ABSTRACT

MANANDHAR, ANJU. The Response of Leaf Expansion and Emergence to Drought Stress in Cowpea. (Under the direction of Dr. Thomas R. Sinclair).

Cowpea (*Vigna unguiculata* L. Walp) is a crop mostly grown in semi-arid regions and drought is a major abiotic stress affecting cowpea production. A better understanding of the response of cowpea leaf area development is needed for progress in crop simulation and selection to maximize crop productivity. Two aspects of leaf area development, leaf expansion and leaf emergence as phyllochron index (PI) were examined under soil drying for six cowpea genotypes. Quantitative relationships between leaf expansion/leaf emergence and fraction of transpirable soil water (FTSW) were found. It was seen that both leaf expansion and leaf emergence declined linearly after soil dried to a threshold FTSW.

The decrease of leaf expansion occurred over a wide FTSW-threshold range of 0.23 to 0.67 across genotypes, and the genotypic differences were consistent across experiments. The average FTSW for cessation of leaf expansion was 0.014 FTSW. Comparison of the response of leaf expansion to transpiration showed that leaf expansion was more sensitive to soil drying than transpiration since both the FTSW threshold for initial decrease in leaf expansion and the FTSW for the cessation of leaf expansion occurred at higher FTSW than transpiration responses. When drought-stress plants were rewatered, leaf expansion recovered within 1 d and transpiration recovered in an average of 2 d.

PI has not been used as a measure for leaf emergence in cowpeas. The assumptions in the calculation of PI were tested for cowpea plants and it was found that PI could be used to estimate the morphological age of cowpea plants. When PI was used to observe the response of leaf emergence to soil drying, leaf emergence declined among genotypes over a range of FTSW-thresholds of 0.28 to 0.53. FTSW-intercepts of -0.03 to 0.06 among genotypes was

seen for the cessation point of leaf emergence. No differences were seen between the response of leaf emergence and leaf expansion to soil drying. Hence, leaf emergence was as sensitive as leaf expansion.

The range of FTSW thresholds and intercepts seen for leaf expansion and leaf emergence suggests that we might be able to select cowpea genotypes with early sensitivity of leaf development to drought stress in dry regions to promote a conservative use of resources and increase the plants chance of survival through the drought period. Genotypes with leaf expansion or emergence that is less sensitive to soil drying can be useful for wetter areas where leaf development can continue to optimize use of resources without being affected by small fluctuations in soil water content.

The Response of Leaf Expansion and Emergence to Drought Stress in Cowpea

by
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DEDICATION

To Cadwell Turnbull, my love, my best friend and partner in marriage. He not only encouraged me but became the best listener, reader and tutor and walked me through my numerous blocks.

BIOGRAPHY

Anju Manandhar is from Kathmandu, Nepal. She left her country in pursuit of a Bachelor's degree in chemistry and biology in La Roche College, Pittsburgh, Pennsylvania. Anju graduated from La Roche College in 2011. Before coming to Raleigh NC, Anju held a range of professional positions including, assistant tutorial coordinator, sales associate, nurse's aide and a canvasser for environmental awareness. She started working in projects related to drought stress response in cowpea as a lab assistant with Dr. Sinclair's research group in 2013. She decided to continue working on the research she was assisting with as a Master's research assistant. She officially started her master's program at North Carolina State University in 2014. Anju is looking forward to starting a PhD position in the Organismic and Evolutionary Biology Department of Harvard University as a student of Dr. Noel Michelle Holbrook.

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CHAPTER 1:

Introduction

Cowpea, *Vigna unguiculata* L. (Walp), is a legume crop that can be grown in a wide geographical range in both the tropics and subtropics for both human consumption and fodder. Cowpea is considered more drought tolerant than other legume crops and is widely grown in the semi-arid sub-Sahelian zone and dry savannas of West and Central Africa (FAO, 2004). Cowpea plants have high rates of nitrogen fixation and seed production under drought conditions compared to other legume crops (Timko, et al., 2008). Additionally they provide a major source of dietary protein upon consumption (Timko, et al. 2008).

In the past decade farmers in West Africa have shifted towards cowpea production in regions prone to both intermediate and terminal drought (Timko, et al., 2008). So it is important to study plant development responses in cowpea to drought stress to understand and predict crop productivity in these regions. Also, differentiating the response of different cowpea genotypes to drought conditions can help guide plant selection based on environment and breeding programs.

Cowpea may be considered more drought tolerant than other crops, but drought is still a large abiotic stress that affects growth and yield. Low water availability influences a range of physiological processes, among which leaf area development is one of the first processes to be affected (Blum, 1996). Aniya and Herzog (2004) showed that specific leaf area and leaf area ratio of cowpea genotypes were reduced as a result of drought stress. But changes in leaf area are determined by both the rate of leaf emergence and leaf expansion. Leaf emergence is

controlled by cell division and leaf expansion by cell enlargement. Even though drought may decrease the rates of both leaf expansion and emergence leading to a decrease in leaf area, it is important to examine the two physiological processes separately to understand how both respond to drought stress. As discussed later, there has been a moderate amount of research to find the response of leaf expansion in various crops including cowpeas. Compared to leaf expansion, there has been even less research to track leaf emergence response to drought and no studies done to track leaf emergence response in cowpea.

Sinclair and Ludlow (1986) refined a proposal by Ritchie (1981) to express plant physiological response as a function of soil water content. This method has recently been used to look at transpiration response to drought stress in various crops including cowpea (Sinclair, et al., 2015). Total transpirable soil water is defined as the amount of water in the soil at pot or field capacity compared to the soil water content when the plant transpiration reaches 10% of maximum transpiration. The soil water content during the soil drying process can be expressed as the fraction of transpirable soil water (FTSW). Sinclair and Ludlow showed that as soil dries progressively, transpiration in crops is initially constant and starts declining below a threshold FTSW.

Many studies done to observe canopy leaf expansion under drought stress condition use the method introduced by Sinclair and Ludlow (1986). The studies done on crops like maize *(Zea mays* L.: Muchow, et al., 1991); chickpea (*Cicer arietinum* L.: Soltani et al. 2000); field peas (*Pisum sativum* L.:Lecoeur, et al., 1996); soybean (*Glycine max* Merr), black gram (*Vigna mungo*) and cowpea (Sinclair, et al., 1987) have shown that leaf expansion response

to soil drying is similar to transpiration response to soil drying, in that leaf expansion also declines linearly as soil dries beyond a threshold FTSW. This pattern of whole plant leaf expansion response of cowpea to soil drying was found by Sinclair et al. (1987) for one genotype. But there has been no further research to examine whether other cowpea genotypes show similar response to drought stress.

Studies of leaf emergence rates are frequently done to understand the phenology of various plants in response to temperature and photoperiod, but not much research has sought to understand leaf emergence response to drought stress (Lecoeur, et al., 1998). While in cowpeas specifically, research has shown the response of leaf emergence under different temperatures and photoperiods (Craufurd, et al., 1997), but no study has examined the response of leaf emergence under drought stress. There were studies done in other crops like field peas (Lecoeur, et al., 1998) which showed that rate of leaf production starts decreasing linearly when there is less than 0.20 FTSW. This study also pointed out that there was high variability in tracking leaf emergence as node number because of uncertainty in measuring leaf numbers and difficulty in distinguishing leaf unfolding stages. There is a need to use an alternative method of measuring leaf emergence to be able to more clearly determine its response to stress on a daily basis.

Erickson and Michelini (1957) developed the Plastochron Index as a means of finding the morphological stage of plant development in terms of node number and length of the newest leaves (plastochron will hence forth be referred to as phyllochron (PI), since phyllochron more specifically refers to the time interval between the emergence of two successive leaves

at the macro scale). PI adds a decimal fraction to the node number of a plant and hence the change in PI gives a continuous number instead of an integer leaf number as a measure of leaf emergence. Vendeland et al. (1982) modified the Erickson and Michelini's equation to find the PI for soybean under drought-stress conditions.

The PI method of measuring leaf emergence has been used by Sinclair (1984) and Randall et al. (1988) to measure the rate of leaf emergence in soybean under drought stress in field conditions. Using PI as a measure of leaf emergence is especially useful when tracking leaf emergence on a daily basis instead of every 2 to 3 days, because the equation adds the changes in the length of the newest leaves to the measure of leaf emergence.

In conclusion, there is little information to be found on the response of cowpea leaf emergence and leaf expansion. The purpose of this thesis is to present research done to address the lack of information about the response to drought of cowpea leaf expansion and leaf emergence. This thesis presents the research in the form of two chapters. Chapter 2 presents studies on the leaf expansion component of cowpea under increasing drought stress and Chapter 3 presents the response of leaf emergence of cowpeas under drought stress.

The research presented in Chapter 2 includes eight genotypes of cowpea that have previously been tested for drought tolerance of transpiration and nitrogen fixation rates (Sinclair, et al., 2015). Hence, the main objective of Chapter 2 was to detect the sensitivity of leaf expansion and it's cessation under drought stress compared to transpiration in cowpea. Since eight genotypes were included in the study, these provided initial information on whether the response in leaf expansion showed any genotypic differences. The experimental

design also allowed the measurement of the response of leaf expansion after fully watering plants following the drought-stress period. Therefore, secondary objectives of this study were to obtain preliminary information on recovery of leaf expansion following rewatering and to observe if there was a genotypic basis for variation in the leaf expansion response.

Chapter 3 examined the response of leaf emergence in the form of PI to soil drying. The modified PI as described by Vendeland et al. (1982) was used. There were two objectives for Chapter 3. (1) Document the response of leaf emergence as PI to soil drying as a function of fraction of transpirable soil water (FTSW). To use PI as a quantitative response of leaf emergence to soil drying, it was important to first ensure PI could be used as a measure of leaf emergence in cowpea. (2) Determine how leaf emergence response differed from leaf expansion response by comparing the FTSW at the points of decline and cessation for both. Also, since several genotypes were tested, it was possible to determine if there were any differences in leaf emergence response among genotypes.

Chapter 4 is included in this thesis to provide a brief summary of the main conclusions of this overall study on leaf development of cowpea. Also, perspective is offered on the key outcomes of this research that might guide future research on cowpea.

CHAPTER 2

Response of leaf expansion and transpiration to soil drying and recovery among cowpea genotypes

Introduction

Vigna unguiculata, commonly referred to as cowpea, is an important legume crop used for food and stover and grown often in semi-arid regions of the tropics and subtropics (Lim, et al, 2012). Cowpeas are often grown under drought-stress conditions; therefore, genotypic differences observed in yield decreases can be very important for late or early season drought (Timko, et al., 2008).

Drought is a major abiotic stress that influences plant processes resulting in losses in crop production. Decreases in current transpiration rates can help save the water available in the soil to allow sustained physiological activity during drought conditions. One approach to achieve decreased transpiration is to decrease leaf area, since a large surface area is the source of transpirational water loss. Canopy leaf expansion, which directly influences leaf area, is often identified as one of the most sensitive processes to water-deficit conditions (Radin, et al., 2010). A decreased leaf area either to save water or as a consequence of drought also means less leaf area for carbon assimilation and the possibility of yield loss. Hence, it is essential to understand the dynamics of response of leaf expansion to developing soil-water deficit.

Research has been done in various crops to understand the decrease in canopy leaf expansion under drought stress conditions. Sinclair and Ludlow (1986) showed that the

response of plant physiological processes can be expressed as a function of soil water available for transpiration. Serraj et al. (1999) showed in soybean [*Glycine max* (L.) Merr.], that leaf expansion decreased linearly after soil water decreased beyond a threshold soil water content. Similar studies on the effects of drought stress on leaf expansion in field peas (*Pisum sativum* L.) (Lecoeur, et al., 1996) and in chickpea (*Cicer arietinum* L; Soltani, et al., 2000) have shown that leaf expansion response can be modeled as a function of FTSW (fraction of transpirable soil water). Sinclair et al.(1987) showed that leaf growth starts declining after FTSW=0.2 was reached in cowpea, soybean and black gram [*Vigna mungo* (L.) Hepper]. Zero leaf expansion was reached when soil water was exhausted to FTSW values of 0.02 in cowpea and black gram and 0.05 in soybean(Sinclair et al, 1987) .

There is little literature exploring the mechanisms of leaf expansion in cowpea under soil drying. Only one genotype of cowpea was tested by Sinclair et al. (1987) and the non-linear model used did not define an FTSW threshold for the exact point of linear decrease in leaf expansion. In cowpea, the sensitivity of leaf expansion to drought stress below the FTSW threshold has not been explored. Also, there is no other research on the point of cessation of leaf expansion. Furthermore, there is also no information on the recovery of cowpea leaf expansion when plants are rehydrated after drought-stress conditions. In regions with intermittent drought, the ability of crops to recover from drought stress could have a major affect the eventual crop productivity.

This research was intended to address this lack of information by focusing on the sensitivity of the decline and cessation of leaf expansion in cowpea under drought stress. Hence, the primary objective of this research with cowpea was to detect the sensitivity of leaf

expansion and cessation under drought stress compared to transpiration. Two secondary objectives were studied to obtain preliminary information on (1) if tolerance or sensitivity during soil drying gives genotypes any advantage in recovery from drought stress after rewatering and (2) possible genotypic differences by studying the drought and recovery responses in eight genotypes.

Materials and Methods

Sinclair et al. (2015) investigated the influence of drought on the decline of transpiration rate among ten cowpea genotypes under progressive soil drying but did not consider leaf expansion. From the ten cowpea genotypes they tested, eight cowpea genotypes (Bambey-21, IT82E-18, IT89KD-288, UC-CB46, IT84S-2049, Mouride, UC-CB27, Suvita2, and IT93KD-503-1) were selected for this study of leaf expansion when subjected to soil drying in a greenhouse experiment. Due to space limitations, only six of the eight genotypes were subsequently tested in two growth-chamber experiments (Bambey-21, IT82E-18, IT89KD-288, IT84S-2049, Mouride and Suvita2).

Experimental conditions

The greenhouse experiment was done at Raleigh, NC (46°35’ N, 39°78’ W) with temperature regulated for cooling at 28^{o}C. Actual temperature and relative humidity in the greenhouse was measured every 5 minutes using data loggers (Lascar Electronics, Erie, PA). Incident photosynthetically active radiation to the greenhouse was obtained from the nearby Lake Wheeler Road Meterological Station (State Climate Office of North Carolina). The plants were sown on 9 May 2014 and the beginning of the dry-down experiment was on 2

June 2014. Supplemental lights were used from 6:00 pm to 10:00 pm Eastern Standard Time to extend the light period to approximately 16 h to help maintain the plants in a vegetative state during the experiment.

The growth chamber experiments were done in a walk-in controlled-environment chamber in the NC State University Phytotron facility. The day/night temperatures were set to 30/24^{o}C. Temperature and relative humidity were also measured in the growth chambers every five minutes using data loggers (Lascar Electronics, Erie, PA). Photosynthetically active radiation in the growth chambers was measured once a month. The first growth chamber experiment had a day length of 16 h. The second growth chamber experiment had a day length of 12h with a 3h dark-period interruption with light from incandescent lamps.

For all experiments, the plants were grown in 20-cm diameter plastic pots with a volume of 4 L filled with loamy soil (69% sand, 18% silt, and 13% clay). The seeds were inoculated with *Bradyrhizobium japonicom* (N-Dure, INTX Microbials, Kentland, IN). Plants were thinned to 1 plant per pot after the first trifoliolate leaf emerged. Any occasional flower that emerged was removed to maintain the plants in a vegetative state.

The plants were grown under well-watered conditions by watering the pots daily with de-ionized water (100 to 200g). During the greenhouse experiment, a nutrient solution (nitrogen 17.9 mM, phosphorus 4.0 mM and potassium 9.0 mM) was prepared using MaxiGro (General Hydroponics, Sebastpol, CA) and applied to the plants once a week. For the growth chamber experiments, the nutrient solution (nitrogen 7.6 mM, phosphorus 0.3 mM, potassium 2.8mM plus micronutrients) as prepared in the phytotron facility was applied

twice a week. Information pertaining to the composition of the nutrient solution can be found in the Phytotron Procedural Manual (Saravitz, 2009).

Dry down treatment

When the plants had four to five trifoliolate leaves, the dry-down treatment was initiated. In the evening before treatments were imposed, all pots were watered until dripping to ensure soil water content was saturated. The pots were allowed to drip over night to permit the soil to drain to pot capacity by the following morning. In the morning, the pots were enclosed in plastic bags tied around the main plant stem to prevent soil water evaporation. Then the pots were weighed to get the pot initial weight.

During the following days of the experiment, all pots were weighed daily to measure water loss by transpiration. In the greenhouse experiment five plants were designated for stress treatment and three plants for well-watered treatment. There were ten stress plant and three well-watered plants in the growth chamber experiments. For the greenhouse experiment and the first chamber experiment, the well-watered pots were rewatered daily to maintain a pot weight of 200 g below their initial weight. For the progressive drought treatment, the soil was allowed to dry as a result of plant transpiration. However, to ensure a more natural development of drought over about two weeks, the stressed pots were watered on each day when their water loss was greater than 100 g so that the net daily water loss was no greater than 100 g. A drought period of about 12 to 14 d was targeted because shorter dry-down periods have been found to give in less consistent results.

Due to high vapor pressure deficit (VPD) during the first chamber experiment (8 d dry-down period) the dry-down period was shorter than desired, so the protocol for the second

chamber experiment was adjusted to extend the experiment. To decrease the transpiration rate, VPD in this second experiment was maintained in the range of 1 to 2 kPa with the use of two humidifiers (Ultrasonic humidifier, Kaz incorporated,Hudson, NY). The criterion for rewatering the water-deficit pots was also changed to achieve a slower rate of dry down. Instead of rewatering the stressed pots to maintain daily water loss to 100 g or less, in this second chamber experiment rewatering was done to keep daily water loss to 60 g or less. The well-watered pots were watered daily to return pot weight to 150 g below their initial weight.

Leaf expansion

Leaf expansion was tracked by measuring the length of each terminal leaflet on each plant every 24 h until the leaflet length was no longer increasing. Corresponding node numbers were also recorded. At the end of the experiment the length of each terminal leaflet and its corresponding trifoliolate leaf area was measured (LI-3100C, Li-cor, Lincoln, NE).

A quadratic relationship between the terminal leaflet length and the area of the corresponding trifoliolate leaf was obtained for each genotype. For example, Figure 2-1 shows this relationship in the genotype IT89KD-288. The relationship between terminal leaflet length and leaf area was used to find the daily leaf area of each plant. The daily leaf area results were used to calculate by difference the leaf total expansion per day for each plant during the experiment.

Recovery

Recovery of transpiration rate and leaf area expansion after severe drought stress was measured in the second chamber experiment. At the end of the dry-drought stress period for each stressed plant, the plant was rewatered so that its pot weight returned to 150 g below initial weight. Pot weight was measured every day and water added for at least 5 days following the rewatering. Also, of each day during the recovery period terminal leaflet lengths were measured to estimate leaf area increase.

Data analysis

The temperature and relative humidity data collected was used to calculate VPD. The average VPD and temperature were found for the daylight hours for the duration of each experiment.

Daily transpiration data were used to calculate the normalized transpiration ratio (NTR) for the plants undergoing stress treatment as described in Serraj et al. (1999). Transpiration rate was calculated from the change in pot weights between successive days. To obtain a transpiration ratio, daily transpiration of each stress plant was divided by the mean transpiration rate of the well-watered plants within each genotype on the same day. This transpiration ratio helped to minimize daily variation as a result of differing environmental conditions. To reduce plant-to-plant variation, daily normalized transpiration rate (NTR) was calculated for each stressed plant by dividing its normalize transpiration ratio by its average transpiration ratio during the first three days of the experiment when the plant was not yet suffering drought. This procedure meant that the initial NTR of all plants was centered on 1.0. The stressed pots were allowed to dry until daily NTR reached a value equal to or less

than 0.10, signifying that transpiration in stress plants was less than 10% of their well-watered condition.

The difference between the initial saturated pot weight and the weight at the end of the drought-stress period was used to calculate the fraction of transpirable soil water (FTSW) in each pot over the course of the dry down.

FTSW= (daily wt. - final wt.)/(initial wt. – final wt.)

Normalized leaf expansion ratio (NLER) was calculated as described by Serraj et al., (1999) similar to NTR. The calculated leaf area was used to find the total expansion per day for each plant during the experiment. The daily expansion ratio for each stressed plant was calculated by using the average expansion observed in the well-watered plants of the same genotype. Normalized leaf expansion ratio for each day for each stress plant was calculated by dividing the daily leaf expansion ratio by the average of expansion seen in the stress plant during the first three days of the experiment.

For each genotype, daily NTR and NLER was compared with FTSW in Graphpad prism 6 (Graphpad software Inc., San Diego, CA) to fit a two-segment linear regression and find the threshold at which each genotype starts declining with decreasing soil water content. These regression results were also used to extrapolate the response of NTR and NLER to find the FTSW values at which each reached zero.

During the recovery period, the transpiration and leaf expansion of recovering plants were normalized against the transpiration and leaf expansion rates of the last two days of the recovery for each plant. A two-segment linear regression using prism was fit for both NTR and NLER against the days of recovery.

Results

The criterion of watering of the drought-stressed pots was effective in extending the average duration of the dry-down period to 13 d during the greenhouse experiment. However, the watering regime in the first growth chamber study resulted in a shortened drought period of 8 d. Adjusting the chamber VPD and rewatering criterion resulted in extending the dry-down period to 11 d during the second growth chamber experiment.

For most genotypes, the plots of both NLER and NTR versus FTSW were well represented by the linear, two-segmented model (Figure 2-2). The initial well-watered phase of the dry-down was a plateau followed by linear decreases- in rate beyond a threshold point.

Environmental Conditions

Differences in the environmental variables of temperature, VPD and light were found among the experiments. Figure 2-3 shows the average daily daytime VPD and temperature during the course of the three experiments. For the greenhouse experiment, the 5 d average VPD covering the time when FTSW thresholds were reached was 2.4 kPa. The first growth chamber experiment had a 5 d average VPD of 2.1 kPa and the second growth chamber experiment had a VPD average of 1.3 kPa. Based on the 95% confidence interval of the averages for each of the three experiments, there was a significant difference in the VPD between the greenhouse and the second growth chamber experiments. There the VPD in the second growth chamber experiment was also significantly different then the VPD in the greenhouse experiment.

The 5-d average temperatures covering the time FTSW thresholds were reached in the experiments were 31.2^{o}C, 29.0^{o}C and 30.3^{o}C for the greenhouse, first growth chamber, and second growth chamber experiments, respectively. Based on a 95% confidence interval, temperature in the greenhouse experiment was significantly higher as compared to the other two experiments. The temperature in the second growth chamber experiment was significantly higher than in the first growth chamber experiment.

The average photosynthetically active photon flux density in the greenhouse experiment ranged from 417 to 690 µmols m^{-2} s^{-1}. The photon flux density for the first and second experiments done in the growth chamber ranged from 520 to 545 µmols m^{-2} s^{-1}.

Leaf expansion

Table 2-1 summarizes the threshold of decrease in leaf expansion. There was substantial variation among genotypes in the FTSW threshold for the decrease in NLER. In the greenhouse experiment, Bambey-21 had the NLER threshold at the lowest FTSW, 0.350. UC-CB27 had the threshold at the highest FTSW at 0.674. In the second growth chamber experiment, the FTSW thresholds for NLER in the range of 0.284 to 0.408 FTSW were obtained for IT82E-18 and Mouride, respectively. In the first growth chamber, the FTSW range of 0.259 to 0.368 for NLER was the smallest with IT84S-2049 reaching the threshold at the lowest FTSW and Suvita 2 reaching the threshold at the highest FTSW.

Based on a 95% confidence intervals for the NLER threshold, IT89KD-288 and Mouride consistently had high thresholds across the three experiments among the tested genotypes. However, no genotype consistently exhibited a low NLER threshold across all three experiments indicating inconsistent drought tolerance in leaf expansion. Bambey 21 and

Mouride showed low thresholds for NLER in the greenhouse and first chamber experiment, but this response was not confirmed in the second chamber experiment.

The FTSW intercepts that showed the zero point of NLER ranged from an FTSW of 0.008 to 0.054 in the greenhouse experiment with an average of 0.014. The FTSW-intercept in the growth-chamber experiment ranged from an FTSW -0.023 to 0.029 with an average of 0.007.

Transpiration

Transpiration response to soil drying was examined as a useful reference for comparing leaf expansion. Table 2-2 presents the FTSW thresholds for decrease in NTR from all experiments. There was considerable difference in the range of the thresholds among genotypes across experiments. The FTSW threshold range in the greenhouse experiment was 0.308 to 0.578. The second growth chamber experiment had a narrower NTR threshold range of 0.202 to 0.331. The first growth chamber experiment in which the duration of the dry-down was short exhibited the smallest threshold range among genotypes of only 0.222 to 0.254.

The FTSW-intercept at which NTR is zero had a range of -0.057 to -0.009 with an average of -0.031 in the greenhouse experiment. In the second growth-chamber experiment the range of FTSW-intercept was from -0.049 to -0.010 with an average of -0.027.

In the greenhouse experiment and second growth chamber experiment, genotypes had similar rankings in FTSW at threshold of decrease in NTR. Only Suvita 2 showed differences in the ranking of threshold between the two experiments. In both experiments, Bambey-21

had the lowest NTR threshold of 0.308 and 0.202 in the greenhouse experiment and in growth chamber experiment 2, respectively. IT84S-2049 had the highest FTSW threshold of 0.331 in growth chamber experiment 2, and one of the highest FTSW thresholds at 0.521 in the greenhouse experiment. FTSW thresholds found in the first growth chamber experiment, however, showed no significant differences among genotypes presumably because of the short drought period.

Comparison of FTSW threshold and intercepts between NLER and NTR

As seen in Figure 2-4, FTSW thresholds for decrease in NLER occurred at higher FTSW than for NTR within genotypes for all experiments. Comparison of the 95% confidence interval between FTSW thresholds of NLER and NTR in the second growth-chamber experiment showed that the threshold point for NLER occurred at a significantly higher FTSW than NTR in Bambey 21 and Mouride. Linear regression when data for all experiments were combined showed that the FTSW thresholds for NLER and NTR were significantly related (R^2=0.75, slope estimate =1.08, and $p<0.0001$). The linear regression between NLER and NTR for the greenhouse experiment showed that variation among genotypes in FTSW threshold for NLER were significantly related to variation of FTSW threshold for NTR with an R^2 of 0.605 and slope with p-value <0.0001. But, FTSW threshold for NLER didn't significantly relate to threshold for NTR in the growth chamber experiments. The limited range of FTSW thresholds for the two growth-chamber experiments may have limited the ability to get a good fit for linear regression between thresholds for NTR versus NLER.

Comparison of the FTSW-intercept of the linear regression segment representing decreasing rates with soil drying between of NLER and NTR offered further indication of relative sensitivity of leaf expansion to drying soil. For all experiments, the FTSW-intercept for NLER was greater than for NTR (Figure 2-5) with a value of 0.016 for NLER and -0.024 (the negative value denotes that the intercept was at less than FTSW = 0) for NTR. The FTSW intercepts for NLER and NTR were significantly different with a p-value<0.0001.Within individual experiments, the zero point of NTR was also lower than NLER with p-values <0.01. For example, in the second growth-chamber experiment, average FTSW intercept for NLER was 0.007 as compared to -0.027 for NTR (p-value of 0.004).

Recovery

Regressions of both NLER and NTR versus days of recovery following watering of the stressed plants were well described by the two linear-segment model (Figure 2-6). There was initially a linear increase of NLER and NTR immediately following rewatering. The increase in NTR and NLER continued to increase until a plateau was reached, which was defined to be the normalized value of 1.0 for the two variables. Recovery of both NTR and NLER occurred rapidly. No differences in days to full recovery were seen among genotypes (Table 2-4). The days required for full recovery of NTR ranged from 2.0 to 2.3 d. The recovery leaf expansion to NLER of 1.0 was calculated to have occurred in a range of 0.7 to 1.1 d.

Since recovery occurred rapidly and no differences were seen in recovery among genotypes, it was not possible to explore correlations within genotypes in response to the dry-down phase in comparison to the recovery phase. Although it was clear that leaf expansion was more sensitive to soil drying and also responded faster to rewatering.

Discussion

Establishment of a large leaf area is important for canopy development but a larger leaf area also means larger transpirational water loss. Leaf expansion is a key physiological process leading to leaf area increase, so the response of leaf expansion to drought conditions is important to understand. The objective of this study was to detect the sensitivity of leaf expansion in cowpea genotypes to soil drying and compare leaf expansion response with transpiration response. This study also explored the ability of leaf expansion to recovery upon rewatering after drought condition. Additionally preliminary information about genotypic differences in leaf expansion response among different genotypes of cowpea was also examined.

For all genotypes tested, leaf expansion response to drought fit the two linear-segment model, with constant leaf expansion until soil water content decreased below a FTSW threshold, after which leaf expansion decreased linearly. The FTSW threshold for the decline in leaf expansion occurred earlier than the decline in NTR (Figure 2-4). Additionally, the average FTSW intercept at which the linear decrease in leaf expansion reached zero was higher than the FTSW intercept for the linear decrease in transpiration. Hence, the stopping point of leaf expansion also occurred earlier than transpiration (Figure 2-5). By both measures of sensitivity of leaf expansion to soil drying, leaf development in cowpea was more sensitive than transpiration to soil drying.

The large range seen in the FTSW thresholds for decline in leaf expansion did show that there was a genotypic basis for the variation in leaf expansion response to soil drying. Bambey 21 consistently had a decline in leaf expansion around 0.30 FTSW. Mouride

consistently had decline in leaf expansion at a higher FTSW of around 0.40 in the greenhouse and the second growth chamber experiment. Suvita 2 and IT89KD-288 also had high FTSW thresholds for leaf expansion among the genotypes tested. The consistent genotypic differences might be valuable for genotype selection for varying environmental conditions. Genotypes with higher FTSW threshold for decline in leaf expansion can have the advantage in arid regions because they will be able to limit the leaf area available for water loss by transpiration. Whereas genotypes that have more tolerant leaf expansion response to soil drying can be useful in wetter areas where it would be advantageous to have genotypes that can continue leaf development and take advantage of the available resources for optimum productivity. Since tracking leaf expansion in this experiment was non-destructive, it is possible to use this method to further phenotype different cowpea cultivars to guide selection in breeding programs.

Full recovery of leaf expansion and transpiration occurred rapidly without discrimination among genotypes. Full recovery of leaf expansion was seen in only about one day even though the plants were severely stressed to NTR ≤ 0.1. Due to this rapidity of recovery, no difference in time to full recovery among genotypes was found. Recovery of leaf expansion was more rapid than transpiration rate recovery, but even transpiration rate recovered within an average of 2 to 3 d. The fast recovery of both leaf expansion and transpiration upon rewatering indicates that cowpea is well suited for sustaining increase in leaf area in dryland regions with intermittent drought.

Another noteworthy observation was the difference seen between the three experiments in the range of FTSW threshold. For both leaf expansion and transpiration the FTSW thresholds occurred at a higher FTSW in the greenhouse experiment. For leaf expansion, the greenhouse experiment had the highest FTSW thresholds with a range of 0.350 to 0.674, the growth chamber experiments had a range of 0.276 to 0.408. Similarly, the FTSW thresholds for transpiration in the greenhouse occurred in the range of 0.308 to 0.578 and in the growth chamber experiments transpiration decline within 0.202 to 0.331 FTSW. But the first growth chamber had the smallest FTSW threshold range of 0.276 to 0.367 for NLER and 0.22 to 0.25 for NTR. The smaller range for thresholds, especially for NTR, in the first growth chamber experiment may be because the duration of the drought stress was too short to allow full expression of inhibited leaf expansion and transpiration earlier in the dry down.

The greenhouse and the second growth chamber experiment both had longer periods of drought stress (13 d and 11 d) but the greenhouse had a range of at higher FTSW thresholds than the second growth chamber experiment. The differences seen in the FTSW thresholds may also be driven by differences in vapor pressure deficit conditions among experiments. The decrease in transpiration under soil drying is associated with partial stomatal closure (Sinclair, et al., 1986). Limited transpiration due to partial closure of stomata under high vapor pressure deficit condition has been seen in crops like soybeans (Sadok, et al., 2009), peanut (*Arachis hypogaea* L.; Shekoofa, et al., 2015), maize (*Zea mays* L.;Messina, et al., 2015), sorghum (*Sorghum bicolor* L.;Shekoofa, et al., 2014). Higher VPD may result in lower turgor in the leaves resulting in both more responsive stomata and in higher threshold for leaf expansion. So, the atmospheric VPD conditions may also affect when decline in

transpiration occurs during soil drying. The greenhouse experiment had higher average VPD than the second growth chamber experiment and this difference higher VPD was associated with a higher FTSW threshold than observed in the growth chamber experiments. Further studies need to consider the VPD conditions to confirm the effect of VPD on the FTSW thresholds for decrease in leaf expansion and transpiration.

Tables:

Table 2-1. Fraction of transpirable soil water (FTSW) at the threshold of decrease in normalized leaf expansion ratio (NLER) during progressive soil drying.
Results are for eight cowpea genotypes in the greenhouse experiment and six genotypes for the growth chamber experiments. Letters following the threshold values indicate differences based on 95%confidence interval of the threshold within each experiment. R^2 of the two-segment regression for each genotype is also included.

Genotype	Threshold	Greenhouse confidence interval	R2	Threshold	Growth chamber experiment 1 confidence interval	R2	Threshold	Growth chamber experiment 2 confidence interval	R2
Bambey-21	0.351a	0.259 - 0.443	0.71	0.310ab	0.284- 0.336	0.96	0.372b	0.343 - 0.400	0.92
IT93K-503-1	0.413a	0.346 - 0.481	0.87						
Mouride	0.464ab	0.395 - 0.533	0.87	0.276a	0.248- 0.304	0.92	0.408b	0.345 - 0.471	0.75
IT82E-18	0.554bc	0.487 - 0.621	0.91	0.342b	0.308 - 0.377	0.93	0.284a	0.247 - 0.321	0.87
IT84S-2049	0.589bc	0.489 - 0.690	0.80	0.259a	0.230 - 0.289	0.92	0.341ab	0.315 - 0.348	0.93
Suvita2	0.647c	0.542 - 0.751	0.83	0.368b	0.338 - 0.397	0.95	0.363b	0.328 - 0.398	0.90
IT89KD-288	0.657c	0.562 - 0.752	0.86	0.367b	0.334 - 0.399	0.95	0.334ab	0.309 - 0.358	0.93
U-CB27	0.674c	0.567 - 0.782	0.83						

Table 2-2. Fraction of transpirable soil water (FTSW) at the threshold of decrease in normalized transpiration ratio (NTR) during progressive soil drying for eight cowpea genotypes.

Results are for eight cowpea genotypes in the greenhouse experiment and six genotypes for the growth chamber experiments. Letters following the threshold values indicate differences based on 95%confidence interval of the threshold within each experiment. R^2 of the two-segment regression for each genotype is also included.

Genotype	Threshold	Greenhouse confidence interval	R^2	Threshold	Growth chamber experiment 1 confidence interval	R^2	Threshold	Growth chamber experiment 2 confidence interval	R^2
Bambey-21	0.308a	0.287 - 0.329	0.98	0.254a	0.238 - 0.271	0.97	0.202a	0.182 - 0.222	0.89
IT93K-503-1	0.346ab	0.321 - 0.370	0.97						
Suvita2	0.393bc	0.366 - 0.419	0.97	0.240a	0.218 - 0.261	0.96	0.329c	0.306 - 0.353	0.94
IT82E-18	0.399c	0.377 - 0.421	0.98	0.249a	0.230 - 0.268	0.96	0.260b	0.233 - 0.286	0.90
Mouride	0.430cd	0.380 - 0.481	0.93	0.249a	0.231 - 0.266	0.97	0.277bc	0.244 - 0.309	0.85
IT89KD-288	0.493de	0.443 - 0.543	0.94	0.222a	0.201 - 0.244	0.94	0.289bc	0.266 - 0.311	0.92
IT84S-2049	0.521de	0.470 - 0.573	0.93	0.251a	0.233 - 0.269	0.97	0.331c	0.303 - 0.359	0.91
UC-CB27	0.578e	0.496 - 0.660	0.85						

Table 2-3. Results of linear regression between fraction of transpirable soil water (FTSW) thresholds for decrease in normalized leaf expansion ratio (NLER) and FTSW thresholds for decrease in normalized transpiration ratio (NTR).
Slope estimates with p-values and R^2 using NLER and NTR thresholds for eight genotypes in the greenhouse experiment and six genotypes for the growth chamber experiments.

	R^2	Slope estimate	P-value
All three experiments	0.747	1.08	<0.0001*
Greenhouse	0.605	1.04	0.023*
Growth chamber experiment 1	0.495	-2.75	0.119
Growth chamber experiment 2	0.002	-0.05	0.926

Table 2-4. Threshold days at which normalized transpiration ratio (NTR) and normalized leaf expansion ratio (NLER) are fully recovered when stress treatments are rewatered to well-watered conditions during the second growth chamber experiment for six cowpea genotypes. Letters following the threshold days indicate differences based on 95%confidence interval within each experiment. The R^2 for the two-segment regression is also included. The threshold days of recovery for IT82E-18 and Mouride was not detected by the regression.

Genotype	NTR Threshold	95% confidence interval	R^2	NLER Threshold	95% confidence interval	R^2
Bambey21	2.17 a	1.92 - 2.41	0.91	1.05 a	0.88 to 1.21	0.83
IT82E-18	1.99 a	1.61 - 2.36	0.92	~ 0.69	(Very wide)	0.70
IT84S-2049	2.33 a	2.14 - 2.53	0.95	1.11 a	0.83 - 1.38	0.66
IT89KD-288	2.25 a	2.05 - 2.46	0.93	1.10 a	0.94 - 1.26	0.84
Mouride	2.00 a	1.62 - 2.38	0.96	~ 0.99	(Very wide)	0.75
Suvita2	2.15 a	1.98 - 2.31	0.95	1.14 a	0.82 - 1.47	0.62

Figures:

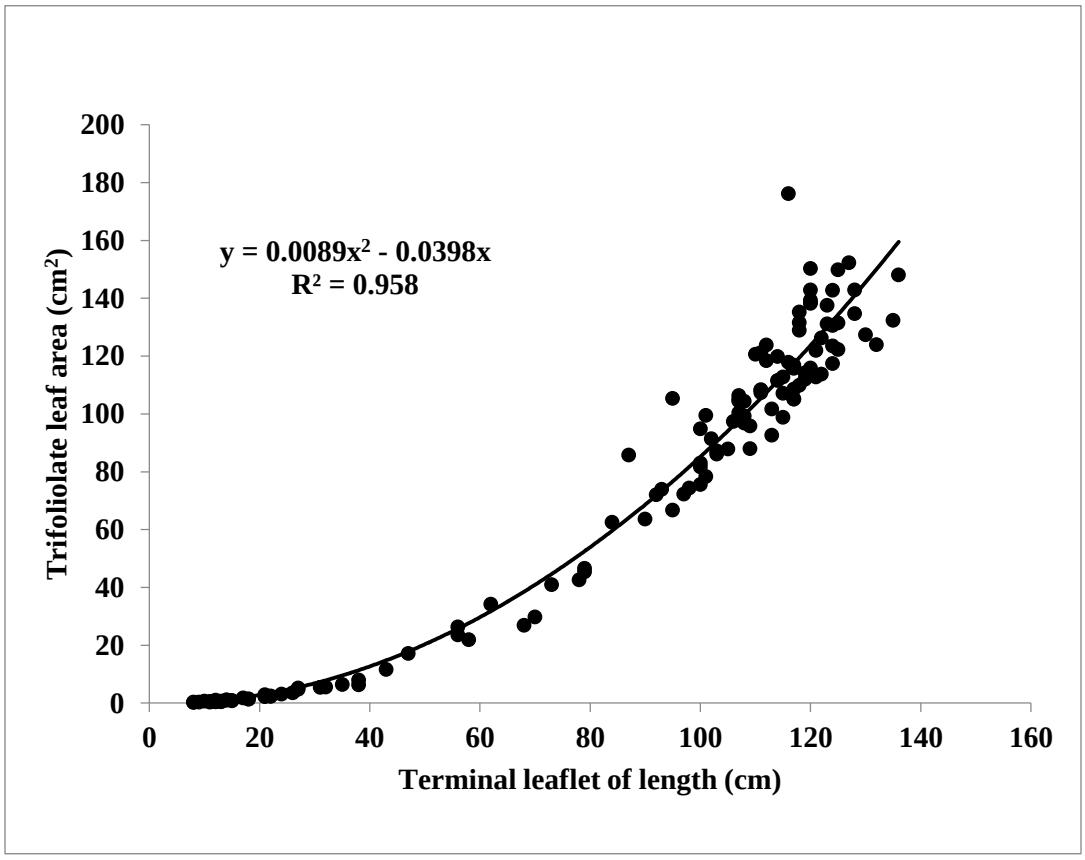
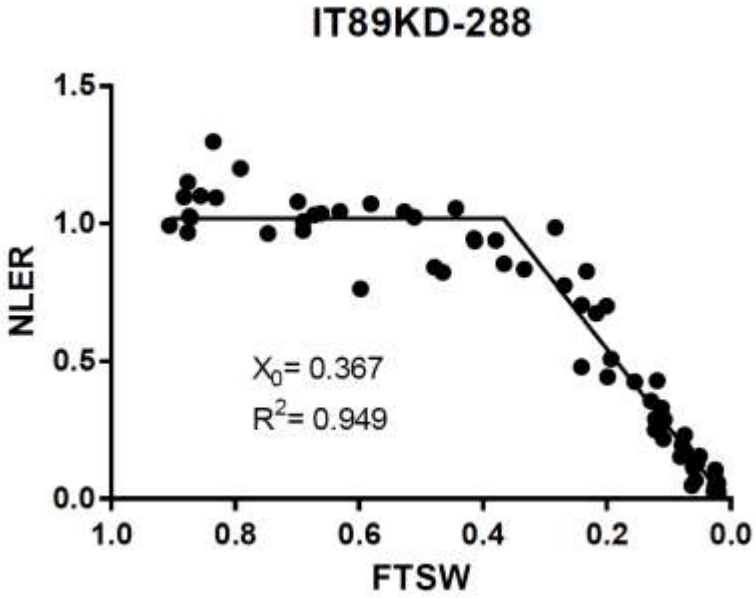
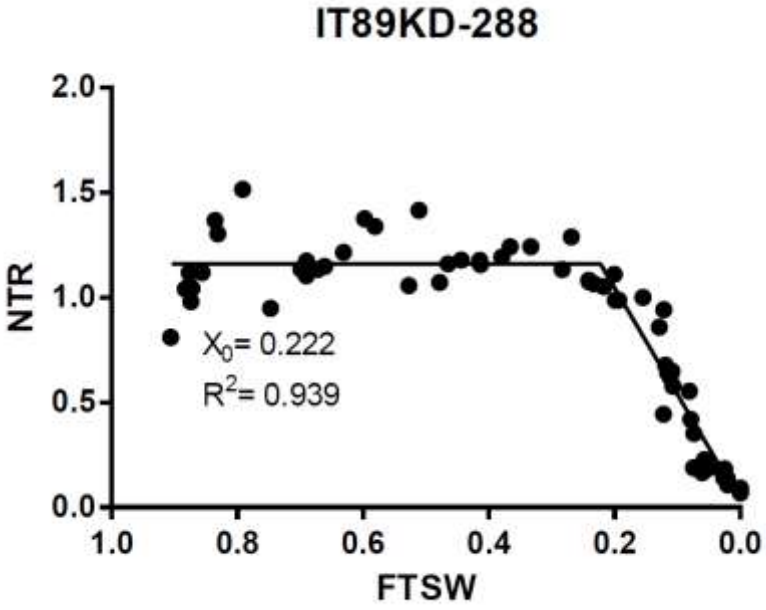


Figure 2-1. The regression between terminal leaflet length and trifoliolate leaf area for a range of leaves from well-watered plants of cowpea genotype IT89KD-288.



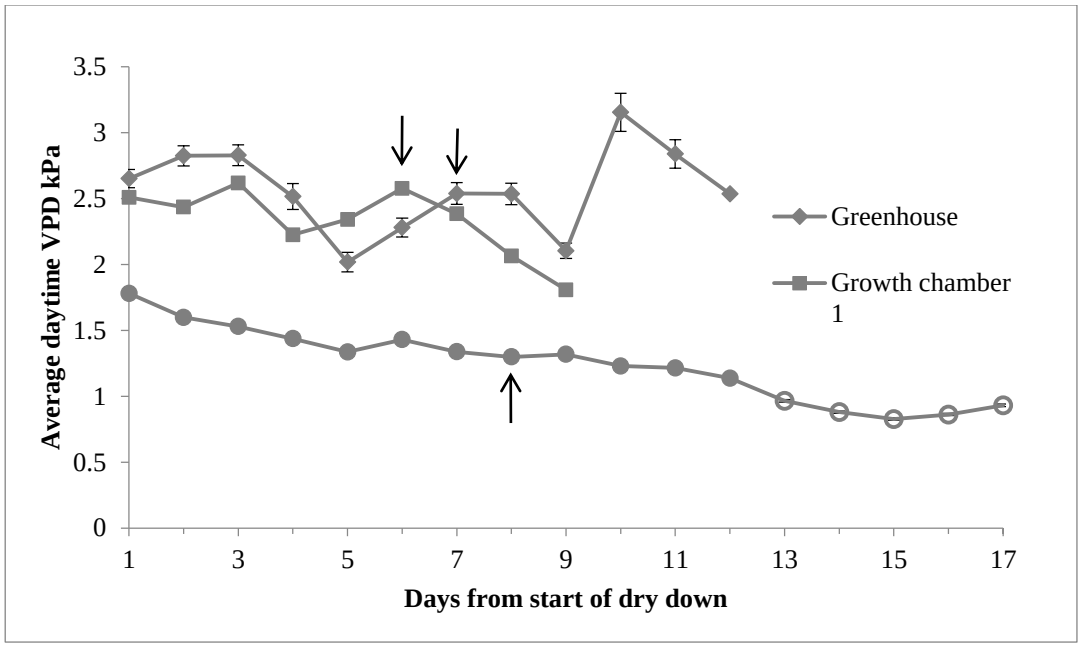
a.



b.

Figure 2-2. The two-segmented regression for fraction of transpirable soil water (FTSW) against (a) normalized leaf expansion ratio (NLER) and (b) normalized transpiration ratio (NTR) for one cowpea genotype.
Each data point represents results for one day for each of the ten plants as the drought stress progresses during the second growth chamber experiment.

a.



b.

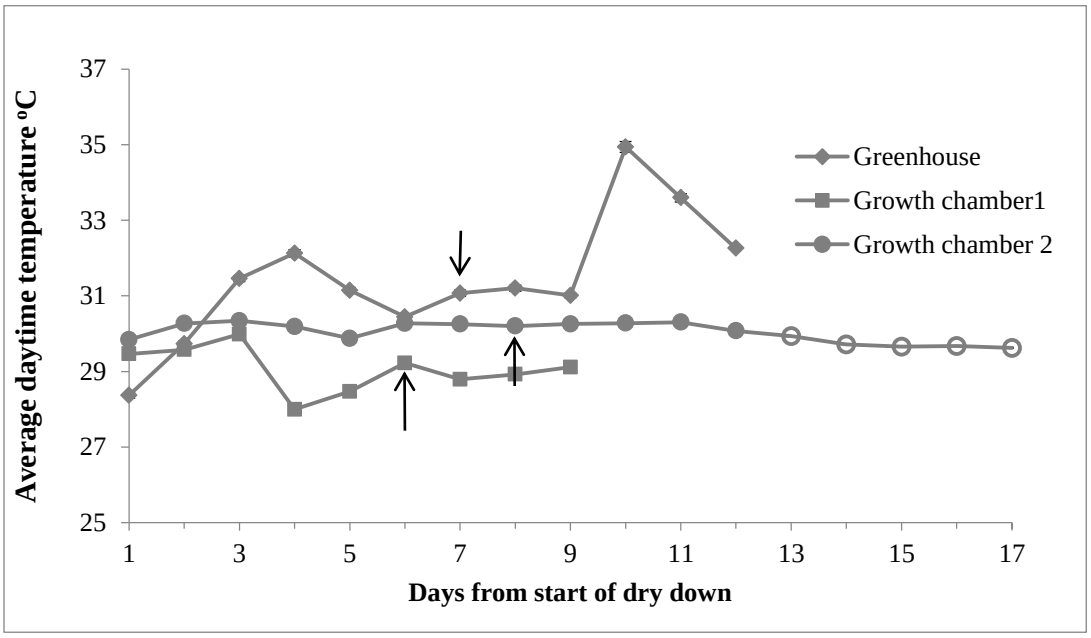


Figure 2-3. Average daytime (a) VPD and (b) temperature during the experiments. Arrows designate average day the plants started reaching the fraction of transpirable soil water (FTSW) thresholds for decrease in normalized transpiration ratio (NTR). Open circles represent the data during the period of recovery done during the second growth chamber experiment.

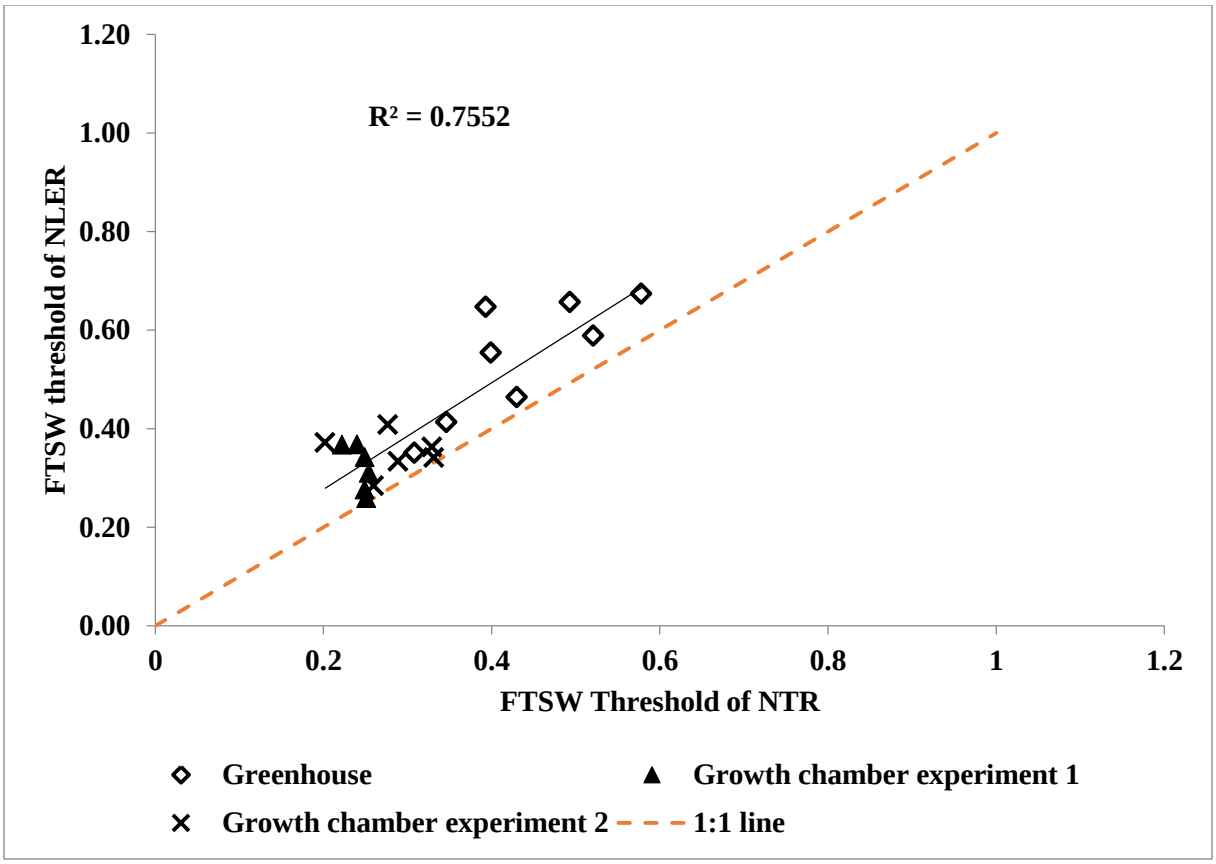


Figure 2-4. Linear regression between fraction of transpirable soil water (FTSW) thresholds for decrease in normalized leaf expansion ratio (NLER) and decrease in normalized transpiration ratio (NTR).
Data points represent eight genotypes in the greenhouse experiment and six genotypes in each growth chamber experiment.

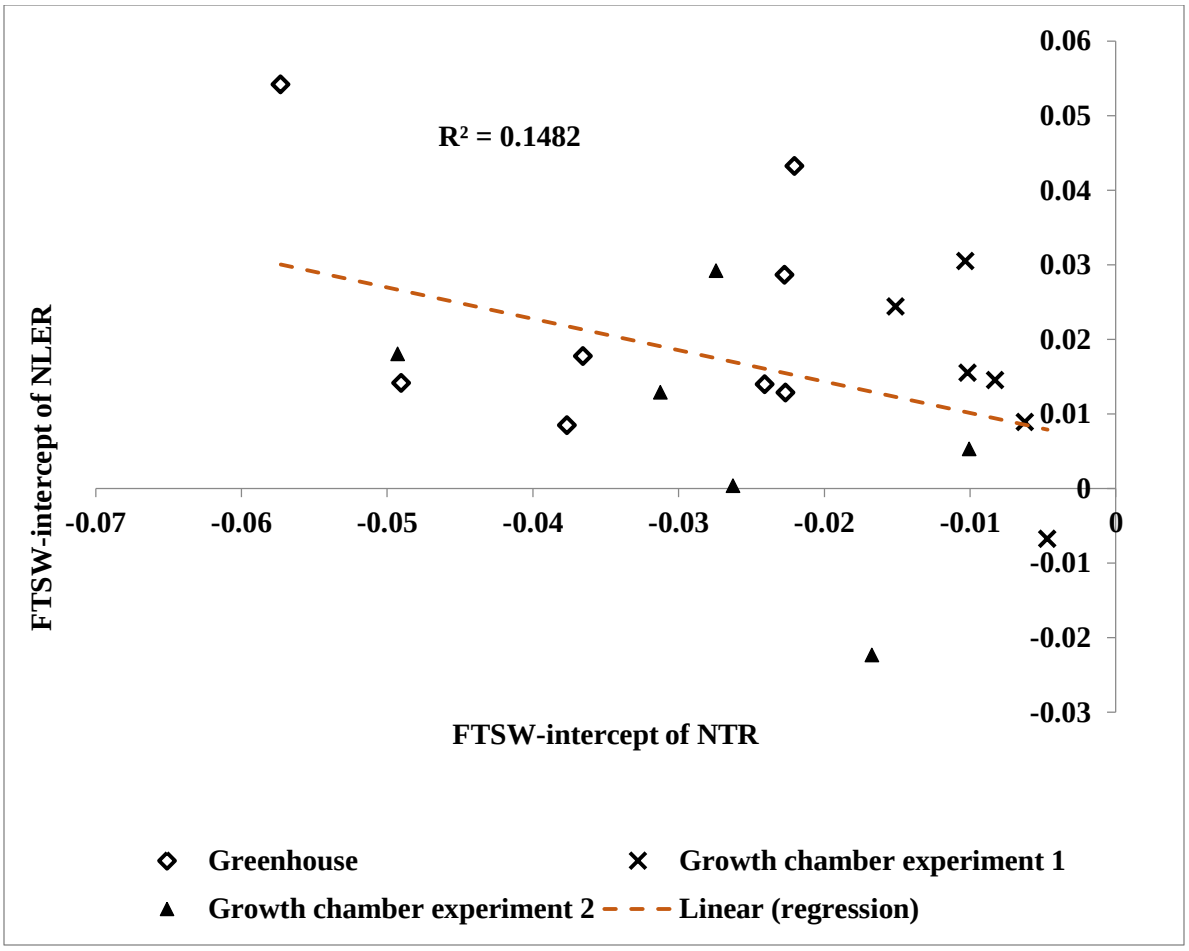
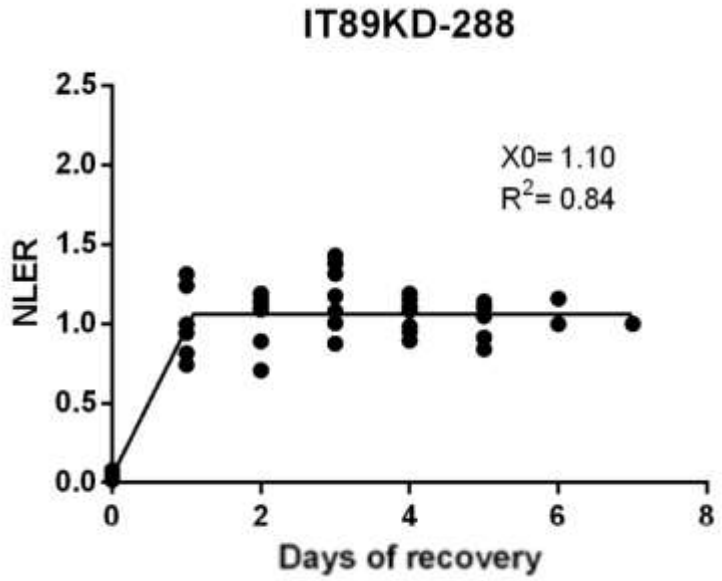
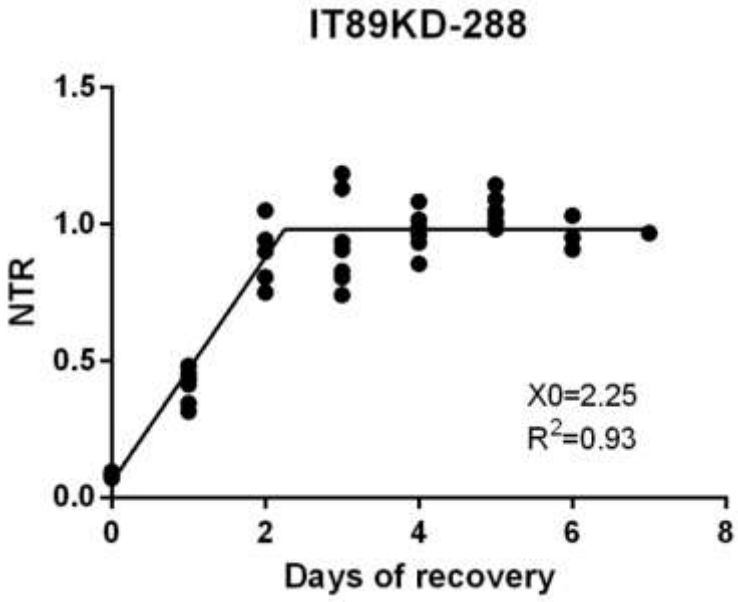


Figure 2-5. Linear regression between fraction of transpirable soil water (FTSW) intercept for zero normalized leaf expansion ratio (NLER) and zero normalized transpiration ratio (NTR).
Data points represent eight genotypes in the greenhouse experiment and six genotypes in each growth chamber experiment.



a.



b.

Figure 2-6. The two-segmented regression for (a) normalized leaf expansion ratio (NLER) and (b) normalized transpiration ratio (NTR) and days of recovery after drought stress plants were rewatered to well-watered conditions for one genotype.
Each data point represents results for one day for each of the ten plants for the second growth chamber experiment.

CHAPTER 3

Comparison of Leaf Emergence (Phyllochron Index) and Leaf Expansion Response to Soil Drying in Cowpea Genotypes

Introduction

Leaf area of a crop defines the amount of photosynthetically active radiation the plant can capture for production of photosynthates, which eventually determines how much plant mass can be accumulated. Decrease in leaf area as a result of drought stress has been known to decrease productivity in many crops, including cowpeas (*Vigna unguiculata*; Pandey, et al., 1984). Since cowpea is commonly grown in the drier regions of the world, including regions of sub-Saharan Africa (Ehlers, et al., 1997), it is important to clearly identify how water-deficit conditions affect leaf area development in cowpeas.

Drought stress has been seen to affect leaf area ratio (plant leaf area divided by plant mass and specific leaf area (leaf area divided by leaf mass) in cowpea (Aniya, et al., 2004). However, these results do not relate directly to leaf area development. Leaf expansion and leaf area index (LAI) were also seen to be affected by drought stress (Pandey, et al., 1984). But it was not clear which processes of leaf area development were affected by drought. More specifically, the separation of the responses of physiological processes described by leaf expansion and leaf emergence to drought effects has not been reported. Since leaf emergence is a result of cell division while leaf expansion occurs in large part due to cell enlargement, water deficit potentially affects both of these processes.

In Chapter 2, cowpea leaf expansion in response to soil drying was found to be readily expressed as a function of soil water content (FTSW: fraction of transpirable soil water). But, there have been virtually no studies that investigated cowpea leaf appearance in response to soil drying. Craufurd et al. (1997) studied how temperature and photoperiod affects leaf appearance in cowpeas but did not consider drought stress. As discussed by Lecour et al. (1998), other than temperature, drought is also a stress factor that can affect leaf initiation rates. A study of leaf appearance response to drought, done in field peas (*Pisum sativum* L.; Lecoeur, et al., 1998), showed that rate of leaf production did decrease as a result of soil drying after a FTSW threshold of about 0.20 was reached. In their study, however, they used a non-linear model to fit the response of leaf production rate to soil drying which made exact definition of the FTSW threshold somewhat ambiguous. Since their results were an initial plateau followed by an approximately linear decrease in leaf production at low FTSW, it seemed likely their data could also be represented using a two-segment linear model. The two-segment linear model would define a threshold FTSW at which rate of leaf production began decreasing linearly with further soil drying.

The study of leaf production rate in field peas was able to show a decrease in leaf production but a large variability was observed because of an uncertainty in determining which new leaves could be considered fully emerged. Vendeland et al. (1982) used an alternative method of measuring the non-discrete quantification of plant morphological age of soybean [*Glycine max* (L.) Merrill]. The key to this approach was to quantify the continuous development of young leaves on the main stem as the Phyllochron Index (PI), which was developed by Erickson and Michelini (1957). The PI defines the node number of a

plant as leaves that have leaf lengths that are greater than or equal to a reference length. The phyllochron index adds a decimal fraction (calculated as a ratio of the two newest leaves) to the node number so that the leaf number/morphological age of the plant is expressed as a continuous value over the course of time and not just discrete node numbers. But, leaf emergence as PI has not been used to quantify the response of leaf emergence to soil drying in cowpeas.

Furthermore, a comparison of leaf emergence and leaf expansion response to drought can help in understanding how water deficit affects each of the two processes involved in leaf area. The study done with field peas (Lecoeur, et al., 1998) showed that leaf production rate was less sensitive to soil drying than leaf expansion. In Chapter 2, leaf expansion response was seen to be more sensitive then transpiration as indicated by a higher FTSW threshold for decline in leaf expansion occurring at a higher FTSW than transpiration. The cessation of leaf expansion also occurred at a higher FTSW than transpiration as the soil dried to very low water content. Since drought response of leaf emergence in cowpea has not been studied, there are no studies that show the response of leaf emergence compared to leaf expansion under water deficit conditions.

The objectives of this research were: (1) To observe the response of leaf emergence as PI to soil drying as a function of fraction of transpirable soil water (FTSW). To find the quantitative response of leaf emergence to soil drying, it was necessary to first ensure PI could be used as a measure of leaf emergence in cowpea. (2) To determine how leaf-emergence response differed from leaf expansion response by comparing the FTSW at the initiation points of decline and cessation for both. Also, since several genotypes were tested,

it was possible to determine if there were any differences in leaf emergence response among genotypes. The resolution of these objectives will make it possible to more accurately predict cowpea development and yield during drought stress, and offer guidance in developing improved genotypes.

Materials and Methods

In Chapter 2, leaf expansion response to soil water-deficit was reported. These experiments involved measuring leaflet length, which provided data to calculate PI. The methodology for the experiments is summarized briefly here.

Eight genotypes were tested in a greenhouse experiment (Bambey-21, IT82E-18, IT89KD-288, UC-CB46, IT84S-2049, Mouride, UC-CB27, Suvita2, and IT93KD-503-1). Subsequently, two growth chamber experiments were done with six genotypes (Bambey-21, IT82E-18, IT89KD-288, IT84S-2049, Mouride and Suvita2). Only the greenhouse experiment and the second growth chamber experiment were used in this study. The first growth chamber experiment was excluded because the drought stress progressed rapidly over only 8 days resulting in a short length for the stress period that was not of sufficient duration to give the needed resolution in the observations of plant development response to drought. The drought in the greenhouse experiment lasted 13 days and the second growth chamber experiment had a drought period of 11 days.

The greenhouse experiment was done in Raleigh, NC (46°35’ N, 39°78’ W) with sowing 9 May 2014. Throughout the growth period and the experimental period, temperature was regulated for cooling at 28^{o}C. Supplemental lights were used from 5:00 pm to 10:00 pm

Eastern Standard Time to extend the day length to approximately 16 h. The dry-down experiment was initiated on 2 June 2014.

The growth chamber experiment was done in a walk-in growth chamber in the North Carolina State University Phytotron facility. The day/night temperature was regulated to 30/24^{o}C. Day length was set to be 12 h with a 3 h light interruption using incandescent lamps. Photosynthetically active radiation in the growth chambers was an average of 533 µmol $m^{-2}s^{-1}$.

For both experiments, seeds inoculated with *Bradyrhizobium japonicom* (N-Dure, INTX Microbials, Kentland, IN) were grown in loamy soil (69% sand, 18% silt, and 13% clay) in 4L plastic pots with a diameter of 20 cm. After the emergence of the 1st trifoliolate leaf, plants were thinned to one plant per pot. To keep the plants in a vegetative stage any occasional flowers were removed.

Dry down experiment

At three weeks following sowing and when the plants had at least 3 to 4 trifoliolate leaves, plants were prepared for the dry-down experiment by watering plants until dripping. In the morning after watering, the pots were put in plastic bags, which were tied around the bottom of the plant's main stem to prevent water loss by evaporation. Then, each pot was weighed to get the initial weight at pot capacity. For the duration of the experiment, pots were weighed every morning to find water loss by transpiration. The well-watered plants were selected so that they would represent the range of plants sizes undergoing stress treatment.

In the greenhouse experiment, the three well-watered plants were watered daily so that pot weight was maintained at 200 g below the initial weight. Five plants per genotypes were given the stress treatment where soil was allowed to dry progressively through water loss by transpiration. To ensure drought stress developed gradually, stress plants were watered if needed so that the pots did not lose more than 100 g water daily.

In the growth chamber experiment, the rewatering conditions were adjusted to achieve an extended period of soil drying. Three well-water plants were watered daily to maintain pots at 150 g below the initial weight. There were ten stress plants for each genotype. The stressed plants were watered so that there was only 60 g of net daily water lose. Additionally, to decrease overall transpiration rates, two humidifiers were placed in the growth chamber to maintain the vapor pressure deficit (VPD) within the range of 1-2 kPa.

The leaf number and length of the corresponding terminal leaflet were measured daily with a ruler until leaflet length stopped increasing for 3 consecutive days. At the end of the experiment, the leaf area (LI-3100C, Li-cor, Lincoln, NE) of each trifoliolate leaf and its corresponding terminal leaflet length were measured destructively.

Data analysis

The level of stress to which each plant was subjected was quantified as fraction of transpirable soil water (FTSW). The FTSW calculation for each pot was based on the total transpirable soil water between pot initial weight and pot weight at the end of the drought period when transpiration had decreased to 10% or less of the well-watered plants. The difference of the daily pot weight from the initial weight was used to calculate FTSW= (daily wt. - final wt.)/(initial wt. – final wt.)

Normalized transpiration rate (NTR) was calculated daily for each stressed plant as described in Chapter 2. Also, the calculation of normalized leaf expansion rate was presented in Chapter 2 using the same approach as for NTR. Briefly, the leaf expansion ratio (LER) for each stressed plant on each day was found between its leaf area expansion and the average leaf area increase of the well-watered plants. The LER of stress plants for each day was normalized by its average LER during the first three days of the experiment when the pot is still under well-watered conditions to obtain daily normalized leaf expansion ratio (NLER).

Leaf emergence (Phyllochron Index)

In the study reported in Chapter 2, measurements of terminal leaflet length allowed PI to be calculated for each stress plant on each day. A modified version of the following equation derived by Erickson and Michelini (1957) was used to find the PI of each plant.

$$PI = n + \frac{\log(L_n) - \log(R)}{PR} \qquad \text{(Eqn 1)}$$

In this equation, the node number (n) is defined as the number of leaves for which the terminal leaflet length is equal to or longer than a reference length R. The value of R was selected from a range of leaf lengths at which all leaves were in an exponential extension of leaflet length. For this study, a reference length of 27 mm was selected after examining leaflet extension of leaves of well-watered plants. The terminal leaflet length of the youngest leaf that is equal to or greater than R is defined as L_n and the leaf with a terminal leaf length smaller than R is defined as L_{n+1}.

Erickson and Michelini (1957) showed that leaves are produced at regular intervals of time. Since the time interval between leaf productions was seen to be constant it followed that the ratio of the two youngest leaves L_n and L_{n+1} measured at regular time intervals was also assumed to be a constant. This ratio between L_n and L_{n+1} was defined as the plastochron ratio (PR).

Vendeland et al. (1982) pointed out that PR is not constant under stress conditions because the rate of leaf production is influenced by drought stress. The average PR of well-watered plants should be used as a constant in the determination of PI of stressed plants. In this study, the plastochron ratio (PR), the log of the ratio of L_n and L_{n+1} is considered to be constant based on observations for well-watered plants.

$$PR = \log \frac{L_n}{L_{n+1}} \qquad \text{(Eqn 2)}$$

The measurements of leaflet length of well-watered plants were analyzed to examine the assumption of constant PR. The analysis of PR was done separately for well-watered plants of each genotype. Average PR for each genotype was determined and Fishers LSD comparison was done to analyze differences in PR of well-watered plants among genotypes.

To observe small increments of change in PI it is important to focus on the newest leaf, L_{n+1}. By definition, leaf L_n is always greater than or equal to R. R is used as a basis of comparison for increase in L_n. Since L_{n+1} defined as the leaf smaller than R, Vendeland et al. (1982) defined a new reference length R' to be able to observe increase in L_{n+1}. R' is derived

from R and PR_{ave} as: R'= $\frac{e^{(PR_{ave})}}{R}$. PI is defined as the ratio of the log difference of L_{n+1} from R' to the PR_{ave} of well-watered plants added to the node number of the plant.

$$PI = n + \frac{\log(L_{n+1}) - \log(R')}{PR_{ave}} \qquad \text{(Eqn 3)}$$

Daily increase in PI for each stress plant was found with the difference in PI between consecutive days (DPI).

Daily values of DPI of the stressed plant of each genotype were graphed as a function of FTSW and a two-segmented linear model was fit to find the FTSW threshold for decrease in DPI. The FTSW-intercepts were found to obtain the zero point at severe stress for DPI in each genotype.

Results

Reference length

It was seen that leaflet lengths did increase exponentially over time until plateauing at a maximum leaflet length. Figure 3-1 shows leaflet extension of a well-watered plant. In all well-watered plants the leaflets were still expanding exponentially at the length of 27mm. Hence it was possible to use R = 27 mm as reference to observe increases in new leaf L_n and consequently R' to observe increases in L_{n+1}.

Plastochron Ratio

Vendeland et al. (1982) and Sinclair (1984) used a constant PR of 0.43 and 0.42, respectively, to determine PI in soybeans under drought condition. The mean PR of the

cowpea genotypes in this study ranged from 0.34 to 0.46 (Table 3-1). Mean PR values for IT89KD-288 differed significantly from Suvita2 and IT82E-18 with $p<0.0001$. IT89KD-288 had the lowest mean PR and IT82E-18 had the highest (Table 3-1).

To ascertain if PR is a constant for well-watered plants within a genotype, linear regressions were done between PR and days from the start of the experiment. The time interval between production of new leaves represented by PR decreased only slightly, but significantly, over the days of the experiment in all genotypes except IT48S-2049 (Table 3-2). For IT84S-2049, linear regression gave a slope estimate of 0.005 which was not significantly different from zero ($p = 0.145$). The correlation coefficient for PR vs. days was low ($R^2 < 0.38$) in all cases (Table 3-2).

To examine if node number affected the ratio of the two newest leaves L_n and L_{n+1}, linear regressions were done for each genotype between PR and node number for well-watered plants. The slope of the regressions were negative for all genotypes, and significant ($p \leq$ 0.0001 to 0.0023;Table 3-3) for all genotypes except IT84S-2049. In the cases of IT82E-18 and Mouride, the R^2 was fairly high with values of 0.64 and 0.63, respectively (Figure 3-2).

To determine if the variation seen in PR over time and development stage resulted in large variation in PI of stressed plants, PR values at the upper and lower limit of a 95% confidence interval of the average PR determined for each genotype were used to make two separate calculations of PI for stressed plants over the dry-down period. As illustrated in Figure 3-3, for the genotype IT82E-18, the two sets of PI values calculated from the differing estimates of PR showed very little difference. Given that the variation in PR had virtually no

impact on PI estimates, the average PR of well-watered plants for each genotype was assumed constant (Table 3-1).

Change in phyllochron index

Although the scatter in the DPI was substantial, the plot of DPI against decreasing FTSW fit a two-segmented linear regression model for all genotypes as represented by Figure 3-4 for IT82E-18. At high FTSW, the values of DPI were a plateau followed by linearly decreasing DPI below a threshold FTSW. The threshold for decrease in DPI, the FTSW intercept and the R-squared values for each genotype are listed in Table 3-4. Even with large variability in the observed data, the regression between FTSW and DPI resulted in R^2 values indicating fairly good fits.

For the greenhouse experiment, the FTSW threshold for decrease in DPI among genotypes was found to be in a range of 0.32 to 0.53 FTSW. But since the 95% confidence interval for each threshold point was so large, no significant differences were found among genotypes. A similar range in FTSW thresholds for DPI was found in the growth chamber experiment (0.28 to 0.53). The confidence interval for each threshold point for decrease in DPI was smaller than the greenhouse experiment, likely a result of the greater number of replicate plants, and allowed comparison among genotypes.

Bambey 21 was seen to have similar FTSW thresholds in both experiments at around 0.30. IT82E-18 and IT84S-2049 also had consistently high thresholds at around 0.45 and 0.53, respectively. Differences among genotypes were seen in the growth chamber experiment with Bambey 21 and Mouride (FTSW threshold: 0.28 and 0.33) being significantly different from Suvita 2 and IT84S-2049 (FTSW threshold: 0.50 and 0.53).

The FTSW intercept which indicates the zero point of DPI ranged from -0.032 to 0.027 in the greenhouse and -0.013 to 0.055 in the growth chamber (Table 3-4). A large variability around the intercept did not allow for a determination of significant confidence intervals for each genotype.

PI and NLER comparison

Comparison of FTSW breakpoint for DPI and FTSW breakpoint for NLER across genotypes showed no correlation (Figure 3-5). Also, the results in Figure 3-5 showed that overall there was no trend to indicate the decrease in DPI occurred at a higher FTSW than the decrease in NLER (linear regression, $p>0.05$). FTSW threshold for DPI happened later than decrease in NLER in first experiment for five genotypes. While in the second experiment DPI was seen to be more sensitive than NLER for four genotypes.

Comparison of the FTSW point at which DPI and NLER reached zero was done by comparing FTSW-intercept of the FTSW vs. DPI regression with the FTSW-intercept of the FTSW vs NLER regression for each genotype. The mean FTSW-intercepts for DPI was not significantly different from the FTSW-intercept for NLER ($p>0.30$). Linear regression of DPI FTSW-intercepts compared to NLER FTSW-intercept did not have significant slope estimates (p-value>0.05) for either experiment. When data from both experiments are combined, a significant linear relationship (p-value=0.017 for slope estimate of 0.86 and R^2=0.39) between the x-intercepts of DPI and NLER was seen.

Discussion

Leaf area development is a key process in understanding crop growth and yield formation. In turn, leaf development can be divided into two activities of leaf emergence, characterized by cell division, and leaf expansion, characterized by cell expansion (Blum, 1996). Both of these activities appear to be sensitive to water-deficit, but it is not clear if there are differences in sensitivity between leaf emergence and expansion. The major objective of this study was to resolve in cowpea the relative sensitivity of the two activities to developing soil water deficit.

It was first necessary to determine whether it was possible to use daily changes in PI as a measure of leaf emergence response to progressive soil drying. The calculation of PI requires the input of PR (Eqn 2). It was found that PR of well-watered plants decreased slightly over time. This response indicated a slight shift in timing of leaf development so that the interval between appearance of successive leaves was decreasing. Nevertheless, it was found that variability in PR had very little impact on the estimates of PI for stress plants (Figure 3-3). These results offered confidence that a constant average PR could be used to effectively calculate plant PI.

Leaf DPI in all the tested genotypes was constant at high FTSW and decreased linearly after a threshold FTSW. The linear decrease in leaf emergence rate occurred at a range of about 0.30 to 0.50 FTSW. These results were obtained in spite of the large variability observed in DPI. Since the daily difference in PI was small as a result of node addition occurring only every 2 to 3 d, DPI values were always less than 1.0 and the results were essentially inherently variable. Nevertheless, the use of DPI as a measure of cowpea leaf

emergence response to soil drying proved to be valuable in describing cowpea leaf area development and phenological development under drought-stress conditions.

The FTSW intercept for the cessation of plant DPI ranged from an FTSW point of 0.055 to -0.032. In this study, the high variability around the FTSW intercept prevented the determination of confidence intervals. But the range of FTSW intercepts indicated the possibility of some genotypes stopping leaf production at an earlier water content, which would give these genotypes a better chance at survival in arid conditions. Since even a small change in FTSW can influence water use, plants stopping leaf production at an earlier water content will be able to conserve resources for a few more days.

Overall, leaf emergence was not less sensitive than leaf expansion in its response to soil drying. The range of FTSW at which DPI declined overlapped with the range of FTSW where NLER started declining. But no clear relationship between thresholds for DPI and thresholds of NLER was seen since the regression between the thresholds of the two responses was not significant. From this study it was found that leaf emergence can be both more sensitive (genotypes) and less sensitive (genotypes) than leaf expansion under progressive soil drying (Figure 3-5). Further, the FTSWs at zero DPI compared to FTSWs at zero NLER also showing that leaf emergence ceased either before or after leaf expansion stops under dry soil. Within the two experiments, cessation of leaf emergence did relate to cessation of leaf emergence (R^2=0.29, p=0.014). Overall, leaf emergence response did not have a significantly different response than leaf expansion to drought stress in cowpea for the tested genotypes.

Distinct genotypic differences in leaf emergence (DPI) in response to soil drying were seen in the growth chamber experiment based on a 95% confidence interval for the FTSW threshold in decreasing DPI. Bambey 21 had consistently low FTSW thresholds around 0.30. IT82E-18 and IT84S-2049 had consistently high FTSW thresholds of 0.45 and 0.53, respectively. These genotypic differences within experiments and similarities among experiments indicate that the FTSW threshold for decrease in leaf emergence is a genotypic trait. For example, since Bambey 21 continued leaf production at lower soil water content, this genotype might be good for less dry regions where the crop can push ahead with canopy development based on an expectation that there will be rain or irrigation before soil drying becomes severe. Whereas, IT82E-18 and IT84S-2049 might be better suited for very dry environments where the sensitivity of leaf emergence to soil drying would allow for conservative water use and the plants would be better positioned for more very severe drought conditions.

Tables:

Table 3-1. Mean phyllochron ratio (PR) for well-watered plants from six cowpea genotypes from the second growth chamber experiment.
Fisher's LSD test was used to find if the PR differed among genotypes. The letters next to the mean values indicate differences in mean.

Genotype	MEAN	LSD
IT89KD288	0.342	a
Bambey21	0.385	ab
Mouride	0.410	ab
IT84S2049	0.432	ab
Suvita2	0.458	b
IT82E18	0.464	b

Table 3-2. Linear regression of phyllochron ratio (PR) versus days from the start of the experiment in well-watered plants for six cowpea genotypes.
The PR of each day for each plant is used to find the slope estimate of the change in PR and the p-value of the slope along with the R^2 values. Only IT84S-2049 does not have a significant slope at α=0.05.

Genotype	R-squared	Slope estimate(1/number)	P value of slope>t
IT84S-2049	0.040	0.005	0.145
Bambey21	0.214	-0.010	0.0011
IT82E-18	0.379	-0.010	<0.0001
IT89KD-288	0.290	-0.011	<0.0001
Mouride	0.324	-0.011	<0.0001
Suvita-2	0.176	-0.011	0.0016

Table 3-3. Linear regression of phyllochron ratio (PR) versus node number of the experiment in well-watered plants for six cowpea genotypes.
The PR and node number of each day for each plant is used to find the slope estimate of the change in PR and the p-value of the slope along with the R^2 values. Only IT84S-2049 does not have a significant slope at α=0.05.

Genotype	R-squared	Slope estimate(1/number)	P value of slope>t
IT84S-2049	0.001	-0.002	0.87
Bambey21	0.210	-0.019	0.0023
IT82E-18	0.636	-0.044	<0.0001
IT89KD-288	0.374	-0.030	<0.0001
Mouride	0.632	-0.045	<0.0001
Suvita2	0.303	-0.031	<0.0001

Table 3-4. Fraction of transpirable soil water (FTSW) at the threshold for decrease in phyllochron index (DPI) and FTSW intercept at the cessation of DPI under soil drying. The results are for six cowpea genotypes in the greenhouse and second growth chamber experiment. Letters following the threshold values indicate differences based on 95% confidence interval of the threshold within each experiment. R^2 of the two-segment regression is also included.

	Greenhouse experiment			Growth-chamber experiment		
Genotype	FTSW threshold	FTSW intercept	R^2	FTSW threshold	FTSW intercept	R^2
Bambey-21	0.395a	0.034	0.78	0.280a	0.008	0.72
Mouride	0.508a	0.026	0.80	0.331ab	0.023	0.67
IT89KD-288	0.411a	-0.013	0.66	0.371abc	-0.032	0.63
Suvita2	0.402a	0.055	0.64	0.496cd	-0.006	0.71
IT82E-18	0.458a	0.029	0.77	0.498bcd	-0.012	0.63
IT84S-2049	0.526a	0.055	0.81	0.5331d	0.027	0.77
IT93K-503-1	0.318a	0.028	0.57			
UC-CB27		0.029	0.71			

Table 3-5 Fraction of transpirable soil water (FTSW) at the threshold for decrease in normalized leaf expansion ratio (NLER) and FTSW intercept at the cessation of NLER under soil drying.
The results are for six cowpea genotypes in the greenhouse and second growth chamber experiment. Letters following the threshold values indicate differences based on 95% confidence interval of the threshold within each experiment. R^2 of the two-segment regression is also included.

	Greenhouse experiment			Growth-chamber experiment		
Genotype	FTSW threshold	FTSW intercept	R^2	FTSW threshold	FTSW intercept	R^2
Bambey-21	0.351a	0.013	0.71	0.372b	0.005	0.92
IT93K-503-1	0.413a	0.029	0.87			
Mouride	0.464ab	0.018	0.87	0.408b	0.029	0.75
IT82E-18	0.554bc	0.014	0.91	0.284a	-0.022	0.87
IT84S-2049	0.589bc	0.014	0.80	0.341ab	0.018	0.93
Suvita2	0.647c	0.043	0.83	0.363b	0.013	0.90
IT89KD-288	0.657c	0.008	0.86	0.334ab	0.0004	0.93
U-CB27	0.674c	0.0542	0.83			

Figures:

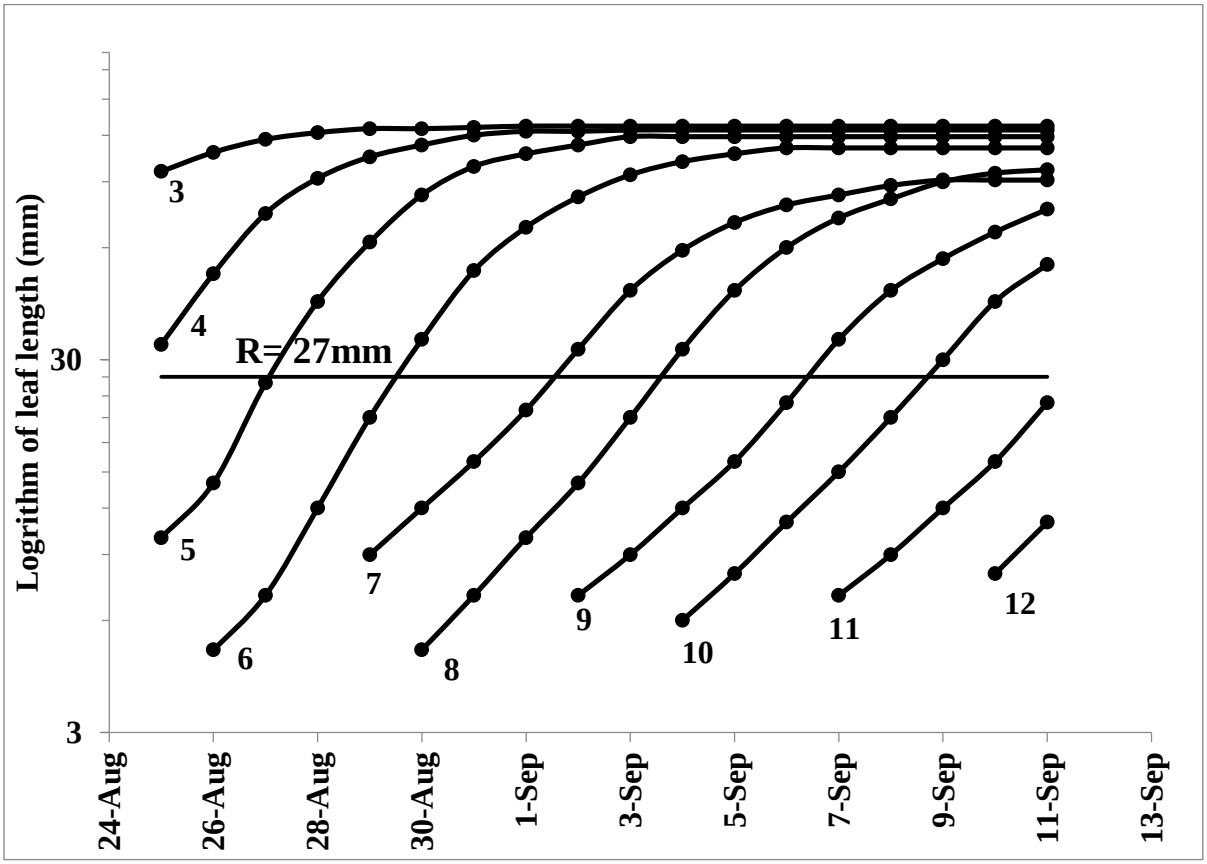


Figure 3-1. Leaf length increase in well-watered plants.
The y-axis represents the logarithm of leaf lengths. Each numbered line follows the development of the leaf with the same serial number.
R denotes the reference length at which all leaves are expanding exponentially. The data represents a well-watered IT82E-18 plant (pot number21).

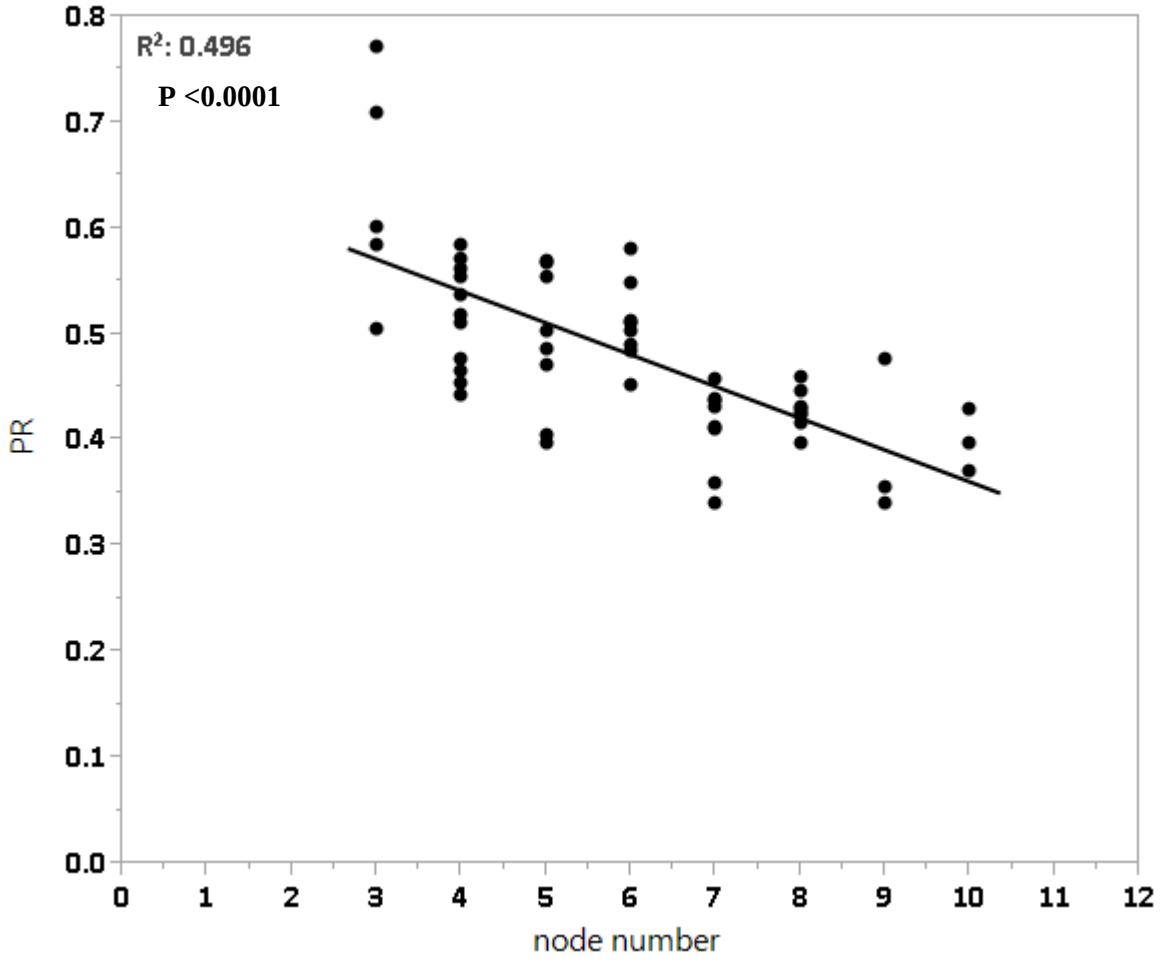


Figure 3-2. Linear regression of the phyllochron ratio (PR) and node number of each day for each of the three well-watered plant of the cowpea genotype IT82E-18.
The R^2 and p-value are included in the figure.

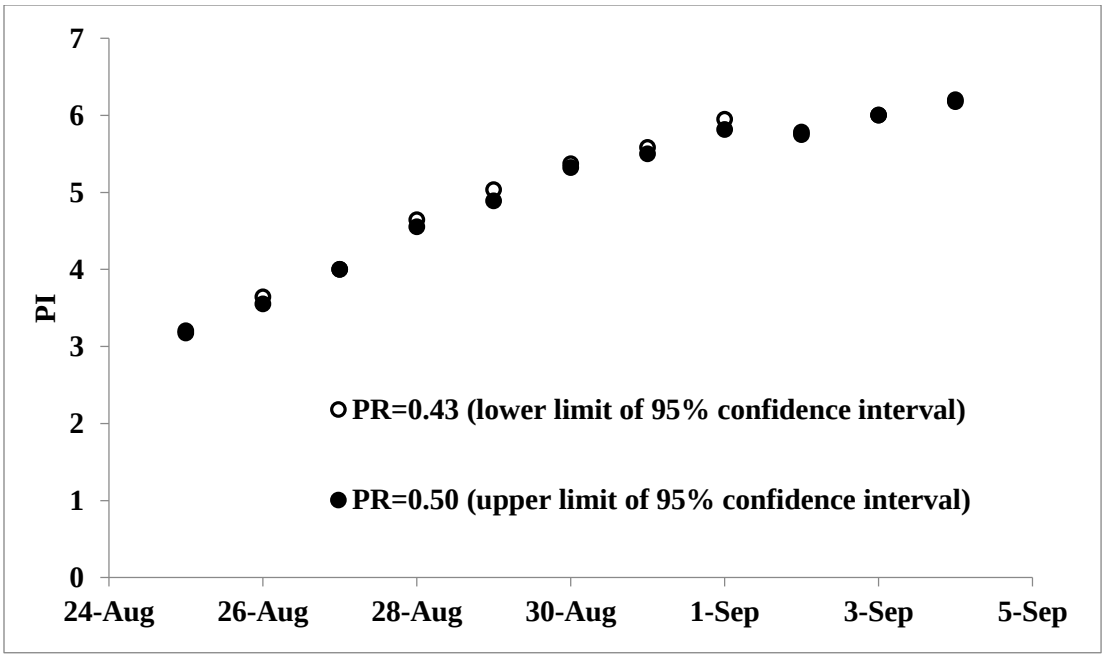
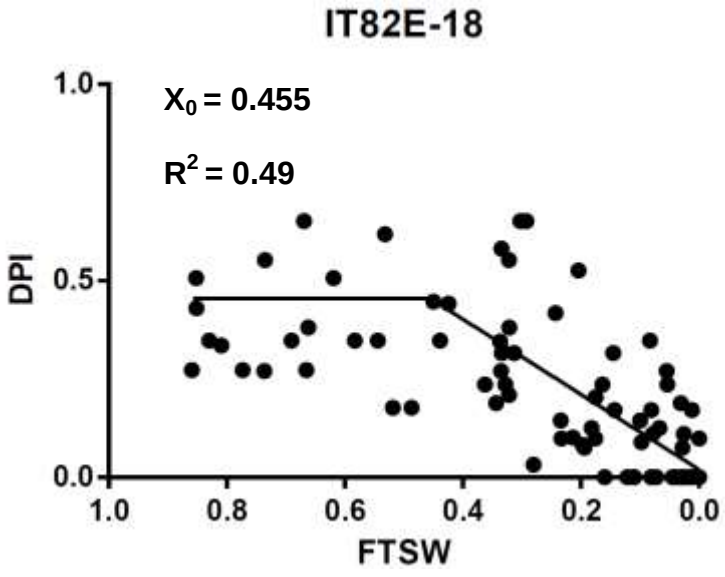
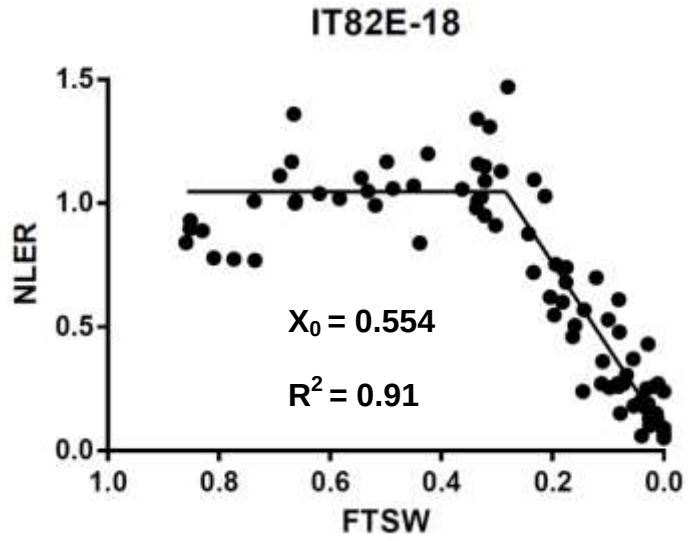


Figure 3-3. Sensitivity of the phyllochron index (PI) of one cowpea plant (IT82E-18, plant number 14) under drought stress to two values of phyllochron ratio (PR).
The two values of PR represent the lower and upper limits of the 95% confidence interval of the mean PR for the well-watered plants of IT82E-18 over the course of the experiment.



a.



b.

Figure 3-4. The two-segmented regression for fraction of transpirable soil water (FTSW) against (a) change in phyllochron ratio (DPI) and (b) normalized leaf expansion ratio (NLER) under soil drying for one genotype.
Each data point represents results for one day for each of the ten plants for the second growth chamber experiment.

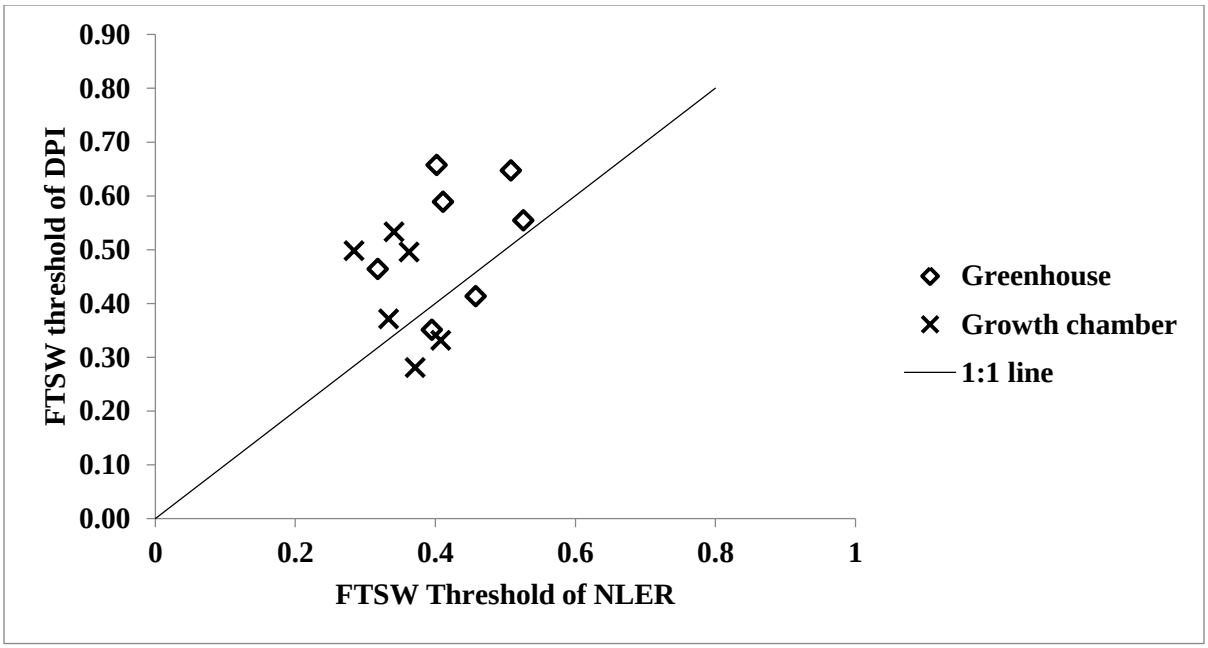


Figure 3-5. Plot of fraction of transpirable soil water (FTSW) thresholds for decrease in phyllochron index (DPI) and decrease in normalized leaf expansion ratio (NLER). Data points represent six genotypes each in the greenhouse experiment growth chamber experiment.

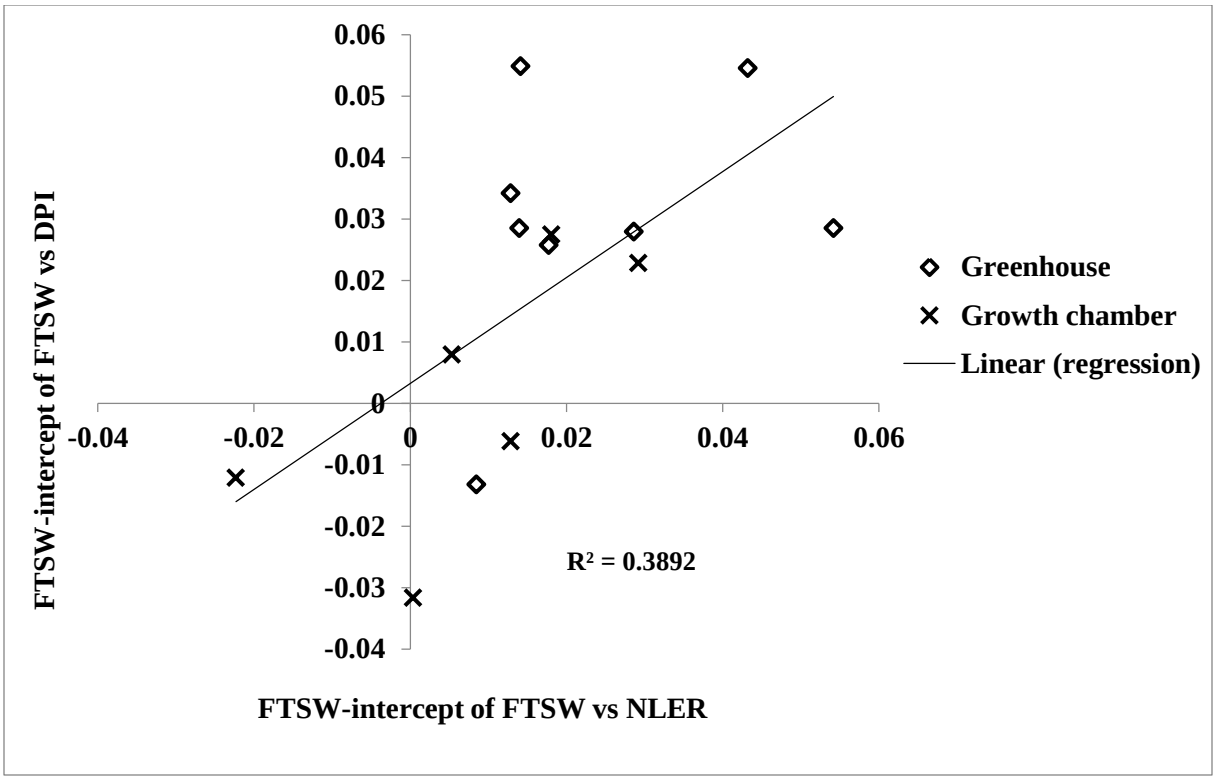


Figure 3-6. Linear regression of fraction of transpirable soil water (FTSW) intercept for zero change in phyllochron index (DPI) and zero normalized leaf expansion ratio (NLER). Data points represent six genotypes each in the greenhouse experiment growth chamber experiment. The R^2 and p-value for the slope estimate are included.

CHAPTER 4

A major means by which drought impacts plant productivity is that it affects the physiological processes associated with leaf area development. Establishment of a large leaf area allows the plant to harvest more photosynthetically active radiation and hence increase productivity. But under drought conditions, continued leaf area development can result in more water loss by transpiration. Hence it is important to understand the dynamics of leaf development response to drought stress. This study focused on the effects of drought on the leaf development of cowpea, since it is a crop popularly grown in semi-arid regions, and crop production is often affected by both intermediate and terminal drought conditions.

Studies have shown that leaf area in cowpea is affected by drought (Aniya, et al., 2004), but these studies do not show how drought affects the two main processes that contribute to leaf area development, namely: leaf expansion and leaf emergence. Leaf expansion occurs as a result of cell enlargement which is sensitive to water stress as the maintenance of turgor pressure (Terry, et al., 1983). Whereas leaf emergence, mostly driven by cell division, is also seen to be sensitive to drought stress (Terry, et al., 1983). Since leaf expansion and leaf emergence are separate physiological processes it is important to understand the consequences of each process as a result of soil drying.

The response of physiological processes like transpiration, nitrogen fixation and even leaf expansion to drought have been studied by finding a quantitative relationship between the trait and soil water content. For example, Sinclair et al. (1987) showed the response of cowpea leaf expansion to decreasing fraction of transpirable soil water. But, their study used

only one genotype of cowpea and there were no data collected to resolve between leaf expansion and leaf emergence response to soil drying. The studies done in this thesis were done to provide the first quantitative documentation of leaf expansion and leaf emergence response in several cowpea genotypes.

The studies presented in the Chapter 2 aimed to characterize cowpea leaf expansion response under progressive soil drying by tracking normalized leaf expansion rate (NLER) from high fraction transpirable soil water (FTSW) to low FTSW. The FTSW threshold for the linear decrease in leaf expansion and the FTSW intercept for the zero point of leaf expansion were found and compared with responses of transpiration in the form of normalized transpiration rate (NTR). The data collected also allowed preliminary observations about the response of leaf expansion and transpiration to rewatering when plants were recovering from drought stress. Also, since several genotypes were tested, we collected preliminary data on genotypic differences in the canopy leaf expansion response.

In Chapter 2, it was found that leaf expansion response under soil drying in all the genotypes tested fit a two-segmented linear model with constant NLER at high FTSW and a linear decrease in NLER is seen after soil water decreases below a threshold FTSW. Leaf expansion was overall more sensitive to soil drying than transpiration, since the FTSW thresholds for decrease in NLER occurred before the FTSW thresholds for decrease in NTR. Additionally, the stopping point of leaf expansion also occurred at an earlier FTSW than the stopping point of NTR, which emphasizes the fact that leaf expansion is more sensitive to drought than transpiration.

The threshold for the linear decrease in leaf expansion and transpiration in cowpea has been used by Sinclair et al. (1987) to predict crop productivity under drought conditions. But Sinclair used 0.2 for the FTSW threshold found from only one genotypes of cowpea. From Chapter 2 of this thesis, it was found that the FTSW threshold for the linear decrease in leaf expansion in cowpea is not a constant across genotypes but had a range of (0.28 to 0.67). The differences in the range of thresholds among experiments also indicated that the atmospheric VPD might also influence the FTSW threshold at which leaf expansion starts to decrease. The greenhouse with higher VPD of 2.4 kPa had FTSW thresholds at a higher soil water content and the second growth chamber experiment with lower VPD of 1.3 kPa had FTSW thresholds at a lower soil water content. More research needs to be conducted to confirm the influence of VPD on the response of cowpea leaf expansion and transpiration. Considering the range of FTSW thresholds and the environmental factors that influences the FTSW threshold is important to improve the methods of predicting crop development and productivity in areas with drought conditions.

The preliminary results on the recovery of leaf expansion and transpiration when plants were rewatered after drought condition showed that leaf expansion recovery in cowpeas is fast. Full recovery from severe stress (NTR $\leq$ 0.1) of leaf expansion occurred (~1 d) more rapidly than the full recovery of transpiration (~2 d). Since the 24 h measurements did not provide enough resolution of the point where the linear increase in NLER and NTR reaches a maximum, more studies might be done to confirm these results. But it is important to note from the study in this thesis that from a production perspective the recovery of leaf expansion was essentially immediate. These traits of cowpea leaf expansion and transpiration recovery

upon rewatering showed the advantage of growing cowpeas in areas with intermediate drought, where even when leaf expansion and transpiration is decreased as a result of drought a rainfall event can help the plants continue the establishment of canopy.

The genotypic differences observed in the decrease of leaf expansion and transpiration can be important in cultivar selection based on the environment for which the cowpea genotypes are being selected. The results in Chapter 2 indicated that Mouride and Suvita 2 had FTSW thresholds at consistently high soil water content. The trait of early decrease in leaf expansion can be useful in dry areas where the plant can conserve water by slowed increase in leaf area and reduced potential surface area for water loss by transpiration. In wetter areas, genotypes like Bambey 21, which leaf expansion that is not sensitive to small fluctuations in soil water, can continue establishment of canopy to maximize the area available for photosynthesis.

Chapter 3 focused on answering the question of how soil drying affects leaf emergence rates in the cowpea genotypes used to study leaf expansion. The study used the leaflet length data to determine daily phyllochron index (PI) as a measure of leaf emergence. The daily increase in PI (DPI) was calculated from PI to develop quantitative relationships showing the response of leaf emergence to decreasing soil water content. The FTSW thresholds for the linear decrease in DPI and the FTSW intercepts for the zero point of DPI were found and compared to the response of NLER to decreasing FTSW. Additionally the differences among genotypes were used to see if leaf emergence response to soil drying could have a genotypic basis.

Firstly, the components of the equation to calculate PI were examined to adapt it for use in detecting leaf emergence in cowpea. The daily determination of PI allowed the status of leaf appearance in the form of a decimal fraction added to the node number to be calculated. The equation for PI initially derived by Erickson and Michelini (1957) and modified by Vendeland et al. (1982) was used to study the effect of drought on newly emerging leaves. This formulation required the determination of a reference leaflet length within the length of exponential extension (R) and a phyllochron ratio (PR). In Chapter 3, it was found that R for cowpea leaf emergence of 27 mm was appropriate for the PI calculation. The well-watered plants were used to explore the assumption that PR was constant (Vendeland et al. 1982) reflecting a constant time interval between the production of new successive leaves. Although it was found that PR decreased slightly over time, it was also determined that the changes in PR had little impact in the determination of PI and DPI (Figure 3-2). It was also seen that average PR differed among genotypes (Table 3-1). Hence for each genotype the average PR of the well-watered plants of that genotype was used to find the change in PI of the stress plants.

Even though the use of PI allowed for the determination of leaf production as a continuous number instead of the discrete units of a node number, there was variability seen in the regression of the daily change in leaf emergence against FTSW. This might be because daily measurements were used to try to separate between decimal increments of PI. To measure larger increments of PI will require measurements at 2-3 d intervals and a longer drought period for better resolution of the changes in leaf emergence.

Even with the variability observed, the use of PI to find the response of leaf emergence to drought allowed determination of FTSW thresholds for linear decrease in leaf emergence and the FTSW intercepts for the zero point of leaf emergence. A range of 0.28 to 0.53 was seen for the FTSW threshold and a range of -0.03 to 0.06 was seen for the FTSW intercepts. The determination of these variables characterizing the response of leaf emergence in cowpea genotypes is important in the prediction of cowpea productivity in drought-affected areas since leaf emergence effects development in both phenology and leaf area.

Chapter 3 also compared leaf emergence response to leaf expansion response in cowpeas to drought stress. When comparing leaf emergence to leaf expansion, no differences were seen in the range of FTSW threshold and FTSW intercepts. Also linear regression between FTSW thresholds of leaf emergence and leaf expansion showed that the two responses to drought were not related. But the regression between FTSW intercepts of leaf emergence and leaf expansion showed is a linear relationship between the stopping points of DPI and NLER (Figure 3-6). In conclusion, cowpea leaf emergence was found to be as sensitive to soil drying as leaf expansion.

Information on the genotypic differences in the FTSW threshold and FTSW intercept of leaf emergence can be important in the consideration of genotypes for selection in breeding for any particular environment. From Chapter 3, the growth chamber experiment showed that the FTSW threshold for decrease in leaf emergence can be a genotypic trait. Also Suvita 2, IT82E-18 and IT84S-2049 consistently had earlier decrease in leaf emergence making it a possible candidate for production in areas with dry environment. While the ability of Bambey

21 to continue producing leaves at lower soil water content gives this genotype the ability to not be affected by small changes in soil water content and suitable for wetter environments.

The FTSW intercept for the stopping point of leaf emergence ranged from -0.032 to 0.055. Even though the variability in the observations doesn't allow for the determination of genotypic differences in the FTSW intercepts the range seen in the stopping point of leaf emergence implies that some genotypes may stop leaf production at an earlier FTSW than the others. Being able to discriminate between genotypes and picking cultivars that have an earlier cessation of leaf emergence can result in less water lose due to a lower leaf area and allow extended time for overall physiological activity.

The results in this thesis bring forth new questions about the response of leaf area development in cowpeas. The differences seen in the threshold for decreases in leaf expansion among the experiments indicate that VPD might have a strong influence in the decrease in response of leaf expansion. It will be interesting to understand how a range of VPDs might influence both leaf expansion and transpiration response in cowpeas under soil drying. At this point there isn't even any study that has explored if cowpea genotypes exhibit the limited transpiration trait at high VPD under well-watered conditions.

Variation of FTSW thresholds and FTSW intercepts for the decrease and cessation of leaf expansion and emergence were seen. But, all of the observations made were for greenhouse and growth chamber studies. Field studies also need to be done to confirm if similar responses to soil drying will be seen in cowpeas. Cowpea genotypes have also exhibited a remarkable ability to recover leaf expansion and transpiration within two days of rewatering.

But these results were for a growth chamber experiment under low VPD conditions. More experiments are necessary to confirm these observations in the field.

The PI is a valuable tool in observing leaf emergence rates. But, researchers have not taken advantage of this method of determining leaf emergence in cowpeas. If adjustments can be made in the measurement methods to reduce the variability in the observations, it can be an efficient means of finding leaf emergence rates in plants in any environment.

Cowpeas are an important crop popularly grown in semi-arid regions by subsistence farmers. It is important to continue research that with help identify traits in cowpea genotypes that might give farmers the ability to increase productivity and reduce risks of yield loss due to drought conditions.

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