

**Soybean Root Growth and Yield Response to Variable Rate Irrigation**

by

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## Abstract

A changing climate and an increasing world population result in a greater need of food. Water secures and increases yield. However, water use and irrigation timing need to be optimized to limit negative effects on natural resources. The objectives of this project are to determine how irrigation affects soybean root development through minirhizotron observation and evaluate yield performance of soybean cultivars under different irrigation regimes.

In 2016, a first experiment with 5 irrigation treatments (0.0cm, 0.9cm, 1.9cm, 2.9cm, 3.8cm) and 8 cultivars indicated different responses of cultivars to irrigation. In 2017, the experiment was repeated with 6 irrigation treatments (dryland, checkbook, sensor, R3, R5, R3+R5) and 6 cultivars and indicated different responses of cultivars to irrigation.

In 2016, yield response showed significance between cultivar and irrigation. 2016 soybean root growth found no significance between treatment, replicate, or depth. In 2017, yield response showed no significant interactions and 2017 root growth found no interactions between treatment, replicate, or depth.

Minirhizotron field research is limited in that data can only be collected from the area surrounding the minirhizotron tube. In regard to yield, response showed significance between cultivar and irrigation during 2016 and no significant interactions in the growing season of 2017. These differences in findings can most likely be attributed to disparities in rainfall during the two growing seasons where 2016 was classified as a dry year and 2017 was classified as a normal year of rainfall.

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## List of Abbreviations

|        |  |
|--------|--|
| ANOVA  | analysis of variance                           |
| AAES   | Alabama agricultural experiment stations       |
| cb     | centibar                                       |
| cm     | centimeter                                     |
| CYMMYT | International Maize & Wheat Improvement Center |
| FC     | field capacity                                 |
| GPD    | growth-phase dependent                         |
| GPS    | global positioning system                      |
| hr     | hour   |
| kPa    | kilopascal                                     |
| mm     | millimeter                                     |
| PAW    | plant available water                          |
| PWP    | permanent wilting point                        |
| R      | reproductive stage                             |
| R1     | production of first flower                     |
| R3     | pod development                                |
| SPAC   | soil-plant-atmosphere-continuum                |
| V      | vegetative                                     |
| V8     | plant ceases to flower                         |
| VE     | emergence                                      |
| VRI    | variable rate irrigation                       |

|        |                 |
|--------|-----------------|
| WP     | wilting point   |
| $\Psi$ | water potential |

## **Introduction**

### **Preface**

World population is currently over 7 billion and is expected to reach beyond 9 billion by 2050 (World Population Review, 2018). As world population continues to grow, resources to sustain abundant life are becoming more scarce. The increasing population has created more developed land and resulted in less arable land that is suitable for agriculture. For that reason, it is critical that nations maximize their food production potential on farms across the world. The most notable improvement in crop yield was achieved by Norman Borlaug, a crops breeder and plant pathologist at the International Maize & Wheat Improvement Center (CYMMYT) (Borlaug, 2007; Easterbrook, 1997). Through his team's effort to increase food production in Mexico, Pakistan, and India, it has been estimated that over a billion lives have been saved from starvation through Borlaug's high yielding, disease resistant, semi-dwarf wheat varieties (Easterbrook, 1997). Agricultural improvement over the past 100 years includes mechanization, tillage and no-tillage implements, pest management tools, pest management without chemicals, breeding, and use of precision agriculture technology.

One of the most limiting factors in crop production is irrigation management, whether it is excessive or limiting (Feres and Soriano, 2007). Over the past 50 years, irrigation methods have included flooding entire fields, furrow irrigation, various types of overhead irrigation, and sub-surface drip irrigation (Cetin and Bilgel, 2002; Sammis, 1980). The primary factor that these methods lacked, with the exception of sub-surface drip, was the ability to vary the amount of water delivered to different areas in the field based on the needs of the crop.

It has been estimated that 70% of the world's fresh water use is for irrigation (Feres and Soriano, 2007; United States Geological Survey, 2016). Efficient water use has become a priority

to ensure food availability for the world's population. Advances in irrigation technology including overhead center pivots, moisture sensors, and plant condition monitors can aid in efficiently meeting the needs of the crop (Camp et al., 1998; Thompson et al., 2007). Rather than applying water at a single, uniform rate across the field, variable rate irrigation (VRI) applies water at different rates along the pivot to better match crop needs. Application patterns and amounts can be altered depending on cultivar, crop type, or changes in soil texture across a field (Booker et al., 2006; Yule et al., 2008). Variable rate irrigation can help utilize the available water supply more efficiently, aid in food production, and reduce losses from applying too much water.

Soybean is the world's fourth highest produced crop and continues to rise in annual production (Hartman et al., 2011; Masuda et al., 2009). Plant stress from drought during the growing season can often be detrimental to soybean production (Bredan and Egli, 2003; Frederick et al., 2001). For example, in Argentina, drought in 2009 caused a 30% decrease in output from the world's third largest soybean producer (Hartman et al., 2011; Masuda et al., 2009). Drought can be catastrophic on one of the world's most valuable crops and must be remedied to maximize its yield (Masuda et al., 2009; Bredan and Egli, 2003).

### **Variable Rate Irrigation (VRI)**

Variable rate irrigation utilizes center pivots and lateral movement systems designed to apply water at varying rates across the field. VRI can be especially useful in a field with multiple crops, crops planted on different dates, or in fields having great soil variability (Booker et al., 2006; Yule et al., 2008). Currently available center pivots can only apply different rates of water across a large area by varying speeds as they move through the field (Booker et al., 2006; Yule et al., 2008). The system can therefore speed up to apply less water in certain areas or slow down

to apply more water in others. Rates are changed on a gross scale within the same field which may help conserve water and reduce irrigation costs (Booker et al., 2006; Yule et al., 2008). Center pivot systems may be as large as an 8km radius and be remotely controlled using global positioning systems (GPS) and cell phone applications (Booker et al., 2006; Kim et al., 2008).

Crop research has shown that water requirements can change throughout the growing season and can be accounted for by utilizing VRI (Evans,2013; Göksoy,2004; Lundstrom,1988). There have been a number of trials that indicate that crops' sensitivity to water stress is dependent upon the age and condition of the crop (Brevedan and Egli, 2003; Evans 2013; Göksoy: 2004; Lundstrom et al.,1988). Research has also shown that water status of the crop changes throughout the growing season and should be accounted for when utilizing VRI (Brevedan and Egli, 2003; Lundstrom et al.,1988). With these factors in mind, it is important to assess the effectiveness and impacts of using VRI to alleviate water stress in soybeans.

### **Root Development**

Research on root development is considered a challenging area given the fragile nature, difficulty, and time involved when working with a plant root system. While both traditional destructive and nondestructive approaches have their limitations, both can be used to evaluate the growth of a plant's root system for virtually any crop (Lowe and Wilson, 1974; Kuchenbuch and Ingram, 2002).

Evaluating the impact of irrigation can be assessed through several facets, one of which is monitoring the development of the crop's root system (Li et al., 2010). Traditional root analysis is a destructive process that consists of manually measuring root growth after uprooting the growing plant (Lowe and Wilson, 1974). Rhizotron systems, using clear plastic screens and cameras, have been used as a non-destructive alternative for viewing and analyzing roots but

limitations exist in expense and intensive labor for creating a working system (Kuchenbuch and Ingram, 2002; Taylor et al., 1991). These systems are typically built below ground and contain a main corridor with acrylic viewing panels that allow viewing into the root systems and microorganisms within the soil. Variations occur and can be built above ground in a large box with windows for viewing the activity within the soil (Huck and Taylor, 1982; Kuchenbuch and Ingram, 2002).

Minirhizotrons have become a useful alternative to rhizotrons in collecting non-destructive root data. This instrument allows for the study of developing roots, in situ, without causing harm to the crop being analyzed. Roots are monitored through pictures taken during the growing season. A camera is fixed to a wire and lowered through a clear tube that runs into the soil and maintains contact with the root system.

### **Drought Stress**

Physiological impact of water stress can vary with crop species, growth stage, and duration (Brevedan and Egli, 2003; Lundstrom et al., 1988). Drought stress during early vegetative stages of soybean growth can aid in its survival. Water deficiencies occurring before the plant's root system has fully developed have been shown to have a shorter impact than a plant experiencing drought in the middle of the growing season (Brevedan and Egli, 2003); Chaves et al., 2002; Doss et al., 1974).

Plant turgor and length of root development are linked to the drought stress of a crop (Baver and Farnsworth, 1941; Mirreh and Ketcheson, 1973). Mirreh and Ketcheson found that the levels of drought stress had noticeable impacts on the roots of corn plants. Plants undergoing more drought stress resulted in longer root growth and lower potentials resulted in shorter and thicker roots (Mirreh and Ketcheson, 1973). The correlation seems to be typical in that stressed

plants result in greater elongation of root growth. The linear correlation of decreasing root growth with decreasing drought stress has been noted as an independent result from soil types (Zou et al., 2001). It should be noted that while higher potentials result in higher root growth, other factors which may vary throughout a field such as air filled porosity and bulk density will influence the extent of this phenomenon (Zou et al., 2001).

### **Research Objectives**

It is hypothesized that different irrigation techniques will have impacts on both root development and crop yield (Brevedan and Egli, 2003; Torrion et al., 2014; Zou et al., 2001). The study focuses on method development for soybean root research and impact of irrigation techniques on yield and root development.

The ability to evaluate soybean root development as impacted by variable rate irrigation is critical for the future success of high-yielding soybean production systems. The objectives are: 1) to determine how irrigation affects soybean root development through minirhizotron observation; and 2) to evaluate yield performance of soybean cultivars under different irrigation regimes.

## Literature Review

### Soybean

Soybean, *Glycine max*, is an oil-seed legume native to China (Hymowitz, 2005; Qiu et al., 2010). It has been reported that soybean cultivars were brought to Korea and Japan some 2000 years ago (Qiu et al., 2010). Soybean became a staple crop for North America during World War II. The soybean is utilized as a protein substitute, a source of edible oil, and found use in fertilization— leading it to an irreplaceable importance in the United States (Hymowitz, 2005; Young, 1979). Research has shown that the amount of annual protein produced by soybeans is enough to meet one third of the world's protein needs (North Carolina Soybean Producers Association, 2014). As a legume, soybeans can “fix” their own nitrogen and are often a part of successful crop rotations.  $N_2$  is unusable by most organisms due to a triple bond between N atoms, resulting in a nearly inert molecule. Through the process of nitrogen fixation, bacteria convert  $N_2$  into usable ammonia. This nitrogen fixation occurs as a result of a symbiotic relationship with nodulating soil bacteria, benefiting surrounding and future crops (Lawn and Brun, 1974; Weisz, 1987).

The world produces 270 million metric tons of soybean annually with over 45 percent produced in the United States (Masuda, et al., 2009). Alabama alone farms 350,000 hectares (ha) of soybean at an average rate of 2.5 tons per ha annually (USDA, 2017). Currently, thirty-one U.S. states produce soybeans with Iowa, Illinois, Indiana, and Minnesota raking as the top producers (North Carolina Soybean Producers Association, 2014).

Soybeans are cultivated in temperate zones and flower as nights become longer, classified as short-day plants. Soybean seed can be various shapes from round to long and flat. Seed color can be green, yellow, brown, or even black, all of which varies by cultivar (Monma,



1994; Yang et al., 2010). Soybean is planted late spring to early summer with physiological maturity being reached early to mid-autumn. Plants will naturally senesce with the leaves beginning to turn yellow and eventually fall off. During these reproductive stages, beginning with the first flower (R1), root systems senesce as the plant focuses on reproductive growth (Huck et al., 1987). The seeds drop in moisture content over time and should be harvested when moisture content is at 13 percent to prevent splitting and cracking (Masuda et al., 2009).

Soybeans are classified into maturity groups by photoperiodism. Depending upon the maturity group classification, under different day lengths the plant will adjust its physiological state causing faster or slower maturity from vegetative to reproductive growth stages (Zhang et al., 2017). Differences in root development under irrigation treatment impact growth trends of the overall root system while singular aspects of the root system, such as taproot or secondary roots, have been shown to show no response (Hoogenboom et al., 1987). Therefore, when conducting soybean root research it is more important to focus on the overall root system development than singular aspects of the root systems. Maturity groups may impact root response to drought stress. Since late maturity groups will maintain vegetative growth longer into the season, it is possible that late season irrigation treatments may impact one cultivar more than another (Huck et al., 1987). When selecting multiple cultivars to determine irrigation impact on root system development, it is then pertinent to select those of similar maturity groups so that soybean root senescence follows a similar timeline.

Irrigation strategies for soybean vary from relying on specific growth stages to applying broader practices like the “checkbook method” where evapotranspiration is considered, automatic weekly application, and observation of wilted plants (Brevedan and Egli, 2003; Li et al., 2011; Lundstrom et al., 1988). The checkbook method takes an initial measurement of the

soil water deficit and balances water input from precipitation and irrigation with water output from plant usage and evapotranspiration to indicate the need for irrigation (Li et al, 2011).

Understanding soybean growth stages has become essential to properly managing the crop, especially with VRI. Soybean growth stages are separated into vegetative (V) and reproductive (R) stages. Soybean generally has six vegetative stages (VE-V5) and eight reproductive stages (R1-R8). Vegetative stages begin with germination and emergence (VE) and end with production of the first flower (R1). Reproductive stages begin with the first flower and lasts until the plant ceases to flower (V8).

### **Water Movement in Soils**

Different soils retain water with variance depending on horizon, climate, and texture. Soil essentially acts similar to a sponge in taking up and retaining water (Azooz and Arshad, 1996; DeBano, 1971). Water movement into the soil is called “infiltration” (Azooz and Arshad, 1996; Govaerts et al., 2007); whereas water movement across the soil surface is called “run-off” (Berndtsson, 2010; Lefrancq, 2017). Infiltration rates are in millimeters per hour ( $\text{mm hr}^{-1}$ ) and may be near  $0 \text{ mm hr}^{-1}$  when soil is extremely high in clay or compacted— meaning that no water is entering the soil (Govaerts et al., 2007). Soils with low rates will often create areas with standing water and result in runoff (Govaerts et al., 2007; Wischmeier and Mannering, 1969). Research has shown that infiltration rates can be increased and runoff decreased when there is organic matter resting on the soil surface (Govaerts et al., 2007). Increasing the surface organic matter aids in water retention, allowing slower evapotranspiration rates and higher infiltration rates (Roose and Bathes, 2001).

Water movement within the soil, called “percolation”, and measured as hydraulic conductivity (Klute and Dirksen, 1986). Hydraulic conductivity varies with soils of different

texture and structure (Bouma, 1982; Mualem, 1976; Rawls, 1998). Hydraulic conductivity is ranked in categories extending from “very slow” ( $0.00-0.42 \mu\text{m sec}^{-1}$ ) to “very rapid” ( $>141 \mu\text{m sec}^{-1}$ ) and is measured at a rate of  $\text{mm hr}^{-1}$  (Klute and Dirksen, 1986). It is through hydraulic conductivity that water moves through the soil and reaches the plant's rooting zone (Mualem, 1976). Water that goes beyond the rooting zone can eventually reach the ground water table (Klute and Dirksen, 1986).

Pore volume and distribution play vital roles in the amount of water that a soil can retain. Water retained in the soil is captured in soil pores where it becomes available to a plant's rooting system, which can be termed as “plant available water” (PAW) (Bouma, 1982; Cassel et al., 1986). Water holding capacity refers to the amount of water held between field capacity (FC) and wilting point (WP) (Karhu et al., 2011). FC is the amount of water that remains in soil after excess water has drained due to evaporation and suction pressure and matric potential is around -33 kPa, typically taking 2 to 3 days of draining (Karhu et al., 2011). FC was often thought to be a constant for a given soil; however, it can be variable through environmental conditions and can change throughout a given year with changes in temperature and utilization of the field (Cassel et al., 1986; Veihmeyer and Hendrickson, 1931). Saturation refers to water content when all pores are filled with water and oxygen has been displaced (Cassel et al., 1986; Veihmeyer and Hendrickson, 1931). Permanent wilting point (PWP) is reached when all plant available water (PAW) has been extracted and matric potential is around -1500 kPa (Cassel et al., 1986; Tolck, 2003). It is at PWP that a plant will wilt and no longer be able to recover, even if the moisture stress is relieved. (Cassel et al., 1986; Tolck, 2003).

## **Irrigation Strategies**

A soil's crop water use is dependent upon the type of crop, growth stage, number of weeks after emergence, and maximum daily temperature (Lundstrom et al., 1988; Salter et al., 1967). Since soybean has a high water requirement (50-70 cm season<sup>-1</sup>) to produce respectable yield, irrigation has become an essential part of the production plan (Masuda 2009; Specht et al., 1999). Water requirements for soybeans vary with growth stage where germination and seedlings need 0.13-0.25 cm day<sup>-1</sup>, vegetative growth needing 0.25-0.51 cm day<sup>-1</sup>, and flowering to pod fill/full canopy requires 0.25-0.76 cm day<sup>-1</sup>. Once the plant reaches physiological maturity and seeds are beginning to mature, the requirement drops to 0.13-0.51 cm day<sup>-1</sup> (Masuda 2009; Specht et al., 1999).

A soil's water holding capacity is the water amount that a soil can hold at FC. Soil texture and constituents cause variability in moisture holding capacity and each soil must be evaluated before scheduling irrigation (Karhu et al., 2011; Payero et al., 2017). When multiple soils are present in the field, the most limiting soil should be evaluated (Li et al., 2011; Payero et al., 2017).

Soybean plants require 50-70 cm of water are required during the growing season to fill their pods and provide good yields. Rainfall alone will not reliably produce good soybean yields in the temperate regions where they are grown (Masuda et al., 2009; Karhu et al., 2011). Growth-phase dependent (GPD) strategies can be developed. Threshold and refill levels as percentages of maximum available soil water can be based upon crop phenology leading to the development of GPD (Hood et al., 1987).

The number of farms in the United States implementing irrigation have been increasing since the 1950's (Nijbroek et al., 2003; USDA, 2017). Irrigation strategies range from blanket applications, uniform strategies, and spatially variable strategies (Cetin and Bilgel, 2002; Harris

and Mapp, 1986; Nijbroek et al., 2003). Blanket strategies prove most useful when focusing on the water needs of the soybean crop in specific growth stages. Since water requirements vary during the crop's life cycle, specific stages should be focused on in an attempt to maximize yield (Brevedan and Egli, 2003; Lundstrom et al., 1988). Physiological impact of water stress is crop dependent and varies upon extent, duration, and type. Deficit water stress early in the season can actually aid in the plant's survival (Chaves et al., 2002). Plants that are exposed to excess light can experience extreme damage from photooxidation due to producing too many highly reactive biological intermediates that harm biological molecules and may slow plant processes (Doss, 1974; Li et al., 2009; Pilon et al., 2015). When a plant is undergoing stress from insufficient water, leaf water potential and stomatal openings may both be decreased resulting in unregulated photosynthesis-related genes and reduced amounts of available CO<sub>2</sub> (Osakabe and Osakabe, 2012; Pilon et al., 2015). If irrigation strategies are based on crop growth stage, equal or higher yields can be produced with more efficient water input (Chaves et al., 2002). Research has shown that focusing on soybean R3 (pod development) growth stage, can result in higher yield than a full-season irrigation strategy (Torrion et al., 2014). Spatially variable irrigation strategies can prove useful in larger fields where the crop or soil changes significantly (Nijbroek et al., 2003).

The checkbook method involves taking local daily maximum air temperatures and estimating the water requirements from crop-specific estimates (Laboski et al., 2001; Payero et al., 2017). Collected data includes initial soil moisture deficit, number of weeks after emergence, daily crop water use, rainfall and irrigation amounts, and periodic in field soil water deficit measurements (Laboski et al., 2001). In addition, pumping capacity (liters per minute) and efficiency of the selected irrigation system must also be accounted for to implement this method.

Initial soil moisture deficit is calculated manually by subtracting current water content from found field capacity (Lundstrom et al., 1988). Multiple samples should be taken in the field at a depth of either 15 or 30 centimeters (Dylla et al., 1980; Lundstrom et al., 1988). Tables developed from the soil moisture deficit are derived from a soil moisture balance sheet. When the moisture deficit is reached, the crop must be irrigated. Irrigation and rainfall adds water to the soil and decreases the deficit while crop water use adds to the deficit (Lundstrom et al., 1988).

### **Water Matric Potential**

Water potential is the energy of water in a unit volume compared to the energy of pure water in measured conditions (Contreras et al., 2017). Water potential ( $\psi$ ) (psi) is the measurement of water movement in plants and soils through various forces such as gravity, osmosis, and capillary action. Though different methods of measuring soil water potential have been used (Cassel et al., 1986; Rezaie-Boroon, 2017), a sensing device known as 'Watermark' sensors (Irrometer, Inc) have been in use since 1975 (Payero et al., 2017). Watermark sensors work on the principle that electrical resistance changes as soil water content and tension changes. In order to obtain a resistance value, a current is applied to the sensor. The resistance is then correlated to kilopascals (kPa) or centibars (cb) of soil water tension which rises as the soil becomes drier (McCann et al., 1992). A gypsum layer acts as a buffer to potential salinity levels often found within agricultural irrigated systems. The Watermark sensor is intended to be a permanent sensor left in the field and can be monitored by attaching a data collection device. Continuous, real time data loggers can be used with these sensors and sent directly to the operator or collected for analysis (Thompson et al., 2007; Vellidis et al., 2008). While other sensors such as a vacuum gauge and mercury-water manometer can collect similar data, they are not cost effective, function over a narrower spectrum of soil water tension, and require large

amounts of manual effort for installation each season (Cassel et al., 1986; Vellidis et al., 2008). Watermark sensors provide a cost-effective way to do so and can wirelessly relay information to a receiving station (Vellidis et al., 2008).

While they are highly effective and inexpensive to use, the Watermark sensors have drawbacks. The sensors cannot respond to in-field changes in potential higher than -10kPa which may prove them non-beneficial in research situations that maintain a potential near 0kPa through the implementation of high irrigation practices (Intrigliolo and Castel, 2004). Beyond this issue, these sensors can have erratic behavior during rapid soil drying and partial rewetting (I.R. McCann et al., 1992). Even with the noted limitations, Watermark sensors have been proven to be useful guides in monitoring relative indications of soil water (Huang et al., 2004; Intrigliolo and Castel, 2004).

A relationship exists between water matric potential, plant turgor, and length of root growth (Baver and Farnsworth, 1941; Mirreh and Ketcheson, 1973). Mirreh and Ketcheson (1973) found that the levels of water potential had noticeable impacts on the roots of corn plants. Higher potentials resulted in longer root growth and lower potentials resulted in shorter and thicker roots (Mirreh and Ketcheson, 1973). The correlation seems to be typical in that higher potentials result in greater elongation of root growth. The linear correlation of decreasing root growth with decreasing matric potentials has been noted as an independent result from soil types (Zou et al., 2001). It should be noted that, while higher potentials result in more root growth, other factors such as air filled porosity and bulk density will influence the extent of this phenomenon (Zou et al., 2001).

### **Soil-Plant-Atmosphere Continuum**

The soil-plant-atmosphere continuum (SPAC) is defined as the pathway for water movement from soil to plants to the atmosphere (Hall and Kaufmann, 1975; Ouyang, 2002). SPAC impacts the changes in matric potential through the nature of water continuously connecting the pathway. Movement of water from the soil, through the plant, and into the atmosphere can increase the soil matric potential and need for irrigation. SPAC is the area near the soil's surface in which water and energy transfer occurs from soil to plants and the atmosphere (Hall and Kaufmann, 1975; Shalhevet et al., 1995). SPAC integrates all components into a productive system in which energy and matter transport processes occur at the same time (Sławiński and Sobczuk 2011). The soil consists of three major constituents which are water, air, and solid matter, all of which can vary with soil texture and structure. A healthy root system must have a perfect balance between the three soil constituents, the most critical being a balance between the liquid and gas phases (Sławiński, C. and Sobczuk, H., 2011).

Soil water impacts the plant by providing oxygen, transport of nutrients, and maintenance of temperature. Aeration allows oxygen to flow into and out of the soil when useable pore space is available and balanced with roots and microbes (Hall and Kaufmann, 1975; Shalhevet et al., 1995). Root growth requires oxygen in the soil and is supplied through diffusion through the soil (Shalhevet et al., 1995). The diffusion gradient associated with the SPAC results from a difference between higher water potential within the leaves of a plant and a lower potential within the atmosphere (Passioura, 1982). Soil physical properties such as texture, structure, and pore space can alter the rate of SPAC, impacting diffusion gradient, and therefore changing irrigation needs of a particular crop (Hudson, 1994; Passioura, 1982; Sławiński, C. and Sobczuk, H., 2011). Diffusion occurs more slowly through water than air making air diffusivity more quickly impact the plant than through water (Shalhevet et al., 1995). Soil water movement is



dependent upon size, orientation, and distribution of pores (Hudson, 1994; Shalhevet et al., 1995). Air diffusion through the soil profile is closely tied to the amount of air-filled pore space and therefore tied to a soil's water retention (Shalhevet et al., 1995). Macropores are air-filled at field capacity and can be taken as an indicator of a soil's ability to diffuse oxygen (Jarvis, 2007; Shalhevet et al., 1995).

SPAC should be considered when determining need for irrigation and quantifying plant stress (Bloom, 2011; Passioura, 1982). Since soybeans require regular irrigation throughout the growing season, it is important to understand the SPAC of a given field in order to create an optimal irrigation prescription. A soil suitable for plant growth must be able to accept and retain irrigation (Shalhevet et al., 1995).

### **Root Analysis**

Traditional root analysis is a destructive process that consists of manually measuring roots after digging up the growing plant (Lowe and Wilson, 1974). Other early research also used destructive techniques that included soil coring, in-season cores, whole root system excavation, and trenching (Johnson et al., 2001). While these methods may still be used, they are being replaced by improved analysis methods such as rhizotron and minirhizotron systems (Black, 2017; Taylor et al., 1970, 1991). Rhizotron systems have been used as a non-destructive method for viewing root growth for the past 45 years (Taylor et al., 1970, 1991). Rhizotron systems are built below ground and contain a main corridor with viewing panels of glass or plastic that allow viewing the root systems within the soil. Variations of rhizotrons occur and can be built above ground in a large box with windows for viewing root activity in the soil (Taylor et al., 1970; Huck and Taylor, 1982). Rhizotrons are effective for collecting root data, but limitations exist in both expense and the intensive labor that must be undertaken to create a

working system (Johnson et al., 2001; Taylor, et al., 1991). As the size of research endeavors increases, space also becomes an issue for building rhizotron boxes in larger studies (Johnson et al., 2001).

Minirhizotrons have been developed as an alternative to rhizotrons for collecting non-destructive root data. A modern minirhizotron system consists of a tube that is inserted into the ground, a color camera to record imagery, and a small computer for viewing. When creating a minirhizotron root system, installation should occur in the crop row soon after germination. Developing root systems can be observed throughout the growing season at varying depths and in a spectrum of conditions. Using root-analysis software, data can be considered when assessing plant health. Minirhizotrons have been utilized in a vast range of research from forests (Joslin and Wolfe, 1999) to deserts (Phillips et al., 2000) to agricultural systems (Volkmar, 1993). Table 1 shows the benefits and problems associated with each root analysis method.

Table 1. Benefits and disadvantages of common methods of root research. Approaches to root research has varied through time. Each method contains both benefits and drawbacks.

| <b>Source</b>                         | <b>Method</b> | <b>Pros</b>                                     | <b>Cons</b>   |
|---------------------------------------|---------------|---|---|
| Lowe and Wilson, 1974                 | Destructive   | Hands-on, accurate results, cheap               | Kills plant, cannot monitor the same plant multiple times         |
| Taylor, Upchurch, and McMichael, 1991 | Rhizotron     | Hands-on, allows for multiple measurements      | Expensive, difficult to recreate field conditions, consumes space |
| Johnson, et al., 2001                 | Minirhizotron | Accurate, extensive data, multiple measurements | Expensive, data analysis software required, time consuming        |

A minirhizotron system will allow the plant to treat the tubes as it would any other soil obstruction or rock and grow around them normally. Installation should have a goal of minimizing soil disturbance in the area around the tube and optimizing soil contact to tube surface (Johnson et al., 2001). Soil:tube contact is necessary in order to maintain a natural environment for the development of roots and their growth in the soil environment. The round tubes are typically made of Plexiglas, glass, acrylic, or plastic and a similar diameter of the determined camera, typically about 8cm (Brown and Upchurch, 1987; Johnson et al., 2001). A common installation schematic can be seen in Figure 1, demonstrating camera function and minirhizotron tube placement in relation to plant.

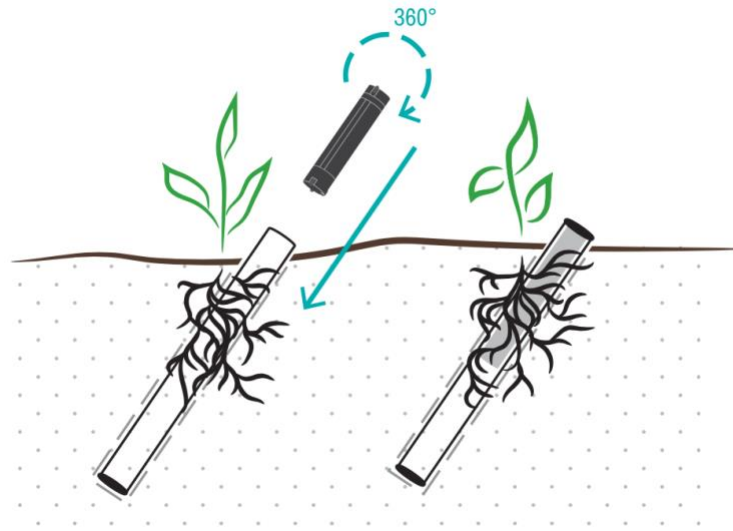


Figure 1. Schematic of 45-degree minirhizotron tube and camera installation ([www.cid-inc.com](http://www.cid-inc.com)). Tube is inserted soon after germination and root systems around tube are captured with rotating camera.

Many options exist for documenting roots in minirhizotron systems including fiber optics with cameras, mirror systems, small video cameras, endoscopes, and boroscope devices (Johnson et al., 2001; Noguchi et al., 2004). More recently, scanners have become useful in collecting root information in minirhizotron systems. Scanners collect images of roots growing around the minirhizotron tubes at specific windows (Dong et al., 2003). Scanner based minirhizotron analysis has been found to be useful when assessing the impact of water limiting factors on root systems (Meier and Leuschner, 2008).

Minirhizotron tubes allow analysis of many important root factors such as root count, diameter, and length. While software options exist for root analysis, with minirhizotron tubes, simply using the images to visually assess root progress can also be done (Shalhevet et al., 1995). Root population can be estimated at each photo depth window by physically counting the

number of roots intersecting a marked transect line within each frame (Shalhevet et al., 1995; Tierney and Fahey, 2002). It is also possible to monitor root growth through a similar approach. Root growth can be noted by comparing initial root counts to the counts after treatment (Shalhevet et al., 1995). While data can be manually recorded using the images, programs are available to expand the types of data possible (Noguchi et al., 2004; Yuditaka-Pessinatti-Ohashi et al., 2013).

## **Materials and Methods**

### **Site Description**

Trials were conducted in Tallassee, Alabama at Auburn University's E.V. Smith Research Center (32°26'14.075" N, 85°54'39.384" W) during the growing seasons of 2016 and 2017. The site consisted of Altavista Silt Loam soils (fine-loamy, mixed, semi-active, thermic, Aquic Hapludults). These soils are moderately well drained and range from Alabama, Georgia, the Carolinas, and Virginia. These soils are highly cultivated and often utilized for corn, cotton, soybean, and small grain crops. Rainfall and temperature data were collected daily and monitored through Alabama Mesonet.

The 2016 trial consisted of five irrigation treatments in 0.4 ha plots with four replicates for each of the eight cultivars. The 2017 trial consisted of six irrigation treatments in 0.4 ha plots with two replicates for each of the six cultivars. In 2016, soybeans were planted on May 17 and harvested on October 19. Planting was delayed in 2017 due to heavy rains and soybeans were planted on July 3 and harvested on November 27, 2017. For both years, the field was strip-tilled and soybeans were planted at a depth of 2.5cm. Cultivar varieties depended on availability for both years and both early and late maturity groups were selected. The 2016 growing season consisted of maturity groups 5 and 9 while 2017 consisted of maturity groups 5, 6, and 7.

### **General Soybean Production Practices**

Eight cultivars were included in 2016 and six cultivars in 2017 based upon availability to the research center (Table 2). Soybeans were planted in 91cm rows in a strip-tilled 40ha field. Planting was delayed in 2017 by frequent, heavy rainfall that prevented field work. Planting rate was 370,500 seed ha<sup>-1</sup> which is a common Alabama production recommendation (ACES, 2013).

Prior to planting both years, weeds were controlled using Gramoxone Inteon (Syngenta ®). Later each season, a “layby” application of Roundup PowerMax (Roundup ®) was made.

Cultivar yield for both growing seasons was determined using an on-board impact (combine) yield monitor. The yield monitor collects data by measuring the impact of force on a plate when the crop comes off the crop elevator. A variable resistor called a potentiometer is mounted in two parts to the combine frame and impact plate. Calibration was done prior to harvest by adding pre-weighed soybean weights to the crop elevator to adjust for accuracy. Yield monitor data was uploaded and overlaid on treatment plots using ArcGIS software. The outer two rows on either side of plots were removed so that yield values were determined from the center of treatment plots to account for non-instantaneous VRI application changes.

### **Variable Rate Irrigation (VRI)**

Irrigation treatments were applied through a ‘Valley 7000’ series center pivot system equipped with VRI capability (Figure 2). During the 2016 growing season, irrigation was applied to all cultivars from May through August (Table 3). The first water application rate was prior to emergence at a rate of 1.27cm to ensure germination and a consistent plant population. Each cultivar was replicated twice and irrigated six times with 0.0cm, 0.9cm, 1.9cm, 2.9cm, and 3.8cm of water (Figure 3).

In 2017, the treatments were further developed based upon common irrigation management practices (Dukes et al., 2003; Kirida, 2002; Li et al., 2011). Irrigation scheduling treatments included: a) soil water sensor; b) checkbook method; c) non-irrigated control; and soybean growth stages d) R3; e) R5; and f) R3+R5 (Figure 4). The checkbook method involved maintaining a daily weather spreadsheet that monitored input of water from precipitation and irrigation and subtracting water output from crop water use and evapotranspiration. When the

soil reached a 50 percent deficit, irrigation was applied to lower the deficit. Through this method, plants were kept from reaching PWP and returned to FC during each treatment. Growth stage treatments were irrigated at R3, R5, and both R3 and R5 respectively. Irrigation amounts were estimated from water retention curve data provided by Web Soil Survey in order to return treatments areas to FC. Watermark sensors were used to monitor matric potential in the soil and in the second year utilized as an irrigation trigger. These were placed at 20, 40, and 60cm depths without knowing the actual depth of the rooting zone or which sensor depth is best for utilization as an irrigation trigger. When matric potentials became more negative than -50 kPa, irrigation was applied to return the area to FC. Daily weather data was collected from the Mesonet station at E.V. Smith. The weather station utilized Campbell Scientific HMP60 for temperature and relative humidity data collection. For precipitation data, a tipping bucket rain gauge, Texas Instruments Model TE525, was utilized. All data were collected by Campbell Scientific CR10X data logger.



Figure 2. Valley 7000 Series pivot irrigation system used for both growing seasons at Auburn's E.V. Smith Research Station in Tallassee, AL ([southplainsvalleyirrigationinc.com](http://southplainsvalleyirrigationinc.com)).



Table 2. Cultivars assessed for yield response to treatment during each growing season. Cultivar selection based upon seed availability. 2016 consisted of a total of eight cultivars and 2017 consisted of a total of six cultivars.

| 2016             | 2017             |
|------------------|------------------|
| Asgrow 5831 R    | Pioneer 52A26 R  |
| Pioneer 52T50 R  | Pioneer 55T81 R  |
| Pioneer 54T94 R  | Asgrow 5831 RR   |
| Asgrow 5533 R    | Asgrow 69X6      |
| Pioneer 55T81 R  | Asgrow 75X6      |
| Pioneer 56T12 S  | Pioneer 76T54 R2 |
| Pioneer 56T29 R2 |                  |
| Pioneer 95Y70 R  |                  |

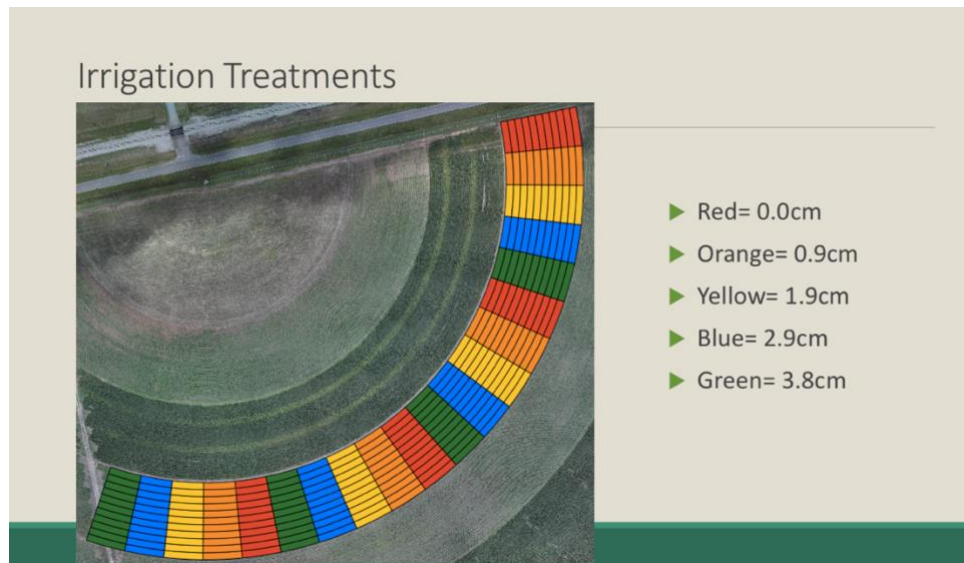


Figure 3. Irrigation treatments (0.0cm, 0.9cm, 1.9cm, 2.9cm, 3.8cm) conducted in 40ha experiment field during 2016 growing season.

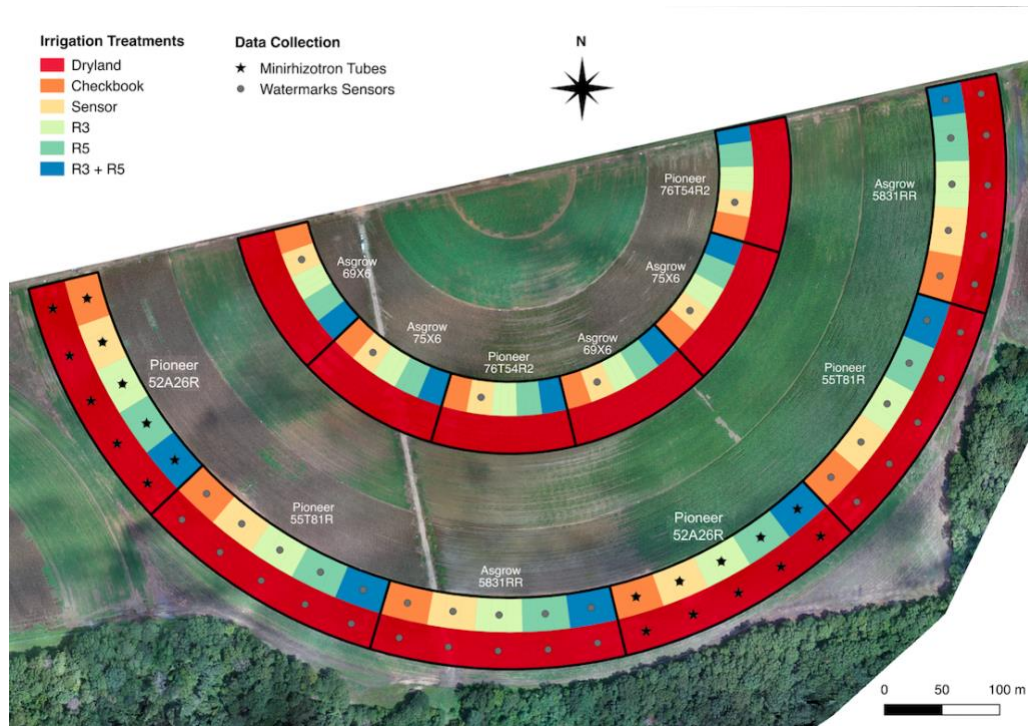


Figure 4. 2017 experimental set up with irrigation treatments (dryland, checkbook, sensor, R3, R5, R3+R5) and data collection (minirhizotron tubes and Watermark Sensors)

## Studying Root Growth Using Minirhizotrons

### 2016 Methods

Minirhizotron tubes were installed after soybean stand was established. At installation, plants were in the V1 growth stage characterized by the full opening of the first trifoliolate and plants were 7cm in height. A tube was placed in replicates of the five different irrigation treatments. To install, a hole was dug in order to put the tube into the ground. An auger or corer was used to minimize soil compaction at tube soil line and to create maximum soil to tube contact (Johnson et al., 2001; McMichael and Taylor, 1987). Before installation, a wire brush was implemented to smooth the newly drilled holes and prevent soil striations from showing up in the later recorded imagery (Box et al., 1989).

Minirhizotrons were created using 7cm wide by 1m long acrylic tubes and rubber end caps (Figure 5). The end to be inserted into the ground was sealed with liquid tape to prevent water infiltration into the tube. Twenty tubes were then installed to one meter depths, 45m apart directly in one soybean row. A 7cm wide auger bit gas auger was used to drill vertical holes into the ground at a 90-degree angle to the soil surface (Bragg et al., 1983; Johnson et al., 2001). Soil inside the drilled holes was brushed gently to improve image clarity and tubes were inserted into the ground adjacent emerged 'Pioneer 52T50 R' soybeans. Minirhizotron tubes were installed after soybean stand was established. At installation, plants were in the V1 growth stage characterized by the full opening of the first trifoliolate and plants were 7cm in height. Root images were collected in 2016 during June, August, and September using a 'CI-600 in-situ root imaging camera' from CID Bio-Science. At the time of data collection, soybean plants were found to be at V4, R5, and R7 growth stages respectively. 'RootSnap' software was selected for image processing and root measurements were recorded.



Figure 5. Full view of minirhizotron tube prior to installation, minirhizotron tube installed at 90-degrees within soybean row, and manually tracing collected image in Rootsnap software.

## **2017 Methods**

Due to the ability to sample the rooting systems of multiple plants at once the tubes were installed at 45-degree angles, using the aforementioned procedure. Tubes were installed in the soybean row (cv. 'Pioneer 52A26 R') with images being collected in July, August, and September. A tube was placed in each irrigation treatment (checkbook, sensor, R3, R5, and R3+R5) within each 'Pioneer 52A26 R' cultivar and in each dryland counterpart. 'Pioneer 52A26 R' treatments were replicated for a total of twenty tube locations. In 2017, it was considered that stomatal conductance as measured through a porometer could be correlated with root growth as literature shows that non-irrigated soybeans produce lower photosynthetic conductance than irrigated (Huck, et al., 1983; Osakabe and Osakabe, 2012; Pilon et al., 2015). Porometer measurements were collected during July, August, and September during the 2017 growing season using a leaf porometer from Decagon Devices. Root images were collected during July, August, and September during the 2017 growing season using the CI-600 in-situ root imaging camera from CID Bio-Science. At the time of data collection, soybean plants were found to be at V4, R4, and R7 growth stages respectively. 'RootSnap' software was selected for image processing and root measurements were collected.

## **Matric Potential Sensors**

Watermark sensors by Irrrometer were used to monitor matric potential at varying depths in the field at each of the selected twenty sites. Each site consisted of three sensors placed at depths of 20cm, 40cm, and 60cm. Sensors were attached to 1.25cm diameter PVC pipe and installed at the three depths. Notches were cut in the tops of the PVC pipe to allow wires to run through and tops were then capped to prevent water from irrigation and rainfall from entering the sensor tubes. A completed sensor can be seen in Figure 6. Holes were drilled with a 1.25cm hand auger and sensors were installed. Sensors were placed at each of the twenty minirhizotron

locations along the ‘Pioneer 52T50 R’ cultivar. Data was collected twice each week in 2016. Final set up of each site with camera tubes and matric potential sensors can be seen in Figure 6.

In 2017 Watermark sensors were again installed at depths of 20cm, 40cm, and 60cm. Within each of the six cultivars, a set of sensors were installed for each of the five irrigation treatments and the dryland counterparts. Each cultivar was replicated twice for a total of sixty-six Watermark locations. Data was collected weekly in 2017.

### **Statistical Analysis**

Analyses of variance (ANOVA) were computed using linear models and based on the method described by Crawley (Crawley, 2013). Data were first analyzed with the full model which was then simplified by removing insignificant effects until obtaining the minimal model. Spatial variance was expected, therefore data was normalized with collection date prior to treatment by computing the differences.



Figure 6. Completed matric potential sensor with attached Watermark sensor. Sensors were placed at 20, 40, and 60cm depths during both growing seasons.

Table 3. Timeline of treatments and events during 2016 growing season. Growing season 2016 began with planting on May 17, 2016 and concluded with harvest on October 19, 2016.

| Date       | Event  |
|------------|--|
| 5/17/2016  | Soybeans planted at 375,000 seeds per ha                         |
| 6/3/2016   | 1.27cm irrigation across field to facilitate stand establishment |
| 6/24/2016  | VRI treatments applied   |
| 7/11/2016  | VRI treatments applied   |
| 7/18/2016  | VRI treatments applied   |
| 7/26/2016  | VRI treatments applied   |
| 7/30/2016  | VRI treatments applied   |
| 8/27/2016  | VRI treatments applied   |
| 10/19/2016 | Harvest  |

## Results and Discussion

### Environmental Conditions

A growing season can be classified as “dry” or “wet” by being one standard deviation below or above the thirty-year precipitation average for the growing season (NOAA, 2017). With a thirty-year average growing season precipitation of 57.9cm (sd=7.1) for the E.V. Smith research station location, 2016 is classified as a “dry” year while 2017 is a normal year, falling within one sd of the thirty-year precipitation average for the growing season.

In 2016, the crop received 35.3cm of precipitation compared to 57.2cm in 2017 (Figure 7). Temperatures in 2016 ranged from 21° to 26°C while 2017 ranged from 11° to 26°C (Figure 7).

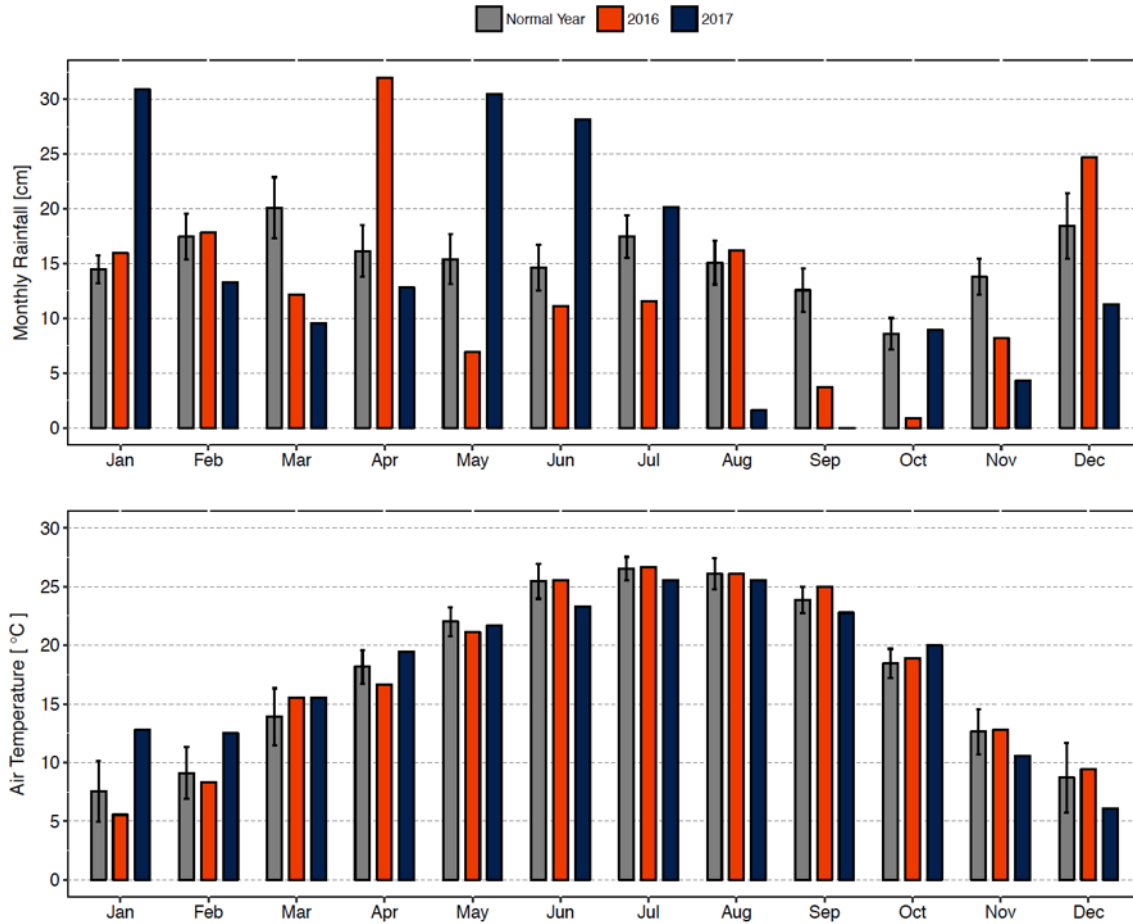


Figure 7. Monthly weather data from 2016 and 2017 with normal values reflecting thirty year averages at site. Error bars indicate  $\pm$  one standard deviation.

### Minirhizotrons and Root Analysis

For 2016 root count, ANOVA revealed no significant interaction between irrigation treatment, replicate, and depth ( $p > 0.05$ ). Further simplification of the model resulted in no significance (Table 4): treatment ( $F(1,56) = 2.59$ ;  $p < 0.01$ ), replicate ( $F(1,56) = 1.36$ ;  $p < 0.01$ ), depth ( $F(2,54) = 2.37$ ;  $p < 0.01$ ). 2016 root count data ranged from 69 to 10 roots observed per viewing window. Roots depleted with both depth and age of plant, but differences were not significant for either irrigated or non-irrigated plants.



For 2017 root count, ANOVA revealed no significant interaction between irrigation treatment, replicate, and depth ( $p > 0.05$ ). Further simplification of the model resulted in significance only between irrigation scheduling treatment ( $F(5,48) = 8.43$ ;  $p < 0.01$ ). Significance between treatments was determined using a post-hoc multiple comparison test. 2017 root count data ranged from 4 to 135 roots observed per viewing window (Figure 8; Table 5). Checkbook produced the highest root count while R3 treatment produced the lowest at both R5 and R7 growth stages. No single treatment resulted in significantly higher counts than all other treatments. In addition to no significance of treatment, root data displayed no trends.

For both years of data, the null-hypothesis that non-irrigated plants would not produce higher root counts than irrigated cannot be rejected. The null-hypothesis that non-irrigated plants would not produce more roots at lower depths than irrigated cannot be rejected as treatment had no significant interaction with depth (Figure 9-12). In 2016, root count generally decreased with depth but differences were not significant for either irrigated or non-irrigated plants. 2017 root count showed no trends in data. While this data is inconsistent with the findings of others (Burch et al., 1978; Hoogenboom et al., 1987; Wang et al., 2014; Zou et al., 2001), minirhizotron root data can only be collected from the area visible to the camera. It is possible that the plant performed similar to previous studies outside of the field of view of the minirhizotron camera. Additionally, it has been reported that minirhizotron systems can alter normal root development and misrepresent data by causing soil disturbance during installation (Joslin and Wolfe, 1999).

Porometer data collected in 2017 showed no correlation between dates and had negligible correlation with root growth (Figure 13; Figure 14). Correlation coefficients varied between -0.43 and 0.61 for irrigated plants and -0.44 and 0.9 for non-irrigated plants. Correlation coefficients indicate no trends in data. Of the six dates taken, the null-hypothesis that non-

irrigated plants would not produce lower photosynthetic conductivity root counts than irrigated cannot be rejected.

Experiments repeated on the same crop, but with data being collected from rhizotron systems noted that non-irrigated plants produced higher root counts with the non-irrigated plants producing more roots at deeper depths ( $p < 0.05$ ) (Hoogenboom, et al., 1987). In the report, a rhizotron system was used and root monitoring occurred by collecting data to a depth of 180cm as roots grew against the glass viewing window. Roots were traced directly against the glass using a wax pencil. With this method, root length and root count could be monitored on a regular basis. Rhizotron systems present more stable conditions than the field conditions of this experiment and create a fuller viewing panel for root growth. Similar research was done on both sorghum and soybean by manually digging the plant up and taking root measurements (Burch et al., 1978). Burch et al. (1978) found that while differences occur between varieties and crop species, irrigated soybeans showed significant increases in root populations both in length and mass at lower soil depths compared to the non-irrigated counterparts ( $p < 0.05$ ). Similar studies on wheat demonstrate the same trends of root populations and densities ( $\text{g m}^{-3}$ ) decreasing as the crop reaches maturity ( $p < 0.05$ ) (Wang et al., 2014). Wang et al. (2014) quantified root populations by densities and collected data by manually uprooting the plant concluding that populations decrease with depth and are impacted by available soil nutrients ( $p < 0.05$ ). Joslin and Wolfe (1999) found that a limitation of the minirhizotron system is that installation and tube disturbances can alter natural root development. Root systems adapt in abnormal growth patterns due to the soil disturbance and foreign object within the soil. The disturbance to the soil for tube installation could explain differences in root growth from expected trends. It should be noted that while soybean maturity groups could potentially impact trends in root development, both years

of data were collected from group five early maturing varieties. This study also varied in that results were limited to only data that could be collected from the specified viewing panels of the minirhizotron camera.

Table 4. Descriptive statistics of 2016 root count at each irrigation treatment (cm) and depth (cm) and associated standard error with N = 4 observations.

|       |       | Irrigation  |             |             |             |             |
|-------|-------|-------------|-------------|-------------|-------------|-------------|
| Depth |       | 0           | 0.9         | 1.9         | 2.9         | 3.8         |
| V4    | 0-20  | 68.8 ± 22.2 | 46.8 ± 21.1 | 41.5 ± 16.4 | 24.6 ± 12.3 | 54.5 ± 28.7 |
|       | 20-40 | 57.0 ± 16.7 | 16.2 ± 5.4  | 19.8 ± 5.2  | 34.8 ± 8.0  | 38.8 ± 19.4 |
|       | 40-60 | 40.5 ± 6.8  | 36.0 ± 10.8 | 28.0 ± 14.3 | 26.8 ± 8.4  | 12.5 ± 4.2  |
| R5    | 0-20  | 76.2 ± 12.4 | 25.8 ± 8.8  | 32.0 ± 11.6 | 52.8 ± 15.5 | 69.0 ± 32.0 |
|       | 20-40 | 24.8 ± 10.0 | 7.8 ± 2.6   | 13.0 ± 3.7  | 15.5 ± 2.9  | 22.2 ± 9.4  |
|       | 40-60 | 7.8 ± 3.3   | 9.8 ± 4.0   | 12.8 ± 7.8  | 13.0 ± 6.1  | 9.5 ± 5.2   |
| R7    | 0-20  | 19.8 ± 3.00 | 20.2 ± 5.40 | 17.2 ± 6.50 | 9.50 ± 2.50 | 15.2 ± 6.00 |
|       | 20-40 | 35.8 ± 11.8 | 12.5 ± 6.00 | 12.0 ± 5.00 | 12.2 ± 1.40 | 20.2 ± 8.30 |
|       | 40-60 | 13.5 ± 5.20 | 13.0 ± 7.30 | 18.8 ± 12.4 | 11.8 ± 3.90 | 8.00 ± 4.20 |

Table 5. Descriptive statistics of 2017 root count at each irrigation treatment and depth (cm) and associated standard error with N = 2 observations for all treatments except dryland where N = 10 observations.

|    |       | Irrigation   |             |              |             |             |              |
|----|-------|--------------|-------------|--------------|-------------|-------------|--------------|
|    | Depth | Checkbook    | Dryland     | R3           | R5          | R3+R5       | Sensor       |
| V4 | 0-14  | 36.0 ± 13.0  | 32.4 ± 9.4  | 121.0 ± 55.0 | 29.5 ± 5.5  | 62.0 ± 50.0 | 83.5 ± 55.5  |
|    | 14-28 | 101.0 ± 65.5 | 41.1 ± 9.5  | 160.0 ± 18.0 | 55.5 ± 15.5 | 53.0 ± 11.0 | 83.5 ± 29.5  |
|    | 28-42 | 68.0 ± 19.0  | 43.9 ± 10.0 | 135.0 ± 32.0 | 55.5 ± 33.5 | 28.5 ± 15.5 | 119.5 ± 17.5 |
| R4 | 0-14  | 69.5 ± 11.5  | 16.9 ± 5.1  | 49.0 ± 8.0   | 28.0 ± 8.0  | 41.5 ± 11.5 | 13.0 ± 8.0   |
|    | 14-28 | 82.0 ± 46.0  | 17.4 ± 5.8  | 67.0 ± 41.0  | 50.5 ± 9.5  | 34.5 ± 3.5  | 11.0 ± 6.0   |
|    | 28-42 | 61.5 ± 16.5  | 20.3 ± 4.8  | 87.5 ± 58.5  | 28.5 ± 8.5  | 12.5 ± 0.5  | 47.0 ± 5.0   |
| R7 | 0-14  | 44.5 ± 23.5  | 15.5 ± 4.4  | 33.0 ± 26.0  | 18.5 ± 11.5 | 9.5 ± 1.5   | 21.0 ± 8.0   |
|    | 14-28 | 41.5 ± 10.5  | 13.1 ± 2.6  | 56.0 ± 37.0  | 20.0 ± 2.0  | 11.0 ± 5.0  | 21.0 ± 18.0  |
|    | 28-42 | 61.0 ± 34.0  | 19.0 ± 3.7  | 54.5 ± 32.5  | 4.0 ± 4.0   | 6.0 ± 2.0   | 30.0 ± 5.0   |

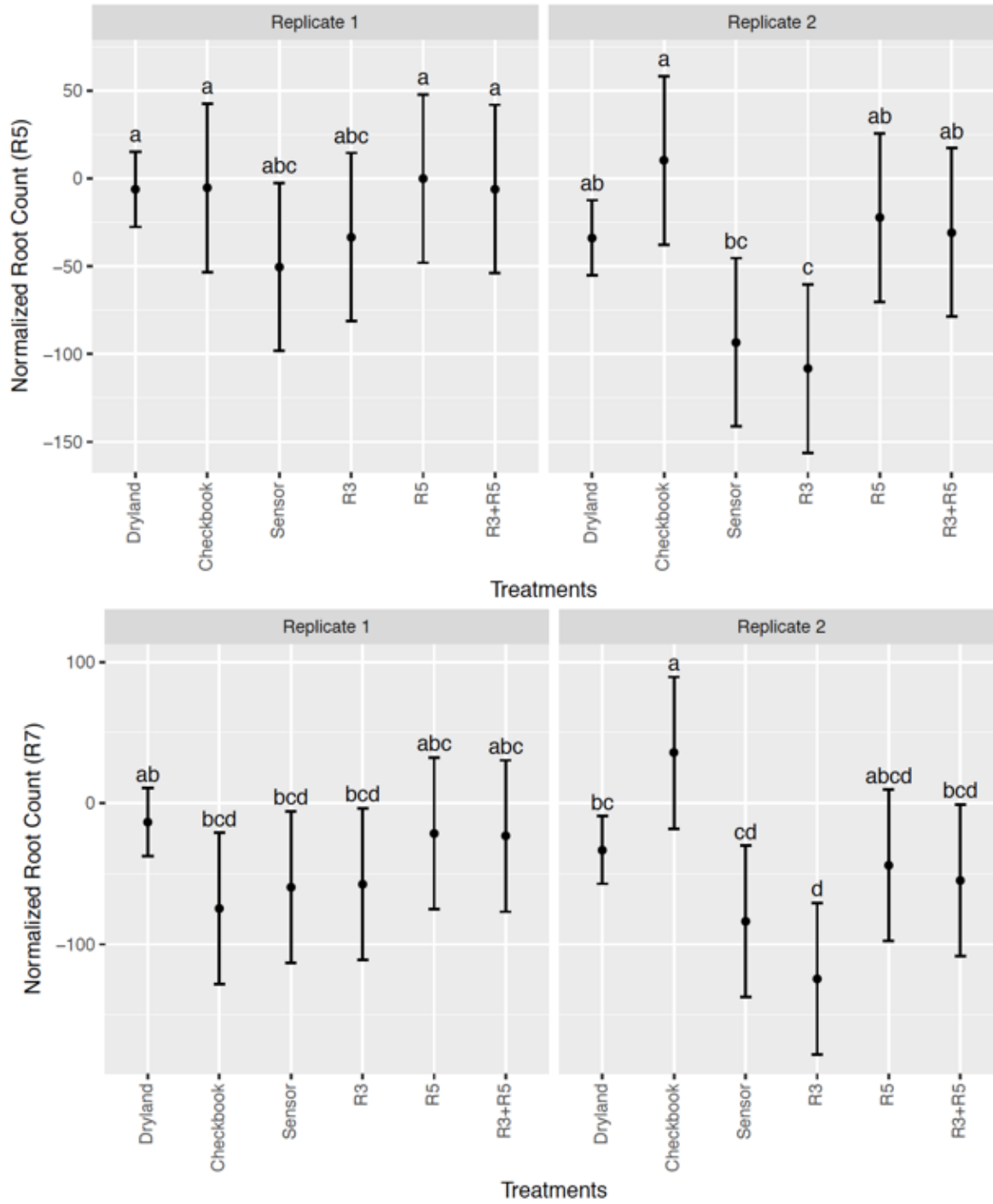


Figure 8. 2017 root count on ‘Pioneer 52A26 R’ soybean plants at R5 and R7 respectively and associated standard error. Significance between treatments was determined using a post-hoc multiple comparison test. Root counts were determined through image analysis using Rootsnap software. Error bars indicate  $\pm$  standard deviation.

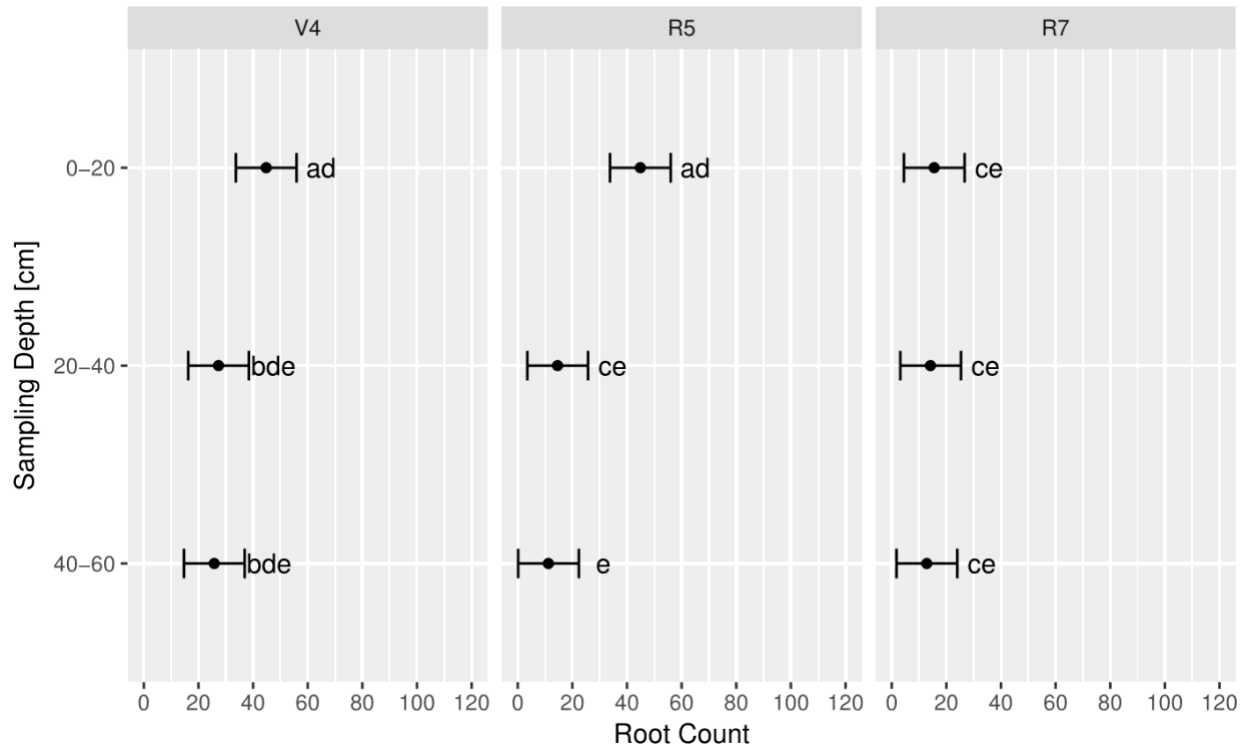


Figure 9. 2016 root count on irrigated ‘Pioneer 52T50 R’ soybean plants at V4, R5, and R7 at 0-20cm, 20-40cm, and 40-60cm depths and associated standard error. Root counts were determined through image analysis using Rootsnap software. Root count decreased with depth and maturity. Error bars indicate  $\pm$  standard deviation.

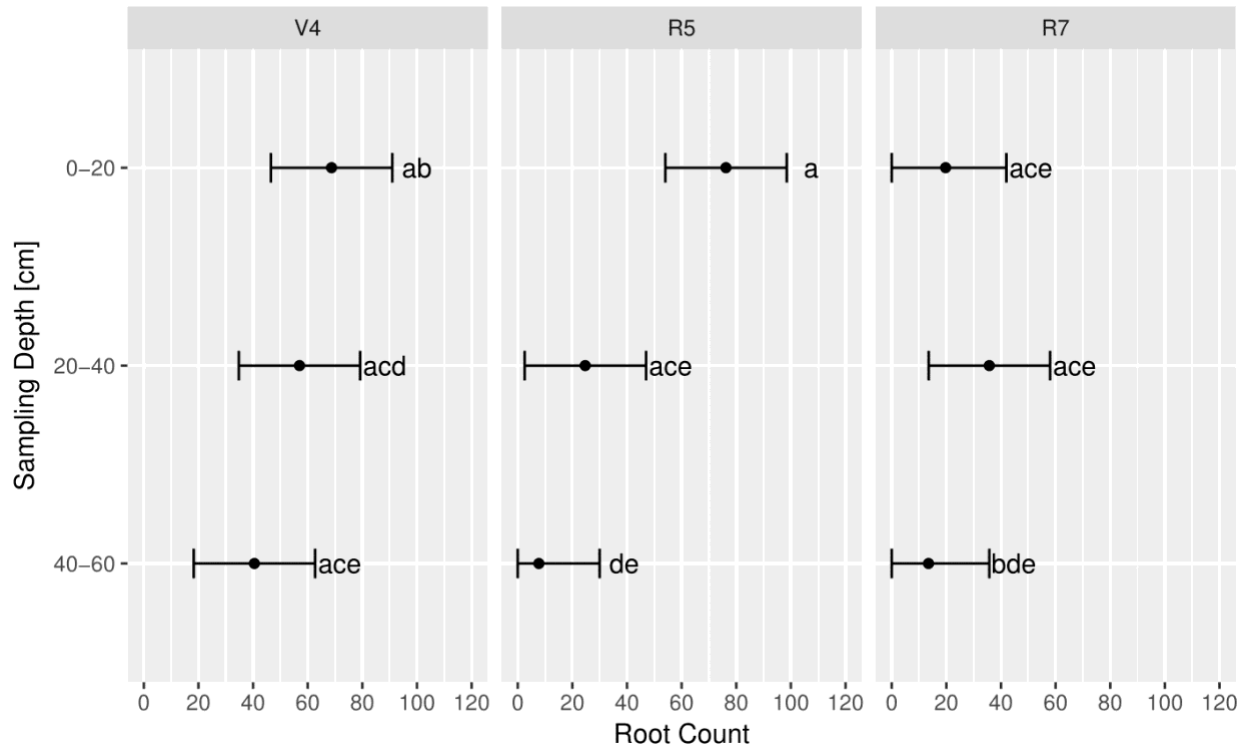


Figure 10. 2016 root count on non-irrigated ‘Pioneer 52T50 R’ soybean plants at V4, R5, and R7 at 0-20cm, 20-40cm, and 40-60cm depths and associated standard error. Root counts were determined through image analysis using Rootsnap software. Root count decreased with depth and maturity. Error bars indicate  $\pm$  standard deviation.

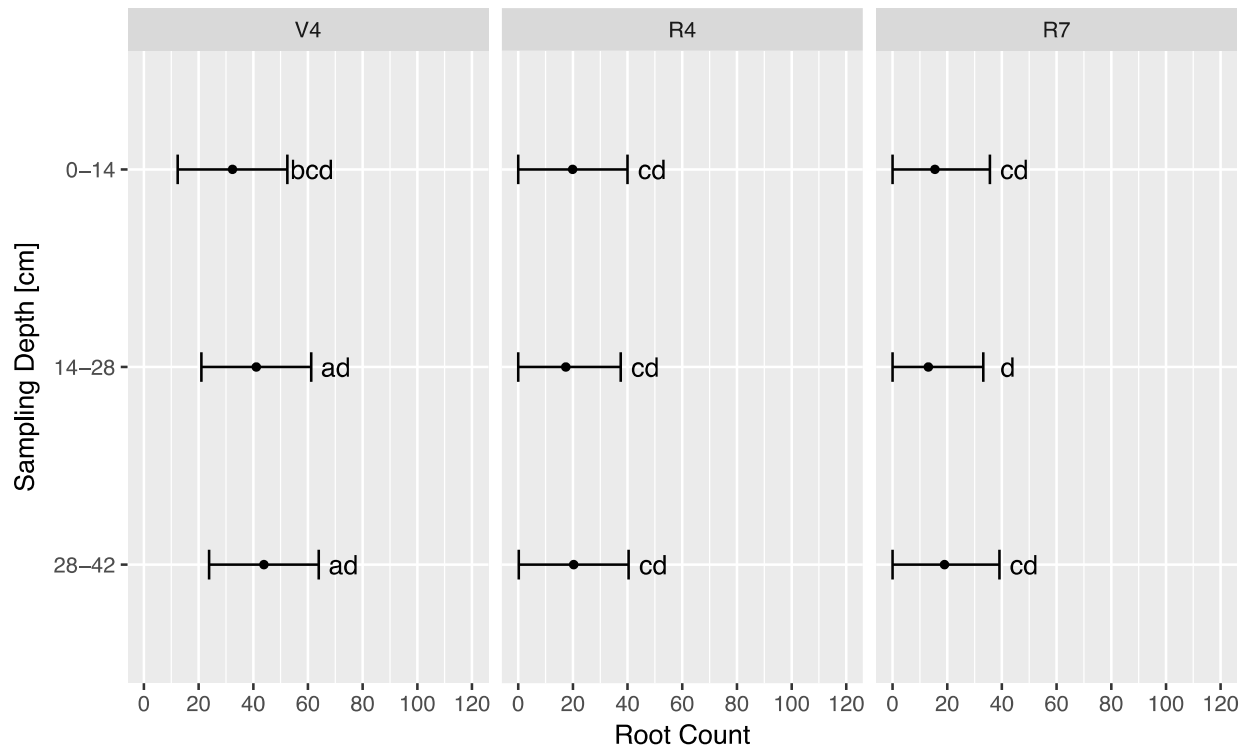


Figure 11. 2017 root count on irrigated ‘Pioneer 52A26 R’ soybean plants at V4, R4, and R7 at 0-14cm, 14-28cm, and 28-42cm depths and associated standard error. Root counts were determined through image analysis using Rootsnap software. Root count varied with depth and maturity. Error bars indicate  $\pm$  standard deviation.



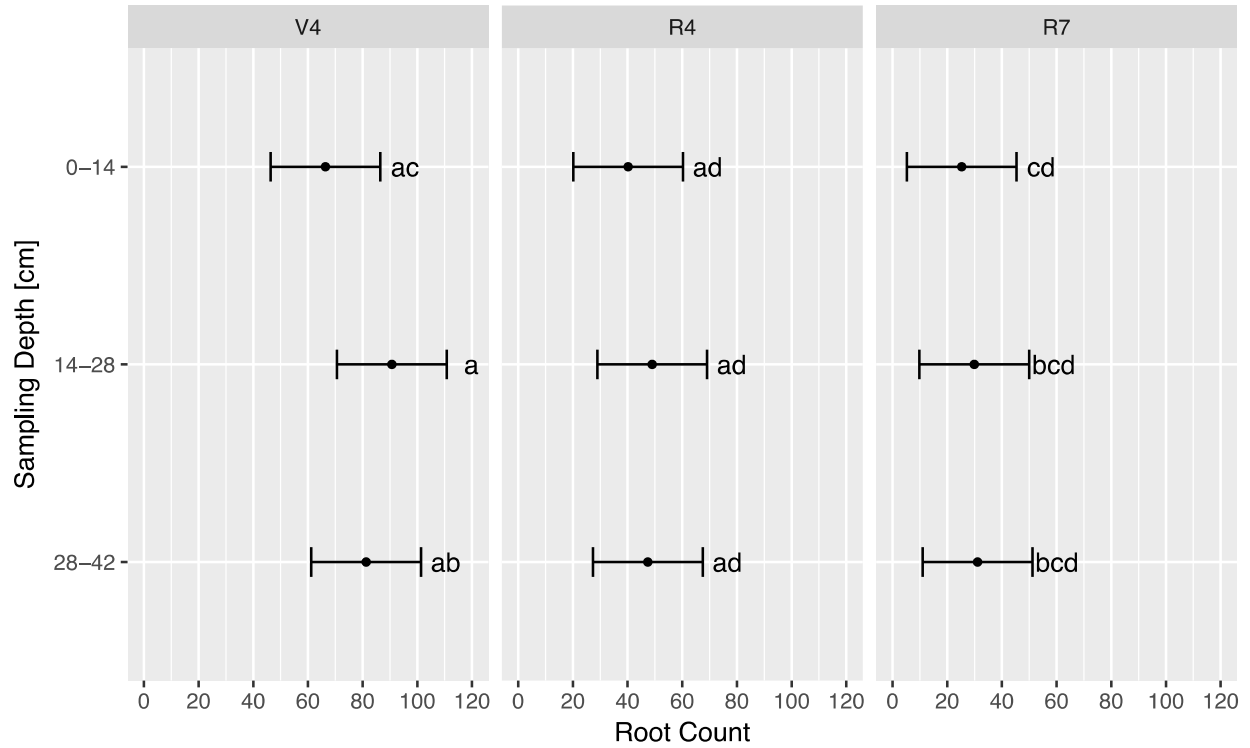


Figure 12. 2017 root count on non-irrigated ‘Pioneer 52A26 R’ soybean plants at V4, R4, and R7 at 0-14cm, 14-28cm, and 28-42cm depths and associated standard error. Root counts were determined through image analysis using Rootsnap software. Root count varied with depth and maturity. Error bars indicate  $\pm$  standard deviation.

### Correlation Coefficients of Selected Data Collection Dates

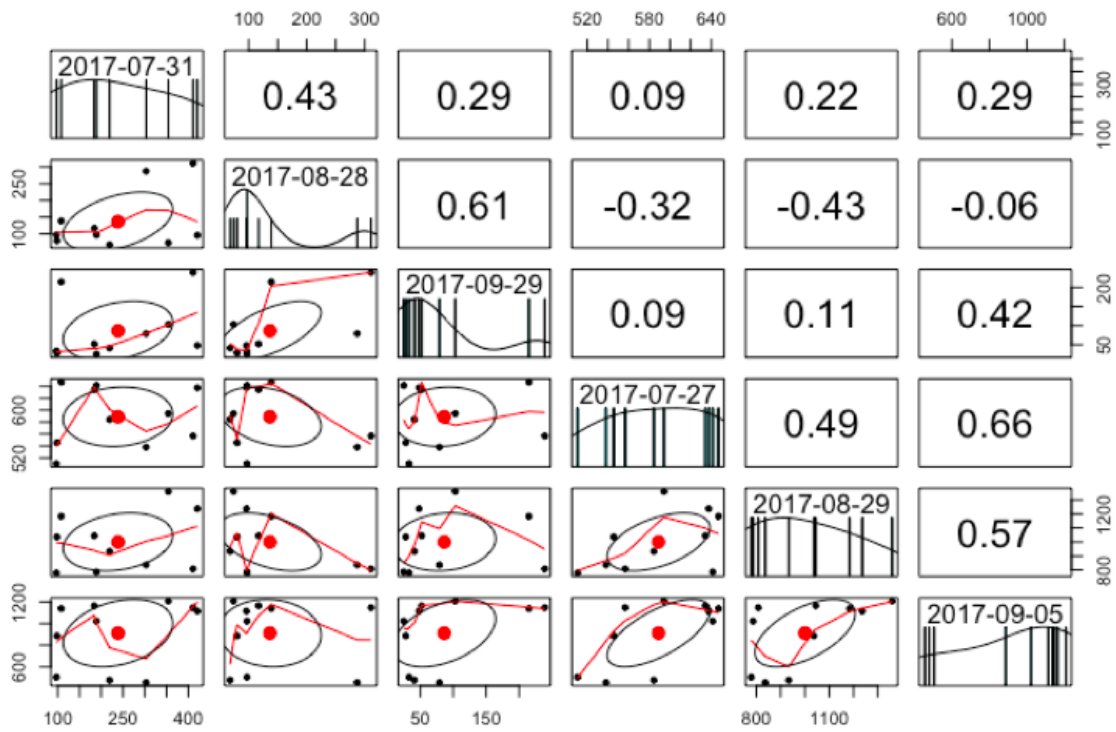


Figure 13. 2017 stomatal conductance data correlated with root count on irrigated plants. Selected dates reflect porometer data collection date followed by closest root data collection date. Scatter plots indicate visual representation of correlation coefficients for corresponding dates. Correlation coefficients indicate no trends in data.

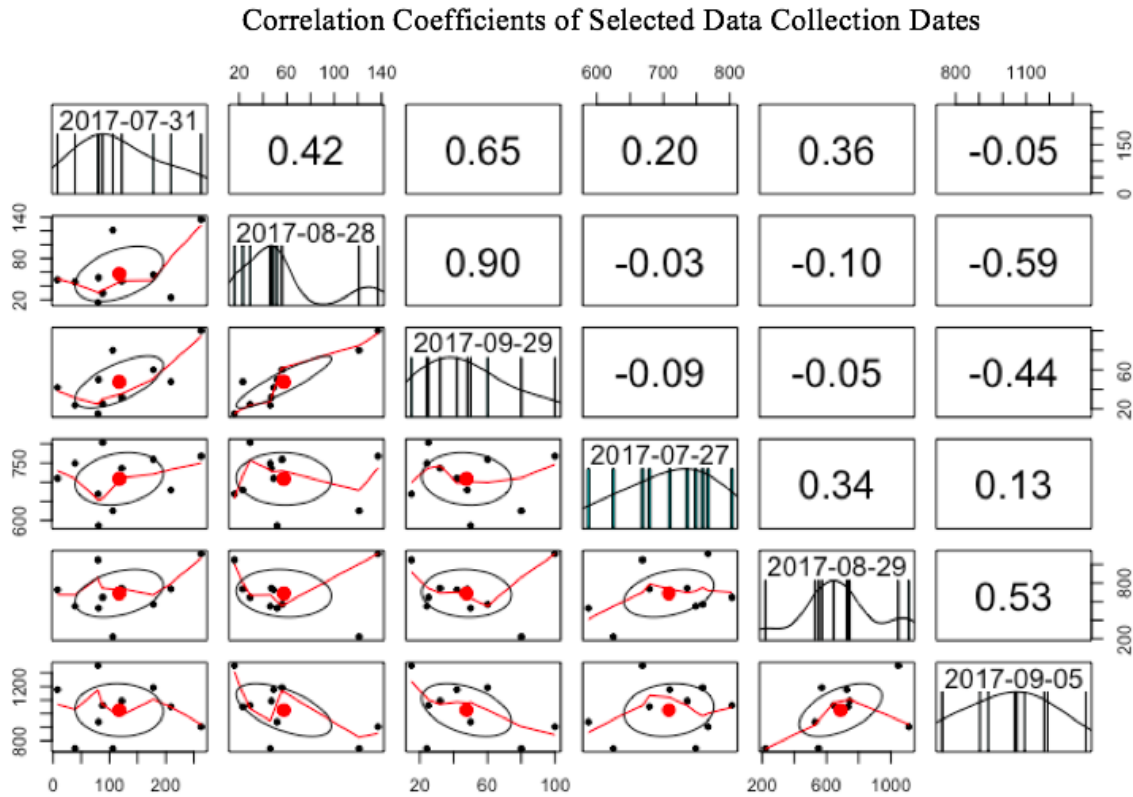


Figure 14. 2017 stomatal conductance data correlated with root count on non-irrigated plants. Selected dates reflect porometer data collection date followed by closest root data collection date. Scatter plots indicate visual representation of correlation coefficients for corresponding dates. Correlation coefficients indicate no trends in data.

### Yield

For 2016 yield, ANOVA analysis effects indicate no significant interaction between cultivar and irrigation, however, both cultivar ( $F(7,148) = 7.70; p < 0.01$ ) and irrigation ( $F(4,148) = 40.50; p < 0.01$ ) were significant. Multiple comparisons ( $p < 0.05$ ) were made between cultivars and between irrigation treatments.

While it was a non-significant interaction, a trend existed between cultivar yield and increased irrigation rates (Table 6). Yield ranged from 3.2t ha<sup>-1</sup> occurring at 0cm treatment for ‘Pioneer 55T81 R’, to 6.4t ha<sup>-1</sup>, occurring at 3.8cm treatment for ‘Pioneer 52T50 R’. ‘Pioneer

52T50 R' produced significantly higher yields than all other cultivars except 'Asgrow 5533 R' at all irrigation treatments (Figure 15). Irrigation was found to have an impact on yield with 1.9, 2.9, and 3.8cm treatments performing significantly better than 0.0 and 0.9cm treatments. Yield increased  $0.7\text{t ha}^{-1}$ ,  $0.4\text{t ha}^{-1}$ ,  $0.3\text{t ha}^{-1}$ ,  $0.2\text{t ha}^{-1}$  respectively from an initial yield of  $3.8\text{t ha}^{-1}$  at 0.0cm treatment. There was no significant difference between 2.9 and 3.8cm treatments (Figure 16). Therefore, the 2.9cm irrigation treatment proved to be the highest irrigation rate for significant gains in yield. Results indicate that while there is no significant interaction between cultivar and irrigation, comparisons can be made between cultivars and between treatments. Irrigation treatments increased yield and yield varied between cultivars.

In 2017 the cultivar and irrigation interaction was not significant. The individual effects of cultivar ( $F(5,52) = 14.64$ ;  $p < 0.001$ ) was significant while irrigation ( $F(5,52) = 2.09$ ;  $p < 0.01$ ) was not. Multiple comparisons ( $p < 0.05$ ) were made between cultivars (Figure 17) and between irrigation treatments (Figure 18).

Yield varied with cultivars and irrigation treatments with 'Pioneer 52A26 R' achieving the highest yield of  $3.2\text{t ha}^{-1}$  and both 'Asgrow 75X6' and 'Asgrow 76T54 R2' achieving the lowest yield at  $1.2\text{t ha}^{-1}$  (Table 7). 'Pioneer 52T50 R' performed significantly better than 'Asgrow 69X6', 'Asgrow 75X6', and 'Pioneer 76T54 R2' ( $p < 0.01$ ). Irrigation was not found to have an impact on yield with only sensor treatments performing significantly higher than R3+R5 treatments.

In 2016 it was found that yield increased with increasing irrigation rates. In 2017, it was found that there was no difference in irrigation scheduling techniques. It is possible that the impact of irrigation scheduling techniques during 2017 may have been masked by receiving a normal year of rainfall (Figure 7).

Matric potential readings monitored throughout both growing seasons report plants consistently closer to PWP in 2016 than 2017 (Figure 19). PWP occurs at -1,500kPa (Cassel et al., 1986; Tolk, 2003). During the 2016 growing season, 2.9cm and 3.8cm irrigation treatments suffered the least in terms of matric potential values. During the 2017 growing season, no treatments exceeded -160kPa, including dryland. Sensor based treatments suffered the least and did not exceed -100kPa throughout the season. Dryland experienced the most negative matric potential values, but did not exceed -160kPa. It is likely that due to the differences in monitored matric potential readings over the two growing seasons, rainfall masked the response to irrigation during the 2017 growing season.

Water requirements for soybeans as reported by Masuda (2009) and Specht (1999) were met with growth stage ( $0.127\text{-}0.254\text{ cm day}^{-1}$ ), vegetative growth ( $0.254\text{-}0.508\text{ cm day}^{-1}$ ), flowering to pod fill/full canopy ( $0.254\text{-}0.762\text{ cm day}^{-1}$ ), and physiological maturity ( $0.127\text{-}0.508\text{ cm day}^{-1}$ ) through rainfall. Brevedan and Egli (2003) and Lundstrom et al. (1988) determined that it is advantageous to focus irrigation on specific growth stages during the reproductive stages of soybean maturity in order to minimize irrigation input and maximize yield. Chaves et. al (2002) found that withholding water in earlier growth stages can create resilience in times of drought compared to those that were watered throughout the entire growing season. This experiment differed in that cultivars were watered throughout the entirety of the growing season in 2016 and cultivars received adequate rainfall during 2017. Torrion et. al (2014) found that focusing irrigation before R3 could be detrimental to yield while irrigating at R3 and later growth stages resulted in higher yields. Torrion reported less rainfall than my experiment in both years, receiving 35.2cm and 48.9cm during the two growing seasons studied— 0.1cm less and 8.3cm less than the respective growing seasons in my experiment. Torrion could have found

stronger irrigation responses due to soybean cultivar selection and less rainfall. While 2016 data supports a significant gain in yield from irrigation, 2017 data did not support the conclusions of Bredan and Egli. Rainfall may have then masked the effects of irrigation on yield.

Table 6. Descriptive statistics of 2016 yield ( $t\ ha^{-1}$ ) for each cultivar at each irrigation treatment (cm) and associated standard error with  $N = 4$  observations.

|                  | Irrigation    |               |               |               |               |
|------------------|---------------|---------------|---------------|---------------|---------------|
|                  | 0             | 0.9           | 1.9           | 2.9           | 3.8           |
| Asgrow 5831 R    | $3.8 \pm 0.2$ | $4.1 \pm 0.2$ | $4.3 \pm 0.2$ | $4.6 \pm 0.2$ | $5.0 \pm 0.2$ |
| Pioneer 52T50 R  | $4.2 \pm 0.6$ | $5.1 \pm 0.2$ | $5.5 \pm 0.1$ | $5.9 \pm 0.1$ | $6.4 \pm 0.1$ |
| Pioneer 54T94 R  | $3.2 \pm 0.4$ | $4.2 \pm 0.2$ | $4.7 \pm 0.2$ | $5.3 \pm 0.2$ | $5.7 \pm 0.1$ |
| Asgrow 5533 R    | $4.2 \pm 0.6$ | $4.9 \pm 0.2$ | $5.0 \pm 0.1$ | $5.4 \pm 0.1$ | $5.5 \pm 0.2$ |
| Pioneer 55T81 R  | $3.2 \pm 0.5$ | $4.3 \pm 0.1$ | $4.9 \pm 0.2$ | $5.2 \pm 0.2$ | $5.1 \pm 0.4$ |
| Pioneer 56T12 S  | $4.0 \pm 0.3$ | $4.6 \pm 0.0$ | $5.0 \pm 0.2$ | $5.3 \pm 0.0$ | $5.3 \pm 0.4$ |
| Pioneer 56T29 R2 | $3.6 \pm 0.3$ | $4.1 \pm 0.2$ | $4.9 \pm 0.1$ | $5.1 \pm 0.2$ | $5.0 \pm 0.6$ |
| Pioneer 95Y70 R  | $3.9 \pm 0.3$ | $4.2 \pm 0.2$ | $4.7 \pm 0.2$ | $4.7 \pm 0.0$ | $4.9 \pm 0.6$ |

Table 7. Descriptive statistics of 2017 yield ( $t\ ha^{-1}$ ) for each cultivar at each irrigation treatment and associated standard error with number of observations.

|                    | Irrigation       |                  |                  |                  |                  |                  |
|--------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                    | Checkbook        | Dryland          | R3               | R5               | R3+R5            | Sensor           |
| Pioneer 52A26<br>R | $2.8 \pm 0.5, 2$ | $2.8 \pm 0.1, 2$ | $2.8 \pm 0.0, 2$ | $2.4 \pm 0.2, 2$ | $2.5 \pm 0.4, 2$ | $3.2 \pm 0.1, 2$ |
| Pioneer 55T81<br>R | $2.3 \pm 0.1, 2$ | $2.3 \pm 0.0, 2$ | $2.3 \pm 0.0, 2$ | $2.1 \pm 0.2, 2$ | $2.1 \pm 0.2, 2$ | $2.3 \pm 0.0, 2$ |
| Asgrow 5831<br>RR  | $2.5 \pm 0.3, 2$ | $2.4 \pm 0.6, 2$ | $2.4 \pm N/A, 1$ | $2.2 \pm N/A, 1$ | $1.0 \pm N/A, 1$ | $2.8 \pm N/A, 1$ |
| Asgrow 69X6        | $2.2 \pm N/A, 1$ | $1.6 \pm 0.4, 2$ | $1.7 \pm 0.6, 2$ | $1.5 \pm 0.9, 2$ | $1.1 \pm 0.6$    | $2.2 \pm N/A, 1$ |
| Asgrow 75X6        | $1.2 \pm 0.7, 2$ | $1.9 \pm 0.1, 2$ | $1.5 \pm 0.1, 2$ | $1.8 \pm 0.2, 2$ | $1.8 \pm 0.2, 2$ | $1.9 \pm 0.1, 2$ |
| Asgrow 76T54<br>R2 | $1.2 \pm 0.6, 2$ | $1.3 \pm 0.1, 2$ | $1.0 \pm 0.0, 2$ | $1.4 \pm N/A, 1$ | N/A              | $1.5 \pm 0.7, 2$ |

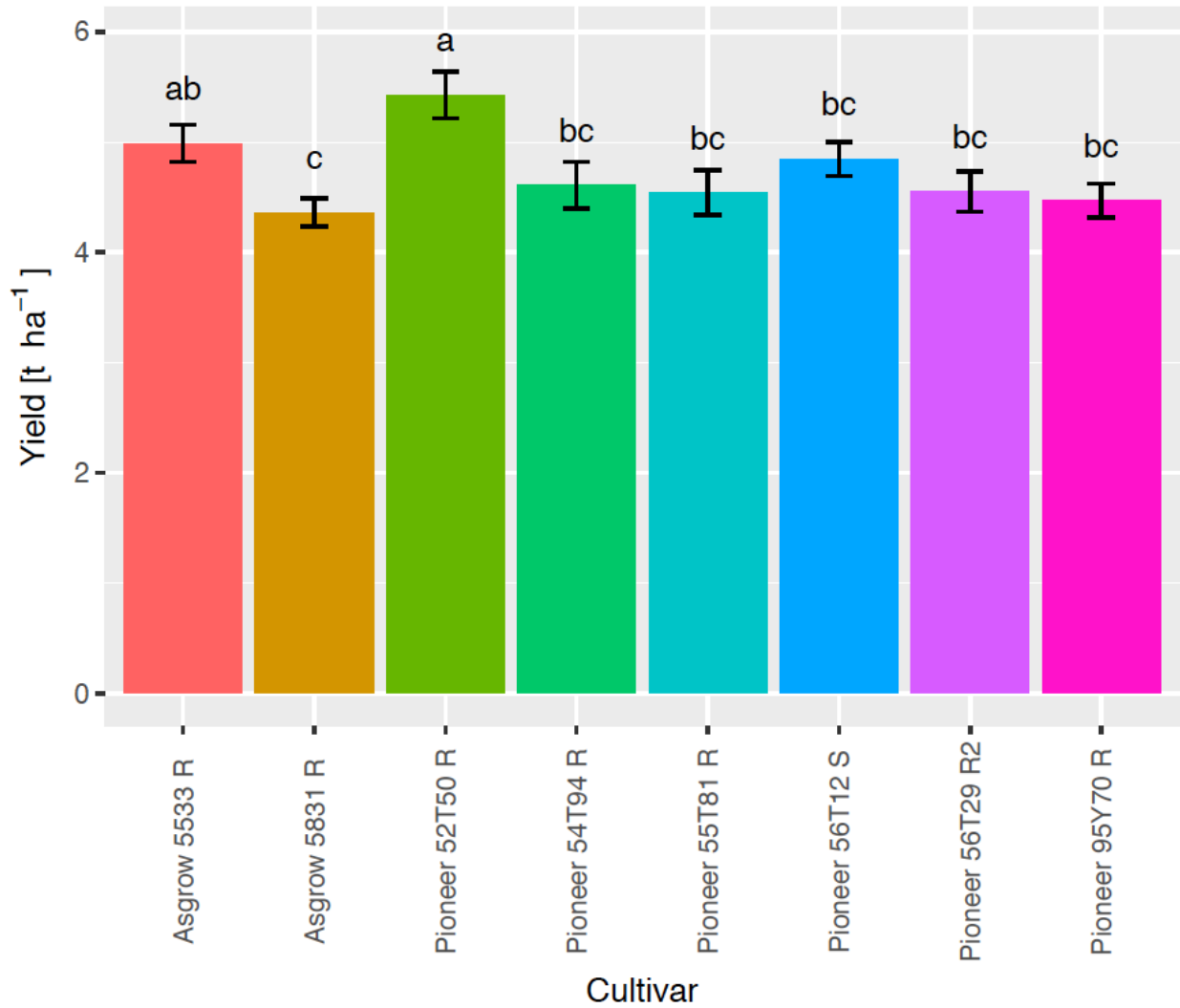


Figure 15. 2016 results of two-way ANOVA and associated standard error between yield and cultivar (N =20,  $\alpha = 0.05$ ). ‘Pioneer 52T50 R’ produced significantly higher yields than all cultivars except ‘Asgrow 5533 R’. Error bars indicate  $\pm$  standard deviation.



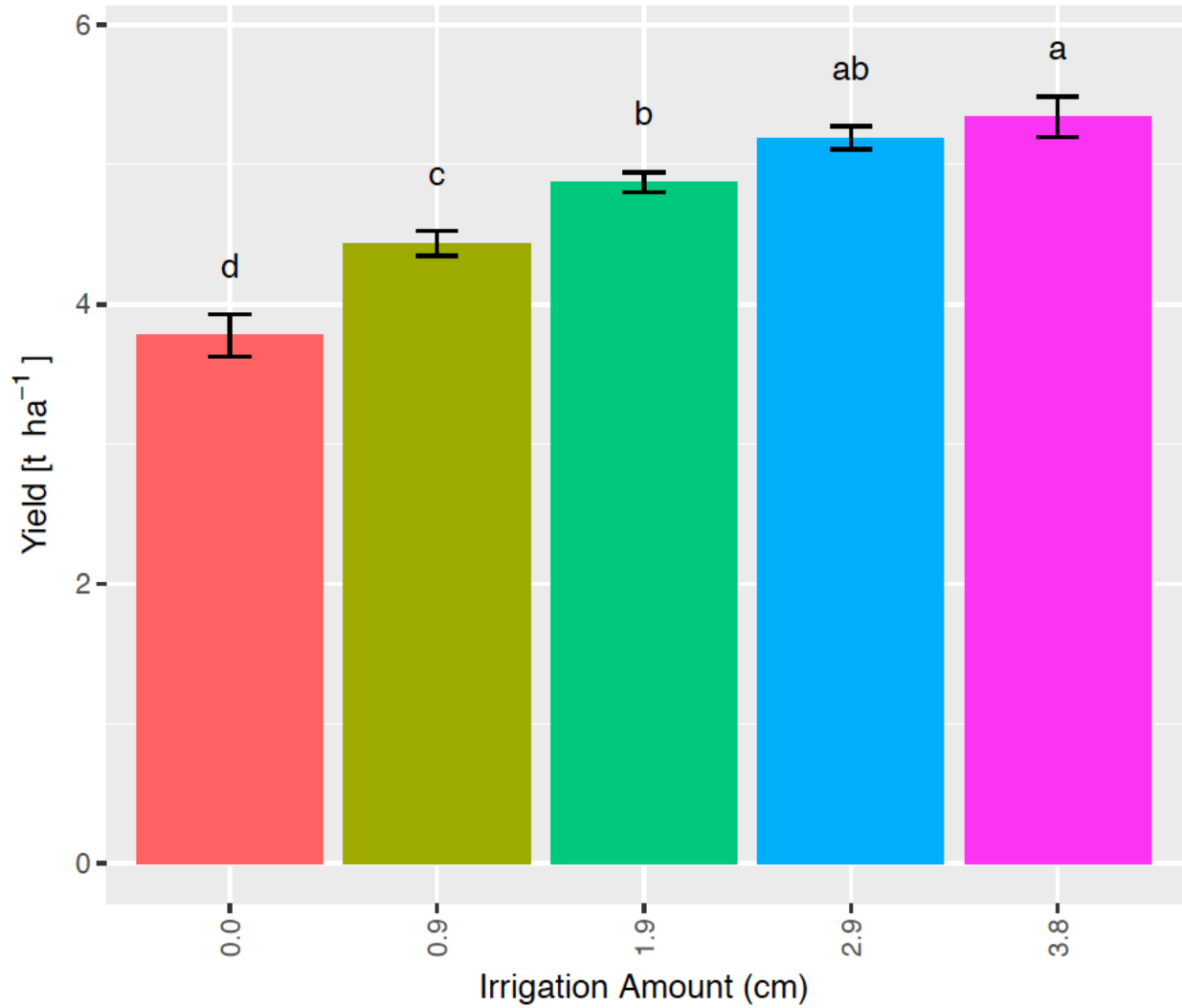


Figure 16. 2016 results of two-way ANOVA and associated standard error between yield and irrigation treatments ( $N = 30$ ,  $\alpha = 0.05$ ). Yield increased with increasing irrigation treatments. There was no significant difference between 2.9 and 3.8cm treatments. Error bars indicate  $\pm$  standard deviation.

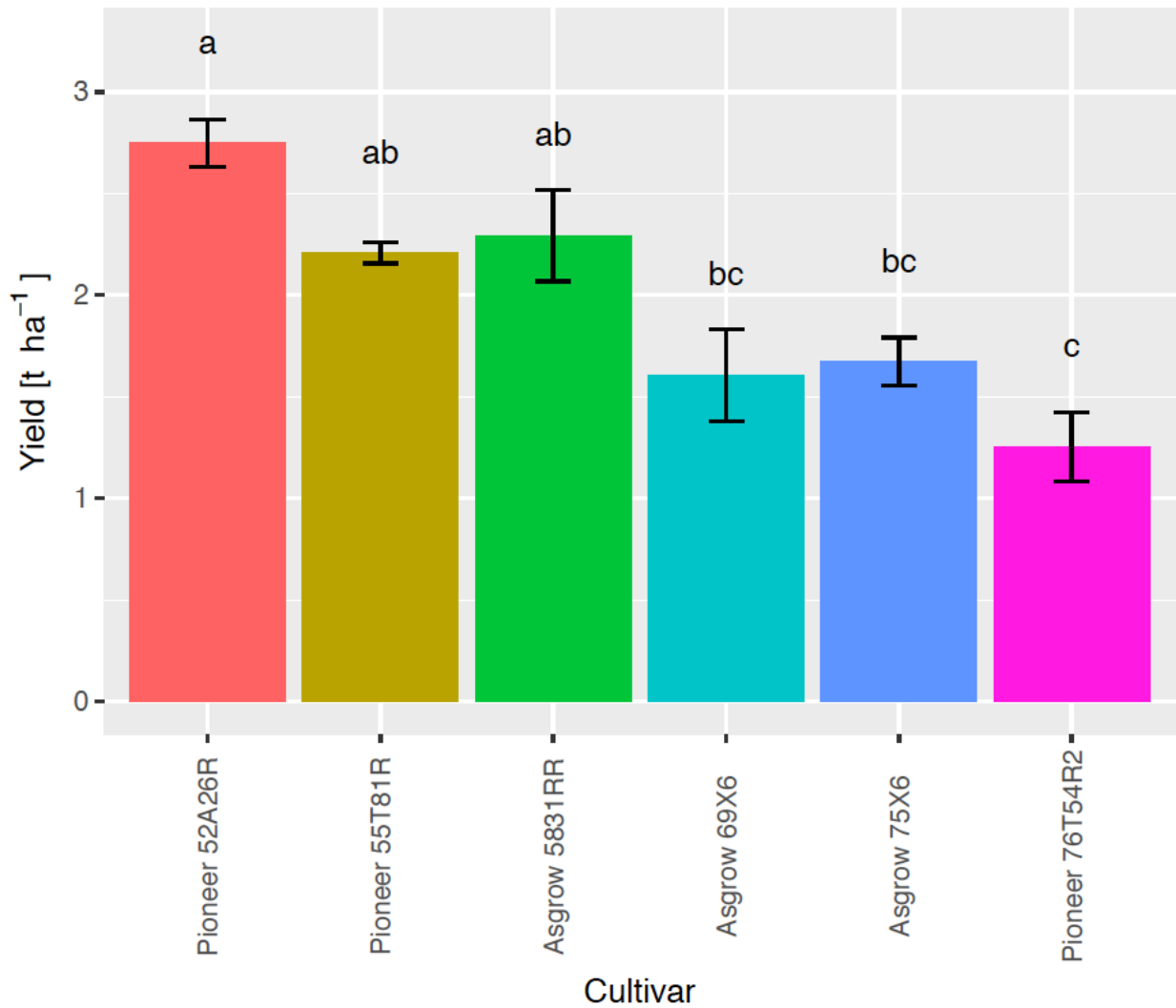


Figure 17. 2017 results of two-way ANOVA and associated standard error between yield and cultivar (N =12,12,8,10,12,9,  $\alpha = 0.05$ ). ‘Pioneer 52T50 R’ produced significantly higher yields than ‘Asgrow 69X6’, ‘Asgrow 75X6’, and ‘Pioneer 76T54R2’. Error bars indicate  $\pm$  standard deviation.

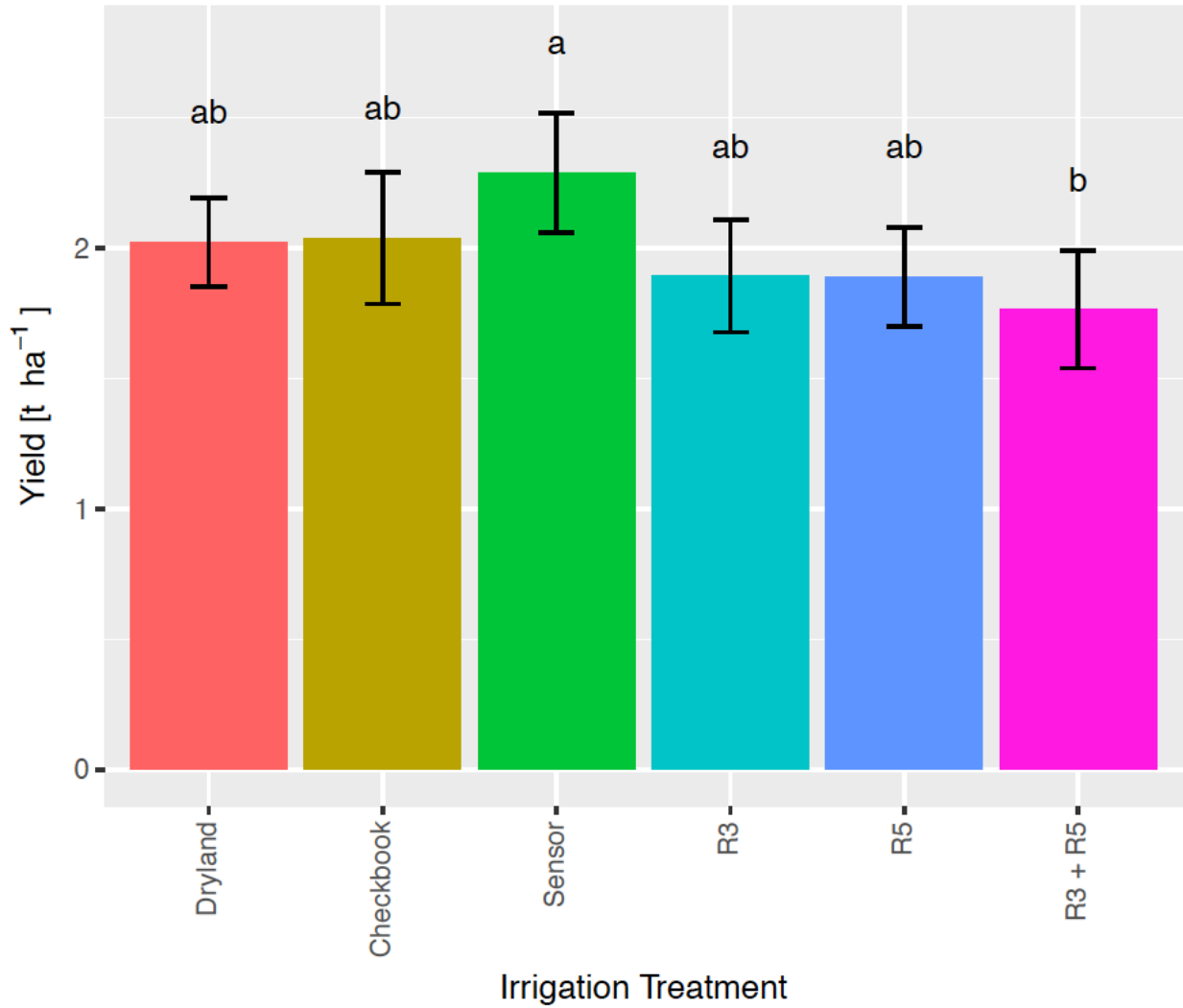


Figure 18. 2017 Results of two-way ANOVA and associated standard error between yield and irrigation treatments (N = 11,12,11,10,9,10,  $\alpha = 0.05$ ). Sensor based treatments performed significantly better than R3+R5 treatments. Error bars indicate  $\pm$  standard deviation.

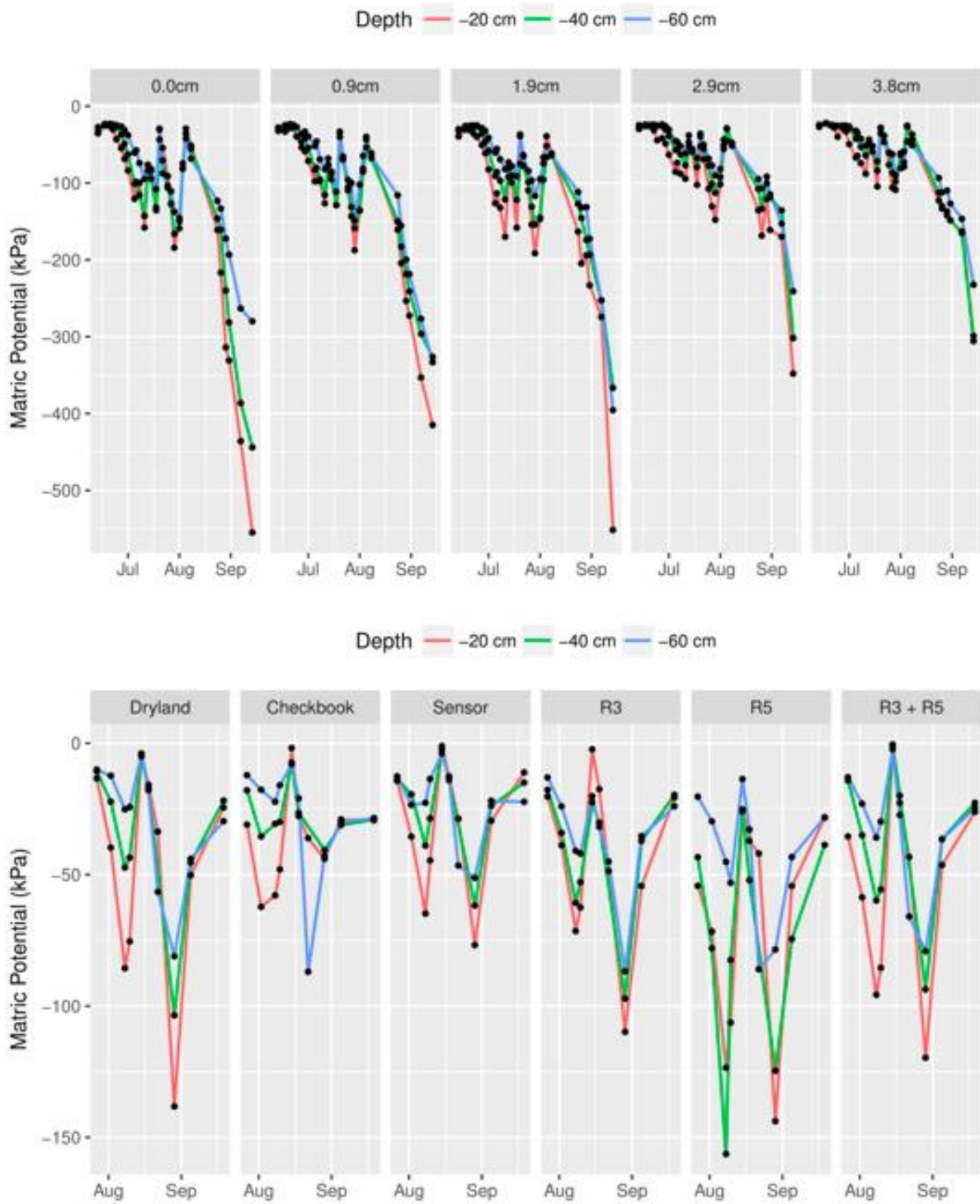


Figure 19. Matric potential sensor readings over 2016 and 2017 growing seasons respectively.

2016 consistently maintained greater water deficit due to receiving less rainfall than 2017.

## Conclusions

In conclusion, soybean root growth is highly variable as no interactions were found between treatment, replicate, or depth. Minirhizotron field research is limited in that data can only be collected from the area surrounding the tube. While results did not follow expectations from literature, it is important to remember that with greater sampling through manually uprooting the plants or utilizing a rhizotron system, it is possible that results would have reflected expectations from literature. Were this experiment repeated, rhizotron systems and manually uprooting plants in the field would allow a better understanding of the accuracy of minirhizotron sampling. Due to the small sample size and the excessive time to process minirhizotron root data, it would not be recommended to use this method for studying irrigation treatment impacts on root development.

In regard to yield, response showed an increase in yield with increasing irrigation rates in 2016. Optimal applications of irrigation treatment may vary depending on cultivar and annual rainfall. Based on the results of 2016, it is evident that soybean cultivars must be considered when developing a successful cropping system. In 2017 yield differences were not found in yield from implementing different irrigation scheduling techniques. It is possible that the impact of irrigation scheduling techniques during 2017 may have been masked by receiving adequate rainfall during the growing season. Additional research is necessary to further define cultivar relationship on soybean yield. In order to maximize in-field crop production, both cultivar and irrigation need should be considered. Future experiments would ensure that the same cultivars were utilized each growing season as well as irrigation treatments. Additional research is necessary to further designate which irrigation regimes are best for yield and individual cultivars.

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