8-2016

The biology and management of waterhemp in Indiana

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Recommended Citation

Heneghan, Joseph M., "The biology and management of waterhemp in Indiana" (2016). *Open Access Theses*. 953.
https://docs.lib.purdue.edu/open_access_theses/953

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Joseph Heneghan

Entitled
The Biology and Management of Waterhemp in Indiana

For the degree of Master of Science

Is approved by the final examining committee:

William G. Johnson Co-chair	
Steven G. Hallett Co-chair	
Kevin D. Gibson	

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Approved by Major Professor(s): William G. Johnson

Approved by: Peter Goldsbrough 5/27/2016
Head of the Departmental Graduate Program Date

THE BIOLOGY AND MANAGEMENT OF WATERHEMP IN INDIANA

A Thesis

Submitted to the Faculty

of

Purdue University

by

Joseph M. Heneghan

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

August 2016

Purdue University

West Lafayette, Indiana

ACKNOWLEDGEMENTS

Completing a Master’s degree has been a challenging task for me and I’d be remiss without thanking those who have helped me reach this point. Thank you to my advisors and committee, Dr. Bill Johnson, Dr. Steve Hallett and Dr. Kevin Gibson, for creating an environment for success and guiding in a way that will be effective long after my time as a student. Thank you to my fellow graduate students, both current and past, for their extensive help with my research as well as our graduate school experience. Finally, thank you to my family for helping and supporting me for my whole life, and especially these past few years.

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ABSTRACT

Heneghan, Joseph M. M.S. Purdue University, August 2016. The Biology and Management of Waterhemp in Indiana. Major Professors: William G. Johnson and Steven G. Hallett.

Waterhemp is a dioecious weed species indigenous to the Midwestern United states yet it has only recently become problematic in agronomic crop production in Indiana. Waterhemp is a small-seeded broadleaf which has increased in prevalence in conjunction with an increase in conservation tillage practices. Waterhemp germinates and emerges from the top 3 cm of soil and is known to exhibit extended periods of continual emergence, longer than most other summer annual weed species that are typically present in agronomic production settings. As a C_4 species, waterhemp then grows rapidly and is capable of producing thousands of seeds, while effectively competing with corn and soybean crops. Corn and soybean yields can be reduced by 50-70% when competing with waterhemp for an entire growing season. There are also many herbicide-resistant biotypes of waterhemp, which create additional management challenges beyond the competitive nature of this weed. The objective of this research was to evaluate the emergence, growth and development, and the influence of tillage and herbicides on waterhemp biology and management in Indiana.

The emergence characteristics of waterhemp were evaluated in a fallow field study where waterhemp emergence was monitored weekly throughout the growing season from three different tillage systems; no tillage, a single tillage event, and two tillage events 30 days apart. Waterhemp densities were low in 2014 and there were no differences in emergence from either tillage system. Higher waterhemp densities in 2015 produced more emergence from both the no-tillage and the two tillage treatments compared to the single tillage treatment. In both years, a flush of emergence was observed after the second tillage event in the two tillage treatment. Waterhemp emergence was first observed on April 24, 2014 and April 16, 2015 and 50% of the total emergence had occurred by May 22, 2014 and May 15, 2015. Waterhemp emergence can be decreased with a single tillage event in high-density waterhemp infestations.

A second field study evaluated waterhemp emergence in a soybean environment. A factorial experiment evaluated the influence of no-tillage and conventional tillage combined with soil residual and foliar herbicides on waterhemp emergence. Soybean were planted on May 8, 2014 and May 14, 2015, and waterhemp emergence was then monitored biweekly throughout the growing season. Wherever soil residual herbicides were utilized, regardless on tillage, there was very little waterhemp emergence. In plots with no residual herbicides, emergence was 152% to 223% greater from the no-tillage treatment. There were no times throughout the season in which weekly emergence was higher in the conventionally tilled plots compared to the no-tillage plots. Waterhemp emerged for 10 and 12 weeks after planting in 2014 and 2015, respectively, with no difference in duration of emergence from either tillage treatment. Soil residual herbicides and conventional tillage were able to decrease waterhemp emergence in soybean.

A third study investigated the growth and development of five waterhemp populations grown in a common garden. Populations from Indiana, Illinois, Missouri, Iowa and Nebraska were established in May, June, and July to simulate a discontinuous germination pattern and were measured weekly for plant height and flowering, and finally harvested for biomass and seed yield. Plant biomass accumulations from the May (1,120 g $plant^{-1}$) and June (1,069 g $plant^{-1}$) establishment dates was greater than the July (266 g $plant^{-1}$) establishment date. There were no differences in the mean biomass accumulation among the five populations in either the May or June establishment but the July establishment ranged from 195 to 338 g $plant^{-1}$ across the populations. Seed yields were higher in the May (926,629 seeds $plant^{-1}$) and June (828,905 seeds $plant^{-1}$) establishment dates than the July (276,258 seeds $plant^{-1}$) establishment date. The Illinois population flowered the latest of all the populations but was also among the tallest in all three establishment dates. The July establishment flowered the quickest after establishment, accumulated the least biomass, and had the fewest, but the largest seeds. This experiment showed the effect of establishment timing on waterhemp growth and development and differences among populations when grown in a common environment.

CHAPTER 1. LITERATURE REVIEW

1.1 Waterhemp Origins and Nomenclature

Waterhemp belongs to the *Amaranthus* family of plants, which are often referred to as the “Pigweeds”. Waterhemp is dioecious and consequently exhibits great genetic diversity and variation (Hager et al. 2000). Waterhemp is indigenous to the Midwestern United States and there are two recognized species in this region; tall waterhemp (*Amaranthus tuberculatus*), and common waterhemp (*Amaranthus rudis*). *A. rudis* is native to the western cornbelt region of present-day Nebraska and Kansas and south to Texas. *A. tuberculatus* is native further east into present-day Indiana and Ohio (Pratt and Clark 2001; Sauer 1967; Trucco and Tranel 2011). These two species are differentiated only by their geographic origin and the manner in which the utricle splits (Waselkov and Olsen 2014). *A. rudis* and *A. tuberculatus* “met in the middle” from Missouri to Illinois to Indiana, and because they were two closely related dioecious species, their hybridization created progeny that is unable to be attributed to one distinct parental species. It has been the subject of debate on whether or not these two species need to be divided into two different species, or if they are part of one single species with extensive variation among its geographic spread (Pratt and Clark 2001). The suggestion has been made to merge the two into one single name, *Amaranthus tuberculatus*, but the name *A. rudis* is still seen and used often.

1.2 Waterhemp Germination and Emergence

Weed seed germination has been characterized for many species in a temperate environment. Temperature, light and moisture are regarded as the main environmental factors that influence germination, with temperature often acting as the primary factor in a temperate region (Baskin and Baskin 1988). Leon et al. (2004) stated similar thoughts, proposing that moisture, oxygen availability and temperature are the most critical environmental factors. Others have suggested that initial spring emergence is often triggered by rapidly rising soil temperatures, with successive emergence events later in the season more reliant on rainfall (Froud-Williams et al. 1984). Refsell and Hartzler (2009) observed this during field trials, noting that after a pause, waterhemp emergence resumed in mid-July following significant rainfall events during the first week of July. Egley and Williams (1991) suggested that rainfall and the resultant higher soil moisture can modify the time of seedling emergence.

Field studies have recorded the initiation of waterhemp germination and emergence with average soil temperatures as low as 12 C (Steckel et al. 2007). However, studies that explicitly study waterhemp germination in controlled environments have discovered that little to no waterhemp emerges below 20 C (Guo and Al-khatib 2003; Steckel et al. 2004). Above 20 C, waterhemp germination increased when exposed to diurnal fluctuations in temperature rather than conditions with constant temperatures. These comparisons were made by comparing a constant temperature to one that fluctuated +/- 40% around the mean temperature, so that both treatments would retain the same mean temperature over a 24 hour period. Of the nine *Amaranthus* species in the experiment, waterhemp was the only one to favorably respond to the diurnal temperature fluctuations at 35 C (Steckel et al. 2004). Above 35 C, germination sharply declined and no waterhemp emergence was observed at temperatures above 48 C.

Waterhemp's discontinuous emergence pattern is one of its most problematic traits. Unlike many other summer annual weeds, waterhemp tends to emerge later in the growing season (Hager et al. 1997). When compared to velvetleaf, giant foxtail, and woolly cupgrass, waterhemp was consistently the last species to emerge (Hartzler et al. 1999). Further, the mean duration of emergence for waterhemp was 53 days, longer than any of the other species present. Leon and Owen (2006) recorded waterhemp emergence occurring over the course of 56 to 70 days in no-tillage areas. Waterhemp occupies a unique niche as one of the last summer annual weed species to emerge, and then continues to emerge well into the summer. Viable waterhemp seed has been shown to persist in the soil for four to five years in separate studies conducted in Iowa and Illinois (Buhler and Hartzler 2001, Steckel et al. 2007). Lengthy periods of emergence and emergence after the conclusion of all planned weed control measures have placed waterhemp in a position to compete in agronomic settings.

1.3 Waterhemp Growth Rate, Crop Competition, and Seed Production

Waterhemp is a C_4 plant that exhibits a rapid growth rate (Elmore and Paul 1983). Waterhemp height gain has been recorded at an impressive 0.11 to 0.16 cm per growing degree day. (Horak and Loughin 2000). This was only slightly less than Palmer amaranth *(Amaranthis palmeri)*, another C_4 *Amaranthus* species, in that same study. A typical July summer day in Indiana can often exceed 30 growing degree days, which could lead to more than 5 cm of growth day^{-1} for a waterhemp plant if a similar growth rate were realized in Indiana.

Waterhemp is able to effectively compete with agronomic crops and decrease yields. Waterhemp has been shown to reduce soybean yields by 43% in Illinois and 56% in Kansas when waterhemp and soybean competed from the time of soybean planting (Bensch et al. 2003; Hager et al. 2002b). Waterhemp can impose even greater yield reduction in corn, with reported losses of 74% in Illinois when competing for an entire growing season (Steckel and Sprague 2004). Yield losses due to waterhemp competition are less when waterhemp emerges later in the growing season, after the crop is established, or is removed very early in the growing season (Hager et al. 2002b; Steckel and Sprague 2004).

Waterhemp's rapid growth and ability to compete with crops goes in conjunction with its high degree of fecundity. Seed yield from a single plant can range from thousands to millions. Sellers et al. (2003) reported an average of 288,950 seeds plant^{-1} in Missouri, Steckel et al. (2003) reported mean yield to be in excess in excess of 1 million seeds plant^{-1} in Illinois, and Hartzler et al. (2004) reported 4.8 million seeds from one single plant in Iowa. Among *Amaranthus* species, waterhemp may have some of the smallest seeds. Sellers et al. (2003) reported the seed yields of six *Amaranthus* species, and common waterhemp produced an average of 3670 seeds g^{-1}; 32-105% more seeds g^{-1} than the other five weedy *Amaranth* species in their study. Sporadic germination, rapid growth, competitive nature, and prolific seed production lay the groundwork for a successful weed.

1.4 Influence of Tillage on Waterhemp Management

Changes in tillage practices, namely the increase in conservation tillage practices, have altered weed dynamics and caused an increase in small-seeded, shallow-germinating weed species (Culpepper 2006). Waterhemp's increased prevalence coincides with an increase in no-tillage acres, especially since the introduction of glyphosate-resistant (GR) crops (Culpepper 2006; Young 2006). Glyphosate originally provided effective control of waterhemp before the first report of GR waterhemp in 2008, although waterhemp often continued to emerge after the last application of glyphosate (Culpepper 2006; Legleiter and Bradley 2008). Total dependence on glyphosate, with decreased utilization of soil residual herbicides and increased no-tillage acres, set the stage for waterhemp to increase in prevalence in agronomic settings (Culpepper 2006; Givens et al. 2009).

Observations such as these have led to the hypotheses about why waterhemp tends to be prevalent in no-till environments (Refsell and Hartzler 2009; Steckel et al. 2004; 2007). Seed on the soil surface would be exposed to greater fluxuations in temperature than seeds that are even slightly buried in the soil profile (Steckel et al. 2004, Stoller and Wax 1973). This further supports the claim that the increase in problematic waterhemp has coincided with the increase in conservation tillage. The environmental conditions created in the no-tillage environment may favor waterhemp germination and emergence. As a result, interest in reevaluating tillage as a cultural management practice targeting waterhemp is growing.

The phenomenon of increased waterhemp prevalence in reduced tillage environments has been investigated in various ways. Leon and Owen (2006) investigated both biotype and tillage type as factors influencing waterhemp emergence. Regardless of biotype, they discovered that waterhemp emergence was four times greater in no tillage areas when compared to moldboard plowed or chisel plowed fields. Refsell and Hartzler (2009) obtained similar results in that three times as many seedlings emerged from no-tillage areas compared to chisel plowed areas. The influence of tillage can also be investigated with a slightly different approach; by investigating seedbank persistence when no new seeds are introduced into the system. With this method it was again observed that emergence was initially higher in the no-tillage areas compared to tilled areas. This declined over time, as the no-tilled areas had no new seed introduced to the germination zone, where in the tilled areas, viable seed that was previously buried was brought into the germination through the seasonal mixing due to the tillage (Egley and Williams 1990; Steckel et al. 2007). One proposed strategy to take advantage of this is to not perform any tillage for at least one year if significant weed seed dispersal has recently occurred on the surface, with the hope of promoting as much weed seed germination as possible and reducing the number of seeds that are introduced to deeper depths, where they may be able to persist longer (Egley and Williams 1990). This strategy would only be successful, however, if complete control were achieved and no new seed rain occurred.

Cognizant of the tendency of waterhemp to flourish in no-tilled environments, interest has been growing in manipulating tillage practices to help manage waterhemp from a cultural standpoint. No-tillage systems were widely adopted because advances in herbicide technology allowed for superior weed control without tillage. This, coupled with reducing erosion and saving on fuel and labor costs, has been the major driver for adapting conservation tillage practices (Triplett and Dick 2008). Continuing this effort yet returning to some tillage to achieve better waterhemp control has been of major interest. Tillage is thought to promote weed seed germination because of favorably impacting the environment around the seeds (Roberts and Dawkins 1967). Evaluating small-seeded broadleaves as a whole, seed near the surface will germinate more readily because of the more favorable conditions (Leuschen et al. 1993). Schweitzer and Zimdahl (1984) reported a 32 to 36% decrease in viable weed seed following cultivation two and four times per year, respectively. Along with a long-term decrease in viable weed seeds, emergence from tilled systems is consistently lower and the duration of waterhemp emerging is often shorter in tilled systems (Leon and Owen 2006; Refsell and Hartzler 2009; Steckel et al. 2007). Considering all of this, waterhemp germination is expected to be higher in no-till systems.

1.5 Herbicidal Control of Waterhemp

The increase in conservation tillage practices was aided by new herbicide technologies (Culpepper 2006, Young 2006). Conservation tillage practices place a higher dependence on chemical weed control and when GR crops were heavily adopted, many soil-applied residual herbicides and integrated weed management strategies were abandoned in favor of post-emergence (POST)-only glyphosate programs (Young 2006). These tillage and herbicide practices inadvertently induced weed shifts in agronomic systems to weeds such as waterhemp, which are able to germinate and emerge on the soil surface and late in the growing season, after POST applications.

Waterhemp has developed resistance to six different modes of action across 18 different US states and Ontario (Heap 2016). Waterhemp’s ability to develop resistances to common agricultural herbicides, aided by its dioecious nature, has propelled it to the forefront of weed management in many states. Much research has been done to both identify what herbicides waterhemp is resistant to and also to identify effective control measures. Waterhemp populations from Missouri have been confirmed to possess multiple resistance to glyphosate, acetolactate synthase (ALS)-inhibitors, and protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Legleiter and Bradley 2008). Waterhemp resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitors has also been confirmed in Illinois, in a single population that had multiple resistance to HPPD-inhibitors as well as photosystem II (PSII)-inhibitors in a continuous seed corn production field (Hausman et al. 2011). Resistance to synthetic auxins has also been documented in Nebraska from a grass seed production environment where 2,4-D did not control waterhemp (Bernards et al. 2012). These documented resistance cases represent six different herbicide families. Similar resistances, from single mode resistance to multiple resistances, have been documented in Kansas, Iowa, Kentucky and Tennessee (Horak and Peterson 1995; McMullan and Green 2011; Patton 2013; Steckel n.d.). Finally, in Illinois, a waterhemp population has been identified that exhibits a five-way resistance, with combined resistance to ALS-inhibitors, PPO-inhibitors, PSII inhibitors, HPPD-inhibitors and synthetic auxins (Evans et al. 2015).

Although waterhemp has many documented herbicide resistances, there are herbicidal management strategies that can be effective. In most cases, resistance occurs due to overreliance on a single mode of action over multiple seasons, and often first surfaces in fields that have been in the same crop for multiple years (Bell et al. 2013; Bernards et al. 2012; Legleiter and Bradley 2008). Most recommended herbicidal management strategies revolve around using varied herbicidal modes of action, along with crop rotation and other cultural practices. Soil applied pre-emergence (PRE) herbicides with residual activity are the cornerstone of management recommendations (Hager et al. 2002a; Legleiter et al. 2009). Utilizing a PRE herbicide in conjunction with a POST application in Missouri soybean production systems increased waterhemp control from 69-100% without a PRE to 77-100% with a PRE, representing a small but significant increase in control, even in a glyphosate-sensitive population (Schuster and Smeda 2007). In cases where the population is at least glyphosate resistant, PRE herbicides are imperative. Without glyphosate, POST options in soybean are limited to glufosinate, PPO-inhibitors and ALS-inhibitors, although ALS-inhibitors are considered obsolete for waterhemp control and PPO overreliance and resistance is already an issue (Nordby et al. 2007). In the absence of a PRE herbicide, weed densities in the (Legleiter et al. 2009) research trial ranged from 51-190 plants m^{-2} with a POST of PPO-inhibitors and glyphosate. When PRE applications were made in conjunction with the same POST applications, the densities ranged from 1-3 plants m^{-2}. When PPO-inhibiting herbicides are still a viable option, timing is critical as to not let the weeds get above target height, and to avoid spraying herbicides with potential for crop injury too late in the season (Jordan et al. 2009). Similarly, with glufosinate, timing is critical, and PRE herbicides should be utilized to extend the early-season window of weed control so that weeds within the target range of 3-4 inches can be sprayed at appropriate crop growth stages for full-season weed control (Jordan et al. 2009; Loux et al. 2010). Herbicidal control options should start with PRE applications and then follow with POST products that are effective for the specific waterhemp population.

1.6 Common Garden Methodology

Common garden experiments allow for direct comparisons between many different plant biotypes. This is done by growing biotypes from different environments in a single, common environment. Common gardens have been successfully used to assess intraspecific variation across many species and many environments (Dorman et al. 2009). Waterhemp variation across the entire region where it is problematic makes it challenging to directly compare results and knowledge from one area to another, yet the growing issue of managing waterhemp in agronomic crops creates the need for continued investigation into waterhemp growth and development across this area. A common garden with waterhemp from different regions would allow for direct comparisons and measurements of populations against each other, and would help determine differences in waterhemp populations.

1.7 Literature Review Summary

Waterhemp is a widespread problem weed with many inherent biological tendencies which have propelled it to the forefront of weed management in many regions. Its inclination to emerge in no-tillage environments and multiple herbicide resistant biotypes has coincided with an increase in conservation tillage and an increased reliance on herbicides, often leading to more waterhemp emergence and less control from herbicide applications. Once established, waterhemp can hinder crop productivity and nearly ensures its future existence with its prolific seed production. Investigations into the effect of tillage have decisively demonstrated that increased tillage can reduce the number or emerged seedlings in a growing season when compared to no-tillage, yet when no further seed additions occur, tillage can continually mix and bring more viable seeds into the germination zone, lengthening the amount of time needed to reduce or exhaust the seedbank.

The forced genetic recombination in every generation of waterhemp increases the likelihood of resistant biotypes existing, and intense selection pressures brought about by shifts away from multi-faceted weed management programs to ones that often rely solely on herbicide applications often allows these biotypes to reproduce and increase in frequency. Herbicide-resistant biotypes severely limit in-crop management options for controlling emerged waterhemp.

Understanding how different waterhemp populations grow and behave when compared to each other may show inherent differences in these populations that span waterhemp’s indigenous range. This baseline biological data may help in developing local management guidelines and transferring knowledge from regions where waterhemp has been problematic for longer periods of time to areas where it is a more recent issue. Research from many states investigating the role of increased tillage and proactive use of soil-residual herbicides indicates that these management tactics have been and will continue to be critical in effectively managing waterhemp in Indiana and across the Midwest.

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CHAPTER 2. WATERHEMP *(AMARANTHUS TUBERCULATUS)* EMERGENCE PATTERNS IN INDIANA

2.1 Abstract

Waterhemp is known to have a discontinuous germination pattern and a tendency to proliferate in no-tillage environments. Research was conducted to evaluate the emergence pattern of waterhemp in a bare ground study in Indiana with three different tillage practices; no tillage, a single tillage event, and two tillage events 30 days apart. Waterhemp densities were low in 2014 and there were no differences in cumulative emergence from any tillage treatment. With greater waterhemp densities in 2015, waterhemp emergence from the single tillage event was lower than the no-tillage and double tillage treatments. In both years, a flush of waterhemp occurred following the second tillage event in the double tillage treatment. Waterhemp emergence was first observed on April 24, 2014 and April 16, 2015 after 107 and 100 growing degree days had accumulated, respectively, and 50% of the total waterhemp had emerged by May 22, 2014 and May 15, 2015 when 279 and 282 growing degree days had accumulated, respectively. Waterhemp emergence was greater with no-tillage and multiple tillage events and therefore, waterhemp emergence can be reduced in high-density settings with a single tillage pass.

Nomenclature: *Amaranthus tuberculatus* (Moq.) Sauer (syn. *rudis*) AMATA

Key words: No-tillage, conventional tillage, weed emergence patterns, discontinuous germination

2.2 Introduction

Waterhemp is a summer annual weed that has one of the longest and latest periods of emergence when compared to other summer annual weeds which infest agronomic crops such as corn and soybean (Hager et al. 1997, Hartzler et al. 1999, Leon et al. 2006). Waterhemp is often still emerging and competing with agronomic crops after all planned weed control measures have taken place (Hager et al. 2002). This discontinuous germination pattern creates late-season weed escapes and often allows for contributions to the soil seedbank (Brewer and Oliver 2007, Hartzler et al. 2004, Steckel and Sprague 2004).

Waterhemp does not readily germinate in cool temperatures. Steckel et al. (2007) reported germination and emergence in field conditions did not occur until average soil temperatures reached 12 C. Guo and Al-khatib (2003) and Steckel et al. (2004) investigated germination in controlled environments and recorded very little germination at an average temperature below 20C. It was also discovered that waterhemp germination increases when the seeds are exposed to diurnal fluctuations in temperatures as opposed to constant temperatures. Waterhemp preferentially germinates and emerges from the top 3 cm of soil and seeds concentrated here are exposed to the greatest fluctuations in temperature, making these conditions favorable to successful waterhemp germination and emergence (Steckel et al. 2004, Stoller and Wax 1973). This phenomenon explains how waterhemp has become more widespread and problematic in agronomic crops in the last 20 to 40 years, in conjunction with an increase in conservation tillage practices (Culpepper 2006, Young 2006). Researchers who studied the effect of tillage on waterhemp emergence and density found more waterhemp is present in no-tillage environments compared to environments where tillage occurs. In separate studies conducted in Iowa, waterhemp emergence was three to four times greater in no-tillage areas than in either moldboard plowed or chisel plowed areas (Leon and Owen 2006, Refsell and Hartzler 2009).

The propensity for waterhemp to germinate and emerge under no-tillage conditions has also spurred interest in investigating this characteristic as a management tool. Waterhemp seed has been found to persist in the soil for four to five years (Buhler and Hartzler 2001, Steckel et al. 2007). If waterhemp seeds in the germination zone germinate but are not allowed to set seed, the seedbank may be exhausted after only a few years if no new seeds are introduced to the germination zone through either soil disturbance or seed rain. Tillage would be counterproductive because it would likely introduce new seeds into the germination zone that were previously deep in the soil profile, and also redistribute seeds deep into the soil profile, where seeds can typically persist longer (Egley and Williams 1990).

Although periodic tillage may introduce fresh seeds into the germination zone and allow for seeds to persist longer in the soil, annual waterhemp emergence has been consistently lower in tilled areas (Egley and Williams 1990, Leon and Owen 2006, Refsell and Hartzler 2009). This is likely due to the continual mixing and dilution of seeds in the germination zone. Some studies have shown that tillage can, however, promote weed seed germination and decrease the number of viable seeds in the soil by favorably impacting the environment around the seed, and this would be especially true for small-seed broadleaves, like waterhemp (Leuschen et al. 1993, Roberts and Dawkins 1967). Schweitzer and Zimdahl (1984) reported a 32 to 36% decrease in viable weed seed following interrow cultivation two and four times per year, respectively.

Understanding the emergence characteristics of waterhemp in Indiana under different tillage regimes could help with management and control of this weed. The objectives of this study were to characterize and document the emergence patterns of waterhemp in Indiana in different tillage systems.

2.3 Materials and Methods

2.3.1 Field Plot Design

The study was conducted at the Meigs South Farm at the Throckmorton Purdue Agricultural Center near Lafayette, IN in 2014 and 2015. The soil type was a Starks-Fincastle complex (fine-silty, mixed, superactive, mesic Aeric Endoaqualf) with a pH of 6.5 and 2.3% organic matter. The trial was established in 2014 on an area where soybean had been grown in 2013 and waterhemp seed had been broadcast in November 2013. In 2015, the trial was conducted in an adjacent area that had been left fallow in 2014, allowing weeds to set and disperse seed. Each plot was 3 m by 3 m and the central 1 m^2 of each plot was used for data collection. The area surrounding the central 1m^2 was maintained as bare ground to minimize interference from surrounding plants.

The experiment consisted of three tillage treatments with four replications of each treatment arranged in a randomized complete block design. Tillage treatments included: no-tillage, a single tillage pass simulating preplant tillage, and two tillage passes, approximately 30 days apart, simulating preplant tillage followed by interrow cultivation. The preplant tillage simulation was performed on April 26, 2014 and April 30, 2015 and the interrow cultivation simulation was performed on May 30, 2014 and June 4, 2015. All tillage was performed with a rear-tine rototiller at a depth of 6 to 9 cm.

2.3.2 Data Collection and Analysis

Newly emerged waterhemp seedlings were counted and removed weekly from the central 1m^2 of each plot from April 17 to October 20, 2014 and April 6 to October 23, 2015. Soil moisture (percent by volume) and soil temperature were recorded at a depth of 3 cm with Spectrum WatchDog 1400 series data loggers with SMEC-300 sensors, and rainfall was recorded with a Spectrum WatchDog 2700 weather station (Spectrum Technologies, 3600 Thayer Court, Aurora, IL 60504) throughout the experiment. Growing degree day (GDD) values were calculated as the mean of the minimum and maximum temperatures minus a base temperature of 10 C and a maximum of 30 C.

Data were analyzed using the PROC GLM procedure with log-transformed data in SAS 9.3 (SAS Institute, Inc., Cary, NC 27513). Weeks with zero waterhemp emergence were excluded from the analysis. Year was found to be a significant factor, therefore data were analyzed separately by year. Means were separated using Tukey’s HSD at a $p = 0.05$ significance level.

2.4 Results and Discussion

2.4.1 Field Emergence

There were substantial differences in waterhemp seedling emergence between 2014 and 2015, which may be due to the recent establishment of waterhemp at the experiment location, and low densities that were present during 2014, the first year of this study (Table 1 and Table 2). There was no experimental activity before the tillage event on May 1 and negligible emergence after June 26 for both years, therefore weekly statistical analysis, found in Table 1, was only performed for this timeframe. Cumulative emergence analysis, found in Table 2, considered all emerged waterhemp seedlings from May 1 to August 20 for both years, when greater than 99% of all seedlings had emerged for the season.

Waterhemp emergence was first recorded on April 24, 2014 and April 16, 2015 when 107 and 100 GDD had accumulated, respectively (Figure 1 and Figure 2). The last emergence cohort from all three treatments in a single week occurred on August 21, 2014 and October 1, 2015 with 1335 and 1888 accumulated GDD, respectively (Figure 1 & data not shown). Waterhemp emerged over a span of 18 weeks in 2014 and 25 weeks in 2015. By May 22, 2014 and May 15, 2015, 50% of the total waterhemp seedlings had emerged, with 279 and 282 accumulated GDD, respectively (Table 1). In both 2014 and 2015, 90% of the total seedlings had emerged by the June 19 observation with 609 and 667 accumulated GDD, respectively (data not shown).

Tillage had one discernable effect in 2014 and three in 2015. First, in 2015, a decrease in emergence occurred after the first tillage event in the plots that had been tilled

(Table 1). Second, in both 2014 and 2015, the second tillage event was followed by a flush of emergence from plots that were tilled a second time, especially in comparison to the single-tillage treatment (Table 1). Cumulatively, emergence in 2015 from the double tillage and no-tillage treatments was higher than from the single tillage treatment. There were no cumulative differences in 2014, presumably due to low waterhemp densities (Table 2).

Decreased waterhemp emergence from single-tilled areas was reported by Leon and Owen (2006) and Refsell and Hartzler (2009). This agrees with our study which showed a decrease in emergence observed after the first tillage event in 2015 and the cumulatively lower emergence observed from the single tillage treatment in 2015. In Iowa, Hartzler et al. (1999) observed comparable durations of emergence but did not see as much early-season emergence compared to our Indiana environment. Hartzler et al. (1999) cited a cool April in 1995 with little GDD accumulation and low rainfall amounts in April 1996 and 1997 that may have delayed emergence.

The emergence cohort observed after the second tillage event may be caused by favorably modifying the germination zone with the tillage event (Roberts and Dawkins 1967). Exposing more seeds to the soil surface more frequently due to tillage is known to exhaust the soil seed bank faster than no-tillage, where seeds are not routinely redistributed in the soil profile, and this tillage-induced emergence event may help reduce seed banks if no new additions occur, but it may also cause more waterhemp to be present later in the growing season if left uncontrolled (Steckel et al. 2007). If annual seedbank additions occur, tillage later in the growing season may be detrimental to waterhemp management due to increased late emergence and few options for control late in the growing season.

2.4.2 Conclusion

This study has shown that waterhemp has shown the ability to behave similarly in the Indiana environment as it has been documented in the Western U.S. Discontinuous germination patterns were evident with both low and high waterhemp density in 2014 and 2015, respectively. A single spring tillage event reduced waterhemp emergence compared to no-tillage event or multiple-tillage events, providing further data that a single tillage pass can be an effective tool in managing waterhemp populations in Indiana. However, intentionally promoting waterhemp emergence with no-tillage or multiple tillage events, which favorably influence the germination zone, may be an effective strategy for reducing the soil seedbank if no further seedbank contributions occur.

2.5 Acknowledgements

The authors would like to thank the members of the Integrated Weed Management labs at Purdue University for their help in completing this research and recognize the United Soybean Board for funding this work.

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Table 2.1: Number of emerged waterhemp seedlings m^{-2} $week^{-1}$ and GDD accumulation for 2014 and 2015[a]

		2014		2015	
Observation Week	Tillage	Seedlings m^{-2}	GDD	Seedlings m^{-2}	GDD
May 8	None	n.d.[b]	181	1186 a[c]	224
	Single	n.d.		229 b	
	Double[d]	-		-	
May 15	None	4 a	246	899 a	282
	Single	8 a		826 a	
	Double	-		-	
May 22	None	86 a	279	1623 a	338
	Single	57 a		399 b	
	Double	-		-	
May 29	None	44 a	356	373 a	418
	Single	48 a		122 b	
	Double	-		-	
June 5	None	n.d.	448	102 a	474
	Single	n.d.		32 b	
	Double	n.d.		-	
June 12	None	10 b	521	500 a	556
	Single	11 b		216 a	
	Double	31 a		480 a	
June 19	None	11 a	609	614 a	667
	Single	8 a		251 b	
	Double	14 a		1065 a	
June 26	None	n.d.	709	102 a	756
	Single	n.d.		31 b	
	Double	n.d.		41 ab	

[a] Data were not pooled across years

[b] No data when there was no waterhemp emergence for a single week

[c] Means followed by the same letter within weeks are not different according to Tukey's HSD at $p = 0.05$

[d] The double tillage event had not occurred during the first four weeks of observation. All counts from this treatment for this timeframe are represented in the single tillage data.

Table 2.2: Cumulative number of emerged waterhemp seedlings m^{-2} in 2014 and 2015[a]

	2014	2015
	——————— Seedlings m^{-2}	——————
No-Tillage	178 a[b]	7280 a
Single Tillage	161 a	2667 b
Double Tillage	172 a	4859 a

[a] Data were not pooled across years

[b] Means followed by the same letter within years are not different according to Tukey's HSD at $p = 0.05$

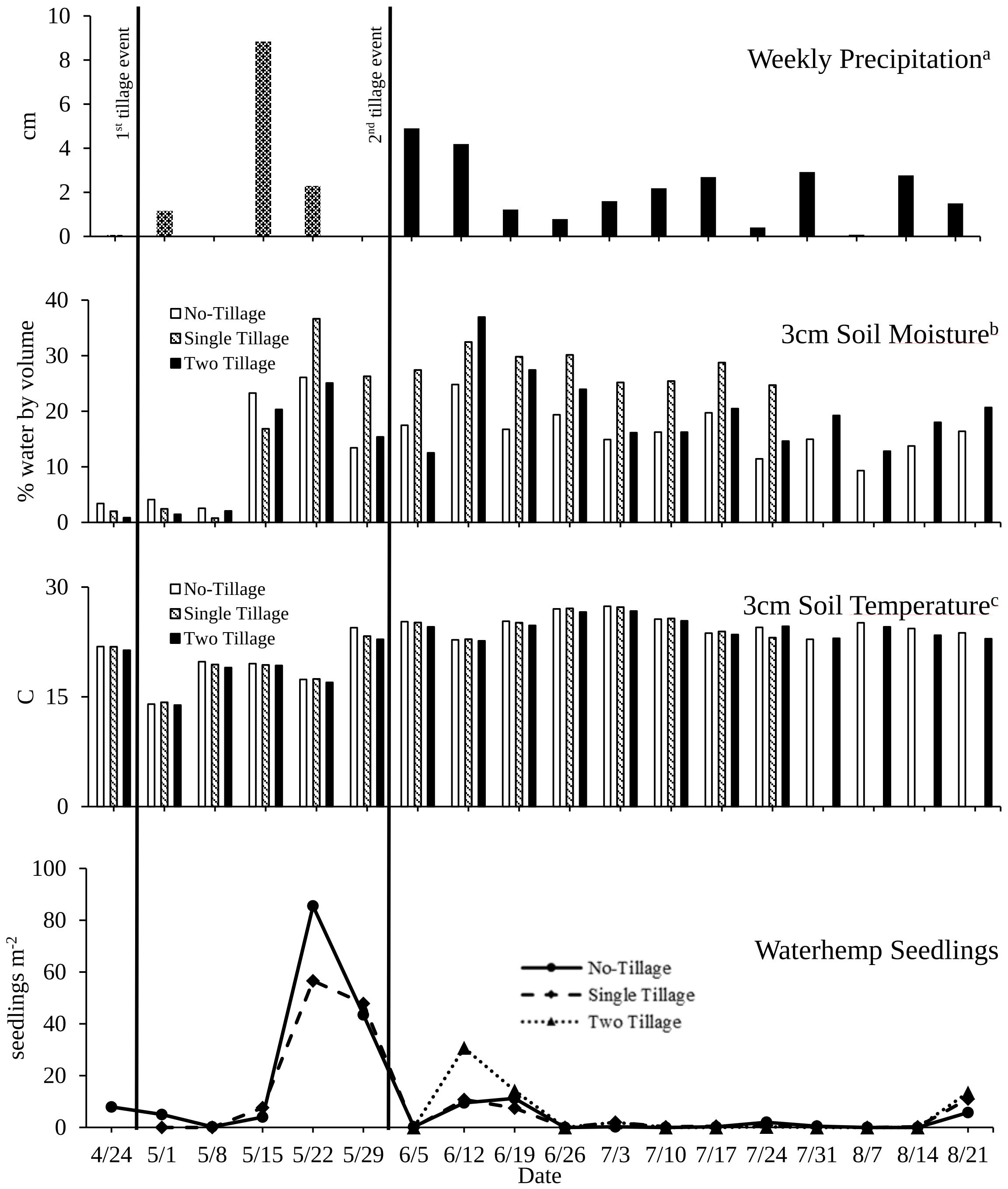

Figure 2.1: Waterhemp seedling emergence, precipitation, 3 cm soil moisture by volume, and 3 cm soil temperature in 2014 in a field study in Indiana.

[a] Regional weather station data was used for 4/24 through 5/29 due to field equipment malfunction.

[b,c] Single tillage soil moisture and temperature was not recorded 7/31 through 8/21 due to equipment malfunction.

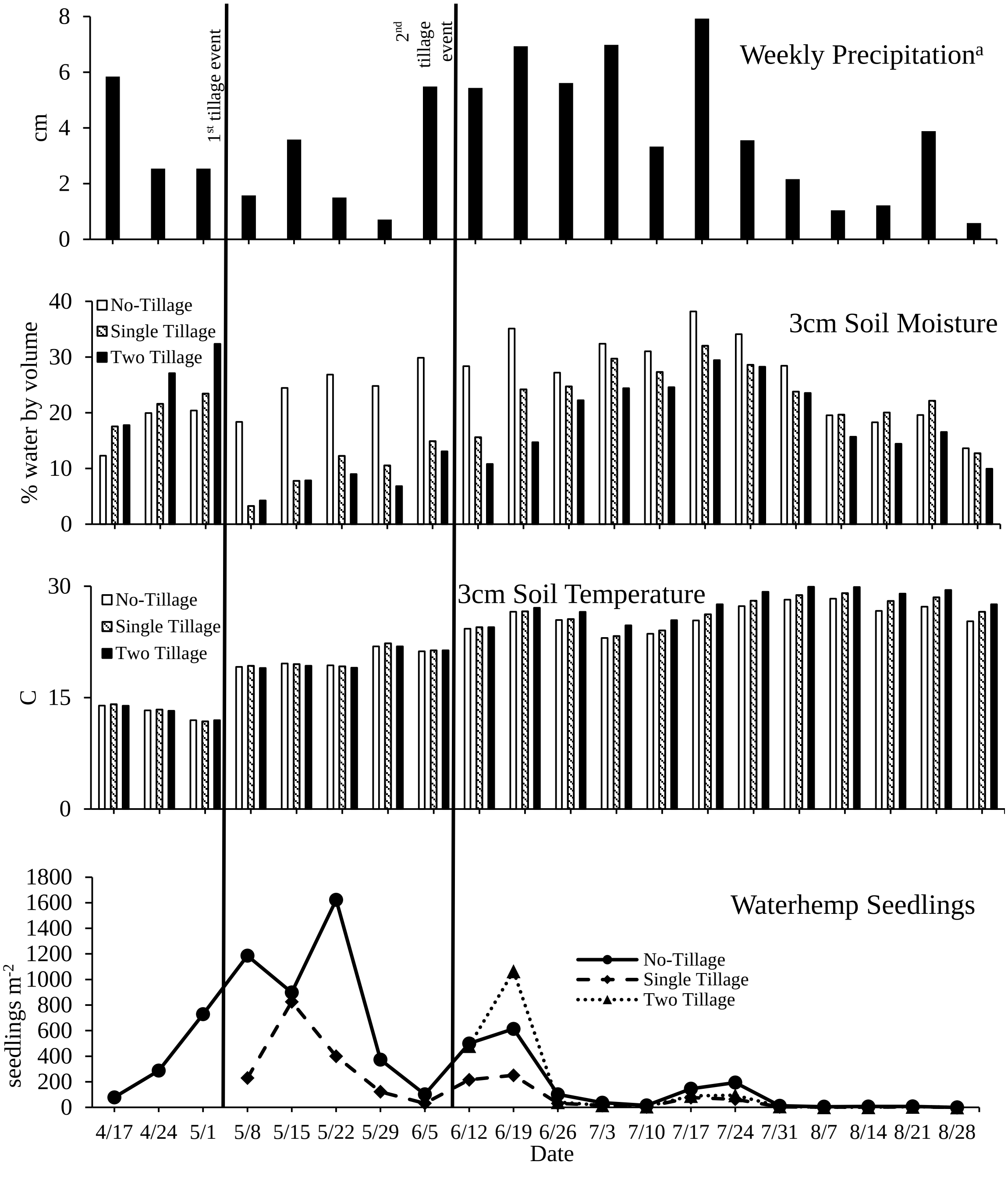

Figure 2.2: Waterhemp seedling emergence, precipitation, 3 cm soil moisture by volume, and 3 cm soil temperature in 2015 in a field study in Indiana.

CHAPTER 3. THE INFLUENCE OF TILLAGE ON WATERHEMP (*AMARANTHUS TUBERCULATUS*) EMERGENCE IN SOYBEAN IN INDIANA

3.1 Abstract

Waterhemp is a problematic weed in soybean production, and its predisposition to flourish in conservation tillage systems has allowed it to grow in prevalence as conservation tillage has been increasingly adopted. Research was conducted to evaluate the influence of no-tillage and conventional tillage combined with soil residual and foliar herbicides on waterhemp emergence in soybean production. Soybean were planted in early May, and waterhemp emergence was monitored throughout the growing season. Waterhemp seedlings emerged for ten and twelve weeks after planting in 2014 and 2015, respectively. The duration of waterhemp emergence was not influenced by tillage. Emergence was negligible where soil residual herbicides were applied. Bi-weekly emergence from conventional tillage plots was never greater than the emergence from no-tillage plots over the course of the whole growing season. Seasonally, seedling emergence from the no-tillage areas was 152% and 223% of the emergence from the conventional tillage treatment in 2014 and 2015, respectively. In Indiana, conventional tillage decreased overall seasonal waterhemp emergence in soybean and decreased in-season biweekly emergence.

Nomenclature: *Amaranthus tuberculatus* (Moq.) Sauer (syn. *rudis*) AMATA; soybean, *Glycine max* (L.) Merr.

Key words: no-tillage, conventional tillage, weed emergence patterns

3.2 Introduction

Waterhemp *(Amaranthus tuberculatus)* is a small-seeded broadleaf summer annual weed with a propensity to flourish in no-tillage and conservation tillage systems (Leon and Owen 2006; Refsell and Hartzler 2009). As conservation tillage practices have increased over the last 20 to 40 years, waterhemp has spread and become more problematic in many crop production areas (Culpepper 2006; Harmon 2015; Nice and Johnson 2005; Young 2006).

In various surveys conducted in Indiana from 1992-2005, crop producers were asked about which weeds were present in their fields and which weeds they perceived as “most problematic”. Waterhemp was not considered to be among the top ten most problematic weeds in Indiana in 1992, 1996 or 2000, but was included in the top ten in 2004 (Gibson et al. 2005; Nice and Johnson 2005). In nine districts surveyed across the state in December 2003, waterhemp was identified as a problematic weed in two of the nine districts by 12 and 13% of farmers, while in the other seven districts, 3% or less of farmers reported problematic levels of waterhemp (Gibson et al. 2005). These findings suggest that waterhemp was not a widespread problem in Indiana in 2003 and 2004, but was beginning to be recognized in areas where it was not previously an issue.

During this time period, changes in tillage practices were also being monitored. No-tillage, defined as, “any direct seeding system, including site preparation, with minimal soil disturbance” increased in Indiana from 9% to 26% and 8% to 57% of planted acres in corn *(Zea mays)* and soybean *(Glycine max)* from 1990-2015, respectively (Harmon 2015). Conservation tillage, defined as, “any system leaving at least 30% residue cover” increased in Indiana from 15% to 40% and 15% to 58% of planted acres in corn and soybean, respectively (Harmon 2015). The time period of when waterhemp began to be recognized as a problematic weed coincides with an increase in conservation tillage practices in Indiana.

Weed seed germination is influenced by temperature, light, soil moisture and the gaseous environment in the soil (Baskin and Baskin 1988; Egley 1986). Waterhemp does not readily germinate in cool temperatures, as Steckel et al. (2007) reported germination and emergence in field conditions did not occur until average soil temperatures of 12 C. Guo and Al-khatib (2003) and Steckel et al. (2004) investigated germination in controlled environments and recorded very little germination at an average temperature below 20 C. They also discovered that waterhemp germination increases when the seeds are exposed to diurnal fluctuations in temperatures as opposed to conditions with constant temperature. The top 3 cm of soil are subject to the greatest fluctuations in temperature, more so than soil deeper in the soil profile (Stoller and Wax 1973). Conservation tillage leaves weed seeds largely distributed on the soil surface, and waterhemp preferentially germinates and emerges from the top 3 cm of soil (Steckel et al. 2007). This phenomenon explains how waterhemp has grown in prevalence in conjunction with an increase in conservation tillage practices. In separate studies conducted in Iowa, waterhemp emergence was three to four times greater from no-tillage areas than from either moldboard plowed or chisel plowed areas (Leon and Owen 2006; Refsell and Hartzler 2009).

Crop canopy can also influence weed seed emergence patterns. Crop canopies can reduce the amount of light that reaches the soil surface and can decrease the amplitude of diurnal temperature fluctuations (Benech-Arnold et al. 1990; Thompson and Grime 1983). Kruk et al. (2006) demonstrated that light penetration and temperature fluctuations changed continually as the crop canopy developed, gradually reducing the amount of light penetration and decreasing the amplitude of the temperature fluctuations. This modification of the germination environment as a crop develops can influence when weed seeds germinate and emerge.

Understanding the emergence characteristics of waterhemp in a soybean environment and what variables may influence season-long emergence could help guide management decisions relating to timing of control. The objective of this study was to characterize the emergence pattern of waterhemp in soybean with varying tillage and herbicide programs in Indiana.

3.3 Materials and Methods

3.3.1 Field Plot Design

The experiment was conducted at the Meigs South Farm at the Throckmorton Purdue Agricultural Center (TPAC) near Lafayette, IN in 2014 and 2015. The soil type was a Starks-Fincastle complex (fine-silty, mixed, superactive, mesic Aeric Endoaqualf) with a pH of 6.5 and 2.3% organic matter. The trial was established on an area where soybean had been grown in 2013, and the same trial space was used in 2015. A burndown of 840 g ai ha^{-1} of paraquat (Gramoxone SL 2.0, Syngenta Crop Protection, LLC, Greensboro, NC 27419) was applied to control any existing vegetation prior trial initiation on May 8, 2014 and May 14, 2015.

The experiment was a split-plot design with two factors and four treatments. Tillage was the main plot and the herbicide treatment was the sub plot. Sub plots were randomized within the whole plot with four replications. The two tillage treatments were no-tillage and conventional tillage. Conventional tillage consisted of chisel plowing at 20 cm depth followed by a field cultivator at 10 cm depth prior to soybean planting. No tillage did not disturb the soil surface before planting. Chisel plowing was performed on December 9, 2013 and March 24, 2015. A late harvest and wet soil conditions did not allow for chisel plowing in the fall of 2014, therefore it was completed in March 2015. Field cultivating was performed on May 6, 2014 and May 14, 2015. Glufosinate-resistant soybean was planted throughout the 3 m by 10 m plot area in 76 cm rows at 345,000 seeds ha^{-1} using Beck 298NL (Beck's Hybrids, Atlanta, IN 46031) on May 8, 2014 and May 14, 2015. The first herbicide treatment consisted of a single POST application of glufosinate at 595 g ai ha^{-1} (Liberty 280 SL, Bayer CropScience, Research Triangle Park, NC 27709) 21 days after planting (DAP). The second was a PRE application of flumioxazin at 90 g ai ha^{-1} (Valor SX, Valent USA Corporation, Walnut Creek, CA 94596) followed by a POST of *s*-metolachlor at 1395 g ai ha^{-1} (Dual II Magnum, Syngenta Crop Protection, LLC, Greensboro, NC 27419) plus glufosinate at 595 g ai ha^{-1} 21 DAP. PRE applications were applied on May 8, 2014 and May 14, 2015 and POST applications were applied on May 29, 2014 and June 4, 2015. All applications were applied with a CO_2-pressurized sprayer through a 3 m boom with eight TeeJet XR11002 flat fan nozzles spaced 38 cm apart and calibrated to apply 140 L ha^{-1} at 0.14 MPa.

3.3.2 Data Collection and Analysis

Two 1 m^2 quadrats were established in the central two rows in each plot and were used for the duration of the study for monitoring waterhemp emergence. Newly emerged waterhemp seedlings were counted and manually removed in each established quadrat every 14 days and plots were monitored from planting until the R6 soybean growth stage. Observations occurred from May 22 to August 22 and from May 27 to September 4 in 2014 and 2015, respectively. Soil temperature and soil moisture were recorded at a 3 cm depth in both tillage treatments using Spectrum WatchDog 1400 series data loggers with SMEC-300 sensors, and rainfall was recorded with a Spectrum WatchDog 2700 weather station (Spectrum Technologies, 3600 Thayer Court, Aurora, IL 60504). Weather data collection began 22 DAP in 2014 due to equipment malfunction but was initiated 0 DAP in 2015. Growing degree day (GDD) values were calculated using air temperatures from the local TPAC weather station and were calculated as the mean of the minimum and maximum temperatures minus a base temperature of 10 C.

Data was analyzed with PROC GLM procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC 27513). Data was subject to square-root transformation data to improve normality. Year was found to be significant, therefore data was analyzed separately by year. Means were separated with Fisher's LSD at a $p = 0.05$ significance level.

3.4 Results and Discussion

3.4.1 Field Emergence

There were substantial differences in waterhemp seedling emergence between 2014 and 2015, which may be due to the recent establishment of waterhemp at the site, and the low densities that were present during the first year of research. Waterhemp emergence occurred for ten weeks after planting (WAP) in 2014 and twelve WAP in 2015, therefore biweekly and cumulative counts were analyzed for ten and twelve WAP in 2014 and 2015, respectively. Tillage did not affect the duration of waterhemp seedling emergence (Table 1). In both years, soil temperature was unaffected by tillage, where soil moisture was consistently lower in the conventionally tilled areas compared to the no-tillage areas (Figure 1 and Figure 2).

Regardless of tillage type, there was minimal waterhemp emergence when PRE flumioxazin (90 g ai ha^{-1}) followed by POST *s*-metolachlor (1395 g ai ha^{-1}) plus glufosinate (595 g ai ha^{-1}) 21 DAP was applied (data not shown). These treatments were excluded from the statistical analysis as there was insufficient count data when compared to the POST-only treatment of glufosinate (595 g ai ha^{-1}) 21 DAP.

In both years, during the biweekly counts, the number of emerged seedlings in the no-tillage plots was always greater than or equal to the number of emerged seedlings from the conventional tillage plots. (Table 1). Through both years, there was no clear trend observed in regard to when more seedlings may emerge in either tillage treatment or if either tillage treatment shifted the period of emergence. There was little emergence and no difference between the tillage treatments eight and ten WAP and beyond in 2014 and 2015, respectively.

In both years, with both high and low waterhemp densities, the cumulative number of emerged seedlings was greater from the no-tillage plots (Table 2). Seedling emergence from the no-tillage areas was 152% and 223% of the emergence from the conventional tillage treatment in 2014 and 2015, respectively. These results are similar with those of Leon and Owen (2006) and Refsell and Hartzler (2009), although they both recorded at least 300% more emergence from the no-tillage compared to conventional tillage.

3.4.2 Conclusion

Redistributing small-seeded weed seeds throughout more of the soil profile with tillage is known to move some of those seeds into conditions unfavorable for germination (Egley and Williams 1991). This relocation from the surface of the soil, where waterhemp is more likely to germinate and emerge, is likely the reason for decreased emergence from the conventional tillage treatment. This annual mixing and redistribution has not been shown to prolong the viability of seed in the seed bank (Steckel et al. 2007). Despite this, it has been suggested that when no further contributions to the seedbank occur, no-tillage may be an effective strategy to encourage the maximum amount of seed to germinate and would consequently reduce the seedbank as fast as possible (Egley and Williams 1990; Steckel et al. 2007). When seed additions occur frequently, tillage may be an effective strategy to decrease the number of seedlings that emerge in a growing season.

3.5 Acknowledgements

The authors would like to thank the members of the Integrated Weed Management labs at Purdue University for their help in completing this research and recognize the United Soybean Board for funding this work.

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Table 3.1: Mean number of emerged waterhemp seedlings m^{-2} and GDD accumulated since planting at various weeks after planting (WAP) in 2014 and 2015[a]

WAP	Tillage	2014		2015	
		Seedlings m^{-2}	GDD	Seedlings m^{-2}	GDD
2	No-tillage	21 a[b]	97	1092 a	131
	Conventional	11 b		792 a	
4	No-tillage	20 a	267	799 a	274
	Conventional	18 a		255 b	
6	No-tillage	11 a	539	999 a	467
	Conventional	5 b		222 b	
8	No-tillage	4 a	625	70 a	613
	Conventional	3 a		17 b	
10	No-tillage	0 a	777	44 a	796
	Conventional	0 a		15 b	
12	No-tillage	n.d.[c]	916	9 a	981
	Conventional	n.d.		5 a	

[a] Data were not pooled across years

[b] Means followed by the same letter within weeks and years are not different according to Fisher’s LSD at $p = 0.05$

[c] No data when there was no emergence for a single week

Table 3.2: Mean number of emerged waterhemp seedlings m^{-2} from each tillage system and total accumulated GDD since planting in 2014 and 2015[a]

	2014		2015	
	Seedlings m^{-2}	Total GDD	Seedlings m^{-2}	Total GDD
No-Tillage	58 a[b]	1260	3000 a	1314
Conventional Tillage	38 b		1345 b	

[a] Data were not pooled across years

[b] Means followed by the same letter within years are not different according to Fisher’s LSD at $p = 0.05$

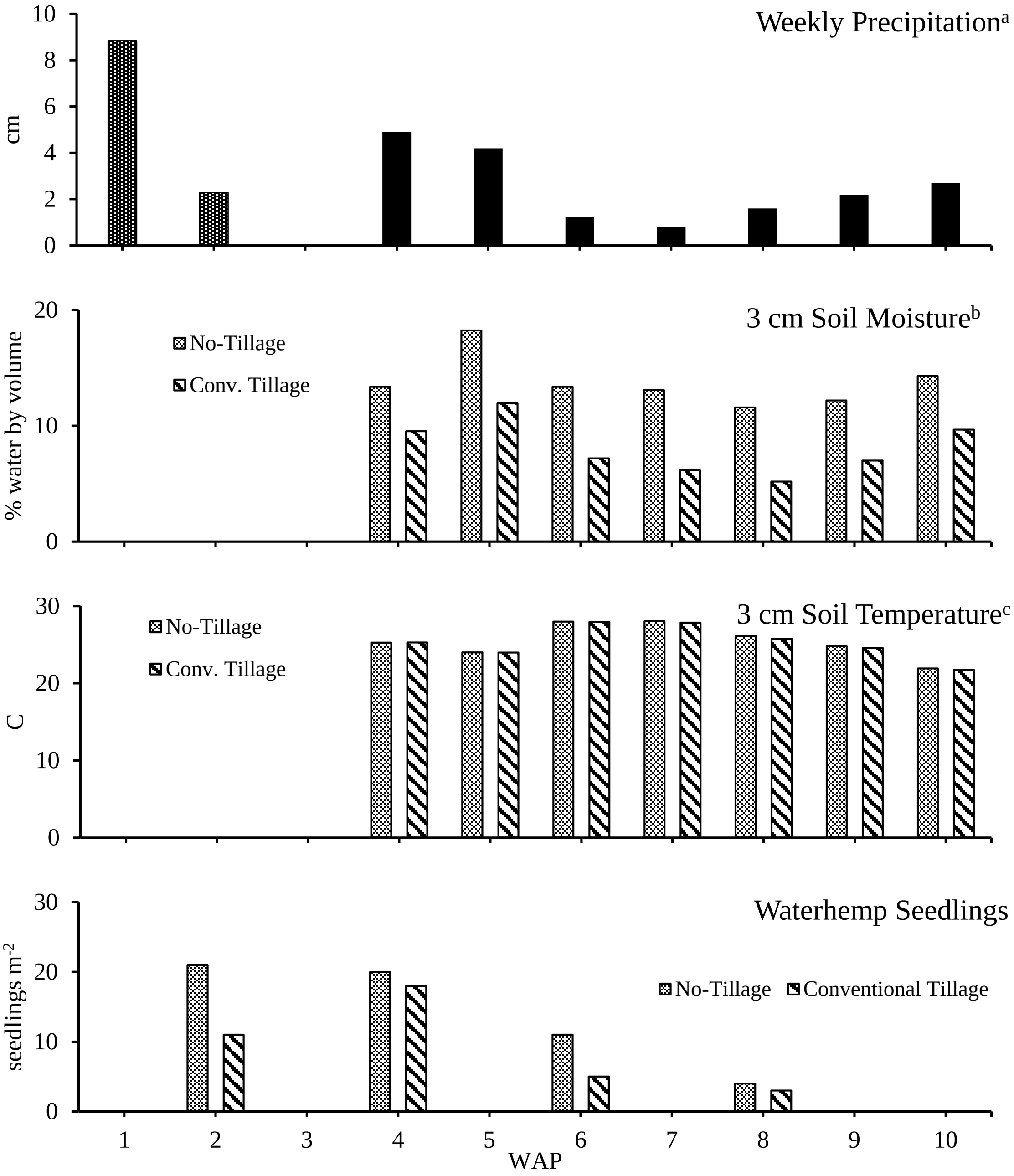

Figure 3.1: Waterhemp seedling emergence, precipitation, 3 cm soil moisture by volume, and 3 cm soil temperature for no-tillage and conventional tillage in a field study in Indiana in 2014.

[a] Regional weather station data used for 1-3 WAP due to field equipment malfunction

[b,c] 3cm soil moisture and temperature was not recorded for 1-3 WAP due to equipment malfunction

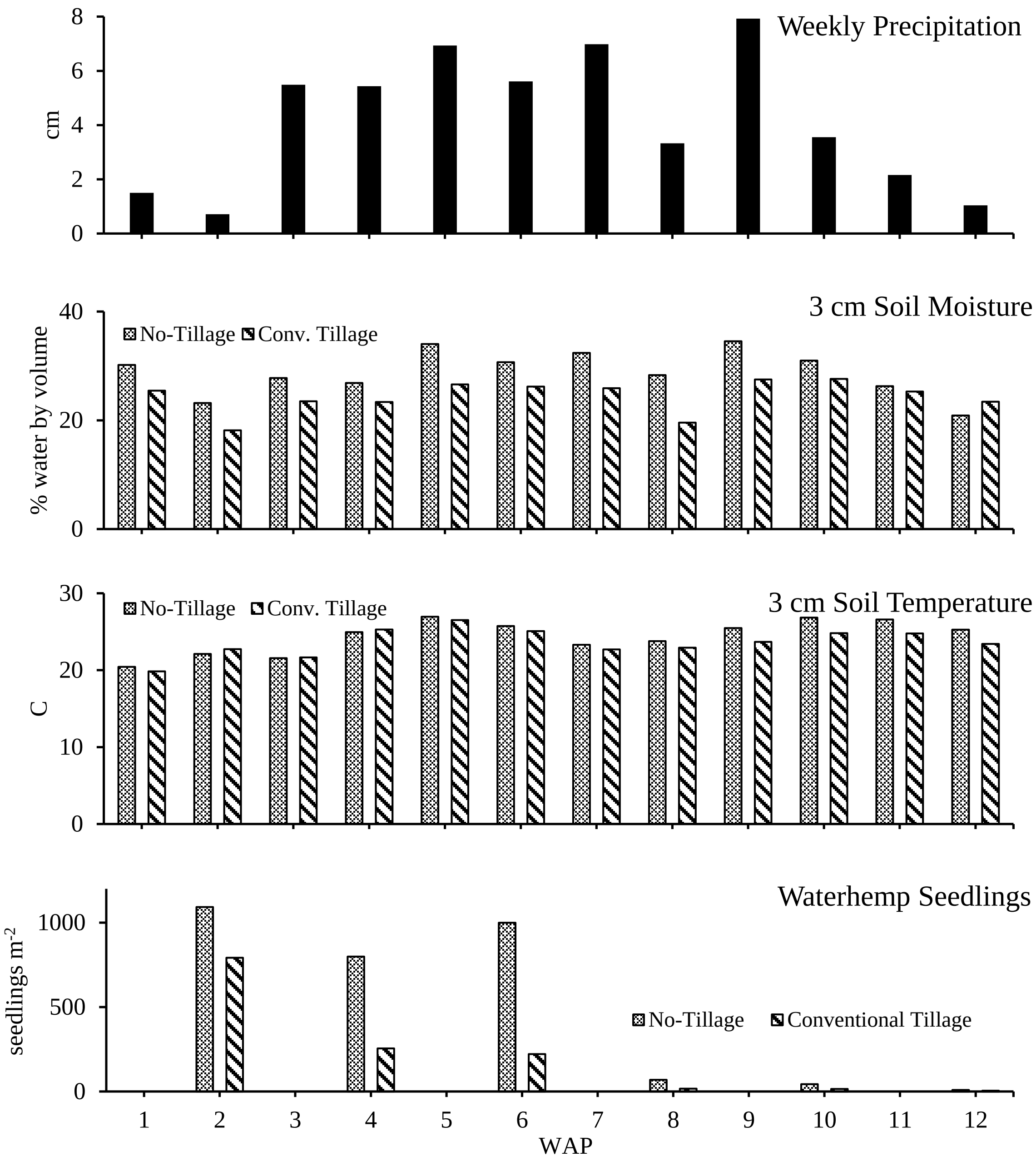

Figure 3.2: Waterhemp seedling emergence, precipitation, 3 cm soil moisture by volume, and 3 cm soil temperature for no-tillage and conventional tillage in a field study in Indiana in 2015.

CHAPTER 4. THE GROWTH AND DEVELOPMENT OF FIVE WATERHEMP *(AMARANTHUS TUBERCULATUS)* POPULATIONS IN A COMMON GARDEN

4.1 Abstract

Waterhemp is a weed indigenous to the Midwestern United States and is problematic in agronomic crop production. This weed is well-suited to inhabit minimally tilled environments and is increasing in prevalence across many agricultural production areas and systems. A common garden experiment was established in Indiana 2014 and 2015 with waterhemp populations from Indiana, Illinois, Missouri, Iowa, and Nebraska to compare the growth and development of waterhemp from these regions. Three establishment dates (May, June, and July) were used each year to simulate discontinuous germination. Mean biomass accumulations from the May (1,120 g plant^{-1}) and June (1,069 g plant^{-1}) establishment dates were higher than from the July (266 g plant^{-1}) establishment date. There were no differences in biomass accumulations between the five populations in the May and June establishment, but ranged from 195 to 338 g plant^{-1} in the July establishment. Mean seed yields were higher from the May (926,629 seeds plant^{-1}) and June (828,905 seeds plant^{-1}) establishment dates compared to the July (276,258 seeds plant^{-1}) establishment. In the May and June establishment, seed yields ranged from 469,939 seeds plant^{-1} to 1,285,556 seeds plant^{-1}. The Illinois population flowered the latest of all the populations yet also grew the tallest. The July establishment flowered the quickest after establishment, accumulated less biomass, and also had the largest seeds.

This study demonstrated differences among waterhemp populations when grown in a common environment and the effect of establishment timing on waterhemp growth and development.

Nomenclature: *Amaranthus tuberculatus* (Moq.) Sauer (syn. *rudis*) AMATA;
Key words: Phenology, photoperiod, male:female ratio, growth modeling

4.2 Introduction

Waterhemp (*Amaranthus tuberculatus)* is a summer annual weed that is indigenous to the Midwestern United States and is becoming more prevalent and problematic in agronomic crop production (Hager et al. 2002; Sauer 1967). Waterhemp's increasing prevalence is partially due to its tendency to emerge from the soil surface and an increase in conservation tillage (Culpepper 2006; Givens et al. 2009; Leon et al. 2006). Two waterhemp species are recognized in the Midwest, *Amaranthus rudis* and *A. tuberculatus*, but these two species are only differentiated by miniscule differences in their seed morphology; therefore it has been proposed that these two species are to be wholly considered as one (Pratt and Clark 2001).

Waterhemp is a dioecious plant, and is consequently an obligate outcrosser, possessing great genetic diversity (Costea et al. 2005; Steckel 2007). The sex ratio of waterhemp and other *Amaranthus* species is variable. Pratt and Clark (2001) report up to a 1:10 ratio of male:female waterhemp plants in Ohio. Bram and Quinn (2000) observed a 1:1 ratio in salt-marsh waterhemp (*A. cannabinus)* in New Jersey until late in the year, when a higher proportion of female plants would occur, but was attributed to male plants having already senesced. Menalled et al. (2004) even observed that the addition of swine compost in a field experiment in Iowa slightly increased the proportion of male waterhemp plants, implying some potential for plasticity. Flowering timing of waterhemp is likened to that of other *Amaranthus* species, in that it is a facultative short-day plant, which flowers in relation to photoperiod (Huang et al. 2000; Wu and Owen 2014).

Waterhemp is notorious for exhibiting a discontinuous germination pattern that extends well into the growing season, beyond the time period of many other summer annual weeds which infest agronomic crops (Hager et al. 1997; Hartzler et al. 1999; Leon et al. 2006). This season-long emergence often allows waterhemp to grow after all weed management actions have been completed, and can oftentimes lead to contributions to the soil seedbank (Brewer and Oliver 2007; Hartzler et al. 2004; Steckel and Sprague 2004). When allowed to develop for a full growing season, waterhemp has demonstrated the ability to produce in excess of one million seeds plant^{-1} in Illinois as Steckel et al. (2003) reported, with Hartzler et al. (2004) reporting an occurrence of over 4.8 million seeds plant^{-1} in Iowa, and in Missouri, Sellers et al. (2003) reported 288,950 seeds plant^{-1} with a slightly delayed start to the season. This high level of fecundity continually adds seeds to the seedbank and ensures their presence, despite only persisting for four to five years in the soil (Buhler and Hartzler 2001; Steckel et al. 2007). Waterhemp seeds are also among the smallest in the *Amaranthus* family. Sellers et al. (2003) reported 3,670 seeds g^{-1} from waterhemp; 32-105% more seeds g^{-1} than the other five *Amaranthus* species in their study.

These biological measures provide a reference to the potential behavior of waterhemp, but the environmental effects of varying locations and seasons do not allow for an absolute reference between all these studies. A common garden experiment which studies multiple populations of interest in a single location can control for these environmental effects and allow for direct comparisons between populations of interest and can help demonstrate inherent differences between populations (Dorman et al. 2009).

The growing issue of managing waterhemp in agronomic crops and the wide geographic spread of waterhemp across the Midwestern United States creates the need for continued investigation into waterhemp growth and development across this geography. Establishing a common garden that includes various waterhemp populations from across the Midwest would allow for concrete comparisons between different populations and may reveal inherent differences in these populations due to local adaption and selection pressures. The objective of this experiment was to establish a common garden with waterhemp populations from Indiana, Illinois, Missouri, Iowa, and Nebraska, USA, and measure their growth and development characteristics in Indiana.

4.3 Materials and Methods

4.3.1 Field Plot Design

The experiment was conducted at the Throckmorton Purdue Agricultural Center (TPAC) near Lafayette, IN in 2014 and 2015. The research area soil type consisted of a Throckmorton silt loam (fine-silty, mixed, superactive, mesic Mollic Oxyaquic Hapludalfs) and an Octagon silt loam (fine-loamy, mixed, active, mesic Mollic Oxyaquic Hapludalfs). The soil pH was 6.7 and 6.9 and organic matter content was 3.0% and 2.9%

in 2014 and 2015, respectively. The 2015 trial area was immediately adjacent to the 2014 trial area to avoid volunteer waterhemp plants.

The experiment was arranged as a split plot design with planting date as the main plot and population as the sub-plot. Three planting dates were used each season in order to simulate a discontinuous emergence pattern: initial spring emergence, 21 days before summer solstice and 21 days after summer solstice. In 2014 the planting dates were May 12, June 2 and July 14. In 2015 the planting dates were May 4, June 1 and July 13. Five populations from different states were used in this experiment; Benton County, IN, Jackson County, IL, Randolph County, MO, Story County, IA and Clay County, NE. Plot size was 3 m by 10 m with three rows of waterhemp planted with 61 cm row spacing, centered within the plots. In order to facilitate successful germination and emergence, the seedlings were initiated in a greenhouse on the previously mentioned planting dates using peat pellets (No. 736 Jiffy Peat Pellets, Hummert International, Earth City, MO) and thinned down to three plants per pellet after emergence. The pellets were then transplanted to the field at the 2 to 4 leaf stage 12 to 15 days after initiation. The seedlings were transplanted to the field by making a 5 cm wide by 5 cm deep cylindrical hole in the soil and then placing the entire peat pellet in the hole and covering it with soil. Transplants were watered at the time of transplanting and were thinned down to thirty plants per plot, or ten plants per row, after successful establishment. The plot areas were weed free at trial initiation and were maintained weed free by mechanical cultivation once the waterhemp plants were established.

4.3.2 Data Collection

Weekly data collection included measuring waterhemp plant heights and monitoring flower emergence. To record plant height, twelve non-adjacent plants, where possible, were permanently marked and the height of these plants was recorded weekly. The height measurements were conducted from transplanting until one week after flowering was complete. The number of flowered plants was also recorded weekly and calculated as a percentage of plants that had flowered. At the completion of flowering, the male to female ratio was recorded in each plot as determined by the reproductive flower structures on each plant. At the conclusion of the growing season, the aboveground biomass from three representative female plants from each plot was harvested four weeks after the collective average of the plots in that planting had surpassed 90% flowering, allowing time for seed maturation yet maintaining seed retention on the plant. Plant biomass and seed yield were quantified from the harvested plants. Plant material was dried in a greenhouse with air temperatures of 20 to 35 C until weight was consistent. Whole plant biomass was recorded, and seed was threshed from the plants, passed through screens, and further cleaned with a vertical column seed blower until foreign matter was removed. The total weight of the seed lots was recorded and three subsamples each consisting of 100 seeds were counted and weighed and the average of these measurements was used to determine the number of seeds g^{-1} and to extrapolate the number of seeds $plant^{-1}$.

4.3.3 Data Analysis

Male to female ratios were compared to an expected 50:50 ratio with a Pearson's chi-square test at $p = 0.05$ with df=1 and $\chi^2 = 3.841$. The remaining analysis was performed with SAS 9.3 (SAS Institute, Cary, NC 27513). Plant biomass and seed yield data were subject to an ANOVA using PROC MIXED at a $p = 0.05$ significance level. Plant biomass data were untransformed, where seed yield data was subject to square-root transformation to achieve normality assumptions. Year was not found to be significant, therefore data were pooled across years. Growing degree day (GDD) values were calculated for the flowering timing data as the mean of the minimum and maximum temperatures minus a base temperature of 10 C using weather from the TPAC local weather station.

Plant height data were fit to a four-parameter growth model:

$$y = c + \frac{d-c}{1+\exp[b*(x-i50)]} \qquad [1]$$

Where y = height (cm) at time x (week), c = maximum height (cm), d = minimum height (cm), $i50$ = inflection point, or time to reach 50% of the final height (weeks after transplanting), and b = rate of change about $i50$. PROC NLMIXED was used to estimate variable values for each individual plot. Model fit was evaluated with root mean square error (RMSE) and modeling efficiency coefficients (EF) (Sarangi et al. 2016). RMSE was calculated with the following equation:

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2\right]^{\frac{1}{2}} \qquad [2]$$

where P_i is the model-predicted value, O_i is the observed value, and n is the number of observations. EF was used instead of R^2 because of the high bias that R^2 calculations have towards highly parameterized models (Sarangi et al. 2016, Spiess and Neumeyer 2010). EF values were calculated with the following equation:

$$EF = 1 - \left[\sum_{i=1}^{n}(O_i - P_i)^2 / \sum_{i=1}^{n}(O_i - \bar{O}_i)^2\right] \qquad [3]$$

where O_i is the observed value, P_i is the model-predicted value, $\bar{O}_i$ is the mean observed value, and n is the number of observations. EF values can range from −∞ to 1, with values closer to 1 indicating a better model fit and more accurate estimates.

Once parameters were estimated, PROC MIXED was used to conduct an ANOVA on the maximum height *(c)*, inflection point *(i50)*, and rate of change *(b)* at a $p = 0.05$ significance level.

4.4 Results and Discussion

4.4.1 Flowering Timing

Waterhemp plants established in May initiated flowering earlier in 2014 compared to 2015, despite a 10 day delay in planting in 2014 than in 2015. In both years, all populations had exceeded 94% flowering by August 14th, with 1258 and 1353 GDD accumulated since March 1st in 2014 and 2015, respectively (Table 1 and Table 2). In both years, the Illinois population was the slowest population to initiate flowering and to achieve 90% flowering.

Waterhemp plants established in June also initiated flowering sooner in 2014 with similar planting dates and GDD accumulations. Despite the earlier start in 2014, flowering from all populations in both years had surpassed 85% by August 14th and 95% by August 21st, with comparable GDD accumulations in both years (Table 1 and Table 2).

Waterhemp plants established in July initiated flowering by August 14th with 1258 and 1353 GDD accumulated in 2014 and 2015, respectively (Table 1 and Table 2). Flowering had exceed 99% by the August 28th observation in 2014 but did not exceed 99% until the September 4th observation in 2015 (Table 1 and Table 2). The July establishment date flowered the soonest after planting and progressed rapidly, presumably induced by late-season environmental triggers such as a shifting photoperiod and decreasing temperatures (Huang et al. 2000; Steckel et al. 2003).

4.4.2 Male to Female Ratio

The male to female ratio was analyzed by establishment date and also as a combined data set. There were few deviations from the expected 50:50 male to female ratio. The Nebraska population established in May and the Illinois population established in June both had a 59:41 male to female ratio, with a *p*-value of 0.039 and 0.005, respectively (Table 3). The Illinois population overall also contained a higher percentage of male to female plants at a 54:46 male to female ratio with a *p*-value of 0.029 (Table 3). This appears to be heavily influenced by the high number of male plants in the June establishment date. All other populations and planting dates did not differ from the expected 50:50 male to female ratio, suggesting no widespread or inherent bias towards either male or female waterhemp plants.

4.4.3 Plant Biomass

Biomass accumulations were analyzed within establishment date as well as across establishment dates, allowing for comparisons among waterhemp populations and time. Plant biomass accumulation within a planting date was only influenced by the waterhemp population in the July establishment date. The Missouri population, with the greatest biomass accumulation, amassed 58% more biomass than the Iowa population, which accumulated the least, with 338 g and 195 g of biomass, respectively (Table 4). Biomass accumulation was drastically reduced in the July establishment date compared to the May or June establishment date. Combining all populations together, the May and June establishment dates accumulated 1120 g and 1069 g of plant biomass, respectively, compared to only 266 g of plant biomass from the July planting date (Table 5). This is due largely to the shorter season and rapid initiation of flowering, and a consequential decrease in vegetative growth from the plants from the July establishment date.

4.4.4 Seed Yield

Seed yield was also analyzed within establishment date as well as across establishment dates. The Indiana and Missouri waterhemp populations yielded the fewest seeds plant^{-1} in the May establishment dates, with a mean yield of 469,939 and 734,651 seeds plant^{-1}, respectively (Table 4). The Nebraska, Illinois and Iowa waterhemp populations yielded the most seeds plant^{-1} in the May establishment dates, with a mean yield of 1,285,556, 1,147,865 and 976,097 seeds plant^{-1}, respectively (Table 4). In the June establishment date, with the exception of the Indiana population, all the other populations were similar in seeds plant^{-1} yield with a range of 793,226 to 1,011,096 seeds plant^{-1} (Table 4). The Indiana waterhemp population yielded the fewest seeds plant^{-1} in the June establishment with a mean yield of 553,594 seeds plant^{-1} (Table 4). In the July establishment date, yields ranged from 204,924 to 338,545 seeds plant^{-1} (Table 4).

When comparing seed yields plant^{-1} across planting dates, the mean yields of 926,629 and 828,905 seeds plant^{-1} from the May and June establishment dates were higher than the yield of 276,258 seeds plant^{-1} from the July establishment date (Table 5). With seed production in excess of one million seeds plant^{-1} from multiple populations in both the May and June establishment dates, the seed yield potential of waterhemp in a full-season setting is undoubtedly a key component of its success as a weed. In addition to this, when considering the late-season July establishment, every population was able to produce greater than 200,000 seeds plant^{-1}. The seed yields from all of these establishment dates demonstrate the prolific seed production capabilities of waterhemp, regardless of when in the season the plant germinates.

Seed size was also quantified, and when comparing across planting dates, seed size was similar in the May and June establishment dates, with a mean 4,473 and 4,504 seeds g^{-1}, respectively (Table 5). The July establishment date produced the largest seeds, with a mean of 3,799 seeds g^{-1}, a number comparable to that reported by Sellers et al. (2003) (Table 5). Within establishment dates, there was no difference in seeds g^{-1} from the populations established in May. The June establishment date ranged from 4,039 to 4,846 seeds g^{-1} and the July establishment date ranged from 3,363 to 4,160 seeds g^{-1}

(Table 4) Despite the drastically lower seed yield plant^{-1} from the July establishment date, the largest seeds were produced by plants from this timing. This phenomenon could be due to the rapid shift from vegetative growth to reproductive growth induced by late-season environmental effects (Steckel et al. 2003; Vengris 1963).

4.4.5 Growth Modeling

The growth of the five populations was modeled for each establishment date using a four-parameter logistic equation. Analysis was separated by establishment date. RMSE values ranged from 208.2 to 1042.8 and EF values ranged from 0.74 to 0.95, signaling a good model fit (Table 6). Dependent on the nature of the measurement, Sarangi et al. (2016) obtained RMSE values of 7.2 to 9,000, with EF values of 0.43 to 0.90, citing high variability leading to the highest RMSE values.

In both the May and June establishment dates, the Illinois population had the greatest estimated maximum height at 302 cm and 349 cm, respectively (Table 6). The Illinois population was also estimated to have the greatest *i50*, at 10.4 weeks and 8.8 weeks after transplanting, respectively, indicating the Illinois population took the greatest amount of time to reach half of its maximum height, despite being the tallest population (Figure 1). This relatively slow yet steady increase in height may be related to the delayed flowering and extended vegetative growth also observed in the Illinois population (Table 1 and Table 2).

In the July establishment, all populations took between 3.3 and 4.2 weeks to reach *i50*, the smallest range of any establishment date (Table 6). The Indiana, Illinois, and Missouri populations predicted *c* values are considerably greater than the Iowa and

Nebraska populations at the July establishment date, and the Iowa and Nebraska populations were observed to flower sooner, especially in 2015 (Table 1 and Table 2). The EF values in the July establishment were the lowest of all three establishments, and this relatively poorer fit is believed to be due to more plant height variability that was observed later in the growing season.

4.4.6 Conclusions

The male to female ratio of waterhemp was largely observed to be a 50:50 ratio in all the populations, despite fluctuations in initiation and duration of flowering among populations. In the May and June establishments there were no differences in the biomass production, however there were differences in seed production, plant height, and growth rate. Seed production from the May and June establishment, which ranged from 469,939 to 1,285,566 seeds plant^{-1}, demonstrated the high fecundity of multiple waterhemp populations when allowed to grow in a common environment with minimal competition. The July establishment date yielded smaller plants with less biomass and seed production, but all populations were still able to produce in excess of 200,000 seeds plant^{-1}. The short season experienced by the waterhemp from the July establishment demonstrated the most uniform flowering and highest *b* of all establishment dates, indicating quick and rapid growth.

These populations exhibited differences in their phenology when they were grown in the same environment, indicating inherent genetic differences and further demonstrating the variability and growth potential of waterhemp.

4.5 Acknowledgements

The authors would like to thank the members of the Integrated Weed Management labs at Purdue University for their help in completing this research. The authors would also like to thank the Purdue Statistical Consulting Service, and particularly Nathan Hankey for his assistance. The authors would like to recognize the United Soybean Board for funding this work.

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Table 4.1: Percentage of flowered plants by date and GDD accumulation from five waterhemp populations grown at a single Indiana location and established on three different dates in 2014.

		Date									
		6/26	7/3	7/10	7/17	7/24	7/31	8/7	8/14	8/21	8/28
		GDD									
Establishment Date	Population	709	807	886	959	1031	1097	1179	1258	1335	1441
		%									
May 14	Indiana	0	1	23	56	80	90	98	100	100	-
	Illinois	0	0	1	8	23	45	87	94	97	-
	Missouri	1	1	16	46	76	86	98	99	99	-
	Iowa	7	11	54	77	92	96	100	100	100	-
	Nebraska	6	18	57	85	94	99	100	100	100	-
June 2	Indiana	-	0	0	18	33	62	96	100	100	-
	Illinois	-	0	0	0	2	5	67	85	98	-
	Missouri	-	1	1	7	15	39	91	99	99	-
	Iowa	-	0	10	36	63	81	98	100	100	-
	Nebraska	-	10	14	38	60	81	99	100	100	-
July 14	Indiana	-	-	-	-	-	-	0	0	96	100
	Illinois	-	-	-	-	-	-	0	0	68	99
	Missouri	-	-	-	-	-	-	0	2	91	100
	Iowa	-	-	-	-	-	-	0	7	99	100
	Nebraska	-	-	-	-	-	-	0	5	90	100

Table 4.2: Percentage of flowered plants by date and GDD accumulation from five waterhemp populations grown at a single Indiana location and established on three different dates in 2015.

		Date								
		7/10	7/17	7/24	7/31	8/7	8/14	8/21	8/28	9/4
		GDD								
Establishment Date	Population	900	988	1085	1184	1267	1353	1444	1509	1605
		%								
May 4	Indiana	0	33	49	96	99	100	100	-	-
	Illinois	0	1	1	35	79	96	99	-	-
	Missouri	0	15	34	96	99	99	99	-	-
	Iowa	0	58	73	99	99	95	95	-	-
	Nebraska	0	41	63	93	95	98	98	-	-
June 1	Indiana	-	0	3	77	99	100	100	-	-
	Illinois	-	0	0	5	44	91	100	-	-
	Missouri	-	0	1	52	91	99	100	-	-
	Iowa	-	0	2	79	100	100	100	-	-
	Nebraska	-	4	6	75	97	98	98	-	-
July 13	Indiana	-	-	-	-	0	15	56	79	100
	Illinois	-	-	-	-	0	0	9	46	100
	Missouri	-	-	-	-	0	13	50	73	100
	Iowa	-	-	-	-	0	44	89	92	100
	Nebraska	-	-	-	-	0	34	68	86	100

Table 4.3: Observed male to female ratio from five waterhemp populations grown in a single Indiana location in 2014 and 2015.

Population	Establishment Timing[a]	Number of Males	Number of Females	Male: Female Ratio	Expected Ratio	χ^2 test statistic[b,c]	*p*-value[c]
Indiana	May	94	114	45:55	50:50	1.735	0.188
	June	129	105	55:45		2.261	0.133
	July	111	123	47:53		0.517	0.472
	Combined	334	342	49:51		0.072	0.788
Illinois	May	118	110	52:48	50:50	0.215	0.642
	June	142	98	59:41		7.704*	0.005*
	July	119	111	52:48		0.213	0.644
	Combined	379	319	54:45		4.987*	0.026*
Missouri	May	109	127	46:54	50:50	1.225	0.268
	June	131	102	56:44		3.365	0.067
	July	114	111	51:49		0.018	0.893
	Combined	354	340	51:49		0.224	0.636
Iowa	May	121	111	52:48	50:50	0.349	0.554
	June	113	102	53:47		0.465	0.495
	July	103	105	50:50		0.004	0.949
	Combined	337	318	52:48		0.494	0.482
Nebraska	May	100	72	59:41	50:50	4.238*	0.039*
	June	105	79	57:43		3.397	0.065
	July	85	100	45:55		1.059	0.303
	Combined	290	251	54:46		2.669	0.102

[a] Data were pooled across years

[b] χ^2 test statistic were calculated with df = 1 and used a critical value of $\chi^2 = 3.841$.

[c] χ^2 test statistic and *p*-vales followed by an asterisk (*) are significant at a $p = 0.05$ level.

Table 4.4: Mean plant biomass and seed yield for five waterhemp populations grown in a single Indiana location in 2014 and 2015.

Establishment Timing[a]	Population	g biomass plant^{-1}		seeds plant $^{-1}$		seeds g^{-1}	
May	Indiana	1004	NS[b]	469939	c[c]	4557	NS
	Illinois	1267	NS	1147865	a	4440	NS
	Missouri	1051	NS	734651	bc	4507	NS
	Iowa	1152	NS	976097	ab	4073	NS
	Nebraska	1125	NS	1285566	a	4861	NS
June	Indiana	1128	NS	553594	b	4502	a
	Illinois	998	NS	919166	a	4415	ab
	Missouri	1059	NS	793226	a	4846	a
	Iowa	973	NS	921913	a	4039	b
	Nebraska	1185	NS	1011096	a	4805	a
July	Indiana	274	b	278103	ab	4160	a
	Illinois	283	ab	338545	a	4055	ab
	Missouri	338	a	328408	a	3670	b
	Iowa	195	c	204924	b	3363	c
	Nebraska	238	bc	231311	b	3805	ab

[a] Data were pooled across years

[b] Non-Significant (NS) population effect

[c] Means followed by the same letter, within establishment date and response variable, are not different at a $p = 0.05$ significance level.

Table 4.5: Mean plant biomass and seed yield for three establishment dates of waterhemp grown in Indiana.

Establishment Timing[a]	g biomass plant^{-1}		seeds plant^{-1}		seeds g^{-1}	
May	1120	a[b]	926629	a	4473	a
June	1069	a	828905	a	4504	a
July	266	b	276258	b	3799	b

[a] Data were pooled across years

[b] Means followed by the same letter, within response variable, are not different at a *p* = 0.05 significance level.

Table 4.6: Estimated maximum height, inflection point, rate of change and goodness of fit (RMSE and EF)[a] from a four-parameter logistic function[b] for five waterhemp populations grown in a single Indiana location in 2014 and 2015.

Establishment Timing[c]	Population	*c*[d]	*i50*[d]	*b*[d]	RMSE	EF
May	Indiana	225 bc	8.4 bc	0.53 b	497.8	0.92
	Illinois	302 a	10.4 a	0.41 c	555.8	0.90
	Missouri	265 ab	8.7 bc	0.46 c	362.8	0.95
	Iowa	217 cd	7.7 cd	0.58 b	436.3	0.93
	Nebraska	189 d	7.4 d	0.67 a	857.9	0.83
June	Indiana	253 bc	6.9 bc	0.62 a	416.3	0.93
	Illinois	349 a	8.8 a	0.49 b	338.4	0.94
	Missouri	282 bc	7.5 bc	0.54 b	509.2	0.92
	Iowa	231 c	6.8 bc	0.64 a	673.7	0.89
	Nebraska	224 c	6.3 c	0.66 a	1042.8	0.82
July	Indiana	126 a	3.6 bc	1.17 b	334.6	0.83
	Illinois	133 a	4.2 a	1.11 b	208.2	0.89
	Missouri	128 a	3.7 b	1.16 b	315.7	0.84
	Iowa	97 b	3.3 d	1.35 a	411.4	0.76
	Nebraska	99 b	3.6 cd	1.24 ab	463.3	0.74

[a] Abbreviations: RSME, root mean square error; EF, modeling efficiency coefficient.

[b] $y = c + (d - c)/(1 + \exp[b * (x - i50)])$, where y = height (cm) at time x (weeks after transplanting), c = maximum height (cm), d = minimum height (cm), $i50$ = inflection point, or time to reach 50% of the final height (weeks after transplanting), and b = rate of change about $i50$.

[c] Data were pooled across years

[d] Values followed by the same letter, within establishment date and response variable, are not different at a $p = 0.05$ significance level.

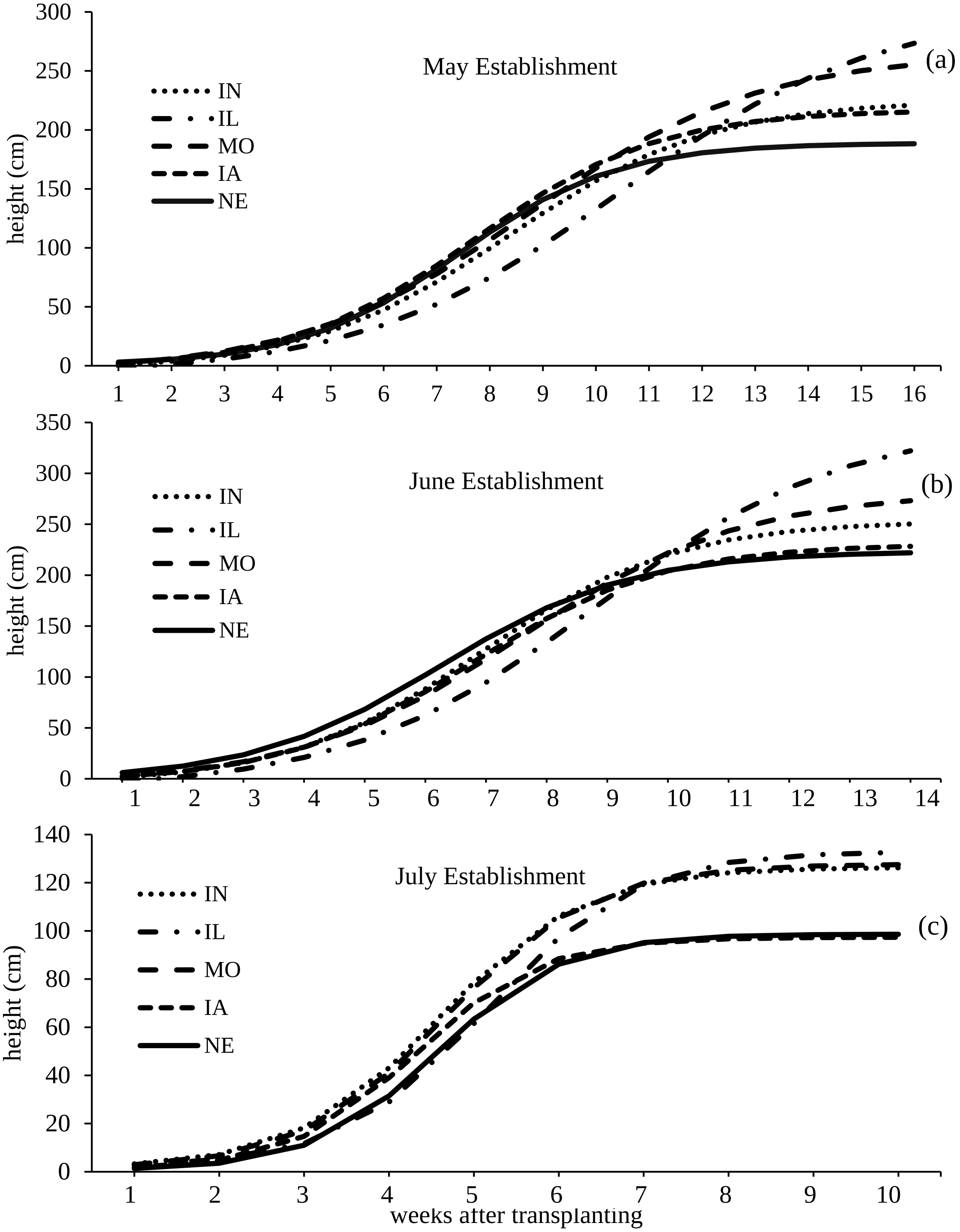

Figure 4.1: The four-parameter logistic modeled growth curves (Equation 1) of five waterhemp populations from (a) May, (b) June, and (c) July, grown in Indiana.