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Drought and Nitrogen Effects on Maize Canopy
Temperature and Stress Indices

David A. Carroll II

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

Drought and Nitrogen Effects on Maize Canopy Temperature and Stress Indices

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Increased water scarcity due to changing climate, population growth, and economic development is a major threat to the sustainability of irrigated agriculture in the Western United States and other regions around the world. Management practices, such as controlled deficit irrigation, that seek to maximize the productivity of a limited water supply are critical. When using controlled deficit irrigation, remote sensing of crop canopy temperature is a useful tool for assessing crop water status and for more precise irrigation management. However, there is potential that nutrient deficiencies could compound the interpretation of water status from leaf temperature by altering leaf color and radiation balance. One objective of this thesis was to evaluate whether nitrogen fertility status of maize interacts with remotely sensed leaf temperature under full and limited irrigation. Another objective was to evaluate the effect of varying irrigation and nitrogen regimes on three water stress indices: Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT). Replicated studies were conducted using maize grown in both the glasshouse and the field. The glasshouse study consisted of combinations of well-watered and drought irrigation and sufficient and deficient nitrogen levels, while the field study consisted of combinations of well-watered, limited or controlled deficit, and drought irrigation and sufficient, sufficient delayed, and deficient nitrogen levels. In the glasshouse, leaf chlorophyll content was reduced moderately by limited irrigation and more so by N deficiency. For most observations in the glasshouse, the remotely sensed leaf temperatures were affected by irrigation, but not by N level. With drought irrigation, leaf temperature averaged 29.0 °C, compared to 27.9 °C for the well-watered treatment. Similar results were observed in the field, illustrating the utility of canopy temperature in detecting water stress and that the measurement was not confounded by N status. It was also found that irrigation had a significant effect on all three water stress indices. For example, in the glasshouse, cumulative DANS was 32.2 for the drought treatment and 15.5 for the well-watered treatment. Similar results were found for other stress index measurements both in the glasshouse and the field. DANS underestimated stress on days when the reference crop was stressed and overestimated stress on low temperature days. DACT risks finding no stress when temperatures are below the canopy threshold temperature of 28.0 °C. Thus, CWSI is the most effective index, given that it takes humidity and air temperature into account. Indices were only weakly related to leaf area, biomass or grain yield, or crop water productivity. Linear regression of Nitrogen Sufficiency Index and its effect on crop growth found significant effects on biomass and grain yield, crop water productivity, and final leaf area. Thus, water stress indices are useful tools in evaluating crop water status, but consideration of other factors, such as nutrient status, must be taken for prediction of crop growth and yield.

Keywords: water stress, deficit irrigation, agriculture, nutrient management

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CHAPTER ONE

Drought and Nitrogen Stress Effects on Maize Canopy Temperature

ABSTRACT

Water scarcity is a major threat to the sustainability of irrigated agriculture. Management practices, such as controlled deficit or limited irrigation, which seek to maximize the productivity of a limited water supply are critical. When using controlled deficit irrigation, evaluating crop water status is vital to precise management of a limited irrigation water resource; remote sensing of crop canopy temperature is a useful tool for assessing crop water status and scheduling irrigation. However, nutrient deficiencies could compound the interpretation of water status from leaf temperature by altering leaf color and radiation balance. The objective of this study is to evaluate whether nitrogen fertility status of maize interacts with remotely sensed leaf temperature under full and limited irrigation. Replicated studies were conducted on maize grown in both the glasshouse and the field. The glasshouse study consisted of combinations of full and limited irrigation and sufficient and deficient nitrogen levels, while the field study consisted of combinations of full, limited, and drought irrigation and sufficient, sufficient delayed, and deficient nitrogen levels. In the glasshouse, leaf chlorophyll concentration was reduced moderately by limited irrigation and dramatically by N deficiency. For most observations in the glasshouse, the remotely sensed leaf temperatures were affected by irrigation treatment, but not by N level. With the drought irrigation treatment, leaf temperature averaged 29.0 °C, compared to 27.9 °C for the well-watered treatment, and these differences were greater than 3.0 °C by the end of the experiment. In the field, leaf chlorophyll concentration and leaf area were influenced by both nitrogen stress and water stress. However, leaf temperature was not affected by nitrogen treatment on any of the measurement dates. As observed in the glasshouse study, leaf temperature in the field study was affected by irrigation treatment, with average temperatures of 29.0 °C and 27.8 °C for the limited and well-watered treatments, respectively. This illustrates the

utility of canopy temperature in detecting water stress and that the measurement was not confounded by crop nitrogen status. By measuring crop canopy temperature, producers can evaluate relative water stress of their crops and plan for more efficient crop water management.

INTRODUCTION

Water is one of the most vital resources for the sustenance of life on earth and the healthy functioning of ecosystems. Water is also essential to the functioning of human societies, which depend on freshwater resources to fulfill municipal, industrial, and agricultural needs. The agriculture sector is the single largest user of water worldwide, using approximately 75% of freshwater resources used by humans on the planet (Wallace, 2000). In many arid and semi-arid regions of the world, water scarcity due to inadequate rainfall is one of the most pressing contemporary challenges for agricultural and food sustainability. In these areas, irrigation has been developed as a strategy for enabling stable, high-yield agricultural production and for avoiding the effects of drought. Despite advances in irrigation technology, water scarcity in many of these regions is a pressing issue due to declining groundwater levels, increasing competition for water by municipal and industrial users, increasing frequency and severity of drought, rapid population growth, and declining water quality due to pollution and salinity (Gleeson et al., 2012; Vörösmarty et al., 2000). Additionally, remote sensing observations from the Gravity Recovery and Climate Experiment (GRACE) satellite mission show that anthropogenic use of groundwater resources is creating varying levels of groundwater stress in many regions of the world (Richey et al., 2014). Innovative strategies for assessing plant water status and scheduling delivery of irrigation water are a key component to the efficient use of limited irrigation water resources.

In recent decades, the agriculture industry has benefitted from advances in technology that have increased the ability of detecting in-field variation at a fine scale, including variations in plant growth, nutrient status, and water status. The development of technologies such as the global positioning system (GPS), geographic information systems (GIS), and in-field and remote

sensing have fueled the growth of potential applications of precision agriculture to manage small-scale variations in crop growth (Zhang et al., 2002). Although much of the research conducted in precision agriculture has traditionally dealt with variable rate seeding and nutrient, pesticide, and herbicide applications, variable-rate site-specific irrigation management technologies have also been developed to increase the efficiency of water applications (Sadler et al., 2005). Precision or variable-rate irrigation can save water by withholding irrigation completely from non-cropped areas of the field, by reducing irrigation rate when plant water status is sufficient, or by fully optimizing the economic value of irrigation water, and these adaptations can be variable across time and space (Sadler et al., 2005). Researchers have used soil moisture sensors to evaluate plant water status through measurement of daily plant-available water content in the soil, as integrated soil moisture data from various regions of a field can then be uploaded to a software-controlled variable-rate automatic irrigation management system (Hedley & Yule, 2009a; Hedley & Yule, 2009b). Variable flow rate sprinklers have also been developed, and tests have shown that such a sprinkler can be used for site-specific applications of irrigation water in center-pivot and lateral-move irrigation systems (King & Kincaid, 2004; King et al., 2005).

In addition to measurement of soil water status, measurement of remotely sensed crop canopy temperature and calculation of various stress indices based on canopy temperature have been widely studied as tools for assessing crop water status (Idso et al., 1981; Jackson et al., 1981; Taghvaeian et al., 2012). These measurements can be employed for spatial management of variable-rate irrigation used in precision agriculture systems. The temperature of a plant leaf is a function of both environmental conditions (solar radiation, air temperature, humidity, wind, etc.), leaf properties (leaf color, leaf area, leaf angle, etc.), and transpiration rates. Transpiration is the

primary plant mechanism for leaf cooling. If a plant closes its stomata during the day in response to water stress, then the transpiration rate will decline, resulting in an increase in leaf temperature. If temperature changes in a crop canopy can be effectively measured and interpreted, the information can then be used to regulate the use and application of limited water supplies (Bausch et al., 2012). Recent advances in remote sensing technology and the development of precision irrigation techniques have created a renewed interest in the application of this technology (Tilling et al., 2007). Beginning in the 1960s, advances in infrared technology led to the development of infrared thermometer (IRT) devices which were readily available for applications in agriculture (Irmak et al., 2000). The theory of infrared thermometry has been discussed (Hatfield, 1990; Gardner & Shock, 1989), and IRT technology has been used to evaluate leaf temperature, which is an effective indicator of plant water status that has been used for irrigation management (Idso et al., 1981).

If remotely sensed canopy temperature is to be widely applied for irrigation management or variable rate irrigation control, researchers must develop an understanding of factors that may confound the interpretation of measured data. For example, plant nutrient status could potentially affect canopy temperature due to effects on leaf color. Nitrogen deficiency is a common occurrence in many crops that results in changes in chlorophyll concentration and leaf color. Several methods exist for evaluating crop nitrogen status, including use of the Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL). However, determination of plant nitrogen status using SPAD may be confounded or significantly affected by crop water status; for example, a study conducted by Martínez and Guiamet (2004) found that leaf chlorophyll concentration determined by SPAD increased from 44.3 units to 47.2 units when the relative water content of plant tissues dropped from 94% to

87.5%. Similarly, a study conducted by Samborski et al. (2009) found that leaf chlorophyll meter threshold values vary significantly between irrigated and non-irrigated crops. Because of the interaction of water stress in determining plant nitrogen status using SPAD, Zhu et al. (2008) developed a normalized SPAD index wherein leaf chlorophyll content measurements are indexed against a non-nitrogen stressed reference crop.

Based on the results described above, it is apparent that measurements of crop nitrogen status are significantly affected by crop water status. Thus, there is also a need to answer the similar question of whether nitrogen deficiency affects remotely sensed leaf temperature. The objective of this study is to determine whether measurement of remotely sensed leaf temperature is significantly affected by crop nitrogen status. This was accomplished by evaluating crop canopy temperature and nitrogen status of deficit and fully irrigated maize. It was hypothesized that N deficiency would result in a measureable decrease in leaf temperature due to lighter green leaf color and lower absorption of solar radiation.

MATERIALS AND METHODS

GLASSHOUSE STUDY

The glasshouse or controlled environment study of water and nutrient status was conducted from February to April, 2014 in Provo, Utah (40° 14' 43" N, 111° 38' 29" W, 1406 m above mean sea level). The glasshouse study consisted of a randomized complete block, full factorial design with three replications of two water levels (well-watered and drought) and two nitrogen levels (sufficient and deficient). Four corn seeds of hybrid Fontenelle 4T105 were planted in each of twelve 11.4-liter pots on February 20, 2014. The growing media was a mixture of equal proportions of two porous ceramic soil conditioners, Turface Athletics MVP and PioneerOne Field Conditioner (Profile Products LLC, Buffalo Grove, IL). The bulk density of

the planting medium was 0.587 g cm^{-3} , with a field capacity volumetric water content of 35%. After planting, the potting media was covered with a 3.0 cm deep layer of perlite to prevent evaporation from the soil surface. All pots were irrigated with a pre-treatment solution until March 27, when corn was at the 5-leaf growth stage, at which point irrigation and nitrogen treatments were initiated for a 21 day treatment period. The pre-treatment solution consisted of a dilute nutrient solution containing all essential plant macro and micronutrients (Geary et al., 2014; Appendix I). Daily evapotranspiration was determined by weighing individual pots every 24 hours and averaging the weight loss across all three replications in a given treatment. For the well-watered irrigation treatment, 100% of measured ET was replaced by irrigation. For the drought irrigation treatment, 60% of the measured ET for the well-watered irrigation treatment was replaced by irrigation. Irrigation solution during the three-week experimental portion of the study was applied as a dilute nutrient solution containing all essential plant nutrients with the exception of nitrogen (Geary et al., 2014; Appendix I). Nitrogen was added to the nutrient solution by adding a volume of 1.0 molar ammonium nitrate solution to create an irrigation solution with a final concentration of either 240 or 30 mg N L^{-1} for the well-watered irrigation/sufficient nitrogen and well-watered irrigation/deficient nitrogen treatments, respectively. The same amounts of N were added to the limited irrigation treatments, although the irrigation volumes were lower.

Leaf temperature was measured daily during the treatment period using an Ex-Series E6 infrared camera (FLIR Systems, Inc., Wilsonville, OR). Each pot was placed individually in front of a black surface and the temperature measurement was taken on the newest fully-expanded leaf. Leaf temperature measurements were taken between 1:00 p.m. and 3:00 p.m. just prior to the daily irrigation. Leaf chlorophyll concentration was measured daily

using a Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL) by averaging three measurements per pot on the youngest fully-expanded leaf (Vig et al., 2012). Leaf area (L.A.) was determined biweekly by measuring the length and width of all leaves on the plant undergoing photosynthesis and then calculating L.A. using the following equation (Kang et al., 2003),

$$L.A. = 0.74 * \sum_{i=1}^n L_i W_i \quad (1.1)$$

where i is the number of individual leaves, L is leaf length, and W is leaf width measured at the widest point.

Analysis of variance was performed for all crop measurements, including leaf chlorophyll concentration, final leaf area, and leaf temperature, using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria). Treatment means were compared using ANOVA analysis with a critical value of 0.1 for determination of significant relationships.

FIELD STUDY

The field component of the study was conducted from May to October, 2014 at a 27 m by 32 m (0.89 ha) outdoor plot located at a glasshouse facility in Provo, Utah (40° 14' 43" N, 111° 38' 29" W, 1406 m above mean sea level). At this site, a 0.45 m deep, homogeneous topsoil layer was artificially created as a mixture of mineral and organic materials and spread over a layer of compacted sub-soil parent material. The soil had a clay loam texture composed of 41% sand, 30% silt, and 29% clay, 1.5% organic matter, and pH of 7.8. The long term average annual precipitation for the study site is 502 mm, with an average annual high temperature of 19°C and an average annual low temperature of 5°C. The study consisted of a randomized complete block, full factorial design with four replications of three irrigation levels (well-watered, drought, and limited/controlled deficit) and three nitrogen levels (sufficient, deficient, and sufficient delayed).

Corn seed of hybrid Fontenelle 4T105 was planted at the study site on May 16, 2014, with a seeding rate equivalent to 7.2 seeds m⁻², placed at a depth of 3 cm below the soil surface in rows spaced 0.75 m apart. Individual plots were 4 rows wide, and all data were collected on the center two rows to avoid irrigation border effects. Irrigation rate for the well-watered irrigation treatment was determined as the difference between evapotranspiration and precipitation. Daily evapotranspiration rates were obtained using an atmometer (Gavilan & Castillo-Llanque, 2009) and multiplying the depth of water evaporated from the atmometer between each irrigation interval by a crop coefficient for maize (Allen et al., 2007). Precipitation and other weather observations were obtained from a weather station located 1.4 km from the study site (40° 15' 18" N and -111° 39' 12" W), and reference evapotranspiration (ET_r) was calculated using the ASCE Standardized Penman-Monteith equation. Irrigation was applied using a calibrated, surface drip-irrigation system (John Deere T-Tape TSX-505-20-125). Drip lines were located adjacent to each maize row and were controlled separately for each irrigation treatment through a PVC-pipe system regulated to a constant pressure of 103 kPa. For the well-watered irrigation treatment, 100% of atmometer-estimated ET was replaced by irrigation, with 50% of this total replaced for the limited irrigation treatment. For the limited irrigation treatment, irrigation was applied to establish the crop, then irrigation was minimal until the 10-leaf growth stage (July 30 or 75 days after sowing), at which point the treatment received the same amount of water as the full irrigation treatment.

Nitrogen was applied as urea fertilizer by surface banding, with the fertilizer applied directly over the irrigation drip tape on each row. Nitrogen was applied on seven separate dates throughout the growing season, namely June 22, June 30, July 5, July 14, July 21, July 28, and August 4, 2014. These dates correspond to 37, 45, 50, 59, 66, 73, and 80 days after sowing. For

the sufficient nitrogen treatment, 22 kg ha⁻¹ N was applied on each of the first six dates, with 48 kg ha⁻¹ N applied on the seventh application date. For the deficient nitrogen treatment, 12 kg ha⁻¹ N was applied on each of the first six dates, with 18 kg ha⁻¹ N applied on the seventh application date. For the sufficient delayed nitrogen treatment, 8 kg ha⁻¹ N was applied on each of the first six dates, with 132 kg ha⁻¹ N applied on the seventh application date. Total nitrogen application rates were 180, 90, and 180 kg ha⁻¹ N for the sufficient, deficient, and sufficient delayed nitrogen treatments, respectively. Phosphorous and potassium were applied uniformly for all treatments over the same seven dates as the urea applications. Weeds were controlled by hand throughout the duration of the experiment.

Leaf temperature was measured weekly during the treatment period using an Ex-Series E6 infrared camera (FLIR Systems, Inc., Wilsonville, OR), with leaf temperature measurements taken on the newest fully expanded leaf. Leaf chlorophyll concentration was measured weekly using a Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL) by measuring the newest fully expanded leaf on a representative plant for each plot (Vig et al., 2012). Leaf area (L.A.) was determined bimonthly by measuring the length and width of all leaves on the plant undergoing photosynthesis and then calculating L.A. using Equation 1.1. Seasonal crop evapotranspiration was calculated as the sum of precipitation and applied irrigation and on the assumption of zero drainage and runoff.

Analysis of variance was performed for all crop measurements, including leaf chlorophyll concentration, final leaf area, and leaf temperature, using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria). Treatment means were compared using ANOVA analysis with a critical value of 0.1 for determination of significant relationships.

RESULTS AND DISCUSSION

IRRIGATION AND EVAPOTRANSPIRATION

Evaluating average total evapotranspiration over the experimental period was important in substantiating differences between irrigation treatments and in calculating crop water productivity. In the glasshouse, irrigation was the main input affecting evapotranspiration, while both irrigation and precipitation affected evapotranspiration totals in the field study. In the glasshouse study, evapotranspiration totaled 120 mm for the well-watered treatment and 74 mm for the drought treatment as determined by daily mass changes and irrigation water additions (Figure 1.1). In the field study, precipitation during the growing season totaled 120 mm (Table 1.1), with effective precipitation calculated as 100 mm. Calculated reference ET (ET_r) totaled 1170 mm and calculated crop ET (ET_c) totaled 780 mm (Figure 1.2). Applied irrigation totaled 700 mm, 500 mm, and 480 mm for the well watered, drought, and limited irrigation treatments, respectively (Figure 1.2; Table 1.1). Seasonal evapotranspiration, based on 90% irrigation efficiency and seasonal effective precipitation, was 730 mm for the well-watered treatment, 550 mm for the drought treatment, and 530 mm for the limited treatment (Figure 1.2; Table 1.1).

LEAF CHLOROPHYLL CONCENTRATION

In the glasshouse, maize leaf chlorophyll concentrations measured by SPAD meter varied with both irrigation and N level (Figure 1.3). Chlorophyll readings were not different between N treatments for the first four days of the treatment period, but then were significantly lower for the N deficient treatment than for the N sufficient treatment (Figure 1.3). By the end of the treatment period, the average chlorophyll content for the N deficient plants was 25.0 units, compared to 37.0 units for the N sufficient plants. When averaged over the treatment period, the chlorophyll content as measured by SPAD was 36.2 units for full irrigation and 34.0 units for

limited irrigation. Limited irrigation plants had lower chlorophyll content than plants with full irrigation on nine of the sixteen measurement dates, but these differences were small and consistent throughout the treatment period. The experiment successfully created differences in plant N status for evaluation of remotely sensed canopy temperature, with differences most obviously due to N treatment (Figure 1.3).

In the field, leaf chlorophyll concentration varied seasonally and was affected by both irrigation and N level (Figure 1.4). Chlorophyll differences between N treatments in the field were predictable. The sufficient and sufficient delayed N treatments exhibited the highest chlorophyll content by the last measurement date, with values of 30.8 units and 30.7 units, respectively. The deficient treatment had a lower final chlorophyll content, with a value of 25.7 units on the last measurement day. Chlorophyll content for the sufficient delayed N treatment was initially significantly lower than the sufficient N treatment, but it rose to approximately the same level as that of the sufficient N treatment after the delayed nitrogen was applied on day 80 (Figure 1.4). The sufficient N treatment exhibited significantly higher leaf chlorophyll concentration than the deficient N treatment, with significant differences on days 56, 59, 66, and 72 ($p = 0.06, 0.05, 0.01, \text{ and } 0.04$, respectively). On day 52, the sufficient delayed N treatment had significantly higher chlorophyll content than the deficient treatment ($p = 0.05$), which was a response to the application of nitrogen to the sufficient delayed treatment on day 80. Although the sufficient delayed treatment had a lower chlorophyll content than the sufficient N treatment during the first part of the growing season, the relationship was only significant on day 75 ($p < 0.01$). Irrigation also affected chlorophyll content, but the differences varied over time. The most interesting observations were towards the end of the growing season, when it was observed that chlorophyll content was lowest for the drought treatment but higher for the limited irrigation and

well-watered treatments, with no significant differences between the limited and well-watered treatments. For example, on day 90, the drought treatment had an average chlorophyll content of 26.3 units, while the limited and well-watered treatments had average chlorophyll contents of 31.2 and 29.8 units, respectively. This suggests that water availability influenced nitrogen uptake. As observed for the glasshouse study, the experiment successfully established significant differences in chlorophyll content based on nitrogen fertilization rate that could be used to evaluate whether this interacts with remote sensing of canopy temperature.

LEAF AREA

In the glasshouse study, both irrigation and N treatments affected the rate of above-ground plant growth, as measured by leaf area development over time (Figure 1.3). Average final leaf area was 1842 cm² pl⁻¹ for N-sufficient plants and 1356 cm² pl⁻¹ for N-deficient plants. Plants with full irrigation reached an average final leaf area of 1951 cm² pl⁻¹, while limited irrigation plants reached a final average leaf area of 1329 cm² pl⁻¹. There was not a significant interaction between irrigation and N treatments on leaf area. Irrigation treatment had a larger effect on plant leaf area than did nitrogen level.

In the field component of the study, both irrigation and N treatments affected the leaf area development of the plant over time (Figure 1.4). Nitrogen treatments were shown to have a significant effect on leaf area. For the last three measurement days during the growing season, the sufficient N treatment exhibited significantly higher leaf area than the deficient N treatment. The sufficient delayed N treatment also exhibited significantly higher leaf area than the deficient N treatment on the last measurement day of the growing season. On the last measurement day, the sufficient N treatment had an average leaf area of 2840 cm² pl⁻¹, while the sufficient delayed and deficient N treatments had average leaf areas of 2720 and 2330 cm² pl⁻¹, respectively.

Irrigation treatments also had a significant effect on average leaf area. The limited irrigation treatment had significantly lower leaf area than did the well-watered treatment early in the growing season, but by the end of the season there was no significant difference in average leaf area between these two treatments. The drought treatment had significantly lower leaf area than the well-watered treatment on two of the five measurement days; one of these days was close to the end of the growing season, at fifty three days into the measurement period.

LEAF TEMPERATURE

Despite the large differences observed in leaf chlorophyll concentration in the glasshouse study, there was no significant N treatment effect on leaf temperature for 11 of the 14 days of comparison, demonstrating that remotely sensed temperature was not sensitive to the difference in leaf color observed due to varying levels of nitrogen stress. During the three days when nitrogen treatment did have an effect on leaf temperature, significant differences were due to water stress differences induced by nitrogen status rather than leaf chlorophyll content. To illustrate this point, leaf temperatures were observed at seven specific times within a single day on April 10 (Figure 1.5). Leaf temperatures were similar among irrigation and nitrogen treatments when taken in the morning or late afternoon, but were greater for limited irrigation and for N sufficient treatments for a measurement taken at 2:00 in the afternoon. The observed differences in leaf temperature between N treatments were due to plant water status for the high N treatment, because the higher amount of nitrogen created plants with greater leaf area and therefore induced greater water stress during the hottest time of the day. Leaf rolling, a response of maize to drought stress, was also more apparent in sufficient N treatments than in deficient N treatments. Unlike nitrogen treatments, there were multiple days of significant difference in leaf temperature between irrigation treatments. For the first six days of the glasshouse study

treatment period, there were no differences observed among leaf temperature measurements. However, beginning seven days after initiation of treatments (25 days after sowing) and for most days thereafter, leaf temperature was significantly greater for the limited irrigation treatment compared to full irrigation. During that time period, leaf temperature averaged 27.9 °C for well-watered plants and 29.0 °C for drought irrigation plants. The average temperature for the drought irrigation treatment is above the critical value of 28.0 °C which has been used by researchers in developing indices to quantify water stress in maize (DeJonge et al., 2015). By nineteen days into the treatment period (39 days after sowing), the limited irrigation treatment plants had leaf temperatures averaging as much as 3.3 °C greater than the full irrigation treatment plants. These observations confirmed that remotely sensed canopy temperature can be an effective tool for assessing plant water status.

Although there were fewer measurement days overall in the field component of the study, the results from the field were in accord with the glasshouse study results. There were no observed differences in leaf temperature among nitrogen treatments despite large differences in chlorophyll content as determined by SPAD meter. There were significant differences between measured leaf temperatures among irrigation treatments. For example, on day 91, the limited irrigation treatment leaf temperature of 33.1 °C was significantly higher than that of the well-watered treatment at 30.8 °C ($p = 0.07$). On day 45, the drought treatment had a significantly higher leaf temperature of 31.1 °C than did the well-watered treatment, with a temperature of 29.0 °C ($p = 0.03$).

Neither the controlled environment nor the field research data support the hypothesis that differences in leaf chlorophyll content as represented by leaf color across nitrogen treatments would lead to corresponding differences in leaf temperature across those treatments. Rather, the

results show that remotely sensed canopy temperature is a robust method for assessing crop water status over varying plant nitrogen levels. Measurement of canopy temperature is a useful tool that has many potential applications, including conservation of irrigation water through precision agriculture. Although canopy temperature was measured on individual plant leaves for this study, remotely sensed temperature obtained on a field scale using aerial imagery can also be used to quantify water stress and plan for more precise management irrigation water resources, which are becoming increasingly limited in many regions of the world.

SUMMARY

The purpose of this study was to evaluate whether plant nitrogen status interacts with remotely sensed leaf temperature of both well-watered and limited irrigated maize. This was done by measuring average chlorophyll concentration level, leaf area, and leaf temperature on maize plants grown in both controlled environment and field studies. In the glasshouse, irrigation treatments included both well-watered and drought treatments, and nitrogen treatments included sufficient and deficient N levels. In the field, irrigation treatments consisted of well-watered, drought, and limited irrigation treatments; the limited irrigation treatment consisted of a controlled deficit irrigation strategy based on growth stage timing. Nitrogen treatments in the field consisted of sufficient, deficient, and sufficient delayed N regimes.

The findings of this study support the use of remotely sensed leaf temperature as a method for determining relative water stress of irrigated crops. It was found that irrigation treatments had a significant effect on leaf temperature in both the glasshouse and the field, with the well-watered treatment averaging 27.9 °C and the limited treatment with an average temperature of 29.0 °C during the study period. These results were confirmed by the field study; average temperatures were higher for the drought and limited irrigation treatments as compared

to the well-watered treatment, with averages of 27.8 °C and 29.0 °C for the well-watered and limited irrigation treatments, respectively. Unlike irrigation treatments, it was found that nitrogen treatments did not have a large effect on leaf temperature. In the glasshouse, there were three days of measurement where the average leaf temperature of sufficient N plants was higher than that of deficient N plants, but this temperature difference was induced by water stress rather than by N stress. In the field, none of the measurement days reported significant differences in leaf temperature among N treatments.

Although nitrogen did not have a significant effect on leaf temperature in either the field or the glasshouse, nitrogen was found to have a significant effect on leaf chlorophyll concentration level. For example, in the glasshouse, average chlorophyll concentration level was 37.2 units for sufficient N plants and 31.9 units for deficient N plants. In the field, average chlorophyll concentration level was 30.8 units for the sufficient N treatment and 25.7 units for the deficient N treatment. This shows that although sufficient N plants had a darker color and hence greater chlorophyll concentration, differences in leaf N status did not interact with leaf canopy temperature. These results suggest that canopy temperature can be used to determine water stress, schedule irrigation, and manage water status of crops without being confounded by differences in plant N status.

FIGURES AND TABLES

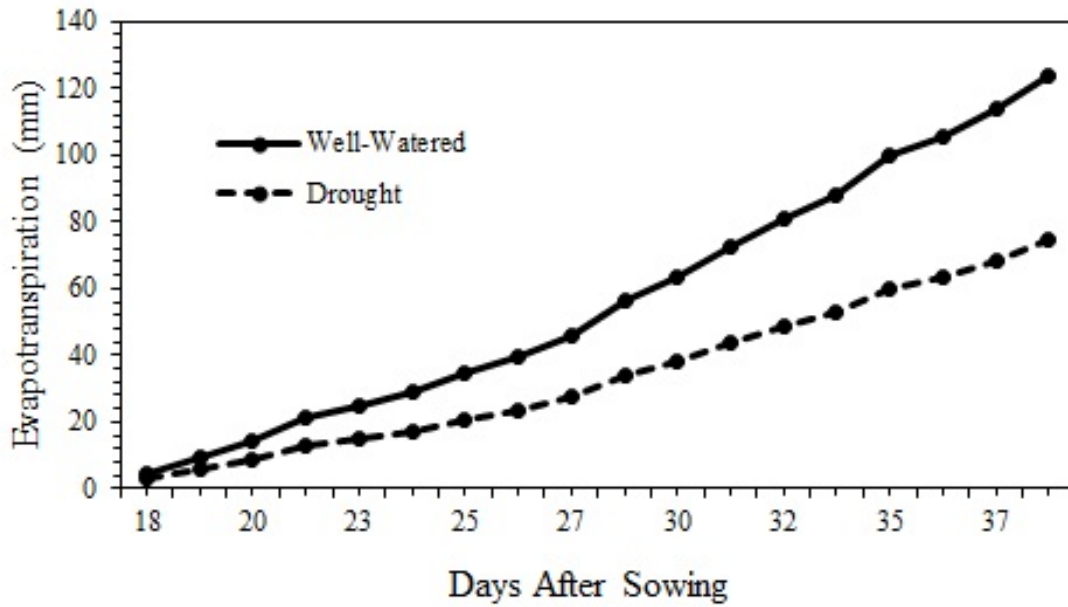


Figure 1.1 Cumulative evapotranspiration for well-watered and drought irrigation treatments throughout the duration of the glasshouse study as determined by daily mass changes and irrigation water additions (March 27-April 16, 2014).

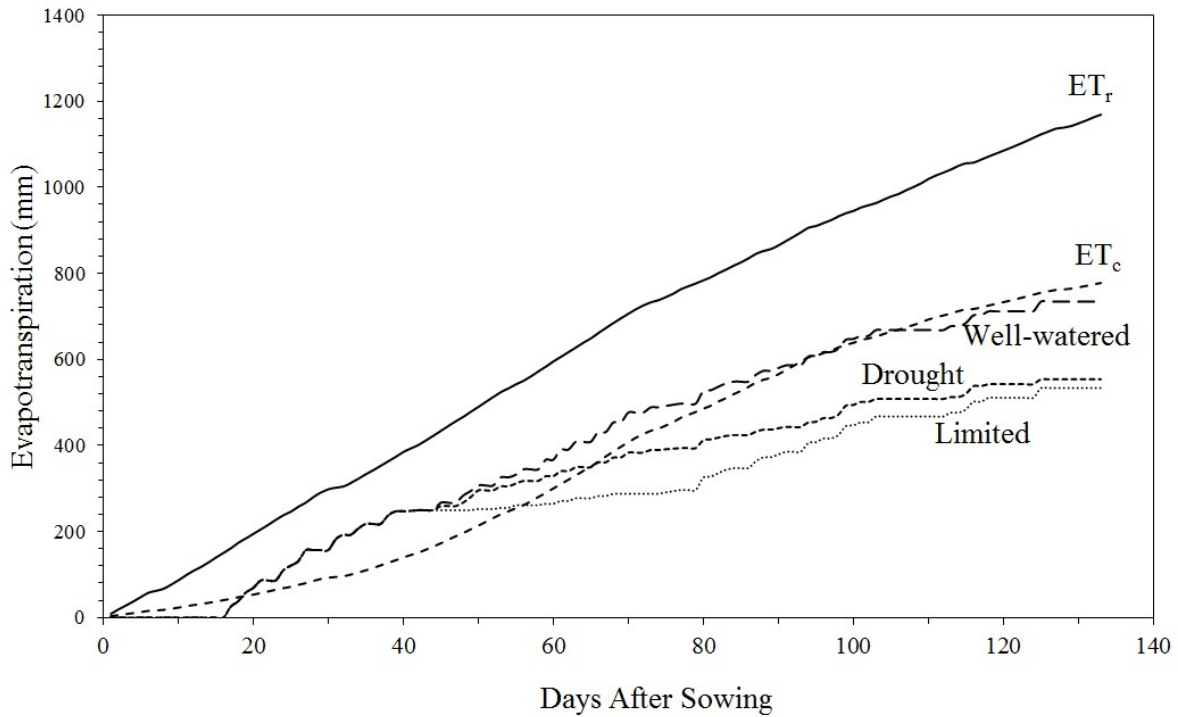


Figure 1.2 Seasonal calculated reference (ET_r) and crop (ET_c) evapotranspiration, and estimated seasonal evapotranspiration (ET) for the well-watered, drought, and limited irrigation treatments in the field study. ET estimates are based on the sum of effective precipitation and applied irrigation.

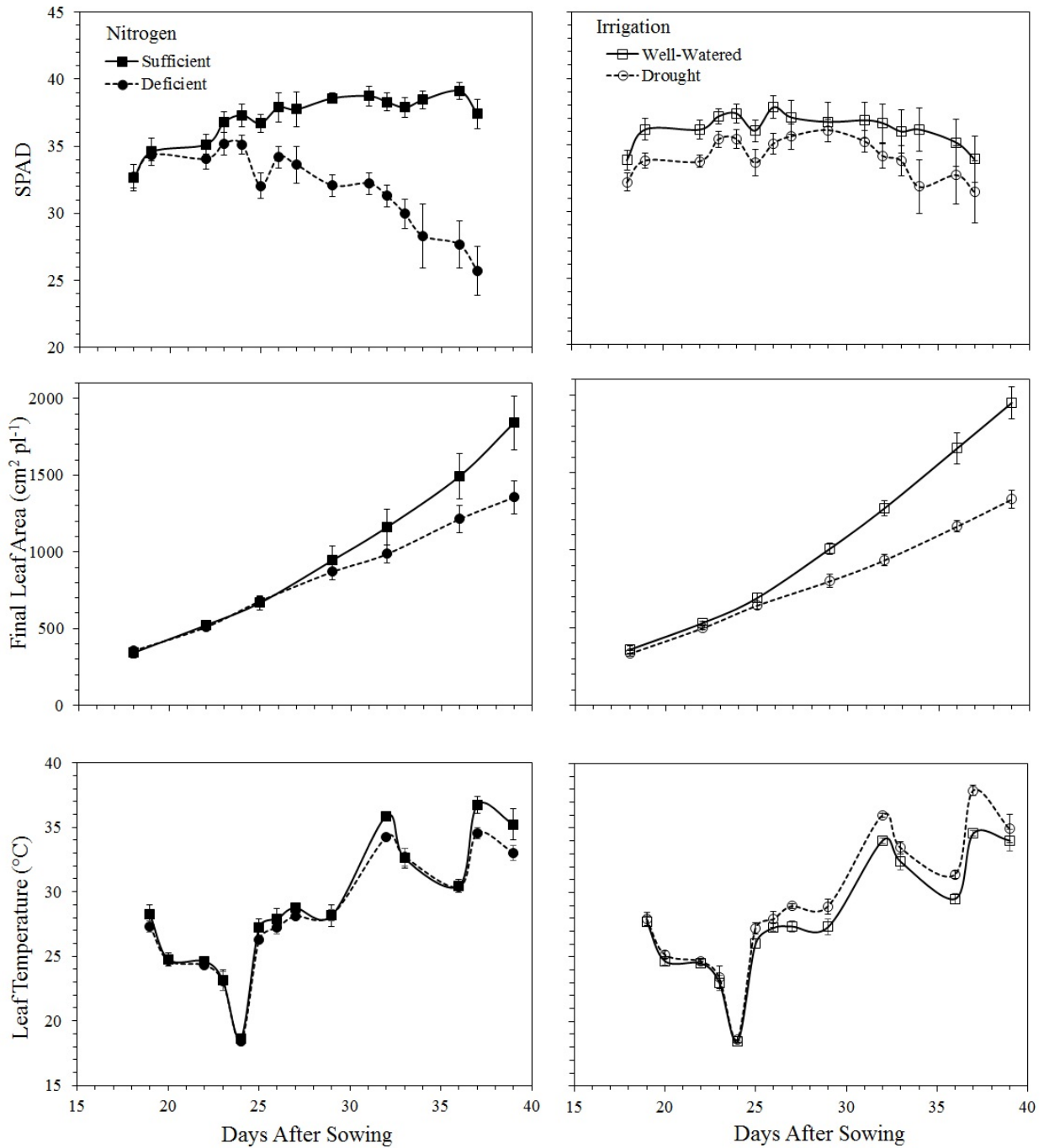


Figure 1.3 The effects of sufficient and deficient nitrogen levels and full and limited irrigation levels on maize leaf chlorophyll concentration measured by SPAD meter (upper figures), leaf area development (middle figures), and on remotely sensed leaf temperature (lower figures) from the glasshouse study. Error bars indicate one standard error of the mean.

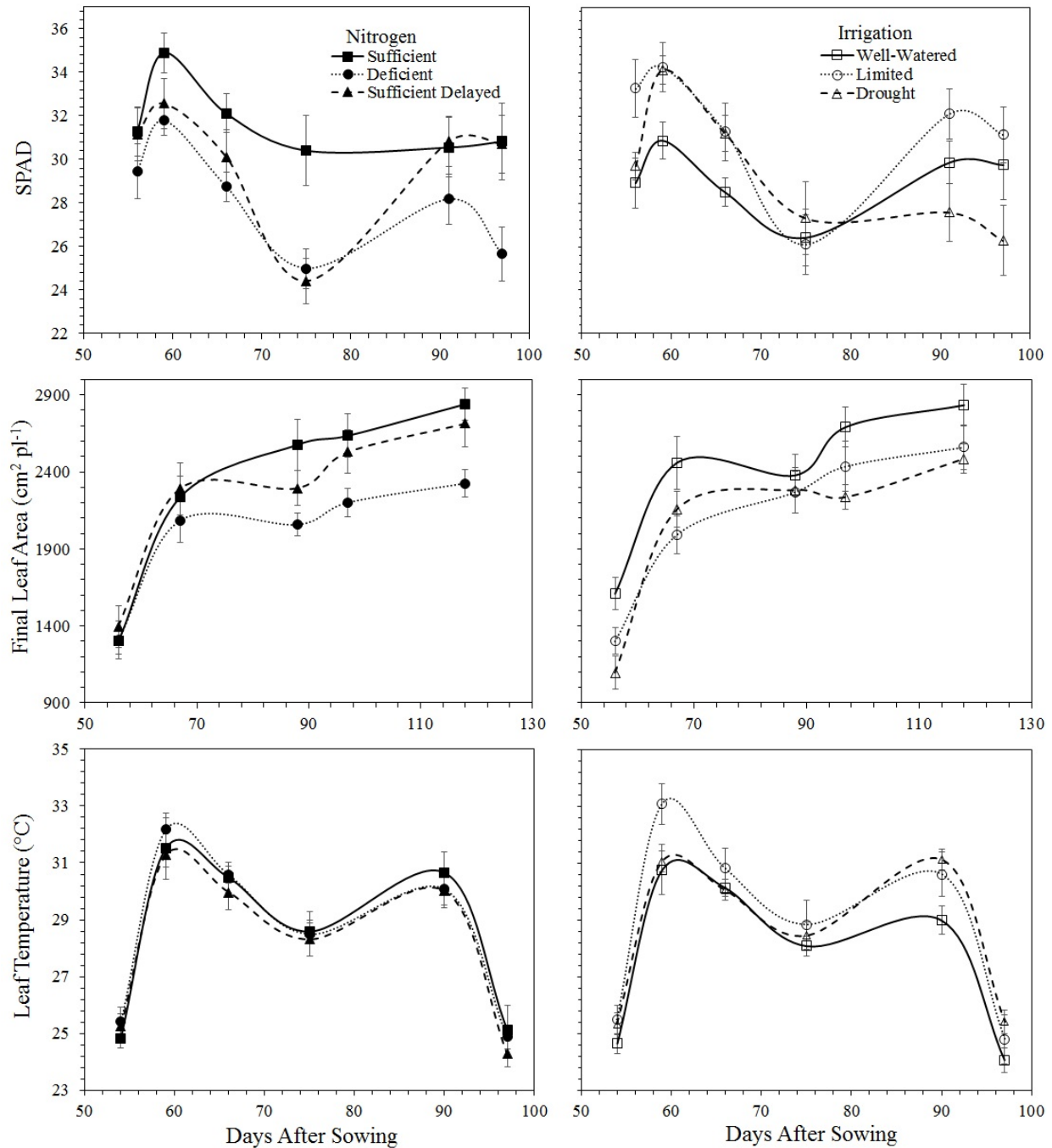


Figure 1.4 The effects of sufficient, deficient, and sufficient delayed nitrogen levels and full, limited, and drought irrigation levels on maize leaf chlorophyll concentration measured by SPAD meter (upper figures), leaf area development (middle figures), and on remotely sensed leaf temperature (lower figures) in the field study. Error bars indicate the standard error of the mean.

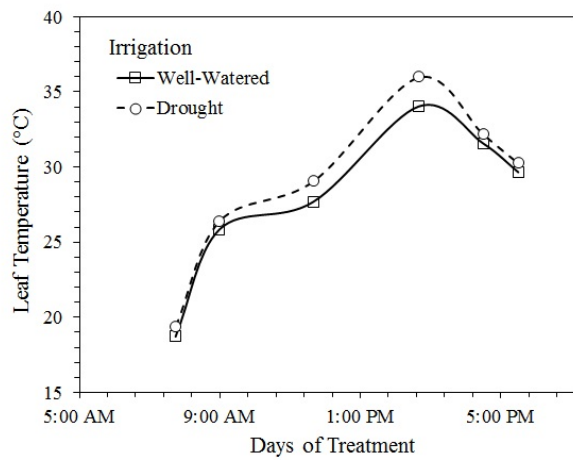
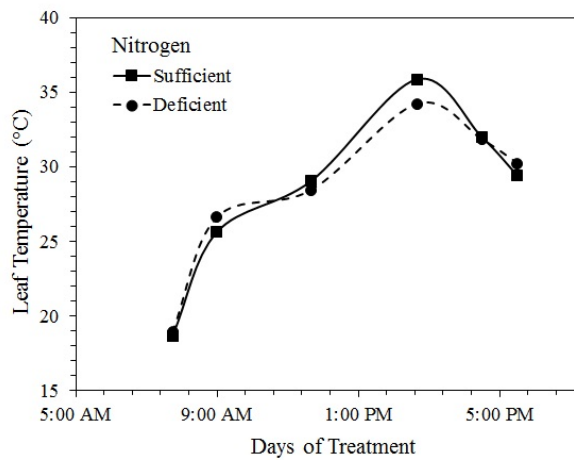


Figure 1.5 The effects of full and limited irrigation and sufficient and deficient nitrogen levels on remotely sensed maize leaf temperatures measured at six times of day on April 10, 2014 in the glasshouse (14 days since treatments began).

Table 1.1 Seasonal irrigation, rainfall, evapotranspiration, and applied nitrogen for both irrigation and nitrogen treatments in the field study.

Irrigation Regime	Nitrogen Regime	Irrigation (mm)	Rainfall (mm)	ET (mm)	Applied N (kg/ha)
Well-Watered	Sufficient	700	120	730	180
	Sufficient delayed	700	120	730	180
	Deficient	700	120	730	90
Drought	Sufficient	500	120	550	180
	Sufficient delayed	500	120	550	180
	Deficient	500	120	550	90
Limited	Sufficient	480	120	530	180
	Sufficient delayed	480	120	530	180
	Deficient	480	120	530	90

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CHAPTER TWO

Comparing Water Stress Indices for Determining Crop Water Status of Maize under Variable Irrigation and Nitrogen

ABSTRACT

Water scarcity is a major threat to the sustainability of irrigated agriculture in many regions of the world; this scarcity is driven by a number of factors, including population and economic growth, increasing severity and intensity of drought, deforestation, overgrazing, and groundwater depletion. Management practices, such as controlled deficit or limited irrigation, are critical in managing limited water supplies and increasing crop water productivity. Remotely sensed crop canopy temperatures can be used to compute a variety of water stress indices in order to assess plant water status and increase the efficiency of irrigation management.

Traditionally, Crop Water Stress Index (CWSI) has been used to evaluate water stress of irrigated crops, but several simplified indices have been developed, including the Degrees Above Non-Stressed (DANS) and Degrees Above Canopy Threshold (DACT) indices. The objective of this study is to evaluate the effect of varying irrigation and nitrogen regimes on CWSI, DANS, and DACT. Another objective is to determine the level of correlation between CWSI and both DANS and DACT in order to evaluate the efficacy of these simplified indices as a surrogate for CWSI. An effort to validate all three indices was made by correlating the cumulative index values to leaf area, grain and biomass yield, and crop water productivity from replicated studies conducted using maize grown in both glasshouse and field conditions. The glasshouse study consisted of combinations of full and limited irrigation and sufficient and deficient nitrogen levels, while the field study consisted of combinations of well-watered, controlled deficit or limited, and drought irrigation and sufficient, sufficient delayed, and deficient nitrogen levels. Irrigation treatment had a significant effect on all three water stress indices. For example, in the glasshouse, cumulative DANS was 32.2 units for the drought treatment and 15.5 units for the well-watered treatment. One weakness of the crop water stress indices is that they do not

discriminate between the impacts of stress occurring at during different growth stages, even though studies have established that stress during the reproductive stages of maize has a larger negative effect on the crop yield. This was evidenced by cumulative stress totals that were higher for the limited or growth stage timed deficit treatment than for the drought or constant rate deficit treatment. For example, cumulative CWSI totals were 0.25, 0.23, and 0.17 units for the limited, drought, and well-watered treatments, respectively. Nitrogen did not have a significant effect on the indices in the field study and on only DACT in the glasshouse study, showing that the stress indices are robust over variable nitrogen levels. Although moderate level of correlation was observed between CWSI and DANS or DACT, CWSI may be advantageous over the other two indices because it is sensitive to differences in humidity. DANS overestimated stress on relatively cool days and underestimated stress on excessively hot days when the reference crop was stressed. CWSI is better at evaluating stress when the canopy temperature is below the 28.0 °C critical temperature used in calculating DACT, since anything below this value is determined to be non-stressed under DACT. This shows the potential advantages of CWSI in predicting water stress under variable conditions in the field. Indices were only weakly related to leaf area, biomass or grain yield, or crop water productivity. Analysis of Nitrogen Sufficiency Index found that plant nitrogen status was a significant predictor of biomass yield and final leaf area in the greenhouse and of grain yield, biomass yield, and crop water productivity in the field. These results suggest that water stress indices are useful tools in evaluating crop water status, but that other factors such as nitrogen status should also be taken into consideration.

INTRODUCTION

Water is one of the most important resources for sustaining human life and the various ecosystems that make up the world. The world's finite freshwater resources must be shared among various users and competing interests, including municipal, industrial, and commercial users, agriculture, and natural ecosystems. Irrigation represents the main agricultural demand on fresh water resources, and irrigated agriculture is an important source of food production worldwide. For example, in the United States in 2007, approximately 57 million acres of land were under irrigation, representing 7.5% of all cropland and pastureland nationwide. However, irrigated farms accounted for 55% of the total value of crop sales during that same year (Taghvaeian et al., 2012). Three quarters of this irrigated land is found in the seventeen westernmost contiguous states. Like the western United States, many other arid and semi-arid regions around the world likewise depend on a steady supply of irrigation water to produce their crops. However, increasing populations in many of these areas are placing pressure on the agriculture industry to divert water resources for urban use. In addition to these factors, climate change may lead to increased variation in the state of water resources around the globe. For example, many developing regions such as the African Sahel and parts of the Middle East and South Asia already experience water stress due to increasing drought severity, growing populations, and subdivision of farmlands. As a result of these ongoing economic, demographic, and environmental changes, growing world demand for food must be met with lower available water resources for agriculture (Pandey et al., 2000). Other factors driving water scarcity include declining groundwater levels, increasing frequency and severity of drought, and pollution and salinification of water resources (Gleeson et al., 2012; Vörösmarty et al., 2000). Additionally, remote sensing observations from the Gravity Recovery and Climate Experiment (GRACE)

satellite mission show that anthropogenic use of groundwater resources is creating varying levels of groundwater stress in many regions of the world (Richey et al., 2014).

As water for irrigation becomes scarcer, developing strategies to improve crop water productivity under drought stress is increasingly more critical in order to preserve the continued viability of irrigated agriculture. One such strategy, referred to as controlled deficit or limited irrigation, involves increasing irrigation efficiency by managing water stress during different crop growth stages through rate and timing of irrigation (DeJonge et al., 2011). Under limited irrigation, water stress is managed by withholding irrigation during non-critical vegetative growth stages while irrigating sufficiently to prevent water stress during critical periods of reproductive growth in order to maintain a significant yield. By withholding water at periods less crucial for harvestable yield, farmers use reduced amounts of water in an optimal fashion, thus minimizing yield losses and improving water productivity of grain crops through slowing the rate of leaf area development and improving the harvest index (Traore et al., 2000; Payero et al., 2006). While the potential benefits of limited irrigation have been documented (DeJonge et al., 2011; Saseendran et al., 2008), further studies that evaluate the interactions of limited irrigation with other factors, such as crop nitrogen status, are needed.

When implementing deficit irrigation strategies, it is very important to develop sound methods for evaluating crop water status and estimating crop evapotranspiration. These measurements enable quantification of water stress, which is essential to optimizing grain yield under deficit irrigation. Beginning in the 1960s, advances in infrared technology led to the development of infrared thermometer (IRT) devices which were readily available for applications in agriculture (Irmak et al., 2000). The theory of infrared thermometry has been thoroughly developed and discussed (Hatfield, 1990; Gardner & Shock, 1989), and IRT

technology has been used to develop several water stress indices. Many researchers and practitioners currently use these stress indices, developed from remote sensing data, to evaluate crop water status and manage application of irrigation water. Infrared thermometry is non-destructive and relatively inexpensive compared to other methods of measuring crop water status. Infrared thermometry is important because canopy temperature can be used to effectively measure water stress. Canopy temperature increases with increasing ambient air temperature and with increasing sunlight intensity due to higher absorption of solar radiation by the leaf. Plants regulate leaf temperature via transpiration, which has a leaf-cooling effect. While it is normal for crop canopy temperature to rise during the afternoon hours and fall throughout the evening and overnight, water-stressed plants will have lower transpiration rates and hence higher crop canopy temperatures relative to well-watered plants. Thus, measurement of canopy temperature has potential utility for quantifying the degree of plant water stress experienced by a crop.

Several indices have been developed to relate canopy temperature to crop water status, which can then be used for irrigation scheduling. One well-used method is the Crop Water Stress Index (CWSI), developed by Idso et al. (1981), which indexes measured canopy temperature against humidity-based maximum and minimum baselines of stress. CWSI can be determined both empirically and theoretically, but the empirical method is more commonly used (Gardner et al., 1992). The use of CWSI for irrigation management has been substantiated (Howell et al., 1984; Reginato & Howe, 1985; Wanjura et al., 1990; Alderfasi & Nielsen, 2001), and CWSI has also been correlated to grain yield (Walker & Hatfield, 1983; Smith et al., 1985). Another more recently developed index that has been used to quantify crop water status is the Degrees Above Non-Stressed (DANS) index, which consists of quantifying the canopy temperature difference between a stressed plant or crop and a non-stressed reference plant or crop. A non-stressed

reference temperature is taken during each measurement period, and the temperature difference between the non-stressed reference and the plant being measured is recorded as the DANS (DeJonge et al., 2015). This index does not require measurement of humidity and air temperature, is much simpler to calculate than CWSI, and was shown to assess crop water status similar to CWSI (DeJonge et al., 2015). Two separate studies found that DANS was as highly correlated as was CWSI to plant parameters such as evapotranspiration, leaf area index, root growth, leaf water potential, fraction of intercepted photosynthetically active radiation (iPAR) and yield in both sunflower and corn (DeJonge et al., 2015; Taghvaeian et al., 2014a). A third index is the Degrees Above Canopy Threshold (DACT) index, which is somewhat similar to DANS in that it deals with quantifying temperature differences. However, instead of taking a non-stressed reference temperature for each measurement period as in DANS, DACT uses a pre-determined canopy threshold or critical temperature for a given crop as the reference standard (DeJonge et al., 2015). While these water stress indices have been documented to assess variation in crop water stress, it is not known how they would perform when water stress is interacting with other sources of biotic or abiotic crop stress. For example, nutrient deficiencies may affect the canopy temperature of a non-water stressed crop, which would then influence the DANS index.

This study uses both controlled environment and field study measurements with known irrigation and nitrogen application treatments to evaluate the effectiveness of CWSI, DANS, and DACT as measurements of crop water status with varying supply of nitrogen. The study also evaluates correlation between water stress indices and crop growth parameters, including leaf area index (LAI), biomass and grain yields, and crop water productivity. It is hypothesized that CWSI, DANS, and DACT will be negatively correlated to measurement of leaf area index and

chlorophyll content, with the continuous drought treatment exhibiting the strongest correlation. It is also hypothesized that there will not be a statistically significant difference in correlation of CWSI, DANS, and DACT to the aforementioned crop parameters.

MATERIALS AND METHODS

GLASSHOUSE STUDY

The study consisted of both controlled environment and field components. The controlled environment component of the study was conducted in a glasshouse during a period from February to April, 2014 in Provo, Utah (40° 14' 43'' N, 111° 38' 29'' W, 1406 m above mean sea level). The study consisted of a randomized complete block, full factorial design with three replications of two water levels (well-watered and drought) and three nitrogen levels (sufficient, intermediate, and deficient). Four corn seeds of hybrid Fontenelle 4T105 were planted in each of twelve 11.4-liter pots on February 20, 2014. The growing media was a mixture of equal proportions of two porous ceramic soil conditioners, Turface Athletics MVP and PioneerOne Field Conditioner (Profile Products LLC, Buffalo Grove, IL). The bulk density of the planting medium was 0.587 g cm⁻³, with a field capacity volumetric water content of 35%. After planting, the potting media was covered with a 3.0 cm deep layer of perlite to prevent evaporation from the soil surface. All pots were irrigated with a pre-treatment solution until March 27, when corn was at the 5-leaf growth stage, at which point irrigation and nitrogen treatments were initiated for a 21 day treatment period. The pre-treatment solution consisted of a dilute nutrient solution containing all essential plant macro and micronutrients (Geary et al., 2014; Appendix I). Daily evapotranspiration was determined by weighing individual pots every 24 hours. For full irrigation treatments, 100% of measured ET was replaced by irrigation. For the limited irrigation treatment, 60% of the measured ET for the full irrigation treatment was

replaced by irrigation. Irrigation solution during the three-week experimental portion of the study was applied as a dilute nutrient solution (Geary et al., 2014; Appendix I) containing all essential plant nutrients with the exception of nitrogen. Nitrogen was added to the nutrient solution by adding a volume of 1.0 molar ammonium nitrate solution to create an irrigation solution with a final concentration of either 240, 135, or 30 mg N L⁻¹ for the full irrigation-sufficient nitrogen, full irrigation-intermediate nitrogen, and full irrigation-deficient nitrogen treatments, respectively. The same amounts of N were added to the lower irrigation volumes corresponding to the drought irrigation treatments.

Leaf temperature was measured daily during the treatment period using an Ex-Series E6 infrared camera (FLIR Systems, Inc., Wilsonville, OR). Each pot was placed individually in front of a black surface and the temperature measurement taken on the newest, fully expanded leaf. Leaf temperature measurements were taken between 1:00 p.m. and 3:00 p.m. just prior to the daily irrigation. Leaf chlorophyll concentration was measured daily using a Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL) by averaging three measurements per pot on the youngest fully expanded leaf. Leaf area (L.A.) was determined biweekly by measuring the length and width of all leaves on the plant and then calculating L.A. using the following equation (Kang et al., 2003),

$$L.A. = 0.74 * \sum_{i=1}^n L_i W_i \quad (2.1)$$

where *i* is the number of individual leaves, *L* is leaf length, and *W* is leaf width measured at the widest point.

FIELD STUDY

Field research was conducted in Provo, Utah (40° 14' 43'' N, 111° 38' 29'' W, 1406 m above mean sea level) at a 0.89 ha site with a 0.45 m deep homogeneous topsoil layer that was

artificially created as a mixture of mineral and organic materials and spread over a layer of compacted sub-soil parent material. The soil mixture had a clay loam texture composed of 41.4% sand, 29.6% silt, and 29% clay, 1.5% organic matter, and a pH of 7.8. The long term average annual precipitation for the study site is 502 mm, with average annual high and low temperatures of 19 °C and 5 °C. The 2014 field study employed a randomized complete block, full factorial design with three irrigation treatments, three nitrogen fertilizer treatments, and four replications. Corn seed of hybrid Fontenelle 4T105 was planted on May 16, 2014. Seeding rate was equivalent to 7.2 seeds m⁻², placed at a depth of 3 cm below the soil surface in rows with a row spacing of 0.75 m. Individual plots were 4 rows wide, with all data collected on the center two rows to avoid irrigation border effects.

All plants were well-watered equally to establish the crop, and irrigation treatments were initiated on June 30, 2014. The irrigation treatments were well-watered, drought, and limited irrigation. The well-watered treatment was managed to minimize water stress. The drought treatment consisted of irrigating at the same timing as the well-watered treatment, but consistently applying 50% of the water applied in the well-watered treatment. The limited irrigation treatment avoided irrigation during the vegetative growth stages (V5-V10) followed by applying the same irrigation amounts as for the well-watered treatment during the reproductive growth stages. Irrigation was applied using a calibrated drip-irrigation system (John Deere T-Tape TSX-505-20-125). Drip lines were placed adjacent to each maize row and were controlled separately for the three irrigation treatments through a PVC-pipe system. Irrigation rate for the well-watered treatment was determined by measuring daily evapotranspiration rates using an atmometer (Irmak et al., 2005) and multiplying by the crop coefficient for maize (Allen et al., 2007).

Nitrogen was applied as urea fertilizer by surface banding method, with the fertilizer applied directly over the irrigation drip tape on each row. Nitrogen was applied on seven separate dates throughout the growing season, namely June 22, June 30, July 5, July 14, July 21, July 28, and August 4, 2014. These dates correspond to 37, 45, 50, 59, 66, 73, and 80 days after sowing. For the sufficient nitrogen treatment, 22 kg ha⁻¹ N was applied on each of the first six dates, with 48 kg ha⁻¹ N applied on the seventh application date. For the deficient nitrogen treatment, 12 kg ha⁻¹ N was applied on each of the first six dates, with 18 kg ha⁻¹ N applied on the seventh application date. For the sufficient delayed nitrogen treatment, 8 kg ha⁻¹ N was applied on each of the first six dates, with 132 kg ha⁻¹ N applied on the seventh application date. Total nitrogen application rates were 180, 90, and 180 kg ha⁻¹ N for the sufficient, deficient, and sufficient delayed nitrogen treatments, respectively. Phosphorous and potassium were applied uniformly for all treatments over the same seven dates as the urea applications. Weeds were controlled by hand throughout the duration of the experiment.

Several parameters were measured weekly throughout the growing season. One plant for each plot was selected for all crop measurements taken throughout the season. Leaf area (L.A.) was determined bimonthly by measuring the length and width of all green leaves on the plant and then calculating L.A. using Equation (2.1). Leaf chlorophyll concentration was measured weekly using a Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Aurora, IL); this was done by measuring the youngest fully expanded leaf on a representative plant for each plot (Vig et al., 2012).

Leaf temperature was measured weekly during the treatment period using an Ex-Series E6 infrared camera (FLIR Systems, Inc., Wilsonville, OR), with leaf temperature measurements taken on the newest, fully expanded leaf. Leaf temperature measurements were taken on six

separate dates throughout the season under sunny conditions between 1:00 pm and 2:00 pm by pointing the camera directly at the newest collared leaf on an individual corn plant, at a distance of approximately four centimeters from the leaf surface.

WATER AND NITROGEN INDICES

Several water stress indices were computed from the canopy temperature data for both the controlled environment and field components of the study. Crop Water Stress Index (CWSI) was calculated for each temperature reading using the empirical method described by Gardner et al. (1992), which consists of the following equation,

$$CWSI = (dT_m - dT_{LL}) / (dT_{UL} - dT_{LL}) \quad (2.2)$$

where dT is the difference between crop canopy temperature and ambient air temperature, and the subscripts m , LL , and UL represent measured, lower-limit, and upper-limit temperature difference measurements. The lower limit represents dT as measured with a well-watered, non-stressed plant at a given humidity level, while the upper limit represents dT as measured with a severely stressed, non-transpiring plant at a given humidity level. The dT_{LL} is a linear function of vapor pressure deficit (VPD) and is referred to as the non-stressed baseline. The dT_{UL} is a linear function of vapor pressure gradient (VPG) and is referred to as the non-transpiring baseline (Taghvaeian et al., 2012). The equations for the non-stressed and non-transpiring baselines are as follows:

$$dT_{LL} = m(VPD) + b \quad (2.3)$$

$$dT_{UL} = m(VPG) + b \quad (2.4)$$

In these equations, m and b represent the respective slopes and intercepts of the linear equations, while VPD and VPG refer to vapor pressure deficit and vapor pressure gradient, respectively. In this study we used $m = -1.9$ and $b = 2.73$ as the dT_{LL} . This lower limit equation was obtained

from a study conducted in Colorado by Taghvaeian et al. (2012). Taghvaeian et al. (2014a) found that empirically determined lower limit baselines can be used in similar geographic regions with comparable climatic conditions. Although Idso et al. (1981) showed that the upper limit (dT_{UL}) is a function of temperature, Gardner et al. (1992) found that variation in dT_{UL} was minimal within the range of normal midday temperatures during the growing season. Because of this, it is possible to assign a constant value for dT_{UL} while introducing a negligible amount of error into CWSI calculations (Gardner et al., 1992). For this study, the upper limit (dT_{UL}) was determined by taking temperature readings on a severely stressed plant twelve times between 9:15 and 15:15 throughout the day on August 8, 2014. The difference between the ambient air temperature and the leaf temperature of the severely stressed plant was computed for all readings and was averaged as the dT_{UL} , which was determined to be 10.0 °C. Ambient air temperature and humidity measurements were obtained from a weather station located 1.4 km from the field site. After the non-stressed baseline (dT_{LL}) was calculated using the vapor pressure deficit and Equation (2.3) and the non-transpiring upper limit was determined as described above, CWSI was calculated using Equation (2.2) for all remotely sensed canopy temperature data in the glasshouse and field studies. Both average and seasonal cumulative CWSI values were also calculated.

The Degrees Above Non-Stressed (DANS) index was calculated by determining the treatment with the lowest average canopy temperature throughout the measurement period for use as the non-stressed reference temperature. The non-stressed reference temperature was then subtracted from each individual crop canopy temperature measurement to calculate DANS, as in the following equation,

$$DANS = T_M - T_{NS} \quad (2.5)$$

where T_{NS} is the non-stressed reference crop canopy temperature and T_M is the measured crop canopy temperature for each individual plant. Cumulative seasonal DANS was computed by summing DANS values from individual measurement dates.

The Degrees Above Canopy Threshold (DACT) index was calculated using 28.0 °C as a reference canopy threshold or critical temperature for maize (DeJonge et al., 2014). The reference canopy threshold was then subtracted from measured crop canopy temperature, as referenced by the following equation,

$$DACT = T_M - T_C \quad (2.6)$$

where T_M is the measured crop canopy temperature and T_C is the reference canopy threshold temperature. When crop canopy temperatures are below the canopy temperature threshold of 28.0 °C, DACT has a value of zero; this differs from CWSI and DANS, which can have negative values (DeJonge et al., 2015). Cumulative DACT was computed by summing DACT values from individual measurement dates.

Relative nitrogen status was determined using Nitrogen Sufficiency Index (NSI). NSI was calculated by dividing the individual leaf chlorophyll concentration measurements for each treatment on a given measurement day by the chlorophyll concentration measurement of an unstressed reference crop on the same day (Zhu et al., 2011). These normalized values were then averaged to give a seasonal average NSI for each treatment, expressed as a decimal between 0 and 1, with a value of 1 indicating no relative nitrogen stress and a value of 0 indicating maximum relative nitrogen stress. The following equation was used,

$$NSI = SPAD_M \div SPAD_{NS} \quad (2.7)$$

where NSI refers to Nitrogen Sufficiency Index, $SPAD_M$ refers to the chlorophyll measurement of a given plant and $SPAD_{NS}$ refers to the measurement of the unstressed reference crop. The

NSI value for each individual date was then averaged over time to produce a seasonal average nitrogen stress values.

To determine biomass and grain yields, several yield components were first measured, including plant and ear densities, which were determined on a 2.5 m length of row in each treatment. Dry stover and grain weights were also determined for 10 representative plants in each treatment, following which kernels per ear and mass per kernel were also determined. Seasonal crop evapotranspiration was estimated as the sum of effective precipitation between planting and physiological maturity and irrigation (assuming an irrigation efficiency of 0.9). This water balance approach assumes zero runoff and drainage. Crop water productivity was determined by dividing average grain yield by average seasonal crop evapotranspiration.

Following measurement of leaf area, chlorophyll content, and crop canopy temperature, calculation of CWSI, DANS, DACT, and NSI, and determination of yield and yield components, statistical analysis was performed using R (R Foundation for Statistical Computing, Vienna, Austria). ANOVA analysis was used to evaluate the effects of irrigation and nitrogen treatments on CWSI, DANS, DACT, and NSI. Linear regression analysis was also performed to determine the effect of each stress index on final leaf area, biomass yield, grain yield, and crop water productivity.

RESULTS AND DISCUSSION

IRRIGATION AND EVAPOTRANSPIRATION

Evaluating average total evapotranspiration over the experimental period was important in substantiating differences between irrigation treatments and in calculating crop water productivity. In the glasshouse, irrigation was the main input affecting evapotranspiration, while both irrigation and precipitation affected evapotranspiration totals in the field study. In the

glasshouse study, evapotranspiration totaled 120 mm for the well-watered treatment and 74 mm for the drought treatment as determined by daily mass changes and irrigation water additions (Figure 2.1). In the field study, precipitation during the growing season totaled 120 mm (Table 2.1), with effective precipitation calculated as 100 mm. Calculated reference ET (ET_r) totaled 1170 mm and calculated crop ET (ET_c) totaled 780 mm (Figure 2.2). Applied irrigation totaled 700 mm, 500 mm, and 480 mm for the well watered, drought, and limited irrigation treatments, respectively (Figure 2.2; Table 2.1). Seasonal evapotranspiration, based on 90% irrigation efficiency and seasonal effective precipitation, was 730 mm for the well-watered treatment, 550 mm for the drought treatment, and 530 mm for the limited treatment (Figure 2.2; Table 2.1).

EFFECT OF IRRIGATION ON STRESS INDICES

In the glasshouse, irrigation treatment was found to have a significant effect on all three water stress indices (Figure 2.3). For example, the cumulative CWSI was 8.7 units for the drought treatment and 6.0 units for the well-watered treatment ($p = 0.009$). The well-watered treatment experienced significantly less water stress than did the drought irrigation treatment throughout the experiment. The well-watered treatment did experience some stress when water use exceeded available water before the daily irrigation events. DANS and DACT showed similar effects of irrigation treatment, with cumulative DANS totals of 32.3 and 15.5 units, respectively ($p = 0.01$) and cumulative DACT totals of 42.6 and 30.4 units for the drought and well-watered treatments, respectively ($p = 0.002$).

In the field, similar results were observed, with irrigation treatments having a significant effect on all three water stress indices (Figure 2.4). Cumulative CWSI averaged 1.0, 1.4, and 1.5 units for the well-watered, drought, and limited treatments, respectively. There was a significant difference between the well-watered and limited treatments ($p = 0.04$) but no significant

difference between the well-watered and drought treatments ($p = 0.16$). Additionally, there was not a significant difference between the drought and limited treatments ($p = 0.80$). Average seasonal cumulative DANS was 15.4, 20.2, and 22.4 units for the well-watered, drought, and limited irrigation treatments, respectively. Overall, DANS followed the same trends of significance as CWSI. Average seasonal cumulative DANS was greater for limited irrigation than for the well-watered treatment ($p = 0.03$), but the drought treatment was not different from either the well-watered ($p = 0.18$) or the limited irrigation treatments ($p = 0.69$). DACT cumulative totals were 7.2, 9.2, and 13.4 units for the well-watered, drought, and limited treatments, respectively. The well-watered treatment was significantly lower than the limited treatment ($p < 0.001$). However, unlike the other indices, the drought treatment was also significantly lower than the limited treatment ($p = 0.03$). There was not a significant difference between drought and well-watered DACT ($p = 0.41$). The limited irrigation treatment exhibited higher overall stress index scores than the drought treatment, even though the limited treatment received more irrigation water than the drought treatment from the onset of reproductive growth stage to harvest date. Given the critical value of $28.0\text{ }^{\circ}\text{C}$ used in calculating DACT, higher overall temperatures during the vegetative growth stages may have inflated water stress levels, which is reflected in the high cumulative total of the limited treatment. The amount of water stress experienced during the vegetative growth stage was such that the limited treatment had higher seasonal cumulative totals for all three stress indices. These higher totals were observed despite the fact that the drought treatment had higher stress index readings than the limited treatment on the last two measurement days of the season, which occurred during the reproductive growth stage. These observations illustrate a weakness of the water stress indices, namely that they do not discriminate the impacts of stress occurring at during different growth

stages. This is in contrast to the fact that that stress during the reproductive stages of maize has a larger negative effect on the crop yield (Traore et al., 2000; Pandey et al., 2000; DeJonge et al., 2000), implying that limited or controlled deficit irrigation should have less overall water stress than drought or consistent deficit irrigation. The results of the DACT index for this study suggest that this index created the widest separation among irrigation treatments during the vegetative growth period and maintained the differences through the rest of the season.

Both glasshouse and field results show differences between irrigation treatments for all three stress indices. Many other studies have shown CWSI to be an effective tool for assessing crop water stress. For example, Irmak et al. (2000) found that greater irrigation water applied to maize resulted in a lower seasonal average CWSI. DeJonge et al. (2014) showed that DANS and DACT correlated well with CWSI and suggested that they might be easier to use than CWSI, given that neither require measurement of air temperature or humidity. A study by Taghvaeian et al. (2014b) was conducted on sunflower and consisted of irrigation treatments with varying levels of water stress, ranging from 58 to 160 mm water applied during the vegetative stages. The study found that average CWSI ranged from 0.05 to 0.59 units and average DANS ranged from -0.09 to 5.69 units between the treatments with the highest and lowest overall evapotranspiration, respectively. These results are in line with the results of the current study, which found that both DANS and CWSI predicted differences in water stress based on irrigation treatment, with lower evapotranspiration leading to higher stress. In a study conducted in northeastern Colorado, Taghvaeian et al. (2014a) used field data to develop baselines for calculating CWSI for maize (Equations 2.3 and 2.4). It was found that the non-stressed baseline developed in their study was similar to those developed in other studies in regions with similar climates (Idso et al., 1982; Taghvaeian et al., 2012). This supports the use of a baseline

developed in northeastern Colorado by Taghvaeian et al. (2012) for use in calculating CWSI in the present study. The same study also included three irrigation treatments, namely full, high frequency deficit, and low frequency deficit irrigation. Average CWSI between 13:00 and 14:00 for the full, high-frequency deficit, and low-frequency deficit irrigation treatments was 0.19, 0.20, and 0.59 units, respectively (Taghvaeian et al., 2014a). These results support the findings of the present study, which show that CWSI, DANS, and DACT were all significantly affected by irrigation treatment.

EFFECT OF NITROGEN ON STRESS INDICES

In the glasshouse, no significant differences were observed among nitrogen treatments on average seasonal cumulative CWSI and DANS. However, significant differences were observed for DACT among nitrogen treatments. Cumulative values of 29.6, 40.0, and 40.0 units were observed for the deficient, intermediate, and sufficient N treatments, respectively (Figure 2.3). The deficient nitrogen treatment was found to have significantly lower cumulative DACT than both the intermediate ($p < 0.04$) and sufficient ($p < 0.04$) N treatments. The intermediate and sufficient N treatments were not different.

In the field, nitrogen treatments were not found to have a significant effect on any of the three water stress indices (Figure 2.4). This is similar to the results obtained from the glasshouse, where only one of the three water stress indices exhibited significantly lower water stress for the deficient N treatment as compared to the intermediate and sufficient N treatments. One possible explanation for the significant differences among nitrogen treatments observed for DACT in the glasshouse study is water deficiency experienced by the sufficient and intermediate nitrogen treatments. Since plants grew faster and larger with increasing N supply, the larger plants in the sufficient and intermediate N treatments may have experience slightly more stress under a

constant irrigation regime than the deficient N treatment. Overall, these results suggest that the stress indices are robust over variable nitrogen levels, both in the field and in a controlled environment.

CORRELATION BETWEEN STRESS INDICES

Similar to the approach taken by DeJonge et al., (2014), daily observations of DANS and DACT stress values from this study were correlated with corresponding daily CWSI stress values to evaluate whether the simpler indices are suitable replacements for CWSI (Figure 2.5). When DANS was correlated with CWSI, observations from individual measurement days had perfect linear relationships. This occurs because the same observed leaf temperature values are being used as the primary input for calculating both indices. However, when observations for all measurement days are plotted together, the individual daily linear relationships do not all align with each other. The misalignment shows that variable weather conditions on measurement days affect CWSI and DANS differently. For example, the glasshouse data points for day 24 have relatively low CWSI values, but the DANS values are comparable to those of other measurement days. On that day, average canopy temperature readings were the lowest observed during the study. Hence, CWSI, which references observed canopy temperature to upper and lower limits, indicated very little stress for any of the plants. DANS, however, references observed canopy temperature against a non-stressed reference crop and still accumulates stress when there are differences between crop and reference temperatures, even if overall canopy temperatures are low for both the measured and reference crops. Another day with misalignment in the relationship between CWSI and DANS was day 32, when CWSI levels were higher than most other measurement days, but DANS levels were not abnormally high. On that day, average canopy temperatures were the highest observed and were high even for the reference crop,

leading to a low DANS. Thus, weaknesses of the DANS index are that it overestimates stress on relatively cool days and underestimates stress on days when the reference crop shows elevated canopy temperature.

When comparing glasshouse observed DACT to CWSI, perfect linear daily relationships also appear, but with this index, there are many days when DACT is zero, the value assigned when observed canopy temperatures did not exceed the 28.0 °C critical reference threshold (DeJonge et al., 2015). For data points with DACT readings of zero, corresponding CWSI values ranged from -1.41 to 1.10. In this sense, CWSI may be advantageous because it estimates water stress readings even when the crop canopy temperature is under the critical level. The non-zero DACT values correlate well with CWSI, except for the observation on day 37. On that day, CWSI readings were lower than on other days, although DACT readings were comparable to those of other measurement days. The ambient air temperature on day 37 was 32.2 °C, which was significantly higher than the air temperature on all other measurement days. Although crop canopy temperatures on day 37 were higher than average, they were still lower than canopy temperatures for day 32, when the ambient air temperature was only 26.7 °C. The greater difference between the air temperature and the crop canopy temperatures on day 37 led to a lower CWSI reading; however, since DACT is not influenced by ambient air temperature, DACT readings were not lower than normal on this day.

Less correlation was observed between CWSI and both DANS and DACT in the field study as compared to the glasshouse study (Figure 2.5). There was some alignment when correlating DANS to CWSI for days 9, 21, 45, and 52, but the data for measurement day 54 are above this cluster and the data for day 75 are below the cluster. On day 54, the average canopy temperature was much higher than the unstressed reference temperature, while on day 75, many

of the individual crop canopy temperatures were lower than the unstressed reference, leading to multiple negative DANS levels on day 75. These varying magnitudes of difference between the reference crop and the experimental treatments affected the position of the data points for these two measurement days. However, the CWSI values vary widely but fairly consistently across measurement days, even when DANS levels are above or below normal for the two days mentioned above. This is likely due to the fact that CWSI uses actual weather conditions to determine stress, including ambient air temperature and vapor pressure, while DANS simply compares the crop canopy temperatures to a reference crop.

When comparing DACT to CWSI from the field study, there was more misalignment and many observations where DACT values were zero (Figure 2.5). As with the CWSI-DANS chart, the data points for days 54 and 75 were at the extremes. One notable difference between the two charts is the steeper slope of the lines for the individual days on the CWSI-DACT chart. This shows that each unit increase in CWSI corresponds to a greater increase in DACT than in DANS. The correlation coefficient between CWSI and DACT ($r = 0.62$) was higher than the correlation coefficient for CWSI-DANS ($r = 0.31$), but neither relationship is well described by a linear fit. DeJonge et al. (2015) showed strong linear fit statistics when correlating CWSI-DACT ($r = 0.92$) and CWSI-DANS ($r = 0.90$) for field studies conducted in Colorado. Their correlations are stronger because of the large volume of aligned data and also because only canopy temperature measurements higher than 29.0 °C were used in the correlations; however, their results also show linear daily correlations that have misalignment (DeJonge et al., 2015).

The interest in DANS and DACT is based on the argument that a simpler index may have a higher likelihood of adoption by producers. DACT is the simplest of the three indices discussed, because it only requires a single canopy temperature measurement, which is then

subtracted from the critical temperature for maize, namely 28.0 °C. However, this method results in a high number of measurements with a DACT of zero, which may mask differences in water stress on days when crop canopy temperatures are below the critical level. This is because the critical temperature in DACT is fixed, whereas CWSI accounts for variations in the critical temperature through use of upper and lower limits, which are based on humidity levels. DANS is also simpler to calculate than CWSI in that all it requires is a canopy temperature measurement and a temperature measurement for a non-stressed reference crop. However, the reference crop used in DANS is responding to local weather conditions; if the reference crop itself becomes water stressed due to high temperatures and low humidity, plants experiencing the same level of stress as the reference crop will be determined to have no stress under DANS. Conversely, in low-stress conditions, plants with temperatures several degrees higher than the reference crop may actually be unstressed, but DANS may still determine these plants as under water stress. This would not be the case for CWSI, which uses a lower baseline equation to determine stress.

While CWSI is a more complicated index that requires greater amounts of data, it has the potential to more accurately reflect water stress levels. This is because CWSI takes into account the effects of ambient air temperature and humidity on upper and lower limits in the difference between crop canopy and air temperature. On days when humidity is low, plants may transpire more than on higher humidity days, leading to greater difference between air temperature and crop canopy temperature. These differences in weather conditions were shown to affect CWSI values on the correlation charts, even when DANS and DACT were still within average ranges. A study conducted by Taghvaeian et al. (2014b) found that DANS and CWSI were highly correlated to each other in measuring sunflower water stress; the authors recommended that DANS could be used as a simpler surrogate for the more complicated CWSI. The results of the

current study suggest that CWSI is a more robust index because it accounts for variations in humidity. With the possibility of using previously established baselines determined empirically in regions with similar climate, it is possible to calculate CWSI in a simple manner (Taghvaeian et al., 2014a). Thus, CWSI is the recommended indexing approach for predicting water stress and scheduling irrigation.

EFFECT OF WATER STRESS INDICES ON YIELD AND YIELD PARAMETERS

Crop water stress indices from the field study were compared with several parameters of crop growth as a means to validate their use. Only the sufficient N treatment averages were used in order to evaluate the effect of drought stress independent of N stress. CWSI ($R^2 = 0.41$) and DANS ($R^2 = 0.40$) showed moderate correlation with the final leaf area (Figure 2.6). Leaf area was inversely related to these indices, with greater water stress resulting in smaller average leaf area. The level of correlation between leaf area and DACT was lower ($R^2 = 0.25$). Similar to the results of this study, Taghvaeian et al. (2014b) showed a strong correlation between both CWSI and DANS to leaf area in sunflower with six controlled deficit irrigation treatments.

A lesser degree of correlation was observed between biomass yield and CWSI ($R^2 = 0.24$), DANS ($R^2 = 0.23$), and DACT ($R^2 = 0.12$), respectively. Very little correlation was observed between any of the three stress indices and grain yield. A relatively low degree of positive correlation was observed between all three stress indices and crop water productivity, with $R^2 = 0.19$, $R^2 = 0.19$, and $R^2 = 0.17$ for CWSI, DANS, and DACT, respectively. The positive relationship between stress indices and crop water productivity means that plants under greater water stress had a higher crop water productivity. In other words, plants under limited or controlled deficit irrigation produce more grain yield per unit of water input than do plants in the

well-watered treatment. However, the low correlation coefficient value suggests that water stress is not the only factor influencing this relationship.

Irmak et al. (2000) found that CWSI was highly correlated to grain yield in deficit-irrigated maize. In that study, maize was irrigated when soil water content dropped to 75%, 50%, and 25% of soil water holding capacity, respectively, and a fourth treatment was not irrigated. Average CWSI was 0.28, 0.19, 0.36, and 0.53 units, and average grain yield was 5.3, 6.1, 4.6, and 0.7 Mg ha⁻¹ for the 0.75%, 0.50%, 0.25%, and non-irrigated treatments, respectively. These results show that in general, lower frequency of irrigation resulted in higher average seasonal water stress and lower grain yield (Irmak et al., 2000). Another study which evaluated the effect of CWSI on cotton and sorghum grain yield found that CWSI was negatively correlated to yield in both cases (Wanjura et al., 1990). These results contrast with those of the present study, which found weak correlation between stress indices and maize grain yield. The weak level of correlation is likely due to the limited or controlled deficit irrigation treatment, which resulted in water stress during non-critical growth stages and which was not present in the study conducted by Irmak et al. (2000). In the present study, reasonable yield prediction depended on accounting for the combinations of water stress severity and timing as well as nitrogen stress. The figures of high nitrogen plots including all three irrigation treatments show that crop water productivity, grain yield, and final leaf area values were above the curve for three of the four individual limited irrigation treatment plants (Figure 2.6). The limited or controlled deficit irrigation plants showed the highest overall stress, even though plants in the drought or consistent deficit irrigation treatment had the lowest crop water productivity, grain yield, and final leaf area (Figure 2.6). This shows that the stress indices are not sensitive to timing of stress, which negatively affects the linear relationship because crop growth is sensitive to timing of stress. This

is one potential problem with the use of stress indices when practicing controlled deficit irrigation.

NITROGEN SUFFICIENCY INDEX

In the greenhouse, both irrigation and nitrogen treatments were found to have a significant effect on average Nitrogen Sufficiency Index (NSI). The well-watered treatment had significantly higher NSI than the drought treatment ($p = 0.009$), with average NSI values of 0.92 and 0.86 units, respectively. The deficient N treatment had significantly lower NSI than both the sufficient treatment ($p < 0.001$) and the intermediate N treatment ($p < 0.001$), with average NSI values of 0.94, 0.92, and 0.81 units for the sufficient, intermediate, and deficient N treatments, respectively. The difference between NSI values for the sufficient and intermediate N treatments was not significant ($p = 0.61$). In the greenhouse, it was found that average NSI had a significant effect on both total biomass ($p = 0.03$) and final leaf area ($p < 0.001$) (Figure 2.7).

In the field, it was found that nitrogen treatment had a significant effect on crop nitrogen status as determined by NSI. The average seasonal NSI was significantly lower for the deficient N treatment than for the sufficient N treatment ($p = 0.007$). There was no significant difference between the sufficient and sufficient delayed N treatments ($p = 0.24$) or the sufficient delayed and deficient N treatments ($p = 0.25$). Average NSI values were 0.83, 0.74, and 0.79 units for the sufficient, deficient, and sufficient delayed N treatments, respectively. It was also found that irrigation treatment did not have a significant effect on NSI, with average values of 0.76, 0.77, and 0.82 units for the well-watered, drought, and limited irrigation treatments, respectively. In the field, average seasonal NSI was found to be a significant predictor of grain yield ($p = 0.002$), biomass yield ($p < 0.001$), and crop water productivity ($p < 0.001$) (Figure 2.8). Based on the results of linear regression, every 0.1 unit increase in NSI in the field study resulted in a 1.6 Mg

ha⁻¹ increase in biomass yield, a 0.4 kg m⁻³ increase in crop water productivity, and a 1.2 Mg ha⁻¹ increase in grain yield, respectively.

Measurements of leaf chlorophyll concentration as determined by SPAD meter were used to calculate the Nitrogen Sufficiency Index (NSI); average leaf chlorophyll concentration for all plants on a given measurement day ranged from 26.6 to 33.1 units in the field. This is lower than the results obtained from a study conducted by Sunderman et al. (1997), which yielded average daily leaf chlorophyll concentration as determined by SPAD ranging from 40.0 to 60.0 units. The higher average leaf chlorophyll concentrations in that study were likely due to the fact that all plants were fully fertilized with 224 kg N ha⁻¹, with treatments consisting of various corn cultivars (Sunderman et al., 1997). Average leaf chlorophyll concentration was lower for the current study because this study incorporated sufficient, deficient, and sufficient delayed nitrogen treatments ranging from 90 to 180 kg N ha⁻¹. Another study conducted by Víg et al. (2012) found leaf chlorophyll concentration as determined by SPAD ranging from 41.6 to 44.6 units between different sections of the leaf canopy on potato plants. These values are closer to those obtained from the current study. A study conducted by Zhu et al. (2011) measured NSI and other nitrogen indices on corn grown under six different nitrogen treatments, ranging from 0 to 250 kg N ha⁻¹. The treatment that received no nitrogen fertilizer had average NSI values of between 0.2 and 0.4 units throughout the growing season. The other treatments had NSI values close to 1.0 unit at the beginning of the season, with more N-deficient treatments dropping off later in the season; for example, the treatment with 50 kg N ha⁻¹ dropped to an average of 0.6 units at the end of the season, while the treatment with 200 kg N ha⁻¹ remained close to 1.0 unit (Zhu et al., 2011). As with the study conducted by Zhu et al. (2011), the results of the present study indicate that nitrogen treatment does have an effect on NSI, with values ranging from 0.66

to 0.81 units for the deficient N treatment and from 0.79 to 0.91 units for the sufficient N treatment. The values for NSI found in this study are comparable to the results obtained from Zhu et al. (2011). These results show that varying rates of nitrogen stress were present in the study and that nitrogen stress along with water stress can account for a greater overall effect on crop water productivity.

A study conducted by Rudnick and Irmak (2013) consisted of a full factorial combination of full and limited irrigation treatments and various N treatments, ranging from 0 kg N ha⁻¹ to 252 kg N ha⁻¹. The results show that an intermediate N treatment, namely 196 kg N ha⁻¹, was optimal for maximizing increases to grain yield above rainfed agriculture when using limited irrigation as a strategy (Rudnick & Irmak, 2013). Another study conducted by Al-Kaisi and Yin (2003) consisted of three irrigation treatments (0.60, 0.80, and 1.00 of estimated evapotranspiration) and four nitrogen treatments (30, 140, 250, and 360 kg N ha⁻¹). Results from the study showed that 0.80 irrigation combined with 140 to 250 kg N ha⁻¹ was the best strategy for maximizing crop water productivity (Al-Kaisi and Yin, 2003). These results further support the premise that nitrogen effects also need to be taken into account when planning for optimal use of a limited irrigation water resource. When using controlled deficit or limited irrigation, nitrogen fertilization rate may also need to be lowered accordingly to optimize grain yield relative to water savings.

SUMMARY

The objective of this study was to evaluate the usage of three water stress indices, namely Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT), in estimating water stress in maize under a variety of irrigation regimes and nitrogen fertilization levels. This was done through both glasshouse and field

studies. The glasshouse study consisted of two irrigation treatments, namely well-watered and drought irrigation, and three nitrogen treatments, namely sufficient, intermediate, and deficient nitrogen. The field study consisted of three irrigation treatments, namely well-watered, drought (consistent deficit irrigation) and limited (growth-stage timed or controlled deficit irrigation), and three nitrogen treatments, namely sufficient, deficient, and sufficient delayed nitrogen. First, it was found that irrigation had a significant effect on all three water stress indices in both the glasshouse and the field. For example, cumulative DANS in the glasshouse was 32.2 units for the drought treatment and 15.5 units for the well-watered treatment. Similar trends were observed for DACT and CWSI in the glasshouse. In the field, cumulative DANS was 22.4 units for the limited treatment and 7.2 units for the well-watered treatment, with similar trends being observed for DACT and CWSI. This shows that the water stress indices were receptive to differences in irrigation regime. However, it was found that stress indices showed higher seasonal cumulative stress levels for the limited irrigation treatment, even though individual measurement days occurring after the onset of anthesis exhibited higher overall stress levels for the drought treatment. This shows a weakness of the stress indices, namely that they do not discriminate between the impacts of stress occurring at during different growth stages, even though studies have established that stress during the reproductive stages of maize has a larger negative effect on the crop yield.

It was found that nitrogen did not have a significant effect on any of the water stress indices in the field, and that significant differences were only observed for the DACT index in the glasshouse study. The differences in DACT values between nitrogen treatments in the glasshouse can be attributed to water stress, as plants with more nitrogen grow larger leaf area and require more water than plants with less nitrogen. These results show that the stress indices

are robust across varying nitrogen levels, illustrating their usefulness in managing water applications even among variable field conditions.

From the correlation plots, it was found that there was a sufficient amount of correlation between CWSI and DANS in both the glasshouse and the field. However, on days where the difference in air temperature and measured crop canopy temperature was greater, CWSI was able to detect lower water stress than DANS. This is because CWSI integrates current weather conditions into the calculation of water stress, while DANS uses a reference crop temperature to determine relative water stress. Thus, weaknesses of the DANS index are that it overestimates stress on relatively cool days and underestimates stress on hot days when the reference crop shows elevated canopy temperature. This shows the potential advantages of using CWSI to predict water stress under varying weather conditions. CWSI was more correlated to DACT in both the glasshouse and the field; however, DACT was the least correlated to crop response variables of all the stress indices. The higher level of correlation between DACT and CWSI can possibly be explained by the fact that all crop canopy temperatures under 28.0 °C were reported as having zero stress under DACT, but not under CWSI. CWSI may be advantageous because it estimates water stress readings even when the crop canopy temperature is under the critical level.

In the field, it was found that none of the three stress indices were highly correlated with grain yield, but there was a considerable amount of correlation to final leaf area, with higher water stress resulting in lower average final leaf area. There was also a modest positive correlation between water stress indices and crop water productivity. For example, in the field, every unit increase in cumulative CWSI resulted in a 0.31 kg m⁻³ increase in crop water productivity. This means that plants experiencing higher levels of water stress in the limited and

drought irrigation treatments had higher overall crop water productivity than did the plants under lower stress in the well-watered treatment.

Linear regressions of the effect of plant nitrogen status on various crop response variables found that Nitrogen Sufficiency Index (NSI) had a significant effect on final leaf area and total biomass in the glasshouse study and on grain yield, biomass yield, and crop water productivity in the field study. These results suggest that water stress indices are useful tools in evaluating crop water status and scheduling irrigation, but that other factors such as nitrogen status should also be taken into consideration in conjunction with water stress. Producers aiming to increase the efficiency of their water inputs should evaluate not only water stress but also nitrogen stress in order to manage their irrigation water more effectively.

FIGURES AND TABLES

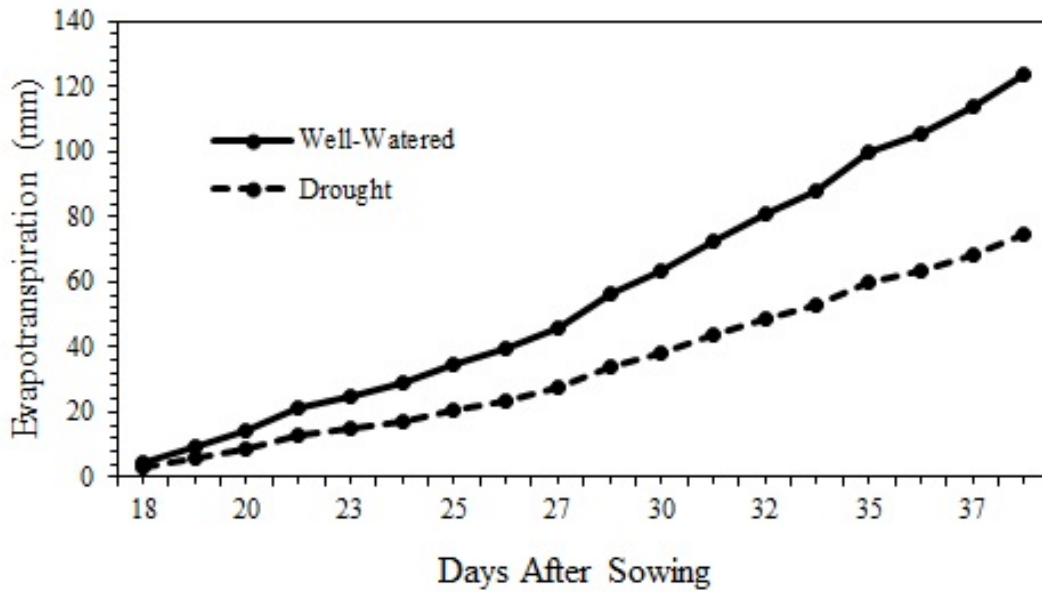


Figure 2.1 Cumulative evapotranspiration for well-watered and drought irrigation treatments throughout the duration of the glasshouse study as determined by daily mass changes and irrigation water additions (March 27-April 16, 2014).

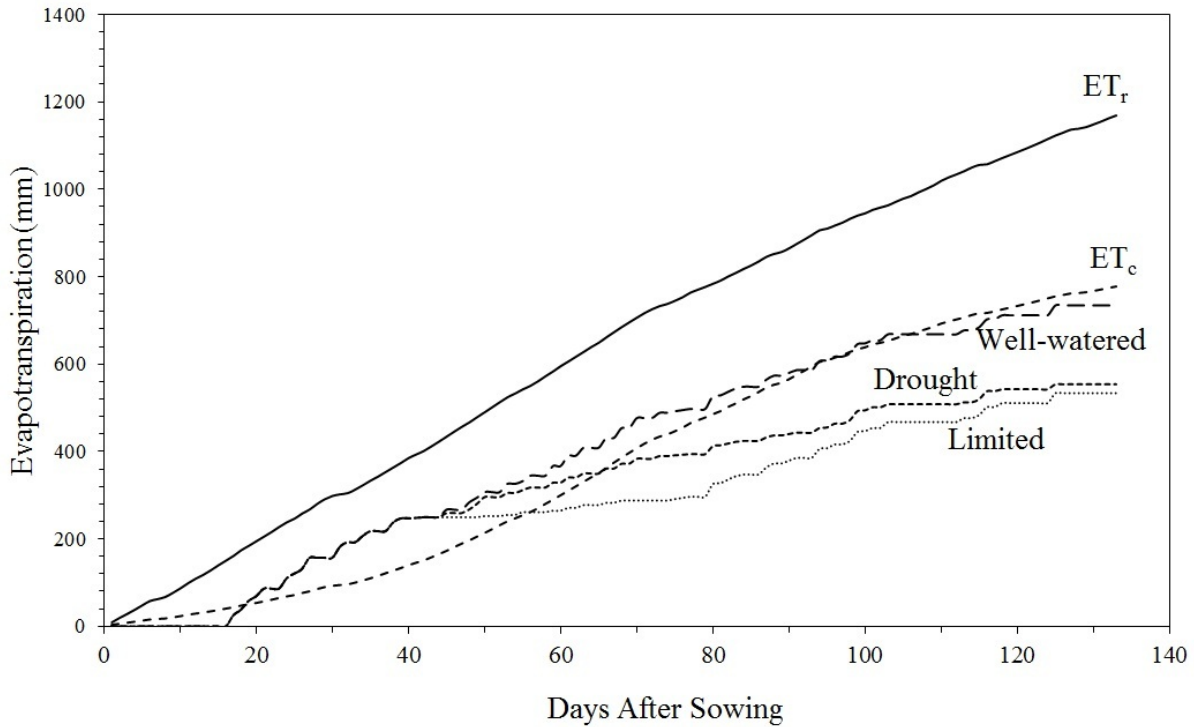


Figure 2.2 Seasonal calculated reference (ET_r) and crop (ET_c) evapotranspiration, and estimated seasonal evapotranspiration for the well-watered, drought, and limited irrigation treatments in the field based on the sum of effective precipitation and applied irrigation treatments in the field based on the sum of effective precipitation and applied irrigation.

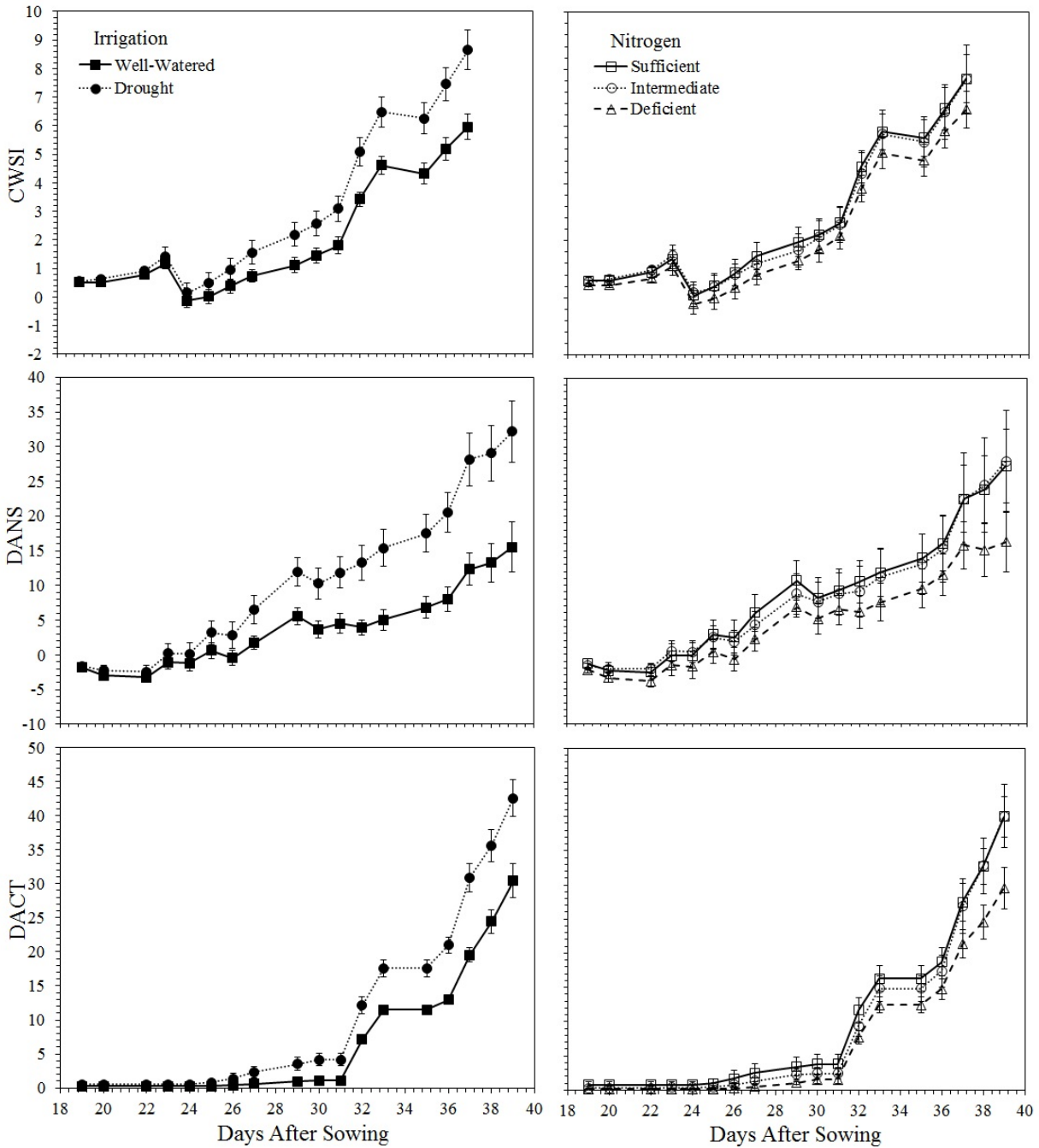


Figure 2.3 The effect of irrigation and nitrogen treatments on cumulative Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT) in the glasshouse.

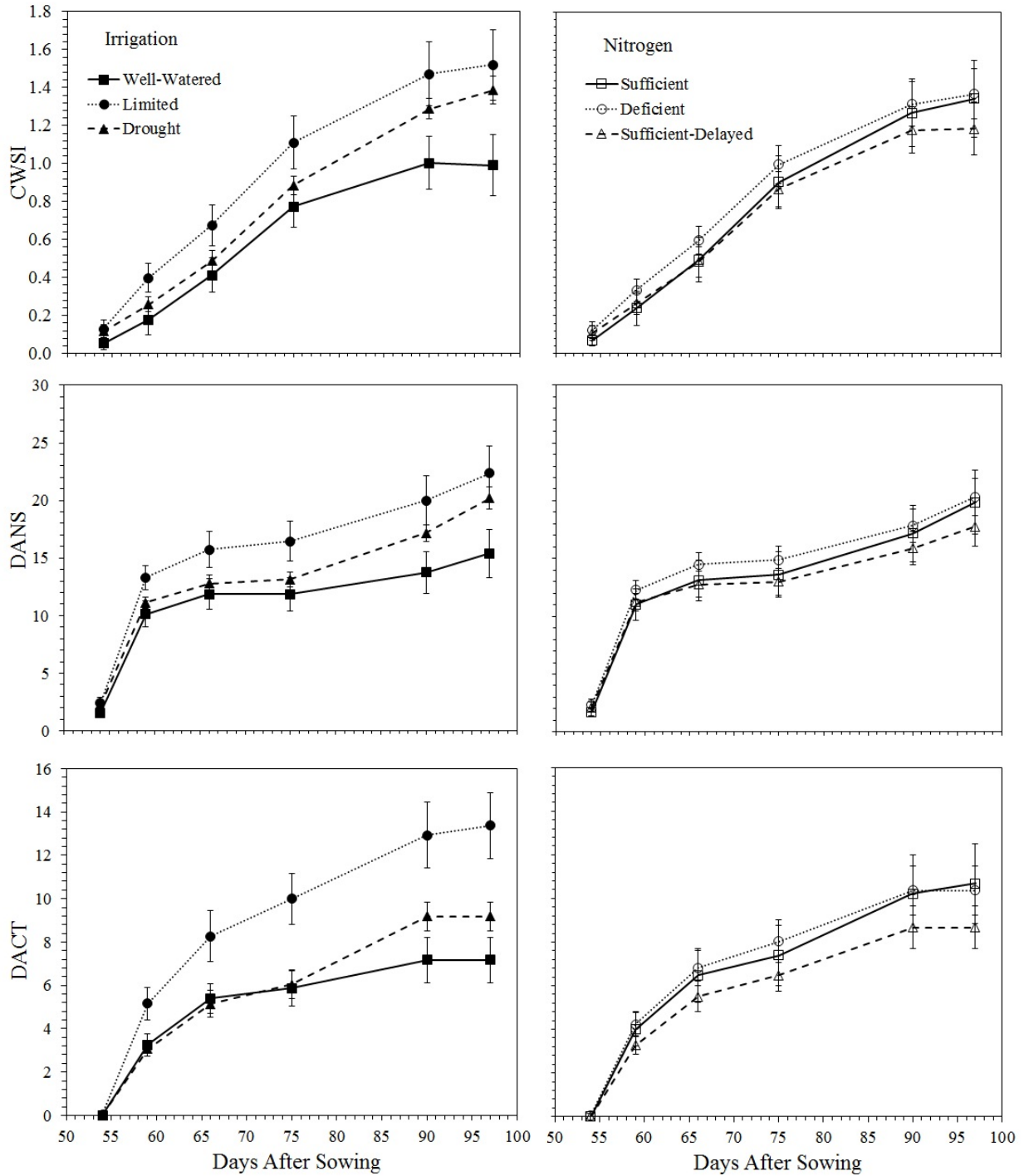


Figure 2.4 The effect of irrigation and nitrogen treatments on cumulative Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT) in the field.

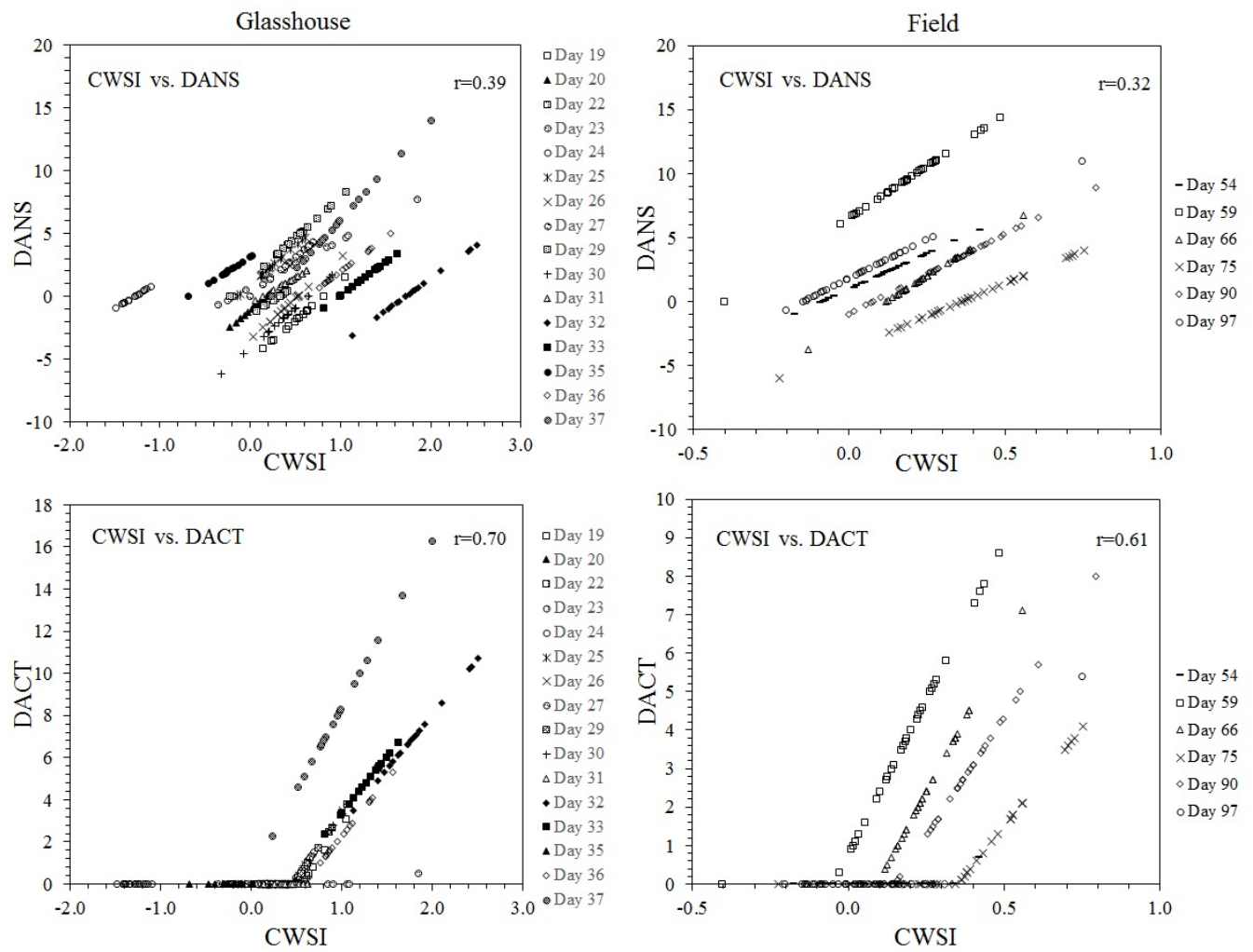


Figure 2.5 Correlation of Crop Water Stress Index (CWSI) to Degrees Above Non-Stressed (DANS) and Degrees Above Canopy Threshold (DACT) from the glasshouse and field studies.

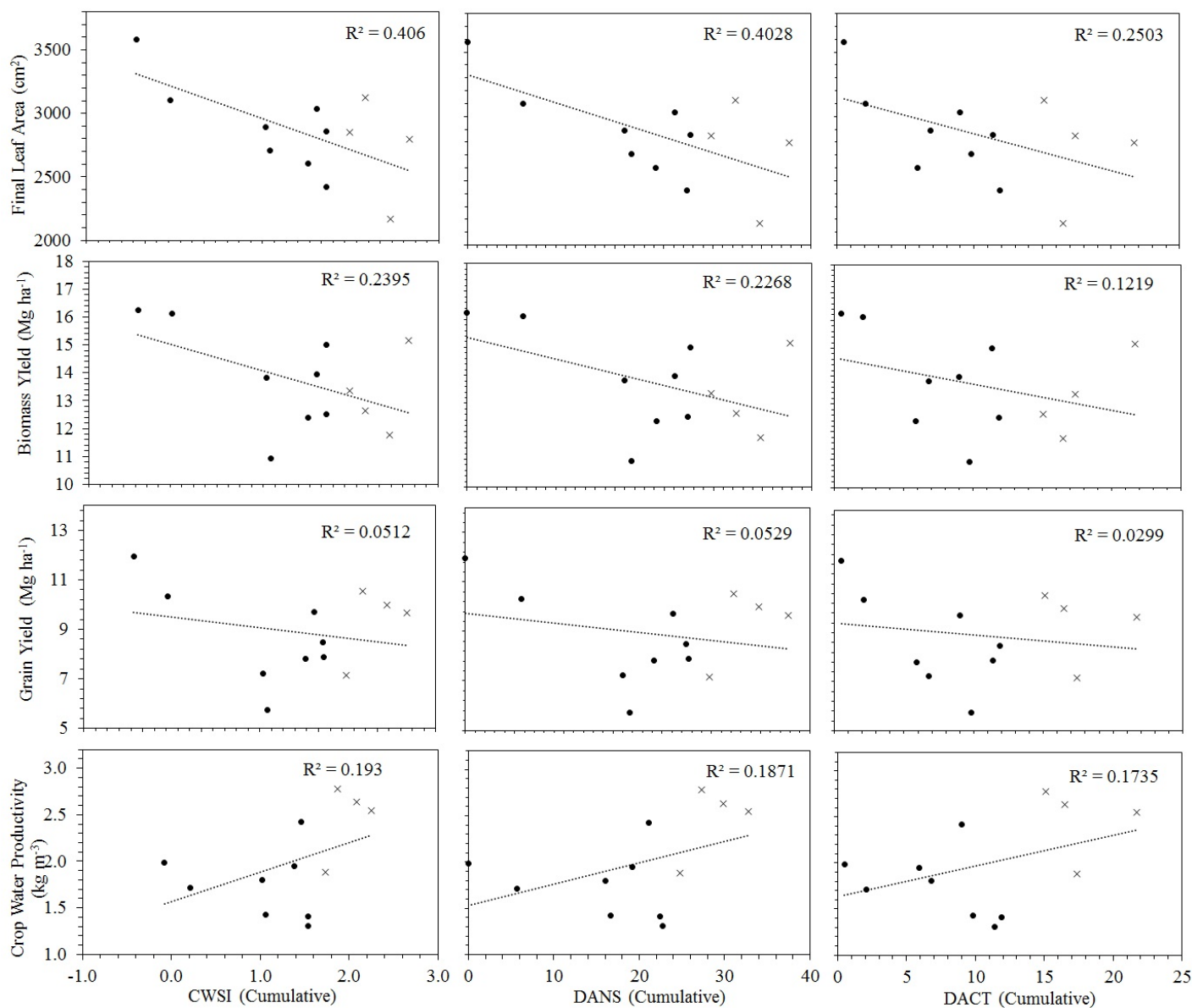


Figure 2.6 Relationships between cumulative Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT) and final leaf area, dry biomass yield, grain yield, and crop water productivity in the field study. The limited irrigation treatment is denoted with an x on the graphs, while the well-watered and drought treatments are denoted with circles.

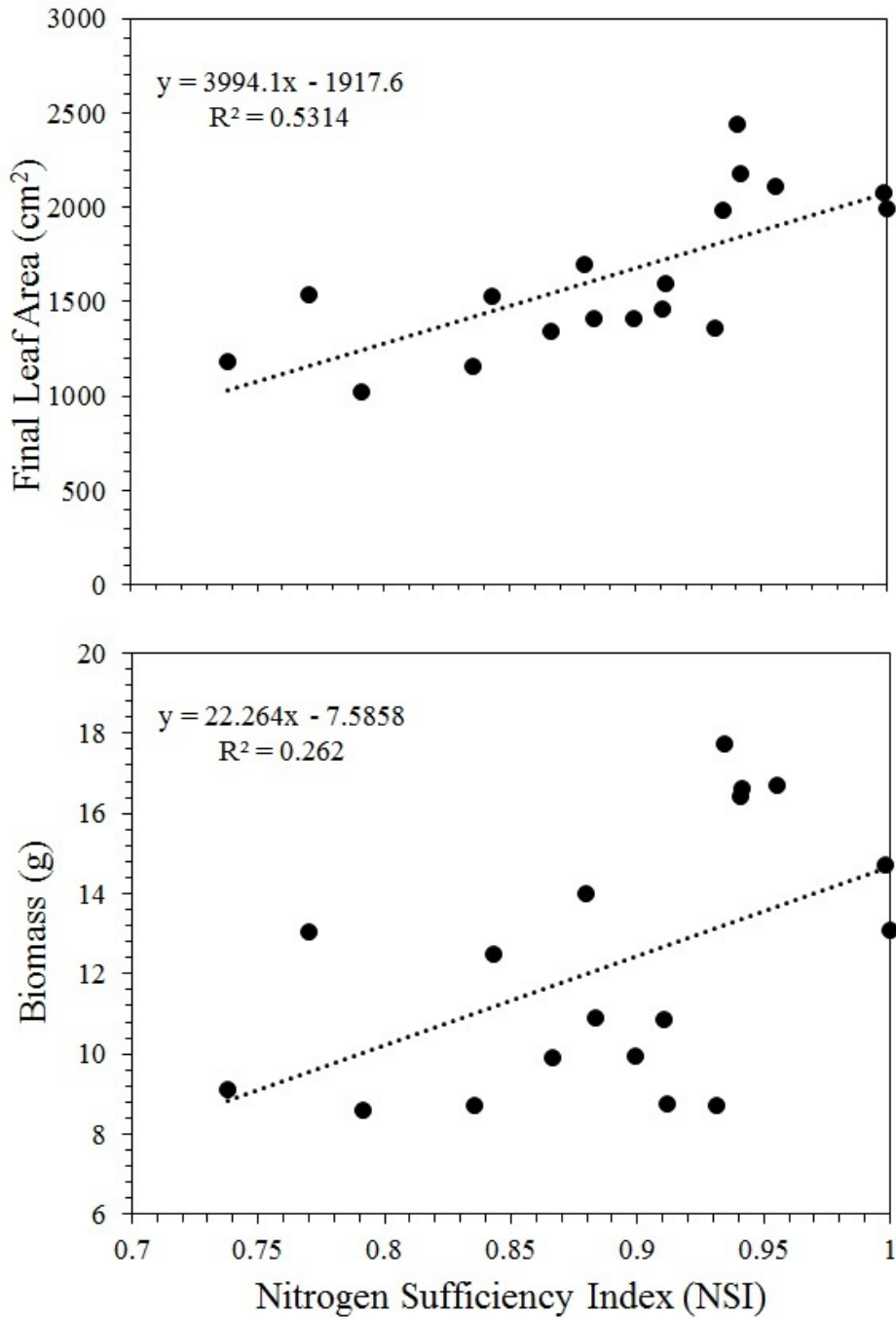


Figure 2.7 Average Nitrogen Sufficiency Index was found to be positively related to average final leaf area and total plant biomass in the glasshouse study.

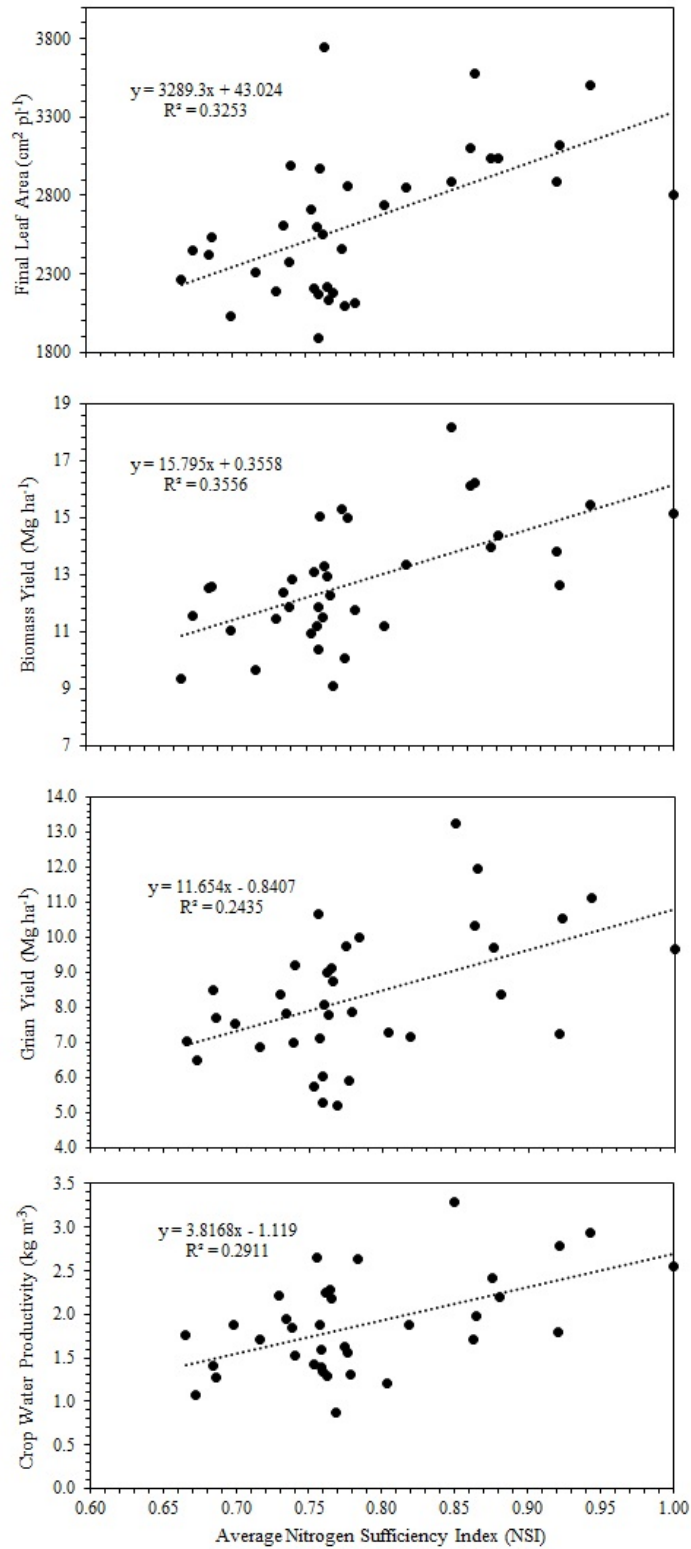


Figure 2.8 Average Nitrogen Sufficiency Index was found to be positively correlated to final leaf area, biomass yield, grain yield, and crop water productivity in the field study.

Table 2.1 Seasonal irrigation, rainfall, evapotranspiration, and applied nitrogen for both irrigation and nitrogen treatments in the field study.

Irrigation Regime	Nitrogen Regime	Irrigation (mm)	Rainfall (mm)	ET (mm)	Applied N (kg/ha)
Well-Watered	Sufficient	700	120	730	180
	Sufficient delayed	700	120	730	180
	Deficient	700	120	730	90
Drought	Sufficient	500	120	550	180
	Sufficient delayed	500	120	550	180
	Deficient	500	120	550	90
Limited	Sufficient	480	120	530	180
	Sufficient delayed	480	120	530	180
	Deficient	480	120	530	90

Table 2.2 Average seasonal Crop Water Stress Index (CWSI), Degrees Above Non-Stressed (DANS), and Degrees Above Canopy Threshold (DACT) values for irrigation and nitrogen treatments in the field and glasshouse studies.

Study	Treatment		CWSI	DANS	DACT
Field	Irrigation	Well-Watered	0.17	2.57	1.19
		Drought	0.23	3.37	1.53
		Limited	0.25	3.73	2.23
	Nitrogen	Sufficient	0.22	3.31	1.78
		Deficient	0.23	3.39	1.73
		Sufficient delayed	0.20	2.96	1.45
Glasshouse	Irrigation	Well-Watered	0.37	0.86	1.69
		Drought	0.54	1.79	2.37
	Nitrogen	Sufficient	0.48	1.52	2.22
		Intermediate	0.48	1.55	2.22
		Deficient	0.41	0.90	1.64

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APPENDIX I

Glasshouse study pretreatment nutrient solution, treatment nutrient solution, and treatment nitrogen solution.

Pretreatment Nutrient Solution				
Solution	ml 18L⁻¹	Molarity	Molecular Weight	g L⁻¹
CaCO ₃	3.86	2.14x10 ⁻³	100.09	0.21
Ca(NO ₃) ₂ *4H ₂ O	38.57	1.00	236.15	236.20
KNO ₃	38.57	1.00	101.10	101.10
MgSO ₄ *7H ₂ O	6.43	1.00	246.48	246.48
KH ₂ PO ₄	4.50	1.00	136.09	136.10
FeCl ₃ *6H ₂ O	2.70	0.18	270.30	48.65
HEDTA	2.70	0.25	278.26	69.50
MES	46.80	0.77	195.24	150.00
Solution C	10.29			
<i>MnCl₂*4H₂O</i>		<i>0.01</i>	<i>197.90</i>	<i>2.34</i>
<i>H₃BO₃</i>		<i>0.03</i>	<i>61.83</i>	<i>2.04</i>
<i>ZnSO₄*7H₂O</i>		<i>3.06x10⁻³</i>	<i>287.56</i>	<i>0.88</i>
<i>CuSO₄*5H₂O</i>		<i>8.01x10⁻⁴</i>	<i>249.69</i>	<i>0.20</i>
<i>Na₂MoO₄*2H₂O</i>		<i>5.21x10⁻⁴</i>	<i>241.95</i>	<i>0.13</i>
Treatment Nutrient Solution				
Solution	ml 18L⁻¹	Molarity	Molecular Weight	g L⁻¹
CaCO ₃	3.86	2.14x10 ⁻³	100.09	0.21
CaCl ₂	9.00	0.50	55.49	27.75
KH ₂ PO ₄	13.50	1.00	136.09	136.09
K ₂ SO ₄	57.86	0.50	174.26	87.13
MgSO ₄ *7H ₂ O	9.64	1.00	246.48	246.48
FeCl ₃ *6H ₂ O	2.70	0.18	270.30	48.65
HEDTA	0.96	0.25	278.26	69.50
Solution C	23.14			
<i>MnCl₂*4H₂O</i>		<i>0.01</i>	<i>197.90</i>	<i>2.34</i>
<i>H₃BO₃</i>		<i>0.03</i>	<i>61.83</i>	<i>2.04</i>
<i>ZnSO₄*7H₂O</i>		<i>3.06x10⁻³</i>	<i>287.56</i>	<i>0.88</i>
<i>CuSO₄*5H₂O</i>		<i>8.01x10⁻⁴</i>	<i>249.69</i>	<i>0.20</i>
<i>Na₂MoO₄*2H₂O</i>		<i>5.21x10⁻⁴</i>	<i>241.95</i>	<i>0.13</i>
Treatment Nitrogen Solution				
Solution	ml 18L⁻¹	Molarity	Molecular Weight	g L⁻¹
NH ₄ NO ₃	1.61	1.00	80.04	80.04

Nutrient Concentrations			
Nutrient	Pretreatment (mg L⁻¹)	Treatment (mg L⁻¹)	Nitrogen (mg L⁻¹)
NO3-N	90.04	0.00	1.30
NH4-N	0.00	0.00	1.30
P	7.74	23.23	
K	93.56	92.16	
S	11.52	68.87	
Ca	85.90	10.04	
Mg	8.68	13.02	
Zn	0.11	0.26	
Fe	1.51	1.51	
Mn	0.37	0.84	
Cu	0.03	0.07	
B	0.20	0.46	
Cl	3.35	21.68	
Mo	0.03	0.06	
Na	0.01	0.03	
HEDTA	10.43	3.73	
MES	390.00	0.00	