Physiological Basis of Herbicide Interaction and Integrated Management of Palmer amaranth (*Amaranthus palmeri*)

by

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M.S., The University of Tennessee Knoxville, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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Abstract

Palmer amaranth is a major threat to many cropping systems in the USA. As a result of selection, Palmer amaranth has evolved resistance to at least six herbicide modes of action including microtubule-, 5-enolpyruvylshikimate-3-phosphate synthase-, acetolactate synthase-, photosystem II-, hydroxyphenylpyruvate dioxygenase-, and protoporphyrinogen oxidase-inhibitors. Dicamba is effective for Palmer amaranth control; however, extensive use of this herbicide increases the likelihood of evolution of resistance to dicamba. The overall objective of this dissertation was to investigate the physiological basis of interaction of herbicides with different modes of action in Palmer amaranth control and evaluate use of integrated approaches to manage Palmer amaranth in field conditions. The specific objectives were to: 1) evaluate the effect of plant height on dicamba efficacy to control Palmer amaranth; 2) investigate the mechanism of resistance to glyphosate in a Palmer amaranth accession from Kansas, and evaluate efficacy of glyphosate and dicamba tank-mix to control this accession; 3) investigate the physiological basis of glyphosate and dicamba interaction in tank-mix to control Palmer amaranth; 4) determine the efficacy of reduced dicamba use on Palmer amaranth control in irrigated corn production; and 5) investigate grain sorghum and Palmer amaranth growth and reproductive attributes in response to sorghum density and nitrogen rate under irrigated conditions. All experiments were repeated and appropriate statistical tests were used for data analyses. The results indicate: a) increased absorption and translocation of dicamba contribute to increased efficacy to control Palmer amaranth at early growth stage; b) tank mixing glyphosate and dicamba had a synergistic effect on Palmer amaranth control; c) rapid absorption of dicamba and increased translocation of glyphosate resulted in increased Palmer amaranth control when applied in combination; d) there is an opportunity to maintain grain yield while effectively controlling Palmer amaranth in irrigated corn with the integration of increased corn plant population density and reduced dicamba application and e) integrating sorghum plant population and nitrogen did not suppress Palmer amaranth in irrigated sorghum, although sorghum grain yield was maintained. The outcome of this dissertation provides several strategies to improve control of Palmer amaranth.

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Dedication

This dissertation is dedicated to my Dad who could not see this completed, and to my family and those who came before me, “for I am because you are.”

Chapter 1 - Review of Literature

Agricultural production is challenged by abiotic and biotic stresses. Abiotic stresses are naturally occurring and unavoidable, and include factors such as temperature, drought, flooding, wind and intense sunlight. Biotic stress on the other hand, can be avoided and is usually caused by pests including insects, pathogens, and weeds. For centuries, humans have acknowledged the importance of these challenges and developed ways to address them. Some developments include breeding crop varieties for abiotic and biotic stress tolerance. Another important development was the discovery of synthetic pesticides. These tools are complementary to each other in addressing both abiotic and biotic stresses. Biotic stresses can be strongly influenced by abiotic stress factors such as drought, temperature or soil fertility. Continuous imposition of abiotic stresses on crops can result in significant yield losses and favor incidence of diseases, insects and weeds as they seem to thrive in conditions where crops would not. According to Muniappan and Heinrichs (2016) and Oerke (2006), these biotic stress factors can also result in significant yield loss in crops. This is especially true of weeds that are estimated to cause greater economic losses than other pests (Oerke, 2006). In total, there are over 30,000 weed species worldwide of which approximately 18,000 are known to cause serious economic losses in agricultural production that are estimated in the order of 10 percent per year globally (Cruz-Garcia and Price, 2012; Chandrasekaran et al., 2013; Vanangamudi et al; 2013; Chakraborty, 2013). In the U.S. alone, weeds are present in over 485 million acres of cropland and almost one billion acres of range and pasture resulting in costs that could approach US$15 to US$20 billion dollars (Inderjit, 2009). Of all weeds that occur in the U.S., Palmer amaranth (*Amaranthus palmeri* L.) is the most troublesome (Anon, 2016).

Palmer amaranth

Palmer amaranth is a troublesome weed throughout the U.S. (USDA-NRCS, 2016). In Kansas, Palmer amaranth is present in approximately 46% of the state's counties (USDA-NRCS, 2016) and has been reported to interfere with production of several crops (Smith et al., 2000; Massinga et al., 2001; Moore et al., 2004). Unlike other weed species, Palmer amaranth can survive harsh conditions and has a deep, extensive root system that allows it to use resources, particularly water and nutrients, more efficiently than most crops (Sosnoskie et al., 2011). The highly competitive nature of Palmer amaranth is further enabled by its prolific seed production, an extended germination and emergence pattern, and a high growth rate and photosynthetic activity (Horak and Loughin, 2000; Ward et al., 2013). Its growth rate is higher than that of other *Amaranthus* species and its photosynthetic rate is three- to four-fold that of corn, cotton and soybean (Horak and Loughin, 2000; Steckel, 2007). Palmer amaranth interference with other crops is dependent on its density and time of emergence (Massinga et al., 2001; Chahal, 2015). For example, when allowed to emerge with corn, 0.5 to 8 Palmer amaranth plants per meter of row were reported to cause 11 to 91% reduction in grain yield. Whereas, when Palmer amaranth plants, at the same density, emerged at later stages of corn growth, four- to seven-leaf, grain yield decreased only 7 to 35% (Massinga et al., 2001). In grain sorghum, 1.58 Palmer amaranth plants per square meter were reported to cause 38 to 63% reduction in yield (Chahal et al., 2015). Similarly, significant yield losses were reported in several other crops due to Palmer amaranth interference (Putman, 2013; Chahal, 2015).

Despite the significance of the impact that weeds have on crops regardless of the length of their interference, current approaches for weed control rely heavily on herbicide-based curative measures. Herbicides are regarded as the foundation of modern agriculture and remain the primary, most effective and widely used approach to control weeds since the mid 1940's when the first modern herbicide, 2,4-D, became available for use in agriculture (Peterson et al., 2016). Weed control options continue to decline due to the evolution of herbicide resistance in weed species, including Palmer amaranth (Nakka, 2016); however, effective herbicide modes of action that confer acceptable weed control are still available. The problem of resistance is particularly acute in Palmer amaranth because many Palmer amaranth populations are resistant to one or several herbicide modes of action (MOA) including microtubule-, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, acetolactate synthase (ALS)-, photosystem II (PS II)-, hydroxyphenylpyruvate dioxygenase (HPPD)- and protoporphyrinogen oxidase (PPO)-inhibitors (Legleiter and Johnson, 2013; Gosset et al., 1992; Brooks, 2013; Heap, 2019).

Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) belongs to synthetic auxins group and is effective for selective control of broadleaf weeds including Palmer amaranth. Like 2,4-D, at low concentrations, dicamba mimics the plant hormone indole-3-acetic acid which is also referred to as auxin or IAA. At high concentrations, however, dicamba is herbicidal and works by stimulating rapid, abnormal, cell elongation and differentiation. This abnormal growth causes blockage of the phloem vascular tissue and destruction of the cambial, phloem cells near meristem. As a result, the cellular transport systems in the plant is disrupted leading to starvation and eventual plant death (Cox, 1994; Ou et al. 2018). Following application, auxinic herbicides are transported into the plant/plant cells by active transporters and then bind to the Transport Inhibitor Response 1 (TIR1)/Auxin-signaling F-Box (AFB) protein components of the Skip, Cullen, Box (SCF)-complex and come together with the Auxin Response Factor (ARF)-repressor also referred to as Aux/IAA transcription factors and cause ubiquitination thus degrading the Aux/IAA-repressor protein and promoting ARF activation followed by transcription of genes related to auxin response. Auxin herbicides are not substrates of Growth Hormone 3 (GH3) protein and, therefore, remain active. Increase in auxin herbicide concentration lead to a cascade of events that ultimately result in eventual plant death (Mithila et al, 2011; Christoffoleti et al., 2015).

Dicamba was introduced in the 1960s and is widely used in a wide variety of crops including corn and grain sorghum pre- and post-emergence. Currently, no case of Palmer amaranth resistance to this herbicide has been reported (Heap, 2019); however, timely applications are strongly recommended. If used improperly, Palmer amaranth can evolve resistance to this herbicide. Recently, researchers from the University of Arkansas successfully selected Palmer Amaranth tolerant to the label-recommended dose of dicamba in a controlled greenhouse study after three generations of exposure to sub lethal doses of the herbicide (Hightower, 2016). This work highlights Palmer amaranth's potential to evolve resistance to this particular herbicide and the need to integrate other control measures.

Herbicide Absorption and Translocation

For any herbicide to be effective, it must be absorbed and translocated to the target site in adequate quantities. Herbicide absorption is influenced by a number of factors including temperature, spray solution properties and plant surface characteristics (Moore, 2005; Ou, 2018). Once inside the plant, herbicides move to their site of action and disrupt several biophysical and biochemical processes; hence, the amount of herbicide translocated and its performance are dependent on the amount of herbicide absorbed. Previous studies investigated absorption and translocation of commonly used herbicides in tank mixes or applied alone in an attempt to further our understanding of the physiological basis of herbicide interaction and fate of herbicide inside the plant in many problem weeds. Ou et al. (2018) observed an antagonistic interaction when glyphosate and dicamba were applied in combination on kochia (*Kochia scoparia*) with faster absorption but reduced translocation compared with solo applications of either herbicide. Nakka et al. (2017) found no difference in absorption and translocation of radiolabeled mesotrione between resistant and susceptible biotypes to mesotrione in Palmer amaranth. Scott (1999) evaluated synergistic herbicide combinations for Virginia buttonweed (*Diodia virginiana*) biomass reduction. Results from his study indicated that combining clopyralid and triclopyr increased translocation of each herbicide at least 26.6%. Lym (1992) investigated how absorption and translocation of fluroxypyr with or without picloram and 2,4-D in leafy spurge is affected by three contrasting plant growth stages. They showed that more fluroxypyr was absorbed in vegetative than flowering or post-flowering plants, but translocation was not affected by plant growth stage. Further, when tank-mixed with either picloram or 2,4-D, fluroxypyr was absorbed and translocated less compared with when applied alone but picloram and 2,4-D are unaffected. Clearly these studies show that absorption and translocation of a particular herbicide is often dependent on tank-mix partner(s), plant growth stage and species. Environmental factors also influence the amount of herbicide absorbed and translocated by plants (Sterling and Lownds, 1992). Therefore, it is important to consider these factors when designing a weed management program.

Integrated Weed (Palmer amaranth) Management

Herbicides are an effective weed management tool. However, with the evolution of herbicide resistance, chemical weed control options are becoming increasingly limited. This is especially true in the case of Palmer amaranth, a weed that has evolved resistance to at least six herbicide modes of action (Heap, 2019). This situation poses significant challenges for the development of effective Palmer amaranth control strategies. Clearly adoption of alternative weed control approaches that reduce selection pressure (Norsworthy, 2012) and maintain acceptable weed control and crop yields are needed (Massinga, 2000). One strategy is the integration of multiple herbicides with effective modes of action in combination with other cultural and or mechanical weed control methods. Incorporation of such practices into existing cropping systems offers an opportunity to reduce the frequency and amount of herbicide applied (Massinga, 2000; Currie and Klocke, 2005) provided that a good crop stand and or canopy establishment is achieved early in the season as this increases crop competitiveness against weeds (Harker et al., 2012). On the other hand, reducing the frequency and amount of herbicide applied would potentially result in environmental and economic benefits while minimizing herbicide selection pressure and delaying resistance (Massinga, 2000; Norsworthy, 2012; Harker et al., 2012).

Previous studies investigated the benefit of increasing crop competitiveness through narrowing row spacing or increasing crop plant population density alone or in conjunction with herbicide(s) (Johnson and Hoverstad, 2002; Fanadzo et al., 2010; Mickelson and Renner, 1997). Fanadzo et al. (2010), reported adequate weed control from a smallholder irrigated maize production system in South Africa when reduced doses of atrazine and narrow rows were combined. Similarly, Sikkema et al. (2008) reported significant yield benefits and acceptable weed control in corn when row spacing was narrowed, crop density increased and herbicide (atrazine+dicamba POST-tank mixed together) dose reduced in a study conducted in Ontario. Conversely, while studying the effect of row spacing and herbicide application timing on weed control and grain yield in corn, Johnson and Hoverstad (2002) found no reduction in weed density and growth due to narrowing row spacing despitesignificant corn yield benefits. Substantial yield benefits and weed control particularly of *Amaranthus* species due to narrowing row spacing in combination with different herbicide programs have also been reported in soybean. For instance, when examining weed control using reduced doses of post-emergence herbicides in soybean, Mickelson and Renner (1997) found greater weed control, yield, and profit when row spacing was narrowed. Similarly, other studies have reported greater soybean yield and greater control of a multiple herbicide resistant waterhemp (*Amaranthus tuberculatus*) when narrowing row spacing, increasing crop plant population density and herbicide were integrated (Schultz et al., 2015).

Other crops that have been evaluated for weed suppression with the integration of chemical and nonchemical weed control tactics include rice (*Oryza sativa*), lima beans (*Phaseolus lunatus*), cotton (*Gossypium hirsutum*) and grain sorghum (*Sorghum bicolor*); however, there were cases in which the findings did not always agree. For example, application of 0, 25, 50 or 75 % less herbicide than the recommended 2 L ha^{-1} of pretilachlor [2-chloro-2',6' diethyl-N-(2-propoxyethyl)-acetanilide] resulted in an adequate weed control without a yield penalty as crop plant population density in rice was increased from 16 to 33 per m row (Aminpanah, 2014). On the other hand, other reports indicated no yield or weed control benefit to narrowing row spacing in lima bean in Delaware and Maryland (Sankula et al., 2001). Similarly, Balkcom et al (2010) found no yield or weed control benefit to narrowing row spacing in a study comparing cotton production across conventional and herbicide tolerant varieties (glyphosate and glufosinate) in contrasting tillage systems in Alabama. In a different study conducted in a nearby location, Aulakh et al. (2011) observed an increase in weed (Palmer amaranth) control due to narrowing row spacing regardless of herbicide program used, but narrowing row spacing alone did not increase yield. Grichar et al. (2004) showed that narrowing row spacing did not result in an increase in sorghum grain yield regardless of herbicide program used but helped suppress weed growth. More recently, Thakur et al. (2016) indicated that weed biomass and density were reduced, and grain sorghum yield increased when pendimethalin or atrazine was applied pre-emergence followed by one hand-weeding 30 days after sowing (DAS) and two hand-weeding 30 and 45 DAS, respectively, under rain-fed conditions. Tollenaar et al. (1994) showed that maize competitiveness against weeds can be enhanced by increasing crop plant population density with an increase in crop planting density from 4 to 10 plants per m row reducing weed biomass by half. Marin and Weiner (2014) showed that crop plant population density, variety and sowing pattern all affected weed biomass and crop yield with highest crop plant population density (10.5 seeds per m row) resulting in greater weed biomass reduction and higher grain yield compared with lower densities (7 and 5 seeds per m row) especially when maize was planted in a grid pattern. Altogether, the findings from these studies highlight the importance of integrating both chemical and nonchemical weed control tactics for an effective weed management especially when dealing with resistant or difficult to control problem weeds. However, clearly no single approach is applicable in all environments. Therefore, it is important to investigate how selected approaches interact when combined.

Justification and Objectives of Research

Herbicides remain the primary and most effective weed management tool. However, their use is limited by the evolution of herbicide-resistant weeds. The problem of resistance is particularly acute in Palmer amaranth because many Palmer amaranth populations are resistant to one or several herbicide MOAs. In the U.S. alone, resistance to at least six herbicide MOA including microtubule-, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, acetolactate synthase (ALS)-, photosystem II (PS II)-, hydroxyphenylpyruvate dioxygenase (HPPD)- and protoporphyrinogen oxidase (PPO)-inhibitors has been reported (Legleiter and Johnson, 2013; Gosset et al., 1992; Brooks, 2013 Heap, 2019). Dicamba is effective for selective control of broadleaf weeds and no case of Palmer amaranth resistance to this herbicide has been reported to date (Heap, 2019). However, with improper use, the likelihood of Palmer amaranth evolving resistance to dicamba increases as more pressure is placed on the herbicide to control a broad spectrum of weeds. Recently, researchers at the University of Arkansas demonstrated the potential of Palmer amaranth evolving resistance to dicamba (Hightower, 2016). This situation is exacerbated by its prolific seed production, high resource use efficiency and competitive nature, season long germination and emergence pattern, and a high growth rate conferred by its C_4 photosynthetic pathway. This situation makes an interesting case to investigate the physiological basis of herbicide interaction and integrated management of Palmer amaranth. The specific objectives of this dissertation are highlighted below.

- Chapter 2: Evaluate the effect of plant height on dicamba efficacy to control Palmer amaranth,

- Chapter 3: Investigate the mechanism, and evaluate the benefit of including dicamba in glyphosate tank-mix to control a suspected glyphosate-resistant Palmer amaranth accession from Kansas,
- Chapter 4: Evaluate the type of interaction when glyphosate and dicamba are applied in combination and determine the physiological basis of the interaction,
- Chapter 5: Test the efficacy of reduced dicamba use on Palmer amaranth control in irrigated corn production, and
- Chapter 6: Investigate grain sorghum and Palmer amaranth growth and reproductive attribute response to crop plant population density and nitrogen rate in an irrigated environment.

References

Aminpanah H (2014) Effects of crop density and reduced rates of pretilachlor on weed control and grain yield in rice. Romanian Agricultural Research 31, 229-238

Anon (2016) WSSA Survey Ranks Palmer Amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in Canada. PRWeb Newswire, pp. PRWeb Newswire, April 5, 2016

Aulakh J, Price A, Balkcom K (2011) Weed Management and Cotton Yield under Two Row Spacings in Conventional and Conservation Tillage Systems Utilizing Conventional, Glufosinate-, and Glyphosate-based Weed Management Systems. Weed Technology 25(4): 542-47

Balkcom KS, Price AJ, Van Santen E, Delaney DP, Boykin DL, Arriaga FJ, Bergtold K, Raper RL (2010) Row spacing, tillage system, and herbicide technology affects cotton plant growth and yield. Field crops research, 117(2-3), 219-225

Brooks R (2013) Palmer Amaranth Pigweed Creeps Farther into the Midwest. Farm Journal; accessed 8/30/2016; available at: http://www.agweb.com/article/palmer_amaranth_pigweed_creeps_farther_into_the_midwest_naa_rhonda_brooks/

Cahoon CW, York AC, Jordan DL, Seagroves RW (2015) Cotton response and Palmer amaranth control with mixtures of glufosinate and residual herbicides. J Cotton Sci, 19, 622-630

Chahal PS, Aulakh JS, Jugulam M, Jhala AJ (2015) Herbicide-Resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the United States — Mechanisms of Resistance, Impact, and Management, Herbicides, Agronomic Crops and Weed Biology, Andrew Price, Jessica Kelton and Lina Sarunaite, IntechOpen, DOI: 10.5772/61512. Available from:

http://www.intechopen.com/books/herbicides-agronomic-crops-and-weed-biology/herbicide-resistant-palmer-amaranth-amaranthus-palmeri-s-wats-in-the-united-states-mechanisms-of-res

Chakraborty S (2013) Plant Molecular Genetics. Scientific Publishers. p 151

Chandrasekaran B, Annadurai K, Kavimani R (2013) Rice Science. Scientific Publishers. p 216

Christoffoleti PJ, Figueiredo MR, Peres LE, Nissen S, Gaines T (2015) Auxinic herbicides, mechanisms of action, and weed resistance: A look into recent plant science advances. Scientia Agricola, 72(4):356-62

Cox C (1994) Dicamba: Factsheet. Journal of Pesticide Reform 14(1):30-35

Cruz-Garcia GS and Price LL (2012) Weeds as important vegetables for farmers. Acta Societatis Botanicorum Poloniae, 81(4).

Currie RS and Klocke N (2005) Impact of a terminated wheat cover crop in irrigated corn on atrazine rates and water use efficiency. Weed Science 53(5): 709-716

Doherty RC, Smith KL, Bullington JA, Meier JR (2009) Palmer Amaranth Control with Dicamba and Glufosinate as Influenced by Weed Size and Herbicide Rate. Summaries of Arkansas Cotton Research 2009. AAES Research Series 582; accessed 10/10/2016, available at: http://arkansasagnews.uark.edu/582-16.pdf

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert Summer Annual. Oecologia 57, 107-12

Fanadzo M, Chiduza C, Mnkeni P (2010) Reduced Dosages of Atrazine and Narrow Rows Can Provide Adequate Weed Control in Smallholder Irrigated Maize (zea Mays L.) Production in South Africa. African Journal of Biotechnology 9(45): 7660-666

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

Goswami S, Gamon J, Vargas S, Tweedie C (2015) Relationships of NDVI, biomass, and leaf area index (LAI) for six key plan species in Barrow, Alaska. Peerj PrePrint, 1–17

Grichar WJ, Besler BA, Brewer KD. (2004) Effect of Row Spacing and Herbicide Dose on Weed Control and Grain Sorghum Yield. Crop Protection 23(3): 263-67

Harker K, O'Donovan J, Blackshaw R, Beckie H, Mallory-Smith C, Maxwell B (2012) Our View. Weed Science 60(2): 143-44

Heap I (2019) The international survey of herbicide resistant weeds. www.weedscience.org

Hightower M (2016) Dicamba resistance in pigweed selected in research greenhouse, not in the field. U of A, Cooperative Extension Service. Accessed 12/12/16, available at: https://www.uaex.edu/media-resources/news/january2016/01-26-2016-Ark-Pigweed-Dicamba.aspx

Inderjit (2009) Management of Invasive Weeds. Dordrecht; London: Springer. Invading Nature; v. 5. Web

Johnson GA, Hoverstad TR (2002) Effect of Row Spacing and Herbicide Application Timing on Weed Control and Grain Yield in Corn (Zea Mays). Weed Technology 16(3): 548-53

Kaushik UK, Shaktawat MS (2005) Effect of row spacing, nitrogen and weed control on growth, yield and nutrient uptake of sorghum (*Sorghum bicolor*). Indian Journal of Agronomy, 50(2): 140-142

Legleiter T, Johnson B (2013) Palmer amaranth biology, identification, and management. Purdue Extension; accessed 08/29/2016, available at: https://www.extension.purdue.edu/extmedia/WS/WS-51-W.pdf

Lym RG (1992) Fluroxypyr absorption and translocation in leafy spurge (Euphorbia esula). Weed Science 40:101-105

Marin C, Weiner J (2014) Effects of density and sowing pattern on weed suppression and grain yield in three varieties of maize under high weed pressure. Weed Research 54, 467-474

Massinga R (2000) Palmer amaranth (*Amaranthus palmeri* S. Wats.) interference in corn (Zea mays L.). Doctoral dissertation. Manhattan, KS: Kansas State University. 14 p

Massinga RA, Currie RS (2002) Impact of Palmer Amaranth (*Amaranthus Palmeri*) on Corn (Zea Mays) Grain Yield and Yield and Quality of Forage. Weed Technology 16(3): 532-36

Massinga RA, Currie RS, Horak MJ, Boyer Jr J (2001) Interference of Palmer amaranth in corn. Weed Sci. 49:202-208

Mickelson JA, Renner KA (1997) Weed control using reduced rates of postemergence herbicides in narrow and wide row soybean. J. Prod. Agric. 1, 431-437. Doi: 10.2134/jpa 1997.0431

Mickelson JA, Renner KA (1997) Weed Control Using Reduced Rates of Postemergence Herbicides in Narrow and Wide Row Soybean. Journal of Production Agriculture 10(3): 431-37

Minnesota Department of Agriculture (2016) Prohibited - Eradicate Noxious Weed Palmer Amaranth - *Amaranthus palmeri* S. Waston. Accessed 08/04/2016, available at: http://www.mda.state.mn.us/plants/pestmanagement/weedcontrol/noxiouslist/palmeramaranth.aspx

Mithila J, Hall JC, Johnson WG, Kelley KB, Riechers DE (2011) Evolution of resistance to auxinic herbicides: historical perspectives, mechanisms of resistance, and implications for broadleaf weed management in agronomic crops. Weed Science 59: 445-457

Moore JW, Murray DS, Westerman RB (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol. 18: 23-29

Moore JW, Murray DS, Westerman RB (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technology, 18(1): 23-29

Muniappan R, Heinrichs EA (2016) Integrated Pest Management of Tropical Vegetable Crops. Springer. Print

Nakka S (2016) Physiological, Biochemical and Molecular Characterization of Multiple Herbicide Resistance in Palmer Amaranth (*Amaranthus Palmeri*). Manhattan, Kan.: Kansas State U, 2016. Web

Nakka S, Godar AS, Wani PS, Thompson CR, Peterson DE, Roelofs J, Jugulam M (2017) Physiological and molecular characterization of hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor resistance in Palmer amaranth (*Amaranthus palmeri* S. Wats.). Frontiers in Plant Science 8:555 DOI: 10.3389/fpls.2017.00555

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. Weed Science 60. Sp1 (2012): 31-62

Oerke EC (2006) Crop losses to pests. Journal of Agricultural Science, 144: 31-43

Ou J (2018) The mechanism(s) and management of dicamba resistance in kochia (Kochia scoparia). Doctoral dissertation. Manhattan, KS: Kansas State University.

Ou J, Thompson CR, Sathlman PW, Bloedow N, Jugulam M (2018) Reduced Translocation of Glyphosate and Dicamba in Combination Contributes to Poor Control of Kochia scoparia: Evidence of Herbicide Antagonism. Scientific reports, 8(1), 5330

Peterson M, McMaster S, Riechers D, Skelton J, Stahlman P (2016) 2,4-D Past, Present, and Future: A Review. Weed Technology, 30(2), 303-345

Putman J (2013) Palmer amaranth control in established alfalfa and documentation of glyphosate-resistant *Amaranthus* species in Kansas. Master's thesis, Manhattan, Kansas State University

Sankula S, VanGessel M, Jr Kee WE, Beste C, Everts K (2001) Narrow Row Spacing Does Not Affect Lima Bean Yield or Management of Weeds and Other Pests. HortScience 36(5): 884-88

Schultz JL, Myers DB, Bradley KW (2015) Influence of Soybean Seeding Rate, Row Spacing, and Herbicide Programs on the Control of Resistant Waterhemp in Glufosinate-Resistant Soybean. Weed Technology 29(2): 169-76

Scott T (1999) Syngergistic Herbicide Combinations for Virginia Buttonweed (Diodia Virginiana L.) Biomass Reduction in Warm Season Turf. Doctoral dissertation. Mississippi: Mississippi State University

Smith D, Baker R, Steele G (2000) Palmer Amaranth (*Amaranthus palmeri*) Impacts on Yield, Harvesting, and Ginning in Dryland Cotton (*Gossypium hirsutum*). Weed Technology, 14(1): 122-126

Sosnoskie LM, Webster TM, Culpepper AS, Kichler J (2011) The biology and ecology of Palmer amaranth: Implications for control. UGA/CPES Research-Extension Circular 1000. Accessed 12/11/2016, available at: http://extension.uga.edu/publications/detail.cfm?number=C1000

Steckel L (2007) The dioecious Amaranthus spp.: Here to stay. Weed Technology 21:567-570

Sterling TM, Lownds NK (1992) Picloram absorption by broom snakeweed (Gutierrezia sarothrae) leaf tissue. Weed Science 40:390-394

Thakur NS, Kushwaha BB, Girothia OP, Sinha NK, Mishra JS (2016) Effect of integrated weed management on growth and yields of rainy-season sorghum (*Sorghum bicolor*). Indian Journal of Agronomy, 61(2): 217-222

Tollenaar M, Dibo AA, Aguilara A, Weise SF, Swanton CJ (1994) Effect of crop density on weed interference in maize. Agronomy Journal, 86(4), 591-595

United States Department of Agriculture, National Conservation Service (USDA-NRCS) (2016) Plants Database. Accessed 9/20/2016, available at plants.usda.gov/core/profile?symbol=AMPA

Vanangamudi K, Bhaskaran M, Balavidhya S, Arthanari (2013) Weed Seed Biology. Scientific Publishers

Ward SM, Webster TM, Steckel LE (2013) Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Technology 27(1):12-27. 2013

Whitson T, Western Society of Weed Science, Western United States Land Grant Universities Cooperative Extension Services (1996) Weeds of the west (5th ed.). Laramie?:

Newark, CA: University of Wyoming; Published in cooperation with the Western Society of Weed Science, Western United States Land Grant Universities Cooperative Extension Services.

Chapter 2 - Increased Absorption and Translocation Contribute to Improved Efficacy of Dicamba to Control Early Growth Stage Palmer amaranth

Abstract

Rapid growth of Palmer amaranth poses a challenge for timely management of this weed. Dose response studies were conducted in 2017 and 2018 under field and greenhouse conditions near Garden City and Manhattan, KS, respectively, to evaluate the efficacy of dicamba to control ≤10 cm tall (day 0)-, 15 cm tall (day 1)-, and 30 cm tall (day 4)-Palmer amaranth. Visual injury rating and reduction in shoot biomass (% of non-treated), and mortality were assessed at four weeks after treatment using a three- and four-parameter log-logistic model, in R software program. Increasing dicamba doses increased Palmer amaranth control regardless of plant height both in the field and greenhouse. Based on the estimates of effective dicamba dose required for 50% control of Palmer amaranth (ED_{50}), delaying application one (15 cm) or four days (30 cm) required a two- and 27-fold increase in the dose of the herbicide to achieve 50% Palmer amaranth control, respectively, under field conditions. However, in the greenhouse, for the same level of Palmer amaranth control, more than one- and two-fold increases in dicamba were required when there was a delay in application in one (15 cm) or four days (30 cm), respectively. Similarly, the effective dose of dicamba required for 50% reduction in Palmer amaranth shoot biomass (GR_{50}) increased more than four- and eight-fold or more than one- and two-fold when dicamba application was delayed by one (15 cm) and four days (30 cm), in the field or in the greenhouse, respectively. To understand the basis of increased efficacy of dicamba in controlling early growth stage of Palmer amaranth, dicamba absorption and translocation studies were conducted. Results indicate a significant reduction in dicamba absorption and translocation with increase in plant height. Results of this research provide evidence that increased absorption and translocation of dicamba results in increased efficacy in improving Palmer amaranth control at early growth stage. Therefore, timely management of Palmer amaranth is crucial and recommended to slow the selection pressure and delay the evolution of resistance to this effective herbicide option.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats.

Keywords: Dicamba absorption and translocation, Plant height, Palmer amaranth, dicamba efficacy

Introduction

Palmer amaranth is a summer, annual broadleaf weed that is native to the desert regions of the southwest United States and northern Mexico (Ward et al., 2013). If uncontrolled, Palmer amaranth is known to interfere with various cropping systems causing massive yield losses (Smith et al., 2000; Massinga et al., 2001; Massinga and Currie, 2002; Bensch et al., 2003; Moore et al., 2004; Burke et al., 2007) that account for millions of dollars annually (Meyer et al., 2015). Weed Science Society of America identified Palmer amaranth as the most troublesome weed in the US (Anon, 2016). Various attributes contribute to Palmer amaranth becoming such a problem weed but, the most important one is its fast growth rate because of C_4 photosynthetic pathway. Palmer amaranth can grow 5 – 7.5 cm per day (Sfiligoj, 2015; Legleiter and Johnson, 2013), and has a growth rate higher than that of other *Amaranthus* species (Horak and Loughin, 2000). Palmer amaranth photosynthetic rate is three to four times higher than that of cotton, soybean and corn (Steckel, 2007). Therefore, Palmer amaranth infestation can result in competition with many crops and other species (Berger et al., 2015; Massinga et al., 2001; Massinga et al., 2003; Morgan et al., 2001).

Palmer amaranth is a problem weed in most parts of Kansas, affecting the sustainability of agricultural production and threatening water availability for irrigation. Palmer amaranth is a common weed in the fallow phase of wheat-fallow-wheat and wheat-sorghum-fallow in western Kansas where the climatic conditions favor its fast growth. Because of its deep rooting system, this weed can effectively compete for water with other species. Therefore, if soil nutrients and moisture are to be conserved for use by the subsequent crop, it is critical that Palmer amaranth is controlled at early growth stages. If Palmer amaranth is to be controlled with foliar-applied herbicides, it is recommended that applications are made when plants are ≤10 cm tall.

Considering its fast growth rate, delaying herbicide application by only a day or two can allow this weed to quickly become too large to control easily. This situation is further exacerbated by the fact that Palmer amaranth has evolved resistance to at least six herbicide modes of action including microtubule-, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, acetolactate synthase (ALS)-, photosystem II (PS II)-, hydroxyphenylpyruvate dioxygenase (HPPD)- and more recently to protoporphyrinogen oxidase (PPO)-inhibitors (Heap, 2019). Dicamba is an effective herbicide to control Palmer amaranth; however, if the risk for resistance to develop is to be mitigated, understanding how plant height at time of herbicide application affects its performance is crucial. We hypothesized that efficacy of dicamba for Palmer amaranth control will decline as Palmer amaranth plant height increases. Therefore, the objective of this study was to evaluate the effect of plant height on dicamba efficacy to control Palmer amaranth.

Materials and Methods

Field Experiments

Two dose-response studies were conducted simultaneously under dryland conditions in adjacent fields at Kansas State University Southwest Research and Extension Center, Garden City, KS in 2018 to evaluate post emergence efficacy of dicamba in the absence of crop competition. Soil at the site was a Richfield silt loam (fine, montmorillonitic, mesic Aridic, Argiustoll) with a slope ≥1%. The site received a total of 55.9 mm of rainfall during August of 2018 and the 30-year average is 63.8 mm (Elliott, 2017). The average daily minimum and maximum air temperatures during August 2018 were 16.7 and 31.1 °C compared to the 30-year average of 24.6 °C (Elliott, 2017). Fields were disked and field cultivated within the last week of July 2018 to ensure a uniform seed distribution in the top 2.5 – 5 cm of soil, the primary zone of weed seed germination, and to stimulate emergence of naturally occurring Palmer amaranth at the site. Plots were established on August 05 2018 when approximately 50% Palmer amaranth emergence was observed. The studies used a randomized complete block experimental design with four replications and with treatments in a split plot arrangement. The main plot treatments consisted of three Palmer amaranth plant heights [≤10 cm tall (day 0), 15 cm tall (day 1), and 30 cm tall (day 4)], and the subplot treatments consisted of dicamba application at seven doses (0, 70, 140, 210, 280, 420, and 560 g ae ha^{-1}). Each subplot was 6 m by 3 m. Dicamba applications were made using a CO_2-pressurized backpack boom sprayer on August 10, 11, and 14 when the wind speed at the site was between 6 and 9 km h^{-1} and approximately 50% of the plants had reached the desired height.

Greenhouse Experiments

Palmer amaranth seed with no known resistance to any commonly used herbicide were collected from the same site as described above [Kansas State University Southwest Research-Extension Center (37º59’38.4” N 100º49’04.8” W), near Garden City, KS] in 2016 were used. Two dose response studies were conducted at Kansas State University, Manhattan, KS during summer 2017 and 2018 to further assess the influence of height on dicamba efficacy to control Palmer amaranth. Seeds of Palmer amaranth were germinated in small trays (25 cm × 15 cm × 2.5 cm) filled with a commercial potting mixture (Miracle-Gro® Moisture Control Potting Mix, CA). Seedlings 2-3 cm tall were transplanted into 6.5 cm × 6.5 cm × 9 cm plots and allowed to grow. The greenhouse was equipped with sodium vapor lamps supplementing 250 µmol m^{-2} s^{-1} of illumination and maintained at 25/20 ºC day/night temperatures and 15/9 h day/night photoperiods. Palmer amaranth seedlings at ≤10 cm tall (day 0), 15 cm tall (day 1), and 30 cm tall (day 4) were treated with varying dicamba doses (0, 70, 140, 210, 280, and 560 g ae ha^{-1}) . All dicamba treatments were applied with a bench-type track sprayer (Generation III, DeVries Manufacturing, RR 1 Box184 Hollandale, MN, USA) equipped with a single moving even flat-fan nozzle tip (8002E TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.85 km h^{-1}. Experiments were conducted in a randomized complete block design with four replications (1 plant per pot), and repeated in time. Mortality and biomass measurements were collected at four weeks after treatment (WAT).

Dicamba Absorption and Translocation Experiments

Two experiments were conducted at Kansas State University, Manhattan, KS in 2017 and 2018. Palmer amaranth seedlings were raised from the same seed that was used in above greenhouse experiment and grown under greenhouse conditions as described above. Three days prior to treatment with ^{14}C dicamba, the seedlings of uniform size were selected and transferred to a growth chamber equipped with fluorescent bulbs capable of delivering 550 µmol m^{-2} s^{-1} photon flux at plant canopy level to acclimate. The growth chamber conditions were maintained at 32.5/22.5 °C day/night, 15/9 h photoperiod, and 60-70% relative humidity. Plants were watered as required until the desired height for treatment was reached. Ten and 30-cm tall plants were treated with 10 one-µl droplets of dicamba (ring-UL-^{14}C)-ethanol solution (11.4 kBq µl^{-1}, specific activity: 2.87 kBq µg^{-1}, BASF Corp.) totaling 3.3 kBq applied on the adaxial surface of the fourth youngest leaf using a Wiretrol® capillary syringe (10 µL, Drummond Scientific Co., Broomall, PA, USA). Non-radiolabeled dicamba was added to the radioactive solution to obtain 560 g ae ha^{-1} of dicamba in a carrier volume of 187 L. All plants were returned to the growth chamber 30 min following treatment. Both 10 and 30 cm plants were harvested 24, 72 and 120 hours after treatment (HAT), and were dissected into three parts: treated leaf (TL), tissue above the treated leaf (ATL) and below the treated leaf (BTL). The TL was washed twice in 20-mL scintillation vials with a 5 mL wash solution (10% ethanol aqueous solution and 0.5% Tween-20) for 1 min at each time and the radioactivity in the rinsate(s) was measured using a liquid scintillation spectrometry (LSS; Beckman Coulter LS6500 Multipurpose Scintillation Counter, Beckman Coulter, Inc. Brea, CA, USA) after adding 15 mL of Ecolite-(R) (MP Biomedicals, LLC. Santa Ana, CA, USA). The dissected plant parts were oven dried at 60 °C for 72 h and then combusted in a biological oxidizer (OXU-501, RJ Harvey Instrument, New York, USA). Radioactivity was then quantified using LSS. Total absorption of [^{14}C] dicamba was determined as: % absorption = (total radioactivity applied – radioactivity recovered in wash solution) × 100/total radioactivity applied.

Herbicide translocation was determined as: % translocation = 100 - % radioactivity recovered in TL, where % radioactivity recovered in TL = radioactivity recovered in TL × 100/radioactivity absorbed. Each treatment included four replications.

Data Collection and Analysis

Visual injury ratings and mortality of Palmer amaranth on a 0 (no control or alive) to 100% (complete control or dead) scale were assessed at 4 WAT. Above-ground plant biomass was collected and oven dried (65 ^{o}C for 3 days) to determine the dry biomass. All data were subjected to analysis of variance to determine the significance of the interaction of Palmer amaranth plant height and dicamba dose on response parameters. If the interaction was not significant at the 5% level, data were pooled one factor to test significance of the other. Specifically, visual injury rantings were subject to nonparametric “kruskal.test” and “TukeyHSD post-hoc.test” in R software for analysis of variance and mean separation, respectively. In addition, visual injury ratings and above-ground plant biomass (% of non-treated; Wortman, 2014), and mortality were regressed over dicamba doses using a three- and four-parameter log-logistic model, respectively, in R software (Ritz et al., 2015; Seefeldt et al., 1995). The lack-of-fit test ($P>0.05$) indicated that the chosen model accurately described the data. Regression analyses were further used to estimate effective dicamba doses required to achieve 50% control (ED_{50}, for visual injury rating; or LD_{50}, for mortality) and shoot biomass reduction (GR_{50}) of Palmer amaranth, and standard errors.

Above-ground plant biomass and ^{14}C dicamba absorption and translocation data were subjected to analysis of variance using the PROC MIXED procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC) to test the significance of the fixed effects (i.e. plant height and dicamba dose) and their interaction. The data of the experiments were also subjected to analysis of variance, and means were separated using Fisher’s protected LSD at α = 0.05 in SAS (SAS, 2011).

Results and Discussion

Field Dose Response

No experiment by treatment interaction was detected; therefore, data were combined across experiments. The data from two-year field experiments suggest increased control of Palmer amaranth with an increase in dicamba dose regardless of plant height at time of spraying. Increase in plant height from ≤10 to 15 and 30 cm tall, however, reduced Palmer amaranth control from 25 to 0, and 99 to 42 and 30%, respectively, with an increase in dicamba dose from 70 to 560 g ae ha^{-1} (Table 2-1). This reduction in control suggests that dicamba efficacy to control Palmer amaranth largely depend on plant height at time of spraying. For instance, when the label-recommended dose of dicamba (560 g ae ha^{-1}) was applied, 99% of ≤10 cm tall Palmer amaranth control was achieved compared to only 42 and 30% of 15 and 30 cm tall Palmer amaranth, respectively, which is less than the level of control (47%) achieved when only one fourth of the label recommended dose of dicamba was sprayed on ≤10 cm tall Palmer amaranth (Table 2-1). Previous studies showed reduced control with increasing plant height at the time of herbicide application in other species including common waterhemp, large crabgrass, giant ragweed, kochia and ivy morning glory (Craigmyle et al., 2013; Cordes et al., 2004; Chahal et al., 2015; Hoss et al., 2003). Furthermore, the effective doses of dicamba required to achieve 50% control (ED_{50}) and shoot biomass reduction (GR_{50}) were greater with an increase in Palmer amaranth plant height at time of dicamba application (Table 2-2). This suggests that more dicamba is required for a desirable control with later Palmer amaranth growth stages. The ED_{50} values increased two- and fourteen-fold, when dicamba was applied on 15 cm and 30 cm Palmer amaranth, respectively, compared to that of ≤10 cm tall plants (Table 2-2). Similarly, the GR_{50} values increased almost five- and seven-fold, when dicamba was applied on 15 cm and 30-cm, Palmer amaranth, respectively, compared to that of ≤10 cm Palmer amaranth (Figure 1; Table 2-2). Chahal et al. (2015) reported an increase in both ED_{50} and GR_{50} values as Palmer amaranth height at time of herbicide application increased from 10 to 20 cm.

Greenhouse Dose Response

No experiment by treatment interaction was detected; therefore, data were combined across experiments. Similar to field observations, Palmer amaranth control was declined on more fully developed plants at time of dicamba application. Palmer amaranth mortality ranged from 0 to 100% for 10-cm tall Palmer amaranth compared to 0 to 87.5% for 15- and 30-cm tall plants, when dicamba dose was increased from 0 to 560 g ae ha^{-1} (Table 2-3). Based on LD_{50} and GR_{50} values determined using fitted log-logistic model, the effective dose of dicamba increased more than one- and two-fold to control 15- and 30-cm Palmer amaranth, respectively, compared with 10-cm Palmer amaranth (Table 2-4). The level of control of > 10 cm tall Palmer amaranth was higher in the greenhouse (mortality; Table 2-3) compared to the field (visual rating; Table 2-1). Additionally, with the exception of 10-cm Palmer amaranth, GR_{50} values of 15- and 30-cm Palmer amaranth obtained from field experiments were relatively greater than those derived from greenhouse experiments (Table 2-2 and 2-4). While our greenhouse data suggest that there is an opportunity to control 15-cm Palmer amaranth with the label recommended dose of dicamba (Figure 2-2), the data from the field studies indicate that this opportunity is very limited and that 15-cm Palmer amaranth is just as difficult to control as 30-cm tall plants (Figure 2-1). These results, therefore, highlight the need to be aware of the effects of the environment of greenhouse studies on cuticle thickness and its effect on comparisons of weed control in field and greenhouse studies underscoring the importance of considering environmental impacts when studying herbicide efficacy. Plant cuticle has been shown to interfere with foliar uptake of pesticides (Kirkwood, 1999). There is a marked difference in cuticle development between field- and greenhouse-grown plants (Hull, 1958) which may explain the discrepancy in response to dicamba between field- and greenhouse-grown Palmer amaranth.

^{14}C Dicamba Absorption and Translocation Experiments

No experiment by treatment interaction was detected; therefore, data were combined across experiments. Differences in ^{14}C dicamba absorption were observed in Palmer amaranth plants at different heights and harvest times (i.e. HAT; Figure 2-3A), $p<0.05$. Palmer amaranth plants treated at 10-cm tall absorbed more ^{14}C dicamba than 30-cm tall plants. This reduction in the amount of ^{14}C dicamba absorbed with increase in plant height may be due to changes in cuticle composition. Older plants or leaves have a relatively more complex and thicker cuticle structure (Kirkwood, 1999). Thicker cuticles are less permeable to foliar-applied pesticides thus reducing the effectiveness of post emergence herbicides (Menendez et al., 2014) by limiting the amount of active ingredient entering the cytoplasm of plant cells. Similarly, at 24 HAT, up to 75 and 60% of ^{14}C dicamba was absorbed in 10- compared to 30-cm Palmer amaranth, respectively. However, maximum ^{14}C dicamba absorption occurred at 120 HAT in Palmer amaranth at both plant heights. Because a plant leaf is not a homogeneous substrate (Menendez et al., 2014), the amount of ^{14}C dicamba absorbed did not always increase with time in 30-cm Palmer amaranth unlike in 10-cm tall plants.

Although more than 50% of ^{14}C dicamba absorbed was translocated out of the treated leaf (TL) over the course of the three sampling times (i.e. 24, 72 and 120 HAT) in both 10- and 30-cm Palmer amaranth, higher and more rapid translocation of ^{14}C dicamba was recorded in 10-cm compared to 30-cm Palmer amaranth (Figure 2-3B). As a result, more ^{14}C dicamba reached above treated leaf (ATL; Figure 2-4A) and below treated leaf (BTL; Figure 2-4C) in 10-cm compared to 30-cm Palmer amaranth. The acropetal translocation exceeded basipetal translocation of ^{14}C dicamba regardless of plant height. Dicamba is a systemic herbicide; however, it is translocated mostly via phloem (Chang and Vanden Born, 1968; Cox, 1994; Ou et al., 2018). Therefore, its movement in the plant is highly dependent on the source-to-sink transport of sugar also referred to as source-sink strength (Lemoine et al., 2013). Hence, to be effective, dicamba mustbe distributed throughout the plant and more importantly the actively growing shoot tissue. This is the reason an increase in translocation to ATL will improve control of sensitive plants with dicamba.

Conclusions

This study demonstrates that a) dicamba efficacy to control Palmer amaranth is greatly influenced by plant height at time of herbicide application and b) an increased absorption and translocation of dicamba at early growth stages contributes to increased efficacy of dicamba to control Palmer amaranth. The results from this study also suggest that opportunities to delay dicamba application for an effective control of Palmer amaranth are very limited. Therefore, timely management of this weed is crucial. Moreover, while this research has demonstrated that dicamba can effectively control Palmer amaranth at its earlier stages of growth, it is important to also consider including other effective herbicide modes of action to broaden the spectrum of weed control and delay evolution of resistance to the few herbicides still effective for Palmer amaranth management.

References

Anon (2016) WSSA Survey Ranks Palmer Amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in Canada. PRWeb Newswire, pp. PRWeb Newswire, April 5, 2016

Bensch CNN, Peterson DJ, Horak MJ (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 1:37–43

Berger S, Ferrell J, Rowland D, Webster T (2015) Palmer Amaranth (*Amaranthus palmeri*) Competition for Water in Cotton. Weed Sci 4:928-935

Burke IC, Schroeder M, Thomas WE, Wilcut JW (2007) Palmer amaranth interference and seed production in peanut. Weed Technology 21:367– 371

Chahal PS, Aulakh JS, Rosenbaum K, Jhala AJ (2015) Growth stage affects dose response of selected glyphosate-resistant weeds to premix of 2, 4-D choline and glyphosate (Enlist Duo™ Herbicide). Journal of Agricultural Science 11:1-10

Chang FY, Vanden Born WH (1968) Translocation of dicamba in Canada thistle. Weed Sci. 1968;16:176–181.

Cordes JC, Johnson WG, Scharf P, Smeda RJ (2004) Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed technol 4:999-1005

Cox C (1994) Dicamba: Factsheet. Journal of Pesticide Reform 14(1):30-35

Craigmyle B, Ellis J, Bradley K (2013) Influence of Weed Height and Glufosinate plus 2,4-D Combinations on Weed Control in Soybean with Resistance to 2,4-D. Weed Technology, 27: 271-280

Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of weed height and glufosinate plus 2, 4-D combinations on weed control in soybean with resistance to 2, 4-D. Weed technol 2:271-80

Elliott J (2017) "Weather Information for Garden City, 2016," Kansas Agricultural Experiment Station Research Reports: Vol. 3: Iss. 5.

Horak MJ, Loughlin TM (2000) Growth analysis of four *Amaranthus* species. Weed Sci. 48:347-3

Hoss NE, Al-Khatib K, Peterson DE, Loughin TM (2003) Efficacy of glyphosate, glufosinate, and imazethapyr on selected weed species. Weed Sci. 51:110–117

Hull HM (1958) The effect of day and night temperature on growth, foliar wax content, and cuticle development of velvet mesquite. Weeds, 6(2):133-142

Kirkwood R (1999) Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. Pesticide Science, 55: 69-77

Legleiter TR, Johnson B (2013) Palmer amaranth biology, identification, and management. West Lafayette, IN: Purdue Extension

Lemoine R, La Camera S, Atanassova R, Dédaldéchamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Coutos-Thévenot P, Maurousset L, Faucher M (2013) Source-to-sink transport of sugar and regulation by environmental factors. Frontiers in plant science, 4:272

Massinga RA, Currie RS (2002) Impact of Palmer Amaranth (*Amaranthus Palmeri*) on Corn (Zea Mays) Grain Yield and Yield and Quality of Forage 1. Weed Technol 3:532-536

Massinga RA, Currie RS, Horak MJ, Boyer J Jr (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202-208

Massinga RA, Currie RS, Trooien TP (2003) Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci. 4:523-31

Menendez J, Rojano-Delgado M, De Prado R (2014) Differences in herbicide uptake, translocation, and distribution as sources of herbicide resistance in weeds. ACS Symposium Series, 1171: 141-157

Meyer CJ, Norsworthy JK, Young BG, Steckel LE, Bradley KW, Johnson WG, Loux MM, Davis VM, Kruger GR, Bararpour MT, Ikley JT (2015) Herbicide program approaches for managing glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus* and *Amaranthus rudis*) in future soybean-trait technologies. Weed Technol 4:716-29

Moore JW, Murray DS, Westerman RB (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (Sorghum bicolor). Weed Technol 1: 23-29

Morgan GD, Baumann PA, Chandler JM (2001) Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. Weed Technol. 3:408-12

Ou J, Thompson CR, Stahlman PW, Bloedow N, Jugulam M (2018) Reduced Translocation of Glyphosate and Dicamba in Combination Contributes to Poor Control of Kochia scoparia: Evidence of Herbicide Antagonism. Scientific reports, 8(1):5330

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. PLoS ONE 10(12): e0146021. https://doi.org/10.1371/journal.pone.0146021

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed technology, 9(2):218-27

Sfiligoj E (2015) Weeds to watch 2015: Resistance remains riling. Croplife 4:10-12

Smith D, Baker R, Steele G (2000) Palmer Amaranth (*Amaranthus palmeri*) Impacts on Yield, Harvesting, and Ginning in Dryland Cotton (*Gossypium hirsutum*) 1. Weed Technol 1:122-126

Steckel L (2007) The dioecious Amaranthus spp.: Here to stay. Weed Technology 21: 567-570

Ward S, Webster T, Steckel L (2013) Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Technol 1: 12-27

Wortman S (2014) Integrating Weed and Vegetable Crop Management with Multifunctional Air-Propelled Abrasive Grits. Weed Technol. 28: 243-252

Table 2-1. Visual estimate of 10-, 15- and 30-cm tall Palmer amaranth control at 4 weeks after treatment with dicamba in a field study conducted at Kansas State University Southwest Research and Extension Center, Garden City, KS in 2018.

Dicamba (g ae ha^{-1})	**Plant height (cm)**		
	≤10 (day 0)	**15 (day 1)**	**30 (day 4)**
	------------------------------ Control (%) ------------------------------		
0	0e	0c	0d
70	25d	0c	0d
140	47c	16b	9c
210	67b	22b	11c
280	77b	24b	13c
420	93a	30ab	20b
560	99a	42a	30a

Dicamba doses followed by the same letter within the same column are not significantly different at 5% level. Control was estimated based on a visual estimate on a scale of 0 (no control) to 100% (complete control).

Table 2-2. Effective dose of dicamba required to achieve 50% control (visual rating; ED_{50}) and shoot biomass reduction (GR_{50}) of 10-, 15- and 30-cm tall Palmer amaranth at 4 weeks after treatment in a field study conducted at Kansas State University Southwest Research and Extension Center, Garden City, KS in 2018.

Plant height (cm)	**ED_{50} (SE)**†	**GR_{50} (SE)**†
	--------------------------------- g ae ha^{-1}	---------------------------------
≤10 (day 0)	188.6 (41.3)	40.4 (27.1)
15 (day 1)	397.7 (347.8)	182.9 (72.7)
30 (day 4)	2,734.2 (1,840.5)	283.5 (82.3)

†ED_{50} and GR_{50} values were obtained by regressing visual weed control ratings and Palmer amaranth dry biomass over dicamba dose using a three-parameter log-logistic model in R software (Ritz et al. 2015; Seefeldt et al. 1995). Values in parentheses are standard error.

Table 2-3. Mortality of 10-, 15- and 30-cm tall Palmer amaranth at 4 weeks after treatment with dicamba in a greenhouse study at Kansas State University, Manhattan, KS in 2017 – 2018.

Dicamba (g ae ha^{-1})	**Plant height (cm)**		
	≤10 (day 0)	**15 (day 1)**	**30 (day 4)**
	-------------------------------- Control (%) --------------------------------		
0	0e	0d	0c
70	0e	0d	0c
140	25d	12.5c	0c
210	62.5c	62.5b	0c
280	87.5b	62.5b	62.5b
560	100a	87.5a	87.5a

Dicamba doses followed by the same letter within the same column are not significantly different at 5% level. Mortality was estimated based on the total number of dead plants per treatment and is on a scale of 0 (no dead plants) to 100% (all plants dead).

Table 2-4. Effective dose of dicamba required to achieve 50% control (mortality; LD_{50}) and shoot biomass reduction (GR_{50}) of 10-, 15- and 30-cm tall Palmer amaranth at 4 weeks after treatment in a greenhouse in 2017 - 2018 at Kansas State University, Manhattan, KS.

Plant height (cm)	**LD_{50} (SE)**	**GR_{50} (SE)**
	-------------------------------- g ae ha^{-1}	--------------------------------
≤10 (day 0)	114.5 (20.1)	47.2 (10.6)
15 (day 1)	143.9 (64.9)	62.5 (12.9)
30 (day 4)	257.6 (98.9)	119.9 (40.0)

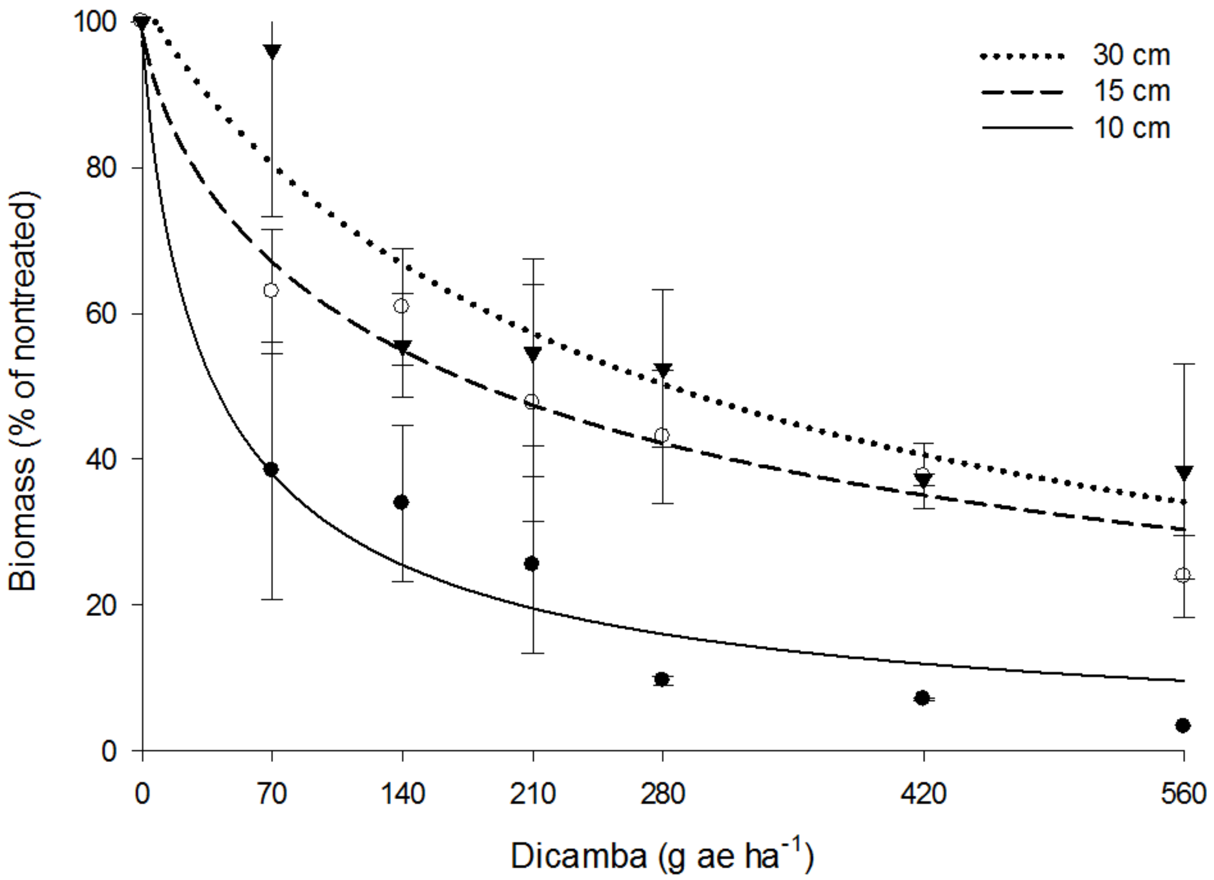

Figure 2-1. Dicamba dose-response of Palmer amaranth at three different heights at 4 weeks after treatment in a field study conducted at Kansas State University Southwest Research and Extension Center, Garden City, KS in 2018.

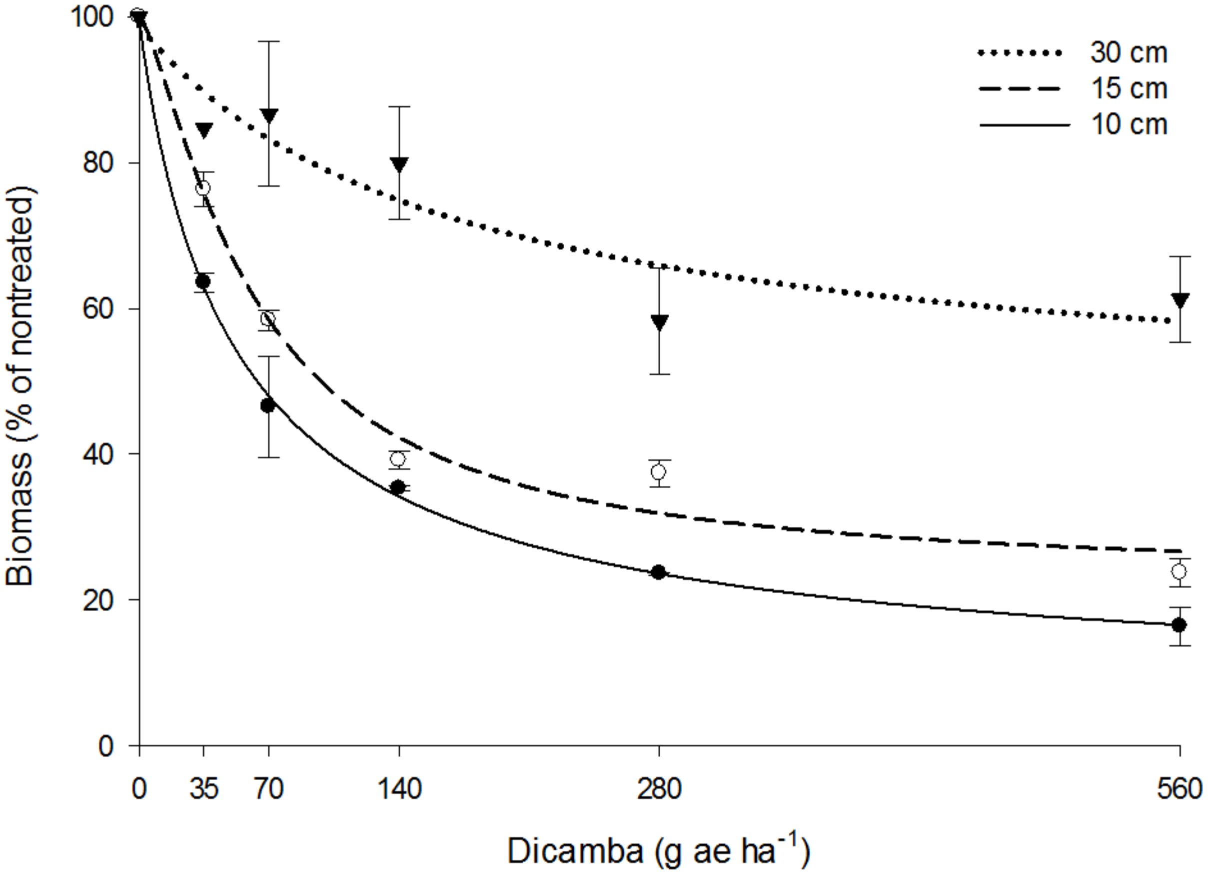

Figure 2-2. Dicamba dose-response of Palmer amaranth at three different heights at 4 weeks after treatment in a greenhouse study at Kansas State University, Manhattan, KS in 2017 – 2018.

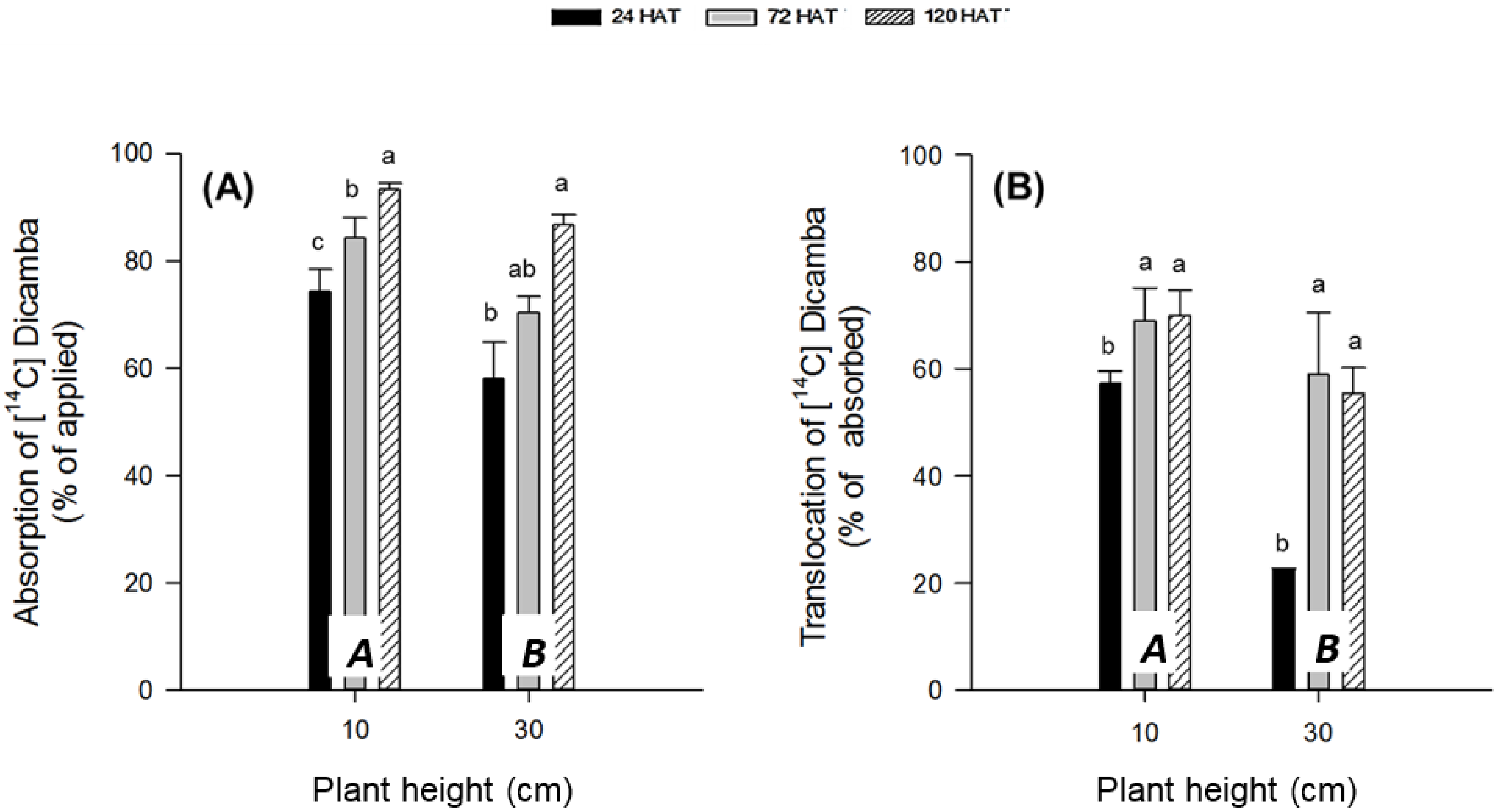

Figure 2-3. Absorption (A) and translocation (B) of ^{14}C dicamba in 10 and 30 cm Palmer amaranth. Lowercase letters indicate difference between hours after treatment (HAT) within the same plant height at 5% level. Italicized uppercase letters indicate difference between plant heights at 5% level. Error bars represent standard error of the mean.

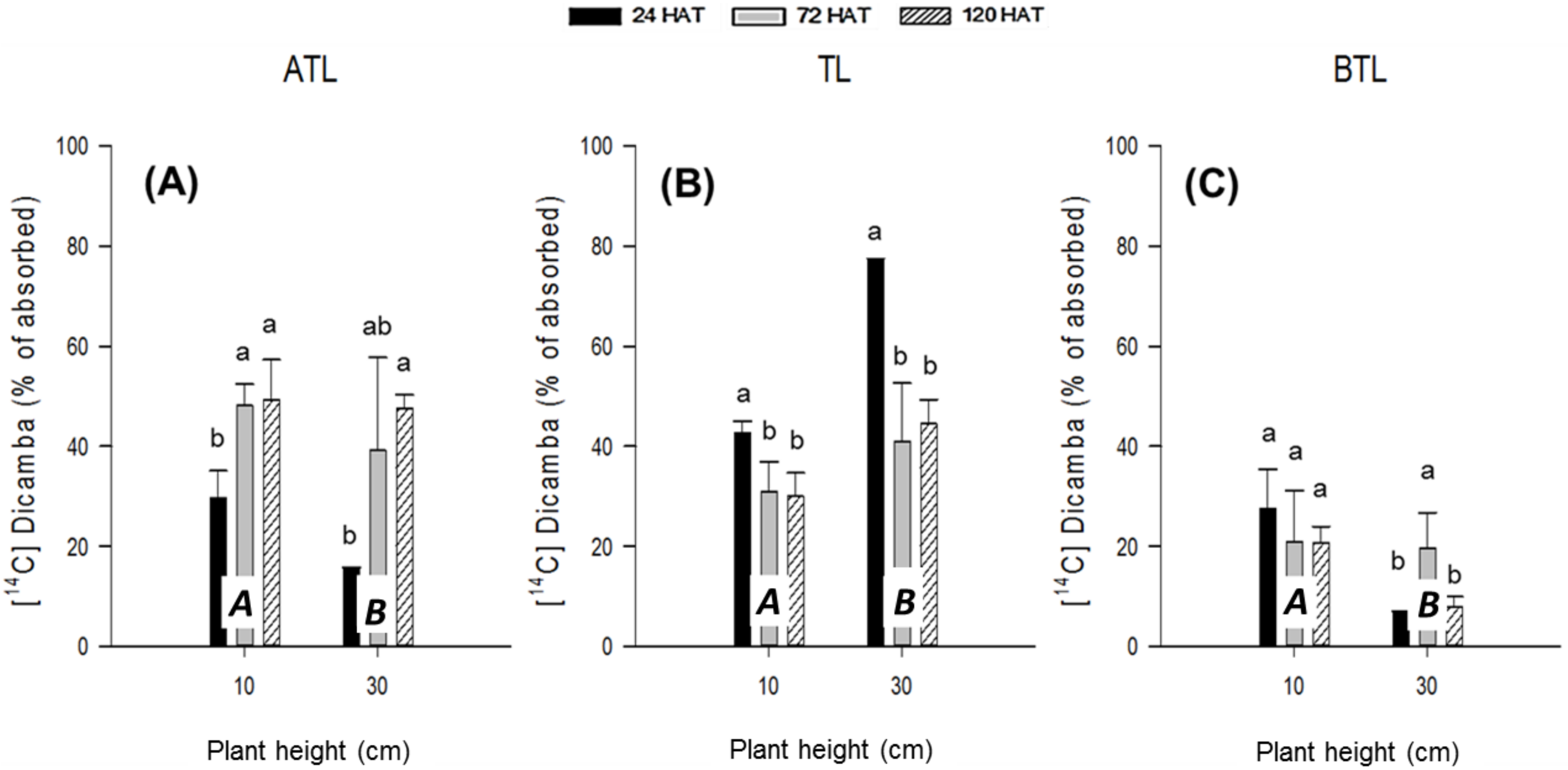

Figure 2-4. Distribution of ^{14}C dicamba to above treated leaf [ATL; (A)], treated leaf [TL; (B)], and below treated leaf [BTL; (C)] in 10 and 30 cm Palmer amaranth. Lowercase letters indicate difference between hours after treatment (HAT) within the same plant height at 5% level. Italicized uppercase letters indicate difference between plant heights at 5% level. Error bars represent standard error of the mean.

Chapter 3 - Tank Mixing of Glyphosate and Dicamba Improves Glyphosate-Resistant Palmer amaranth Control: Evidence of Herbicide Synergism

Abstract

Glyphosate resistance in weeds has increased at an alarming rate. However, if used wisely, glyphosate can still provide effective control of many weed species. The objective of this chapter was to investigate the mechanism of glyphosate resistance, and to determine if tank-mixing of dicamba and glyphosate improves the control of a glyphosate-resistant Palmer amaranth accession (PC) from Kansas. A known glyphosate-susceptible Palmer amaranth accession (FC), also from Kansas was included for comparison. Both the PC and FC accessions did not survive the label recommended dose of dicamba (560 g ae ha^{-1}) applied alone or in combination with glyphosate. The PC accession, on the other hand, survived the label recommended dose of glyphosate (840 g ae ha^{-1}) when applied alone but not in combination with dicamba. The effective dose of glyphosate required to reduce Palmer amaranth shoot biomass by 50% (GR$_{50}$) was 147.9 g ae ha^{-1} glyphosate for PC accession which was 2-fold greater than that of the FC accession. To understand the mechanism that enabled PC to survive the label recommended dose of glyphosate, quantitative PCR analysis was performed to determine the copy number of the *EPSPS* (5-enolpyruvylshikimate 3-phosphate synthase) gene, the molecular target of glyphosate, in both PC and FC accessions using appropriate endogenous controls. Results indicate that the PC accession had a relatively higher number of *EPSPS* copies (35-48) compared to FC (2-3) accession. These results show that elevated *EPSPS* copy number contributes to glyphosate resistance in the PC accession; however, tank mixing dicamba and glyphosate showed a synergistic effect on control of this Palmer amaranth accession. Therefore, tank mixing dicamba and glyphosate can help manage glyphosate-resistance in Palmer amaranth.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats.

Keywords: Palmer amaranth, dicamba, glyphosate, tank mix, synergistic effect, control

Introduction

Following the first report of Palmer amaranth resistance to glyphosate recorded from an accession collected from a glyphosate-resistant cotton field in Georgia, U.S. (Culpepper et al., 2006), many other cases of glyphosate resistance in Palmer amaranth have been documented (Heap, 2019). This alarming increase in glyphosate resistance has been attributed to an over reliance on simplified, single mode-of-action 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS inhibitors), herbicide programs. To this date, however, glyphosate remains the most popular and widely used pesticide in the U.S. and globally due to increasing availability and adoption of glyphosate-tolerant crops such as soybean, corn and cotton (Benbrook, 2016). Other factors such as the increasing adoption of no-till and conservation tillage practices, a competitive market price of glyphosate in relation to other herbicide products, and an exponential increase of herbicide-resistant weeds also have contributed to its continually increasing use (Benbrook, 2016).

Palmer amaranth is remarkably prone to evolving herbicide resistance (Ward et al., 2013). A recent survey by the Weed Science Society of America ranked Palmer amaranth as the most troublesome weed in the U.S. (WSSA, 2016). At present, glyphosate-resistant Palmer amaranth populations have been documented in at least 50% of the U.S. states including Kansas (Heap, 2019) where it interferes with production of a number of crops including wheat, sorghum, corn and soybean making it one of the most economically damaging glyphosate-resistant weeds in the country (Ward et al., 2013).

Although glyphosate resistance has increasingly become a major threat to agriculture, depending on the level of resistance and plant growth stage, post emergence glyphosate applications are still effective in controlling problem weed, including glyphosate-resistant

Palmer amaranth provided that other compatible herbicides with effective modes of action are added as discussed elsewhere (Norsworthy et al., 2012). For example, Merchant et al. (2014) reported up to 86% increase in control of glyphosate-resistant Palmer amaranth in Enlist cotton when glyphosate and 2,4-D were mixed. In the same study, however, control was improved to 95 and 96% when sequential applications of the two herbicides were followed by either pendimethalin or fomesafen, respectively. Wiggins et al. (2015) reported greater than 95% control of glyphosate-resistant Palmer amaranth in corn 28 days after glyphosate was applied with either atrazine alone or combined with S-metolachlor and mesotrione. The benefit of adding other effective herbicide modes of action to glyphosate applications when controlling glyphosate-resistant Palmer amaranth was also reported in Enlist soybeans (Miller and Norsworthy, 2016). Miller and Norsworthy (2016) also reported glyphosate-resistant Palmer amaranth control ranging from 66 to 82, 69 to 86, and 94 to 98% 14 days after glyphosate was applied early post with 2,4-D choline, and both glyphosate and 2,4-D choline were applied early and mid-post or following pre residual herbicides such as sulfentrazone with chloransulam, and flumioxazin with or without chlorimuron, respectively. The objectives of this study were to investigate the mechanism and evaluate the benefit of including dicamba in glyphosate tank-mix to control a suspected glyphosate-resistant Palmer amaranth accession from Kansas.

Materials and Methods

Plant Material and Growth Conditions

Two Palmer amaranth accessions consisting of fully matured seeds were collected from two distinct locations in Kansas, one in 2015 and another in 2016. The first one, designated as PC, was collected from a farm field located in Pawnee County, KS where Palmer amaranth had been reported to survive the label recommended dose of glyphosate (840 g ae ha^{-1}), and the second, designated as FC, was collected from a farm field located in Finney County, KS with no known resistance to any commonly used herbicide. Both PC and FC accessions were hand-threshed, cleaned with an air-propelled column blower, and composited into two separate samples. The samples were then kept in a paper bag and stored at 4 ^{o}C until used.

Seeds from each Palmer amaranth sample were germinated in small trays (25 cm × 15 cm × 2.5 cm) filled with a commercial potting mixture (Miracle-Gro® Moisture Control Potting Mix, CA) in a greenhouse. Seedlings 2-3 cm tall were transplanted into 6.5 cm × 6.5 cm × 9 cm plots and allowed to grow. The greenhouse was equipped with sodium vapor lamps supplementing 250 µmol m^{-2} s^{-1} of illumination and maintained at 25/20 ^{o}C day/night temperatures and 15/9 h day/night photoperiod.

Dicamba and Glyphosate Dose Response Study

Dose-response studies were conducted in summer 2017 and 2018 in a greenhouse at Kansas State University, Manhattan, KS. Actively growing Palmer amaranth seedlings (10 cm tall) from both accessions, PC and FC, were treated separately with dicamba at doses of 0, 35, 70, 140, 280, and 560 g ae ha^{-1} or glyphosate at doses of 0, 53, 105, 210, 420, and 840 g ae ha^{-1} with 2.5% (w/v) ammonium sulfate. All dicamba and glyphosate treatments were applied with a bench-type track sprayer (Generation III, DeVries Manufacturing, RR 1 Box184 Hollandale, MN, USA) equipped with a single moving even flat-fan nozzle tip (8002E TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.85 km h^{-1}. Experiments were conducted in a randomized complete block design with four replications (1 plant per pot). Biomass measurements were collected at four weeks after treatment (WAT) to estimate biomass sensitivity of FC and PC accessions to both dicamba and glyphosate. In addition, the number of plants that survived the label recommended dose of each herbicide were estimated and reported as mortality in percent.

Genomic DNA Extraction and Determination of *EPSPS* Copy Number

EPSPS copy number was determined to understand the mechanism conferring glyphosate resistance to the PC accession. Genomic DNA was extracted to assess *EPSPS* copy number of three plants from FC and PC accessions, respectively, and two plants of a known resistant (RC) and susceptible (SC) accessions were used as checks. The plants were grown as described previously under plant material and growth conditions. For each of nine plants, fresh leaf tissue (~100 mg) was collected in 50-ml falcon tubes, flash frozen in liquid nitrogen and stored at -80 ^{o}C for genomic DNA extraction. Genomic DNA was extracted using OMEGA bio-tek E.Z.N.A. Plant DNA Kit (Omega Bio-tek, Inc., GA) following the instructions provided by the manufacture. Quantification of DNA was done using a Nano Drop 1000 spectrophotometer (Thermoscientific., DE). Genomic DNA was diluted in a 1:5 ration and used for EPSPS copy number determination in a Quantitative PCR (StepOnePlusTM real-time detection system, Thermo Fisher Scientific) reaction. The qPCR reaction mix (14 µL) consisted of 8µL of PowerUpTM SYBR® Green Master Mix (Applied Biosystems), 2 µL each of forward and reverse primers (5 µM), and 2 µL of genomic DNA. The 96-well qPCR reaction plate was set up with three technical replicates for each plant of the two accessions (PC and FC) and the two checks (RC and SC). The qPCR conditions were 95 ^{o}C for 10 min, 40 cycles of 95 ^{o}C for 30 s, and an annealing at 60 ^{o}C for 1 min. The forward and reverse primers used for amplifying the EPSPS were:

- Forward 5'-ATGTTGGACGCTCTCAGAACTCTTGGT-3'
- Reverse 5'-TGAATTTCCTCCAGCAACGGCAA-3'.

β-tubulin was used as a reference gene (Godar et al., 2015), with the primers:

- Forward 5'-ATGTGGGATGCCAAGAACATGATGTG-3'
- Reverse 5'-TCCACTCCACAAAGTAGGAAGAGTTCT-3'.

Dicamba and Glyphosate Efficacy Study

Efficacy studies were conducted in summer 2017 and 2018 at Kansas State University, Manhattan, KS. Actively growing seedlings (10-cm tall) from both FC and PC accessions were treated with seven combinations of dicamba and glyphosate to test the efficacy of adding dicamba to glyphosate application(s) in controlling glyphosate-resistant Palmer amaranth. Treatments consisted of 560 g ae ha^{-1} dicamba, 560 g ae ha^{-1} dicamba + 53 g ae ha^{-1} glyphosate, 560 g ae ha^{-1} dicamba + 210 g ae ha^{-1} glyphosate, 840 g ae ha^{-1} glyphosate, 840 g ae ha^{-1} glyphosate + 35 g ae ha^{-1} dicamba, 840 g ae ha^{-1} glyphosate + 140 g ae ha^{-1} dicamba, and a check with no herbicide applied. All treatments were applied with a bench-type track sprayer (Generation III, DeVries Manufacturing, RR 1 Box184 Hollandale, MN, USA) equipped with a single moving even flat-fan nozzle tip (8002E TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.85 km h^{-1} as described in the dose response study. Experiments were conducted in a randomized complete block design with four replications (1 plant per pot). Mortality and biomass measurements were collected at four WAT.

Data Analysis

No interaction was detected between study year and treatments; therefore, data were pooled. Mortality data were presented in percent, and biomass either as percent of non-treated or regressed over dicamba and glyphosate dose using four- and three-parameter log logistic model in R (Ritz et al., 2015; Seefeldt et al., 1995). The lack-of-fit test ($P>0.05$) indicated that the chosen model accurately described the data. Regression analyses were further used to estimate effective dicamba and glyphosate doses required to cause 50% Palmer amaranth shoot biomass reduction (GR_{50}). The EPSPS copy numbers were measured relative to the known susceptible (SC), and the data were subjected to the formula for fold induction ($2^{-\Delta\Delta Ct}$) (Pfaffl, 2001).

Results and Discussion

Dicamba and Glyphosate Dose Response Study

Palmer amaranth seedlings (10 cm tall) from both FC and PC accessions were screened with the label recommended doses of dicamba (560 g ae ha^{-1}) and glyphosate (840 g ae ha^{-1}) to determine the frequency of resistance in each accession prior to determining their respective level of resistance in the dose response assay. FC did not survive the label recommended dose of dicamba or glyphosate 4 WAT. PC, on the other hand, survived the label recommended dose of glyphosate (100%) but not of dicamba 4 WAT. PC did not survive four times the label recommended dose of glyphosate (data not shown). These results confirmed that the PC Palmer amaranth infesting the farm field in Pawnee County, KS does indeed survive the label recommended dose of glyphosate.

In general, greater reduction in overall Palmer amaranth shoot biomass occurred in response to dicamba compared with glyphosate (Figure 3-1). PC had greater shoot biomass reduction in response to dicamba application compared with FC (Figure 3-1A). Conversely, reduction in shoot biomass in response to glyphosate was greater in FC than PC (Figure 3-1B). Dose-response analyses suggest that PC has relatively higher sensitivity to dicamba but lower sensitivity to glyphosate than FC.

Based on the fitted log-logistic model, the dicamba dose required to cause 50% reduction in Palmer amaranth shoot biomass (GR_{50}) for FC and PC accessions were 47.1 and 25.8 g ae ha^{-1}, respectively, suggesting that the FC accession withstands 1.8 times more dicamba than its PC counterpart despite both not surviving the field recommended dose of dicamba. The glyphosate dose required to cause 50% reduction in Palmer amaranth shoot biomass (GR_{50}) for FC and PC accessions was 72.3 and 147.9 g ae ha^{-1}, respectively, suggesting that the PC accession that survived the label recommended dose of glyphosate withstands two times more glyphosate than its FC counterpart (Table 3-2). Steckel et al. (2008) reported a low level of resistance to glyphosate of up to 1.5- and 5-fold in glyphosate-resistant Palmer amaranth accessions from Tennessee. Culpepper et al. (2006) reported up to 6.2-fold resistance level to glyphosate in a glyphosate-resistant Palmer amaranth accession from central Georgia. More recently, Chahal et al. (2017) reported a much higher level of resistance to glyphosate of up to 37- to 40-fold in a glyphosate-resistant Palmer amaranth accession from south central Nebraska. In contrast to the above reports, more dramatic findings have been reported elsewhere. For example, a resistance level 30 to 50 times the one reported here has been reported in glyphosate-resistant Palmer amaranth accessions from Cowley County, in South Central KS (Putman, 2013) a distinct location from where the suspected glyphosate-resistant Palmer amaranth accession evaluated in the current analysis was collected. Norsworthy et al. (2008) also reported a similar resistance level of up to 39.5 to 57.5-fold the one reported in this study in a glyphosate-resistant Palmer amaranth accession form Arkansas. This variation in the level of resistance could be due to several factors (Jasieniuk et al., 1996). This includes the intensity of selection pressure that varies from field to field. Overall, these findings suggest that the PC accession may have been exposed to a less intense glyphosate selection.

Dicamba and Glyphosate Efficacy Study

FC and PC Palmer amaranth accessions response to dicamba and glyphosate applied in combination is presented as percent mortality and biomass in relation to non-treated 4 WAT, Table 3-2. The label recommended dose of dicamba (560 g ae ha^{-1}) controlled 100% of both FC and PC accessions. Due to no differences in control, there was no value in adding glyphosate to dicamba except for biomass reduction. In contrast, the label recommended dose of glyphosate

(840 g ae ha^{-1}) which controlled the FC Palmer amaranth accession 100%, completely failed to control the PC accession (0% control), further confirming that the PC Palmer amaranth accession does indeed survive the label recommended dose of glyphosate. However, unlike the addition of glyphosate to dicamba which provided no additional benefit in controlling FC and PC Palmer amaranth except biomass suppression, there was value in adding dicamba to glyphosate application(s) to control both accessions. For example, when one sixteenth of the label recommended dose dicamba (35 g ae ha^{-1}) was mixed with the label-recommended dose of glyphosate, 50% control of the PC Palmer amaranth accession was achieved. Complete control of this Palmer amaranth accession was achieved when a quarter of the label recommended dose of dicamba (140 g ae ha^{-1}) was mixed with the label recommended dose of glyphosate. In terms of biomass sensitivity, addition of increasing doses of dicamba to the label recommended dose of glyphosate resulted in greater biomass reduction in FC than PC (Table 3-2). Based on the dose response curve analysis, the earlier accession (FC) also exhibited a relatively higher biomass sensitivity to glyphosate than PC (Figure 3-1B). These findings suggest that the addition of dicamba to glyphosate was effective against the PC Palmer amaranth accession. The benefit of tank mixing herbicides with different modes or sites of action to manage herbicide resistant weeds has been documented elsewhere (Johnson and Gibson, 2006; Beckie and Reboud, 2009). In this study, however, we found that adding dicamba to glyphosate application(s) has a synergistic effect on control of a Palmer amaranth accession resistant to glyphosate.

EPSPS Copy Number

EPSPS copy numbers ranged from 2 to 3 for the FC accession and 36 to approximately 48 for the PC accession (Figure 3-2). The number of *EPSPS* copies obtained from FC did not differ from that of SC (1-2) which suggests that FC is equally susceptible to glyphosate. The number of copies obtained from PC, on the other hand, differed from that of SC as well as RC (84 to111 EPSPS copy number) falling exactly in the middle between known susceptible and resistant accessions. This elevated number of EPSPS copies helps explain PC's ability to survive up to three times the label recommended dose of glyphosate (840 g ae^{-1}) when applied alone. Previously, it has been shown that Palmer amaranth plants carrying more than 30 EPSPS gene copies were able to survive the label recommended dose of glyphosate (Gaines et al., 2011; Varanasi et al., 2015). However, it has been reported previously that Palmer amaranth plants carrying even as few as 6 to 8 EPSPS gene copies were able to survive field-recommended doses of glyphosate (Mohseni-Moghadam et al., 2013). Previous studies indicated that differences in the number of EPSPS gene copies conferring glyphosate resistance might be more inter than intraspecific (Nandula et al., 2014; Chatham et al., 2015; Saragi, 2016;).

Conclusions

This study confirmed that the Palmer amaranth accession from Pawnee County, survived the label recommended dose of glyphosate due to elevated EPSPS gene copy number. However, tank mixing dicamba and glyphosate had a synergistic effect on control of this glyphosate resistant Palmer amaranth accession from Pawnee County, KS. Results from this study showed that tank mixing dicamba and glyphosate can help mitigate glyphosate resistance in Palmer amaranth.

References

Beckie H, Reboud X (2009) Selecting for Weed Resistance: Herbicide Rotation and Mixture. Weed Technology, 23(3):363-370

Benbrook C (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe, 28(1):1-15

Chahal P, Varanasi V, Jugulam M, Jhala A (2017) Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Nebraska: Confirmation, EPSPS Gene Amplification, and Response to POST Corn and Soybean Herbicides. Weed Technology, 31(1):80-93

Chatham LA, Bradley KW, Kruger GR, Martin JR, Owen MD, Peterson DE, Mithila J, Tranel PJ (2015) A multistate study of the association between glyphosate resistance and EPSPS gene amplification in waterhemp (*Amaranthus tuberculatus*). Weed Science, 63(3):569-77

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 Science, 54(4):620-626

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proceedings of the National Academy of Sciences, 107(3):1029-34

Godar AS, Varanasi VK, Nakka S, Prasad PV, Thompson CR, Mithila J (2015) Physiological and molecular mechanisms of differential sensitivity of Palmer amaranth (*Amaranthus palmeri*) to mesotrione at varying growth temperatures. PloS One, 10:0126731

Heap I (2019) The International Survey of Herbicide Resistant Weeds. Online. Internet. Accessed January 5, 2019. Available www.weedscience.org

Jasieniuk M, Brulebabel A, Morrison I (1996) The evolution and genetics of herbicide resistance in weeds. Weed Science, 44(1):176-193

Johnson W, Gibson K (2006) Glyphosate-Resistant Weeds and Resistance Management Strategies: An Indiana Grower Perspective. Weed Technology, 20(3):768-772

Merchant R, Culpepper A, Eure P, Richburg J, Braxton L (2014) Controlling Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Cotton with Resistance to Glyphosate, 2,4-D, and Glufosinate. Weed Technology, 28(2):291-297

Miller M, Norsworthy J (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 Technology, 30(2):366-376

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 science, 61(4):517-525

Nandula VK, Wright AA, Bond JA, Ray JD, Eubank TW, Molin WT (2014) EPSPS amplification in glyphosate- resistant spiny amaranth (*Amaranthus spinosus*): a case of gene transfer via interspecific hybridization from glyphosate- resistant Palmer amaranth (*Amaranthus palmeri*). Pest management science, 70(12):1902-9

Norsworthy J, Griffith G, Scott R, Smith K, Oliver L (2008) Confirmation and Control of Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technology, 22(1):108-113

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW (2012) Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. Weed Science, 60(Sp1):31-62

Plaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research, 29:2002-2007

Putman JA (2013) Palmer amaranth control in established alfalfa and documentation of glyphosate-resistant *Amaranthus* species in Kansas. Master's thesis. Manhattan, KS: Kansas State University

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. PLoS ONE 10(12): e0146021. https://doi.org/10.1371/journal.pone.0146021

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed technology, 9(2):218-27

Steckel L, Main C, Ellis A, Mueller T (2008). Palmer Amaranth (*Amaranthus palmeri*) in Tennessee Has Low Level Glyphosate Resistance. Weed Technology, 22(1):119-123

Varanasi VK, Betha S, Thompson CR, Jugulam M (2015) Target and non-target site multiple herbicide resistance in Palmer amaranth (*Amaranthus palmeri*) from Kansas. Page 81 in Proceedings of the 70th North Central Weed Science Society. Indianapolis, IN: North Central Weed Science Society

Ward S, Webster T, Steckel L (2013) Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Technology, 27(1):12-27

Wiggins M, McClure M, Hayes R, Steckel L (2015) Integrating Cover Crops and POST Herbicides for Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) Control in Corn. Weed Technology, 29(3):412-418.

[WSSA] Weed Science Society of America (2016) WSSA Survey Ranks Palmer amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in

Canada. http://wssa.net/2016/04/wssa-survey-ranks-palmer-amaranth-as-the-most-troublesome-weed-in-the-u-s-galium-as-the-most-troublesome-in-canada/. Accessed January 5, 2019

Table 3-1. Effective dicamba and glyphosate doses required to achieve 50% reduction (GR_{50}) of Palmer amaranth shoot biomass in a greenhouse dose response study at Kansas State University, Manhattan, KS in 2017 – 2018.

		Estimate	
Palmer amaranth accession	**Herbicide**	**GR_{50} (SE)**[†]	**RI**[††]
		g ae ha^{-1}	
FC	Dicamba	47.1 (10.6)	1.8
	Glyphosate	72.3 (32.9)	--
PC	Dicamba	25.8 (5.5)	--
	Glyphosate	147.9 (59.9)	2.0

[†] SE is the absolute standard error of the mean

[††] RI (resistance index) is the ratio of GR_{50} of the resistant or less sensitive to GR_{50} of the susceptible or more sensitive Palmer amaranth accession.

Table 3-2. Control of 10-cm Palmer amaranth (mortality) 4 weeks after treatment with dicamba and glyphosate alone or in combination in a greenhouse dose response study at Kansas State University, Manhattan, KS in 2017 – 2018.

		Palmer amaranth control			
		FC accession		PC accession	
Herbicide	Rate	Mortality	Biomass (SE)[†]	Mortality	Biomass (SE)[†]
	(g ae ha^{-1})	Control (%)			
Control	0	0	100.00 (0.063)	0	100.00 (0.260)
Dicamba	560	100	26.13 (0.046)	100	3.56 (0.026)
Dicamba+glyphosate	560+52.5	100	20.52 (0.034)	100	3.17 (0.014)
	560+210	100	19.37 (0.044)	100	3.27 (0.018)
Glyphosate	840	100	26.80 (0.278)	0	35.99 (0.094)
Glyphosate+dicamba	840+35	100	17.72 (0.368)	50	21.95 (0.075)
	840+140	100	4.11 (0.029)	100	21.17 (0.063)

[†] Biomass values are % of non-treated. SE is the absolute standard error of the mean.

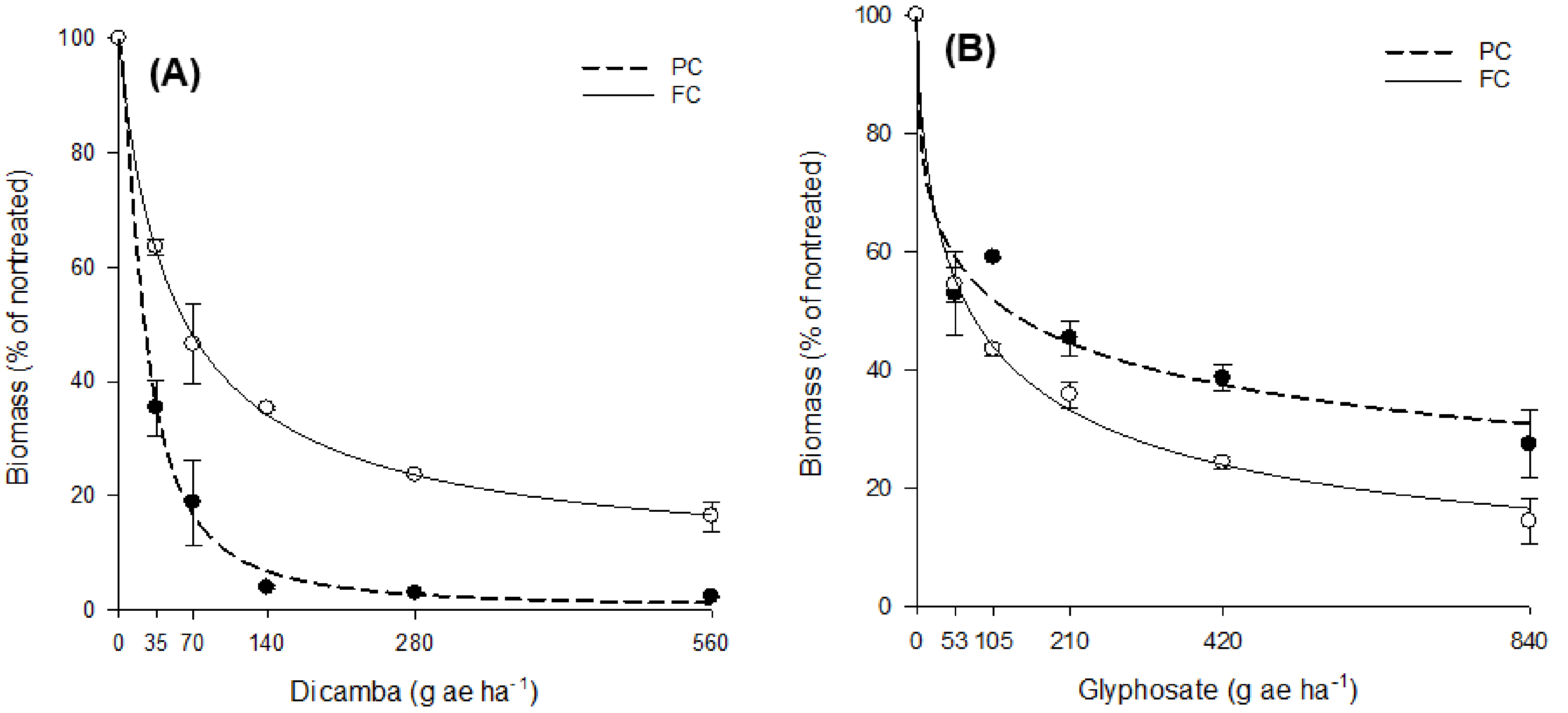

Figure 3-1. Suspected glyphosate-resistant (PC) and susceptible (FC) Palmer amaranth response to dicamba (A) and glyphosate (B) 4 weeks after treatment in a greenhouse dose response study at Kansas State University, Manhattan, KS in 2017 – 2018. PC represents the Pawnee County accession, and FC represents the Finney County accession.

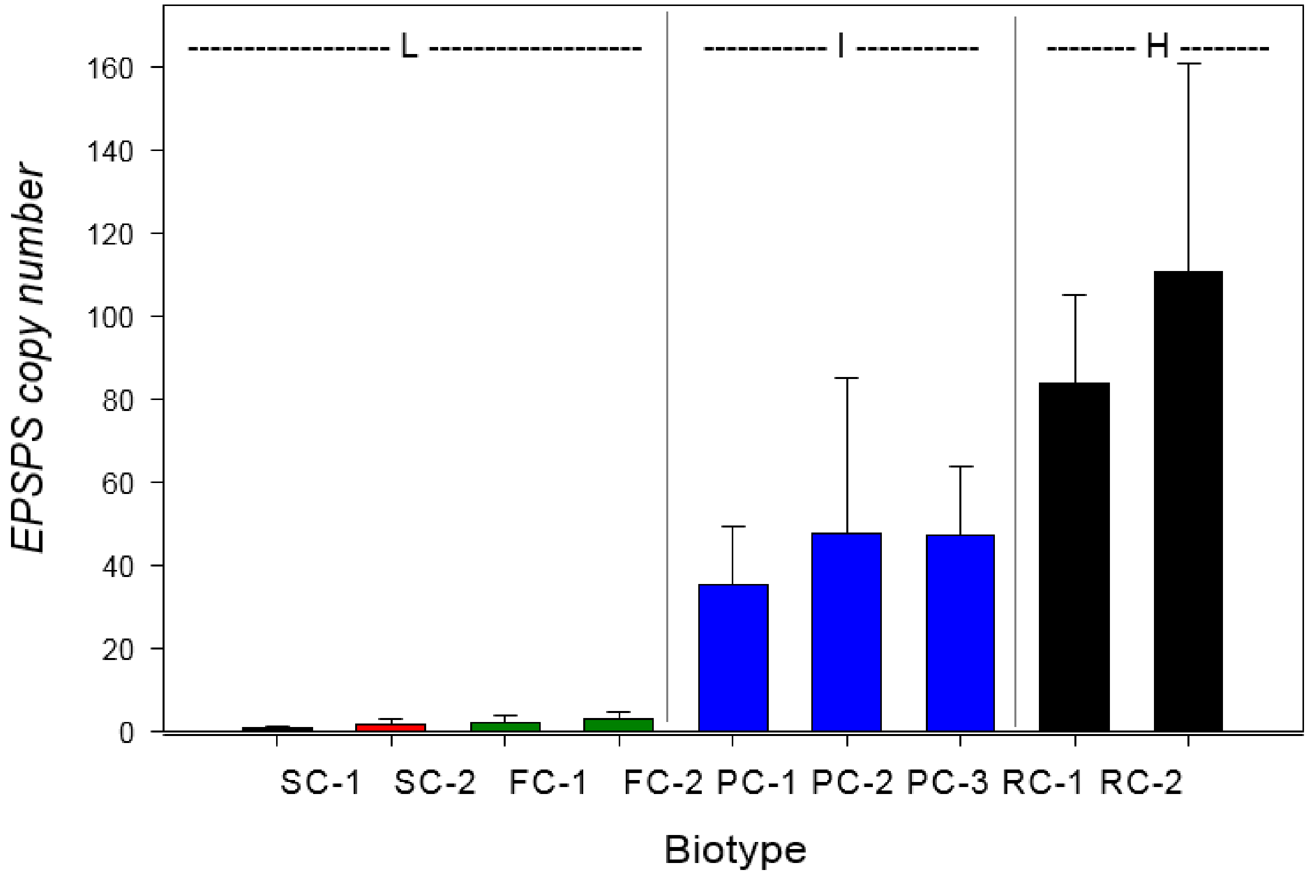

Figure 3-2. EPSPS gene copies in Palmer amaranth. L, I and H represent the plants with low (<5), intermediate (35–50) and high (>48) *EPSPS* copy number, respectively. SC and RC represent previously known glyphosate-susceptible and -resistant Palmer amaranth biotypes, respectively, used as checks. FC and PC represent Finney and Pawnee county accessions, respectively. Error bars represent standard error of the means.

Chapter 4 - Rapid Absorption of Dicamba and Increased Translocation of Glyphosate in Tank-Mixes Improve Palmer amaranth Control

Abstract

Dicamba and glyphosate have been reported to interact antagonistically when used to control several weed species including *Avena fatua*, *Sorghum halepense*, *Kochia scoparia* and *Echinochloa crus-galli*. This research was conducted to evaluate how a Palmer amaranth accession from Kansas with no known resistance to any commonly used herbicides responds to dicamba and glyphosate alone or in combination. The dicamba and glyphosate-dose response studies were conducted by applying the two herbicides either alone or in combination on the Palmer amaranth accession from KS. The GR_{50} values (dose required to reduce shoot biomass by 50%) of dicamba and glyphosate were determined as 47.1 and 72.3 g ae ha^{-1}, respectively, suggesting that the Palmer amaranth accession is 1.5 times more sensitive to dicamba than glyphosate. However, complete control of Palmer amaranth occurred when both dicamba (560 g ae ha^{-1}) and glyphosate (840 g ae ha^{-1}) were applied at the label-recommended dose. More importantly, complete control was obtained with less than the label-recommended doses when these two herbicides were applied in combination. To understand the physiological basis of the synergism of dicamba and glyphosate when tank-mixed, experiments were conducted using ^{14}C dicamba or glyphosate. The results suggested rapid absorption of dicamba and increased translocation of glyphosate when they were tank-mixed than when applied separately. The results of this study demonstrate a synergistic interaction when dicamba and glyphosate were tank-mixed resulting in greater translocation of glyphosate and improved control of Palmer amaranth.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats.

Keywords: Palmer amaranth, dicamba, glyphosate, efficacy, absorption, translocation

Introduction

Glyphosate (n-phosphonomethyl glycine) is an integral component of many cropping systems in the U.S. (Benbrook, 2016). However, there is a rapid and steady increase in resistance to this herbicide in many weed species (Heap, 2019). This makes weed control, especially in no-till systems more challenging. Farmers have adopted the practice of tank-mixing other herbicides with glyphosate such as dicamba (3,6-dichloro-o-anisic acid), an auxinc herbicide that has proven effective in controlling difficult to control and herbicide-resistant weeds (Spaunhorst and Bradley, 2013; Joseph, 2014; Brachtenbach, 2015; Byker et al., 2017; Underwood et al., 2017; Ou, 2018; Zimmer et al., 2018). Despite this practice, several studies have reported that dicamba interferes with glyphosate activity on a number of weed species. For example, Huff (2010) reported antagonism (≤40% control) when a combination of dicamba and glyphosate was sprayed at low rates on johnsongrass (*Sorghum halepense*), barnyardgrass (*Echinoclhoa crus-galli*), large crabgrass (*Digitaria sanguinalis*), broadleaf signalgrass (*Urochloa platyphylla*), sicklepod (*Senna obtusifolia*), hemp sesbania (*Sesbania herbacea*), prickly sida (*Sida rhombifolia*), and pitted morning glory (*Ipomoea lacunose*) due to reduced translocation. Similarly, O'Sullivan and O'Donovan (1980) reported reduced weed control in wheat, barley and wild oats when glyphosate and dicamba were applied in combination. Barrett (1968) also reported significant reduction in johnsongrass control when glyphosate was applied in combination with dicamba due to reduced absorption and translocation. More recently, Ou et al. (2018) reported reduced control of a kochia (*Kochia scoparia*) accession from Kansas when glyphosate and dicamba were combined due to dicamba interfering with glyphosate translocation. Another example of antagonism involving dicamba and glyphosate was reported recently by Meyer (2018) who observed a reduction in glyphosate absorption in barnyard grass when glyphosate and dicamba were applied in combination. Others have reported an additive or synergistic effect on weed control when dicamba and glyphosate were applied in combination. For example, Joseph et al. (2018) observed an additive effect on 5 cm tall Palmer amaranth control when glyphosate and dicamba were mixed. Flint and Barret (1989) observed a synergistic effect on field bindweed control from a combined application of the two herbicides. How these two herbicides interact depends on a number of factors particularly weed species (O'Sullivan and O'Donovan, 1980; Meyer, 2018; Ou et al., 2018). Therefore, the objectives of this study were, using a Palmer amaranth accession from Kansas with no known resistance to any commonly used herbicides, to a) evaluate the type of interaction when glyphosate and dicamba are applied in combination and b) determine the physiological basis of the interaction.

Materials and Methods

Plant Material and Growth Conditions

Seed from a Palmer amaranth accession with no known resistance to any commonly used herbicides (including glyphosate and dicamba) was collected from a farm field at Kansas State University Southwest Research-Extension Center near Garden City, KS. The seed was kept in a paper bag and stored at 4 ^{o}C until used. Seed was germinated in small trays (25 cm x 15 cm x 2.5 cm) filled with a commercial potting mixture (Miracle-Gro® Moisture Control Potting Mix, CA) in a greenhouse. Seedlings 2-3 cm tall were transplanted into 6.5 cm × 6.5 cm × 9 cm plots and allowed to grow for herbicide dose-response, and radiolabeled herbicide absorption and translocation experiments. The greenhouse was equipped with sodium vapor lamps supplementing 250 µmol m^{-2} s^{-1} of illumination and maintained at 25/20 ^{o}C day/night temperatures and 15/9 h day/night photoperiod.

Dicamba and Glyphosate Dose Response Studies

Two separate dose response studies were conducted in summer 2017 and 2018 at Kansas State University, Manhattan, KS. Actively growing Palmer amaranth seedlings (10-cm tall) were treated separately with dicamba (no adjuvants) at doses of 0, 35, 70, 140, 280, and 560 g ae ha^{-1} or glyphosate at doses of 0, 53, 105, 210, 420, and 840 g ae ha^{-1} with 2.5% (w/v) ammonium sulfate. All herbicide treatments were applied with a bench-type track sprayer (Generation III, DeVries Manufacturing, RR 1 Box184 Hollandale, MN, USA) equipped with a single moving flat-fan nozzle tip (8002E TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.85 km h^{-1}. Each experiment was conducted in a randomized complete block design with four replications (1 plant per pot) and repeated twice in time. Biomass measurements were collected at four weeks after treatment (WAT) to estimate shoot biomass sensitivity to both dicamba and glyphosate on dry weight-basis. In addition, the dose of each herbicide required to reduce shoot biomass by 50% (GR_{50}) was estimated.

Dicamba and Glyphosate Tank-Mix Studies

Studies were conducted in summer 2017 and 2018 at Kansas State University, Manhattan, KS to evaluate control of Palmer amaranth by combined application of glyphosate and dicamba. Actively growing Palmer amaranth seedlings (10-cm tall) were treated with seven combinations of dicamba and glyphosate. Treatments consisted of 560 g ae ha^{-1} dicamba, 560 g ae ha^{-1} dicamba + 53 g ae ha^{-1} glyphosate, 560 g ae ha^{-1} dicamba + 210 g ae ha^{-1} glyphosate, 840 g ae ha^{-1} glyphosate, 840 g ae ha^{-1} glyphosate + 35 g ae ha^{-1} dicamba, 840 g ae ha^{-1} glyphosate + 140 g ae ha^{-1} dicamba, and a check with no herbicide applied. All treatments were applied with a bench-type track sprayer (Generation III, DeVries Manufacturing, RR 1 Box184 Hollandale, MN, USA) equipped with a single moving flat-fan nozzle tip (8002E TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.85 km h^{-1}. Experiments were conducted in a randomized complete block design with four replications (1 plant per pot) and repeated twice in time. Mortality and biomass measurements were collected at four WAT.

Dicamba and Glyphosate Absorption and Translocation Studies

To understand the physiological basis of dicamba and glyphosate interaction in a tank-mix, ^{14}C dicamba and glyphosate absorption and translocation studies were conducted in Palmer amaranth in 2017 and 2018. Palmer amaranth seedlings were raised as described above for dose-response studies and transferred to a growth chamber three days prior to treatment with ^{14}C herbicides to acclimate to chamber conditions. The growth chamber was equipped with fluorescent bulbs capable of delivering 550 µmol m^{-2} s^{-1} photon flux at plant canopy level, and was maintained at 32.5/22.5 ºC day/night, 15/9 h photoperiod, and 60-70% relative humidity. Plants were watered as required throughout the experiment. Four treatments were evaluated including dicamba (560 g ae ha^{-1}) and glyphosate (840 g ae ha^{-1}) applied at their label recommended doses and two versions of a combination of the label recommended dose of glyphosate with a quarter of the label recommended dose of dicamba (140 g ae ha^{-1}) with one version containing radiolabeled dicamba and the other containing radiolabeled glyphosate. The dicamba-glyphosate combination was chosen based on the data from the dicamba and glyphosate tank-mix study. Specifically, the first treatment consisted of dicamba (ring-UL-^{14}C) ethanol solution (11.4 kBq μl^{-1}, specific activity: 2.87 kBq μg^{-1}, BASF Corp.) with non-radiolabeled dicamba added to the radioactive solution to obtain 560 g ae ha^{-1} of dicamba in a carrier volume of 187 L; the second treatment consisted of dicamba (ring-UL-^{14}C) ethanol solution (11.4 kBq μl^{-1}, specific activity: 2.87 kBq μg^{-1}, BASF Corp.) with non-radiolabeled dicamba and glyphosate added to the radioactive solution to obtain 140 and 840 g ae ha^{-1} of dicamba and glyphosate, respectively, in a carrier volume of 187 L; the third treatment consisted of [phosphonomethyl-^{14}C]-glyphosate (3.7 kBq μL^{-1}, specific activity: 2.04 kBq μg^{-1}, PerkinElmer, Inc., Boston, MA, USA) with non-radiolabeled glyphosate added to the radioactive solution to obtain 840 g ae ha^{-1} of glyphosate in a carrier volume of 187 L; and the fourth and last treatment consisted of [phosphonomethyl-^{14}C]-glyphosate (3.7 kBq μL^{-1}, specific activity: 2.04 kBq μg^{-1}, PerkinElmer, Inc., Boston, MA, USA) with non-radiolabeled glyphosate and dicamba added to the radioactive solution to obtain 840 and 140 g ae ha^{-1} of glyphosate and dicamba, respectively, in a carrier volume of 187 L. Each treatment totaled 3.3 kBq. AMS at 2.5% (w/v) was added to every treatment containing glyphosate. For each treatment, 10 one-µl droplets of solution were applied on the adaxial surface of the fourth youngest leaf of four uniform plants using a Wiretrol®

capillary syringe (10 µL, Drummond Scientific Co., Broomall, PA, USA). All plants were returned to the growth chamber 30 min after treatment. Plant tissue was harvested 24, 72 and 120 hours after treatment (HAT) and dissected into treated leaf (TL), and tissue above (ATL) and below treated leaf (BTL). The TL was washed twice in 20-mL scintillation vials with 5 mL of a wash solution (10% ethanol aqueous solution and 0.5% Tween-20) for 1 min at each time. The radioactivity in the rinsate was measured using liquid scintillation spectrometry (LSS; Beckman Coulter LS6500 Multipurpose Scintillation Counter, Beckman Coulter, Inc. Brea, CA, USA) after adding 15 mL of Ecolite-R (MP Biomedicals, LLC. Santa Ana, CA, USA). The dissected plant parts were oven dried at 60 ^{o}C for 72 h and then combusted in a biological oxidizer (OXU-501, RJ Harvey Instrument, New York, USA). Radioactivity in each plant part was estimated using LSS. Total absorption of [^{14}C]-herbicide was determined as % absorption = (total radioactivity applied – radioactivity recovered in wash solution) × 100/total radioactivity applied. Herbicide translocation was determined as: % translocation = 100 - % radioactivity recovered in TL, where % radioactivity recovered in TL = radioactivity recovered in TL × 100/radioactivity absorbed. The experiment was repeated twice in time.

Data Analysis

Shoot dry biomass as percent of non-treated was regressed over dicamba and glyphosate dose using a four- and three-parameter log logistic model in R (Ritz et al., 2015; Seefeldt et al., 1995). The lack-of-fit test (P>0.05) indicated that the chosen model accurately described the data. Regression analyses were further used to estimate effective dicamba and glyphosate doses required to reduce Palmer amaranth dry shoot biomass by 50% (GR_{50}). Mortality and shoot biomass data were also presented as percent of non-treated. Absorption and translocation data were subjected to analysis of variance and means separated using Fisher’s protected LSD at α = 0.05 in SAS (SAS, 2011).

Results and Discussion

Dicamba and Glyphosate Dose Response Study

The data from 2017 and 2018 were combined because no treatment by year interaction was detected for any response variable. In general, Palmer amaranth control increased with increasing doses of dicamba and glyphosate. However, Palmer amaranth response varied with herbicide (Figure 1). The GR_{50} values were estimated as 47.1 and 72.3 g ae ha^{-1} for dicamba and glyphosate, respectively, suggesting that this Palmer amaranth accession is 1.5-fold more sensitive to dicamba than glyphosate (Table 4-1). Differential response of Palmer amaranth to different herbicides has been reported previously. For example, Bond et al. (2006) found greater reduction in Palmer amaranth shoot biomass in response to fomesafen than glyphosate and pyrithiobac treatment. Similarly, Kohrt and Sprague (2017) reported variation in Palmer amaranth biomass accumulation in response to various hydroxyl-phenylpyruvate dioxegenase (HPPD)-inhibiting herbicides and atrazine. They reported greater reduction in Palmer amaranth biomass accumulation when treated with tolpyralate and tembotrione than topramezone, mesotrione and atrazine. Although this level of variation in sensitivity to glyphosate is normal, documenting the effects of inclusion of other effective modes of action of herbicides, such as dicamba in combination with glyphosate might prevent evolution of resistance to these herbicides in this Palmer amaranth accession.

Dicamba and Glyphosate Tank-Mix Studies

No interaction was detected between the study, year and treatments for any response variable; therefore, data were pooled. Palmer amaranth response to dicamba and glyphosate applied in combination is presented as biomass reduction relative to non-treated plants at 4 WAT (Table 4-2). When glyphosate or dicamba were applied separately at their respective label recommended doses, shoot biomass was reduced to 26.13 and 26.80% relative to the non-treated control. However, when either a one-sixteenth (52.5 g ae ha^{-1}) or quarter (210 g ae ha^{-1}) of the label recommended dose of glyphosate was added to the labelled dose of dicamba (560 g ae ha^{-1}), the shoot biomass was reduced to 20.52 and 19.37% of control, respectively. On the other hand, when either a one-sixteenth (35 g ae ha^{-1}) or quarter (140 g ae ha^{-1}) of the label recommended dose of dicamba was added to labelled rate of glyphosate (840 g ae ha^{-1}), biomass was reduced to 17.72 and 4.11% of control, respectively. This suggests that while adding reduced rates of glyphosate to dicamba may have some additive effects, the opposite (adding reduced doses of dicamba to glyphosate) seems to display more of a synergistic effect on Palmer amaranth control. The benefit of mixing herbicides with different modes or sites of action has been emphasized in several studies (Johnson and Gibson, 2006; Beckie and Reboud, 2009). Although these findings highlight the value of including dicamba in glyphosate applications to manage Palmer amaranth, it is important to also investigate how the proportion of each herbicide in a tank mixture affects their interaction.

Dicamba and Glyphosate Absorption and Translocation Studies

No interaction was detected between the study, experiment run and treatments for any response variable; therefore, data were pooled. More [^{14}C] dicamba was absorbed at 24 and 72 HAT when dicamba was applied in combination with glyphosate than alone (Figure 4-2A). For example, when dicamba was applied alone, only 74.3 and 84.3% of [^{14}C] dicamba was absorbed at 24 and 72 HAT, respectively. Whereas, when dicamba was applied in combination with glyphosate, 91.4 and 97.1% of [^{14}C] dicamba was absorbed, respectively, at the same time points. However, after 120 HAT, there was no difference in [^{14}C] dicamba absorbed between dicamba applied alone (93.3%) or in combination with glyphosate (95.8%; Figure 4-2A). This suggests that dicamba was absorbed faster when tank mixed with glyphosate. The rapid absorption of dicamba could be due to the enhancement effect of ammonium sulfate on the apoplastic pH which makes dicamba more lipophilic (Ou et al., 2018); however, this hypothesis was not tested. However, an opposite pattern was observed with translocation of [^{14}C] dicamba. More [^{14}C] dicamba was translocated at 24 and 72 HAT when applied alone than in combination with glyphosate (Figure 4-3A). For example, when applied alone, 57.3 and 69.1% of [^{14}C] dicamba was translocated at 24 and 72 HAT, respectively. Conversely, when applied in combination with glyphosate, only 39.1 and 48.8% of [^{14}C] dicamba was translocated at 24 and 72 HAT, respectively. Similar to absorption, however, dicamba translocation at 120 HAT did not differ between dicamba applied alone (69.9%) or in combination with glyphosate (70.9%; Figure 4-3A). Thus, the translocation of dicamba appears to be slower when glyphosate is added to the mixture. Additionally, more [^{14}C] dicamba was translocated upward to above treated leaf (Figure 4-4A) and downward to below treated leaf (Figure 4-4C) at 24 and 72 HAT when dicamba was applied alone than in combination with glyphosate which had more [^{14}C] dicamba retained in the treated leaf (Figure 4-4B). Similarly, Ou et al. (2018) found that more [^{14}C] dicamba was retained in the treated leaf and less moved to above and below treated leaf when dicamba was applied in combination with glyphosate than alone on kochia. This was likely due to glyphosate-induced physiological alterations in plants (Ou et al, 2018).

The amount of [^{14}C] glyphosate absorbed by Palmer amaranth increased over time regardless of whether glyphosate was applied alone or in combination with dicamba (Figure 4-2A). Less [^{14}C] glyphosate was absorbed at 24, 72 and 120 HAT when glyphosate was applied in combination with dicamba than when it was alone (Figure 4-2B). For example, when glyphosate was applied in combination with dicamba, only 64.9, 65.6 and 75.4% of [^{14}C] glyphosate was absorbed at 24, 72 and 120 HAT, respectively. Whereas when glyphosate was applied alone, 74.6, 79.4 and 82.9% of [^{14}C] glyphosate was absorbed at 24, 72 and 120 HAT, respectively. These data suggest that the addition of dicamba to glyphosate may have reduced [^{14}C] glyphosate absorption. Interestingly, the slow absorption (≤65.6% of applied at 24 and 72 HAT) and a rapid change in the amount of [^{14}C] glyphosate absorbed when glyphosate was applied alone or in combination with dicamba (13.8% at 72 HAT to 7.3% at 120 HAT) also implies that there is a possibility that glyphosate absorption was still occurring beyond 72 HAT. Ou et al. (2018) reported a continued [^{14}C] glyphosate absorption in kochia up to 168 HAT regardless of whether glyphosate was applied alone or in combination with dicamba. Conversely, in this study translocation of [^{14}C] glyphosate was faster and significantly increased when glyphosate was applied with dicamba than alone (Figure 4-3B). For example, when glyphosate was applied with dicamba, 36.6% of [^{14}C] glyphosate was absorbed at 24 HAT compared to only 16.3% when applied alone.

Translocation of [^{14}C] glyphosate increased from 43.9% when glyphosate was applied alone to 56.3% when glyphosate was applied with dicamba 120 HAT (Figure 4-3B). This, suggests that, translocation of [^{14}C] glyphosate is not only faster but greater when dicamba is added with glyphosate. As a result, more [^{14}C] glyphosate was translocated more rapidly to above treated leaf (Figure 4-5A) and less was retained in the treated leaf (Figure 4-5B) when glyphosate was applied in combination with dicamba than alone. This is especially important because glyphosate distribution in the plant is influenced by the source-to-sink strength (Sandberg et al., 1980). Thus, an increase in its upward translocation and or accumulation above the treated leaf will result in the herbicide being more effective.

Conclusions

In summary, the results from this study suggest that tank mixing dicamba and glyphosate has a synergistic interaction on Palmer amaranth control. The physiological mechanism attributed to this synergistic interaction appears to be a rapid absorption of dicamba and increased translocation of glyphosate. Taken together, these results suggest that tank mixing dicamba and glyphosate offer an opportunity to mitigate the risk of evolution of herbicide resistance in Palmer amaranth. Future work should investigate if the proportion of herbicides in a tank mixture affects how they interact while considering the possibility of selecting for resistant individuals.

References

Barrett M (1989) Antagonism of Glyphosate Toxicity to Johnsongrass (*Sorghum halepense*) by 2,4-D and Dicamba. Weed Science, 37(5):700-705

Benbrook C (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe, 28(1):1-15

Bond JA, Oliver LR, Stephenson IV DO (2006) Response of Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate, fomesafen, and pyrithiobac. Weed Technology, 20(4):885-892

Brachtenbach DA (2015) Kochia scoparia response to dicamba and effective management practices for soybeans. Doctoral dissertation. Manhattan, KS: Kansas State University

Byker HP, Soltani NE, Robinson DH, Sikkema PC, Van Wely AB, Lawton M (2017) Single and sequential applications of dicamba for the control of glyphosate-resistant common ragweed in glyphosate-and dicamba-resistant soybean. Canadian Journal of Plant Science, 98(3):552-556

Heap I (2019) The International Survey of Herbicide Resistant Weeds. Online. Internet. Accessed January 5, 2019. Available www.weedscience.org

Huff JA (2010) Interactions of Glyphosate and Dicamba in Controlling Key Weed Species. Doctoral dissertation. Oxford, MS: Mississippi State University

Joseph DD (2014) Evaluation of 2, 4-D and dicamba based herbicide programs for weed control in soybean. Doctoral dissertation. Clemson, SC: Clemson University

Joseph DD, Marshall MW, Sanders CH. Efficacy of 2, 4-D, Dicamba, Glufosinate and Glyphosate Combinations on Selected Broadleaf Weed Heights. American Journal of Plant Sciences, 9(06):1321

Kohrt JR, Sprague CL (2017) Response of a Multiple-Resistant Palmer Amaranth (*Amaranthus palmeri*) Population to Four HPPD-Inhibiting Herbicides Applied Alone and with Atrazine. Weed Science, 65(4):534-545

Meyer CJ (2018) Mitigating the Risk for Glufosinate Resistance. Doctoral dissertation. Fayetteville, AR: University of Arkansas. Available https://scholarworks.uark.edu/etd/3081

O'Sullivan P, O'Donovan J (1980) Interaction between glyphosate and various herbicides for broadleaved weed control. Weed Research, 20(4):255-260

Ou J, Thompson CR, Stahlman PW, Bloedow N, Jugulam M (2018) Reduced Translocation of Glyphosate and Dicamba in Combination Contributes to Poor Control of *Kochia scoparia*: Evidence of Herbicide Antagonism. Scientific reports, 8(1):5330

Ou, J (2018) The mechanism (s) and management of dicamba resistance in kochia (*Kochia scoparia*). Doctoral dissertation. Manhattan, KS: Kansas State University

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. PLoS ONE 10(12): e0146021. https://doi.org/10.1371/journal.pone.0146021

Sandberg CL, Meggitt WF, Penner D (1980) Absorption, translocation and metabolism of 14C-glyphosate in several weed species. Weed Research, 20:195-200

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed technology, 9(2):218-27

Spaunhorst D, Bradley K (2013) Influence of Dicamba and Dicamba plus Glyphosate Combinations on the Control of Glyphosate-Resistant Waterhemp (*Amaranthus rudis*). Weed Technology, 27(4):675-681

Underwood M, Soltani N, Hooker D, Robinson J, Sikkema C, Vink P, Swanton (2017) Benefit of tank mixing dicamba with glyphosate applied after emergence for weed control in dicamba- and glyphosate-resistant soybean. Canadian Journal of Plant Science, 97(5):891-901

Zimmer MG, Young B, Johnson W (2018) Weed Control with Halauxifen-Methyl Applied Alone and in Mixtures with 2,4-D, Dicamba, and Glyphosate. Weed Technology, 32(5):597-602.

Table 4-1. Effective dicamba or glyphosate dose required to reduce Palmer amaranth shoot biomass by 50% (GR_{50}) in a greenhouse dose response study at Kansas State University, Manhattan, KS in 2017 and 2018.

Herbicide	**GR_{50} (SE)**
	------------ g ae ha^{-1} ------------
Dicamba	47.1 (10.6)
Glyphosate	72.3 (32.9)

Table 4-2. Palmer amaranth control at 4 weeks after treatment when dicamba and glyphosate are applied alone or in combination in a greenhouse efficacy study at Kansas State University, Manhattan, KS in 2017 and 2018.

Herbicide	**Rate**	**Control** **Mortality**	 **Biomass (SE)**[†]
	(g ae ha^{-1})	---------------------------- %	--------------------------
Control	0	0	100.00 (0.063)
Dicamba	560	100	26.13 (0.046)
Dicamba+glyphosate	560+52.5	100	20.52 (0.034)
	560+210	100	19.37 (0.044)
Glyphosate	840	100	26.80 (0.278)
Glyphosate+dicamba	840+35	100	17.72 (0.368)
	840+140	100	4.11 (0.029)

[†] Biomass values are % of non-treated. SE is the absolute standard error of the mean.

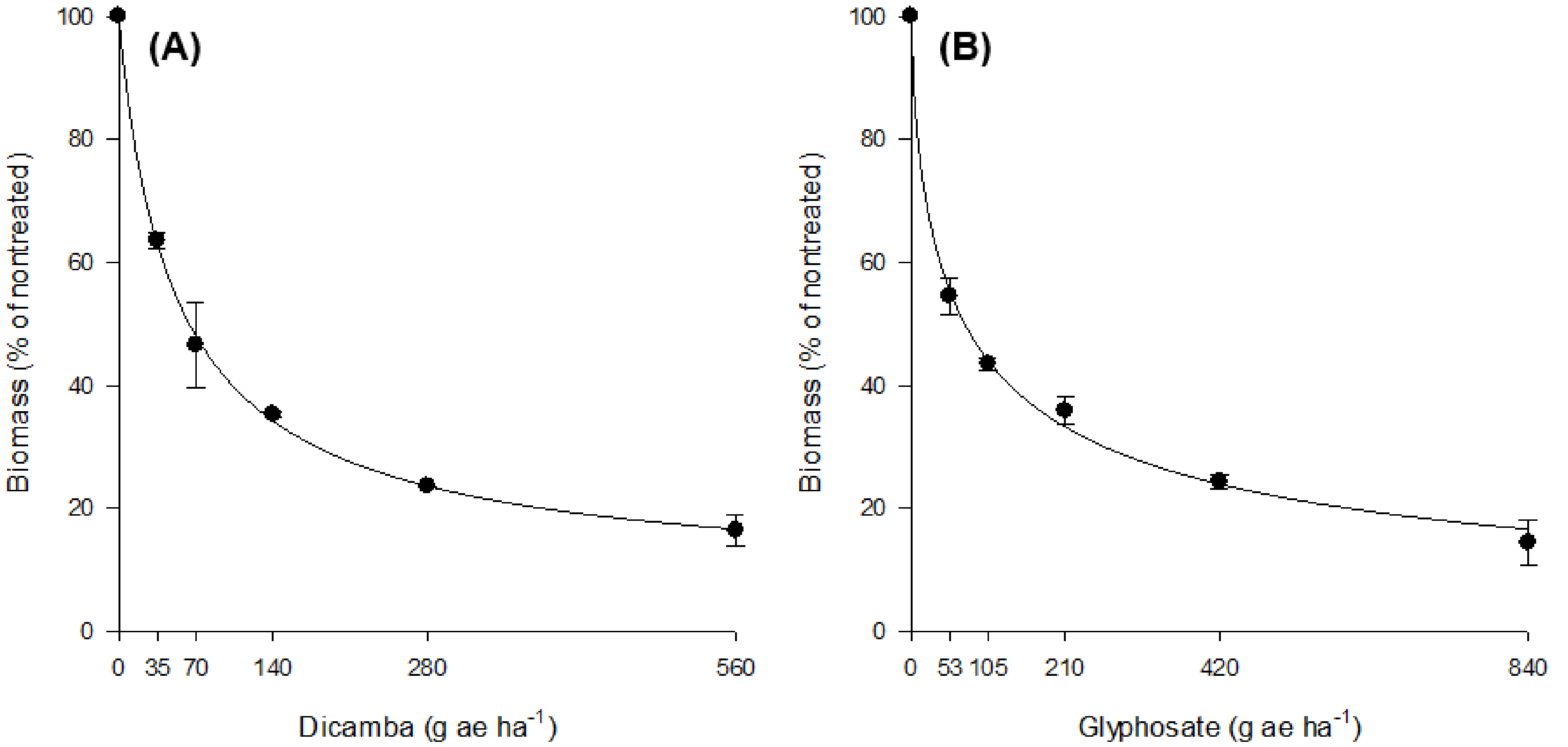

Figure 4-1. Palmer amaranth response to dicamba (A) or glyphosate (B) in a greenhouse study at Kansas State University, Manhattan, KS in summer 2017 and 2018.

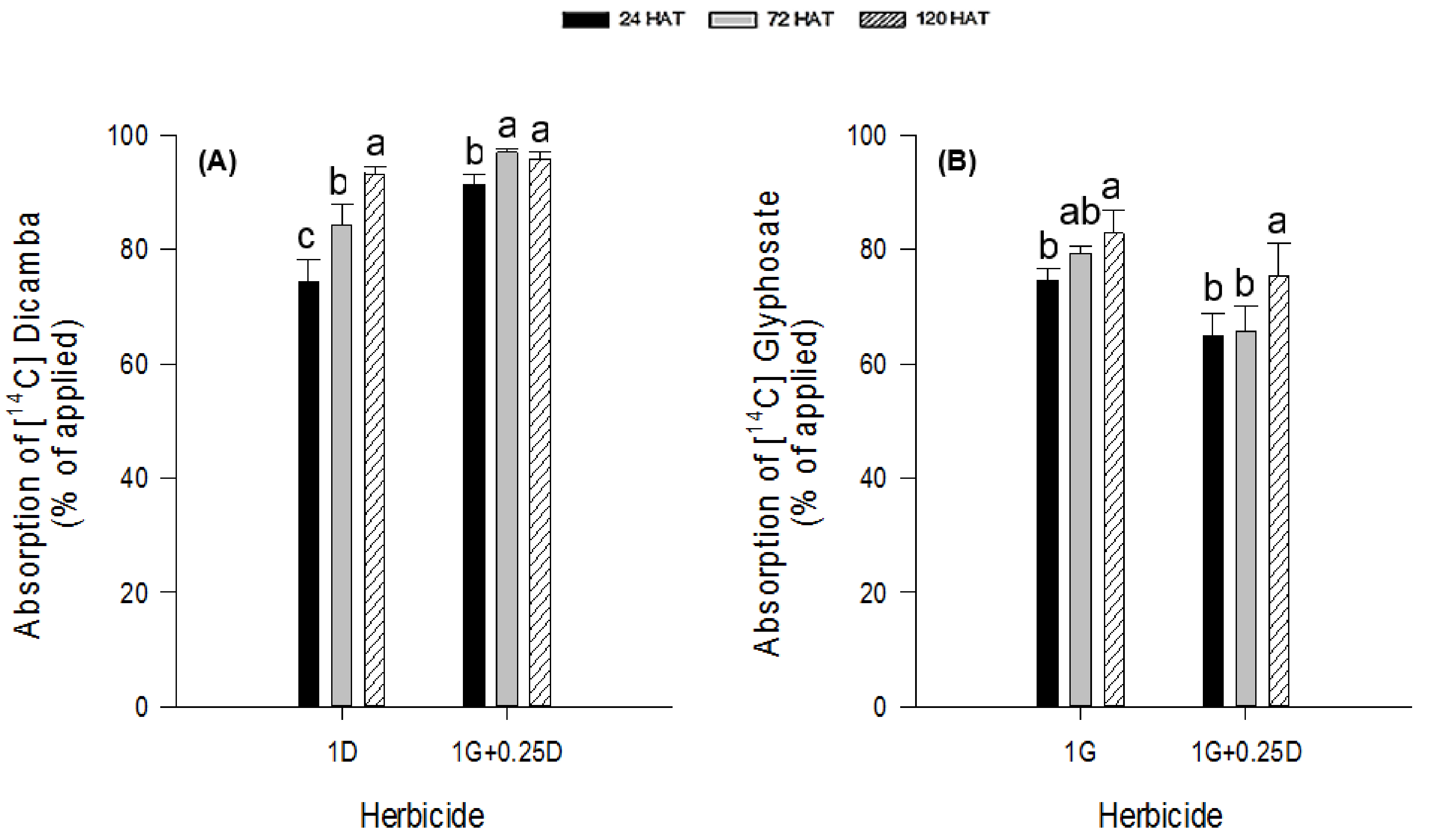

Figure 4-2. Palmer amaranth absorption of [^{14}C] dicamba applied alone (1D) or in combination with glyphosate (1G+0.25D) (A). Figure 2B illustrates the absorption of [^{14}C] glyphosate applied alone (1G) or in combination with dicamba (1G+0.25D) in Palmer amaranth. Bars represent hours after treatment (HAT), and error bars are standard error of the mean (n=8). Lowercase letters indicate significant difference between hours after treatment within the same treatment at α=0.05.

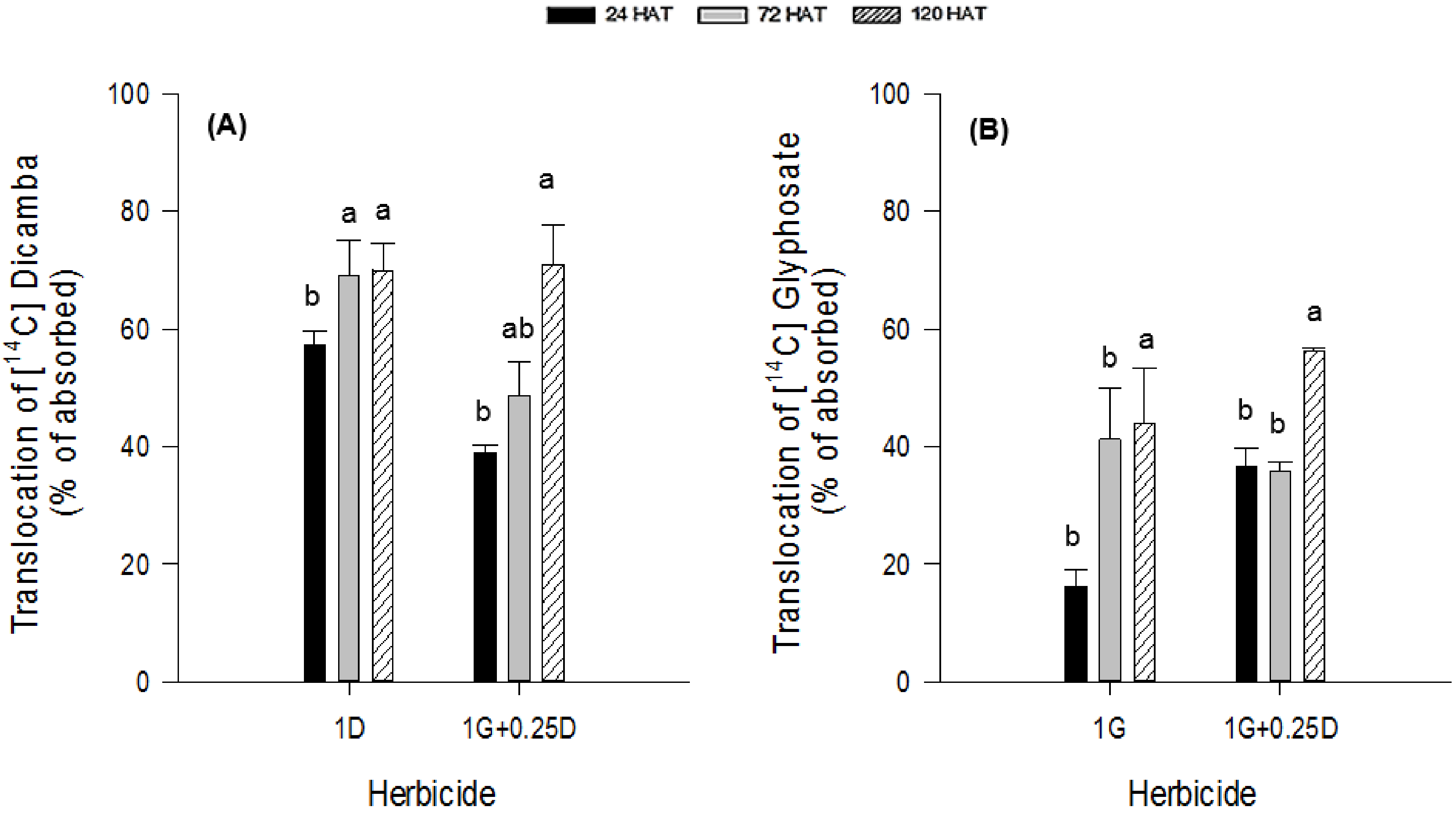

Figure 4-3. Palmer amaranth translocation of [^{14}C] dicamba applied alone (1D) or in combination with glyphosate (1G+0.25) (A). Figure 3B illustrates the translocation of [^{14}C] glyphosate applied alone (1G) or in combination with dicamba (1G+0.25D) in Palmer amaranth. Bars represent hours after treatment (HAT), and error bars standard error of the mean (n=8). Lowercase letters indicate significant difference between hours after treatment within the same treatment at α=0.05.

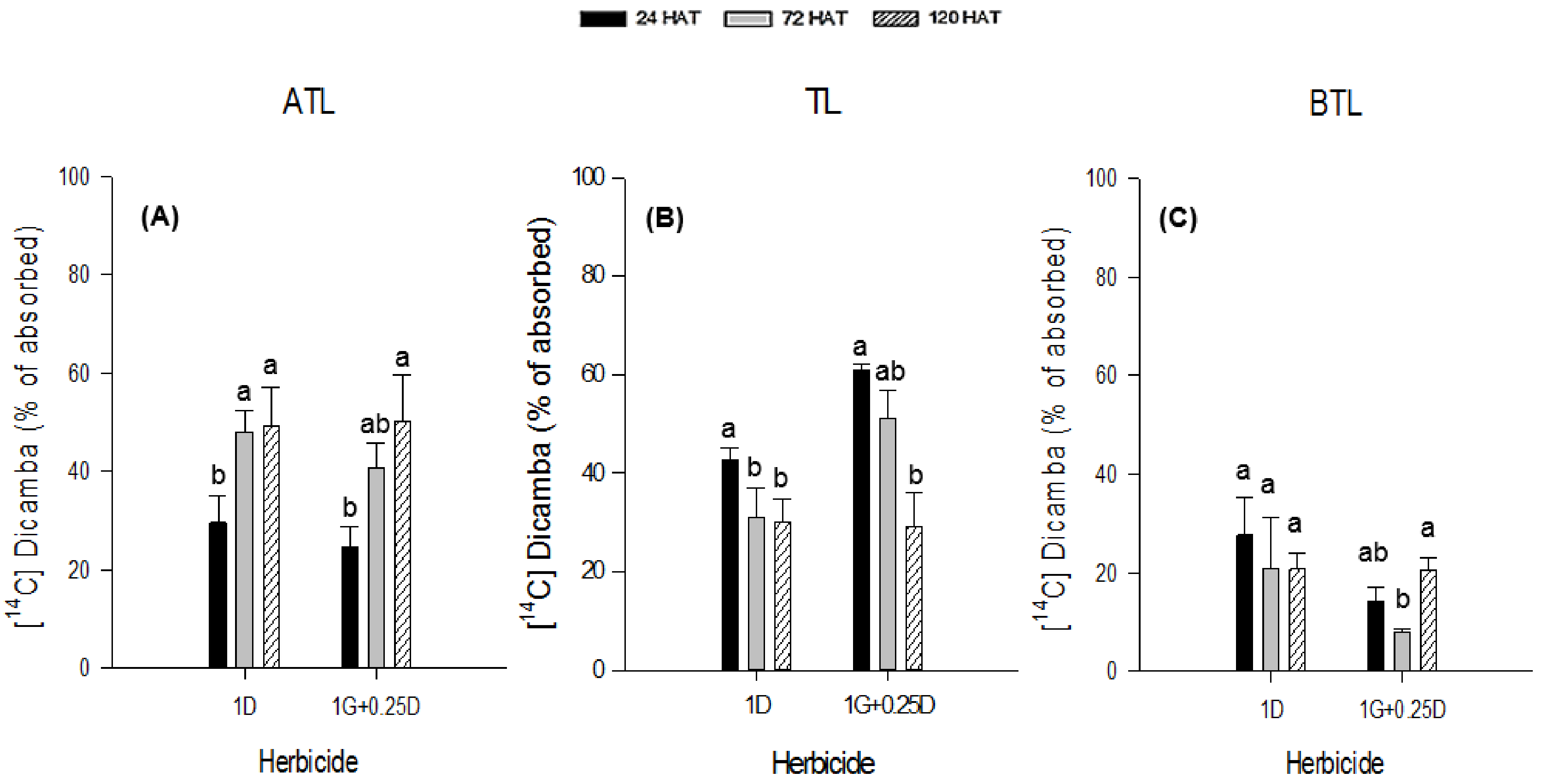

Figure 4-4. Palmer amaranth translocation of [^{14}C] dicamba applied alone (1D) or in combination with glyphosate (1G+0.25D) to above and below treated leaf (ATL and BTL; (A) and (C), respectively), retained in the treated leaf (TL; (B). Bars represent hours after treatment (HAT), and error bars standard error of the mean (n=8). Lowercase letters indicate significant difference between hours after treatment within the same treatment at α=0.05.

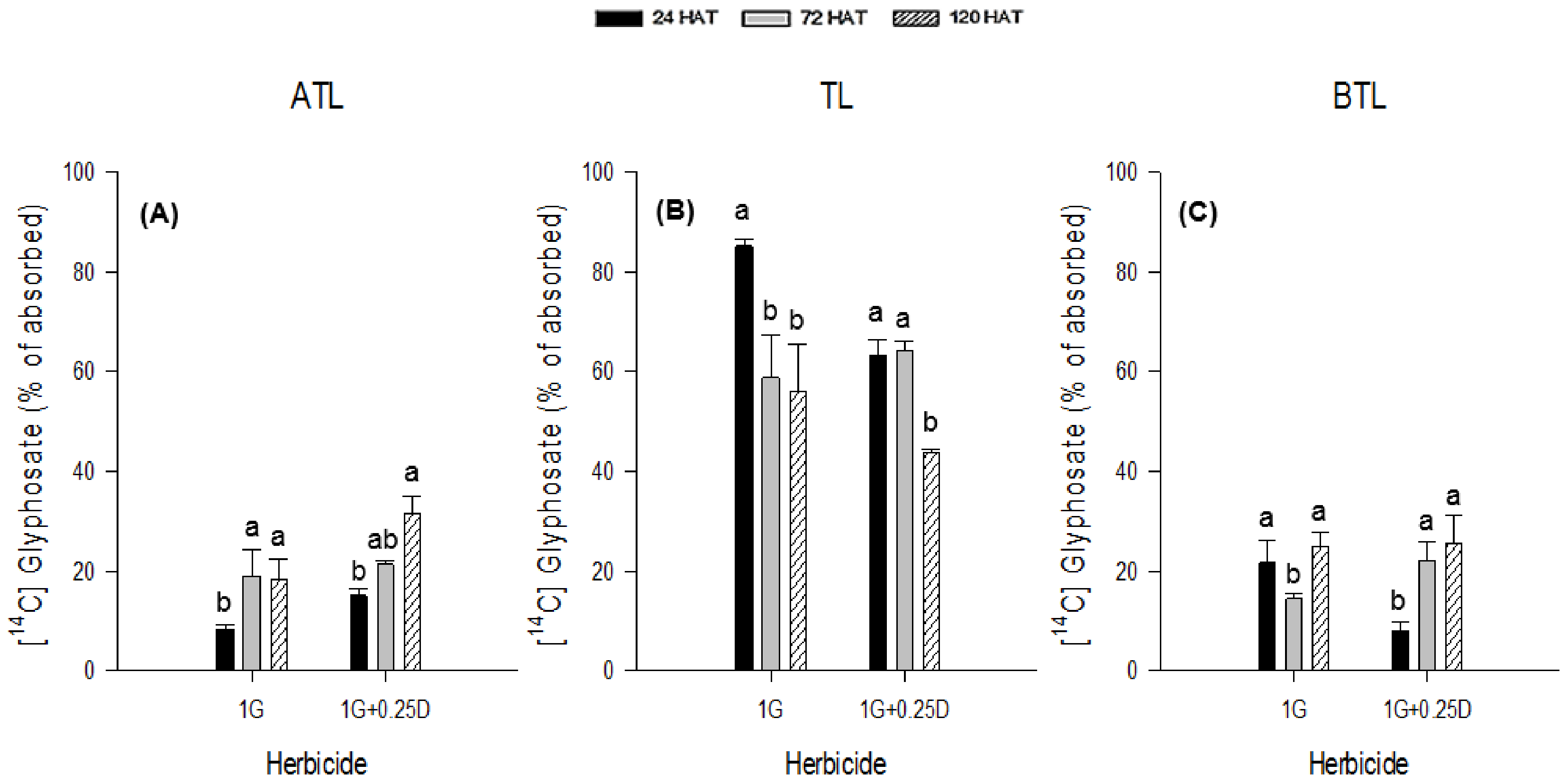

Figure 4-5. Palmer amaranth translocation of [^{14}C] glyphosate applied alone (1G) or in combination with dicamba (1G+0.25D) to above and below treated leaf (ATL and BTL; (A) and (C), respectively), retained in the treated leaf (TL; (B)). Bars represent hours after treatment (HAT), and error bars standard error of the mean (n=8). Lowercase letters indicate significant difference between hours after treatment within the same treatment at α=0.05.

Chapter 5 - Interaction Effects of Irrigated Corn Plant Population and Dicamba Dose on Palmer amaranth Control

Abstract

Field studies were conducted in 2017 and 2018 near Garden city, KS to examine the effect of interaction between crop plant population and dicamba dose on control of Palmer amaranth in irrigated corn. The experiments used a randomized complete block design with four replicates and a split-plot arrangement of treatments. Main plots consisted of corn planted at five plant population densities (49,400; 61,700; 74,100; 86,400 and 98,800 plants ha^{-1}) and sub-plots consisted of six dicamba doses [(70, 140, 210, 280, 4200, and 560 g ae ha^{-1}) applied as late-POST (~V6)], a weedy-check, and a weed-free check. Palmer amaranth was broadcast-seeded uniformly in all experimental units at ~535 g ha^{-1} before corn planting. There was an interaction effect of corn plant population and dicamba dose on corn grain yield and Palmer amaranth height, density, and biomass accumulation. Palmer amaranth control increased with increasing corn plant population and dicamba dose, but the dicamba dose required to reduce Palmer amaranth growth declined as corn plant population increased. The effective dose of dicamba required to reduce Palmer amaranth growth by 50% (GR_{50}) as corn plant population increased from 49,400 to 98,800 plants ha^{-1} ranged from 331.096 to 423.299, 144.471 to 241.947, and 112.482 to 253.410 g ae ha^{-1}, for Palmer amaranth height, density and shoot biomass respectively. Similarly, increasing corn plant population and dicamba dose increased corn grain yield at sub-label doses of dicamba; however, there was no benefit of increasing corn plant population when the label-recommended dose of dicamba (560 g ae ha^{-1}) was applied. This study shows that there is an opportunity to maintain grain yield of corn while effectively controlling Palmer amaranth in irrigated corn with the integration of crop plant population and dicamba.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; Corn, *Zea Mays* L.

Key words: Corn, Dicamba, Palmer amaranth, interaction

Introduction

Palmer amaranth (*Amaranthus palmeri*) is a major constraint to crop production in the North American Great Plains including Kansas (Rule, 2007; Nakka, 2016; McCall, 2018) where corn acreage is second to wheat (Kansas Department of Agriculture, 2019). Due to wide spread irrigation and recent increase in non-irrigated acres, the western part of the state accounts for most of the land under corn production (Kansas Department of Agriculture, 2016). Palmer amaranth interferes with corn production in Kansas (Massinga et al., 2001). Its deep and extensive root system allows the weed to use resources particularly water and nutrients more efficiently than most crops (Sosnoskie et al., 2011). Palmer amaranth's competitive ability is further complemented by its high photosynthetic activity which is higher than other C_4 species (Ward et al., 2013) including corn (Steckel, 2007). Massinga et al., 2001 reported 11 to 91 percent reduction in grain yield when Palmer amaranth infestation at 0.5 to 8 plants m^{-1} row competed with corn from its emergence. Others have reported similar yield reductions in corn and other crops (Brooks, 2013; Putman 2013; Chahal 2015).

Although producers have effectively controlled Palmer amaranth in the past, its propensity to evolve resistance to herbicides has begun to limit weed control options. Dicamba (3,6-dichloro-2-methoxybenzoic acid) has been proven effective for control of several broadleaf weeds (Soltani et al., 2016; Ganie and Jhala, 2017) including difficult-to-control species (Norsworthy et al., 2011) such as Palmer amaranth (Inman et al., 2016; Underwood et al., 2017). This is an especially viable option because no cases of Palmer amaranth resistance to dicamba have been reported yet (Heap, 2019). Despite its effectiveness, dicamba's susceptibility to drift remains a major concern even with the availability of formulations developed to reduce its off-target movement (Osipitan et al., 2019). In this research, we tested the efficacy of reduced dicamba doses on Palmer amaranth control in irrigated corn production. We hypothesized that the integration of reduced dicamba dose in conjunction with increased corn plant population density could provide acceptable Palmer amaranth control while maintaining grain yield.

Materials and Methods

Experimental Site

Field experiments were conducted at the Kansas State University Southwest Research-Extension Center near Garden City, KS (37°59’38.4” N, 100°49’04.8” W) in 2017 and 2018 growing seasons. The site was irrigated and had been in a fully irrigated corn-wheat cropping sequence since 2004. The climate at the site is semi-arid, and the soils are predominantly Richfield silt loam (fine, montmorillonitic, mesic Aridic Argiustoll) with <1% slope.

Experimental Design and Management

Fields were disked and then the final seedbed was prepared with a field cultivator before planting to corn ‘DKC62-97RIB’ (Monsanto Co., St. Louis, MO) on May 23rd in 2017 and May 6th in 2018. The studies used a randomized complete block experimental design (RCBD) with four replicates and a split-plot arrangement of treatments. Main plot treatments consisted of five corn plant population densities (49,400; 61,700; 74,100; 86,400 and 98,800 plants ha^{-1}), and subplot treatments consisted of six dicamba doses [(70, 140, 210, 280, 420, and 560 g ae ha^{-1}) applied as late-POST near the V6 developmental stage of corn with 0.25% (v/v) nonionic surfactant], a weedy-check, and weed-free check. All experimental units were broadcast-seeded with Palmer amaranth at 535 g ha^{-1} (~1,078,293 seeds per ha) before corn planting to ensure a uniform infestation across all experimental units. The main plots were 61 m-long with six rows spaced 76 cm apart. The subplots were 7.62 m-long with six rows spaced 76 cm apart. Weed-free plots were hand-hoed as required throughout the growing season. All other weed species were removed from the entire plot area by hand hoeing. Irrigation water was supplied (with no more than two 25-mm irrigation events weekly) as required throughout the growing seasons at non-yield limiting rates based on locally derived models.

Data Collection and Analysis

Data were collected from the two central rows of each subplot and included Palmer amaranth height (m), density (number of plants m^{-2}) and shoot biomass (kg m^{-2}) on a dry weight basis; and corn grain yield. Both corn and Palmer amaranth measurements were performed at corn physiological maturity. Corn grain was combine-harvested from the two central rows of each subplot in October of each year, and yield was adjusted to 15.5% moisture.

Data were analyzed using the PORC MIXED procedure for analysis of variance (ANOVA) in SAS 9.3 (SAS Institute, Inc., Cary, NC) and means were separated using Fisher's Protected Least Significant Difference test at $\alpha = 0.05$ when the ANOVA indicated treatment effects were significant. Year effect and the significance of interaction between year, corn plant population and dicamba dose also were tested at $\alpha = 0.05$. If the year effect and or interaction of year with treatment were not significant, data were pooled. The interaction between corn plant population and dicamba dose on Palmer amaranth height and density, and shoot biomass was further tested in R 3.4.1 (R Core Team, 2017) using a three- and four-parameter log-logistic regression model, respectively, to determine the effective dose of dicamba required to reduce Palmer amaranth height, density and shoot biomass by 50% (ED_{50}) (Knezevic et al., 2007). The lack-of-fit test ($P>0.05$) indicated that the chosen model accurately described the data.

Results and Discussion

Analyses of data from 2017 and 2018 suggested the year effect was not significant ($p>0.05$). However, a two-way interaction effect was detected between corn plant population and dicamba dose on Palmer amaranth height, density and shoot biomass, as well as on corn grain yield, $p<0.05$. Therefore, data were analyzed for the effect of corn plant population within each dicamba dose and vice versa.

Palmer Amaranth Height

Results showed reduction in Palmer amaranth height reduced with increasing corn plant population and dicamba dose (Figure 5-1). However, increasing dicamba dose resulted in a greater reduction in Palmer amaranth height than increasing corn plant population (Table 5-1). For example, Palmer amaranth height was reduced by 9.2% from 1.42 to 1.29 m when the corn plant population was increased from 49,400 to 98,800 plants ha^{-1} in non-treated corn. In Contrast, when dicamba was applied at the label recommended dose (560 g ae ha^{-1}), Palmer amaranth height was reduced at least 37.2% relative to Palmer amaranth height in non-treated corn regardless of corn plant population. Furthermore, unlike dicamba dose, increasing corn plant population did not produce a clear trend for reduction in Palmer amaranth height. However, the greatest reduction in Palmer amaranth height was recorded when the label recommended dose of dicamba was applied to the highest corn plant population of 98,800 plants ha^{-1}. This lack of a clear trend in the reduction of Palmer amaranth height with increasing corn plant population was observed across all dicamba doses including non-treated corn. Despite this unclear trend, the greatest reduction in Palmer amaranth height was observed when dicamba was applied at the label recommended dose and corn planted at 98,800 plants ha^{-1}. Similarly, the effective dose of dicamba required to reduce Palmer amaranth height by 50% (ED_{50}) was also reduced with an increase in corn plant population (Table 5-5). Based on the fitted log-logistic model, the ED_{50} value for corn plant population at 49,400 and 98,800 plants ha^{-1} was 423.299 and 404.666 g ae ha^{-1}, respectively. There was considerable variation in this response at the intermediate corn plant populations.

Palmer Amaranth Density

Increases in corn plant population and dicamba dose reduced the density of Palmer amaranth (Figure 5-2) with a greater reduction with increasing dicamba dose rather than corn plant population (Table 5-2). For example, Palmer amaranth population density in non-treated corn was reduced by 37.5% from 13.6 to 8.5 plants m^{-2} when corn plant population was increased from 49,400 to 98,800 plants ha^{-1}. This effect was masked by dicamba application. When dicamba was applied at one-eighth (70 g ae ha^{-1}) of the label recommended dose, Palmer amaranth population density was reduced at least 9.7% relative to non-treated corn regardless of corn plant population. This masking effect was even more pronounced when dicamba was applied at the label recommended dose (560 g ae ha^{-1}), with Palmer amaranth population density reduced at least by 67% relative to non-treated corn regardless of corn plant population. The corn plant population of 98,800 plants ha^{-1} had the lowest Palmer amaranth density (1.49 plants m^{-2}), but the greatest reduction (79.5%) in Palmer amaranth population density as dicamba dose was increased was observed when corn was planted at 49,400 plants ha^{-1}. These results suggest that although corn population had positive benefits, dicamba application had a greater impact on Palmer amaranth density at lower corn plant populations. It is also possible that a better spray coverage associated with a more open crop canopy might have had confounding effects. Based on the fitted log-logistic model, the effective dose of dicamba required to reduce Palmer amaranth population density by 50% (ED_{50}) was estimated at 144.4 and 209.7 g ae ha^{-1} for corn plant populations of 49,400 and 98,800 plants ha^{-1}, respectively (Table 5-5). As with the Palmer amaranth population density response to corn population density, the ED_{50} values for Palmer amaranth population density did not consistently increase with corn plant population density despite the clear upward trend. This suggests that more dicamba was needed for an effective reduction in Palmer amaranth population density as corn plant population increased.

Palmer amaranth Shoot Biomass

Palmer amaranth shoot biomass decreased with increasing corn plant population and dicamba dose (Figure 5-3). As was seen in Palmer amaranth density, increasing dicamba dose resulted in a greater reduction in Palmer amaranth shoot biomass than increasing corn plant population (Table 5-3). For example, increasing corn plant population from 49,400 to 98,800 plants ha^{-1} in non-treated corn reduced Palmer amaranth shoot biomass only 6.7% from 1.63 to 1.52 kg m^{-2}, respectively. Whereas when dicamba was applied at one-eighth or half (70 and 280 g ae ha^{-1}, respectively) of the label recommended dose, Palmer amaranth shoot biomass was reduced at least 24.5 and 48.3%, respectively, regardless of corn plant population. The greatest reduction in Palmer amaranth shoot biomass, however, occurred when the label recommended dose of dicamba was applied in conjunction with a corn plant population of 98,800 plants ha^{-1}, a 95.7% reduction in shoot biomass relative to non-treated corn planted at 49,400 plants ha^{-1}. Unlike dicamba, increasing corn plant population did not produce a consistent trend in reduction of Palmer amaranth shoot biomass except when dicamba was applied at 140 g ae ha^{-1}. Based on the fitted log-logistic model, 61,700 and 74,100 corn plants ha^{-1} had greater effective doses of dicamba required to reduce Palmer amaranth shoot biomass by 50% (ED_{50}) than 49,400, 86,400 and 98,800 plants ha^{-1}(Table 5). This difference in ED_{50} values between corn plant populations suggests that increasing crop plant population may have reduced the spray coverage but appears to increase weed biomass suppression which is why ED_{50} values were smallest at 49,400, 86,400 and 98,800 plants ha^{-1}. Crespo et al. (2016) also reported that increasing dicamba dose from 0 to ≥560 g ae ha^{-1} rapidly decreased Palmer amaranth shoot biomass regardless of Palmer amaranth populations tested.

Corn Grain Yield

Although the benefit of increasing corn plant population was not evident in non-treated corn, the results of this study showed an increase in grain yield with increasing corn plant population and dicamba dose. However, increasing dicamba dose resulted in greater corn grain yield than increasing corn plant population. For example, when corn plot was kept weed-free, increasing plant population from 49,400 to 98,800 plants ha^{-1} increased grain yield only 24.8% from 10.10 to 12.60 Mg ha^{-1}. Conversely, when dicamba application was increased from 0 to 560 g ae ha^{-1}, corn grain yield was increased at least 43.3% regardless of corn plant population. The greatest increase in grain yield, however, was observed when dicamba was applied at the label-recommended dose (560 g ae ha^{-1}) in conjunction with a corn plant population of 98,800 plants ha^{-1}. However, dicamba application at the label-recommended dose was not always required to achieve corn grain yield similar to that obtained from corn that was maintained weed-free throughout the growing season regardless of crop plant population. Increasing corn plant population up to 98,800 plants ha^{-1} did not always result in a significant increase in grain yield for each dose of dicamba applied. Abuzar et al. (2011) also reported no grain yield benefit to increasing corn plant population beyond 60,000 plants ha^{-1}. In contrast, Robinson and Conley (2007) showed that an increasing crop plant population can have advantages such as quicker canopy closure, greater light interception and lower weed competition. However, they did not always show an increase in yield. Others also reported marginal to no benefit(s) in increasing corn plant population due to interplant competition for resources (Sangakkara et al., 2004). According to Sangoi (2001), interplant competition for resources can lead to a decline in grain yield if the increase in corn plant population is beyond the optimum.

Conclusions

This study showed that acceptable corn grain yield and Palmer amaranth control are possible with the integration of cultural practices and weed control tactics. The interaction between corn plant population and dicamba dose on Palmer amaranth significantly reduced Palmer amaranth height, density, and biomass while at the same time maintaining grain yield at levels close or equal to that of season-long weed-free corn. However, increasing corn plant population alone or in combination with dicamba did not always provide a consistent reduction in Palmer amaranth. Although increasing crop plant population density could reduce spray coverage, its benefits seemed to overcome this effect and increase Palmer amaranth suppression. These findings highlight the need to include pre-emergence herbicides in weed control programs. Even when other best management practices that increase crop competition are considered, an aggressive herbicide program is still required when dealing with species with season-long germination and emergence patterns like Palmer amaranth.

References

Abuzar MR, Sadozai GU, Baloch MS, Baloch AA, Shah IH, Javaid T, Hussain N (2011) Effect of plant population densities on the yield of maize. The Journal of Animal & Plant Sciences, 21(4):692-695

Brooks R (2013) Palmer amaranth pigweed creeps farther into the Midwest. Farm Journal; accessed 8/30/2016; available https://www.agweb.com/article/palmer_amaranth_pigweed_creeps_farther_into_the_midwest_naa_rhonda_brooks/

Chahal PS, Aulakh JS, Jugulam M, Jhala AJ (2015) Herbicide-Resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the United States—mechanisms of resistance, impact, and management. In Herbicides, Agronomic Crops and Weed Biology. InTech.

Crespo RJ, Wingeyer AB, Borman CJ, Bernards ML (2016) Baseline Sensitivity of Nebraska Waterhemp and Palmer Amaranth to Dicamba and 2, 4-D. Agronomy Journal, 108(4):1649-1655

Inman MD, Jordan DL, York AC, Jennings KM, Monks DW, Everman WJ, Bollman SL, Fowler JT, Cole RM, Soteres JK (2016) Long-term management of Palmer amaranth (*Amaranthus palmeri*) in dicamba-tolerant cotton. Weed Science, 64(1):161-169

Kansas Department of Agriculture (2016) Kansas Farm Facts. Retrieved from http://agriculture.ks.gov/docs/default-source/ag-marketing/kansas-farm-facts-2016.pdf?sfvrsn=4

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: The concept and data analysis. Weed Technology, 21:840-848. Doi: 10.1614/WT-06-161.1

Massinga RA, Currie RS, Hoak MJ, Boyer Jr J (2001) Interference of Palmer amaranth in corn. Weed Science, 49:202-208

McCall C (2018) Integrating cover crops and herbicides for horseweed and Palmer amaranth management in no-till soybean. Master's thesis. Manhattan, Kan.: Kansas State University

Nakka (2016) Physiological, Biochemical and Molecular Characterization of Multiple Herbicide Resistance in Palmer Amaranth (*Amaranthus Palmeri*). Doctoral dissertation. Manhattan, Kan.: Kansas State University

Norsworthy JK, Riair D, Jha P, Scott RC (2011) Confirmation, control and physiology of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in Arkansas. Weed Technology 25:430-435. DOI:10.1614/WT-D-10-00155.1

Osipitan OA, Scott JE, Knezevic SZ (2019) Glyphosate-Resistant Soybean Response to Micro-Rates of Three Dicamba-Based Herbicides. Agrosystems, Geosciences & the Environment, 2(1).

Putman J (2013) Palmer amaranth control in established alfalfa and documentation of glyphosate-resistant *Amaranthus* species in Kansas. Master's thesis. Manhattan, Kan.: Kansas State University

Robinson AP, Conley SP (2007) Plant populations and seeding rates for soybeans. West Lafayette, IN: Purdue University Publication No. AY-217-W. Soybean Production Systems: Purdue Extension, 3.

Rule D (2007) Corn and Palmer amaranth interactions in dryland and irrigated environments. Doctoral dissertation. Manhattan, Kan.: Kansas State University.

Sangakkara UR, Bandaranayake PS, Gajanayake JN, Stamp P (2004) Plant populations and yield of rainfed maize grown in wet and dry seasons of the tropics. Maydica, 49: 83-88

Sangoi L (2001) Understanding plant density effect of maize growth and development: an important issue to maximize grain yield. Ciência Rural, 31(1):159-168

Soltani NH, Sikkema PE, Nurse R (2016) Response of glyphosate-resistant soybean to dicamba spray tank contamination during vegetative and reproductive growth stages. Canadian Journal of Plant Science, 96(1):160-164

Sosnoskie LM, Webster TM, Culpepper AS, Kichler J (2011) The biology and ecology of Palmer amaranth: Implications for control. UGA/CPES Research-Extension Circular 1000. Accessed 12/11/2016, available http://extension.uga.edu/publications/detail.cfm?number=C1000

Steckel L (2007) The dioecious *Amaranthus* spp.: Here to stay. Weed Technology 21:567-570

Underwood MG, Soltani NE, Robinson DC, Hooker DH, Sikkema PJ, Swanton CP, Vink J (2017) Weed control, environmental impact, and net revenue of two-pass weed management strategies in dicamba-resistant soybean. Canadian Journal of Plant Science, 98(2):370-379

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): A review. Weed Technology 27(1):12-27

Zahoor AG, Jhala AJ (2017) Interaction of 2,4-D or Dicamba with Glufosinate for Control of Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.) in Glufosinate-Resistant Maize (*Zea mays* L.). Frontiers in Plant Science, 8:1207

Table 5-1. Corn plant population and dicamba dose interaction on Palmer amaranth height. SE is standard error of the mean. LSD is least significant difference at 5% level.

Corn	Palmer amaranth height (SE)							
	Dicamba (g ae ha^{-1})							
	0	70	140	210	280	420	560	LSD$_{.05}$
Plants ha^{-1}	-- m --							
49,400	1.42 (0.02)	0.96 (0.03)	0.91 (0.03)	0.84 (0.02)	0.78 (0.02)	0.77 (0.03)	0.59 (0.05)	0.092
61,700	1.45 (0.08)	1.06 (0.06)	0.93 (0.03)	0.85 (0.03)	0.74 (0.04)	0.74 (0.03)	0.58 (0.04)	0.134
74,100	1.46 (0.08)	1.03 (0.04)	0.92 (0.03)	0.84 (0.03)	0.78 (0.02)	0.69 (0.06)	0.63 (0.04)	0.134
86,400	1.39 (0.07)	1.15 (0.09)	0.90 (0.02)	0.84 (0.04)	0.77 (0.04)	0.65 (0.06)	0.53 (0.02)	0.161
98,800	1.29 (0.15)	1.14 (0.07)	0.96 (0.07)	0.89 (0.04)	0.73 (0.03)	0.71 (0.06)	0.48 (0.05)	0.228
LSD$_{.05}$	ns	0.188	ns	ns	ns	ns	0.124	

ns - indicates non-significant difference at $\alpha = 5\%$.

Table 5-2. Corn plant population and dicamba dose interaction on Palmer amaranth density. SE is standard error of the mean. LSD is least significant difference at 5% level.

Corn	Palmer amaranth density (SE)							
	Dicamba (g ae ha^{-1})							
	0	70	140	210	280	420	560	LSD$_{.05}$
Plants ha^{-1}	-- Plants m^{-2} --							
49,400	13.64 (1.48)	8.73 (1.00)	7.34 (1.03)	5.45 (0.12)	4.83 (0.65)	3.92 (0.89)	2.79 (0.67)	2.715
61,700	11.50 (1.66)	7.85 (0.72)	5.73 (0.42)	5.29 (0.71)	3.75 (0.45)	3.75 (0.25)	2.09 (0.44)	2.343
74,100	9.56 (1.08)	6.73 (0.80)	6.20 (0.98)	4.88 (1.01)	4.59 (0.590	3.19 (0.85)	2.33 (0.62)	2.549
86,400	7.57 (0.76)	6.22 (0.91)	5.03 (0.70)	3.97 (0.41)	3.24 (0.35)	2.63 (0.31)	2.50 (0.00)	2.859
98,800	8.52 (2.03)	7.69 (1.01)	5.66 (0.84)	4.16 (0.22)	3.00 (0.45)	2.66 (0.46)	1.49 (0.54)	2.859
LSD$_{.05}$	4.445	ns	ns	ns	1.542	ns	ns	

ns - indicates non-significant difference at $\alpha = 5\%$.

Table 5-3. Corn plant population and dicamba dose interaction on Palmer amaranth biomass. SE is standard error of the mean. LSD is least significant difference at 5% level.

Corn	Palmer amaranth biomass (SE)							
	Dicamba (g ae ha^{-1})							
	0	70	140	210	280	420	560	$LSD_{.05}$
Plants ha^{-1}	---------- kg m^{-2} ----------							
49,400	1.63 (0.16)	1.23 (0.11)	0.89 (0.13)	0.61 (0.08)	0.48 (0.02)	0.36 (0.06)	0.32 (0.09)	0.298
61,700	1.51 (0.11)	1.01 (0.06)	0.79 (0.07)	0.55 (0.04)	0.42 (0.03)	0.29 (0.03)	0.13 (0.04)	0.180
74,100	1.43 (0.11)	0.89 (0.04)	0.74 (0.06)	0.53 (0.08)	0.38 (0.05)	0.26 (0.15)	0.19 (0.04)	0.197
86,400	1.33 (0.21)	0.82 (0.070	0.57 (0.08)	0.36 (0.05)	0.24 (0.020	0.19 (0.01)	0.09 (0.01)	0.270
98,800	1.52 (0.08)	0.87 (0.13)	0.57 (0.06)	0.39 (0.08)	0.31 (0.04)	0.20 (0.03)	0.07 (0.02)	0.209
$LSD_{.05}$	ns	0.265	0.255	0.195	0.105	0.140	0.146	

ns - indicates non-significant difference at α = 5%.

Table 5-4. Corn plant population and dicamba dose interaction on corn grain yield. SE is standard error of the mean. LSD is least significant difference at 5% level.

Corn	Corn grain yield (SE)								
	Dicamba (g ae ha^{-1})								
	0	70	140	210	280	420	560	Weed-free	LSD$_{.05}$
Plants ha^{-1}	---------- Mg ha^{-1} ----------								
49,400	5.16 (0.14)	5.69 (0.50)	6.00 (0.77)	6.73 (0.46)	7.22 (0.56)	8.67 (0.05)	10.04 (0.61)	10.10 (0.12)	1.55
61,700	5.90 (0.03)	6.68 (0.08)	7.51 (0.44)	8.60 (0.20)	8.93 (0.47)	8.96 (0.41)	10.41 (0.27)	11.14 (0.39)	1.08
74,100	6.18 (0.49)	6.81 (0.79)	8.55 (0.02)	10.15 (0.83)	10.08 (0.08)	10.99 (0.65)	10.97 (0.26)	11.35 (0.23)	1.68
86,400	5.37 (0.39)	8.23 (0.21)	9.54 (0.41)	9.91 (0.34)	9.84 (0.20)	10.24 (0.71)	11.08 (0.63)	11.88 (0.36)	1.44
98,800	3.68 (0.02)	7.47 (0.54)	8.65 (0.78)	9.71 (1.02)	10.07 (0.62)	11.01 (0.24)	11.38 (0.35)	12.60 (0.17)	1.84
LSD$_{.05}$	1.05	1.813	2.04	2.36	1.60	1.76	ns	1.01	

ns - indicates non-significant difference at $\alpha = 5\%$.

Table 5-5. Effective dicamba dose (ED_{50}) required to reduce Palmer amaranth height, density, and shoot-biomass in various corn plant populations by 50%. ED_{50} values were estimated using Palmer amaranth height, density and shoot-biomass (dry weight basis). SE is standard error of the mean**.**

Palmer amaranth attribute	**Corn (plants ha^{-1})**	**ED_{50} (SE)**
		--------- g ae ha^{-1}-------
Height (m)	49,400	423.299 (95.572)
	61,700	349.956 (55.504)
	74,100	357.518 (63.785)
	86,400	331.096 (39.540)
	98,800	404.666 (43.263)
Density (m^{-2})	49,400	144.471 (27.407)
	61,700	153.590 (32.138)
	74,100	223.941 (54.184)
	86,400	241.947 (64.031)
	98,800	209.674 (35.735)
Biomass (kg m^{-2})	49,400	126.634 (23.390)
	61,700	253.410 (87.921)
	74,100	212.988 (37.029)
	86,400	112.482 (36.185)
	98,800	116.281 (60.025)

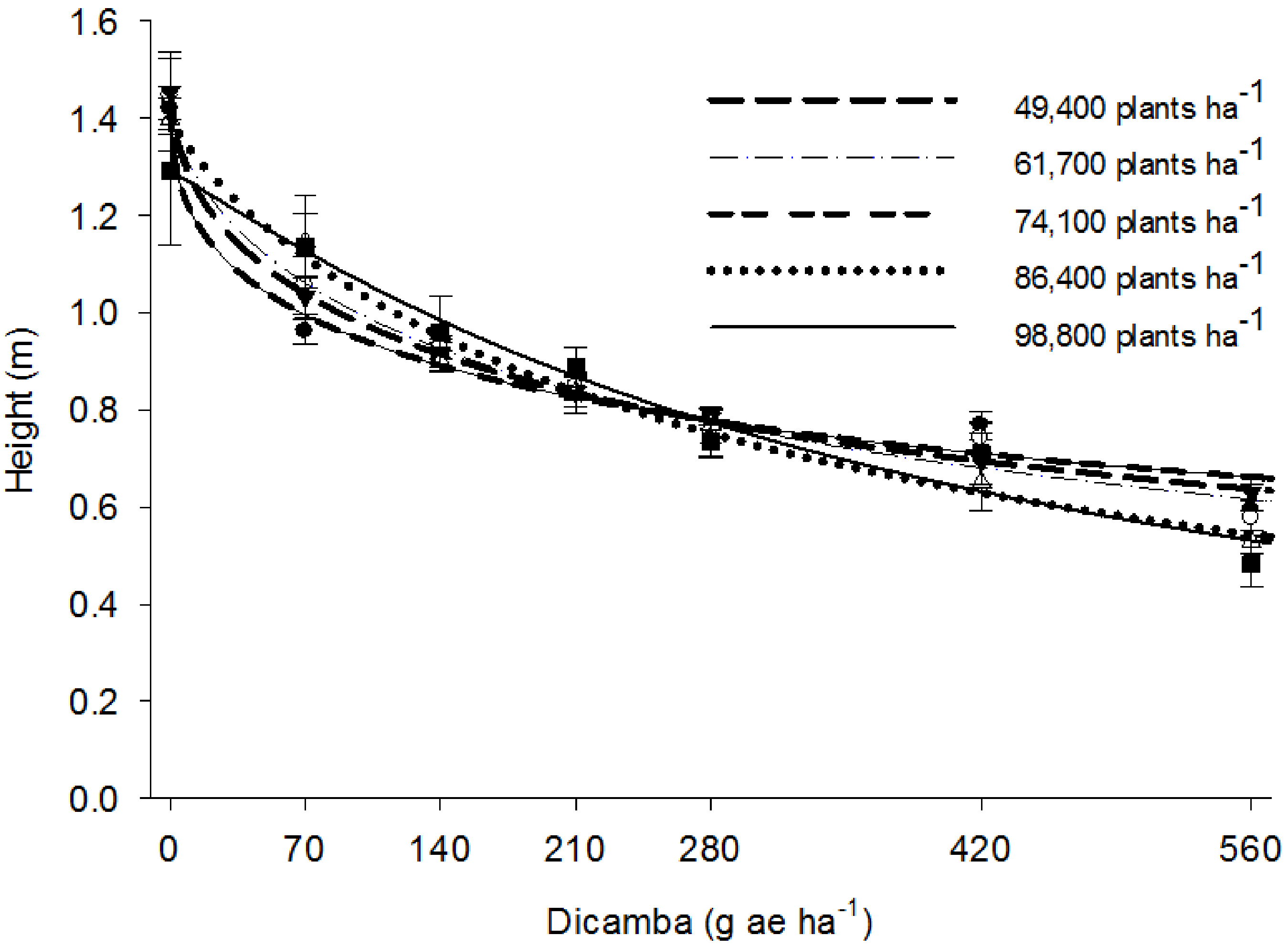

Figure 5-1. Corn plant population and dicamba dose interaction effect on Palmer amaranth height.

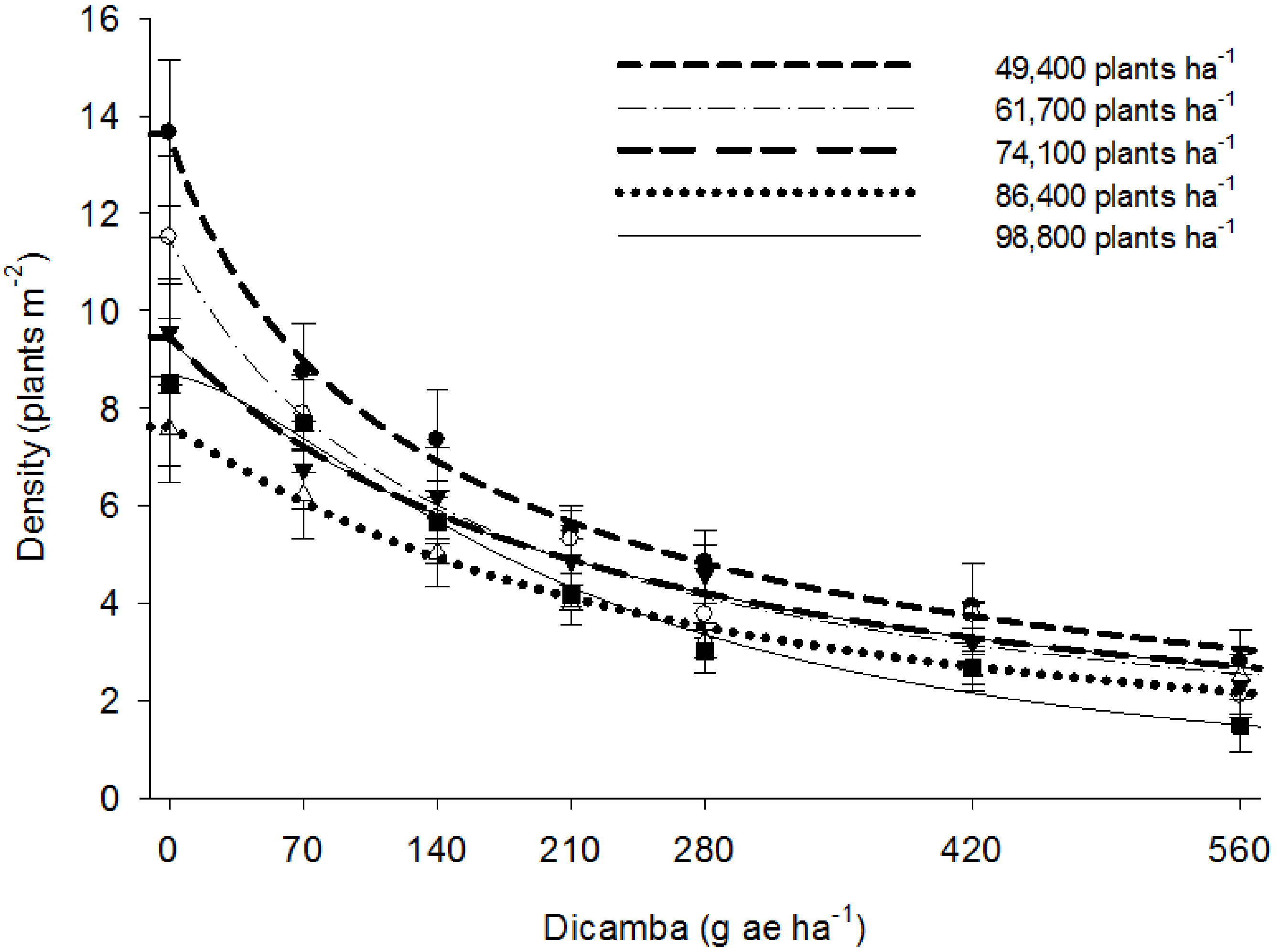

Figure 5-2. Corn plant population and dicamba dose interaction effect on Palmer amaranth density.

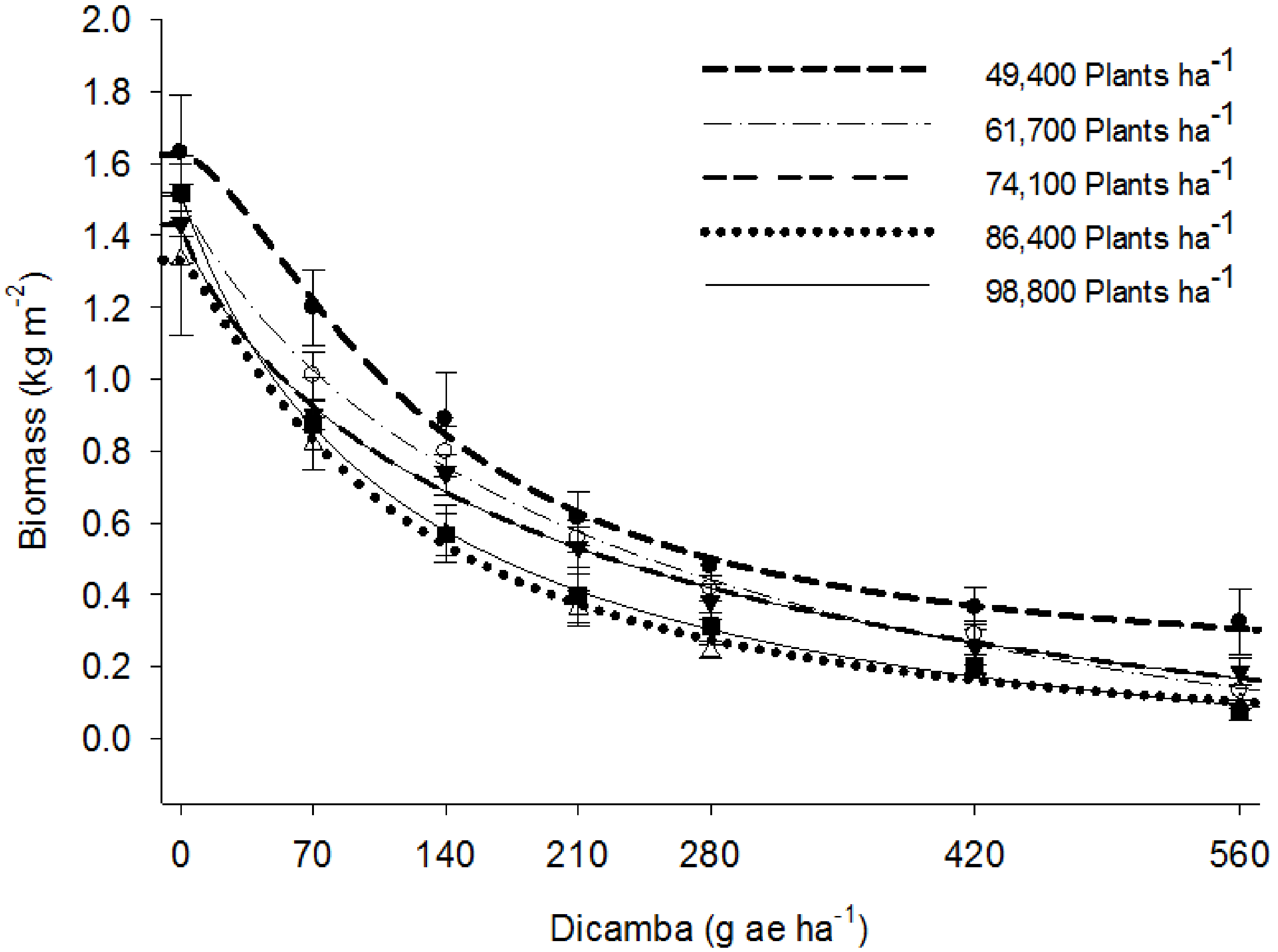

Figure 5-3. Corn plant population and dicamba dose interaction effect on Palmer amaranth shoot biomass.

Chapter 6 - Grain Sorghum and Palmer amaranth Response to Cultural Practices

Abstract

Palmer amaranth is a problem weed in sorghum. Field studies were conducted in 2016 and 2017 near Garden City, KS, to measure grain sorghum and Palmer amaranth's growth and reproductive attribute response to crop plant population density and nitrogen rate. Treatments comprised weed-free and weedy sorghum as main plots, three sorghum population densities as sub-plots, and three nitrogen rates as sub-sub-plots within a randomized complete block design with a split-split-plot treatment arrangement. Weed-free sorghum out-yielded weedy sorghum by 42%. Increasing sorghum population density did not increase sorghum grain yield or height but increased head number. Increasing nitrogen rate did not increase sorghum grain yield or height but decreased sorghum height due to greater weed competition. Similarly, Palmer amaranth density, height and biomass were not affected by increasing sorghum population density and nitrogen rate. Results suggest that despite the opportunity to maintain grain yield, increasing sorghum population and nitrogen rate in combination did not improve Palmer amaranth control.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; Sorghum, Sorghum bicolor (L.) Moench.

Keywords: Grain sorghum, Palmer amaranth, sorghum population density, nitrogen rate

Introduction

Sorghum (*Sorghum bicolor*) is a major crop in Kansas (Kansas Department of Agriculture, 2019). The majority of its production is confined to the western and southern parts of the state where the climate is warmer and drier. These conditions also favor Palmer amaranth (*Amaranthus palmeri*), the most troublesome weed in the U.S. (Anon, 2016). In Kansas, Palmer amaranth has been confirmed in at least 46 percent of the state's counties (USDA-NRCS, 2016) and has been found to reduce crop yield in several cropping systems including soybean, corn, cotton and sorghum (Smith et al., 2000; Massinga et al., 2001; Massinga and Currie, 2002; Bensch et al., 2003; Moore et al., 2004; Burke et al., 2007).

Limited herbicide options are available for weed control in grain sorghum, increasing the difficulty of managing problematic weeds like Palmer amaranth can be difficult (Ehleringer, 1983; Brooks, 2013). This difficulty is attributed to its extended germination and emergence patterns that result in multiple flushes of emergence, fast growth and adaptability, C_4 photosynthetic pathway, and high resource use efficiency (Ehleringer, 1983; Brooks, 2013). This is exacerbated by Palmer amaranth's propensity to evolve herbicide resistance. To date, Palmer amaranth has evolved resistance to at least six herbicide modes of action that are commonly used in the U.S. including microtubule-, 5-enolpyruvylshikimate-3-phosphate synthase-, acetolactate synthase-, photosystem II-, hydroxyphenylpyruvate dioxygenase-, and protoporphyrinogen oxidase-inhibitors (Heap, 2018). This makes its control even more challenging.

Because of the limited post-emergence herbicide options for grain sorghum, most Palmer amaranth plants emerging from late flushes (after crop emergence and establishment) are often poorly controlled or are left uncontrolled during the sorghum growing season thus adapting and becoming better at utilizing resources under traditional cropping systems. In Kansas, the recommended plant population density for irrigated sorghum is 100,000 plants ha^{-1}. Nitrogen recommendations, on the other hand, vary with expected yield, soil texture and cropping sequence (Handbook, G. S. P. 1998). According to Radosevich et al. (2007), changing practices usually gives crops an advantage over weeds, including increasing crop plant population density or seeding rate. For example, Norsworthy and Oliver (2001) have demonstrated that increasing soybean seeding rate can increase the crop's ability to suppress weeds while maintaining or increasing yield. Similar findings were reported in wheat (Olsen et al., 2005) and more recently in rye cover crop (Ryan, 2011). Hence, we hypothesized that the integration of high sorghum population density in conjunction with a greater nitrogen rate would give sorghum an early-season advantage over Palmer amaranth and reduce its impact on sorghum growth and reproductive attributes.

Chemical weed control tactics for Palmer amaranth control have been extensively studied (Whitaker et al., 2011; Barnett et al., 2013; Meyers et al., 2013; Putman, 2013; Merchant et al., 2014; Reddy et al., 2014; Reed et al., 2014; Cahoon et al., 2015; Hay, 2017; Thompson, 2018). However, Palmer amaranth resistance to current herbicide modes of action continues to increase. This increase in Palmer amaranth resistance to herbicides (Heap, 2018) poses challenges for sorghum growers as herbicide options for effective in-crop use are scarce. Therefore, the objective of this study was to investigate grain sorghum and Palmer amaranth growth and reproductive attribute response to crop plant population density and nitrogen rate in an irrigated environment.

Materials and Methods

Experimental Site

Studies were conducted in adjacent fields in 2016 and 2017 at the Kansas State University Southwest Research-Extension Center (37°59’38.4” N 100°49’04.8” W) near Garden City, KS. Soil at the site was a Richfield silt loam (fine, montmorillonitic, mesic Aridic Argiustoll) with <1% slope. The site had been in a fully irrigated corn-corn-wheat cropping sequence since 2004 and received a seasonal (May – October) total rainfall of 325 mm in 2016 and 297 mm in 2017 compared to a 30-year average of 356 mm (Elliot 2017). The average daily air temperature during the same period was 21.4 and 19.4 °C in 2016 and 2017, respectively, compared to a 30-year average of 20.4 °C (Elliot, 2017).

Experimental Design and Management

Fields were disked and the final seedbed was prepared with a single pass of a field cultivator before planting. Palmer amaranth seeds with no known resistance to any herbicide, collected from Kansas State University Southwest Research-Extension Center (37°59’38.4” N 100°49’04.8” W) in 2014, were broadcast-seeded at 535 g ha^{-1} prior to planting to ensure a uniform population in the primary zone of weed seed germination (top 2.5-5 cm). Sorghum ‘DK3707’ (Monsanto Co., St. Louis, MO) was planted on June 20th and May 24th in 2016 and 2017, respectively.

The experimental design consisted of a randomized complete block design (RCBD) with four replicates and treatments arranged in a split-split plot. Main plot treatments consisted of weed-free and weedy sorghum infested with Palmer amaranth, sub-plot treatments consisted of three sorghum populations densities [low (158,200 plants ha^{-1}); medium (222,300 plants ha^{-1}) and high (296,400 plants ha^{-1})], and sub-sub-plot treatments consisted of three nitrogen rates [0, 112 and 224 kg ha^{-1}; urea top-dressed at 2 weeks after planting (WAP)]. Weed-free sorghum was hand-hoed as required throughout the growing season to remove all weeds, and weedy sorghum was kept free of weeds other than Palmer amaranth. All experimental units were PRE-treated with half-rates of atrazine (560 g ai ha^{-1}) and dicamba (280 g ae ha^{-1}) to ensure a weed-free seed bed at crop emergence, but still allow later season establishment of Palmer amaranth to evaluate effects of cultural practices. The impact of reduced rates of atrazine on Palmer amaranth has been shown in a previous study (Currie and Klocke 2005). The experiments were irrigated using an overhead sprinkler irrigation system to ensure that water never limited yield throughout the growing season. The first irrigation event supplied about 13 mm of water applied immediately after PRE herbicide application, for herbicide activation. Subsequent irrigation events did not exceed two weekly, with each supplying 25 mm of water. Each sub-sub-plot was 10 m long with four rows spaced 76 cm apart.

Data Collection and Analysis

Data were collected from the two center rows of each sub-sub plot. Grain yield was combine harvested from the two center 10-m rows, and all other measurements were collected from the central square meter area in each sub-sub plot. Response variables recorded were: sorghum height (m), head-number (m^{-1} row), and grain yield (Mg ha^{-1}); and Palmer amaranth number (m^{-2}), height (m), and biomass (kg m^{-2}). Sorghum and Palmer amaranth measurements were collected a week before harvest (October 31st in 2016 and October 2nd in 2017). Data were analyzed using the PROC GLIMMIX procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC) for analysis of variance (ANOVA) and means were separated using Fisher's Protected Least Significant Difference test at $\alpha = 0.05$ when the ANOVA indicated that treatment effects were significant. Significance of interactions between weedy vs. weed-free, sorghum population density, and nitrogen rate also were tested at $\alpha = 0.05$.

Results and Discussion

The results from 2016 and 2017 were combined for analysis. No two-way interaction between weedy vs. weed-free, sorghum population density and nitrogen rate was detected for corn grain yield, height and biomass as well as Palmer amaranth density, height and biomass. Therefore, each main treatment effect was evaluated across levels of the other main effects.

Grain Sorghum Response to in-season Weeding, Sorghum Population Density and Nitrogen Rate

Weed-free sorghum had significantly greater yield than weedy-sorghum (Table 6-1). Previous studies have shown differential crop response to Palmer amaranth depending on the weed density and time of its emergence. For example, Massinga et al. (2001) detected no yield reduction when Palmer amaranth emerged four to five weeks after corn was planted. Similarly, Bensch et al. (2003) observed no yield penalty when Palmer amaranth emerged from three to six weeks after soybean was planted. More recently, MacRae et al. (2013) reported no yield losses when Palmer amaranth emerged six to eight weeks after cotton was planted. In this study, however, Palmer amaranth consistently emerged approximately five WAP. Results showed that 2-3 Palmer amaranth plants m^{-2} emerging as sorghum entered eight-leaf stage, caused 42% yield reduction. Increasing sorghum population density and nitrogen rate did not increase grain yield regardless of Palmer amaranth presence (Table 6-1). This finding is inconsistent with previous reports (Bayu et al., 2005; Fernandez et al., 2012; Besançon et al., 2017) and could be due, in part, to viable tillering and head size adjustment in low and medium sorghum densities (data not shown). Previous reports on grain sorghum response to nitrogen are inconsistent. For example, while the findings of this study may agree with those of Unruh (2013), they disagree with the reports of others (Peterson and Varvel, 1989; Kaizzi et al., 2012; Riffel, 2012). The lack of grain sorghum yield response to nitrogen could be attributed to the high residual nitrogen at this site (data not shown).

Sorghum head number was impacted by Palmer amaranth presence with weed-free sorghum producing more heads than weedy-sorghum (Table 6-1). This difference in the number of sorghum heads is reflected in the difference in grain yield between weedy- and wee-free-sorghum. Sorghum head number m^{-1} increased with greater sorghum plant population density but not with nitrogen rate (Table 6-2). Similarly, sorghum height was impacted by Palmer amaranth's presence with taller plants produced in weed-free sorghum compared to weedy sorghum (Table 6-1). Sorghum height did not increase with increasing sorghum population but decreased with increasing nitrogen rate (Table 6-2). This reduction in sorghum height with increasing nitrogen could be due to greater Palmer amaranth height and biomass with increasing nitrogen, highlighting the efficiency of this weed in using resources (i.e. water, light, nutrients and or space) under competition. Berger et al. (2015) reported more water removed from the soil profile by Palmer amaranth compared to cotton when both were grown under competition. Massinga et al. (2001) reported reduction in corn water use efficiency when Palmer amaranth was grown in competition with corn. Likewise, Ruf-Pachta et al. (2013) found similar nitrogen concentrations in corn and Palmer amaranth plant tissues when both were grown together under contrasting nitrogen environments suggesting that they respond similarly to soil nitrogen.

Palmer amaranth Response to Sorghum Population Density and Nitrogen Rate, and its impact on Sorghum

Palmer amaranth density, height and biomass were not impacted by increasing sorghum population density and nitrogen rate (Table 6-2). Hewitt (2015) also detected no differences in Palmer amaranth weed biomass among varying sorghum populations. Although the benefit of integrating high sorghum population density and nitrogen rate for Palmer amaranth control was not evident in this study, the opposite was reported elsewhere. For example, Besançon et al. (2017) observed increased Palmer amaranth control and a significant reduction in the weed's biomass when sorghum was planted at a population density ≥297,000 plants ha^{-1}. Unruh (2013) observed an increase in early-season Palmer amaranth growth with increasing nitrogen rate. These examples highlight the lack of single-factor effect and the need to integrate multiple approaches when dealing with such a problematic weed.

There was significant difference in sorghum grain yield, head number m^{-1} and height between weedy- and weed-free sorghum. This difference corresponds to 9, 21 and 42% reduction in weedy-sorghum height, head number and grain yield, respectively, relative to weed-free sorghum (Figure 6-1). This suggests, therefore, that 2-3 Palmer amaranth plants m^{-2} emerging as grain sorghum enters eight-leaf stage can cause up to 42% yield loss in irrigated grain sorghum; however, the impact can change depending on weed density and timing of emergence (Massinga et al., 2001). The importance of timing of weed emergence and density for interference with agronomic crops has been thoroughly reviewed (Smith et al., 2000; Massinga et al., 2001; Moore et al., 2004). Chahal et al. (2015) reported yield losses varying from 54% and 91% in cotton and corn, respectively, with Palmer amaranth densities not exceeding 10 or 8 plants m^{-2}, respectively. In sorghum, yield losses up to 63% have been reported at a Palmer amaranth density of 13.7 plants m^{-2} (Moore et al., 2004).

Conclusions

Results from this study show that while there is an opportunity to maintain sorghum grain yield by increasing sorghum population and nitrogen rate in combination, it does not suppress Palmer amaranth. Furthermore, 2-3 Palmer amaranth plants m^{-2} emerging as sorghum enters eight-leaf stage can cause up to 42% yield loss in irrigated grain sorghum. Therefore, integration of increasing sorghum population density and nitrogen rate with use of full rates of effective herbicide (both PRE and POST) modes of action and other best weed management practices (Norsworthy et al., 2012) should be considered when controlling both early- and late-emerging Palmer amaranth in irrigated grain sorghum.

References

Anon (2016) WSSA Survey Ranks Palmer Amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in Canada. PRWeb Newswire, pp. PRWeb Newswire, April 5, 2016

Barnett K, Culpepper A, York A, Steckel L (2013) Palmer Amaranth (*Amaranthus palmeri*) Control by Glufosinate plus Fluometuron Applied Postemergence to WideStrike® Cotton. Weed Technol 2: 291-297

Bayu W, Rethman NFG, Hammes PS (2005) Growth and Yield Compensation in Sorghum (*Sorghum Bicolor* L. Moench) as a Function of Planting Density and Nitrogen Fertilizer in Semi-arid Areas of Northeastern Ethiopia. S Afr J Plant Soil 2:76-83

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 1:37-43

Berger S, Ferrell J, Rowland D, Webster T (2015) Palmer Amaranth (*Amaranthus palmeri*) Competition for Water in Cotton. Weed Sci 4:928-935

Besançon TE, Heiniger RW, Weisz RJ, Everman W (2017) Grain Sorghum and Palmer Amaranth (*Amaranthus palmeri*) Response to Herbicide Programs and Agronomic Practices. Weed Technol 6:781-792

Brooks R (2013) Palmer Amaranth Pigweed Creeps Farther into the Midwest. Farm J. http://www.agweb.com/article/palmer_amaranth_pigweed_creeps_farther_into_the_midwest_naa_rhonda_brooks/. Accessed: August 30, 2016

Burke IC, Schroeder M, Thomas WE, Wilcut JW (2007) Palmer amaranth interference and seed production in peanut. Weed Technol 21:367– 371

Cahoon C, York A, Jordan D, Everman W, Seagroves R, Culpepper A, Eure P (2015) Palmer Amaranth (*Amaranthus palmeri*) Management in Dicamba-Resistant Cotton. Weed Technol 4:758-770

Chahal PS, Aulakh JS, Jugulam M, Jhala AJ (2015) Herbicide-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the United States—Mechanisms of resistance, impact, and management. In Herbicides, Agronomic Crops and Weed Biology. In Tech.

Currie RS, Klocke NL (2005) Impact of a Terminated Wheat Cover Crop in Irrigated Corn on Atrazine Rates and Water Use Efficiency. Weed Sci 5:709-716

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert Summer Annual. Oecol 57:107-12

Elliott, J. (2017) "Weather Information for Garden City, 2016," Kansas Agricultural Experiment Station Research Reports: Vol. 3: Iss. 5.

Fernandez C, Fromme D, Grichar W (2012) Grain Sorghum Response to Row Spacing and Plant Populations in the Texas Coastal Bend Region. Int J Agron 2012: 6

Handbook, G. S. P. (1998) Cooperative Extension Service. Kansas State University, Manhattan, Kansas. 8 p

Hay MM (2017) Control of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) in double crop soybean and with very long chain fatty acid inhibitor herbicides. Master’s thesis. Manhattan, KS: Kansas State University.

Heap I (2018) The International Survey of Herbicide Resistant Weeds. Online. Internet. www.weedscience.org. Accessed: April 23, 2018

Hewitt CA (2015) Effect of row spacing and seeding rate on grain sorghum tolerance of weeds. Master’s thesis. Manhattan, KS: Kansas State University. 34 p

Kaizzi KC, Byalebeka J, Semalulu O, Alou I, Zimwanguyizza W, Nansamba A, Musinguzi P, Ebanyat P, Hyuha T, Wortmann SC (2012) Sorghum response to fertilizer and nitrogen use efficiency in Uganda. Agron J 1:83-90

Kansas Department of Agriculture (2019) Kansas Agriculture's Economic Impact. Retrieved from http://agriculture.ks.gov/about-kda/kansas-agriculture MacRae AW, Webster TM, Sosnoskie LM, Culpepper AS, Kichler JM (2013) Cotton yield loss potential in response to length of Palmer amaranth (*Amaranthus palmeri*) interference. J Cotton Sci 17:227-232

Massinga RA, Currie RS (2002) Impact of Palmer Amaranth (*Amaranthus Palmeri*) on Corn (Zea Mays) Grain Yield and Yield and Quality of Forage 1. Weed Technol 3:532-536

Massinga RA, Currie RS, Horak MJ, Boyer J Jr (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202-208

Merchant R, Culpepper A, Eure P, Richburg J, Braxton L (2014) Controlling Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Cotton with Resistance to Glyphosate, 2,4-D, and Glufosinate. Weed Technol 2:291-297

Meyers S, Jennings K, Monks D (2013) Herbicide-Based Weed Management Programs for Palmer Amaranth (*Amaranthus palmeri*) in Sweetpotato. Weed Technol 2:331-340

Moore JW, Murray DS, Westerman RB (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol 1: 23-29

Norsworthy JK, Oliver L (2001) Effect of Seeding Rate of Drilled Glyphosate-Resistant Soybean (*Glycine max*) on Seed Yield and Gross Profit Margin 1. Weed Technol 2:284-292

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60(SP1): 31-62

Olsen J, Kristensen L, Weiner J, Griepentrog HW (2005) Increased density and spatial uniformity increase weed suppression by spring wheat. Weed Res 4:316-321

Peterson TA, Varvel G (1989) Crop yield as affected by rotation and nitrogen rate .2. Grain-sorghum. Agron J 5: 731-34

Putman JA (2013) Palmer amaranth control in established alfalfa and documentation of glyphosate-resistant *Amaranthus* species in Kansas. Master's thesis. Manhattan, KS: Kansas State University.

Radosevich SRR, Holt JSS, Ghersa CMM (2007) Ecology of Weeds and Invasive Plants: Relationship to Agriculture and Natural Resource Management: 3rd edn, John Wiley and Sons

Reddy SS, Stahlman PW, Geier PW, Bean BW, Dozier T (2014) Grain sorghum response and Palmer amaranth control with postemergence application of fluthiacet-methyl. Int J Pest Manage 3:147-152

Reed J, Keeling J, Dotray P (2014) Palmer Amaranth (*Amaranthus palmeri*) Management in GlyTol® LibertyLink® Cotton. Weed Technol 4:592-600

Riffel JD (2012) Yield Response and Economic Impact of Variable-rate Nitrogen Applications in Grain Sorghum. Master's thesis. Manhattan, KS: Kansas State University. 38 p

Ruf-Pachta E, Rule D, Dille J (2013) Corn and Palmer amaranth (*Amaranthus palmeri*) Interactions with Nitrogen in Dryland and Irrigated Environments. Weed Sci 2: 249-258

Ryan MR, Curran WS, Grantham AM, Hunsberger LK, Mirsky SB, Mortensen DA, Nord EA, Wilson DO (2011) Effects of Seeding Rate and Poultry Litter on Weed Suppression from a Rolled Cereal Rye Cover Crop. Weed Sci 3:438-444

Smith D, Baker R, Steele G (2000) Palmer Amaranth (*Amaranthus palmeri*) Impacts on Yield, Harvesting, and Ginning in Dryland Cotton (*Gossypium hirsutum*) 1. Weed Technol 1:122-126

Thompson NR (2018) Palmer amaranth (*Amaranthus palmeri*) control in double-crop dicamba/glyphosate resistant soybean (Glycine max) and dicamba and 2, 4-D efficacy on Palmer amaranth and common waterhemp (*Amaranthus rudis*). Master's thesis. Manhattan, KS: Kansas State University

United States Department of Agriculture, National Conservation Service (USDA-NRCS) (2016) Plants Database. plants.usda.gov/core/profile?symbol=AMAPA. Accessed: September 20, 2016

Unruh BJ (2013) Influence of nitrogen on weed growth and competition with grain sorghum. Master's thesis. Manhattan, KS: Kansas State University. 27 p

Ward S, Webster T, Steckel L (2013) Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Technol 1:12-27

Whitaker JR, York AC, Jordan DL, Culpepper AS, Sosnoskie LM (2011) Residual herbicides for palmer amaranth control. J Cotton Sci 1:89-99

Table 6-1. Weedy vs. weed-free sorghum plant height, head number and grain yield compared.

Sorghum	Grain sorghum attribute (SE)		
	Plant height (m)	Head number (m^{-2})	Grain yield (Mg ha^{-1})
Weedy	1.18 (0.02)	16.54 (0.90)	3.76 (0.27)
Weed-free	1.29 (0.01)	21.00 (0.85)	6.51 (0.13)
$LSD_{.05}$	0.03	2.39	0.62

SE - is standard error of the mean; and LSD – is least significant difference.

Table 6-2. Grain sorghum and Palmer amaranth response to sorghum population density and nitrogen rate. Low, medium and high represent 158,200, 222,300 and 296,400 plants ha^{-1}, respectively.

	Grain sorghum attribute (SE)			Palmer amaranth attribute (SE)		
Treatment	Plant height (m)	Head number (m^{-2})	Grain yield (Mg ha^{-1})	Plant height (m)	Density (plants m^{-2})	Biomass (kg m^{-2})
Sorghum population density (plants ha^{-1})						
Low	1.21 (0.01)	15.3 (0.80)	5.02 (0.32)	1.66 (0.01)	2.60 (0.30)	0.34 (0.05)
Medium	1.25 (0.02)	18.8 (1.10)	5.28 (0.35)	1.69 (0.01)	2.50 (0.45)	0.31 (0.05)
High	1.24 (0.01)	22.1 (1.20)	5.47 (0.32)	1.55 (0.01)	1.98 (0.29)	0.29 (0.06)
$LSD_{.05}$	ns	2.92	ns	ns	ns	ns
Nitrogen rate (kg ha^{-1})						
0	1.26 (0.01)	19.38 (1.12)	5.21 (0.33)	1.50 (0.01)	2.36 (0.37)	0.26 (0.04)
112	1.23 (0.01)	18.55 (1.09)	5.27 (0.32)	1.67 (0.01)	2.52 (0.45)	0.34 (0.06)
224	1.21 (0.01)	18.39 (1.18)	5.28 (0.35)	1.72 (0.01)	2.23 (0.39)	0.34 (0.06)
$LSD_{.05}$	0.04	ns	ns	ns	ns	ns

SE - is standard error of the mean; LSD – is least significant difference; and ns - indicates non-significant difference at $\alpha = 5\%$.

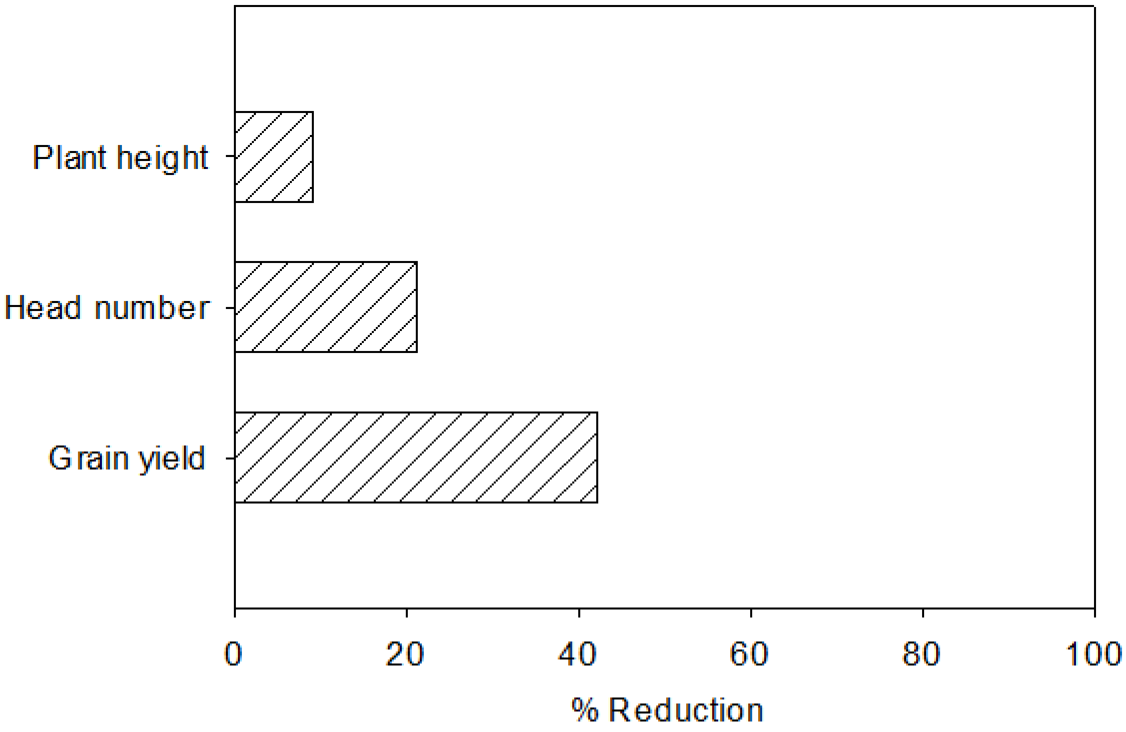

Figure 6-1 Reduction in weedy-sorghum height (m), head number (m-1 row) and biomass (kg m^{-2}) relative to weed-free sorghum. Dashed line indicates 50% reduction in sorghum height, head number and grain yield.

Chapter 7 - Summary, Conclusions, Implications and Limitations

Herbicides remain the primary, and most effective and widely used option to control weeds since the mid 1940's when 2,4-D became available for use in agriculture (Peterson et al., 2016). However, chemical weed control options continue to decline due to the evolution of herbicide resistance in weeds. The problem of resistance is particularly acute in Palmer amaranth (Heap, 2019). Over the past several years, Palmer amaranth has evolved resistance to at least six herbicide modes of action in the U.S. alone (Heap, 2019). Of all the agriculturally important weeds in the U.S., Palmer amaranth has been ranked as the most troublesome species (Anon, 2016). This research investigated the physiological basis of interaction of herbicides with different modes of action in Palmer amaranth control and evaluated use of integrated approaches to manage this weed in field conditions. Specifically, the research objectives were to: 1) evaluate the effect of plant height on dicamba efficacy to control Palmer amaranth; 2) investigate the mechanism of resistance to glyphosate in a Palmer amaranth accession from Kansas, and evaluate efficacy of glyphosate and dicamba tank-mix to control this accession; 3) investigate the physiological basis of glyphosate and dicamba interaction in tank-mix to control Palmer amaranth; 4) determine the efficacy of reduced dicamba use on Palmer amaranth control in irrigated corn production; and 5) investigate grain sorghum and Palmer amaranth growth and reproductive attributes in response to sorghum density and nitrogen rate under irrigated conditions. Results of this dissertation research suggest that: a) dicamba efficacy to control Palmer amaranth is greatly influenced by plant height at time of herbicide application and b) an increased absorption and translocation of dicamba at early growth stages of Palmer amaranth contributes to increased efficacy of dicamba to control this weed; c) the Palmer amaranth accession from Pawnee County survived the label recommended dose of glyphosate due to elevated EPSPS gene copy number; however, tank mixing dicamba and glyphosate had a synergistic effect on control of this glyphosate resistant Palmer amaranth accession from Pawnee County, KS; and d) the physiological mechanism attributed to this synergistic interaction appears to be a rapid absorption of dicamba and increased translocation of glyphosate. Results from this research also indicate that e) acceptable corn grain yield and Palmer amaranth control are possible with the integration of greater corn plant population density and reduced dicamba dose; however, f) increasing sorghum plant population density and nitrogen rate in combination did not suppress Palmer amaranth.

Taken together, the outcome of this dissertation provides several strategies to improve Palmer amaranth control. Also, this study highlights the importance of timely management of Palmer amaranth and the benefit of integrating multiple approaches to control this weed. Although this research has demonstrated that dicamba can effectively control Palmer amaranth, early in its growth stage, it is important to also consider including other effective herbicide modes of action to broaden the spectrum of weed control and delay evolution of resistance. Furthermore, this study showed that glyphosate could still be used to control glyphosate-resistant Palmer amaranth provided that dicamba is used in tank-mixes; however, the proportion of herbicides in the tank mixture seems to affect how they interact. In addition, this study found that Palmer amaranth could be controlled with integration of crop plant population density and reduced herbicide dose in corn but not in sorghum even when higher nitrogen rates were applied. Therefore, it is crucial that other best management practices (Norsworthy et al., 2012) that increase crop competition are considered when dealing with Palmer amaranth.

In terms of limitations, this study lacked contrasting Palmer amaranth accessions in evaluating the effect of plant growth stage on dicamba efficacy to control Palmer amaranth and in investigating the physiological basis of glyphosate and dicamba interaction in tank-mix to control this weed. These limitations guide directions for future research. For instance, further research is needed to address similar research questions in different Palmer amaranth accessions. This will increase the generality of the findings of this study. Another limitation of this study is associated with lack of contrasting Palmer amaranth emergence flushes in investigating grain sorghum and Palmer amaranth response to sorghum plant population density and nitrogen rate under irrigated conditions. Therefore, this limitation should also be considered in future research.

References

Anon (2016) WSSA Survey Ranks Palmer Amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in Canada. PRWeb Newswire, pp. PRWeb Newswire, April 5, 2016

Heap I (2019) The International Survey of Herbicide Resistant Weeds. Online. Internet. Accessed January 5, 2019. Available www.weedscience.org

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60(SP1): 31-62

Peterson M, McMaster S, Riechers D, Skelton J, Stahlman P (2016) 2,4-D Past, Present, and Future: A Review. Weed Technology, 30(2), 303-345