Nodes 7+	0.199	0.174	0.165	0.170	0.166	0.169
Nodes 4-6	0.199	0.151	0.166	0.149	0.161	0.157
Nodes 1-3	0.208	0.150	0.165	0.164	0.159	0.159
Total Plant	0.202*	0.156	0.165	0.163	0.162	0.161*
			Number of Beans			
Nodes 7+	21	17	18	28	27	22.5
Nodes 4-6	31	40	23	18	39	30
Nodes 1-3	27	22	44	28	26	30
Plant Total	79	79	85	74	92	82.5
			Total Bean Mass (grams)			
Nodes 7+	4.18	2.96	2.97	4.76	4.48	3.79
Nodes 4-6	6.17	6.04	3.82	2.68	6.28	4.71
Nodes1-3	5.62	3.30	7.26	4.59	4.13	4.82
Total Plant	15.97	12.30	14.05	12.03	14.89	13.32

Table 2. Harvest measurements for 2015 Field Trail. Superscript letters denote differences which are significant at α=0.01. Test weight, moisture and seed size are not reflective of harvest conditions.

2015 Field Trial – Yield Results						
Treatment	Plot ID	Harvest Wt.	Test Weight	Moisture	Seed Number	Yield[§]
		g $1.52m^{2-1}$	kg hl^{-1}	%	Seeds kg^{-1}	kg ha^{-1}
Irrigated	AI1	920.6	69.9	6.4	6,067	6450.3
	BI1	868.1	69.1	6.6	6,544	6082.4
Population	CI1	882.0	70.1	6.6	6,684	6179.8
247,000 seeds	DI1	745.6	69.9	6.5	6,631	5224.1
ha^{-1}	EI1	1027.2	68.5	6.5	6,618	7197.2
	Avg.				6,509 ^{A}	6226.7 ^{A}
Irrigated	AI2	778.3	70.0	6.5	6,277	5453.2
	BI2	839.1	70.0	6.5	6,176	5879.2
Population	CI2	957.7	70.1	7.0	6,123	6710.2
494,000 seeds	DI2	945.2	69.9	6.3	5,959	6622.6
ha^{-1}	EI2	693.2	69.4	6.4	6,038	4857.0
	Avg.				6,115 ^{A}	5904.4 ^{A}
Rain-fed	AD1	524.2	69.5	6.5	7,836	3672.8
	BD1	573.6	70.5	6.5	7,564	4019.0
Population	CD1	427.2	71.6	6.6	7,968	2993.2
247,000 seeds	DD1	506.6	69.8	6.5	7,309	3549.5
ha^{-1}	ED1	591.0	70.8	6.6	7,558	4140.9
	Avg.				7,647 ^{B}	3675.1 ^{B}
Rain-fed	AD2	539.4	71.3	6.9	7,968	3779.3
	BD2	471.4	69.9	6.4	7,326	3302.9
Population	CD2	422.8	71.5	6.5	7,496	2962.4
494,000 seeds	DD2	373.9	71.0	6.5	7,412	2619.8
ha^{-1}	ED2	695.2	70.3	6.5	7,215	4871.0
	Avg.				7,483 ^{B}	3507.1 ^{B}

§ Yield corrected to represent a harvest moisture of 13.0% water by mass.

Table 3. Measurements taken via LI-COR 6400 one week prior to initiation of experiment sampling at R4. Photosynthetic levels are in excess of rates recorded from other weeks during this trial and observed literature. Aerial application of Priaxor fungicide occurred 18 hours before data collection.

2015 Field Trial - Priaxor Fungicide Treatment					
Plot Replicate	Photosynthesis umol CO_2 $m^{-2}s^{-1}$	Conductance mol H_2O $m^{-2}s^{-1}$	Fv'/Fm'	Transpiration mmol H_2O $m^{-2}s^{-1}$	Leaf RWC %
AI1	57	1.07	0.519	12.2	0.84
BI1	64.5	1.31	0.574	12.6	0.77
CI1	65.7	1.47	0.568	12.6	0.85
DI1	45.1	1.17	0.528	11.8	0.74
EI1	58.2	1.08	0.575	11.3	0.84
AI2	63.2	1.62	0.541	13.1	0.69
BI2	53.7	1.32	0.557	12.5	0.66
CI2	64.9	1.44	0.563	13.3	0.77
DI2	61.5	1.21	0.539	13.4	0.75
EI2	59.3	1.43	0.525	13.6	0.84
Replicate Avg.	59.3	1.31	0.549	12.6	0.78

Figure 1. Images depict sampling methods for growth chamber data collections of bean size (thickness) and soil moisture (volumetric water content).

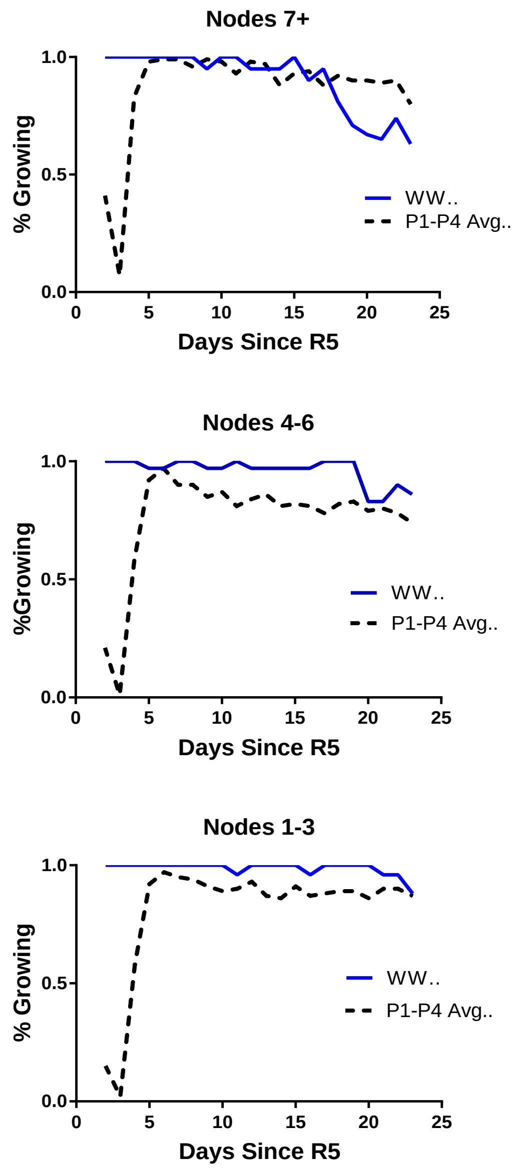


Figure 2. Lines indicate the percentage of soybeans experiencing positive growth in volume relative to the number of viable beans that the plant initiated prior to water stress. Proximity of line for the average percent growing of soybean amongst drought stress treatments (P1-P4 Avg.) in relation to line for percent growing of well-watered check (WW) is an indicator of the ability of the soybean to recover after the period of stress. Data depicted includes beans which aborted. Rates of growth were initially smoothed via single Hanning (0.25 day prior 0.5 current day 0.25 day post).

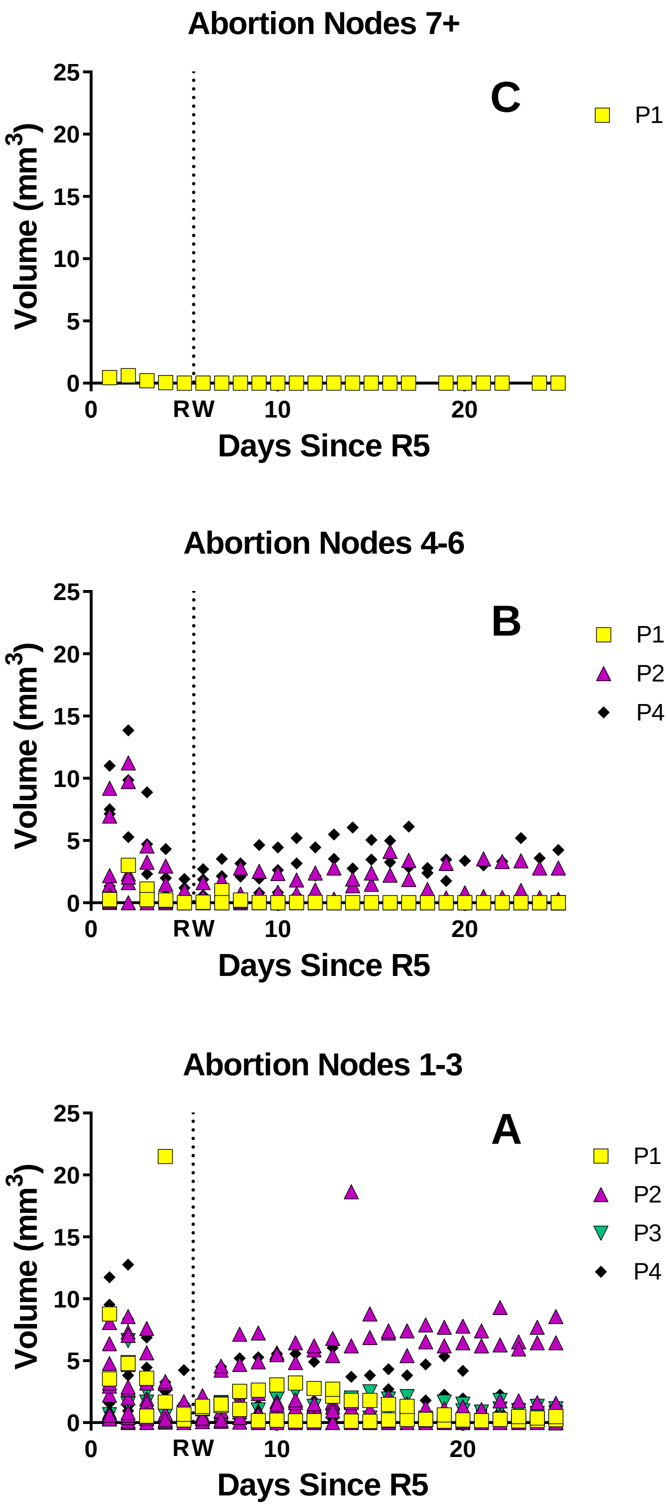


Figure 3. Volume of individual beans that aborted after the period of drought stress. (*RW – point of re-watering for stressed plants)*

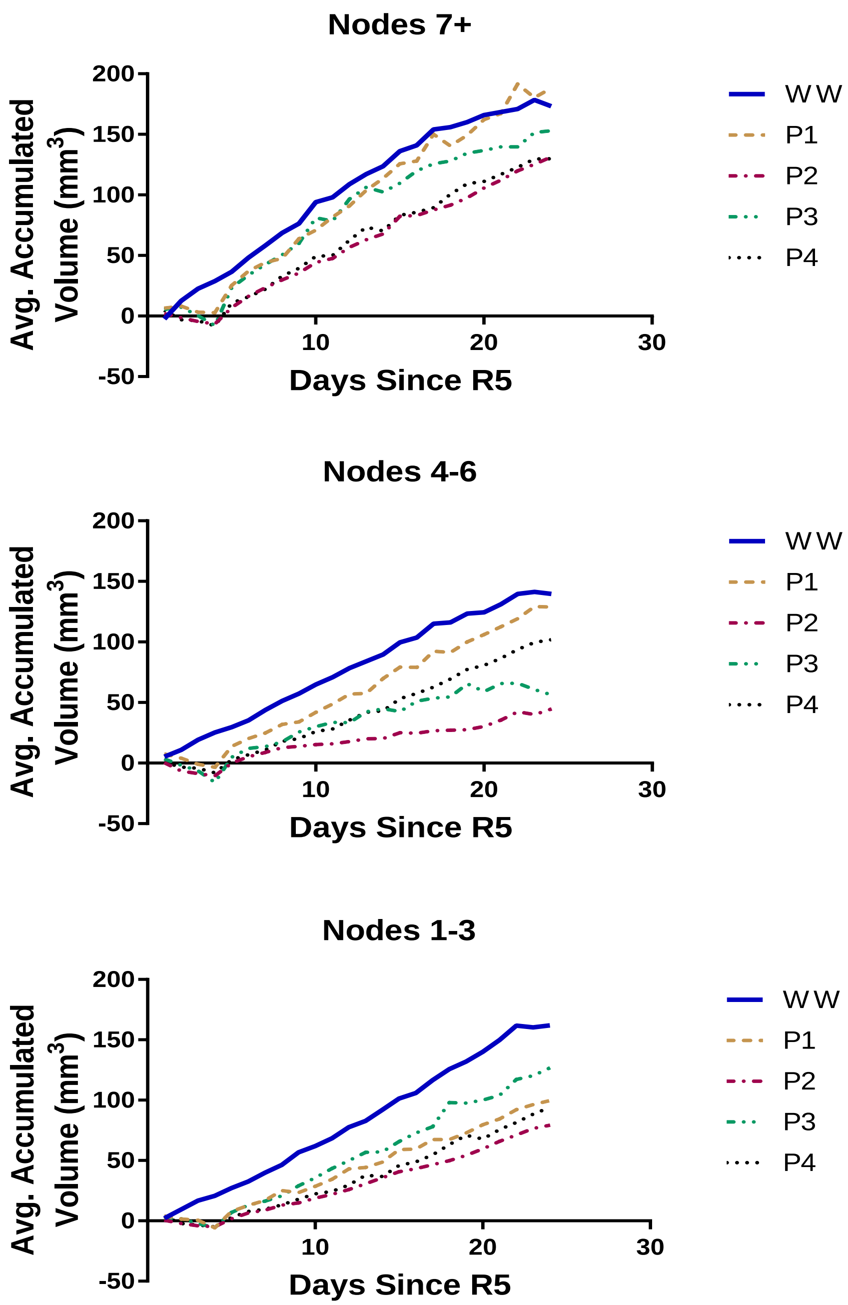


Figure 4. Lines indicate the average accumulate volume for the individual beans within each node as growth progresses from R5. The drought stress treatments (P1-P4) all show injury with initial loss of volume. The most basal node group (nodes 1-3) show the largest loss in bean volume accumulated after stress. Drought stress treatments recover closer to the accumulated volume of beans in the well-watered check (WW) with nodes located higher in the canopy.

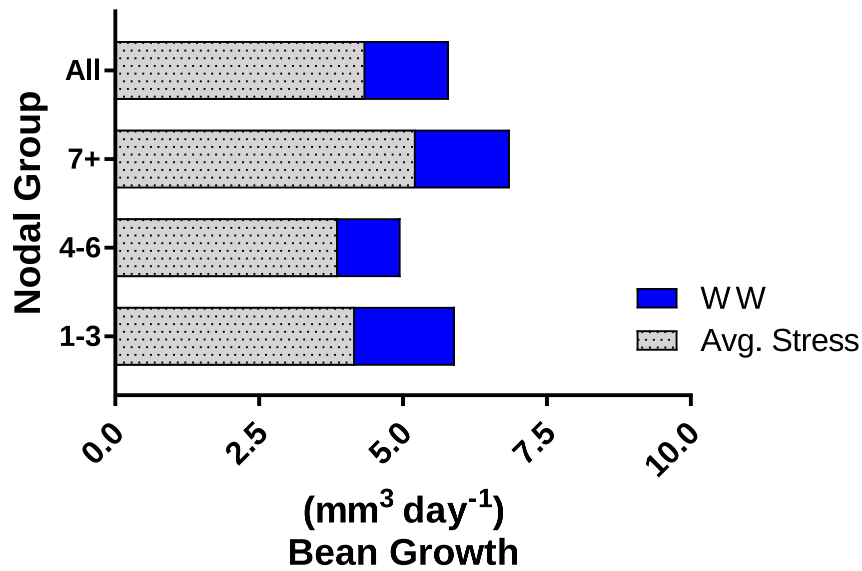


Figure 5. Growth rates were calculated during a five-day period spanning from ten days after the first sampling date, until ten days before the final sampling date. The composite average growth rate for beans on the plants that had experienced drought stress is 25% less than the growth rate achieved by the control which never experienced stress. This growth is occurring during a time when all plants are considered to be free from water stress. The calculations for growth rate excluded beans which were deemed aborted. The elevated growth rates in the upper nodes are likely a product of low pod density per node.

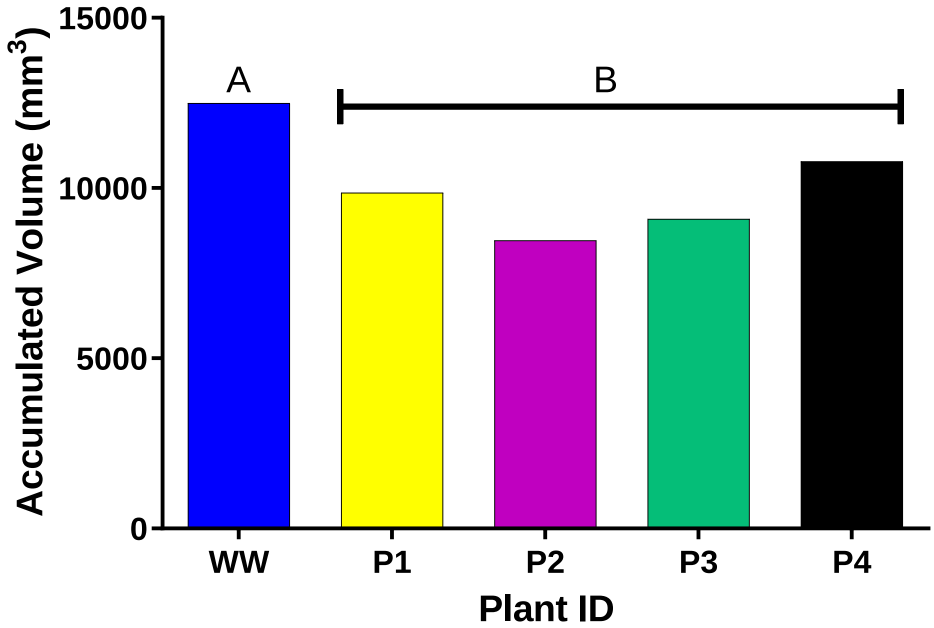


Figure 6. Accumulated volume for all soybeans on a plant from the initiation of stress (Early R5) to the end of data collection (Late R6). A four-day drought resulted in a lasting reduction of soybean volume.

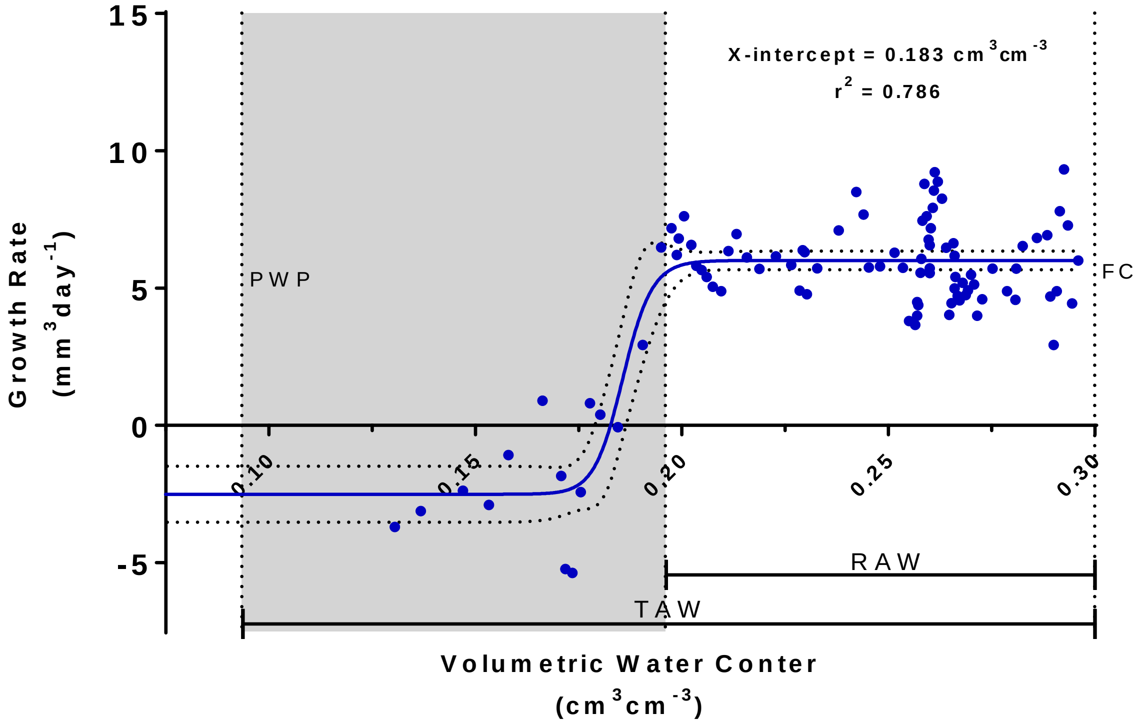


Figure 7. Average daily growth rate for all beans on plant, exhibited for soybeans experiencing varying water conditions. X-intercept indicates point where greater stress results in the loss of bean volume. Dotted line indicates 95 % confidence interval. (RAW = 0.5 plant available water, PWP = permanent wilting point -1500 kPa, FC = field capacity -10 kPa). Data was smoothed via single Hanning (0.25 day prior 0.5 current day 0.25 day post).

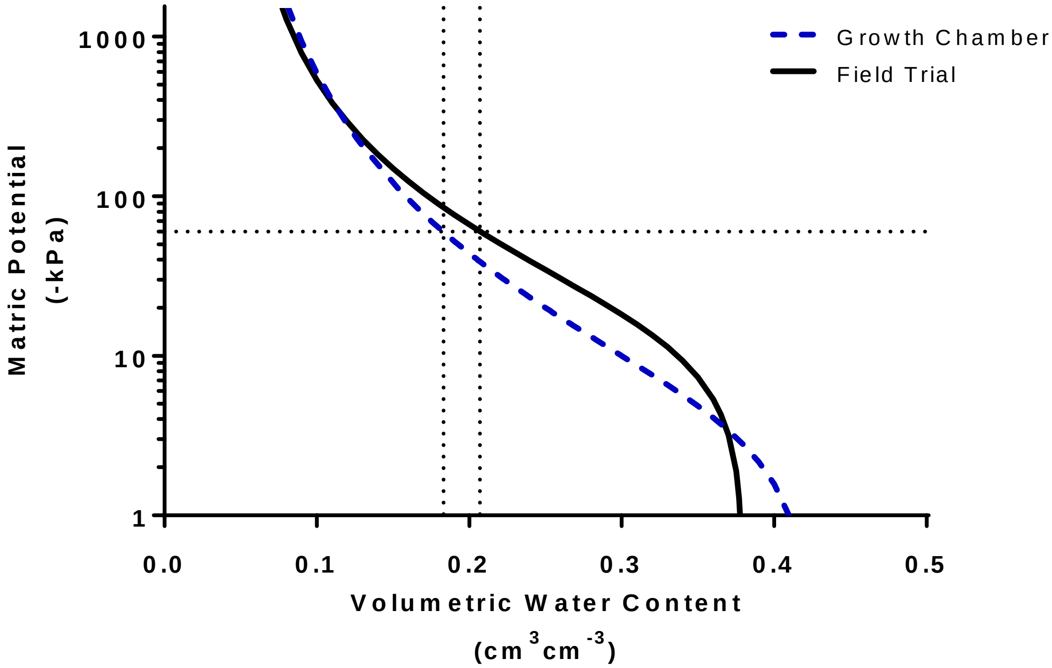


Figure 8. Soil water retention curves for the growth chamber soil medium and the top 20 cm. of field trial profile. Vertical dotted line at X = 0.18 cm^3 cm^{-3} represents the determined critical volumetric water content for the onset of water stress in the growth chamber medium. The matric potential for which this volumetric water content corresponds is -60 kPa. Utilizing -60 kPa as the critical matric potential value across soils, the corresponding critical volumetric water content for the field trial occurs at volumetric water content ~0.20 cm^3 cm^{-3}.

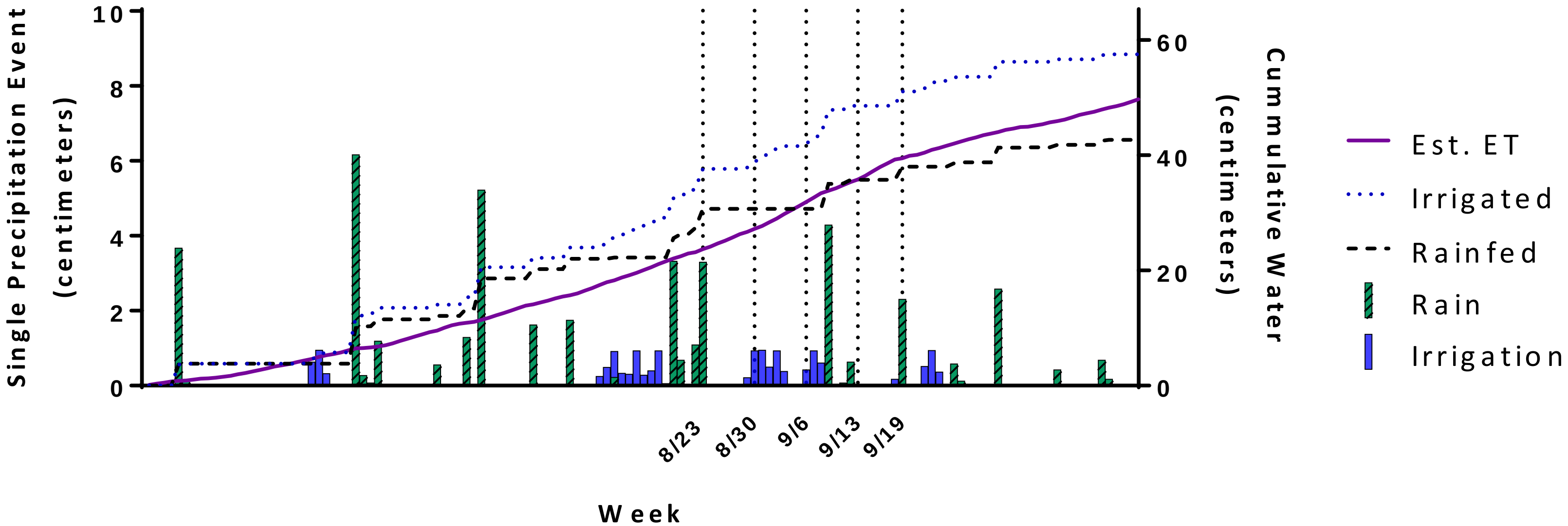


Figure 9. Bars depict single precipitation events provided through rainfall or irrigation. Dotted lines represent the accumulation of these precipitation events throughout the growing season. Estimated ET (evapotranspiration) is approximated from weather data recorded at the closest Mesonet weather station (Blackwell, OK). Rain events are approximates from the Blackwell, OK Mesonet weather station while irrigation events were record at the center pivot system.

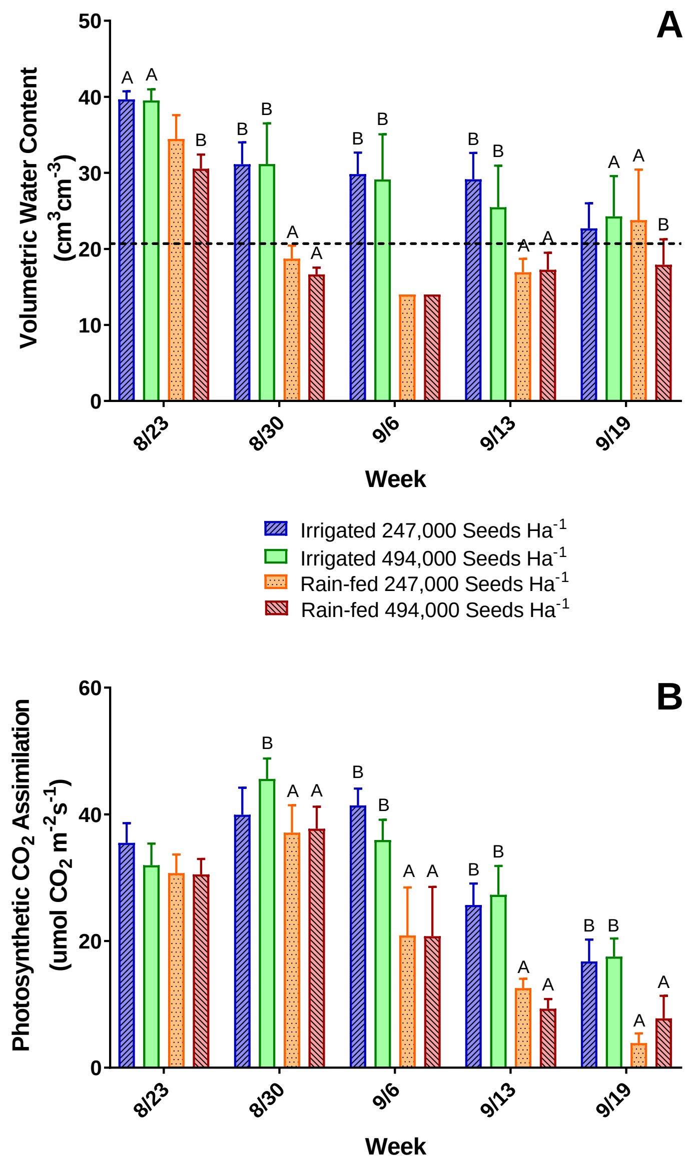
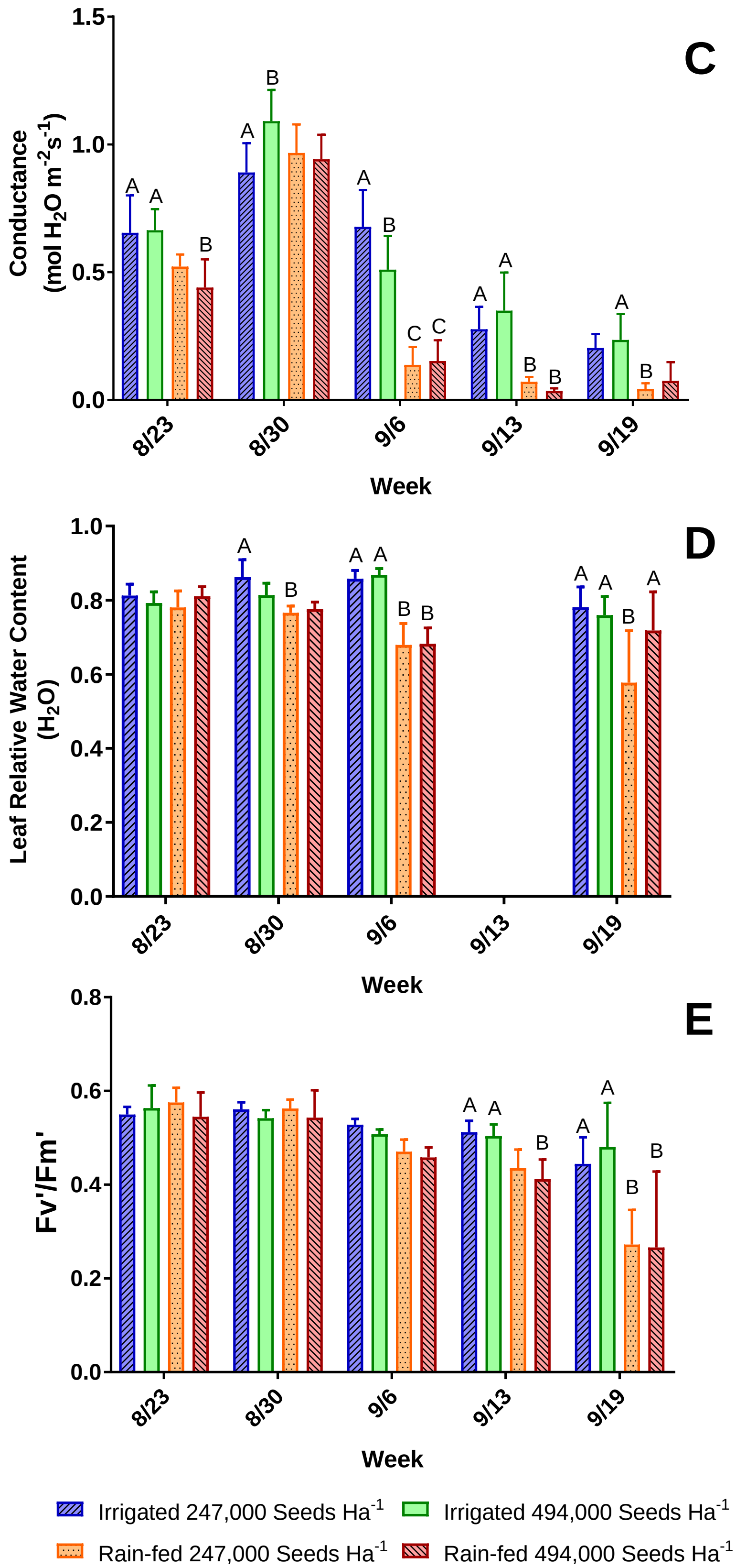


Figure 10. A) Average volumetric water content for plots within treatment (only single measurement recorded for water content on week 9/6 due to inability to insert probes into soil). B) Photosynthetic carbon dioxide assimilation rate recorded on the youngest fully expanded leaf in an upper node utilizing a LI-COR 6400. Significant differences in assimilation rate coincide with the occurrence of water contents at or below the critical value determined in growth chamber study. Figures 10C, 10D & 10E represent additional plant metrics. Bars indicated mean and standard deviation with letters denoting significant difference p-values <0.05.


C
Conductance (mol H_2O $m^{-2}s^{-1}$)
Week
D
Leaf Relative Water Content (H_2O)
Week
E
Fv'/Fm'
Week
Irrigated 247,000 Seeds Ha^{-1}
Irrigated 494,000 Seeds Ha^{-1}
Rain-fed 247,000 Seeds Ha^{-1}
Rain-fed 494,000 Seeds Ha^{-1}

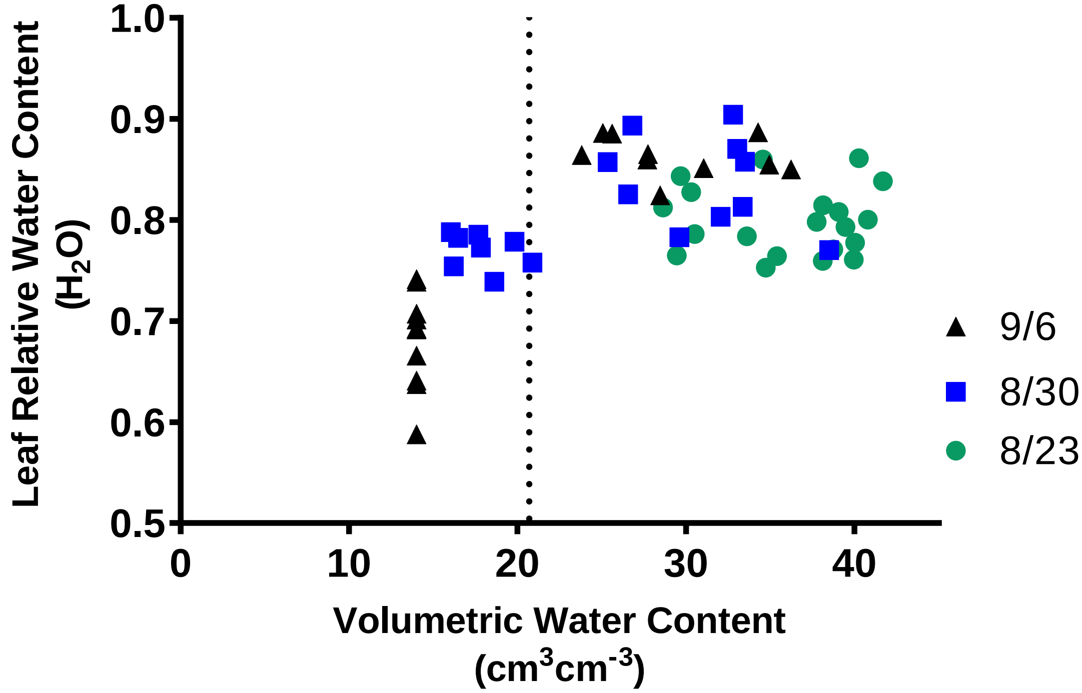


Figure 11. Point distribution of measured leaf relative water content percentages with the associated soil moisture conditions that the plant was experiencing display a downward trend with decreasing soil moisture. This begins after conditions dry to near the predetermined critical soil moisture content. Dry values from week 9/6 are assumed at a volumetric water content of 14.0% as conditions were too dry to allow for ground penetration and sampling with the soil moisture probe. The value 14.0% was the driest attainable sample.

VITA

Patrick James Curl

Candidate for the Degree of

Master of Science

Thesis: SEED GROWTH AND PHOTOSYNTHESIS DYNAMICS OF SOYBEAN EXPERIENCING DROUGHT STRESS

Major Field: Plant and Soil Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in May 2017.

Completed the requirements for the Bachelor of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in December 2014.

Experience:

Employed by Curl Farms and Seed as co-owner/operator, Braman, OK. August 2011 – Current.

Professional Memberships:

American Society of Agronomy 2014 – Present
Crop Science Society of America 2014 – Present
Soil Science Society of America 2014 – Present