BIOLOGY AND MANAGEMENT OF MULTIPLE- (GLYPHOSATE, ALS, AND ATRAZINE) RESISTANT PALMER AMARANTH IN MICHIGAN

By

Jonathon Kohrt

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences – Doctor of Philosophy

2017

ABSTRACT

BIOLOGY AND MANAGEMENT OF MULTIPLE- (GLYPHOSATE, ALS, AND ATRAZINE) RESISTANT PALMER AMARANTH IN MICHIGAN

By

Jonathon Kohrt

Palmer amaranth (*Amaranthus Palmeri* S. Wats.) was first identified in Michigan in 2010, it is a non-native pigweed species that has been detrimental to row crop production throughout the southern and Great Plains regions of the United States. In 2013, the failure of atrazine to control a suspected glyphosate- and ALS-resistant Palmer amaranth population identified in Barry County, MI prompted further investigation into the possibility that this Palmer amaranth population was resistant to three different herbicide sites of action. Field, greenhouse, and laboratory experiments were conducted in 2013, 2014, and 2015 to quantify the levels of resistance, identify the mechanisms, study the biology, and identify possible management strategies for Palmer amaranth in Michigan corn production systems. The resistance factor (RF) values for the suspected multiple-resistant Barry County (MR) Palmer amaranth population were 12, 43, and 9X for POST applications of glyphosate, thifensulfuron, and atrazine, respectively, compared with a known susceptible population. The MR population was also highly resistant to PRE applications of atrazine, RF = 112X. These results confirmed that this population was resistant to three different herbicide sites of action. Laboratory experiments identified target-site based resistance for glyphosate and the ALS-inhibiting herbicides via gene amplification and amino acid substitution, respectively. Resistance to atrazine was not target-site mediated, with no observed nucleotide substitutions within the *psbA* gene, leading us to believe that atrazine resistance in this population may be metabolism-based. In the field, sole reliance on a PRE or single site of action POST herbicide application did not provide season-long control of Palmer

amaranth. Several one-pass EPOS weed management programs effectively managed multiple-resistant Palmer amaranth. However, these programs must contain at least two effective herbicide sites of action with foliar activity tank-mixed with a residual herbicide for season-long control. The most consistent and effective management strategies for control of this multiple-resistant Palmer amaranth population in corn were two-pass herbicide programs, PRE followed by POST. These strategies included at least one effective herbicide site of action PRE and two effective foliar sites of action POST plus a soil residual herbicide for season-long Palmer amaranth control. The HPPD-inhibiting herbicides will be a major component of a Palmer amaranth management program in corn. The effectiveness of the POST HPPD-inhibiting herbicides for Palmer amaranth control were tolpyralate > tembotrione = topramezone > mesotrione. The addition of atrazine to mesotrione and tembotrione was synergistic for Palmer amaranth control. This suggests that even in an atrazine-resistant Palmer amaranth population the addition of atrazine to some of the HPPD-inhibiting herbicides would be beneficial for Palmer amaranth control, especially as Palmer amaranth size increases. In a three year crop rotation experiment, Palmer amaranth emergence started at ~281 GDD_{10} (late May/early June) and did not cease until September in central Michigan. The total number as well as the duration of Palmer amaranth emergence was greater in corn than in soybean. The initial growth rate of Palmer amaranth was greatest for early emerging cohorts in corn, however those that emerged 2 wks later in the season were more competitive in soybean. Seed production declined with each successive cohort and was greatest for early emerging Palmer amaranth in soybean with >64,000 seeds $plant^{-1}$. Palmer amaranth that emerged in August after wheat harvest produced seed that added to the soil seedbank. When corn is included in the rotation the availability of more

effective herbicide sites of action paired with reduced Palmer amaranth growth and lower seed production can lead to greater success in managing resistant Palmer amaranth populations.

ACKNOWLEDGEMENTS

I would like to thank Dr. Christy Sprague for giving me the opportunity to be a part of the weed science team and for serving as major advisor. Her guidance has been invaluable in both educational and professional development throughout graduate studies here at Michigan State University. I would also like to thank Dr. Karen Renner, Dr. Christina DiFonzo, and Dr. Kurt Steinke for serving on my guidance committee.

I would like to give special thanks to Gary Powell and Erin Hill for their assistance in the setup and design of the field and greenhouse aspects of my project. This research would have been very difficult to conduct without the help of my fellow graduate students: Dr. David Powell, Amanda Harden, Amanda Goffnett, and Kelsey Rogers. It would have been impossible to complete this research without the help from all of the undergraduates: Megan Tomlin, Chris Bauer, Chelsea Bonthuis, Kalvin Canfield, R.J. Lee, Taylor Truckey, Kelly White, Dennis Erickson, Jerrid Smith, Cody Tyrell, Mike Schulze, Jake Hall, Mitchell Ackerman, Haonan Qi, and Matt Singer. Special thanks to the Michigan Corn Marketing Board and Project GREEEN for the funding to conduct this research.

I also owe a great deal of gratitude to my friends and family. Without their support none of this would have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

REVIEW OF LITERATURE

Introduction

The genus *Amaranthus* is comprised of over 70 species both native and non-native to the United States (U.S.). However, only a select few are problematic in U.S. crop production systems. The most common species are redroot pigweed (*Amaranthus retroflexus* L.), Powell amaranth (*Amaranthus powellii* S. Wats.), spiny amaranth (*Amaranthus spinosus* L.), smooth pigweed (*Amaranthus hybridus* L.), common waterhemp (*Amaranthus tuberculatus* Moq. Sauer.), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Bensch et al. 2003; Knezevic et al. 1994; Gossett and Toler 1999; Grichar 1994; Hager et al. 2002; Massinga et al. 2001; Moolani et al. 1964; Schweizer and Lauridson 1985; Toler et al. 1996). The majority of these detrimental species are monoecious (male and female structures on the same plant), while common waterhemp and Palmer amaranth are dioecious (male and female structures on separate plants) (Bryson and DeFelice 2010). Although all of these species are troublesome in row crop production and are distributed throughout the U.S. and Canada, few have been as detrimental in recent history as Palmer amaranth.

Biology of Palmer amaranth

Palmer amaranth is a C_4 Sonoran Desert annual indigenous to the Southwestern U.S. and Northern Mexico, and the most successful *Amaranthus* species to establish itself as a weedy species in artificial habitats (Eleringer 1983, Sauer 1957). Within six years of being observed in South Carolina in 1989, Palmer amaranth was the most problematic weed in cotton (*Gossypium*

hirsutum L.) in both North and South Carolina (Webster and Coble 1997). By 2009, Palmer amaranth was ranked in the top 10 most troublesome weeds in corn (*Zea mays*), soybean (*Glycine max*), and cotton in several southern states (Webster and Nichols 2012). The development of herbicide resistance likely contributed to the spread and success of Palmer amaranth as a weedy species throughout most of the Southern and Great Plains regions of the U.S. (Horak and Peterson 1995; Gossett et al. 1992). While Palmer amaranth remains a major weed problem in these regions, it has recently spread throughout the Midwest (Sellers et al. 2003) and was first identified in Michigan in 2010 (Sprague 2011).

Germination. The inherent ability of Palmer amaranth to germinate rapidly under favorable temperatures in the presence of moisture is one characteristic that has makes it a successful weed species (Ehleringer 1983). Temperatures for initial and peak germination vary widely for the different *Amaranthus* species (Guo and Al-Khatib 2003; Steckel et al. 2004; Steinmaus et al. 2000). Steinmaus et al. (2000) reported that the minimum temperature requirement for Palmer amaranth germination was approximately 17 C. While initial germination can occur under cooler soil temperatures, Palmer amaranth favors warmer conditions for germination. Palmer amaranth germination was 8 and 83% when temperatures were alternated ± 40% at 5 and 30 C, respectively (Steckel et al. 2004). Peak germination occurred when temperature was alternated from 32 to 38 C; when temperatures were alternated from 45 to 50 C no germination occurred (Guo and Al-Khatib 2003).

Emergence. Similar to germination, time to emergence also differs among the *Amaranthus* spp. with Palmer amaranth emergence occurring within 5 d of planting, while other *Amaranthus* spp.

may take up to 17 d (Sellers et al. 2003). 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) indicating that, regardless of climate, Palmer amaranth has the ability to emerge throughout the growing season. This rapid and continued emergence may require the use of residual herbicides to manage Palmer amaranth throughout the growing season. Including different crops and cultural practices into a crop rotation may help to reduce Palmer amaranth emergence. Soybean canopy can reduce Palmer amaranth emergence >70% (Jha and Norsworthy 2009). Palmer amaranth emergence can also be influenced by burial depth, with greater emergence occurring when seeds were buried ≤ 1.3 cm (Keeley et al. 1987). The decline in emergence is likely related to the light requirement and phytochrome-mediated responses associated with Palmer amaranth germination. Jha et al. (2010) found that once soybean canopied, far-red light increased which inhibited Palmer amaranth germination. Further research needs to be conducted to determine the influence of other crops on the emergence of Palmer amaranth in cooler climates.

Growth rate, Biomass Accumulation, and Seed Production. Other characteristics that can attribute to the competitiveness of Palmer amaranth with other weeds and crops are its rapid growth rate, biomass accumulation, and abundant seed production. Palmer amaranth grew at a quicker rate and accumulated more biomass than other *Amaranthus* spp., including redroot pigweed, common waterhemp, and tumble pigweed (*Amaranthus albus* L.) (Horak and Loughin 2000). Palmer amaranth has also been reported to be 45 and 600% taller than common waterhemp and redroot pigweed, respectively (Sellers et al. 2003). The rapid growth rate of

Palmer amaranth makes it an ideal indicator species for herbicide applications when dealing with mixed *Amaranthus* populations (Horak and Loughin 2000).

Climate and time of establishment greatly influence Palmer amaranth seed production. At optimal emergence times Palmer amaranth produced 250,000, 446,000, 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). Seed production of Palmer amaranth declines as plants emerge later in the growing season. In California, seed production was reduced 90% when plants were established in August compared with May (Keeley et al. 1987). A 50% reduction in seed production was observed when Palmer amaranth was established 6 wk after initial cohort planting in Georgia (Webster and Grey 2015). In addition to time of emergence, the presence of a crop at the time of Palmer amaranth establishment also influences seed production. When established at the time of cotton planting Palmer amaranth seed production was reduced by 30%, compared with plants grown in the absence of cotton (Webster and Grey 2015). At a density of 8 plants m^{-2}, seed production was reduced from 514,000 to 91,000 seeds m^{-2} when Palmer amaranth emergence was delayed until the 7-leaf stage in corn (Massinga et al. 2001). Seed production was reduced 97% when Palmer amaranth emerged in V3 to V6 soybean compared, with plants that emerged from soybean planting to the V3 stage (Jha et al. 2008). If Palmer amaranth is not controlled throughout the growing season seed rain will add to the soil seedbank, perpetuating the problem. Little information is available on how cropping system and Palmer amaranth emergence time influences the growth and seed production of Palmer amaranth in the cooler climate of Michigan.

Competition. Palmer amaranth readily competes with crops for water, light, and nutrients resulting in a negative impact on yield. Corn yield was reduced up to 91% when 8 Palmer amaranth plants m^{-2} competed with corn throughout the growing season (Massinga 2001). When Palmer amaranth emergence was delayed until V4 to V7 corn, yield was reduced by <35% (Massinga et al. 2001). Competition from Palmer amaranth at densities ranging 0.33 to 10 plants m^{-1} row reduced soybean yield from 17 to 64% (Klingaman and Oliver 1994). The ability of Palmer amaranth to effectively compete and reduce yield even after crop establishment may be due to high photosynthetic capacity and the ability to acclimate to shade. Palmer amaranth has adapted to tolerate and maintain growth under high light and temperature environments, with 90% of peak photosynthetic rate occurring between 36 and 46^{o} C (Eleringer 1983). However, photosynthetic rate is highly dependent on temperature. Photosynthesis occurred at 50% of the maximum rate at 25^{o} C (Eleringer 1983), indicating that Palmer amaranth may not be able to effectively compete with crops in a cooler climate like Michigan. To compensate for shading, Palmer amaranth, can alter leaf area and increase chlorophyll content to maintain growth and effectively compete with crops (Jha et al. 2008).

Herbicide Resistance

In addition to Palmer amaranth's biological characteristics, the propensity at which Palmer amaranth develops resistance to different herbicides has perpetuated it as a problem weed. Palmer amaranth has developed resistance to several herbicide sites of action including: acetolactate synthase inhibitors (ALS) (Group 2), microtubule inhibitors (Group 3), photosystem II (PSII) (Group 5), Protoporphyrinogen oxidase inhibitors (PPO) (Group 14), and 4-hydroxyphynelpyruvate dioxygenase inhibitors (HPPD) (Group 27) (Gossett 1992, Horak and

Peterson 1995, Heap 2016). Resistance to ALS-inhibiting herbicides in Palmer amaranth has been reported to be as high as 2,800 times the use rate of imazethapyr (Sprague et al. 1997). While not widespread 6, 14, and 23 times the use rate were required to achieve the same level of control in susceptible Palmer amaranth compared with resistant populations for dinitroaniline, atrazine, and HPPD-inhibiting herbicides, respectively (Gossett et al. 1992; Jhala et al. 2014). Perhaps one of the most important and widespread resistances in Palmer amaranth was the development of glyphosate resistance. One of the major contributors to the development of herbicide resistance was the rapid adoption of glyphosate-resistant (GR) crops. This adoption of GR crops led to multiple applications of a single herbicide site of action glyphosate (Group 9), which increased selection pressure for resistant weed biotypes (Young 2006; Owen 2008; Vencill et al. 2012). The first case of glyphosate-resistant Palmer amaranth was reported in Georgia in 2005 (Culpepper et al. 2006). This population survived applications of glyphosate in the field at 12 times (10 kg ae ha^{-1}) the normal use rate. Palmer amaranth resistant to glyphosate has since spread to 23 other states including Michigan (Heap 2016). The magnitude of glyphosate resistance within these populations ranges from 1.5 to 115 times the rate of glyphosate required to achieve 50% control in a susceptible population (Norsworthy et al. 2008; Steckel et al. 2008). In addition to resistance to a single herbicide site of action, there are several populations demonstrating resistance to multiple herbicide sites of action (Heap 2016). In Michigan, there are three different confirmed resistance profiles in Palmer amaranth ranging from single site of action glyphosate or ALS to multiple sites glyphosate and ALS within a single population. In addition to these there is a population suspected of being resistant to three herbicide sites of action: glyphosate, ALS-inhibitors, and atrazine. There have been several reported instances of populations of Palmer amaranth resistant to glyphosate and ALS-inhibitors,

however there has only been one other documented case of Palmer amaranth resistant to glyphosate, ALS-inhibitors, and atrazine (Heap 2016). This population was found in Georgia and there has been little information published. Common waterhemp, a close relative of Palmer amaranth has developed resistance to five different herbicide sites of action in a single Illinois population (Evans et al. 2015). The development of resistance, specifically multiple resistance, drastically limits the options for control.

Herbicide Resistance Mechanisms. The primary mechanisms in which resistance to herbicides is conferred in weeds has been categorized into five mechanisms: altered-target site, metabolism-based, reduced absorption/translocation, sequestration into vacuoles, and gene amplification (Heap 2014). Altered target-site resistance is the most common mechanism of resistance for various herbicides in several weed species. In Palmer amaranth and other *Amaranthus* spp., the primary mechanism for resistance to ALS-inhibiting herbicides is due to an altered target site via amino acid substitution within the ALS enzyme (Foes et al. 1998; Franssen et al. 2001; Sprague et al. 1997). Betha et al. (2015) reported that a proline to serine change at site 197 in a Kansas population of Palmer amaranth. The majority of instances of atrazine resistance have been attributed to either amino acid substitutions at the D1 protein that have been found in other *Amaranthus* spp., and in some instances non-target site mediated forms of resistance have been reported (Foes et al. 1998; Patzoldt et al. 2003). In a Kansas population of triazine-resistant Palmer amaranth it was determined that resistance was non-target site based and possibly glutathione-s-transferase conjugation (Betha et al. 2015). In 2010, Gaines et al. (2010) identified gene amplification of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme as a novel mechanism for glyphosate-resistance in Palmer amaranth. In the Palmer amaranth

population that they studied from Georgia expression of the EPSPS enzyme relative to the ALS enzyme ranged from 1.0 to 1.3 for the susceptible population and was 5 to >160 copies of the EPSPS enzyme in the resistant population (Gaines et al. 2010). Since 2010, gene amplification has been confirmed as the mechanism of resistance to glyphosate in populations of Palmer amaranth in Mississippi, North Carolina, and New Mexico (Chandi et al. 2012; Ribeiro et al. 2013; Mohseni-Moghadam et al. 2013).

Palmer amaranth Management

In the Northern Corn Belt the majority of Palmer amaranth populations are resistant to glyphosate and/or ALS-inhibiting herbicides. This poses significant challenges for the development of effective Palmer amaranth management strategies. With limited herbicide options available in soybean, planting corn may provide farmers the greatest opportunity to effectively managing Palmer amaranth. One reason is that there are a greater number of effective herbicide site of action groups available in corn versus soybean. With Palmer amaranth being a relatively new problem in major corn producing regions of the U.S., little research has been conducted on effective management strategies in corn. The majority of research has been focused on multiple-resistant Palmer amaranth management in cotton, soybean, and peanut (Ward et al. 2013). However, in corn Johnson et al. (2012) found that atrazine, *s*-metolachlor, and isoxaflutole applied preemergence (PRE) alone and in combination with other corn herbicides was able to provide 78 to 100% control of herbicide-susceptible Palmer amaranth, 8 wks after application. The addition of atrazine to low rates of pyroxasulfone and *s*-metolachlor improved Palmer amaranth control when applied PRE (Geier et al. 2006). Atrazine at 2.24 kg ha^{-1} applied postemergence (POST) controlled herbicide-susceptible Palmer amaranth 96%, 28 d

after treatment (Stephenson et al. 2015). The addition of the ALS-inhibitor, thiencarbazone-methyl, to tembotrione did not improve Palmer amaranth control. However, the addition of either atrazine, glyphosate, or glufosinate to the combination improved control 4 to 6% (Stephenson et al. 2015). Others have reported that the use of HPPD-inhibiting herbicides, a common site of action in corn production, can effectively control resistant Palmer amaranth with isoxaflutole, tembotrione, and mesotrione providing 90, 92, and 97% control 21 to 28 DAT, respectively (Norsworthy et al. 2008; Schuster et al. 2008; Stephenson et al. 2015).

In order to maintain high levels of Palmer amaranth control POST, application timing as pertaining to weed height must be considered. Corbett et al. (2004), observed greater levels of Palmer amaranth control when glufosinate was applied to 2 to 5 cm weeds compared with 8 to 10 cm. Control of Palmer amaranth was reduced when herbicide applications were delayed for 8 d after intended application timing (Eure et al. 2013). The success of the previously described management strategies is primarily due to the susceptibility of the Palmer amaranth populations to specific herbicide site of action groups. However, when managing multiple-resistant populations of Palmer amaranth, management strategies need to be based on the use of multiple effective herbicide sites of action. These strategies will likely need to include multiple herbicide applications, such as PRE followed by (fb) POST programs, due to Palmer amaranths prolonged emergence patterns and fast growth rate. Little information is available on detailed management strategies for multiple-resistant Palmer amaranth in corn.

Interaction of HPPD-Inhibitors and Atrazine

Combinations of two or more herbicides can yield either an additive, synergistic, or antagonistic response. These responses are determined by calculating a predicted value and

comparing it to an expected value of the herbicide combination (Colby 1967; Flint et al. 1988; Gowing 1960). If the observed value is significantly greater than the calculated predicted value then the herbicide combination is deemed to be synergistic. A combination is antagonistic if the observed value is significantly less than predicted. If the values of the observed and predicted are equal then the combination is additive. Previous research has shown that antagonism can occur between mesotrione and sulfonylurea herbicides. The addition of mesotrione to nicosulfuron reduced green foxtail (*Setaria viridis* L.) control up to 23% compared with nicosulfuron alone (Schuster et al. 2008). The mechanisms by which antagonism can occur has been attributed to reduced absorption, reduced translocation, and physiological interactions within the plant (Hart and Wax 1996; Green 1989; Schuster et al. 2007). A well-documented instance of herbicide synergism has been observed with the 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides applied in combination with atrazine (Abendroth et al. 2006, Armel et al. 2007, Hugie et al. 2008, Woodyard et al. 2009a). The modes of action for atrazine and the HPPD-inhibitors are complementary to each other which leads to an increase in activity when they are applied in combination.

Herbicides that inhibit the HPPD enzyme are members of the isoxazole, pyrazole, pyrazolone and triketone chemical families. HPPD-inhibiting herbicides stop the conversion of 4-hydroxyphenylpyruvate to homogentisate, which leads to the depletion of plastoquinone and α-tocopherol (Grossmann and Ehrhadt 2007; Mitchell et al. 2001; Pallett et al. 1998; Schulz 1993). Plastoquinone is an enzyme cofactor for phytoene desaturase and depletion results in a loss of carotenoid production, causing the bleaching of new tissue (Mitchell et al 2001; Schulz et al. 1993).

As a member of the *s*-triazine chemical family atrazine is a photosynthetic inhibitor functioning within PSII, and has both PRE and POST herbicidal activity. In PRE and POST applications, atrazine is rapidly absorbed via passive diffusion (Thompson and Slife 1970; Price and Blake 1983). Movement of atrazine is primarily conducted within the xylem in the transpiration stream, and once in the leaves movement is acropetal following the transpiration stream (Jachetta et al. 1986; Thompson and Slife 1970). Initial injury symptoms from atrazine applied either PRE or POST is localized chlorosis leading to necrosis on leaf margins. Ultimately herbicidal activity of atrazine and plant death is caused by the binding of atrazine over plastoquinone to the Q_B region of the D1 protein in the electron transport chain of PSII (Hess 2000, Pfister 1981). This binding results in the production of singlet oxygen and triplet chlorophyll which results in lipid peroxidation of cell membranes (Hess 2000).

The synergism between atrazine and the HPPD-inhibitors may be attributed to the indirect effect of plastoquinone and α-tocopherol depletion caused by the HPPD inhibitors (Kruk et al. 2005; Trebst et al. 2002). This synergism has been observed in giant ragweed (*Ambrosia trifida* L.), common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* M.), common waterhemp, and redroot pigweed (Abendroth et al. 2006; Hugie et al. 2008; Woodyard et al. 2009a; Woodyard et al. 2009b). Synergism between PSII and HPPD-inhibitors has been observed in triazine-resistant redroot pigweed and velvetleaf, however the extent of this interaction is dependent on whether the mechanism of resistance is altered-target site or metabolism based (Woodyard et al. 2009b). The majority of previous research has been focused on the interaction of mesotrione and atrazine; little information is available on the potential synergism with the other classes of HPPD-inhibitors and atrazine. While previous research has

shown the presence of synergism with HPPD-inhibitors and atrazine in other triazine-resistant weed species, it is unknown whether this interaction exists in triazine-resistant Palmer amaranth.

Cultural Practices for Depletion of Soil Seedbank

Management of herbicide resistance should include cultural practices to reduce the abundance of these species within the soil seedbank, and should not be limited to only herbicide-based programs (Norsworthy et al. 2012). Declines in the soil seedbank can be attributed to cultural practices such as crop rotation and tillage, or through natural processes like seed predation and seed mortality over time (Ball and Miller 1990; Bellinder et al. 2003; Buhler et al. 2001; Cardina et al. 2002; Davis et al. 2005; Sonoskie et al. 2013). Rotating crops from year to year can influence weed species densities and the soil seedbank based on the different management practices (Ball and Miller 1990, Buhler et al. 2001, Cardina et al. 2002 Davis et al. 2005, Bellinder et al. 2003). The pairing of an herbicide program along with tillage can also reduce soil seedbank densities (Ball and Miller 1990, Bellinder et al. 2003, Davis et al. 2005). The use of continuous corn or a corn and soybean rotation reduced shepherd's-purse (*Capsella bursa-pastoris* L. Medik), Pennsylvania smartweed (*Polygonum penslvanicum* L.), corn speedwell (*Veronica arvensis* L.), yellow woodsorrel (*Oxalis stricta* L.), spotted spurge (*Chamaesyce maculate* L. Small), and redroot pigweed compared with a corn, oats, and hay rotation (Cardina et al. 2002). Herbicide availability and tillage practices associated with corn may be one reason for relatively low increases in the weed seedbank compared with rotations that included rye and legume crops (Bellinder et al. 2003). In monoculture cropping systems few well adapted species will dominate the seedbank, whereas varying levels of competition, allelopathy, soil disturbance, and management strategies associated with diverse cropping

systems can increase diversity and reduce the size of the seedbank (Buhler et al. 1997; Cardina et al. 2002). Seed predation can also influence the soil seedbank. Seed predation from insects and rodents resulted in a Palmer amaranth seedbank decline of 66 and 75%, respectively (Sosnoskie et al. 2013). Sosnoskie et al. (2013), also reported that in a Georgia Palmer amaranth population seed burial depth can affect viability, with 9 and 22% of Palmer amaranth remaining viable after 3 years at 1 cm and 40 cm burial depths, respectively. In Michigan, one year after burial there was no difference between burial depth and seed viability (Powell 2014). Powell (2014) concluded that the Palmer amaranth seedbank could be reduced anywhere from 50 to 90% in one year if no seed was produced. These results indicate that significant reductions can be made in a short amount of time to the Palmer amaranth seedbank if best management practices are utilized to reduce Palmer amaranth seed production.

LITERATURE CITED

LITERATURE CITED

Abendroth JA, Martin AR, Roeth FW (2006) Plant response to combinations of mesotrione and photosystem II inhibitors 1. Weed Technol 20.1:267–274

Armel GR, Rardon PL, McComrick MC, Ferry NM (2007) Differential response of several carotenoid biosynthesis inhibitors in mixtures with atrazine. Weed Technol 21.4:947–953

Ball DA, Miller SD (1990) Weed seed population response to tillage and herbicide use in three irrigated cropping sequences. Weed Sci 511–517

Bellinder RR, Dillard HR, Shah DA (2003) Weed seedbank community responses to crop rotation schemes. Crop Protect 23.2:95–101

Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37–43

Bryson CT, DeFelice MS, eds (2010) Weeds of the Midwestern United States & Central Canada. Athens, GA: University of Georgia Press. Pp 34–39.

Buhler DD, Hartzler RG, Forcella F (1997) Implications of weed seedbank dynamics to weed management. Weed Sci 45:329–336

Buhler DD, Kohler KA, Thompson RL (2001) Weed seed bank dynamics during a five-year crop rotation 1. Weed Technol 15.1:170–176

Cardina JC, Herms P, Doohan DJ (2002) Crop rotation and tillage system effects on weed seedbanks. Weed Sci 50.4:448–460

Chandi A, Milla-Lewis SR, Giacomini D, Westra P, Preston C, Jordan DL, York AC, Burton JD, Whitaker JR (2012) Inheritance of evolved glyphosate resistance in a North Carolina Palmer amaranth (*Amaranthus palmeri*) biotype. Int J Agron DOI:10.1155/2012/176108

Colby SR (1967) Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 20–22

Corbett JL, Askew SD, Thomas WE, Wilcut JW (2004) Weed efficacy evaluations for bromoxynil, glufosinate, glyphosate, pyrithiobac, and sulfosate. Weed Technol 18:443–453

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54.4:620–626

Davis AS, Renner KA, Gross KL (2005) Weed seedbank and community shifts in a long-term cropping systems experiment. Weed Sci 53.3:296–306

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. Oecologia 57:10–112

Eure PM, Jordan DL, Fisher LR, York AC (2013) Efficacy of herbicides when spray solution application is delayed. Int J Agron DOI: 10.1155/2013/782486

Evans CM, Tranel P, Riechers DE, Davis AS, Maxwell D, Gonzini L, Hager AG (2015) Characterization of a novel five-way resistant population of waterhemp *Amaranthus tuberculatus*. Page 61 *in* Proceeding of the 70th Annual Meeting of the North Central Weed Science Society. Indianapolis, IN: North Central Weed Science Society

Flint JL, Cornelius PL, Barrett M (1988) Analyzing herbicide interactions: a statistical treatment of Colby's method. Weed Technol 304–309

Foes MJ, Tranel PJ, Wax, LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 514–520

Franssen AS, Skinner DZ, Al-Khatib K, Horak MJ, Kulakow PA (2001) Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci 49.5:598–606

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107.3:1029–1034

Geier PW, Stahlman PW, Frihauf JC (2006) KIH-485 and s-metolachlor efficacy comparisons in conventional and no-tillage corn. Weed Technol 20.3:622–626

Gossett BJ, Toler JE (1999) Differential control of Palmer amaranth (*Amaranthus palmeri*) and pigweed (*Amaranthus hybridus*) by postemergence herbicides in soybean (*Glycine max*) Weed Technol 13:165–168

Gossett BJ, Murdock EC, Toler JE (1992) Resistance of Palmer amaranth (*Amaranthus palmeri*) to the dinitroaniline herbicides. Weed Technol 587–591

Gowing DP (1960) Comments on tests of herbicide mixtures. Weeds 8.3:379–391

Green JM (1989) Herbicide antagonism at the whole plant level. Weed Technol 3:217–226

Grichar WJ (1994) Spiny amaranth (*Amaranthus spinosus* L.) control in peanut (*Arachis hypogaea* L.). Weed Technol 8:199–202.

Grossmann K, Ehrhardt T (2007) On the mechanism of action and selectivity of the corn herbicide topramezone: a new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag Sci 63.5:429–439

Guo P, Al-Khatib K (2003) Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). Weed Sci 51.6:869–875

Hager AG, Wax LM, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci 50:607–610

Hart SE, Wax LM (1996) Dicamba antagonizes grass weed control with imazethapyr by reducing foliar absorption. Weed Technol 10:828–834

Heap I (2014) Herbicide resistant weeds. Pages 281-301 *in* Pimentel D, Peshin R, eds. Integrated Pest Management. Netherlands: Springer

Heap I (2016) The international survey of herbicide resistant weeds. www.weedscience.com. Accessed: February 5, 2016.

Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci 48.2:160–170

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus species.* Weed Sci 48.3:347–355

Horak MJ, Peterson DE (1995) Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol 9:192–195

Hugie JA, Bollero GA, Tranel PJ (2008) Defining the rate requirements for synergism between mesotrione and atrazine in redroot pigweed (*Amaranthus retroflexus*). Weed Sci 56.2:26–-270

Jachetta JJ, Appleby AP, Boserma L (1986) Apoplastic and symplastic pathways of atrazine and glyphosate transport in shoots of seedling sunflower. Plant Physiol 82:1000–1007

Jha P, Norsworthy JK (2009) Soybean canopy and tillage effects on emergence of Palmer amaranth (*Amaranthus palmeri*) from a natural seed bank. Weed Sci 57.6:644–651

Jha P, Norseworthy JK, Riley MB, Bridges Jr W (2010) Annual changes in temperature and light requirements for germination of Palmer amaranth (*Amaranthus palmeri*) seeds retrieved form the soil. Weed Sci 58:426–432

Jha P, Norsworthy JK, Bridges Jr W, Riley MB (2008) Influence of glyphosate timing and row width on Palmer amaranth (*Amaranthus palmeri*) and pusley (*Richardia spp.*) demographics in glyphosate-resistant soybean. Weed Sci 56.3:408–415

Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of triazine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38

Johnson WG, Chahal GS, Regehr DL (2012) Efficacy of various corn herbicides applied preplant incorporated and preemergence. Weed Technol 26.2:220–229

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 199–204

Klingaman TE, Oliver LR (1994) Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). Weed Sci 523–527

Knezevic SZ, Weise SF, Swanton CJ (1994) Interference of redroot pigweed (*Amaranthus retroflexus*) in corn (*Zea mays*). Weed Sci 42:568–573

Kruk J, Holländer-Czytko H, Oettmeier W, Trebst A (2005) Tocopherol as singlet oxygen scavenger in photosystem II. Journal of Plant Phys 162.7:749–757

Massinga RA, Curie RS, Horak MJ, Boyer Jr J (2001) Interference of Palmer amaranth in corn. Weed Sci 49.2:202–208

Menges RM (1987) Weed seed population dynamics during six years of weed management systems in crop rotations on irrigated soil. Weed Sci 328–332

Mengistu LW, Christoffers MJ, Lym RG (2005) A *psbA* in *Kochia scoparia* (L) Schrad from railroad rights-of-way with resistance to diuron, tebuthiuron and metribuzin. Pest Manag Sci 61:1035–1042

Mitchell G, Barlett DW, Fraser TEM, Hawkes TR, Holt DC, Townson JK, Wichert RA (2001) Mesotrione: a new selective herbicide for use in maize. Pest Manag Sci 57.2:120–128

Mohseni-Moghadam M, Schroeder J, Ashigh J (2013) Mechanism of resistance and inheritance in glyphosate resistant Palmer amaranth (*Amaranthus palmeri)* populations from New Mexico, USA. Weed Sci 61:517–525

Moolani KM, Knake EL, Slife FW (1964) Competition of smooth pigweed with corn and soybeans. Weeds 126–128

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgis, NR, Witt, WW, Barrett M (2012) Reducing risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62

Owen MDK, Zelaya IA (2005) Herbicide-resistant crops and weed resistance to herbicides. Pest Manag Sci 61.3:301–311

Pallett KE, Little JP, Sheeky M, Veerasekaran P (1998) The mode of action of isoxaflutole: I. Physiological effects, metabolism, and selectivity. Pesticide Biochemistry and Phys 62.2:113–124

Patzoldt, WL, Tranel PJ, Hager AG (2002) Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot 21:707–712

Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq) Sauer that is not site-of-action mediated. Pest Manag Sci 59:1134–1142

Pfister K, Steinback KE, Gardner G, Arntzen (1981) Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. Proc Natl Acad Sci 78.2:981–985

Powell DK (2014) Biology and management of multiple (glyphosate/ALS)-resistant Palmer amaranth in Michigan soybean. Ph.D dissertation. East Lansing, MI: Michigan State University. 122 p

Price TP, Blake NE (1983) Characterization of atrazine accumulation by excised velvetleaf (*Abutilon theophrasti*) roots. Weed Sci 31:14–19

Ribeiro DN (2013) Mechanisms and variability of glyphosate resistance in *Amaranthus palmeri* and *Ipomoea lacunose* Ph.D dissertation. Starkville, MS: Mississippi State University. 127 p

Sauer J (1957) Recent migration and evolution of the dioecious amaranths. Evolution 11–31

Schulz A, Ort O, Beyer P, Kleinig H (1993) SC-0051, a 2-benzoyl-cyclohexane-1, 3-dione bleaching herbicide, is a potent inhibitor of the enzyme p-hydroxyphenylpyruvate dioxygenase. FEBS letters 318.2:162–166

Schuster CL, Al-Khatib K, Dille JA (2007) Mechanism of antagonism of mesotrione on sulfonylurea herbicides. Weed Sci 55:429–434

Schuster CL, Al-Khatib K, Dille JA (2008) Efficacy of sulfonylurea herbicide when tank mixed with mesotrione. Weed Technol 22:222–230

Schweizer EE, Lauridson TC (1985) Powell amaranth (*Amaranthus powellii*) interference in sugarbeet (*Beta vulgaris*). Weed Sci 33:518–520

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus species* in Missouri. Weed Sci 51.3:329–333

Sonoskie LM, Webster TM, Culpepper AS (2013) Glyphosate resistance does not affect Palmer amaranth (*Amaranthus palmeri*) seedbank longevity. Weed Sci 61:283–288

Sprague CL (2011) Glyphosate-resistant Palmer amaranth confirmed in Michigan. http://msuweeds.com/newsletter-holder/emerging-weed-issues/glyphosate-resistant-palmer-amaranth-confirmed-in-southwest-michigan/. Accessed July 11, 2016

Sprague CL, Stoller EW, Wax LM, Horak MJ (1997) Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci 192–197

Steckel LE, Main CL, Ellis AT, Mueller TC (2008) Palmer amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technol 22:119–123

Steckel LE, Sprague CL, Stoller EW, Wax LM (2004) Temperature effects on germination of nine *Amaranthus* species. Weed Sci 52:217–221

Steinmaus SJ, Prather TS, Holt JS (2000) Estimation of base temperatures for nine weed species. J of Exper Bot 51.343:275–286

Stephenson IV DO, Bond JA, Landry RL, Edwards HM (2015) Weed management in corn with postemergence applications of tembotrione or thiencarbazone:tembotrione. Weed Technol 29.3:350–358

Thompson L, Jr, Slife FW (1970) Root and Foliar absorption of atrazine applied postemergence to broadleaf weeds. Weed Sci 18:349–351

Toler JE, Guice B, Murdock EC (1996) Interference between Johnsongrass (*Sorghum halepense*), smooth pigweed (*Amaranthus hybridus*), and soybean (*Gylcine max*). Weed Sci 44:331–338

Trebst A, Depka B, Holländer-Czytko H (2002) A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. FEBS letters 516.1:156–160

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci 60:2–30

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

Webster TM, Coble HD (1997) Changes in the weed species composition of the Southern United States: 1974 to 1995. Weed Technol 11:308–317

Webster TM, Grey TL (2015) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) morphology, growth, and seed production in Georgia. Weed Sci 63:264–272

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145–157

Whaley CM, Wilson HP, Westwood JH (2007) A new mutation in plant *ALS* confers resistance to five classes of ALS-inhibiting herbicides. Weed Sci 55:83–90

Woodyard AJ, Bollero GA, Riechers DE (2009a) Broadleaf weed management in corn utilizing synergistic postemergence herbicide combinations. Weed Technol 23:513–518

Woodyard AJ, Hugie JA, Riechers DE (2009b) Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci 57.4:369–378

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops 1. Weed Technol 20.2:301–307

CHAPTER 2

CONFIRMATION OF A THREE-WAY (GLYPHOSATE, ALS, AND ATRAZINE) HERBICIDE-RESISTANT POPULATION OF PALMER AMARANTH IN MICHIGAN

Abstract

The failure of PRE and POST applications of atrazine to control Palmer amaranth in recent field studies prompted further investigation to determine if this population had evolved resistance to multiple herbicide sites of action including, glyphosate (Group 9), thifensulfuron (Group 2), and atrazine (Group 5). Greenhouse and laboratory experiments were conducted to: 1) confirm the presence of a 3-way resistant Palmer amaranth population, 2) determine the resistance factor (RF) for herbicide site of action group, and 3) determine the molecular basis for resistance in this population. In the greenhouse, the combination of glyphosate + thifensulfuron + atrazine at 1.26 + 0.0044 + 1.12 kg ai ha^{-1} provided 55% control of the suspected multiple-resistant (MR) Palmer amaranth population and 93% control of the known susceptible population (S). The decreased sensitivity of the MR population compared with the S population at labeled use rates of these herbicides indicated that this population was likely resistant to three different herbicide site of action groups. The RF values for POST applications of glyphosate, thifensulfuron, and atrazine were 12.2, 42.9, and 9.3X, respectively, for the MR Palmer amaranth population relative to the S population. The RF value for atrazine PRE for the MR population was 112.2X. Laboratory experiments confirmed that the mechanisms for ALS-inhibitor and glyphosate resistance in the MR Palmer amaranth population were target site based, via amino acid substitution and amplified *EPSPS* copy number, respectively. For the ALS-inhibitor resistance there was a Pro to Leu substitution at site 197 and for the glyphosate resistance there was a >50-fold increase in *EPSPS* copy number. There were no nucleotide changes in the *psbA* gene, therefore atrazine

resistance in this population was not target-site mediated. Atrazine resistance in this population could possibly be metabolism based. The development of multiple resistance in Palmer amaranth populations poses significant management challenges to growers.

Nomenclature: Atrazine; glyphosate; thifensulfuron; Palmer amaranth, *Amaranthus palmeri* S. Wats.

Keywords: 3-way resistance; gene amplification; mechanism of resistance; molecular analysis; resistance factor.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a C_4 Sonoran Desert annual indigenous to the Southwestern U.S. and Northern Mexico, and the most successful *Amaranthus* species to establish itself as a weedy species in artificial habitats (Eleringer 1983; Sauer 1957). Within six years of being identified in South Carolina in 1989, Palmer amaranth became the most problematic weed in cotton (*Gossypium hirsutum* L.) in both North and South Carolina (Webster and Coble 1997). By 2009, Palmer amaranth was ranked as one of the Top 10 most-troublesome weeds in corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and cotton in several states of the Southeastern U.S. (Webster and Nichols 2012). The development of herbicide resistance likely contributed to the spread and success of Palmer amaranth as a weedy species throughout most of the southern and Great Plains regions of the U.S. (Horak and Peterson 1995; Gossett et al. 1992). While Palmer amaranth remains a major problem in those regions, it has recently spread into the Midwest (Sellers et al. 2003) and was first identified in Michigan in 2010 (Sprague 2011).

The propensity at which Palmer amaranth develops resistance to different herbicides has perpetuated it as a problem weed. Herbicide resistance in Palmer amaranth is not new. The first

reported case of herbicide-resistance in Palmer amaranth was identified in South Carolina in 1989 (Gossett et al. 1992). Populations from two South Carolina counties evolved resistance to trifluralin, a dinitroaniline (Group 3) herbicide. These populations had varying levels of resistance to five other dinitroaniline herbicides. By 1993, atrazine (Group 5) resistance was reported in a Texas population of Palmer amaranth (Heap 2016), but since then, triazine-resistant populations have been reported only in three other states. The inability for triazine-resistant Palmer amaranth to establish and become widespread may be due to reproductive fitness penalties often associated with triazine resistance in other *Amaranthus* species (Sibony and Rubin 2003; Soltani et al. 2008).

As Palmer amaranth expanded north in the mid-1990s, it rapidly developed resistance to the widely used ALS-inhibiting (Group 2) herbicides in several states (Heap 2016; Horak and Peterson 1995). Selectivity, low use rate, and the ability to control a broad-spectrum of weed species both pre- and post-emergence led to the rapid adoption and extensive use of ALS-inhibiting herbicides (Tranel and Wright 2002). Resistance to the ALS-inhibiting herbicides is so widespread in Illinois that all populations of common waterhemp (*Amaranthus rudis*), a closely related species to Palmer amaranth, are assumed resistant (Tranel and Wright 2002; Patzoldt et al 2002). To date, populations of Palmer amaranth demonstrating resistance to ALS-inhibiting herbicides has been reported in 12 states (Heap 2016).

The advent of glyphosate-resistant crops in the mid-1990s provided growers with an effective option to control weeds resistant to other herbicide sites of action (Shaner 2000). The rapid adoption of glyphosate-resistant crops led to the abandonment of preemergence (PRE) herbicides and the sole reliance on multiple applications glyphosate (Group 9) for weed control (Young 2006; Owen 2008; Shaner 2000; Vencill et al. 2012). This over-reliance on a single site selected

for the development of glyphosate-resistant biotypes and weed population shifts towards to more tolerant species (Young 2006; Owen 2008; Vencill et al. 2012). The first case of glyphosate-resistant Palmer amaranth was reported in Georgia in 2005 (Culpepper et al. 2006). This population survived applications of glyphosate in the field at 12 times (10 kg ae ha^{-1}) the normal use rate. Glyphosate-resistant Palmer amaranth has since been reported in 27 other states, including Michigan (Heap 2016).

In addition to the development of resistance to a single herbicide site of action, Palmer amaranth has developed resistance to multiple herbicide sites of action. One of the most prevalent instances of multiple resistance in populations of Palmer amaranth is resistance to glyphosate and ALS-inhibiting herbicides. Populations of Palmer amaranth resistant to both glyphosate and ALS-inhibitors have been identified in eight states including Georgia, Mississippi, Tennessee, South Carolina, Arizona, Illinois, Florida, Delaware, and Michigan (Heap 2016; Nandula et al. 2012; Sosnoskie et al. 2011). Other cases of multiple resistance that have been reported in Palmer amaranth are: protoporphyrinogen oxidase (PPO)-inhibitors (Group 14) + glyphosate (IL, TN), atrazine + 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Group 27) (NE), atrazine + glyphosate (NE), ALS-inhibitors + atrazine + HPPD-inhibitors (KS), and ALS-inhibitors + atrazine + glyphosate (GA) (Heap 2016). The development of resistance multiple herbicide sites of action drastically limits the options for Palmer amaranth control.

The primary mechanisms by which weeds develop resistance to herbicides are categorized into five mechanisms: altered-target site, metabolism-based, reduced absorption/translocation, sequestration into vacuoles, and gene amplification (Heap 2014). Altered target-site resistance is the most common mechanism of resistance for various herbicides in several weed species. In

Palmer amaranth and other *Amaranthus spp*., the primary mechanism for resistance to ALS-inhibiting herbicides is an altered target site via amino acid substitution within the ALS enzyme (Foes et al. 1998; Franssen et al. 2001). Betha et al. (2015) reported that ALS resistance in KS population of Palmer amaranth was due to a proline to serine change at site 197. An altered target site was reported as the primary mechanism for atrazine resistance in smooth pigweed (*Amaranthus hybridus* L.), common waterhemp, kochia (*Kochia scoparia* L.), and Powell amaranth (*Amaranthus powellii* S. Wats) (Diebold et al. 2003; Foes et al. 1998; Foes et al. 1999; Maertens et al. 2004), attributed to an amino acid substitution of glycine for serine at position 264 of the D1 protein. However, non-target site based triazine resistance has been reported for populations of tall waterhemp and velvetleaf (*Abutilon theophrasti* Medic) (Anderson and Gronwald 1991; Patzoldt et al. 2003). To date, the only identified mechanism of glyphosate resistance in Palmer amaranth is the over production of the target enzyme 5-enolpyruvlshikimate-3-phosphate synthase (EPSPS) (Chandi et al. 2012; Gaines et al. 2010; Ribeiro et al. 2013; Mohseni-Moghadam et al. 2013). This means that glyphosate cannot saturate the system and stop normal enzyme function, resulting in plant survival.

The failure of atrazine to control a newly-identified population of Palmer amaranth in Michigan that was suspected to be resistant to glyphosate and ALS-inhibiting herbicides in field experiments in 2013 led to the following research objectives: 1) confirm the presence of resistance to glyphosate, an ALS-inhibitor (thifensulfuron), and atrazine in a single Palmer amaranth population, 2) establish resistance factors (RF) for herbicide site of action, and 3) establish the molecular basis for resistance to these herbicide sites of action.

Materials and Methods

Seed Collection and Preparation. Seed heads from the suspected multiple-resistant population were collected in fall 2013 from a field in Barry County, MI (MR) (42.702467°N; -85.524992°W) and threshed. Since Palmer amaranth is not native to Michigan, seed for the known susceptible population was obtained from Dr. Larry Steckel, University of Tennessee (S). Seed from both populations were treated with a 50% sulfuric acid and water solution for 4 min, rinsed, and then exposed to gibberellic acid at a concentration of 0.15 g L^{-1} of water for 6 h to enhance germination.

Initial Screen for Three-Way Resistance. Approximately 15 seeds of the MR and S Palmer amaranth populations were planted 0.75 cm deep in 10 x 10 cm pots filled with potting media (Suremix Perlite, Michigan Gower Products, Inc., Galesburg, MI). Seedlings were grown in the greenhouse at 25 ± 5 C and sunlight was supplemented to provide a total midday light intensity of 1,000 µmol m^{-2} s^{-1} photosynthetic photon flux at plant height in a 16 h day. Plants were watered and fertilized as needed to promote optimum plant growth. After emergence, pots were thinned to one Palmer amaranth plant pot^{-1}. When plants were approximately 10 cm tall, a mixture of glyphosate (Roundup PowerMAX, Monsanto Co., St. Louis, MO) + thifensulfuron (Harmony, DuPont Crop Protection, Wilmington, DE) + atrazine (AAtrex 4L, Syngenta LLC, Greensboro, NC) was applied at 1.26 + 0.0044 + 1.12 kg ai ha^{-1} with a single nozzle (8001E, TeeJet Technologies, Wheaton, IL) track sprayer calibrated to deliver 187 L ha^{-1} at 193 kPa of pressure. These rates represented the 1X field use rates for these herbicides. Spray grade ammonium sulfate (AMS) (Actamaster, Loveland Products, Inc., Loveland, CO) at 2% w w^{-1} and 1% v v^{-1} of crop oil concentrate (COC) (Herbimax, Loveland Products Inc., Loveland, CO) was

added to this treatment. Palmer amaranth control was evaluated 14 days after treatment (DAT) on a scale of 0 to 100, with 0 indicating no Palmer amaranth control and 100 indicating complete plant death. Aboveground biomass was harvested 14 DAT and dried at 60 C for 7 d and weighed.

Resistance Factor. Greenhouse experiments were conducted to determine the resistance factor (RF) (Equation 1) of the suspected multiple-resistant Michigan Palmer amaranth population (MR) to postemergence applications of glyphosate, thifensulfuron, and atrazine, and to preemergence applications of atrazine. The suspected resistant Michigan Palmer amaranth population was compared with a known susceptible population (S) to determine the dose required for 50% growth reduction (GR_{50}).

$$Resistance\ Factor = \frac{GR_{50}resistant}{GR_{50}susceptible} \quad \text{[EQ. 1]}$$

Dose-Response to Postemergence Herbicides. Palmer amaranth planting, greenhouse growing conditions, and herbicide application for this experiment were the same as described above. Herbicide applications of the isopropylamine salt of glyphosate (Buccaneer, Tenkoz Inc., Alpharetta, GA), thifensulfuron, and atrazine were made when Palmer amaranth averaged 10 cm in height. Application rates ranged from 1/32 to 2X the labeled rate for the S population and 1/4 to 32X the labeled rate for the suspected resistant MR population. The 1X rates for each herbicide were: 1.26 kg ae ha^{-1} of glyphosate, 0.0044 kg ai ha^{-1} of thifensulfuron, and 1.12 kg ai ha^{-1} of atrazine. Herbicide rates were selected to provide a range of responses from no control to complete plant death. All atrazine treatments contained 1% v v^{-1} COC. Glyphosate and thifensulfuron treatments each included NIS at 0.5% v v^{-1} and AMS at 2% w w^{-1}. Non-treated

controls for each Palmer amaranth population (MR and S) were also included in the experiment. Palmer amaranth control was evaluated 14 DAT on a scale of 0 to 100. Aboveground biomass was harvested 14 DAT and dried at 60 C for 7 d and weighed.

Dose-Response to Preemergence Atrazine. Twenty-five seeds of the MR and S Palmer amaranth populations were planted at a depth of 0.75 cm in 10 x 10 cm pots filled with a steam sterilized Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalfs) soil composed of 78.1, 13.3, and 8.6% sand, silt, and clay, respectively, with a pH of 7.6 and 2.7% organic matter. Prior to planting and herbicide application, pots were watered to near field capacity. Atrazine was applied to the soil surface at rates ranging from 1/8 to 8X for the S population and at 1/2 to 32X for the MR population, with the 1X equal to 1.12 kg ai ha^{-1}. After herbicide application, pots were placed in the greenhouse (see greenhouse conditions listed above), and the soil surface for each pot was watered uniformly to incorporate the herbicide. To minimize herbicide leaching all subsequent watering was done through sub-irrigation. A single application of a 50 mL of a 20-20-20 fertilizer solution was applied as a drench to the soil surface 14 DAT to maintain normal plant growth. Emergence counts were taken weekly and aboveground biomass was harvested 28 DAT. Biomass was dried at 60 C for 7 d and weighed.

Statistical Analysis. Each experiment was arranged in a randomized complete block design with six (initial screen and postemergence experiment) or seven (preemergence experiment) replications and conducted twice. Dry weights from each experiment were converted to a percent of the non-treated control for each population (MR and S). In order to keep the results objective, only the dry weight data was used to determine the GR_{50} values. Data for each

experiment was analyzed using nonlinear-regression in SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA). The herbicide dose required to reduce Palmer amaranth biomass (growth) by 50% (GR_{50}) was then calculated for each population-herbicide combination using the log-logistic model (Burgos et al. 2013) (Equation 2):

$$y = c + \frac{d-c}{1+\left(\frac{x}{GR50}\right)^b} \quad \text{[EQ. 2]}$$

Where *d* equals the upper limit, *c* is the lower limit, and *b* is the relative slope around the GR_{50}. Deviations from the model are indicated by R^2 values and standard errors for the GR_{50} values are presented. The resistance factors were calculated for each population-herbicide combination (Equation 1).

Molecular Basis for Resistance. *Plant Material and DNA Extraction.* Suspected multiple-resistant (MR) and susceptible (S) Palmer amaranth plants were grown as described above in the postemergence experiment. When plants from the MR population reached 10 cm in height atrazine was applied at 18 kg ha^{-1} + 1% v v^{-1} COC, or 16X the normal use rate to select for atrazine-resistant plants. Glyphosate and thifensulfuron were not applied to these plants since there was less variability in the whole plant responses to these herbicides. Young newly-emerging leaf tissue (approximately 150 mg) was harvested 21 d after atrazine was applied to the MR population from four individual plants from the MR and S populations. Harvested leaf tissue was immediately frozen in liquid nitrogen and stored at -20 C until extraction for genomic DNA (gDNA). Palmer amaranth gDNA was extracted for each individual plant using the Qiagen DNeasy Mini Kit (Qiagen, Valencia, CA), and quantified using a nanodrop spectrometer (NanoDrop 2000c, Thermo Fisher Scientific Inc., Waltham, MA).

ALS and psbA Gene Isolation and Sequencing. The *ALS* and *psbA* genes were isolated and sequenced to determine if ALS and atrazine resistance in the MR Palmer amaranth population was conferred through nucleotide changes leading to amino acid substitution at the target site. Primer selection and methods for polymerase chain reaction (PCR) and sequencing were based off previous research conducted by Betha et al. (2015), Mengistu et al. (2005), and Whaley et al. (2007). Primers for amplifying approximately a 2 kb section of the *ALS* gene were designed by Whaley et al. (2007) and based on the *Amaranthus spp.* sequence (GenBank Accession U55852). Primers used for amplification of the *ALS* gene in Palmer amaranth are listed in Table 2.1 as *ALS* forward 1 and *ALS* reverse 1. Amplification of a 576 bp region of the *psbA* gene was done with primers designed by Mengistu et al. (2005). Primers used for amplification of the *psbA* gene are listed in Table 2.1 as *psbA* forward 1 and *psbA* reverse 1. Each PCR reaction for both *ALS* and *psbA* amplification contained 2 µL of gDNA, 10 µM of each forward and reverse primers, 10 mM deoxynucleotide triphosphates (DNTP's), 0.5 µL Phusion® high-fidelity DNA polymerase (New England Biolabs Inc., Ipswich, MA), 10 µL of supplied 5x buffer, and nuclease-free water to a final volume of 50 µL. Two separate thermoprofiles were designed for the amplification of the *ALS* and *psbA* genes. Reactions for the *ALS* genes were subjected to 30 s at 98 C, 10 s at 98 C, 30 s at 60 C, 90 s at 72 C, 34 cycles of 98 C, and a final 10:00 min at 72 C. Reactions for the *psbA* genes were subjected to 30 s at 98 C, 10 s at 98 C, 30 s at 55 C, 30 s at 72 C, 34 cycles of 98 C, and a final 10:00 min at 72 C. PCR products were quantified using gel electrophoresis. Prior to sequencing, PCR products were purified using the Wizard® SV gel and PCR clean-up kit (Promega Co., Madison, WI), and concentrations were measured using a nanodrop spectrometer. Eight and four separate sequencing reactions were conducted for the *ALS* and *psbA* gene, respectively, for each of the four biological replicates for the MR and S Palmer

amaranth populations. To ensure complete coverage and overlap of 2 kb *ALS* region, *ALS* forward and reverse primers 1 - 3 were used (Table 2.1). Since the *psbA* region was only 576 bp, only a single set of forward and reverse primers was necessary (Table 2.1). Sanger sequencing (Applied Biosystems™ 3730 XL, Thermo Fisher Scientific Inc., Waltham, MA) reactions contained 1 µL of 100 ng purified PCR product, 3 µL of forward or reverse primers, and biologically pure water brought up to a final volume of 12 µL. Sequences were aligned and compared using Sequencher™ 5.4.1 software (Gene Codes Corporation, Ann Arbor, MI). Additional sequence alignment was done with clustalW analysis and peptide sequences and numbering was obtained with translate tools available through ExPASy (ExPASy: SIB bioinformatics resource portal, http://www.expasy.org).

EPSPS Copy Number. Real-time quantitative polymerase chain reaction (qPCR) was used to determine if glyphosate resistance in the MR Palmer amaranth population was due to amplification of the *EPSPS* gene. Quantification of the EPSPS gene was determined by comparing the relative copy number of the *EPSPS* gene to the *ALS* gene. The primers used in the qPCR assay were identical to the ones described by Gaines et al. (2010) and Giacomini et al. (2014). These primers are listed as *ALS* forward and reverse 4 and *EPSPS* forward and reverse 1 in Table 2.1. Dilution series of the primers were not conducted, since previous research has shown high efficiencies with these primer sets (Gaines et al. 2010; Giacomini et al. 2014). The reactions for qPCR were setup containing 3 µL of gDNA (2 ng μL^{-1}) from the two Palmer amaranth populations, 2x SYBR® Green Master Mix (Applied Biosystems™, Thermo Fisher Scientific Inc.), 10 µM of each forward and reverse primer, and distilled water to bring the final reaction volume to 15 µL. The negative controls contained 7.5 µL of the 2x SYBR® Green

Master Mix and 7.5 µL of distilled water. All reactions for the four biological replicates of the MR and S populations were run in triplicate with the following thermoprofile on QuantStudio™ 7 Flex real-time PCR system (Applied Biosystems™, Thermo Fisher Scientific Inc.): 10 min of 95 C, 40 cycles of 95 C for 30 s, 1 min at 60 C, and melt curve analysis to check for primer dimers.

Threshold cycles (*C*t) were calculated using QuantStudio real-time PCR software version 1.2 (Applied Biosystems™, Thermo Fisher Scientific). Relative copy number of the *EPSPS* gene compared to the *ALS* gene was calculated using a modification of the $2^{-\Delta\Delta Ct}$ method (Gaines et al 2010; Livak and Schmittgen 2001). Estimated *EPSPS* copy number was determined by finding the change in *C*t values (Equation 3), and calculating the $2^{\Delta Ct}$.

$$\Delta Ct = (Ct, ALS - Ct, EPSPS) \quad \text{[EQ. 3]}$$

Results and Discussion

Initial Screen for Three-Way Resistance. The initial screen showed that the combination of glyphosate + thifensulfuron + atrazine failed to control the MR population of Palmer amaranth. Previous research has shown complete control of other Palmer amaranth populations with these herbicides applied alone, at or below the rates used in this experiment (Chandi et al. 2013; Horak and Peterson 1995; Norsworthy et al. 2008). Control with all three herbicides applied in combination was 55 and 93% 14 DAT for the MR and S populations, respectively. Biomass reduction from the combination was 7.15X greater in the S population compared with the MR population. The results from this initial screen in the greenhouse confirm the preliminary observations from previous field trials, of a lack of sensitivity in the MR population to glyphosate, ALS-inhibitors, and atrazine. This multiple-resistance of glyphosate, ALS-

inhibitors, and atrazine in Palmer amaranth is not widespread, and has only been reported in one other population in Georgia (Heap 2016). To date, there has been little published on the Georgia population.

Resistance Factor. *ALS Resistance.* Thifensulfuron applied at half (0.002 kg ai ha^{-1}) of the normal field use rate or greater provided near complete control of the S Palmer amaranth population (Figure 2.1). However, there were some plants that survived the higher application rates, indicating that this population may not be completely susceptible to thifensulfuron. Even with this minor variability in control of the susceptible population, the dose of thifensulfuron required to reduce biomass of the S population 50% was 0.00014 kg ai ha^{-1} (Table 2.2). The GR_{50} value for the suspected ALS-resistant population (MR) was 0.006 kg ai ha^{-1} of thifensulfuron, indicating the RF for the MR population to be 42.9X (Table 2.2). The level of resistance in this population is lower than what has been previously reported for other populations of ALS-resistant Palmer amaranth (Sprague et al. 1997). The population investigated by Sprague et al. (1997) was highly resistant to ALS-inhibiting herbicides, with an RF of >3700 for thifensulfuron. There have been other reports of varying levels of ALS resistance between populations. For example, suspected ALS-resistant populations of Palmer amaranth from Mississippi and Georgia treated with pyrithiobac had RF values of 8 and 303X, respectively (Nandula et al. 2012; Sosnoskie et al. 2011). The difference in resistance levels may be attributed to the sensitivity of the susceptible population to ALS-inhibiting herbicide used the in screening process. Even with the lower resistance level of the MR population, complete control with thifensulfuron was not observed with 32X (0.14 kg ai ha^{-1}), the normal field use of rate of thifensulfuron and biomass was only reduced 70% at this rate (Figure 2.1).

Glyphosate Resistance. The dose response analysis showed that the S Palmer amaranth population was more sensitive to glyphosate compared with the MR population (Figure 2.2). The 1X (1.26 kg ae ha^{-1}) rate of glyphosate completely controlled the S population, while the 1X rate of glyphosate only reduced Palmer amaranth biomass 10% for the MR population. Glyphosate applied at 16X (13.5 kg ae ha^{-1}) the labeled rate reduced Palmer amaranth biomass 95% for the MR population. The GR_{50} values were 0.094 and 1.14 kg ae ha^{-1} for the S and MR populations, respectively (Table 2.2). The RF value of 12X for the MR population falls within the range of the RF values of 5 to 115X that have been previously reported for other populations of glyphosate-resistant Palmer amaranth (Culpepper et al. 2006; Norsworthy et al. 2008; Steckel et al. 2008). These results demonstrate that the MR population is resistant to both glyphosate and ALS-inhibiting herbicides, including thifensulfuron. The first populations of documented glyphosate- and ALS-resistant Palmer amaranth were found in Georgia and Mississippi in 2008 (Nandula et al. 2012; Sosnoskie et al. 2011). Since then, several other Palmer amaranth populations have been documented as having multiple resistance to glyphosate and ALS-inhibiting herbicides. This multiple-resistance can limit options for Palmer amaranth control and in some cases, the only option for POST management is the use of glufosinate in glufosinate-resistant crops.

Atrazine Resistance POST. Atrazine applied POST at 1.12 kg ai ha^{-1} (1X) reduced Palmer amaranth biomass 89% for the S population (Figure 2.3). This dose falls in the range of rates that Jhala et al. (2014) reported for the effective dose to reduce Palmer amaranth biomass 90% (ED_{90}) in two atrazine-sensitive Palmer amaranth populations. To reduce Palmer amaranth biomass 90% for the MR population, atrazine needed to be applied at 32X (35.90 kg ai ha^{-1}) the

normal use rate (Figure 2.3). The GR_{50} values for the S and MR Palmer amaranth populations were 0.13 and 1.20 kg ai ha^{-1}, respectively, resulting in a RF of 9X (Table 2.2). The RF value for the MR population is similar to RF values (9 to 14X) reported by Jhala et al. (2014) in a Nebraska Palmer amaranth population resistant to atrazine. However, the RF for atrazine in the MR Palmer amaranth population is lower than what has previously been reported for triazine-resistant smooth pigweed and tall waterhemp, where RFs were greater than 100X (Foes et al. 1998; Maertens et al. 2003). The mechanism for atrazine resistance in these populations was reported as target site mediated. The lower RF observed in the MR population may indicate that the mechanism for resistance in this population may not be target-site based, and another mechanism for atrazine resistance, such as metabolism, may be responsible. Metabolism has been reported as another mechanism for triazine resistance in velvetleaf, Palmer amaranth, and common waterhemp (Anderson and Gronwald 1991; Betha et al. 2015; Patzoldt et al. 2003).

Atrazine Resistance PRE. In addition to the MR population being less sensitive to atrazine applied POST, it was also less sensitive to atrazine applied PRE compared with the S population. The 1X (1.12 kg ai ha^{-1}) rate of atrazine reduced biomass of the S Palmer amaranth population 98%, while rates as high as 32X (35.90 kg ai ha^{-1}) the normal use rate failed to reduce biomass in the MR population >60% (Figure 2.4). Palmer amaranth is generally quite susceptible to PRE applications of atrazine (Johnson et al. 2012). Atrazine applied PRE failed to control several other weed species, such as common groundsel (*Senecio vulgaris* L.), common lambsquarters (*Chenopodium album* L.), hood canarygrass (*Phalaris paradoxa* L.), rigid ryegrass (*Lolium rigidum* Gaudin), and blackgrass (*Alopecurus myosuroides* Huds.) that have displayed resistance to atrazine applied POST (Fuerst et al. 1986; Ryan 1970; Yaacoby et al. 1986). The GR_{50} values

atrazine applied PRE for the S and MR Palmer amaranth populations were 0.035 and 3.93 kg ai ha^{-1}, respectively (Table 2.2). The RF for the MR population was 112.2X for atrazine applied PRE. This RF was 12 times greater than the RF for atrazine POST, showing that this population has a much higher level of resistance to PRE applications of atrazine than when it is applied POST. A possible explanation for the higher RF for atrazine PRE could be the rapid detoxification of atrazine via glutathione conjugation in the stem prior to movement into the leaves when atrazine is absorbed by the roots. This has been reported for velvetleaf where atrazine was metabolized at a higher rate in stem tissue compared with leaves, and stem metabolism has also been reported as an important mechanism for soybean (*Glycine max* L Merr.) tolerance to metribuzin (Fedtke and Schmidt 1983; Gronwald et al. 1989).

Molecular Basis for Resistance. *ALS-Inhibitors.* Previous research has shown that a single nucleotide change leading to amino acid substitution is responsible for the majority of ALS-resistance in tall waterhemp, redroot pigweed, smooth pigweed, Powell amaranth, and Palmer amaranth (Diebold et al. 2003; Foes et al. 1998; Patzoldt and Tranel 2007; Sibony et al. 2001; Whaley et al. 2007). This paired with the level of resistance expressed by the MR Palmer amaranth population in the dose response experiments, prompted molecular analysis to establish the alteration conferring resistance in this population of Palmer amaranth. Resistance to ALS-inhibitors in *Amaranthus spp.* have been well documented. Amino acid substitutions reported to cause resistance to the ALS-inhibitors in *Amaranthus spp*. have been found at six locations within the ALS enzyme: $alanine_{122}$ (Ala), $proline_{197}$ (Pro), Ala_{205}, $aspartate_{376}$ (Asp), $tryptophan_{574}$ (Trp), and $serine_{653}$ (Ser) (Ashigh et al. 2009; Heap 2016; Huang et al. 2016; McNaughton et al. 2005; Tranel and Wright 2002). All amino acid numbering is normalized to

the *Arabidopsis thaliana* sequence. In the MR Palmer amaranth population, there were several nucleotide changes at multiple locations within the *ALS* enzyme. With one exception, all polymorphisms resulted in an amino acid change and all others were silent mutations, resulting in no amino acid changes. In the MR population there was a cytosine to thymine change at position 574 (Table 2.3). This change allowed for a Pro to Leu amino acid substitution at Pro_{197} relative to the *Arabidopsis thaliana* numbering. This mutation has not been identified in other ALS-resistant Palmer amaranth populations, however the Pro to Leu substitution was previously reported to confer resistance to sulfonylurea herbicides (i.e., thifensulfuron) in redroot pigweed at similar levels as observed here with the MR population (Heap 2016; Sibony et al. 2001). The MR population was only screened with thifensulfuron, a sulfonylurea herbicide, for ALS-resistance, so cross-resistance to other classes of ALS-inhibiting herbicides was not determined. However, the Pro to Leu substitution was reported to cause low to high RF in redroot pigweed to the imidazolinone, trizolopyrimadine, and pyrimidinylthiobenzoic acid classes of ALS-inhibiting herbicides, in addition to the sulfonylurea herbicides (Sibony et al. 2001). This indicates a strong likelihood that the MR population would demonstrate cross resistance to four of the five classes of ALS-inhibiting herbicides.

Glyphosate Resistance. Weed resistance to glyphosate has been shown to be due to multiple mechanisms. In populations of horseweed (*Conyza Canadensis* L Cronq.) and rigid ryegrass, glyphosate resistance is conferred through reduced translocation and vacuole sequestration (Koger and Reddy 2005; Ge et al. 2009; Lorraine-Colwill et al. 2002). Similar to evolved resistance in weeds to ALS-inhibitors, populations of goosegrass (*Eleusine indica* L. Gaertn.), rigid ryegrass, and Italian ryegrass (*Lolium perenne* L. *ssp. multiflorum* (*Lam.)* Husnot) have

expressed target-site resistance to glyphosate with amino acid substitutions at Pro_{106} (Perez-Jones et al. 2007; Powles and Preston 2006; Wakelin and Preston 2006). The novel mechanism of resistance that has been attributed to conferring resistance to glyphosate in Palmer amaranth populations from Georgia, North Carolina, and New Mexico is over expression of the target enzyme EPSPS (Chandi et al. 2012; Gaines et al. 2010; Ribeiro et al. 2013; Mohseni-Moghadam et al. 2013). Based on these previous reports and the RF for the MR Palmer amaranth population, a molecular analysis was conducted to determine if gene amplification was the mechanism of glyphosate resistance in this population. The qPCR results indicated that the susceptible S population had only one copy of the EPSPS gene (Figure 2.5), while the number of copies ranged from 47 to >100 copies of EPSPS enzyme relative to ALS enzyme in the resistant MR population. The number of EPSPS copies in the MR population fell within the range of 5 to >160 genomic copies reported by Gaines et al. (2010). Gaines et al. (2010) reported that shikimate, the normal substrate for EPSPS, accumulation was minimal illustrating normal enzyme function with 65 or more copies of EPSPS in Palmer amaranth. Resistance to glyphosate increases as EPSPS copy number increases, however only 30 to 50 copies are necessary to survive normal field use rates of glyphosate (Gaines et al. 2011). All of the plants tested from the MR population fall within or above this range, with 75% of the plants having >60 genomic copies of the EPSPS enzyme. The results from qPCR and dose response experiments confirm that that MR population has moderate to high levels of resistance to glyphosate that is widely distributed within the population.

Atrazine Resistance. Target-site resistance with an amino acid substitution of Gly for Ser at position 264 of the D1 protein, has been reported as the primary mechanism for triazine

resistance in smooth pigweed, common waterhemp, kochia, and Powell amaranth (Diebold et al. 2003; Foes et al. 1998; Foes et al. 1999; Maertens et al. 2004). This amino acid substitution like most target-site based resistances confers a high level of resistance to atrazine. For example, this substitution was reported in an Illinois atrazine-resistant common waterhemp population that had a RF of 185X (Foes et al. 1998). There have been reports of other amino acid substitutions at Phe_{211}, Val_{219}, and Ala_{251} confering resistance to the Photosystem II (PSII) herbicides (i.e., atrazine) at a lower RF than the Gly to Ser_{264} substitution (Devine and Shukla 2000; Mengistu et al. 2000). The RF values reported for these other amino acid substitutions are similar to the one reported here for the MR population. Molecular analysis was conducted to determine if an amino acid substitution was present within the region of the *psbA* gene causing atrazine resistance in the MR population of Palmer amaranth. Evaluation of the *psbA* gene showed no nucleotide polymorphisms within the sequenced region. The absence of polymorphisms and the variability in expression of resistance, indicate that the mechanism of resistance for atrazine is most likely metabolism based. Non-target site triazine resistance, possibly via glutathione-S-transferase conjugation, was recently reported in a Kansas Palmer amaranth population and in other *Amaranthus spp.* (Betha et al. 2015; Ma et al. 2013).

This research confirms that a Palmer amaranth population found in Michigan is resistant to three different herbicide sites of action: glyphosate (Group 9), thifensulfuron an ALS-inhibiting herbicide (Group 2), and to PRE and POST applications of atrazine (Group 5). While this resistance profile was documented with one other Palmer amaranth population in Georgia 2010 (Heap 2016), this is the first report of RF values and the possible mechanisms of resistance for this type of three-way resistance. The addition of atrazine resistance to the already wide-spread resistance to glyphosate and ALS-inhibiting herbicides will make management of Palmer

amaranth more of a challenge in corn. Atrazine applied both PRE and POST has been an effective tool for Palmer amaranth management (Johnson et al. 2012; Norsworthy et al. 2008; Wiggins et al. 2015). Without atrazine, glyphosate, or the ALS-inhibiting herbicides for Palmer amaranth control, farmers will rely heavily on HPPD-inhibiting (Group 27) herbicides both PRE and POST, the long chain fatty acid inhibitors (Group 15) PRE, glufosinate (Group 10) POST, and the plant growth regulating herbicides (Group 4) POST. The sole reliance on these herbicides applied alone is not a sustainable approach to management, especially since there are recent documented cases of HPPD-resistance in Palmer amaranth (Jhala et al. 2014, Heap 2016), and a case of tall waterhemp with reported resistance to five different herbicide sites of action (Evans et al. 2015). Integrated approaches that include crop rotation, tillage, the use of both PRE and POST herbicide applications with overlapping residuals, tank-mixtures of herbicides with multiple effective sites of action, and perhaps the incorporation of cover crops will be needed to manage multiple-resistant Palmer amaranth populations.

APPENDIX

APPENDIX

CHAPTER 2 TABLES AND FIGURES

Table 2.1. List of oligonucleotide primers used for PCR, gene sequencing, and qPCR of the *ALS, psbA, and EPSPS* genes.

Primer	Sequence
ALS forward 1	5'TCCTCGCCGCCCTCTTCAAATC
ALS forward 2	5'GTCCGGGTGCTACTAATCTTGTTT
ALS forward 3	5'TTGCTAGTACTTTAATGGGGTTGG
ALS forward 4	5'GCTGCTGAAGGCTACGCT
ALS reverse 1	5'CAGCTAAACGAGAGAACGGCCAG
ALS reverse 2	5'GCATCTGGTCGAGCAACAGCAG
ALS reverse 3	5'GTCACTCGATCATCAAACCTAACC
ALS reverse 4	5'GCGGGACTGAGTCAAGAAGTG
psbA forward 1	5'CTCCTGTTGCAGCTGCTACT
psbA reverse 2	5'GAGGGAAGTTGTGAGC
EPSPS forward 1	5'ATGTTGGACGCTCTCAGAACTCTTGGT
EPSPS reverse 2	5'TGAATTTCCTCCAGCAACGGCAA

Table 2.2. GR_{50}[a] values, standard errors (±S.E.) and resistance factors (RF) for suspected resistant (MR) and susceptible (S) Palmer amaranth populations following preemergence and postemergence applications of atrazine, glyphosate, and thifensulfuron.

Herbicide	Population	GR_{50}[a]	± S.E.	RF[b]
		kg ai ha^{-1}		
Atrazine (PRE)	MR	3.927	7.99	112.2X
	S	0.035	0.02	
Atrazine (POST)	MR	1.206	0.2181	9.3X
	S	0.13	0.0235	
Glyphosate	MR	1.143	0.79074	12.2X
	S	0.094	0.00696	
Thifensulfuron	MR	0.006	0.00093	42.9X
	S	0.00014	0.00003	

[a] GR_{50} = required dose to reduce Palmer amaranth dry biomass 50%.

[b] Resistance factor $= \frac{GR_{50}\text{ (MR resistant)}}{GR_{50}\text{ (S susceptible)}}$

Table 2.3. Nucleotide and amino acid polymorphisms conferring ALS-resistance in the suspected multiple-resistant (MR) Michigan population of Palmer amaranth.

	Nucleotide and amino acid polymorphisms[a]	
Population	Codon 573-575	Amino acid 197
Susceptible (S)	CCC	Proline
Resistant (MR)	CTC	Leucine

[a] Polymorphisms denoted by nucleotide position within the codon. Amino acid position numbering normalized to *Arabidopsis thaliana.*

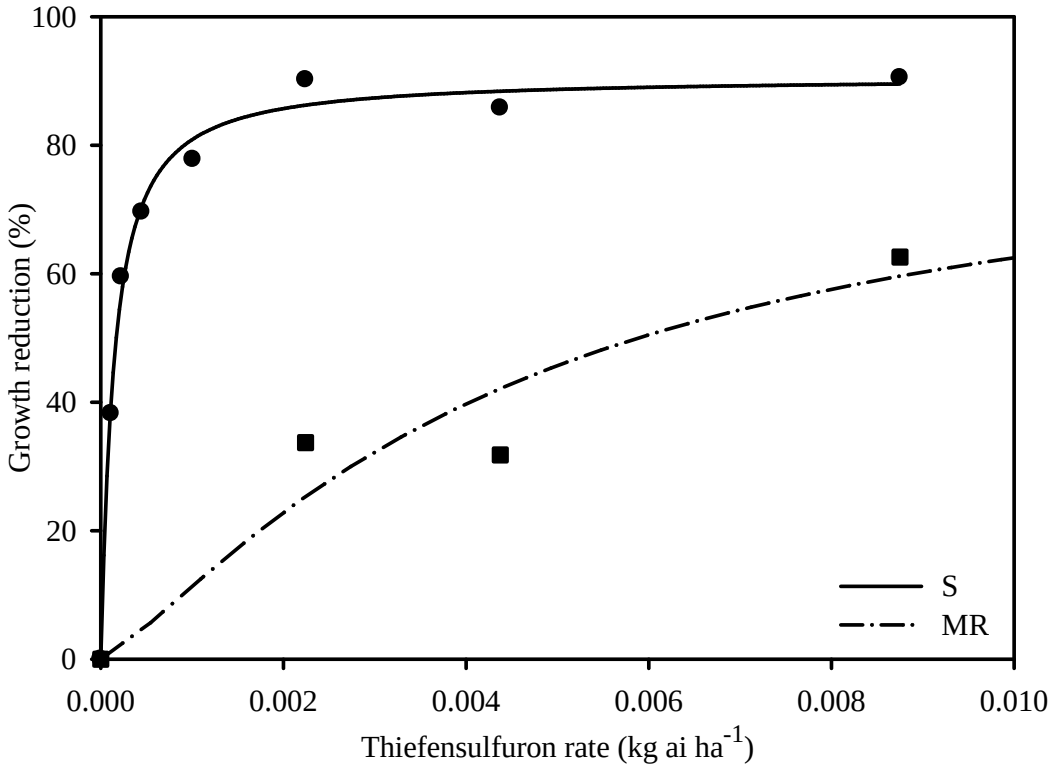

Figure 2.1. Biomass growth reduction of Palmer amaranth populations in response to applications of thifensulfuron. Fitted lines were calculated with the 3-parameter log-logistic model: S (susceptible), $y=90.5/(x/0.14)^{1.08}$, $R^2 = 0.79$; MR (suspected multiple-resistant), $y=84.8/(x/5.96)^{1.26}$, $R^2 = 0.77$. Means for the S population are represented by (●) and means for MR population are represented by (■).

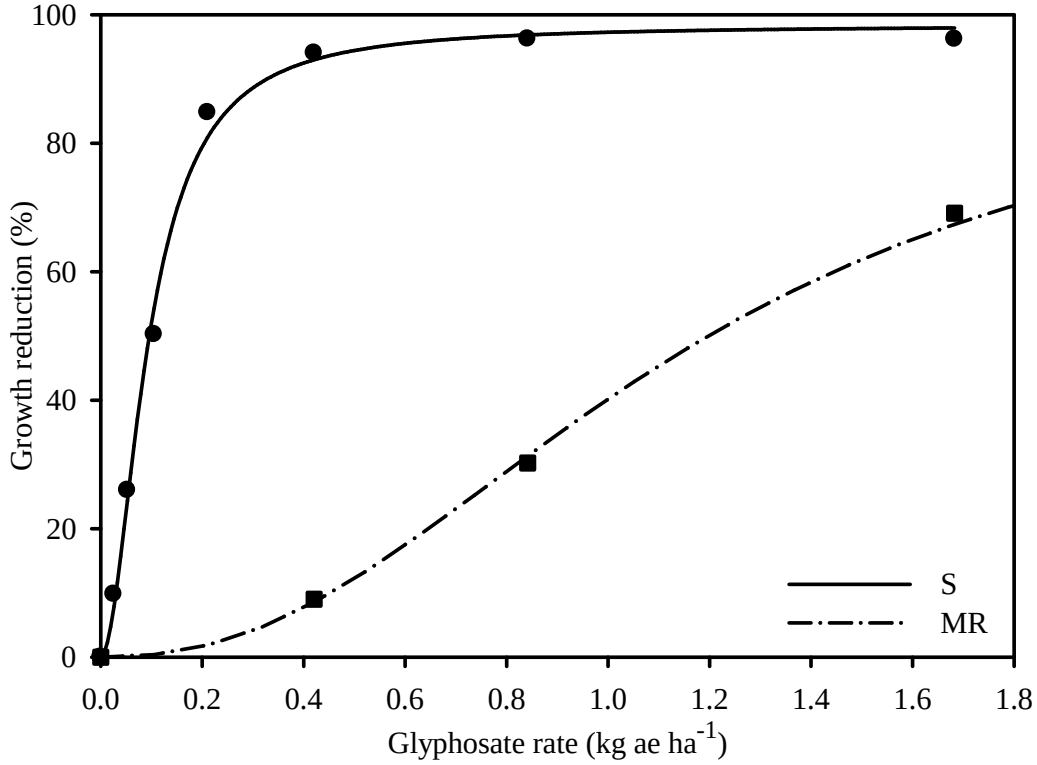

Figure 2.2. Biomass growth reduction of Palmer amaranth populations in response to applications of glyphosate. Fitted lines were calculated with the 3-parameter log-logistic model: S (susceptible), $y=90.5/(x/0.14)^{1.08}$, $R^2 = 0.79$; MR (suspected multiple-resistant), $y=84.8/(x/5.96)^{1.26}$, $R^2 = 0.77$. Means for the S population are represented by (●) and means for MR population are represented by (■).

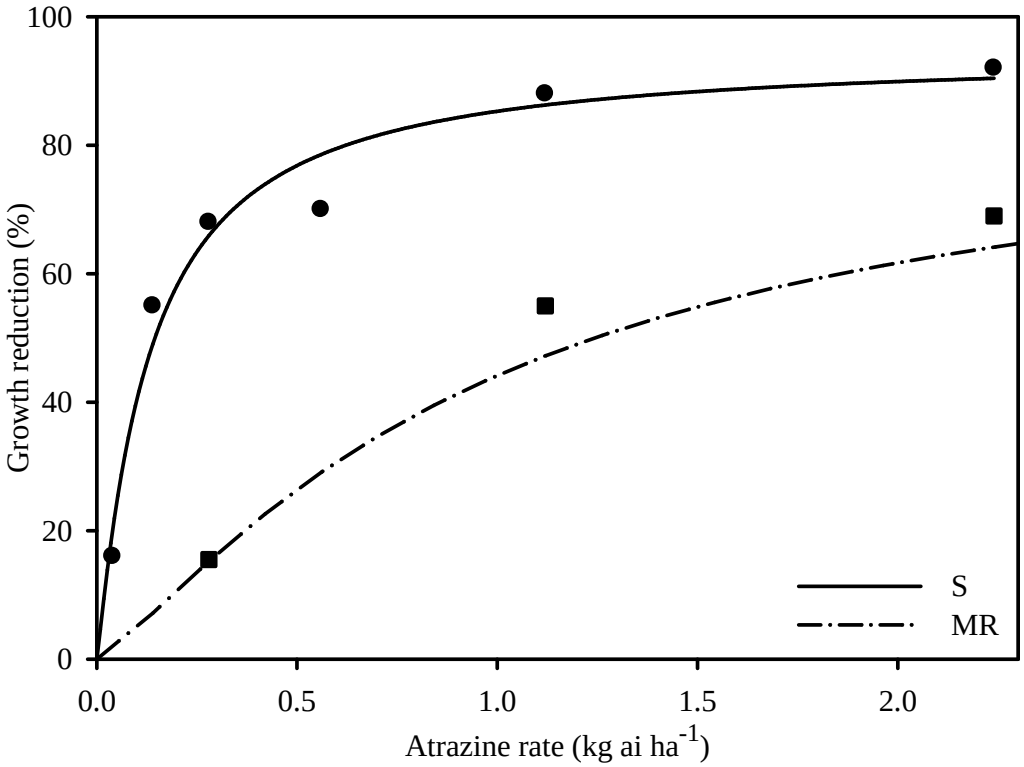

Figure 2.3. Biomass growth reduction of Palmer amaranth populations in response to postemergence (POST) applications of atrazine. Fitted lines were calculated with the 3-parameter log-logistic model: S (susceptible), $y=90.5/(x/0.14)^{1.08}$, $R^2 = 0.79$; MR (suspected multiple-resistant), $y=84.8/(x/5.96)^{1.26}$, $R^2 = 0.77$. Means for the S population are represented by (●) and means for MR population are represented by (■).

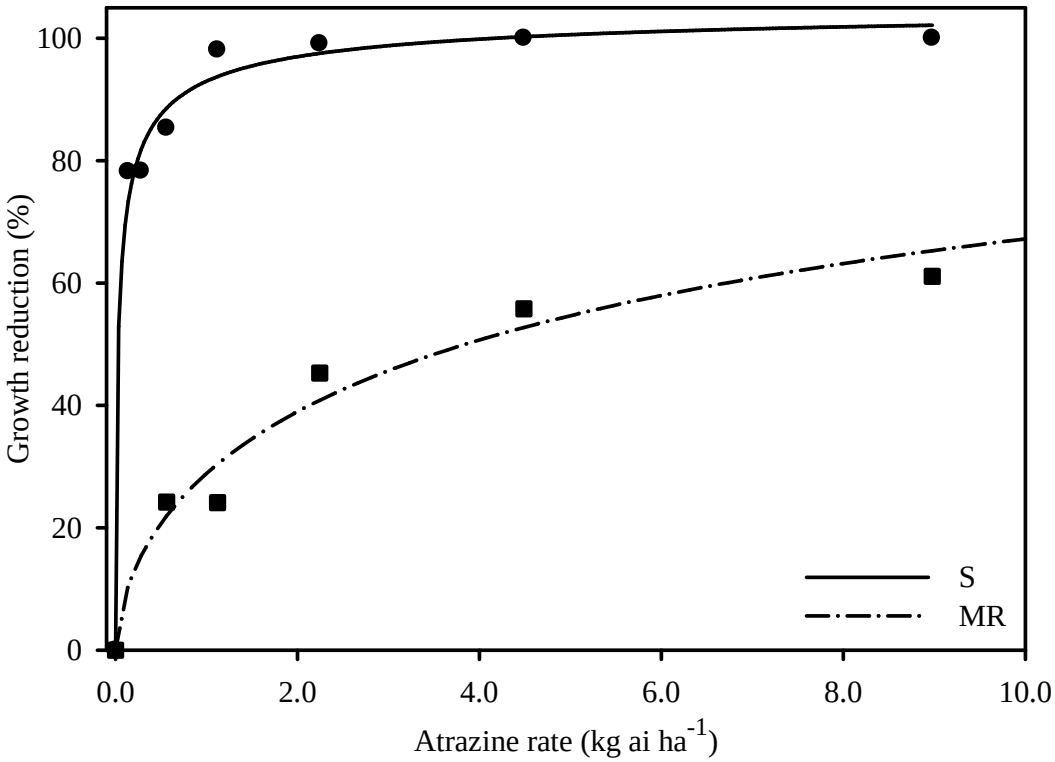

Figure 2.4. Biomass growth reduction of Palmer amaranth populations in response to preemergence (PRE) applications of atrazine. Fitted lines were calculated with the 3-parameter log-logistic model: S (susceptible), $y=90.5/(x/0.14)^{1.08}$, $R^2 = 0.79$; MR (suspected multiple-resistant), $y=84.8/(x/5.96)^{1.26}$, $R^2 = 0.77$. Means for the S population are represented by (●) and means for MR population are represented by (■).

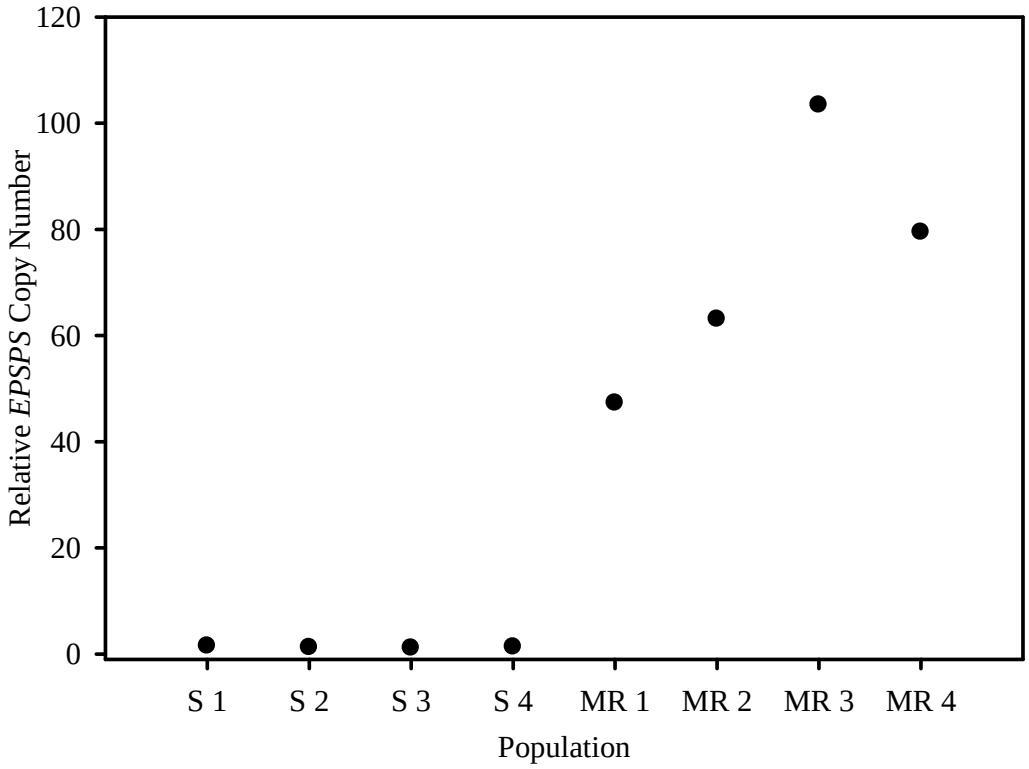

Figure 2.5. EPSPS copy number relative to *ALS enzyme* in susceptible (S) and suspected multiple-resistant (MR) populations of Palmer amaranth. Relative copy number determined using real-time qPCR with methods described by Gaines et al. (2010).

LITERATURE CITED

LITERATURE CITED

Anderson MP, Gronwald JW (1991) Atrazine resistance in a velvetleaf (*Abutilon theophrasti*) biotype due to enhanced glutathione *S*-transferase activity. Plant Physiol 96:104–109

Ashigh J, Corbett CL, Smith PJ, Laplante J, Tardif FJ (2009) Characterization and diagnostic tests of resistance to acetohydroxyacid synthase inhibitors due to an $Asp_{376}Glu$ substitution in *Amaranthus powellii*. Pestic Biochem and Physol 95:38–46

Betha S, Thompson CR, Peterson DE, Jugulam M (2015) Mechanism(s) of resistance to PS II- and ALS-inhibitors in mesotrione-resistant Palmer amaranth. Pages 36-37 *in* Proceedings of 70th annual meeting of the North Central Weed Science Society Indianapolis, IN: North Central Weed Science Society

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norseworthy JK, Ritz C (2013) Review: Conformation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4–20

Chandi A, Jordan DL, York AC, Milla-Lewis SR, Burton JD, Culpepper AS, Whitiker JR (2013) Interference and control of glyphosate-resistant and –susceptible Palmer amaranth (*Amaranthus palmeri*) populations under greenhouse conditions. Weed Sci 61:259–266

Chandi A, Milla-Lewis SR, Giacomini D, Westra P, Preston C, Jordan DL, York AC, Burton JD, Whitaker JR (2012) Inheritance of evolved glyphosate resistance in a North Carolina Palmer amaranth (*Amaranthus palmeri*) biotype. Int J Agron DOI:10.1155/2012/176108

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54.4:620–626

Devine MD, Shukla A (2000) Altered target sites as a mechanism of herbicide resistance. Crop Prot 19:881–889

Diebold RS, McNaughton KE, Lee EA, Tardif FJ (2003) Multiple resistance to imazethapyr and atrazine in Powell amaranth (*Amaranthus powellii*). Weed Sci 51:312–318

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. Oecologia 57:10–112

Evans CM, Tranel P, Riechers DE, Davis AS, Maxwell D, Gonzini L, Hager AG (2015) Characterization of a novel five-way resistant population of waterhemp *Amaranthus tuberculatus*. Page 61 *in* Proceeding of the 70th Annual Meeting of the North Central Weed Science Society. Indianapolis, IN: North Central Weed Science Society

Fedtke C, Schmidt RR (1983) Behavior of metribuzin in tolerant and susceptible soybean varieties. Pages 177–182 *in* Miyamoto J, Kearney PC, Eds. Pesticide chemistry: Human welfare and the environment, volume 3. Pergamon, Elmsford, New York

Foes MJ, Tranel PJ, Wax, LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 514–520

Foes MJ, Liu L, Vigue G, Stoller EW, Wax LM, Tranel PJ (1999) A kochia (*Kochia scoparia*) biotype resistant to triazine and ALS-inhibiting herbicides. Weed Sci 47:20–27

Franssen AS, Skinner DZ, Al-Khatib K, Horak MJ, Kulakow PA (2001) Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci 49.5:598–606

Fuerst EP, Barrett M, Penner D (1986) Control of triazine-resistant common lambsquarters (*Chenopodium album*) and two pigweed species (*Amaranthus spp.*) in corn (*Zea mays*). Weed Sci 34:440–443

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci 107.3:1029–1034

Gaines TA, Shaner DL, Ward SM, Leach JE, Preston C, Westra P (2011) Mechanism of resistance of evolved glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*). J Agric Food and Chem 59:5886–5889

Ge X, d'Avignon DA, Ackerman JJ, Sammons RD (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag Sci 66:345–348

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci 62:29–37

Gossett BJ, Murdock EC, Toler JE (1992) Resistance of Palmer amaranth (*Amaranthus palmeri*) to the dinitroaniline herbicides. Weed Technol 6:587–591

Gronwald JW, Anderson RN, Yee C (1989) Atrazine resistance in velvetleaf (*Abutilon theophrasti*) due to enhanced atrazine detoxification. Pestic Biochem Physiol 34:149–163

Heap I (2014) Herbicide resistant weeds. Pages 281-301 *in* Pimentel D, Peshin R, eds. Integrated Pest Management. Netherlands: Springer

Heap I (2016) The international survey of herbicide resistant weeds. www.weedscience.com. Accessed: June 24, 2016.

Horak MJ, Peterson DE (1995) Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol 9:192–195

Huang Z, Chen J, Zhang C, Huang H, Wei S, Zhou X, Chen J, Wang X (2016) Target-site basis for resistance to imazethapyr in redroot amaranth (*Amaranthus retroflexus* L.) Pestic Biochem and Physol 128:10–15

Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of triazine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38

Johnson WG, Chahal GS, Regehr DL (2012) Efficacy of various corn herbicides applied preplant incorporated and preemergence. Weed Technol 26:220–229

Koger CH, Reddy KN (2005) Role of absorption and translocation in the mechanism of glyphosate resistance in horseweed (*Conyza canadensis*). Weed Sci 55:84–89

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Lorraine-Colwill DF, Powles SB, Hawkes TR, Hollinshead PH, Warner SAJ, Preston C (2002) Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. Pestic Biocehm and Physio 74:62–72

Ma R, Kaundun SS, Tranel PJ, Riggins CW, McGinness DL, Hager AG, Hawkes T, McIndoe E, Riechers DE (2013) Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. Plant Phys 163:363–377

Maertens KD, Sprague CL, Tranel PJ, Hines RA (2004) *Amaranthus hybridus* populations resistant to atrazine and acetolactate synthase-inhibiting herbicides. Weed Res 44:21–26

McNaughton KE, Letarte J, Lee EA, Tardif FJ (2005) Mutations in *ALS* confer herbicide resistance in redroot pigweed (*Amaranthus retroflexus*) and Powell amaranth (*Amaranthus powellii*). Weed Sci 53:17–22

Mengistu LW, Mueller-Warrant GW, Liston A, Barker RE *psbA* mutation ($valine_{219}$ to isoleucine) in *Poa annua* resistant to metribuzin and diuron. Pest Manag Sci 56:209–217

Mengistu LW, Christoffers MJ, Lym RG (2005) A *psbA* in *Kochia scoparia* (L) Schrad from railroad rights-of-way with resistance to diuron, tebuthiuron and metribuzin. Pest Manag Sci 61:1035–1042

Mohseni-Moghadam M, Schroeder J, Ashigh J (2013) Mechanism of resistance and inheritance in glyphosate resistant Palmer amaranth (*Amaranthus palmeri)* populations from New Mexico, USA. Weed Sci 61:517–525

Nandula VK, Reddy KN, Koger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribeiro DN (2012) Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Sci 60:179–188

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

Owen MDK (2008) Weed species shifts in glyphosate-resistant crops. Pest Manag Sci 64.4:377–387

Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq) Sauer that is not site-of-action mediated. Pest Manag Sci 59:1134–1142

Patzoldt, WL, Tranel PJ, Hager AG (2002) Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot 21:707–712

Patzoldt WL, Tranel PJ (2007) Multiple ALS mutations confer herbicide resistance in waterhemp (*Amaranthus tuberculatus*) Weed Sci 55:421–428

Perez-Jones A, Park K-W, Polge N, Colquhoun, Mallory-Smith CA (2007) Investigating the mehanisms of glyphosate resistance in *Lolium multiflorum*. Planta 226:395–404

Powles SB, Preston C (2006) Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. Weed Technol 20:282–289

Ribeiro DN (2013) Mechanisms and variability of glyphosate resistance in *Amaranthus palmeri* and *Ipomoea lacunose* Ph.D dissertation. Starkville, MS: Mississippi State University. 127 p

Ryan GF (1970) Resistance of common groundsel to simazine and atrazine. Weed Sci 18:614–616

Sauer J (1957) Recent migration and evolution of the dioecious amaranths. Evolution 11:11–31

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus species* in Missouri. Weed Sci 51.3:329–333

Sibony M, Michel A, Haas HU, Rubin B, Hurle, K (2001) Sulfometuron-resistant *Amaranthus retroflexus*: cross-resistance and molecular basis for resistance to acetolactate synthase-inhibiting herbicides. Weed Res 41:509–522

Sibony M, Rubin B (2003) The ecological fitness of *Amaranthus retroflexus* and *A. blitoides* resistant to acetolactate synthase (ALS) inhibitors and atrazine. Weed Res 43.1:40–47

Shaner DL (2000) The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Manag Sci 56:320–326

Soltani N, Vyn JD, Swanton CJ, Weaver S, Sikkema PH (2008) Growth and fitness of triazine-susceptible and triazine-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*). Weed Biol Manag 8:73–77

Sosnoskie LM, Kichler JM, Wallace RD, Culpepper AS (2011) Multiple resistance in Palmer amaranth to glyphosate and pyrithiobac confirmed in Georgia. Weed Sci 59:321–325

Sprague CL (2011) Glyphosate-resistant Palmer amaranth confirmed in Michigan. http://msuweeds.com/newsletter-holder/emerging-weed-issues/glyphosate-resistant-palmer-amaranth-confirmed-in-southwest-michigan/. Accessed July 11, 2016

Sprague CL, Stoller EW, Wax LM, Horak MJ (1997) Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci 192–197

Steckel LE, Main CL, Ellis AT, Mueller TC (2008) Palmer amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technol 22:119–123

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700–712

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci 60:2–30

Wakelin AM, Preston C (2006) A taget-site mutation is present in a glyphosate-resistant *Lolium rigidum* population. Weed Res 46:432–440

Webster TM, Coble HD (1997) Changes in the weed species composition of the Southern United States: 1974 to 1995. Weed Technol 11:308–317

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145–157

Whaley CM, Wilson HP, Westwood JH (2007) A new mutation in plant *ALS* confers resistance to five classes of ALS-inhibiting herbicides. Weed Sci 55:83–90

Wiggins MS, McClure MA, Hayes RM, Steckel LE (2015) Integrating cover crops and POST herbicides for glyphosate-resistant Palmer amaranth control in corn. Weed Technol 29:412-418

Yaacoby T, Schonfeld M, Rubin B (1986) Characteristics of atrazine-resistant biotypes of three grass weeds. Weed Sci 34:181–184

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops 1. Weed Technol 20.2:301–307

CHAPTER 3

HERBICIDE MANAGEMENT STRATEGIES IN CORN FOR A THREE-WAY (GLYPHOSATE, ALS, AND ATRAZINE) HERBICIDE-RESISTANT PALMER AMARANTH POPULATION

Abstract

Three different field experiments were conducted from 2013-2015 in Barry County, MI to evaluate the effectiveness of PRE, POST, and one- (EPOS) and two-pass (PRE followed by POST) herbicide programs for management of multiple-resistant Palmer amaranth in corn. The Palmer amaranth population at this location has demonstrated resistance to glyphosate (Group 9), ALS-inhibiting herbicides (Group 2), and atrazine (Group 5). In the PRE only experiment, control with the soil-applied products varied by year. In 2013, rainfall exceeded 17 cm 30 days after treatment (DAT) causing the failure of several PRE herbicides to control Palmer amaranth 45 DAT. Atrazine at 1.12 and 2.24 kg ai ha^{-1} applied PRE failed to control Palmer amaranth control in all three years of the experiment, confirming that this population was resistant atrazine. The only herbicides that consistently provided ~80% or greater control PRE were pyroxasulfone and the combination of mesotrione + *s*-metolachlor. However, none of these treatments provided season-long Palmer amaranth control. In the POST only experiment, only topramezone provided >85% Palmer amaranth control 14 DAT. Of the 19 herbicide programs studied all but three programs provided ≥88% Palmer amaranth control at corn harvest. Herbicide programs that did not control Palmer amaranth relied on only one effective herbicide site of action and in one case did not include a residual herbicide POST for late-season Palmer amaranth control. Some of the EPOS treatments were effective for season-long Palmer amaranth control; however, application timing will be critical and the inclusion of a residual herbicide component necessary for controlling Palmer amaranth. The programs that consistently provided the highest levels of

season-long Palmer amaranth control were PRE followed by POST herbicide programs that relied on a minimum of two effective herbicide sites of action and usually included a residual herbicide for late-season Palmer amaranth control.

Nomenclature: Atrazine; glyphosate; mesotrione; pyroxasulfone; *s*-metolachlor; corn, *Zea mays* L.; Palmer amaranth, *Amaranthus palmeri* S. Watts.

Keywords: Herbicide sites of action; multiple-resistance; Palmer amaranth control.

Introduction

The genus *Amaranthus* is comprised of over 70 species that are both native and non-native to the United States (U.S.). However, only a select few are problematic in U.S. crop production systems. Redroot pigweed (*Amaranthus retroflexus* L.), Powell amaranth (*Amaranthus powellii* S. Wats.), spiny amaranth (*Amaranthus spinosus* L.), smooth pigweed (*Amaranthus hybridus* L.), common waterhemp (*Amaranthus tuberculatus* Moq. Sauer.), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) are the most common of these problematic species (Bensch et al. 2003; Knezevic et al. 1994; Gossett and Toler 1999; Grichar 1994; Hager et al. 2002; Massinga et al. 2001; Moolani et al. 1964; Schweizer and Lauridson 1985; Toler et al. 1996). Redroot pigweed, Powell amaranth, spiny amaranth, and smooth pigweed are monoecious (male and female structures on the same plant), while common waterhemp and Palmer amaranth are dioecious (male and female structures on separate plants) (Bryson and DeFelice 2010). Although all of these species are broadly distributed and troublesome in row crop production, but few have been as detrimental in recent history as Palmer amaranth.

Some of the attributes that make Palmer amaranth a troublesome weed are season-long emergence, rapid growth, and prolific seed production (Ehleringer 1983; Horak and Loughin

2000; Keeley et al. 1987). In Michigan, Palmer amaranth emerges from mid-May through September (Powell 2014). This extended emergence pattern makes it difficult to achieve season-long Palmer amaranth control with soil-applied, preemergence (PRE) herbicides. Following emergence, Palmer amaranth grows more rapidly and accumulates more biomass than other *Amaranthus* spp. (Horak and Loughin 2000). This makes it difficult to time postemergence (POST) herbicide applications for Palmer amaranth control. Palmer amaranth produced 250,000, 446,000, and 613,000 seeds plant^{-1} in Missouri, Georgia, and California, respectively, when it emerged at optimal times without inter- or intra-specific competition (Keeley et al. 1987; Sellers et al. 2003; Webster and Grey 2015). This prolific seed production allows Palmer amaranth to remain and replenish the soil seedbank if left uncontrolled. If Palmer amaranth competes from crop emergence, yield can be reduced up to 91 and 64% in corn and soybean, respectively (Klingaman and Oliver 1994; Massinga 2001). Delaying Palmer amaranth emergence can reduce competition, however yield can still be impacted by as much as 35% when Palmer amaranth competed with corn starting at V4 to V7 (Massinga et al. 2001). The rapid growth rate of Palmer amaranth allow for it to effectively compete with crops for light, water, and nutrients (Horak and Loughin 2000; Massinga et al. 2003; Wiese 1968).

In addition to biological characteristics, Palmer amaranth's propensity to develop resistance to herbicides has perpetuated it as a problem weed. Currently, Palmer amaranth is resistant to six herbicide sites of action (Heap 2016), including glyphosate. The first case of glyphosate-resistant Palmer amaranth was reported in Georgia in 2005 (Culpepper et al. 2006). Since then, glyphosate-resistant Palmer amaranth has spread to 23 other states, including Michigan (Heap 2016). The magnitude of glyphosate resistance ranges from 1.5 to 115-times the rate of glyphosate required to achieve 50% control in a susceptible population (Norsworthy et al. 2008;

Steckel et al. 2008). In addition to resistance to single herbicide sites of action, there are several populations with resistance to multiple herbicide sites of action (Heap 2016). One of the most prevalent is resistance to glyphosate and ALS-inhibiting herbicides. Populations of Palmer amaranth resistant to both glyphosate and ALS-inhibitors were identified in eight states, including Michigan (Heap 2016; Nandula et al. 2012; Sosnoskie et al. 2011). In Michigan, there are four confirmed resistance profiles in Palmer amaranth, ranging from single site of action, glyphosate or ALS-inhibiting herbicides, to multiple herbicide sites of action, glyphosate + ALS-inhibitors, within a single population. In addition to these populations, there is a population in Michigan with confirmed resistant to three different herbicide sites of action, glyphosate + ALS-inhibitors + atrazine (Chapter 2). Herbicide resistance in Palmer amaranth poses significant challenges for the development of management strategies.

With the limited number of effective herbicide options available for Palmer amaranth control in soybean, planting corn may provide farmers the greatest opportunity to manage this weed. Herbicides that control herbicide-susceptible and glyphosate and ALS-resistant Palmer amaranth in corn include: photosystem II inhibitors (Group 5), glufosinate (Group 10), long-chain fatty acid inhibitors (Group 15), and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting (Group 27) herbicides (Geier et al. 2006; Johnson et al. 2012; Norsworthy et al. 2008; Schuster et al. 2008; Stephenson et al. 2015). The success of the previously described management strategies is primarily due to the susceptibility of the Palmer amaranth populations to specific herbicide sites of action. However, when managing Palmer amaranth populations resistant to three-herbicide sites of action, management strategies need to be based on the use of effective herbicides. These strategies will likely need to include multiple herbicide applications, due to Palmer amaranth's prolonged emergence and rapid growth rate. Palmer amaranth is a relatively

new problem in the major corn producing regions of the U.S; the majority of research has focused on management of multiple-resistant Palmer amaranth in cotton, soybean, and peanut (Ward et al. 2013). Therefore, the objectives of this research were to: 1) evaluate the effectiveness of several PRE herbicides applied alone, in combination with atrazine, and commercially available premixes, 2) evaluate the effectiveness of POST applied herbicides, and 3) develop and evaluate one- and two-pass herbicide programs for the control of multiple-resistant Palmer amaranth in corn.

Materials and Methods

Three separate field experiments were conducted in a commercial corn production field with a known population of multiple-resistant Palmer amaranth in Barry County, MI (42.702467°N; -85.524992°W) in 2013, 2014, and 2015. This population was suspected to be resistant to glyphosate and ALS-inhibiting herbicides. After control failures with atrazine in 2013, this population was confirmed resistant to three herbicides site of actions: glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2). All experiments were arranged in a randomized complete block design with four replications. Plot size for each treatment was 3 m wide by 10 m long. The soil type was a combination of Oshtemo sandy loam and a Boyer loamy sand composed of 73.0, 15, and 12% sand, silt, and clay, respectively, with a pH of 7.0 and 2.2% organic matter (OM). Field preparation included fall chisel plowing followed by two-passes of a soil finisher in the spring. Corn was planted at 67,950 seeds ha^{-1} in 76 cm rows; planting dates and corn hybrid information can be found in Table 3.1. Precipitation and temperature data were obtained from a nearby weather station operated by Michigan State University (MSU Enviroweather 2016).

Evaluation of Preemergence (PRE) Herbicides. Field experiments were conducted to evaluate the control of multiple-resistant Palmer amaranth with soil-applied PRE herbicides. Herbicide treatments were applied to the soil surface after planting (0-2 d) using a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at a pressure of 207 kPa through 11003 AIXR flat-fan nozzles (TeeJet, Spraying Systems Co., Wheaton, IL 60187) (Table 3.1). Herbicide product information and treatments for this experiment can be found in Tables 3.2 and 3.3, respectively. A non-treated control treatment was included as a comparison.

Corn injury and Palmer amaranth control were evaluated at 30, 45, 60, and 72 days after planting (DAP). Evaluations were based on a scale of 0 to 100%, with 0 being no control and 100 indicating complete control. Aboveground Palmer amaranth biomass was harvested from two 0.25 m^{-2} quadrats, 45 DAP in 2013 (June 30) and 60 DAP in 2014 (July 18) and 2015 (July 13). Palmer amaranth biomass was dried at 60 C for approximately 1 wk then weighed and percent biomass reduction was calculated using equation 1.

$$y = (100 - (\frac{sample\ dry\ weight}{nontreated\ control\ dry\ weight} \times 100)) \quad \text{[EQ. 1]}$$

Evaluation of Postemergence (POST) Herbicides. A field experiment was conducted only in 2013 and 2014 evaluated the effectiveness of POST herbicides for multiple-resistant Palmer amaranth control in corn. Herbicide treatments were applied when Palmer amaranth was between 7.5 and 10 cm tall, using the equipment described above. Application dates can be found in Table 3.1. Herbicide product information and treatments for this experiment can be found in Tables 3.2 and 3.4, respectively. A non-treated control treatment was included in this experiment as a comparison. Crop injury and weed control were evaluated 7 and 14 DAT, on a scale from 0 to 100% with 0 representing no control and 100 indicating plant death.

Evaluation of Herbicide Programs for Palmer amaranth Control in Corn. Field experiments were conducted to evaluate several weed control programs for control of multiple-resistant Palmer amaranth in corn. These programs consisted of: 1) two POST herbicide applications, early POST (EPOS) followed by (fb) POST, 2) PRE fb POST, and 3) one-pass EPOS options. The herbicide programs examined are listed in Table 3.5 and herbicide product information can be found in Table 3.2. Spray grade ammonium sulfate (AMS) (Actamaster, Loveland Products, Inc., Loveland, CO) at 2% w w^{-1} was added to all EPOS and POST herbicide treatments. Herbicide applications were made using the same equipment as described above. Herbicide application dates can be found in Table 3.1. PRE herbicides were applied to the soil surface after planting (0-2 d) and POST herbicides applied when the majority of plots had Palmer amaranth at 7.5 cm. The one-pass EPOS applications were made when Palmer amaranth was 5 to 7.5 cm in height.

Corn injury and Palmer amaranth control were evaluated 14 d after the EPOS (DAEP) and POST (DAPO) and at harvest. Evaluations were based on a scale of 0 to 100 described above. Aboveground Palmer amaranth biomass was harvested from two 0.25 m^{-2} quadrats, 14 DAPO. Palmer amaranth biomass was dried at 6 C for approximately 1 wk, weighed, and percent biomass reduction calculated using equation 1.

Statistical Analysis. Statistical analysis was conducted using SAS 9.4 (SAS Institute, Cary, NC). Each experiment was analyzed separately. Assumptions of normality of residuals and homogeneity of variances were confirmed using PROC UNIVARIATE and analysis of variance (ANOVA) was conducted using PROC MIXED. The statistical model included herbicide treatment and year as fixed effects and replication as a random effect for the PRE and POST

herbicide experiments. Data were combined over years when there was not a treatment by year interaction. For the herbicide programs experiment, the statistical model included herbicide programs as a fixed effect and replications and years as random effects in the model. Levene's test for homogeneity of variances indicated that the variance was unequal between years for this experiment. Therefore, data was analyzed using the REPEATED statement and the GROUP = option; GROUP = year was used to compensate for differences in variance and the degrees of freedom was adjusted for unequal variances with DDFM = SATTERTH option in the MODEL statement. For all experiments, multiple comparisons among the means were made using *t*-tests when herbicide treatments were found to be statistically significant at 0.05 levels.

Results and Discussion

Evaluation of PRE Herbicides. Little to no corn injury was observed with any of the PRE herbicide treatments (data not shown). Overall Palmer amaranth control from the PRE herbicide treatments was lower in 2013 than in 2014 and 2015. This may have been attributed to the higher amounts of rainfall within 10 d of planting and the PRE applications. In 2013, 9.3 cm of rain fell compared with 1.71 and 1.57 cm in 2014 and 2015, respectively, within this timeframe. Therefore, the 2013 results are presented separately from the combined 2014 and 2015 results. The small seed size of Palmer amaranth generally favors germination from shallow depths (≤ 1.3 cm) in the soil (Keeley et al. 1987). The rainfall in 2013 could have accelerated herbicide dissipation and increased leaching of some of the herbicides below the Palmer amaranth germination zone, resulting in lower control in 2013 as compared with 2014 and 2015.

Atrazine at 1.1 and 2.2 kg ai ha^{-1} failed to control Palmer amaranth (Table 3.3). Palmer amaranth control and biomass reduction with atrazine was amongst the poorest in 2013 with both

rates, and even though 2.2 kg ai ha^{-1} provided slightly better control 72 DAP and higher biomass than 1.1 kg ai ha^{-1} of atrazine in 2014-2015, Palmer amaranth control was only 51% and unacceptable. Atrazine applied PRE has generally been an effective tool for Palmer amaranth control in corn. Johnson et al. (2012) reported that atrazine PRE at 1.68 kg ai ha^{-1} controlled Palmer amaranth >98%, 8 wks after application (WAP). After the failure to control this population with atrazine in 2013, greenhouse testing confirmed that this population was resistant to PRE atrazine (resistance factor = 112-fold).

Isoxaflutole was the only other herbicide active ingredient applied alone or in combination with atrazine that failed (<60%) to control Palmer amaranth in all three years (Table 3.3). While cases of HPPD-resistance in Palmer amaranth have been documented (Jhala et al. 2014, Heap 2016), Palmer amaranth control from mesotrione was amongst the highest in 2014-2015, and was just slightly lower than the best treatments in 2013. This suggests that this population is not resistant to the HPPD-inhibiting herbicides and that isoxaflutole is not an effective herbicide for Palmer amaranth control at this location. Greater levels of Palmer amaranth control with isoxaflutole have been previously reported (Johnson et al. 2012; Stephenson and Bond 2012). Isoxaflutole is a pro-herbicide that needs to be converted to the more soluble compound diketonitrile for activity (Pallett et al. 1998). The sorption of isoxaflutole to the soil is influenced by soil OM and pH, with sorption decreasing as soil pH increases from 4.5 to 8.5 (Pallett et al. 2001). The rate at which isoxaflutole converts to diketonitrile increases as soil moisture increases, increasing the possibility of leaching or the conversion to the inactive benzoic acid (Taylor-Lovell et al. 2002). The relatively high soil pH and low OM at this location paired with higher rainfall after application in 2013 could have led to the rapid conversion of unbound isoxaflutole to diketonitrile, leading to increased leaching or the degradation of isoxaflutole and

diketonitrile complexes outside of the Palmer amaranth germination zone. These factors may have contributed to the overall reduction in Palmer amaranth control observed with isoxaflutole at this location compared with previous reports.

Control of Palmer amaranth with saflufenacil applied alone or in combination with atrazine or dimethenamid-P was drastically different between 2013 and 2014-2015 (Table 3.3). In 2013, Palmer amaranth biomass was reduced <50% with these treatments and provided no Palmer amaranth control with saflufenacil alone or tank-mixed with atrazine. In 2014-2015, 70-85% visual control and a reduction in Palmer amaranth biomass between 82-87% occurred with the same treatments. Saflufenacil doesn't bind strongly to soil particles; the majority of the herbicide remains in the soil solution which could make it prone to leaching following heavy rainfall in the 10 d after application in 2013 (Papiernik et al. 2012).

Palmer amaranth control with the long chain fatty acid inhibitors (Group 15), acetochlor, pyroxasulfone, and s-metolachlor, varied by active ingredient and year. Pyroxasulfone was the most consistent at controlling Palmer amaranth of the three products applied alone. Pyroxasulfone at 0.18 kg ai ha^{-1} provided 83% Palmer amaranth control 45 DAP, and was amongst the treatments with the greatest control in 2013 (Table 3.3). Control with acetochlor and s-metolachlor was 33 and 20%, respectively, 45 DAP. In 2014-2015, Palmer amaranth control was greater with all three products; 77% with s-metolachlor and 89 and 91% with acetochlor and pyroxasulfone (0.24 kg ai ha^{-1}), respectively, 72 DAP. Palmer amaranth biomass reduction 60 DAP followed similar trends as control 72 DAP in 2014-2015. Previous research showed that in saturated soils, acetochlor and metolachlor dissipate more rapidly than pyroxasulfone (Mueller et al. 1999; Westra et al. 2014). The differences in Palmer amaranth

control between years was likely due to greater dissipation of *s*-metolachlor and acetochlor compared to pyroxasulfone.

The addition of atrazine at 1.1 kg ai ha^{-1} to any of the single active ingredient tested did not improve Palmer amaranth control over the single active ingredient alone, with the exception of mesotrione 72 DAP in 2014-2015 (Table 3.3). The lack of improved control with these combinations was expected due to the decreased sensitivity of this Palmer amaranth population to atrazine. However, the greater Palmer amaranth control 72 DAP in 2014-2015 from the PRE application of the atrazine and mesotrione combination should be examined further. Others reported synergistic responses from the addition of atrazine to mesotrione applied POST to atrazine-resistant velvetleaf and redroot pigweed (Woodyard et al. 2009).

Mesotrione and s-metolachlor applied alone, tank-mixed with atrazine, or tank-mixed with bicyclopyrone and atrazine were amongst the treatments that provided the greatest Palmer amaranth control (Table 3.3). Palmer amaranth control 45 DAP in 2013 and 72 DAP in 2014-2015 with this combination was greater than control with either mesotrione or s-metolachlor alone, indicating that both mesotrione and s-metolachlor were contributing to control.

Overall the results from the three years of this experiment, Palmer amaranth control was inconsistent from PRE only herbicide treatments. In 2013, none of the PRE treatments provided greater than 85% control beyond 45 DAP. Palmer amaranth control was more consistent and lasted longer in 2014-2015. Of the 20 PRE herbicide treatments examined, there were 10 treatments that provided similar levels of Palmer amaranth control (89-98%) 72 DAP in 2014-2015. However, none of these treatments provided complete control for the entire growing season and all would likely need an effective POST herbicide treatment for season-long control. Treatments that included pyroxasulfone or the combination of mesotrione and s-metolachlor

provided the greatest and most consistent Palmer amaranth control in all three years of this experiment. However, relying on a single site of action (i.e., pyroxasulfone) for Palmer amaranth control will increase the selection pressure for additional resistances. Tank-mixing herbicides with other sites of action (i.e., saflufenacil), while not always the most consistent, may help reduce selection pressure on a single site of action.

Evaluation of POST Herbicides. There was no corn injury from any of the POST herbicide treatments (data not shown). Due to a significant year-by-treatment interaction, Palmer amaranth control results are presented separately by year. The majority of the POST herbicide treatments in 2013 failed to provide adequate Palmer amaranth control 14 DAT. Topramezone was the only herbicide that provided greater than 85% control (Table 3.4). Control with all others was less than 65%. In 2014, five of the nine herbicide treatments evaluated provided greater than 90% control 14 DAT. These treatments included; topramezone, dicamba, dicamba + diflufenzopyr, and glufosinate.

Palmer amaranth control was lowest with glyphosate, atrazine at 0.56 and 1.12 kg ai ha^{-1}, and 2,4-D amine in both years of the study (Table 3.4). Glyphosate and atrazine applied POST historically provided excellent Palmer amaranth control (Bond et al. 2006; Jhala et al. 2014; Norsworthy et al. 2008). The failure to effectively control Palmer amaranth in both years of this experiment illustrates the resistance in this population to both atrazine and glyphosate. While this population was not tested for resistance to 2,4-D, the lower control observed with 2,4-D amine was most likely due to an ineffective dose. Miller and Norsworthy (2016) reported similar Palmer amaranth control results when 2,4-D choline was applied at similar acid equivalent rates

in 2,4-D resistant soybean. Palmer amaranth control was greatest when 2,4-D choline was applied at 1.1 kg ae ha^{-1}, twice the amount that can be applied in corn.

Palmer amaranth population densities at the time of application may help explain the contrasting differences in Palmer amaranth control between 2013 and 2014. Palmer amaranth populations were 10-fold greater in 2013 (484) compared with (43) plants m^{-2} in 2014. The relatively poor control of Palmer amaranth in 2013 with several of the POST herbicides could be attributed to a lack of spray coverage and possible plant stresses associated with higher Palmer amaranth populations. Previous research has shown that the lack of spray coverage can lead to inconsistent control of annual weeds, particularly with contact herbicides like glufosinate (Eubank et al. 2008; Steckel et al. 1997). The higher levels of control observed with some of the treatments in 2014 may have been somewhat inflated due to the lower Palmer amaranth population. Farmers who rely on a single POST herbicide applications will most likely be faced with Palmer amaranth population densities similar to what was observed in 2013. Topramezone provided the most consistent control over the two years, however, it was not completely effective. Results suggest that an effective PRE herbicide will be needed to reduce Palmer amaranth populations prior to a POST application.

Evaluation of Herbicide Programs for Palmer amaranth Control in Corn. None of the herbicide programs examined resulted in significant corn injury (data not shown). Palmer amaranth control was 87% 14 DAEP with three of the five EPOS herbicide treatments (Table 3.5). Each of the effective treatments contained a HPPD-inhibiting herbicide. Previous research showed topramezone, tembotrione, and mesotrione POST can effectively control Palmer amaranth (≥90%) (Jhala et al. 2014; Norsworthy et al. 2008; Schuster et al. 2008; Stephenson et

al. 2015). Palmer amaranth control from the PRE herbicides at the time of the POST application ranged between 73 and 85%. All POST treatments, with the exception of glyphosate alone, following a PRE herbicide application provided greater than 90% Palmer amaranth control 14 DAPO. Palmer amaranth control was 58% and biomass was reduced only 48% when glyphosate was applied POST following a PRE application of *s*-metolachlor + atrazine. The EPOS treatment of acetochlor + clopyralid + flumetsulam + glyphosate also failed to provide a high level of Palmer amaranth control and only reduced biomass 83% 14 DAPO. At harvest, these programs only provided 34% and 69% Palmer amaranth control, respectively. Additionally, at harvest the EPOS program of atrazine + tembotrione + thiencarbazone-methyl + glyphosate provided slightly lower control (83%) than several of the other programs evaluated. Due to the resistance profile of this Palmer amaranth population, tembotrione would have been the sole component of this treatment contributing to Palmer amaranth control. Tembotrione is a highly effective HPPD-inhibitor for management of Palmer amaranth, and a synergistic response has been reported when applied in combination with atrazine in atrazine-resistant Palmer amaranth (Chapter 4). However, the lower level of Palmer amaranth control at harvest with this treatment was most likely due to lack of the addition of a residual herbicide to control later emerging Palmer amaranth.

The majority of EPOS programs provided similar Palmer amaranth control as the PRE fb POST programs. The EPOS programs that provided the greatest Palmer amaranth control all contained a HPPD-inhibiting herbicide plus atrazine for POST control and a Group 15 herbicide (i.e., *s*-metolachlor, pyroxasulfone) for residual Palmer amaranth control. However, due to Palmer amaranth's extended emergence pattern and rapid growth, relying on a one-pass EPOS program may not be the most consistent long-term strategy, especially when managing a

multiple-resistant Palmer amaranth population. If the EPOS program fails to control Palmer amaranth, options for rescue treatments become extremely limited. The two-pass POST programs of glufosinate (EPOS) fb glufosinate (POST) provided control comparable to several of the PRE fb POST and EPOS programs, controlling 88% of Palmer amaranth at harvest (Table 3.5). While this program provided good Palmer amaranth control, it would increase selection pressure for glufosinate resistance from the repeated application of a single herbicide site of action.

We conclude that one of the most effective and consistent management strategies to control multiple-resistant Palmer amaranth is a PRE fb POST herbicide program approach. While some of the PRE and POST only treatments provided good control of Palmer amaranth, complete season-long control was not achieved. Herbicide programs that contained effective herbicide sites of action both PRE and POST were among the most consistent programs. Strategies should include at least one effective herbicide site of action PRE and two effective foliar sites of action POST plus a soil residual herbicide to control Palmer amaranth for the entire growing season.

APPENDIX

APPENDIX

CHAPTER 3 TABLES

Table 3.1. Planting dates, hybrids, and herbicide application dates for PRE, POST, and herbicide program experiments to control multiple-resistant Palmer amaranth in corn in Barry County, MI (2013-2015).

	PRE experiment			POST experiment		Herbicide program experiment		
	2013	2014	2015	2013	2014	2013	2014	2015
Planting date	May 16	May 19	May 16	May 16	May 19	May 16	May 19	May 14
Corn hybrid[a]	DKC 48-12	DKC 48-12	P0157 AM	DKC 48-12	DKC 48-12	DKC 48-12	DKC 48-12	P0157 AM
PRE application date	May 18	May 19	May 18	—	—	May 18	May 19	May 18
EPOS application date	—	—	—	—	—	June 6	June 5	June 4
POST application date	—	—	—	June 14	June 9	June 21	June 26	June 22

[a] Company information: DKC 48-12, Dekalb, Monsanto Company, St. Louis MO; P0157 AM, Dupont Pioneer, Johnston, IA.

Table 3.2. Herbicide product, application rates and timings, and manufacturer information for herbicide treatments used for Palmer amaranth control in corn in Barry County, MI (2013-2015).

Trade name	Active ingredients	Rates	Timings [a]	Manufacturer[b]
		kg ai or ae ha^{-1}		
2,4-D Amine 4	2,4-D amine	0.56	POST	Winfield Solutions
AAtrex 4L	atrazine	1.12, 1.68, 2.24	PRE, EPOS, POST	Syngenta Crop Protection
Armezon	topramezone	0.018	POST, EPOS	BASF Corporation
Acuron	atrazine + bicyclopyrone + mesotrione + s-metolachlor	0.84 + 0.05 + 0.20 + 1.8	PRE	Syngenta Crop Protection
Balance Flexx	isoxaflutole	0.11	PRE	Bayer CropScience
Bicep II Magnum	atrazine + s-metolachlor	1.82 + 1.41	PRE	Syngenta Crop Protection
Callisto	mesotrione	0.21	PRE	Syngenta Crop Protection
Callisto Xtra	atrazine + mestrione	0.67 + 0.1	POST	Syngenta Crop Protection
Capreno	thiencarbazone-methyl + tembotrione	0.015 + 0.076	EPOS	Bayer CropScience
Clarity	dicamba	0.56	POST	BASF Corporation
Dual II Magnum	s-metolachlor	1.4	PRE	Syngenta Crop Protection
Halex GT	glyphosate + mesotrione + s-metolachlor	1.05 + 0.1 + 1.05	POST, EPOS	Syngenta Crop Protection
Harness	acetochlor	1.79	PRE	Monsanto Company
Harness Xtra	acetochlor + atrazine	1.4 + 1.73	PRE	Monsanto Company
Laudis	tembotrione	0.092	POST	Bayer CropScience
Lexar EZ[c]	atrazine + mesotrione + s-metolachlor	1.46 + 0.18 + 1.46	PRE	Syngenta Crop Protection
Liberty 280SL	glufosinate	0.6	POST, EPOS	Bayer CropScience
Lumax EZ	atrazine + mesotrione + s-metolachlor	0.7 + 0.18 + 1.8	PRE	Syngenta Crop Protection
Roundup PowerMax	glyphosate	0.84	POST, EPOS	Monsanto Company
Sharpen	saflufenacil	0.08	PRE	BASF Corporation
Status	dicamba + diflufenzapyr	0.14 + 0.056	POST	BASF Corporation
TripleFLEX	acetochlor + clopyralid + flumetsulam	0.31 + 0.13 + 0.04	EPOS	Monsanto Company
Verdict	dimethenamid-P + saflufenacil	0.66 + 0.075	PRE	BASF Corporation
Warrant	acetochlor	1.26	POST	Monsanto Company
Zemax	mesotrione + s-metolachlor	0.19 + 1.9	PRE	Syngenta Crop Protection
Zidua[d]	pyroxasulfone	0.18/0.24	PRE	BASF Corporation

[a] Abbreviations: PRE, preemergence application; POST, postemergence application; EPOS, early postemergence application.

Table 3.2 (cont'd)

[b] Manufacturer information: Winfield Solutions, LLC, St. Paul, MN, www.winfield.com; Syngenta Crop Protection, LLC, Greensboro, NC, www.syngenta.com; BASF Corporation, Research Triangle Park, NC, www.basf.com; Bayer CropScience, Research Triangle Park, NC, www.cropscience.bayer.com; Monsanto Company, St. Louis, MO, www.monsanto.com.

[c] The Lexar EZ (atrazine + mesotrione + s-metolachlor) rate was lowered to 0.73 + 0.09 + 0.73 kg ai ha^{-1} when mesotrione was applied POST in the programs experiment to stay within the maximum allowed mesotrione rate per season.

[d] The Zidua application rate was 0.18 kg ai ha^{-1} in 2013 and increased to 0.24 kg ai ha^{-1} for 2014 and 2015.

Table 3.3. Multiple-resistant Palmer amaranth control in corn with preemergence herbicides 45 and 72 days after planting (DAP) and Palmer amaranth biomass reduction for 2013 and 2014-2015 in Barry County, MI.

		Palmer amaranth control				
		45 DAP		72 DAP	Palmer amaranth biomass[a]	
Treatment	Rate	2013	2014-2015	2014-2015	2013	2014-2015
	kg ai ha^{-1}	%			% reduction	
atrazine	1.1	0 f[b]	24 g	13 h	22 g	30 f
atrazine	2.2	3 f	58 e	51 fg	30 fg	65 de
acetochlor	1.8	33 cd	91 ab	89 a-c	43 ef	91 ab
isoxaflutole	0.11	5 f	64 de	59 f	53 de	76 cd
mesotrione	0.21	68 b	87 ab	79 cd	83 b	94 ab
pyroxasulfone[c]	0.18/0.24[c]	83 a	94 ab	91 ab	94 a	97 a
s-metolachlor	1.4	20 e	82 bc	77 de	46 ef	76 cd
saflufenacil	0.75	0 f	75 cd	70 ef	18 g	87 a-c
acetochlor + atrazine	1.8 + 1.1	40 c	93 ab	90 ab	55 de	91 ab
isoxaflutole + atrazine	0.11 + 1.1	20 e	49 f	43 g	45 ef	62 e
mesotrione + atrazine	0.21 + 1.1	70 b	97 a	97 a	81 b	99 a
pyroxasulfone[c] + atrazine	0.18/0.24 + 1.1	85 a	97 a	98 a	95 a	99 a
s-metolachlor + atrazine	1.4 + 1.1	40 c	84 bc	83 b-d	64 cd	98 a
saflufenacil + atrazine	0.75 + 1.1	3 f	83 bc	80 c-e	47 ef	83 bc
pyroxasulfone[c] + saflufenacil	0.18/0.24 + 0.75	79 ab	97 a	96 a	96 a	98 a
mesotrione + *s*-metolachlor	0.19 + 1.9	79 ab	96 a	92 ab	93 a	98 a
dimethenamid-P + saflufenacil	0.66 + 0.075	37 cd	86 bc	81 cd	78 bc	82 bc
mesotrione + *s*-metolachlor + atrazine	0.19 + 1.9 + 0.7	80 ab	97 a	98 a	88 ab	99 a
mesotrione + *s*-metolachlor + atrazine	0.19 + 1.4 + 1.5	84 a	98 a	94 ab	95 a	98 a
bicyclopyrone + mesotrione + *s*-metolachlor + atrazine	0.05 + 0.19 + 1.8 + 0.84	—	93 ab	90 ab	—	86 a-c

[a] Palmer amaranth biomass was collected 45 DAP in 2013 and 60 DAP in 2014 and 2015. Biomass reduction was calculated as y = (100 – ((sample dry weight / non-treated control dry weight) * 100)).

[b] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

[c] The pyroxasulfone rate was increased to 0.24 kg ai ha^{-1} for 2014 and 2015 from 0.18 kg ai ha^{-1} in 2013.

Table 3.4. Multiple-resistant Palmer amaranth control in corn with postemergence herbicides 7 and 14 days after treatment (DAT) in Barry County, MI.

		Palmer amaranth control			
		2013		2014	
Herbicide treatment[a]		7 DAT	14 DAT	7 DAT	14 DAT
	kg ai ha^{-1}	%		%	
atrazine + COC	0.56	28 d[a]	15 cd	68 bc	68 b
atrazine + COC	1.12	47 c	26 cd	70 bc	72 b
dicamba	0.56	64 b	55 b	68 bc	91 a
dicamba + diflufenzopyr + NIS + AMS	0.14 + 0.06	63 b	64 b	74 bc	94 a
glufosinate + AMS	0.6	90 a	23 cd	96 a	95 a
glyphosate + AMS	0.87	0 e	8 d	68 bc	66 b
topramezone + MSO + AMS	0.018	81 a	88 a	78 b	96 a
2,4-D amine	0.56	62 b	30 c	62 c	68 b

[a] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

[b] Adjuvant information: COC = crop oil concentrate at 1% v v^{-1} (Herbimax, Loveland Products Inc., Loveland, CO), AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO), NIS = non-ionic surfactant at 0.25% v v^{-1} (Activator 90, Loveland Products Inc., Loveland, CO), MSO = methylated seed oil at 1% v v^{-1} (SuperSpread, Wilbur-Ellis Co., San Francisco, CA).

Table 3.5. Evaluation of herbicide programs for the management of multiple-resistant Palmer amaranth in corn for 2013-2015 in Barry County, MI.

Treatment[b]	Timing[c]	Rate	Palmer amaranth control			Biomass[a]
			14 DAEP	14 DAPO	at Harvest[d]	14 DAPO
		kg ai ha^{-1}	%			% reduction
glufosinate fb glufosinate	EPOS POST	0.6 fb 0.6	73 e[e]	90 b-d	88 ab	100 a
acetochlor + clopyralid + flumetsulam + glyphosate	EPOS	0.31 + 0.13 + 0.04	76 e	80 e	69 c	83 b
atrazine + mesotrione + s-metolachlor + glyphosate + COC	EPOS	1.12 + 0.1 + 1.05 + 1.05 + 1% v v^{-1}	87 a	93 a-d	92 ab	99 a
atrazine + tembotrione + thiencarbazone-methyl + glyphosate + COC	EPOS	1.12 + 0.015 + 0.076 + 0.84 + 1% v v^{-1}	87 a	89 cd	84 b	96 a
atrazine + topramezone + pyroxasulfone + glyphosate + MSO	EPOS	1.68 + 0.018 + 0.18 + 0.84 + 1% v v^{-1}	87 a	96 a	95 a	100 a
acetochlor fb glufosinate	PRE POST	1.79 fb 0.6	76 e	91 a-d	87 ab	97 a
atrazine + s-metolachlor fb glufosinate	PRE POST	1.82 + 1.41 fb 0.6	84 a-c	94 a-c	93 a	99 a
atrazine + s-metolachlor fb tembotrione + glufosinate	PRE POST	1.82 + 1.41 fb 0.092 + 0.6	83 a-d	94 a-c	92 ab	99 a
atrazine + s-metolachlor fb glyphosate	PRE POST	1.82 + 1.41 fb 0.84	79 b-d	58 f	34 d	48 c
atrazine + s-metolachlor fb atrazine + mesotrione + COC	PRE POST	1.82 + 1.41 fb 0.67 + 0.1 + 1% v v^{-1}	79 b-d	95 ab	94 a	100 a
atrazine + s-metolachlor fb mesotrione + s-metolachlor + glyphosate + NIS	PRE POST	1.82 + 1.41 fb 0.1 + 1.05 + 1.05 + 0.25% v v^{-1}	85 ab	93 a-d	88 ab	97 a
acetochlor + atrazine fb atrazine + topramezone + glyphosate + MSO	PRE POST	1.4 + 1.73 fb 0.56 + 0.018 + 0.84 + 1% v v^{-1}	78 de	94 a-c	92 ab	100 a
atrazine + isoxaflutole fb acetochlor + glufosinate	PRE POST	1.12 + 0.11 fb 1.26 + 0.6	80 b-d	93 a-d	95 a	99 a

Table 3.5 (cont'd)

Treatment	Timing	Rate	Palmer amaranth control			Biomass
			14 DAEP	14 DAPO	at Harvest	14 DAPO
		kg ha^{-1}	%			% reduction
dimethenamid-p + saflufenacil fb dicamba + diflufenzopyr + glyphosate	PRE POST	0.66 + 0.075 fb 0.14 + 0.056 + 0.84	83 a-d	92 a-d	93 a	96 a
dimethenamid-p + saflufenacil fb dicamba + diflufenzopyr + tembotrione + glyphosate	PRE POST	0.66 + 0.075 fb 0.14 + 0.056 + 0.014 + 0.84	73 e	91 a-d	94 a	100 a
dimethenamid-p + saflufenacil fb dicamba + diflufenzopyr + tembotrione + glufosinate	PRE POST	0.66 + 0.075 fb 0.14 + 0.056 + 0.092 + 0.6	83 a-d	95 a-c	94 a	100 a
atrazine + mesotrione + s-metolachlor fb acetochlor + glufosinate	PRE POST	1.46 + 0.18 + 1.46 fb 1.26 + 0.6	85 ab	95 a-c	93 a	100 a
atrazine + mesotrione + s-metolachlor fb atrazine + tembotrione + COC	PRE POST	1.46 + 0.18 + 1.46 fb 0.56 + 0.092 + 1% v v^{-1}	79 b-d	95 a-c	92 ab	100 a
atrazine + mesotrione + s-metolachlor fb mesotrione + s-metolachlor + glyphosate + NIS	PRE POST	0.73 + 0.09 + 0.73 fb 0.1 + 1.05 + 1.05 + 0.25% v v^{-1}	84 a-c	95 ab	90 ab	99 a

[a] Palmer amaranth biomass reduction was calculated as y = (100 – ((sample dry weight / non-treated control dry weight) * 100)).
[b] Adjuvant information: COC = crop oil concentrate at 1% v v^{-1} (Herbimax, Loveland Products Inc., Loveland, CO), NIS = non-ionic surfactant at 0.25% v v^{-1} (Activator 90, Loveland Products Inc., Loveland, CO), MSO = methylated seed oil at 1% v v^{-1} (SuperSpread, Wilbur-Ellis Co., San Francisco, CA). All treatments contained AMS = ammonium sulfate at 2% w w^{-1} (Actamaster, Loveland Products Inc., Loveland, CO).
[c] Abbreviations: EPOS = early postemergence; PRE = preemergence; POST = postemergence; 14 DAEP = 14 d after EPOS, 27 to 38 d after PRE, and at POST; 14 DAPO = 14 d after POST.
[d] Weed control was evaluated just prior to corn harvest.
[e] Means followed by the same letter within a column are not statistically different at α = 0.05.

LITERATURE CITED

LITERATURE CITED

Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37–43

Bond JA, Oliver LR, Stephenson DO, IV (2006) Response of Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate, fomesafen, and pyrithiobac. Weed Technol 20:885–892

Bryson CT, DeFelice MS, eds (2010) Weeds of the Midwestern United States & Central Canada. Athens, GA: University of Georgia Press. Pp 34–39.

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54.4:620–626

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri,* a sonoran desert summer annual. Oecologia 57:10–112

Eubank TW, Poston DH, Nandula VK, Koger CH, Shaw DR. Reynolds DB (2008) Glyphosate-resistant horseweed (*Conyza Canadensis*) control using glyphosate-, paraquat-, glufosinate-based herbicide programs. Weed Tecnol 22:16–21

Geier PW, Stahlman PW, Frihauf JC (2006) KIH-485 and s-metolachlor efficacy comparisons in conventional and no-tillage corn. Weed Technol 20.3:622–626

Gossett BJ, Toler JE (1999) Differential control of Palmer amaranth (*Amaranthus palmeri*) and pigweed (*Amaranthus hybridus*) by postemergence herbicides in soybean (*Glycine max*) Weed Technol 13:165–168

Grichar WJ (1994) Spiny amaranth (*Amaranthus spinosus* L.) control in peanut (*Arachis hypogaea* L.). Weed Technol 8:199–202.

Hager AG, Wax LM, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci 50:607–610

Heap I (2016) The international survey of herbicide resistant weeds. www.weedscience.com. Accessed: February 5, 2016.

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus species.* Weed Sci 48.3:347–355

Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of triazine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38

Johnson WG, Chahal GS, Regehr DL (2012) Efficacy of various corn herbicides applied preplant incorporated and preemergence. Weed Technol 26.2:220–229

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Klingaman TE, Oliver LR (1994) Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). Weed Sci 42:523–527

Kohrt JR (2016) Biology and management of multiple- (glyphosate, ALS, and atrazine)-resistant Palmer amaranth in Michigan. Ph.D dissertation. East Lansing, MI: Michigan State University. 150 p

Knezevic SZ, Weise SF, Swanton CJ (1994) Interference of redroot pigweed (*Amaranthus retroflexus*) in corn (*Zea mays*). Weed Sci 42:568–573

Massinga RA, Curie RS, Horak MJ, Boyer Jr J (2001) Interference of Palmer amaranth in corn. Weed Sci 49.2:202–208

Massinga RA, Currie RS, Trooien TP (2003) Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci 51:523–531

Michigan State University Enviro-weather (2016) weather station network. https://www.enviroweather.msu.edu. Accessed: May 15, 2016

Miller MR, Norsworthy JK (2016) Evaluation of herbicide programs for use in a 2,4-D-resistant soybean technology for control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) Weed Technol 30:366-376

Moolani KM, Knake EL, Slife FW (1964) Competition of smooth pigweed with corn and soybeans. Weeds 12:126–128

Mueller TC, Shaw DR, Witt WW (1999) Relative dissipation of acetochlor, alachlor, metolachlor, and SAN 582 from three surface soils. Weed Technol 13:341–346

Nandula VK, Reddy KN, Koger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribeiro DN (2012) Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Sci 60:179–188

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

Pallett KE, Little JP, Sheeky M, Veerasekaran P (1998) The mode of action of isoxaflutole: I. Physiological effects, metabolism, and selectivity. Pesticide Biochemistry and Phys 62.2:113–124

Pallett KE, Cramp SM, Little JP, Veerasekaran P, Crudace AJ, Slater AE (2001) Isoxaflutole: the background to its discovery and the basis of its herbicidal properties. Pest Manag Sci 57:133–142

Papiernik SK, Koskinen WC, Barber BL (2012) Low sorption and fast dissipation of the herbicide saflufenacil in surface soils and subsoils of an eroded prairie landscape. J Agric Food Chem 60:10936–10941

Powell DK (2014) Biology and management of multiple (glyphosate/ALS)-resistant Palmer amaranth in Michigan soybean. Ph.D dissertation. East Lansing, MI: Michigan State University. 122 p

Schuster CL, Al-Khatib K, Dille JA (2008) Efficacy of sulfonylurea herbicide when tank mixed with mesotrione. Weed Technol 22:222–230

Schweizer EE, Lauridson TC (1985) Powell amaranth (*Amaranthus powellii*) interference in sugarbeet (*Beta vulgaris*). Weed Sci 33:518–520

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus species* in Missouri. Weed Sci 51.3:329–333

Sosnoskie LM, Kichler JM, Wallace RD, Culpepper AS (2011) Multiple resistance in Palmer amaranth to glyphosate and pyrithiobac confirmed in Georgia. Weed Sci 59:321–325

Steckel GJ, Wax LM, Simmons FW, Phillips WH, II (1997) Glufosinate efficacy on annual weeds is influenced by rate and growth stage. Weed Technol 11:484–488

Steckel LE, Main CL, Ellis AT, Mueller TC (2008) Palmer amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technol 22:119–123

Stephenson DO IV, Bond JA (2012) Evaluation of thiencarbazone-methyl- and isoxaflutole-based herbicide programs in corn. Weed Technol 26:37–42

Stephenson DO IV, Bond JA, Landry RL, Edwards HM (2015) Weed management in corn with postemergence applications of tembotrione or thiencarbazone:tembotrione. Weed Technol 29.3:350–358

Taylor-Lovell S, Sims GK, Wax LM (2002) Effects of moisture, temperature, and biological activity on the degradation of isoxaflutole in soil. J Agric Food Chem 50:5626–5633

Toler JE, Guice B, Murdock EC (1996) Interference between Johnsongrass (*Sorghum halepense*), smooth pigweed (*Amaranthus hybridus*), and soybean (*Gylcine max*). Weed Sci 44:331–338

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

Webster TM, Grey TL (2015) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) morphology, growth, and seed production in Georgia. Weed Sci 63:264–272

Westra EP, Shaner DL, Westra PH, Chapman PL (2014) Dissipation and leaching of pyroxasulfone and *s*-metolachlor. Weed Technol 28:72–81

Wiese (1968) Rate of weed root elongation. Weed Sci 16:11–13

Woodyard AJ, Hugie JA, Riechers DE (2009) Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci 57.4:369–378

CHAPTER 4

RESPONSE OF A MULTIPLE-RESISTANT PALMER AMARANTH POPULATION TO FOUR HPPD-INHIBITING HERBICIDES APPLIED ALONE AND WITH ATRAZINE

Abstract

Control of multiple- (glyphosate, ALS, and atrazine) resistant Palmer amaranth populations in corn will rely heavily on the use of POST 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides. Therefore, field and greenhouse experiments were conducted to: 1) evaluate the level of Palmer amaranth control with four HPPD-inhibitors alone and in combination with atrazine at two application timings, and 2) investigate the joint-activity of HPPD-inhibiting herbicides and atrazine in atrazine-resistant (AR) and atrazine-susceptible (AS) Palmer amaranth populations. Control of the AR Palmer amaranth population varied among the HPPD-inhibiting herbicides with tolpyralate > tembotrione = topramezone > mesotrione based GR_{50} values in the greenhouse. In the field Palmer amaranth control was lower when the HPPD-inhibiting herbicides, with the exception of tolpyralate, were applied to 15 cm versus 8 cmtall Palmer amaranth. Tolpyralate controlled Palmer amaranth 95% or greater at both application timings. The addition of atrazine at 560 g ai ha^{-1} improved Palmer amaranth control with mesotrione and topramezone at the 8 cm application timing and with mesotrione and tembotrione at the 15 cm application timing. In the greenhouse, the joint activity of mesotrione and atrazine and tembotrione and atrazine was synergistic with both the AR and AS Palmer amaranth populations. To initiate a synergistic response in the AR population with tembotrione 8X the rate of atrazine was needed compared with the AS population. Synergistic responses with mesotrione were detected with all the atrazine rates for the AS population and for atrazine rates of 280 to 2,240 g ai ha^{-1}. Only additive responses were observed when atrazine was applied with

tolpyralate and topramezone, indicating that the triketones are more susceptible to joint activity in the form of synergism compared with the benzopyrazoles. When faced with an AR population Palmer amaranth, the addition of atrazine to HPPD-inhibitors may increase the overall success of weed management due to joint activity.

Nomenclature: Atrazine; mesotrione; tembotrione; tolpyralate; tembotrione; corn, *Zea mays* L.; Palmer amaranth, *Amaranthus palmeri* S. Wats.

Key words: HPPD-inhibitors; joint activity; multiple-resistance; synergism.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats) is one of the most troublesome weeds in agronomic row crop production, due to season-long emergence, rapid growth rate, and prolific seed production (Horak and Loughin 2000; Keeley et al. 1987). In addition to its innate biological characteristics, the development of resistance to several different herbicide sites of action that makes Palmer amaranth difficult to control. Currently in the United States, Palmer amaranth populations have developed resistance to six different herbicide sites of action (Heap 2016). In addition to populations that are only resistant to a single site of action, there are several populations that have developed resistance to multiple herbicide sites of action which restricts the number of herbicides that farmers can use to manage this weed. In 2013, a population of Palmer amaranth found in Michigan was confirmed to be resistant to three herbicide sites of action groups: Group 9 (glyphosate), Group 2 (ALS-inhibitors), and Group 5 (atrazine) (Chapter 2), limiting the number of herbicide options available for Palmer amaranth control in corn.

The 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Group 27) are potential postemergence options for control of multiple herbicide-resistant Palmer amaranth in corn. Mesotrione, tembotrione, topramezone and the new herbicide active ingredient, tolpyralate, are herbicides that inhibit the HPPD-enzyme and may potentially control multiple-resistant Palmer amaranth (Grossmann and Ehrhardt (2007); Mitchell et al. 2001; Witschel 2008). HPPD-inhibiting herbicides control sensitive species by stopping the conversion of 4-hydroxyphenylpyruvate to homogentisate, which leads to the depletion of plastoquinone and α-tocopherol (Grossmann and Ehrhadt 2007; Mitchell et al. 2001; Pallett et al. 1998; Schulz 1993). Plastoquinone is a key enzyme cofactor for phytoene desaturase, ultimately leading to the inhibition of carotenoid biosynthesis, which results in the bleaching of new tissue (Mitchell et al 2001; Schulz et al. 1993). The inhibition of α-tocopherol results in membrane destruction and degradation of the D1 protein, due to the plant's inability to quench reactive oxygen species and triplet chlorophyll produced by photosystem II (PSII) (Kruk et al. 2005; Trebst et al. 2002). Combinations of two or more herbicides can yield either an additive, synergistic, or antagonistic response. These responses are determined by calculating a predicted value and comparing it to an expected value of the herbicide combination (Colby 1967; Flint et al. 1988; Gowing 1960). If the values of the observed and predicted are equal then the combination is additive. If the observed value is significantly greater than the calculated predicted value then the herbicide combination is deemed to be synergistic and if the observed value is significantly less than predicted the combination is antagonistic. Antagonistic responses have been observed with several herbicide tank-mixtures (Damalas and Eleftherohorinos 2001; O'Donovan and O'Sullivan 1982; Schuster et al. 2008; Selleck and Baird 1981). For example, previous research has shown that antagonism can occur between the HPPD-inhibiting herbicide mesotrione and

sulfonylurea herbicides (Schuster et al. 2008). The addition of mesotrione to nicosulfuron reduced green foxtail (*Setaria viridis* L.) control up to 23% compared with nicosulfuron alone. The mechanisms by which antagonism can occur has been attributed reduced absorption, reduced translocation, and physiological interactions at the target site within the plant (Hart and Wax 1996; Green 1989; Schuster et al. 2007).

A well-documented instance of a synergistic response with combinations of two herbicides has been with the HPPD-inhibiting herbicide, mesotrione and the PSII-inhibiting herbicide, atrazine (Abendroth et al. 2006; Armel et al. 2007; Hugie et al. 2008; Woodyard et al. 2009a). This synergistic response was observed in giant ragweed (*Ambrosia trifida* L.), common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* M.), common waterhemp (*Amaranthus rudis* Sauer), and redroot pigweed (*Amaranthus retroflexus* L.) (Abendroth et al. 2006; Hugie et al. 2008; Woodyard et al. 2009a; Woodyard et al. 2009b), and has also been observed in atrazine-resistant redroot pigweed and velvetleaf (Woodyard et al. 2009b). However, the extent of synergistic responses in the atrazine-resistant weed species was dependent on whether the mechanism of resistance was altered-target site or metabolism based. The majority of previous research has been focused on the interaction of mesotrione and atrazine. Currently, there is no information available on the potential synergistic effects of atrazine with the newer HPPD-inhibiting herbicides, tembotrione, topramezone, and tolpyralate. While previous research has shown that synergistic responses can occur with mesotrione and atrazine in other atrazine-resistant weed species, it is unknown whether this interaction will exist in the multiple-resistant Palmer amaranth population found in Michigan. Therefore, the objectives of this research were to: 1) evaluate Palmer amaranth control with four HPPD-inhibiting herbicides alone and in combination with atrazine at two different application timings,

and 2) characterize the response of an atrazine-resistant and atrazine-sensitive Palmer amaranth population to combinations of HPPD-inhibiting herbicides and atrazine.

Materials and Methods

Field Experiment. Field experiments were conducted in 2013 and 2015 in a commercial corn field in Barry County, MI (42.702467°N; -85.524992°W). The Palmer amaranth population at this location was confirmed to be resistant to glyphosate, ALS-inhibiting herbicides, and atrazine though previous greenhouse screenings (Chapter 2). The soil type was a combination of an Oshtemo sandy loam and a Boyer loamy sand composed of 73% sand, 15% silt, and 12% clay, with a pH of 7.6 and 2.2% organic matter. Field preparation at this location was fall chisel plow followed by two-passes of a soil finisher in the spring. Corn (DKC 48-12, Dekalb, Monsanto Company, St. Louis, MO.) was planted on May 20, 2013 and May 14, 2015 at 67,950 seeds ha^{-1}. The Palmer amaranth population at this location was relatively high with 484 and 334 plants m^{-2} in 2013 and 2015, respectively.

The experiment was conducted as a two-factor randomized complete block design with four replications. Factor A was application timing and Factor B was herbicide treatment. Each plot was 3 m wide by 9 m long. Herbicide treatments were applied when Palmer amaranth was 8 and 15 cm in height. Mesotrione, tembotrione, tolpyralate, and topramezone were applied alone and in combination with atrazine. Table 4.1 provides a complete listing of product information, application rates, and adjuvants for each herbicide treatment. Herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at a pressure of 207 kPa through 11003 AIXR flat-fan nozzles (TeeJet, Spraying Systems Co., Wheaton, IL 60187).

Crop injury and weed control were evaluated 7, 21, and 28 days after treatment (DAT) for the 8 cm application timing, and at 7 and 21 DAT for the 15 cm timing. Evaluations were based on a scale from 0 to 100% with 0 representing no control or crop injury and 100 indicating complete control or total plant death. At the conclusion of the visual evaluations, aboveground Palmer amaranth biomass was harvested from two random 0.25 m^2 quadrats in each plot. Biomass was dried for approximately 7 d at 65 C, and then weighed to calculate the percent Palmer amaranth biomass reduction compared with the non-treated control plots.

Statistical analysis was conducted using SAS® 9.4 (SAS institute Inc. Carey, NC 27513). Assumptions of normality of residuals and homogeneity of variances were confirmed using the PROC UNIVARIATE. Analysis of variance was conducted using PROC MIXED to test for main effects (application timing and herbicide treatment), their interactions, and interactions between years. Since year interactions were not significant, data was combined over both years of the experiment. Multiple comparisons among the means were conducted using t-tests when data was found to be statistically significant at 0.05 levels.

Joint activity between atrazine and each HPPD-inhibiting herbicide were evaluated using the model described by Gowing (1960) (Equation 1).

$$E = A + \left(\frac{B(100-A)}{100}\right) \quad \text{[EQ. 1]}$$

Where *E* is the expected value of the herbicide combination, *A* is the observed percent control by herbicide atrazine, and *B* is the observed percent control by the HPPD-inhibiting herbicide. The expected values and observed values for each herbicide combination were compared using t-tests in PROC MIXED. If the observed value was significantly less than the expected value the combination was antagonistic; if the observed value was significantly greater than the expected

value the combination was synergistic; and if there was no significant difference between the observed and expected values the combination was additive.

Greenhouse Experiments. Seed heads from the multiple herbicide-resistant Palmer amaranth population from Barry County, MI (42.702467°N; -85.524992°W) were collected in fall 2013 and threshed. We will refer to this population as atrazine-resistant (AR) throughout the remainder of the text. Seed from a susceptible Palmer amaranth population was obtained from Dr. Larry Steckel, University of Tennessee and will be referred to as our atrazine-sensitive population (AS). Seed from both populations were treated with a 50% sulfuric acid and water solution for 4 min, rinsed, and then exposed to gibberellic acid at a concentration of 0.15 g L^{-1} of water for 6 h to enhance germination. Approximately 15 Palmer amaranth seeds of the AR and AS populations were planted 0.75 cm deep in 10 x 10 cm pots filled with potting media (Suremix Perlite, Michigan Gower Products, Inc., Galesburg, MI). Seedlings were grown in the greenhouse at 25 ± 5 C and sunlight was supplemented to provide a total midday light intensity of 1,000 µmol m^{-2} s^{-1} photosynthetic photon flux at plant height in a 16 h day. Plants were watered and fertilized as needed to promote optimum plant growth. After emergence, pots were thinned to one Palmer amaranth plant pot^{-1}.

Differential Response of Palmer amaranth with Four HPPD-Inhibiting Herbicides. Mesotrione, tembotrione, tolpyralate, and topramezone were applied at rates ranging from 0.125 to 4X, 0.063 to 2X, 0.031 to 2X, and 0.063 to 2X, respectively, when the AR Palmer amaranth population was 10 cm tall. The 1X rates of mesotrione, tembotrione, tolpyralate, and topramezone were 105, 92, 39, and 18 g ai ha^{-1}, respectively. Tembotrione, tolpyralate, and topramezone treatments were

applied with methylated seed oil (MSO) (SuperSpread, Wilbur-Ellis Co., San Francisco, CA) at 1% v v^{-1} and 1% w w^{-1} of spray grade ammonium sulfate (AMS) (Actamaster, Loveland Products, Inc., Loveland, CO). Mesotrione treatments were applied with 1% v v^{-1} crop oil concentrate (COC) (Herbimax, Loveland Products Inc., Loveland, CO) and 1% w w^{-1} AMS. Herbicide treatments were applied using a single-nozzle track sprayer equipped with an 8001E TeeJet flat-fan nozzle (Teejet Technologies, Wheaton, IL) calibrated to deliver 187 L ha^{-1} at 193 kPa of pressure. Weed control was evaluated 14 DAT using a 0 to 100% scale, 0 representing no control and 100 equaling plant death. Aboveground biomass was harvested 14 DAT, dried for 7 d at 60 C, and weighed. Dry weights were converted to the percent of the non-treated control pots. All treatments were replicated five times and the experiment was repeated in time.

Data was analyzed using nonlinear-regression in SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA). The herbicide dose required to provide 50% control and reduce Palmer amaranth biomass (growth) by 50% (GR_{50}) was calculated for each herbicide using the log-logistic model (Equation 2) (Burgos et al. 2013):

$$y = c + \frac{d-c}{1+\left(\frac{x}{GR50}\right)^{b}} \qquad \text{[EQ. 2]}$$

Where *d* equals the upper limit, *c* is the lower limit, and *b* is the relative slope around the GR_{50}. Deviations from the model are indicated by r^2 values. Differences in GR_{50} values between the HPPD-inhibitors were detected using the extra sum of squares principle for non-linear regression (Lindquist et al 1996).

Joint Activity of HPPD-Inhibiting Herbicides with Atrazine. Atrazine was applied at rates ranging from 0.031 to 2X (0.04 to 2.24 kg ai ha^{-1}) for the susceptible (AS) population and 0.25 to 32X (0.28 to 35.89 kg ai ha^{-1}) for the resistant (AR) population. These rates were applied alone

and in combination with mesotrione at 35 g ai ha^{-1}, tembotrione at 11.5 g ai ha^{-1}, tolpyralate at 2.5 g ai ha^{-1}, and topramezone 2.25 g ai ha^{-1}. Rates of the HPPD-inhibiting herbicides selected were closely related to the GR_{50} responses from the previous experiment with the AR population. The 1X atrazine rate was 1.12 kg ai ha^{-1}, regardless of population. Crop oil concentrate at 1% v v^{-1} plus AMS at 1% w w^{-1} was included when atrazine was applied alone or in combination with mesotrione. Methylated seed oil at 1% v v^{-1} plus AMS at 1% w w^{-1} was added to the treatments that included tembotrione, tolpyralate, and topramezone. Similar to the previous experiment, all herbicide treatments were applied using a single-nozzle track sprayer equipped with an 8001E TeeJet flat-fan nozzle calibrated to deliver 187 L ha^{-1} at 193 kPa of pressure. Weed control evaluations were made 14 DAT on a scale of 0 to 100. All treatments were replicated six times and the experiment was repeated in time.

Data was analyzed in SAS 9.4 using a method described by Flint et al. (1988) to evaluate herbicide interactions. The expected values for the herbicide combinations were derived from the equation developed by Colby in 1967 (Equation 3).

$$E = \frac{XY}{100} \quad \text{[EQ. 3]}$$

Where *E* represents the expected growth reduction of the herbicide combination, *X* and *Y* is the observed growth reduction with atrazine and HPPD-inhibitor applied at specific rates, respectively. Flint et al. (1988), developed a statistical test using ANOVA for Colby's equation to determine whether herbicide interactions were additive, antagonistic, or synergistic. All data was log-transformed to account for heterogeneity and allow for slope comparison of the HPPD-inhibitors and atrazine applied alone and in combination to determine herbicide interaction (Flint et al. 1988; Hugie et al. 2008; Woodyard et al 2009b). Slope estimate comparisons were

conducted using PROC MIXED and a series of contrast statements to determine whether the herbicide combinations were additive, antagonistic, or synergistic.

Results and Discussion

Palmer amaranth Control in the Field with HPPD-Inhibiting Herbicides Alone and with Atrazine. Corn injury was minimal (<3%) with all of the treatments examined (data not shown). Atrazine applied alone was one of the least effective treatments for Palmer amaranth control, regardless of Palmer amaranth height at the time of application (Table 4.2). Postemergence applications of atrazine are generally effective for Palmer amaranth control (Norsworthy et al. 2008; Stephenson et al. 2015). Atrazine plus glyphosate applied POST provided 95% control of a glyphosate-resistant Palmer amaranth population in Tennessee, 28 DAT (Wiggins et al. 2015). Seed samples collected from the field where our experiments were conducted showed that the Palmer amaranth population that we were examining had a resistance factor of 9.3X for POST applied atrazine compared with an atrazine-sensitive Palmer amaranth population (Chapter 2). Even though we consider this population to be atrazine resistant, control of this population was variable and slightly higher than we expected (61%) for a resistant population when atrazine was applied to 8 cm tall Palmer amaranth. The observations of live Palmer amaranth plants that survived the POST atrazine application next to dead plants indicates that this population may still be segregating toward an atrazine-resistant population in the field. Palmer amaranth control was <20% when atrazine was applied to 15 cm tall Palmer amaranth. Atrazine-resistant Palmer amaranth populations have also been reported in Georgia, Kansas, Nebraska, and Texas (Heap 2016).

Of the four HPPD-inhibiting herbicides applied; tolpyralate provided the greatest control (≥95%), regardless of Palmer amaranth height at the time of application (Table 4.2). Tembotrione also provided good control of Palmer amaranth (91%) when it was applied at the 8 cm application timing. However, control was significantly lower (59%) when tembotrione was applied to 15 cm tall Palmer amaranth. Applications of mesotrione and topramezone were not as effective at controlling Palmer amaranth as tolpyralate or tembotrione at the 8 cm application timing and only tolpyralate effectively controlled Palmer amaranth (95%) at the 15 cm timing. Biomass results were less indicative of the differences in the response of Palmer amaranth to the HPPD-inhibiting herbicides when compared with the control evaluations applied at the 8 cm timing, even though similar trends were observed (Table 4.2). However, biomass results adequately reflected the differences observed when the HPPD-inhibiting herbicides were applied to 15 cm tall Palmer amaranth; tolpyralate reduced Palmer amaranth biomass (98%) more than tembotrione and topramezone, and mesotrione was the least effective of the HPPD-inhibitors at reducing Palmer amaranth biomass. Variability in the Palmer amaranth growth and populations across the plots may have skewed the biomass data, since biomass was sampled from two random 0.25 m^{-2} quadrats per plot. Each quadrat would not have had the same number of initial plants. Possibly flagging a known number of plants at the time of application and harvesting those plants for biomass would have provided more representative results.

The addition of atrazine to mesotrione and topramezone improved Palmer amaranth control from 69 to 97% and 77 to 97%, respectively, at the 8 cm application timing (Table 4.2). Adding atrazine to tolpyralate and tembotrione did not improve Palmer amaranth control over these HPPD-inhibiting herbicides applied alone, possibly due to the already high levels of control. When Palmer amaranth was 15 cm tall an improvement in control and percent biomass reduction

was also observed when atrazine was added mesotrione and tembotrione. Even with this improved control, the combination of mesotrione plus atrazine failed to adequately control (65%) larger Palmer amaranth plants. Adding atrazine to topramezone did not improve Palmer amaranth control at the later application timing and control was only around 70% with topramezone alone or in combination with atrazine. Tolpyralate with or without the addition of atrazine provided the greatest Palmer amaranth control ($\geq$95%) when it was applied to 15 cm tall Palmer amaranth.

The improved Palmer amaranth control with the addition of atrazine with some of the HPPD-inhibiting herbicides was somewhat unexpected, since this population has been shown to be resistant to atrazine. Synergistic responses were observed with the combination of mesotrione and atrazine at both application timings, topramezone and atrazine at the 8 cm application timing, and tembotrione plus atrazine at the 15 cm application timing (Table 4.3). Weed height at application has previously been reported to influence the detection of synergism (Abendroth et al. 2006). This population of Palmer amaranth has demonstrated resistance to atrazine that is suspected to be metabolism based (Chapter 2). Synergism has been observed with atrazine and the HPPD-inhibitors in atrazine-resistant weed species, however the level at which the synergism occurs is dependent on mechanism resistance. In metabolism based atrazine-resistant velvetleaf synergistic interactions were present when atrazine and mesotrione were applied as a tank-mixture; whereas in an altered target-site resistant redroot pigweed synergism occurred with both tank-mixtures and when atrazine was applied PRE and mesotrione was applied POST (Woodyard et al. 2009b). The lack of synergism in the metabolism-based velvetleaf when atrazine was applied PRE followed by mesotrione POST was likely due to atrazine being detoxified prior to mesotrione application.

Differential Response of Atrazine-Resistant Palmer amaranth (AR) with Four HPPD-Inhibiting Herbicides in the Greenhouse. Dose response experiments were conducted to compare the response of Palmer amaranth to the HPPD-inhibiting herbicides: mesotrione, tembotrione, tolpyralate, and topramezone. Of the four HPPD-inhibiting herbicides examined, Palmer amaranth was the least sensitive to mesotrione. The doses required to control and reduce Palmer amaranth biomass by 50% (GR_{50} values), 14 DAT, were 0.23X (24.2 g ai ha^{-1}) and 0.12X (12.6 g ai ha^{-1}) the labeled use rates, respectively (Tables 4.4 and 4.5). These doses are in the range of GR_{50} values reported by Abendroth et al. (2011) and Jhala et al. (2014) for control of Palmer amaranth populations sensitive to HPPD-inhibiting herbicides. According to GR_{50} values for dry Palmer amaranth biomass, Palmer amaranth responses to tembotrione, tolpyralate, and topramezone were similar, with GR_{50} values ranging from 0.03 to 0.05X the labeled use rates of these products (Table 4.5). However, GR_{50} values for Palmer amaranth control indicated that Palmer amaranth was slightly more sensitive to applications of tolpyralate than tembotrione or topramezone (Table 4.4). These results are consistent with results that we observed in the field, where tolpyralate was amongst the most effective and mesotrione was the least effective of the HPPD-inhibiting herbicides for Palmer amaranth control, regardless of application timing (Table 4.2). Palmer amaranth control with tembotrione and topramezone fell somewhere in between tolpyralate and mesotrione, depending on Palmer amaranth height.

Joint Activity of HPPD-Inhibiting Herbicides with Atrazine on Atrazine-Sensitive and Atrazine-Resistant Palmer amaranth Populations in the Greenhouse. *Mesotrione.* In the atrazine-sensitive (AS) Palmer amaranth population, a synergistic response was detected when a constant rate of 35 g ai ha^{-1} of mesotrione was tank-mixed with all rates of atrazine ranging from

40 to 2,240 g ai ha^{-1} (Table 4.6). The strongest synergistic response, as indicated by the estimate values, occurred when 35 g ai ha^{-1} of mesotrione was applied in combination with 560 g ai ha^{-1} of atrazine. Palmer amaranth control with this combination was 23% higher than expected. Synergistic responses were also detected in the atrazine-resistant (AR) population. The combination of the single rate of mesotrione with atrazine at rates ranging from 280 to 2,240 were all synergistic and the strongest response occurred when atrazine was at 2,240 g ai ha^{-1}, 2X the labeled atrazine rate. When atrazine was applied the higher rates from 4,480 to 35,900 g ai ha^{-1} (4 to 32X) the response in the AR population was additive. The additive response with atrazine applied form the 4 to 32X rate could possibly be due to an interaction of mesotrione with and the clay-based formulation of atrazine at these high rates. A visible white film coated the leaf surface when atrazine was applied at the 32X rate, which may have restricted mesotrione uptake.

Tembotrione. Synergistic responses were also detected in the atrazine-sensitive (AS) and atrazine-resistant (AR) Palmer amaranth populations when a constant rate of tembotrione (11.5 g ai ha^{-1}) was applied with atrazine (Table 4.7). Unlike the synergistic responses observed with mesotrione, a much narrower range of atrazine rates triggered the synergistic response with tembotrione. Atrazine rates ranging from 140 to 1,120 g ai ha^{-1} triggered the synergistic responses in the AS population and only 1,120 and 2,240 g ai ha^{-1} of atrazine triggered the synergistic responses in the AR population. Additive responses were observed at both the lowest and highest atrazine rates tested in both populations. With the exception of atrazine applied at 35,900 (32X) in the AR population, all other additive responses had a negative slope estimate, indicating the potential for synergism. To date, we are unware of any research that has tested the

combination of tembotrione and atrazine for synergistic responses. However, several researchers have reported improved and less variable weed control in several weed species, including redroot pigweed and Palmer amaranth, when various rates of atrazine were applied with tembotrione compared with tembotrione applications alone (Stephenson et al. 2015; Williams et al. 2011).

Tolpyralate. All responses to the combination of the single rate of tolpyralate with atrazine in both the AS and AR Palmer amaranth populations were not significant and therefore additive, with the exception of the combination with the highest rate of atrazine 35,900 g ai ha^{-1} (32X) in the AR population (Table 4.8). The strong antagonistic response (estimate = 2.1) at this rate may possibly be due to reduced absorption of the HPPD-inhibitor, tolpyralate. Atrazine applied at 32X the labeled rate caused a white film over the leaf's surface, possibly due to the clay based formulation. This clay film may have resulted in the binding of tolpyralate on the leaf's surface, resulting in reduced absorption which could lead to decreased levels of the herbicide reaching the target site in the plant. The herbicidal activity of tolpyralate is similar to that of other HPPD-inhibitors, however our results indicate that it may function at a different site within the carotenoid biosynthesis pathway than the triketone herbicides, mesotrione and tembotrione. Differential responses of synergism and antagonism, have been observed with atrazine combinations with herbicides that inhibit different sites within the carotenoid biosynthesis pathway (Armel et al. 2007). Since tolpyralate is pro-herbicide and needs to be metabolized to become herbicidally active (Jeanmart et al. 2015), the rate of metabolism to the active form may occur at different rates within different plant species, similar to conversion of the pro-herbicide isoxaflutole to the active diketonitrile (Pallet et al. 1998). If metabolism of tolpyralate occurs too

rapidly in Palmer amaranth, and the herbicidal activity does not coincide with that of atrazine, synergism may be lost, regardless of atrazine susceptibility.

Topramezone. Similar to tolpyralate, no synergistic responses were observed with the combination of the single rate of 2.25 g ha^{-1} of topramezone and atrazine at any rate for both the AS and AR Palmer amaranth populations (Table 4.9). These results are contrary to the synergistic response that we observed in the field when 18 g ai ha^{-1} of topramezone was applied with 560 g ai ha^{-1} atrazine to the 8 cm tall AR Palmer amaranth population (Table 4.3). The presence of negative slopes within some of the atrazine rates and topramezone combinations indicate that there is potential for a synergistic response between these herbicides (Table 4.9). The combination of topramezone with the highest rate of atrazine (32X) on the AR Palmer amaranth population showed a strong antagonistic response (estimate = 1.7) and was likely due to possible reductions in topramezone absorption, similar to what was observed with tolpyralate. Topramezone and tolpyralate are members of the benzolpyrazole chemical family (Witschel et al. 2009; Wood 2016). Benzolpyrazole herbicides are considered the most herbicidally active members of the known HPPD-inhibiting herbicides (Almsick 2009; Witschel 2008). The potency of these herbicides for inhibition of the HPPD enzyme compared with the triketone herbicides, mesotrione and tembotrione, may have led to the lack synergistic responses with topramezone and tolpyralate.

The majority of previous research conducted on synergism has only focused on one of HPPD-inhibiting herbicides, mainly mesotrione in combination with atrazine (Abendroth et al. 2006; Hugie et al. 2008; Woodyard et al. 2009a; Woodyard et al. 2009b). The novel aspect of this research is showing the differential control of Palmer amaranth and joint-activity of four

different HPPD-inhibiting herbicides applied alone and in combination with atrazine. Differential levels of Palmer amaranth control between the HPPD-inhibitors were observed in both the field and greenhouse, with mesotrione being the least effective on Palmer amaranth. The GR_{50} values from the greenhouse dose response experiments indicate that tolpyralate > tembotrione = topramezone > mesotrione for HPPD-inhibitor activity for Palmer amaranth control 14 DAT. Weed height at application also influenced Palmer amaranth control with the HPPD-inhibiting herbicides. Control was lower when HPPD-inhibitors were applied to 15 cm tall Palmer amaranth compared with the 8 cm application timing with all of the HPPD-inhibitors, except for tolpyralate, which provided >95% control. Even though this population of Palmer amaranth has demonstrated atrazine resistance, the addition of atrazine to some of the HPPD-inhibiting herbicides improved control at both application timings. The less effective HPPD-inhibitors like mesotrione showed the greatest increases in control. This increase in control can be attributed to synergistic interactions between some of the HPPD-inhibitors and atrazine. The joint-activity of atrazine and HPPD-inhibitors in the field was synergistic for mesotrione and topramezone at the 10 cm timing and for tembotrione and mesotrione at the 15 cm timing; all other combinations were additive. In the greenhouse, synergism was detected in both AS and AR populations with mesotrione and tembotrione. While trends for synergism in the greenhouse were present with 3 of the 4 HPPD-inhibitors, they were only significant with mesotrione and tembotrione, the members of the triketone chemical family. This indicates that the independent physical chemical properties, potency, and speed of activity associated with different HPPD-inhibiting herbicides can influence the joint-activity with atrazine in both resistant and susceptible populations. Armel et al. (2007) demonstrated similar results showing that joint activity can vary within herbicides within the same mode of action. Previous research has

suggested that joint-activity between HPPD-inhibitors and atrazine is due to the depletion of plastoquinone and α-tocopherol caused by inhibition of the HPPD enzyme (Armel et al. 2005; Kruk et al. 2005; Trebst et al. 2002). Plastoquinone is the normal substrate for the Q_B binding site of the D1 protein PSII, and depletion could cause an increase in atrazine binding (Hess 2000, Pfister 1981). This binding results in the production of singlet oxygen and triplet chlorophyll which results in lipid peroxidation of cell membranes (Hess 2000). Therefore, the inhibition of α-tocopherol, an important plant antioxidant, can exacerbate the lipid peroxidation caused by the degradation of PSII due to the plant's inability to quench reactive oxygen species and triplet chlorophyll produced by PSII (Armel et al. 2005; Kruk et al. 2005; Trebst et al. 2002).

The results from this research show that even when faced with an AR population of Palmer amaranth, atrazine can still be an effective tool for weed management. Applying atrazine in combination with the HPPD-inhibitors can generate a synergistic or additive increase for the control of AS and AR weed species. These responses can be helpful in controlling AR Palmer amaranth, especially as Palmer amaranth size increases.

APPENDIX

APPENDIX

CHAPTER 4 TABLES AND FIGURES

Table 4.1. Herbicide information for all treatments applied to 8 and 15 cm tall multiple-resistant Palmer amaranth in Barry County, MI in 2013 and 2015.

Herbicide treatments	Adjuvants[a]	Herbicide rates	Trade names	Manufacturer[b]
		g ai ha^{-1}		
atrazine	COC	560	AAtrex 4L	Syngenta Crop Protection
mesotrione	COC + AMS	105	Callisto	Syngenta Crop Protection
tembotrione	MSO + AMS	92	Laudis	Bayer CropScience LP
tolpyralate	MSO + AMS	40	SL-573	ISK Biosciences, Corp
topramezone	MSO + AMS	18	Armezon	BASF Corporation
mesotrione + atrazine	COC + AMS	105 + 560	Callisto + AAtrex 4L	Syngenta Crop Protection
tembotrione + atrazine	MSO + AMS	92 + 560	Laudis + AAtrex 4L	Bayer CropScience + Syngenta
tolpyralate + atrazine	MSO + AMS	40 + 560	SL-573 + AAtrex 4L	ISK Biosciences + Syngenta
topramezone + atrazine	MSO + AMS	18 + 560	Armezon + AAtrex 4L	BASF Corporation + Syngenta

[a] COC = crop oil concentrate at 1% v v^{-1}, Herbimax, Loveland Products Inc., Loveland, CO; AMS = ammonium sulfate at 1% w w^{-1}, Actmaster, Loveland Products Inc., Loveland, CO; MSO = methylated seed oil at 1% v v^{-1}, SuperSpread®, Wilbur-Ellis Co., San Francisco, CA.

[b] Manufacturer information: Syngenta Crop Protection, LLC, Greensboro, NC; Bayer CropScience LP, Research Triangle Park, NC; ISK Biosciences, Corp, Concord, OH; BASF Corporation, Research Triangle Park, NC.

Table 4.2. Interaction of weed height and HPPD-inhibiting herbicides applied with and without atrazine on atrazine-resistant Palmer amaranth[a] control and biomass reduction in the field, 21 DAT.

			Palmer amaranth	
Weed height	Herbicide treatment	Rate	Control	Biomass[c]
		g ai ha^{-1}	—— % ——	— % reduction —
8 cm	atrazine	560	61 c[b]	77 bc
	mesotrione	105	69 bc	92 a
	tembotrione	92	91 a	98 a
	tolpyralate	40	96 a	98 a
	topramezone	18	77 b	96 a
	mesotrione + atrazine	105 + 560	97 a	100 a
	tembotrione + atrazine	92 + 560	96 a	100 a
	tolpyralate + atrazine	40 + 560	98 a	100 a
	topramezone + atrazine	18 + 560	97 a	100 a
15 cm	atrazine	560	18 e	47 d
	mesotrione	105	34 d	68 c
	tembotrione	92	59 c	82 b
	tolpyralate	40	95 a	98 a
	topramezone	18	68 bc	84 b
	mesotrione + atrazine	105 + 560	65 c	88 ab
	tembotrione + atrazine	92 + 560	88 ab	97 a
	tolpyralate + atrazine	40 + 560	98 a	100 a
	topramezone + atrazine	18 + 560	70 bc	94 a

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).
[b] Means followed by the same letter are not statistically significant at an α value of 0.05.
[c] Biomass was collected from two 0.25m^2 quadrats per plot and samples were dried and biomass was converted to a percent of the non-treated control plots.

Table 4.3. Joint activity of the combination of HPPD-inhibiting herbicides and atrazine on atrazine-resistant Palmer amaranth[a] control in the field, 21 DAT. Data were combined over years.

Weed height	Herbicide treatment	Rate	Palmer amaranth control		Response[c]
			Expected[b]	Observed	
		g ai ha^{-1}	——— % ———	——— % ———	
8 cm	mesotrione + atrazine	105 + 560	85	97	Synergistic
	tembotrione + atrazine	92 + 560	95	96	Additive
	tolpyralate + atrazine	40 + 560	97	98	Additive
	topramezone + atrazine	18 + 560	89	97	Synergistic
15 cm	mesotrione + atrazine	105 + 560	39	65	Synergistic
	tembotrione + atrazine	92 + 560	55	88	Synergistic
	tolpyralate + atrazine	40 + 560	97	98	Additive
	topramezone + atrazine	18 + 560	66	70	Additive

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).

[b] Expected control values were calculated using the Gowing (1960) equation $E = A + (\frac{B(100-A)}{100})$.

[c] A synergistic response occurred when the observed control value was statistically greater than then the expected control value at $\alpha \leq 0.05$.

Table 4.4. Equations, R^2 values, and GR_{50} values calculated from dose response experiments in the greenhouse to compare the differential response of four HPPD-inhibiting herbicides on atrazine-resistant Palmer amaranth[a] control, 14 DAT.

Herbicide	Log-logistic model	R^2	GR_{50} values[b]
			—— X dose (g ai ha^{-1}) ——
Mesotrione	$y=89.4/(x/0.23)^{1.93}$	0.91	0.23X (24.2) a
Tembotrione	$y=99.5/(x/0.11)^{1.47}$	0.93	0.10X (9.2) b
Tolpyralate	$y=89.0/(x/0.07)^{2.23}$	0.90	0.07X (2.7) c
Topramezone	$y=93.7/(x/0.10)^{1.48}$	0.96	0.11X (2.0) b

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).
[b] GR_{50} values followed by the same letter are not significantly different at $\alpha \leq 0.05$.

Table 4.5. Equations, R^2 values, and GR_{50} values calculated from dose response experiments in the greenhouse to compare the differential response of four HPPD-inhibiting herbicides on atrazine-resistant Palmer amaranth[a] dry weight, 14 DAT.

Herbicide	Log-logistic model	R^2	GR_{50} values[b]
			—— X dose (g ai ha^{-1}) ——
Mesotrione	$y=13.96+(100-13.96)/(x/0.12)^{-3.55}$	0.91	0.12X (12.6) a
Tembotrione	$y=7.32+(100-9.32)/(x/0.03)^{-1.09}$	0.94	0.03X (2.80) b
Tolpyralate	$y=12.88+(100-12.88)/(x/0.05)^{-2.47}$	0.88	0.05X (1.95) b
Topramezone	$y=12.26+(100-12.26)/(x/0.04)^{-1.58}$	0.88	0.04X (0.72) b

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).
[b] GR_{50} values followed by the same letter are not significantly different at $\alpha \leq 0.05$.

Table 4.6. Joint activity of mesotrione and atrazine applied in combination for control of atrazine-sensitive (AS) and atrazine-resistant[a] (AR) Palmer amaranth biotypes in the greenhouse, 14 DAT. Herbicide joint activity was determined by comparing the slope of the log-transformed dose response of atrazine alone compared with that obtained from atrazine combined with a constant rate of mesotrione (Flint et al. 1988; Hugie et al. 2008).

	Herbicide rate (g ai ha^{-1})		Palmer amaranth control (%)				
Population	Mesotrione	Atrazine	Expected[b]	Observed	P value[c]	Estimate[d]	Response
Sensitive (AS)	35	40	56	91	0.0001	-1.9390	Synergistic
	35	140	60	91	0.0001	-1.7438	Synergistic
	35	280	70	90	0.0001	-1.6063	Synergistic
	35	560	73	96	0.0001	-2.3982	Synergistic
	35	1,120	83	97	0.0001	-1.9616	Synergistic
	35	2,240	93	98	0.0010	-1.3357	Synergistic
Resistant (AR)	35	280	69	86	0.0323	-1.3197	Synergistic
	35	1,120	82	96	0.0165	-1.4725	Synergistic
	35	2,240	83	97	0.0013	-2.0926	Synergistic
	35	4,480	87	97	0.6223	-0.2881	Additive
	35	8,980	90	98	0.1919	-0.7731	Additive
	35	35,900	98	98	0.2424	0.7002	Additive

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).

[b] Expected control values were calculated using the $E = \frac{XY}{100}$ explained by Colby (1967).

[c] P values $\leq$ 0.05 are indicative of a significant response.

[d] Significant negative slope estimates indicate a synergistic response, significant positive slope estimates indicate an antagonistic response and non-significant slope estimates equal an additive response.

Table 4.7. Joint activity of tembotrione and atrazine applied in combination for control of atrazine-sensitive (AS) and atrazine-resistant[a] (AR) Palmer amaranth biotypes in the greenhouse, 14 DAT. Herbicide joint activity was determined by comparing the slope of the log-transformed dose response of atrazine alone compared with that obtained from atrazine combined with a constant rate of mesotrione (Flint et al. 1988; Hugie et al. 2008).

	Herbicide rate (g ai ha^{-1})		Palmer amaranth control				
Population	Tembotrione	Atrazine	Expected[b]	Observed	P value[c]	Estimate[d]	Response
Sensitive (AS)	11.5	40	64	75	0.1331	-0.5120	Additive
	11.5	140	64	84	0.0010	-1.2308	Synergistic
	11.5	280	73	89	0.0041	-1.0319	Synergistic
	11.5	560	75	92	0.0018	-1.1488	Synergistic
	11.5	1,120	86	94	0.0272	-0.7897	Synergistic
	11.5	2,240	93	96	0.4918	-0.2308	Additive
Resistant (AR)	11.5	280	76	88	0.0699	-1.1243	Additive
	11.5	1,120	83	95	0.0241	-1.4213	Synergistic
	11.5	2,240	79	96	0.0047	-1.8271	Synergistic
	11.5	4,480	89	97	0.2403	-0.7160	Additive
	11.5	8,980	85	99	0.0750	-1.0875	Additive
	11.5	35,900	97	97	0.1601	0.8612	Additive

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).

[b] Expected control values were calculated using the $E = \frac{XY}{100}$ explained by Colby (1967).

[c] P values $\leq$ 0.05 are indicative of a significant response.

[d] Significant negative slope estimates indicate a synergistic response, significant positive slope estimates indicate an antagonistic response and non-significant slope estimates equal an additive response.

Table 4.8. Joint activity of tolpyralate and atrazine applied in combination for control of atrazine-sensitive (AS) and atrazine-resistant[a] (AR) Palmer amaranth biotypes in the greenhouse, 14 DAT. Herbicide joint activity was determined by comparing the slope of the log-transformed dose response of atrazine alone compared with that obtained from atrazine combined with a constant rate of mesotrione (Flint et al. 1988; Hugie et al. 2008).

	Herbicide rate (g ai ha^{-1})		Palmer amaranth control				
Population	Tolpyralate	Atrazine	Expected[b]	Observed	P value[c]	Estimate[d]	Response
Sensitive (AS)	2.5	40	59	58	0.8572	0.0512	Additive
	2.5	140	62	61	0.9266	0.0270	Additive
	2.5	280	71	69	0.7079	0.1069	Additive
	2.5	560	74	69	0.6312	0.1390	Additive
	2.5	1,120	86	82	0.5095	-0.1912	Additive
	2.5	2,240	93	83	0.3730	0.2556	Additive
Resistant (AR)	2.5	280	46	56	0.7707	-0.2069	Additive
	2.5	1,120	66	69	0.9752	0.0214	Additive
	2.5	2,240	63	58	0.5914	0.3819	Additive
	2.5	4,480	83	68	0.1186	1.1171	Additive
	2.5	8,980	81	80	0.5919	0.3762	Additive
	2.5	35,900	95	82	0.0065	2.1000	Antagonistic

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).

[b] Expected control values were calculated using the $E = \frac{XY}{100}$ explained by Colby (1967).

[c] P values $\leq$ 0.05 are indicative of a significant response.

[d] Significant negative slope estimates indicate a synergistic response, significant positive slope estimates indicate an antagonistic response and non-significant slope estimates equal an additive response.

Table 4.9. Joint activity of topramezone and atrazine applied in combination for control of atrazine-sensitive (AS) and atrazine-resistant[a] (AR) Palmer amaranth biotypes in the greenhouse, 14 DAT. Herbicide joint activity was determined by comparing the slope of the log-transformed dose response of atrazine alone compared with that obtained from atrazine combined with a constant rate of mesotrione (Flint et al. 1988; Hugie et al. 2008).

Biotype	Herbicide rate (g ai ha^{-1})		Palmer amaranth control		P value[c]	Estimate[d]	Response
	Topramezone	Atrazine	Expected[b]	Observed			
Sensitive (AS)	2.25	40	68	59	0.3917	0.2658	Additive
	2.25	140	68	70	0.9884	-0.0046	Additive
	2.25	280	76	76	0.7927	-0.0800	Additive
	2.25	560	79	80	0.3864	-0.2688	Additive
	2.25	1,120	87	90	0.1453	-0.4573	Additive
	2.25	2,240	94	93	0.8873	0.0437	Additive
Resistant (AR)	2.25	280	69	82	0.3462	-0.6768	Additive
	2.25	1,120	85	81	0.7952	-0.1826	Additive
	2.25	2,240	80	88	0.3311	-0.6891	Additive
	2.25	4,480	89	95	0.9068	-0.0812	Additive
	2.25	8,980	90	92	0.5830	-0.3816	Additive
	2.25	35,900	99	87	0.0171	1.7638	Antagonistic

[a] This Palmer amaranth population has been confirmed resistant to glyphosate, ALS-inhibiting herbicides, and atrazine (Chapter 2).

[b] Expected control values were calculated using the $E = \frac{XY}{100}$ explained by Colby (1967).

[c] P values ≤ 0.05 are indicative of a significant response.

[d] Significant negative slope estimates indicate a synergistic response, significant positive slope estimates indicate an antagonistic response and non-significant slope estimates equal an additive response.

LITERATURE CITED

LITERATURE CITED

Abendroth JA, Martin AR, Roeth FW (2006) Plant response to combinations of mesotrione and photosystem II inhibitors. Weed Technol 20:267–274

Almsick AV (2009) New HPPD-inhibitors – a proven mode of action as a new hope to solve current weed problems. Outlooks Pest Manag. 20:27–30

Armel GR, Hall GJ, Wilson HP, Cullen N (2005) Mesotrione plus atrazine mixtures for control of Canada thistle (*Cirsium arvense*). Weed Sci 53:202–211

Armel GR, Rardon PL, McComrick MC, Ferry NM (2007) Differential response of several carotenoid biosynthesis inhibitors in mixtures with atrazine. Weed Technol 21.4:947–953

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norseworthy JK, Ritz C (2013) Review: Conformation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4–20

Colby SR (1967) Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20–22

Damalas CA, Eleftherohorinos IG (2001) Dicamba and atrazine antagonism on sulfonylurea herbicides used for Johnsongrass (*Sorghum halepense*) control in corn (*Zea mays*). Weed Technol 15:62–67

Flint JL, Cornelius PL, Barrett M (1988) Analyzing herbicide interactions: a statistical treatment of Colby's method. Weed Technol 2:304–309

Gowing DP (1960) Comments on tests of herbicide mixtures. Weeds 8.3:379–391

Green JM (1989) Herbicide antagonism at the whole plant level. Weed Technol 3:217–226

Grossmann K, Ehrhardt T (2007) On the mechanism of action and selectivity of the corn herbicide topramezone: a new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag Sci 63.5:429–439

Hart SE, Wax LM (1996) Dicamba antagonizes grass weed control with imazethapyr by reducing foliar absorption. Weed Technol 10:828–834

Heap I (2016) The international survey of herbicide resistant weeds. www.weedscience.com. Accessed: February 5, 2016

Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci 48.2:160–170

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus species.* Weed Sci 48.3:347–355

Hugie JA, Bollero GA, Tranel PJ (2008) Defining the rate requirements for synergism between mesotrione and atrazine in redroot pigweed (*Amaranthus retroflexus*). Weed Sci 56.2:26–-270

Jeanmart S, Edmunds AJF, Lamberth C, Pouliot M (2015) Synthetic approaches to the 2010-2014 new agrochemicals. Bioorg Med Chem 24:317-341

Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of triazine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Kohrt JR (2016) Biology and management of multiple- (glyphosate, ALS, and atrazine)-resistant Palmer amaranth in Michigan. Ph.D dissertation. East Lansing, MI: Michigan State University. 149 p

Kruk J, Holländer-Czytko H, Oettmeier W, Trebst A (2005) Tocopherol as singlet oxygen scavenger in photosystem II. Journal of Plant Phys 162.7:749–757

Lindquist JL, Mortenson DA, Clay SA, Schmenk R, Kells JJ, Howatt D, Westra P (1996) Stability of corn (*Zea mays*) velvetleaf (*Abutilon theophrasti*) interference relationships. Weed Sci 44:309–313

Mitchell G, Barlett DW, Fraser TEM, Hawkes TR, Holt DC, Townson JK, Wichert RA (2001) Mesotrione: a new selective herbicide for use in maize. Pest Manag Sci 57.2:120–128

Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113

O'Donovan JT, O'Sullivan (1982) The antagonistic action of 2,4-D and bromoxynil on glyphosate phytotoxicity to barley (*Hordeum vulgare*). Weed Sci 30:30–34

Pallett KE, Little JP, Sheeky M, Veerasekaran P (1998) The mode of action of isoxaflutole: I. Physiological effects, metabolism, and selectivity. Pesticide Biochemistry and Phys 62.2:113–124

Pfister K, Steinback KE, Gardner G, Arntzen (1981) Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. Proc Natl Acad Sci 78.2:981–985

Schulz A, Ort O, Beyer P, Kleinig H (1993) SC-0051, a 2-benzoyl-cyclohexane-1, 3-dione bleaching herbicide, is a potent inhibitor of the enzyme p-hydroxyphenylpyruvate dioxygenase. FEBS Letters 318.2:162–166

Schuster CL, Al-Khatib K, Dille JA (2007) Mechanism of antagonism of mesotrione on sulfonylurea herbicides. Weed Sci 55:429–434

Schuster CL, Al-Khatib K, Dille JA (2008) Efficacy of sulfonylurea herbicide when tank mixed with mesotrione. Weed Technol 22:222–230

Selleck GW, Baird DD (1981) Antagonism with glyphosate and residual herbicide combinations. Weed Sci 29:185–190

Stephenson DO IV, Bond JA, Landry RL, Edwards HM (2015) Weed management in corn with postemergence applications of tembotrione or thiencarbazone:tembotrione. Weed Technol 29.3:350–358

Trebst A, Depka B, Holländer-Czytko H (2002) A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. FEBS Letters 516.1:156–160

Williams MM, Boydston R, Peachey E, Robinson D (2011) Significance of atrazine as a tank-mix partner with tembotrione. Weed Technol 25:299-302

Wiggins MS, McClure MA, Hayes RM, Steckel LE (2015) Integrating cover crops and POST herbicides for glyphosate-resistant Palmer amaranth control in corn. Weed Technol 29:412-418

Witschel M (2009) Design synthesis and herbicidal activity of new iron chelating motifs for HPPD-inhibitors. Bioorg Med Chem 17:4221-4229

Wood A (2016) Compendium of herbicide common names. http://www.alanwood.net/pesticides/index. Accessed: September 15, 2016

Woodyard AJ, Bollero GA, Riechers DE (2009a) Broadleaf weed management in corn utilizing synergistic postemergence herbicide combinations. Weed Technol 23:513–518

Woodyard AJ, Hugie JA, Riechers DE (2009b) Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci 57.4:369–378

CHAPTER 5

INFLUENCE OF CROP, CROP ROTATION, AND MANAGEMENT STRATEGY ON PALMER AMARANTH EMERGENCE, GROWTH, REPRODUCTION, AND DEPLETION OF THE SOIL SEEDBANK

Abstract

Palmer amaranth, a non-native weed species to Michigan, was first identified in a Michigan soybean field in 2010. Several factors, including crop rotation, weed management practice, and time of emergence can influence the growth, reproductive potential, and persistence of Palmer amaranth. In 2013, a 3-year experiment was initiated to study the emergence, growth and fecundity of Palmer amaranth in Michigan cropping systems. The crop rotations included continuous corn (C-C-C), corn-soybean-corn (C-S-C), soybean-corn-soybean (S-C-S), and corn-soybean-wheat (C-S-W). Winter wheat delayed Palmer amaranth emergence until late July following harvest. In two of three years Palmer amaranth emerged over a longer period of time in corn as compared with soybean, and total Palmer amaranth emergence was greater in corn, due to rapid early emergence possibly influenced by nitrogen fertilizer application at V3-V4 corn. Palmer amaranth emergence was reduced by 95% in 2015 compared with 2013, suggesting a rapid decline in the soil seedbank over the three year period. The relative growth rate (RGR) of the early-emerging cohort was greater during the first 3 wks of growth in corn compared with soybean. However, for the mid- and late-emerging cohorts the RGR was greater in soybean than corn throughout the growing season. The excessive lateral branching in soybean as opposed to the primarily vertical growth in corn, allowed Palmer amaranth to accumulate more biomass and produce more seed for the early- and mid-emergence cohorts. Palmer amaranth seed production was >64,000 seeds plant^{-1} for plants emerging 2 wks after soybean planting; seed production declined to 40 seeds plant^{-1} for the late cohort in corn. Variability in the soil seedbank numbers

resulted in few differences in final Palmer amaranth seedbank as compared with the initial seedbank in 2013 in the corn-soybean rotations. Not controlling Palmer amaranth in the wheat phase of the C-S-W rotation increased the seedbank by >100,000 seeds m^{-3}, illustrating the necessity of implementing Palmer amaranth management practices after wheat harvest. The non-native weed species, Palmer amaranth, has adapted to northern climates, with emergence beginning at ~281 GDD_{10} in later May and early June. Palmer amaranth emergence continued for ~1216 GDD (through August), and all cohorts produced viable seed. To reduce 95% of viable seed production, Palmer amaranth emergence must be delayed approximately 6 weeks after mid-May corn and soybean planting to allow crop competition to reduce Palmer amaranth growth and fecundity.

Nomenclature: Corn, *Zea mays* L.; Palmer amaranth, *Amaranthus palmeri* S. Wats.; soybean, *Glycine max* (L.) Merr.; wheat, *Triticum aestivum* L.

Key words: Emergence; relative growth rate; reproduction; seed production; soil seedbank.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.) was first identified in Michigan in 2010. It is a non-native pigweed species that is detrimental in row crop production throughout the Southern and Great Plains regions of the United States (Jha et al. 2008; Massinga 2001; Sprague 2011). One of the characteristics that makes Palmer amaranth a successful weed species is the inherent ability to germinate rapidly and emerge throughout the growing season (Ehleringer 1983). Palmer amaranth germination tends to occur at higher temperatures compared with other annual weed species. Of nine weedy species studied, Palmer amaranth had one of the highest temperature requirements (~17 C) for initial germination (Steinmaus et al. 2000). Palmer

amaranth attained complete germination in less than one day, whereas it took several other *Amaranthus* species 3 to 8 d to reach 50% germination when temperatures were alternated ± 40% around 30 C. Time to emergence can also occur more rapidly for Palmer amaranth than other *Amaranthus* spp. In Missouri, initial Palmer amaranth and common waterhemp emergence occurred within 5 and 17 d of seeding, respectively (Sellers et al. 2003). In non-crop situations, Palmer amaranth emergence occurred from March through October in California and from mid-May through September in Michigan (Keeley et al. 1987; Powell 2014). Rapid and continued emergence of Palmer amaranth may require the use of residual herbicides to manage this weed throughout the growing season. Crop rotation and other cultural practices may also reduce Palmer amaranth emergence. Jha and Norsworthy (2009) reported a 70% or greater reduction in Palmer amaranth emergence when grown in the presence of a soybean canopy, likely due to the light requirement and phytochrome-mediated responses associated with Palmer amaranth germination. In research by Jha et al. (2010), far-red light in the soybean canopy inhibited Palmer amaranth germination. The influence of maize (*Zea mays* L.), cotton *(Gossypium hirsutum* L.*)* and other crop canopies on Palmer amaranth emergence has not been reported in the literature.

Other characteristics that contribute to the competitiveness of Palmer amaranth include a rapid growth rate, rapid biomass accumulation, and abundant seed production. Palmer amaranth grew faster and accumulated more biomass than other *Amaranthus* spp., including redroot pigweed (*Amaranthus retroflexus* L.), common waterhemp (*Amaranthus rudis* Sauer), and tumble pigweed (*Amaranthus albus* L.) in Kansas research (Horak and Loughin 2000). Palmer amaranth was 45 and 600% taller than common waterhemp and redroot pigweed, respectively, in Missouri (Sellers et al. 2003).

Climate and time of establishment influence Palmer amaranth seed production. Palmer amaranth produced 250,000, 446,000, 613,000 seeds plant^{-1} in Missouri, Georgia, and California, respectively, in early-emerging cohorts when there was no inter- or intra-specific competition (Keeley et al. 1987; Sellers et al. 2003; Webster and Grey 2015). Seed production was reduced by 90% when plants established in August compared with May in California (Keeley et al. 1987). In Georgia, Palmer amaranth seed production was reduced 50% when establishment was delayed by 6 wk compared with the initial cohort planting (Webster and Grey 2015), and in Kansas, seed production from 8 plants m^{-2} was reduced from 514,000 to 91,000 seeds m^{-2} when emergence was delayed until the 7-leaf stage in corn (Massinga et al. 2001). Seed production in South Carolina was reduced 97% when Palmer amaranth emerged between V3 and V6 soybean compared with plants that emerged from soybean planting to the V3 stage (Jha et al. 2008a). Little information is available on Palmer amaranth emergence timing, growth and fecundity in northern climates.

Management of Palmer amaranth and other weeds should include cultural practices and not be limited to herbicides alone (Norsworthy et al. 2012). Cultural practices such as crop rotation and tillage, or ecosystem processes including seed predation and seed decay may reduce the persistence of Palmer amaranth seedbanks (Ball and Miller 1990; Bellinder et al. 2003; Buhler et al. 2001; Cardina et al. 2002; Davis et al. 2005; Sonoskie et al. 2013). Crop rotation changes management practices, which in turn changes the niche for weed species emergence and competition with the crop (Ball and Miller 1990; Buhler et al. 2001; Cardina et al. 2002; Davis et al. 2005; Bellinder et al. 2003). The pairing of a herbicide program along with tillage can reduce soil seedbank densities (Ball and Miller 1990, Bellinder et al. 2003, Davis et al. 2005), and usually more diverse crop rotations reduce the size of the seedbank compared with monocultures

(Buhler et al. 1997). Tillage practices and the herbicides used in corn may be a reason for relatively low increases in the weed seedbank compared with rotations that include rye, hay and legume crops (Bellinder et al. 2003). In monoculture cropping systems, a few well adapted species will dominate the seedbank, whereas management strategies and predation associated with diverse cropping systems can increase weed diversity and reduce the seedbank (Buhler et al. 1997; Cardina et al. 2002). Sosnoskie et al. (2013) reported that Palmer amaranth burial depth affected seed viability in Georgia; 9 and 22% of Palmer amaranth seed remained viable after 3 years at 1 and 40 cm burial depths, respectively. In Michigan, there was no influence of burial depth on seed viability after one year (Powell 2014). Powell concluded that the Palmer amaranth seedbank could be reduced from 50 to 90% in one year if no further seed was produced. This suggests that a significant reduction in the Palmer amaranth seedbank can occur in a short time period if management practices are utilized that stop Palmer amaranth seed production.

The objectives of our research were to determine the duration of emergence, the relative growth rate, and the reproductive development of Palmer amaranth in Michigan. In addition to the biological characteristics of Palmer amaranth, the use of crop rotation paired with weed management strategy was investigated as a strategy for depleting the Palmer amaranth seedbank.

Materials and Methods

A three-year field study was initiated in 2013 in a commercial no-till production field with a known herbicide (glyphosate, ALS, and atrazine)-resistant Palmer amaranth population in Barry County, MI (42.702467°N; -85.524992°W). The soil type at this location was a combination of Oshtemo sandy loam and a Boyer loamy sand composed of 73.0, 15, and 12% sand, silt, clay, respectively, with a pH of 6.7 and 2.2% organic matter. Four crop rotations were established for

a three-year period. The rotations were: three years of continuous corn (C-C-C), corn-soybean-corn (C-S-C), soybean-corn-soybean (S-C-S), and corn-soybean-wheat (C-S-W) with each crop rotation replicated four times. Plot size was 6 m wide by 12 m long, and each plot was divided into three sub-plots. Subplot treatments consisted of: 1) weed-free (WF), 2) best management practice (BMP), and 3) weedy (W) control plots. The BMP and WF subplots were 3 m wide by 7.5 m long and the W plots were 6 m wide by 4.5 m long. The duration of Palmer amaranth emergence and the influence of crop rotation on the soil seedbank were evaluated in the BMP and WF plots. Palmer amaranth growth and development were evaluated in the W plots. Planting information can be found in Table 5.1. Urea (46-0-0) was broadcast surface-applied at 90 kg ha^{-1} in early spring to wheat (Feekes 3), and at 168 kg ha^{-1} to corn at the V3-V4 growth stage. Temperature and precipitation data were monitored throughout the growing season at a nearby long-term weather station that is part of the Michigan State University weather monitoring system (MSU Enviroweather 2016).

BMP and WF Treatments. The BMP treatments for corn and soybean consisted of preemergence (PRE) followed by (fb) postemergence (POST) herbicide applications. PRE herbicide applications were made immediately after planting and POST herbicides were applied when Palmer amaranth was 10 cm tall. The BMP for wheat was a POST herbicide application in early spring when wheat was at Feekes stage 4. Herbicide products and applications rates for the BMP treatments are listed in Table 5.2. The WF plots were kept weed-free with applications of glufosinate (Liberty 280SL, Bayer CropScience LP, Research Triangle Park, NC) and/or hand-weeding in the corn and soybean years of the rotation and by hand-weeding in wheat. Herbicide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 187 L

ha^{-1} at a pressure of 207 kPa through 11003 AIXR flat-fan nozzles (TeeJet, Spraying Systems Co., Wheaton, IL 60187).

Palmer amaranth Emergence. Two 0.25 m^2 permanent quadrats $plot^{-1}$ were established at the beginning of each season in the WF and BMP subplots. Each week throughout the growing season newly emerged Palmer amaranth plants were counted and removed. Total emergence was calculated and fit to a logistic curve. Time was converted to growing degree days (GDD) each year starting January 1, using a base temperature of 10 C. Cumulative weekly emergence was regressed against growing degree days (GDD) using the Gompertz equation (Equation 1; Forcella et al. 2000).

$$Y = 100 \times \exp[-B \times \exp(-K \times X)] \quad \text{[Eq. 1]}$$

Where *Y* is the cumulative emergence, *B* is the GDD prior to emergence, *K* is the rate of emergence, and *X* is GDD accumulation. Differences in emergence between crops and years were determined using the extra sum-of-squares principle for non-linear regression analysis (Linquist et al. 1996). In order to measure Palmer amaranth cumulative and total emergence in the absence of herbicides, measurements were taken in the WF plots. The BMP plots were used to determine how management practices influenced Palmer amaranth emergence in corn and soybean.

Palmer amaranth Growth, Development, and Seed Production. In the W plots, 10 plants $plot^{-1}$ for three different Palmer amaranth cohorts (early, mid, and late) were flagged at 2-wk intervals beginning at 200-300 GDD. Additional Palmer amaranth plants were removed from around the marked plants to reduce intra-specific competition. Palmer amaranth height was

measured weekly (2014 and 2015) or biweekly (2013). The relative growth rate (RGR) for each cohort in each crop was calculated using the height data (Equation 2).

$$RGR = \frac{[\ln H_2 - \ln H_1]}{[T_2 - T_1]} \quad \text{[Eq. 2]}$$

H_2 represents plant height at time 2, H_1 is plant height at time 1, T_2 is the GDD for the ending height measurement, and T_1 is the GDD for the initial height measurement.

Palmer amaranth development was also assessed weekly in 2014 and 2015, and biweekly in 2013. Plant stages recorded were: first sight of male and female flowering structures, pollination (onset of anthers for male plants), flowering (onset of pistils for female plants), and stages of seed maturity. Seed maturity was assessed by taking small subsamples of the seed head and evaluating for the presence of brown immature seed or black mature seed. At plant maturity, aboveground biomass was harvested, dried at 60 C for approximately 1 wk, and then weighed. Male plants were deemed mature when pollination ceased and female plants were harvested 3-wks after the onset of black seed. Plants that never matured were harvested just prior to crop destruction. Seed heads from the female plants were hand threshed and seed was separated from the chaff by sieving through a 500 µ screen and air-column separator (Seedburo Equipment Co., Des Plains, IL). Total seed number was determined by dividing the total clean sample weight by the 100 seed weight for three subsamples and then multiplying by 100 (Equation 3).

$$Total\ Seed\ Number = \frac{Total\ Sample\ Weight}{100\ Seed\ Weight\ avg} \times 100 \quad \text{[Eq. 3]}$$

Data for RGR, Palmer amaranth height, time to reproductive stages, end-of-season biomass, and seed production were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Assumptions of normality of residuals and homogeneity of variances were confirmed using PROC UNIVARIATE and analysis of variance (ANOVA) was conducted using PROC MIXED. Palmer amaranth biomass and seed production data were square-root and log-transformed,

respectively, as suggested by Box-Cox analysis (Box and Cox 1964) using PROC TRANSREG. The statistical model included the main effects of crop, cohort, and their interactions as fixed effects. Years and replication were considered random effects in the model. Mean separation for the main effects of crop and cohort, and their interaction was conducted with multiple *t*-tests and were found to be statistically significant at α level of 0.05.

Palmer amaranth Soil Seedbank. At the establishment of the experiment in the spring of 2013, three soil cores (10.4 cm diameter by 15 cm depth) were taken in the BMP and WF subplots. These plots were sampled again at the conclusion of the experiment in the fall of 2015. Soil samples were stored in the freezer at -20 C until sample processing. Palmer amaranth seed was separated into different soil fractions by wet sieving samples through a 2,000 and 500 µ screens. Samples were then air dried and Palmer amaranth seed was extracted from the remaining soil using a method previously described by Malone (1967). This procedure uses a solution of 10 g sodium hexametaphosphate, 5 g sodium bicarbonate, and 25 g magnesium sulfate in 200 mL of water. Each sample was agitated for 2 min in the solution, decanted through a 500 µ sieve, and rinsed. This process was repeated 3 times or until all the seed had been removed. Samples were air dried, and seed was enumerated by hand counting seed in 0.5 g of the total sample and extrapolating the total seed number (Equation 4).

$$\text{Seed number} = \frac{Total\ sample\ weight}{0.5} \times Seed\ number\ in\ 0.5\ g \qquad \text{[Eq. 4]}$$

All data for the 2013 seedbank was pooled over management practice to establish a base or initial Palmer amaranth seedbank and compared with 2015. Soil seedbank data were analyzed for differences between the different crop rotations within the BMP and WF management practices with ANOVA using PROC MIXED in SAS 9.4. Each management practice was

analyzed separately with rotation as a fixed effect and replication as random. Mean separation for the influence of crop rotation on the Palmer amaranth soil seedbank was conducted with multiple t-tests at α level 0.05. To show the variability of Palmer amaranth within the soil seedbank data are presented in box-plots as seeds m^{-3}.

Results and Discussion

Palmer amaranth Emergence. *Corn and Soybean.* Initial emergence of Palmer amaranth in corn and soybean was similar for all three years, occurring on May 24 (260 GDD), May 28 (260 GDD), and June 8 (323 GDD) in 2013, 2014, and 2015, respectively. Similar results for Palmer amaranth initial emergence in Michigan were reported by Powell (2014). Emergence did not occur until the minimum soil temperature was >17 C, similar to the temperature requirements reported by Stienmaus et al. (2000) for Palmer amaranth germination. Ideal corn planting dates in central Michigan are the first two weeks in May; ideal soybean planting dates are May 8 through May 20, suggesting that Palmer amaranth would begin emerging within the first three and two weeks following corn and soybean emergence, respectively.

The duration of Palmer amaranth emergence in corn and soybean were measured in the WF sub-plots. In 2013, a significant rainfall event caused flooding forcing the experiment to be relocated, shortly after initial Palmer amaranth emergence. Corn and soybean were replanted June 6. Warm temperatures coupled with high soil moisture caused high numbers of Palmer amaranth plants to emerge prior to reestablishment of the experiment. This initial emergence, accounted for 40 and 85% of the total Palmer amaranth emergence for corn and soybean, respectively, in 2013 (Figure 5.1a). The duration of Palmer amaranth emergence in 2013 was greater in corn compared with soybean; 95% of total emergence had occurred by July 24 (829

GDD) 24 d (223 GDD) after soybean (Table 5.3). In 2014, there was no difference in the duration of Palmer amaranth emergence in corn compared with soybean (Figure 5.1b). The duration of Palmer amaranth emergence from 10 to 95% was 863 GDD (87 d) and 768 GDD (77 d) for corn and soybean, respectively, with 95% emergence occurring by Aug. 26 (Table 5.3). Similar to 2013, the duration of Palmer amaranth emergence was greater in corn compared with soybean in 2015 (Figure 5.1c). Palmer amaranth continued to emerge for an additional 181 GDD (17 d) longer in corn compared with soybean, lasting until July 27 (Table 5.3). Palmer amaranth emergence ceased on Sept. 18, Sept. 9, and Sept. 23 in 2013, 2014, and 2015, respectively.

In two of three years, the duration of and the total emergence of Palmer amaranth was influenced by crop. Total emergence was greater in corn compared with soybean (Figures 5.2a-c) in 2013, 2014, and 2015, and more Palmer amaranth emerged in corn early in the season (400 – 800 GDD). Nitrogen fertilizer was broadcast applied as urea (46-0-0) in corn at the V3 growth stage, possibly influencing Palmer amaranth emergence. Sweeney et al. (2008) reported an increase in common lambsquarters (*Chenopodium album* L.) emergence in the presence of nitrogen, however redroot pigweed emergence was not influenced. Cumulative Palmer amaranth emergence in soybean plateaued prior to complete canopy closure, suggesting that another crop management factor, possibly shading Pfr, may influence emergence patterns in crop. In research by Jha et al. (2010) reduced Pfr below the soybean canopy reduced Palmer amaranth emergence compared with the absence of crop. In 2014, lower amounts and less frequent rainfall events within two weeks after planting may have reduced overall soybean growth leaving a more open soybean canopy and more Pfr for continued Palmer amaranth emergence similar to what was observed in corn.

A sharp decline in total Palmer amaranth emergence occurred over the three years of this experiment, regardless of the crop or rotation (Figure 5.2a-c). There was >95% reduction in Palmer amaranth emergence when the 2013 and 2015 emergence data were compared. This suggests a relatively non-persistent seedbank for Palmer amaranth in Michigan, and supports previous seed viability results reported by Powell (2014). Palmer amaranth emergence was also influenced by weed management practice. The mean for total emergence of Palmer amaranth pooled over crop rotation was 137 and 36 plants in 0.5 m^{-2} for the WF and BMP management practices, respectively (P = 0.0286), suggesting that herbicides with residuals in the BMP management strategy reduced Palmer amaranth emergence by up to 74%. The influence of crop rotation on Palmer amaranth emergence was evaluated in the WF and the BMP management strategy in the C-C-C and C-S-C rotations. These two rotations were chosen because they both started and ended with corn in the rotation. Palmer amaranth emergence in the WF plots in 2015 was similar in the C-C-C and C-S-C rotations, averaging 5 and 11 plants 0.5 m^{-2}, respectively. However, in the BMP treatment, Palmer amaranth emergence declined more in the C-C-C compared with the C-S-C rotation, averaging 0.75 and 18.5 plants 0.5 m^{-2}, respectively (P = 0.0452). This suggests that planting continuous corn is the best strategy for Palmer amaranth management if residual herbicides are applied.

Wheat. Winter wheat was only present in the last year of the experiment. Wheat was planted in the fall of 2014 and was 45 cm tall and at Feekes stage 7 by the end of May when Palmer amaranth typically emerged in corn and soybean. Wheat was very effective in delaying the emergence of Palmer amaranth; little to no emergence occurred until wheat senescence and harvest in late July (Figure 5.1c). Ten percent of the total cumulative emergence occurred on

August 16 (1089 GDD), 3 weeks after wheat harvest (Table 5.3); emergence of Palmer amaranth continued until the end of September. This data suggests that wheat can suppress Palmer amaranth emergence until harvest, however, fall management practices must be implemented to control Palmer amaranth emergence in August and September and to stop seed production.

Palmer amaranth Relative Growth Rate, Height, and Reproductive Development. *Corn and Soybean.* The emergence cohort influenced Palmer amaranth RGR and final height. RGR was greater initially (the first three weeks) for the early-emerging cohort in corn compared with soybean (Table 5.4), likely due to the application of nitrogen fertilizer to V3-V4 corn. The opposite trend in RGR was observed for the mid-emerging cohort (Figure 5.3 and Table 5.4); RGR was two times greater in soybean compared with corn (Table 5.4). The mid cohort grew above the soybean canopy prior to closure; the mid cohort could not effectively compete for light in the corn canopy. Furthermore, the N application in corn may have had less influence on RGR in the mid cohort because light resources were reducing Palmer amaranth nutritional demands. The early cohort (emergence 2 wks after corn and soybean planting) grew to ~180 cm in both corn and soybean (Table 5.5). Palmer amaranth was much shorter in the later-emerging cohorts in both crops (Figure 5.3). The average final height for the late-emerging (~6 wks after planting) Palmer amaranth cohort was 18 cm (Table 5.5).

Palmer amaranth reached the reproductive stage more rapidly in soybean (441 GDD, 42 d) compared with corn (506 GDD, 49 d), when combined over years and cohorts (Table 5.6). In soybean the early- and mid-emerging cohorts were able to grow above the canopy, while all cohorts in corn were unable to grow above the corn canopy. In previous research, Palmer amaranth, common waterhemp, and redroot pigweed life history stages were delayed when

plants were grown in shade (Jha et al. 2008b; McLachlan et al. 2003; Steckel et al. 2003). Resources were allocated to the main stem rather than to lateral branches or reproductive structures, suggesting that the corn canopy may delay Palmer amaranth reproductive development. Alternatively, a delay in the reproductive development of Palmer amaranth in corn may have been caused by additional nitrogen from fertilization in corn that may have prolonged Palmer amaranth's vegetative growth. Furthermore, Palmer amaranth development from floral initiation to the presence of mature seed did not differ between corn and soybeans (Table 5.6).

The time of cohort emergence also influenced the development of Palmer amaranth when data were pooled over crop and year. Initiation of reproductive structures occurred more rapidly (406 GDD 38 d) with the early emerging cohort compared with the mid and late emerging cohorts (Table 5.7). This change from the vegetative to reproductive stages for the early cohort, aligns with the decrease in day length that has been speculated to trigger reproductive stages in Palmer amaranth and other *Amaranthus spp.* (Keeley et al. 1987; Huang et al. 2000; Norsworthy et al. 2016). The delay in the transition to the reproductive stage in the mid and late cohorts may be due to the slowed growth rate of Palmer amaranth under the crop canopy. Contrary to those that emerge early, the development of reproductive structures for Palmer amaranth that emerged later in the season may be dependent on the quantity and quality of light, rather than day length. Little information is available on the reproductive phenology of Palmer amaranth at different emergence times. Generally, once the reproductive structures have developed, fewer GDD's are required for the later emerging Palmer amaranth to reach the reproductive growth stages and the onset of mature black seed (Table 5.7). The more rapid development in the later-emerging cohorts could be attributed to the greater reproductive effort required for the early cohorts due to plant size and total number of inflorescences on the lateral branches. Reproductive efforts in

redroot pigweed increased with plant height and lateral branching (Wang et al. 2006). The total time from flagging to mature seed production was 750 (72 d), 795 (81 d), 709 GDD (80 d) for the early, mid, and late cohorts, combined over crop and year (Table 5.7), which is approximately 10 to 11 weeks from emergence until mature seed production. Mature seed from Palmer amaranth emerging in June and July will be produced in late August through September in Michigan. In California, viable Palmer amaranth seed was produced 8 to 12 and 5 to 7 wks after emergence for early- and late-emergence timings, respectively, in the absence of crop competition (Keeley et al. 1987). The longer length of time for seed production in our research may be attributed to the cooler temperatures of Michigan compared with California (longer time for GDD to accumulate), coupled with crops competing for resources in our research.

Wheat. With only one year of wheat data and little to no emergence of Palmer amaranth during the time of cohort flagging, results for wheat were viewed as anecdotal and will be discussed separately from corn and soybean. In wheat, Palmer amaranth plants were marked with flags 2 wks (August 6) and 5 wks (August 27) after wheat harvest (WAH), 5 to 7 wks after the last cohort was flagged in corn and soybeans. Palmer amaranth RGR was similar between the two cohorts (data not shown); mean height for the 2 and 5 WAH cohorts was 67 and 16 cm plant^{-1}, respectively.

Palmer amaranth Dry Weight and Seed Production. *Corn and Soybean.* The time of Palmer amaranth emergence and the presence of a crop influenced final dry weight and seed production. Palmer amaranth dry weight decreased with each successive cohort (Table 5.4), and dry weight was 6 to 8 times greater in soybean compared with corn for the early and mid cohorts,

respectively (Table 5.4). Palmer amaranth had excessive lateral branching in soybean, whereas growth was mainly vertical with little branching in corn. Similar trends in common waterhemp dry weight production in corn and soybean were previously reported (Uscanga-Mortera et al. 2007). Decreases in Palmer amaranth dry weight with later emergence times have also been previously reported in the absence of a crop (Keeley et al. 1987; Horak and Loughin 2000; Sellers et al. 2003).

The early and late cohorts of Palmer amaranth produced >64,000 and 40 seeds plant^{-1}, respectively (Table 5.4). In both corn and soybean, Palmer amaranth seed production was reduced by as much as 99% in the late compared with the early cohorts (Table 5.4). Previous research has shown that the presence of a crop and the time of emergence influence seed production. Palmer amaranth that was established at the time of cotton planting produced 30% less seed compared with plants grown in the absence of cotton (Webster and Grey 2015), and delaying Palmer amaranth emergence reduced seed production in corn (Massinga et al. 2001) and soybean (Jha et al. 2008).

Palmer amaranth seed production was 6 and 14 times greater for the early and mid cohorts respectively, in soybean compared with corn, due to the increased lateral branching and inflorescences in the early-emerging cohort (Table 5.4). Taller plants and increased branching in the mid cohort allowed for greater seed production in soybean compared with corn. Similar trends were observed in the seed production of common waterhemp with different emergence times in corn and soybean (Uscanga-Mortera et al. 2007). Palmer amaranth seed production in corn and soybean has previously been reported to be as high as 514,000 and 211,400 seeds m^{-2}, respectively (Jha et al. 2008; Massinga et al. 2001). However, when the Palmer amaranth density was reduced to 0.5 plants m^{-1}, seed production in corn was reported as 140,000 seeds m^{-2}

(Massinga et al. 2001). The greatest mean seed production of Palmer amaranth in our experiment was 64,257 seeds plant^{-1} for early-emerging Palmer amaranth in Michigan soybean (Table 5.4). This is much lower than what was previously reported from other areas, however individual plants in this experiment produced >350,000 seeds plant^{-1}, indicating the potential for severe infestations of Palmer amaranth the following year if the weed is allowed to emerge in later May and grow to maturity.

Wheat. In wheat, Palmer amaranth dry weight was 22 and 2 g plant^{-1} for plants emerging at 2 and 5 WAH, respectively. The 2 and 5 WAH cohorts produced >20,000 and 27 viable seeds plant^{-1}, respectively. While these observations are derived from only 1-year of data, they suggest the need for Palmer amaranth control strategies following wheat harvest to prevent seed production.

Soil Seedbank Reduction. The soil seedbank for Palmer amaranth at this location was highly variable, which led to the inability to detect differences in the seedbank in the rotations where wheat was not included (Figures 5.4 and 5.5). The soil seedbank in 2013 ranged from 13,000 to 300,000 Palmer amaranth seeds m^{-3}. Previous research has documented that accurate soil seedbank determination can be highly variable depending on sampling method (Gross 1990). However, variability in seed distribution at our research location may have also been caused by the manure application that introduced Palmer amaranth in this field creating greater densities of Palmer amaranth seeds in some areas within the field. At the conclusion of the 3-year crop rotations the Palmer amaranth soil seedbank ranged from 0 to >690,000 and 0 to >130,000 seeds m^{-3} for the BMP and WF plots, respectively (Figures 5.4 and 5.5). Palmer amaranth was not

allowed to disperse seed in the WF treatment, and it was expected that the seedbank would have declined over the three- year period in this treatment.

There was an increase of Palmer amaranth in the soil seedbank with the BMP management practice in the C-S-W rotation (Figure 5.4). The soil seedbank increased 3X from 43,726 in 2013 to 145,734 seeds m^{-3} following the 3-year rotation of C-S-W. Palmer amaranth produced seed following wheat harvest. The seed bank was sampled in late October 2015 and August emerging Palmer amaranth had produced seed following wheat harvest.

In conclusion, Palmer amaranth is well-adapted to the cooler climate and crop rotations in Michigan. Palmer amaranth produced viable seeds in corn, soybean, and following wheat when not managed. Palmer amaranth emerged in late May (281 GDD) and emergence continued throughout the growing season. Winter wheat can effectively delay emergence until crop senescence, however management practices such as tillage, frost-seeded clover, or herbicide applications in wheat stubble need to be implemented to maintain any benefit from integrating wheat into the rotation. Palmer amaranth can successfully produce seed, regardless of emergence time in corn and soybean; however, total seed number was reduced with each successive emergence cohort. Both RGR and seed production were greater for the mid cohort in soybean compared with corn. Therefore, it is important to monitor and employ timely control measures for the mid-cohort emerging Palmer amaranth in soybean.

Soybean in our research was planted at a 76 cm row spacing; previous research has shown a 35% reduction in Palmer amaranth seed production when grown in narrow rows compared with wide rows (Jha et al. 2008). Decreasing the soybean row spacing from 76 cm may help manage Palmer amaranth in Michigan. The early cohort in corn was very competitive and had a faster RGR and delayed time to reproductive stage compared with soybean. Broadcast nitrogen

fertilizer at V3 in corn, just prior to the first cohort emergence, may increase the emergence and competitiveness of Palmer amaranth in corn.

Crop rotation and management practice did not have a significant influence on the Palmer amaranth seedbank, except for in the C-S-W rotation. Several other studies have reported that crop rotation can influence the composition of the soil seedbank of various weed species (Ball 1992; Buhler et al 2001; Cardina et al. 2002). To reduce the chances of cross-plot contamination this research was conducted in no-till. Previous research has shown that in no-till production systems 90% of the seedbank will be within the top 5 cm (Swanton et al. 1999). The variability in the soil seedbank distribution paired with the methodology used for enumeration may have contributed to our inability to detect changes in the seedbank. Regardless, Palmer amaranth emergence in this no-till production system declined over the three-year period in all of the crop rotations by >95%. Therefore, not allowing Palmer amaranth to produce seed could result in up to 278 fewer plants 0.5 m^{-2} emerging after a three-year period in rotations that include corn.

APPENDIX

APPENDIX

CHAPTER 5 TABLES AND FIGURES

Table 5.1. Planting information for the long-term crop rotation study in Barry County, MI.

	2013	2014	2015
Corn[a]			
Planting date	June 6	May 28	May 18
Hybrid[b]	DKC 48-12	P 9807 AM	P 9807 AM
Population	79,000 seeds ha^{-1}	79,000 seeds ha^{-1}	79,000 seeds ha^{-1}
Soybean[a]			
Planting date	June 6	May 28	May 18
Variety[b]	DF 9251 LL	MCIA2365 LL	MCIA2512 LL
Population	420,000 seeds ha^{-1}	420,000 seeds ha^{-1}	420,000 seeds ha^{-1}
Wheat[a]			
Planting date	—	October 17	—
Variety[b]	—	Sunburst	—
Population	—	5.2 million seeds ha^{-1}	—

[a] Corn and soybean were planted in 76 cm row widths and wheat was planted in 19 cm rows.
[b] Company information: DKC 48-12; Dekalb, Monsanto Company, St. Louis, MO; P 9807 AM, DuPont Pioneer, Johnston, IA; DF 9251 LL, D.F. Seeds, Inc., Dansville, MI; MCIA2365LL, MCIA2512LL, and Sunburst, Michigan Crop Improvement Assoc., Lansing, MI;

Table 5.2. Best management practice (BMP) herbicide programs for multiple-resistant Palmer amaranth control used in corn, soybean, and wheat in Barry County, MI.

Crop	Timing[b]	Treatment	Rate (kg ai ha^{-1})	Trade name[a]
Corn	PRE	*s*-metolachor + atrazine	1.4 + 1.79	Bicep II Magnum
	POST	mesotrione + atrazine + COC + AMS	0.11 + 0.67 + 1% v v^{-1} + 1% w w^{-1}	Callisto Xtra
Soybean	PRE	flumioxazin	0.07	Valor SX
	POST	glufosinate + *s*-metolachlor + AMS	0.6 + 1.42 + 1% w w^{-1}	Liberty + Dual II Magnum
Wheat	POST	2,4-D amine + pyrosulfotole + bromoxynil + NIS + AMS	0.56 + 0.04 + 0.12 + 0.25% v v^{-1} + 1% w w^{-1}	2,4-D Amine + Huskie

[a] Manufacturer information: Bicep II Magnum, Syngenta Crop Protection, LLC, Greensboro, NC; Callisto Xtra, Syngenta Crop Protection, LLC, Greensboro, NC; COC, crop oil concentrate, Herbimax, Loveland Products, Inc., Loveland, CO; AMS, ammonium sulfate, Actamaster, Loveland Products, Inc., Loveland, CO; Valor SX, Valent Corporation, Walnut Creek, CA; Liberty 280SL, Bayer CropScience LP, Research Triangle Park, NC; Dual II Magnum, Syngenta Crop Protection, LLC, Greensboro, NC; 2,4-D Amine 4, Winfield Solutions, LLC, St. Paul, MN; Huskie, Bayer CropScience, Research Triangle Park, NC; NIS, non-ionic surfactant, Activator 90, Loveland Products, Inc., Loveland, CO.

[b] Abbreviations: PRE, preemergence; POST, postemergence.

Table 5.3. Gompertz[a] equation parameters and growing degree days (GDD) for cumulative emergence of Palmer amaranth in corn, soybean, and wheat for 2013, 2014, and 2015 in Barry County, MI.

		Parameters			GDD[b] to X% emergence		
Year	Crop	B	K	R^2	10%	50%	95%
2013	Corn	3.9	0.005	0.65	—	332	829
	Soybean	0.25	0.003	0.30	—	—	606
2014	Corn	9.0	0.004	0.75	313	592	1176
	Soybean	13.8	0.005	0.74	363	606	1131
2015	Corn	39.5	0.008	0.72	370	533	862
	Soybean	227.7	0.01	0.94	361	454	672
	Wheat	146,239	0.01	0.93	1089	1220	1482

[a] Gompertz equation for cumulative emergence: $Y = 100 \times \exp(-B \times \exp(-K \times X))$, where Y is cumulative emergence, B is growing degree day (GDD) lag time, K is rate of emergence, and X is GDD accumulation.

Table 5.4. Total relative growth rate[a] (RGR) and the RGR for the first 3 and 5 wks after flagging Palmer amaranth for early, mid, and late emergence cohorts in corn and soybean in Barry County, MI. Data are combined over 2013, 2014, and 2015.

		Palmer amaranth growth period		
Crop	Cohort	First 3 wks	First 5 wks	Total
		RGR cm cm^{-1} wk^{-1}		
Corn	Early	1.13 a[b]	0.81 a	0.52 a
	Mid	0.42 d	0.33 c	0.24 d
	Late	0.24 e	0.18 d	0.20 e
Soybean	Early	1.03 b	0.81 a	0.53 a
	Mid	0.80 c	0.62 b	0.42 b
	Late	0.43 d	0.30 c	0.23 c

[a] Relative growth rate for plant height was calculated as (ln Height2 – ln Height1) / (Time2-Time1).

[b] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 5.5. Palmer amaranth height, dry weight, and seed production for early, mid, and late cohorts in corn and soybean in Barry County, MI. Data are combined over 2013, 2014, and 2015.

Crop	Cohort	Final height	Dry weight[a]	Seed production[b]
		cm plant^{-1}	g plant^{-1}	number plant^{-1}
Corn	Early	176 a[c]	28 b	10,849 b
	Mid	34 c	1 d	350 c
	Late	12 d	0.1 e	40 c
Soybean	Early	182 a	178 a	64,257 a
	Mid	95 b	8 c	4,862 b
	Late	23 cd	0.2 e	458 c

[a] Means for the dry weight data are the back-transformed values from log-transformed data.
[b] Means for Palmer amaranth seed production are the back-transformed values from square-root transformed data.
[c] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 5.6. Growing degree days[a] (GDD) and days required for the reproductive development of Palmer amaranth in corn and soybean. Data are combined over years and pooled over cohort emergence time.

	GDD to	GDD from reproduction to				GDD from flagging to
Crop	Reproduction	Pollen	Flower	Immature seed	Mature seed	Mature seed
	GDD (days)[b]					
Corn	506 (49) a[c]	141 (15) a	84 (10) a	216 (23) a	277 (32) a	783 (81) a
Soybean	441 (42) b	129 (14) a	93 (10) a	213 (23) a	280 (32) a	721 (74) b

[a] Growing degree days were calculated using a base temperature of 10 C from the time of flagging for each emergence cohort as GDD = (((Temp$_{max}$ + Temp$_{min}$)/2) – base temp).

[b] Values within the parentheses represent the number of days required to accumulate the number of GDD's; all mean separations were done with the GDD values.

[c] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

Table 5.7. Growing degree days[a] (GDD) and days required for the reproductive development of early, mid, and late Palmer amaranth emergence cohorts. Data are combined over years and pooled over corn and soybean crop.

	GDD to	GDD from reproduction to				GDD from flagging to
Cohort	Reproduction	Pollen	Flower	Immature seed	Mature seed	Mature seed
	GDD (days)[b]					
Early	406 (38) b[c]	157 (16) a	103 (10) a	284 (27) a	344 (34) a	750 (72) a
Mid	513 (51) a	147 (15) a	90 (10) ab	209 (23) b	282 (30) b	795 (81) a
Late	500 (49) a	102 (12) b	72 (10) b	151 (20) c	209 (31) c	709 (80) a

[a] Growing degree days were calculated using a base temperature of 10 C from the time of flagging for each emergence cohort as GDD = (((Temp$_{max}$ + Temp$_{min}$)/2) – base temp).

[b] Values within the parentheses represent the number of days required to accumulate the number of GDD's; all mean separations were done with the GDD values.

[c] Means followed by the same letter within a column are not statistically different at $\alpha = 0.05$.

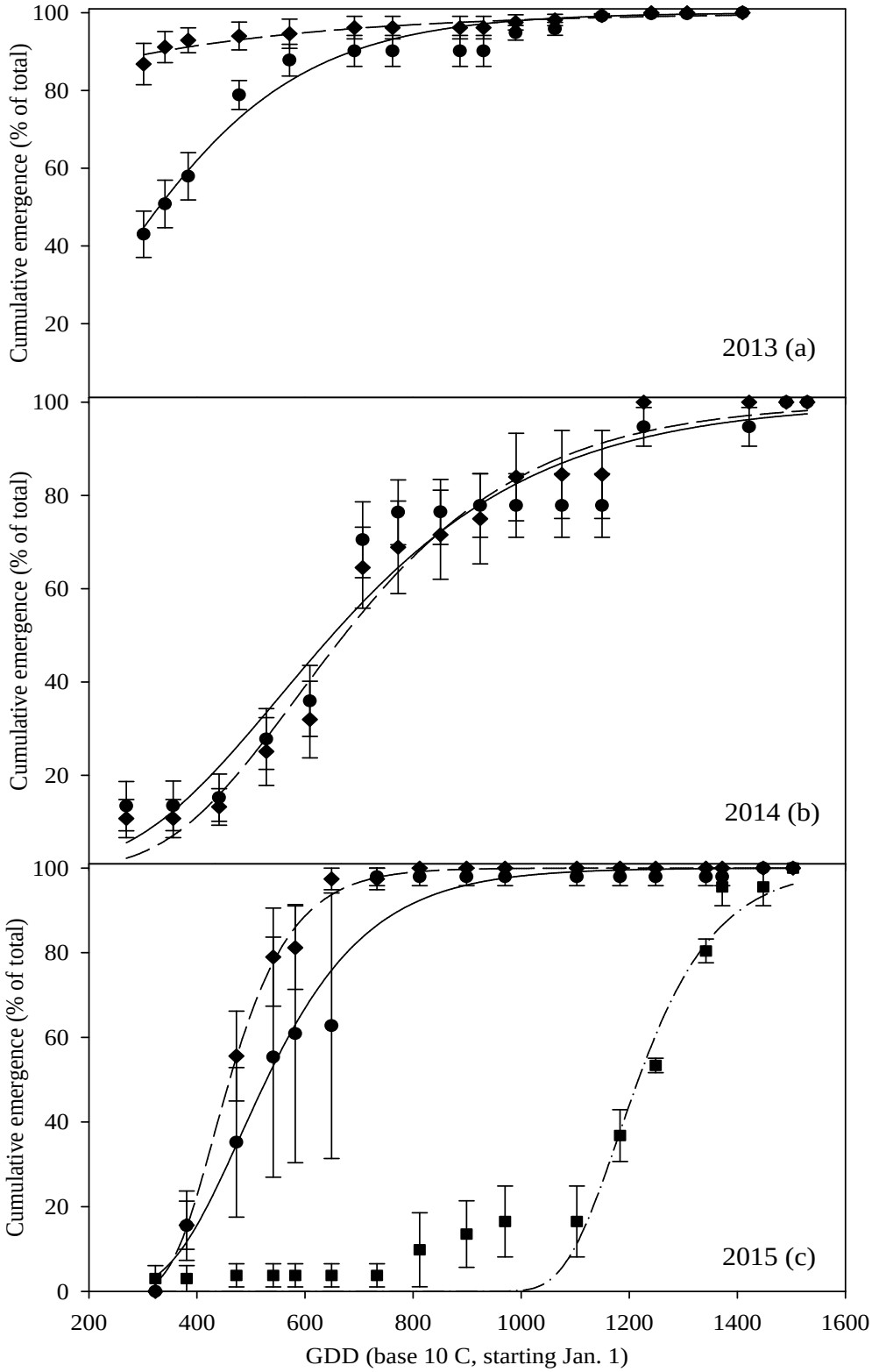

Figure 5.1a-c. Cumulative Palmer amaranth emergence as a percent of total emergence in corn (◆), soybean (●), and wheat (■) in 2013 (a), 2014 (b), and 2015 (c) and bars represent standard error of emergence. Regression parameters are listed in Table 5.3.

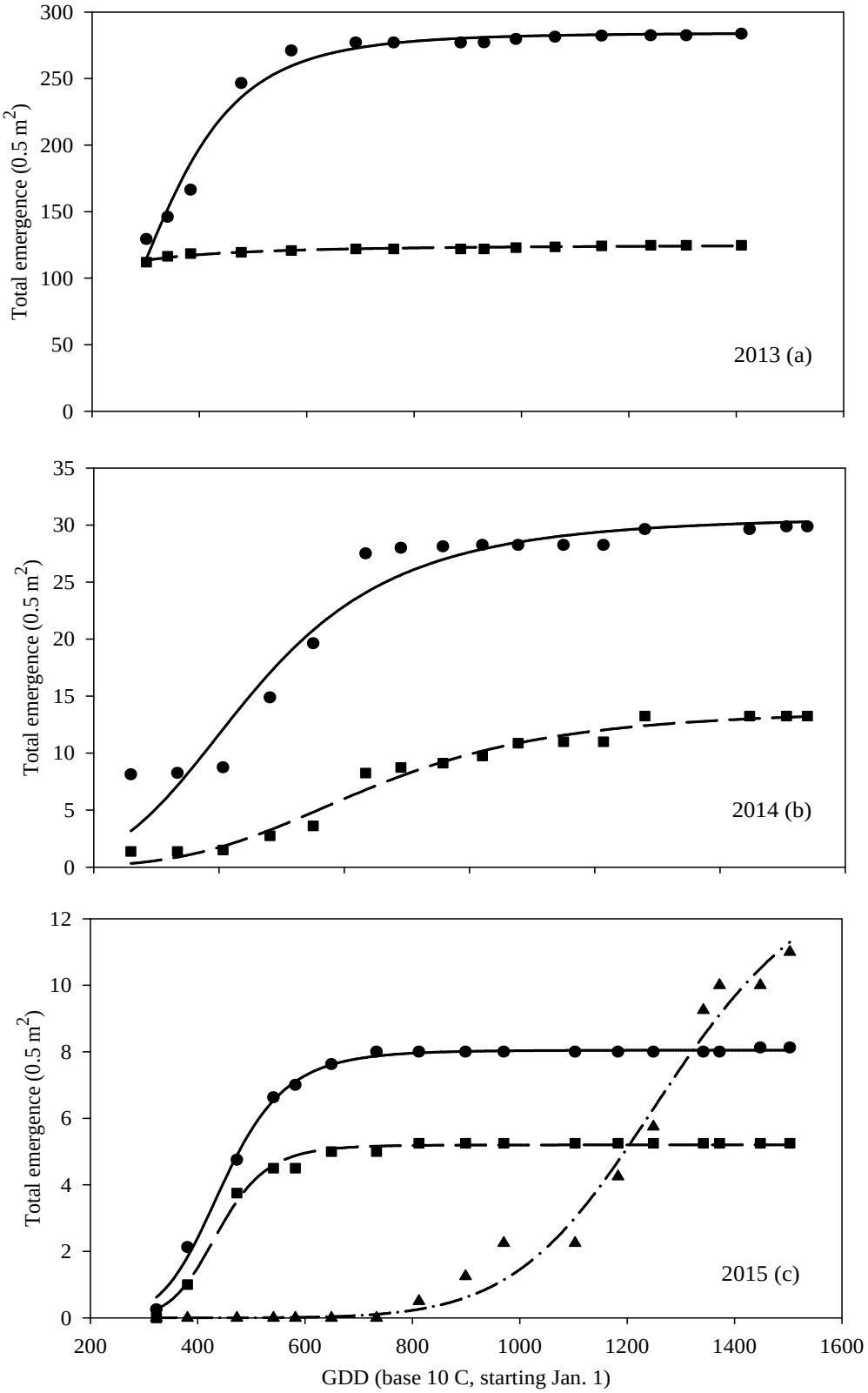

Figure 5.2a-c. Total Palmer amaranth emergence in corn (◆), soybean (●), and wheat (■) in 2013 (a), 2014 (b), and 2015 (c).

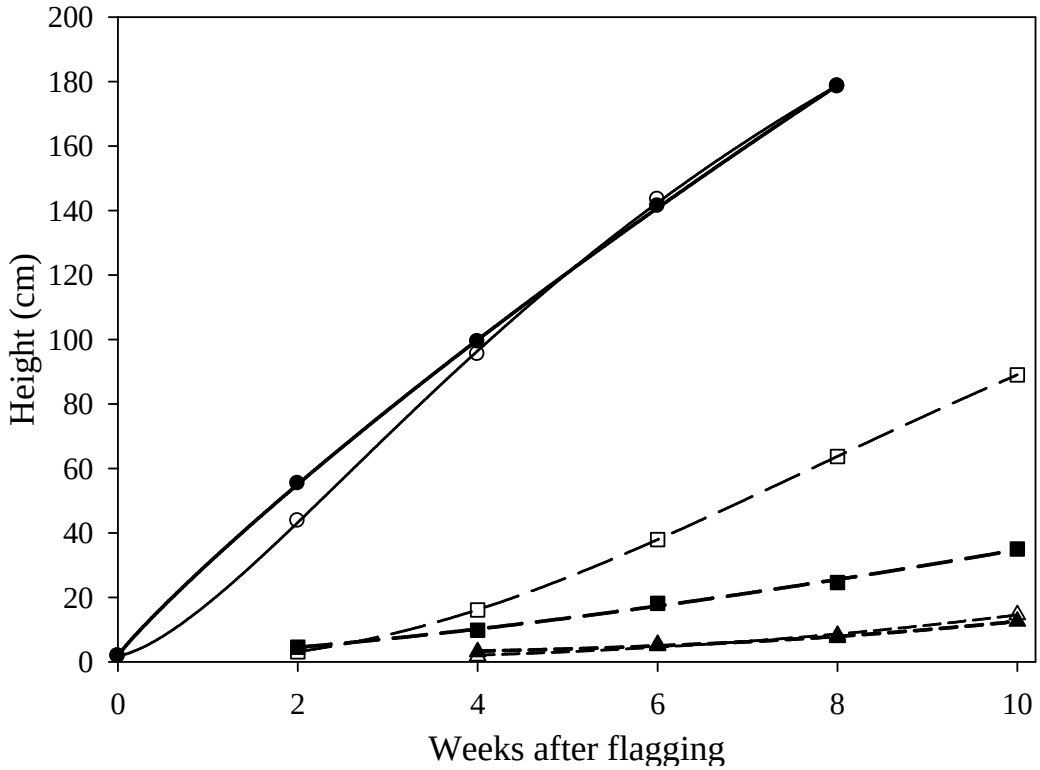

Figure 5.3. Cumulative growth of Palmer amaranth as influenced by crop and emergence time, with 0 weeks representing the time of flagging for the early emergence time. Lines for Palmer amaranth growth in weeks were fitted using the 4-parameter log-logistic model. Early cohort corn (●), early cohort soybean (○), mid cohort corn (■), mid cohort soybean (□), late cohort corn (▲), and late cohort soybean (△).

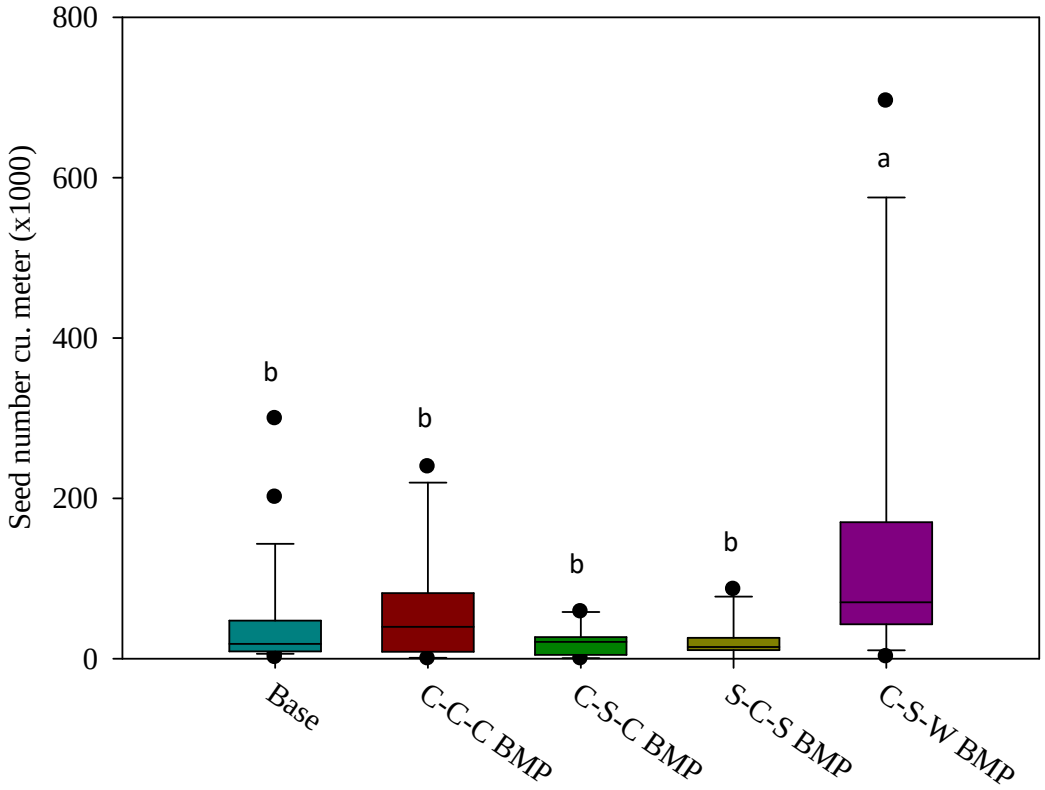

Figure 5.4. Palmer amaranth seed distribution after three years in the best management practice (BMP) plots for four different crop rotations. The base plot represents the initial Palmer amaranth soil seedbank. The letters above indicate significant differences; plots followed by the same letter are not significantly different at $\alpha = 0.05$.

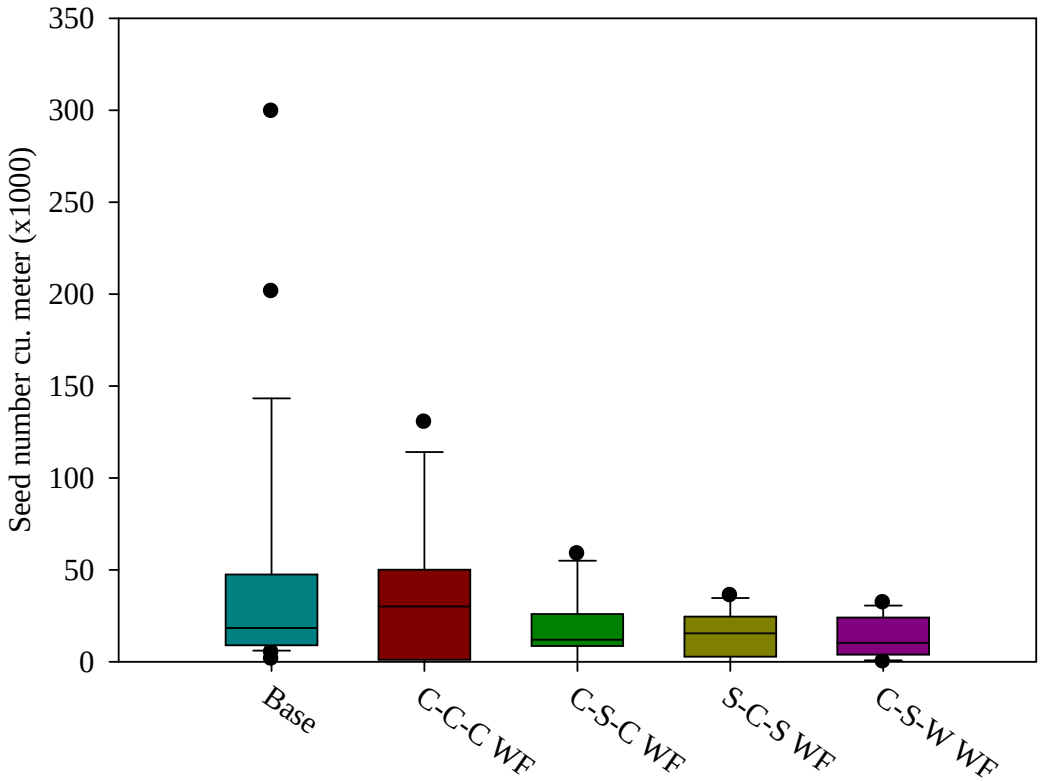

Figure 5.5. Palmer amaranth seed distribution after three years in the weed-free plots for four different crop rotations. The base plot represents the initial Palmer amaranth soil seedbank.

LITERATURE CITED

LITERATURE CITED

Ball DA (1992) Weed seedbank response to tillage, herbicides, and crop rotation sequence. Weed Sci 40:654–659

Ball DA, Miller SD (1990) Weed seed population response to tillage and herbicide use in three irrigated cropping sequences. Weed Sci 38:511–517

Bellinder RR, Dillard HR, Shah DA (2003) Weed seedbank community responses to crop rotation schemes. Crop Protection 23.2:95–101

Box GEP, Cox DR (1964) An analysis of transformation (with discussion). J R Stat Soc B 26:211–252

Buhler DD, Hartzler RG, Forcella F (1997) Implications of weed seedbank dynamics to weed management. Weed Sci 45:329–336

Buhler DD, Kohler KA, Thompson RL (2001) Weed seed bank dynamics during a five-year crop rotation 1. Weed Technol 15.1:170–176

Cardina JC, Herms P, Doohan DJ (2002) Crop rotation and tillage system effects on weed seedbanks. Weed Sci 50.4:448–460

Davis AS, Renner KA, Gross KL (2005) Weed seedbank and community shifts in a long-term cropping systems experiment. Weed Sci 53.3:296–306

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. Oecologia 57:10–112

Forcella F, Benech-Arnold RL, Sanchez R, Ghersa CM (2000) Modeling seedling emergence. Field Crops Res 67:123–139

Gross KL (1990) A comparison of methods for estimating seed numbers in the soil. J Ecol 78:1079–1093

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus species*. Weed Sci 48.3:347–355

Huang JZ, Shrestha A, Tollenaar M, Deen W, Rahimian H, Swanton CJ (2000) Effects of photoperiod on the phenological development of redroot pigweed (*Amaranthus retroflexus* L.). Can J Plant Sci 80:929–938

Jha P, Norseworthy JK, Riley MB, Bridges Jr W (2010) Annual changes in temperature and light requirements for germination of Palmer amaranth (*Amaranthus palmeri*) seeds retrieved form the soil. Weed Sci 58:426–432

Jha P, Norsworthy JK (2009) Soybean canopy and tillage effects on emergence of Palmer amaranth (*Amaranthus palmeri*) from a natural seed bank. Weed Sci 57.6:644–651

Jha P, Norsworthy JK, Bridges Jr W, Riley MB (2008a) Influence of glyphosate timing and row width on Palmer amaranth (*Amaranthus palmeri*) and pusley (*Richardia spp.*) demographics in glyphosate-resistant soybean. Weed Sci 56.3:408–415

Jha P, Norsworthy JK, Riley MB, Bielenberg DG, Bridges Jr W (2008b) Acclimation of Palmer amaranth (*Amaranthus palmeri*) to shading. Weed Sci 56:729–734

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Lindquist JL, Mortenson DA, Clay SA, Schmenk R, Kells JJ, Howatt D, Westra P (1996) Stability of corn (*Zea mays*) velvetleaf (*Abutilon theophrasti*) interference relationships. Weed Sci 44:309–313

Malone CR (1967) A rapid method for enumeration of viable seeds in soil. Weeds 15:381–382

Massinga RA, Curie RS, Horak MJ, Boyer Jr J (2001) Interference of Palmer amaranth in corn. Weed Sci 49.2:202–208

McLachlan SM, Tollenaar M, Swanton CJ, Weise SF (1993) Effect of corn-induced shading on dry matter accumulation, distribution, and architecture of redroot pigweed (*Amaranthus retroflexus*). Weed Sci 41:568–573

Michigan State University Enviro-weather (2016) weather station network. https://www.enviroweather.msu.edu. Accessed: May 15, 2016

Norsworthy JK, Schrage BW, Barber TL, Schwartz LM (2016) Emergence date influences growth and fecundity of Palmer amaranth in cotton. J Cotton Sci 20:263–270

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgis, NR, Witt, WW, Barrett M (2012) Reducing risks of herbicide resistance: best management practices and recommendations. Weed Sci spec iss 31–62

Powell DK (2014) Biology and management of multiple (glyphosate/ALS)-resistant Palmer amaranth in Michigan soybean. Ph.D dissertation. East Lansing, MI: Michigan State University. 122 p

Schweizer EE, Lauridson TC (1985) Powell amaranth (*Amaranthus powellii*) interference in sugarbeet (*Beta vulgaris*). Weed Sci 33:518–520

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus species* in Missouri. Weed Sci 51.3:329–333

Sonoskie LM, Webster TM, Culpepper AS (2013) Glyphosate resistance does not affect Palmer amaranth (*Amaranthus palmeri*) seedbank longevity. Weed Sci 61:283–288

Sprague CL (2011) Glyphosate-resistant Palmer amaranth confirmed in Michigan. http://msuweeds.com/newsletter-holder/emerging-weed-issues/glyphosate-resistant-palmer-amaranth-confirmed-in-southwest-michigan/. Accessed July 11, 2016

Steckel LE, Sprague CL, Hager AG, Simmons FW, Bollero GA (2003) Effects of shading on common waterhemp (*Amaranthus rudis*) growth and development. Weed Sci 51:898–903

Steinmaus SJ, Prather TS, Holt JS (2000) Estimation of base temperatures for nine weed species. Journal of Experimental Botany 51.343:275–286

Swanton CJ, Shrestha A, Knezevic SZ, Roy R, Ball-Coelho BR (1999) Influence of tillage on vertical seedbank distribution in a sandy soil. Can J Plant Sci 80:455–457

Sweeney AE, Renner KA, Laboski C, Davis A (2008) Effect of fertilizer nitrogen on weed emergence and growth. Weed Sci 56:714–721

Uscanga-Morta E, Clay SA, Forcella F, Gunsolus J (2007) Common waterhemp growth and fecundity as influenced by emergence date and competing crop. Agron J 99:1265–1270

Wang T, Zhou D, Wang P, Zhang H (2006) Size-dependent reproductive effort in *Amaranthus retroflexus:* the influence of planting density and sowing date. Can J Bot 84:485–492

Webster TM, Grey TL (2015) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) morphology, growth, and seed production in Georgia. Weed Sci 63:264–272