MANAGEMENT CONSIDERATIONS FOR LATE-SEASON ESCAPES OF PALMER AMARANTH AND COMMON WATERHEMP IN TEXAS COTTON

A Thesis

by

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ABSTRACT

Herbicide resistance in weeds is an emerging problem across Texas, and a strong
emphasis on weed seedbank management is vital for addressing this problem. Escaped weeds
present in the late-season at crop harvest (weeds that escape control measures early-season
and/or the ones that recruit after control interventions have been terminated) can tremendously
contribute to seedbank addition, but little is known on the level of seed input from such escapes,
particularly that of Palmer amaranth and common waterhemp - two of the most problematic and
herbicide resistance prone weed species in cotton production in Texas. Moreover, effective
methods to reduce viable seed production from late-season escapes also need to be developed. A
state-level survey was conducted to quantify seed production in late-season escapes of Palmer
amaranth (*Amaranthus palmeri* S. Wats) and common waterhemp (*A. tuberculatus* (Moq.) Sauer)
across important cotton production regions in Texas (objective 1). The survey revealed that
Palmer amaranth densities and seed production were greater in the High Plains region (6.4 to
13.9 million seed ha^{-1}), whereas waterhemp was predominant in the Blacklands and the Upper
Gulf Coast regions, with 12.9 and 9.8 million seeds ha^{-1}, respectively. In addition, the three most
common weeds documented in Texas cotton were Palmer amaranth, Texas millet [*Urochloa
texana* (Buckl.)] and common waterhemp. Further, experiments were carried out in College
Station and Lubbock to understand the effect of different cotton defoliants on the viable seed
production potential of Palmer amaranth, when applied at different maturity stages (green
inflorescence, white seed, brown seed and black seed) (objective 2). Results indicated that
paraquat, MSMA, diuron and glufosinate provided the greatest seed mortality compared to a
number of other defoliants evaluated. Findings suggest that certain defoliants may be used to
provide the dual benefit of crop harvest aid as well as reducing the seed viability of late-season

weed escapes. Future experiments in a controlled environment could provide more insights on

the impact of these desiccants on seed viability of Palmer amaranth.

DEDICATION

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This work was supervised by a thesis committee consisting of Dr. Muthukumar Bagavathiannan (Chair), Dr. Peter Dotray (Department of Soil and Crop Sciences), and Dr. Greg Sword (Department of Entomology).

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

I.1. Introduction

Farmers typically manage weeds by applying herbicides before and during the growing

seasons. Despite these efforts, a significant population of weeds survive traditional management

practices and present during the late-growing season as escapes. Additionally, certain weed

species can germinate after crop-harvest (post-harvest recruits) and thus escape crop competition

and management interventions. The late-season weed escapes include the weeds that escape

management within the crop as well as the ones that recruit after crop harvest (Bagavathiannan

and Norsworthy 2012). Weeds that survive/escape management during the growing season and

present at crop harvest as late-season escapes can result from either the weeds that survive

management practices (i.e. early-season survivors) or the ones that emerge after all management

practices have been terminated (i.e. late-emerging weeds). The potential for weed seedbank

replenishment can result from one of four pools on the basis of dispersal status and location: 1)

undispersed, remaining on the mother plant, 2) dispersed in the current year and collected by

harvest machinery, 3) dispersed in the current year, lying on the soil surface, and 4) dispersed in

a previous year and persisting within the soil seed bank (Davis 2008). Late-season escapes refer

to the first of these pools – the undispersed seeds remaining on the mother plants.

Late-season escapes are often a result of inadequate herbicide rate, poor spray coverage,

absence of an adjuvant, application at inappropriate weed sizes, herbicide interactions, and/or

any unfavorable environmental conditions leading to reduced weed control (Hartzler 2001;

Jordan et al. 1997). Another notable reason for these escapes is that some emergence patterns of

weed species allow for high population densities after crop and weed emergence herbicide

applications (POST) or emerge after applications (Johnson et al. 2004b). Late-season weed

escapes are often ignored because they rarely cause crop yield penalties (Bagavathiannan and Norsworthy 2012). Growers typically make weed control decisions based on an economical threshold (ET), which evaluates the short-term financial benefits of controlling certain weed densities, rather than long-term economic benefits of reducing weed seedbank inputs and future weed infestations. The ET approach warrants weed management only if the estimated yield reductions are greater than the cost of control. Thus, weed management actions based on the ET model rarely encourage the control of late-season weed escapes (Bauer and Mortensen 1993). While these late-season escapes may not cause yield reductions in the current year, seed production from these escapes can replenish soil seedbank and lead to future weed management expenses.

An important concern with allowing seed production in the late-season escapes is that these escapes may harbor individuals with rare resistance alleles and favor the evolution of herbicide resistance (Norsworthy et al. 2012). It is unknown what direct correlation, if any, exists between the level of late-season escapes and herbicide resistance (Bagavathiannan and Norsworthy 2012). However, simulation models have shown that the risk of herbicide resistance increases proportionally with the seedbank size (Bagavathiannan et al. 2013; Neve et al. 2011) and thus high levels of seedbank replenishment caused by late-season escapes may in turn increase the risk of resistance evolution.

Texas leads the US as the top cotton producing state (USDA 2017) and late-season weed control measures have been limited in Texas cotton production due to a lack of short-term economic incentives. As a result, seedbank addition from late-season escapes can be substantial across different cotton production regions. Important cotton production regions in Texas include the Texas High Plains, Lower Gulf Coast, Upper Gulf Coast, Central and the Blacklands region.

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The High Plains is the largest cotton producing area, with three to four million acres of cotton planted in May and harvested from October to December (TAMU 2017). The area of cotton in the Blacklands ranges from 90,000 to 150,000 acres and is planted from late March through April and harvested during late August/September. The Lower Gulf Coast region produces 450,000 to 500,000 acres of cotton with planting in late February through March and harvested in late July/early August. The Upper Gulf coast region is similar to the Lower Gulf Coast region in planting and harvest timing, with about 250,000 to 300,000 acres. The Central Texas region west of San Antonio (also known as the Winter Garden) produces 6,000 to 10,000 acres of cotton with planting in March and harvest in August.

In Texas cotton production, Palmer amaranth (*Amaranthus palmeri* S. Wats.) and waterhemp (*A. tuberculatus* (moq.) Sauer) are two priority weeds that influence the selection of weed management programs. Both of these troublesome species have season-long emergence, rapid growth, and prolific seed production (Ehleringer 1983; Horak and Loughin 2000; Keeley et al. 1987). Amaranthus species are considered critical weed species in other cropping systems such as sorghum [*Sorghum bicolor* (L.) Moench)*, soybean [*Glycine max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) as well (Ferrell et al. 2015; Ciampitti et al. 2017). The options available for managing late-season escapes are limited especially when the weeds have already transitioned to reproductive development stage. The efficacy of herbicides typically declines as the weed size increases (McAfee and Baumann 2017).

For late-season weed escapes, the management goal is to minimize viable seed production. It is unclear what herbicide options can be effective in reducing weed seed viability. In cotton, desiccants/defoliants are used to facilitate harvest operations; many of these chemicals have herbicidal activity, but their ability to affect weed seed viability is unknown. Given the

importance of Palmer amaranth and waterhemp as troublesome weeds in Texas row crops, this research specifically focuses on these two weed species. The overall goal of this project was to understand seedbank addition potential from Palmer amaranth and waterhemp prior to cotton harvest, as well as understand the effect of various harvest aid applications on seed viability of Palmer amaranth.

I.2. Review of Literature

I.2.1. Weedy Amaranthus species

I.2.1.1. Background

In the US there are over 70 native and non-native species in the genus *Amaranthus*. Within this genus, only a few species are problematic in the U.S. crop production systems. The most common Amaranthus species in the Midwest and Southern U.S. are Palmer amaranth, common waterhemp, spiny amaranth (*A. spinosus* L.) and tumble pigweed (*A. albus)*. Redroot pigweed (*Amaranthus retroflexus* L.) and Powell amaranth (*A. powellii* S. Wats.) are found all over the U.S., whereas smooth pigweed (*A. hybridus* L.) is typically found in the eastern half of the U.S. (Bensch et al. 2003; Hager et al. 2002; Massinga et al. 2001; Schweizer and Lauridson 1985).

The plants belonging to the genus Amaranthus are characterized by the C_4 photosynthetic pathway that exhibit high growth potential. Palmer amaranth and common waterhemp set themselves apart as dioecious species (male and female structures on separate plants), while the rest of the *Amaranthus* species occurring in the U.S. are monoecious (male and female structures on the same plant). Special attention is paid on Palmer amaranth and common waterhemp which, in 2016, were ranked among the top 5 of the most troublesome weeds in various cropping systems across the U.S. (WSSA 2017). Another challenge presented by these weeds is that some

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of them can hybridize and transfer resistance alleles across different species. There was one such

confirmation of hybridization in Mississippi where a Palmer amaranth biotype transferred

glyphosate resistance alleles to spiny amaranth, ultimately exhibiting the resistance trait (Bennett

2017).

I.2.1.2. Palmer amaranth

I.2.1.2.1. Origin and distribution

Palmer amaranth is a Sonoran Desert annual that derives from the Southwestern U.S. and

New Mexico, and is the most successful Amaranthus species to adapt to a range of environments

(Eleringer 1983, Sauer 1957). Abundant seed production, extended emergence patterns, and

rapid growth rate are characteristics that escalate the ability of Palmer amaranth to become a

problematic weed in row crop production throughout the southern U.S. and beyond (Powell

2014). In 2010, glyphosate and ALS-inhibitor resistant Palmer amaranth was confirmed in

Michigan (Sprague 2011), only eight years after its first confirmation in Georgia in 2002

(Culpepper et al. 2006). In Texas, Palmer amaranth shows an ability to adapt to a wide range of

environmental conditions, including the High Plains, Central Texas, Lower Gulf Coast, among

others. Currently, Palmer amaranth is found in at least 30 states in the U.S. (USDA 2017) and

has been confirmed resistant to various herbicides that collectively inhibit at least 6 herbicide

sites of action: acetolactate synthase (ALS), dinitroanilines (microtubules), EPSPS, 4-

hydroxyphenylpyruvate dioxygenase (HPPD), PPO, and triazine herbicides (photosystem-II, Site

A) (Chahal et al. 2015, Heap 2018).

I.2.1.2.2. Emergence

Palmer amaranth seedling emergence occurs within 5 d of planting, while other

Amaranthus spp. may take up to 17 d (Sellers et al. 2003). Typically, under non-crop situations,

Palmer amaranth emergence has been reported to occur from March through October in California and from mid- May through September in Michigan (Keeley et al. 1987; Powell 2014). In Pendleton, SC Palmer amaranth emergence can begin in early May and continue until mid-July (Jha and Norsworthy 2009). In College Station and Lubbock, TX Palmer amaranth seedling emergence can occur as early as February and March, respectively, and continue until the first killing frost in October or November (unpublished data). The ability of Palmer amaranth to emerge for such an extended period allows it to escape multiple management interventions, resulting in substantial late-season escapes and seedbank addition.

I.2.1.2.3. Growth and reproduction

Palmer amaranth has a rapid growth rate of 0.21 cm per growing degree unit that allows it to accumulate more biomass than other *Amaranthus* spp., including redroot pigweed (0.12 cm), common waterhemp (0.16 cm), and prostrate pigweed (0.09 cm) (Horak and Loughin 2000). The growth rate of common row crops such as cotton are generally slower (National Cotton Council 2018) compared to that of Palmer amaranth. Just two wks after planting, Palmer amaranth can produce 65% more biomass than that of redroot pigweed, common waterhemp, spiny amaranth, tumble pigweed, or smooth pigweed (Sellers et al. 2003). Research by the authors showed that Palmer amaranth can grow 45 and 600% taller than common waterhemp and redroot pigweed, respectively.

Palmer amaranth produced 250,000, 446,000 and 613,000 seeds plant^{-1} in Missouri, Georgia and California, respectively, when there was no inter- or intra-specific plant competition (Keeley et al. 1987; Sellers et al. 2003; Webster and Grey 2015). These seeds typically replenish the weed seedbank and increase weed management challenges to the grower in subsequent years.

I.2.1.3. Common waterhemp

I.2.1.3.1. Origin and distribution

Common waterhemp is indigenous to the Midwestern U.S. and is also considered a troublesome weed because of its rapid growth, high seed production, and an ability to outcross with other Amaranthus species such as Palmer amaranth and smooth pigweed (Franssen et al. 2001; Trucco et al. 2005; Wetzel et al. 1999). Waterhemp is widespread in the U.S. Midwest, being indigenous to the state of Illinois (Worthle et al. 2014). Reports showed that common waterhemp was the most problematic weed in the states of Missouri and Illinois for corn farmers at the turn of the century (Hager et al. 2002). Waterhemp populations with resistance to glyphosate have been confirmed in Nebraska, Iowa, Kansas, Missouri, Illinois, and Kentucky (Chatham et al. 2015). Though waterhemp is most common in the Midwest, it is a significant weed issue in areas outside of this region from Texas to Maine (Nordby et al. 2017). In Texas, waterhemp is predominantly found in the Southeast, specifically in the Upper Gulf coast and Blacklands regions. The high genetic diversity of common waterhemp enables it to adapt to repetitive applications of herbicides with the same site of action (Hager et al. 1997; Nordby et al. 2007). Due to the high degree of genetic similarity and frequent hybridization between the two waterhemp species (common, tall), multiple botanists have grouped them into one "waterhemp" species, *Amaranthus tuberculatus* (Pratt and Clark 2001).

I.2.1.3.2. Emergence

Common waterhemp exhibits prolonged emergence periodicity similar to that of Palmer amaranth. The emergence pattern of common waterhemp is one of its most problematic traits since it tends to emerge later in the growing season (Hager et al. 1997). Common waterhemp occupies a formidable position as one of the last summer annual weed species to emerge, and then continues to emerge well into the summer (Heneghan 2016). In a study conducted by

Heneghan (2016), the mean duration of emergence for common waterhemp was 53 d longer than any other species present in the study site. Leon and Owen (2006) recorded common waterhemp emergence over the course of 56 to 70 d in no-till areas. Temperature, light, moisture and oxygen are regarded as important environmental factors that influence germination, with temperature often acting as the primary factor in a temperate region (Baskin and Baskin 1988). Initial spring emergence is credited to rising soil temperatures, but common waterhemp emergence was found to be significant in mid-July following substantial rainfall events. Egley and Williams (1990) suggested that rainfall and the resulting high soil moisture can alter the timing of weed seedling emergence.

I.2.1.3.3. Growth and reproduction

Common waterhemp has an aggressive growth rate that can be almost 2.5 cm day^{-1} during the growing season (Horak and Loughin 2000; Nordby et al. 2017). This rapid growth rate allows common waterhemp to be highly competitive with crops. Heneghan (2016) reported that corn and soybean yield was reduced by 50-70% when common waterhemp competed for an entire growing season in Indiana. Like many plants in the Amaranthaceae family, seed production from a single plant can range from thousands to millions. A single waterhemp plant produced an average of 288,950 seeds plant^{-1} in Missouri (Sellers et al. 2003), more than 1 million seeds plant^{-1} in Illinois (Steckel et al. 2003), and 4.8 million seed in Iowa (Heneghan 2016).

I.2.2. Weed management thresholds

The concept and utilization of ET originated in the 1950's by relating the population density of the pest compared to the anticipated crop yield loss caused in a single season. This threshold began as a tool in managing arthropods, then extended to nematodes and eventually

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other pests, including weeds, as integrated pest management became an established norm. Benefits of using the ET model are that it can reduce current-season costs and promote farmland biodiversity (Bagavathiannan and Norsworthy 2012; Franke et al. 2009). One major flaw of traditional weed management solely based on ET and the critical weed-free period (time where weeds can cause the largest yield losses) is that these concepts rarely warrant control of late-season escapes because they are unlikely to cause crop yield reduction for the current season (Baumann et al. 1993). Weed management decisions based on a single-season profitability can be detrimental since this approach ignores the contribution of weed seed production to seedbank replenishment which can increase future weed management costs. Long-term weed infestations and likelihood for the evolution of herbicide resistance are not considered by the ET model.

I.2.3. Soil seedbank replenishment

A major input of seedbank replenishment comes from weed escapes that persist at harvest, despite earlier weed management interventions. Late-season escapes are a major contributor to seedbank persistence. As harvesting occurs, the weed seed has either already shattered or is dispersed by the harvester. Mechanical disturbance redistributes weed seeds to the soil surface, increasing spread and replenishment of the soil seedbank (Walsh and Powles 2014; Bagavathiannan and Norsworthy 2012). An escaped population of 12 Palmer amaranth plants ha^{-1} can result in a seedbank addition of about 5 million seeds ha^{-1} (Culpepper and Sosnoskie 2011).

The consequences of weed seedbank can continue from previous years when management was less regimented. Residual seedbank populations can be sufficient to ensure persistence even after preventing seed production for several years (Schwiezer and Zimdahl 1984). Menges (1987) reported that maintenance of a whole-season weed-free condition for six

continuous yrs reduced Palmer amaranth seedbank population by 98%, but the prevailing 2% consisted of about 18 million seeds ha^{-1}.

I.2.4. Management of late-season escapes

In addition to typical pre-weed and crop emergence (PRE) and POST applications, herbicides are sometimes applied prior to harvesting to facilitate crop harvest. In cotton, desiccants/defoliants are typically applied as harvest aids to enable timely cotton harvest. However, many weed species are not effectively controlled by herbicide/desiccant applications at this later stage and the weeds often continue to develop and mature (Bennett and Shaw 2000a). Desiccants (chemicals which cause the green foliage to lose water, expediting the drying process) or defoliants (substances that cause leaves or foliage to drop from the plant) (Fishel 2018) may still reduce seed viability, but the impact on seed viability is unknown and needs to be investigated. Multiple desiccants or defoliants with herbicidal activity may be used to minimize leaf material at the time of cotton harvest. The specific mechanisms of desiccant/defoliant action on weed seed viability may range from herbicide translocation to reproductive tissues to indirect impact of plant stress and death.

Harvest weed seed control (HWSC) is an alternative weed control practice that helps reduce seedbank input from escaped weeds, that may include herbicide-resistant weeds. Here, harvest weed seed control tactics combine cultural and mechanical techniques and specifically focus on minimizing weed seedbank replenishment at harvest time. These practices, not yet widely used in the U.S., include chaff carts, narrow-windrow burning, Harrington Seed Destructor, bale-direct systems, and other means of targeting the chaff during harvest (Schwartz et al. 2016). These practices present an opportunity to collect and destroy any non-shed weed seeds prior to their return to the soil seedbank (Walsh 2001). It is important to note that there has

been no HWSC option currently developed for cotton production systems. Other options that may be used in crops such as cotton include crop topping or hand chopping of escapes. Crop-topping is the concept of applying herbicides to reduce seed viability or seedling fitness by use of late-season POST application of broad spectrum herbicides in crops (Steadman et al. 2006; Walsh 2001). In Australia, crop-topping is used primarily to control weeds in legume crops, but it could be utilized in a range of production systems (Douglas 2017).

I.2.5. Desiccants/defoliants

Application of desiccants or defoliants is common in cotton production to aid harvesting. This application allows for less debris or trash to obstruct harvest equipment and avoid contamination of lint. Desiccants cause the green foliage to lose water and accelerate the drying process, whereas the defoliants cause the foliage to drop from the plant (Fishel 2018). Herbicide mechanism of action indicates how a selected herbicide acts within the plant. Dormancy is triggered by an accumulation of abscisic acid (ABA) (Kermode 2005), and it is likely that herbicide stress might be influencing seed dormancy. In particular, I hypothesize that herbicides with systemic activity may have more time to influence seed dormancy compared to the ones that provide contact activity.

Synthetic auxins and auxin transport inhibitors (WSSA Group 4) are typically used for controlling broadleaf weeds in grass crops and pastures. Generally known as plant growth regulators, these synthetic auxins (e.g. 2,4-D, dicamba) are systemic compounds that affect cell wall plasticity and nucleic acid metabolism (Shaner 2014). By absorption through the roots and foliage, they can translocate to the meristematic tissue and interfere with cell division (University of California 2018). Synthetic auxins subsequently affect plant growth and stimulate ethylene

evolution, which often causes the plant to bend and twist (i.e. epinastic symptoms) (Shaner 2014).

Photosynthetic inhibitors, such as diuron (Group 7), control multiple broadleaf and select grass weeds. These herbicides inhibit photosynthesis by binding to the D1 protein of the photosystem II complex in chloroplast thylakoid membranes (Shaner 2014). Though all PSII Inhibitors bind to the D1 protein, the binding occurs at three different attachment sites; diuron attaches at the Site A (Shaner 2014). Ultimately, these herbicides block the electron transport and stop CO_2 fixation and production of ATP and $NADPH_2$ needed for plant growth. Indirect effect on other processes is what ultimately causes plant death, rather than solely photosynthate depletion. Halting electron transport in PSII promotes the formation of highly reactive molecules that catalyze a chain reaction. Results cause lipid and protein membrane destruction and membrane leakage, allowing cells and cell organelles to dry and rapidly disintegrate (University of California 2018).

Glyphosate (Group 9) inhibits the enzyme 5-enlopyruvate-shikimate-3-phosphate synthase (EPSPS) in the shikimic acid biosynthesis pathway (Shaner 2014). This broad spectrum, systemic herbicide creates a depletion of aromatic amino acids such as tryptophan, tyrosine, and phenylalanine that cannot be produced without the EPSPS enzyme (University of California 2018). Lack of these amino acids affect plant growth (Shaner 2014). While plant death does occur from an inhibition of the EPSPS enzyme, the actual sequence of phytotoxic processes are unclear (Shaner 2014).

Glufosinate (Group 10) is a broad-spectrum postemergence herbicide that controls most annual grasses and broadleaves. It is a contact herbicide with limited translocation throughout the plant (University of California 2018). Glufosinate inhibits glutamine synthetase, the enzyme that

converts glutamate and ammonia to glutamine and the accumulation of ammonia in the plant destroys cells (Shaner 2014).

Pyraflufen ethyl (Group 14) is a contact herbicide and inhibits the protoporphyrinogen oxidase (PPO) enzyme. PPO inhibitors have herbicidal activity mostly on broadleaf plants, but some PPO inhibitor herbicides can have activity on grasses. PPO is an enzyme in the chloroplast that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX) (Shaner 2014). Depletion of PPIX, a precursor molecule for both chlorophyll (needed for photosynthesis) and heme (needed for electron transfer chain), results in the formation of highly reactive molecules that attack and destroy lipids and protein membranes which desiccate and disintegrate rapidly.

Monosodium methanearsonate (MSMA) is a commonly used contact herbicide in warm-season climates on grasses. It is an organic arsenical placed in Group 17, where the mechanism of action remains unknown.

Paraquat (Group 22) is a photosystem I (PSI) electron diverter, and is a broadspectrum contact herbicide. These bipyridylium herbicides are light activated and accept electrons from PSI and, through reduction, form an herbicide radical (Shaner 2014). This radical subsequently reduces other molecules to form extremely reactive and dangerous molecules that destroy membrane lipids, chlorophyll, and cell membranes (University of California 2018).

I.3. Rationale and objectives

The specific objectives of this project were: 1) survey the density of escapes and seedbank addition potential by late-season Palmer amaranth and waterhemp escapes in cotton fields prior to crop harvest, and determine the problematic weed species infesting different cotton

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producing regions in Texas; and 2) evaluate the impact of various desiccants/defoliants on seed viability of Palmer amaranth when applied at various seed developmental stages. In order to quantify the potential of weed seedbank addition, a field survey was carried out across the following important cotton producing regions of Texas: High Plains, Blacklands, Central Texas, Lower Gulf Coast and Upper Gulf Coast. The High Plains region was divided into three sub-regions: upper High Plains (north of Amarillo), central High Plains (around Lubbock), and lower High Plains (south of Lubbock). The surveys have focused on the two most problematic weed species, Palmer amaranth and common waterhemp, within these cotton cropping regions.

Given that producers apply various harvest aids at the time of crop maturity to facilitate crop harvest, the impact of these chemicals on weed seed viability was evaluated to determine whether specific harvest aids can also contribute to Palmer amaranth management. Determining the impact of these harvest aids when applied at different seed developmental stages of Palmer amaranth is also important. These stages include pre-embryo development stage (green), white seed, brown seed, and black seed. This knowledge is expected to allow for a better understanding of the choice of desiccant and optimal growth stages of application to minimize viable seed production. The inflorescence of Palmer amaranth matures from the bottom towards top, allowing for up to 4 different seed maturity stages (black, brown, white, and green inflorescence) to be present at a given time. Depending on the level of maturity/age of the plant, one or more stages can represent the majority of the inflorescence (e.g. at later stages of development, a majority of the inflorescence is expected to have black seeds).

CHAPTER II

LATE-SEASON SURVEYS TO UNDERSTAND DOMINANT WEEDS AND SEEDBANK

ADDITION POTENTIAL OF PALMER AMARANTH AND COMMON WATERHEMP IN

TEXAS COTTON

II.1. Introduction

Widespread adoption of herbicide-resistant crops has allowed growers to apply broad-spectrum postemergence (POST) herbicides for effective weed management; however, weeds may survive management interventions (i.e. early-season survivors) or escape management practices by emerging later in the season (i.e. late-emerging weeds) and present at crop harvest as escapes. Weeds survive herbicide applications often as a result of inadequate herbicide rate, poor spray coverage, absence of an adjuvant, application at inappropriate weed sizes, herbicide interactions, and/or any unfavorable environmental conditions leading to reduced weed control (Hartzler 2001; Jordan et al. 1997). These survivors may include herbicide-resistant individuals. Further, the ability of certain weeds to exhibit prolonged emergence periodicity (i.e. extending the distribution of emergence times) allow some cohorts to escape management by emerging after all control measures have been terminated (Johnson et al. 2004b). For example, Jha and Norsworthy (2009) documented the extended emergence of Palmer amaranth (*Amaranthus palmeri* S. Wats.) from early May to as late as mid-July in Pendleton, SC. Likewise, in a study conducted by Heneghan (2016), the mean duration of emergence for common waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer) was 53 days longer than any other species present in the study site.

In cotton production in Texas, Palmer amaranth and common waterhemp are two important weed species (WSSA 2018; Webster 2005). These are prolific seed producers. It is

reported that an average of 12 Palmer amaranth escapes ha^{-1} can result in a seedbank addition of about 5 million seeds ha^{-1} (Culpepper and Sosnoskie 2011). Also, without strenuous competition for resources, Palmer amaranth has been recorded to produce 250,000 to 613,000 seeds plant^{-1} (Keeley et al. 1987; Sellers et al. 2003; Webster and Grey 2015). Under ideal growing conditions, a single Palmer amaranth plant can produce over 2 million seed (Smith et al. 2011). In Missouri, a single waterhemp plant produced an average of 288,950 seeds plant^{-1} (Sellers et al. 2003), and as great as 4.8 million seeds plant^{-1} was reported in Iowa (Heneghan 2016). Despite such significant levels of fecundity and seedbank addition potential, the late-season weed escapes are often ignored because they rarely cause yield penalty in the current crop (Bagavathiannan and Norsworthy 2012), although financial implications can still occur from harvesting difficulties and dockage due to weed contamination of commodities (Smith et al. 2000).

Farmers typically make weed control decisions based on the economic threshold (ET) approach (Wiles 2004), which evaluates financial costs versus yield when controlling a certain density of weeds, rather than a long-term ecological threshold that is based on reducing future weed infestations. The ET approach warrants weed management only if the estimated yield reductions are greater than the cost of control (e.g. Bosnic and Swanton 1997). Thus, weed management actions based on the ET model rarely encourage the control of late-season weed escapes (Bauer and Mortensen 1993). While these late-season escapes may not cause yield reductions in the current year, seed production from these escapes can lead to the need of future weed management inputs. Moreover, these escapes may harbor resistance-endowing alleles and thereby increase the risk of herbicide resistance evolution (Bagavathiannan and Norsworthy 2012).

The potential for weed seedbank replenishment can result from one of four pools on the basis of dispersal status and location: 1) undispersed, remaining on the mother plant, 2) dispersed in the current year and collected by harvest machinery, 3) dispersed in the current year, lying on the soil surface, and 4) dispersed in a previous year and persisting in the soil seedbank (Davis 2008). Management approaches for the late-season escapes focus on the first of these pools - the undispersed seeds that remain on the escaped plants at the time of harvest. Knowledge of the level of seedbank addition potential from such weed escapes can help justify required management efforts. Weed escapes often are documented through pre-harvest weed surveys. In Western Canada, late-season weed surveys periodically have been carried out with the goal of establishing the nature and extent of weed infestations (e.g. Leeson et al. 2005). Moreover, such weed surveys also provide valuable insights into problematic and emerging weed issues at regional scales (Schweizer and Lybecker 1998; Webster and MacDonald 2001). Research and extension agencies as well as the broader agricultural industry can utilize the results of weed surveys to develop suitable weed management plans.

Though Palmer amaranth and waterhemp are important weed species in Texas cotton and known to exhibit high fecundity, little is known on the level of late-season weed escapes and seedbank addition potential from these escapes. Moreover, knowledge also is limited on the most problematic weeds infesting different cotton producing regions of Texas. The objective of this study was to conduct a state-level field survey to estimate the density of and seed production potential by late-season Palmer amaranth and common waterhemp escapes prior to cotton harvest, and identify the most problematic weed species infesting cotton fields across Texas.

II.2. Materials and Methods

Field surveys were conducted during fall of 2016 and 2017 across the following major cotton producing regions of Texas: High Plains, Gulf Coast, Central Texas, and Blacklands (Figure 1). The High Plains (HP) region was divided into three sub-regions: Upper HP, Central HP, and Lower HP, and the Gulf Coast (GC) was divided into two zones: Upper GC and Lower GC.

A semi-stratified survey methodology was followed using an adapted protocol described by Bagavathiannan and Norsworthy (2016). In each region, 10 to 20 cotton fields randomly were identified for sample collection, and a total of 84 and 134 cotton fields were surveyed in 2016 and 2017, respectively, across the major cotton producing regions in Texas described above. Fields (appendix 1) were pre-selected randomly on Google® map using the ITN Converter software (version 1.88; Benichou Software) and further loaded in a GPS device (TomTom International, BV) to navigate to the survey sites. If a cotton field was not present in a pre-selected survey site, the first cotton field along the survey route leading to the next survey site was used for sampling.

At each survey site, average densities of naturally occurring Palmer amaranth and common waterhemp were documented by counting the number of plants in three representative 1.0 m^2 quadrats placed randomly between two cotton rows. Observations also were carried out on the approximate percent area (visual estimates) infested in each field by Palmer amaranth and/or common waterhemp escapes on an average hectare basis. The Palmer amaranth and/or common waterhemp plants present in the three 1.0 m^2 quadrats were clipped at the base and gently placed in paper bags, and the samples were dried at 50°C for 48 hrs. Seed heads were mechanically thrashed using a Test Mark Industries Soil Grinder (SA-1800®) and cleaned with a

Seedburo South Dakota Seed Blower®. The thrashed seeds were counted using a Data Technologies SJR® seed counter. Further, top-three weed species infesting each survey site was identified by walking in a zig-zag manner between the cotton rows; ranking was carried out based on visual density. The top-three weed species also were recorded in randomly selected cotton fields in between two survey sites. A total of 187 such sites were visited across the different survey regions (High Plains:70; Central:15: Gulf Coast:102).

II.3. Statistical Analysis

Seed production data for Palmer amaranth and common waterhemp were analyzed using the R software (R Core Team 2016). Box-plots were generated using the R program to illustrate the distribution of Palmer amaranth and waterhemp seed production potential across different regions. Data pertaining to the top-three escaped weeds in cotton in each region of Texas were used to determine the most frequently occurring weeds at a regional- as well as the state-level, following the procedure described by Sarangi and Jhala (2018). Three, two, and one frequency points were assigned to rank #1, #2, and #3 of the weed species in each field, respectively, and the relative frequency points were calculated for each weed species by using the equation 1:

$$RP = \sum_{r=1}^{3} \frac{FX}{n} \qquad [1]$$

where, F is the frequency of a particular rank (r) assigned to a certain weed species, X is the frequency points associated with that particular rank, and n is the total number of fields surveyed. The top-three escaped weeds were presented at the regional and state levels.

II.4. Results and Discussion

Regional distribution and rankings of the weed escapes

The five most frequently escaped weeds were identified in each region and presented in Figure 1. Palmer amaranth was the top occurring weed in the High Plains (HP Upper, Central,

and Lower) and Lower GC regions and also was listed in the top-five escaped weeds in

Blacklands and Central Texas regions (Fig. 1). During the field survey, Palmer amaranth

occurred in all seven cotton producing regions visited, though less frequently in the Upper GC

(5% of the surveyed fields in the region) (Fig. 2). A stakeholder survey conducted by the Weed

Science Society of America (WSSA) showed that Palmer amaranth was the most common weed

in cotton production systems in the U.S. (Van Wychen 2017). Moreover, Garetson et al. (2017)

reported that about 31% of the surveyed Palmer amaranth biotypes in the Texas High Plains

were resistant to glyphosate, which may have contributed to weed escapes. Reports showed that

Palmer amaranth escapes were commonly observed in cotton fields (Dotray et al. 1996;

Sosnoskie and Culpepper 2014). Merchant et al. (2014) noted that glyphosate-resistant Palmer

amaranth plants escaping PRE herbicides or early-season tillage are difficult to manage and such

situations can lead to substantial weed escapes late-season.

Common waterhemp, the second most common Amaranthus species in the state (Table 1)

was identified as the most frequent weed in the Upper GC and the third most frequent weed in

the Blacklands region (Fig. 1). Waterhemp appears to be adapted to the high rainfall, relatively

more moist areas of the Upper GC (approx. 125 cm annual average precipitation), and the

southern parts of the Blacklands (~100 cm). These observations confirm the survey results of

Garetson et al. (2017) who also reported the occurrence of common waterhemp predominantly in

the Upper GC and Blacklands regions of Texas. Moreover, widespread glyphosate resistance was

noted in these waterhemp populations (Garetson et al. 2017), supporting the frequency rankings

obtained in this study. However, waterhemp was not noticed throughout the High Plains and was

only found in very low frequencies in Central Texas (4% of the surveyed fields in the region)

and Lower GC (7%) regions (Fig. 2). It also was noted that the co-occurrence of both species in a

given production field was rare, with only about 7% occurrence in the Upper GC and 13% occurrence in the Blacklands region (Fig. 3). Our observations suggest that there are distinct ecological niches between Palmer amaranth and waterhemp in Texas.

Among the other weed species, ragweed parthenium (*Parthenium hysterophorus* L.) was the most frequent weed in the Central Texas region. It also was the second most common weed in the Lower GC region. Texas millet (*Urochloa texana* (Buckley) R.D. Webster) was the most commonly occurring weed in Blacklands, the second most frequent weed in the Central HP and Upper GC regions, and the third most common weed in the Lower UC region (Fig. 1). Further, bindweeds (*Convolvulus* spp.) were commonly observed throughout the HP, with the second, third, and fifth ranking for occurrence in the Upper, Central, and Lower HP regions, respectively. Specific species was not identified in each field due to possible species mixtures.

The frequency calculations for weed escapes in cotton across the entire state (Table 2) have revealed that Palmer amaranth, Texas millet, common waterhemp, ragweed parthenium and barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) were the top five most frequent weeds (Table 2). Among all weeds, the relative frequency points of Palmer amaranth was considerably greater (1.7 out of the maximum possible points of 3.0) than the other weed escapes recorded in this survey, which illustrates the widespread occurrence of this species in cotton production fields in Texas. It is possible that the prolonged emergence pattern of Palmer amaranth and common waterhemp has resulted in seedlings emerging after the POST herbicide applications, leading to substantial late-season escapes. A similar survey conducted among crop consultants in Arkansas in 2006 has ranked Palmer amaranth among the most problematic weeds in cotton (Norsworthy et al. 2007). It is the most problematic weed in soybean (*Glycine max* (L.) Merr) across the Midsouth in a recent survey conducted by Schwartz-Lazaro et al. (2018), and this

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trend is expected to be similar for cotton. Additionally, in our survey, sprangletops (*Leptochloa*

spp.), silverleaf nightshade (*Solanum elaeagnifolium* Cav), bindweed, Texas blueweed

(*Helianthus ciliaris* DC) and johnsongrass (*Sorghum halepense* (L) Pers.) also were listed among

the top-ten commonly found weed escapes in Texas cotton (Table 1).

Seed addition potential of Palmer amaranth in Texas cotton

  Results showed that the seed addition potential from escaped Palmer amaranth plants was

the greatest in the High Plains region (Upper: 13.9 million seeds ha^{-1}, Lower: 7.3 million ha^{-1}

and Central: 6.4 million ha^{-1}), and the lowest in the Central Texas region (2.4 million ha^{-1})

(Table 3; Fig. 3). The seed addition data pertains only to the fields where weed escapes were

observed. The escaped Palmer amaranth densities ranged from 405 to 3,543 plants ha^{-1}

depending on the survey region (Table 2). In some cases, weed density and total seed production

per hectare didn't follow the same trend, which could be attributed to size differences of Palmer

amaranth plants and resulting differences in fecundity. The area infested with Palmer amaranth

escapes in individual cotton fields was the greatest in the Lower Gulf Coast region (9.4% area

coverage), followed by the High Plains region (ranging from 5.1 to 8.1%) (Table 2).

  The lowest area (2.4%) of late-season weed infestation in a survey field, the lowest

densities of escapes (405 plants ha^{-1}), and the lowest seed production (2.4 million seeds ha^{-1})

were observed in Central Texas region. This could be either due to adoption of effective Palmer

amaranth management strategies by cotton growers in this region or potential ecological factors

that limit the persistence of Palmer amaranth; based on our observations, the earlier is most

likely. Our findings corroborate Norsworthy et al. (2016) who reported that glyphosate-only

POST treatment resulted in Palmer amaranth escapes of 25 to 43 plants m^{-2} with 101,000 to

407,000 seeds m^{-2}; however, PRE followed by POST herbicide programs drastically reduced

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Palmer amaranth densities (< 0.75 plants m^{-2}) and seed production ($< 2,800$ seeds m^{-2}). Thus, the density of escaped weeds and seed addition potential can be associated with the robustness of weed management programs practiced in a given field.

In the High Plains, cotton is often grown in monoculture, especially in the Lower and Central High Plains regions, with cotton-corn rotation being predominant in the Upper High Plains region. The majority of cotton grown in recent years in the region, until the introduction of dicamba-tolerant cotton technology in 2017, were glyphosate-resistant cultivars. Repeated applications of glyphosate in glyphosate-resistant crops has imposed a great selection pressure on the weed population, which has likely contributed to the widespread evolution and spread of glyphosate-resistant Palmer amaranth in the High Plains as well as other cotton producing regions in Texas (Garetson et al. 2017). High soil seedbank levels as a result of glyphosate resistance, coupled with a prolonged seedling emergence pattern could have contributed to weed escapes. It also was noted that in the High Plains, Palmer amaranth was observed in fewer fields in 2017 compared to 2016, with 67 and 44% (Upper High Plains), 88 and 35% (Central High Plains), and 93 and 65% (Lower High Plains) in 2016 and 2017, respectively (Fig 2). This difference may be attributed to the widespread adoption of dicamba-tolerant cotton (XtendFlex®) technology, which was reported to have been planted in over 70% of the cotton acreage in the Texas High Plains in 2017 (Steadman 2017).

Seed addition potential of common waterhemp in Texas cotton

The results of this survey showed that late-season escapes of common waterhemp were observed in the Blacklands, Central Texas, and the Gulf Coast regions (Table 1; Fig. 1). The infested area in a field ranged between 2.0 and 7.3% depending on the region surveyed (Table 2). Escaped common waterhemp density was the greatest (1,037 plants ha^{-1}) in the Upper Gulf

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Coast region and the lowest (200 plants ha^{-1}) in the Lower Gulf Coast region. This follows the trend observed by Garetson (2017), where the greatest waterhemp infestations occurred in the Upper Gulf Coast region. With respect to seedbank addition from waterhemp escapes, the largest seedbank inputs (12.9 million seeds ha^{-1}) were documented in the Blacklands region and the lowest (0.2 million seeds ha^{-1}) in the Central Texas region (Table 2; Fig. 5). The Upper Gulf Coast region has intensive row-crop production, with cotton, corn and soybean being important crops. Multiple resistance to the acetolactate synthase-inhibitors and glyphosate is widespread in waterhemp populations across this region (Garetson et al. 2017). High levels of field infestations (i.e. area coverage of escapes) and substantial densities of escaped weeds in this region suggest high soil seedbank levels, which in turn increase the chances of weed escapes following herbicide applications.

Implications for Management

The long-term goals of a management program for annual weeds should directly be related to the reduction of seedbank inputs, and eventually the size of the soil seedbank (Davis 2006; Bagavathiannan and Davis 2018). Amaranthus species are known for their prolific seed production, and this survey has demonstrated that Palmer amaranth and common waterhemp escapes can add in excess of 13 million seeds ha^{-1} in Texas cotton under current management scenarios. Even the lowest seed addition levels observed (~200,000 seeds ha^{-1}) are significant because assuming a 10% seedling emergence (Neve et al. 2011) and two effective herbicide applications with a field-level efficacy of 98% each, a minimum of 8 seedlings ha^{-1} will escape control measures. With a nominal seed production potential of 50,000 seeds plant^{-1}, the total seed returning to the soil seedbank will be about 400,000 ha^{-1}, indicating a continued raise in soil seedbank size. The scenario described above is a best-case scenario, assuming effective field-

level control. However, several application, environmental and biological factors, and their interactions, can lead to more weed escapes in production fields. In order to be sustainable, the seedbank size must follow a declining trend or at least maintained at the current levels and not increase over-time. Thus, these late-season escapes warrant control to minimize seedbank size and the risk of herbicide resistance evolution.

A number of management strategies can be adopted to address late-season weed escapes. An effective strategy starts with reducing weed densities and escapes by developing and implementing a robust, diversified weed management program. For example, Chahal et al. (2018) showed that application of overlapping residual herbicides can provide season-long control of Palmer amaranth. Similarly, Neve et al. (2011) and Norsworthy et al. (2016) noted that POST applications of foliar-active herbicides tank-mixed with residual herbicides are necessary to reduce Palmer amaranth escapes in cotton. Integration of non-chemical tactics such as crop rotation and cover crops can augment the herbicide options in reducing weed escapes and seedbank addition (Bagavathiannan and Davis 2018).

The escapes of problematic weeds such as *Amaranthus* sp., that still exist in the late-season after implementation of robust in-field weed management, can be targeted with harvest-time weed seed control (HWSC) strategies. Implementation of HWSC refers to a host of strategies that can be applied at the time of crop harvest to reduce viable weed seed adding to the soil seedbank (Walsh et al. 2017). These practices, not yet widely used in the U.S., include chaff carts, narrow-windrow burning, Harrington Seed Destroyer, bale-direct systems, and other means of targeting the chaff during harvest (Walsh et al. 2013). The above tactics present an opportunity to collect and destroy any non-shed weed seeds prior to their return to the soil seedbank. However, none of these options are ideal for cotton as of now, given the current

design of the harvest machinery. Other options such as crop topping can be employed. Crop-topping is the concept of applying herbicides to reduce seed viability or seedling fitness by use of late-season POST application of broad spectrum herbicides in crops (Steadman et al. 2006, Walsh 2001). Overall, implementation of chemical/non-chemical practices to target weed seeds at the time of cotton harvest is imperative to minimize seedbank inputs.

II.5. Conclusions

Given the high fecundity and season-long emergence capacity, Palmer amaranth and common waterhemp are considered two of the most problematic weeds in row-crops in the US. The late-season weed survey conducted in this study in major cotton producing regions in Texas revealed that Palmer amaranth was the most frequently found escaped weed in Texas cotton and waterhemp was the second most prevalent broadleaf weed. This survey also showed that escaped plants of Palmer amaranth and common waterhemp can greatly contribute to seedbank addition, with seed production reaching in excess of 13 million seeds ha^{-1}. Few management techniques currently focus on minimizing seedbank replenishment from late-season escapes because these escapes do not cause significant current-season yield reductions and thus are not seen as a threat. Developing robust weed management programs that include diversified herbicide options and non-chemical strategies will be vital in reducing the number of escapes for these troublesome weeds. Given that weed escapes are often unavoidable due to various field-level factors, developing effective tactics to reduce viable seed production from these troublesome weed escapes is imperative. Though HWSC research has been carried out recently on crops such as soybean (e.g. Norsworthy et al. 2016), such tactics have not been investigated in cotton. The overall production system and harvesting of cotton is very different from other row-crops, and more research is necessary in developing suitable HWSC tactics in cotton.

Table 1. Rankings of the most frequently found weed escapes in Texas cotton

Rank	Weed	Frequency Points*
1	Palmer amaranth	1.7
2	Texas Millet	0.8
3	Common waterhemp	0.7
4	Ragweed Parthenium	0.5
5	Barnyardgrass	0.5
6	Sprangletops	0.4
7	Silverleaf nightshade	0.3
8	Field Bindweed	0.2
9	Texas blueweed	0.2
10	Johnsongrass	0.2

*The frequency points were calculated using the equation:

$$RP = \sum_{r=1}^{3} \frac{FX}{n}$$

where, F is the frequency of a particular rank (r) assigned to a certain weed species, X is the frequency points (3 for r#1, 2 for r#2, and 1 for r#3) associated with that particular rank, and n is the total number of fields surveyed. The maximum possible relative frequency points for weed species is 3.0.

Table 2. Plant density and seedbank return by escaped Palmer amaranth and common waterhemp in Texas cotton

Weed	Region†	% area infested in a field*	Weed density*	Total seed*
			——— #plants ha^{-1}———	—— #seeds (million) ha^{-1}——
Palmer amaranth	Blacklands	4.5 ± 1.6	971 ± 380	3.3 ± 1.3
	Central Texas	2.4 ± 0.3	405 ± 83	2.4 ± 1.5
	Upper HP	6.4 ± 0.9	757 ± 103	13.9 ± 3.3
	Central HP	5.1 ± 1.1	1,248 ± 427	6.4 ± 2.5
	Lower HP	8.1 ± 2.5	1,444 ± 403	7.3 ± 2.1
	Lower GC	9.4 ± 3.4	3,543 ± 1799	4.1 ± 1.8
Common waterhemp	Blacklands	4.9 ± 1.3	673 ±245	12.9 ± 4.6
	Central Texas	2.0 ± 1.0	800 ± 500	0.2 ± 0.02
	Upper GC	7.3 ± 1.9	1,037 ± 226	9.8 ± 3.6
	Lower GC	2.0 ± 0.1	200 ± 10	0.3 ± 0.02

* Values are presented as mean ± standard error of mean

†Abbreviations: High Plains (HP), Gulf Coast (GC)

Figure 1. Map depicting top late-season weed escapes in major cotton producing regions of Texas. Pie chart showing frequency points of late-season escapes surveyed in 2016 and 2017.
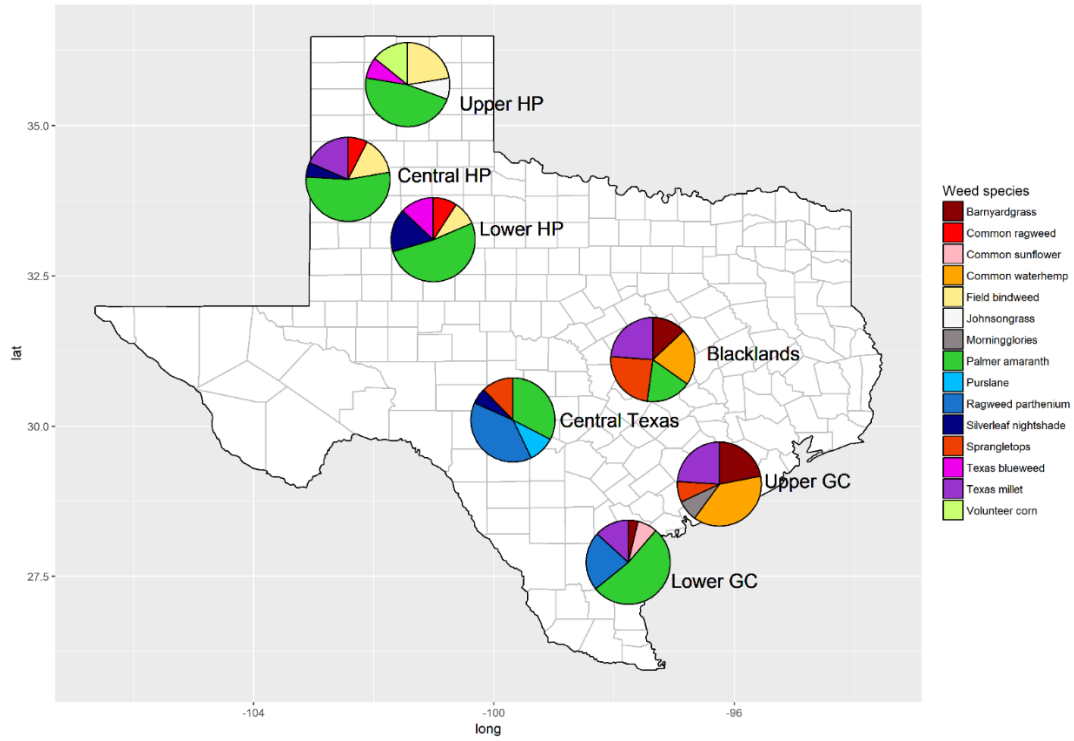
Abbreviations: High Plains (HP), Gulf Coast (GC)

Figure 2. Fields with Palmer amaranth and common waterhemp escapes (% occurrence) in major cotton producing regions in Texas*
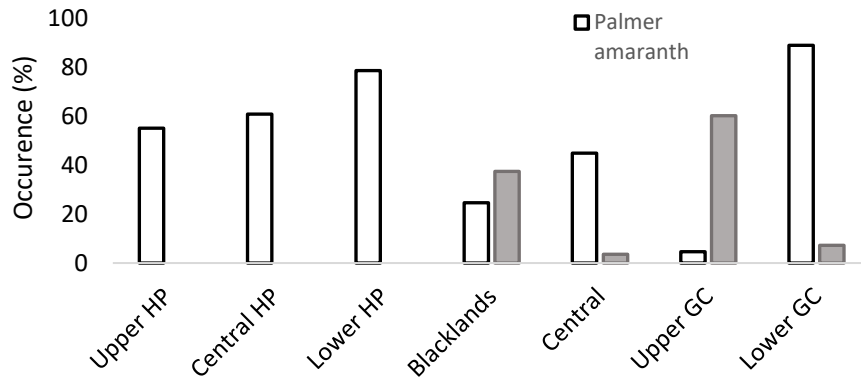
*Survey in the Lower Gulf Coast region was conducted only in 2017

Abbreviations: High Plains (HP), Gulf Coast (GC)

Figure 3. Occurrence (%) of Palmer amaranth in the High Plains in 2016 and 2016
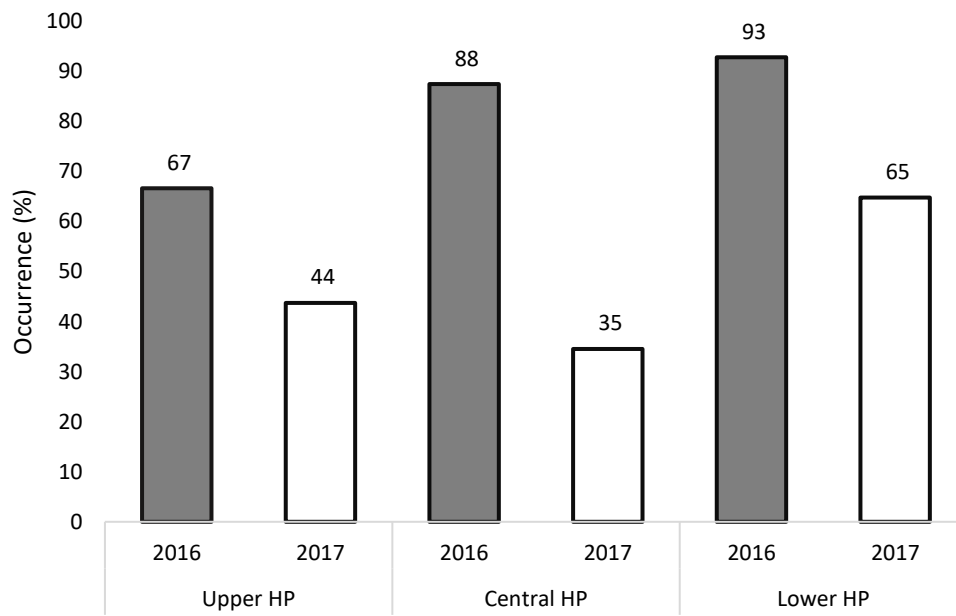
Abbreviations: High Plains (HP)

Figure 4. Boxplot presenting potential seed rain from late-season Palmer amaranth escapes prior to cotton harvest in major cotton producing regions in Texas.

Palmer amaranth
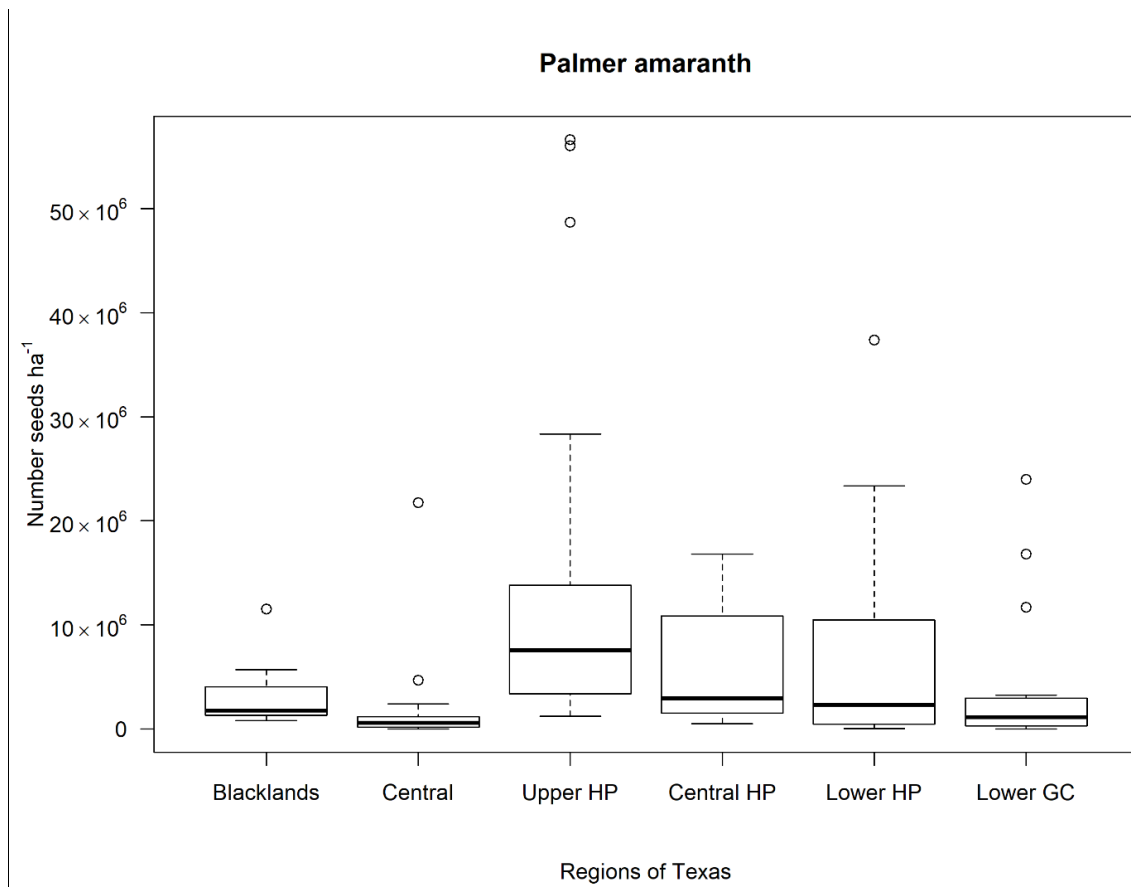
*Abbreviations: High Plains (HP), Gulf Coast (GC)

Figure 5. Boxplot presenting potential seed rain from late-season common waterhemp escapes prior to cotton harvest in Texas.
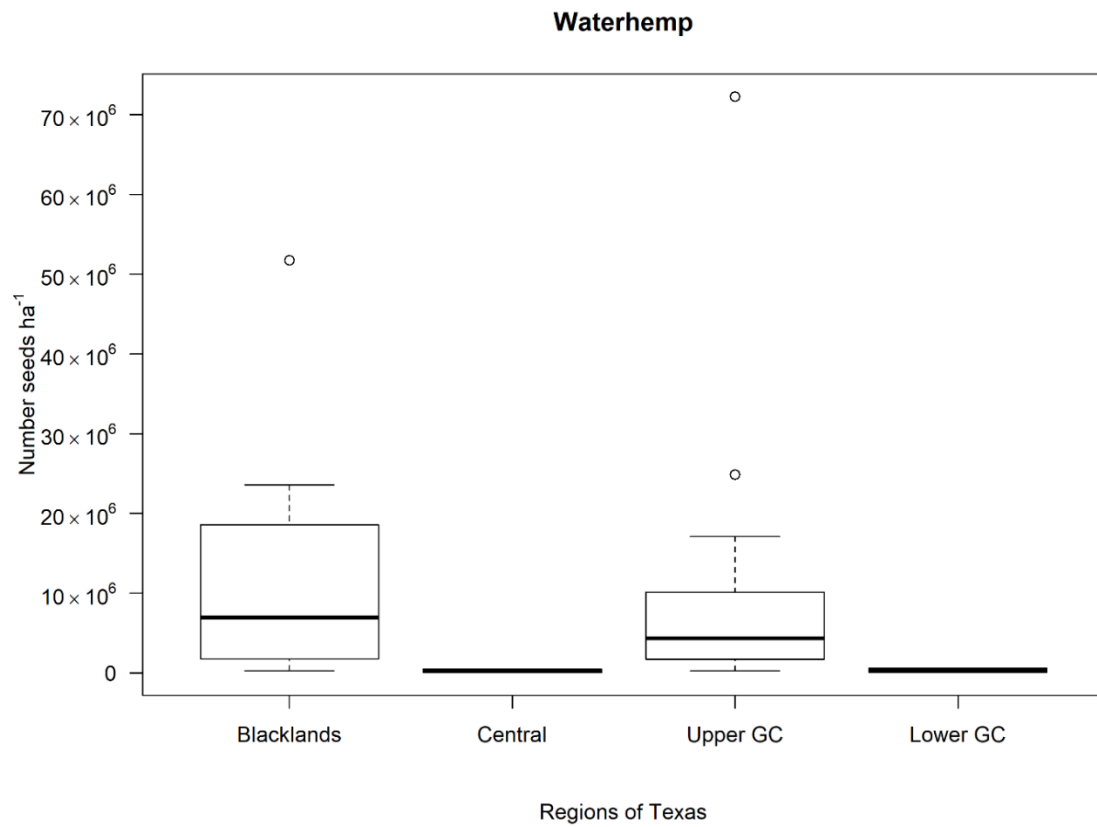
*Abbreviations: High Plains (HP)

CHAPTER III

EFFECT OF DEFOLIANTS USED IN COTTON ON SEED VIABILITY OF PALMER
AMARANTH

III.1. Introduction

Historically, weed management has focused on preventing seedling establishment and

growth, but has placed limited emphasis on minimizing seed return from weed escapes or

maximizing soil seedbank depletion (Anderson 2007; Cardina et al. 1999; Gallandt 2006). Late-

season escapes (weeds that survive earlier season control efforts and/or the ones that emerge

after all control measures have been stopped) are major contributors to seedbank replenishment,

which eventually leads to the persistence of weeds in future years. Weed survival to herbicide

applications, apart from any pre-existing resistance, can result from inadequate herbicide rate,

poor spray coverage, absence of an adjuvant, application at inappropriate weed sizes, herbicide

interactions, and/or any unfavorable environmental conditions leading to reduced weed control

(Hartzler 2001; Jordan et al. 1997). In addition, escapes also can result from prolonged weed

seedling emergence patterns, with considerable emergence after all postemergence (POST)

herbicide applications are completed (Johnson et al. 2004b). These late-season recruits can

contribute significantly to seedbank replenishment (Jha and Norsworthy 2009).

Palmer amaranth is the most commonly occurring weed in cotton production fields in

Texas. It is considered a troublesome weed species throughout the southern United States (U.S.)

and other parts of the country (Van Wychen 2017), with a high tendency for evolving herbicide

resistance. As of now, this species has evolved resistance to six different herbicide sites of action

(SOA), with several populations exhibiting multiple resistance to at least 3 different SOA (Heap

2018). The success of Palmer amaranth as one of the most troublesome weeds in row crops can

be attributed in part to its ability to recruit over an extended period. Palmer amaranth emergence occurred from March through October in California (Keeley et al. 1987), and from May to September in Michigan (Powell 2014). In College Station, TX, Palmer amaranth emerged as early as February and prolonged seedling emergences were observed until the first killing frost in November (unpublished data). In SC, Palmer amaranth seedling emergence occurred over a 10-wk period from early May to mid-July (Jha and Norsworthy 2009). The extended emergence pattern of Palmer amaranth typically allows for weed escapes during the late-season.

Palmer amaranth is a prolific seed producer and, coupled with its extended emergence, escaped plants of this species can add substantial amount of seed to the seedbank. A single plant is capable of producing in excess of 2 million seeds when growing under ideal conditions (Smith et al. 2011). Seed production under competition with agronomic crops for resources, which is more practical to encounter when plants are late recruits, can still range from 250,000 to 613,000 seeds plant^{-1} (Keeley et al. 1987, Sellers et al. 2003). Even though Palmer amaranth exhibits high seedbank loss (Bagavathiannan and Norsworthy 2013), a small proportion of the seedbank can still stay viable for several years (Menges 1987) and contribute to the persistence of the species. It is thus imperative to reduce seedbank input from escaped Palmer amaranth plants.

A number of harvest-time weed seed control (HWSC) strategies can be employed for minimizing viable seed input from Palmer amaranth (Walsh and Powles 2014; Norsworthy et al. 2016). For cotton, however, the existing harvest methodology is not conducive for the mechanical weed seed collection and destruction options described by Walsh and Powles (2014). A chemical practice, known as crop topping, has been used in Australia to reduce viable seed production by late-season escapes (Steadman et al. 2006; Walsh 2001), and this approach can be tested on Palmer amaranth in cotton production in the U.S. In fact, farmers typically apply a

chemical desiccant to defoliate cotton and facilitate the harvesting process. Such applications also improve harvest efficiency by impacting the concurrently occurring weed escapes and minimizing their interference with the harvest machinery (Sunil and Shaw 1992). These chemical defoliants also may have the potential to impact the seed viability of weed escapes, but little is known on this prospect.

This experiment incorporates 8 different desiccants/defoliants. Synthetic auxins (WSSA Group 4) (e.g. 2,4-D, dicamba) are systemic compounds that affect cell wall plasticity and nucleic acid metabolism (Shaner 2014). Photosynthetic inhibitors, such as diuron (Group 7), inhibit photosynthesis by binding to the D1 protein of the photosystem II complex in chloroplast thylakoid membranes (Shaner 2014). Ultimately, these herbicides block the electron transport and stop CO_2 fixation and production of ATP and $NADPH_2$ needed for plant growth. Glyphosate (Group 9) inhibits the enzyme 5-enlopyruvate-shikimate-3-phosphate synthase (EPSPS) in the shikimic acid biosynthesis pathway (Shaner 2014). This broad spectrum, systemic herbicide creates a depletion of aromatic amino acids such as tryptophan, tyrosine, and phenylalanine that cannot be produced without the EPSPS enzyme (University of California 2018). Glufosinate (Group 10) is a contact herbicide with limited translocation throughout the plant (University of California 2018). It inhibits glutamine synthetase, the enzyme that converts glutamate and ammonia to glutamine and the accumulation of ammonia in the plant destroys cells (Shaner 2014).

Pyraflufen ethyl (Group 14) is a contact herbicide and inhibits the protoporphyrinogen oxidase (PPO) enzyme that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX) (Shaner 2014). Depletion of PPIX, a precursor molecule for both chlorophyll (needed for photosynthesis) and heme (needed for electron transfer chain), results in the formation of

highly reactive molecules that attack and destroy lipids and protein membranes which desiccate and disintegrate rapidly. Monosodium methanearsonate (MSMA) (Group 17) is an organic arsenical, the mechanism of action of which remains unknown. Paraquat (Group 22) is a photosystem I (PSI) electron diverter, and is a broadspectrum contact herbicide.

Given that application of chemical defoliants is a common practice in cotton, it would be valuable to evaluate the impact of these chemicals on the seed viability of Palmer amaranth escapes. Determining the impact of these defoliants when applied at different seed maturity stages of Palmer amaranth [i.e. green inflorescence (pre-embryo development), white seed, brown seed and black seed] also is important. Therefore, the specific objective of this experiment was to evaluate the impact of various defoliants on seed viability of Palmer amaranth, applied at various seed developmental stages. This knowledge is expected to allow for an informed selection of the defoliant to achieve the dual benefit of assisting cotton harvest, while minimizing viable weed seed production by Palmer amaranth escapes.

III.2. Materials and Methods

Plant Material and Treatments

This experiment was conducted in two locations, College Station, TX (spring and fall 2017) and Lubbock, TX (fall 2017) on naturally occurring populations of Palmer amaranth. The experimental design was split-plot with four replications. The defoliant (9 levels, Table 3) was considered as the main-plot factor and the Palmer amaranth seed developmental stage (4 levels: green inflorescence, white seed, brown seed and black seed) was regarded as the sub-plot factor. Within each main-plot, 16 plants (4 at each seed developmental stage x 4 replications) randomly were selected. Palmer amaranth seed development progresses from the bottom of the

inflorescence to the top and it is possible that a single inflorescence has different seed

developmental stages at a given time. The location of the inflorescence at a required seed

developmental stage was identified and tagged using colored ribbons. Overall, a total of 640

plants were tagged and treated with defoliants for each site.

The desiccants were applied at label recommended rates (labeled use rates) described in

Table 4 using a CO_2 pressurized backpack sprayer fitted with XR8002 nozzles calibrated to

deliver the spray liquid at a carrier volume of 140 liter ha^{-1} traveling at 4.8 km hr^{-1}. The hand-

clipping was achieved by locating the desired seed development stage and then snipping portion

of the inflorescence on the same day as the remaining treatments were applied. This allowed for

understanding of how mechanical chopping, and in situ seed development stages would be

affected. The samples were harvested at 28 d after treatment by carefully clipping the portion of

the inflorescence that was tagged for each treatment. The inflorescences were air dried in an

oven at 50 C for 72 hrs, and subsequently thrashed manually. The seeds were carefully examined

to determine the total number of fully developed and aborted seeds produced following the

defoliant application. The aborted seeds include those that were physiologically emaciated or

shriveled.

Germination and Viability Testing

The fully developed seeds (50 seeds each) were evaluated for germination in 9-cm

diameter Petri dishes containing moistened filter paper (Whatman No. 1) and incubated in a

growth chamber at a day/night temperature regime of 30/28 C. Germinated seedlings were

recorded for 10 d. The ungerminated seeds were subjected to a tetrazolium (2,3,5

triphenyltetrazolium chloride) seed viability test. The seeds were immersed in 1% tetrazolium

chloride solution in Petri dishes and incubated at 30 C for 48 hrs. The imbibed seeds were

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crushed to observe the presence of a stained embryo. When imbibed by the living tissue, the tetrazolium chloride salt reduces to a reddish, water insoluble compound, thus turning viable embryos pink or light red (Borza et al. 2007).

III.3. Statistical Analysis

Data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc, Cary, NC). Defoliant treatments and seed developmental stages were considered as fixed effects, whereas year, location, replication, and all their interactions were considered random effects. Prior to ANOVA, normality and homogeneity of variance were tested using PROC UNIVARIATE in SAS; data transformation was not necessary. Means were separated using the Fisher's protected LSD at $\alpha = 0.05$.

III.4. Results and Discussion

Seed Mortality

The greatest seed mortalities were noted with hand-clipping at the white seed stage (97%); MSMA at the brown (96%), green (93.5%) and white (93%) stage; and diuron at white seed stage (94%). Timings for producers to apply at these individual seed stages can be a challenge, but the estimates from this study provides an average seed mortality values expected with a defoliant when multiple seed maturity stages were present at the time of application. When Palmer amaranth is a seedling, the green inflorescence has no seeds yet, but white seeds soon develop in the florets. Immature inflorescence and early seed stages (e.g. white seed stage) may not have sufficient nutrients or internal development to withstand disruption, and this might be why the efficacy of herbicides declines as the weed size increases and further development occurs. Most postemergence (POST) herbicides are recommended to be applied to ≤10 cm tall weeds (McAfee and Baumann 2017).

Being an indeterminate crop, Palmer amaranth is able to switch between vegetative and reproductive growth and back again without having ceased vegetative growth beforehand. This allows for an inflorescence to develop continuously, whether on the main raceme or side branches. As the plant continues to mature, the seed colors range from white to black. It is unknown what specifically separates brown and black stages in terms of viability, but these stages may have enough development when compared to the two immature stages (green inflorescence and white seed stage), as late-season herbicide applications seem to induce less mortality and less changes in viability (Fig 6). If applied at a more unified, early inflorescence stage of Palmer amaranth, glufosinate, 2,4-D, and dicamba may reduce seedbank replenishment (Jha and Norsworthy 2012). This suggests that timing of herbicide applications is important.

Given the prolonged seedling emergence capacity of Palmer amaranth, it is possible to have plants emerging continuously. In late-season, when many plant growth stages are present, maturity of an individual raceme may vary, making it possible for all stages of seed development to be present in a single seed head before it fully matures and begins shattering seeds. Results show that high levels of overall seed mortality can be achieved using paraquat (87%), MSMA (86%), glufosinate (86%), and diuron (83%) (Fig 5). This information provides producers an opportunity to achieve weed seed mortality using defoliants that are being used anyways as harvest aids.

Seed Dormancy

Seed viability is defined by its capacity to germinate and produce a normal seedling during favorable conditions (Borza et al. 2007) and seeds that are considered dormant remain viable for long periods of time with delayed germination. These seeds can become dormant, resulting from various interactions or combinations of environmental, edaphic, physiological, and genetic

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factors (Dyer 1995). Although specific processes controlling dormancy are unknown, dormancy characteristics are thought to be triggered during the maturation process of the seed. Common agronomic practices influence weed seed dormancy and germination by altering the microenvironmental and edaphic conditions surrounding seeds in the soil. Mechanical practices (tillage, planting, harvesting, etc.) can affect light penetration, soil fertility, temperature, and soil water content. Through the application of various defoliants at specific seed developmental stages, we were able to observe changes in seed dormancy.

When compared to the hand clipping treatment, all herbicides showed an increase in dormancy with the greatest impact being comparative between pyraflufen-ethyl (PPO), 2,4-D (growth regulator), MSMA (unkown SOA), glufosinate (Glutamine inhibitor) and diuron (PSII inhibitor, site A) (Fig 7). The breadth of SOA that these herbicides represent, and the lack of difference between them suggests that using a variety of desiccants that affect photosynthetic, or other metabolic, processes did not result in differences in levels of affected seed dormancy. Paraquat (PSI electron diverter) application resulted in the lowest seed dormancy, followed by dicamba (growth regulator) both of which are contact herbicides. However, this trend of contact herbicide on dormancy does not continue as pyraflufen-ethyl, another chemical with contact activity, caused the greatest levels of dormancy. When compared solely on seed development stages, no dormancy differences were found between the black, brown, and white seed stages (Fig 8).

Differences in seed dormancy when applications were made at the green inflorescence stage suggests that herbicide interaction with the plant at this early stage might be influencing internal interactions and thereby affect dormancy levels.

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The tetrazolium chloride test allowed for differentiation between viable and nonviable seeds. Thus, seeds that germinated and tested positive in the tetrazolium test were recorded as viable (Jha and Norsworthy 2012). Based on previous research, reduction in viability following herbicide applications might be due to inhibition of seed development resulting in immature embryo or dead seeds or to the abscission of floral structures (Isaacs et al. 1989; Steadman et al. 2006). In a similar study completed on Palmer amaranth in Arkansas, the effect of glyphosate on seed viability reduction did not differ from treatments of glufosinate or 2,4-D (Jha and Norsworthy 2012). Glyphosate has shown some variability in how it affects seed development, as noted in a study completed using a late-season POST application in sicklepod (*Senna obtusifolia* (L.) Irwin and Barneby) (Shane and Lawrence 1997). Glyphosate reduced sicklepod seed production and emergence by 84% when applied at blooming or early fruit developmental stage, but reductions were less than 33% when applied at the initiation of seed development. Future research in a controlled greenhouse environment will help establish the trend between herbicides and specific seed development stages at application.

Contact vs Systemic

Some of the defoliants used in this experiment were contact, while others were systemic. Contact herbicides affect the parts of the plant that they physically come into contact, whereas systemic herbicides are adsorbed by roots or foliage and are typically translocated to locations distant from the uptake site. In this experiment, pyraflufen-ethyl, paraquat and MSMA are contact chemicals, while the remaining ones are all systemic (glufosinate has some level of systemic activity). Results showed that the defoliants with contact activity, especially paraquat and MSMA, had the greatest impact on Palmer amaranth seed viability. Among the defoliants with systemic activity, diuron had the greatest impact (Fig 7). In an experiment conducted on

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sicklepod in Arkansas, regardless of herbicide used, rate, or application timing, viability was greater than >90% with all treatments (Taylor and Oliver 1997). Research conducted by Jha and Norsworthy (2012) only used systemic herbicides and found that all herbicides applied at the first sign of inflorescence reduced seed viability of glyphosate-resistant Palmer amaranth.

III.5. Conclusions

This study demonstrates that applications of certain defoliants, especially the ones with strong contact activity, can effectively reduce viable seed production in Palmer amaranth escapes. This information will help growers make informed decisions on defoliant selection. However, these late-season applications should not be considered as a stand-alone practice, but rather a component of an integrated weed management strategy (IWM) to minimize weed seedbank replenishment potential. An effective IWM strategy will include diverse over-the-top and soil residual herbicides as well as non-chemical tactics with a goal of reducing the number of weed escapes and further minimizing viable seed production by the escapes. Managing late-season weed escapes is typically not economical short term, but choosing a suitable defoliant that will be applied anyways as a harvest aid can be economically attractive when additional benefits are observed following application. We did notice inconsistencies in the activities of some of the defoliants across the different field environments. Additional research in a controlled environment might shed more light on the activities of specific defoliants tested in this study.

Table 3. List of defoliants/desiccants used and application rates

No.	Defoliant common name	Trade name	Application rate (kg ai/ha)	Rate (Product/ ha)	Adjuvant
1	Paraquat	Gramoxone®	2.247	4.67 L/ha^{-1}	NIS (0.25% v/v)
2	Glyphosate	Roundup Powermax®	6.180	2.33 L/ha^{-1}	NIS (0.25% v/v)
3	Glufosinate	Liberty®	2.629	2.33 L/ha^{-1}	NIS (0.25% v/v)
4	Dicamba	Clarity®	4.494	1.17 L/ha^{-1}	-
5	2,4-D	Weedar 64®	4.270	1.17 L/ha^{-1}	-
6	Pyraflufen-ethyl	ET®	0.234	0.073 L/ha^{-1}	COC (1% v/v)
7	MSMA	MSMA 6 Plus®	6.742	2.81 L/ha^{-1}	-
8	Diuron	Direx®	4.494	2.33 L/ha^{-1}	NIS (0.25% v/v)
9	Hand clipping	-	-	-	-

Figure 6. Seed mortality (%) of Palmer amaranth as influenced by various defoliant/desiccant treatments applied at four different seed maturity stages
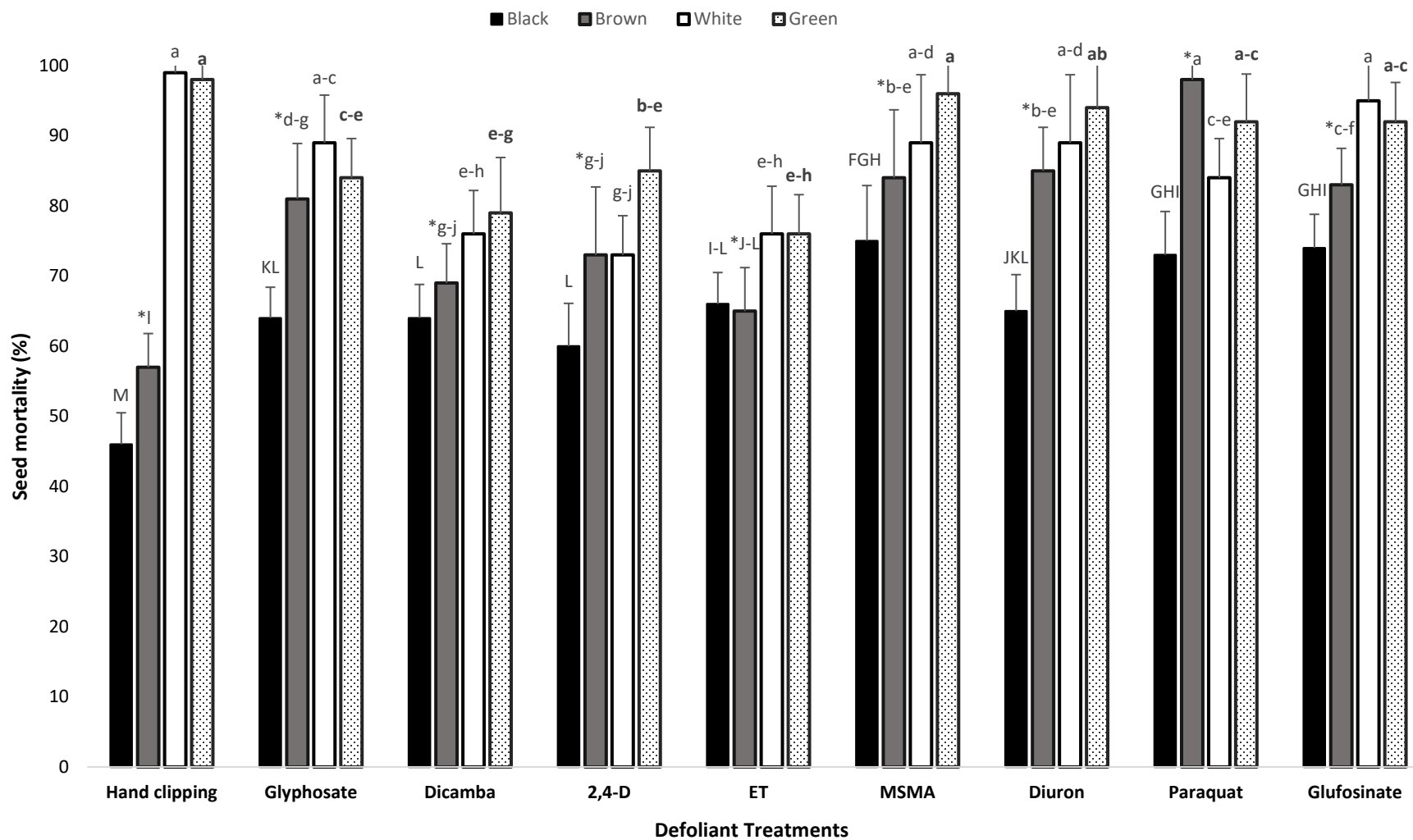
Figure 7. The main effect of defoliant choice on the dormancy level (%) of viable Palmer amaranth seeds
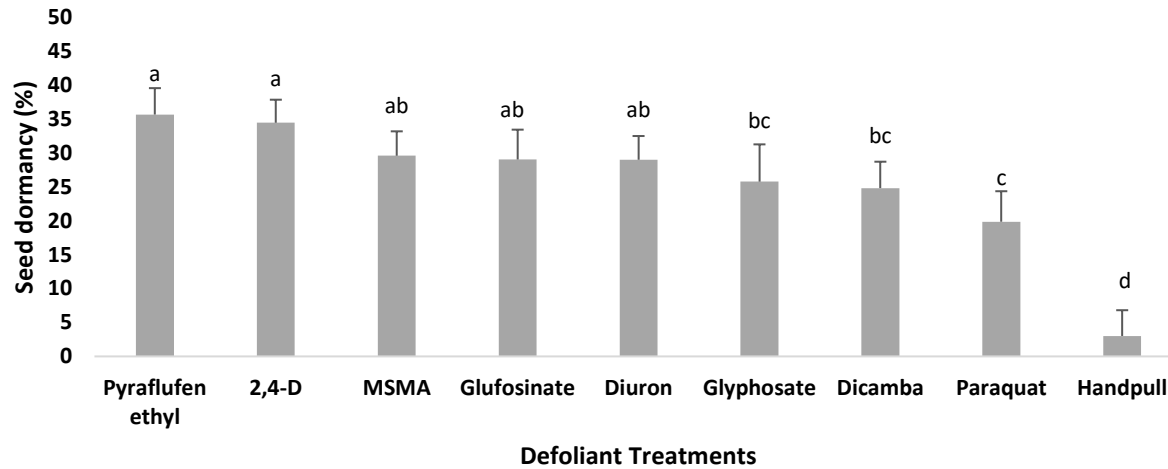
Figure 8. The main effect of seed maturity stage at defoliant application on the dormancy level (%) of viable Palmer amaranth seeds
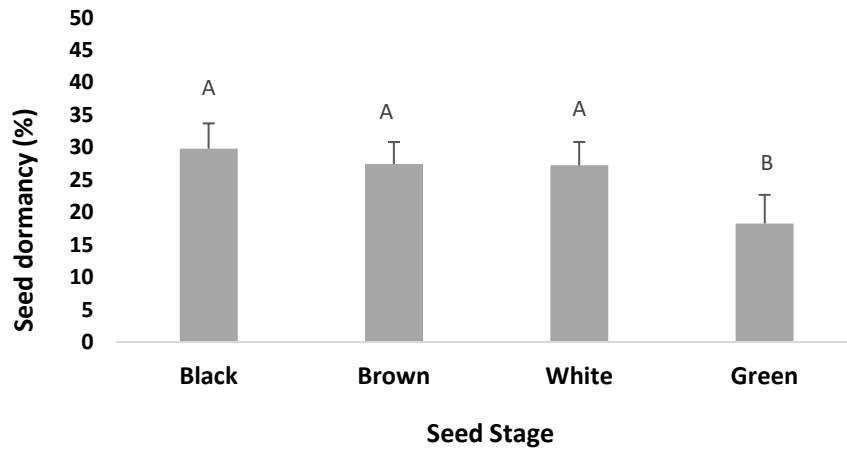
CHAPTER IV

SUMMARY AND CONCLUSIONS

Current management practices in Texas cotton often overlook late-season escapes since they do not cause yield reductions. Significant Palmer amaranth seedbank additions exist in the Texas High Plains, the Lower Gulf Coast region, and in the Blacklands, while common waterhemp seedbank additions were the greatest in the Upper Gulf Coast region followed in the Blacklands. Allowing the weed escapes to reproduce and replenish the soil seedbank can increase the risk of herbicide resistance evolution. With the loss of important herbicide families due to resistance, multiple IWM tools must be considered to preserve the utility of the remaining herbicides that are still effective. Preliminary success was witnessed in 2017, when high reductions in Palmer amaranth escapes occurred in the High Plains, possibly due to the widespread adoption of the dicamba resistant cotton (XtendFlex®) technology. Minimizing the number of weed escapes through diversified, integrated weed management is critical in reducing weed seedbank sizes to a manageable level and also thwarting the risk of herbicide resistance evolution. Management practices that reduce viable seed production in the late-season escapes must be an integral component of any IWM strategy targeting problematic and prolific weeds such as Palmer amaranth. For cotton, selection of a suitable defoliant, especially among the ones that exhibit strong contact activity, may provide dual benefits of defoliation along with a reduction in viable weed seed rain from the Palmer amaranth escapes. In closing, weed management programs for annual weeds must place a strong emphasis on soil seedbank management in order to achieve long-term cropping system and herbicide sustainability.

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APPENDIX

1. List of survey sites for both 2016 and 2017

Year	Date	Region	Field	Latitude	Longitude
2016	Sept 1st	Blacklands	1	30.707131	-96.552426
2016	Sept 1st	Blacklands	2	30.716116	-96.556209
2016	Sept 1st	Blacklands	3	30.37041	-96.21226
2016	Sept 1st	Blacklands	4	30.48621	-96.36005
2016	Sept 1st	Blacklands	5	30.50589	-96.38915
2016	Sept 1st	Blacklands	6	30.520685	-96.39891
2016	Sept 1st	Blacklands	7	31.15754	-96.55079
2016	Sept 1st	Blacklands	8	31.17044	-96.54915
2016	Sept 1st	Blacklands	9	31.18022	-96.56909
2016	Sept 1st	Blacklands	10	31.25455	-97.03137
2016	Sept 1st	Blacklands	11	31.25955	-97.02248
2016	Sept 1st	Blacklands	12	31.2765	-97.0294
2017	Sept 1st	Blacklands	13	30.42953	-97.18418
2018	Sept 1st	Blacklands	14	30.40089	-97.27472
2016	Sept 1st	Blacklands	15	-97.30282	-97.30282
2016	Sept 1st	Blacklands	16	30.33006	-97.30281
2016	Sept 7th	Central	1	29.14879	-99.37355
2016	Sept 7th	Central	2	29.151	-99.36538
2016	Sept 7th	Central	3	29.1794	-99.30644
2016	Sept 7th	Central	4	29.18264	-99.27811
2016	Sept 7th	Central	5	29.173	-99.27685
2016	Sept 7th	Central	6	29.17673	-99.27605
2016	Sept 7th	Central	7	29.17239	-99.27687
2016	Sept 7th	Central	8	29.19907	-99.21644
2016	Sept 7th	Central	9	29.20211	-99.1488
2016	Sept 7th	Central	10	29.20224	-99.12605
2016	Sept 7th	Central	11	29.15775	-98.52511
2016	Sept 7th	Central	12	29.15754	-98.51324
2016	Sept 7th	Central	13	29.19492	-98.47213
2016	Sept 7th	Central	14	29.28853	-95.50077
2016	Sept 8th	Upper Gulf Coast	1	29.34658	-95.48096
2016	Sept 8th	Upper Gulf Coast	2	29.33471	-95.50029
2016	Sept 8th	Upper Gulf Coast	3	29.33428	-95.50729

2016	Sept 8th	Upper Gulf Coast	4	29.3341	-95.51332
2016	Sept 8th	Upper Gulf Coast	5	29.34404	-95.52518
2016	Sept 8th	Upper Gulf Coast	6	29.37855	-95.5761
2016	Sept 8th	Upper Gulf Coast	7	29.37073	-96.01996
2016	Sept 8th	Upper Gulf Coast	8	29.27354	-96.04329
2016	Sept 8th	Upper Gulf Coast	9	29.18929	-95.59597
2016	Sept 8th	Upper Gulf Coast	10	29.20076	-95.46243
2016	Sept 8th	Upper Gulf Coast	11	29.24.049	-95.44688
2016	Sept 8th	Upper Gulf Coast	12	29.24472	-95.45215
2016	Sept 8th	Upper Gulf Coast	13	29.25645	-95.45357
2016	Sept 8th	Upper Gulf Coast	14	29.48542	-95.712884
2016	Sept 8th	Upper Gulf Coast	15	29.31809	-95.82812
2016	Oct 20th	Upper High Plains	1	36.0663	-102.23065
2016	Oct 20th	Upper High Plains	2	36.17951	-102.0349
2016	Oct 20th	Upper High Plains	3	36.17951	-102.04562
2016	Oct 20th	Upper High Plains	4	36.17319	-102.03749
2016	Oct 20th	Upper High Plains	5	36.16599	-102.03739
2016	Oct 20th	Upper High Plains	6	36.15307	-102.03734
2016	Oct 20th	Upper High Plains	7	36.08552	-101.57254
2016	Oct 20th	Upper High Plains	8	36.07075	-101.57276
2016	Oct 20th	Upper High Plains	9	34.2751	-101.46664
2016	Oct 20th	Upper High Plains	1	34.19707	-101.44264
2016	Oct 20th	Upper High Plains	2	34.19704	-101.44261

Year	Date	Region	Number	Latitude	Longitude
2016	Oct 20th	Upper High Plains	3	34.17039	-101.42555
2016	Oct 20th	Upper High Plains	4	34.16666	-101.4254
2016	Oct 20th	Upper High Plains	5	34.16418	-101.43583
2016	Oct 20th	Upper High Plains	6	34.16205	-101.43543
2016	Oct 20th	Upper High Plains	7	34.13382	-101.4667
2016	Oct 20th	Upper High Plains	8	34.09978	-101.47
2016	Oct 20th	Upper High Plains	9	34.052	-101.50145
2016	Oct 21st	Lower High Plains	1	33.28858	-102.0365
2016	Oct 21st	Lower High Plains	2	33.28185	-102.04692
2016	Oct 21st	Lower High Plains	3	33.2857	-102.04782
2016	Oct 21st	Lower High Plains	4	33.27641	-102.0554
2016	Oct 21st	Lower High Plains	5	33.24692	-102.09907
2016	Oct 21st	Lower High Plains	6	33.20449	-102.12279
2016	Oct 21st	Lower High Plains	7	33.12869	-102.1646
2016	Oct 21st	Lower High Plains	8	33.14234	-102.16469
2016	Oct 21st	Lower High Plains	9	33.10968	-102.3366
2016	Oct 21st	Lower High Plains	10	33.08433	-102.44973
2016	Oct 21st	Lower High Plains	11	33.07383	-102.15818
2016	Oct 21st	Lower High Plains	12	33.10857	-102.14266
2016	Oct 21st	Lower High Plains	13	33.26903	-101.40122
2016	Oct 21st	Lower High Plains	14	33.2688	-101.4207
2016	Oct 22nd	Central High Plains	1	34.20473	-101.44462
2016	Oct 22nd	Central High Plains	2	34.19707	-101.44264

2016	Oct 22nd	Central High Plains	3	34.19704	-101.44261
2016	Oct 22nd	Central High Plains	4	34.17039	-101.42555
2016	Oct 22nd	Central High Plains	5	34.16666	-101.4254
2016	Oct 22nd	Central High Plains	6	34.16418	-101.43583
2016	Oct 22nd	Central High Plains	7	34.16205	-101.43543
2016	Oct 22nd	Central High Plains	8	34.13382	-101.4667
2016	Oct 22nd	Central High Plains	9	34.09978	-101.47
2016	Oct 22nd	Central High Plains	10	34.052	-101.50145
2016	Oct 22nd	Central High Plains	11	33.59549	-101.5174
2016	Oct 22nd	Central High Plains	12	33.28858	-102.0365
2016	Oct 22nd	Central High Plains	13	34.20473	-101.44462
2016	Oct 22nd	Central High Plains	14	34.14009	-101.4377
2016	Oct 22nd	Central High Plains	15	34.10537	-101.45852
2016	Oct 22nd	Central High Plains	16	34.10536	-101.45352
2017	July 25th	Lower Gulf Coast	1	97.794380	-28.24132
2017	July 25th	Lower Gulf Coast	2	97.787740	-28.24316
2017	July 25th	Lower Gulf Coast	3	97.827520	-28.25749
2017	July 25th	Lower Gulf Coast	4	97.814030	-28.19074
2017	July 25th	Lower Gulf Coast	5	97.803490	-28.17838
2017	July 25th	Lower Gulf Coast	6	97.769360	-28.15382
2017	July 25th	Lower Gulf Coast	7	97.868170	-27.89752
2017	July 25th	Lower Gulf Coast	8	97.861390	-27.45241
2017	July 25th	Lower Gulf Coast	9	97.740810	-27.57962

2017	July 26th	Lower Gulf Coast	10	97.637180	-27.57575
2017	July 26th	Lower Gulf Coast	11	97.846710	-27.61065
2017	July 26th	Lower Gulf Coast	12	97.754010	-27.66085
2017	July 26th	Lower Gulf Coast	13	97.457210	-27.63744
2017	July 26th	Lower Gulf Coast	14	97.503280	-27.66332
2017	July 26th	Lower Gulf Coast	15	97.656480	-27.94999
2017	July 26th	Lower Gulf Coast	16	97.597520	-27.97901
2017	July 26th	Lower Gulf Coast	17	97.571360	-27.95482
2017	July 26th	Lower Gulf Coast	18	97.556570	-27.94239
2017	July 26th	Lower Gulf Coast	19	97.522750	-27.95078
2017	July 26th	Lower Gulf Coast	20	97.522170	-27.97956
2017	Aug 7th	Central	1	99.647830	-29.24643
2017	Aug 7th	Central	2	99.644750	-29.26188
2017	Aug 7th	Central	3	99.595840	-29.31496
2017	Aug 7th	Central	4	99.456060	-29.29464
2017	Aug 7th	Central	5	99.449890	-29.29455
2017	Aug 7th	Central	6	99.446580	-29.456
2017	Aug 7th	Central	7	99.441680	-29.29449
2017	Aug 7th	Central	8	99.374860	-29.31437
2017	Aug 7th	Central	9	99.360770	-29.33761
2017	Aug 7th	Central	10	99.347700	-29.31633
2017	Aug 8th	Central	11	99.206560	-29.33657
2017	Aug 8th	Central	12	99.274610	-29.27385
2017	Aug 8th	Central	13	99.196820	-29.32894
2017	Aug 8th	Central	14	99.199530	-29.3146
2017	Aug 8th	Central	15	99.199530	-29.3146
2017	Aug 8th	Central	16	99.137110	-29.30779
2017	Aug 8th	Central	17	99.140280	-29.30776
2017	Aug 8th	Central	18	99.167980	-29.30727
2017	Aug 8th	Central	19	98.856030	-29.34943
2017	Aug 8th	Central	20	98.864080	-29.34586
2017	Aug 16th	Upper Gulf Coast	1	28.814313	-96.696195

2017	Aug 16th	Upper Gulf Coast	2	28.859198	-96.645574
2017	Aug 16th	Upper Gulf Coast	3	28.884418	-96.659965
2017	Aug 16th	Upper Gulf Coast	4	28.996874	-96.63007
2017	Aug 16th	Upper Gulf Coast	5	28.996654	-96.630541
2017	Aug 16th	Upper Gulf Coast	6	29.010490	-96.574009
2017	Aug 16th	Upper Gulf Coast	7	29.064264	-96.51462
2017	Aug 16th	Upper Gulf Coast	8	29.076029	-96.510769
2017	Aug 16th	Upper Gulf Coast	9	29.070131	-96.414258
2017	Aug 16th	Upper Gulf Coast	10	29.247961	-96.306576
2017	Aug 17th	Upper Gulf Coast	11	29.249346	-93.304008
2017	Aug 17th	Upper Gulf Coast	12	29.270672	-96.316321
2017	Aug 17th	Upper Gulf Coast	13	29.303899	-96.199699
2017	Aug 17th	Upper Gulf Coast	14	29.347184	-96.090506
2017	Aug 17th	Upper Gulf Coast	15	29.355262	-96.045988
2017	Aug 17th	Upper Gulf Coast	16	29.399073	-96.1479
2017	Aug 17th	Upper Gulf Coast	17	29.504344	-96.072443
2017	Aug 17th	Upper Gulf Coast	18	29.569626	-96.076214
2017	Aug 17th	Upper Gulf Coast	19	29.573015	-96.083404
2017	Aug 17th	Upper Gulf Coast	20	29.577169	-96.069863
2017	Sept 6th	Blacklands	1	30.611021	-96.318495
2017	Sept 6th	Blacklands	2	30.611021	-96.318495
2017	Sept 6th	Blacklands	3	30.704955	-96.551759
2017	Sept 6th	Blacklands	4	30.789745	-96.599119
2017	Sept 6th	Blacklands	5	30.797840	-96.597018
2017	Sept 6th	Blacklands	6	30.815026	-96.594414
2017	Sept 6th	Blacklands	7	31.147947	-96.823767
2017	Sept 6th	Blacklands	8	30.601573	-97.301396

2017	Sept 6th	Blacklands	9	30.604454	-97.286817
2017	Sept 6th	Blacklands	10	30.572919	-97.334788
2017	Sept 7th	Blacklands	11	30.570375	-97.33863
2017	Sept 7th	Blacklands	12	30.500311	-97.400701
2017	Sept 7th	Blacklands	13	30.499345	-97.388307
2017	Sept 7th	Blacklands	14	30.520937	-97.362209
2017	Sept 7th	Blacklands	15	30.608158	-97.388078
2017	Sept 7th	Blacklands	16	30.607811	-97.398723
2017	Sept 7th	Blacklands	17	30.653394	-97.383267
2017	Sept 7th	Blacklands	18	30.745563	-97.358165
2017	Sept 7th	Blacklands	19	30.752247	-97.380008
2017	Oct 20th	Lower High Plains	1	30.795213	-97.427751
2017	Oct 20th	Lower High Plains	2	32.863383	-102.79493
2017	Oct 20th	Lower High Plains	3	32.908175	-102.79476
2017	Oct 20th	Lower High Plains	4	32.915027	-102.75641
2017	Oct 20th	Lower High Plains	5	32.915113	-102.66525
2017	Oct 20th	Lower High Plains	6	32.925429	-102.46811
2017	Oct 20th	Lower High Plains	7	32.920757	-102.4681
2017	Oct 20th	Lower High Plains	8	32.875094	-102.46816
2017	Oct 20th	Lower High Plains	9	32.907957	-102.41631
2017	Oct 20th	Lower High Plains	10	32.912022	-102.26121
2017	Oct 20th	Lower High Plains	11	32.912229	-102.12277
2017	Oct 20th	Lower High Plains	12	32.872627	-102.09813
2017	Oct 20th	Lower High Plains	13	32.751297	-101.96732
2017	Oct 20th	Lower High Plains	14	32.852763	-101.89224
2017	Oct 20th	Lower High Plains	15	33.151329	-101.7891
2017	Oct 20th	Lower High Plains	16	33.469261	-102.07803
2017	Oct 20th	Lower High Plains	17	33.460515	-102.09214

2017	Oct 20th	Lower High Plains	18	33.326285	-102.21229
2017	Oct 20th	Lower High Plains	19	33.311807	-102.23091
2017	Oct 20th	Lower High Plains	20	33.051429	-102.68111
2017	Oct 21st	Upper High Plains	1	35.868939	-102.38108
2017	Oct 21st	Upper High Plains	2	35.867581	-102.34179
2017	Oct 21st	Upper High Plains	3	35.866473	-102.32147
2017	Oct 21st	Upper High Plains	4	35.866745	-102.23485
2017	Oct 21st	Upper High Plains	5	35.869948	-102.26411
2017	Oct 21st	Upper High Plains	6	36.139651	-102.6481
2017	Oct 21st	Upper High Plains	7	36.139779	-102.66582
2017	Oct 21st	Upper High Plains	8	36.136435	-102.6255
2017	Oct 21st	Upper High Plains	9	36.171767	-102.6888
2017	Oct 21st	Upper High Plains	10	36.168900	-102.87532
2017	Oct 21st	Upper High Plains	11	36.082358	-102.76524
2017	Oct 21st	Upper High Plains	12	36.082039	-102.74924
2017	Oct 21st	Upper High Plains	13	36.079269	-102.74924
2017	Oct 21st	Upper High Plains	14	36.284436	-102.15198
2017	Oct 21st	Upper High Plains	15	36.259137	-102.15184
2017	Oct 23rd	Central High Plains	1	34.264269	-101.70903
2017	Oct 23rd	Central High Plains	2	34.270488	-101.70855
2017	Oct 23rd	Central High Plains	3	34.277821	-101.70848
2017	Oct 23rd	Central High Plains	4	34.278370	-101.68993
2017	Oct 23rd	Central High Plains	5	34.448113	-101.76471

2017	Oct 23rd	Central High Plains	6	34.470615	-101.7684
2017	Oct 23rd	Central High Plains	7	34.538520	-102.0881
2017	Oct 23rd	Central High Plains	8	34.538928	-102.13757
2017	Oct 23rd	Central High Plains	9	34.539829	-102.19648
2017	Oct 23rd	Central High Plains	10	34.307967	-102.30442
2017	Oct 23rd	Central High Plains	11	34.011155	-102.33127
2017	Oct 23rd	Central High Plains	12	34.010983	-102.36555
2017	Oct 23rd	Central High Plains	13	33.810934	-102.08585
2017	Oct 23rd	Central High Plains	14	33.824592	-102.05091
2017	Oct 23rd	Central High Plains	15	33.824117	-101.85396
2017	Oct 23rd	Central High Plains	16	33.794977	-101.85414
2017	Oct 23rd	Central High Plains	17	33.727069	-101.84233
2017	Oct 23rd	Central High Plains	18	33.687162	-101.75009
2017	Oct 23rd	Central High Plains	19	33.694436	-101.73743
2017	Oct 23rd	Central High Plains	20	33.665582	-101.3325