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Characterization and Management of Auxin-Resistant Palmer amaranth in Tennessee

Delaney C. Foster
University of Tennessee, Knoxville, dfoste37@vols.utk.edu

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I am submitting herewith a dissertation written by Delaney C. Foster entitled "Characterization and Management of Auxin-Resistant Palmer amaranth in Tennessee." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Lawrence E. Steckel, Major Professor

We have read this dissertation and recommend its acceptance:

Heather Kelly, Thomas C. Mueller, Avat Shekoofa

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Characterization and Management of Auxin-Resistant Palmer amaranth in Tennessee

A Dissertation Presented for the
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ABSTRACT

Palmer amaranth has a long history of evolving resistance to herbicides to the point it has become a significant row crop production obstacle. Cotton and soybean growers were offered new technologies in 2016, expanding in-crop herbicide options to include dicamba or 2,4-D. Within three years of commercialization, dicamba use in these crops increased ten-fold and growers began to report Palmer amaranth escapes in west Tennessee auxin-tolerant production systems. A survey of Palmer amaranth escapes in dicamba and 2,4-D-tolerant cotton and soybean fields in Tennessee was conducted in the fall of 2021 with the objective of determining if poor control was due to environmental phenomenon or development of auxin resistance in west Tennessee. Field experiments were conducted across Tennessee in locations where growers witnessed poor control following these herbicides and in Georgia and Texas to characterize potentially resistant populations and compare these with known susceptible populations. Greenhouse findings confirmed three Palmer amaranth accessions with relative resistance factor to dicamba between 1.85-2.49 and one population from Lauderdale County, Tennessee with a relative resistance factor of 14.25. In field studies across multiple locations in Tennessee, the labelled rate of dicamba or 2,4-D controlled Palmer amaranth ≤60%. The addition of malathion insecticide in field experiments did not improve Palmer amaranth control, ruling out certain cytochrome p-450's as a resistance mechanism.

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INTRODUCTION

Weeds are considered one of the most detrimental pests to crop production. Weeds cause an average yield loss of 34% if not controlled properly (Oerke 2006). According to the North American 2019 survey of most common and troublesome weeds in broadleaf crops, Palmer amaranth [*Amaranthus palmeri* S. Watson] and morningglory [*Ipomoea* spp] were the top two most common and most troublesome weeds in cotton [*Gossypium hirsutum* L.] and soybean [*Glycine max* L. Merr.] production (Van Wychen 2019). Weeds can be controlled chemically [through use of herbicides], mechanically, and culturally. In 1960, herbicides accounted for 18% of United States pesticide use; however, that number grew to 76% by 2008 because of new herbicide chemistries and advances in herbicide tolerant crop genetics (Fernandez-Cornejo et al. 2014).

Herbicides have become a popular weed control method for cotton and soybean growers, but sole-reliance does not properly steward new technology, resulting in herbicide resistance (Young 2006). Palmer amaranth has become a problematic weed in broadleaf crop production, confirming resistance to eight different herbicide modes of action since 1989 (Heap 2021). In Tennessee, reports of Palmer amaranth escaping dicamba and 2,4-D applications in auxin-based cropping systems became notably more prevalent in 2019. It was determined that in many cases, these herbicides were applied timely to small [<10cm] Palmer amaranth and control was not consistent as in previous years. In 2020, the first dicamba-resistant Palmer amaranth was confirmed in Tennessee (Heap 2021).

Synthetic Auxin Herbicides

Dicamba and 2,4-D are synthetic auxin herbicides [Herbicide Resistance Action Committee Group 4], part of a class of chemistries that mimic the plant hormone indole-3-acetic acid [auxin] (HRAC 2021). Globally, auxin herbicides rank third amongst most used herbicide chemistries behind ALS and EPSP synthase inhibitors (Busi et al. 2018) and among the auxins, dicamba and 2,4-D are the two most used herbicides in the group on a basis of treated acres (Todd et al. 2020).

Dicamba and 2,4-D were first registered for use in the United States in 1962 and 1945, respectively (Peterson et al. 2016; Timmons 2005). They are widely used, effective herbicides at controlling a number of broadleaf weed species. In west Tennessee, where agriculture is dominated by row-crops, many areas have received more than 3.75 pounds per square mile of dicamba each year since 2000 (USGS 2021). This is largely due to 75 to 85% of Tennessee cotton, corn (*Zea mays* L.), and soybean acres being farmed in no-till production systems for several decades, and dicamba has historically been used in many burndown applications (USDA NASS 2018). Much of middle Tennessee agriculture is encompassed by pastures, where 2,4-D has been used extensively year after year at a rate of >28 pounds per square mile each year for the past two decades (USGS 2021).

In 2016, transgenic events became commercially available in cotton and soybeans when 2,4-D [Enlist™] and dicamba [XtendFlex®] tolerant varieties launched. These technologies were developed through the insertion of the AAD-1 [aryloxyalkanoate dioxygenase-1] transgene and dicamba monooxygenase gene, respectively, which result in herbicide detoxification (Behrens et al. 2007; Braxton et al. 2017; Inman et al. 2016). The following year, low volatility herbicide formulations received Federal 3 label status for use in these new technologies. Since 2017, over-the-top applications of low volatile dicamba salts and 2,4-D have been labelled for use in tolerant cotton and soybean varieties, further increasing the amount of these herbicides used during the growing season. These technologies improved control of many troublesome weed species such as glyphosate-resistant Palmer amaranth, waterhemp [*Amaranthus rudis*], and horseweed [*Conyza canadensis*] (Cahoon et al. 2015; Flessner et al. 2015; Spaunhorst and Bradley 2013). Use of these herbicides comes with the risk of off-target movement and tank contamination, which can be detrimental to sensitive crops such as peanut [*Arachis hypogaea* L.], non-tolerant cotton and soybean, and specialty crops at low concentrations (Culpepper et al. 2018).

Palmer amaranth

Palmer amaranth originated in the dry southwestern United States and Mexico, but has travelled far from its home and is now present across the entire southern United States (Sauer 1950; Steckel 2007). In a 2019 survey, Palmer amaranth was ranked as the most common and most troublesome weed species among all broadleaf crops, fruits, and vegetables (Van Wychen 2019). Since its first known case of herbicide resistance in 1989, Palmer amaranth has developed resistance to eight modes of action including ALS inhibitors, auxin mimics, EPSP synthase inhibitors, HPPD inhibitors, microtubule assembly inhibitors, photosystem II binders, PPO inhibitors, and long chain fatty acid inhibitors (Heap 2021). Palmer amaranth is a highly competitive, dioecious weed whose high fecundity and obligate outcrossing results in large genetic diversity (Steckel 2007; Ward et al. 2013). Among the four most common Amaranthus species, Palmer amaranth has the greatest leaf number, dry matter, and fastest growth rate per growing degree days (Horak and Loughin 2000). It was also determined that Palmer amaranth has the highest germination rate of the *Amaranthus* species (Steckel et al. 2004). Additionally, one female Palmer amaranth plant can produce over half a million seeds each year, replenishing the seed bank continuously (Keeley et al. 1987; Ward et al. 2013).

Cytochrome P-450

Cytochrome P450s are a class of enzymes belonging to the largest family involved in oxygen-dependent hydroxylation reactions (Pandian et al. 2020). Cytochrome P450s are present in the majority of life processes, but are one of the main contributors to oxidation based metabolism in plants (Mizutani and Sato 2011). These enzymes, specifically cytochrome P-450 monooxygenase, contribute to herbicide detoxification by adding an oxygen atom onto herbicide compounds to make them more hydrophilic and broken down more easily in subsequent metabolic reactions; overexpressing just one of the many cytochrome P-450 genes can confer herbicide resistance (Hirose et al. 2005; Hu et al. 2009).

Interactions between certain cytochrome P450 inhibiting organophosphate [OP] insecticides with herbicides have shown to increase crop injury in cases where crop tolerance was mediated by metabolism of the herbicide. In corn, many herbicide labels restrict the use of these pesticides in conjunction with one another. For example, Kapusta and Krausz (1992) determined that the insecticide terbufos increased corn yield but had an adverse effect on yield when applied in close timing with nicosulfuron. More recent research shows that foliar or in-furrow applications of organophosphate insecticide chlorpyrifos increased injury and decreased grain yield when used in conjunction with HPPD-inhibitor based premixed herbicides in corn (Steckel et al. 2015). It is because of previous suggested research that organophosphate insecticides such as malathion have become good candidates to indicate possible nontarget site metabolic resistance mechanisms in weeds (Kumar et al. 2020; Varanasi et al. 2019). In 2020, Shyam et al. determined that treatment of malathion with 2,4-D applications reversed herbicide resistance to the auxin.

Auxin Resistance

Herbicide tolerance and resistance are two terms often times used interchangeably, but in truth have different meanings. Herbicide resistance is defined as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type" while tolerance is "the inherent ability of a species to survive and reproduce after herbicide treatment; this implies that there was no selection or genetic manipulation to make the plant tolerant, it is naturally tolerant" (Technology Notes 1998). Globally, there are 505 unique cases of herbicide resistant weeds, and that number continues to grow each year (Heap 2021). The first documented case of dicamba resistance in the United States was kochia [*Kochia scoparia*] in 1994 (Heap 2021). Currently, there are only three weed species with confirmed resistance to dicamba in the United States: kochia, prickly lettuce [*Lactuca serriola*], and Palmer amaranth. 2,4-D, on the other hand, has a longer history and several more species confirming resistance. In 1957, spreading dayflower [*Commelina diffusa*] became the first herbicide resistant weed in the United States, conferring resistance to 2,4-D. Since then, six other weed species in the United States have developed resistance to this herbicide (Heap 2021).

In 2019, the University of Tennessee Extension Service received no fewer than 170 calls from growers reporting weed control failures in both Xtend® and Enlist™ cropping systems. A survey of fields that contained escapes in 2019 suggested that 65% of the time, the culprit was Palmer amaranth. Seed was collected from fields of concern in 2019 for dose response screening and field trials were conducted in 2020 to examine the response of potentially tolerant Palmer amaranth populations to dicamba and 2,4-D and resistance has been confirmed.

References

Behrens M, Mutlu N, Chakraborty S, Dumitru R, Jiang W, LaVallee B, Herman P, Clemente T, Weeks D (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. Science 316:1185-1188

Braxton L, Richburg J, York A, Culpepper A, Haygood R, Lovelace M, Perry D, Walton L (2017) Resistance of Enlist™ (AAD-12) cotton to glufosinate. Weed Technol 31:380-386

Busi R, Goggin DE, Heap IM, Horak MJ, Jugulam M, Masters RA, Napier RM, Riar DS, Satchivi NM, Torra J, Westra P, Wright TR (2018) Weed resistance to synthetic auxin herbicides. Pest Manage Sci 74:2265-2276

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 29:758-770

Culpepper A, Smith J, Prostko E (2018) Visual Sensitivity Scale for Dicamba and 2,4-D. Pages 1-2 UGA Weed Science: University of Georgia Weed Science

Fernandez-Cornejo J, Nehring R, Osteen C, Wechsler S, Martin A, Vialou A (2014) Pesticide use in U.S. agriculture: 21 selected crops, 1960-2008 *in* Agriculture USDo, ed.

Flessner ML, McElroy JS, McCurdy JD, Toombs JM, Glenn R. Wehtje, Burmester CH, Price AJ, Ducar JT (2015) Glyphosate-resistant horseweed (*Conyza canadensis*) control with dicamba in Alabama. Weed Technol 29:633-640

Heap I (2021) The International Survey of Herbicide Resistant Weeds. www.weedscience.org

Herbicide Resistance Action Committee (2021) Global Herbicide Classification Lookup

Hirose S, Kawahihashi H, Ozawa K, Shiota N, Inui H, Ohkawa H, Ohkawa Y (2005) Transgenic rice containing human CYP2B6 detoxifies various classes of herbicides. J of Agricultural and Food Chemistry 53:3461-3467

Horak M, Loughin T (2000) Growth analysis of four Amaranthus species. Weed Sci 48:347-355

Hu J, Tranel PJ, Stewart NN, Yuan JS (2009) Molecular and genomic mechanisms of non-target-site herbicide resistance. Pages 149-153 *in* Stewart CN, ed. Weedy and Invasive Plant Genomics

Inman M, Jordan D, York A, Jennings K, Monks D, Everman W, Bollman S, Fowler J, Cole R, Soteres J (2016) Long-term management of Palmer amaranth (*Amaranthus palmeri*) in dicamba-tolerant cotton. Weed Sci 64:161-169

Kapusta G, Krausz RF (1992) Interaction of terbufos and nicosulfuron on corn (*Zea mays*). Weed Technol 6:999-1003

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

Kumar V, Liu R, Currie RS, Jha P, Morran S, Gaines T, Stahlman PW (2020) Cross-resistance to atrazine and metribuzin in multiple herbicide-resistant kochia accessions: confirmation, mechanism, and management. Weed Technol 35:539-546

Mizutani M, Sato F (2011) Unusual P450 reactions in plant secondary metabolism. Arch of Biochemistry and Biophysics 507:194-203

Oerke E (2006) Crop losses to pests. J Agric Sci 144:31-43

Pandian BA, Sathishraj R, Djanaguiraman M, Prasad PVV, Jugulam M (2020) Role of cytochrome P450 enzymes in plant stress response. Antioxidants 9:454

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D past, present, and future: a review. Weed Technol 30:303-345

Sauer J (1950) The Grain Amaranths: A survey of Their History and Classification. Annals of the Missouri Botanical Garden 1990 37:561-632

Shyam C, Borgato EA, Peterson DE, Dille JA, Jugulam M (2020) Predominance of metabolic resistance in a six-way-resistant Palmer amaranth (*Amaranthus palmeri*) population. Front Plant Sci 11

Spaunhorst DJ, Bradley KW (2013) Influence of dicamba and dicamba plus glyphosate combinations on the control of glyphosate-resistant waterhemp (*Amaranthus rudis*). Weed Technol 27:675-681

Steckel L, Sprague C, Stoller E, Wax L (2004) Temperature effects on germination of nine Amaranthus species. Weed Sci 52:217-221

Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567-570

Steckel LE, Stewart SD, Steckel S (2015) Corn response to POST-applied HPPD-inhibitor based premix herbicides with in-furrow and foliar-applied insecticides. Weed Technol 29:18-23

Technology Notes (1998) "Herbicide resistance" and "herbicide tolerance" defined. Weed Technol 12:789

Timmons F (2005) A history of weed control in the United States and Canada. Weed Sci 18:294-307

Todd OE, Figueiredo MRA, Morran S, Soni N, Preston C, Kubeš MF, Napier R, Gaines TA (2020) Synthetic auxin herbicides: finding the lock and key to weed resistance. Plant Sci 300

USDA NASS (2018) Tennessee Tillage Systems Accessed 10/2/20. https://www.nass.usda.gov/Statistics_by_State/Tennessee/Publications/Special_Surveys/tillage2018.pdf

USGS (2021) Estimated Annual Agricultural Pesticide Use. National Water-Quality Assessment Project

Van Wychen L (2019) 2019 Survey of the most common and troublesome weeds in broadleaf crops, fruits, and vegetables in the United States and Canada- Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx

Varanasi VK, Brabham C, Korres NE, Norsworthy JK (2019) Nontarget site resistance in Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] confers cross-resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Technol 33:349-354

Ward S, Webster T, Steckel L (2013) Palmer amaranth (Amaranthus palmeri): a review. Weed Technol 27:12-27

Young B (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

CHAPTER I

CONFIRMATION OF DICAMBA-RESISTANT PALMER AMARANTH IN TENNESSEE

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Abstract

Palmer amaranth has a long history of evolving resistance to herbicides to the point it has become a significant row crop production obstacle. A survey of Palmer amaranth escapes in dicamba-tolerant cotton and soybean fields in Tennessee was conducted in the fall of 2021 with the objective of determining if poor control was due to environmental phenomenon or development of dicamba resistance in west Tennessee. A greenhouse dicamba dose response screen was conducted on 15 Tennessee accessions. Three accessions with relative resistance factor between 1.85-2.49 and one population from Lauderdale County, Tennessee with a relative resistance factor of 14.25 were found. The Lauderdale County 1 population developed a much higher dicamba resistance level than all others evaluated and can no longer be effectively controlled using dicamba. The history of Palmer amaranth escaping dicamba in the Lauderdale County 1 location from 2019 to 2021 in the field and in preliminary greenhouse screens would suggest that the dicamba-resistance has passed between generations. This research documents the first findings of Palmer amaranth control failures, in cotton and soybean fields, due to the evolution of dicamba resistance.

Introduction

From 2012 to 2022, United States growers planted over 75 million acres of soybeans [*Glycine max* (L.) Merr.] and 10 million acres of cotton [*Gossypium hirsutum* L.] each year (USDA NASS 2022). Weeds are the largest threat to United States soybean and cotton production, with the potential to decrease yields by ≥34% if left uncontrolled (Oerke 2006). In 2016, new transgenic cultivars became commercially available for soybean and cotton producers, with tolerance to 2,4-D or dicamba in addition to glufosinate and glyphosate, increasing the number of over-the-top herbicide options for growers. These technologies were developed through the insertion of the AAD-1 (aryloxyalkanoate dioxygenase-1) transgene and dicamba monooxygenase gene, respectively, resulting in herbicide detoxification (Behrens et al. 2007; Braxton et al. 2017; Inman et al. 2016). The following year, low volatility herbicide formulations of 2,4-D and dicamba received Federal 3 label status for use in these new soybean and cotton technologies. These two auxinic herbicides selectively control broadleaf weeds such as Palmer amaranth, and when applied in a timely manner are effective at controlling weeds postemergence (Cahoon et al. 2015; Manuchehri et al. 2017).

Prior to 2017, total dicamba use in the United States was estimated at less than 6 million kg per year (USGS 2021). Since the commercialization of dicamba-tolerant crops and subsequent labeling of the herbicide for in-crop use, over 15 million kg of dicamba is now applied across the United States; 10 out of 15 million kg applied in the country were used in cotton and soybeans in 2019. This is nearly ten times the amount used in these cropping systems prior to 2017. The state of Tennessee accounts for approximately 5% of this dicamba use, despite planting less acres in soybeans and cotton compared with other states. Over-reliance on a specific herbicide site of action can lead to increased selection pressure for herbicide-resistant biotypes (Beckie and Rebound 2009; Powles et al. 1997). A survey and seed collection of Palmer amaranth escapes in dicamba-tolerant cotton and soybean fields in Tennessee was conducted in the fall of 2021 with the objective of determining if poor control was due to environmental phenomenon or development of dicamba-resistant Palmer amaranth in west Tennessee.

Materials & Methods

A preliminary field screen for dicamba resistance was conducted at the Lauderdale County 1 site in 2020 and 2021. Additionally, in 2020 a field screen was conducted at the Gibson 2 location. The herbicide applications were made when Palmer amaranth reached 10 cm in height. Treatments consisted of dicamba applied at 0.56 (1X), and 1.12 (2X) kg ae ha^{-1}. Dicamba was applied using a CO_2-pressurized backpack sprayer equipped with Turbo TeeJet Induction 11002 (TeeJet® Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 220 kPa. In addition, a preliminary greenhouse screen of the Lauderdale 1, Lauderdale 2 and Gibson 3 Palmer amaranth was conducted in the spring of 2020. In that screen, dicamba was applied as described earlier but only at one rate (0.56 kg ha^{-1}) to 10 cm tall Palmer amaranth. In each of these screens, control of Palmer amaranth was ≤50% following timely applications of dicamba, prompting the larger survey and dose-response experiment.

A greenhouse dose response experiment was conducted in 2021 and 2022 at the West Tennessee AgResearch and Education Center in Jackson, TN (35.632003°N, -88.855874°W). Palmer amaranth seed from fifteen locations across west Tennessee where dicamba failures were reported was collected in the fall of 2021 (Table 1). Specific field history for most individual commercial field locations is unknown, however extensive use of dicamba for the past 2 decades in burndown due to widespread no-till practices across the state and more recently in-crop use in

Xtend® crops suggests heavy dicamba use regardless of location (USDA NASS 2018). The specific field history for the Lauderdale County 1 location is known and consisted of Xtend® cotton planted from 2016 to 2021. Gibson County 1 and 2 were planted to Xtend® cotton from 2016 to 2020, Enlist® cotton in 2021, and back to Xtend cotton in 2022. In 2019, both growers noticed a small area of escaped Palmer amaranth after multiple applications of dicamba at 0.56 kg ha^{-1}. Seed was collected from these fields after being brought to the authors' attention by Extension Agricultural agents or crop consultants and a preliminary greenhouse screen for dicamba resistance was conducted in 2020 prior to the survey at hand (results not shown).

Seeds from all 15 west Tennessee survey sites were processed and stored at 4C for four weeks prior to initiation of greenhouse trials. A known susceptible population of Palmer amaranth purchased from Azlin Seed Services (112 Lilac Dr., Leland, MS 38756) was included for comparison. Palmer amaranth seeds were sprinkled on top of pre-moistened potting mix (Sta-Green Moisture Max Potting Mix) in 28 cm by 55 cm by 6 cm greenhouse trays (Greenhouse Megastore, Danville, IL). Seeds were covered with 0.5 cm potting mix and overhead watered. Trays were kept moist throughout the experiment using bottom irrigation and supplemental lighting was used to ensure a 16 h photoperiod; daytime temperature was set to 33C and nighttime temperature was 26C. Once plants emerged, Palmer amaranth were thinned to one plant per 30 cm^2 or approximately 50 per tray. Trays were arranged in a randomized complete block design. The experiment was repeated two times with three replications, or trays, per population in each run.

Herbicide treatments were applied using a stationary greenhouse spray chamber (Devries Manufacturing, Hollandale, MN) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 200 kPa from a boom set up with two Turbo TeeJet Induction 11002 (TeeJet® Technologies, Glendale Heights, IL). The herbicide application was made when Palmer amaranth reached 10 cm in height. Treatments consisted of dicamba (Xtendimax® with VaporGrip® Technology, Bayer CropScience, St. Louis, MO) applied at 0.14 (0.25X), 0.28 (0.5X), 0.56 (1X), and 1.12 (2X) kg ae ha^{-1}. The 1X rate was based on the XtendiMax label where 0.56 kg ha^{-1} (Anonymous a 2022) is designated as the labeled over-the-top use rate for tolerant cotton and soybeans. Plants were placed in the greenhouse after application and grown for 21 d, after which the number of dead and alive plants per flat were counted to calculate a percent mortality (control) and fresh weight of surviving plants was measured in grams.

Percent control and fresh weights were subjected to analysis of variance using the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Care, NC) with Tukey's HSD at $\alpha = 0.05$ for means separation. Location, herbicide rate, and location*herbicide rate interactions were tested for significance. Single degree of freedom contrast statements were conducted to compare each suspected resistant population with the susceptible check by rate. Percent control was fit to a 3-parameter sigmoidal curve using SigmaPlot 14.5 (Systat Software Inc, San Jose, CA) as suggested by Thornley and Johnson (1990), where parameter a describes the upper limit of control, parameter b estimates the slope, and parameter c represents the EC_{50} rate (Equation 1). The EC_{50} value was then subjected to analysis of variance using the same methodology as the percent control and fresh weight values. Both replication and run were considered random effects in the model. Relative resistance factor was then calculated by dividing the EC_{50} estimate for each population by the EC_{50} estimate for the susceptible population.

$$y = a/(1+\exp(-(\text{rate}-c)/b)) \qquad \text{Equation 1}$$

Results & Discussion

Contrast statements used to compare the response of fifteen Palmer amaranth accessions from Tennessee with a known susceptible check following increasing rates of dicamba showed a decrease in control at 0.14 kg ae ha^{-1} for ten of fifteen accessions (Table 2). Four Tennessee accessions (Carroll, Lauderdale 1, Lauderdale 2, and Dyer Counties) were not controlled as effectively at the 0.28 kg ae ha^{-1} rate. When using the 1X field rate (0.56 kg dicamba ha^{-1}), Lauderdale 1 (1%), Lauderdale 2 (72%), Tipton (81%), and Gibson 3 (80%) County accessions exhibited less control than the susceptible check (95%). At 1.12 kg ae ha^{-1}, dicamba only controlled Palmer amaranth 20%, 79% and 82% at Lauderdale 1, Madison 1 and Dyer counties, respectively, while control in the susceptible check was 100%.

Dicamba dose response curves suggest that Palmer amaranth populations in Tennessee are segregating based on their relative susceptibility to dicamba (Figure 1). There are eight accessions that responded with higher tolerance or resistance to dicamba. Of those eight accessions, three showed less control at rates 2 to 4 times above the 0.56 kg ha^{-1} rate. The Lauderdale County 1 population represented by the grey line showed an order of magnitude greater resistance to dicamba than all other accessions.

The EC_{50} value for the susceptible check was 0.1262, indicating that this amount of dicamba ha^{-1} would control 50% of the population (Table 3). Four Tennessee Palmer amaranth accessions had higher EC_{50} values than the susceptible check: Carroll County (0.2338), Lauderdale County 1 (1.7978), Lauderdale County 2 (0.3140), and Dyer County (0.2398). The relative resistance factor for Carroll, Lauderdale 2, and Dyer counties was between 1.85-2.49 while the relative resistance factor for the Lauderdale County 1 accession was 14.25, indicating that this population has developed a high level of resistance and can no longer be effectively controlled using dicamba. These results are consistent with reports from the grower who manages this field. Lauderdale and Tipton Counties in Tennessee have been the epicenter for Palmer amaranth resistance to herbicides in previous years and is where the first glyphosate and one of the first PPO-resistant Palmer amaranth populations were discovered in the state (Steckel et al. 2008; Copeland et al. 2018).

Fresh weight of surviving plants was measured 21 d after application. At less than 0.56 kg ae ha^{-1}, an increase in biomass was observed in some accessions compared with the nontreated control of those same accessions. Because the location*rate interaction was not significant for fresh weights, but location was significant, fresh weight was averaged for each location and compared with the susceptible check (Table 4). Lauderdale County 1 (106%) and Carroll County (40%) were the only accessions to exhibit higher overall biomass as a % of the nontreated control compared with the susceptible check (20%). This data supports the control results with the Lauderdale County 1 population showing an actual biomass increase after a dicamba application compared with the same population not treated.

These data document a segregating population of Palmer amaranth to dicamba in Tennessee. It ranged from 11 accessions with control similar to the susceptible check to three accessions (Caroll, Dyer, Lauderdale 2) showing resistance ratios of of 1.85 to 2.49. The Lauderdale 1 accession is confirmed highly resistant with a resistant ratio of 14.25. Another step to confirm resistance is documenting heritability of the resistance between generatons. The history of Palmer amaranth escaping dicamba in the Lauderdale 1 location from 2019 to 2021 in the growers field, preliminary field research and in this greenhouse dose response would indicate that the dicamba-resistance has passed between generations. This demonstrates the dicamba-resistance allele or alleles were passed from the 2019 Palmer amaranth generation to the 2020 and the 2021 generations. This research documents the first findings of Palmer amaranth control failures, in cotton and soybean fields, due to the evolution of dicamba resistance.

Dicamba-resistance in Palmer amaranth greatly limits control options in cotton and soybean. Glyphosate-resistant Palmer amaranth was first documented in Tennessee in 2008 (Steckel et al. 2008). By 2013, the glyphosate-resistant biotype had become the predominant biotype in west Tennessee and was becoming established in middle Tennessee (Steckel 2013). Recent documentation of glufosinate-resistant Palmer amaranth (Priess et al. 2022) in the Arkansas county adjacent to Lauderdale County, Tennessee calls into question if the XtendFlex trait (Bayer CropScience, St. Louis, MO) that provides cotton and soybean resistance to dicamba, glyphosate and glufosinate will be a viable weed management tool for this weed in future years.

Future research should be conducted to determine if dicamba-resistant Palmer amaranth populations are cross-resistant to 2,4-D. In additon, research designed to assess the mechanism or mechanisms of resistance with the Lauderdale 1 population will be conducted. Finally, weed management research needs to be conducted to detemermine how best to integrate herbicides and non-chemical tactics to better control these Palmer amaranth populations.

References

Anonymous a (2022) XtendiMax herbicide label. Accessed 8/8/22 https://www.cdms.net/ldat/ldH7U008.pdf

Beckie HJ and Rebound X (2009) Selecting for weed resistance: herbicide rotation and mixture. Weed Technol 23:363-370

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. Sci 316:1185-1188

Braxton LB, Richburg JS, York AC, Culpepper AS, Haygood RA, Lovelace ML, Perry DH, Walton LC (2017) Resistance of Enlist™ (AAD-12) cotton to glufosinate. Weed Technol 31:380-386

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758-770

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018) Distribution of PPX2 mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Technol 32:592-596

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 Sci 64:161-169

Manuchehri MR, Dotray PA, Keeling JW (2017) Enlist weed control systems for Palmer amaranth (*Amaranthus palmeri*) management in Texas High Plains cotton. Weed Technol 31:793-798

Oerke EC (2006) Crop losses to pests. J Agri Sci 144:31–43

Powles SB, Preston C, Bryanand B, Jutsum R (1997) Herbicide resistance: impact and management. Adv Agron 58:57–93

Priess GL, Norsworthy JK, Godara N, Mauromoustakos A, Butts TR, Roberts TL, Barber T (2022) Confirmation of glufosinate-resistant Palmer amaranth and response to other herbicides. Weed Technol 36:368-373

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

Steckel LE (2013) Glyphosate-resistant Palmer amaranth becoming a bigger issue in Middle Tennessee. UTcrops New Blog. Accessed 8/8/2022 https://news.utcrops.com/2013/06/glyphosate-resistant-palmer-amaranth-becoming-a-bigger-issue-in-middle-tn/

Thornley JHN, Johnson IR (1990) The logistic growth equation. Pages 78–82 in Plant and Crop Modeling. A Mathematical Approach to Plant and Crop Physiology. Oxford, UK: Clarendon Press

USDA NASS (2018) 2018 Tennessee Tillage Systems https://www.nass.usda.gov/Statistics_by_State/Tennessee/Publications/Special_Surveys/tillage2018.pdf Accessed 8/8/2022

USDA NASS (2022) Charts and maps, acreage by year field crops https://www.nass.usda.gov/Charts_and_Maps/

USGS (2021) Estimated Annual Agricultural Pesticide Use. https://www.usgs.gov/media/images/estimated-annual-agricultural-pesticide-use Accessed 2/7/22

Appendix

Table 1. Palmer amaranth accessions screened for dicamba resistance.

Location	Coordinates	
	°N	°W
Gibson 1	35.7889	-88.7967
Madison 1	35.7849	-88.9171
Crockett 1	35.8262	-89.0456
Carroll	35.9221	-88.6462
Crockett 2	35.7816	-89.1327
Madison 2	35.6321	-88.8557
Lauderdale 1	35.7123	-89.9175
Gibson 2	35.7815	-88.8516
Lauderdale 2	35.7204	-89.8771
Shelby	35.3421	-89.8051
Dyer	36.0701	-89.534
Tipton	35.6204	-89.6151
Gibson 3	35.87	-89.0458
Lauderdale 3	35.7183	-89.8544
Lauderdale 4	35.7158	-89.9187
Check		

Table 2. Contrast statements comparing % Palmer amaranth mortality between 15 accessions with a susceptible population following increasing rates of dicamba.

Location	0.14 kg dicamba ha^{-1}		0.28 kg dicamba ha^{-1}		0.56 kg dicamba ha^{-1}		1.12 kg dicamba ha^{-1}	
	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value
Gibson 1	35	0.0005	68	0.1295	91	0.3877	98	0.8453
Madison 1	60	0.3181	92	0.4823	100	0.8743	79	0.0372
Crockett 1	54	0.1077	82	0.8039	92	0.4577	99	0.9200
Carroll	25	<.0001	62	0.0432	89	0.2681	93	0.4015
Crockett 2	44	0.0091	72	0.2297	100	0.8743	88	0.1765
Madison 2	47	0.0186	66	0.0869	96	0.7704	95	0.5882
Lauderdale 1	5	<.0001	3	<.0001	1	<.0001	20	<.0001
Gibson 2	43	0.0075	69	0.1430	93	0.4847	89	0.2057
Lauderdale 2	21	<.0001	41	0.0002	72	0.0014	92	0.3702
Shelby	61	0.3331	77	0.5064	91	0.3779	100	0.9748
Dyer	15	<.0001	54	0.0052	85	0.1206	82	0.0444
Tipton	45	0.0128	68	0.1161	81	0.0310	97	0.7246

Table 2 continued

Location	0.14 kg dicamba ha^{-1}		0.28 kg dicamba ha^{-1}		0.56 kg dicamba ha^{-1}		1.12 kg dicamba ha^{-1}	
	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value
Gibson 3	50	0.0412	68	0.1258	80	0.0263	97	0.7218
Lauderdale 3	64	0.5381	91	0.5845	98	0.9953	100	0.9819
Lauderdale 4	70	0.5548	84	0.2781	99	0.6997	100	1.0000
Check	64		96		95		100	

Table 3. Response of Tennessee Palmer amaranth accessions to dicamba in 2022.

Location	EC50[ab]		RRF
	kg ae ha^{-1}		
Gibson 1	0.1945	cd	1.55
Madison 1	0.1301	d	1.03
Crockett 1	0.1312	d	1.04
Carroll	0.2338	bc	1.85
Crockett 2	0.1776	cd	1.41
Madison 2	0.1792	cd	1.42
Lauderdale 1	1.7978	a	14.25
Gibson 2	0.1638	cd	1.30
Lauderdale 2	0.3140	b	2.49
Shelby	0.1246	d	0.98
Dyer	0.2398	bc	1.90
Tipton	0.2063	cd	1.64
Gibson 3	0.1676	cd	1.33

Table 3 continued

Location	EC50[ab]		RRF
	kg ae ha^{-1}		
Lauderdale 3	0.1210	d	0.96
Lauderdale 4	0.1133	d	0.90
Check	0.1262	d	1
P=0.0064			

[a] Abbreviations: EC50, half-maximal effective concentration; RRF, relative resistance factor

[b] Means not followed by a common letter are significantly different ($p<0.05$)

Table 4. Palmer amaranth fresh weights 21 d following dicamba application.

Location	Fresh weight	
	% of NT control[ab]	
Gibson 1	23	c
Madison 1	18	c
Crockett 1	21	c
Carroll	40	b
Crockett 2	25	c
Madison 2	24	c
Lauderdale 1	106	a
Gibson 2	23	c
Lauderdale 2	30	bc
Shelby	32	bc
Dyer	26	c
Tipton	23	c
Gibson 3	31	bc
Lauderdale 3	21	c
Lauderdale 4	20	c
Check	20	c

[a] Abbreviations: NT, nontreated.

[b] Means not followed by a common letter are significantly different ($p<0.05$)

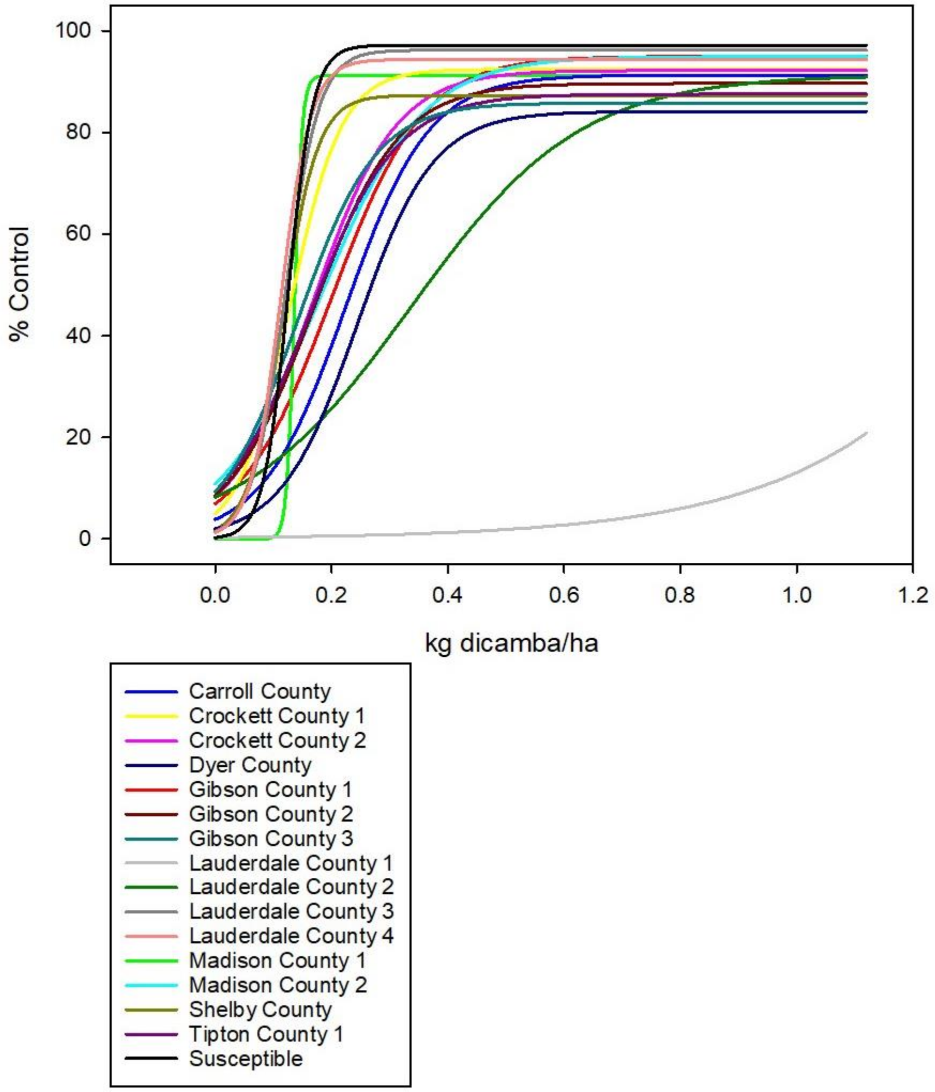

Figure 1. Dicamba dose response by 15 Tennessee accessions. The responses of Palmer amaranth to increasing rates of dicamba as described by Equation 1: y = a/(1+exp(-(rate-c)/b)) where parameter a described the upper limit of control, parameter b estimates the slope, and parameter c represents the EC_{50} rate.

CHAPTER II

DICAMBA-RESISTANT PALMER AMARANTH DOCUMENTED IN TENNESSEE: EXAMINATION OF A POSSIBLE RESISTANCE MECHANISM

A version of this chapter was originally published by Delaney C. Foster, Peter A. Dotray, Stanley Culpepper, and Lawrence E. Steckel:

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Abstract

Cotton and soybean growers were offered new technologies in 2016, expanding in-crop herbicide options to include dicamba or 2,4-D. Within three years of commercialization, dicamba use in these crops increased ten-fold and growers began to report Palmer amaranth escapes in west Tennessee dicamba-tolerant production systems. In 2020, Palmer amaranth seed was collected from eight Tennessee locations where growers witnessed poor control following dicamba. In 2021, field experiments were conducted in Georgia, Tennessee, and Texas to evaluate the efficacy of dicamba in cotton production systems and to examine if malathion insecticide, a cytochrome P450 inhibitor, would improve weed control and not reduce cotton yield when applied in conjunction with the dicamba application. Greenhouse experiments were conducted to evaluate Palmer amaranth control. Palmer amaranth sourced in 2020 escaped dicamba in the greenhouse at 1, 2 and 4 times the labeled rate. There was 15 to 26% Palmer amaranth survival rate exhibited by five populations to the labeled dicamba rate in the greenhouse. These findings were reenforced in the field when research on three of those populations in 2021 showed 55% control with the labeled dicamba rate and 69% control with 2 times the labeled rate. This demonstrates the dicamba resistance allele or alleles were passed between generations. The addition of malathion did not reverse dicamba resistance from populations collected from Tennessee. This result was not consistent in the Macon County or Worth County, GA locations where malathion improved dicamba control of 15 to 38 cm tall Palmer amaranth. Cotton injury was observed when malathion was applied in combination with dicamba. These data further document that dicamba-resistant Palmer amaranth has evolved in Tennessee. It also shows that malathion insecticide, a cytochrome P-450 inhibitor, did not reverse the dicamba resistance in Tennessee Palmer amaranth populations.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.) originated in the dry southwestern U.S. and Mexico, but is now present across the entire southern U.S. and in some Midwestern states such as Illinois and Minnesota (Sauer 1950; Steckel 2007). In a 2019 survey by the Weed Science Society of America, Palmer amaranth was ranked as the most common and most troublesome weed species among all broadleaf crops, fruits, and vegetables (Van Wychen 2019). Since its first known case of herbicide resistance in 1989, Palmer amaranth has developed resistance to eight modes of action including ALS inhibitors (WSSA Group 2), auxin mimics (WSSA Group 4), EPSP synthase inhibitors (WSSA Group 9), HPPD inhibitors (WSSA Group 27), microtubule assembly inhibitors (WSSA Group 3), photosystem II binders (WSSA Group 5), PPO inhibitors (WSSA Group 14), and long chain fatty acid inhibitors (WSSA Group 15) (Heap 2022).

Herbicides are the most effective and economical approach to control weeds in cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* (L.) Merr.), but over-reliance on limited modes of action has resulted in the rapid development of herbicide resistant weeds (Young 2006). Cotton and soybean growers were offered new technologies in 2016, expanding in-crop herbicide options including dicamba or 2,4-D, but these technologies are not without their own resistance development challenges. The first documented case of dicamba resistance in the U.S. was kochia (*Kochia scoparia* L.) in 1994 (Heap 2022). Currently, kochia, prickly lettuce (*Lactuca serriola* L.), and Palmer amaranth have been confirmed with dicamba resistance in the U.S. (Heap 2022). With a longer history of use, several more weed species have confirmed resistance to 2,4-D. In 1957, spreading dayflower (*Commelina diffusa* Burm. F.) became the first herbicide resistant weed in the U.S., conferring resistance to 2,4-D (Heap 2022). Today, five other weed species in the U.S. have developed resistance to this herbicide, including Palmer amaranth, tall waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), buckhorn plantain (*Plantago lanceolata* L.), wild carrot (*Daucus carota* L.), and prickly lettuce (*Lactuca serriola* L.).

In west Tennessee, where row crop production averages 1.3 million ha each year, over 94,000 kg dicamba have been applied on average annually from 1992 to 2019 (USGS 2021). This is due largely to approximately 85% of Tennessee cotton, corn (*Zea mays* L.), and soybean ha being farmed using no-till production relying on dicamba as a part of the preplant burndown program for decades (USDA NASS 2018). Much of middle Tennessee agriculture is encompassed by pastures, where 2,4-D has been used extensively year after year at a rate of >49 g ha^{-1} annually for the past two decades (USGS 2021). While historical use of dicamba and 2,4-D likely contribute to resistance issues, the use of these herbicides has increased 10-fold in cotton and soybean production systems since the commercialization of tolerant varieties in 2016. In Georgia and Texas, tillage is much more extensively used in row crop production, which has led to less reliance on auxin herbicides prior to planting compared with Tennessee (USDA NASS 2018; USGS 2021).

Cytochrome P-450s are a class of enzymes belonging to the largest family involved in oxygen-dependent hydroxylation reactions (Pandian et al. 2020). Cytochrome P-450s are present in the majority of life processes and are one of the main contributors to oxidation-based metabolism in plants (Mizutani and Sato 2011). These enzymes, specifically cytochrome P-450 monooxygenase, contribute to herbicide detoxification by adding an oxygen atom onto herbicide structures to make them more hydrophilic, thereby facilitating degradation more easily in subsequent metabolic reactions; overexpressing just one of the many cytochrome P-450 genes can confer herbicide resistance (Hirose et al. 2005; Hu et al. 2009). Indeed, the dicamba resistance in Xtend® (Bayer CropScience, St. Louis, MO) cotton is garnered from dicamba-monooxygenase, a cytochrome P-450 (Behrens et al. 2007).

Interactions between certain cytochrome P-450 inhibiting organophosphate insecticides with herbicides have shown to increase crop injury in cases where crop tolerance was mediated by metabolism of the herbicide. In corn, many herbicide labels restrict the use of these pesticides to be used in conjunction with one another. Two examples are the herbicides Halex® GT and Accent® Q (Anonymous a and b 2022). This was documented initially by Kapusta and Krausz (1992) who determined that the insecticide terbufos increased corn yield but had an adverse effect on yield when applied in close timing with nicosulfuron. More recent research shows that foliar or in-furrow applications of the organophosphate insecticide chlorpyrifos increased injury and decreased grain yield when used in conjunction with HPPD-inhibitor based premixed herbicides in corn (Steckel et al. 2015).

Previous research has suggested that organophosphate insecticides such as malathion are good candidates to indicate possible nontarget site metabolic resistance mechanisms in weeds (Kumar et al. 2020; Varanasi et al. 2019). Cytochrome P-450 inhibitors prevent the hydroxylation of herbicides by P-450 enzymes, thereby slowing degradation and increasing the half-life of the herbicide within plants (Kreuz and Fonne-Pfister 1992). Shyam et al. (2020) determined that treatment of malathion 30 min prior to 2,4-D applications reversed herbicide resistance to the auxinic herbicide. In recent years, popular press has written stories on metabolic- driven herbicide resistance being mitigated with the use of an organophosate insecticide tying up cytochrome P-450 (Benjamin 2017).

The objectives of these studies were to (1) examine if eight Tennessee, two Texas, and two Georgia Palmer amaranth populations have evolved dicamba resistance, (2) explore a possible mechanism of resistance within Palmer amaranth to auxin herbicides by applying a cytochrome P-450 inhibitor (malathion) in conjunction with dicamba in both field and greenhouse environments, and (3) evaluate cotton response to a known cytochrome P-450 inhibitor malathion to determine if the addition to malathion would be a viable control option for dicamba-resistant Palmer amaranth in cotton.

Materials & Methods

Greenhouse Experiment Screening for Dicamba Resistance

In 2021, a greenhouse experiment was conducted four times at the Texas A&M AgriLife Research Center in Lubbock, TX (33.692106, -101.823311). Palmer amaranth seed was collected in the fall of 2020 from eight Tennessee locations and two Texas locations as noted in Table 5. After collection, seed was threshed, cleaned, and stored at 4C for at least one month prior to planting. Seeds were sprinkled on top of moist potting mix (Berger BM6, Edmond, OK) in pots with 11.4 cm by 11.4 cm by 9.5 cm dimensions; seeds were lightly covered with potting mix and watered. Pots were placed on benches with supplemental lighting to ensure a daily photoperiod of 14 h. Daytime temperature setpoint was 32C and nighttime temperature was set to 27C. Soil was kept moist with overhead irrigation until emergence at which time plants were thinned to 4 to 5 plants per pot. Treatments were applied in a stationary greenhouse spray chamber equipped with Turbo TeeJet Induction nozzles calibrated to deliver 140 L ha^{-1} at 4.8 kph using 200 kPa when plants reached a height of 8 to 10 cm.

The background of these Tennessee fields where the seed was collected consisted of extensive use of dicamba for the past 2 decades in burndown before planting and more recently where dicamba was used in Xtend® crops (USDA NASS 2018). The Bedford County field was planted to Xtend® soybean from 2017 to 2019 when the growers observed very poor control of 8 to 14 cm Palmer amaranth with 0.56 kg ha^{-1} of dicamba. The field history at the Madison County site from 2017 to 2020 was soybean research centered around dicamba-based systems. In 2020, very poor control (<60%) occurred with two applications of dicamba applied at a rate of 0.56 kg ha^{-1} (data not shown). The other 6 sites were all planted to Xtend® cotton from 2017 to 2020. Growers managing these sites began experiencing poor Palmer amaranth control with dicamba in either 2019 or 2020. Specifically, seed was collected from the Palmer amaranth escapes in 2019 at the Bedford County, Gibson County, TN 2, Crockett County, TN 1 and TN 2 locations in the fall of 2019 and underwent a preliminary greenhouse screen for resistance to dicamba. The results showed more than 10% survivors from these locations following dicamba at the 0.56 kg ha^{-1} rate and was more than the susceptible check. In 2020, research was conducted in the field using 0.56 and 1.16 kg ha^{-1} dicamba rates on 5 to 10 cm tall Palmer amaranth at the Gibson County, TN 2 and Crockett County TN 1 locations. In those studies, only 50 and 60% Palmer amaranth control was acquired with those two respective rates.

The treatment design was a complete factorial (malathion timing * dicamba rate) within a randomized complete block study design. The first factor of timing consisted of malathion at 2 kg ai ha^{-1} applied 24 h or 1 h before dicamba application, tank mixed with dicamba, or a dicamba alone control. The second factor was herbicide rate, which consisted of dicamba applied at 0.56 (1X), 1.12 (2X) and 2.24 (4X) kg ae ha^{-1} field rates. Survival rate of Palmer amaranth was calculated by counting the number of dead and alive plants in each plot and fresh weight of live plants was measured 21 d after application. Data were analyzed using the GLIMMIX procedure (2014 Version 9.4, SAS Institute Inc., Cary, NC) for ANOVA and Fisher’s LSD at $\alpha = 0.05$.

Single degree-of-freedom contrast statements were conducted to compare each location with the known susceptible check.

Field Experiments Determining the Influence of Malathion on Dicamba Activity

The field experiment was conducted at three locations in Tennessee, two locations in Georgia, and one location in Texas. At all locations, a complete factorial (malathion timing * dicamba rate) within a randomized complete block study design was implemented. In Georgia and Tennessee, the first factor of timing consisted of malathion at 2 kg ai ha^{-1} applied 24 h or 1 h before dicamba application, tank mixed with dicamba, or a dicamba alone control. The malathion rate was based on Shyam et al. (2020) research, which determined 2 kg ha^{-1} reversed auxin herbicide resistance in a Kansas population of Palmer amaranth. However, this rate is above what is recommended in row crops (Stewart et al. 2022). In Texas, treatment structure was identical except there was no 1 h before dicamba applications. The second factor at all locations was herbicide rate, which consisted of dicamba applied at 0.56 (1X), 1.12 (2X) and 2.24 (4X) kg ae ha^{-1} field rates. All herbicides were applied using a CO_2-pressurized backpack sprayer equipped with AIXR 11002 nozzles (TeeJet® Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 220 kPa except in Texas where TTI 11002 nozzles and 193 kPa spray pressure were used.

Tennessee field experiments were conducted in 2021 at three locations. The first location was the West Tennessee AgResearch and Education Center in Madison County (WTREC) (35.632003°N, -88.855874°W). The field history at this site is described previously. The second location was a grower’s field site in Madison County (35.781542°N, -88.851567°W). The field history consisted of Xtend® cotton planted from 2016 to 2019 followed by Enlist® cotton in 2020. In 2019, the grower noticed areas of escaped Palmer amaranth after multiple applications of dicamba at 0.56 kg ha^{-1}. Seed was collected from these Palmer amaranth plants in the fall of 2019 and screened for resistance to dicamba. There were 10% survivors from this location following dicamba at the 0.56 kg ha^{-1} rate. In 2020, a preliminary screen was conducted in the field and only 50 and 55% control was observed with 0.56 and 1.16 kg ha^{-1} dicamba rates, respectively, when applied to 10 to 14 cm tall Palmer amaranth. The third location was on a grower's field in Lauderdale County (35.715428°N, -89.918452°W). The field history was similar to the Madison County site where Xtend® cotton was planted from 2016 through 2020. Again, the grower noticed Palmer amaranth escapes in 2019 after multiple dicamba applications of 0.56 kg ha^{-1}.

The WTREC site was equipped with lateral overhead irrigation while the Madison and Lauderdale County sites were rain-fed. Each location consisted of a native Palmer amaranth population with no crop present. Once the initial flush of Palmer amaranth emerged, pyroxasulfone at 0.12 kg ai ha^{-1} was applied broadcast over the study to suppress new flushes of weeds. In addition, clethodim at 0.28 kg ai ha^{-1} was applied to control native junglerice (*Echinochloa colona* L.). Plot size was 3 m wide by 9 m in length. At the time of treatment application, Palmer amaranth was 10 cm in height.

Georgia field experiments were conducted in 2021 at a grower's field site in Macon County (32.423478°N, -84.128571°W) and at the University of Georgia's Coastal Plains Experiment Station Ponder Farm in Worth County (31.505°N, -83.6508°W). The field history at both these sites was cotton weed control research where dicamba had provided Palmer amaranth control >80%. Both sites were equipped with overhead irrigation. Cotton (Bayer CropScience St. Louis, MO) was planted on April 27 at Macon County and on May 17 at Worth County with native Palmer amaranth populations present. Plot size was 2 m wide by 8 m in length. Dicamba application was targeted for a crop stage of 3 to 5 leaf, not weed height; therefore, the height of Palmer amaranth at the time of dicamba application was 15 cm at Macon County and up to 38 cm at Worth County when treated. The Palmer amaranth was much larger at both Georgia locations compared with Tennessee or Texas due to logistics and weather delay. At 7 and 18 d after treatment application, *S*-metolachlor (1.0 kg ai ha^{-1}) was applied over the entire study to prevent additional Palmer amaranth emergence. In addition, clethodim at 0.28 kg ha^{-1} was applied to control native annual grasses. Cotton production practices followed those for GA (Hand et al. 2022).

The field experiment in Texas was conducted at the Texas Tech University New Deal Research Farm in Lubbock County (33.730881°N, -101.734796°W). The field history at this site was cotton weed control research where dicamba had provided >90% Palmer amaranth control. This site was equipped with subsurface drip irrigation. Cotton was planted on June 5, 2021 and plots were maintained weed-free throughout the season using trifluralin at 1.12 kg ai ha^{-1} preplant incorporated, prometryn at 1.12 kg ai ha^{-1} preemergence, glyphosate at 1.4 kg ae ha^{-1} + glufosinate at 0.88 kg ai ha^{-1} + ammonium sulfate at 3.4 kg ha^{-1} postemergence, cultivation, and hand-weeding. Treated plot size was 2 m wide by 9 m in length. Cotton was 8 leaf at the time of treatment applications.

Palmer amaranth control at the Tennessee and Georgia locations was evaluated visually using a 0 to 100% scale (Frans et al. 1986) as well as counting the number of surviving plants per m^2 at 14 to 28 d after herbicide treatment (DAT). Cotton injury was rated on a 0 to 100% scale with 0% providing no injury and 100% providing complete crop death at 5, 9, and 14 DAT. Cotton lint yield was collected per plot using a spindle picker at the GA locations and a cotton stripper at New Deal. Data were analyzed using the GLIMMIX procedure (2014 Version 9.4, SAS Institute Inc., Cary, NC) for ANOVA and Fisher's LSD at $\alpha = 0.05$.

Greenhouse Experiment Screening for Dicamba Resistance

In 2021, a greenhouse experiment was conducted four times at the Texas A&M AgriLife Research Center in Lubbock, TX (33.692106, -101.823311) as previously described. The treatment design was identical to the field experiment conducted in Georgia and Tennessee with the 1, 2, and 4X rate of dicamba applied with no malathion, in mixture with malathion, or 1 or 24 h after malathion. Survival rate of Palmer amaranth was calculated by counting the number of dead and alive plants in each tray and fresh weight of live plants was measured 21 d after application. Data were analyzed using the GLIMMIX procedure (2014 Version 9.4, SAS Institute Inc., Cary, NC) for ANOVA and Fisher's LSD at $\alpha = 0.05$. Single degree-of-freedom contrast statements were used to compare biotypes from each location in the greenhouse. Overall, control of Palmer amaranth was better in the greenhouse than in the field. This would be consistent with previous researchers who reported improved control with herbicides in the greenhouse compared with the field (Combellack 1982; Perkins et al. 2020). Since control was better in the greenhouse than observed in the field, an $\alpha = 0.1$ was used for separation.

Results & Discussion

Palmer amaranth Greenhouse Experiment

In the greenhouse experiment, malathion did not influence Palmer amaranth control by dicamba regardless of rate. Thus, dicamba rate by seed population were the only factors evaluated when determining Palmer amaranth response. When dicamba was applied at 0.56 kg ha^{-1}, five Tennessee Palmer amaranth populations had a greater survival percentage when compared with the known susceptible Lubbock County, TX 1 population (Table 6). These

Tennessee populations were Crockett County, TN 1 (15%)(P=0.0997) and 2 (18%)(P=0.0324), Bedford County (26%)(P=0.0021), Gibson County, TN 2 (18%)(P=0.0371) and Carroll County, TN (16%)(P=0.0730). At 1.12 kg ha^{-1} of dicamba, the Crockett County, TN 1 (14%)(P=0.0461), and Madison County, TN (13%)(0.0571) populations had a greater percentage of survivors when compared with Lubbock County, TX 1 (2%).

Palmer amaranth fresh weight as a percentage of the nontreated control showed similar results where greater weights were recorded in the Bedford County (16%)(P=0.0765) and Carroll County, TN (18%)(P=0.0215) populations when compared with Lubbock County, TX 1 (8%) following dicamba at 0.56 kg ae ha^{-1} (Table 7).

Palmer amaranth Control Field Experiments

Weed control studies were separated by location in Georgia with differing results likely influenced by Palmer amaranth size at the time of application. No differences among locations were noted in Tennessee and data were combined across three locations.

Palmer amaranth was controlled 73, 92, and 97% with the 1, 2, and 4X rate of dicamba, respectively at Macon County, GA at 28 DAT (Table 8). The addition of malathion increased Palmer amaranth control by 10 to 16% when dicamba was applied at the 0.56 kg ae ha^{-1} 1X rate. Higher use rates of dicamba provided at least 92% control thus the benefit from malathion likely would not be detectable. Palmer amaranth density 14 DAT was almost 74,000 plants ha^{-1} in the nontreated control. Dicamba alone at the 1X rate decreased density by 82% and the addition of malathion 24 h or 1 h before dicamba further decreased Palmer amaranth density to 89 to 90% of the control. Malathion timing did not decrease density when dicamba was applied at 1.12 or 2.24 kg ha^{-1}.

At Worth County, GA, Palmer amaranth control increased by 15 to 25% when malathion was applied 1 h before or in mixture with the 1X rate of dicamba (Table 8). At the 1.12 kg ha^{-1} dicamba rate (2X), malathion applied 24 h or 1 h before the herbicide increased Palmer amaranth control 9 to 12% compared with the herbicide alone. All dicamba-malathion combinations at the 2.24 kg ae ha^{-1} dicamba rate were similar to the herbicide applied alone. Palmer amaranth density at Worth County was 17,346 plants ha^{-1} in the nontreated control 28 DAT. Only the addition of malathion applied 1 h before dicamba at 0.56 kg ha^{-1} decreased Palmer amaranth density compared with the same rate of the herbicide alone.

In Tennessee, Palmer amaranth control was not affected by malathion timing but was influenced by dicamba rate. Palmer amaranth was controlled 55, 69, and 85% with the 1, 2, and 4X rate of dicamba, respectively (Table 8). Palmer amaranth density in the nontreated control was >166,000 plants ha^{-1} 21 DAT. The labeled rate of dicamba (0.56 kg ha^{-1}) did not reduce Palmer amaranth populations compared with the nontreated populations. Applying malathion 24 h prior to dicamba at the labeled rate was the only insecticide timing to decrease Palmer amaranth density (86,000 plants ha^{-1}). This result was similar to response shown by the Macon County Palmer amaranth population. The 1.121 kg ha^{-1} rate of dicamba reduced the Palmer amaranth population 53% compared with the 0.56 kg ha^{-1} rate. The 2.24 kg ha^{-1} rate did not reduce Palmer amaranth populations compared with the 1.12 kg ha^{-1} dicamba rate.

These results were consistent with reports of Palmer amaranth control failures by the Tennessee farmers who managed those fields. The Texas Palmer amaranth populations were susceptible to dicamba. The first research on the Georgia populations would indicate that one location is suceptible to dicamba and another may be evolving dicamba resistance. As noted earlier, the Palmer amaranth at application for the Georgia populations were quite large (15 to 38 cm); therefore, weed size rather than dicamba resistance evolution may be the reason for poor control. Additional research on the Palmer amaranth at this site would be warranted as malathion did improve control.

Previous research by Shyam et al. (2020) indicated that 2,4-D resistance in Palmer amaranth was mitigated when malathion was mixed with 2,4-D and applied to the resistant biotype. In PPO-resistant Palmer amaranth populations, malathion followed by (fb) fomesafen or saflufenacil partially reversed resistance; however, malathion fb flumioxazin or acifluorfen had no affect on Palmer amaranth compared with those herbicides applied alone (Varansi et al. 2018; 2019). Similarly, in ALS-resistant Palmer amaranth, 2,000 g ha^{-1} malathion applied 1 h before chlorsulfuron reduced plant biomass by 50% when compared with the herbicide applied alone (Nakka et al. 2017). Similar results have been reported in other weed species as well. When malathion was mixed with imazamox and quinclorac and applied to resistant junglerice populations, resistance was mitigated (Wright et al. 2018). Christopher et al. (1994) reported higher mortality from chlorsulfuron in both susceptible and resistant *Lolium rigidum* Gaud. populations when the mechanism of resistance was confirmed as metabolic.

Cotton Response Field Experiments

Cotton response field experiments were separated by location due to location interactions ($p<0.0001$). In Macon County, GA, dicamba at the 1, 2, and 4X rate injured cotton 16, 23, and 41% at 5 DAT, respectively (Table 9). Applying malathion 24 h before dicamba did not influence injury compared with dicamba alone, regardless of rate. Malathion applied 1 h before dicamba applications increased crop injury when applied at the 2 and 4X rate while the tank mixture showed 9 to 15% more injury at the 1 and 2X dicamba rates, respectivly. Injury was generally similar at 9 DAT but by 14 DAT, differences in injury were only observed when comparing dicamba rates with no affect from malathion. Cotton visual injury was not detectable by 21 DAT (data now shown). Plant height was measured and malathion treatment had no influence on cotton height; however, there was a trend for shorter cotton when the 4X rate of dicamba was applied (data not shown). Cotton lint yield was not influenced by crop response or malathion but rather by Palmer amaranth control obtained with dicamba. Both higher use rates of dicamba noted higher yields when compared with the 1X dicamba rate; no yield was obtained from the non-treated control.

In Worth County, GA, malathion had less influence on cotton response from dicamba (Table 10). At 5 DAT, injury differences were only observed with increasing rates of dicamba ranging from 17 to 28%. By 9 DAT, injury response was similar to that observed at 5 d except the tank mix of 1.12 kg ha^{-1} of dicamba with malathion was 9% more injurious than the respective rate of dicamba alone. By 14 DAT, visual injury was no longer detectable (data not shown). Plant height was measured and herbicide treatment had no influence on cotton height; however, competition from Palmer amaranth decreased cotton height in the nontreated control late in the season (data not shown). Cotton lint yield was not influenced by malathion, dicamba rate, Palmer control among herbicide treatments, or crop response. Yield from the nontreated control was 0 kg ha^{-1} and yields were improved by all herbicide treatments (721 to 1115 kg ha^{-1}).

In Texas, cotton injury was greatest 5 DAT (Table 11). Dicamba at the 1, 2, and 4X rate injured cotton 10, 18, and 30%, respectively. Applying malathion 24 h before dicamba increased injury 8 to 12% with the 1 and 2X rate of dicamba. Malathion mixed with dicamba at the 1, 2, and 4X rates increased injury 16, 9, and 5%, respectively, compared with herbicide alone 5 DAT. Injury observations at 9 DAT were generally similar to those at 5 DAT. By 14 DAT, differences in injury were only observed when comparing dicamba rate. Injury was no longer visually detectable by 21 DAT (data now shown). Differences in cotton yield were not observed in this weed-free experiment.

Similar studies have shown that malathion applied in combination with other herbicides such as pyrithiobac increased cotton injury with no adverse affects on yield (Allen and Snipes 1995; Snipes and Seifert 2003; Minton et al. 2005). Postemergence applications of malathion tank mixed with trifloxysulfuron applied to 4- to 5-leaf cotton increased phytotoxicity 10% at 4 DAT compared with trifloxysulfuron alone (Minton et al. 2008). Insecticides in the same organophosphate class as malathion, such as dimethoate, produced similar results when applied in conjunction with pyrithiobac and glufosinate where visual injury increased with the addition of the insecticide, but cotton yield was not adversely affected (Costello et al. 2005, Steckel et al. 2012). Another possible explanation for the cotton injury would be that dicamba resistance in cotton is derived with a P-450 inhibitor dicamba monooxygenase (Behrens et al. 2017). As such, the cotton injury could possibly be from the high rate of malathion de-activating dicamba monoxygenase allowing dicamba to injure the cotton. However, the injury observed was a leaf burn not the typical epinasty associated with dicamba. The authors would suggest the high rate of malathion acted like a surfactant which caused the leaf burn.

The recommended rate of malathion insecticide in Tennessee cotton from emerged to blooming is 1.4 kg ai ha$^{-1,}$, a lower rate and later in the season than applications in these studies (Stewart et al. 2022). While visual injury was observed, cotton lint yield was not reduced by the addition of malathion. In dicamba-susceptible Palmer amaranth populations, the addition of malathion to the labeled rate of dicamba improved control, but as herbicide rate increased, the benefit of adding malathion diminished. In dicamba-resistant Palmer amaranth populations, malathion did not improve control. Therefore, this research suggests that adding malathion does not reverse dicamba resistance in Palmer amaranth and the insecticide increased crop injury.

These results document dicamba failing to control Palmer amaranth sourced in 2020 in the greenhouse at 1, 2 and 4 times the labeled rate. Moreover, the 15 to 26% Palmer amaranth survival rate exhibited by five populations to the labeled dicamba rate in the greenhouse documents that Palmer amaranth at those locations has evolved dicamba resistance. Another step to confirm resistance is documenting heritability of the resistance between generations. The history of Palmer amaranth escaping dicamba was suggested when research in three of those fields in 2021 showed just 55% control with the labeled dicamba rate and 69% control with 2 times the labeled rate. This indicated that dicamba resistance was passed down from the 2019 Palmer amaranth generation to the 2020 and 2021 generations. These findings are consistent with and reinforce other research in Tennessee that documented dicamba resistance in Palmer amaranth.

The addition of malathion did not reverse dicamba resistance from populations collected from Tennessee. However, this does not necessarily rule out metabolic resistance or cytochrome P-450s being a key player in dicamba resistance due to the hundreds of P-450 enzymes and other metabolic enzymes present in plants (Bak et al. 2011; Jun et al. 2015). This result was not consistent in the Macon County or Worth County, GA locations where malathion did improve control of large (15 to 38 cm) Palmer amaranth.

Future research needs to be conducted to determine how well established the dicamba-resistant biotype of Palmer amaranth has become in Tenneessee and if these populations are cross-resistant to 2,4-D. Moreover, more detailed analysis of the relative dicamba resistance ratio of these populations should be conducted. In addition, research designed to assess the mechanism or mechanisms of resistance should be conducted on these biotypes. Finally, weed management research needs to be conducted to determine how best to integrate herbicides and non-herbicide tactics to better control these Palmer amaranth populations.

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References

Allen RL, Snipes CE (1995) Interactions of foliar insecticides applied with pyrithiobac. Weed Technol 9:512–517

Anonymous a (2022) Halex GT herbicide label. Accessed 2/7/22 http://www.cdms.net/ldat/ld8DT001.pdf

Anonymous b (2022) Accent Q herbicide label. Accessed 2/7/22 http://www.cdms.net/ldat/ld3OS000.pdf

Bak S, Beisson F, Bishop G, Hamberger B, Hofer R, Paquette S, Werck-Reichhart D (2011) Cytochromes P-450. The Arabidopsis Book 9: e0144

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jian WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: Enlarging and preserving biotechnology-based weed management strategies. Science 316:1185-1188

Benjamin C (2017) Insecticide shown to reverse metabolic herbicide resistance. Weed Smart. Accessed 7/26/22 https://www.weedsmart.org.au/content/insecticide-shown-to-reverse-metabolic-herbicide-resistance/

Christopher JT, Preston C, Powles SB (1994) Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. Pesticide Biochem and Physiol 49:172-182

Combellack JH (1982) Loss of herbicides from ground sprayers. Weed Res 22:193-204

Costello RW, Griffin JL, Leonard BR, Miller DK, Church GE (2005) Pyrithiobac and insecticide coapplication effects on cotton tolerance and broadleaf weed and thrips (Frankliniella spp.) control. Weed Technol 19:430-436

Frans R, Talbert R, Marx D, Crowley H (1986) Experimental design and techniques for measuring and analyzing plant response to weed control practices *in* Camper ND eRMiWS, ed. Southern Weed Science Society. Champaign, IL

Hand, C, Culpepper S, Harris G, Kemerait B, Liu Y, Perry C, Hall D, Porter W, Roberts P, Smith A, Virk S, Bag S, Sintim H (2022) 2022 Georgia Cotton Production Guide. Athens, GA: The University of Georgia. 146 p

Heap I (2022) The International Survey of Herbicide Resistant Weeds. www.weedscience.org Accessed 2/7/22

Hirose S, Kawahihashi H, Ozawa K, Shiota N, Inui H, Ohkawa H, Ohkawa Y (2005) Transgenic rice containing human CYP2B6 detoxifies various classes of herbicides. J Agricultural Food Chem 53:3461-3467

Hu J, Tranel PJ, Stewart NN, Yuan JS (2009) Molecular and genomic mechanisms of non-target-site herbicide resistance. Pages 149-153 *in* Stewart CN, ed. Weedy and Invasive Plant Genomics. Iowa: Blackwell Publishing

Jun X, Xin-yu W, Wang-zhen G (2015) The cytochrome P-450 superfamily: Key players in plant development and defense. J Integrative Agriculture 14:1673-1686

Kapusta G, Krausz RF (1992) Interaction of terbufos and nicosulfuron on corn (*Zea mays*). Weed Technol 6:999-1003

Kreuz K, Fonne-Pfister R (1992) Herbicide-insecticide interaction in maize: Malathion inhibits cytochrome P450-dependent primsulfuron metabolism. Pest Biochem and Physiol 43:232-240

Kumar V, Liu R, Currie RS, Jha P, Morran S, Gaines T, Stahlman PW (2020) Cross-resistance to atrazine and metribuzin in multiple herbicide-resistant kochia accessions: Confirmation, mechanism, and management. Weed Technol 35:539-546

Minton BW, Senseman SA, Cothren JT, Chandler JM, Wells JW (2005) Cotton response to CGA-362622 applied alone and in combination with selected insecticides. Weed Technol 19:244-250

Minton B, Matocha M, Senseman S (2008) The influence of malathion on trifloxysulfuron absorption and translocation in cotton. J Cotton Sci 12:48-52

Mizutani M, Sato F (2011) Unusual P-450 reactions in plant secondary metabolism. Arch Biochemistry and Biophysics 507:194-203

Nakka S, Thompson CR, Peterson DE, Jugulam M (2017) Target site-based and non-target site based resistance to ALS inhibitors in Palmer amaranth (*Amaranthus palmeri*). Weed Sci 65:681-689

Pandian BA, Sathishraj R, Djanaguiraman M, Prasad PVV, Jugulam M (2020) Role of cytochrome P-450 enzymes in plant stress response. Antioxidants 9:454

Perkins CM, Mueller TC, Steckel LE (2020) Survey of glyphosate-resistant junglerice (Echinochloa colona) acession in dicamba-resistant crops in Tennessee. Weed Technol 35:410-418

Sauer J (1950) The grain amaranths: A survey of their history and classification. Annals of the Missouri Botanical Garden 1990 37:561-632

Shyam C, Borgato EA, Peterson DE, Dille JA, Jugulam M (2020) Predominance of metabolic resistance in a six-way-resistant Palmer amaranth (*Amaranthus palmeri*) population. Front Plant Sci 11

Snipes C, Seifert S (2003) Influence of malathion timing on cotton (*Gossypium hirsutum*) response to pyrithiobac. Weed Technol 17:266-268

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

Steckel LE, Stephenson D, Bond J, Stewart SD, Barnett KA (2012) Evaluation of WideStrike Flex cotton response to over-the-top glufosinate tank mixtures. J Cotton Sci 16:88-95

Steckel LE, Stewart SD, Steckel S (2015) Corn response to POST-applied HPPD-inhibitor based premix herbicides with in-furrow and foliar-applied insecticides. Weed Technol 29:18-23

Stewart S, Brown S, Steckel S, Williams M, McClure A (2022) 2022 Insect Control Recommendations for Field Crops. University of Tennessee Extension PB1768 https://extension.tennessee.edu/publications/Documents/PB1768.pdf Accessed 3/22/22

USGS (2021) Estimated Annual Agricultural Pesticide Use. https://www.usgs.gov/media/images/estimated-annual-agricultural-pesticide-use Accessed 2/7/22

USDA NASS (2018) 2018 Tennessee Tillage Systems https://www.nass.usda.gov/Statistics_by_State/Tennessee/Publications/Special_Surveys/tillage2018.pdf Accessed 2/7/2022

Van Wychen L (2019) 2019 Survey of the most common and troublesome weeds in broadleaf crops, fruits, and vegetables in the U.S. and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx Accessed 2/7/22

Varanasi VK, Brabham C, Norsworthy JK (2018) Confirmation and characterization of non-target site resistance to fomesafen in Palmer amaranth (*Amaranthus palmeri*). Weed Sci 66:702–709

Varanasi VK, Brabham C, Korres NE, Norsworthy JK (2019) Nontarget site resistance in Palmer amaranth (*Amaranthus palmeri* (S.) Wats.) confers cross-resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Technol 33:349-354

Wright AA, Rodriguez-Carres M, Sasidharan R, Koski L, Peterson DG, Nandula VK, Ray JD, Bond JA, Shaw DR (2018) Multiple herbicide–resistant junglerice (*Echinochloa colona*): Identification of genes potentially involved in resistance through differential gene expression analysis. Weed Sci 66:347–354

Young B (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

Appendix

Table 5. Location coordinates for Palmer amaranth populations collected in fall 2020 to determine potential resistance to dicamba in the greenhouse.

Location	Latitude	Longitude
	°N	°W
Bedford County, TN	35.4415	-86.6373
Carroll County, TN	36.0790	-88.6824
Crockett County, TN 1	35.7814	-89.1329
Crockett County, TN 2	35.7854	-89.1567
Gibson County, TN 1	35.8702	-89.0480
Gibson County, TN 2	35.7889	-88.7964
Gibson County, TN 3	35.9668	-89.0044
Lubbock County, TX	33.6896	-101.8201
Lubbock County, TX 2	33.7311	-101.7337
Madison County, TN	35.6310	-88.8575

Table 6. Contrast statements comparing survival rate of Palmer amaranth populations vs a known susceptible population (Lubbock County, TX 1) following increasing rates of dicamba.

	0.56 kg ha^{-1}		1.121 kg ha^{-1}		2.24 kg ha^{-1}	
Location	Survival rate	P-value	Survival Rate	p-value	Survival Rate	p-value
	-------%-------		---------%---------		---------%---------	
Lubbock County, TX 1	3		2		5	
Bedford County, TN	26	0.0021	8	0.3398	4	0.8090
Carroll County, TN	16	0.0730	9	0.2073	4	0.8123
Crockett County, TN 1	15	0.0997	14	0.0461	1	0.1902
Crockett County, TN 2	18	0.0324	5	0.5990	3	0.5926
Gibson County, TN 1	12	0.3039	8	0.3217	7	0.5772
Gibson County, TN 2	18	0.0371	7	0.4001	0	0.1281
Gibson County, TN 3	9	0.4327	6	0.4654	1	0.1918
Lubbock County, TX 2	10	0.3481	7	0.4174	3	0.4576
Madison County, TN	7	0.5934	13	0.0571	1	0.1783

Table 7. Contrast statements comparing fresh weight of Palmer amaranth populations vs a known susceptible population (Lubbock County, TX 1)

Location	0.56 kg dicamba ha^{-1}		1.121 kg dicamba ha^{-1}		2.24 kg dicamba ha^{-1}	
	Fresh weight[a]	P-value	Fresh weight	P-value	Fresh weight	P-value
	-------%-------		-------%-------		-------%-------	
Lubbock County, TX 1	8		8		9	
Bedford County, TN	16	0.0765	4	0.0725	5	0.0051
Carroll County, TN	18	0.0215	10	0.1305	8	0.3147
Crockett County, TN 1	8	0.9329	7	0.6502	3	<.0001
Crockett County, TN 2	8	0.9606	5	0.1113	5	0.0063
Gibson County, TN 1	12	0.4792	6	0.5564	6	0.0263
Gibson County, TN 2	15	0.1274	6	0.2636	5	0.0009
Gibson County, TN 3	12	0.3345	8	0.5726	7	0.1696
Lubbock County, TX 2	15	0.1239	10	0.1377	7	0.1700
Madison County, TN	8	0.8845	10	0.2104	6	0.0437

[a] Expressed as % of the nontreated control

Table 8. Palmer amaranth control 28 d following dicamba applications with and without malathion.

		Macon County, GA		Worth County, GA		Tennessee[1]	
Dicamba Rate	Malathion Timing	28 DAT	14 DAT	28 DAT		28 DAT	21 DAT
kg ae ha^{-1}		%	plants ha^{-1}	%	plants ha^{-1}	%	plants ha^{-1}
Nontreated Control		N/A	73,906 a	N/A	17,346 a	N/A	167,000 a
0.56	No Malathion	73 f	13,324 b	56 f	4,097 b	55 c	156,000 ab
	24 h	86 de	8,210 c	76 cde	3,581 bc	57	86,000 cd
	1 h	89 cd	7,482 c	81 bcd	2,261 ef	56	110,000 abc
	Tankmix	83 e	14,341 b	71 e	4,173 b	55	100,000 bcd
1.12	No Malathion	92 bc	3,764 cd	75 de	2,807 cde	69 b	73,000 cd
	24 h	95 ab	3,354 cd	84 bc	2,746 cde	70	107,000 abcd
	1 h	94 abc	2,367 d	87 ab	1,851 ef	70	91,000 cd
	Tankmix	93 abc	5,326 cd	81 bcd	3,551 bcd	69	82,000 cd
2.24	No Malathion	97 ab	940 d	88 ab	2,579 def	85 a	61,000 cd
	24 h	99 a	774 d	93 a	1,972 ef	83	45,000 d
	1 h	99 a	819 d	93 a	1,714 f	87	45,000 d

Table 8 continued

		Macon County, GA		Worth County, GA		Tennessee[1]	
Dicamba Rate	Malathion Timing	28 DAT	14 DAT	28 DAT		28 DAT	21 DAT
kg ae ha^{-1}		%	plants ha^{-1}	%	plants ha^{-1}	%	plants ha^{-1}
	Tankmix	98 a	1,563 d	89 ab	2,504 ef	84	46,794 d
p-values							
Rate		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Malathion Timing		0.0021	0.0144	<.0001	0.0056	0.5492	0.1166
Rate*Malathion Timing		0.0052	<.0001	0.0587	<.0001	0.9705	0.0009

[1] Tennessee data combined across three locations.

Table 9. Cotton response to dicamba as influenced by malathion at Macon County, GA.

		Cotton Injury			Lint Yield
Dicamba Rate	Malathion Timing	5 DAT	9 DAT	14 DAT	
kg ae ha^{-1}		-----------------%-----------------			kg ha^{-1}
0.56	No Malathion	16 e	18 f	18 b	362 b
	24 h	19 de	19 ef	15	374
	1 h	24 de	26 cd	23	354
	Tankmix	25 d	21 def	16	371
1.12	No Malathion	23 de	25 de	20 ab	519 a
	24 h	25 d	24 def	19	530
	1 h	40 bc	33 bc	21	501
	Tankmix	38 c	36 ab	21	561
2.24	No Malathion	41 bc	35 ab	24 a	570 a
	24 h	46 ab	40 a	21	568
	1 h	50 a	40 a	28	503
	Tankmix	41 bc	35 ab	23	594

Table 9 continued

		Cotton Injury			Lint Yield
Dicamba Rate	Malathion Timing	5 DAT	9 DAT	14 DAT	
p-values					
Rate		<.0001	<.0001	0.0386	<.0001
Malathion Timing		<.0001	0.0043	0.1887	0.3972
Rate*Malathion Timing		0.0264	0.0348	0.9500	0.7192

Table 10. Cotton response to dicamba as influenced by malathion at Worth County, GA.

Dicamba Rate	Malathion Timing	Cotton Injury 5 DAT	 9 DAT	Lint Yield
kg ae ha^{-1}		----------%----------		kg ha^{-1}
0.56	No Malathion	17 c	12 c	721
	24 h	16	15 bc	781
	1 h	16	16 bc	961
	Tankmix	18	14 c	931
1.12	No Malathion	21 b	13 c	871
	24 h	23	13 c	994
	1 h	21	16 bc	1,115
	Tankmix	21	22 a	961
2.24	No Malathion	28 a	24 a	934
	24 h	28	23 a	931
	1 h	28	23 a	1,044
	Tankmix	30	20 ab	988

Table 10 continued

		Cotton Injury		Lint Yield
Dicamba Rate	Malathion Timing	5 DAT	9 DAT	
p-values				
Rate		<.0001	<.0001	0.0681
Malathion Timing		0.2413	0.298	0.0618
Rate*Malathion Timing		0.7813	0.023	0.9213

Table 11. Cotton response to dicamba as influenced by malathion at New Deal, TX.

		Cotton Injury			Lint Yield
Dicamba Rate	Malathion Timing	5 DAT	9 DAT	14 DAT	
kg ae ha^{-1}		-----------------%-----------------			kg ha^{-1}
Nontreated		N/A	N/A	N/A	1409
0.56	No Malathion	10 g	5 d	3 c	1572
	24 h	22 e	19 c	6	1396
	Tankmix	26 d	18 c	3	1441
1.12	No Malathion	18 f	16 c	10 b	1378
	24 h	26 d	24 b	11	1328
	Tankmix	27 cd	23 b	13	1445
2.24	No Malathion	30 bc	23 b	17 a	1287
	24 h	32 ab	31 a	18	1373
	Tankmix	35 a	34 a	15	1315
p-values					

Table 11 continued

		Cotton Injury			Lint Yield
Dicamba Rate	Malathion Timing	5 DAT	9 DAT	14 DAT	
Rate		<.0001	<.0001	<.0001	0.1796
Malathion Timing		<.0001	<.0001	0.1991	0.7584
Rate*Malathion Timing		0.0007	0.0265	0.3424	0.4457

CHAPTER III

WEED HEIGHT INFLUENCES EFFICACY OF 2,4-D AND DICAMBA IN AUXIN-RESISTANT PALMER AMARANTH POPULATIONS IN TENNESSEE

Abstract

In 2017, the University of Tennessee Extension Service began receiving observations of auxin herbicides failing to control Palmer amaranth in dicamba and 2,4-D-tolerant cotton and soybean fields. The University of Tennessee Extension Service determined that while occasionally applications were made to large (>10 cm) weeds in a manner not recommended by the label, explaining some failures, the majority of dicamba and 2,4-D applications were made to small, actively growing Palmer amaranth plants and the weed survived the herbicide application. Experiments were initiated in grower’s fields where herbicide failures were observed to determine the impact of weed height on Palmer amaranth control following applications of dicamba or 2,4-D at increasing rates and determine if reported failures were because of possible selection for herbicide resistance. While weed height at the time of application had a significant effect on Palmer amaranth control with auxin herbicides, control was still unacceptable in the field at the labelled rates of dicamba and 2,4-D at <10 cm tall weeds (48% and 53%, respectively). This research is the first to show Palmer amaranth that is resistant to both 2,4-D and dicamba.

Introduction

Weeds are one of the most detrimental pests to crop production, potentially causing an average yield loss of 34% across 6 major crops if not controlled properly (Oerke 2006). Herbicides have become the most effective control method for cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L. Merr.) growers, but over-reliance on a select few modes of action has resulted in rapid development of herbicide resistance (Young 2006). The most common and troublesome weed species in United States cotton and soybean production is Palmer amaranth (*Amaranthus palmeri* S. Wats.), a weed native to the southwestern United States which has developed resistance to eight different herbicide modes of action (Van Wychen 2019; Heap 2022). If left uncontrolled, Palmer amaranth can cause significant crop losses due to decreased yield and harvest inefficiencies due to mechanical interference (Smith et al. 2000).

New herbicide-tolerant cotton and soybean technologies became commercially available in 2016, allowing growers to apply dicamba or 2,4-D over-the-top in these production systems where previously these herbicides could only be used prior to planting. Just two years after these herbicides were labeled for in-crop use, growers in Tennessee began experiencing herbicide failures on Palmer amaranth from these chemistries. The University of Tennessee Extension Service determined that while occasionally applications were made to large weeds in a manner not recommended by the label, explaining some failures, the majority of dicamba and 2,4-D applications were made to small, actively growing Palmer amaranth plants and the weed indeed survived the herbicide application.

As Palmer amaranth and other weeds become larger throughout the season, they become more difficult to control. Everitt and Keeling (2007) determined that higher rates of dicamba or 2,4-D were needed to control horseweed (*Erigeron canadensis* L.) and Russian thistle (*Salsola* *iberica* (Sennen & Pau) Botsch. Ex Czerep.) as plant height increased from 3- to 8-, 10- to 15-, and 25- to 46- cm. Similar results were observed when 2,4-D was applied to 30- or 60- cm red morningglory (*Ipomoea coccinea*) where greater control was achieved when the same rate of herbicide was applied to smaller weeds and increasing the herbicide rate was needed to control larger morningglories (Siebert et al. 2004).

Once environmental factors are ruled out in causing herbicide failures, resistance should be investigated as the cause. Auxin herbicides have been an effective option for selective weed control since the 1940's and together, dicamba and 2,4-D have nine weed species with confirmed resistance in the United States (Peterson et al. 2016; Heap 2022). Palmer amaranth resistant to 2,4-D in conservation tillage field on a research station in Kansas was found to be 6- to 11- fold resistant to the herbicide with a metabolic-based resistance mechanism (Shyam et al. 2022). This type of non-target site resistance can predispose Palmer amaranth populations to evolve resistance to other herbicides both within the same and across different herbicide chemistries (Shyam et al. 2020).

To answer the question, why auxin herbicides failed to control Palmer amaranth in dicamba and 2,4-D tolerant cotton and soybean fields, experiments were initiated in grower's fields where herbicide failures were observed to determine the impact of weed height on Palmer amaranth control following applications of dicamba or 2,4-D at increasing rates and determine if reported failures were because of weed size or possible development of herbicide resistance.

Materials & Methods

Non-crop field experiments were conducted at 6 site-years in 2021 and 2022 at the West Tennessee AgResearch and Education Center (35.632003°N, -88.855874°W) in Madison

County, at a grower's field site in Lauderdale County (35.715428°N, -89.918452°W), and at a grower's field site in Madison County (35.781542°N, -88.851567°W). All field sites were non-irrigated. Treatments were arranged as a complete factorial (weed height * herbicide rate) within a randomized complete block design with 3 or 4 replications. Weed height consisted of Palmer amaranth which averaged 10, 20, or 30 cm at the time of application. Herbicide rate included dicamba applied at 0.28 (1/2X), 0.56 (1X), 1.12 (2X) and 2.24 (4X) kg ae ha^{-1} field rates or 2,4-D applied at 0.53 (1/2X), 1.06 (1X), 2.12 (2X), and 4.24 (4X) hg ae ha^{-1}. The reason for those rates was that the herbicide rates specified by the XtendiMax® and Enlist One® labels are 0.56 and 1.06 kg ae ha^{-1}, respectively (Anonymous A and B). All herbicide applications used a CO_2-pressurized backpack sprayer equipped with TTI 11002 nozzles (TeeJet® Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 220 kPa.

Experiments were initiated when Palmer amaranth reached 10 cm in height and subsequent applications were made when Palmer amaranth reached 20 cm and 30 cm in height. Once experiments began, either pyroxasulfone at 0.12 kg ai ha^{-1} or *S*-metolachlor at 1.07 kg ai ha $^{-1}$ was applied to control new flushes of weeds. As needed, clethodim at 0.28 kg ai ha^{-1} was applied to control native junglerice (*Echinochloa colona* L.) and goosegrass (*Eleusine indica* L.) populations.

Palmer amaranth control was visually evaluated 21 days after each application using a 0 to 100% scale (Frans et al. 1986), where 0 = no control and 100 = complete plant necrosis and the number of surviving plants was counted within a random m^2 of each plot. Data were analyzed using the GLIMMIX procedure (2014 Version 9.4, SAS Institute Inc., Cary, NC) for analysis of variance and Tukey's HSD at alpha = 0.05. Year was considered a random effect to broaden the inference space and account for environmental variability when making a recommendation

(Blouin et al. 2011; Carmer et al. 1989; Moore and Dixon 2014). Location was also considered a random effect due to the similarity of Palmer amaranth response across locations ($p > 0.05$).

Results & Discussion

Palmer amaranth control

Height, rate, and height*rate interactions were significant for both dicamba ($p<0.0001$ for all three variables) and 2,4-D ($p<0.0001$ height and rate, $p=0.0349$ height*rate) Palmer amaranth control experiments (Table 12). When applied to ≤10 cm Palmer amaranth, 0.56 kg dicamba ha^{-1} provided 48% control (Table 12). This is the labeled rate of herbicide and recommended weed height on the Xtendimax® herbicide label (Anonymous A). The greatest level of control was achieved following an application of 1.12 or 2.24 kg dicamba ha^{-1} to ≤10 cm Palmer amaranth (63-81%), which was double or quadruple the labeled rate. Similar results were observed when dicamba was applied to 20 cm Palmer amaranth at 2.24 kg ae ha^{-1} (77%).

Results were similar for weed height at time of 2,4-D application. At the maximum single-application labeled rate of Enlist One®, 1.06 kg ae ha^{-1}, ≤10cm Palmer amaranth was controlled 53% (Table 1) (Cite Enlist Label). Greater control was observed when 2.12 or 4.24 kg 2,4-D ha^{-1} were applied to ≤10 cm Palmer amaranth (76-84%) or 4.24 kg 2,4-D ha^{-1} was applied to 20 cm weeds (67%).

Palmer amaranth density

Palmer amaranth height and herbicide rate were both significant ($p<0.05$) for dicamba and 2,4-D experiments when measuring weed density; however, height*rate interactions were not significant at the same p-value (Table 13). Regardless of herbicide or rate, Palmer amaranth control increased as weed height at the time of application decreased. Regardless of weed height, 0.28 or 0.56 kg dicamba ha^{-1} did not decrease Palmer amaranth density compared with the nontreated; similar results were observed with the 0.53 kg 2,4-D ae ha^{-1} rate.

As weed height increased, Palmer amaranth became more difficult to control, though applications at weed height of 10 cm, which is directed by the labeled, did improve control (10 cm 53% vs 20 cm 39%) it was still much less than is acceptable in the field. Increasing the rate of dicamba mitigated the poor control across weed heights; however, this tactic would not be recommended to growers because the rate of herbicide needed to control such large Palmer amaranth would not follow label directions, increase grower input costs, and possibly increase off target movement due to volatilization.

The results from this research agreed with previous studies that larger weeds are harder to control with 2,4-D and dicamba (Everitt and Keeling 2007; Siebert et al. 2004). These results differed from those studies as higher rates of 2,4-D and dicamba on large Palmer amaranth only improved control marginally. These data reaffirm previous reported research that Palmer amaranth in Tennessee is dicamba resistant (Foster and Steckel 2022). This result would agree with research first reported in Kansas that confirmed 2,4-D resistant Palmer amaranth (Shyam et al. 2022; Shyam et al. 2020). The 2,4-D resistant Palmer amaranth in those studies was not also dicamba resistant. This research is the first to show Palmer amaranth that is resistant to both 2,4-D and dicamba. The researchers in Kansas reported that the Palmer amaranth that was resistant to 2,4-D was also resistant to five other herbicide modes of action and that the resistance mechanism was metabolic based, which can allow weeds to more quickly evolve resistance to other herbicides as well.

References

Anonymous a (2022) XtendiMax herbicide label. Accessed 10/9/22 https://www.cdms.net/ldat/ldH7U008.pdf

Anonymous b (2022) Enlit One herbicide label. Accessed 10/9/22 https://www.cdms.net/ldat/ldE27016.pdf

Blouin D, Webster E, Bond J (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer S, Nyquist W, Walker W (1989) Least significant differences for combined analyses of experiments with two- or three- factor treatment designs. Agron J 81:665-672

Everitt JD, Keeling JW (2007) Weed control and cotton (Gossypium hirsutum) response to preplant applications of dicamba, 2,4-D, and diflufenzopyr plus dicamba. Weed Technol 21:506-510

Frans R, Talbert R, Marx D, Crowley H (1986) Experimental design and techniques for measuring and analyzing plant response to weed control practices *in* Camper ND, ed. Southern Weed Science Society. Champaign, IL

Heap I (2022) The International Survey of Herbicide Resistant Weeds. www.weedscience.org Accessed 2/7/22

Moore K, Dixon P (2014) Analysis of combined experiments revisited. Agron J 107:763-771

Oerke EC (2006) Crop losses to pests. J Agri Sci 144:31–43

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D past, present, and future: a review. Weed Technol 30:303–345

Shyam C, Peterson DE, Jugulam M (2022) Resistance to 2,4-D in Palmer amaranth (*Amaranthus palmeri*) from Kansas is mediated by enhanced metabolism. Weed Sci. 70:390-400

Shyam C, Borgato EA, Peterson DE, Dille JA, Jugulam M (2020) Predominance of metabolic resistance in a six-way-resistant Palmer amaranth (Amaranthus palmeri) population. Front Plant Sci 11:614-618

Siebert JD, Griffin JL, Jones CA (2004) Red morningglory (*Ipomoea coccinea*) control with 2,4-D and alternative herbicides. Weed Technol 18:38-44

Smith DT, Baker RV, Steele GL (2000) Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol 14:122-126

Van Wychen L (2019) 2019 Survey of the most common and troublesome weeds in broadleaf crops, fruits, and vegetables in the U.S. and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx Accessed 2/7/22

Young B (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

Appendix

Table 12. Palmer amaranth control at 10, 20, or 30 cm in height following increasing rates of dicamba or 2,4-D.

Herbicide	Height	Rate	Control		Herbicide	Height	Rate	Control	
	cm	kg ae ha^{-1}	%			cm	kg ae ha^{-1}	%	
Dicamba	10	0.28	36	def	2,4-D	10	0.53	40	efg
		0.56	48	cd			1.06	53	de
		1.12	63	b			2.12	76	ab
		2.24	81	a			4.24	84	a
	20	0.28	28	ef		20	0.53	27	gh
		0.56	37	de			1.06	39	fg
		1.12	58	bc			2.12	55	cd
		2.24	77	a			4.24	67	bc
	30	0.28	23	f		30	0.53	24	h
		0.56	29	ef			1.06	29	gh
		1.12	40	de			2.12	40	efg
		2.24	45	cd			4.24	53	def
p-values	<0.0001	<0.0001	<0.0001			<0.0001	<0.0001	0.0349	

Table 13. Palmer amaranth density at 10, 20, or 30 cm in height following increasing rates of dicamba or 2,4-D.

Dicamba				2,4-D			
Height	Density	Rate	Density	Height	Density	Rate	Density
cm	1,000 plants ha^{-1}	kg ae ha^{-1}	1,000 plants ha^{-1}	cm	1,000 plants ha^{-1}	kg ae ha^{-1}	1,000 plants ha^{-1}
Nontreated	295 a	0	295 a	Nontreated	267 a	0	267 a
10	172 b	0.28	274 a	10	140 c	0.53	215 ab
20	196 b	0.56	239 ab	20	191 b	1.06	199 bc
30	280 a	1.12	192 bc	30	199 b	2.12	166 bcd
		2.24	156 c			4.24	128 d
p-values	<0.0001		<0.0001		0.0004		<0.0001

CHAPTER IV

MANAGING AUXIN-RESISTANT PALMER AMARANTH WITH SEQUENTIAL APPLICATIONS OF DICAMBA OR 2,4-D WITH AND WITHOUT GLUFOSINATE

Abstract

Palmer amaranth (*Amaranthus palmeri* S. Wats.) that is resistant to glyphosate and PPO-inhibitors remains a constant threat to cotton and soybean production systems in Tennessee. Recently, dicamba and 2,4-D resistant Palmer amaranth has been reported in west Tennessee, further complicating weed management systems in the area. Bareground experiments were conducted in 2021 and 2022 to determine the best timing between sequential applications and in what order 2,4-D or dicamba should be used with glufosinate in a systems approach to control resistant Palmer amaranth. Palmer amaranth control increased when the interval between postemergence herbicide applications decreased from 21 to 7 days. At the 7 day interval in a dicamba-based system, the order of herbicides did not affect Palmer amaranth control, but with 2,4-D-tolerant systems greatest control was achieved when 2,4-D was applied first followed by either 2,4-D or glufosinate.

Introduction

Soybean (*Glycine max* L. Merr.) and cotton (*Gossypium hirsutum* L.) are Tennessee's two most important row crop commodities having a total farm gate value of more than $1.2 billion (USDA-NASS 2021). Palmer amaranth (*Amaranthus palmeri* S. Wats.) that is resistant to glyphosate and PPO-inhibitors remains a constant threat to these important production systems (Heap 2022; Steckel et al. 2008; Copeland et al. 2018). Palmer amaranth is a weed native to the dry southwestern Americas that has adapted to thrive in many warm climates across the United States (Sauer 1950). If left uncontrolled, Palmer amaranth can severely decrease cotton and soybean yields and impede harvest efficiency (Morgan et al. 2001; MacRae et al 2013; Smith et al. 2000).

In 2017, more options for postemergence herbicides became available when Xtendimax® and Enlist One® received registration for use over-the-top in dicamba or 2,4-D tolerant crops. These herbicide chemistries have come with their own resistance issues. In 2020, a 2,4-D resistant Palmer amaranth was reported on a research farm in Kansas (Shyam et al. 2020). During the same time period, growers in Tennessee began reporting both dicamba and 2,4-D failures in their auxin-tolerant soybean and cotton fields. It was determined that some populations in west Tennessee were resistant to both herbicides (Cite our article once it's published).

Weed management strategies used by soybean and cotton growers often utilize a multi-pass system approach, applying a preemergence herbicide at planting and postemergence herbicide(s) throughout the season to control troublesome weed species. Sequential applications of dicamba or 2,4-D with glufosinate can be effective at controlling small (≤10 cm) Palmer amaranth (Ogden and Dotray 2021; Ogden and Dotray 2022; Smith et al. 2019); however, such research has not been conducted on auxin- or glufosinate- resistant Palmer amaranth populations. Therefore, the objective of this study was to determine the best timing between sequential applications and in what order 2,4-D or dicamba should be used with glufosinate to control Palmer amaranth.

Materials & Methods

A bareground field experiment was initiated in 2021 at the West Tennessee AgResearch and Education Center (35.632003°N, -88.855874°W) (WTREC) and at a grower's field in Lauderdale County (35.715428°N, -89.918452°W) and 2022 at WTREC and a grower's field site in Madison County (35.781542°N, -88.851567°W). The experiment was performed with treatments in a randomized complete block design with 3 or 4 replications. The initial herbicide application was made when Palmer amaranth reached an average of 10 cm in height and sequential applications were made either 7 or 21 days later. Herbicide treatments are described in Table 14. All herbicide applications used a CO_2-pressurized backpack sprayer equipped with TTI 11002 nozzles or AIXR 11002 nozzles for glufosinate treatments (TeeJet® Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 220 kPa. Once experiments began, either pyroxasulfone at 0.12 kg ai ha^{-1} or *S*-metolachlor at 1.07 kg ai ha $^{-1}$ was applied to control new flushes of weeds. As needed, clethodim at 0.28 kg ai ha^{-1} was applied to control native junglerice (*Echinochloa colona* L.) and goosegrass (*Eleusine indica* L.) populations.

Palmer amaranth control was visually evaluated 21 days after the sequential application using a 0 to 100% scale (Frans et al. 1986), where 0 = no control and 100 = complete plant necrosis and the number of surviving plants was counted within a random m^2 of each plot. Data were analyzed using the GLIMMIX procedure (2014 Version 9.4, SAS Institute Inc., Cary, NC) for analysis of variance and Tukey's HSD at alpha = 0.05. Year was considered a random effect to broaden the inference space and account for environmental variability when making a recommendation (Blouin et al. 2011; Carmer et al. 1989; Moore and Dixon 2014). Location was also considered a random effect due to the similarity of Palmer amaranth response across locations (p >0.05).

Results & Discussion

Palmer amaranth control

The number of days between application had a significant effect on Palmer amaranth control 21 days after the sequential application (p=0.0035) (Table 15). Overall, treatments with a 7- day interval between postemergence applications provided 8% better control than treatments with a 21- day interval. For treatments including dicamba with a 7- or 21- day interval, there was no difference between dicamba + glyphosate followed by (fb) dicamba + glyphosate or glufosinate and glufosinate fb dicamba + glyphosate, indicating that it did not matter the order of herbicides (Table 16). At both the 7- and 21-day intervals when 2,4-D was used in conjunction with glufosinate, 2,4-D applied first provided better control than glufosinate applied first. One application of 2,4-D did not provide adequate control of Palmer amaranth, however one application of dicamba + glufosinate provided similar control to sequential treatments. This application cannot be recommended because glufosinate is not an approved tank-mix partner for dicamba due to volatility concerns (Anonymous 2022).

Palmer amaranth density

Overall, there was a difference in Palmer amaranth density when herbicides were applied at a 7- versus 21- day interval ($p<0.0001$) (Table 15). These results were similar to visual ratings. At the 7- day interval, there were more than 86,000 plants ha^{-1} while waiting 21 days between applications increased that number to over 158,000 plants ha^{-1}. All herbicide combinations with a 7- day interval decreased Palmer amaranth density compared with the nontreated control (313,003 plant ha^{-1}) (Table 17). At the 21- day interval, only dicamba + glyphosate fb dicamba + glyphosate or glufosinate decreased Palmer amaranth density compared with the nontreated control. While weed control was lowest following 2,4-D alone, Palmer amaranth density was comparable to all applications with a 7- day interval.

It is noteworthy that the 2,4-D treatment resulted in 152,000 plants ha^{-1} density. The 7-day sequential reduced that population 50%. However, for the 21- day interval the densities were no different than 2,4-D alone. Visually the Palmer amaranth that survived the initial dicamba or 2,4-D herbicide application typically ranged in response from growing very little after application to almost complete recovery. The timing of that recovery varied across the population as well but typically showed immediate regrowth from lower lateral growing points. These data suggest that the 21- day interval is more than enough time for these Palmer amaranth populations to recover enough to better withstand the follow-up herbicide application.

Similarly, Randell et al. (2020) reported that shorter intervals between two glufosinate applications provided better Palmer amaranth control than longer intervals greater than 10 days. Ogden et al. (2021 & 2022) found that when using 2,4-D, the order of herbicides did not matter as long as Palmer amaranth were <10 cm in height but when using dicamba, the auxin applied first followed by glufosinate was the best option. These results would suggest that shortening the interval between herbicide applications, regardless of whether growers are utilizing a dicamba or 2,4-D tolerant production system, to 7 days would increase control of auxin-resistant Palmer amaranth. While the Palmer amaranth populations in these experiments were resistant to dicamba and 2,4-D, multiple applications of these herbicides were able to provide some control of these weeds.

Given that even with the better 7- day sequential treatments provided no better than 81% to 89% control resulting in 64,000 to 84,000 surviving Palmer amaranth ha^{-1} suggests that relying solely on these herbicides for Palmer amaranth control will not be a sustainable weed management strategy. Rather, an integrated weed management approach that incorporates preemergence and postemergence herbicides used in a season-long system along with cultural practices such as cover crops, tillage, and crop rotation will be needed for consistent weed control.

References

Anonymous (2022) XtendiMax herbicide label. Accessed 10/13/22 https://www.cdms.net/ldat/ldH7U008.pdf

Blouin D, Webster E, Bond J (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer S, Nyquist W, Walker W (1989) Least significant differences for combined analyses of experiments with two- or three- factor treatment designs. Agron J 81:665-672

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018) Distribution of PPX2 mutations conferring PPO-inhibitor resistance in palmer amaranth populations of Tennessee. Weed Technol 32:592-596

Frans R, Talbert R, Marx D, Crowley H (1986) Experimental design and techniques for measuring and analyzing plant response to weed control practices *in* Camper ND, ed. Southern Weed Science Society. Champaign, IL

Heap I (2022) The International Survey of Herbicide Resistant Weeds. www.weedscience.org Accessed 10/13/22

MacRae A, Webster T, Sosnoskie L, Culpepper A, Kichler J (2013) Cotton yield loss potential in response to length of palmer amaranth (*Amaranthus palmeri*) interference. J Cotton Sci 17:227–232

Moore K, Dixon P (2014) Analysis of combined experiments revisited. Agron J 107:763-771

Morgan G, Baumann P, Chandler J (2001) Competitive impacts of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. Weed Technol 15:408–412

Ogden GF, Dotray PA (2021) Using glufosinate in sequential application systems with dicamba on Palmer amaranth. J of Exp Ag International 43:10-20

Ogden GF, Dotray PA (2022) Managing Palmer amaranth (*Amaranthus pameri*) with sequential applications of glufosinate and 2,4-D. J of Exp Ag International 44:180-187

Randell TM, Hand LC, Vance JC, Culpepper AS (2020) Interval between sequential glufosinate applications influences weed control in cotton. Weed Technol 34:528-533

Sauer J (1950) The Grain Amaranths: A survey of their history and classification. Ann Mo Bot Gard 1990 37:561–632

Shyam C, Borgato EA, Peterson DE, Dille JA, Jugulam M (2020) Predominance of metabolic resistance in a six-way-resistant Palmer amaranth (*Amaranthus palmeri*) population. Front Plant Sci 11:614-618

Smith DT, Baker RV, Steele GL (2000) Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol 14:122-126

Smith A, Soltani N, Kaastra AJ, Hooker DC, Robinson DE, Sikkema PH (2019) Annual weed management in isoxaflutole-resistant soybean using a two-pass weed control strategy. Weed Technol 33:411–425

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

[USDA-NASS] U.S. Department of Agriculture, National Statistics Service (2021) 2021 State Agriculture Overview – Tennessee https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=tennessee

Appendix

Table 14. Treatments for dicamba and 2,4-D sequential intervals experiments.

Initial Herbicide(s)	Rate	Sequential Herbicide(s)	Rate	Interval between applications
	kg ae or ai ha^{-1}		kg ae or ai ha^{-1}	d
Dicamba + Glyphosate	0.56 + 1.26	Dicamba + Glyphosate	0.56 + 1.26	7
Dicamba + Glyphosate	0.56 + 1.26	Glufosinate	0.88	7
Glufosinate	0.88	Dicamba + Glyphosate	0.56 + 1.26	7
2,4-D + Glyphosate	1.06 + 1.26	2,4-D + Glyphosate	1.06 + 1.26	7
2,4-D + Glyphosate	1.06 + 1.26	Glufosinate	0.88	7
Glufosinate	0.88	2,4-D + Glyphosate	1.06 + 1.26	7
Dicamba + Glyphosate	0.56 + 1.26	Dicamba + Glyphosate	0.56 + 1.26	21
Dicamba + Glyphosate	0.56 + 1.26	Glufosinate	0.88	21
Glufosinate	0.88	Dicamba + Glyphosate	0.56 + 1.26	21
2,4-D + Glyphosate	1.06 + 1.26	2,4-D + Glyphosate	1.06 + 1.26	21
2,4-D + Glyphosate	1.06 + 1.26	Glufosinate	0.88	21
Glufosinate	0.88	2,4-D + Glyphosate	1.06 + 1.26	21

Table 14 continued

Initial Herbicide(s)	Rate	Sequential Herbicide(s)	Rate	Interval between applications
	kg ae or ai ha^{-1}		kg ae or ai ha^{-1}	d
2,4-D	1.06			-
Dicamba + Glufosinate	0.56 + 0.88			-

Table 15. P-values for % Palmer amaranth control and density comparing sequential interval timings.

	Control		Density	
	Interval		Interval	
	-----d-----	-----%-----	-----d-----	plants ha^{-1}
	7	80 a	7	86436 b
	21	72 b	21	158726 a
p-value	P=0.0035		P<0.0001	

Table 16. Palmer amaranth control following sequential applications of dicamba or 2,4-D with and without glufosinate.

Initial Herbicide(s)	Sequential Herbicide(s)	Interval	Control	Letter
		----d----	-----%-----	
Dicamba + Glyphosate	Dicamba + Glyphosate	7	85	ab
Dicamba + Glyphosate	Glufosinate	7	83	ab
Glufosinate	Dicamba + Glyphosate	7	82	abc
2,4-D + Glyphosate	2,4-D + Glyphosate	7	77	abcd
2,4-D + Glyphosate	Glufosinate	7	89	a
Glufosinate	2,4-D + Glyphosate	7	67	bcde
Dicamba + Glyphosate	Dicamba + Glyphosate	21	81	abc
Dicamba + Glyphosate	Glufosinate	21	81	abc
Glufosinate	Dicamba + Glyphosate	21	62	cde
2,4-D + Glyphosate	2,4-D + Glyphosate	21	71	abcd
2,4-D + Glyphosate	Glufosinate	21	81	abc
Glufosinate	2,4-D + Glyphosate	21	57	de
2,4-D		-	49	e
Dicamba + Glufosinate		-	75	abcd

Table 17. Palmer amaranth density following sequential applications of dicamba or 2,4-D with and without glufosinate.

Initial Herbicide(s)	Sequential Herbicide(s)	Interval	Density	Letter
		----d----	plants ha^{-1}	
Nontreated			313,000	a
Dicamba + Glyphosate	Dicamba + Glyphosate	7	85,000	bcd
Dicamba + Glyphosate	Glufosinate	7	79,000	bcd
Glufosinate	Dicamba + Glyphosate	7	83,000	bcd
2,4-D + Glyphosate	2,4-D + Glyphosate	7	72,000	bcd
2,4-D + Glyphosate	Glufosinate	7	64,000	cd
Glufosinate	2,4-D + Glyphosate	7	134,000	bcd
Dicamba + Glyphosate	Dicamba + Glyphosate	21	57,000	d
Dicamba + Glyphosate	Glufosinate	21	111,000	bcd
Glufosinate	Dicamba + Glyphosate	21	205,000	ab
2,4-D + Glyphosate	2,4-D + Glyphosate	21	195,000	abc
2,4-D + Glyphosate	Glufosinate	21	189,000	abcd

Table 17 continued

Initial Herbicide(s)	Sequential Herbicide(s)	Interval	Density	Letter
		----d----	plants ha^{-1}	
Glufosinate	2,4-D + Glyphosate	21	195,000	abc
2,4-D		-	152,000	bcd
Dicamba + Glufosinate		-	84,000	bcd

CONCLUSION

Dicamba and 2,4-D are important tools for cotton and soybean growers in the battle against glyphosate and PPO-resistant Palmer amaranth biotypes. Identifying and characterizing auxin resistance is an important step in controlling resistant Palmer amaranth biotypes and preserving the life of these chemistries. The first objective of this research was to document the level of dicamba resistance through a survey of problem fields and dose response assay in the greenhouse. Data documented a segregating population of Palmer amaranth resistant to dicamba in Tennessee. It ranged from 11 accessions with control similar to the susceptible check to three accessions (Caroll, Dyer, Lauderdale 2) showing resistance ratios of of 1.85 to 2.49. The Lauderdale 1 accession is confirmed highly resistant with a resistant ratio of 14.25. Another step to confirm resistance is documenting heritability of the resistance between generatons. The history of Palmer amaranth escaping dicamba in the Lauderdale 1 location from 2019 to 2021 in the growers field, preliminary field research and in this greenhouse dose response would indicate that the dicamba resistance has passed between generations. This demonstrates the dicamba resistance allele or alleles were passed from the 2019 Palmer amaranth generation to the 2020 and the 2021 generations. This research documents the first findings of Palmer amaranth control failures, in cotton and soybean fields, due to the evolution of dicamba resistance.

The second objective was to explore possible resistance mechanisms within Palmer amaranth to auxin herbicides by applying a cytochrome P-450 inhibitor (malathion) in conjunction with dicamba and evaluate crop response from this mixture.

The addition of malathion did not reverse dicamba resistance from populations collected from Tennessee. However, this does not necessarily rule out metabolic resistance or cytochrome P-450s being a key player in dicamba resistance due to the hundreds of P-450 enzymes and other metabolic enzymes present in plants. This result was not consistent in the Macon County or Worth County, GA locations where malathion did improve control of large (15 to 38 cm) Palmer amaranth. While visual injury was observed, cotton lint yield was not reduced by the addition of malathion. In dicamba-susceptible Palmer amaranth populations, the addition of malathion to the labeled rate of dicamba improved control, but as herbicide rate increased, the benefit of adding malathion diminished. In dicamba-resistant Palmer amaranth populations, malathion did not improve control. Therefore, this research suggests that adding malathion does not reverse dicamba resistance in Palmer amaranth and the insecticide increased crop injury.

The third objective was to determine the impact of weed height on Palmer amaranth control following applications of dicamba or 2,4-D at increasing rates and determine if reported failures in grower’s fields were because of weed size or possible development of herbicide resistance. Results showed that larger weeds are harder to control with 2,4-D and dicamba, and in auxin-resistant Palmer amaranth populations, control only improved marginally with increased rates of these herbicides. Field research in this study was the first to confirm Palmer amaranth that is resistant to both 2,4-D and dicamba.

The final objective was to investigate the best timing between sequential applications and in what order 2,4-D or dicamba should be used with glufosinate to control auxin-resistant Palmer amaranth. These results would suggest that shortening the interval between herbicide applications, regardless of whether growers are utilizing a dicamba or 2,4-D tolerant production system, to 7 days would increase control of auxin-resistant Palmer amaranth. While the Palmer amaranth populations in these experiments were resistant to dicamba and 2,4-D, multiple applications of these herbicides were able to provide some control of these weeds.

Palmer amaranth continues to be a problematic weed for cotton and soybean growers across the United States. With herbicide resistance cases on the rise, an integrated weed management approach that incorporates both preemergence and postemergence herbicides with cultural practices such as cover crops or tillage will be needed for weed control.

VITA

Delaney Caitlin Foster was born and raised in Perry, Georgia. She obtained a Bachelor of Science degree in Crop and Soil Sciences from Abraham Baldwin Agricultural College in Tifton, Georgia. She fell in love with weed science through an undergraduate research assistant position with Dr. Stanley Culpepper at the University of Georgia. Delaney went on to pursue a Master of Science degree in Plant and Soil Science with a concentration in crop protection from Texas Tech University. She is currently a PhD student at the University of Tennessee and upon completion of her degree plants to pursue a degree as an agronomist in the agriculture industry.