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**EVALUATING EVAPOTRANSPIRATION AND MANAGEMENT OF
GLYPHOSATE-RESISTANT PALMER AMARANTH (*AMARANTHUS
PALMERI* S. WATSON)**

by

Jasmine M. Mausbach

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EVALUATING EVAPOTRANSPIRATION AND MANAGEMENT OF
GLYPHOSATE-RESISTANT PALMER AMARANTH (*AMARANTHUS PALMERI* S.
WATSON)

Jasmine Mausbach, M.S.

University of Nebraska, 2021

Advisors: Amit J. Jhala, Suat Irmak

Palmer amaranth (PA) is the most problematic weed in agronomic cropping systems in the United States. Acetolactate synthase (ALS) inhibitor-/glyphosate-resistant (GR) PA has been confirmed in Nebraska and is widespread in several counties. Soybean resistant to isoxaflutole/glufosinate/glyphosate has been developed to provide additional herbicide sites of action for control of herbicide-resistant weeds. The objectives of this study were to evaluate herbicide programs for control of ALS inhibitor/GR PA and their effect on PA density and biomass, as well as soybean injury and yield in isoxaflutole/glufosinate/glyphosate-resistant soybean. A PRE herbicide fb glufosinate controlled PA 80%–99% 21 d after late-POST in 2018 and reduced density 89%–100% in 2018 and 58%–100% in 2019 at 14 d after early-POST.

Weed-crop competition models offer a significant tool for understanding and predicting crop yield losses due to crop-weed interference. Within current empirical models, weed biological characteristics are unknown, which limits understanding of weed growth in competition with crops and how that competition affects crop growth

parameters. The objective of this study was to determine the effect of center-pivot and subsurface drip irrigation on the average evapotranspiration (ET_a) of PA grown in corn, soybean, and fallow in south central Nebraska. Results suggest irrigation affects subplot ET_a differences early in the growing season, but crop system and progression of plant growth with available water have a greater effect on ET_a differences than irrigation type later in the growing season. Thus, crop management will likely have greater effects on PA ET_a values than irrigation practices alone. This study provides base data on weed evapotranspiration and its relation to weed morphological features for future use in mechanistic weed-crop competition models.

Velvetleaf is another troublesome broadleaf weed that competes with agronomic crops for resources such as soil moisture. The objective of this study was to determine the effect of degree of water stress on the growth and fecundity of velvetleaf using soil moisture sensors under greenhouse conditions. The results of this study demonstrate that velvetleaf can survive $\geq 50\%$ field capacity (FC) continuous water stress conditions, although with reduced leaf number, plant height, and growth index compared to 75% and 100% FC.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

Introduction

Palmer amaranth. Palmer amaranth is a summer annual broadleaf weed belonging to the Amaranthaceae family and is one of the dioecious species among pigweeds (Steckel 2007). Human activities in the 20th century such as agricultural development, within- and between-field operations, and seed and equipment transportation have led Palmer amaranth to spread to the northern United States (Costea et al. 2004; 2005; Culpepper 2006). Since the first report of Palmer amaranth beyond its native habitat in the southwest United States, it has become one of the most problematic and troublesome weeds in agronomic cropping systems in the United States (Culpepper et al. 2010; Ward et al. 2013). A multistate growers' survey conducted in 2005–2006 reported that pigweeds were one of the three most problematic weeds in glyphosate-resistant corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production fields in the Midwest (Kruger et al. 2009). A statewide survey of Nebraska stakeholders in 2015 found that Palmer amaranth ranked fourth out of the top five most difficult to control weeds in the Panhandle and West Central Nebraska (Sarangi and Jhala 2018). More recently, a statewide survey of Nebraska stakeholders found that Palmer amaranth was considered the most difficult to control in corn and soybean cropping systems.

Several factors have enabled Palmer amaranth to become such a dominant and difficult-to-control weed, including its rapid growth rate (Ehleringer and Forseth 1980; Ehleringer 1985), prolific seed production (Keeley et al. 1987; Massinga et al. 2001), and ability to tolerate adverse environmental conditions, including disease, genetic

abnormalities (Franssen et al. 2001), and water stress (Chahal et al. 2018). Horak and Loughin (2000) reported that Palmer amaranth had the highest plant dry weight, leaf area, water-use efficiency, and growth rate (0.10–0.21 cm per growing degree day) compared to redroot pigweed (*Amaranthus retroflexus* L.), tumble pigweed (*Amaranthus albus* L.), and waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] in a two-year field study in Kansas. Palmer amaranth also has greater root length and root biomass compared with most crops, allowing it to occupy a larger soil volume and obtain soil nutrients (Wright et al. 1999). As a dioecious species, Palmer amaranth is an obligate outcrossing, wild pollinated species (Sosnoskie et al. 2012), resulting in wide genetic diversity that can lead to the spread of herbicide-resistant alleles (Jhala et al. 2021; Oliveira et al. 2018). Due to its prolific seed production, aggressive growth habit, and ability to evolve resistance to commonly used herbicides, it is vital to control Palmer amaranth early in the growing season by integrating mechanical, cultural, and chemical practices, including PRE herbicides with multiple sites of action (de Sanctis et al. 2021; Norsworthy et al. 2012).

Velvetleaf. Velvetleaf (*Abutilon theophrasti*) is regarded as a troublesome broadleaf weed (Spencer 1984), causing grain yield losses in fields of corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], sorghum [*Sorghum bicolor* (L.) Moench], and cotton (*Gossypium hirsutum* L.) (Behrens 1979; Colton and Einhellig 1980; Eaton et al. 1976; Hagood et al. 1980; Higgins et al. 1984; Oliver 1979; Spencer 1984; USDA 1970). A multistate growers' survey conducted in 2005–2006 reported velvetleaf as one of the three most problematic weeds in GR corn and soybean cropping systems in the Midwest (Kruger et al. 2009). A statewide survey of Nebraska stakeholders in 2015 found that

velvetleaf ranked the fourth most difficult to control weed in corn and soybean production fields (Sarangi and Jhala 2018).

Velvetleaf possesses a number of characteristics that contribute to its success as a weed, including rapid root growth and the ability to produce sugars at a relatively efficient rate in low sunlight, allowing growth under partially shaded crop canopies (Roeth 1987). Velvetleaf produces 700–17,000 seeds plant⁻¹ that have high viability and can persist in the soil up to 50 years (Anderson et al. 1985; Chandler and Dale 1974; Khedir and Roeth 1981). In addition, velvetleaf has a sporadic and continuous germination pattern (Burnside et al. 1981; Roeth 1987); robust seedling vigor (Hartgerink and Bazzaz 1984); allelopathic effects (Bhowmik and Doll 1982; Colton and Einhellig 1980; Elmore 1980; Gressel and Holm 1964; Sterling 1987a, 1987b); is a host to several crop pests and pathogens (Hepperley et al. 1980; Jacques and Peters 1971); and has reduced susceptibility to some herbicides used in corn and soybean production (Jhala et al. 2021), such as dicamba (de Sanctis and Jhala 2021).

Herbicide Resistance. Globally, glyphosate is the most widely used agricultural pesticide and is used extensively in glyphosate-resistant (GR) canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), sugarbeet (*Beta vulgaris* var. *saccharifera*), and soybean in the United States (Heap and Duke 2018). Since the commercialization of GR crops, particularly GR corn and soybean in the midwestern United States and GR cotton in the southern United States, continuous use of glyphosate multiple times in a year, along with a decline in the use of residual herbicides (Culpepper 2006; Young 2006), has resulted in the evolution of GR weeds (Beckie 2006). As of 2020, 50 weeds have been confirmed resistant to glyphosate worldwide (Heap 2021),

including six broadleaf weeds such as common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), kochia [*Bassia scoparia* (L.) A. J. Scott], horseweed (*Erigeron canadensis* L.), waterhemp, and Palmer amaranth in Nebraska (Jhala 2017a). Palmer amaranth resistant to glyphosate was first confirmed in Georgia in 2004 (Culpepper et al. 2006). Since then, GR Palmer amaranth has been confirmed in 28 states in the United States (Heap 2021; Heap and Duke 2018). Palmer amaranth resistant to ALS inhibitors was first confirmed in Kansas in 1994 and since then has been confirmed in 14 states in the United States (Heap 2021; Sprague et al. 1997). Palmer amaranth resistant to glufosinate, another commonly used herbicide, was recently confirmed in Arkansas (Barber et al. 2021).

Palmer amaranth has evolved resistance to herbicides from at least eight herbicide sites of action: microtubule-, acetolactate synthase (ALS)-, 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS)-, photosystem (PS) II, hydroxyphenylpyruvate dioxygenase (HPPD)-, very long chain fatty acid (VLCFA)-, protoporphyrinogen oxidase (PPO)-, and synthetic auxin inhibitors (Heap 2021). In Nebraska, Palmer amaranth biotypes with multiple herbicide-resistance to HPPD- and PS II-inhibitors, as well as EPSPS- and PS II-inhibitors were confirmed in 2014 and 2016, respectively (Chahal et al. 2017; Jhala et al. 2014). In Kansas, a population of PA resistant to five herbicide sites of action including synthetic auxin-, EPSPS-, ALS-, PS II-, and HPPD-inhibitors was confirmed (Kumar et al. 2019). Herbicide-resistant Palmer amaranth reduces herbicide options for growers and can cause major crop yield losses if not controlled early in the growing season. Annually, weeds cause an estimated loss of more than US\$100 billion and a 10% yield loss on a global scale (Appleby et al. 2000). In light of these losses, it is clear that a greater

understanding of crop-weed interactions is necessary to develop cost-effective and sustainable weed management practices.

LibertyLink GT27™ Soybean. In order to address the growing need to control GR weeds in cropping systems, multiple-herbicide-resistant soybean traits have been developed. The recently available isoxaflutole/glufosinate/glyphosate-resistant soybean (LibertyLink GT27™) provides an opportunity to use isoxaflutole applied PRE alone or in mixture with other residual herbicides for early-season weed control. It also provides an opportunity to use glufosinate as a contact, POST herbicide for control of emerged broadleaf and grass weeds (Jhala et al. 2017). Norsworthy et al. (2008) reported 99% control of GR Palmer amaranth with glufosinate. Wiesbrook et al. (2001) found that glufosinate in sequential applications improved control of broadleaf weeds over a single application. Glufosinate applied early-POST (EPOST) resulted in 73% Palmer amaranth control compared to 76% control with glufosinate applied EPOST and late-POST (LOST) (Hoffner et al. 2012). Glufosinate applied EPOST resulted in 71% control and a sequential LPOST application provided 76% control of GR waterhemp in glufosinate-resistant soybean in Nebraska (Jhala et al. 2017). An additional option for POST control of GR Palmer amaranth in glufosinate-resistant soybean is glufosinate mixed with residual herbicides such as acetochlor, pyroxasulfone, or *S*-metolachlor (Aulakh and Jhala 2015). This mixture provides foliar and residual control of Palmer amaranth through overlapping residual activity.

Weed and Crop Water Demands. Weed species compete with crops for a variety of environmental resources, including water, which is one of the most limiting factors for optimum crop production (Benjamin and Nielsen 2006). Weed-crop competition models

offer a significant tool for understanding and predicting crop yield losses due to crop-weed interference. However, within these models, weed biological characteristics are unknown, limiting our understanding of weed growth in competition with crops and how that competition affects crop growth parameters. In terms of weed-crop competition, two main principles are apparent: (1) the first plant to occupy a given area has an advantage over later emerging plants, and (2) more aggressive plant species typically dominate in an intermixed community of weeds and crops (Singh et al. 2020). In general, weed species with similar growth patterns to crops are more competitive than weeds with a dissimilar growth pattern. Conversely, the competitive ability of crops depends on many factors, such as (1) crop type and cultivar or variety selection, sowing date, row spacing and tillage practices; (2) weed density and composition; (3) soil and climatic factors; and (4) crop rotation (Singh et al. 2020). The time and method of irrigation may also impact weed-crop competition as weeds are also benefitted during irrigation. As the inherent ability of crops to compete against weeds is weakened by climatic and soil stresses (Mohler 2004), various farm management practices, including irrigation, can be adjusted in such a way to hinder weed growth.

Nebraska growers lead the U.S. in irrigated acres at ~10 million acres [4.1 million ha], with roughly half a million irrigated acres added each year (USDA 2019). Sprinkler systems, particularly center pivot irrigation (CPI) systems, represent ~80% of Nebraska's irrigation, while gravity and drip systems represent ~20% and 0.05%, respectively (USDA 2010). In CPI and gravity irrigation systems, a certain portion of water withdrawn is returned as surface water or groundwater, although much is consumed by evapotranspiration (ET). In comparison to CPI systems, ET from drip systems like

subsurface drip irrigation (SDI) has been shown to be 10% lower in certain areas. The reduction in ET of SDI systems translates to additional water for transpiration, potentially resulting in increased crop yield and weed biomass if competitive weeds are present (Odhiambo and Irmak 2015). Developing irrigation management strategies based on available soil water requires knowledge of weed and crop response to water deficit, which can be obtained through modeling (Paredes et al. 2014), relating biomass production to ET. The effects of crop ET rates on crop yield are well known – the challenge is determining the effect of weed ET on weed morphological features (i.e., biomass, leaf area index, plant height).

Objectives

1. Evaluate herbicide programs for control of ALS inhibitor/GR Palmer amaranth and their effect on Palmer amaranth density and biomass, as well as soybean injury and yield in isoxaflutole/glufosinate/glyphosate-resistant soybean.
2. Determine the effect of center-pivot and subsurface drip irrigation on the average evapotranspiration of Palmer amaranth grown in corn, soybean, and fallow in south central Nebraska.
3. Determine the effect of degree of water stress on the growth and fecundity of velvetleaf using soil moisture sensors under greenhouse conditions.

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CHAPTER 2:
**CONTROL OF ACETOLACTATE SYNTHASE INHIBITOR/GLYPHOSATE-
RESISTANT PALMER AMARANTH (*AMARANTHUS PALMERI*) IN
ISOXAFLUTOLE/GLUFOSINATE/GLYPHOSATE-RESISTANT SOYBEAN**

Mausbach JM, Irmak S, Sarangi D, Lindquist J, Jhala AJ (2021) Control of acetolactate synthase inhibitor/glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in isoxaflutole/glufosinate/glyphosate-resistant soybean. Weed Technology (In press)

Abstract

Palmer amaranth is the most problematic and troublesome weed in agronomic cropping systems in the United States. Acetolactate synthase (ALS) inhibitor- and glyphosate-resistant (GR) Palmer amaranth has been confirmed in Nebraska and it is widespread in several counties. Soybean resistant to isoxaflutole/glufosinate/glyphosate has been developed to provide additional herbicide sites of action for control of herbicide-resistant weeds. The objectives of this study were to evaluate herbicide programs for control of ALS inhibitor/GR Palmer amaranth and their effect on Palmer amaranth density and biomass, as well as soybean injury and yield in isoxaflutole/glufosinate/glyphosate-resistant soybean. Field experiments were conducted in 2018 and 2019 in a grower's field infested with ALS inhibitor/GR Palmer amaranth near Carleton, Nebraska. Isoxaflutole applied alone or mixed with sulfentrazone/pyroxasulfone, flumioxazin/pyroxasulfone, or imazethapyr/saflufenacil/pyroxasulfone provided similar control (86%–99%) of Palmer amaranth 21 d after PRE (DAPRE). At 14 d after early-POST (DAEPOST), isoxaflutole applied PRE and PRE followed by (fb) POST controlled Palmer amaranth 10% and 63%,

respectively. Glufosinate applied EPOST provided 75%–96% control with in both years. A PRE herbicide fb glufosinate controlled Palmer amaranth 80%–99% 21 d after late-POST (DALPOST) in 2018 and reduced density 89%–100% in 2018 and 58%–100% in 2019 at 14 DAEPOST. No soybean injury was observed from any of the herbicide programs tested in this study. Soybean yield in 2019 was relatively higher due to higher precipitation compared with 2018 with generally no differences between herbicide programs. This research indicates that herbicide programs are available for effective control of ALS inhibitor/GR Palmer amaranth in isoxaflutole/glufosinate/glyphosate-resistant soybean.

Introduction

ALS inhibitor- and/or GR Palmer amaranth has been observed in several corn/soybean production fields in south-central and west-central Nebraska, in addition to alfalfa (*Medicago sativa* L.), corn, and sugarbeet fields in western Nebraska (Vieira et al. 2018). To address the growing need to control GR weeds in cropping systems, multiple-herbicide-resistant soybean traits have been developed. For example, isoxaflutole/glufosinate/glyphosate-resistant soybean has been developed to provide additional herbicide sites of action for control of herbicide-resistant weeds, primarily GR weeds; however, herbicide programs need to be developed and tested that provide season-long control of GR Palmer amaranth in this multiple herbicide-resistant soybean. The objectives of this research were to: (1) evaluate isoxaflutole- and glufosinate-based herbicide programs for control of ALS inhibitor/GR Palmer amaranth in isoxaflutole/glufosinate/glyphosate-resistant soybean, and (2) evaluate the effect of

herbicide programs on Palmer amaranth density and biomass, as well as soybean injury and grain yield.

Materials and Methods

Field Experiments. Field experiments were conducted in 2018 and 2019 in a grower's field near Carleton, NE (40.30°N, 97.67°W). The field had a GR corn-soybean rotation with reliance on glyphosate for weed control in a no-till production system for the last 10 years and confirmed to have an ALS inhibitor/GR Palmer amaranth population (Chahal et al. 2017) [(multiple herbicide-resistant (MHR) Palmer amaranth here after)]. The soil at the experimental site was silt loam (montmorillonitic, mesic, Pachic Argiustolls) with a pH of 6.0 and 19% sand, 63% silt, 18% clay, and 2.6% organic matter content. Winter annual weeds were controlled with glyphosate at 900 g ae ha⁻¹, 2,4-D ester at 560 g ae ha⁻¹, and liquid ammonium sulfate at 3% v/v two weeks prior to establishing an experiment. A soybean cultivar resistant to isoxaflutole/glufosinate/glyphosate was planted in a no-till seedbed at 345,800 seeds ha⁻¹ in rows spaced 76 cm apart. Soybean was planted on May 10 in 2018 and May 6 in 2019. Individual experimental plot dimensions were 3 m wide by 9 m long. The experimental site was in a rainfed environment with no supplemental irrigation. The precipitation received during both growing seasons are listed (Table 2-1).

Treatments were arranged in a randomized complete block design with four replications. Herbicide programs evaluated to control MHR Palmer amaranth consisted of PRE, EPOST, LPOST, and PRE fb POST herbicide programs (Table 2-2). A nontreated control was included for comparison. Herbicides were applied with a handheld CO₂-pressurized backpack sprayer equipped with AIXR 110015 flat fan nozzles (TeeJet®

Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL) calibrated to deliver a 140 L ha⁻¹ flow rate at 276 kPa at a constant speed of 4.8 km h⁻¹. Glufosinate was mixed with liquid ammonium sulfate at 3% v/v (Anonymous 2017) and was applied with XR 11005 flat fan nozzles (TeeJet® Technologies, Spraying Systems Co., P.O. Box 7900, Wheaton, IL). The PRE herbicides were applied after soybean planting on the same day (i.e., May 10) in 2018, and four d after soybean planting (i.e., May 10) in 2019. The EPOST herbicides were applied 31 d after PRE (DAPRE) herbicides were applied.

Palmer amaranth was 1–8 cm tall depending on herbicide program. Soybean was at the first to second trifoliolate (V1–V2 growth stage). The LPOST herbicides were applied 20–22 DAEPOST herbicide applications. Palmer amaranth was 8–25 cm tall depending on the herbicide program. Palmer amaranth plant height was variable because new plants had emerged and some plants had been partially controlled by the EPOST herbicide.

Data Collection. Palmer amaranth control was assessed visually at 21 DAPRE, 14 DAEPOST, and 14 and 28 DALPOST herbicide applications on a scale of 0%–100% (0% indicating no control of Palmer amaranth and 100% indicating complete control).

Palmer amaranth densities were recorded 21 DAPRE, 14 DAEPOST, 14 DALPOST, and 28 DALPOST by counting the number of Palmer amaranth plants in one 0.5 m² quadrat placed randomly between two center soybean rows in each plot. Soybean injury was assessed visually at 14 DAPRE, 14 DAEPOST, 14 and 28 DALPOST herbicide applications based on a scale of 0%–100% (0% indicating no soybean injury and 100% indicating complete plant death). Palmer amaranth plants counted during density ratings were clipped at the soil surface, placed into paper bags, and placed in an oven at 65°C until they reached a constant weight. Aboveground biomass was converted into percent

biomass reduction and was compared with the nontreated control using the following equation (Wortman 2014):

$$\% \text{ Biomass reduction} = \left[\frac{\bar{C} - B}{\bar{C}} \right] * 100$$

where \bar{C} is the biomass of the nontreated control and B is the biomass of an individual treatment plot. Soybean was harvested from the center two rows in each plot using a plot combine. Grain yield was adjusted to 13% moisture content and converted into kg ha^{-1} .

Statistical Analysis. Data were subjected to ANOVA using the PROC MIXED procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Data were tested for normality with the use of PROC UNIVARIATE. Palmer amaranth control, density, and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with the mean separation based on the transformed data. Year and herbicide treatments were considered fixed effects, while replication was considered a random effect in the model. If year-by-treatment interaction was non-significant, data from both years were combined. However, if the year-by-treatment interaction was significant, data were analyzed separately by year. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Tukey Kramer's pairwise comparison test.

Results and Discussion

Year-by-treatment interaction for MHR Palmer amaranth control 21 DAPRE was not significant ($P > 0.05$); therefore, data were combined for both years. Palmer amaranth control estimates 14 DAEPOST and 28 DALPOST, Palmer amaranth density, and soybean yield were significant ($P \leq 0.05$); therefore, data were presented separately for

both years. No soybean injury was observed from any herbicide program (data not shown), indicating that the herbicides evaluated in this study are safe to use in isoxaflutole/glufosinate/glyphosate-resistant soybean when applied according to label instructions. Schultz et al. (2015) also reported that isoxaflutole is safe to use in isoxaflutole-resistant soybean.

Temperature and Precipitation. The 2018 growing season started off warmer than average, with temperatures of 20.6°C and 25.0°C for May and June, respectively, compared with 14.8°C and 21.8°C in 2019 (Table 2-1). Monthly precipitation varied from the 30-yr average of 135 mm in May and 115 mm in June in both years. Below-average precipitation occurred in 2018, with 78 and 96 mm in May and June, respectively, while above-average precipitation was observed throughout the 2019 growing season (Table 2-1).

Palmer amaranth Control. The PRE herbicides evaluated in this study controlled MHR Palmer amaranth 86%–99% 21 DAPRE (Table 2-3). Although similar to other PRE herbicide spray timings, pyroxasulfone/sulfentrazone, flumioxazin/pyroxasulfone, and imazethapyr/pyroxasulfone/saflufenacil controlled Palmer amaranth 97%–99%. The contribution of the ALS-inhibiting herbicide (i.e., imazethapyr) was minimal; rather, the VLCFA-inhibitor (i.e., pyroxasulfone) and PPO-inhibitor (i.e., saflufenacil) primarily contributed to the control. Shyam et al. (2021) reported similar findings 14 DAPRE with imazethapyr/pyroxasulfone/saflufenacil, where Palmer amaranth control ranged from 87%–97% in a two yr study in 2,4-D choline/glufosinate/glyphosate-resistant soybean. Sarangi and Jhala (2019) reported at least 98% Palmer amaranth control 14 and 28

DAPRE with imazethapyr/dimethenamid-P/saflufenacil and flumioxazin/pyroxasulfone. Isoxaflutole applied PRE controlled Palmer amaranth 86%–89% 21 DAPRE (Table 2-3); however, variable control of Palmer amaranth has been reported with isoxaflutole in the literature. Meyer et al. (2016) and Johnson et al. (2012) reported at least 87% Palmer amaranth control with isoxaflutole 28 DAPRE. In contrast, Spaunhorst and Johnson (2016) reported 57%–70% GR Palmer amaranth control 21 DAPRE. Greater control with isoxaflutole occurred in a higher rainfall year, indicating the importance of moisture for herbicide activation (Spaunhorst and Johnson 2016). Isoxaflutole requires 12.7–25.4 mm of irrigation or rain to activate, although too much water can cause the herbicide to become diluted and leach, reducing efficacy (Jhala 2017b). If moisture is adequate, isoxaflutole can provide 14–21 d of residual activity for Palmer amaranth control (Chahal et al. 2015).

Palmer amaranth control varied between years with PRE fb EPOST herbicide programs (Table 2-3). As an EPOST application, glufosinate applied alone controlled MHR Palmer amaranth 95%–96% in 2018 and 75% in 2019. Glufosinate mixed with isoxaflutole controlled Palmer amaranth 92%–95% in 2018 and 85%–94% in 2019 (Table 2-3). Shyam et al. (2021) reported 88% Palmer amaranth control 14 DAEPOST with glufosinate. Conversely, Chahal and Jhala (2015) found that glufosinate in single and sequential applications provided 53%–76% and 56%–77% waterhemp control, respectively. Sequential glyphosate applications provided no control of MHR Palmer amaranth in this study, indicating that the population is highly resistant to glyphosate (Table 2-3). Chahal et al. (2017) reported 37- to 40-fold level of glyphosate resistance in

MHR Palmer amaranth at this research site; therefore, no control with glyphosate was expected.

At 28 DALPOST, isoxaflutole applied PRE or in sequential applications (PRE fb EPOST) controlled MHR Palmer amaranth 10% and 53% in 2018, respectively, while providing no control in 2019 (Table 2-3). This indicates isoxaflutole applied alone at 105 g ai ha⁻¹ will not provide effective control later in the growing season and that mixture with other herbicide(s) is needed to achieve economically acceptable control. In this study isoxaflutole was applied at 105 g ai ha⁻¹; however, it can be applied in a range of 140–210 g ai ha⁻¹ in a single application with a season maximum of 210 g ai ha⁻¹ (Anonymous 2020). Relatively lower rate of use in this study is because the study was conducted before isoxaflutole was label approved in 2020. In addition, isoxaflutole is primarily a residual herbicide with limited foliar activity; therefore, effective control of emerged Palmer amaranth at the time of application should not be expected. Janak and Grichar (2016) reported similar findings of 51% Palmer amaranth control with a single application of isoxaflutole 101 DAPRE. When mixed with metribuzin, isoxaflutole has been shown to provide 97%–98% control of redroot pigweed (*Amaranthus retroflexus*) and Powell amaranth (*Amaranthus powellii*) (Smith et al. 2019). With the exception of isoxaflutole, PRE fb POST herbicide programs provided 80%–99% MHR Palmer amaranth control in 2018 and 76%–99% control in 2019 at 28 DALPOST (Table 2-3). Whitaker et al. (2010) reported greater than 80% late-season control of GR Palmer amaranth with a PRE application of flumioxazin/*S*-metolachlor fb fomesafen; however, less than 30% late-season control was achieved with flumioxazin/*S*-metolachlor without a POST application of fomesafen. A single herbicide application is less likely to provide

season-long control of Palmer amaranth – a PRE followed by a POST herbicide program is required for effective Palmer amaranth control and reducing Palmer amaranth seedbank (Norsworthy et al. 2012).

Palmer amaranth Density and Biomass. Palmer amaranth density and biomass were affected by herbicide programs (Table 2-4). At 14 DAEPOST isoxaflutole reduced MHR Palmer amaranth density 0% in 2018 and 48% in 2019, while PRE fb EPOST applications of isoxaflutole reduced density 49% in 2018 and 53% in 2019. Similarly, Meyer et al. (2016) reported 62% Palmer amaranth density reduction with isoxaflutole applied PRE. Meyer et al. (2015) reported 78%–93% Palmer amaranth density reduction with flumioxazin/pyroxasulfone in soybean in a multi-year, multi-state study, while Sarangi et al. (2017) reported 91% and 98% density reduction of GR waterhemp with flumioxazin/pyroxasulfone and imazethapyr/dimethenamid-P/saflufenacil, respectively.

PRE herbicides fb glufosinate reduced MHR Palmer amaranth density at least 85% in 2018 and 2019. Similar findings were reported by Shyam et al. (2021) and Norsworthy et al. (2016). EPOST applications of glufosinate reduced MHR Palmer amaranth density 89% in 2018 and 58% in 2019, while glufosinate mixed with isoxaflutole reduced Palmer amaranth density 63%–100% in 2018 and 85%–94% in 2019 (Table 2-4). Chahal and Jhala (2015) reported 50% density reduction of waterhemp with an EPOST application of glufosinate and 83% density reduction of waterhemp with EPOST fb LPOST applications of glufosinate 45 DALPOST in glufosinate-resistant soybean in Nebraska.

At 14 DALPOST in 2019, PRE herbicide(s) fb glufosinate applied EPOST reduced MHR Palmer amaranth biomass 49%–97% compared to 95% biomass reduction

with PRE herbicide fb EPOST and LPOST applications of glufosinate (Table 2-4). Aulakh and Jhala (2015) reported 79%–88% weed biomass reduction with PRE applications of dimethenamid-P/saflufenacil, or imazethapyr/sulfentrazone fb glufosinate. Shyam et al. (2021) reported 100% Palmer amaranth biomass reduction with imazethapyr/pyroxasulfone/saflufenacil fb glufosinate and 99% biomass reduction with EPOST fb LPOST applications of glufosinate in 2,4-D choline/glufosinate/glyphosate-resistant soybean. Single or sequential applications of isoxaflutole resulted in no biomass reduction due to poor Palmer amaranth control (Table 2-4). Chahal and Jhala (2015) reported 80%–91% biomass reduction with a single POST application of glufosinate and 92%–95% biomass reduction with sequential POST applications of glufosinate in glufosinate-resistant soybean. Overall, a PRE herbicide with multiple sites of action fb glufosinate has consistently provided > 90% Palmer amaranth control and > 90% Palmer amaranth density and biomass reduction in most studies.

Soybean Yield. Year-by-treatment interaction was significant ($P \leq 0.05$); therefore, yield data are presented separately for both years (Table 2-4). Soybean yield in 2019 was higher compared to 2018 due to higher precipitation in 2019 that provided sufficient moisture for soybean growth and development (Table 2-1). Isoxaflutole mixed with pyroxasulfone/sulfentrazone applied PRE fb glufosinate resulted in the highest soybean grain yield of 2,294 kg ha⁻¹ in 2018, which was comparable with several herbicide programs (Table 2-4). In 2019, several herbicide programs resulted in similar soybean yield in the range of 3,139–4,227 kg ha⁻¹ (Table 2-4). Shyam et al. (2021) reported soybean yields with similar PRE herbicides used in combination with glufosinate.

Practical Implications

A new soybean trait resistant to isoxaflutole/glufosinate/glyphosate has been commercially available since the 2019 growing season in the United States. Results of this study suggest that herbicide programs are available for effective control of MHR Palmer amaranth in isoxaflutole/glufosinate/glyphosate-resistant soybean. No soybean injury was observed with any of the herbicide programs evaluated in this study, including isoxaflutole applied in sequential applications. Isoxaflutole (Alite™ 27) was registered in 2020 for application in isoxaflutole-resistant soybean; however, use of this herbicide is limited to certain counties in a few states. For example, isoxaflutole (Alite™ 27) is labeled for application in only four southwest counties (Chase, Dundy, Hitchcock, and Red Willow) in Nebraska (Anonymous 2020). In addition, isoxaflutole cannot be applied on coarse-textured soils (e.g., sandy, sandy loam, loamy sand) with less than 1.5% organic matter content, limiting the use of this herbicide. The majority of soybean in Nebraska is grown in the eastern region, so while growers can plant isoxaflutole/glufosinate/glyphosate-resistant soybean in this region, they cannot use isoxaflutole (Alite™ 27) due to label restrictions (Anonymous 2020). Therefore, adoption of soybean resistant to isoxaflutole/glufosinate/glyphosate in Nebraska will likely be very limited.

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Table 2-1. Monthly mean air temperature and total precipitation during the 2018 and 2019 growing seasons (March to October), along with the 30-yr average at the research site near Carleton, Nebraska.^a

Month	Mean air temperature (°C)			Total precipitation (mm)		
	2018	2019	30-yr average	2018	2019	30-yr average
March	4.5	1.1	4.6	23.6	85.6	45.2
April	5.9	11.8	10.6	26.4	16.0	66.3
May	20.6	14.6	16.4	78.0	172.7	135.4
June	25.0	21.8	22.3	96.0	153.2	115.1
July	24.7	25.1	24.9	95.5	137.2	105.2
August	23.3	23.1	23.7	92.2	154.9	94.0
September	20.6	22.6	19.1	153.4	120.4	66.0
October	10.6	9.6	12.1	99.8	118.1	58.4

^aData were obtained from National Oceanic and Atmospheric Administration (NOAA 2019).

Table 2-2. Herbicides, application timings, and rates used for control of acetolactate synthase (ALS) inhibitor and glyphosate-resistant Palmer amaranth in isoxaflutole/glufosinate/glyphosate-resistant soybean in field experiments conducted near Carleton, Nebraska in 2018 and 2019.

Herbicide program ^a	Trade name	Application timing	Rate (g ae or ai ha ⁻¹)	Manufacturer
Isoxaflutole	Alite 27	PRE	105	BASF Corporation
Isoxaflutole fb	Alite 27 fb	PRE fb	105	BASF Corporation
isoxaflutole	Alite 27	early POST	105	BASF Corporation
Glufosinate	Liberty	Early POST	657	BASF Corporation
Glufosinate fb	Liberty fb	Early POST fb	657	BASF Corporation
glufosinate	Liberty	late POST	657	BASF Corporation
Isoxaflutole fb	Alite 27 fb	PRE fb	105	BASF Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Pyroxasulfone/sulfentrazone fb	Authority Supreme fb	PRE fb	292	FMC Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Pyroxasulfone/sulfentrazone + isoxaflutole fb	Authority Supreme + Alite 27 fb	PRE fb	292 + 105	FMC Corporation + BASF Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Flumioxazin/pyroxasulfone fb	Fierce fb	PRE fb	160	Valent
glufosinate	Liberty	early POST	657	BASF Corporation
Flumioxazin/pyroxasulfone + isoxaflutole fb	Fierce + Alite 27 fb	PRE fb	160 + 105	Valent + BASF Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Imazethapyr/pyroxasulfone/saflufenacil fb	Zidua PRO fb	PRE fb	215	BASF Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Imazethapyr/pyroxasulfone/saflufenacil + isoxaflutole fb	Zidua PRO + Alite 27 fb	PRE fb	215 + 105	BASF Corporation
glufosinate	Liberty	early POST	657	BASF Corporation
Isoxaflutole + glufosinate	Alite 27 + Liberty	Early POST	105 + 657	BASF Corporation
Isoxaflutole fb	Alite 27 fb	PRE fb	105	BASF Corporation
glufosinate fb	Liberty fb	early POST fb	657	BASF Corporation
glufosinate	Liberty	late POST	657	BASF Corporation
Isoxaflutole + glufosinate fb	Alite 27 + Liberty fb	Early POST fb	105 + 657	BASF Corporation
isoxaflutole + glufosinate	Alite 27 + Liberty	late POST	105 + 657	BASF Corporation
Glufosinate fb	Roundup PowerMAX fb	Early POST fb	1,260	Bayer CropScience
glyphosate	Roundup PowerMAX	late POST	1,260	Bayer CropScience

^aGlufosinate was mixed with ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) at 4.2 kg ha⁻¹.

Table 2-3. Effect of herbicide programs on acetolactate synthase (ALS) inhibitor- and glyphosate-resistant Palmer amaranth control in isoxaflutole/glufosinate/glyphosate-resistant soybean 21 d after PRE (DAPRE), 14 d after early-POST (DAEPOST), and 28 d after late-POST (DALPOST) herbicide application in field experiments conducted near Carleton, Nebraska in 2018 and 2019.^{a,b,c}

Herbicide program	Application timing	Rate g ae or ai ha ⁻¹	Palmer amaranth control ^d				
			21 DAPRE ^e		28 DALPOST		
			%				
			2018/2019	2018	2019	2018	2019
Isoxaflutole	PRE	105	89 a	41 c	38 c	10 d	2019
Isoxaflutole fb	PRE fb	105	86 a	63 b	10 d	53 c	0 e
isoxaflutole	early POST	105					
Glufosinate fb	Early POST fb	657	- ^e	95 a	75 b	80 ab	36 cd
glufosinate	late POST	657	- ^e	96 a	75 b	99 a	85 ab
Isoxaflutole fb	PRE fb	105	88 a	97 a	95 ab	80 ab	34 cd
glufosinate	early POST	657					
Pyroxasulfone/sulfentrazone fb	PRE fb	292	98 a	98 a	99 a	91 ab	89 ab
glufosinate	early POST	657					
Pyroxasulfone/sulfentrazone + isoxaflutole fb	PRE fb	292+105	99 a	96 a	99 a	95 ab	88 ab
glufosinate	early POST	657					
Flumioxazin/pyroxasulfone fb	PRE fb	160	97 a	99 a	99 a	99 a	93 ab
glufosinate	early POST	657					
Flumioxazin/pyroxasulfone + isoxaflutole fb	PRE fb	160+105	95 a	99 a	99 a	97 ab	91 ab
glufosinate	early POST	657					
Imazethapyr/pyroxasulfone/saflufenacil fb	PRE fb	215	99 a	99 a	98 a	98 a	78 ab
glufosinate	early POST	657					
Imazethapyr/pyroxasulfone/saflufenacil + isoxaflutole fb	PRE fb	215+105	92 a	99 a	98 a	93 ab	61 bc
glufosinate	early POST	657					
Isoxaflutole + glufosinate	Early POST	105+657	- ^e	95 a	85 ab	76 b	29 de
Isoxaflutole fb	PRE fb	105	88 a	94 a	92 ab	97 ab	93 a
glufosinate fb	early POST fb	657					
glufosinate	late POST	657					
Isoxaflutole + glufosinate fb	Early POST fb	105+657	- ^e	92 a	94 ab	95 ab	89 ab
isoxaflutole + glufosinate	late POST	105+657					
Glyphosate fb	Early POST fb	1,260	- ^e	0 d	0 d	0 d	0 d
glyphosate	late POST	1,260					

^aYear-by-treatment interaction for Palmer amaranth control 14 DAPRE was not significant; therefore, data were combined across years.

^bYear-by-treatment interaction for Palmer amaranth control 14 DAEPOST and 28 DALPOST was significant; therefore, data are presented separately for both years.

^cAbbreviations: DAEPRE, d after PRE herbicide application, DAEPOST, d after early-POST herbicide application, DAEPOST, d after late-POST herbicide application.

^dMeans presented within each column with no common letter(s) are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

^ePOST herbicides were not applied at the time of evaluation 21 DAPRE.

Table 2-4. Effect of herbicide programs on glyphosate-resistant Palmer amaranth density reduction and biomass reduction and isoxaflutole/glufosinate/glyphosate-resistant soybean yield in field experiments conducted near Carleton, Nebraska in 2018 and 2019.^a

Herbicide program	Application timing	Rate g ae or ai ha ⁻¹	Palmer amaranth density reduction ^{b,d}		Biomass reduction ^{c,d}		Soybean yield ^{b,d} kg ha ⁻¹
			%		%		
			14 DAEPOST	14 DAEPOST	14 DALPOST	14 DALPOST	
Nontreated control	-	-	2018 0	2019 0	2019 0	2018 955 b	2019 2,129 c
Isoxaflutole	PRE	105	0 c	48 d	0 d	990 b	2,707 bc
Isoxaflutole fb	PRE fb	105	49 b	53 cd	0 d	1,112 ab	2,160 c
isoxaflutole	early POST	105					
Glufosinate fb	Early POST	657	89 a	58 bcd	41 c	1,480 ab	2,038 c
Glufosinate	Early POST fb	657	92 a	78 abcd	90 a	1,697 ab	4,227 a
glufosinate	late POST	657					
Isoxaflutole fb	PRE fb	105	95 a	92 abc	49 bc	1,037 b	2,665 bc
glufosinate	early POST	657					
Pyroxasulfone/sulfentrazone fb	PRE fb	292	100 a	100 a	87 ab	1,751 ab	3,974 ab
glufosinate	early POST	657					
Pyroxasulfone/sulfentrazone + isoxaflutole fb	PRE fb	292 + 105	100 a	100 a	95 a	2,294 a	4,022 ab
glufosinate	early POST	657					
Flumioxazin/pyroxasulfone fb	PRE fb	160	100 a	100 a	92 a	1,935 ab	4,050 ab
glufosinate	early POST	657					
Flumioxazin/pyroxasulfone + isoxaflutole fb	PRE fb	160 + 105	100 a	100 a	97 a	2,118 ab	3,630 ab
glufosinate	early POST	657					
Imazethapyr/pyroxasulfone/saflufenacil fb	PRE fb	215	100 a	99 ab	84 ab	2,027 ab	3,658 ab
glufosinate	early POST	657					
Imazethapyr/pyroxasulfone/saflufenacil + isoxaflutole fb	PRE fb	215 + 105	100 a	99 ab	83 ab	1,461 ab	3,370 abc
glufosinate	early POST	657					
Isoxaflutole + glufosinate	Early POST	105 + 657	100 a	85 abcd	59 abc	1,571 ab	2,033 c
Isoxaflutole fb	PRE fb	105	95 a	87 abcd	95 a	1,975 ab	4,282 a
glufosinate fb	early POST fb	657; 657					
glufosinate	late POST						
Isoxaflutole + glufosinate fb	Early POST fb	105 + 657	63 ab	94 abc	96 a	1,019 b	3,139 abc
isoxaflutole + glufosinate	late POST	105 + 657					
Glyphosate fb	Early POST fb	1,260	0 c	0 e	0 d	954 b	2,129 c
glyphosate	late POST	1,260					

^aAbbreviations: DAEPOST, d after early-POST herbicide application; DALPOST, d after late-POST herbicide application.

^bYear-by-treatment interaction for glyphosate-resistant Palmer amaranth density and soybean yield were significant; therefore, data were not combined across the two years.

^cBiomass reduction data is only available for 2019.

^dMeans presented within each column with no common letter(s) are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

CHAPTER 3:
**EVAPOTRANSPIRATION OF PALMER AMARANTH (*AMARANTHUS*
PALMERI S. WATSON) IN CORN, SOYBEAN, AND FALLOW UNDER
SUBSURFACE DRIP AND CENTER-PIVOT IRRIGATION SYSTEMS**

Abstract

Palmer amaranth (*Amaranthus palmeri* S. Watson) is a major biotic constraint in agronomic cropping systems in the United States due to its rapid growth rate and ability to tolerate adverse climatic conditions, among other characteristics. Weed-crop competition models offer a significant tool for understanding and predicting crop yield losses due to crop-weed interference. Research is currently dominated by empirical studies where crop yield loss and weed threshold values are predicted in response to variable weed density or biomass in certain environmental conditions. However, within these models, weed biological characteristics are unknown, which limits understanding of weed growth in competition with crops under different irrigation methods and how that competition affects crop growth parameters. The objective of this study was to determine the effect of center-pivot irrigation (CPI) and subsurface drip irrigation (SDI) on the average evapotranspiration (ET_a) of Palmer amaranth grown in corn, soybean, and fallow in south central Nebraska. Field experiments were conducted in 2019 and 2020 at South Central Agricultural Laboratory near Clay Center, NE. Twelve Palmer amaranth plants were alternately transplanted one meter apart in the middle two rows of corn, soybean, and fallow subplots under CPI and SDI. Corn, soybean, and fallow subplots without Palmer amaranth were included for comparison. Watermark Granular Matrix soil

moisture sensors were installed at 0.3-, 0.6-, 0.9-m depths next to or between three Palmer amaranth and crop plants in each subplot. Soil matric potential data were collected hourly from the time of Palmer amaranth transplanting to crop harvest. Results suggest irrigation affects subplot ET_a differences early in the growing season, but crop system and progression of plant growth with available water have a greater effect on ET_a differences than irrigation type later in the growing season. Although there were irrigation differences in Palmer amaranth ET_a in fallow subplots, growers typically do not irrigate fallow fields. Thus, crop management will likely have greater effects on Palmer amaranth ET_a values than irrigation practices alone. This study provides baseline information about Palmer amaranth evapotranspiration and its relation to Palmer amaranth morphological features (i.e., growth index, biomass, and total leaf area) for future use in mechanistic weed-crop competition models.

Introduction

Research is currently dominated by empirical studies where crop yield loss (or crop yield) and weed threshold values are predicted in response to variable weed density or biomass in certain environmental conditions. Complex empirical models have been developed by considering variables such as multiple weed species in simultaneous competition (Firbank and Watkinson 1985; Pantone and Baker 1991; Park et al. 2002; Diggle et al. 2003), timing of weed emergence (Cousens et al. 1987; Neve et al. 2003), and weeds with multiple emergence patterns (Peltzer et al. 2012). However, within these models, weed biological characteristics are unknown, limiting our understanding of weed growth in competition with crops and how that competition affects crop growth

parameters. Crop-weed competition is a complex phenomenon, and for predictive purposes, a detailed mechanistic model offers greater insights than an empirical model. Mechanistic models take into account all underlying morphological and physiological processes and their dependence on each other with respect to external forces and time (Singh et al. 2020). However, morphological and physiological plasticity in weed species is a challenge for studies/models that have been developed on the basis of weed growth. Research on weed biology and ecology has been conducted, although additional studies conducted in a systematic way and under different locations/environmental conditions are needed to elucidate simulation models and thus weed management decisions (Van Acker 2009; Chauhan and Johnson 2010).

The objective of this research was to determine the effect of center-pivot and subsurface drip irrigation on the average evapotranspiration of Palmer amaranth grown in corn, soybean, and fallow in south central Nebraska.

Materials and Methods

Plant Materials. Glyphosate-resistant Palmer amaranth seed were germinated in 11.4 cm deep square plastic pots in a University of Nebraska-Lincoln greenhouse maintained at 18/24 °C day/night temperatures with a 14-hr photoperiod. Glyphosate-resistant seed was used so that other weeds could be controlled after Palmer amaranth was transplanted in the field. In order to ensure the Palmer amaranth plants were glyphosate-resistant, glyphosate at 64 fl oz/acre mixed with liquid ammonium sulfate at 3% v/v was sprayed on 10–12 cm tall Palmer amaranth plants using an AIXR Teejet nozzle that applied the herbicide mixture at a rate of 140.3 L ha⁻¹ at 1.0 m s⁻¹ using a chamber track sprayer.

Palmer amaranth plants survived the glyphosate application, signifying the seed source was truly glyphosate-resistant.

Experimental Design, Site Description, and Crop Management. Field experiments were conducted in the 2019 and 2020 growing seasons at the University of Nebraska-Lincoln South Central Agricultural Laboratory near Clay Center, Nebraska (40.57°N, 98.12°W). The soil at the experimental site is a Hastings silt loam, a well-drained upland soil with water holding characteristics of 0.34 m³ m⁻³ field capacity, 0.14 m³ m⁻³ permanent wilting point, and 0.53 m³ m⁻³ saturation point. Typical effective rooting depth of field corn in the experimental site is 1.2 m. Total available water holding capacity of the soil profile is 240 mm 1.2 m⁻¹ (Irmak 2010). The 30-year average rainfall in the area during the growing season (May to August) is 112.4 mm, with significant annual and growing season variability in both timing and magnitude (de Sanctis and Jhala 2021). The 2019 and 2020 growing season weather data are presented in Figure 3-1. The experiment used a split-plot design with irrigation as the whole-plot factor. Two methods of irrigation were used, including center-pivot (CPI) and subsurface drip irrigation (SDI). The CPI field was irrigated using a four-span hydraulic and continuous move system (T-L Irrigation, Hastings, Nebraska). The SDI field was irrigated with drip lines installed 0.4 m below the soil surface. The 257 m long laterals were centered in the inter-row area of every other plant with drip emitters spaced about 0.46 m apart along the laterals (Net-afim-USA, Fresno, California). Subplots consisted of six cropping systems, including corn, corn with Palmer amaranth, soybean, soybean with Palmer amaranth, fallow, and fallow with Palmer amaranth. Soybean, corn, and fallow plots without Palmer amaranth were included for comparison. Each subplot measured 3 m wide by 9 m long, with four

rows of corn or soybean in the subplots containing these crops. The field was rolling stalk chopped without tillage practice. A broadcast application of 11-52-0 NPK at 168 kg ha⁻¹ and an in-furrow injection of 32-0-0 NPK at 201 kg ha⁻¹ were applied before crops were sown. Dekalb DKS 60-87RIB corn was planted at a depth of 5.0 cm and at a rate of 34,000 seeds acre⁻¹ [85,000 seeds ha⁻¹]. NK S29-K3X soybean was planted at a depth of 3.8 cm and at a rate of 150,000 seeds acre⁻¹ [375,000 seeds ha⁻¹]. A premix of saflufenacil/imazethapyr/pyroxasulfone (Zidua PRO herbicide) was applied at 6 fl oz/acre to soybean, a premix of atrazine/bicyclopyrone/mesotrione/S-metolachlor (Acuron herbicide) was applied at 2.5 qt/acre to corn and fallow, and a POST application of glyphosate at 32 fl oz/acre was applied across all subplots for control of existing weeds. Once Palmer amaranth plants reached a height of 18–25 cm in the greenhouse, twelve Palmer amaranth were alternately transplanted one meter apart in the middle two rows of each subplot. There were 36 Palmer amaranth plants under each irrigation system for a total of 72 sample units each year of the study.

Measurement of Soil Water Status and Irrigation Management. Watermark Granular Matrix Sensors (Irrometer Co, Riverside, California) were installed next to or between three Palmer amaranth plants and crop plants in each subplot to measure soil matric potential (SMP) on an hourly basis. The sensors were buried at 0.3-, 0.6-, and 0.9-m depths and data were collected from the Palmer amaranth transplant date to shortly before crop harvest in both years. A total of 45 and 54 sensors were installed across the subplots of each irrigation system in 2019 and 2020, respectively. The sensors were connected to Watermark Monitor dataloggers (Irrometer Co, Riverside, California). SMP

measurements were converted to percent volumetric soil water content (VWC) using predetermined soil water retention curves for the same field (Irmak et al. 2016):

$$\theta_v = (3 \times 10^{-6} \times \text{SMP}^2) - (0.0013 \times \text{SMP}) + 0.3764$$

where θ_v is the volumetric soil water content (% vol or $\text{m}^3 \text{m}^{-3}$), and SMP is the soil matric potential (kPa). VWC was converted to total soil water (TSW) by adding the VWC values at each sensor depth and multiplying by a conversion value of 3.048 (ft to mm). The TSW in the complete monitored soil profile (0–0.9 m) reflects the daily integration of soil moisture detected at individual incremental depths throughout the profile. Sensor data were used to determine crop and Palmer amaranth evapotranspiration using the soil water balance approach and for irrigation timing. Irrigation was initiated under CPI and SDI when the average of the top 0.9 m SMP values was approximately 100 to 110 kPa (Irmak et al. 2012, 2016), or when the soil-water in the crop root zone was depleted by 40% to 45% below field capacity (Kukul and Irmak 2019). The depletion criterion of 40% to 45% TSW was implemented to prevent water stress. Irrigation timing and amount for the 2019 and 2020 growing seasons are presented (Table 3-1).

Seasonal Actual Evapotranspiration Using Soil-Water Balance. Crop and Palmer amaranth actual evapotranspiration (ET_a , mm) were calculated using a general soil-water balance equation:

$$P + I + U + R_{\text{on}} = R_{\text{off}} + D + \Delta\text{SWS} + \text{ET}_a$$

where P is precipitation (mm), I is irrigation water applied (mm), U is upward soil moisture flux (mm), R_{on} is surface run-on within the field (mm), R_{off} is surface runoff from individual treatments (mm), ΔSWS is change in soil water storage in the root zone soil profile (mm), and D is deep percolation below the crop root zone (mm). Deep

percolation was estimated by a soil water balance approach using a program written in Microsoft Visual Basic (Bryant et al. 1992). The inputs to the program are daily weather data (including precipitation, air temperature, relative humidity, wind speed, and incoming shortwave radiation), initial water content of the soil profile at crop emergence, irrigation date and amount, and crop- and site-specific information such as planting date, maturity date, maximum rooting depth, and soil parameters. The program calculated daily ET_a and the water balance in the crop root-zone using the two-step approach ($ET_a = K_c \times ET_o$) where ET_o is evapotranspiration of a grass reference crop, and K_c is the crop coefficient. In the program, ET_o is calculated using weather data as the input to the Penman-Monteith equation (Monteith 1965; Monteith and Unsworth 1990), and K_c is used to adjust the estimated ET_o for the reference crop to that of the desired crops at different growth stages and environments (Kukal and Irmak 2019). The daily soil water balance equation used for calculating deep percolation is:

$$D_j = \max(P_j - R_j + I_j - ET_{aj} - CD_{j-1}, 0)$$

where D_j is deep percolation on day j (mm), P_j is precipitation on day j (mm), R_j is precipitation and/or irrigation runoff from the soil surface on day j (mm), I_j is irrigation depth on day j (mm), ET_{aj} is crop or Palmer amaranth actual evapotranspiration on day j (mm), and CD_j is root zone cumulative depletion depth at the end of day $j-1$, estimated using the two-step approach (Bryant et al. 1992; Payero et al. 2009). R_{off} from individual treatments was estimated using the USDA-NRCS curve number method. According to the silt loam soil at the experimental site and the known land use, slope, and conservation tillage, curve number $C = 75$ was used (USDA-NRCS 1985). Assuming U and R_{on} are

negligible, the soil water balance equation was reduced to the following form for calculating crop and Palmer amaranth ET_a :

$$ET_a = P + I - R_{\text{off}} - D \pm \Delta SWS$$

Although the soil water balance approach is widely used for calculating ET_a , this approach may have drawbacks if the R_{off} and/or D values are not accurately quantified, resulting in erroneous ET_a calculations. This is of particular concern in humid and subhumid climates where R_{off} and/or D is greater than in arid or semiarid climates, where potential for runoff is minimal. Lysimetry and other surface energy balance type instruments (i.e., eddy covariance system) may provide more robust ET_a values since they do not need to account for R_{off} and/or D ; however, their use in determining individual plot/treatment or replication-scale ET_a is not feasible. The soil water balance approach can be applied to each plot or replication of a given treatment, a requirement of this study.

Growth Index, Plant Biomass, and Total Leaf Area Measurements. Three Palmer amaranth plants were selected and sampled at four removal timings according to soybean growth stage. Removal timings occurred at V4, R1, R3, and R5 soybean growth stages in 2019, and at R1, R3, R5, and R6 soybean growth stages in 2020. Growth index, plant biomass, and total leaf area were determined at these removal timings. Growth index was calculated using the following equation (Irmak et al. 2004; Sarangi et al. 2015):

$$GI (cm^3) = \pi \times (w/2)^2 \times h$$

where w is the width of the plant calculated as an average of two widths, one measured at the widest point and another at 90° to the first; and h is the plant height measured from the soil surface to the shoot apical meristem. After plant height and width measurements

were taken, leaves were counted and removed from each Palmer amaranth plant to measure total leaf area using a leaf area meter (LI-3100C Area Meter, Li-Cor, Lincoln, NE). Palmer amaranth plants were stored separately in paper bags and oven-dried at 65°C for 7 d to obtain dry biomass.

Statistical Analysis. TSW values were analyzed using the area under the curve (AUC) function in R (R Foundation for Statistical Computing, Vienna, Austria). AUC values were calculated for each treatment group [irrigation type x (crop system x Palmer amaranth)] and replicate combination. AUC values obtained in R were then analyzed in SAS with linear models using AUC as the response variable; irrigation, crop, and Palmer amaranth as explanatory variables; and the split plot design in the random statement to account for any design effects that could cause variability across AUC values. ET_a values were analyzed using regression models in SAS with irrigation, crop, Palmer amaranth, and day as fixed effects. The terms irrigation, crop, and Palmer amaranth were treated as categorical, while day was treated as a quantitative regression variable. The main effect terms of irrigation, crop, and Palmer amaranth were initially analyzed to find significant differences ($P \leq 0.05$) between intercept terms for the regression lines. All categorical terms and their interactions were then interacted with a day linear term and day quadratic terms. The regression model was simplified by using the Type I table of fixed effects to remove terms one at a time that were not significant in relation to the ET response. Regression models were then run for each significant term in the reduced model. Prediction plots were also obtained by looking at a scatter plot of the raw data with an overlay of the regression lines from the analysis models. For the initial and reduced regression models, random terms were fit to account for variability due to experimental

design effects and for repeated measures across the days. A SP(POW) covariance structure was fit to both models since the day term is quantitative and unequally spaced, accounting for the repeated measurements between days. In order for the covariance parameter estimation and parameter estimation to run smoothly, ET_a values were scaled down by a factor of ten. Additionally, regression model parameters were obtained to find predicted ET_a values across regression lines. Regression lines for each crop system with and without Palmer amaranth under each irrigation type in 2019 were found using the parameter estimates in the following equation:

$$\begin{aligned} & \text{Predicted Avg Scaled } ET_{Irr \times Crop} \\ & = (irr + crop) - (day(irr) \times irr) * Days + (day * day(irr) \times irr) \\ & \quad * Days^2 \end{aligned}$$

Regression lines for each crop system under each irrigation type were separated out by the presence of Palmer amaranth or no Palmer amaranth in 2020 and were found using the parameter estimates in the following equation:

$$\begin{aligned} & \text{Predicted Avg Scaled } ET_{Irr,Crop,PA} \\ & = (irr * crop + crop * PA) + (day(crop * PA)) * Days - (day \\ & \quad * day(crop * PA)) * Days^2 \end{aligned}$$

Growth index, plant biomass, and total leaf area were analyzed as response variables in SAS. Irrigation type, crop system, and sampling date were fit as class variables for all models analyzed. Variable main effects and interactions were analyzed for significance ($P \leq 0.05$). Random terms were included in the models to account for the split plot design. More specifically, the random terms accounted for differences between replicates, for differences between the split plot levels, and for the correct degrees of

freedom within the models. Beginning with the largest interaction, if significant terms in the Type III effects of fixed effects table were found, differences between variable levels within these interactions were analyzed. Simple effect tests were run to find differences between specific levels while holding the levels of other variables in the interaction constant. If no interactions were significant, main effects were analyzed.

Results and Discussion

Total Soil Water. According to the Type III test of fixed effects for variable interactions and main effects in 2019, there were no statistical differences in the total soil water (TSW) of crop system with and without Palmer amaranth under center-pivot (CPI) and subsurface drip irrigation (SDI). However, there were differences that could impact the total water usage of a grower that has Palmer amaranth in their field and a grower who does not. According to Figure 3-2A,B, crop systems with Palmer amaranth had lower TSW than crop systems without Palmer amaranth under CPI and SDI, although TSW differences between crop systems with and without Palmer amaranth were greater under SDI. These results suggest that Palmer amaranth does influence TSW within corn and soybean systems and that early-season control is needed for optimum water savings in these crops. Irrigation as a main effect caused no statistical differences in TSW, although crop system as a main effect did cause differences in TSW in 2019 (Table 3-2). The largest difference in TSW occurred between corn and fallow systems (2,060 mm), followed by differences between corn and soybean systems (1,490 mm) under both irrigation types (Table 3-3).

Similarly in 2020, there were no statistical differences in the TSW of crop systems with and without Palmer amaranth under CPI or SDI. Unlike 2019, where crop systems with Palmer amaranth had lower TSW than crop systems without Palmer amaranth (Figure 3-2A,B), crop systems with Palmer amaranth in 2020 had similar or higher TSW values compared to crop systems without Palmer amaranth (Figure 3-3A,B). The only notable difference in TSW between crop systems with and without Palmer amaranth occurred under SDI between fallow systems in 2020 (Figure 3-3B). Irrigation as a main effect caused no differences in TSW, although crop system as a main effect did cause differences in TSW (Table 3-2). Differences in TSW between corn and fallow (6,870 mm) and soybean and fallow (5,470 mm) were even more pronounced than in 2019 (Table 3-3).

Scaled Actual Evapotranspiration. In 2019, there was a crop system effect on the intercepts of the reduced model (Table 3-4). Whereas corn systems with and without Palmer amaranth had the lowest TSW, corn systems with and without Palmer amaranth had the highest evapotranspiration (ET_c) rates, followed by soybean with and without Palmer amaranth, and then fallow with and without Palmer amaranth having the lowest ET_c rates (Figure 3-4A). There was also an irrigation by day interaction (Table 3-4). CPI had higher ET_a rates than SDI at the beginning of the growing season and ET_a differences leveled out 40 d after sensor installation (Table 3-5; Figure 3-5). These results suggest that irrigation affects ET_a differences across crop systems with and without Palmer amaranth early in the growing season, but that crop system and progression of plant growth with available water have a greater effect on ET_a differences under CPI and SDI.

In 2020, crop and Palmer amaranth effects were greater compared to the 2019 growing season. The reduced model indicated linear and quadratic interactions between crop system, irrigation and crop system, and day \times day \times crop system \times Palmer amaranth (Table 3-4). In terms of the day \times day \times crop system \times Palmer amaranth interaction, statistical differences in ET_a between crop systems with and without Palmer amaranth at the beginning of the growing season were not indicated, although there were differences. At 24 d after sensor installation through the remainder of the growing season, differences in ET_a between fallow systems with and without Palmer amaranth were detected (Table 3-6). Overall, Palmer amaranth cumulative evapotranspiration (ET_c) rates were higher under SDI than CPI, with the largest ET_c differences occurring in fallow systems (Table 3-7; Figure 3-6). These results suggest the presence of Palmer amaranth influenced fallow system ET_a more so than corn or soybean systems under both irrigation types. In terms of irrigation and crop system interactions, the largest ET_a differences occurred between soybean and fallow (32.1 mm) and corn and fallow (26.8 mm) under SDI, followed by differences in ET_a between corn and fallow (17.4 mm) and soybean and fallow (16.4 mm) under CPI (Table 3-8). These results suggest a slight irrigation effect and major crop effect on ET_a across crop systems with and without Palmer amaranth.

Growth Index, Plant Biomass, and Total Leaf Area. In 2019 and 2020, irrigation type did not statistically influence Palmer amaranth growth index. However, there was a sampling date \times crop system interaction in both years (Table 3-7). In 2019, Palmer amaranth growth index values in fallow were statistically greater than corn and soybean systems at the third and fourth sampling dates (Figure 3-7A,B). Similarly in 2020, Palmer amaranth growth index values in fallow were statistically greater than corn and soybean

systems at the fourth sampling date (Figure 3-7C,D). In addition, there was a sampling date \times irrigation type \times crop system interaction in 2020, although this three-way interaction was likely the result of the sampling date \times crop system interaction (Table 3-7). These results suggest crop system has a major effect on Palmer amaranth growth index, with greater growth index values of Palmer amaranth in fallow systems. There was also a slight irrigation effect in 2020, indicating that SDI may result in greater Palmer amaranth growth index. In terms of the sampling date effect, Palmer amaranth has aggressive growth characteristics; thus, a difference in Palmer amaranth growth index over time in well-watered conditions was expected.

Irrigation type did not statistically influence Palmer amaranth biomass in 2019 and 2020. However, as expected, there was a sampling date \times crop system interaction in both years (Table 3-8). Similar to Palmer amaranth growth index results, Palmer amaranth biomass was greater in fallow compared to corn and soybean systems at the third and fourth sampling dates (Figure 3-8A). These results are supported by the fact that Palmer amaranth growth over time directly affects Palmer amaranth biomass accumulation over time. In 2019, there was sampling date \times irrigation type interaction, although this interaction was likely driven by the Palmer amaranth biomass differences across sampling dates (Table 3-8; Figure 3-8B). In 2020, there were sampling date \times irrigation type \times crop system, sampling date \times crop system, and irrigation type \times crop system interactions. Irrigation as a main effect did not statistically influence Palmer amaranth biomass, so the sampling date \times irrigation type \times crop system; and irrigation type \times crop system interactions were likely driven by the sampling date and crop system effects (Table 3-8).

In 2019 and 2020, irrigation type did not statistically influence Palmer amaranth total leaf area. In 2019, there were no variable interactions, indicating that irrigation type, crop system, nor sampling date caused differences in Palmer amaranth total leaf area (Table 3-9; Figure 3-9A,B). Despite the lack of statistical differences of Palmer amaranth total leaf area in corn, soybean and fallow under both irrigation types, Palmer amaranth total leaf area was greatest in fallow, but only at the third and fourth sampling dates (Figure 3-9A,B). However, in 2020, there was a sampling date \times irrigation type \times crop system interaction that was likely driven by the sampling date \times crop system interaction (Table 3-9). Similar to Palmer amaranth growth index and biomass results, Palmer amaranth total leaf area was greater in fallow systems compared to corn systems at the second, third, and fourth sampling dates, and soybean systems at the third and fourth sampling dates (Figure 9C-D). These results are supported by Palmer amaranth growth index and biomass results given the knowledge that Palmer amaranth growth index over time directly affects biomass accumulation and total leaf area over time.

Practical Implications

This is the first study that evaluates the actual evapotranspiration (ET_a) of Palmer amaranth in multiple crop systems under center-pivot (CPI) and subsurface drip irrigation (SDI) with the goal to find an irrigation effect on Palmer amaranth ET_a . Irrigation contributes to differences in ET_a between crop systems with and without Palmer amaranth early in the growing season, where crop systems with and without Palmer amaranth had higher ET_a rates under CPI compared to SDI. However, the irrigation effect is likely overcome by differences in crop ET_a , later in the growing season. Thus, irrigation and crop management may influence Palmer amaranth ET_a rates. In regard to total soil water

(TSW), crop system, irrigation type, and Palmer amaranth influenced ET_a rates, with lower TSW in crop systems with Palmer amaranth and lower TSW in center-pivot crop systems. Irrigation type did not cause noticeable differences among Palmer amaranth growth index, plant biomass, and total leaf area values. This study provides base data on Palmer amaranth evapotranspiration and its relation to Palmer amaranth morphological features (i.e., growth index, biomass, and total leaf area) for future use in mechanistic weed-crop competition models. Further research on weed evapotranspiration should be conducted at more locations under varying climatic conditions to build a robust database of evapotranspiration for important agronomic weed species such as Palmer amaranth, waterhemp, and horseweed.

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Table 3-1. Center-pivot and subsurface drip irrigation date and amount in the 2019 and 2020 growing seasons at South Central Agricultural Laboratory in Clay Center, NE.

Center-pivot irrigation		Subsurface drip irrigation	
Date	Amount --mm--	Date	Amount --mm--
2019		2019	
7/31	32	8/2	32
2020		2020	
7/13	32	7/15	32
7/28	32	7/29	32
8/7	32	8/7	32
8/17	32	8/17	32
8/24	32	8/24	32
9/1	32	9/1	32

Table 3-2. Type III tests of fixed effects for irrigation type \times crop system \times Palmer amaranth main effects and interactions for total soil water in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Effect	2019				2020			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Irrigation	1	4	3.09	0.1536	1	4	2.80	0.1697
Crop	2	8	4.58	0.0473	2	8	23.60	0.0004
Irrigation*Crop	2	8	0.15	0.8595	2	8	2.64	0.1314
Palmer	1	8	0.81	0.3936	1	12	0.49	0.4985
Irrigation*Palmer	1	8	0.05	0.8363	1	12	0.25	0.6260
Crop*Palmer	1	8	0.76	0.4093	2	12	2.69	0.1080
Irrigation*Crop*Palmer	1	8	0.00	0.9495	2	12	1.40	0.2836

*Significance at $P \leq 0.05$

Table 3-3. Least squares means T grouping for total soil water by crop system under center-pivot and subsurface drip irrigation in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

2019				2020		
Crop	Estimate --mm--			Crop	Estimate --mm--	
Fallow	39941		A	Fallow	32208	A
			A			
Soybean	39368	B	A	Soybean	26739	B
		B				B
Corn	37882	B		Corn	25339	B

*Least squares means ($\alpha = 0.05$) with the same letter are not significantly different

Table 3-4. Type I tests of fixed effects for the irrigation type \times crop system \times day main effects and interactions in the reduced models for actual evapotranspiration in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Effect	2019			
	Num DF	Den DF	F Value	Pr > F
Irrigation	1	4	10.17	0.0333
Crop	2	10	5.53	0.0241
Day	1	416	167.42	<.0001
Day*Irrigation	1	416	7.15	0.0078
Day*Day	1	416	1.55	0.2142
Day*Day*Irrigation	1	416	6.85	0.0092
	2020			
Irrigation	1	4	1.74	0.2570
Crop	2	8	42.25	<.0001
Irrigation*Crop	2	8	3.64	0.0750
PA_None	1	15	1.63	0.2206
Crop*PA_None	2	15	2.27	0.1377
Day	1	384	154.37	<.0001
Day*Crop	2	384	12.03	<.0001
Day*PA_None	1	384	0.05	0.8201
Day*Crop*PA_None	2	384	0.18	0.8331
Day*Day	1	384	19.66	<.0001
Day*Day*Crop	2	384	0.73	0.4834
Day*Day*PA_None	1	384	0.63	0.4267
Day*Day*Crop*PA_Non	2	384	3.21	0.0413

*Significance at $P \leq 0.05$

Table 3-5. Least squares means T grouping for actual evapotranspiration by irrigation type and day in 2019 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

2019			
Irrigation	Day	Estimate --mm--	
Pivot	1.00	5.8416	A
SDI	1.00	3.0755	B
Pivot	8.00	5.4170	A
SDI	8.00	3.1554	B
Pivot	24.00	4.4940	A
SDI	24.00	3.2035	B
Pivot	31.00	4.1111	A
SDI	31.00	3.1658	B
Pivot	40.00	3.6376	A
SDI	40.00	3.0647	A
Pivot	47.00	3.2838	A
SDI	47.00	2.9451	A
Pivot	58.00	2.7535	A
SDI	58.00	2.6849	A
SDI	66.00	2.4402	A
Pivot	66.00	2.3876	A
SDI	74.00	2.1487	A
Pivot	74.00	2.0383	A
SDI	83.00	1.7649	A
Pivot	83.00	1.6652	A
SDI	89.00	1.4762	A
Pivot	89.00	1.4282	A
Pivot	98.00	1.0902	A
SDI	98.00	0.9938	A

*Least squares means ($\alpha=0.05$) with the same letter are not significantly different

Table 3-6. Actual evapotranspiration simple effect comparisons of crop system with and without Palmer amaranth by day in 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Simple Effect Comparisons of Crop*PA None Least Squares Means By Crop											
Simple Effect Level --Crop--	PA_None	PA_None	Day	Estimate --mm--	Standard Error	DF	t Value	Pr > t	Alpha	Lower	Upper
Corn	None	PA	1.00	-0.03075	1.0669	15	-0.03	0.9774	0.05	-2.3048	2.2433
Fallow	None	PA	1.00	0.5122	1.0669	15	0.48	0.6381	0.05	-1.7618	2.7863
Soybean	None	PA	1.00	-0.1530	1.0669	15	-0.14	0.8879	0.05	-2.4271	2.1210
Corn	None	PA	8.00	-0.04122	0.8215	15	-0.05	0.9606	0.05	-1.7921	1.7097
Fallow	None	PA	8.00	-0.2187	0.8215	15	-0.27	0.7937	0.05	-1.9696	1.5322
Soybean	None	PA	8.00	0.1216	0.8215	15	0.15	0.8842	0.05	-1.6293	1.8726
Corn	None	PA	24.00	-0.06850	0.5737	15	-0.12	0.9065	0.05	-1.2913	1.1543
Fallow	None	PA	24.00	-1.4452	0.5737	15	-2.52	0.0236	0.05	-2.6680	-
Soybean	None	PA	24.00	0.5593	0.5737	15	0.97	0.3451	0.05	-0.6635	1.7821
Corn	None	PA	31.00	-0.08192	0.5761	15	-0.14	0.8888	0.05	-1.3099	1.1461
Fallow	None	PA	31.00	-1.7875	0.5761	15	-3.10	0.0073	0.05	-3.0155	-
Soybean	None	PA	31.00	0.6676	0.5761	15	1.16	0.2647	0.05	-0.5604	1.8956
Corn	None	PA	40.00	-0.1005	0.6053	15	-0.17	0.8703	0.05	-1.3906	1.1896
Fallow	None	PA	40.00	-2.0538	0.6053	15	-3.39	0.0040	0.05	-3.3439	-
Soybean	None	PA	40.00	0.7323	0.6053	15	1.21	0.2450	0.05	-0.5577	2.0224
Corn	None	PA	47.00	-0.1160	0.6178	15	-0.19	0.8536	0.05	-1.4329	1.2009
Fallow	None	PA	47.00	-2.1258	0.6178	15	-3.44	0.0036	0.05	-3.4427	-
Soybean	None	PA	47.00	0.7249	0.6178	15	1.17	0.2590	0.05	-0.5921	2.0418
Corn	None	PA	58.00	-0.1421	0.5975	15	-0.24	0.8153	0.05	-1.4157	1.1315
Fallow	None	PA	58.00	-1.9998	0.5975	15	-3.35	0.0044	0.05	-3.2734	-
Soybean	None	PA	58.00	0.6108	0.5975	15	1.02	0.3229	0.05	-0.6628	1.8843
Corn	None	PA	66.00	-0.1625	0.5603	15	-0.29	0.7758	0.05	-1.3568	1.0318
Fallow	None	PA	66.00	-1.7248	0.5603	15	-3.08	0.0076	0.05	-2.9191	-
Soybean	None	PA	66.00	0.4492	0.5603	15	0.80	0.4352	0.05	-0.7451	1.6435
Corn	None	PA	74.00	-0.1841	0.5379	15	-0.34	0.7369	0.05	-1.3305	0.9624
Fallow	None	PA	74.00	-1.2952	0.5379	15	-2.41	0.0294	0.05	-2.4417	-
Soybean	None	PA	74.00	0.2215	0.5379	15	0.41	0.6863	0.05	-0.9249	1.3680
Corn	None	PA	83.00	-0.2097	0.6009	15	-0.35	0.7319	0.05	-1.4905	1.0710
Fallow	None	PA	83.00	-0.6274	0.6009	15	-1.04	0.3130	0.05	-1.9081	0.6534
Soybean	None	PA	83.00	-0.1137	0.6009	15	-0.19	0.8525	0.05	-1.3944	1.1671
Corn	None	PA	89.00	-0.2277	0.7246	15	-0.31	0.7577	0.05	-1.7720	1.3167
Fallow	None	PA	89.00	-0.07347	0.7246	15	-0.10	0.9206	0.05	-1.6178	1.4709
Soybean	None	PA	89.00	-0.3837	0.7246	15	-0.53	0.6042	0.05	-1.9280	1.1607
Corn	None	PA	98.00	-0.2558	1.0298	15	-0.25	0.8072	0.05	-2.4507	1.9390
Fallow	None	PA	98.00	0.9203	1.0298	15	0.89	0.3856	0.05	-1.2746	3.1152
Soybean	None	PA	98.00	-0.8584	1.0298	15	-0.83	0.4176	0.05	-3.0533	1.3365

*Significance at $P \leq 0.05$

Table 3-7. Type III tests of fixed effects for irrigation type × crop system × sampling date main effects and interactions for Palmer amaranth growth index in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Effect	2019				2020			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Irrigation	1	4	0.23	0.6560	1	4	0.91	0.3937
Crop	2	8	18.27	0.0010	2	8	13.60	0.0027
Irrigation*Crop	2	8	0.25	0.7870	2	8	2.71	0.1266
Date	3	36	21.78	<.0001	3	36	8.47	0.0002
Date*Irrigation	3	36	0.13	0.9436	3	36	1.28	0.2970
Date*Crop	6	36	8.81	<.0001	6	36	6.09	0.0002
Date*Irrigation*Crop	6	36	0.17	0.9840	6	36	2.35	0.0515

*Significance at $P \leq 0.05$

Table 3-8. Type III tests of fixed effects for irrigation type × crop system × sampling date main effects and interactions for Palmer amaranth biomass in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Effect	2019				2020			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Irrigation	1	4	0.38	0.5710	1	4	1.83	0.2473
Crop	2	8	50.99	<.0001	2	8	58.56	<.0001
Irrigation*Crop	2	8	0.31	0.7406	2	8	8.03	0.0122
Date	3	35	45.01	<.0001	3	36	34.64	<.0001
Date*Irrigation	3	35	2.54	0.0726	3	36	3.44	0.0269
Date*Crop	6	35	24.75	<.0001	6	36	24.55	<.0001
Date*Irrigation*Crop	6	35	1.10	0.3816	6	36	7.03	<.0001

*Significance at $P \leq 0.05$

Table 3-9. Type III tests of fixed effects for irrigation type × crop system × sampling date main effects and interactions for Palmer amaranth total leaf area in 2019 and 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

Effect	2019				2020			
	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Irrigation	1	4	0.98	0.3776	1	4	0.21	0.6741
Crop	2	8	1.73	0.2372	2	8	18.72	0.0010
Irrigation*Crop	2	8	0.99	0.4113	2	8	0.04	0.9568
Date	3	36	1.55	0.2184	3	36	8.25	0.0003
Date*Irrigation	3	36	1.03	0.3894	3	36	0.46	0.7116
Date*Crop	6	36	1.41	0.2384	6	36	6.00	0.0002
Date*Irrigation*Crop	6	36	1.02	0.4291	6	36	2.27	0.0589

*Significance at $P \leq 0.05$

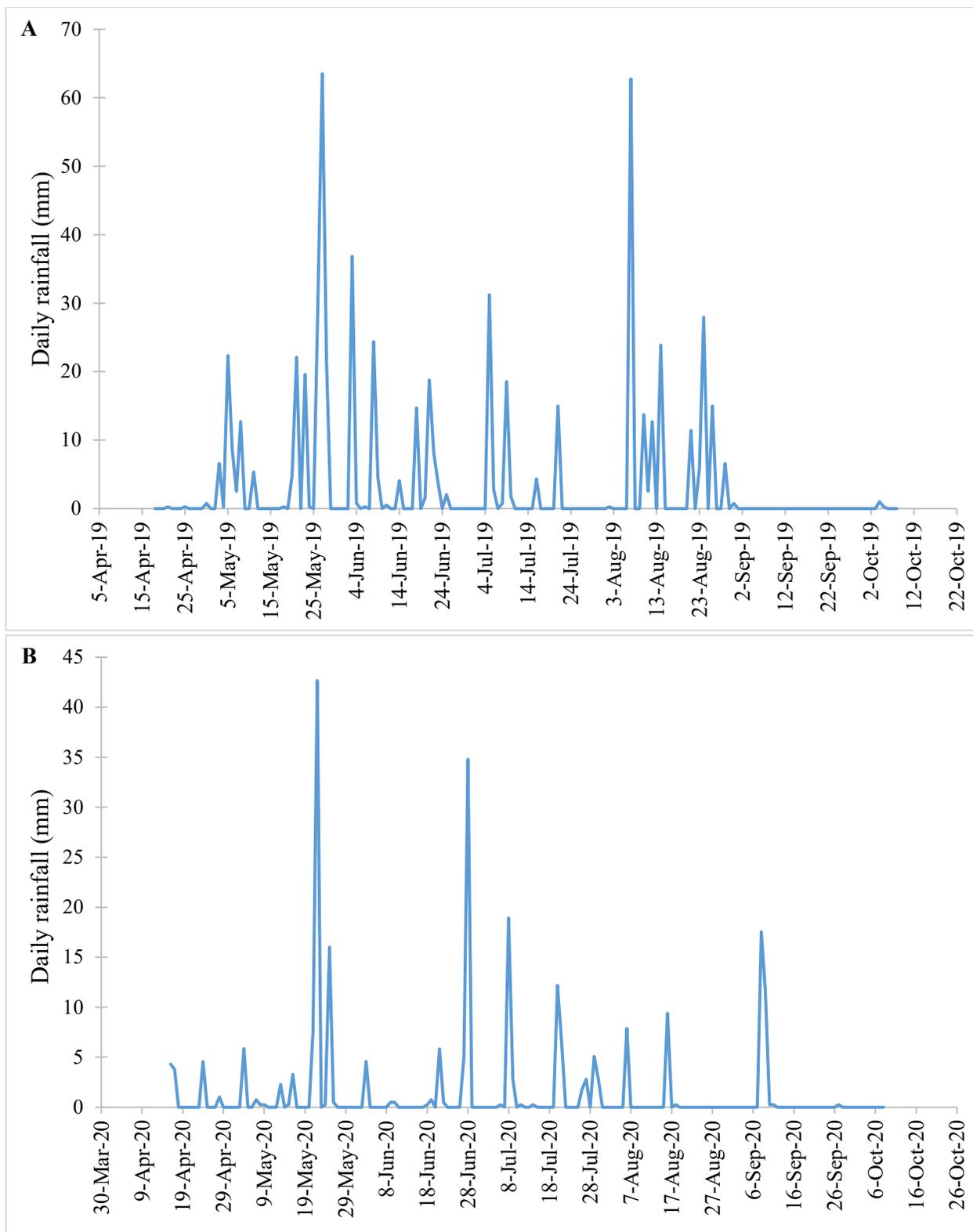
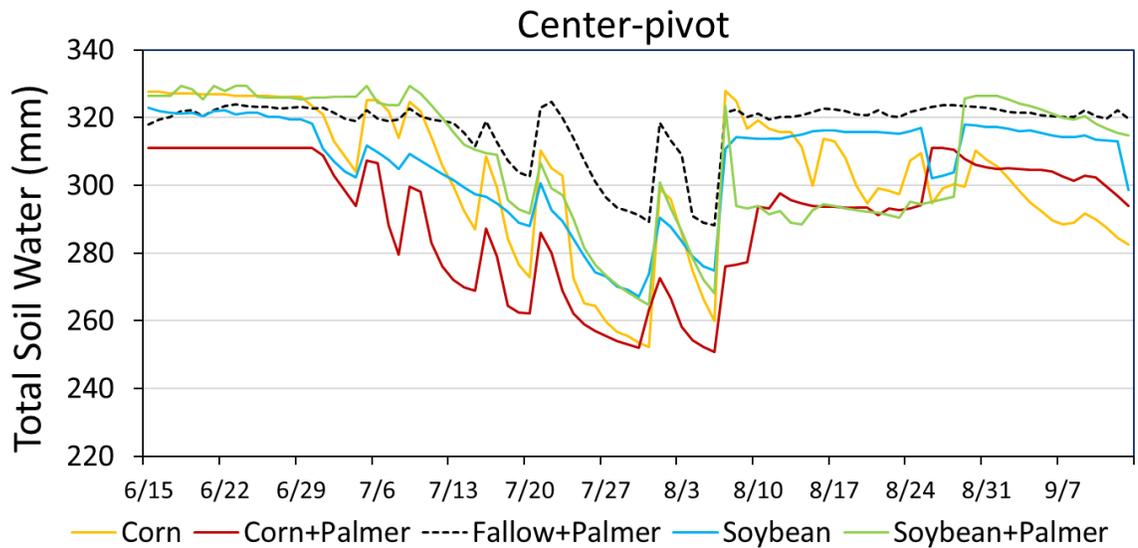


Figure 3-1. Daily rainfall in the (A) 2019 and (B) 2020 growing seasons at South Central Agricultural Laboratory near Clay Center, NE

A



B

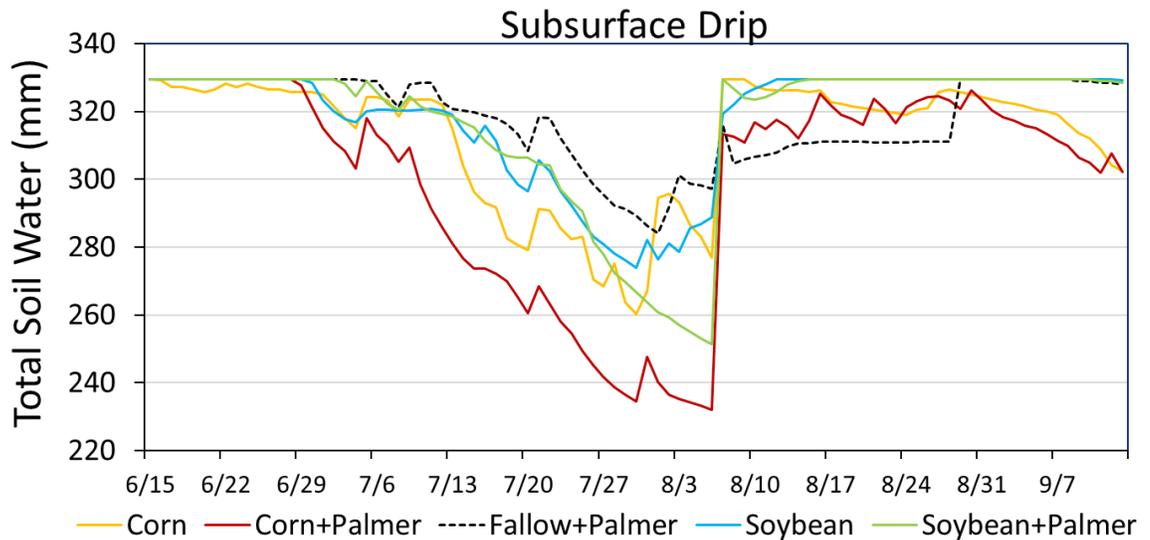


Figure 3-2. Average total soil water results of crop systems under (A) center-pivot and (B) subsurface drip in 2019 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

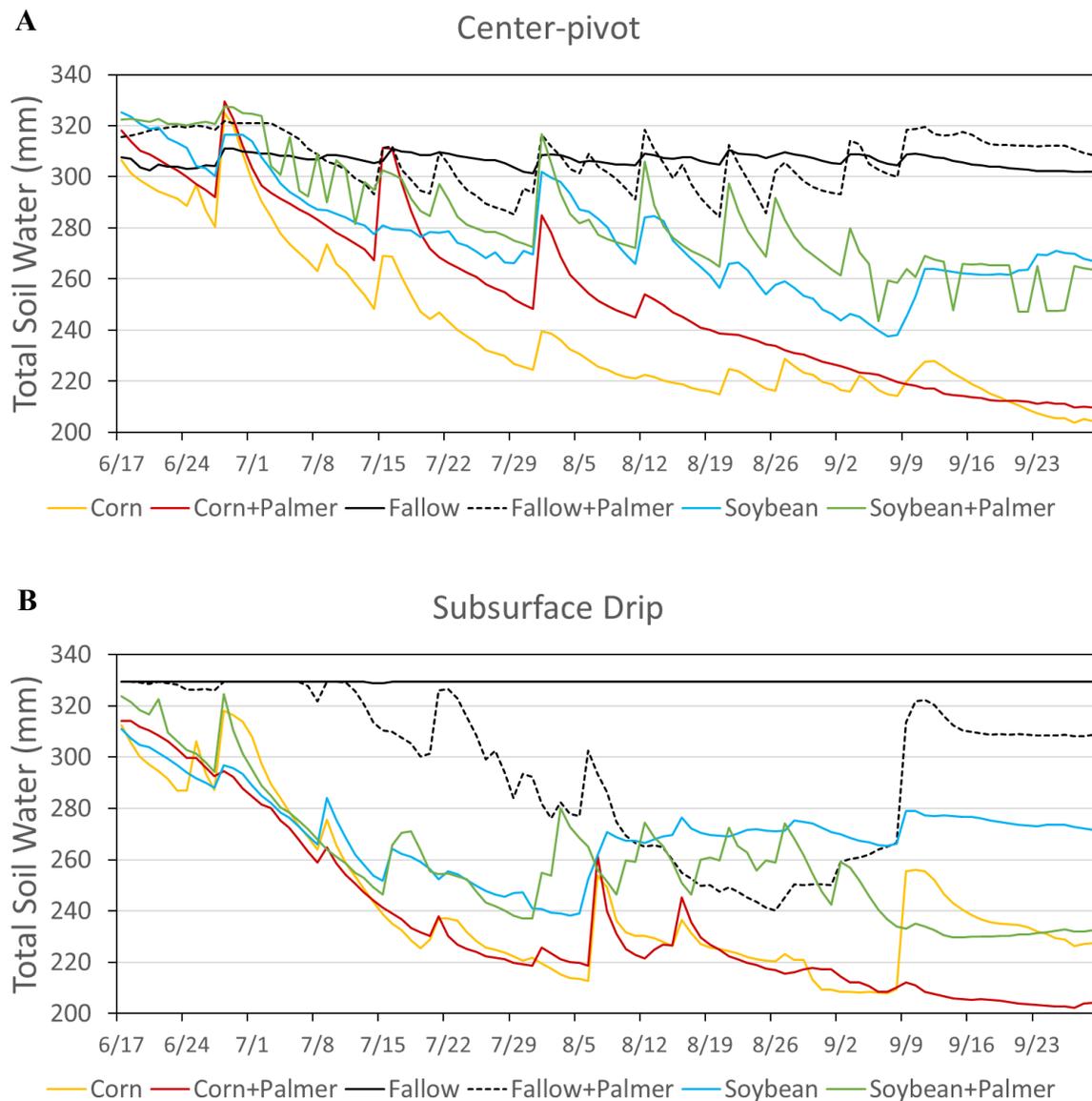


Figure 3-3. Average total soil water results of crop systems under (A) center-pivot and (B) subsurface drip in 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

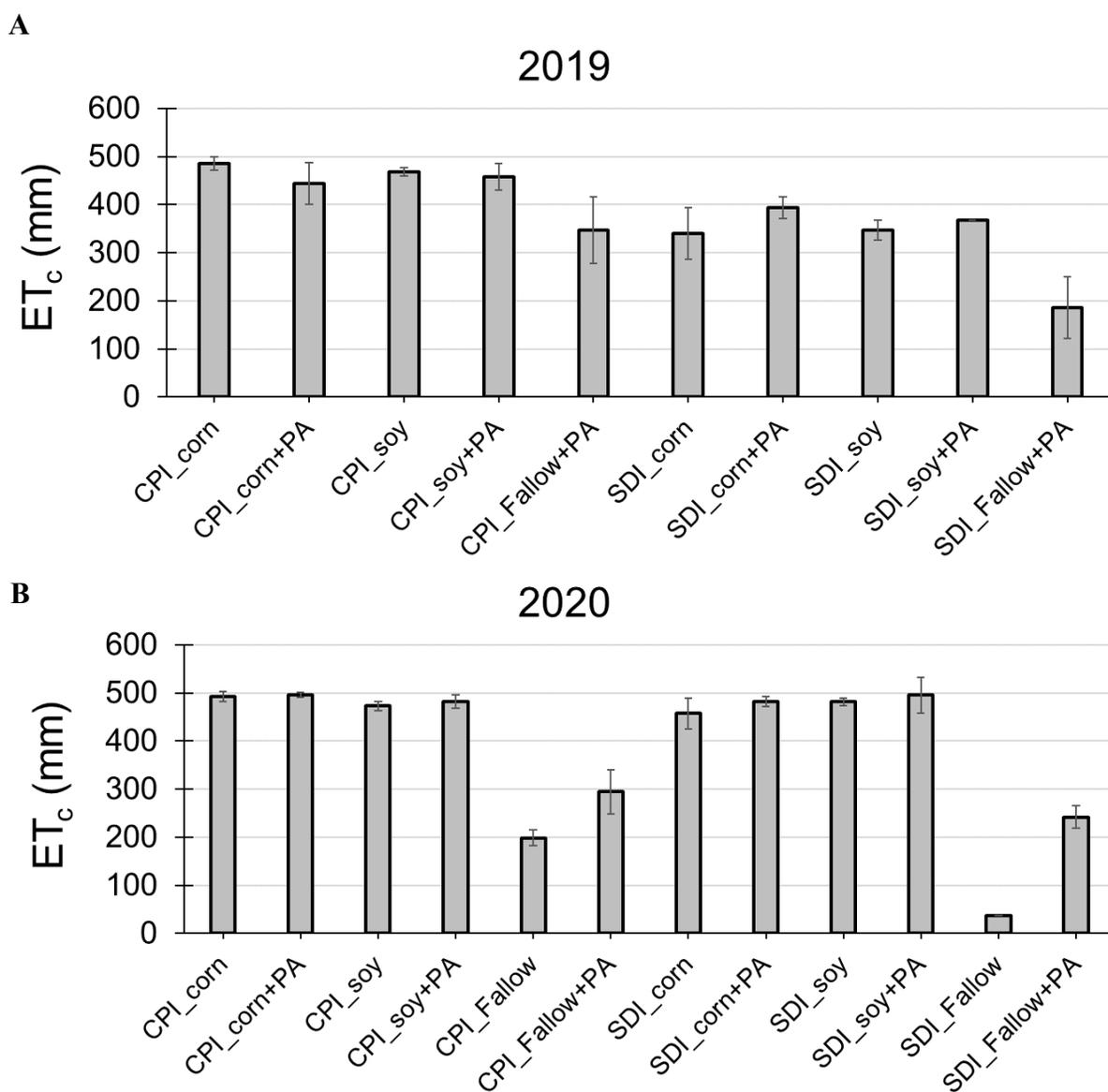


Figure 3-4. Actual evapotranspiration by crop system and irrigation type in (A) 2019 and (B) 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

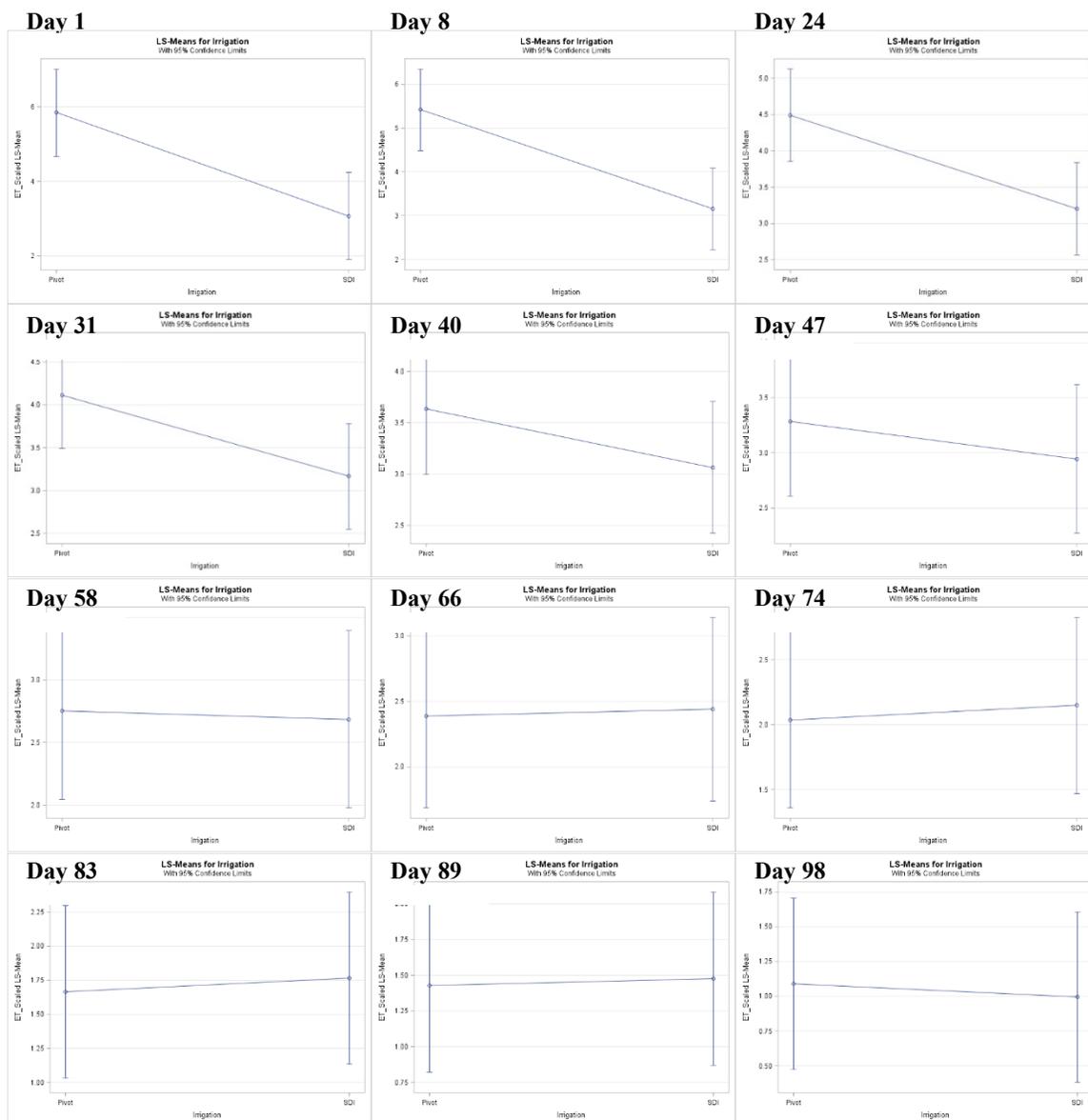


Figure 3-5. Least squares means plots of actual evapotranspiration by irrigation from day 1 to day 98 after sensor installation in 2019 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

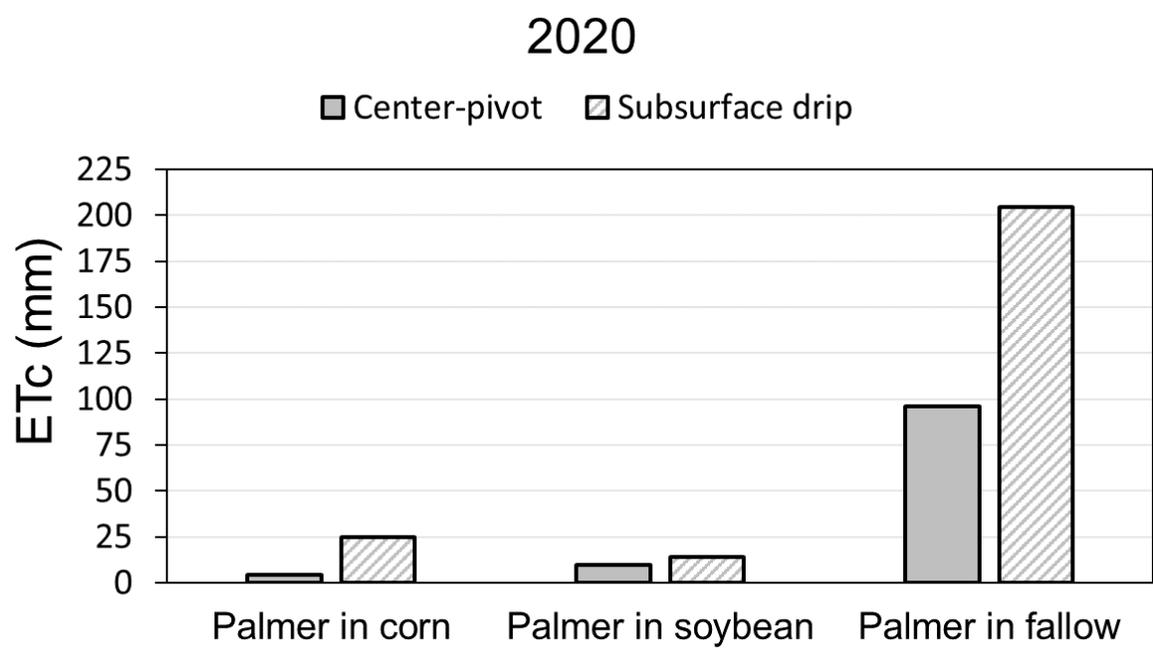


Figure 3-6. Cumulative evapotranspiration of Palmer amaranth in corn, soybean, and fallow systems under center-pivot and subsurface drip irrigation in 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

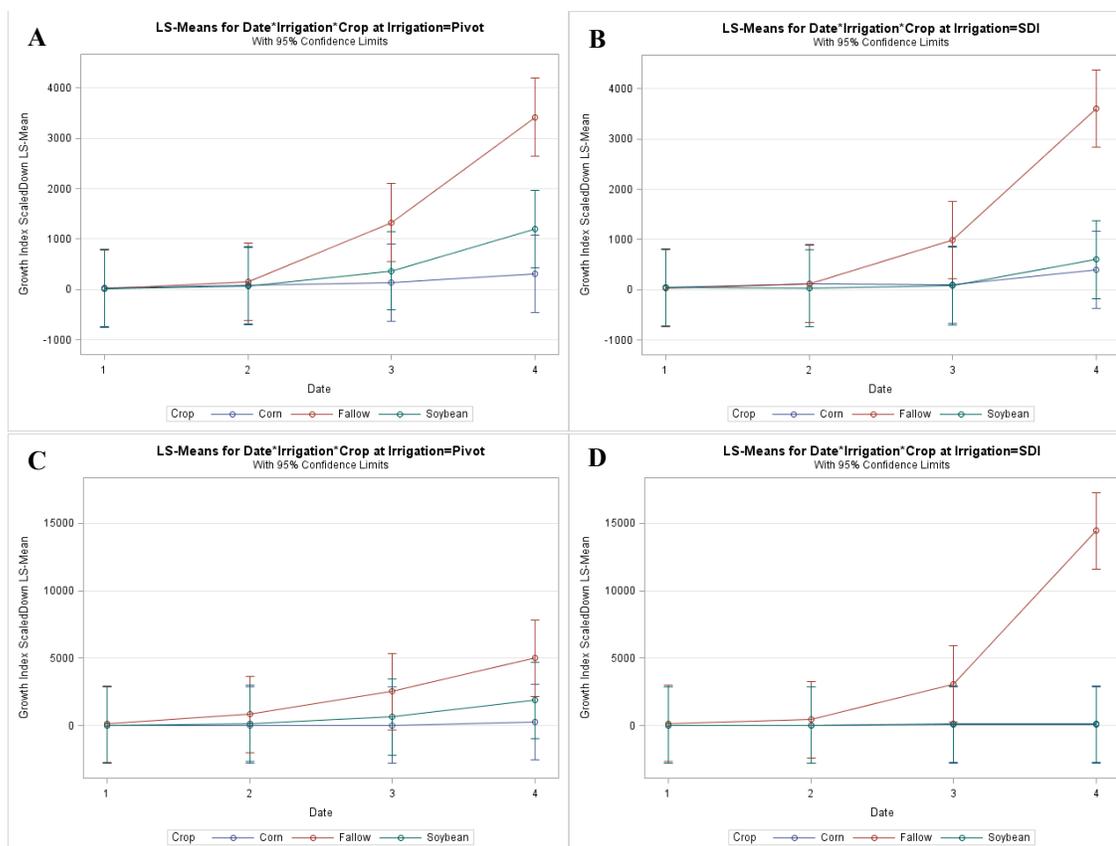


Figure 3-7. Mean interaction plots for Palmer amaranth growth index by crop type and sampling date in (A) center-pivot plots in 2019, (B) subsurface drip plots in 2019, (C) center-pivot plots in 2020, and (D) subsurface drip plots in 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

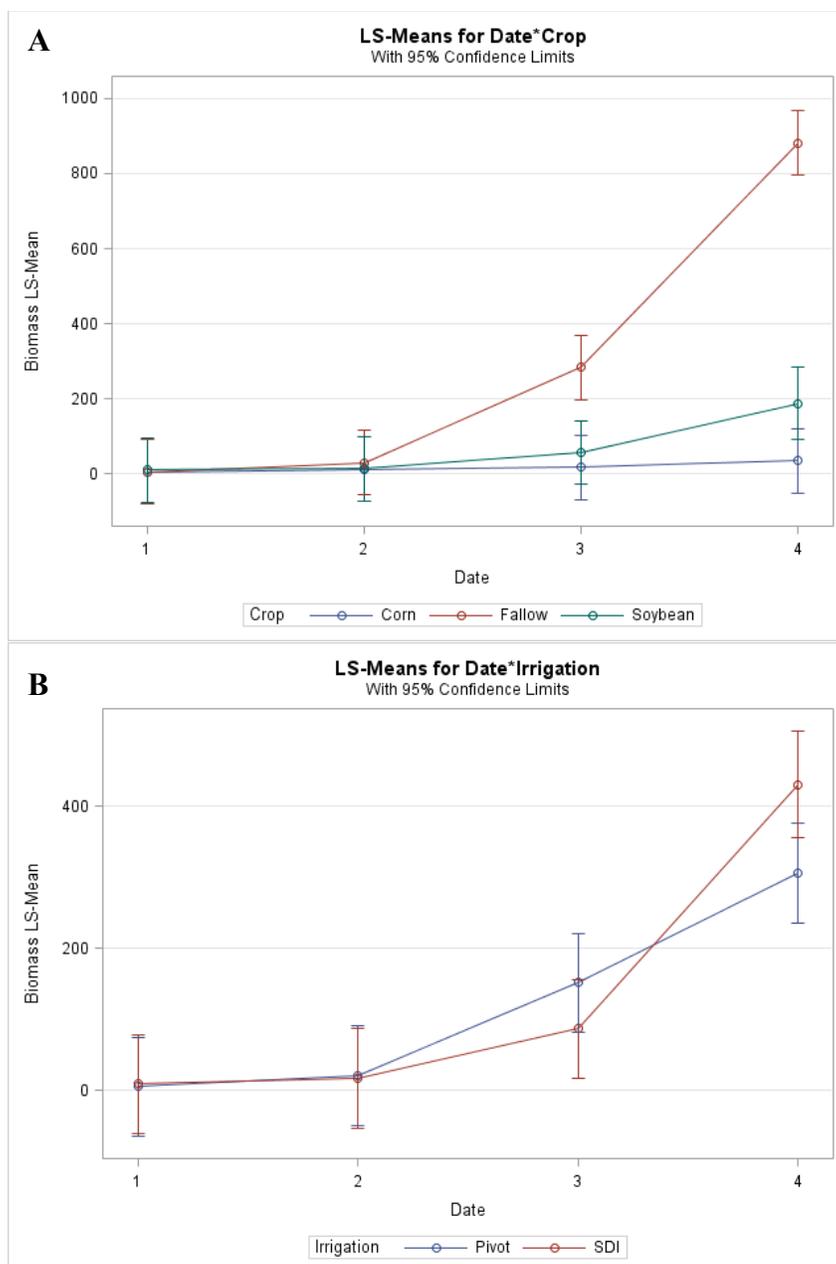


Figure 3-8. Mean interaction plots for 2019 Palmer amaranth biomass by (A) sampling date and crop type and (B) sampling date and irrigation type in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

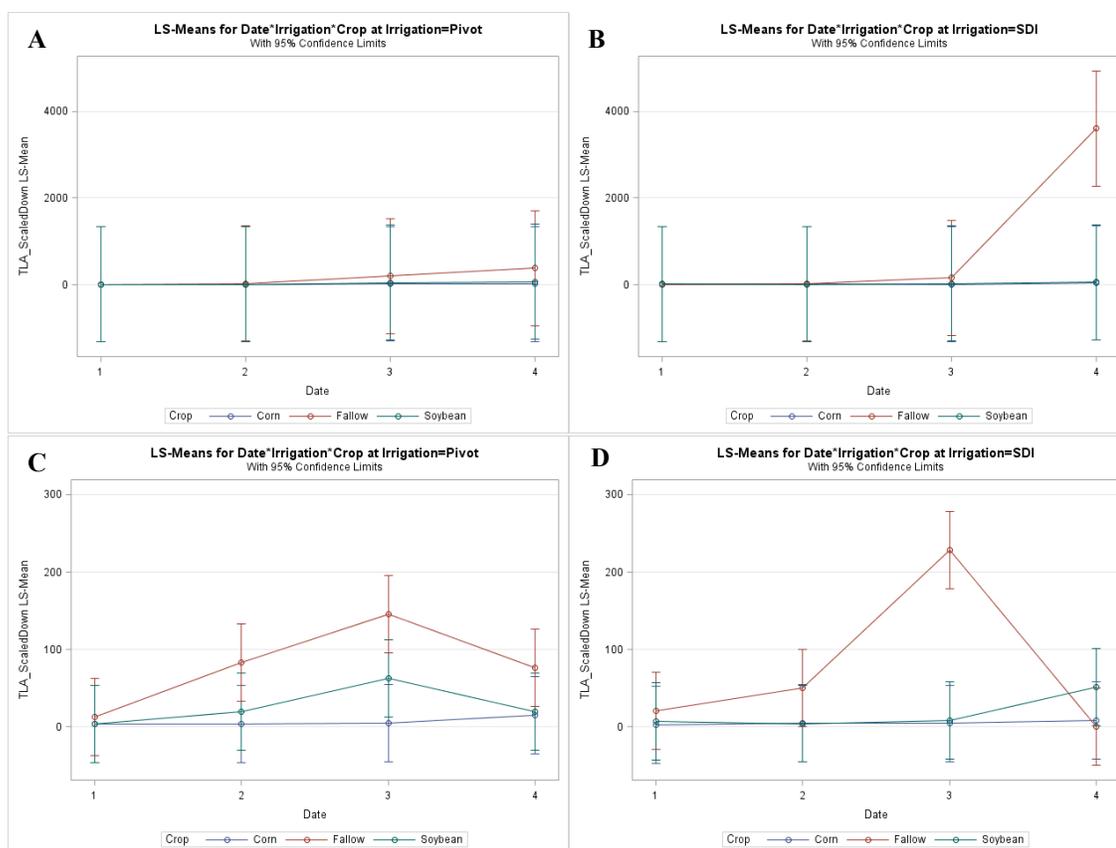


Figure 3-9. Mean interaction plots for Palmer amaranth total leaf area by crop type and sampling date in (A) center-pivot plots in 2019, (B) subsurface drip plots in 2019, (C) center-pivot plots in 2020, and (D) subsurface drip plots in 2020 in a study to determine evapotranspiration of Palmer amaranth in corn, soybean, and fallow under subsurface drip and center-pivot irrigation systems at Clay Center, NE.

CHAPTER 4:
EFFECT OF DEGREE OF WATER STRESS ON GROWTH AND FECUNDITY
OF VELVETLEAF (*ABUTILON THEOPHRASTI*)
USING SOIL MOISTURE SENSORS

Abstract

Velvetleaf (*Abutilon theophrasti* L.) is a troublesome broadleaf weed that competes with agronomic crops for resources such as soil moisture. Water stress can affect the ability of weed species to grow and produce seeds. The objective of this study was to determine the effect of degree of water stress on the growth and fecundity of velvetleaf using soil moisture sensors under greenhouse conditions. Velvetleaf seeds collected from a corn/soybean field were grown in silty clay loam soil and plants were maintained at 100%, 75%, 50%, and 25% soil field capacity (FC) corresponding to no-, light-, moderate-, and high-water stress conditions, respectively. Water was regularly added to pots based on soil moisture levels detected by a Decagon 5TM sensor to maintain the desired water stress level required by treatment. Plants maintained at 100% FC had the maximum number of leaves (28 leaves plant⁻¹), followed by 21 and 15 leaves plant⁻¹ at 75% and 50% FC, respectively. Velvetleaf at 100% and 75% FC achieved maximum plant height (108 to 123 cm) compared with 83 cm at 50% FC. Velvetleaf maintained at 75% FC had the greatest growth index (79,907 cm³) followed by 72,197 cm³ at 100% FC and 64,256 cm³ at 50% FC. Seed production was similar at 100%, 75%, and 50% FC (288 to 453 seeds plant⁻¹) compared with 2 seeds plant⁻¹ at 25% FC. This is because the majority of plants maintained at 25% FC did not survive more than 77 d after

transplanting. Seed germination was 96%–100% at 100%, 75%, and 50% FC compared to 20% germination at 25% FC. The results of this study demonstrate that velvetleaf can survive $\geq 50\%$ FC continuous water stress conditions, although with a reduced leaf number, plant height, and growth index compared to 75% and 100% FC.

Introduction

Throughout Nebraska's agricultural history, natural disasters such as drought have had an adverse effect on crop yields and the economy (USDA 2020; Wu et al. 2013). In the early mid-2000s and in 2012, Nebraska dealt with severe drought resulting in reduced crop yields (Wu et al. 2013). More recently, in August of 2020, Nebraska began experiencing drought conditions, and by October of 2020, 34 counties in Nebraska were eligible for emergency loans for drought relief (USDA 2020). Recognition of drought periods is important because weed species such as velvetleaf compete with crops for a variety of environmental resources, including water, which is one of the most limiting factors for optimum crop production (Benjamin and Nielsen 2006). Water stress can negatively affect the growth and productivity of crops and associated weed species, though the outcomes of competition for water depend on the crop and weed species' abilities to survive under water stress conditions (Begg and Turner 1976; Patterson 1995). Compared to C₄ weed species such as *Amaranthus palmeri* and *Portulaca oleracea* that have water stress resistance mechanisms (e.g., drought avoidance, drought tolerance, drought recovery, or drought escape), C₃ weed species such as velvetleaf are not able to maintain the same level of growth and development under water stress conditions (Kumar et al. 1984; Pearcy and Ehleringer 1984; Sung and Krieg 1979; Ward et al. 2001). Hinz

and Owen (1994) found that velvetleaf under water stress conditions caused leaf water and osmotic potential to decrease linearly over time. Munger et al. (1987a; 1987b) indicated that as leaf water potential decreased in velvetleaf plants, stomatal conductance, photosynthetic, and transpiration rates decreased.

In addition to growth and development, seed germination is an important component of weed establishment and is influenced by environmental factors such as water availability, water temperature, light quality, and light duration during seed development (Baskin and Baskin 1998; Fenner 1991). Velvetleaf seed germination is sensitive to varying degrees of water stress and was completely inhibited by an osmotic potential of -600 kPa (Sadeghloo et al. 2013; Xiong et al. 2018). Despite these findings, scientific literature is not available on the effect that water stress throughout velvetleaf's growth period has on growth and fecundity. Bathke et al. (2014) projected a 5%–10% decrease in soil moisture for Nebraska under a high emissions scenario, indicating the potential for increased water stress and plant water competition in Nebraska plant populations. Despite projected increases in precipitation events in the eastern Great Plains, soil moisture is expected to decrease most near the soil surface due to evaporative loss from warmer temperatures (Bathke et al. 2014; Berg et al. 2016). While some plant developmental processes in leaves, roots, and reproductive structures are conserved across species, most plant responses are variable within and between species and are dependent on the developmental stage (Gray and Brady 2016).

Research evaluating a plant's response to water stress is typically performed under greenhouse or controlled environment conditions. The plants are often grown in pots to maintain certain water stress levels or soil field capacity (FC) for a limited growth

period or until the plant has reached maturity. A common method for maintaining desired FC in similar studies has been to weigh pots regularly to determine water lost from the soil and then add the appropriate amount of water (Chandi et al. 2013; Chauhan 2013; Chauhan and Johnson 2010; Earl 2003; Sarangi et al. 2015). However, it is not possible to determine the weight of the plant and pot separately using this method, resulting in inaccurate soil water content (Chahal et al. 2018). This could result in errors when adding water, especially as plants accumulate more biomass. Moreover, this approach is time-consuming and labor intensive since the pots must be lifted and weighed at regular intervals until completion of the study. By incorporating soil moisture sensors such as Decagon 5TM sensors (Decagon Devices, 2365 NE Hopkins Court, Pullman, WA), the labor required to weigh and add water to pots can be reduced. The Decagon 5TM sensor is a frequency-domain reflectometry sensor that measures soil water content directly as percent volume, determining soil moisture stress in real time with increased accuracy (Chahal et al. 2018). Soil moisture sensors allow researchers to measure soil water content more frequently and maintain FC within a narrow, predetermined range (Irmak et al. 2016) in loam and silt-loam soils (Paudel et al. 2016; Zhu 2016). Thus, the objective of this study was to determine the effect of degree of water stress on growth and fecundity of velvetleaf using soil moisture sensors.

Materials and Methods

Plant Materials. Velvetleaf seeds were collected from fields under corn-soybean rotation at the South Central Agricultural Laboratory in Clay County, Nebraska (40.57°N,

98.14°W). The seeds were stored in a refrigerator at 5°C until used in this study.

Velvetleaf seeds were planted in germination trays containing silty clay loam soil with a particle size distribution of 53% silt, 28% clay, 19% sand, and 2% organic matter content and a pH of 6.7. The soil used in this study was collected from a field near Lincoln, NE with no herbicide use in the last 10 yr. Germination trays were kept under greenhouse conditions at the University of Nebraska-Lincoln maintained at 27/21°C day/night temperatures. Overhead metal-halide lamps with 600 mmol photon m⁻² s⁻¹ light intensity were used to provide supplemental light in the greenhouse to maintain a 16-h photoperiod. Velvetleaf seedlings 6 to 8 cm in height were transplanted into round, free-draining pots (20-cm diam and 30-cm ht) containing 10 kg of the same soil used in the germination trays, with one plant per pot. Pots were already at the desired moisture stress level of 100%, 75%, 50%, and 25% FC when velvetleaf seedlings were transplanted, minimizing the risk of transplant shock. Treatments were arranged in a completely randomized design with six replications.

Soil Water Content. The soil used in this study had a permanent wilting point and saturation point of 17.7% and 34.7% volumetric, respectively. The soil had a bulk density of 1.4 g cm⁻³ and a volumetric FC of 39.2% based on soil test reports (American Agricultural Laboratory, Inc., McCook, NE). Gravimetric FC was 28% and was calculated using the following equation (Hillel 1998):

$$\theta g = \theta v / \rho_b$$

where θg is the percent gravimetric soil water content, θv is the percent volumetric soil water content, and ρ_b is the soil bulk density in grams per cubic cm.

The study included four soil water stress treatments: 100%, 75%, 50%, and 25% of the soil FC, corresponding to no-, light-, moderate-, and high-water stress levels, respectively (Chahal et al. 2018; Sarangi et al. 2015). Soil water content in the pots was measured using Decagon 5TM soil moisture sensors and Em50 data loggers (Figure 4-1). The sensors were installed at a 45° angle at a 15 cm depth in each pot. Because the soil had a gravimetric FC of 28%, 2.8 L of water (28% of 10 kg soil) was added to each pot at 12 and 4 d before transplanting in 2019 and 2020, respectively, to maintain 100% gravimetric FC. Likewise, 2.3 L (75% of 2.8 L), 1.6 L (50% of 2.8 L), and 0.9 L (25% of 2.8 L) of water were added to maintain 75%, 50%, and 25% soil FC, respectively, with a range of ±2% actual volumetric water content set for water stress treatments. Soil moisture data from Decagon data loggers were recorded once a day, and the required amount of water was added evenly on top of the soil to maintain treatment soil FC.

Data Collection. Velvetleaf height, number of leaves per plant, and growth index were determined at 7-d intervals beginning 7 d after transplanting (DAT) until plants were harvested upon maturity at 84 DAT during both years. Growth index can be defined as a quantitative indicator of plant growth rate used to compare plants grown under different soil water conditions and was calculated using the following equation (Irmak et al. 2004; Sarangi et al. 2015):

$$GI (cm^3) = \pi \times (w/2)^2 \times h$$

where w is the width of the plant calculated as an average of two widths, one measured at the widest point and another at 90° to the first; and h is the plant height measured from the soil surface to the shoot apical meristem (Sarangi et al. 2015). Upon maturity, leaves

were counted and removed from each stem to measure the total leaf area for each plant using a leaf area meter (LI-3100C Area Meter, Li-Cor, Lincoln, NE).

During harvest, plant stems were clipped at the soil surface and roots were removed from the pots; stems and roots were washed with water in a container and air-dried for 24 h. The leaves, shoots, and roots from each plant were stored separately in paper bags and oven-dried at 65°C for 7 d. Seed heads were collected and the number of seeds per plant counted; seeds were then weighed and stored in the dark at room temperature until used in germination tests. Seed dormancy was interrupted in velvetleaf seeds by soaking them in boiling water for five seconds (Sadeghloo et al. 2013). Fifty seeds from each plant were placed on a piece of moist Whatman No. 4 filter paper (GE Healthcare UK, Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, UK) in a petri dish. Petri dishes were stored for 21 d in a growth chamber maintained at 35/28°C day/night temperatures with a 16-h photoperiod, and an appropriate amount of water were added each day to keep the filter paper wet. Fluorescent bulbs were used to produce a light intensity of 85 mmol m⁻² s⁻¹. The total number of germinated seeds was counted and converted to percent germination based on the total seed number in each petri dish.

Statistical Analysis. Three parameter log-logistic models were fit to velvetleaf height, leaf number per plant, and growth index using the drc package in R (R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007):

$$Y = \left\{ \frac{d}{1 + \exp[b(\log x - \log e)]} \right\}$$

where Y is plant height, leaf number per plant, or growth index; x is days after transplanting; d is the estimated maximum plant height, leaf number per plant, or growth index; e is the time taken to achieve 50% of plant height, leaf number per plant, or

growth index; and b represents the relative slope around the parameter e . A t -test was used to determine whether the water stress treatments significantly affected maximum estimates, rate of change, and time taken to achieve 50% of plant height, leaf number per plant, or growth index. Velvetleaf stem, leaf, and root biomass per plant (g), total leaf area per plant (cm²), seed number per plant, and percent seed germination were subjected to ANOVA and LSD tests using the agricolae and LSD procedures in R, respectively. Experimental year and replication were considered fixed effects to determine whether velvetleaf stem, leaf, aboveground and root biomasses, total leaf area, seed number, and percent seed germination were significant by year or replication, and whether there was a year by replication interaction. Velvetleaf stem, aboveground, and root biomass were significant by year, so ANOVA and LSD tests were performed for these parameters by year, while leaf biomass, total leaf area, seed number, and percent seed germination were grouped together by year. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$.

Results and Discussion

Leaf Number. Velvetleaf responded to increasing water stress by senescing the oldest leaves, resulting in a reduced number of leaves with increasing water stress level (Table 4-1). Similarly, Schmidt et al. (2011) reported senescence of older leaves in velvetleaf under drought conditions. Velvetleaf maintained at 100% FC had a maximum of 28 leaves plant⁻¹, followed by 21 and 15 leaves plant⁻¹ at 75% and 50% FC, respectively (Table 4-1, Figure 4-2A). Chadha et al. (2019) reported similar results in prickly lettuce (*Lactuca serriola* L.), where leaf numbers were higher in 100% (52 leaves plant⁻¹) and

75% FC (49 leaves plant⁻¹) compared to 50% FC (41 leaves plant⁻¹). In contrast, Mahajan et al. (2018) reported a similar leaf number plant⁻¹ in two African turnip weed (*Sisymbrium thellungii* O.E. Schulz) biotypes at 100%, 75%, and 50% FC. Kaur et al. (2016) also reported a similar number of leaves plant⁻¹ in giant ragweed (*Ambrosia trifida* L.) at 100%, 75%, and 50% FC. A significant reduction in leaf number in *Sisymbrium thellungii* (Mahajan et al. 2018) and *Ambrosia trifida* L. (Kaur et al. 2016) at 25% FC were reported, similar to the results of this study, where velvetleaf plants maintained at 25% FC had a maximum of 7 leaves plant⁻¹ before plant death (Figure 4-2A). The log-logistic model estimated that velvetleaf grown at 100%, 75%, and 50% FC took a similar amount of time [3.3 to 4.3 weeks after transplanting (WAT)] to achieve 50% of maximum leaf number. Similarly, there was no difference in the time it took for *Ambrosia trifida* L. to achieve 50% of maximum leaf number at 100%, 75%, and 50% FC (6 to 9 WAT) (Kaur et al. 2016).

Plant Height. Velvetleaf maintained at 100% and 75% FC achieved a height of 108 cm and 123 cm compared with a height of 83 cm at 50% FC (Table 4-1, Figure 4-2B). These results suggest that available soil moisture at $\geq 75\%$ FC is sufficient to achieve maximum velvetleaf height and that a visible decrease in plant height at 50% FC could be a result of reduced cell enlargement due to low turgor pressure at 50% FC water stress level (Farooq et al. 2009; Jaleel et al. 2009). Similar results were reported by Chadha et al. (2019), in which *Lactuca serriola* had the greatest plant height at 75% FC (115 cm) and 100% FC (104 cm) compared with 77 cm at 50% FC. Kaur et al. (2016) also reported that *Ambrosia trifida* L. had the greatest plant height at 75% FC (140 cm) and 100% FC (125 cm) compared with 112 cm at 50% FC. In contrast, Mahajan et al. (2018) reported the

greatest plant height at 50% FC (65 cm) compared to 75% FC (53 cm) and 100% FC (56 cm) of the St. George biotype of *Sisymbrium thellungii*. Karimi et al. (2015) reported the greatest plant height in sweetleaf (*Stevia rebaudiana* Bertoni) at 90% FC, and decreased height with increasing water stress up to 45% FC. The model estimated that velvetleaf grown at 100%, 75%, and 50% FC took a similar amount of time (5.7 to 6.2 WAT) to achieve 50% of maximum plant height. Similarly, Kaur et al. (2016) reported that *Ambrosia trifida* L. grown at 100%, 75%, and 50% FC took 6 WAT to reach 50% of maximum plant height.

Plant Growth Index. Velvetleaf maintained at 75% FC had the greatest growth index (79,907 cm³) compared with a growth index of 72,197 cm³ at 100% FC and 64,256 cm³ at 50% FC (Table 4-1, Figure 4-2C). Results suggest that available soil moisture at 75% FC is sufficient for maximum growth of velvetleaf, and that available soil moisture at 100% FC might actually hinder plant growth due to root saturation (Ashraf 2012). Similarly, Kaur et al. (2016) reported that *Ambrosia trifida* L. maintained at 75% FC had the greatest growth index (588 cm³), followed by 416 cm³ at 100% FC and 274 cm³ at 50% FC. The time to achieve 50% of maximum growth index was similar across water stress levels (4.6 to 5.7 WAT) (Table 4-1). In contrast, Kaur et al. (2016) reported that the time for *Ambrosia trifida* L. to achieve 50% of maximum growth index was longer at 75% FC (6 WAT) compared to 100% and 50% FC (4 WAT).

Velvetleaf maintained at 25% FC did not survive more than 77 DAT during both years, although one plant produced a small number of seeds; therefore, root, leaf, and stem biomass, total leaf area, number of seeds per plant, and percent seed germination are presented (Table 4-2). The permanent wilting point of soil used in this study was 17.7%

by volume, corresponding to 45.2% FC. The soil water available to velvetleaf plants at 25% FC was below the permanent wilting point that resulted in plant death.

Plant Stem and Root Biomass. Year by treatment interactions were significant for stem and root biomass; therefore, data were separated (Table 4-2). Velvetleaf plants maintained at 75% FC were the tallest; however, they resulted in similar root, stem, or leaf biomass as 100% FC during both years. In 2019, stem biomass was similar (10–14 g plant⁻¹) at 100%, 75%, and 50% FC and root biomass was reduced at 100% FC (1.5 g plant⁻¹) and 25% FC (0.08 g plant⁻¹) compared with 2.4 to 3.4 g plant⁻¹ at 75% and 50% FC. Similarly, no differences were reported in *Ambrosia trifida* L. (Kaur et al. 2016) and *Sisymbrium thellungii* (Mahajan et al. 2018) stem biomass at 100%, 75%, and 50% FC. Other studies reported that velvetleaf (Vaughn et al. 2016) and *Stevia rebaudiana* (Karimi et al. 2015) aboveground biomass increased as water supply increased and was generally greatest at full transpiration and 90% FC, respectively. Studies also reported that velvetleaf (Vaughn et al. 2016) and *Ambrosia trifida* L. (Kaur et al. 2016) root biomass were greatest at full transpiration and 100% FC, respectively, but that was not the case in this study, as root biomass was reduced at 100% FC in 2019. Root biomass at 100% FC was likely reduced due to waterlogging of the soil, inhibiting root system elongation and potentially leading to adventitious root formation (Ashraf 2012; Steffens and Rasmussen 2016). In 2020, stem biomass (0.05 to 0.3 g plant⁻¹) and root biomass (0.01 to 0.07 g plant⁻¹) were reduced at 50% and 25% FC compared to stem biomass (1.2 to 1.3 g plant⁻¹), and root biomass (0.16 to 0.17 g plant⁻¹) at 100% and 75% FC. Chadha et al. (2019) reported similar results in which aboveground biomass of *Lactuca serriola* was greatest at 100% and 75% FC (19.4 to 22.4 g plant⁻¹) compared to 50% and 25% FC

(17.2 to 17.5 g plant⁻¹). On the contrary, Karkanis et al. (2011) reported no effect on root biomass due to water stress.

Total Leaf Area. The total leaf area per plant increased with increasing water stress, with the highest value at 50% FC (86.7 cm² plant⁻¹) and the value reduced to 5.5 cm² plant⁻¹ at 25% FC (Table 4-2). However, total leaf area values were statistically similar (56 to 86.7 cm² plant⁻¹) across 100%, 75%, and 50% FC. In contrast, Chadha et al. (2019) reported the greatest leaf area of *Lactuca serriola* at 75% FC, followed by 50%, 100%, and 25% FC. Vaughn et al. (2016) and Manivannan et al. (2007) reported reduced total leaf area with decreased water availability in velvetleaf and *Helianthus annuus*.

Seed Production. Water stress influenced the number of velvetleaf seeds produced per plant. Velvetleaf at 75% FC produced the highest number of seeds (453 seeds plant⁻¹), followed by 100% (406 seeds plant⁻¹), 50% (288 seeds plant⁻¹), and 25% FC (2 seeds plant⁻¹) (Table 4-2). Results suggest that although velvetleaf plant growth may be reduced by 50% FC, a considerable number of seeds are still produced. Thus, early-season control of velvetleaf is crucial for avoiding a large infestation later in the growing season. Similarly, Kaur et al. (2016) reported that seed production of *Ambrosia trifida* L. was influenced by degree of water stress, with the highest number of seeds produced at 75% FC, followed by 100%, 50%, and 25% FC. In contrast, Chadha et al. (2019), Chahal et al. (2018), Mahajan et al. (2018), and Sarangi et al. (2015) reported decreased seed production with increased water stress in *Lactuca serriola*, *Sisymbrium thellungii*, waterhemp (*Amaranthus tuberculatus*), and Palmer amaranth (*Amaranthus palmeri* S. Watson), respectively, indicating their sensitivity to water stress compared with velvetleaf.

Seed Germination. Velvetleaf seed germination was similar (96% to 100%) at 100%, 75%, and 50% FC compared with 20% germination at 25% FC. Similarly, Chahal et al. (2018) reported no difference in *Amaranthus palmeri* seed germination at 100%, 75%, and 50% FC, and no seeds were produced at 25% FC, signifying that *Amaranthus palmeri* seed production is more sensitive to water stress than velvetleaf. In contrast, Chadha et al. (2019) reported no difference in the germination ability of *Lactuca serriola* seeds produced under water stress conditions, demonstrating a higher tolerance to water stress compared to velvetleaf. These findings imply that velvetleaf can survive and produce viable offspring at water stress levels as low as 50% FC, prompting the need for early-season control.

Practical Implications

This is the first study that evaluates the response of velvetleaf to the degree of water stress using soil moisture sensors that more frequently and accurately maintain a precise level of water stress throughout the growth period. Plant height and leaf number per plant were sensitive to water stress than total leaf area, stem, leaf, and root biomass, seed production, and seed germination. Seeds of velvetleaf used in this study were collected from a field under continuous corn-soybean rotation in Clay County, NE. The growth characteristics of velvetleaf observed in this study could vary if velvetleaf biotypes were collected from different cropping systems or rotations. Waselkov et al. (2020) found that agriculturally prevalent *Amaranthus tuberculatus* from the Mississippi Valley and Plains regions had higher relative performance than waterhemp from the Northeast region, where waterhemp is less of an agricultural weed. The results of this study could also vary under field conditions because velvetleaf plants were not able to

grow to their full potential due to limited pot size and pest infestation under greenhouse conditions. In 2020, stem and root biomass were significantly lower compared to 2019, likely due to white fly (*Aleyrodidae*) infestation. In addition, a single velvetleaf plant was grown in each pot without inter- or intra-specific competition; thus, plants growing with crops might produce flowers earlier or later in the growing season depending on the competitive nature of the crops, resulting in higher or lower seed formation. Water stress treatments were imposed throughout the growth period in this study, while duration of water stress can also play an important role in determining velvetleaf's growth response. Therefore, it is expected that velvetleaf grown under field conditions will have a better chance of survival and higher seed production due to possibly limited periods of water stress because of rain/irrigation compared with the continuous water stress conditions imposed in this study.

Water stress may also influence the duration of the critical weed-free period for various crops. Light to moderate water stress (75% to 50% FC) would not likely impact the critical weed-free period of velvetleaf in crops, although high water stress (25% FC) might reduce the critical weed-free period of velvetleaf compared with saturated conditions (Coble et al. 1981; Jackson et al. 1985). Velvetleaf is a temperate climate species and is typically absent from environments where dry climate and high evapotranspiration rates restrict growth. Munger et al. (1987a) and Vaughn et al. (2016) have shown that crops such as soybean and corn, respectively, have higher transpiration efficiency (TE) compared with velvetleaf under short-term water stress conditions. The higher TE of corn and soybean is likely due to earlier leaf senescence in velvetleaf during short-term water stress; however, the velvetleaf response to long-term water stress may

be advantageous, where early conservation of available soil moisture and early leaf senescence could result in maintaining enough water for transpiration later and potentially producing seeds (Schmidt et al. 2011). Hagood et al. (1980) reported a greater reduction in soybean growth due to velvetleaf competition during a dry year compared to a wet year, indicating potential competition for moisture between two species. The growth characteristics of velvetleaf at $\leq 75\%$ FC in this study could indicate its competitive ability under long-term water stress conditions. For these reasons, this information could be useful for evaluating weed-crop interaction using competition models, as well as for developing climate simulation models to understand the effect of drought, rising atmospheric CO₂ concentrations, rising global temperatures, reductions in annual soil and groundwater recharge, and increasing frequency of extreme weather events on crop and weed species.

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Table 4-1. Parameter estimates and test of lack of fit at 95% level for the three-parameter log-logistic function^a fit to velvetleaf leaves per plant, plant height, and growth index under differing degrees of water stress at 84 d after treatment (DAT) in a greenhouse study at University of Nebraska-Lincoln.

Water stress treatment ^{b,c}	$d \pm SE^{b,c,d}$	e (weeks) ^{c,d}	$b^{e,d}$	Lack of fit ^e
Leaves plant ⁻¹				
100% FC (no water stress)	28 ± 7 a	4.3 ± 1.3 a	- 2.4 ± 1.6 a	1.0
75% FC (light water stress)	21 ± 1.4 b	3.4 ± 0.47 a	- 4.9 ± 2.5 a	0.3
50% FC (moderate water stress)	15 ± 1.8 c	3.3 ± 0.6 a	- 2.5 ± 1.2 a	0.4
Plant height (cm)				
100% FC (no water stress)	108 ± 10.7 a	5.7 ± 0.4 a	- 4.7 ± 1.7 a	0.9
75% FC (light water stress)	123 ± 12.3 a	6.0 ± 0.4 a	- 4.8 ± 1.6 a	0.9
50% FC (moderate water stress)	83 ± 11.6 b	6.2 ± 0.6 a	- 4.8 ± 2.1 a	0.9
Growth index (cm ³) ^f				
100% FC (no water stress)	72,197 ± 8,310 b	5.1 ± 0.4 ab	- 6.6 ± 3.1 a	0.9
75% FC (light water stress)	79,907 ± 7,072 a	4.6 ± 0.3 a	- 10.1 ± 5.6 a	0.9
50% FC (moderate water stress)	64,256 ± 8,398 c	5.7 ± 0.4 ab	- 8.3 ± 4.5 a	1.0

^a $Y = \{d / [1 + \exp\{b(\log x - \log e)\}]\}$, where Y is the leaves per plant, plant height, or growth index; x is days after transplanting; d is the estimated maximum leaves per plant, plant height, or growth index; e is the time taken to achieve 50% of leaves per plant, plant height, or growth index; and b is the relative slope around parameter e .

^bAbbreviation: FC, field capacity; SE, standard error.

^cOnly one velvetleaf plant maintained at 25% soil FC survived more than 77 DAT, and the three-parameter log-logistic model did not provide a good fit for leaves per plant, plant height, or growth index; therefore, data are not presented.

^dMeans within columns with no common letter(s) are significantly different at $P \leq 0.05$.

^eA test of lack of fit at the 95% level was not significant for any of the curves tested for the water stress treatments, indicating that the fitted model was correct.

^fGrowth index = $\pi * (w/2)^2 * h$, where w is the width of the plant calculated as an average of two widths; and h is the plant height measured from the soil surface to the apical meristem.

Table 4-2. Effect of degree of water stress on velvetleaf biomass, seed production, and seed germination at 84 d after treatment (DAT) in a greenhouse study at University of Nebraska-Lincoln using soil moisture sensors.

Water stress treatment ^a	Stem biomass ^b		Leaf biomass ^b		Aboveground biomass ^b		Root biomass ^b		Total leaf area ^b		Seed number ^b		Seed germination ^b
	2019	2020	2019-20	2020	2019	2020	2019	2020	2019-2020	2020	2019-2020	2020	2020
	g plant ⁻¹												%
100% FC (no water stress)	10.1 a	1.3 a	0.51 a	2.2 a	10.3 a	2.2 a	1.5 bc	0.16 a	56 a	406 ab	98.8 a		
75% FC (light water stress)	14 a	1.2 a	0.5 a	1.8 a	14.4 a	1.8 a	3.4 a	0.17 a	74.1 a	453 a	100 a		
50% FC (moderate water stress)	10.2 a	0.3 b	0.44 ab	0.4 b	10.9 a	0.4 b	2.4 ab	0.07 b	86.7 a	288 b	96.7 a		
25% FC (high water stress)	0.2 b	0.05 b	0.04 b	0.05 b	0.3 b	0.05 b	0.08 c	0.01 b	5.5 b	1.5 c	20 b		

^aAbbreviation: FC, field capacity.

^bMeans within columns with no common letter(s) are significantly different at $P \leq 0.05$.

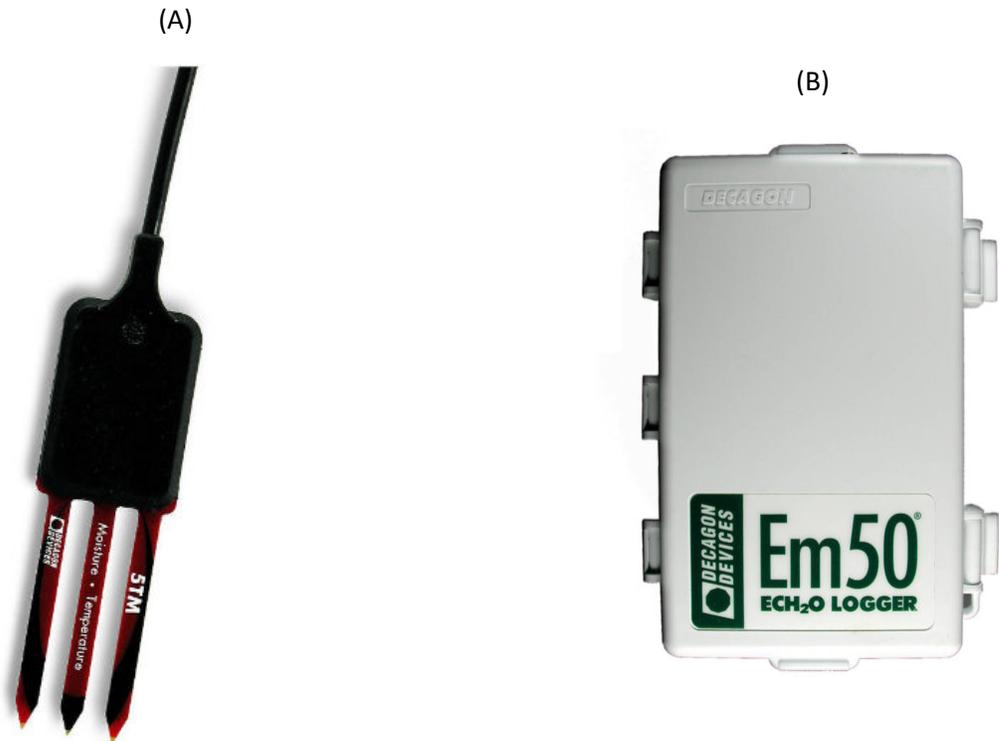


Figure 4-1. Soil moisture content in pots was measured using (A) Decagon 5TM moisture sensors and (B) Em50 data loggers to determine degree of water stress on velvetleaf in a greenhouse study conducted at the University of Nebraska–Lincoln.

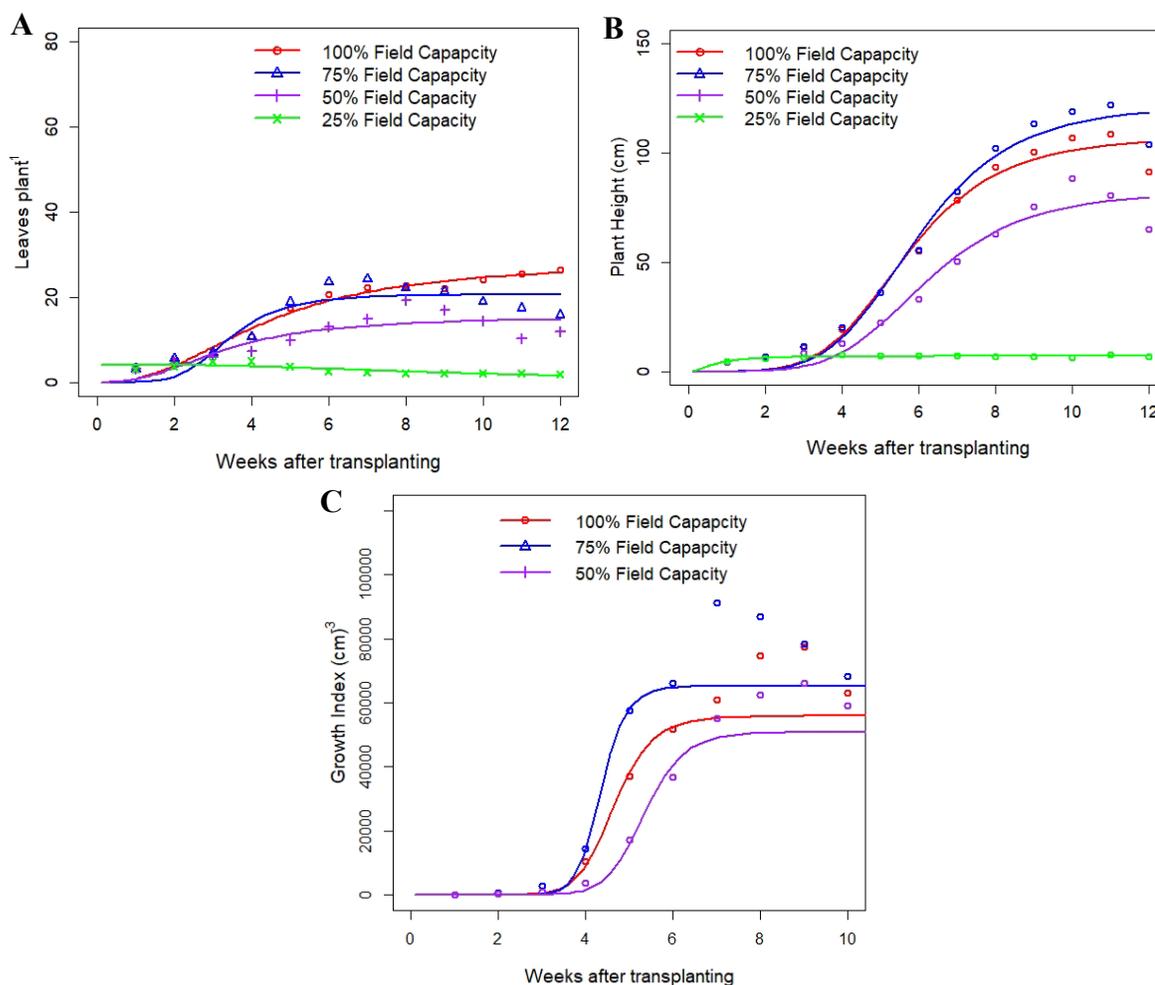


Figure 4-2. Effect of degree of water stress on (A) leaves per plant, (B) plant height, and (C) growth index of velvetleaf after 84 DAT during both years. 100%, 75%, 50%, and 25% field capacity (FC) treatments correspond to no-, light-, moderate-, and high-water stress, respectively. Only one velvetleaf plant maintained at 25% FC survived more than 77 DAT during both years, and the three-parameter log-logistic model did not provide a good fit for leaves per plant, plant height, or growth index; therefore, curves are presented for 25% FC, although only for visual sake.