RESPONSE OF SIMULATED STRESS OF SOYBEAN PRODUCTION SYSTEMS:

GROWTH PARAMETERS AND YIELD

By

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RESPONSE OF SIMULATED STRESS OF SOYBEAN PRODUCTION SYSTEMS: GROWTH PARAMETERS AND YIELD

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Abstract: Soybean (*Glycine max*) hectares planted in western Oklahoma have recently increased by 75.9%, but soybean-growing challenges are unknown in the region, which has historically been dominated by small-grain production. To overcome latent yield limitations, it is critical to recognize soybean physiological response to stressors and potential for recovery. Thus, two studies were conducted to evaluate challenges associated with producing soybean in western Oklahoma, or dryland systems in low precipitation regions, and how to measure soybean response in those conditions.

The first study was designed to study the physiological changes from simulated stress during critical yield determination stages by removal of reproductive structures at R2, R3, and R5 on cumulative mainstem locations. Trials were conducted in 2018 and 2019 in Oklahoma. Field study results indicated significant yield recovery potential when stress was imposed at R2 and R3. Significant impact was observed on seed weight and seed number that occurred when stress was imposed at R5. Moreover, when significant pod removal is experienced at R5, soybean has potential to develop green-stem. This physiological reaction, due to a source to sink imbalance in favor of the source, retaining assimilates and leaving plants unharvestable as a reaction to intolerable stressors.

Furthermore, to determine the physiological response of moisture stress during high water requirement stages of R2, R3, and R5, a growth chamber study was conducted. The effect of moisture stress duration of 7 or 14 days was significant, with all plants experiencing 14 days of moisture stress reducing yield potential. In correlation with the field study, it was also found that soybean reproductive stages R3 and R5, especially, experienced yield impact.

It was concluded that impact of stress on yield is minimal at R2, increases at R3, and is greatest at R5. This can be attested to the fact that moisture stress influences seed growth to some extent at all stages, but specifically during R5 with the greatest yield impact by seed number reduction. Soybean reaching genetic potential in such dryland systems can only be achieved by physiological response to stressors and mitigation of yield losses.

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CHAPTER I

INTRODUCTION

Soybean (*Glycine max*) is an oilseed crop that is used as a biofuel feedstock, livestock feed, and a protein source in the human diet. The continued increased demand for soybeans is a direct response of its multitudes of use, with world soybean production surpassing 290 million megagrams in the 2014-2015 growing seasons (FAO, 2016) and its value reaching 20 billion dollars (Goldsmith, 2008). Soybean grain yield is defined as the average mass of individual seeds from the mean number of plants per unit area and the area soybean production covers is quantified in hectares. Present global soybean production spans over 75.5 million hectares (FAO, 2019) and is concentrated to the United States with over 108 million megagrams, followed by Brazil and China (FAOSTAT, 2017).

Soybean is the dominant oilseed crop grown in the United States with 36.3 and 32.1 million hectares harvested in 2018 and 2019 alike (USDA-NASS, 2019). Improvement of production practices and increased genetic vigor has allowed soybean hectares to expand (Miller et al., 2002), with Oklahoma ranking 40th at 242.8 thousand hectares harvested in 2018 (USDA-NASS, 2018) and covering 210.4 thousand hectares harvested in 2018 (USDA-NASS, 2018) and covering 210.4 thousand planted hectares in 2019 (USDA-NASS, 2019). The dominant soybean counties in Oklahoma are Kay, Grant, and Garfield that collectively harvested around 110 thousand hectares and yield over a million bushels annually (USDA-NASS, 2017). While soybean has historically been farmed to the east central and northeastern regions of Oklahoma, hectares of production are stretching to the west. Soybean hectares planted in the western half of the state has increased by 75.9% from 2012 to 2016 (USDA-NASS, 2012, 2013, 2014, 2015, 2016), but soybean growing challenges are unknown in the region historically dominated by small-grain production.

Western Oklahoma experiences cool nights, similar to the Midwest, but temperature extremes and lack of consistent precipitation wedge a gap in production discrepancy and sets it apart from traditional soybean production regions in the United States. Moisture stress alone can be very impactful on soybean production; however, low plant available moisture often couples high temperatures during the summer months in the southern Great Plains. High temperatures and lack of available moisture commonly occur simultaneously and the ability of soybean to tolerate, adapt, and recover from these stresses directly reflects crop performance (Prasad and Staggenborg, 2008).

To overcome yield limitations of limited rainfall, temperature, and other production challenges such as insects and disease, it is critical to recognize soybean physiological response to these stressors and the potential for recovery. Thus, this study was developed to evaluate the challenges associated with producing soybean in western Oklahoma and how to measure soybean response in those conditions. The first objective was to determine the physiological response of imposed drought stress during high water requirement stages in a growth chamber study. When water scarcity occurs in a specific developmental phase, the yield component of the soybean ontogeny at that stage is reduced to the extent of the timing and severity of the stress (Purcell and Specht, 2004). Therefore, it was intended for the second objective, to evaluate the physiological changes from simulated stress during critical yield determination stages in a field study by removal of reproductive structures at certain times and plant locations. This simulated stress could be from production challenges of drought, heat, insects, or diseases. Determining soybean yield recovery from these simulated stressors and the relationship of yield loss prevention is the third objective of this study. Soybean reaching their genetic potential in the emerging production region of western Oklahoma can only be achieved by the physiological response of soybean to given stressors and mitigation of potential yield losses.

CHAPTER II

REVIEW OF LITERATURE

Historically, the reason soybean hectares have been concentrated to the eastern portion of Oklahoma can be partially credited to a more suitable climate, where consistent rainfall patterns and high relative humidity allows soybean to proliferate. Western Oklahoma also has different attributes of a high producing soybean region that is only now being capitalized. The contrast of the two Oklahoma soybean growing regions begins with climatic conditions that shift from humid subtropical in the east to semi-arid in the west (Arndt, 2003). Yearly precipitation ranges, on average, from 43 to 142 centimeters from the western panhandle to the southeast part of the state (Arndt, 2003). The frequency of annual rainfall events follow the same trend with 115 days in the east to 45 days in west, with this variability of rainfall events in the western half of the state certainly attributing to an increased dryland drought susceptibility (Arndt, 2003).

Vegetation and soils shift as an effect of the differing climatic conditions with eastern Oklahoma soils developing under oak-hickory-pine forests and tall grasses, steep slopes and ridges while soils of western Oklahoma are influenced by grasses, scrub oaks, cedars, and shrubs (NRCS, 2006). With that, accumulation of organic matter from grassland prairies, deeper profiles and typically less erosion in eastern Oklahoma soils create a good medium for plant growth. At the same time, the silt loam soils found throughout western Oklahoma are inherently some of the most productive in the state.

Productive soils are an important component for crop performance, but growth and development can easily be hindered by drought and heat stress. These conditions become major limiting factors for soybean production, mainly due to the physiology of the plant. In high light and temperature environments, adapted photosynthesis and improved water use efficiency makes C4 species more productive than C3 species (Ehleringer and Cerling, 2002). A result of the two different photosynthetic pathways, C4 species have 50% higher photosynthetic efficiency, which is advantageous in hot and dry climates (Wang et al., 2012). Soybean is a C3, while grain sorghum and corn, commonly grown in western Oklahoma, are C4. Grain sorghum is more adapted to semi-arid climates as a drought-tolerant crop species. While corn is also a C4 crop, its higher water requirement results in it not being as favorable in drought prone regions (Pugnaire et al., 1999).

When climatic conditions favor evaporative water loss, C4 plants are able to minimize stomatal conductance with the aid of Rubisco, a cellular carbon concentrating mechanism (van der Kooi et al., 2016), and acquire sufficient CO_2 even when the stomata are partially closed (Gowik and Westhoff, 2011). This efficient pathway of CO_2 fixation in C4 plants gives an evolutionary benefit over C3 species (Pearcy and Ehleringer, 1984; Sage et al., 1999), with approximately 30% increase in yield under dry conditions (van der Kooi et al., 2016). Higher water use efficiencies promoted by C4 photosynthesis at a given photosynthetic capacity are capable at high temperatures by prevention of oxygenation and CO_2 limitations (Pearcy and Ehleringer, 1984). Photosynthesis is the process most sensitive to heat events by increased evaporative demand, causing stomatal closure and elevated photorespiration, ultimately decreasing net photosynthesis (DeBoeck et al., 2010; Zinn et al., 2010). Decreases in photosynthesis during heat events can be attributed to the alteration of the enzymatic properties of Rubisco and carbon dioxide's solubility to oxygen, both of which favor RuBP being oxidized to Rubisco (Siebers et al., 2015). Thus, causing the already inefficient photosynthetic pathway of C3 species to undergo photorespiration, thereby decreasing net photosynthesis by nearly 40% in high temperature and dry conditions (Ehleringer et al., 1991). The chance of increased moisture loss starts at the photosynthetic pathway as it is restricted to the bundle sheath cells within the leaf, decelerating the rate of photosynthesis, while stomates stay open for longer periods of time to compensate for this inefficiency (Ehleringer and Cerling, 2002).

Photosynthesis is influenced by moisture stress creating resistances to CO_2 absorption (Shaw and Laing, 1966), with absorption ceasing when half of the maximum leaf water content is lost (Brilliant, 1924). Maintaining sufficient leaf water content keeps epidermal stomates open for atmospheric carbon dioxide to diffuse to carboxylation sites found in leaf mesophyll cell chloroplasts which is critical for biomass production via photosynthesis (Purcell and Specht, 2004). However, leaving stomates open escalates drought susceptibility as water evaporating from cell walls diffuses from within the leaf to the atmosphere through the epidermal orifices. The amount of water lost from the leaf, as quantified by the mass or moles of C or CO_2, reflects transpiration efficiency (Purcell and Specht, 2004). Transpiration is sourced from leaf conductance for gas exchange, and this process can function at lower levels in C4 species, giving an advantage in efficiency. However, as atmospheric CO_2 levels continue to rise, C3 species will likely increase transpiration efficiency to a similar rate (Purcell and Specht, 2004). As commonly grown western Oklahoma crops are C4 species, soybean as a C3 will pose a different growing challenge of optimizing photosynthetic activity and efficiently utilizing available water in the semi-arid climate.

Water availability in the root zone can quickly become a limiting factor in yield by decreased crop water use, or evapotranspiration (Foroud et al., 1992). Crop growth is limited to the amount of water the crop transpires because it also controls the accumulation of plant biomass from photosynthesis (Purcell and Specht, 2004). Crop yield is reduced by decreased evapotranspiration from limited available water supply (Foroud et al., 1992), while extent of yield loss is stress period time and length dependent (Doss et al., 1974).

Plant response to moisture stress has adverse effects on vegetative growth as insufficient plant water supply increases respiration, decreases photosynthesis, and declines available carbohydrates (Shaw and Laing, 1966). For the same reason, moisture stress will decrease plant size because water is the major constituent of active tissue and essential for maintaining turgidity for cell enlargement and growth (Newman and Kramer, 1963). The internal water balance of plants controls the relative rates of water loss and absorption in the plant, and thus determines the quantity of biomass accumulation (Kramer et al., 1963). Muchow et al. (1986) concluded that when water was withheld from soybean, leaf senescence escalated and the lowered leaf area index decreased photosynthetically active radiation (PAR) use efficiency. This decline in PAR use efficiency was directly linked to decreases in stomatal conductance and leaf expansion (Muchow et al., 1986). In turn, decreases in leaf area expansion hinders crop biomass accumulation with the greatest impact being before canopy closure has been reached (Prasad and Staggenborg, 2008). Crop growth rate depends on the expansion of leaf area to capture solar energy for photosynthesis, which is then converted into dry matter (Andriani et al., 1991; Board and Harville, 1996). Yield components are minimally influenced by moisture stress until reproduction (Purcell and Specht, 2004).

Water uptake requirements begin to increase as late vegetative and early reproductive stages occur sequentially, for indeterminate soybean, with continued growth of the terminal bud and axillary raceme flower initiation (Carlson and Lersten, 2004; Kranz et al., 2012; Woodworth, 1932). Soybean seed yield originates from the number of flowers a plant produces during an estimated period of 20 to 40 days (Hansen and Shibbles, 1978). The number of flowers produced during this period, R1 to R2, directly relates to yield, and inversely, the number of aborted flowers limits yield. Board and Tan (1995) demonstrated that assimilatory capacity, or source strength, affects branch and node number as well as pods per reproductive node on main stems and branches from beginning flowering to pod fill. To support peak biomass and flower production, water redistributes assimilates around the plant with assimilate supply being mediated by photosynthesis (Brevedan and Elgi, 2003; Elgi, 2010).

A deficient water supply causes flowers to abscise from insufficient supply of photosynthetic assimilates to reproductive organs (Raper and Kramer, 1987). Developing pistils and stamens can be impaired by stress during anthesis (Carlson and Lersten, 1987) with ovule function being more sensitive than pollen production (Kokubun et al., 2001). However, the most reproductive loss happens after fertilization in an early embryonic development stage as a result of inadequate water supply (Kokubun et al., 2001), signifying the influence of stress timing on yield loss and water stress (Frederick et al., 1990; Shaw and Laing, 1966). Studies by Shaw and Laing observed that stress at R1 allowed later recovery of pods when stress was alleviated before the end of R2 (Shaw and Laing, 1966). Stress during R2 greatly reduced seed set with inadequate time for compensation, demonstrating that peak moisture uptake begins at R1 and continues through R2 (Eck et al., 1987; Foroud et al., 1993; Shaw and Laing, 1996). Length of the stress period is also influential as Shaw and Laing (1996) found stress throughout R1-R2 had maximum reduction in seed set, followed by stress during late R2, and early R1. This dynamic of water stress timing continues to influence successive soybean reproductive stages.

Soybean flowering is highly asynchronous, extending the flowering period through R3 to increase pod survival to maturity (Elgi and Bruening, 2006). Location influences the order of flower development from nodes on the main raceme, to secondary and tertiary branches, and sub-branches (Elgi and Bruening, 2006). Pods from early flowers, starting on lower nodes, are sinks that consume most of the assimilate supply (Heithholt et al., 1986), leaving limited resources for late flowers developing simultaneously at the top of the mainstem (Elgi and Bruening, 2006). Consequently, late developing pods also have a decreased chance of survival (Heithholt et al., 1986) because of inadequate assimilate supply (Bruening and Elgi, 1999). A study by Frederick et al. found stresses near R3 decreased branch vegetation growth and ultimately decreased soybean seed yield (Frederick et al., 2001). Impact of moisture stress at R3 limits biomass, restricting the creation of additional nodes to supply yield, and at the same time influences the survival of late developing flowers and pods by competition with larger sinks for resources.

During R5, no additional flowers are being produced to mitigate loss from aborted pods and low seed weight (Eck et al., 1987). Drought occurring during late reproduction shortens seed-fill rate (Thomas and Raper, 1977), accelerates leaf senescence (Specht et al., 1986; Muchow et al., 1986), and directly affects the production of biomass and assimilate; forcing the plant to rely on stored assimilate to support grain growth (Purcell and Specht, 2004). Meckel et al. (1984) suggests that moisture stress decreases seed fill duration and may also be a factor of yield loss in drought stressed soybean. Stress during R5 has high potential yield loss by significantly reducing yield components of seed number and seed weight (Brevedan and Elgi, 2003; Foroud et al., 1992). Water uptake requirements make likelihood of drought stress the greatest during flowering by the need to support both biomass production and reproductive structures. While soybean at R5 require less water to conduct typical functions, the high physiological impact of drought stress during this stage results in severe yield loss (Foroud et al., 1993). Soybean sensitivity to water deficit is minimal during vegetative through R2, increases at R3, and is most sensitive at R5 (Kranz et al., 2012).

Soybean vulnerability to drought stress during seed development complicates management of production systems with limited irrigation. Foroud et al. (1992) found that withholding irrigation water at R5 greatly reduced seed yield and significantly impacted seed number and weight. This finding is similar to that of Eck et al. (1987) in which restricting water from the end of pod development to pod fill, or early R5, had the maximum impact on yield reduction. This does not exactly correlate with water uptake requirements as maximum moisture uptake peaks at 0.8 centimeters average daily evapotranspiration during R2 and early R3 (Shaw and Laing, 1996; Eck et al., 1987; Kranz et al., 2012). In high soybean production regions with a fully irrigated soybean crop, 50 to 66 centimeters of total water is applied (Kranz et al., 2012). With western Oklahoma receiving a historical average of only 43 centimeters of rainfall during the growing season (Arndt, 2003), and limited available water for irrigation if applicable, drought is certainly a major production discrepancy separating such dryland soybean systems from more prolific production regions.

Soybean tolerance to stressors at a given growth stage and duration should also account for heat events. The ability of soybean to produce flowers for twenty to thirty days, along with the response of increased pod set, gives the plant potential to recover yield from stressful temperatures (Siebers et al., 2015). However, this is not the same case during R5 as high temperatures disrupt seed development. As a result, crop quality is reduced by shriveled seeds and remaining normal seeds exhibiting lower quality properties (Elgi et al., 2006; Spears et al., 1997). Excessive heat occurring during early R3 reduced yield by 10% (Siebers et al., 2015) but soybean can compensate for the loss of pods by increasing seed weight (Munier-Jolain et al., 1998). Mann and Jaworski (1970) linked high temperatures, near 40°C, to decreased photosynthetic rates causing severe pod drop. This temperature extreme is not common for prolonged periods in western Oklahoma, but these temperatures can occur in short increments during the months of June, July, and August (Arndt, 2003), which typically are associated with timing of R3 and R5. These short periods of high temperatures can still significantly alter yields (Mann and Jaworski, 1970). High temperature events, which are frequently coupled with moisture stress, can compound unfavorable and uncontrollable environmental stresses in dryland soybean production in low precipitation regions.

Reductions of not only yield, but also decreased grain quality and quantity result from drought stress. The success of a mature seed is negatively impacted by reduced assimilate supply demoting seed growth (De Souza et al., 1997). Water stress accelerates leaf senescence, shortening the length of R5 by significantly earlier physiological maturity, ultimately reducing final seed size and resulting in lower yields (De Souza et al., 1997; Vieira et al., 1992), with drought stress during R5 having the biggest impact by decreased seed numbers (Smicklas et al., 1989). An experiment conducted by De Souza et al. (1997) found that water stress rapidly decreased leaf chlorophyll content as well as the quantity of C and N in the seed, all of which are partial factors in determining the extent of yield loss from stress during R5. The remobilization of C and N from leaves and other vegetative parts to the developing seed is a major contributor of yield (De Souza et al., 1997) and when demand of these nutrients exceeds supply, photosynthesis becomes limited and senescence begins prematurely (Purcell and Specht, 2004). Water is needed to move assimilates from sources to sinks and concurrently produce assimilates to support grain growth (Purcell and Specht, 2004). The amount of water the crop transpires controls growth and yield while the crops ability to acquire sufficient C and N quantifies the concentration of oil and protein in the seed (Purcell and Specht, 2004). As defined by Rupe and Luttell, grain quality is the type and amount of oil and protein from the physical and chemical properties of the soybean seed (Rupe and Luttrell, 2008). Oil and protein synthesize and accumulate in the seed during R5 and deposit rapidly, spanning from 20-40 to 70 days after flowering (Rose, 1988). This is also a sensitive growth stage to drought stress (Foroud et al., 1992), with extent of oil and protein content loss per seed being capable of reducing more than half of total seed weight (Rose, 1998). Seed weight reduction is the yield response to severity and duration of a given moisture stress event, with stress during late R5 also impacting oil content (Rose, 1998). Oil content of soybean seed is nearly half of protein content, indicating that moisture stress has a greater impact on oil content, critically during the R5 stage (Rose, 1998).

Understanding the potential underlying mechanisms of yield loss by water stress is of importance when considering the final commodity. Additional growing challenges Oklahoma producers could face, such as insect and disease pressures and patterns, decrease grain quality and quantity to the same extent. According to Koenning (2007), insect and disease losses each averaged approximately 9% in the southern United States in 2006. Shifting hectares to soybean production can radically change the entomological dynamics of arthropod species, indicating that insect niches travel to occupy unfilled feeding regions (Turnipseed and Kogan, 1976).

Developing soybean areas are initially inhabited by accumulations of euryphagous species such as grasshoppers and stink bugs (Turnipseed and Kogan, 1976). The first invaders of emerging soybean territories are often grasshoppers, *Orthoptera (acrididae)*, that defoliate plant tissue with their chewing mouthparts and can increase susceptibility to disease (Turnipseed and Kogan, 1976; Rupe and Luttrell, 2008). Stink bug species, *Nezara viridula*, for example are increasing pressure in the southern United States with potential correlation to early-season indeterminate soybean cultivar production (McPherson et al., 1993; Rupe and Luttrell, 2008). Pressure of this insect can impact soybean directly from seed quality and yield damage to soybean seed from punctures, discoloration, or shriveling and indirectly as a vector of *Nematospora coryli* (McPherson et al., 1993; Rupe and Luttrell, 2008; Turnipseed and Kogan, 1976).

Examples of species that shift to introduced soybean hectares are the bean leaf beetle, *C. trifurcata*, and the soybean looper, *Chrysodeixis includens* (Turnipseed and Kogan, 1976). The bean leaf beetle moves from wild legumes to introduced soybean (Turnipseed and Kogan, 1976) with both larvae and adults causing soybean injury by defoliation, with 50% defoliation as the threshold for economic losses (Heatherly and Hodges, 1998). Soybean loopers were found in both cotton and soybean hectares in Louisiana by Kogan and Cope, illustrating the relationship of soybean looper infestation in these two crops, from adult moths proliferating on cotton nectar and the caterpillars feeding on soybean tissue (Jensen et al., 1974; Kogan and Cope, 1974). Soybean loopers typically move from pastures to soybean fields later in the growing season. In soybean and cotton production areas, female soybean loopers produce significantly more eggs with access to cotton nectar (Heatherly and Hodges, 1998). The concentration of pasture and cotton hectares in western Oklahoma could impose a soybean looper infestation in nearby soybean fields. Although not considered a major threat to soybean, they do contribute to defoliation injury by consuming leaf tissue (Heatherly and Hodges, 1998). While the patterns and influence of these insects are unknown, the effect of yield on defoliation timing has been well documented. Soybean has the capability to recover from insect damage with no yield loss up to 40% defoliation before reproduction and 25% defoliation after full bloom and 80% damage at pod development gives potential to compensate yield (Rupe and Luttrell, 2008), but may require different insecticides and new integrated pest management practices for optimal control.

On the other hand, cool nights in western Oklahoma with humidity forming under the soybean canopy could be optimal for disease development. An example of possible disease pressure is Frogeye leaf spot, caused by the fungus *Cercospora sojina* (Heatherly and Hodges, 1998). This disease develops in warm, humid conditions and targets leaves as they develop until the entire plant is infected; advancing onto stems and pods under favorable conditions (Heatherly and Hodges, 1998).

According to Koenning, insect and disease losses each averaged approximately 9% in the southern United States in 2006 (Koenning, 2007). The implications of insect and disease pressures in the developing soybean hectares in western Oklahoma are unknown, so new integrated pest management practices must be created and implemented to prevent additional yield losses. The eastern side of Oklahoma is well established in soybean production, and thus is knowledgeable of these growing season challenges and equipped to handle a given stressor imposed on the soybean crop. It is of interest to recognize the unique soybean production regions of Oklahoma to optimize and adapt practices to combat biotic stressors, such as insects and diseases, which may also threaten the progression of dryland grown soybean.

In late-season soybean, the detrimental physiological response to an interference of the source-sink ratio, known as green-stem, is well documented (Elgi and Bruening, 2014; Harbach et al., 2016a; Hill et al., 2006; Hobbs and Hill, 2006). Reduction of pod load in late-season soybean by any mechanism has been indirectly related to the onset of green-stem (Elgi and Bruening, 2014). Causal agents of this symptomology involve viral infection, insect pressures and low soil moisture content (Elgi and Bruening, 2006; Hill et al., 2006). Green-stem restricts physiological maturity by retaining moisture in vegetative tissue while pods and seeds mature and approach appropriate harvest moisture levels (Hill et al., 2006). This phenomena also signifies that pod and seed maturation does not require vegetative maturation (Elgi and Bruening, 2014). Factors that alter pod numbers create a physiological unbalance, slowing the movement of C and N from the biomass to pods with more available resources in the vegetative tissue than can be supplied to the lessened sink (Miceli et al., 1995; Wittenback, 1983). Vegetative tissue remains green from high concentrations of starch and N being directed into vegetative tissue rather than depositing these photosynthetic assimilates into developing seeds (Miceli et al., 1995). A pod removal study by Harbach et al. (2016b) found lower pods on the mainstem attributed to disrupting senescence signals by accumulation of photosynthetic assimilates in the stems and these seeds exhibited a higher moisture content.

It is hypothesized that green-stem is initiated by a stress that disrupts the source-sink relationship in favor of the source, such as aborting pods reducing the pod load, and further developed by factors of the environment, production practices, and genetics (Harbach et al., 2016a). Biotic stressors such as insect pressure have been shown to onset green stem (Boethel et al., 2000, Harbach et al., 2016b; Hobbs et al., 2006). Green stink bug infestations during later reproductive stages, above pesticide application thresholds, significantly impacted seed yield and quality (Boethel et al., 2000). Feeding on seeds resulted in underdeveloped or aborted seeds (Miller et al., 1977), directing greater quantities of photosynthate to vegetative tissues and consequently delaying maturity (Boethel et al., 2000).

Development of green-stem can be furthered by environmental conditions of drought (Xavier et al., 2017; Harbach et al., 2016a; Fulai et al., 2003). Interactions with the environment and green-stem have been demonstrated (Xavier et al., 2017) and contributed specifically to precipitation events that determined the degree of green-stem (Harbach et al., 2016a). The relationship between rainfall and late season soybean reproductive stages are positively correlated with higher rainfall events decreasing the incident of green-stem and drought conditions restricting assimilate flow (Harbach et al., 2016a). Depending on the extent of the stress, yield is impacted by increased seed loss and difficulty at the time of harvest. In some cases of extreme late season stress, high incidences of lost yield in high yielding environments (Harbach et al., 2016a, 2016b) recorded detrimental risks of high seed moisture levels, lodging, mechanical damage, and shattering (TeKrony et al., 1987). Delayed senescence by green-stem can be partially alleviated by harvest aids or a hard frost reducing moisture levels in the vegetative tissue (Harbach et al., 2016b). Farmer practice to mitigate stress factors of insecticide applications, monitoring late season rainfall patterns, and planting date management are critical in avoidance of yield losses by green-stem on late soybean reproductive stages (Harbach et al., 2016b)

CHAPTER III

RESPONSE OF SOYBEAN GROWTH AND YIELD TO REPRODUCTIVE STRUCTURE ALTERATION

Soybean can often experience several stressors in-season; however, with dryland production systems in low precipitation regions, the increased chance of abiotic and biotic stressors experienced by soybean can alter the number of retained reproductive structure that ultimately contribute to yield. Thus, this field study was conducted to evaluate the physiological changes from simulated stress during critical yield determining stages of R2, R3, and R5. Trials evaluated three different cumulative removal locations at these stages that represented different magnitudes of potential stressors. Removal of the reproductive structures simulated production challenges that western Oklahoma soybean may experience. Yield recovery from these stressors and the relationship to yield loss prevention was of interest. Sites of Bixby and Perkins, OK were chosen for soil and climatic differences. The Bixby site received irrigation and thus reflected irrigated systems while the Perkins site represented dryland soybean production. Treatments had individual proximal checks of the same dimensions for physiological comparison and data evaluation.

To overcome yield limitations and production challenges western Oklahoma soybean production may face, it is critical to recognize soybean physiological response to these stressors and the potential for recovery. In this study, it was concluded that moderate stress during early-season growth stages of R2 and R3 does not impact yield and can be recovered, while minor stress, R5:T, or extensive, R5:W, was detrimental to yield. The repercussions of theses stressors differs in physiological response and illustrates the direct impact stress has on plant function.

In summary, R2:W treatments at both sites and within years was able to recover yield components of seed number and seed weight by later developing flowers. When advancing to R3, stress experienced on the bottom of the mainstem, R3:B, had a greater yield impact than when stress experienced on the top of the mainstem, R3:T. However, soybean was still able to recover yield potential of seed weight and seed number at R3 by later-developing flowers. The largest observed yield impact was with treatments R5:T and R5:W, highlighting the importance of management of late-season stressors for dryland soybean grown in low precipitation regions. The physiological phenomena of green-stem from large pod-load reduction by extensive stress furthered the call for mitigation of late-season stress.

Late-season management for maximized genetic potential should be emphasized in dryland soybean production regions. Impact of stress on yield is minimal at R2, increases at R3, and is greatest at R5. The late season stressors producers may face that are controllable such as insects and disease, and moisture stress; and uncontrollable such as heat stress and hail damage, should be recognized and managed when possible for yield loss mitigation.

METHODOLOGY

3.1 Field Experiment

3.1.1 Locations, Soils, and Environment

Field trials were conducted at the Mingo Valley Research Station (35◦ 57’ 52.3” N, 95◦ 51’ 38.6” W) near Bixby, OK and the Cimarron Valley Research Station (35◦ 59’ 09.1” N, 97◦ 02’ 47.1” W) near Perkins, OK in 2018 and 2019. The soil at the Bixby and Perkins locations were both Mollisols. The soil series in Bixby was a mix of Wynona silty clay (fine-silty, mixed, active, thermic Cumulic Epiaquolls) and a Mason silt loam (fine-silty, mixed, active, thermic Pachic Argiudolls) (Soil Survey Staff-NRCS, 2019). Perkins was a Teller fine sandy loam (fine-loamy, mixed, active, thermic Udic Argiustolls) (Soil Survey Staff- NRCS, 2019). The Bixby site received 40.6 cm of water as irrigation throughout the season in both 2018 and 2019, while the Perkins site represented dryland production. Rainfall in the 2018 growing season rainfall accumulated 57.9 and 57.8 cm in Bixby and Perkins, respectively (Oklahoma Mesonet, 2018). In 2019, both locations received above average rainfall totals of 80.8 cm in Bixby and 86.5 cm in Perkins during the growing season (Oklahoma Mesonet, 2019). A critical rainfall period in Perkins in 2019 was noted by only 1.9 cm of rainfall in July, with 1.0 cm occurring in a single event later in the month (Oklahoma Mesonet, 2019). Temperatures and rainfall for each year are given in Figures 3.1-3.4.

3.1.2 Experimental Design

Trials evaluated three treatment timings and three different removal areas. The treatment timings included : R2 (full bloom), R3 (pod development), and R5 (seed fill). Pod removal solely occurred on the mainstem. For 2018, at R2, R3, and R5, the removal occurred on the whole plant (W), middle third (M), and bottom third (B) and occurred on the whole plant (W), middle third (M), and top third (T) in 2019. Plots were 6.1 meter long by 1.5 meter wide blocks and the plot design consisted of two rows per non-treated check (NTC) and one row per treatment. At both sites, the treatments and all interactive effects were replicated four times. Each treatment was induced on a smaller target area of a 3.1 meter by 0.8 meter section (3 m^2) within each plot as to have a more homogeneous region for the treatments. Mainstem nodes were counted at the treatment timings for cumulative treatment location accuracy and sectioned with marking tape. Treatments had individual proximal checks of the same dimensions for physiological comparison and data evaluation. In 2018 and 2019, Perkins was planted at 260,000 seeds ha^{-1} to reflect a dryland planting population. Due to increased yield potential with irrigation, planting density was increased to 308,881 seeds ha^{-1} in Bixby for both trial years. Stems per each 3 m^2 treatment area were recorded and adjusted according to average planting density of each site. In 2019 at the Perkins location, frequent early rainfall and flooding early required replanting to achieve consistent populations.

Table 3.1. Planting date and harvest date for field trials conducted in Bixby and Perkins, OK in 2018 and 2019.

Location	Planting Date	Harvest Date
Bixby 2018	22 May	29 October
Perkins 2018	24 May	07 November
Bixby 2019	21 May	28 October
Perkins 2019	15 May	12 September

3.1.3 Field Methodology

The soybean variety by Pioneer (Pioneer P48A6OX; Pioneer; Johnston, IA), an indeterminate, maturity group IV variety, that contained the RoundUp Ready Xtend trait was planted in each site year. Plots were established using a Monosem (Monosem Inc.;

Edwardsville, Kansas) planter in 2018 and 2019. Before planting, the seed was treated with Vault SP for Soybeans Inoculant (Vault SP; BASF; Ludwigshafen, Germany) to supplement the development of the bacterial component in soybean root nodules, *Bradyrhizobium Rhizobium japonicum*. A post-emergent herbicide application of 4483.4 g ha^{-1} active ingredient Glyphosate and 1541.2 g ha^{-1} active ingredient Dicamba was applied at Perkins and Bixby in 2018. In 2019, post-emergent herbicide application of 2241.7 g ha^{-1} active ingredient Glyphosate at Perkins and 3082.34 g ha^{-1} active ingredient Glyphosate at Bixby were made with rates reflecting optimal weed control. For control of in-season pests, primarily Southern Green Stinkbugs (*Nezara viridula*) and Soybean Pod Worm (*Helicoverpa zea*), at Perkins and Bixby in the 2019 growing season, 1169.2 g ha^{-1} of Chlorantraniliprole (Prevathon; FMC Corporation; Philadelphia, PA) and 584.6 g ha^{-1} of Lambda-cyhalothrin, Chlorantraniliprole (Besiege; Syngenta; Basel, Switzerland) were applied. Desiccation was done at both trial locations in 2018 and 2019 using Paraquat applied at 1169.2 g ha^{-1}. No added phosphorous and potassium fertilizer was applied based on soil test recommendations (Table 3.1).

3.1.4 Treatments, 2018

Treatments were designed to evaluate the effect of an imposed stress on soybean plants at three critical growth stages (referred to as stage), full bloom (R2), pod development (R3), and seed fill (R5). Induction of stress was simulated by removing reproductive structures on the mainstem at the cumulative treatment locations. Within each removal period, cumulative treatment locations (referred to as location) were top (T) and bottom half (B) of the mainstem. The third treatment experienced cumulative treatment location (location) in entirety, or whole (W) mainstem. Thus, the treatment structure by growth stage:location was R2:T, R2:B, R2:W, R3:T, R3:B, R3:W, R5:T, R5:B, and R5:W.

3.1.5 Treatments, 2019

A similar treatment structure was induced in 2019, with one minor difference. Similar to 2018, removal of mainstem reproductive structure occurred at R2, R3, and R5 growth stages and was conducted solely on the primary stem. However, cumulative treatment location on the plant differed, with removal occurring on top third (T), middle third (M), and whole mainstem (W). Naming of the treatments will follow a similar pattern, stage:location, with the same stage but different locations, of R2:T, R2:M, R2:W, R3:T, R3:M, R3:W, R5:T, R5:M, R5:W.

3.1.6 Plant Measurements

In-field growth and physiological measurements of leaf surface temperature, growth stages, and field notes were taken weekly throughout the project period. Weekly leaf surface temperature readings were taken with a Spectrum Temperature Meter (IR Temp Meter; Spectrum Technologies, Inc.; Aurora, IL) and compared to the NTC. Additionally, growth stages and any physiological differences were noted weekly for both the treated and non-treated subplots. As a measure of delayed senescence, Canapeo (Canapeo App; Oklahoma State University; Stillwater, OK) was used to determine the percent green canopy remaining in each treatment at both locations prior to harvest in 2019.

At maturity, a subsample of three plants were collected from each treatment plot as well as NTC at each site and used to average plant height (height), number of mainstem node (node), aborted beans per plant, and number of beans per pod. The number of pods on plant mainstem versus branch (MVB) was of interest and thus counted on treatment plants separately. The MVB measure was created by subtracting the number of pods on the mainstem from that on the branches. A positive number indicated more pods were on the mainstem, while a negative number signified pod numbers were concentrated on the branches, and was analyzed as such. At harvest, plants were hand harvested and threshed using a Kincaid thresher (18" Heavy Duty Bundle Thresher; Kincaid; Haven, KS). Plot weights were used to estimate yield on a per hectare basis. An electric seed counter (Electric Counter; Model 8502; The Old Mill Company; Savage, MD) was used to count the number of seeds (SN) from each treatment area. Protein and oil content by treatment, in comparison to the NTC, were evaluated with a diode array analyzer (DA 7200 NIR Analysis System; Perten Instruments; Hägersten, Sweden).

3.1.7 Statistical Analysis

Statistical analysis was performed using SAS v9.4 (SAS Institute Inc., Cary, NC) to determine the impact of imposed stress at given reproductive stages and mainstem locations on soybean yield, seed number, location of pods, plant height, mainstem nodes, and oil and protein content. Imposed soybean stress stages (stage) and cumulative treatment location (location), as well as their interactive effects (stage:location), were designated as fixed variables, while replication, site, year, and their interactions were treated as a random effect. Due to variability, analysis between sites and years were analyzed separately. Analysis of variance was conducted with Procedure Mixed, using a Fishers Protected LSD as a means separation test. All analysis was done with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Weather

Climate in Bixby and Perkins is not as inherently different than regions of eastern and western Oklahoma in terms of soil type, annual precipitation, and temperature that separates the historic and emerging soybean production regions (Oklahoma Mesonet, 2018; Oklahoma Mesonet, 2019). However, the amount of precipitation and average daily temperatures, coupled with the fact Bixby was under lateral irrigation and Perkins was dryland production, did assist Bixby in outperforming Perkins in both site years.

In 2018, both sites were similar in the amount of rain received in growing season months of May, July, and September. In June, Perkins experienced approximately double the precipitation, at 15 centimeters (Figure 3.2), while the same held true for Bixby in the month of August with 15 centimeters received (Figure 3.1). The average daily temperatures at the two sites were fairly similar across the growing season period (Figure 3.1, Figure 3.2).

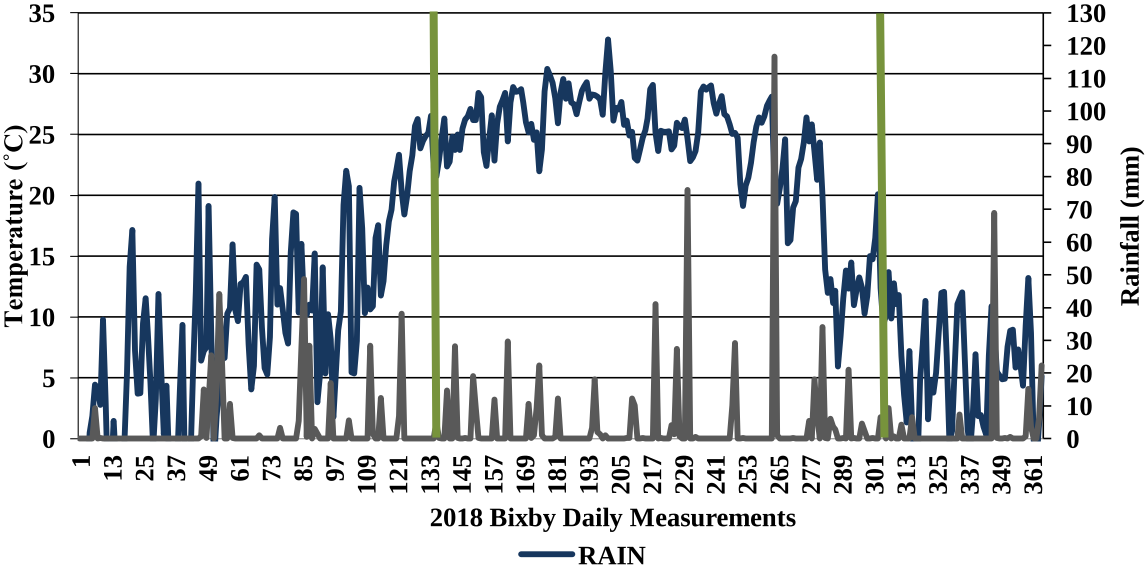

Figure 3.1. Average daily temperature (TAVG) and rainfall (RAIN) at Bixby, OK in 2018. Green line indicates the growing season.

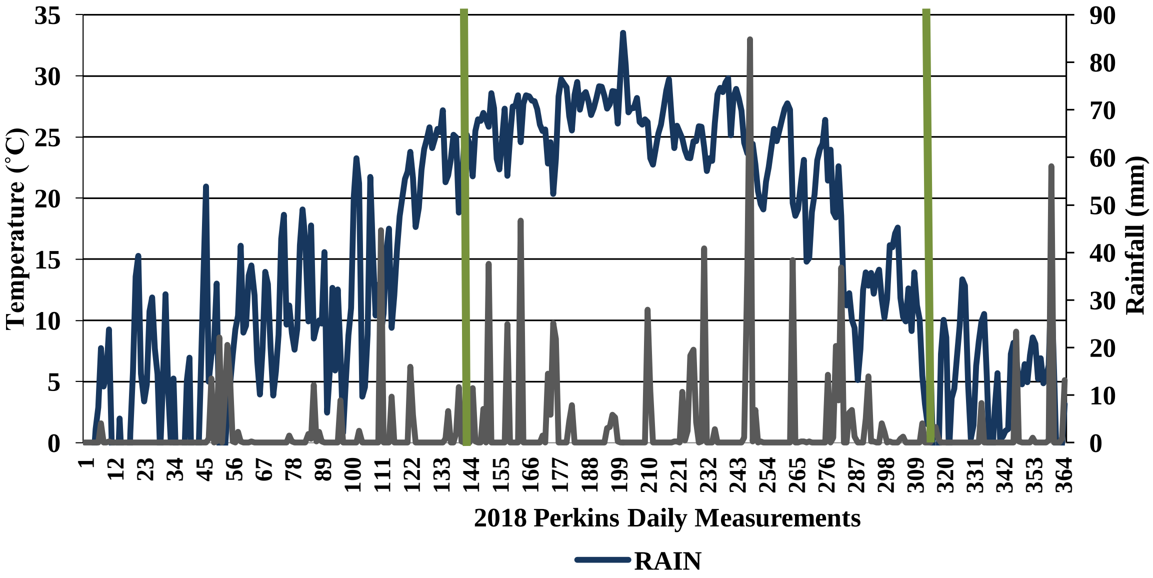

Figure 3.2. Average daily temperature (TAVG) and rainfall (RAIN) at Perkins, OK in 2018. Green line indicates the growing season.

In 2019, Bixby average daily rainfall was observed to be consistent throughout the growing season, with a large amount of 33 centimeters received in May to support early season vigor once planted (Figure 3.3). Rainfall in Perkins was more sporadic, with 40 centimeters in May, then 12 centimeters in June, to nearly the entire month of July enduring a moisture stress with only 2 centimeters received (Figure 3.4). As July corresponded to critical reproductive growth stages, we suspected this stress caused the large yield differences between the two locations in 2019 (Shaw and Laing, 1996; Eck et al., 1987; Kranz et al., 2012). Similar to the previous trial year, both sites experienced similar average daily temperatures during the growing season (Figure 3.3, Figure 3.4).

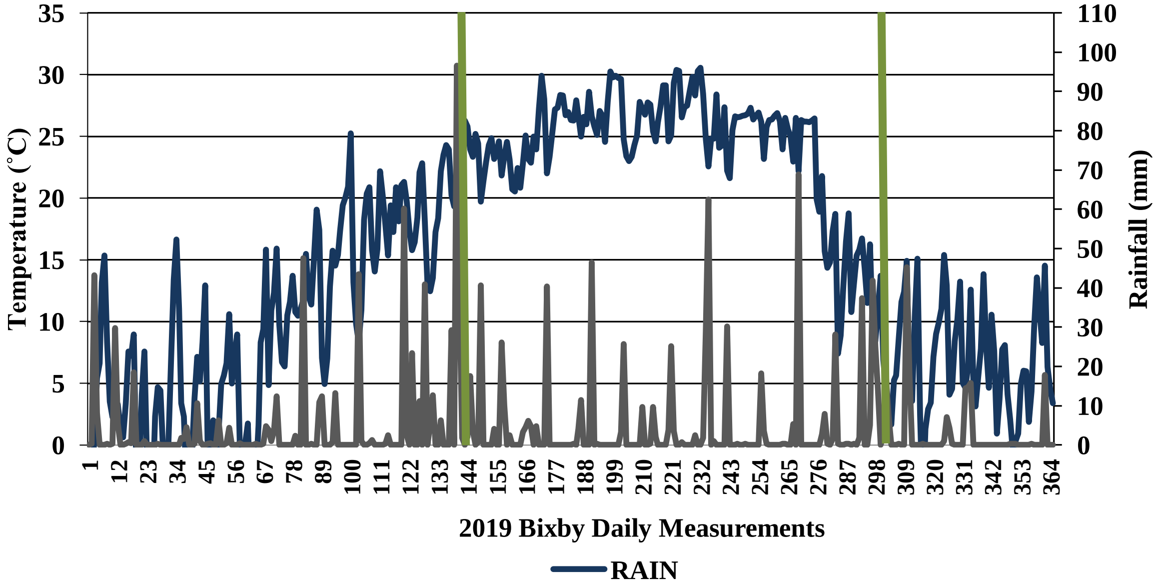

Figure 3.3. Average daily temperature (TAVG) and rainfall (RAIN) at Bixby, OK in 2019. Green line indicates the growing season.

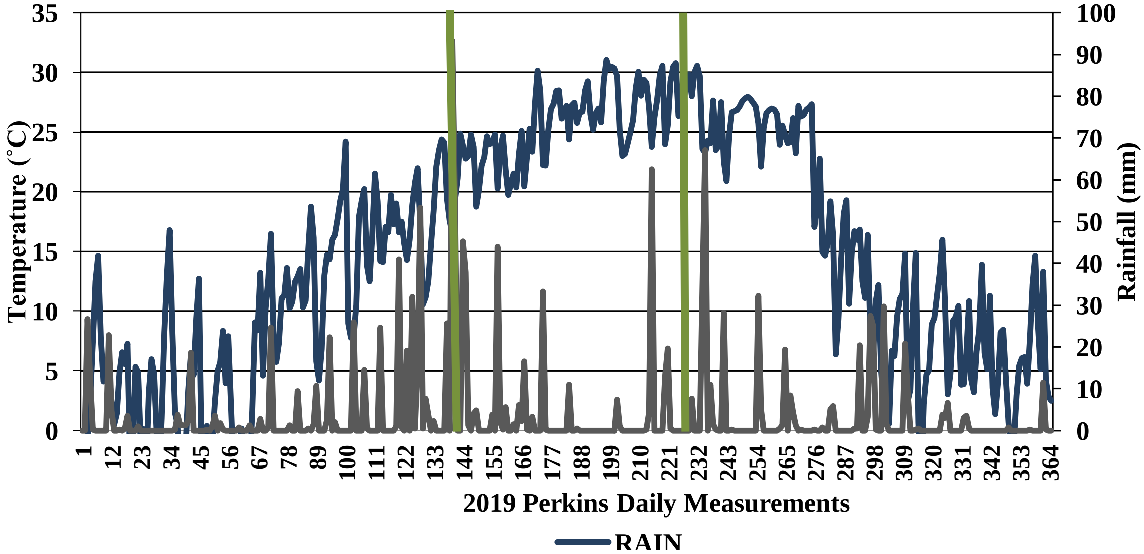

Figure 3.4. Average daily temperature (TAVG) and rainfall (RAIN) at Perkins, OK in 2019. Green line indicates the growing season.

In 2018, trial sites in Bixby and Perkins, OK yielded 4,773 and 3,016 kg ha^{-1}, respectively (Figure 3.5, Figure 3.6). While similar dry conditions in July were experienced at Bixby, irrigation was able to compensate. This influence of environment and irrigated versus dryland soybean production systems resulted in yield differences between the locations in both years. Reduced precipitation received in dryland soybean production systems is certainly an associated challenge that, when coupled with historically higher temperatures, can impact crop performance at all reproductive stages (Prasad and Staggenborg, 2008; Kokubun et al., 2001; Shaw and Laing, 1996; Meckel et al., 1984). In this study, the interaction of stage:location treatments on yield at both sites was of interest, and showed significant impact on crop performance (Table 3.2, Table 3.3).

Table 3.2. Analysis of variance *p*-values for soybean yield response as affected by reproductive structure removal at treatment growth stages and cumulative treatment locations at Bixby, OK in 2018.

Effect	**D.F**[a]	**YIELD**[b]
Stage	33	<0.01
Location	33	<0.01
Stage by Location	27	<0.01

[a] Degrees of freedom.

*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05.

Table 3.3. Analysis of variance *p*-values for soybean yield response as affected by reproductive structure removal at treatment growth stages and cumulative treatment locations at Perkins, OK in 2018.

Effect	**D.F**[a]	**YIELD**[b]
Stage	33	<0.01
Location	33	<0.01
Stage by Location	27	<0.01

[a] Degrees of freedom.

*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05.

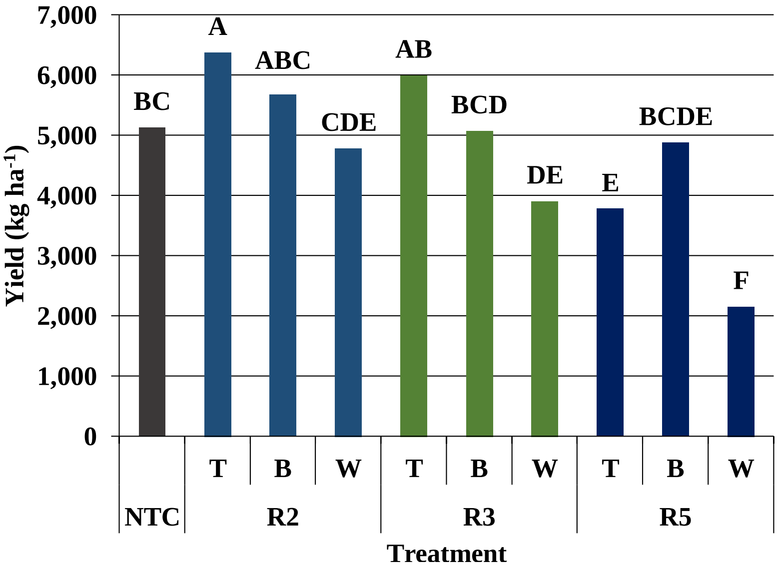

Figure 3.5. Soybean yield response as affected by cumulative treatment location at given soybean reproductive stages at Bixby, OK in 2018.

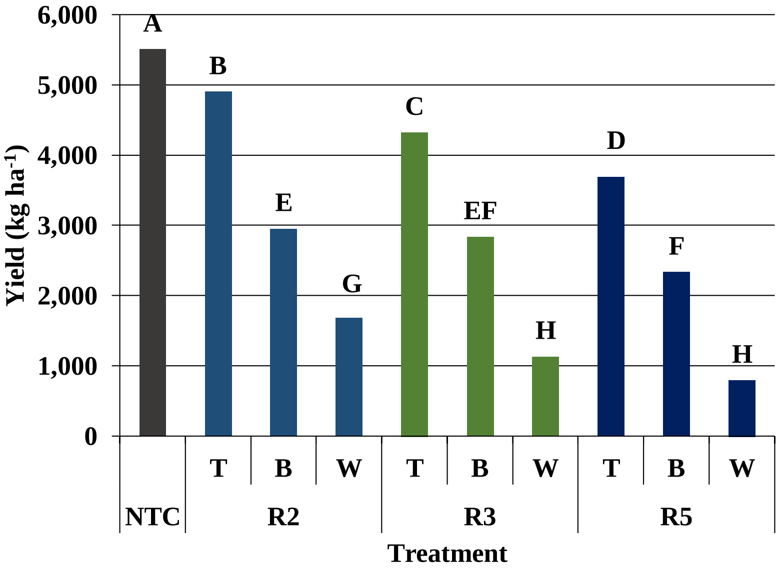

Figure 3.6. Soybean yield response as affected by cumulative treatment location at given soybean reproductive stages at Perkins, OK in 2018.

At the Bixby site, when the highest amount of removal occurred at R2, R2:W, the impact on yield was not significantly different from the NTC at 5,127 kg ha^{-1} and 4,780 kg ha^{-1}, respectively (Figure 3.5). An even smaller separation of yield from the NTC was observed with R2:B at 5,676 kg ha^{-1} (Figure 3.5). Meanwhile, the R2:T treatment yielded 6,372.61 kg ha^{-1}, a significant increase of 1,245.52 kg ha^{-1} above the NTC (Figure 3.5).

Soybean flowering period extends to 40 days, and as soybean yield is a function of the number of flowers produced over this time (Hansen and Shibbles, 1978). It is remarkable, but not surprising, that later flowering nodes or nodes on branches allowed reproductive recovery of R2:T, R2:B, and R2:W treatments (Figure 3.5), (Shaw and Laing, 1966). This demonstrated the ability of soybean to recover from stress during early flowering, yield can be compensated at R2 from realized soybean production stressors. However, the R2:W treatment at the Perkins site told a different story as yield of 1,681 kg ha^{-1} significantly differed from the NTC at 5,506 kg ha^{-1} (Figure 3.6). The soybean growth stage R2 is a unique time of supporting both biomass and flower production in indeterminate soybean, and full flower production in determinate soybean, marking the need for peak moisture uptake (Carlson and Lersten, 2004; Kranz et al., 2012; Woodworth, 1932; Shaw and Laing, 1996). Insufficient water at R2 reduces available photosynthetic assimilates to support early embryonic development (Raper and Kramer, 1987; Kokubun et al., 2001). At this time, precipitation at the Perkins site was well below this requirement to support yield, or provide the potential to recover, from the imposed stress at R2 with only 10 millimeters received (Figure 3.2, Figure 3.6). This difference in available water supply demonstrated that water is certainly a yield limiting factor at R2 and the likelihood of moisture stress is greatest at this time as both biomass production and reproductive structures require support in indeterminate soybean.

The soybean growth stage R3, the primary focus of the plant is to supply assimilates to pods from early flowers (Heithholt et al., 1986). At Bixby, treatments R3:T and R3:B, at 5,995 kg ha^{-1} and 5,0712 kg ha^{-1}, showed no significant difference from the R3:W treatment or the NTC (Figure 3.5). The Perkins site exhibited a significant yield separation from the NTC, of 5,506 kg ha^{-1}, to R3:T at 4,321 kg ha^{-1} (Figure 3.6). Then, R3:T significantly differed from the 2,836 kg ha^{-1} and 1,131 kg ha^{-1} yields of treatments R3:B and R3:W, respectively (Figure 3.6). Moreover, the significant separation in yield between the R3:T, R3:B, and R3:W treatments indicates the impact of the area where soybean stress is experienced, and the extent of that stress (Table 3.3). The location of flowers on the soybean plant influences flower development order, from bottom to top along the mainstem; as such, order of developing pods begins on the lower nodes and are large sinks at this reproductive stage (Elgi and Bruening, 2006). Meanwhile, upper mainstem nodes are still experiencing the extended flowering period (Hansen and Shibbles, 1978). The dynamic of reproductive structure development being disrupted when stress is experienced at a different locations on the plant ultimately plays a large role in yield (Table 3.2, 3.3). Stress on the bottom of the mainstem had a higher impact than stress on the top of the mainstem, which is comparable to moisture stress that initially impacts the bottom developing pods and insect pressures that primarily target the top of the mainstem (Figure 3.6). This agrees with findings of Spollen et al. (1986) and Wiebold et al. (1981) that the lower one third of the soybean canopy is most prone to reproductive losses. Thus, providing an example to prioritizing management practices to mitigate yield loss when producing dryland soybean in low precipitation regions. At the same time, a peak in temperatures around and above 30˚C occurred during this stage. This temperature extreme for short periods is common in most parts of Oklahoma and historically coincides with R3 and R5 growth stages (Arndt, 2003). Although no effect on yield was observed, high temperatures can cause seeds to shrivel and reduce crop quality (Elgi et al., 2006; Spears et al., 1997). This is another production challenge, often coupled with moisture stress, that could compound detrimental effects in dryland soybean produced in low precipitation regions.

At R5, no additional flowers are being produced to mitigate yield losses (Eck et al., 1987). This lack of available recovery in dryland soybean was exhibited by the R5:B treatment at Perkins as yield dropped to 2,331 kg ha^{-1} (Figure 3.6). Meanwhile, the Bixby site R5:B treatment was able to retain yield at 4,876 kg ha^{-1}, which was not significantly different from the NTC (Figure 3.6). Again, this proves the capabilities of plant response to stress in irrigated versus dryland soybean production systems. A yield impact was also experienced by R5:T treatments as yield dropped to 3,775 kg ha^{-1} at Bixby (Figure 3.5) and 3,684 kg ha^{-1} at the Perkins site (Figure 3.6). These yield responses place an importance on late season soybean management to protect developing seeds against stressors that can impact yield with no potential for yield recovery.

Yield 2019

In 2019, trial sites were again separated by yield with 5,002 kg ha^{-1} at Bixby and 3,837 kg ha^{-1} at Perkins which can be attributed to the fact Bixby soybean was under lateral irrigation while Perkins reflected dryland soybean production. Bixby average daily rainfall was steady throughout the growing season (Figure 3.3), while Perkins was more sporadic and experienced a period of drought in July (Figure 3.4). Again, the interaction of stage:location treatments on yield at both sites was of interest, and showed significant impact (Table 3.4, Table 3.5).

Table 3.4. Analysis of variance *p*-values for soybean yield response as affected by reproductive structure removal at treatment growth stages and cumulative treatment locations at Bixby, OK in 2019.

Effect	D.F[a]	HEIGHT[b]	NODE[c]	MVB[d]	SN[e]	CANAPEO[f]	YIELD[g]
Stage	33	0.94	0.21	<0.01	<0.01	<0.01	<0.01
Location	33	0.01	0.61	0.29	0.06	0.10	<0.01
Stage by Location	27	0.20	0.17	<0.01	<0.01	<0.01	<0.01

[a] Degrees of freedom.
[b] Average plant height.
[c] Number of mainstem nodes.
[d] Mainstem versus branch.
[e] Seed number.
*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05.

Table 3.5. Analysis of variance p-values for soybean yield response as affected by reproductive structure removal at treatment growth stages and cumulative treatment locations at Perkins, OK in 2019.

Effect	D.F[a]	HEIGHT[b]	NODE[c]	MVB[d]	SN[e]	CANAPEO[f]	YIELD[g]
Stage	33	<0.01	0.89	<0.01	<0.01	<0.01	0.18
Location	33	0.46	0.64	<0.01	0.04	0.31	<0.01
Stage by Location	27	<0.01	0.82	<0.01	<0.01	<0.01	<0.01

[a] Degrees of freedom..
[b] Average plant height.
[c] Number of mainstem nodes.
[d] Mainstem versus branch.
[e] Seed number.
*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05

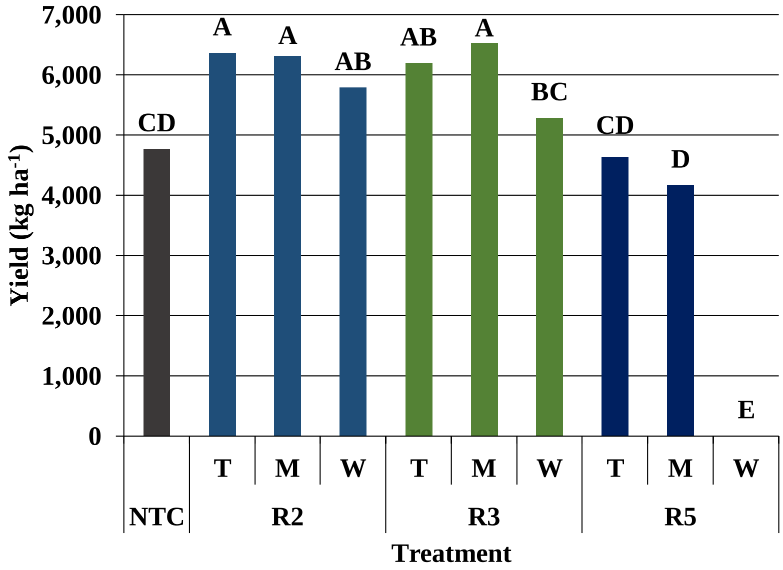

Figure 3.7. Soybean yield response as affected by cumulative treatment location at given soybean reproductive stages at Bixby, OK in 2019.

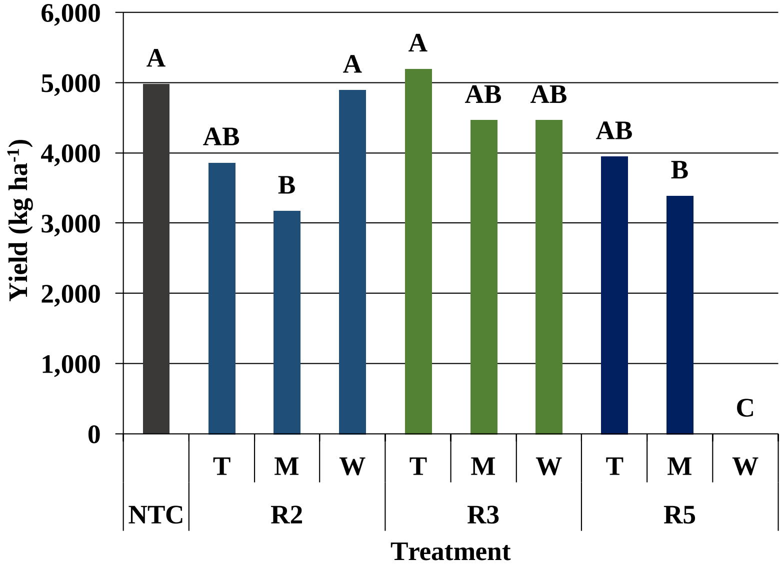

Figure 3.8. Soybean yield response as affected by cumulative treatment location at given soybean reproductive stages at Perkins, OK in 2019.

All R2 treatments at Bixby yielded significantly higher than the NTC of 4,768 kg ha^{-1}; with R2:T, R2:M, and R2:W producing 6,362 kg ha^{-1}, 6,307 kg ha^{-1}, and 5,786 kg ha^{-1}, respectively (Figure 3.7). At the Perkins site, R2:T with 3,862 kg ha^{-1} and R2:M at 3,174 kg ha^{-1}, did not significantly differ from the NTC (Figure 3.8). Similarly, with the

2018 site year, the asynchronous manner of soybean flowering allows early season yield recovery. Rainfall during R2 was adequate at Bixby (Figure 3.3) and Perkins (Figure 3.4), allowing both sites to perform at genetic potential.

The treatment of focus at R3 for 2019 was R3:M. Pod location on the middle portion of the mainstem has an advantage of receiving assimilate supply (Katsunori et al, 1995). These pods have also been shown as high contributors to yield by a high pod-setting ratio and increased number of seeds per pod (Katsunori et al, 1995). Treatment R3:M at Bixby yielded significantly higher than the NTC at 6,520 kg ha^{-1} (Figure 3.7). At the same time, Perkins R3:M soybean was not significantly separated from the NTC with 4,465.11 kg ha^{-1} (Figure 3.8). This yield recovery can be compared to findings of Spollen et al. (1986) that abscission probability of upper nodes decreased when middle node reproductive structures were removed. The mechanism intra-raceme competitive ability remains unknown, but could be related to differences in the time of floral initiation and remobilization of photosynthate from this reduction of sink strength (Herbert and Litchfield, 1982; Frederick et al., 2001). It has been shown that the upper one third of the soybean plant is one of the most productive regions (Ahmed et al., 2010), but abscission probability increases with increasing position number (Spollen et al., 1986). The R5:T treatment had no significant yield impact at Bixby and Perkins, with 4,638 kg ha^{-1} and 3,949 kg ha^{-1} produced, in comparison to their respective NTC of 4,768 kg ha^{-1} and 4,982 kg ha^{-1} (Figure 3.7, Figure 3.8). Although the top mainstem region is prone to abscission when stress is experienced, the loss of these developing seeds does not affect final yield (Figure 3.7, Figure 3.8). This may be due to the fact that seed weight capabilities were maximized by mobilization of photosynthates from the upper leaves to support the remaining pods (Herbert and Litchfield, 1982). In terms of the total number of seeds available for filling on a soybean plant, these upper fruiting positions have a lesser impact on overall yield contributions in terms of what brings dividends at the elevator.

However, the R5:W treatment told a different story of the importance of late season management. At both sites, the late-season reduction of pod load resulted in green-stem, a physiological response from this alteration of the source-sink ratio. The removal or loss of pods, thus a reduced sink, slows the movement of C and N from the vegetative tissue to pods as the stress response favors the source (Miceli et al., 1995; Wittenback W.A., 1983). The occurrence of green-stem prevents vegetative tissue from exuding moisture by accumulation of photosynthetic assimilates of starch and N (Figure 3.9, Figure 3.10, Figure 3.17), (Miceli et al., 1995). The impact of stress at R5 on the percent of retained vegetative tissue at the time of harvest was significant at both sites (Table 3.4, Table 3.5). Treatment R5:W at Bixby and Perkins had 18.86% and 22.09% vegetative tissue at the time of harvest, compared to the standard physiological maturity of the NTC at 1.43% and 1.52% respectively (Figure 3.9, Figure 3.10). This vegetative tissue did not reach physiological maturity at either site despite a desiccant application and frost (Figure 3.3, Figure 3.4). Thus, plants affected by green-stem are unharvestable from this reaction to intolerable stressors (Figure 3.7, Figure 3.8). Late-season stressors that western Oklahoma producers experience such as insects, disease, moisture stress, environment, and hail that influences the source-sink ration has this capability (Conley et al., 2009; Harbach et al., 2016b; Hill et al., 2006; Hobbs et al., 2006; Boethel et al., 2000; Xavier et al., 2017). With insect and disease losses each averaging approximately 9% in the southern United States, viral infection, and low soil moisture content, the threat of green-stem makes late season management of these factors of upmost importance (Boethel et al., 2000; Harbach et al., 2016b; Hobbs et al., 2006; Koenning, 2007). The potential for the onset of green-stem from these stressors in western Oklahoma soybean and dryland soybean produced in low precipitation regions can experience certainly signifies the importance of late season soybean management for optimal production (Harbach et al., 2016b).

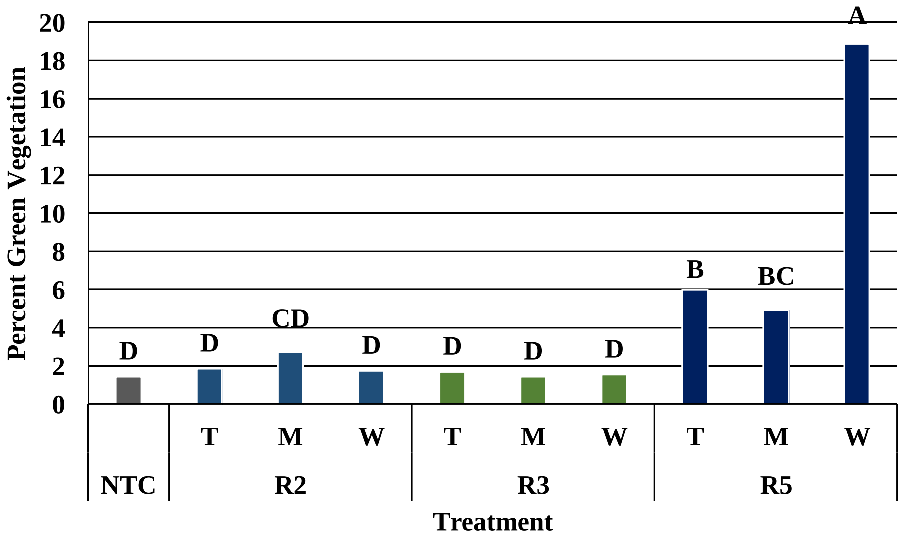

Figure 3.9. Canapeo readings prior to harvest indicate percent of remaining vegetative tissue at Bixby, OK in 2019.

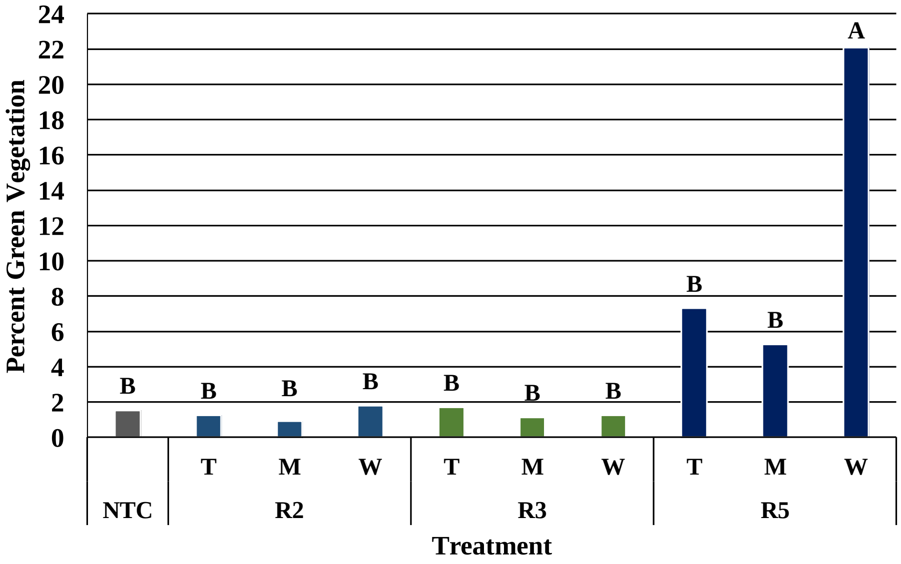

Figure 3.10. Canapeo readings prior to harvest indicate percent of remaining vegetative tissue at Perkins, OK in 2019.

Seed Number

In 2019, additional data was collected within the $3m^2$ treatment area to increase understanding of the physiological response to the imposed stress and the impact on yield quantity and quality. As seed number is a contributing factor to yield, the interaction of stage:location was of interest and overall found to be significant at both locations (Table 3.4, Table 3.5). The R2:W treatment did not significantly differ in comparison to NTC at Bixby (Figure 3.11) and Perkins (Figure 3.12), separated by 468 and 1,164 seeds. The larger separation in seed number at Perkins could be attributed to decreased precipitation at the R3 growth stage (Figure 3.4) because moisture stress during seed fill can decrease seed numbers (Smicklas et al., 1989). As yield was also not significantly different at both sites (Figure 3.7, Figure 3.8), it can be assumed the R2:W treatment can sufficiently recover yield components of seed number and seed weight from even extensive stress the plant may experience early in the growing season. This yield recovery potential by seed number carried to R3 as R3:M treatments at Bixby (Figure 3.11) and Perkins (Figure 3.12) with 7,518 seeds and 7,765 seeds produced, respectively, and not significantly different from their respective NTC. Seed number dropped substantially with the R5:T treatment, separated from the NTC by 2,169 seeds at Bixby (Figure 3.11) and 1,866 seeds at Perkins (Figure 3.12). Extensive stress at R5 significantly impacted yield in terms of seed number, with no chance for reproductive recovery (Eck et al., 1987). As this stage requires high water uptake to support seed fill, assimilates that sequester seed growth can be easily limited by moisture and heat stress. Moreover, maintain the stability of these upper fruiting positions at R5 becomes increasingly important for yield loss mitigation (Herbert and Litchfield, 1982).

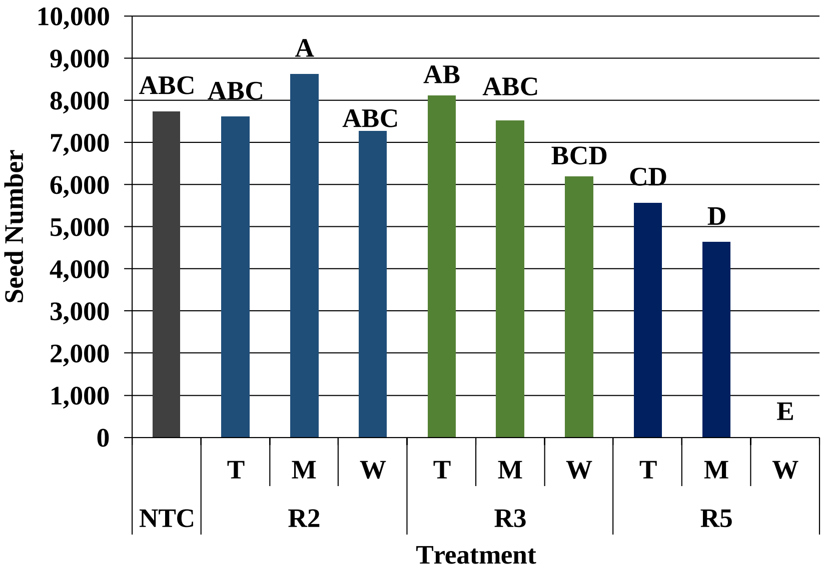

Figure 3.11. Influence of stage and location of simulated stress on seed number per treatment area in Bixby, OK in 2019.

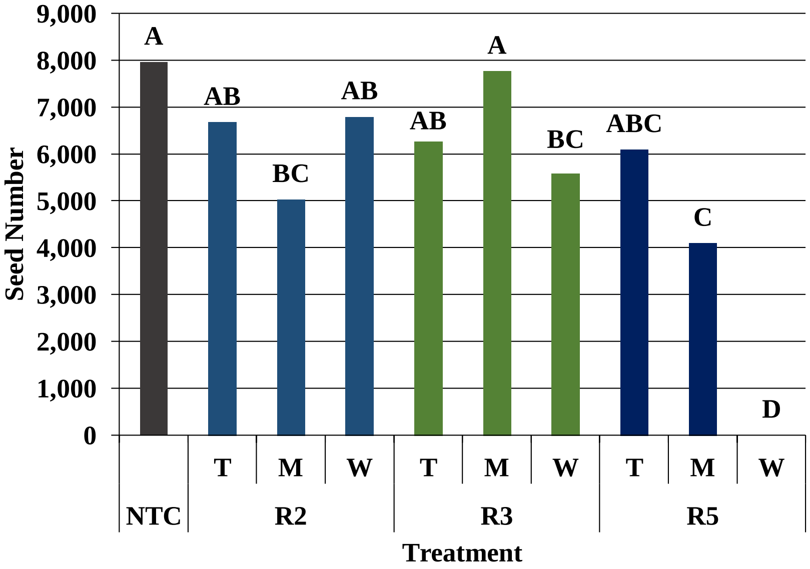

Figure 3.12. Influence of stage and location of simulated stress on seed number per treatment area in Perkins, OK in 2019.

Parameters of soybean seed quality and quantity are influenced by environmental stressors, illustrating the importance of soil moisture control and maintaining soybean productivity (De Souza et al., 1997; Vieira et al., 1992; Smicklas et al., 1989; Siebers et al., 2015). Therefore, these potential mechanisms of yield loss must be considered in management practices to prevent such yield limiting factors. If irrigation is limited, water should be applied to meet soybean water uptake requirements when rainfall is insufficient (Kranz et al., 2012). In dryland soybean production, practices such as no till or cover crops that decrease evaporation and retain soil moisture could assist with growing season plant available moisture (Arndt, 2003). With the soybean growth stage R5 in mind as the time maximum impact on yield reduction is experienced, farmer practice should prioritize this time in water management schedules and practices (Foroud et al., 1992; Eck et al., 1987). It should also be noted that the physiological response of green-stem to extensive stress at R5 resulted in unharvestable plants at both sites, and is reflected by a zero seed number (Figure 3.11, Figure 3.12).

Mainstem versus Branches

The comparison of pods on the main stem versus branches (MVB) was of interest to evaluate physiological response of treatments. Pods on branches was subtracted from number of pods on the mainstem to observe any physiological stress response in the 3m^2 treatment areas. A positive number indicated more pods were on the mainstem while a negative number signified pod numbers were concentrated on the branches. A near-zero number signified equality of pod numbers on the mainstem and branches. It is important to note that this parameter does not give any indication of pod number produced and a near zero estimate for MVB does not mean lower number of pods being developed but near equal being produced on mainstem and branches.

According to Frederick et al. (2001), optimal branch vegetative growth is indicative of high seed yields. The number of pods located on the mainstem for R2:W was not significantly different from the NTC at Bixby (Figure 3.13) and Perkins (Figure

3.14). As this treatment represents a full removal of reproductive structures early in the season, this data illustrates both yield (Figure 3.7, Figure 3.8) and seed number (Figure 3.11, Figure 3.12) were able to recover yield, specifically on the mainstem, from this early-season stress.

The treatment R3:M also showed no significant difference from the NTC of pods retained on the mainstem at Bixby (Figure 3.13) and Perkins (Figure 3.14). This provides further confirmation that mid-season stress is not detrimental to yield as mainstem reproductive structures can still be recovered and retained.

Because of limited stress, as long as the variety possesses branching nature, soybean under irrigation has been shown to produce more yield on the branches in comparison to dryland soybean (Frederick et al., 2001). The R5:T treatment at Bixby shifted pod numbers primarily to the branches but still maintained a yield of 4,637 kg ha^{-1} that was not significantly different from the NTC of 4,768 kg ha^{-1} (Figure 3.7). This suggests irrigation allowed pods on branches to capitalize on the increased available photosynthates to mitigate yield loss experienced on the mainstem (Figure 3.3). Mainstem soybean assimilatory capacity, or source strength, affects pod retention per reproductive mainstem node (Board and Tan, 1995). Moreover, it has been demonstrated that mainstem seed yield is higher than branch yield for soybean grown with no irrigation (Frederick et al., 2001). This explains how R5:T treatment at Perkins (Figure 3.12) was not significantly different from the NTC on number of retained mainstem pods. Plant height and number of mainstem nodes was also of interest, but found to not be significant at either site (Table 3.4, Table 3.5). An observed physiological response was increased branching on these treatment plants (Figure 3.17). This finding agrees with that of

Frederick et al. (2001) when stress, in the form of drought, largely increased the number and length of formed branches.

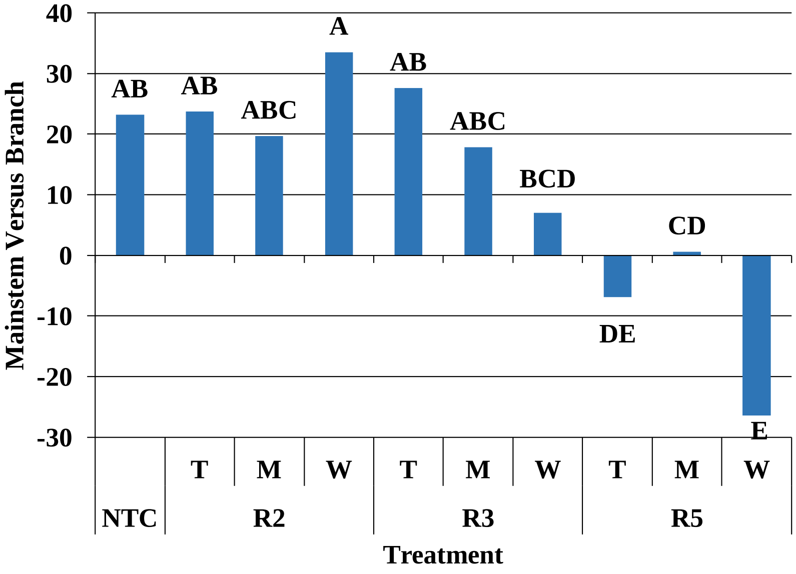

Figure 3.13. Influence of final pod location by stage and location of simulated stress. Positive values indicate more pods on the mainstem at physiological maturity while negative values, calculated as the difference, signifies more pods on the branch of treatment plants in Bixby, OK in 2019.

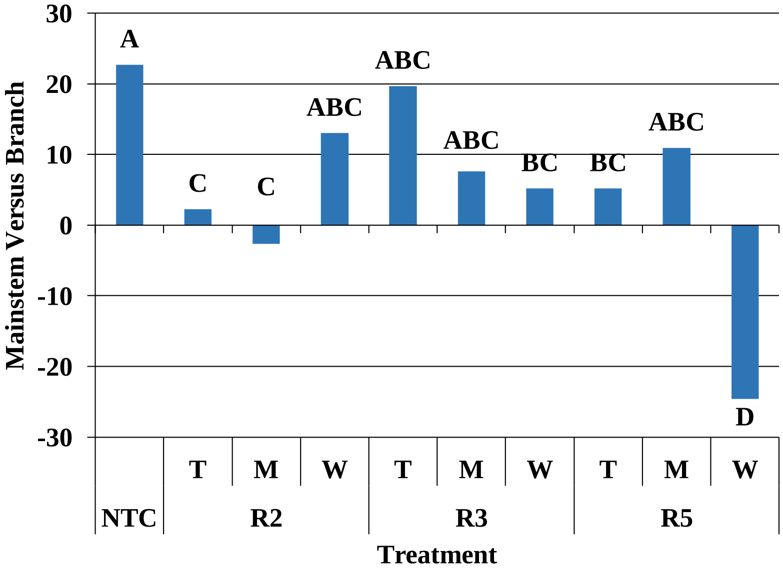

Figure 3.14. Influence of final pod location by stage and location of simulated stress. Positive values indicate more pods on the mainstem at physiological maturity while negative values, calculated as the difference, signifies more pods on the branch of treatment plants in Perkins, OK in 2019.

Protein and Oil Content

There was not a significant difference found with protein and oil content in the treatment interaction (Table 3.7, Table 3.8). The only observation was the protein content of the seed increased with the R2:M treatment, and with this increase in protein, a slight decrease in oil was the result (Figure 3.14), (Hicks and Pendleton, 1969). Overall, from this, it can be gained that protein and oil content are relatively unaffected by stress and as such will not impact the grower's bottom line in terms of seed quality.

Table 3.6. Seed quality parameters for soybean seed quality response as affected by mainstem reproductive structure removal location at given soybean reproductive stages in Bixby, OK in 2019.

Effect	**D.F**[a]	**PROTEIN**[b]	**OIL**[c]
Stage	33	0.43	0.03
Location	33	0.53	0.22
Stage by Location	27	0.58	0.09

*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05.

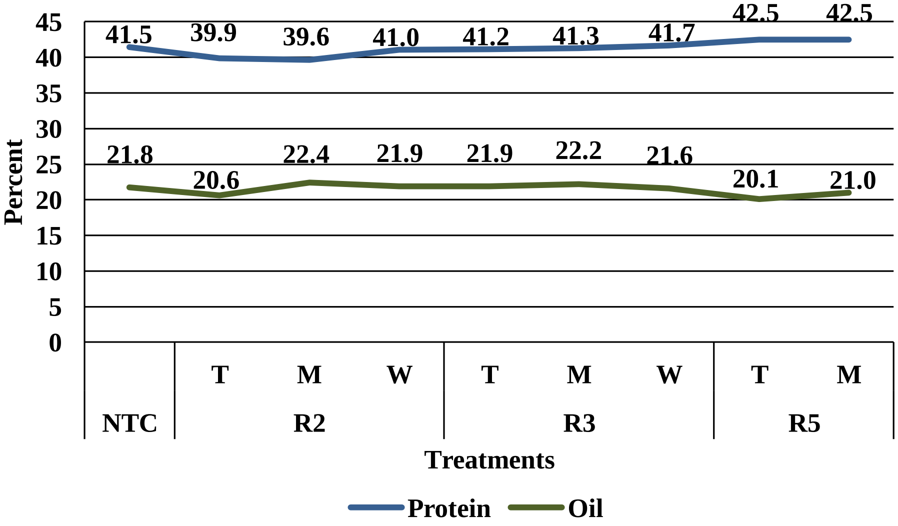

Figure 3.15. Protein and Oil content of treatment plants in comparison to the NTC at Bixby, OK in 2019.

Table 3.7. Seed quality parameters for soybean seed quality response as affected by mainstem reproductive structure removal location at given soybean reproductive stages in Perkins, OK in 2019.

Effect	D.F[a]	PROTEIN[b]	OIL[c]
Stage	33	0.08	0.24
Location	33	0.31	0.79
Stage by Location	27	0.10	0.59

*Conducted with Procedure Mixed using a Fishers Protected LSD as a means separation test at α = 0.05.

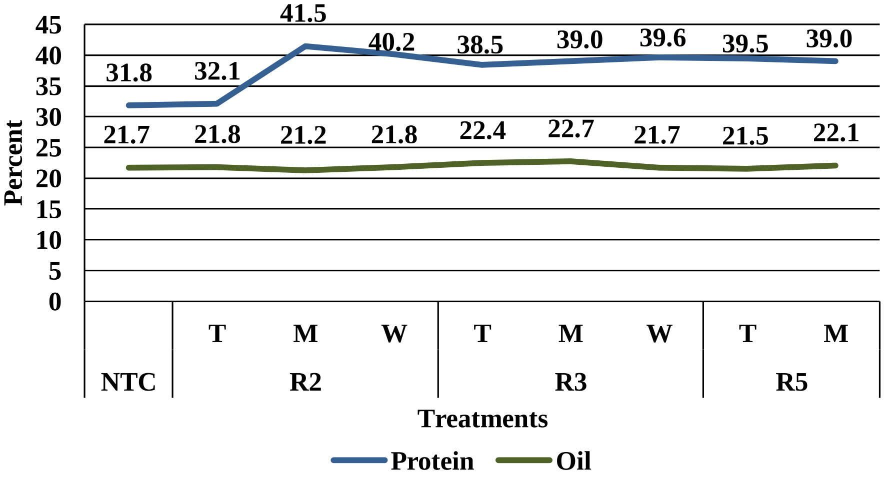

Figure 3.16. Protein and Oil content of treatment plants in comparison to the NTC at Perkins, OK in 2019.

CONCLUSIONS

To overcome yield limitations and production challenges of dryland soybean production systems in low precipitation regions, it is critical to recognize soybean physiological response to these stressors and the potential for recovery. In this study, it was concluded that moderate stress during early-season growth stages of R2 and R3 did not consistently negatively impact yield and can be recovered, while stress of a marginal, R5:T, or extensive, R5:W, magnitude is detrimental to yield. The repercussions of theses stressors differs in physiological response and illustrates the direct impact stress has on plant function.

In summary, R2:W treatments at both sites and within years was able to recover by later developing flowers. It should be noted that the high water uptake requirements at this reproductive stage could impact crop performance in dryland soybean production in low precipitation regions. When advancing to R3, location becomes important as stress experienced on the bottom of the mainstem, R3:B, holds a higher impact than that experienced on the top of the mainstem, R3:T. Moisture stress is first experienced on the bottom of the plant and this lower canopy region is also most prone to reproductive losses, making efficient plant water use key for survival of developing pods to contribute to final yield. Meanwhile, the middle portion of the mainstem is a large sink, but as shown by R3:M, does not influence final yield as this reduction in sink strength remobilizes phosynthates to be capitalized by retained pods. The largest observed yield impact was R5:T, highlighting the need for management of late-season stressors with dryland soybean grown in low precipitation regions. Thus, the impact of stress on yield is minimal at R2, increases at R3, and is greatest at R5. The physiological phenomena of green-stem from large pod-load reduction by extensive stress at R5 furthered the call for mitigation of late-season stress.

The yield component of seed number followed a similar trend. The R2:W treatments were able to sufficiently recover this yield component, again showing early-season stress can be recovered, with this potential extending to R3:M treatments. However, when stress is experienced at R5:T or R5:W, seed numbers are significantly impacted. More specifically, stressors impact pod retention at R5 can decrease seed number under the high potential for physiological impact by decreased evapotranspiration and nutrient remobilization. Management of dryland soybean in low precipitation regions should place priority on the late growing season when yield losses from stressors have potential to be severe on seed number.

Physiological responses of pod location on the mainstem did not differ with R2:W and R3:M treatments. This shows the ability of the asynchronous soybean flowering period to recover and retain lost mainstem reproductive structures. When pods were removed for the R5:T treatment, available water became the determining factor of retaining remaining pods to contribute to yield. Later irrigation at Bixby provided growth and development opportunities for branch pods that closed the yield gap when mainstem stress was experienced. Perkins, reflecting dryland soybean production, was also not significantly different from the NTC, but lost yield potential in branch development by limited available water.

Seed quality was not significantly impacted by treatments and thus concludes that impact of these stressors is experienced highest with yield, then with seed number, with pod location and seed quality to a lesser extent. As soybean production is driven by yield, emphasis should be placed on late-season management to maximize genetic potential in dryland soybean production in low precipitation regions.

The capabilities of plant response and yield recovery was evident in irrigated versus dryland soybean production systems as demonstrated by the Perkins and Bixby sites. Soybean was affected in terms of seed weight, seed number, and physiological response, by environmental and imposed stressors that certainly could be experienced in dryland production systems. The production gap dryland soybean in low precipitation regions experiences can be partially attributed to the limited ability of soybean to mitigate yield loss from these events. Here, plant available water is the yield limiting factor. As dryland soybean production systems are restricted by received precipitation under these circumstances, management of other stressors soybean may experience in the growing season is critical for yield retention. Management to mitigate stress factors should consider planting date, insecticide and fungicide applications, herbicide programs, and monitoring season rainfall patterns for avoidance of yield losses. The compounding effect of a combination of these abiotic and biotic stressors can greatly impact yield by losses of both seed number and seed weight, especially later in the growing season when water uptake requirements are high and reproductive structure development is most sensitive to altered plant dynamics. Here, farmer practice in dryland soybean produced in low precipitation regions must make late season management of upmost importance for yield loss mitigation of these factors. This is true especially when the source-sink relationship alters the sink under stress at R5 and the physiological response of green-stem with retained vegetative tissues leaving the plants unharvestable and detrimental to contributions at the grain elevator. The onset of green-stem is exacerbated under moisture stress conditions that restrict assimilate flow, thus placing further emphasis on the need for increased late season management in dryland soybean production systems in low precipitation regions.

Figure 3.17. Branching was an observed physiological response from R2:Whole removal treatments.

Figure 3.18. Drone depiction of the green-stem syndrome in R5:Top and R5:Whole removal treatments.

CHAPTER IV

EVALUATION OF SOYBEAN POD RECOVERY FROM IMPOSED MOISTURE STRESS

In this study, the specific impact of simulated moisture stress by stage:duration treatments on yield was of interest and the objective was to determine the physiological response of imposed drought stress during high water requirement stages. Treatments included three growth stages, R2, R3, and R5 and two moisture stress durations of 7 and 14 days. Each treatment was replicated four times.

Moisture stress at R2:7 did not impact yield or seed number, nor restrict vegetative growth. The turnover of flower production during this period allowed for full yield recovery when short-term moisture stress was experienced. The flowering period of soybean extends up to R3, with moisture stress impairing reproductive structure development and a yield restriction begins to be created. A slight but non-significant decrease in seed weight and seed number was observed at R3:7, demonstrating flowers produced after the moisture stress period could recover some lost yield from previously aborted flowers. When stress was experienced for an additional seven days, R3:14, a significant yield lost was noted. Thus, the impact of moisture stress is dependent on duration. This was also observed to be dependent on time, or growth stage, as R5:7 experienced the most significant impact on seed number and seed weight.

Understanding the potential underlying mechanisms of yield loss by water stress is of importance when accounting for the final commodity. Especially considering the yield impact of moisture stress duration is significant, soybean water management in western Oklahoma of upmost importance. When water scarcity occurs in a specific developmental phase, the yield component of the soybean ontogeny at that stage is reduced to the extent of the timing and severity of the stress. This impact was demonstrated in the yield separation and physiological response of plants experiencing fourteen days of moisture stress in comparison to the tolerable duration of seven days.

METHODOLOGY

4.1 Growth Chamber Experiment

4.1.1 Settings and Soils

The study was conducted in a controlled environment growth chamber (Percival Modular Control Systems, Boone, IA) to evaluate the physiological effect of moisture limiting stress on soybean. To mimic the light spectrum of the sun and serve as the main energy source, metal halide and high-pressure sodium bulbs were placed alternately, with additional 400 nm (blue) LED lights positioned in between to reduce stem elongation (Shimizu et al., 2006). A preliminary study was conducted to determine the optimal growing conditions to mitigate diverse growing effects in the controlled environment growth chamber. In the initial study, the maximum temperature was increased from 27.8°C in -16.7°C increments as soybean growth progressed. The higher temperature threshold resulted in soil moisture temperature variation and irregular growth habits of the soybean. The climate was adjusted to only experience variation occurring in correlation with temperatures and lengths during the night at a minimum of 20.0 °C, with an otherwise static environment of 27.8 °C. Day length was set to mimic the light conditions in Northern Oklahoma, with light ranges initiating the growing season at 14 hours of sunlight and concluding maturity at 11 hours. Humidity and carbon dioxide levels fluctuated as humidity ranged 20-40% and CO_2 from 500-700 ppm. These CO_2 concentrations are expected under 2040-2080 conditions which could influence drought stress tolerance as high CO_2 levels are detected in mature leaves and signaled to immature leaves which then alters stomatal development (Ainsworth and Rogers, 2007).

The soil medium was extracted from a field in Stillwater, OK (36◦ 8’ 2.67’ N, 97◦ 6’ 22.158” W). The soil type was a clay loam with a bulk density of 1.21 g cm^{-3}.

4.1.2 Plant Propagation

A maturity group IV soybean variety (Pioneer P48A6OX) was selected to represent the major maturity group of soybean grown in Oklahoma. Decagon soil moisture sensors (5TE) were inserted horizontally two inches from the bottom and edge of a 37 L pot in five pots as they were packed with the soil medium and watered in layers to reduce the loss of soil structure. Two seeds were planted per pot in the sixteen pre-watered pots. At VC (unrolled unifoliate leaves) they were thinned to one plant and remained well watered from VC-R5 (unrolled unifoliate leaves to pod-fill).

4.1.3 Experimental Design

Treatments included three water-limiting periods and two lengths of moisture stress. The three periods included R2 (Full-flower; when flowers were present in the top four nodes of the plant), R3 (Pod development; when a fully developed pod was found on the top four nodes), and R5 (Seed Fill; when seeds touched in inner member of the pods in the top four nodes). During each of these growth stages (stage), moisture was limited for either 7 or 14 days (duration). Thus, the treatment structure was by stage:duration was R2:7 days, R2:14 days, R3:7 days, R3:14 days, R5:7 days, R5:14 days. Additionally, a well-watered control was used, which maintained adequate soil moisture through the evaluation period. Each treatment was replicated four times. Due to space limitations, the experiment had two spatial replications (two replications each evaluation period) and two temporal replications (two replications during two distinct evaluation periods. At the initiation of R5 (beginning seed), pod measurements were taken with digital calipers on each bean from the pod proximal to distal end, pod length, and pod width over the period of thirty days with measurements taken ten times. Mainstem node, branch number, node on branch, and pod within the node cluster were labeled to create a single plant coordinate system for identification of individual pods for measurements. At the conclusion of each treatment, plants were re-watered to levels between field capacity and saturation and remained well watered until plant maturity. The number of pods per mainstem node and branch were recorded for each plant, which did not include shriveled pods (<15 mm). A pod was denoted as shriveled if it was twisted and small and contained no mature seeds. Pods were removed from each plant and final digital caliper measurements taken. Seeds were manually shelled, location classified as individual branch or mainstem, counted, and air dried at room temperature.

4.1.4 Soil Moisture Measurements

Soil samples were taken directly from the soybean pots for basic soil physical property measurements. From the soil sample measurements, soil hydraulic properties were estimated to develop a soil moisture retention curve using the Rosetta pedotransfer function (Schaap et al., 2001) within HYDRUS/1D. The Rosetta pedotransfer function converted gathered volumetric water content measurements of the soil via Decagon 5TE probes (Decagon Devices, Pullman, WA) taken in 60-minute intervals. Sensor output review and commands occurred in the ECH$_2$O Utility software program with data recorded by a Decagon Em 50 ECH$_2$O data logger. Particle-size analysis with a hydrometer determined soil texture (Gee and Dani, 2002). Tempe cells determined volumetric water content at field capacity (cm^3/cm^3 value) while volumetric water content at permanent wilting point (cm^3 cm^{-3} value) was found by using a pressure plate (Dane and Jan, 2002). Corresponding tension values were obtained from utilizing these parameters in the van Genuchten water retention curve equation (Van Genuchten, 1980).

4.1.5 Statistical Analysis

Statistical analysis was performed using SAS v9.4 (SAS Institute Inc., Cary, NC) to determine the effect of drought stress timing and duration. Drought stress timing and duration were designated as fixed variable while replication and run were treated as random effects. Two trial runs were conducted for this experiment and analyzed together. Four replications represented experimental treatments of stress timing and duration. Analysis of variance was conducted using Procedure Mixed, with a Fisher's Protected LSD used to estimate means separation. All analysis was done with $\alpha = 0.05$.

Figure 4.1. A coordinate system was developed to track the growth of individual pods over time.

Figure 4.2. Digital calipers were utilized to measure pod growth parameters such as length (shown) and width.

RESULTS AND DISCUSSION

Yield

In this study, the impact of simulated moisture stress by stage:duration treatments on yield was of interest. When moisture stress was experienced at R2:7, yield did not significantly differ from the NTC at 10.8 g plant^{-1}, but did exhibit higher yields at 16.5 g plant^{-1} (Figure 4.3). This finding agrees with Shaw and Laing (1966) of short-term stress during early flowering allowing later recovery of pods.

However, a deficient water supply results in flower abscission and impaired development of pistils, stamens, and ovules (Carlson and Lersten, 1987; Raper and Kramer, 1987). Insufficient supply of photosynthetic assimilates to support these structures from inadequate plant available water is the culprit of this reproductive loss (Kokubun et al., 2001). Studies by Frederick et al. (1990) and Shaw and Laing (1966), indicate stress timing and duration directly influence yield loss. As moisture stress creates resistances to CO_2 absorption for photosynthesis and completely ceases when half of the maximum water content is lost, the difference of two time periods of moisture stress was of interest (Brilliant, 1924; Shaw and Laing, 1966). This sensitivity to moisture stress during reproductive fertilization and influence of stress duration was demonstrated with the yield separation of R3:7 and R3:14 treatments. Treatment R3:7 compared to the NTC was significantly different with a yields of 6.6 g plant^{-1} and 10.8 g plant^{-1}, respectively (Figure 4.3). As a result of moisture stress at R3:7, 4.2 g plant^{-1} of yield potential was lost by flower abscission and impaired reproductive structure development (Figure 4.3). This stress during late flowering to early pod development exhibits a reduction in seed set with inadequate time for compensation (Eck et al., 1987; Foroud et al., 1993; Shaw and Laing, 1996). The growth stage R3 also has high water uptake requirements to support peak biomass production and reproductive structures (Carlson and Lersten, 2004) and consequently, when moisture stress duration was extended an additional seven days for R3:14, yield decreased to 1.0 g plant^{-1} (Figure 4.3). This finding coincides with that of Foroud et al. (1992) and Doss et al. (1974) that crop yield is reduced by evapotranspiration from limited water supply, with the extent of yield loss being period time and length dependent (Doss et al., 1974; Foroud et al., 1992).

Moisture stress during late reproductive stages is coupled with high potential for yield loss by impact of yield components of seed number and seed weight (Brevedan and Elgi, 2003; Foroud et al., 1992). Yield of the R5:7 treatment was 4.9 g plant^{-1} and significantly different from the NTC of 10.8 g plant^{-1} (Figure 4.3). Less water is needed to support plant functions at R5, but substantial yield loss can be caused when moisture stress is experienced due to decreased evapotranspiration from limited plant available water to supply (Foroud et al., 1993). These findings are in agreeance with Kranz et al. (2012) that soybean sensitivity to moisture stress is minimal during flowering, increases at pod development, and is most sensitive at seed fill.

Table 4.1. Analysis of variance *p*-values for yield response as affected by periods of moisture stress at given soybean reproductive stages in growth chamber runs in 2018 and 2019.

Effect	**D.F**[a]	**MVB**[b]	**SN**[c]	**YIELD**[d]
Stage	24	0.80	0.16	0.05
Duration	28	0.42	0.13	0.01
Stage by Duration	28	0.85	0.05	0.01

[a] Degrees of freedom.
[b] Mainstem versus branch.
[c] Seed number.

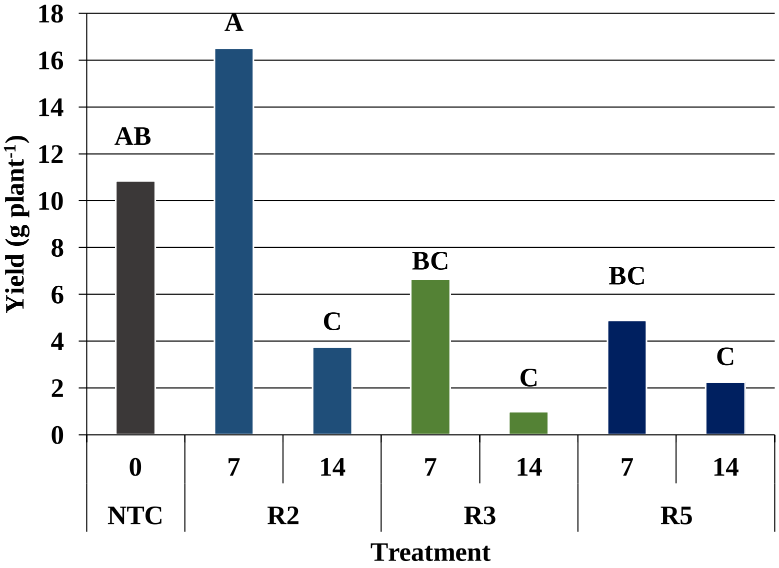

Figure 4.3. Influence of stage and duration of imposed moisture stress on soybean yield.

Seed Number

Studies by Brevedan and Elgi (2003) and Foroud et al. (1992) found yield loss from moisture stress was specifically from a reduction in seed number. Similar to the g plant^{-1} response to the R2:7 treatment, seed number reached 124; significantly higher than the NTC with 69 seeds produced (Figure 4.4). Sensitivity to moisture stress did not influence yield at this stage, which demonstrates the ability of early-season plant recovery.

The plants under the R3:7 treatment experienced enough moisture stress to significantly decrease seed number compared to the NTC with 51 and 69 seed produced per plant, respectively. Under fourteen days of water stress at R3 (R3:14), the plants reflected a significant seed weight loss of 39.9 g plant^{-1} and reduction in seed number to 29 seeds per plant in comparison to the NTC (Figure 4.4). Soybean at late flowering and early pod development are at the peak moisture uptake requirement of 0.8 cm average daily evapotranspiration (Shaw and Laing, 1996; Eck et al., 1987; Kranz et al., 2012). In high soybean production regions with fully irrigated systems, 50.8 to 66.4 centimeters of total water is applied at this time (Kranz et al., 2012). For soybean produced in western Oklahoma and other dryland production regions, this period of the growing season is often coupled with periods of moisture and heat stress and this water uptake requirement will not be met (Figure 3.4), (Arndt, 2003). The success of seed weight and seed number is a function of the supply of assimilates to seed growth, which can be greatly hindered under moisture stress (De Souza et al., 1997). Moreover, the duration of moisture stress at R3 is critical as seven days to fourteen days of stress creates a higher yield loss potential.

During R5, moisture stress has the greatest impact in number of seeds that survive to maturity and contribute to yield (Smicklas et al., 1989). Soybean sensitivity to moisture stress is most sensitive at R5 and this was demonstrated with the significant seed number loss of 43 seeds when the R5:7 was only able to retain 25 seeds per plant (Figure 4.4). It has been demonstrated that withholding water at R5 greatly affected both seed number and seed weight contributions to yield (Foroud et al., 1992). Moisture stress at R5 shortens assimilate supply that is mediated by plant water availability, directly impacting seed weight (Thomas and Raper, 1977; Vieira et al., 1992). In limited irrigation systems, vulnerability to moisture stress complicates management but places priority on R5 for water management to lock late-season yield potential.

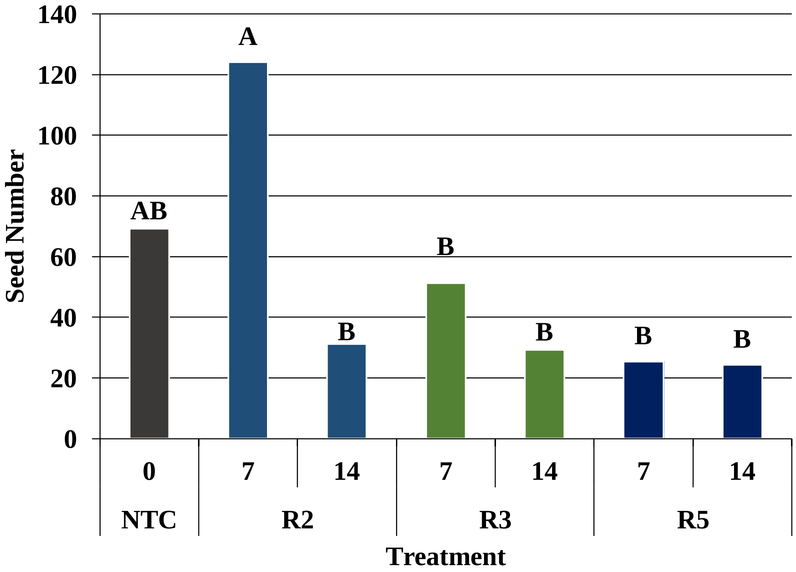

Figure 4.4. Influence of stage and duration of imposed moisture stress on number of seeds contributing to yield.

Mainstem versus Branches

The number of pods located on the branches were subtracted from the mainstem to compare and analyze this physiological impact. A positive number indicated a higher number of pods on the mainstem contributed to yield, while a negative number signified pods were concentrated on the branches. A near-even number signifies equality of the two.

Plant biomass production, or vegetative growth, and number of available pod bearing nodes is related to photosynthetic efficiency, which is limited by the amount of water the crop transpires (Purcell and Specht, 2004). Thus, vegetative growth is mediated by the internal water balance of plants and the control of water loss and absorption (Kramer et al., 1963). Insufficient plant water supply decreases photosynthesis, along with active tissue responsible for cell turgidity and enlargement, and adversely affects vegetative growth (Shaw and Laing, 1996).

Crop growth limitation in terms of node was not experienced with the R2:7 treatment as a branching potential proliferated by producing 15 more pods located on branches than the mainstem (Figure 4.5). Compared to the NTC, an average of 12 pods on branches contributed to yield. From this, it can be assumed short periods of moisture stress at R2 have no effect on vegetative growth and branching capabilities.

The maintenance of plant internal water balance was also observed when moisture stress was experienced for a short period at R3. Treatment R3:7 was able to maintain an average of 10 additional pods on the branches than the mainstem compared to the NTC with 12 additional branch pods (Figure 4.5). However, this did not hold true when an additional seven days of moisture stress was imposed. The potential of branch located pods greatly decreased as more pods on the mainstem of R3:14 plants contributed to yield (Figure 4.5). Seven additional days decreased available plant moisture and thus, mobilization of assimilates to supply these pods on secondary and tertiary locations. Because of this, assimilates were directed to the larger sink of pods developing on the mainstem.

When moisture stress is experienced at R3, biomass production is limited along with the potential for additional nodes to supply yield (Frederick et al., 2001). At the same time, the survival of late developing flowers on upper nodes are at resource disadvantage to developing pods that are a larger sink for the limited assimilates (De Souza et al., 1997). Due to this, a shift in more pods produced on branches to the mainstem began to decline at R3 and branch pod capacity greatly reduced at R5 (Figure 4.5). With the R5:7 plants producing an even number of pods on the mainstem and branches, coupled with a diminished overall yield, it can be assumed yield was impacted by moisture stress on the mainstem and the branches at the same magnitude (Figure 4.5).

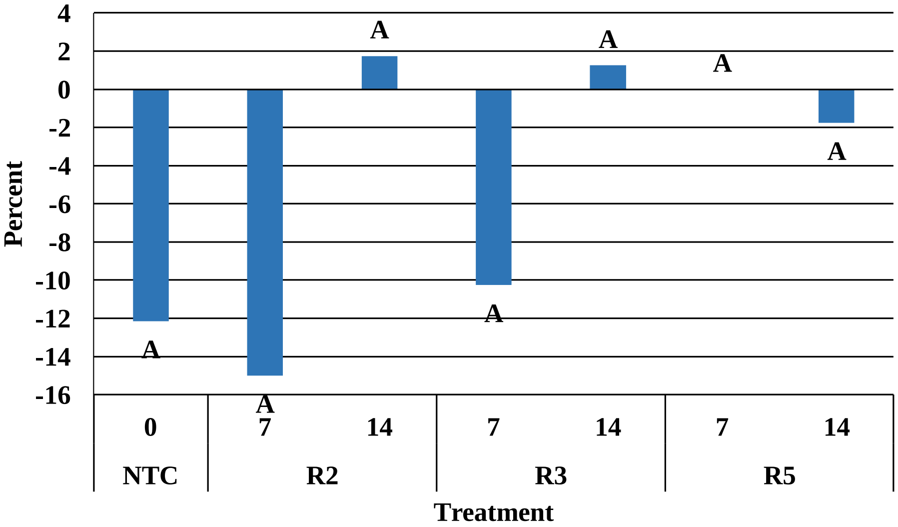

Figure 4.5. Influence of final pod location by stage and location of simulated stress. Positive values indicate more pods on the mainstem at physiological maturity while negative values, calculated as the difference, signifies more pods on the branch on treatment plants.

CONCLUSIONS

Understanding the potential underlying mechanisms of loss by water stress is important when accounting for the final commodity of yield. Especially, considering the yield impact of moisture stress duration is significant, soybean water management is of upmost importance. As western Oklahoma historically receives only 43.2 centimeters of rainfall during the growing season, and limited availability of irrigation water if applicable, moisture stress could certainly be a yield limiting factor of western Oklahoma soybean production.

Crop yield directly correlates with evapotranspiration efficiency during limited plant water supply periods, with the extent of yield loss depending on the period and length of moisture stress. This impact was demonstrated in the yield separation and physiological response of plants experiencing fourteen days of moisture stress in comparison to the tolerable duration of seven days. Especially considering this yield impact from moisture stress duration is significant, soybean water management of thresholds is critical.

In dryland soybean production in low precipitation regions, plant water use efficiency of received rainfall is of upmost importance for a successful crop. Water uptake requirements to maintain assimilatory capacity for optimal pod retention was found to be minimum at R2, increased at R3, and substantial at R5. As irrigation is not applicable in such dryland systems, management farm economics must emphasize practices to conserve soil moisture and protect against external losses throughout the growing season. Avoidance of yield losses from moisture stress can be reduced by decreasing plant competition with wider row spacing or reduced populations, controlling weed pressures, and protection from insect injury. The utilization of winter cover crops to encourage retention of soil moisture and enhance fertility could also be of great benefit to dryland soybean production systems. With R5 being the most critical time to avoid moisture stress, late season rainfall patterns should be monitored and planting date adjusted to correlate with the received precipitation around R5 to optimize dryland soybean produced in low precipitation regions. Farmer practice to mitigate stress factors that could onset moisture stress, while working with the environmental factors of the region, will protect plant available water throughout the growing season. More importantly, this will decrease the duration of the moisture stress dryland soybean experiences and allow genetic potential to be attainable in the low precipitation regions of Oklahoma and beyond.

CHAPTER V

CONCLUSIONS

Soybean production hectares are continuing to expand under dryland conditions in more moisture limited regions, but the growing challenges of these new production systems are unknown. These systems are known for not only limited but inconsistent rainfall but also potentially temperature extremes. Furthermore, as soybean spread into newer regions, new and novel pests could provide additional stressors. All of these stressors can result in stress during reproductive growth, which can result in damage to the reproductive structures. Soybean response to the known and unknown stressors and mitigation of potential yield losses is crucial for maximization of genetic potential in the emerging production region.

When stress, in the form of imposed stress or moisture stress, was experienced at R2, there was no impact on plant contribution to yield. Components of yield, plant physiology, and seed quality were not significantly affected by flower removal or moisture stress periods. The recovery of yield from both imposed stress and moisture stress expressed the vigor of R2 soybean and the benefit of an extended flowering period to handle substantial impact. This stage requires the largest amount of water to support yield, which may impact soybean that experience a long period between rainfall events and a common occurrence in western Oklahoma and other dryland soybean systems in low precipitation regions.

Pod development is a period in the growing season that is especially sensitive to stressors. There was overall effect on yield in the field and growth chamber studies and thus illustrated the ability of soybean to tolerate stress at R3 with late-developing flowers providing potential for yield recovery. Stress imposed on the top and middle of the mainstem did not affect yield and can be related to soybean recovery potential from stressors. However, stress on the bottom of the mainstem was significant. This impact on the bottom of the mainstem places an emphasis is mitigation of moisture stress at R3, which is also initially experienced at the bottom of the mainstem. In the growth chamber study, this was demonstrated by the significant loss of yield when moisture stress was experienced at seven days. When this moisture stress was experienced for an additional seven days, seed weight, seed number, and plant physiology was altered. From this, it can be concluded that crop yield is reduced from limited water supply and is dependent on period time and length. Soybean production in western Oklahoma must consider both the impact of moisture stress at this stage and the duration of the event on yield. High temperatures can cause seeds to shrivel and reduce crop quality at R3 and is another production challenge, that is often coupled with moisture stress, that can compound stress in western Oklahoma soybean and other dryland production systems in low precipitation regions.

The greatest impact on yield was observed at R5 in both the field and growth chamber studies. Thus, it can be concluded, the impact of stress on soybean yield is minimal during R2, increases at R3, and is greatest at R5. Yield components of seed weight and seed number were significantly impacted at R5 with no potential for recovery, unlike prior stages. Plant vegetation was also influenced by decreased plant height and branching. Reduction of pod load by instances such as insects, disease, hail, and low soil moisture that interfere with the source-sink ratio are causal agents of green-stem. Mitigation of late-season stress by prioritizing management of dryland soybean grown in low precipitation at R5 has been proven as crucial to prevent the detriment of green-stem and to retain yield. Producers must implement practices that help manage stress, such as insecticide and fungicide applications at threshold, monitoring rainfall patterns, and planting date decisions are critical to avoid such losses during late-season soybean reproductive stages.

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APPENDICES

Table 7.1. Bixby Soil Test Report.

Routine Test	**Values**
pH:	6.6
NO3-N (lbs/Acre) Surface:	3
Soil Test P Index:	48 (24 ppm)
Soil Test K Index:	198 (99 ppm)

Table 7.2. Perkins Soil Test Report.

Routine Test	**Values**
pH:	5.7
NO3-N (lbs/Acre) Surface:	4
Soil Test P Index:	64 (32 ppm)
Soil Test K Index:	223 (111.5 ppm)

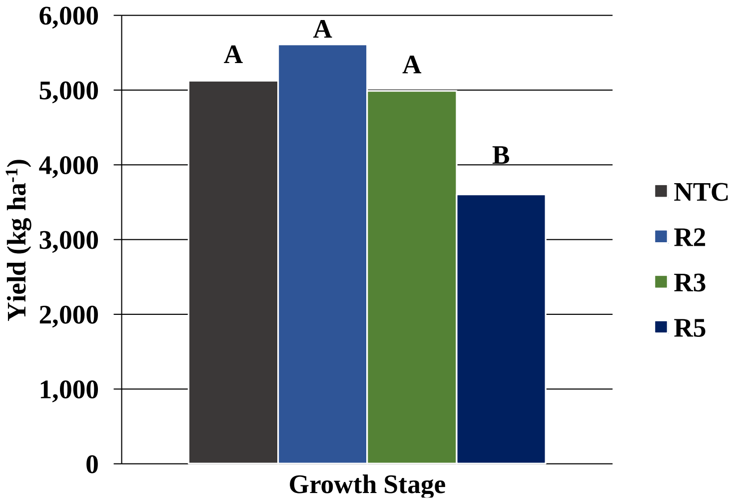


Figure 7.1. Influence of treatment timing on Bixby yield in 2018.

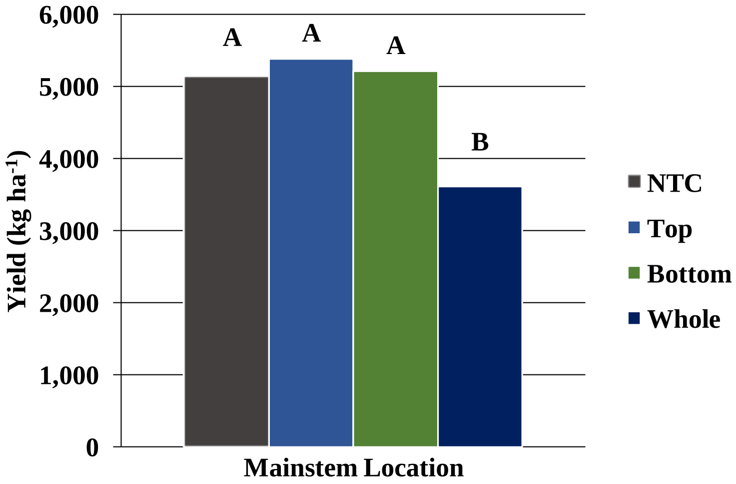


Figure 7.2. Influence of cumulative treatment location on Bixby yield in 2018.

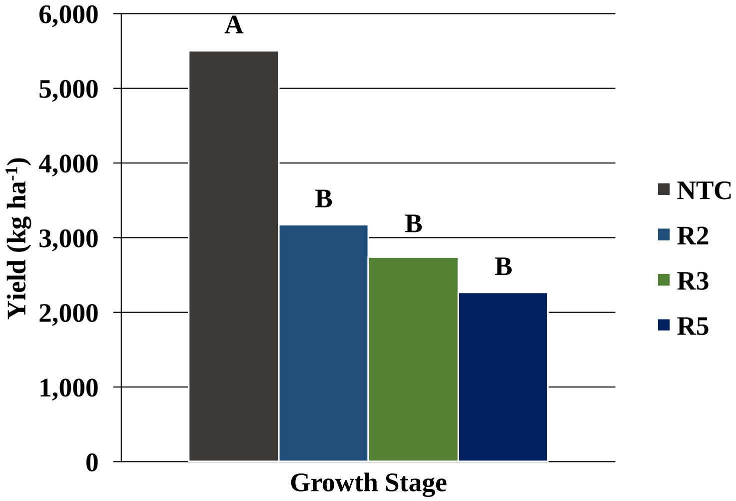


Figure 7.3. Influence of treatment timing on Perkins yield in 2018.

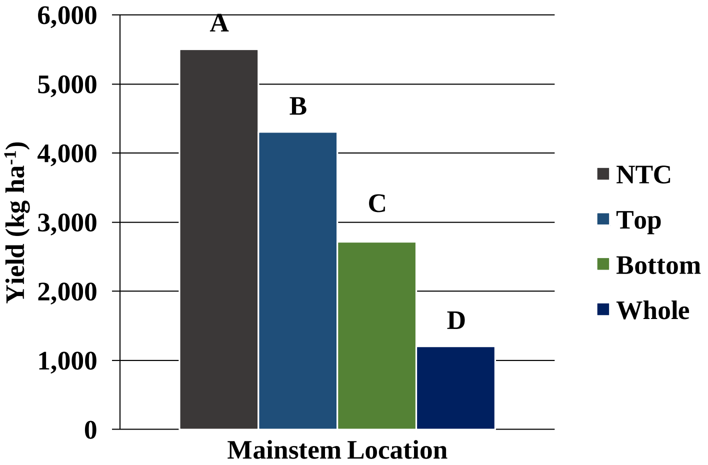


Figure 7.4. Influence of cumulative treatment location on Perkins yield in 2018.

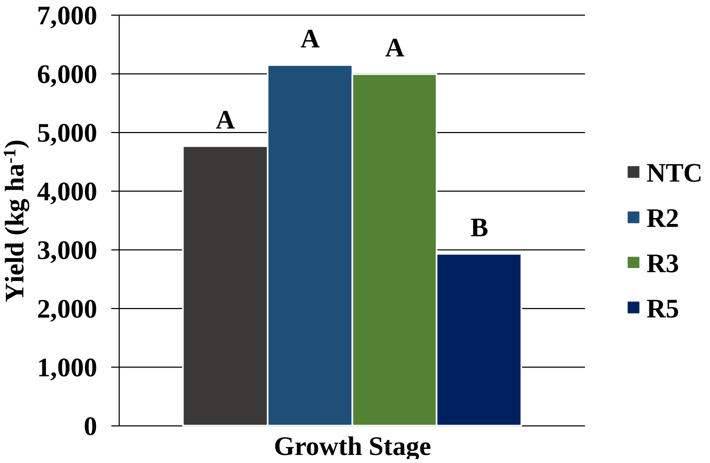


Figure 7.5. Influence of treatment timing on Bixby yield in 2019.

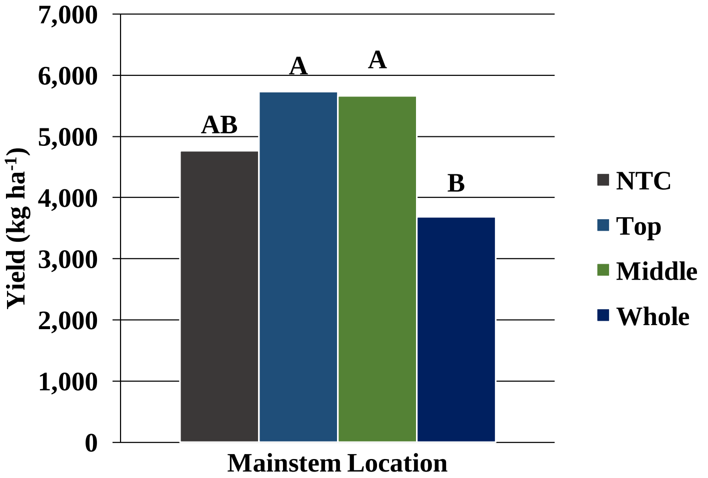


Figure 7.6. Influence of cumulative treatment location on Bixby yield in 2019.

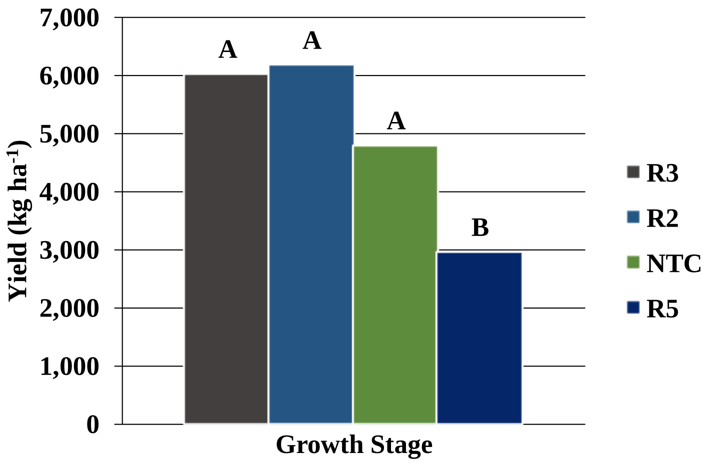


Figure 7.7. Influence of treatment timing on Perkins yield in 2019.

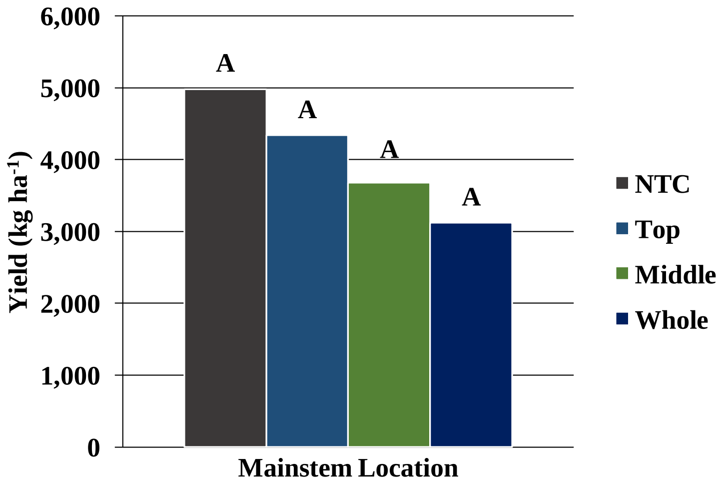


Figure 7.8. Influence of cumulative treatment location on Perkins yield in 2019.

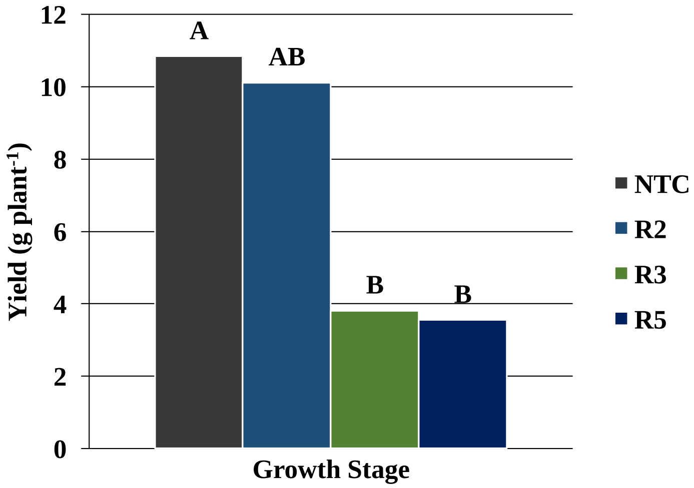


Figure 7.9. Influence of treatment timing on growth chamber plant yield.

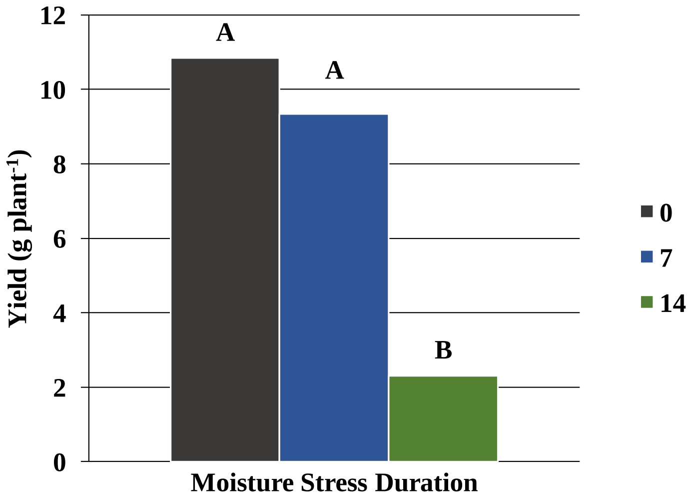
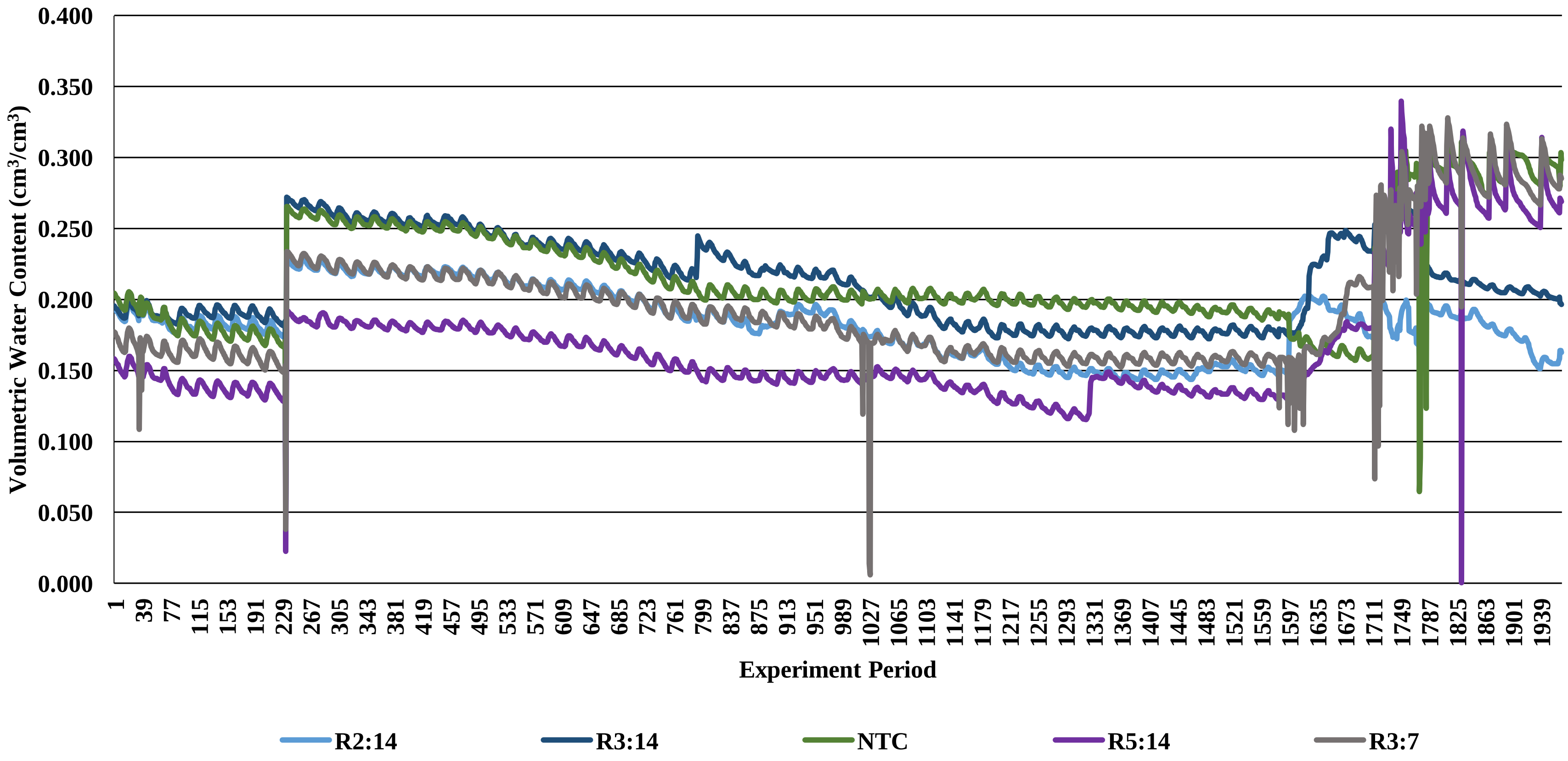


Figure 7.10. Influence of moisture stress duration on growth chamber plant yield.

Figure 7.11. PatEM50 soil moisture sensor measurements of volumetric water content in five treatment pots.



VITA

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Biographical:

Education:

Completed the requirements for the Master of Science in Plant & Soil Science at Oklahoma State University, Stillwater, Oklahoma in May, 2020.

Completed the requirements for the Bachelor of Science in Plant & Soil Science at Oklahoma State University, Stillwater, Oklahoma in 2018.

Experience:

Undergraduate Researcher for Dr. Lofton, Stillwater, OK.
August 2016-May 2018.

Helena Chemical Company Intern, Goshen, IN, and Culver, OR.
May 2015-June 2017.

Professional Memberships:

American Society of Agronomy 2016-Present
Crop Science Society of America 2016-Present
Soil Science Society of America 2016-Present
Weed Science Society of America 2019-Present